OPCW

Scientific Advisory Board

Sixteenth Session
4 – 6 April 2011

SAB-16/1
6 April 2011
Original: ENGLISH

REPORT OF THE SIXTEENTH SESSION OF THE SCIENTIFIC ADVISORY BOARD

1. AGENDA ITEM ONE – Opening of the session

The Scientific Advisory Board (SAB) met for its Sixteenth Session from 4 to 6 April 2011 at the OPCW Headquarters in The Hague, the Netherlands. The session was opened by the Chairperson of the SAB, Philip Coleman of South Africa. Mahdi Balali-Mood of the Islamic Republic of Iran served as Vice-Chairperson. A list of participants appears as Annex 1 to this report.

2. AGENDA ITEM TWO – Election of the Chairperson and of the Vice-Chairperson of the Scientific Advisory Board[1]

By consensus, the SAB members re-elected Philip Coleman as the Chairperson of the SAB for a term of one year. Mahdi Balali-Mood was re-elected as Vice-Chairperson for a term of one year.

3. AGENDA ITEM THREE – Adoption of the agenda

The SAB adopted the following agenda for its Sixteenth Session:

1. Opening of the session
2. Election of the Chairperson and Vice-Chairperson of the Scientific Advisory Board
3. Adoption of the agenda
4. *Tour de table* to introduce Scientific Advisory Board Members
5. Welcome address by the Director-General
6. Overview of developments at the OPCW since the last session of the Scientific Advisory Board

[1] In accordance with paragraph 1.1 of the "Rules of Procedure for the Scientific Advisory Board and the Temporary Working Groups of Scientific Experts" (EC-XIII/DG.2, dated 20 October 1998).

7. Establishment of a drafting committee

8. Report of the fifth meeting of the temporary working group on sampling and analysis

9. Scheduled chemicals, including ricin and saxitoxin

10. Incapacitating chemical agents

11. Novel toxic compounds

12. Captive use of Schedule 1 chemicals

13. Production by synthesis

14. Convergence of chemistry and biology

15. Plan for compiling the report of the Scientific Advisory Board on developments in science and technology

16. Outreach to the scientific community

17. Possible contribution of the Scientific Advisory Board to the Conference on International Cooperation and Chemical Safety and Security

18. Future work of the Scientific Advisory Board

19. Any other business

20. Adoption of the report

21. Closure of the session

4. AGENDA ITEM FOUR – *Tour de table* to introduce Scientific Advisory Board members

The meeting was opened with a *tour de table* in order to introduce new SAB members to existing SAB members; the new members are: Abdullah Saeed Al-Amri of Saudi Arabia, Neivy Fernández Manresa of Cuba, Slawomir Neffe of Poland, Paula Vanninen of Finland, and Nan Zhang of China.

5. AGENDA ITEM FIVE – Welcome address by the Director-General

5.1 The Director-General welcomed the members of the SAB, and in particular, the new members. The Director-General expressed to Alberto Fratadocchi of Italy, Godwin Ogbadu of Nigeria, and Valery Kukhar of Ukraine, whose terms of office on the SAB will finish in August 2011, his deep appreciation for their commitment and contribution to the work of this Board. The Director-General encouraged the temporary working group on sampling and analysis to continue its work, which is of great importance for the OPCW. The Director-General expressed his appreciation to

the members of the temporary working group and its Chairperson, as well as to the heads of the correspondence groups, for their dedicated work and commitment.

5.2 As regards the question of the convergence of chemistry and biology, the Director-General stated that he believed that the SAB is the right body to conduct a thorough study, which would include an assessment of the feasibility of production of Schedule 1 compounds using biologically mediated processes. The Director-General went on to say that he was also looking forward to receiving the contributions of the SAB on this topic, together with its recommendations on the potential implications of the convergence of chemistry and biology for the Chemical Weapons Convention (hereinafter "the Convention").

5.3 In regard to outreach to the scientific community, the Director-General expressed his expectation that the SAB, in response to his letter to its Chairperson, would provide advice and counsel that would assist the Technical Secretariat (hereinafter "the Secretariat") to understand how to reach out to the scientific community and develop and enhance those relationships. In this respect, he believed that efforts need to be redoubled to finalise a code of conduct, a matter that has been pending for some time.

6. AGENDA ITEM SIX – Overview of developments at the OPCW since the last session of the Scientific Advisory Board

The Secretary gave a presentation to the SAB on developments at the OPCW since the Fifteenth Session of the SAB (which was held from 12 to 14 April 2010). The members were informed about the status of destruction of Category 1 chemical weapons as at 28 February 2011, on the state of play of the membership of the Convention, and on efforts made towards promoting universality. The SAB was briefed on the funding of its activities in 2011 and 2012, and on the financial status of its trust fund.

7. AGENDA ITEM SEVEN – Establishment of a drafting committee

The SAB established a drafting committee, composed of four of its members, to prepare a draft report of its Sixteenth Session.

8. AGENDA ITEM EIGHT – Report of the fifth meeting of the temporary working group on sampling and analysis

8.1 The SAB received the report of the fifth meeting of the temporary working group on sampling and analysis, held on 5 and 6 November 2010 (see Annex 2). Robin Black, Chairperson of the group, presented the key findings, conclusions, and recommendations, which are summarised below. The topics discussed were: on-site analysis procedures; sample preparation for aqueous samples; emerging techniques that have possible applications to on-site analysis; toxin analysis (saxitoxin and ricin); and identification criteria for trace analysis in investigations of alleged use (IAUs) of chemical weapons (environmental and biomedical samples).

8.2 Shortening of on-site sample preparation time for the analysis of aqueous samples continues to be a high priority for the Secretariat. The OPCW Laboratory has developed a procedure that involves absorption of aqueous samples onto Tenax tubes,

on-tube derivatisation, and analysis by thermal desorption gas chromatography-mass spectrometry (GC-MS). The temporary working group agreed that this appeared to be the most promising alternative procedure reported, subject to further validation. The temporary working group and the OPCW Laboratory will request other laboratories to assist in assessing the procedure for its applicability and robustness.

8.3 The temporary working group remained of the view that hollow-fibre liquid-phase microextraction is also a promising technique for preparing aqueous samples. However, assessment in a small number of laboratories suggests that it is less robust and would require greater operator training than the Tenax tube procedure. Other procedures that target a narrower range of analytes, e.g. the use of 1-(diazomethyl)-3,5-bis(trifluoromethyl)benzene to derivatise phosphonic acids directly in aqueous solution, should also be considered for use in appropriate scenarios.

8.4 The temporary working group supported further investigation of fast GC to shorten on-site analysis time. Four laboratories, including the OPCW Laboratory, have investigated fast GC procedures for scheduled compounds. Although good resolution has been maintained with faster GC programmes, there has been some variation in retention indices (RIs), compared to those in the OPCW Central Analytical Database (OCAD).

8.5 The temporary working group remained of the view that solid-phase microextraction (SPME) has shortcomings with regard to on-site OPCW analysis (in terms of robustness and cost), although it is a useful technique in other scenarios. Molecularly imprinted polymers (MIPs) were considered inappropriate for on-site sample preparation.

8.6 An informal experimental survey conducted by the temporary working group has provided useful data on which to base criteria for the identification of saxitoxin. The temporary working group has proposed draft criteria for the SAB to consider.

8.7 A trial proficiency test conducted by the Robert Koch Institute, under the auspices of the Global Health Security Action Group, has provided useful data on which to base criteria for the identification of ricin. Draft criteria will be circulated to the temporary working group members for comment, and for consultation with other laboratories that have the appropriate expertise.

8.8 The temporary working group endorsed the report of the first OPCW confidence-building exercise on biomedical samples. The exercise represents significant progress in broadening expertise in this area. The temporary working group supported the OPCW Laboratory's proposal to hold a short workshop on biomedical samples and a second confidence-building exercise in 2011 and 2012. Following the workshop, which was held at the OPCW on 11 February 2011, the Director-General issued a formal notice of the intention to hold a second exercise in January 2012 (S/908/2011, dated 8 March 2011).

8.9 The temporary working group recommended that the criteria for identification of trace levels of drugs in urine, as used by the World Anti-Doping Agency, should be adapted by the Secretariat, with appropriate modification, to the trace analysis of

environmental and biomedical samples in the context of IAUs of chemical weapons. VERIFIN and the OPCW Laboratory were requested to compose draft criteria for circulation. It is recommended that these criteria should be assessed when they are applied during an analysis of biomedical samples carried out as part of the second OPCW confidence-building exercise, and during an analysis of environmental samples undertaken as a voluntary exercise supplementing a proficiency test.

8.10 Experiences of a Member State mobile laboratory during the recent ASSISTEX 3 exercise highlighted shortcomings in procedures and training. The role of such laboratories in OPCW activities in the context of Article X of the Convention requires clarification.

8.11 The SAB endorsed the report of the temporary working group, and proposed that both it and the temporary working group on sampling and analysis be briefed when they next meet on what further progress has been made in regard to Schedule 2 inspections, and in relation to the effectiveness of sampling and analysis during the 2010 ASSISTEX 3 exercise.

8.12 The SAB noted the importance of exercises to test and refine the procedures to be used for off-site analysis in cases of IAUs of chemical weapons or challenge inspections. A complete exercise may be warranted—one that covers areas from on-site sample collection to the submission to the Director-General of reports from designated laboratories.

9. AGENDA ITEM NINE – Scheduled chemicals, including ricin and saxitoxin

9.1 Robert Mathews of Australia provided a summarised version of a presentation that had been provided during the Eighth Session of the SAB on the evolution of the way salts of scheduled chemicals have been treated, beginning with the negotiations on this issue during the Geneva Conference on Disarmament in the 1980s. It is apparent from the record of the negotiations that the treatment of salts of scheduled chemicals was, in fact, considered by the negotiators, and that the inclusion of some (but not other) salts had remained controversial until the very end of those negotiations. Related to this issue was the question of what constitutes saxitoxin, which is listed in Schedule 1, together with the Chemical Abstracts Service (CAS) registry number of the dihydrate (free base). This situation is of little help when it comes to assessing which form or forms of the molecule are actually considered as being included in the schedules of chemicals.

9.2 A survey of the literature on the matter shows how the understanding of the molecular structure of saxitoxin has evolved over the past decades. Since the elucidation of the structure, the term “saxitoxin” has been variously used to describe the dihydrochloride of the molecule, or the free base, or its dication. More recently (and since the conclusion of the negotiations of the Convention), the nomenclature has become more specific, and distinguishes between saxitoxin dihydrochloride and saxitoxin (di)hydrate. From the record of negotiations, it appears that what negotiators wanted to include in the schedules was the form of saxitoxin that had been weaponised in the past (the agent TZ, which is a salt), and other forms of weaponisable saxitoxin. It should be mentioned, as a side comment, that the issue of what constitutes saxitoxin

shows again that the CAS registry numbers given in the Convention cannot be considered to have regulatory power. They are essentially identification aids.

9.3 The SAB re-considered the draft saxitoxin fact sheet that it had prepared and distributed at its Fourteenth Session, and which had been adjusted as a result of drafting suggestions made by SAB members during the Fifteenth Session. Further drafting suggestions were made at this session.

9.4 It was agreed that the draft saxitoxin fact sheet should be finalised, preferably by a correspondence group, prior to or at the Seventeenth Session of the SAB.[2]

9.5 It was also recommended that the same group prepare a similar fact sheet for ricin.

10. AGENDA ITEM TEN – Incapacitating chemical agents

10.1 Some scientific aspects about incapacitating chemical agents were presented by Robin Black. There is no definition of incapacitating chemical agents in the context of the Convention. The following was suggested by the presenter as a working definition solely for the purposes of the presentation: "An agent that renders an individual incapable of concerted physical and/or mental action (in the particular context of use), but whose effects are perceived to be temporary and readily reversible". Riot control agents (RCAs), which are defined by the Convention, are usually distinguished on the basis that they target peripheral sensory organs, but their effects rapidly dissipate when exposure is terminated.

10.2 It was noted that the term "non-lethal agent" is commonly used, but was considered inappropriate and misleading, since the toxicity of chemicals is a matter of dosage.

10.3 Some of the problems of incapacitating chemical agents were summarised, particularly in regard to their safe use in hostage situations. Chemicals considered to have high safety margins on the basis of LD_{50}/ED_{50} ratios, in the context of pharmaceutical use, can have very low safety margins when factors such as maximum coverage, minimal casualties, variability in human response, uneven dissemination, and a need for the rapid onset of action are considered. Furthermore, pharmaceutical companies usually publish toxicity data that have been obtained by means of experimentation with small rodent species—information that may not extrapolate to higher species. In particular, there are large differences in how various species react in terms of the toxicity of opioids. It was also emphasised that it is not just what incapacitating chemical agent is used for law enforcement purposes, but how it is used.

10.4 A summary was provided on some of the types of chemicals/pharmaceuticals that have been considered as incapacitating chemical agents (according to open literature sources). Most incapacitating chemical agents emerged from drug programmes in the 1960s and 1970s, and are centrally acting compounds that target specific neuronal pathways in the brain. BZ, a glycollate psychotomimetic, is the only centrally acting incapacitating chemical agent that has been identified as having been weaponised as a

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[2] Comprising Robin Black, Philip Coleman, Herbert De Bisschop, Robert Mathews, Stefan Mogl, Jean-Claude Tabet, and Paula Vanninen.

chemical-warfare agent, and is listed in Schedule 2 of the Annex on Chemicals. The most recent attention has focused on opioids of the fentanyl class. These are in clinical use as analgesics/anaesthetics, and in veterinary use for immobilising large animals. They are reported to have been components of the agent used in ending the siege of a Moscow theatre in 2002.

11. AGENDA ITEM ELEVEN – Novel toxic compounds

11.1 The Chairperson provided an introduction to the topic of new toxic compounds that are not included in the schedules of chemicals. This has been attracting increasing attention in recent years, particularly among non-governmental organisations (NGOs). Although very little information has appeared in the public domain, there have been claims that a new class of nerve agents, known as "Novichoks", has been developed. In December 2008, a former defence scientist published a book, which included information on structures reported to be those of the new agents. Some of these structures meet the criteria for Schedule 2 B4 (S2 B4); however, all others are non-scheduled chemicals. The author claimed that the toxicity of certain "Novichok" agents may exceed that of VX.

11.2 In a discussion of the issue, SAB members emphasised that, to date, there has been no confirmation of the author's claims, nor has any peer review been undertaken in regard to the information on these chemicals in the scientific literature on this subject. The SAB noted that those chemicals containing a phosphorus atom to which is bonded one methyl, ethyl or propyl (normal or iso) group but not further carbon atoms would be declarable as S2 B4 chemicals above a threshold of one tonne. The SAB asked the Secretariat if such chemicals had ever been declared. The Secretariat replied that it has no record of any such declarations. With respect to non-scheduled chemicals relevant to "Novichoks", it was noted that for declarations under the other chemical production facility (OCPF) regime, the name of the chemical is not required, and therefore, the Secretariat would not be able to determine (from declarations received) if there are facilities producing "Novichok" agents that are also non-scheduled chemicals.

11.3 The SAB also noted more extensive reporting in the 1990s of nerve agents of the GV class, which were reported to have high toxicity and low stability. The existence of highly toxic carbamates, reported in the 1980s, was also noted. The SAB expressed the opinion that the OPCW, as the implementing body for the Convention, should expand its knowledge of such chemicals in order to assist States Parties to fulfill their obligations under the Convention, and particularly in regard to Articles IX and X.

12. AGENDA ITEM TWELVE – Captive use of Schedule 1 chemicals

12.1 A presentation was given by Steve Wade, Head of the Declarations Branch, outlining the results of consultations held at the OPCW on a case involving the production of a Schedule 1 chemical in a captive use situation.

12.2 In 2010, Denmark identified a process in use at a pharmaceutical company, which used HN2 as a captive intermediate in the production of a drug product. Recognising the implications of this in terms of the Convention, Denmark submitted a request to

the Executive Council (hereinafter "the Council") (EC-62/NAT.6, dated 17 September 2010), seeking a decision from the Council on the following:

(a) an understanding of the meaning of a chemical weapons production facility (CWPF) in relation to Part VI of the Verification Annex of the Convention (hereinafter "the Verification Annex"), and of the concept of captive use; and

(b) an amendment to the production limits, as envisaged in the decision by the Conference of the States Parties (hereinafter "the Conference") on captive use of Schedule 1 chemicals (C-10/DEC.12, dated 10 November 2005).

12.3 The Council requested a legal opinion from the Secretariat and also undertook informal consultations to consider this issue. During consultations, France presented a non-paper on an alternate synthesis route that did not involve Schedule 1 chemicals. As a consequence, Denmark withdrew its request to the Council. At its next session, the Council decided that no further action was required on the proposals. Steve Wade then raised the question as to whether there might be other facilities using HN2 as an intermediate. In addition, there is a broader question concerning whether there are other commercially significant processes producing other Schedule 1 chemicals as captive intermediates.

12.4 Following the presentation of the Secretariat, Herbert De Bisschop provided an update to his 2005 study which was included in the SAB report (S/528/2005, dated 1 November 2005). The current study focused on possible commercial chemistry routes that could be in use producing nitrogen mustards as captive use intermediates. Literature citations, based on patents and other publications, regarding the use of nitrogen mustards in reactions (including salts) revealed a number of potential applications in the pharmaceutical industry. However, the problem remains of detecting captive use in industry, since patents may not give the full picture. There is no assurance that commercial processes will use the respective patent or use it as described. As a rule, the production of organic compounds requires a licence that is obtained from a government agency (such as the European Medical Association for Pharmaceuticals). The licence contains the full description of the real process route that takes place during synthesis. Therefore, it is very difficult to obtain a full picture of captive use of nitrogen mustard in commercial use without a comprehensive search of the licence databases in all countries.

12.5 From a chemical point of view, finding alternative process routes (not involving nitrogen mustards) is not expected to be a problem. However, for pharmaceutical applications, the company may have to repeat toxicity studies and possibly clinical trials, if there is a change in the impurity profile of the product during the new synthesis route. This can be a costly and time-consuming process in the development stage for marketing a drug product.

12.6 The SAB recommends that the Secretariat develop a communication plan to inform States Parties and industry associations of the implications (including the definition in subparagraph 8(b)(i) of Article II, and the possible need to amend production limits in Part VI of the Verification Annex, as regulated in decision C-10/DEC.12, which is related to the production of Schedule 1 chemicals in a captive use situation and, in particular, nitrogen mustards).

13. AGENDA ITEM THIRTEEN – Production by synthesis

13.1 With reference to the draft report of the Vice-Chairman of the cluster on chemical-industry and other Article VI issues, it was proposed that the Council request the Director-General to refer the issue to the SAB, with the request that it should keep it under its consideration, and to report to the Council as and when appropriate.[3]

13.2 Robert Mathews provided the SAB with a historical briefing on the term "produced by synthesis", which is used in subparagraphs 1(a) and (b) of Part IX of the Verification Annex. There was no agreement during the negotiations in Geneva whether the OCPF regime should cover the production of chemicals using biologically mediated processes, and the term "produced by synthesis" was used as a creative ambiguity. The interpretation of the term "produced by synthesis" can have an impact as to whether a facility producing discrete organic chemicals (DOCs) is considered declarable as an OCPF. The key issue in the implementation of Part IX of the Verification Annex is whether biologically mediated processes can also be considered as processes involving chemical synthesis, and thus be covered by the term "produced by synthesis". This subject was subsequently discussed in the Preparatory Commission, without agreement being reached, at least in part because OCPF inspections were not scheduled to start until 2000. The matter has still not been agreed upon by States Parties.

13.3 A report was prepared by the SAB in 1999 addressing the term "production by synthesis". The report concluded that from a scientific standpoint, it is no longer possible to make a clear distinction between "chemical" and "biological and biologically mediated" processes. The emphasis should be on the product rather than on the process. This report was considered by policy-making organs and a draft decision was prepared for the Conference at its Fourth Session (C-IV/DEC/CRP.22, dated 28 June 1999). The draft decision was not adopted. This resulted in a more narrow classical interpretation of chemical synthesis, which did not include biologically mediated processes.

13.4 As a result, the current practice of the Secretariat is that biologically mediated processes are not considered to be included in terms of DOC production, and therefore declarations are not required under Part IX of the Verification Annex.

13.5 The SAB discussed to what extent biologically mediated processes are being utilised in commercial-scale facilities.

13.6 The SAB determined that it is not in a position to make a full assessment without further study of current chemical-industry practice, where there appears to be an increase in the use of biologically mediated processes. Bill Kane offered to undertake a review of the matter and report to the SAB at its next session in November 2011.

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[3] Extract from EC-62/4, dated 5 October 2010 (subparagraph II.1(ii)): "**Current status:** At the Industry Cluster consultations on 22 June 2010 it was proposed that the Council request the Director-General to refer the issue to the SAB with the request that it should keep the issue under its consideration, and to report to the Council as and when appropriate".

14. AGENDA ITEM FOURTEEN – Convergence of chemistry and biology

14.1 Following a short briefing from the Chairperson, the SAB discussed the following aspects of the convergence of chemistry and biology: The fundamental scientific aspects of this issue and its implications for the operation of the Convention; and the importance of continued interaction between SAB members and experts from the biological sciences.

14.2 It was recognised that there has always been a relationship between the fundamental sciences of chemistry and biology. At the molecular level, biological systems have always obeyed the laws of chemistry, just as at the atomic and subatomic levels, chemicals have always obeyed the laws of physics. What is changing is the rapidly growing understanding by scientists of the fundamental chemistry of living systems—a result of the work of interdisciplinary teams using more sophisticated equipment and experimentation. One view expressed within the SAB was that a number of different issues were being covered under the general umbrella term of "convergence", specifically: The increasing use of biologically mediated processes (catalysts, naturally occurring organisms and genetically modified organisms) for the production of chemicals (biosynthesis); the more recent development of the chemical synthesis of replicating organisms (which has taken place with small viruses so far); and recombinant DNA technology that allows replacement of the original genome in bacterial cells with synthetically produced genomes, to produce bacteria with new capabilities (synthetic biology).

14.3 These advances promise many benefits to humankind, including more efficient food production, improvements to medicines and to health care, the generation of renewable energy sources, and the enhancement of pollution management. However, it was also recognised that these developments could potentially be misused, for example, to produce toxic chemicals and toxins through "biologically mediated processes". It was recognised that it would be important to have access to expertise from the biotechnology industry to assist the SAB in reaching an assessment of this rapidly developing area, and to help it gain an understanding of how toxins are produced through biologically mediated processes. The SAB recommended the establishment of a temporary working group to further explore the convergence of chemistry and biology, and the implications of this for the Convention. In order to obtain a better appreciation of current and future capabilities, the working group should include experts in biology and also individuals knowledgeable about industrial-scale biotechnology production.

14.4 The SAB recommended increasing the dialogue between the different stakeholders of the Convention, so that the necessary inputs to assist in negotiations will be generated with sufficient time to build consensus on complex issues that might imply changes to the Convention. The dialogue should include members of the SAB, as well as biological experts involved in the consideration of advances in science and technology relevant to the Biological Weapons Convention (BWC). One possible early step was the suggestion that the InterAcademy Panel (IAP)[4] and some of the other relevant scientific unions in the life sciences, such as the International Union of Biochemistry and Molecular Biology (IUBMB) and the International Union of Microbiological

[4] Website = http://www.interacademies.net/

Societies (IUMS), be invited to participate in the forthcoming IUPAC[5]/OPCW meeting, which will be considering the developments in science relevant to the Convention, in advance of the Third Special Session of the Conference of the States Parties to Review the Operation of the Chemical Weapons Convention (hereinafter "the Third Review Conference") (see paragraph 15.3 below).

15. AGENDA ITEM FIFTEEN – Plan for compiling the report of the Scientific Advisory Board on developments in science and technology

15.1 The Secretary of the SAB, Patrice Palanque, introduced a tentative plan of activities for the SAB for its report to the Director-General on developments in science and technology relevant to the Convention. The plan describes the preparations that should be undertaken by the SAB between its Sixteenth and Twentieth Sessions; in addition, the plan is based on the assumption that the Third Review Conference will take place in 2013, and any relevant recommendations will be considered by the SAB at its Twenty-First Session. It is projected that the SAB will conduct two sessions per year from 2011 to 2013.

15.2 In this context, the SAB agreed to hold its Seventeenth Session from 21 to 23 November 2011. The session should be preceded by the convening of the sixth meeting of the temporary working group on sampling and analysis on 17 and 18 November 2011.

15.3 The Secretary also informed the SAB that the OPCW had entered into discussion with the IUPAC to support it in a review of developments in science and technology that are relevant to the Convention. The IUPAC and the OPCW are planning, as happened before the First and Second Special Sessions of the Conference of the States Parties to Review the Operation of the Chemical Weapons Convention, to conduct a technical workshop to identify relevant developments. It is planned that this workshop will take place in early 2012, possibly at the Spiez Laboratory in Switzerland. Dates and a venue have yet to be confirmed between the OPCW and the IUPAC. It was suggested that the workshop should take place prior to the Eighteenth Session of the SAB, which should be held at the end of April 2012.

15.4 Because the workshop is scheduled only two months following the Seventeenth Session of the SAB in November, the SAB decided to establish a correspondence group to propose a list of topics that it wishes to have discussed. The coordinator of this correspondence group is Patrice Palanque.

16. AGENDA ITEM SIXTEEN – Outreach to the scientific community

16.1 The Chairperson commenced discussion of this item by referring the SAB to the letter from the Director-General, which highlighted the view from within his Office that the Board should intensify links with the scientific community, and develop these links in a more sustained way.

16.2 The SAB recalled the OPCW/IUPAC outreach/codes of conduct workshop, held in 2005, which resulted in the development of an IUPAC Code of Conduct and a number

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5 IUPAC = International Union of Pure and Applied Sciences

of teaching modules based on the general ethical principles of chemistry; these modules are available on the IUPAC website.[6]

16.3 A number of SAB members outlined various education and outreach activities that they have been involved in, including postgraduate courses. Other SAB members mentioned that their efforts to provide presentations and courses related to enhancing education about and awareness of the Convention had not materialised. The important role the National Authority plays in encouraging such activities was noted.

16.4 The Chairperson of the SAB requested SAB members to provide their input in response to the letter from the Director-General within four weeks, in order for him to draft a reply.

17. AGENDA ITEM SEVENTEEN – Possible contribution of the Scientific Advisory Board to the Conference on International Cooperation and Chemical Safety and Security

The Director-General requested the SAB to consider its possible contribution to the upcoming Conference on International Cooperation and Chemical Safety and Security, which will be held in September. The Director-General has established a task force and the SAB was given an overview of its work by Malik Ellahi from the Secretariat. A Note by the Secretariat providing further information on the organisation of the Conference has been released and has been made available to members of the SAB via its Portal site. Members of the SAB were supportive and willing to provide input and support to this conference and will respond with specific offers when the programme is available.

18. AGENDA ITEM EIGHTEEN – Future work of the Scientific Advisory Board

18.1 The SAB also agreed that the detailed roadmap for the future work of the SAB should be conducted by correspondence. This roadmap should include a detailed plan of actions for the Board to undertake and reports that should be written before the Third Review Conference. It will be further discussed at the next session of the SAB. The importance of the roadmap was emphasised, since there is only limited time to prepare documentation for the Third Review Conference.

18.2 The SAB recommended discussing the following topics at its next session, which will be held from 21 to 23 November 2011:

(a) the report of the sixth meeting of the temporary working group on sampling and analysis, to be held the week before the Seventeenth Session of the SAB;

(b) the fact sheets on saxitoxin and ricin;

(c) the feedback received from the Secretariat on the question of captive use of Schedule 1 chemicals;

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6 Website = http://multiple.kcvs.ca/

(d) the convergence of chemistry and biology: Consideration of the report of the first meeting of the temporary working group on the convergence of chemistry and biology (if the group is able to be established before the next session of the SAB);

(e) the update and finalisation of the roadmap specifying the work the SAB should undertake in the future (during 2012 and 2013); and

(f) outreach to the scientific community.

19. AGENDA ITEM NINETEEN – Any other business

19.1 There was some discussion concerning the length of the SAB meeting, and it was suggested that a flexible approach to the length of meetings should be adopted, depending on the agenda and on the views of the Chairperson and the Secretary to the SAB.

19.2 The Secretary of the SAB, Patrice Palanque, informed the Board that this was his last meeting and that he was leaving the Secretariat in the autumn. The Chairman officially thanked Patrice Palanque for all his work for the SAB and his continuous efforts to improve its work and enhance its internal and external interaction. All members of the SAB wished him all the best in the future.

20. AGENDA ITEM TWENTY – Adoption of the report

The SAB considered and adopted the report of its Sixteenth Session.

21. AGENDA ITEM TWENTY-ONE – Closure of the session

The Chairperson closed the session at 20:15 on 6 April 2011.

Annexes:

Annex 1: List of Participants in the Sixteenth Session of the Scientific Advisory Board

Annex 2: (English only, unedited): Report of the Fifth Meeting of the SAB Temporary Working Group on Sampling and Analysis

Annex 1

LIST OF PARTICIPANTS IN THE SIXTEENTH SESSION OF THE SCIENTIFIC ADVISORY BOARD[7]

	Participant	State Party
1.	Djafer Benachour	Algeria
2.	Alejandra Graciela Suárez	Argentina
3.	Robert Mathews	Australia
4.	Herbert De Bisschop	Belgium
5.	Nan Zhang	China
6.	Neivy Fernández Manresa	Cuba
7.	Paula Vanninen	Finland
8.	Jean-Claude Tabet	France
9.	Michael Geist	Germany
10.	Devendra Kumar Dubey[8]	India
11.	Mahdi Balali-Mood	Iran (Islamic Republic of)
12.	Alberto Breccia Fratadocchi	Italy
13.	José González Chávez	Mexico
14.	Muhammad Zafar-Uz-Zaman	Pakistan
15.	Slawomir Neffe	Poland
16.	Igor V. Rybalchenko	Russian Federation
17.	Abdullah Saeed Al-Amri	Saudi Arabia
18.	Slavica Vučinić	Serbia
19.	Philip Coleman	South Africa
20.	Stefan Mogl	Switzerland
21.	Valery Kukhar	Ukraine
22.	Robin Black	United Kingdom of Great Britain and Northern Ireland
23.	William Kane	United States of America

7 Shuzo Fujiwara of Japan, and Godwin Ogbadu of Nigeria did not participate in the Sixteenth Session of the SAB.

8 Devendra Kumar Dubey joined the session on Wednesday 6 April 2011.

Annex 2

REPORT OF THE FIFTH MEETING OF THE SAB TEMPORARY WORKING GROUP ON SAMPLING AND ANALYSIS, THE HAGUE, THE NETHERLANDS 18 – 19 NOVEMBER 2010

1. Introduction

1.1 The Temporary Working Group (TWG) on Sampling and Analysis (S&A) of the Scientific Advisory Board (SAB) held its fifth meeting on 18 and 19 November 2010 at the OPCW Headquarters in The Hague.

1.2 The meeting was chaired by Robin Black on behalf of the SAB.

1.3 The list of participants in the meeting is given in Appendix 1. With reference to rule 2 of the rules of procedure of the SAB, the meeting was attended by Mrs Helma Spruit (18 November) and Mr Rob Groeneveld (19 November) of the Netherlands, and Mr Rahman Mirzaei of the Islamic Republic of Iran.

1.4 The following agenda was adopted:

(a) Opening of the meeting by the Director-General

(b) Adoption of the agenda (Chairman of the Temporary Working Group on Sampling and Analysis)

(c) Sample preparation for aqueous solutions of degradation products

(d) Applications of molecularly-imprinted polymers (MIPs) (at the request of the SAB)

(e) Emerging techniques with possible applications for on-site analysis: Fast GC, SPME, LC-MS, miniaturisation, others

(f) Toxin analysis (ricin and saxitoxin), off site and on site. (Martin Schaer, Sten-Åke Fredriksson):

i) Results / discussion of ricin round robin

ii) Results / discussion of saxitoxin experimental data survey

iii) Recommended provisional criteria for identification of ricin & saxitoxin

(g) Discussion of the Report of the First OPCW Confidence-Building Exercise on Biomedical Sample Analysis (Robin Black)

(h) Criteria for trace analysis in investigations of alleged use (Paula Vanninen)

(i) Any other business

(j) Summary of conclusions and recommendations

(k) Closure of the meeting.

2. Opening of the meeting by the Director General

2.1 The Director-General welcomed the members of the TWG and thanked them for the good work performed to date.

2.2 He noted that the TWG had a full agenda that would cover a wide spectrum of important topics, including: faster procedures for on-site analysis; application of molecularly-imprinted polymers; criteria for trace analysis; methods for the identification of saxitoxin and ricin; and biomedical samples.

2.3 He reassured the members of his continued support for the important work performed by the TWG and SAB. He emphasised that this work enhanced the OPCW's capabilities and helped the Organisation better understand the impact of scientific advances on the Convention, thus allowing implementation practices to be adjusted accordingly.

2.4 The Director-General concluded by informing the TWG that the OPCW would be hosting a conference in September 2011 to celebrate the International Year of Chemistry 2011. The theme of the conference would be international cooperation, including chemical safety and chemical security.

3. Sample preparation for aqueous solutions of degradation products

3.1 A major limitation of on-site GC-MS analysis as currently performed by inspectors is the time and equipment required for the identification of polar degradation products and precursors of CW agents in aqueous samples. The current operating procedure requires concentration of aqueous samples to dryness prior to derivatisation, a procedure which is lengthy and requires additional equipment.

Use of Tenax tubes

3.2 Oliver Terzic from the OPCW Laboratory described a promising new procedure for the rapid analysis of aqueous samples (Appendix 2). Small aliquots of aqueous or mixed solvent samples are absorbed onto Tenax tubes, the water is removed by a short period of heating under a stream of helium, and polar compounds are converted on-tube to their trimethylsilyl derivatives. Analysis is performed using full-scan thermal desorption GC-MS. The procedure is technically simple and fast, and requires only a few microlitres of sample and derivatising agent. It could also be used for concentrating samples, thus decreasing limits of detection. Volatile low polarity compounds and derivatised polar compounds are analysed in a single GC-MS run. Compared to the current on-site sample preparation and analysis procedure, the time required for removal of water is reduced from 3 hours to 5 minutes, and overall analysis time from 6 hours to 25 minutes. Furthermore, the new procedure utilises

equipment already used for on-site analysis, and reduces the logistic burden of equipment compared to the current procedure. It has been successfully applied to several proficiency test samples.

3.3 The TWG members agreed that this appears to be a promising improvement to the current procedure, subject to further validation. The TWG and the OPCW Laboratory encourage other laboratories to assist in assessing the procedure for applicability with regard to analytes and for robustness.

Alternative approaches

3.4 The TWG discussed alternative approaches to aqueous sample preparation. At its second meeting, held in Madrid in 2007, the TWG discussed a number of approaches, including hollow fibre liquid phase microextraction, on-tube solid phase extraction or microextraction, aqueous or mixed phase derivatisation, and molecularly imprinted polymers. Hollow fibre liquid phase microextraction was judged to be the most promising and broadly applicable of these techniques. However, a disadvantage is that it requires a moderate degree of operator training, and a number of laboratories have experienced experimental difficulties, particularly with regard to loss of the extracting solvent. The TWG members retain the view that this technique merits further investigation, although the Tenax tube method outlined above appears to be more robust and easier to adopt.

3.5 The TWG reaffirmed its view that solid phase microextraction (SPME) is insufficiently robust, particularly with regard to 'dirty' samples, to justify further investigation for on-site analysis. Moreover, the sampling fibres used are expensive. SPME can, however, be a very useful technique for environmental sampling in other scenarios, and in biomedical sample analysis.

3.6 The TWG members expressed the view that rapid procedures developed for a narrower range of analytes, such as alkyl alkylphosphonic acids (degradation products of nerve agents), should not be dismissed, nor should they be constrained by the choice of derivative. An example is a rapid procedure reported by FOI Sweden, which converts phosphonic acids to their bis-3,5-trifluoromethylbenzyl derivatives directly in aqueous solution.

3.7 Armando Alcaraz proposed that laboratories use proficiency test samples to assess the robustness and utility of new methods/techniques, in parallel with the recommended procedures.

4. Applications of molecularly imprinted polymers (MIPs)

4.1 At its fifteenth session, the SAB was given an excellent overview of applications of molecularly imprinted polymers (MIPs) by Professor Pernelle of the Conservatoire National des Arts et Métiers (CNAM). This included applications of MIPs to chemical defence in the areas of detection, on-site monitoring and decontamination. MIPs are sometimes described as synthetic antibodies and are produced by preparing a polymer in the presence of an analyte as a template; the analyte is then removed by extraction. MIPs have been a rapidly expanding area of interest and Professor

Pernelle expressed the view that they should find applications in CWC-related analytical procedures, as well as in detector/sensor applications. There were, however, questions from a number of SAB members about the limitations of MIPs. For example, a major limitation of first-generation MIPs for sample extraction was their lack of applicability directly to aqueous samples, although recent developments appear to have partly overcome this limitation. The SAB therefore requested that the TWG discuss the applicability of MIPs to CWC-related analytical procedures.

4.2 Anne Bossée presented a summary of the experiences of the DGA laboratory, France, with using MIPs for sample preparation of alkyl methylphosphonic acids (Appendix 3). A major problem was inconsistency in the selectivity of MIPs between different batches of similarly prepared material. Template bleed was also a potential problem unless an expensive deuterated analogue was used as a template in preparing the MIP. The recent development of sol gels to produce MIPs that can be used to extract analytes directly from aqueous solutions was also considered to be problematic, although it has been successful for much narrower ranges of target analytes, e.g. in the pharmaceutical field. The DSO laboratory, Singapore, and the DSTO laboratory, Melbourne, Australia, had experienced similar problems with inconsistent selectivity of MIPs. DSTO had found that MIPs as sensor components were quite sensitive to environmental conditions and interferences.

4.3 The view of the TWG was that MIPs were insufficiently rugged, and too time consuming to produce, to be considered for generic CWC-related analysis. It was also noted that, for most OPCW applications, analysis for a broad range of scheduled chemicals and/or their degradation products is required and sample preparation using an MIP would filter many of these out.

5. Emerging techniques with possible application for on-site analysis

Fast GC

5.1 Hugh Gregg, Head of the OPCW Laboratory, reported on the current status of the shorter GC procedure developed by the OPCW Laboratory. The procedure, which approximately halves GC run-time, had not yet been adopted for on-site analysis because of some variance in retention indices compared to those in the OCAD. Philip Coleman stated that work on fast GC in the Protechnik Laboratory in South Africa had been promising. Work undertaken with fast GC at VERIFIN in Finland and Dstl in the United Kingdom of Great Britain and Northern Ireland had been reported on at previous meetings of the TWG. Dstl had experienced some problems with retention index variation.

Other developments

5.2 The TWG members were not aware of any other emerging techniques, or miniaturisation of instrumentation such as LC-MS, that had reached a degree of maturity and robustness to be considered for on-site analysis.

6. Toxin analysis

Ricin

6.1 Sten-Åke Fredriksson, FOI, Sweden, presented an overview of a trial proficiency test on the analysis of ricin-containing samples, with the aid of material provided by Dr Brigitte Dorner, Robert Koch Institute (RKI), Berlin. The test was conducted during October 2009 and was coordinated by the RKI under the auspices of the Global Health Security Action Group [GHSAG Laboratory Network, within the Global Health Security Initiative (GHSI)].

6.2 The main objective of the test was to obtain information on the performance of existing methods as used by expert laboratories, and to promote the exchange of information between laboratories. Seventeen laboratories from 12 countries participated, including some OPCW designated laboratories.

6.3 Six liquid samples, five spiked with ricin and one unlabelled blank, were prepared at RKI and sent to participating laboratories. The spiked samples contained purified ricin or a crude *R. communis* extract in the mg/ml to pg/ml-range. Bovine serum albumin was added to the low concentration samples as a stabiliser. A consensus reference standard of ricin was made available to the participants for quality assurance purposes.

6.4 One of the main conclusions of the exercise was that a combination of methods was necessary to demonstrate the presence of ricin in all of the samples. The immunological methods (ELISA) and functional assays demonstrated positive responses down to pg/ml concentration. At higher concentrations (μg/ml), mass spectrometric methods correctly identified ricin and agglutinin (RCA120) in the samples.

6.5 Four laboratories correctly identified all samples and 11 laboratories correctly identified the crude *R. communis* extract. Experimental results obtained by the Swedish Defence Research Agency Laboratory, FOI CBRN Defence and Security, were presented to the TWG (Appendix 4).

6.6 Provisional criteria for the identification of ricin, previously discussed by the TWG, were applied retrospectively by Mr Fredriksson to 13 voluntary 'self-evaluation' reports obtained from the participating laboratories. It was considered that the data reported by 8 laboratories would meet the criteria for identification. The techniques used by these laboratories were based mainly on screening by ELISA for the intact toxin, combined with LC-MS/MS in SRM or product ion scan modes, or MALDI MS/MS product ion scans, of peptides produced by enzymatic digestion.

6.7 Some changes to the draft criteria were subsequently suggested to allow the inclusion of a functional assay as a screening technique, and to relax the tolerance for mass accuracy from ± 0.2 to 0.8 Da in order to accommodate the use of less expensive MS instrumentation. These draft criteria will be circulated to TWG members for comment. TWG members are requested to consult with experts outside the group on the validity of these criteria. The US member, Armando Alcaraz, was requested to

compare the draft criteria with those used by forensic groups within the US, if this data is available.

Saxitoxin

6.8 Martin Schaer, Spiez Laboratory, Switzerland, presented the results of the recent informal experimental data survey undertaken for the TWG between June and September 2010 (Appendix 5). Five laboratories provided experimental data. Although saxitoxin is a low molecular mass toxin, it cannot be analysed by GC-MS. Based on the data supplied, the following proposals were made for the identification of saxitoxin.

6.9 To meet OPCW requirements for two methods of identification, two groups of methods were considered, one set essentially for screening, and the second set for confirmation. The first group consisted of lateral flow immunoassay (LFA), qualitative ELISA, LC/fluorescence and ^{1}H-NMR if obtainable. Confirmatory assays were based exclusively on LC-MS/MS, either as a product ion scan or in SRM mode. The minimum requirement proposed is two SRM transitions with product ion ratio tolerances ≤ 20% of a reference chemical analysed under similar experimental conditions. Single stage LC-MS was not considered appropriate because of the limited information it provides. Furthermore, very few laboratories use LC-MS instruments without MS/MS capability. LC-high resolution MS was added to the list of acceptable methods, although no laboratories reported high resolution data.

6.10 The proposal was considered to have the following advantages:

a) no new evaluation system would be required by the OPCW laboratory;

b) criteria for chromatographic retention time, NMR and LC-MS/MS data should be no different from other scheduled chemicals; and

c) laboratories would have a choice of biochemical or chromatographic method as the first analytical method; LC-MS/MS then provides unequivocal identification.

6.11 It should be noted that this proposal would require laboratories to have LC-MS/MS instrumentation to unequivocally identify saxitoxin. The OPCW Laboratory would need to consider how LFA and ELISA assays should be reported. Additional criteria for these immunoassays and LC-MS/MS would need to be incorporated into the OPCW Laboratory Work Instruction for the Evaluation of OPCW Proficiency Tests.

7. Discussion of the report of the first OPCW confidence-building exercise on biomedical sample analysis

7.1 The SAB recommended to the Director-General in 2007 that a series of confidence-building exercises be held as a prelude to initiating a process towards a separate designation system for biomedical samples. This recommendation was accepted by the Director-General. The intention of the Technical Secretariat to

proceed in developing an OPCW capability for biomedical sample analysis was noted by the Executive Council (March 2007).

7.2 Robin Black provided a summary of the first confidence-building exercise, held from November 2009 to January 2010, with 22 participating laboratories from 17 Member States (Appendix 6). Samples were prepared by the TNO Health, Security and Safety Laboratory, Rijswijk, the Netherlands, and the results evaluated by Dstl, Porton Down, UK. Samples of commercial synthetic urine (chosen to avoid any problems associated with the transport of biological samples) were spiked with urinary metabolites of nerve agents or sulfur mustard at concentrations of 100 or 10 ng/ml. The samples were analysed using liquid and gas chromatography combined with single stage or tandem mass spectrometry. The most sensitive and selective methods were provided by LC-MS/MS or GC-MS/MS in selected reaction monitoring (SRM) mode, or high resolution single stage LC-MS in extracted ion mode. For alkyl methylphosphonic acids and thiodiglycol, perfluorinated derivatives using selective chemical ionisation gave greater selectivity and signal to noise in comparison to silyl derivatives. The use of commercial synthetic urine caused some unexpected problems in the analysis of one of the sulfur mustard metabolites.

7.3 The results successfully demonstrated a broader capability for the analysis of urinary metabolites of Schedule 1 agents than had previously been shown. They have also provided a starting point for discussion of criteria for identification at trace levels. Evidence of system or sample contamination was observed in more than half of the laboratories, particularly for GC-MS and GC-MS/MS analysis. This is an important problem, which needs to be addressed by the laboratories. A meeting was held with participating laboratories to discuss the results of the exercise on 25 March 2010. The TWG endorsed a recommendation that a second exercise and a workshop be held in 2011. Synthetic urine will not be used in future exercises.

8. Criteria for trace analysis in investigations of alleged use

8.1 Trace analysis, i.e. samples where generic full scan GC-MS or LC-MS analysis is unlikely to provide adequate signal to noise or resolution from extraneous components, is an important component of analytical investigations into alleged use of chemical weapons, for both environmental and biomedical samples. If trace analysis is to be used by the OPCW it is important to have written criteria for trace analysis, in line with other international bodies such as the World Anti-Doping Agency (WADA) and the European Commission.

8.2 Paula Vanninen, VERIFIN, Finland, gave a presentation on **"**Criteria for trace analysis in investigations of alleged use", (Appendix 7) based mainly on the criteria for chromatographic and mass spectral data used by WADA. Although WADA's identification criteria (Appendix 8) are for human urine samples, they should be adaptable to the trace analysis of environmental samples. It was agreed that criteria proposed for trace analysis of environmental and biomedical samples should be mutually consistent and, as much as possible, consistent with criteria currently used by the OPCW laboratory for non-trace analysis. Relatively minor modifications would need to be made to the WADA criteria for consistency with current OPCW criteria for proficiency tests, for example, minimum signal to noise ratio should be 5:1

instead of 3:1. Some further consideration may be required as to what should be regarded as a diagnostic ion, for example, the status of isotopic and low mass peaks. The WADA document stipulates tolerances for the relative intensities of ions (see Table 1, Appendix 8), compared to the same ions acquired from a spiked positive control urine sample, reference collection sample, or reference material. Similar reference materials may be more problematic to obtain for some types of matrices relevant to investigations into alleged use of chemical weapons, e.g. soil, and urine from traumatised casualties. Differences in matrix composition can lead to greater variance in retention times and ion ratios. It was recommended that criteria should be drafted by the OPCW Laboratory and VERIFIN, taking into account current OPCW criteria for proficiency testing. Some additional flexibility in criteria may be desirable for biomedical samples to accommodate the identification of more than one biomarker of exposure for the same agent.

8.3 Draft criteria will be sent to laboratories for comment. The TWG supported a proposal that these criteria be tested for environmental samples as a voluntary supplementary exercise, added to an OPCW proficiency test. Draft criteria for biomedical samples should be incorporated into the second confidence-building exercise on biomedical sample analysis.

9. Any other business

Notes on the performance of mobile laboratories during exercise ASSISTEX 3

9.1 A presentation on mobile laboratory performance was given by Francesco Pilo, Provincial Firefighters Headquarters, Venice, Italy, based on his experience with the conduct of on-site analysis during ASSISTEX 3 (see Appendix 8). Techniques used in the mobile laboratory included fast GC-MS, GC-MS, FTIR and IC. A major problem highlighted during the exercise was the response time from receipt of sample to issuing a report. This was a minimum of 3.5 hours and a maximum of 7 hours, which was too long to assist in the management of the emergency. Another major problem was that many of the samples (up to 50%) were not usable because of sampling errors, problems during sample decontamination, and loss of information. Mr Pilo also noted the lack of definition of acceptance criteria for results from the mobile laboratory.

9.2 The TWG expressed the view that the role of the mobile laboratory needs to be clearly defined in different OPCW scenarios in the context of Article X. Additional points made during discussion were the key role of field detection techniques in providing initial information to assist in the management of the emergency, and in collecting the most useful samples for analysis. The importance of regular training was emphasised in the use of portable detection equipment, sampling, ensuring chain of custody, and sample preparation and analysis.

Progress on the 2010 edition of the VERIFIN 'Blue Book' on 'Recommended Operating Procedures for CWC-Related Analysis'

9.3 Paula Vaninnen outlined the progress being made in compiling the new edition of the VERIFIN 'Blue Book' (Appendix 9). The previous edition was issued in 1994 and

formed the basis of the recommended operating procedures used in OPCW proficiency tests and inspections. The new edition will be web-based (Wiki style) and significantly more extensive in its coverage than the 1994 edition. Chapters are being drafted by scientists from at least 10 designated laboratories, including TWG members, as well as from the OPCW Laboratory. Additional contributions were requested on a number of topics, either as authors or reviewers. A review meeting will be held at the OPCW on 9 February 2011, and a workshop will take place in Helsinki on 16 and 17 November 2011.

10. Summary and conclusions

10.1 Shortening of on-site sample preparation time for the analysis of aqueous samples continues to be a high priority for the Technical Secretariat. The OPCW Laboratory has developed a procedure which involves absorption of aqueous samples onto Tenax tubes, on-tube derivatisation, and analysis by thermal desorption GC-MS. The TWG agreed that this appears to be the most promising alternative procedure reported, subject to further validation. The TWG and OPCW Laboratory request other laboratories to assist in assessing the procedure for applicability and robustness.

10.2 The TWG remained of the view that hollow fibre liquid phase microextraction is also a promising technique for aqueous sample preparation. However, assessment in a small number of laboratories suggests that it is less robust and would require greater operator training than the Tenax tube procedure.

10.3 The TWG supported further investigation of fast GC to shorten on-site analysis time. The TWG remained of the view that solid phase microextraction (SPME) has shortcomings with regard to on-site OPCW analysis, although is a useful technique in other scenarios.

10.4 An informal experimental survey conducted by the TWG has provided useful data on which to base criteria for the identification of saxitoxin. The TWG has proposed draft criteria for consideration by the SAB.

10.5 A trial proficiency test conducted by the Robert Koch Institute, under the auspices of the Global Health Security Action Group, has provided useful data on which to base criteria for the identification of ricin. Draft criteria will be circulated to TWG members for comment, and for consultation with other laboratories with appropriate expertise.

10.6 The TWG endorsed the report of the first OPCW confidence-building exercise on biomedical samples. The exercise represents significant progress in broadening expertise in this area. The TWG supported the OPCW laboratory proposal to hold a short workshop on biomedical samples and a second confidence-building exercise in 2011.

10.7 The TWG recommended that the criteria for identification of trace levels of drugs in urine, as used by WADA, should be adapted, with appropriate modification, to the trace analysis of environmental and biomedical samples in the context of investigations of alleged use of chemical weapons. VERIFIN and the OPCW

Laboratory were requested to compose draft criteria for circulation. It is recommended that these should be assessed for biomedical samples as part of the second OPCW confidence-building exercise, and for environmental samples as a voluntary exercise supplementary to a proficiency test.

10.8 The experiences of a mobile laboratory during the recent ASSISTEX 3 exercise had highlighted shortcomings in procedures and training. The role of such laboratories in OPCW activities in the context of Article X requires clarification.

11. Closure of the meeting

The Chairperson closed the meeting at 16:50 on 19 November 2010.

Appendices:

Appendix 1: List of Participants in the Fifth Meeting of the Temporary Working Group on Sampling and Analysis
Appendix 2: Screening of Chemical Warfare Agents, Their Degradation Products, Impurities and Precursors in Liquid Samples/Extracts Using Tenax Tubes and Thermal Desorption-Gas Chromatography-Mass Spectrometry
Appendix 3: Overview on MIPs Experiments at DGA MNRBC
Appendix 4: ELISA and Real Time PCR Detection Combined With LC-MS/MS Identification in the Ricin Proficiency Test
Appendix 5: Results of the Experimental Data Survey and Updated Identification Criteria
Appendix 6: The First OPCW Confidence-Building Exercise on Biomedical Sample Analysis
Appendix 7: Criteria for Trace Analysis in Investigations of Alleged Use
Appendix 8: WADA Technical Document – TD2010IDCR
Appendix 9: Notes about Performance of Mobile Labs During Exercise ASSISTEX 3
Appendix 10: Updating of the ROPs Within International Cooperation

Appendix 1

LIST OF PARTICIPANTS IN THE FIFTH MEETING OF THE TEMPORARY WORKING GROUP ON SAMPLING AND ANALYSIS

	Participant	State Party
1.	Robert Mathews	Australia
2.	Jiří Matoušek	Czech Republic
3.	Paula Vaninnen	Finland
4.	Jean-Claude Tabet	France
5.	Anne Bossée	France
6.	Ralf Trapp	Germany
7.	Mehran Babri	Iran (Islamic Republic of)
8.	Francesco Pilo	Italy
9.	José Luz González Chávez	Mexico
10.	Mui Tiang Sng	Singapore
11.	Philip Charles Coleman	South Africa
12.	Sten Åke Fredriksson	Sweden
13.	Martin Schär	Switzerland
14.	Robin Black[9]	United Kingdom of Great Britain and Northern Ireland
15.	Armando Alcaraz	United States of America

9 Chairman of the TWG.

Appendix 2

SCREENING OF CHEMICAL WARFARE AGENTS, THEIR DEGRADATION PRODUCTS, IMPURITIES AND PRECURSORS IN LIQUID SAMPLES/EXTRACTS USING TENAX TUBES AND THERMAL DESORPTION–GAS CHROMATOGRAPHY–MASS SPECTROMETRY

Screening of chemical warfare agents, their degradation products, impurities and precursors in liquid samples/extracts using Tenax tubes and thermal desorption–gas chromatography–mass spectrometry

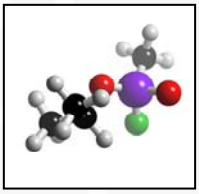
Oliver Terzic,
Inspector Analytical Chemist

ORGANISATION FOR THE PROHIBITION OF CHEMICAL WEAPONS

Outline

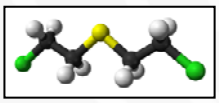
- Introduction: OPCW on-site analytical procedures
- Objectives of the laboratory study
- Method development
- Method application
- Conclusions

OPCW On-Site Sample Preparation Procedures



- Devised to cover great variety of organic compounds in different matrices

- Compounds of different reactivity, polarity and solubility
- Compounds containing phosphorus, sulphur, nitrogen, arsenic, chlorine, or fluorine heteroatom(s)
- Compounds which are non-volatile or elute poorly on GC



Sample Matrices

- S&A on industrial inspections
  - Neat or diluted samples
  - Mixtures, slurries
  - Solids or liquids (aqueous or organic)
  - Solutions containing polymer(s)
  - Solutions made of mixture of solvents of different polarity, emulsions
  - Exact sample composition unknown (proprietary information)

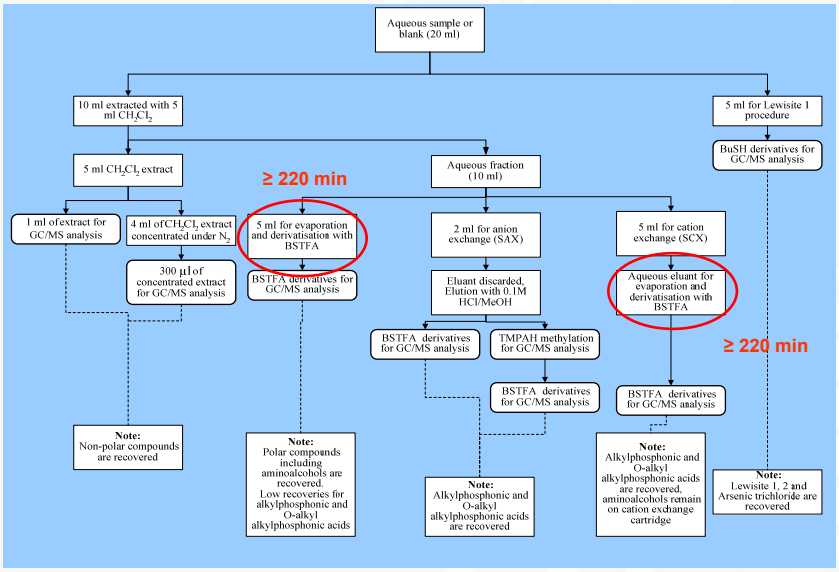
- S&A on challenge inspections and investigations of cases of alleged use of CW
  - Any kind of samples possible, including environmental or waste samples
  - Analyte concentrations may range from 100% down to parts per billion

Complex Analytical Task
= Complex Analytical Procedures

- Tedious and time consuming on-site analysis

- Several extraction and derivatization steps
- Sample preparation fractions analyzed in the separate GC-MS runs, each preceded with a method blank run
- Aqueous samples and extracts "bottle-neck" of the on-site analysis
- Reduces the number of samples that can be analysed in the restricted inspection time

Preparation of Aqueous Samples (*QDOC-LAB-WI-SP2*)



Objectives of The Laboratory Study

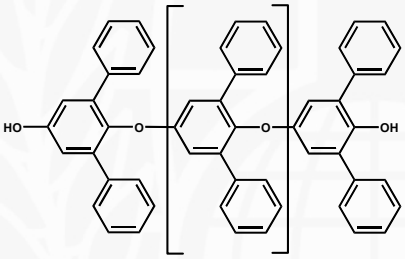
- Development of new, simpler and faster method for analytical screening of liquid samples/extracts, especially aqueous based solutions and organic-aqueous mixtures
- Available (approved) OPCW on-site analytical equipment and chemicals used for the method
- Reduction of the mission logistic burden and waste generated

Working Hypothesis

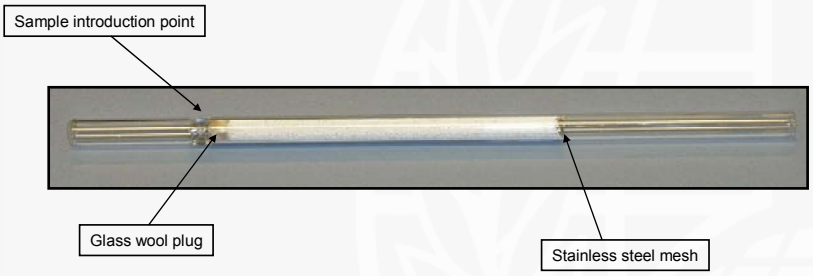
- Tenax thermal desorption (TD) tube as:
  - depository for the more polar/less volatile CWA degradation products and precursors
  - trap for more volatile compounds
  - solid support for the derivatisation

Why Tenax?

- 2,6-diphenyl-p-phenylene
- extremely low affinity for water
- high heat tolerance (> 375°C)
- chemical inertness
- fast elution and sharp peaks



Preparation of the TD Tubes

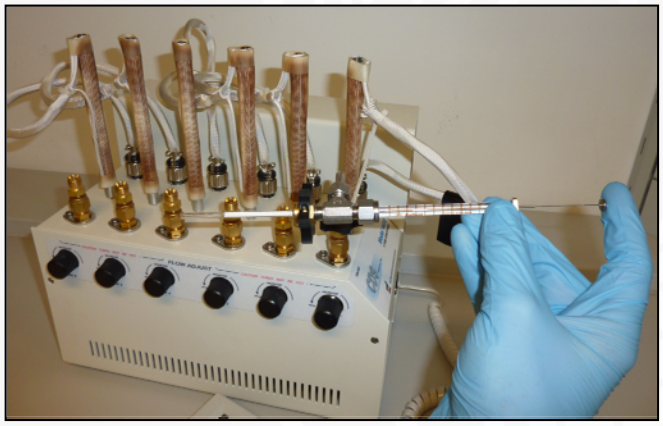


Tenax beads (~ 0.07 g), 60/80 mesh, packed into borosilicate GC liner (1 ring restriction, 88mm length, 3 mm ID) in between glass wool and stainless steel plug

TD Tube Preparation

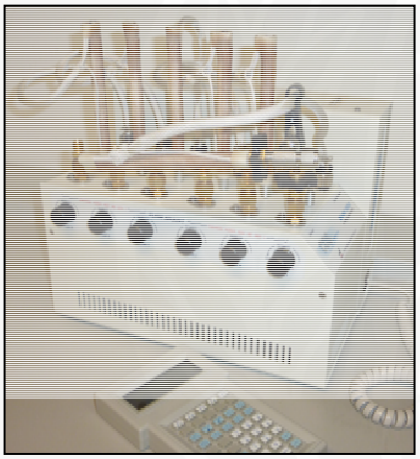
Placing the TD tube into the spiking attachment of tube conditioner

TD Tube Spiking for Deactivation



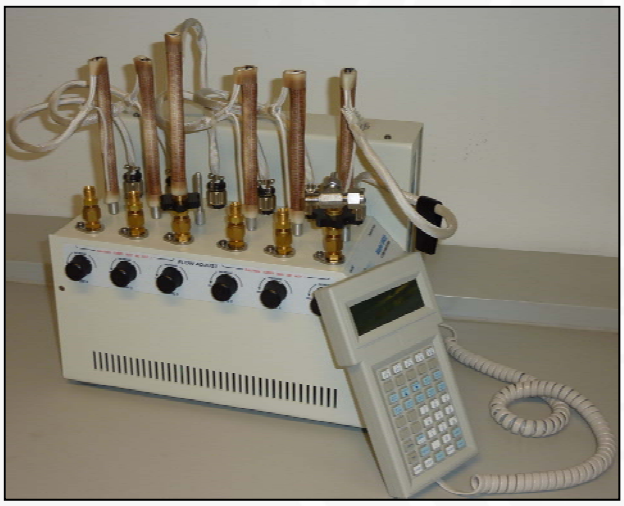
5 µl BSTFA, 60 ml/min He flow

TD Tube Deactivation



3 min 60°C, 60 ml/min He flow

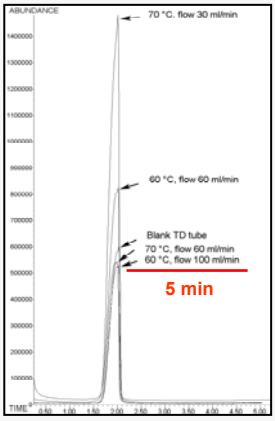
TD Tube Conditioning



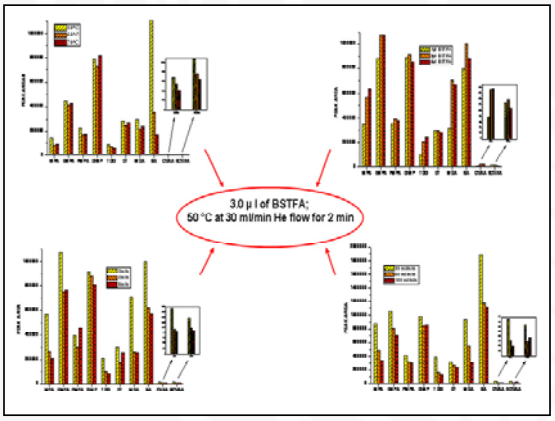
120 min, 330 °C, 100 ml/min He flow

Sample Preparation Optimization Studies

Water/solvent removal



In-tube derivatisation: 10 compounds; conc. 10 ppm in water; 2 µl spike



Sample Preparation

Sample injection: 1-10 µl, 100 ml/min He flow

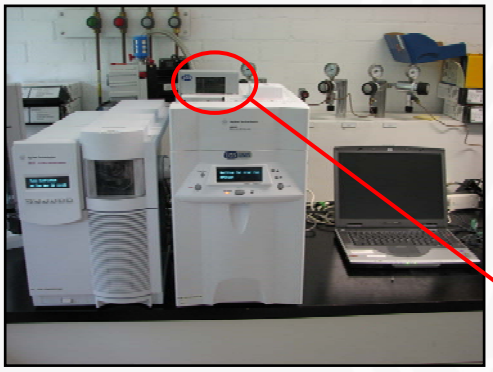
Pushing the sample toward Tenax: 2 min, 100 ml/min He flow

Water / solvent removal at 60°C for 5 min

Injection of 3 µl BSTFA, He flow 30 ml/min

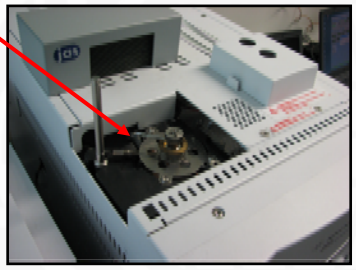
2 min heating at 50°C

Sample Analysis

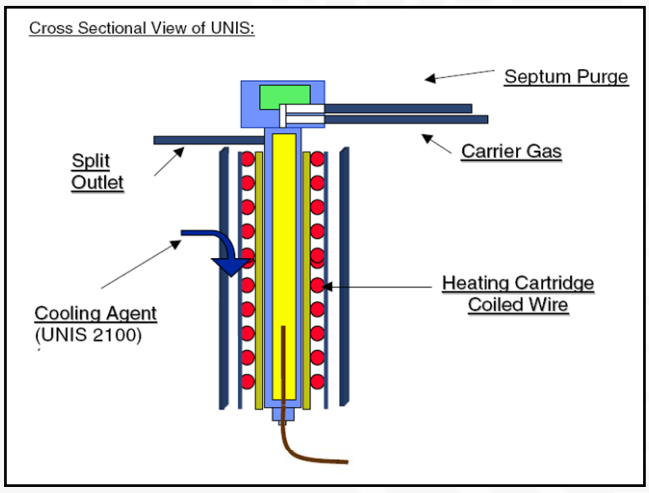


Agilent 6850 GC/ 5975 Inert MSD

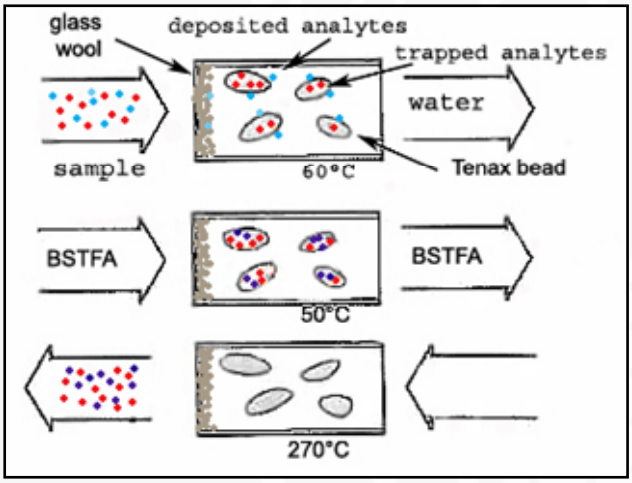
Peltier element + UNIS inlet system



UNIS inlet system

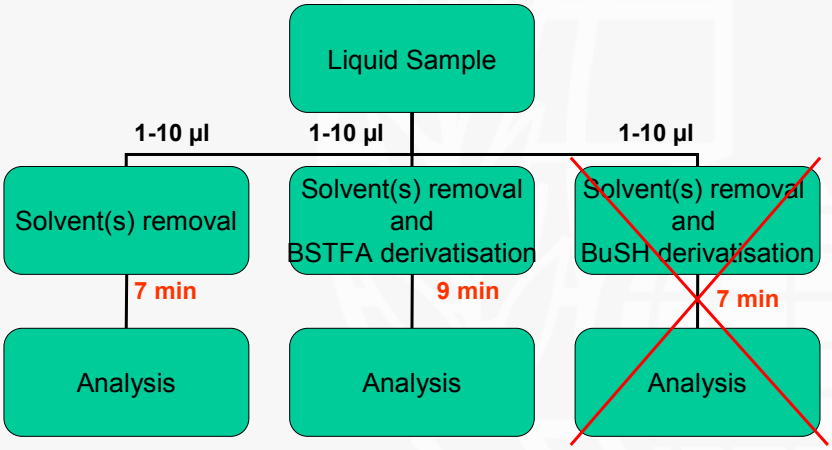


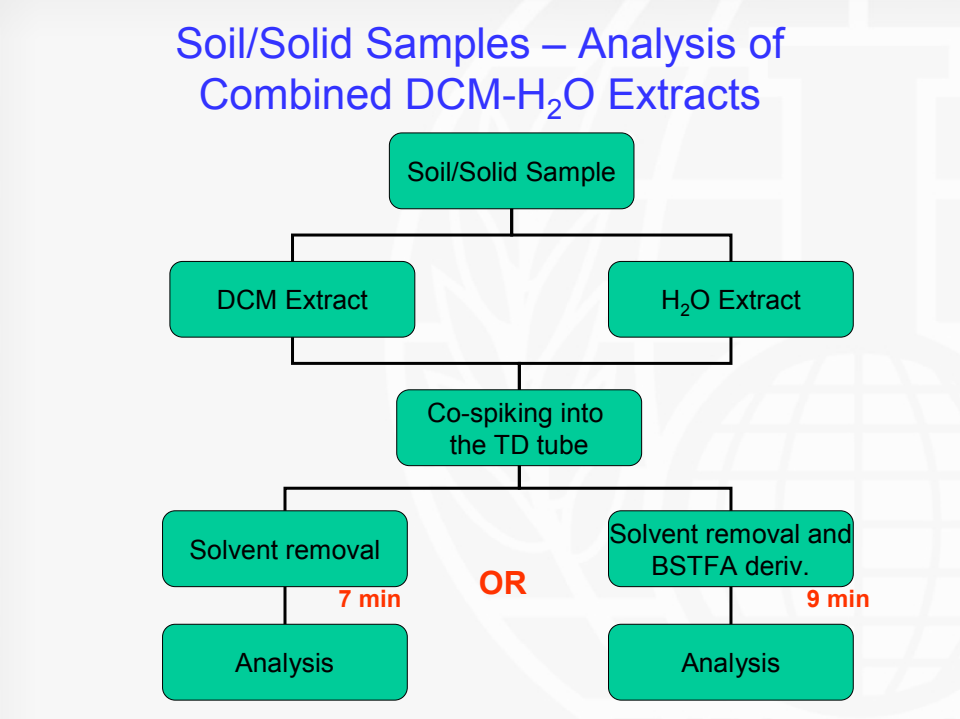
Simplified Process

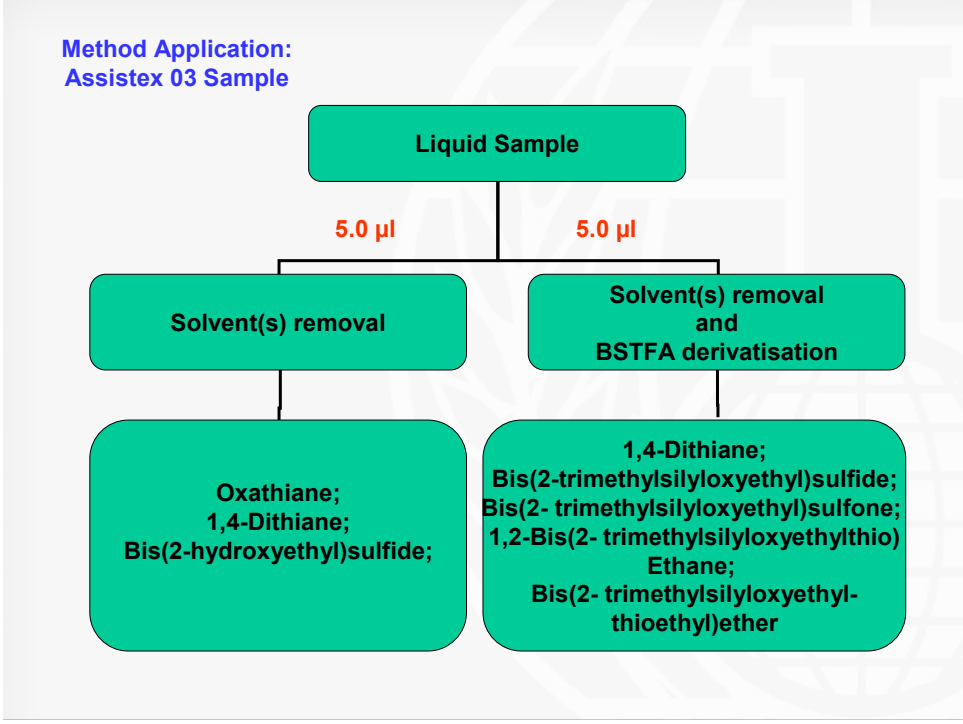


- *More polar / less volatile compounds*
- *Less polar / more volatile compounds*
- *TMS derivatives of more polar / less volatile compounds*

Analysis Scheme for Liquid Samples




Soil/Solid Samples – Analysis of Combined DCM-H2O Extracts
Soil/Solid Sample
DCM Extract
H2O Extract
Co-spiking into the TD tube
Solvent removal
7 min
Analysis
OR
Solvent removal and BSTFA deriv.
9 min
Analysis

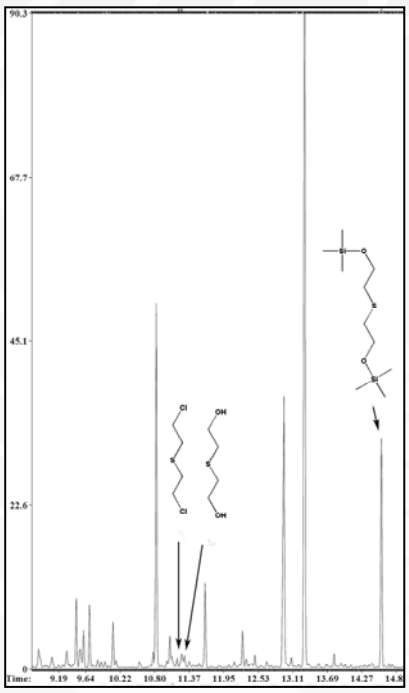

Method Application:
Assistex 03 Sample
Liquid Sample
5.0 µl
5.0 µl
Solvent(s) removal
Solvent(s) removal and BSTFA derivatisation
Oxathiane;
1,4-Dithiane;
Bis(2-hydroxyethyl)sulfide;
1,4-Dithiane;
Bis(2-trimethylsilyloxyethyl)sulfide;
Bis(2- trimethylsilyloxyethyl)sulfone;
1,2-Bis(2- trimethylsilyloxyethylthio) Ethane;
Bis(2- trimethylsilyloxyethyl-thioethyl)ether

Method Application: Toxic Chemical Training Aqueous Sample

HD spiked water

❑ Sample preparation:

- 8.0 µl spike
- 2 min purge at 100 ml/min He flow
- 5 min solvent removal at 60 ° C and 100 ml/min He flow
- 3.0 µl BSTFA spike
- 2 min at 50 °C and 30 ml/min He flow

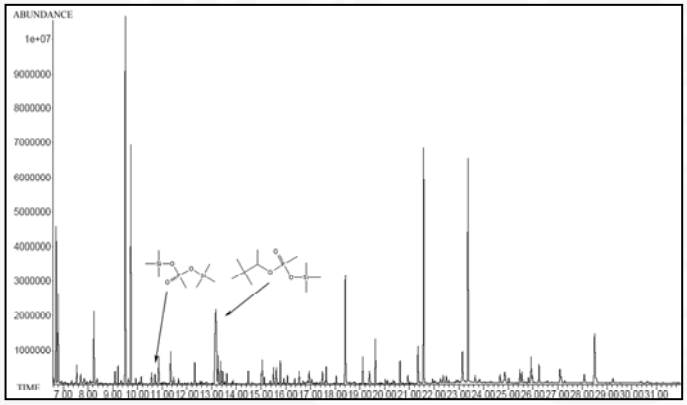


Method Application: 23rd Official OPCW Proficiency Test LW sample

Component	CWC Sched.	Conc. (µg/ml)
3,3-Dimethyl-2-butanol	2.B.14	10
Pinacolyl methylphosphonic acid	2.B.04	10
Deionised water	-	Solvent (20 % v/v)
Methanol	-	Solvent (80 % v/v)

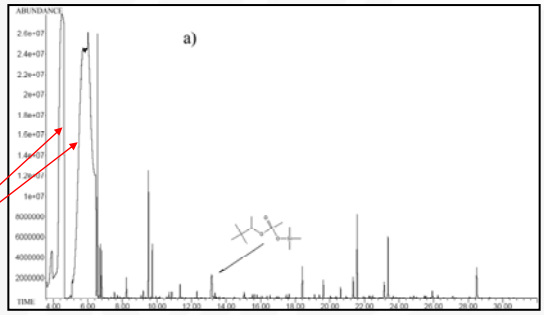
❑ **Sample preparation:**

- 2.0 µl spike
- 2 min purge at 100 ml/min He flow
- 5 min solvent removal at 60 ° C and 100 ml/min He flow
- 3.0 µl BSTFA spike
- 2 min at 50 °C and 30 ml/min He flow

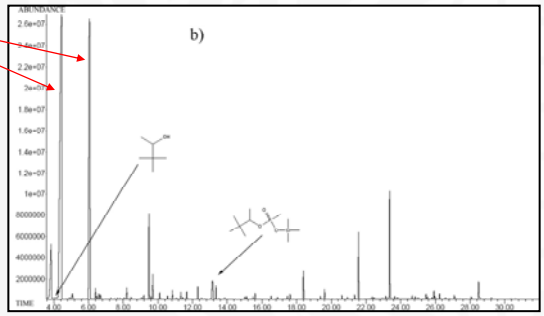


Purging of the excess of the derivatizing reagent

a) Standard procedure



Derivatizing reagent related peaks



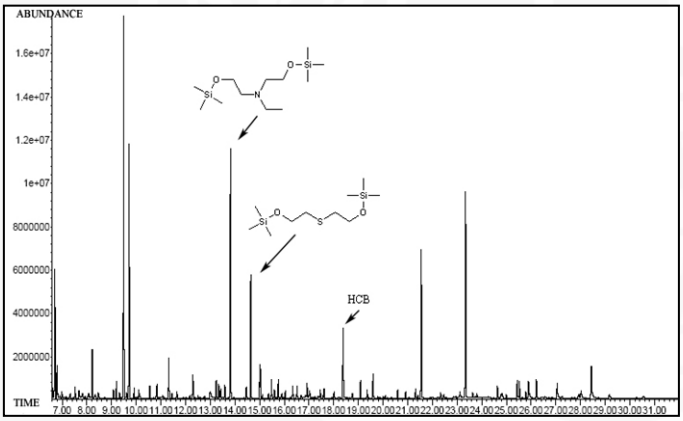
b) 5 min additional purge at 60 °C and 100 ml/min He flow

Method Application: 25th Official OPCW Proficiency Test 226 sample

Component	CWC Sched.	Conc. (µg/ml)
Bis(2-hydroxyethyl)sulphide (TDG)	2.B.13	20 ±0 .2
N-Ethyldiethanolamine	3.B.15	20 ± 0.2
Deionised water	-	Solvent (50 % v/v)
Acetonitrile	-	Solvent (50 % v/v)
Calcium Chloride Dihydrate	-	1000 ± 10
Sodium Sulfate Anhydrous	-	1000 ± 10

❑ Sample preparation:

- 2.0 µl spike
- 2 min purge at 100 ml/min He flow
- 5 min solvent removal at 60 ° C and 100 ml/min He flow
- 3.0 µl BSTFA spike
- 2 min at 50 °C and 30 ml/min He flow

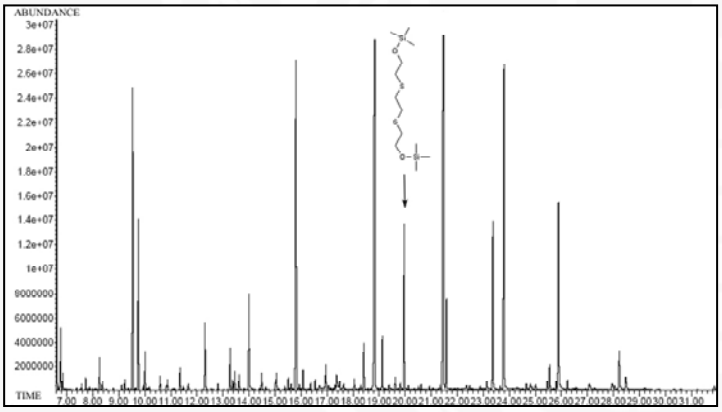


Method Application:
25th Official OPCW Proficiency Test 215 sample

Component	CWC Sched.	Conc. (µg/ml)
1,2-Bis(2-hydroxyethylthio)ethane	- (sesquimustard deg. pr.)	20 ±0 .2
Polyethylene glycol 200 (PEG 200)	-	0.5 ‰
Deionised water	-	Solvent (50 % v/v)
Dichloromethane	-	1 ‰

❑ Sample preparation:

- 2.0 µl spike
- 2 min purge at 100 ml/min He flow
- 5 min solvent removal at 60 ° C and 100 ml/min He flow
- 3.0 µl BSTFA spike
- 2 min at 50 °C and 30 ml/min He flow

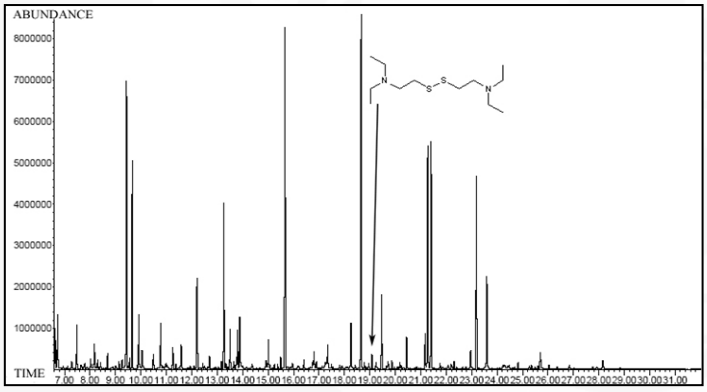


Method Application:
26th Official OPCW Proficiency Test 801 sample

Component	CWC Sched.	Conc. (µg/ml)
Bis-(2-N, N-diethylaminoethyl)disulfide	- (Russian VX degr. pr.)	10
(2-chloroethyl)phosphonic acid, (Etephon)	-	10
Deionised water	-	Solvent
O,S-dimethyl acetylphosphoramidothioate, (Acephate)	-	10
Butylphosphonic acid	-	10
Polyethylene glycol 200 (PEG 200)	-	0.05%

❑ Sample preparation:

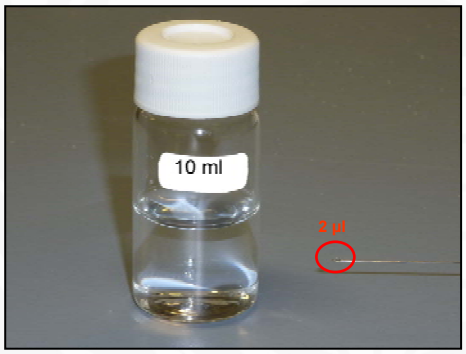
- 2.0 µl spike
- 2 min purge at 100 ml/min He flow
- 5 min solvent removal at 60 ° C and 100 ml/min He flow
- 3.0 µl BSTFA spike
- 2 min at 50 °C and 30 ml/min He flow



Conclusions

- Use of Tenax TA packed tube and in-tube silylation followed by TD-GC- full scan MS has shown to be a viable method for qualitative analysis of chemical warfare agents, their degradation products, precursors and impurities
- The method offers numerous advantages such as:

• Drastic reduction in the amount of sample required (enables analysis of diluted drop-size samples)



Conclusions

• Drastic decrease in the sample preparation and analysis time

OPCW Sample Preparation Method AQ samples	In-Tenax tube derivatization	Standard
Water removal time	5 min	180 min
Derivatisation time	2 min	30 min
Overall sample preparation time	25 min	> 360 min
Number of GC runs (40 min/run)	1-3 for sample + 1-3 for method blank	4-6 for sample + 4-6 for method blank

Conclusions

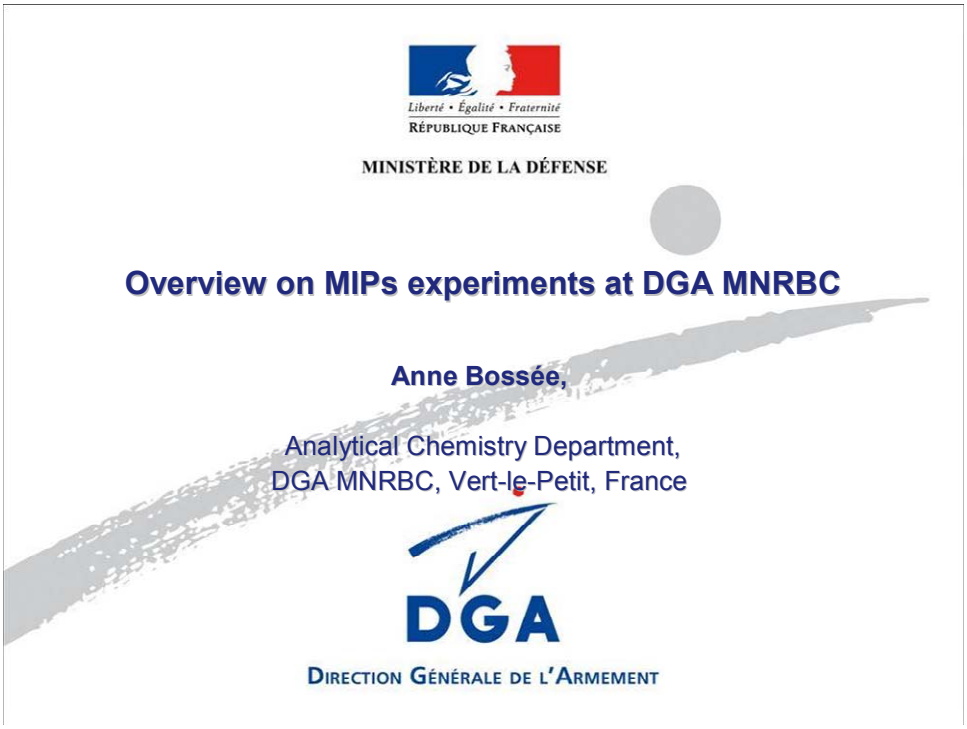
- Effective concentration of the analytes for higher sensitivity (ppb levels)
- For majority cases, no other sample treatment required (for example filtration, separation of the solvents or drying of organic extracts)
- Less contamination and carry-over of compounds like alkyl phosphonic acids
- Good for samples containing non-volatile background material (e.g. polymers)
- Excellent results with wet organic solutions or solutions composed of a mixture of solvents of different polarity
- Beside for aqueous solutions, applicable for wipe sample extracts, soil or other solids extracts, and water soluble solids

Conclusions

- Relatively simple to perform (user friendly)
- Major reduction in the amounts of dangerous goods to take on inspection & the waste generated
- Logistic burden further reduced by the use of the same equipment as for air sampling
- Already approved equipment used with the method
- Extends the number of the samples hat can be collected and analysed on the mission

Appendix 3

OVERVIEW ON MIPs EXPERIMENTS AT DGA MNRBC



Summary

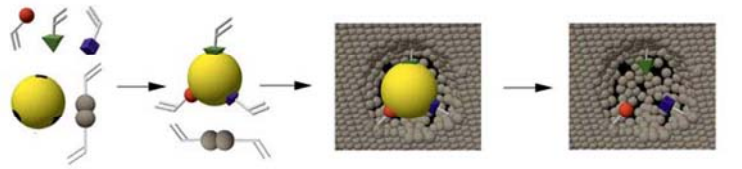
- Context / goal
- Experiments performed in the lab
  - Selectivity results on AMPAs
  - Reproducibility
- Advantages/drawbacks of Molecularly Imprinted Polymer (MIP)
- Perspectives

Context - Goal

- Study to improve aqueous sample treatment in our lab
  - Academic partner: Paris-ESPCI (LECA -V. Pichon) – PhD student (synthesis done in ESPCI and MIP evaluation in DGA)
  - Goal : Development of a sample treatment more selective, less time consuming for the identification of alkyl alkylphosphonic acids at low concentration level (ppb) in complex matrices
  - New technique : Molecularly imprinted solid-phase extraction technique
  - Matrix clean up using molecularly imprinted polymers similar to immuno-extraction: specific recognition of a template molecule.

- Strategy
  - MIP synthesis using pinacolyl methylphosphonic acid (PMPA) as template
    Pinacolyl moiety large enough (R = C_6) to create a cavity able to recognize a large number of OPs degradation products.
  - Experiments on EMPA and evaluation of selectivity performances by affinity differences comparison with a non-imprinted polymer (NIP)

Synthesis steps of MIP



Choice of -Template -Monomer(s) -Cross linker -Solvent

Complex formation between monomer(s) and template

Polymerization around the template

Template release release of interaction sites

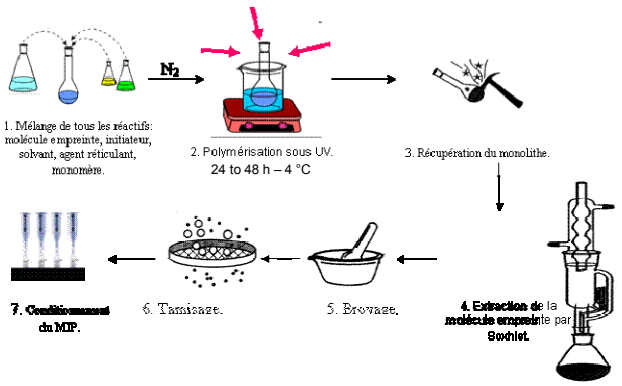
- Synthetic support with cavities presenting a specific spatial and functional recognition for the template molecule or its structural analogues

Synthesis steps of MIP

- Different parameters to optimize
  - Basic or acid monomer with a polar template : hydrogen binding interaction
  - Cross-linker: 3D structure of MIP and impact on non-specific interactions
  - Polymerization solvent :
    - semi-polar , aprotic for hydrogen binding
    - to avoid polar interactions between solvent and monomer or template leading to complex dissociation
  - Polymerization initiator
  - Duration and temperature for polymerization : low temperature for complex stabilization

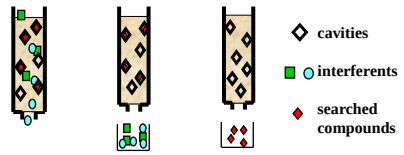
Synthesis steps of MIP

3-4 days for synthesis and conditioning a MIP



How does it work ?

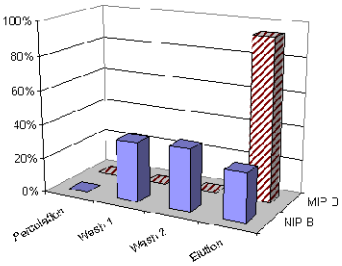
- Similar use as immuno sorbents
  - 3 steps :Percolation, Washing, Elution



- Selectivity characterization by comparison with a NIP
  - Synthesis steps of a NIP : exactly same steps without template

Selectivity for EMPA

- Different syntheses, the best with :
  - Monomer : MAA (methacrylic acid)
  - Cross-linker : TRIM (trimethylpropane trimethacrylate)
  - Polymerization solvent : CH_3CN

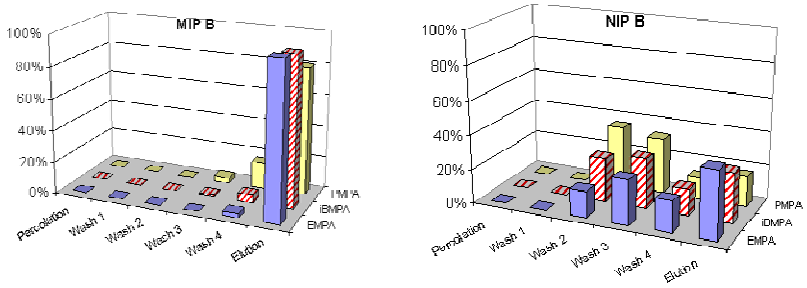


Step elution of EMPA (2 µg/5 mL) from MIP/NIP B. Percolation with CH3CN; Wash 1 and 2 : 5 mL of MeCN/MeOH (95/5) (v/v); Elution with 5 mL water

Journal of Chromatography A Vol 1108, 2006, p. 7 Sophie Le Moullec, Arlette Bégos, Valérie Pichon, and Bruno Bellier

- Good selectivity : EMPA recovery : NIP: 34 ± 4 % and MIP :87 ± 6 %,
- Elution in 5 mL : time to concentrate
- Percolation in water not possible, need to introduce a SPE step before

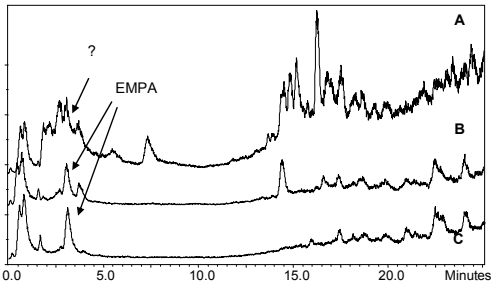
Application to other AMPAs



Percolation: 5mL CH_3CN; 2 µg of each compound - Wash : 2,5 mL of MeCN/MeOH (95/5, v/v).
Elution: 5 mL H_2O

- Selectivity confirmed for iBMPA and PMPA :
  - iBMPA : 94% (MIP) – 29% (NIP)
  - PMPA : 80% (MIP) - 18% (NIP)
  - Partial loss of iBMPA and PMPA during last wash : less interaction compared to EMPA-MIP

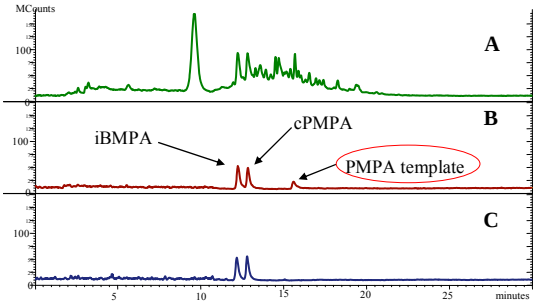
Selectivity for soil extract



LC-MS total ion current chromatograms: A) Soil extract fortified with EMPA ; B) Soil extract fortified with EMPA and purified on MIP B ; C) Standard solution of EMPA

- H_2O extraction, treatment on SPE OASIS HLB, elution with CH_3CN, percolation on MIP, elution with CH_3CN/MeOH, concentration
- removal of the more polar compounds eluted at the beginning of the chromatogram
- Easier detection and quantification of EMPA in complex matrix.

Selectivity for other AMPAs in soil extract



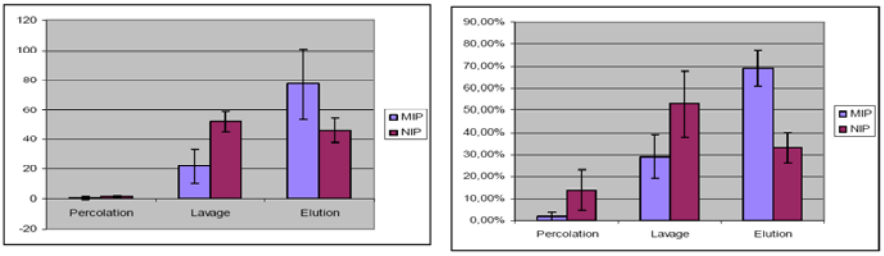
soil extract: 25mL
spiking at 5 µg for each
(1 µg/g soil if 50% recovery)

LC-MS chromatograms in full scan mode of the analysis of a soil extract spiked with iBMPA and cPMPA treated using Oasis HLB (A) or Oasis HLB and MIP (B) and of a standard solution of each compound at 10 µg mL^{-1} (C).

- Template release ➡ false positive risk

Reproducibility

- 2 other MIPs produced in the same conditions by 2 others analysts



Repeatability for 6 extractions of 1mL CH_3CN fortified by 1 µg EMPA

- Reproducibility for the elution step relatively similar but small decrease of selectivity and high CV
  - 87% MIP 0 – 34% NIP 0
  - 80% MIP 1 – 45% NIP 1
  - 70% MIP 2 – 35% NIP 2
- ➡ Selectivity for MIP 1 and MIP 2 not satisfactory

Conclusion

- Advantages
  - Reusable
  - Low cost
  - Easy to synthesize
  - Large capacity
  - Selectivity
  - Stable (pH, temperature)

- Drawbacks
  - Time consuming for synthesis and sample treatment
  - Reproducibility difficult to obtain and loss of specificity
  - Template release (solution: use a deuterated template, but not low cost)
  - No direct treatment of aqueous samples

Perspectives

- Study on MIP abandoned
  - In our lab
  - For AMPAs by our academic partner ; continued for other templates (pharmaceutical compounds, …)

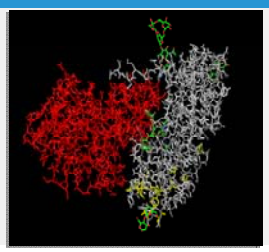
- New methodology for molecularly imprinted polymer applicable for aqueous sample treatment : sol gel (MIS) ?
  - Synthesis in water
  - As for MIP, results obtained with the first cartridge of sol-gel very promising but problem of repeatability
  - But hydrolysis of silica phase after pure water samples (pH=6)

- Study on hollow fiber (PhD student in the academic partner)

Appendix 4

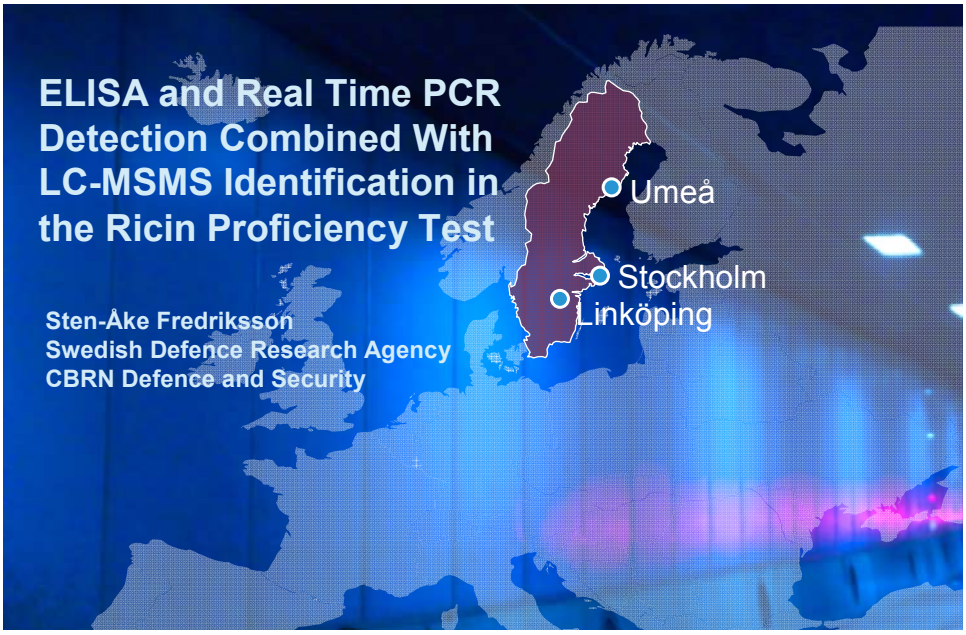
ELISA AND REAL TIME PCR DETECTION COMBINED WITH LC-MS/MS IDENTIFICATION IN THE RICIN PROFICIENCY TEST

ELISA and Real Time PCR Detection Combined With LC-MSMS Identification in the Ricin Proficiency Test



Sten-Åke Fredriksson
Swedish Defence Research Agency
CBRN Defence and Security

FOI



FOI

RKI proficiency test on ricin

Methods at FOI

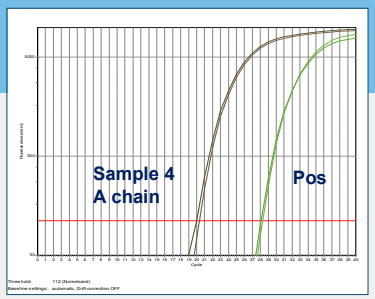
- Capillary gel electrophoresis
- Immunoaffinity - MALDI MS
- Real Time PCR screening
- ELISA screening and quantification
- Affinity purification - enzymatic digestion & LC-MSMS identification
- LC-MRM quantification

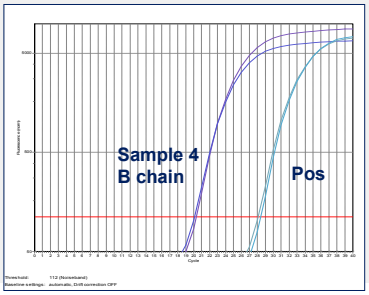
Ricin Real Time PCR

- DNA preparation: QIAamp DNA mini kit (Qiagen)
- 2x PerfeCTa SYBR mix (Quanta Bioscience)
- Positive control: *Ricinus communis* carmencita castor bean crude extract
- 3 primer pairs for ricin chain A
- 5 primer pairs for ricin chain B

1. He.X et al.J.Agric.Food Chem., Vol.55, No, 2007
2. FOI (unpublished)

- Eppendorf RealPlex 2 thermal cycler





Real Time PCR Results

Results Real Time PCR: mean number of cycles to threshold value (n=2)

	Primer							
	Chain A[1] R/F	Chain A2[1] R/F	Chain A3[1] R/F	Ricin 1[2] F/R	Ricin 2[2] F/R	Ricin 3[2] F/R	Ricin 4[2] F/R	Ricin 5[2] F/R
Sample 1	-	-	-	-	-	-	-	-
Sample 2	-	-	-	-	-	-	-	-
Sample 3	-	-	-	-	-	-	-	-
Sample 4	20.5	20.5	20.2	21.7	20.4	20.2	20.3	20.7
Sample 5	-	-	34.3/-	-	33.3/-	32.5	34.5	35.3
Sample 6	-	34.5/-	-	-	35.4/-	35.7/-	34.4/-	-
Positive control	27.4	28.4	28.2	29.3	30.1	28.3	27.7	28.9

1. He X. *et al.* J. Agric. Food Chem. Vol. 55, 6897, 2007
2. FOI CBRN, unpublished

ELISA consensus results

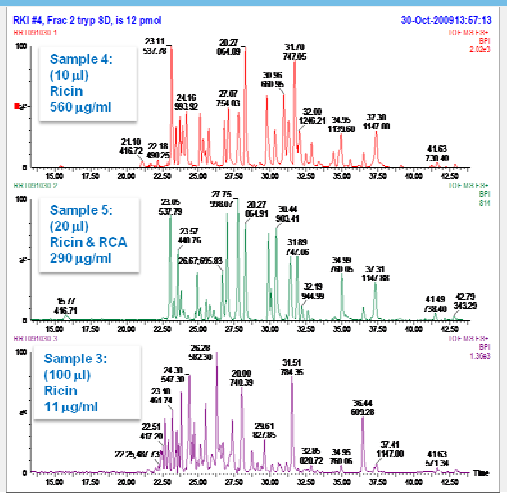
	Ricin concentration (µg/ml)	Ricin total amount (pmol/sample)
Sample 1	0.00098	0.015
Sample 2	0	0
Sample 3	9.6	150
Sample 4	445	6950
Sample 5	1018	15900
Sample 6	0.00009	0.002

1 ml samples in PBS, samples 1, 2, 3 and 6 also contained serum albumin 1 mg/ml

Affinity enrichment LC-MS of trypsin digests

ELISA-positive samples
- concentrated by affinity chromatography
- digested with trypsin
- analyzed by LC-MS and LC-MSMS

Solvent enhanced enzymatic digestion: 40 µl 200 mM ammonium bicarbonate pH 8.3, 50% methanol. Modified trypsin (Promega) 2h@37 °C



FOI

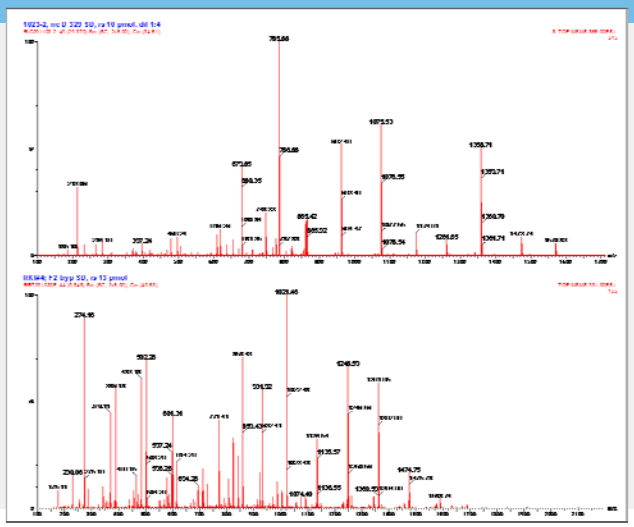
Selection of peptides for MS/MS

A chain T19: 864.93^{2+}
SAPDPSVITLENSWGR

also found in RCA_{120}

B chain T18: 931.51^{2+}
NDGTILNLY**S**GLVLDVR

RCA120 & Ricin E:
NDGTILNLY**N**GLVLDVR



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Ricin D chain B trypsin digest peptides

T# [a]	Ricin D peptide sequence [b]	Mass	Observed sequence ions
T2	IVGR	444.29+	y''_1-y''_3, b_2-b_3, a_2
T3-ss-T5	**NGLCVDVR FHNGNAIQLWPCK**	**800.68³⁺**	$[M+3H-H_2O]^{3+}$, $[T_B3\text{-ss-}T_B5y''_3\text{-}T_B5y''_4]^{3+}$, $[T_B3\text{-ss-}T_B5b_{12}]^{3+}$, $[T_B5\text{-ss-}T_B3y''_3]^{2+}$, T_B3; y''_1-y''_2, T_B5; b_2-b_3, b_6-b_{10}, i_{10}
T4	DGR	[346.16]	
T6	**SNTDANQLWTLK**	**695.85²⁺**	y''_1-y''_{10}, b_2-b_9, $[M+2H-H_2O]^{2+}$
T7	R	[174.11]	
T8	**DNTIR**	**618.32⁺**	y''_1-y''_2, y''_4, b_2-b_3, TI,
T9	SNGK	[404.20]	
T10	**CLTTYGYSPGVYVMIYDCNTAATDATR**	**983.10³⁺**	y''_1-y''_{19}, i_9/i_{16}, PG, PGV, PGV, PGVY, PGVYV, PGVYVM, PGVYVMI, PGVYVMIY, $[M+3H-H_2O]^{3+}$, ([y''-b]²⁺) #
T11	**WQIWDNGTIINPR**	**997.77³⁺**	y''_2-y''_{12}, b_2-b_{10}, INP, T11, $T11^{2+}$, $[T_B11+HexNAc+2H]^{2+}$, $[T_B11+2HexNAc+2H]^{2+}$, $[T_B11+2HexNAc+Hex+2H]^{2+}$-$[T_B11+HexNAc+4Hex+2H]^{2+}$, HexNAc, (HexNAc-$Hex_x$)-(HexNAc-$Hex_x$)
T12	**SSLVLAATSGNSGTTLTVQTNIYAVSQGWLPTNNTQ PFVTTIVGLYGLCLQANSGQVWIEDCSSEK**	**[6936.75]**	
T13	AEQQWALYADGSIRPQQNR	744.37³⁺	b_2-b_6, y''_1, y''_5, $y''_{9}{}^{2+}$-$y''_{17}{}^{2+}$, DG, QQ, EQ, EQQ-NH_3, QQ-NH_3, QQWA-NH_3
T14-ss-T16	**DNCLTSDSNIR ILSCGPASSGQR**	**804.30³⁺**	$[T_B14\text{-ss-}T_B16y''_9\text{-}y''_{11}]^{2+}$, $[T_B16\text{-ss-}T_B14y''_9]^{2+}$, T_B14; y''_1-y''_8, T_B16; y''_1-y''_8, b_2-b_3, a_2
T15	ETVVK	575.34⁺	y''_1-y''_4, b_2-b_3
T17	WMFK	611.30⁺	y''_1-y''_3, b_2, a_2
T18	**NDGTILNLYSGLVLDVR**	**931.51²⁺**	y''_1-y''_5, y''_7-y''_9, b_2, b_3, b_5-b_9, i_9, LN/NL, SGL
T19	**ASDPSLK**	**359.19²⁺**	y''_1-y''_3, b_3, PS/SD-H_2O, PS-CO, PS- H_2O, SL-CO, DP- H_2O, DP, PSL- H_2O, DPS-H_2O/SDP-H_2O, PSL,
T20	**QIILYPLHGDPNQIWLPLF**	**1139.13²⁺**	y''_2-y''_5, y''_{16}, b_{14}-b_{15}, $b_{14}{}^{2+}$-$b_{18}{}^{2+}$

Ricin-specific peptides in bold

Sample 3: Ricin-specific peptides selected for MSMS

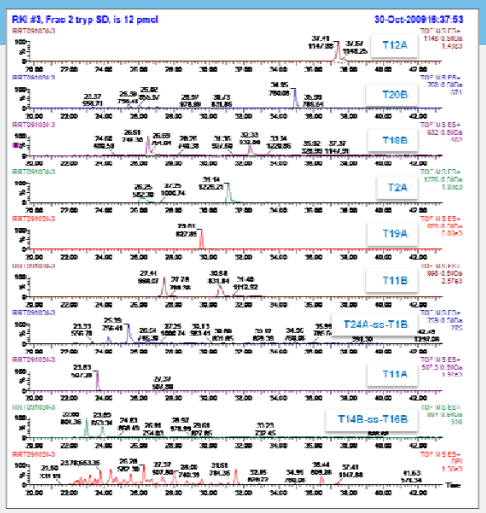
LCMSMS: Waters CapLC-Qtof Ultima

nanoLC: 100 mm 75 um i.d.

PepMap C18.

Gradient: 5-60% MeCN 0.2% formic acid over 30 min

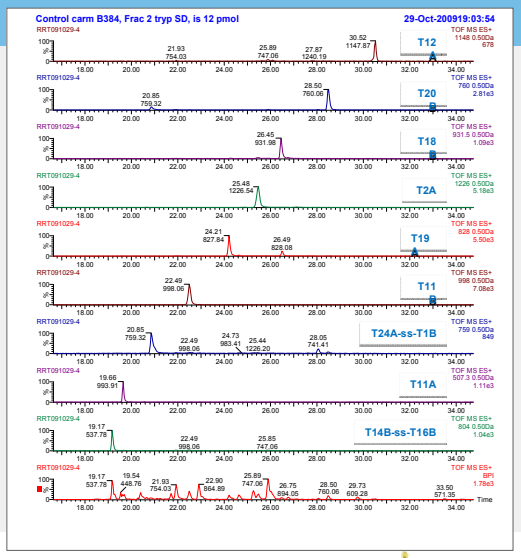
Precursor ions programmed in time segments



Control crude extract: Ricin-specific peptides selected for MSMS

LCMSMS: Waters CapLC-Qtof Ultima
nanoLC: 100 mm 75 um i.d PepMap C18.
Gradient: 5-60% MeCN 0.2% formic acid over 30 min

Precursor ions programmed in time segments

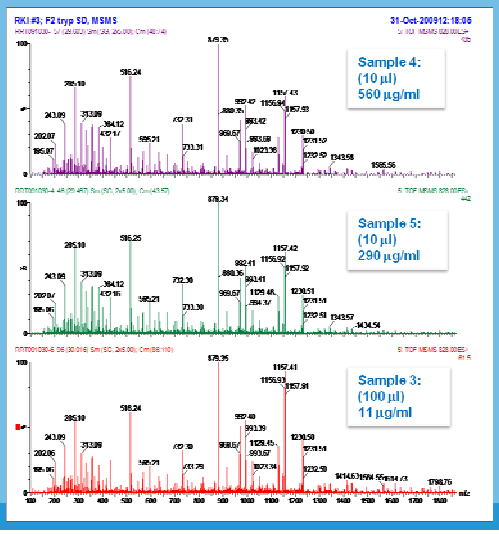


MSMS spectrum T9 Ricin chain A

in Sample 3,4 and 5:

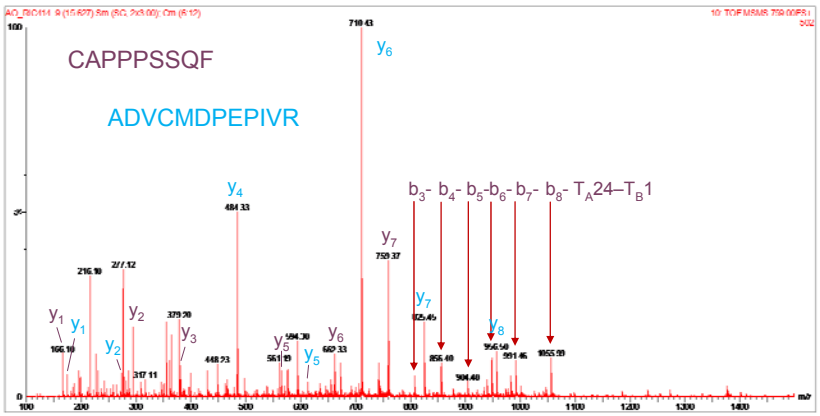
Product ion spectrum of *m/z* 827.64^{4+} (M: 3306.53)

AGNSAYFFHPDNQEDAEAI
THLFTDVQNR
Mw 3306.52



Intact A-B disulfide bond indicates active ricin

Chain A-B disulfide linked peptide: $(T_A24{-}SS{-}T_B1)^{2+}$



Result summary

LCMS & MSMS: Summary of qualitative results

		Qualitative (no. of matching product ions[1])								
	Sample Volume (ul)	T14B -ss- T16B	T11A	T24A -ss- T1B	T11B	T19A	T2A	T18B	T20B	T12A
Sample 3	100	>4	4	>4	>4	>4	>4	>4	>4	>4
Sample 4	20	>4	>4	>4	>4	>4	>4	>4	>4	>4
Sample 5	10	>4	>4	>4	>4	>4	>4	>4	>4	>4

1. Preliminary identification criteria for ricin:
Retention time ±0.2 min;
4 matching product ions/spectrum

Proposed ricin identification criteria

Method A \ Method B	MALDI MS ESI MS	LC-MS	MALDI MS/MS ESI MS/MS	LC-MS/MS MRM	LC-MS/MS
Lateral Flow Assay	A: Positive B: Molecular weight of min. 3 peptides, Δm<0.2, S/N>10	A: Positive B: Ret.time ±0.2 min. Mol. weight, min. 3 peptides, Δm<0.2, S/N>5	A: Positive B: MSMS spectrum min. 2 product ions, Δm<0.2, S/N>10	A: Positive B: : Ret.time ±0.2 min. Min. 3 peptides, min. 2 MRM transitions, S/N>5	A: Positive B: : Ret.time ±0.2 min. MSMS spectrum min. 2 product ions, Δm<0.2, S/N>10
Electrophoresis Chromatography	A: Ret.time ±0.2 min. B: Molecular weight of min. 3 peptides, Δm<0.2, S/N>10	A: Ret.time ±0.2 min. B: Ret.time ±0.2 min. Mol. weight, min. 3 peptides, Δm<0.2, S/N>5	A: Ret.time ±0.2 min. B: MSMS spectrum min. 2 product ions, Δm<0.2, S/N>10	A: Ret.time ±0.2 min. B: Ret.time ±0.2 min. Min. 3 peptides, min. 2 MRM transitions, S/N>5	A: Ret.time ±0.2 min. B: : Ret.time ±0.2 min. MSMS spectrum min. 2 product ions, Δm<0.2, S/N>10
MALDI MS Molecular weight	A: 64000 ±1000 B: Molecular weight, min. 3 peptides, Δm<0.2, S/N>10	A: 64000 ±1000 B: Ret.time ±0.2 min. Mol. weight, min. 3 peptides, Δm<0.2, S/N>5	A: 64000 ±1000 B: MSMS spectrum min. 2 product ions, Δm<0.2, S/N>10	A: 64000 ±1000 B: Ret.time ±0.2 min. Min. 3 peptides, min. 2 MRM transitions, S/N>5	A: 64000 ±1000 B: MSMS spectrum min. 2 product ions, Δm<0.2, S/N>10
ELISA	A: Positive B: Molecular weight. Min 3 peptides, Δm<0.2, S/N>10	A: Positive B: Ret.time ±0.2 min. Mol. weight, min. 3 peptides, Δm<0.2, S/N>5	A: Positive B: MSMS spectrum min. 2 product ions, Δm<0.2, S/N>10	A: Positive B: : Ret.time ±0.2 min. Min. 3 peptides, min. 2 MRM transitions, S/N>5	A: Positive B: : Ret.time ±0.2 min. MSMS spectrum min. 2 product ions, Δm<0.2, S/N>10
PCR	A: Positive B: Molecular weight. Min 3 peptides, Δm<0.2, S/N>10	A: Positive B: Ret.time ±0.2 min. Mol. weight, min. 3 peptides, Δm<0.2, S/N>5	A: Positive B: MSMS spectrum min. 2 product ions, Δm<0.2, S/N>10	A: Positive B: : Ret.time ±0.2 min. Min. 3 peptides, min. 2 MRM transitions, S/N>5	A: Positive B: : Ret.time ±0.2 min. MSMS spectrum min. 2 product ions, Δm<0.2, S/N>10

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Ricin chain A trypsin digest peptides

T# [a]	Peptide sequence [b]	Mass	Observed sequence ions
T1	IFPK	504.32[+]	y''_1-y''_3, b_2, a_2, FP
T2	**QYPIINFTTAGATVQSYTNFIR**	**1225.90**[3+]	y''_1-y''_{11}, b_5, T_A2^{2+}, $[T_A2–HexNAc+2H]^{2+}$, HexNAc, $(HexNAcHex_{1-3})$
T3	AVR	[344.22]	
T4	GR	[231.13]	
T5	**LTTGADVR**	**416.73**[2+]	y''_1-y''_2, b_2/DV, a_2/AD, a_4/TGAD, GAD
T6	HEIPVLPNR	537.81[2+]	y''_1, y''_3-y''_8, b_1-b_3, a_1-a_3, PV
T7	**VGLPINQR**	**448.77**[2+]	y''_1-y''_7, b_2, PL/LP, NQ, GLP, PIN
T8	**FILVELSNHAELSVTLALDVTNAYVVGYR**	**1069.57**[3+]	y''_1-y''_{14}, b_{18}^{2+}-b_{21}^{2+}, b_{23}^{2+}-b_{28}^{2+}, $[M+3H-H_2O]^{3+}$,
T9	**AGNSAYFFHPDNQEDAEAITHLFTDVQNR**	**827.63**[4+]	y''_1, y''_4- y''_{12}, y''_9^{2+}-y''_{12}^{2+}, y''_{20}^{2+}- y''_{21}^{2+}, y''_{22}^{3+}-y''_{28}^{3+}, b_3-b_5, NS, PD-H_20, FH, GNSA, GNSA-NH_3, QED-NH_3, FFH
T10	**YTFAFGGNYDR**	**655.79**[2+]	y''_1, y''_4-y''_{10}, b_4, i_1, i_2/i_9
T11	**LEQLAGNLR**	**507.29**[2+]	y''_1- y''_2, y''_3-y''_8, b_2, AGN-CO
T12	**ENIELGNGPLEEAISALYYYSTGGTQLPTLAR**	**1147.58**[3+]	y''_1-y''_{14}, b_4-b_5, b_{14}, b_{17}, b_{17}^{2+}-b_{21}^{2+}, b_{24}^{2+}- b_{27}^{2+}, $[M+3H-H_2O]^{3+}$
T13	**SFIICIQMISEAAR**	**791.41**[2+]	y''_1, y''_3-y''_{12}, b_2-b_6, a_2
T14	FQYIEGEMR	586.77[2+]	y''_1-y''_2, y''_4-y''_8
T15	TR	[275.16]	
T16	IR	[287.20]	
T17	YNR	[452.23+]	y''_1, a_1
T18	R	[174.11]	
T19	SAPDPSVITLENSWGR	864.93[2+]	y''_2-y''_{14}, y''_{12}^{2+}, y''_{14}^{2+}, b_3-b_6, PD/ DP, PDPSVI
T20	LSTAIQESNQGAFASPIQLQR	1130.09[2+]	y''_1-y''_9, y''_{11}-y''_{16}, b_4-b_7, b_{10}, b_{12}-b_{15}
T21	R	[174.11]	
T22	NGSK	[404.20]	
T23	**FSVYDVSILIPIIALMVYR**	**738.09**[3+]	y''_1-y''_{11}, SI-H_20, IP/PI, YDVS-H_20/SVYD-H_20, VSILI-CO, VYDVS-H_20/SVYDV-H_20, VSILIPIIA-H_20
T24A-ss-T1B	ADVCMDPEPIVR [T_B1] CAPPPSSQF [T_A24]	759.01[3+]	[T_B1+T_A24b_3], [T_B1+T_A24b_3-T_A24b_8], T_B1; y''_1, y''_4-y''_8, T_B24; y''_1-y''_3, y''_5-y''_7, SS, PP, SQ, PPPS, APPPSSQ

Ricin-specific peptides in bold

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Method specifications

Method	
ELISA	MDL down to 0.003 ng/ml (46 amol/ml) Ricin/RCA120 crossreactivity
Lateral Flow Assay	Microgram/ml MDL Ricin/RCA120 crossreactivity
PCR	Highly sensitive/specific for DNA
Functional Assay	Cytotoxicity cell test Adenine release; MS measurement; fmol MDL
Electrophoresis/IEF	Microgram MDL (CBB stain)
MALDI MS	MW +/- 500; pmol sensitivity
MALDI PMF	amol sensitivity
LC-MRM	Low fmol sensitivity
LC-MSMS	fmol sensitivity
MALDI MSMS	fmol sensitivity

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Methods in the Ricin PT

Lab #	ELISA	LFA	PAGE/IEF	PCR	Func. assay	MALDI Mw	MALDI PMF	LC-MRM	LC-MSMS	MALDI MSMS
	Provisional id.			Confirmed id.				Unambiguous id.		
1	x					x				
2	x x									
3	x		x							
4	x		x				x			x
5	x									
6			x					x	x	
7	x	x								x
8	x			x	x		x			
9									x	
10		x								
11	x			x					x	
12	x	x					x		x	
13					x			x	x	

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Methods in the Ricin PT

Method	# labs.	
ELISA	10	Commercial/In house antibodies
Lateral Flow Assay	3	Commercial test kits
PCR	2	In house primers
Functional Assay	2	Cytotoxicity cell test Adenine release - MS measurement
Electrophoresis/IEF	4	Gel electrophoresis (PAGE & IEF PAGE)
MALDI MS	1	Molecular weight
MALDI PMF	4	Peptide mass fingerprint (PMF)
LC-MRM	3	Retention time # peptides, # transitions/peptide
LC-MSMS	4	Retention time Product ion spectrum, # peptides
MALDI MSMS	2	Product ion spectrum, # peptides

RKI International Proficiency Test on Ricin, 2009

FOI

The Ricin Group at FOI

Calle Nilsson

Elisabet Artursson

Mona Byström

Ulla Eriksson

Anders Östin

Tomas Bergström

Sten-Åke Fredriksson

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Considerations on MS methods

- Number of enzymatic digest peptides
- Peptides from both A & B chains
- Specificity of peptides (MALDI & LCMS PMF)
- Proteomic approach vs. comparison with reference standard
- Specificity of LC-MRM transitions
- Retention time and mass accuracy
- Coupled techniques for low conc and complex samples
  - PAGE – In gel digest - Mass spec (Shevchenko et al. Anal. Chem . 1996)
  - Immunoaffinity - Digest – Mass spec (Duriez et al J.Proteome Res. 2008; Kull et al. Anal. Chem. 2010)

Appendix 5

RESULTS OF THE EXPERIMENTAL DATA SURVEY AND UPDATED IDENTIFICATION CRITERIA

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Saxitoxin (STX)

Results of the Experimental Data Survey and Updated Identification Criteria

Meeting of the SAB TWG on Sampling and Analysis
The Hague, 18/19 November, 2010

Martin Schaer
SPIEZ LABORATORY, Switzerland

Schweizerische Eidgenossenschaft
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Confederazione Svizzera
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STX- Identification Criteria 2009

Method **B** / Method **A**	**LC/ESI-MS**	**LC/ESI/MS/MS 1 MRM-Transition**	**LC/ESI-MS/MS**	**LC/ESI/MS/MS 2 MRM-Transitions**
Lateral Flow Assay	**A**: Positive **B**: MS full scan: $[M+H]^+$ 1 structure specific ion, $\Delta m<0.2$ Da, no intensity restriction	**A**: Positive **B**: Ret. time ±0.2 min 1 MRM-Transition S/N ≥ 5	**A**: Positive **B**: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	**A**: Positive **B**: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value
ELISA	**A**: Positive **B**: MS full scan: $[M+H]^+$ plus 1 structure specific ion, $\Delta m<0.2$ Da, no intensity restriction	**A**: Positive **B**: Ret. time ±0.2 min 1 MRM-transition S/N ≥ 5	**A**: Positive **B**: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	**A**: Positive **B**: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value
LC/Fluorescence	**A**: Ret. time ±0.2 min, S/N ≥ 5 **B**: MS full scan: $[M+H]^+$ plus 1 structure specific ion, $\Delta m<0.2$ Da, no intensity restriction	**A**: Ret. time ±0.2 min, S/N ≥ 5 **B**: Ret. time ±0.2 min 1 MRM-transition S/N ≥ 5	**A**: Ret. time ±0.2 min, S/N ≥ 5 **B**: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	**A**: Ret. time ±0.2 min, S/N ≥ 5 **B**: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value

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STX- Identification Criteria 2009

Confirmation level	Minimal Points Sum for combination of Methods A and B
Unambiguous identification	≥ 9
Confirmed	≥ 7
Provisional	≥ 3
No Confirmation	< 3

	No LC/MS method	LC/ESI-MS	LC/ESI/MS/MS 1 MRM-Transition	LC/ESI-MS/MS	LC/ESI/MS/MS 2 MRM-Transitions
No LC/FI or immunoassay method	0	3	4	5	6
Lateral Flow Assay	3	6	7	8	9
ELISA	4	7	8	9	10
LC/Fluorescence	6	9	10	11	12

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STX- Identification Criteria 2009

Situation at the end of the last TWG meeting in 2009:

1. Criteria (e.g. mass accuracy ±0.2 amu)?
2. How to report assay results?
3. Where to draw the line between „sufficient“ and „not sufficient“ data for unambiguous identification?
4. No point system. Stick to the current evaluation scheme.

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STX- Experimental Data Survey

It was decided to organize an experimental data survey…

…to be able to derive draft criteria from the data for consideration by the TWG and to answer the open questions.

2nd June	Templates, Instructions and Results SL sent to all TWG members
15 September	Deadline for reports
	Last contribution arrived 2nd November….;-)

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STX- Experimental Data Survey

Method	Experiment	STX "CRM-STX-e" LOT-#: 20060419	Questions	To Report
ELISA	Assay using ELISA kit	Blank 20ppb	How to report ELISA results? Which ELISA kits will be used?	Positive/negative Calibration curve
LFA	LFA kit	0.1, 0.2 0.3, 0.5, 1ppm	How to report LFA results? Which LFA kits will be used?	Positive/negative LOD Pictures of Strips
LC/Fluorescence	LC run with fluorescence detection	Blank 0.5ppb	S/N calculation feasible? LOD-determination Derivatization	S/N (Blank) S/N (0.5ppb) LOD (S/N=3)
LC/MS	Full scan	Blank 1ppm	Mass accuracy Structure specific ions	Spectrum m/z-values of specific fragments
LC/MS	MS/MS	0.1ppm 1ppm	Mass accuracy Structure specific ions	Spectrum m/z-values of specific fragments Ion intensities
LC/MS	MRM (1 Transition)	0.1ppm 1ppm	Suitable MRM transition S/N possible criteria?	S/N-values
LC/MS	MRM (2 Transitions)	0.1ppm 1ppm	Suitable MRM-transitions	Transitions Intensity ratios Area ratios

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STX- Identification Criteria Proposal

Method	DSO	dstl	FOI	SL	VERIFIN	Contributions
ELISA	✓			✓		2
LFA	✓			✓		2
LC/Fluorescence				✓		1
LC/MS	✓	✓ + HILIC	✓	✓		5
LC/MS/MS	✓	✓ + HILIC	✓	✓	✓	6
LC/MS (1MRM)	✓	✓ + HILIC		✓		4
LC/MS (2MRM)	✓	✓ + HILIC	✓	✓	✓	6

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STX- Identification Criteria Proposal

First of all:

Thank you very much for your contributions!

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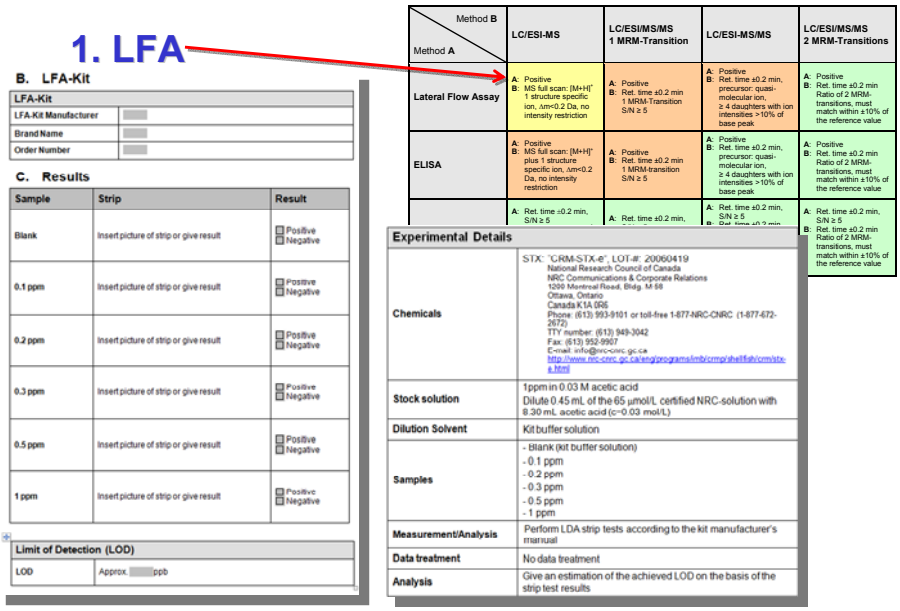
STX- Identification: ELISA



1. LFA

B. LFA-Kit

LFA-Kit	
LFA-Kit Manufacturer	
Brand Name	
Order Number	

C. Results

Sample	Strip	Result
Blank	Insert picture of strip or give result	Positive / Negative
0.1 ppm	Insert picture of strip or give result	Positive / Negative
0.2 ppm	Insert picture of strip or give result	Positive / Negative
0.3 ppm	Insert picture of strip or give result	Positive / Negative
0.5 ppm	Insert picture of strip or give result	Positive / Negative
1 ppm	Insert picture of strip or give result	Positive / Negative

Limit of Detection (LOD)	
LOD	Approx. ppb

Method A \ Method B	LC/ESI-MS	LC/ESI/MS/MS 1 MRM-Transition	LC/ESI-MS/MS	LC/ESI/MS/MS 2 MRM-Transitions
Lateral Flow Assay	A: Positive B: MS full scan: [M+H]+ 1 structure specific ion, Δm<0.2 Da, no intensity restriction	A: Positive B: Ret. time ±0.2 min 1 MRM-Transition S/N ≥ 5	A: Positive B: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	A: Positive B: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value
ELISA	A: Positive B: MS full scan: [M+H]+ plus 1 structure specific ion, Δm<0.2 Da, no intensity restriction	A: Positive B: Ret. time ±0.2 min 1 MRM-transition S/N ≥ 5	A: Positive B: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	A: Positive B: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value
	A: Ret. time ±0.2 min, S/N ≥ 5	A: Ret. time ±0.2 min,	A: Ret. time ±0.2 min, S/N ≥ 5	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value

Experimental Details	
Chemicals	STX: "CRM-STX-e", LOT-#: 20060419 National Research Council of Canada NRC Communications & Corporate Relations 1200 Montreal Road, Bldg. M-58 Ottawa, Ontario Canada K1A 0R6 Phone: (613) 993-9101 or toll-free 1-877-NRC-CNRC (1-877-672-2672) TTY number: (613) 949-3042 Fax: (613) 952-9907 E-mail: info@nrc-cnrc.gc.ca http://www.nrc-cnrc.gc.ca/eng/programs/imb/crmg/shellfish/crm/stx-a.html
Stock solution	1ppm in 0.03 M acetic acid Dilute 0.45 mL of the 65 µmol/L certified NRC-solution with 8.30 mL acetic acid (c~0.03 mol/L)
Dilution Solvent	Kit buffer solution
Samples	- blank (kit buffer solution) - 0.1 ppm - 0.2 ppm - 0.3 ppm - 0.5 ppm - 1 ppm
Measurement/Analysis	Perform LDA strip tests according to the kit manufacturer's manual
Data treatment	No data treatment
Analysis	Give an estimation of the achieved LOD on the basis of the strip test results

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STX- Identification: LFA by SL

A. LFA-Kit

LFA-Kit	
LFA-Kit Manufacturer	JELLET RAPID TESTING LTD.; 4654 Route #3, Chester Basin, Nova Scotia, Canada, www.jellet.ca
Brand Name	JELLETT RAPID TEST for PSP
Order Number	-

Sample	Strip	Result
Blank		☐ Positive ☒ Negative
0.1 ppm		☐ Positive ☒ Negative
0.2 ppm		☐ Positive ☒ Negative
0.3 ppm		☒ Positive ☐ Negative
0.5 ppm		☒ Positive ☐ Negative
1 ppm		☒ Positive ☐ Negative

Limit of Detection (LOD)	
LOD	Approx. 300ppb

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STX- Identification: LFA by DSO

B. LFA-Kit

LFA-Kit	
LFA-Kit Manufacturer	JELLET RAPID TESTING LTD.: 4654 Route #3. Chester Basin, Nova Scotia, Canada, www.jellet.ca
Brand Name	JELLETT RAPID TEST for PSP
Order Number	Batch 40000-01Dec09

G. Discussion/Conclusion

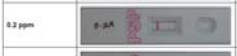
As stated in Section D, we carried out follow up investigations on the LFA kit analyses.

The stock solution was prepared as per TWG's instuctions but replacing acetic acid with deionised water. In addition, the concentration of the LFA samples were calculated based on MW = 372.2 g/mol

From these analyses, the LOD of the sample is at 0.075 ppm. In addition, the 1 ppm sample from the deionised water stock provided a positive LFA result. This indicated that the test kit is sensitive to the samples' pH shifts.

From our analyses, we feel that the LFA kit is easy to use even for a new operator and the binary answer provided by the kit is useful as a screening method. The results must be complemented by a confirmatory method such as tandem mass spectrometry to constitute an identification. However, the LOD values should not be used as an identification criteria due to its variability.

E. Results from Further Investigations

Sample* (stock prepared in water)	Strip	Result
Blank		☐ Positive ☒ Negative
0.01 ppm		☐ Positive ☒ Negative
0.02 ppm		☐ Positive ☒ Negative
0.05 ppm		☐ Positive ☒ Negative
0.075 ppm		☒ Positive ☐ Negative
0.1 ppm		☒ Positive ☐ Negative
0.2 ppm		☒ Positive ☐ Negative
0.3 ppm		☒ Positive ☐ Negative
0.5 ppm		☒ Positive ☐ Negative
1 ppm		☒ Positive ☐ Negative

* Duplicate analyses (Set A and B) was carried out with reproducible results. Only data from Set A was shown.

F. Achieved LOD	
LOD	75 ppb

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STX- Identification: LFA

Qualitative

- Blank (Positive or „Not detected“)
- Sample (Positive or „Not detected“)
- Reference (Positive or „Not detected“)
- Report LOD

Pos:

\+ Simple (for participant and evaluation lab)

Neg:

-Low significance

-Variability

LFA: Proposal for identification criteria

- Blank (Positive or „Not detected“)
- Sample (Positive or „Not detected“)
- Reference (Positive or „Not detected“)
- Report LOD (in spite of variability)
- Report concentration?

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STX- Identification: ELISA

2. ELISA

Method A \ Method B	LC/ESI-MS	LC/ESI/MS/MS 1 MRM-Transition	LC/ESI-MS/MS	LC/ESI/MS/MS 2 MRM-Transitions
Lateral Flow Assay	A: Positive B: MS full scan: $[M+H]^+$ 1 structure specific ion, $\Delta m<0.2$ Da, no intensity restriction	A: Positive B: Ret. time ±0.2 min 1 MRM-Transition S/N ≥ 5	A: Positive B: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	A: Positive B: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value
ELISA	A: Positive B: MS full scan: $[M+H]^+$ plus 1 structure specific ion, $\Delta m<0.2$ Da, no intensity restriction	A: Positive B: Ret. time ±0.2 min 1 MRM-transition S/N ≥ 5	A: Positive B: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	A: Positive B: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value
LC/Fluorescence	A: Ret. time ±0.2 min, S/N ≥ 5 B: MS full scan: $[M+H]^+$ plus 1 structure specific ion, $\Delta m<0.2$ Da, no intensity restriction	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min 1 MRM-transition S/N ≥ 5	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value

Experimental Details	
Chemicals	STX: "CRM-STX-e", LOT-#: 20060419 National Research Council of Canada NRC Communications & Corporate Relations 1200 Montreal Road, bldg. M-58 Ottawa, Ontario, Canada K1A 0R6 Phone: (613) 993-9101 or toll-free 1-877-NRC-CNRC (1-877-672-2672), TTY number: (613) 949-3042 Fax: (613) 952-9907 E-mail: info@nrc-cnrc.gc.ca http://www.nrc-cnrc.gc.ca/eng/programs/imb/comp/shellfish/crm/stx-e.html
Stock solution	1ppm in 0.03 M acetic acid Dilute 0.45 mL of the 65 µmol/L certified NRC-solution with 8.30 mL acetic acid (c=0.03 mol/L)
Dilution Solvent	Solvents/solutions contained in the ELISA-Kit
Samples	- Blank (manufacturers dilution solvent/solution) - 20 ppb
Measurement/Analysis	- Calibration curve (According to the manufacturer's manual) 2 Samples (repeat measurements 3 times)
Data treatment	According to the manufacturer's manual
Analysis	- Calculate calibration curve - Test positive or negative? - Calculate concentration of STX

B. ELISA-Kit Information

ELISA-Kit	
Manufacturer	
Type	Order Number

C. Results

Parameter	Result
Calibration Curve (use standards included in the ELISA-kit if possible)	Insert graph of calibration curve with calibration sample from kit
Blank	☐ Positive ☐ Negative
20 ppb	☐ Positive ☐ Negative ☐ Calculated amount: ± ppb

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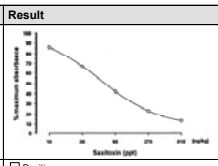
STX- Identification: ELISA

A. ELISA-Kit Information

ELISA-Kit	
Manufacturer	r-biopharm AG, Darmstadt, Germany
Type	RIDASCREEN®FAST PSP SC Order Number: R1905

B. Results

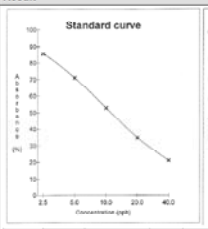
Parameter	Result
Calibration Curve (use standards included in the ELISA-kit if possible)	
Blank	☐ Positive ☒ Negative
20 ppb	☒ Positive ☐ Negative Calculated amount: Not determined

Qualitative

B. ELISA-Kit Information

ELISA-Kit	
Manufacturer	r-biopharm AG, Darmstadt, Germany
Type	RIDASCREEN®FAST PSP SC Order Number: R1905

C. Results from TWG survey

Parameter	Result
Calibration Curve (standards included in the ELISA-kit were used)	 Standards Conc (ppb) / A [Mean] 2.5 1.573 (Coeff. of Var.: 0.0%) 5.0 1.305 (Coeff. of Var.: 0.0%) 10.0 0.971 (Coeff. of Var.: 0.0%) 20.0 0.640 (Coeff. of Var.: 0.0%) 40.0 0.388 (Coeff. of Var.: 0.0%) Conc (ppb) | Abs at 450nm (mean of 3 meas) | B/B0 (%) | Deviation (%) | Measured Conc (ppb) Standard: 0 (Std) 1.839 100 0 2.5 1.573 85.5 0 5 1.305 71 0 10 0.971 52.8 0.1 20 0.640 34.8 0.1 40 0.388 21.1 0.1 Sample: 20, Rep 1 0.458 24.9 32.41 20, Rep 2 0.443 24.1 33.86 20, Rep 3 0.435 23.7 34.67
Blank	☐ Positive ☒ Negative
20 ppb	☒ Positive ☐ Negative ☒ Calculated amount: 33.65 ± 1.23 ppb

Quantitative

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STX- Identification: ELISA

Qualitative

- Blank (Positive or „Not detected“)
- Sample (Positive or „Not detected“)
- Reference (Positive or „Not detected“)
- Report LOD

Pos:
+ Simple (for participant and evaluator)

Neg:
- Low significance

Quantitative

- Show absorption of Reference vs. Sample
- Show Blank (Positive or „Not detected“)
- Show Sample (Positive or „Not detected“)
- Reference (Positive or „Not detected“)
- Calculate amounts
- SDD ±??ppb
- MD (Mean difference)

Pos:
+ Simple (for participant and evaluator)

Neg:
-Evaluation difficult
-MD and SSD need several participants

Proposal for identification criteria:

- Qualitative
- Blank (Positive or „Not detected“)
- Sample (Positive or „Not detected“)
- Reference (Positive or „Not detected“)
- Report LOD

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STX- Identification: LC/Fluorescence

3. LC/Fluorescence

B. Instructions

Experimental Details	
Chemicals	STX: "CRM-STX-e", LOT-#: 20060419
Stock solution	1 ppm in 0.03 M acetic acid
Dilution solution	0.03 M acetic acid
Samples	Calibration samples -Blank, i.e. 0.03 M acetic acid - 0.5 ppb in 0.03 M acetic acid
Sample Preparation	No sample preparation except: - Dilution - Derivatization
Derivatization	Peroxide derivatization according to DIN EN 14526, See (1).
Measurements	Repeat each measurement 3 times. Excitation wavelength λ=340nm Emission wavelength λ=400nm
Data treatment	No data treatment
Analysis	S/N should be calculated using: - Instrument specific software, or - Manually, by using the formula given below - Give mean value of S/N and relative standard deviation - Calculate LOD for S/N=3

Method B / Method A	LC/ESI-MS	LC/ESI/MS/MS 1 MRM-Transition	LC/ESI-MS/MS	LC/ESI/MS/MS 2 MRM-Transitions
Lateral Flow Assay	A: Positive B: MS full scan: [M+H]+ 1 structure specific ion, Δm<0.2 Da, no intensity restriction	A: Positive B: Ret. time ±0.2 min 1 MRM-Transition S/N ≥ 5	A: Positive B: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	A: Positive B: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value
ELISA	A: Positive B: MS full scan: [M+H]+ plus 1 structure specific ion, Δm<0.2 Da, no intensity restriction	A: Positive B: Ret. time ±0.2 min 1 MRM-transition S/N ≥ 5	A: Positive B: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	A: Positive B: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value
LC/Fluorescence	A: Ret. time ±0.2 min, S/N ≥ 5 B: MS full scan: [M+H]+ plus 1 structure specific ion, Δm<0.2 Da, no intensity restriction	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min 1 MRM-transition S/N ≥ 5	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value

Parameter	Result
Calibration Curve	Insert graph of calibration curve together with linear regression
Chromatograms: - Blank - 0.5 ppb sample (please show x-axis)	Show one chromatogram of the 0.5 ppb measurement together with S/N-calculation
S/N (Blank)	S/N = [ ] (n= [ ]) Calculated with: ☐ Software: specify [ ] ☐ Manually using baseline range and peak height Please indicate used noise-range in chromatogram ☐ Other: [ ]
S/N (0.5 ppb sample)	S/N = [ ] (n= [ ]) Calculated with: ☐ Software: specify [ ] ☐ Manually using baseline range and peak height Please indicate used noise-range in chromatogram ☐ Other: [ ]
LOD (S/N=3)	LOD= [ ] ppb

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STX- Identification: LC/Fluorescence

A. Instrument and Method

Instrument: Liquid Chromatography	
Manufacturer	DIONEX
Type	HPLC
Autosampler	ASI-100
Pump	P580 LPG
Column-oven	STH-585
Degasser	-
Column	Manufacturer: Agilent Type: ODS-Hypersil Material: C18 Dimension: 4.6x250mm Particle size: 5 µm

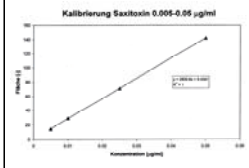
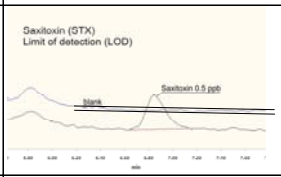
Instrument: Fluorescence Detector	
Manufacturer	DIONEX
Type	RF-2000
Additional Information	Excitation wavelength λ=340nm Emission wavelength λ=400nm

Method	Parameters/Procedure
Peroxide- Derivatization	Method and/or reference: Peroxide derivatization according to DIN EN 14526
LC	Solvent A: Acetonitril Solvent B: Ammoniumformiate-buffer Flow: 0.75 mL/min Temperature: 25 °C Gradient:

Time [min]	%A	%B
0	0	100
2	0	100
17	100	0
25	100	0
26	0	100
35	0	100

Injection Volume: 25 µL
Other:

A. Results

Parameter	Result
Calibration Curve	
Chromatograms: - Blank - 0.5 ppb sample (please show x-axis)	
S/N (Blank)	S/N = 0 (No signal) Calculated with: ☐ Software: specify ☐ Manually using baseline range and peak height Please indicate used noise-range in chromatogram ☐ Other:.....
S/N (0.5 ppb sample)	S/N = 8.5±12% (N=3) Calculated with: ☐ Software: specify ☒ Manually using baseline range and peak height Please indicate used noise-range in chromatogram ☐ Other:.....
LOD (S/N=3)	LOD = 0.2 ppb

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STX- Identification: LC/Fluorescence

- Blank
- Sample
- Reference

Pos:

+ Same as
  Work Instruction for the Evaluation of the Results of OPCW Proficiency Tests, QDOC/LAB/WI/PT03, Attachment 1, §3
+ Pre-column derivatisation is PSP specific

Neg:

-

LC/Fluorescence: Proposal for identification criteria

•Same as in
Work Instruction for the Evaluation of the Results of OPCW Proficiency Tests, QDOC/LAB/WI/PT03, Attachment 1, §3
•Additional method to biochemical methods?

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STX- Identification: LC/MS

4. LC/MS

Sample	Result
Blank	Insert TIC spectrum
1 ppm	Insert TIC spectrum
	Insert mass spectrum. Use 1 decimal place for m/z-label precision

Structure specific ions	$m/z_{experimental}$	$m/z_{theoretical}$	Origin ([M+H]$^+$, fragment ion, structure, etc.)
	Monoisotopic m/z-value		

Method B / Method A	LC/ESI-MS	LC/ESI/MS/MS 1 MRM-Transition	LC/ESI-MS/MS	LC/ESI/MS/MS 2 MRM-Transitions
Lateral Flow Assay	A: Positive B: MS full scan: [M+H]$^+$ 1 structure specific ion, Δm<0.2 Da, no intensity restriction	A: Positive B: Ret. time ±0.2 min 1 MRM-Transition S/N ≥ 5	A: Positive B: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	A: Positive B: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value
ELISA	A: Positive B: MS full scan: [M+H]$^+$ plus 1 structure specific ion, Δm<0.2 Da, no intensity restriction	A: Positive B: Ret. time ±0.2 min 1 MRM-transition S/N ≥ 5	A: Positive B: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	A: Positive B: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value
LC/Fluorescence	A: Ret. time ±0.2 min, S/N ≥ 5 B: MS full scan: [M+H]$^+$ plus 1 structure specific ion, Δm<0.2 Da, no intensity restriction	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min 1 MRM-transition S/N ≥ 5	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value

Experimental Details	
Chemicals	STX: "CRM-STX-e", LOT-#: 20080419 National Research Council of Canada NRC Communications & Corporate Relations 1200 Montreal Road, Bldg. M-58 Ottawa, Ontario Canada K1A 0R6 Phone: (613) 993-9101 or toll-free 1-877-NRC-CNRC (1-877-672-2672) TTY number: (613) 949-3042 Fax: (613) 952-9907 E-mail: info@nrc-cnrc.gc.ca http://www.nrc-cnrc.gc.ca/eng/programs/imb/crmp/shellfish/crm/stx-e.html
Stock solution	1 ppm in 0.03 M acetic acid Dilute 0.45 mL of the 65 µmol/L certified NRC-solution with 8.30 mL acetic acid (c=0.03 mol/L)
Dilution solution	Acetic acid (c=0.03 mol/L)
Samples	- Blank, i.e. 0.03 M acetic acid - 1 ppm in 0.03 M acetic acid
Sample Preparation	Dilution
Measurement	Full scan mode
Data treatment	Smoothing, centroiding, background subtraction etc. as for PT reporting
Analysis	Show TIC and mass spectrum

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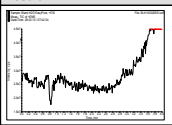
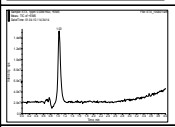
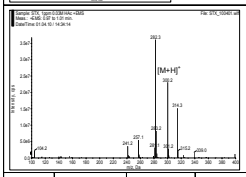
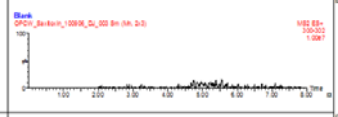
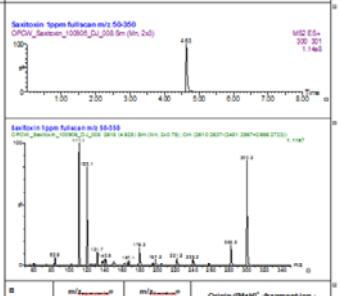
STX- Identification: LC/MS

Linear Ion Trap - *AB 3200QTrap*

Sample	Result
Blank	
1 ppm	
	

Structure specific ions	$m/z_{experimental}$	$m/z_{theoretical}$	Origin ([M+H]$^+$, fragment ion, structure, etc.)
	Monoisotopic m/z-value		
	300.2	300.1	[M+H]$^+$
	282.3	282.1	Fragment: -H_2O
	257.1	-	?
	241.2	-	?

Triple Quad - *WATERS Xevo TQ*

Sample	Result
Blank	
1 ppm	

Structure specific ions	Monoisotopic m/z-values		Origin ([M+H]$^+$, fragment ion, structure, etc.)
	300.2	300.1	(M+H)$^+$
	282.2	282.1	(M+H-H_2O)$^+$

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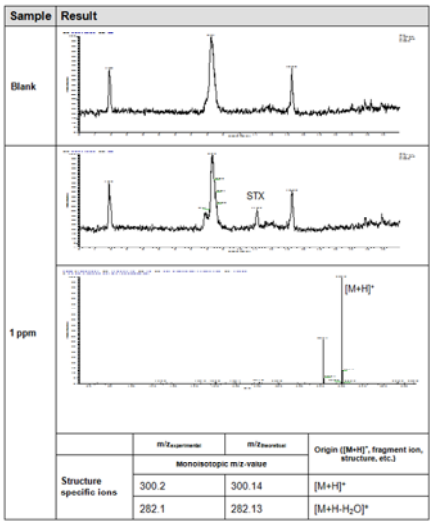
STX- Identification: LC/MS

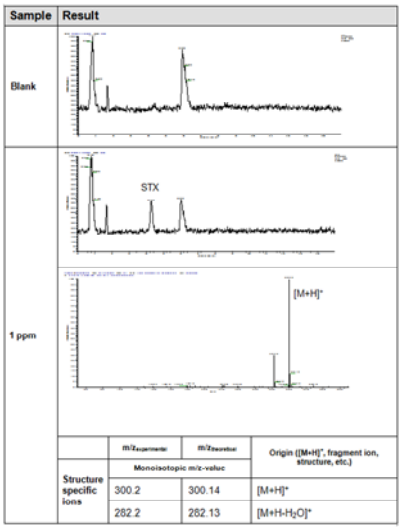
Ion Trap – *ThermoFinnigan LCQ Deca XP Plus*

C18



Structure specific ions	$m/z_{experimental}$ (Monoisotopic m/z-value)	$m/z_{theoretical}$ (Monoisotopic m/z-value)	Origin ([M+H]+, fragment ion, structure, etc.)
	300.2	300.14	$[M+H]^+$
	282.1	282.13	$[M+H-H_2O]^+$

HILIC



Structure specific ions	$m/z_{experimental}$ (Monoisotopic m/z-value)	$m/z_{theoretical}$ (Monoisotopic m/z-value)	Origin ([M+H]+, fragment ion, structure, etc.)
	300.2	300.14	$[M+H]^+$
	282.2	282.13	$[M+H-H_2O]^+$

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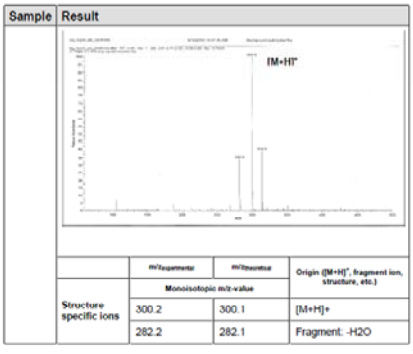
STX- Identification: LC/MS

Ion Trap

Thermo Fisher Scientific - LCQ Fleet



Structure specific ions	$m/z_{experimental}$ (Monoisotopic m/z-value)	$m/z_{theoretical}$ (Monoisotopic m/z-value)	Origin ([M+H]+, fragment ion, structure, etc.)
	300.2	300.1	$[M+H]^+$
	282.2	282.1	Fragment: -H2O

Comments / Additional Information

In full scan mode, an interference peak at 0.8 min was detected when acetic acid was used as the sample blank. This interference peak was also observed in 1 ppm STX sample (acetic acid is used as the dilution solution). This accounts for 2 separate peaks in 1 ppm sample's TIC (STX peak was observed at 0.7 min). We used acetic acid as the sample blank as instructed in the sample report, although a water blank was shown in the sample report instead. With water as our sample blank, there will be no interference peak (see chromatogram above).

The MS spectrum obtained was also slightly different. Using our LCQ Fleet, the base peak was m/z of 300.2. This differs from analysis using QTrap in the sample report (base peak: m/z of 282.3). This illustrates that different ion trap instruments will produce different MS spectra.

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STX- Identification: LC/MS

MS	Ionisation	LC	[M+H]⁺ Theoretical m/z	[M+H]⁺ Experimental m/z	[M+H]⁺ Δm	Base peak m/z	Fragment m/z
Ion Trap *QTrap*	ESI	C18	300.1	300.2	+0.1	282.3	282.3
Triple Quad *Xevo TQ*		BEH AMIDE		300.2	+0.1	300.2	282.2
Ion Trap LCQ *Deca XP Plus*		C18		300.2	+0.1	300.2	282.1
		HILIC		300.2	+0.1	300.2	282.2
Ion Trap *LCQ Fleet*		C18		300.2	+0.1	300.2	282.2

4 of 5 labs report $[M+H]^+$ and 1 fragment ion. Other fragment ion intensities <10%.
All Δm are within ±0.2 amu.

LC/MS: Proposal for identification criteria

- **LC**: According to *Work Instruction for the Evaluation of the Results of OPCW Proficiency Tests, QDOC/LAB/WI/PT03, Attachment 1, §3*
- **MS**: Present $[M+H]^+$ plus 1 specific fragment ion in a mass spectrum. Mass accuracy ±0.2 amu (absolute and versus reference). Reference and sample.

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STX- Identification: LC/MS/MS

5. LC/MS/MS

Sample	Result
0.1 ppm	Fragment ions: $m/z_{Experimental}$ | Intensity [%] Basepeak=100% | $m/z_{Experimental}$ | Intensity [%] Basepeak=100%
1 ppm	Fragment ions: $m/z_{Experimental}$ | Intensity [%] Basepeak=100% | $m/z_{Experimental}$ | Intensity [%] Basepeak=100%

Method B / Method A	LC/ESI-MS	LC/ESI/MS/MS 1 MRM-Transition	LC/ESI-MS/MS	LC/ESI/MS/MS 2 MRM-Transitions
Lateral Flow Assay	A: Positive B: MS full scan: $[M+H]^+$ 1 structure specific ion, Δm<0.2 Da, no intensity restriction	A: Positive B: Ret. time ±0.2 min 1 MRM-Transition S/N ≥ 5	A: Positive B: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	A: Positive B: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value
ELISA	A: Positive B: MS full scan: $[M+H]^+$ plus 1 structure specific ion, Δm<0.2 Da, no intensity restriction	A: Positive B: Ret. time ±0.2 min 1 MRM-transition S/N ≥ 5	A: Positive B: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	A: Positive B: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value
LC/Fluorescence	A: Ret. time ±0.2 min, S/N ≥ 5 B: MS full scan: $[M+H]^+$ plus 1 structure specific ion, Δm<0.2 Da, no intensity restriction	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min 1 MRM-transition S/N ≥ 5	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value

Experimental Details	
Chemicals	STX: "CRM-STX-e", LOT-#: 20060419 National Research Council of Canada 1200 Montreal Road, Bldg. M-58 Ottawa, Ontario Canada K1A 0R6 Phone: (613) 993-9101 or toll-free 1-877-NRC-CNRC (1-877-672-2672) TTY number: (613) 943-3042 Fax: (613) 952-9607 E-mail: info@nrc-cnrc.gc.ca http://www.nrc-cnrc.gc.ca/eng/programs/imb/cmp/shellfish/cmi/stx-e.html
Stock solution	1 ppm in 0.03 M acetic acid Dilute 0.45 mL of the 65 µmol/L certified NRC-solution with 8.30 mL acetic acid (c=0.03 mol/L)
Dilution solution	Acetic acid (c=0.03 mol/L)
Samples	- 0.1 ppm in 0.03 M acetic acid - 1 ppm in 0.03 M acetic acid
Sample Preparation	Dilution
Measurement	- MS/MS-Mode (ion trap: MS^2 or trapping mode) - Precursor ion: $[M+H]^+$, m/z 300
Data treatment	Spectra be smoothed, centroided, etc. as for PT reports
Analysis	List fragmentions to be used for identification with intensities >20% of base peak

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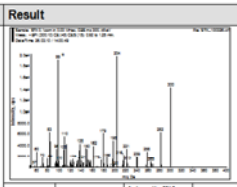
STX- Identification: LC/MS/MS

Linear Ion Trap - *AB 3200QTrap*

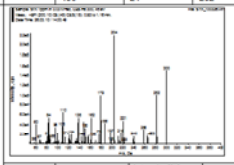
Sample	Result

0.1 ppm



Fragment ions	m/z Experimental	Intensity [%] Basepeak=100%	m/z Experimental	Intensity [%] Basepeak=100%
	83	24	162	21
	108	21	180	22
	110	27	204	100
	114	29	258	22
	138	24	282	26

1 ppm

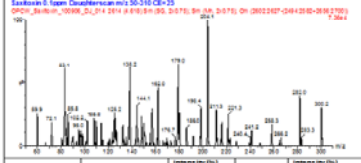


Fragment ions	m/z Experimental	Intensity [%] Basepeak=100%	m/z Experimental	Intensity [%] Basepeak=100%
	83	30	179	48
	84	26	180	24
	110	37	204	100
	137	26	221	20
	138	30	282	36
	148	23		
	162	25		

Triple Quad - *WATERS Xevo TQ*

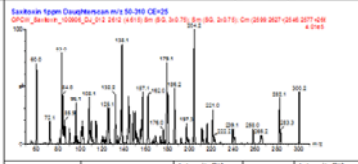
Sample	Result

0.1 ppm



Fragment ions	m/z Experimental	Intensity [%] Basepeak=100%	m/z Experimental	Intensity [%] Basepeak=100%
	83.1	61	179.0	68
	138.2	64	198.4	37
	144.1	33	204.1	100
	157.0	30	282.0	39
	162.0	51		

1 ppm



Fragment ions	m/z Experimental	Intensity [%] Basepeak=100%	m/z Experimental	Intensity [%] Basepeak=100%
	60.0	68	157.1	50
	83.0	78	162.0	45
	96.1	36	179.1	74
	108.1	44	186.2	46
	132.2	41	204.2	100
	138.1	84	221.1	31
	144.1	42	282.1	38

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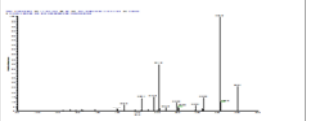
STX- Identification: LC/MS/MS

Ion Trap - *ThermoFinnigan LCQ Deca XP Plus*

C18

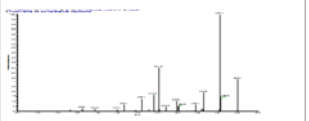
Sample	Result

0.1 ppm



Fragment ions	m/z Experimental	Intensity [%] Basepeak=100%	m/z Experimental	Intensity [%] Basepeak=100%
	282.0	100		
	221.0	50.3		

1 ppm

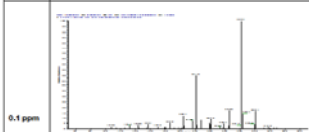


Fragment ions	m/z Experimental	Intensity [%] Basepeak=100%	m/z Experimental	Intensity [%] Basepeak=100%
	282.1	100		
	221.0	45.8		

HILIC

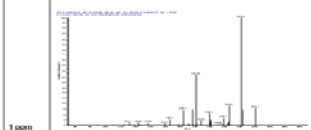
Sample	Result

0.1 ppm



Fragment ions	m/z Experimental	Intensity [%] Basepeak=100%	m/z Experimental	Intensity [%] Basepeak=100%
	282.1	100		
	266.0	20.7		
	221.0	52.7		

1 ppm



Fragment ions	m/z Experimental	Intensity [%] Basepeak=100%	m/z Experimental	Intensity [%] Basepeak=100%
	282.1	100		
	221.0	47.2		

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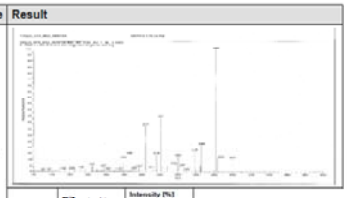
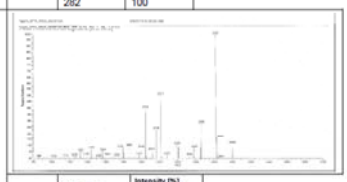
STX- Identification: LC/MS/MS

Ion Trap
Thermo Fisher Scientific - LCQ Fleet

Sample	Result
0.1 ppm	
Fragment ions	

m/z Experimental	Intensity [%] Basepeak=100%
204	38
221	44
266	22
282	100

Sample	Result
1 ppm	
Fragment ions	

m/z Experimental	Intensity [%] Basepeak=100%
204	40
216	23
221	50
266	28
282	100

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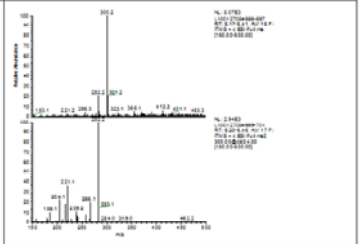
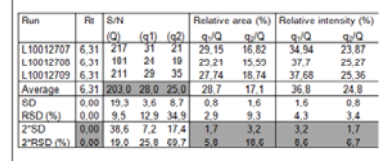
STX- Identification: LC/MS/MS

Linear Ion Trap
Thermo Fisher Scientific - LXQ

Sample	Result
1 ppm	
Fragment ions	

m/z Experimental	Intensity [%] Basepeak=100%	m/z Experimental	Intensity [%] Basepeak=100%
186 (q_3)	10		
204 (q_2)	21		
221(q_1)	36		
282 (Q)	100		

Transitions		t_R [min]
1. Transition	m/z 282→221 (q_1/Q)	6.3 min
2. Transition	m/z 282→204 (q_2/Q)	
3. Transition	m/z 282→186 (q_3/Q)	

Sample	Signals and Ratios R_I and R_A
1 ppm in HCl	

Run	Rt	S/N			Relative area (%)		Relative intensity (%)	
		(Q)	(q_1)	(q_2)	q_1/Q	q_2/Q	q_1/Q	q_2/Q
L10012707	6.31	217	31	21	29.15	16.82	34.94	23.87
L10012708	6.31	181	24	19	29.21	15.59	37.7	25.27
L10012709	6.31	211	29	35	27.74	18.74	37.68	25.36
Average	6.31	203.0	28.0	25.0	28.7	17.1	36.8	24.8
SD	0.00	19.3	3.6	8.7	0.8	1.6	1.6	0.8
RSD (%)	0.00	9.5	12.9	34.9	2.9	9.3	4.3	3.4
2*SD	0.00	38.6	7.2	17.4	1.7	3.2	3.2	1.7
2*RSD (%)	0.00	19.0	25.8	69.7	5.8	18.6	8.6	6.7

1 ppm in eluent buffer	

Run	Rt	S/N				Relative area (%)			Relative Intensity (%)		
		Q	q_1	q_2	q_3	q_1/Q	q_2/Q	q_3/Q	q_1/Q	q_2/Q	q_3/Q
L10012703	6.29	342	71	40	33	33.53	19.51	8.20	32.38	22.99	13.88
L10012704	6.28	387	72	39	25	33.08	18.28	7.79	34.03	23.12	10.01
L10012705	6.29	284	56	35	16	32.46	19.09	5.84	39.82	25.18	9.63
Average	6.29	337.7	66.3	38.0	24.7	33.0	19.0	7.3	35.4	23.8	11.2
SD	0.01	51.6	9.0	2.6	8.5	0.5	0.6	1.3	3.9	1.2	2.4
RSD (%)	0.09	15.3	13.5	7.0	34.5	1.6	3.3	17.3	11.0	5.2	21.0
2*SD	0.01	103.3	17.9	5.3	17.0	1.1	1.3	2.5	7.8	2.5	4.7
2*RSD (%)	0.18	30.6	27.0	13.9	69.0	3.3	6.6	34.7	22.1	10.3	42.1

Ratio (mean±relSD)	
1 ppmin HCl	1 ppmin eluent buffer
Intensity ratio: (q_1/Q) R_A=36.8 ± 4.3 % (N=3)	Intensity ratio: (q_1/Q) R_A=35.4 ± 11 % (N=3)
Area ratio: (q_1/Q) R_I=28.7 ± 2.9 % (N=3)	Area ratio: (q_1/Q) R_I=33.0 ± 1,6 % (N=3)

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STX- Identification: LC/MS/MS

MS	Collision Energy [eV]	Fragment1		Fragment2		Fragment3		Fragment4		Fragment5	
		m/z	Rel. Int. % of Base peak	m/z	Rel. Int. % of Base peak	m/z	Rel. Int. % of Base peak	m/z	Rel. Int. % of Base peak	m/z	Rel. Int. % of Base peak
Ion Trap	45eV	282.2	36	204.1	100	179.1	48	138.1	30	110.0	37
Triple Quad	25eV	282.1	38	204.2	100	179.1	74	138.1	84	83	78
Ion Trap	24%/25%	282.1	100	221.0	46/47	-	-	-	-	-	-
Ion Trap	22eV	282	100	266	28	221	50	216	23	204	40
Linear Ion	34eV(?)	282	100	221	36	204	21	186	10	-	-

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STX- Identification: LC/MS/MS

- 3 of 5 labs detect 5 or more fragment ions with intensities > 10% of base peak. All Δm are within ±0.1 amu.
- All labs find at least 3 fragment ions with relative abundance > 20%.
- m/z 282 is base peak for 3 labs. Two labs see m/z 204 as base peak.
- VERIFIN points out to use m/z 282 as quantifier ion for MRM-experiments.
- Resolution was unit or 0.7 amu (FWHM)

LC/MS: Proposal for identification criteria

•**LC**:
According to *Work Instruction for the Evaluation of the Results of OPCW Proficiency Tests, QDOC/LAB/WI/PT03, Attachment 1, §3*

•**MS**:
Same as *Work Instruction for the Evaluation of the Results of OPCW Proficiency Tests, QDOC/LAB/WI/PT03, Attachment 2, §4.3*

•Minimum unit resolution

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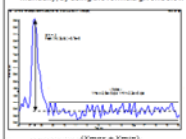
STX- Identification: LC/SRM (1 Transition)

6. LC/SRM

Experimental Details	
Chemicals	STX: "CRM-STX-e", LOT-#: 200604119 National Research Council of Canada NRC Communications & Corporate Relations 1200 Montreal Road, Bldg. M-58, Ottawa, Ontario, Canada K1A 0R6 Phone: (613) 993-9101 or toll-free 1-877-NRC-CNRC (1-877-672-2672) Fax: (613) 952-9907 E-mail: info@nrc-cnrc.gc.ca http://www.nrc-cnrc.gc.ca/eng/programs/imb/cmp/shellfish/crm/stx-e.html
Stock solution	1 ppm in 0.03 M acetic acid Dilute 0.45 mL of the 65 µmol/L certified NRC-solution with 8.30 mL acetic acid (c=0.03 mol/L)
Dilution solution	Acetic acid (c=0.03 mol/L)
Samples	- 0.1 ppm in 0.03 M acetic acid - 1 ppm in 0.03 M acetic acid
Sample Preparation	Dilution
Measurement	- 1 SRM/MRM transition: Choose m/z-pair for Q1 and Q3, respectively, with sufficiently high intensity. Care must be taken not to choose the ammonia or water loss peaks for the MRM experiment because that would result in a less than unique transition. - Use the same transition for all samples - Optimize SRM/MRM-transition parameters - Repeat measurement 3 times
Data treatment	No data treatment
Analysis	S/N should be calculated using: - Instrument specific software, or - Manually, by using the formula given below  $S/N = \frac{(Peak\ Int. - \frac{(Ymax + Ymin)}{2})}{(Ymax - Ymin)}$ - Calculate mean value and relative standard deviation of S/N

Method B / Method A	LC/ESI-MS	LC/ESI/MS/MS 1 MRM-Transition	LC/ESI-MS/MS	LC/ESI/MS/MS 2 MRM-Transitions
Lateral Flow Assay	A: Positive B: MS full scan: [M+H]+ 1 structure specific ion, Δm<0.2 Da, no intensity restriction	A: Positive B: Ret. time ±0.2 min 1 MRM-Transition S/N ≥ 5	A: Positive B: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	A: Positive B: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value
ELISA	A: Positive B: MS full scan: [M+H]+ plus 1 structure specific ion, Δm<0.2 Da, no intensity restriction	A: Positive B: Ret. time ±0.2 min 1 MRM-transition S/N ≥ 5	A: Positive B: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	A: Positive B: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value
LC/Fluorescence	A: Ret. time ±0.2 min, S/N ≥ 5 B: MS full scan: [M+H]+ plus 1 structure specific ion, Δm<0.2 Da, no intensity restriction	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min 1 MRM-transition S/N ≥ 5	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value

Sample	SRM/MRM transition	t_R [min]	S/N (mean value ± relSD[1])
0.1 ppm	Q1: m/z Q3: m/z		S/N= ± % (N=)
1 ppm			S/N= ± % (N=)

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STX- Identification: LC/SRM (1 Transition)

Linear Ion Trap *AB 3200QTrap* Triple Quad *WATERS Xevo TQ*

Sample	SRM/MRM transition	t_R [min]	S/N (mean value ± relSD[1])
0.1 ppm	Q1: m/z 300 Q3: m/z 204	1.0	S/N=43±14% (N=3)
1 ppm			S/N=291±4% (N=3)

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STX- Identification: LC/SRM (1 Transition)

Ion Trap - *ThermoFinnigan LCQ Deca XP Plus*

C18

Sample	SRM/MRM transition	t_R [min]	S/N (mean value ± relSD[1])
0.1 ppm	m/z 300.1 → m/z 221.0 ± 1	11.05	S/N=253±0.23% → (N=3)
1 ppm			S/N=2352±10.2% → (N=3)

Comments / Additional Information

Noise level was difficult to measure owing to almost complete lack of baseline noise, even on expanded baseline. Small differences in noise lead to large differences in S/N.

HILIC

Sample	SRM/MRM transition	t_R [min]	S/N (mean value ± relSD[1])
0.1 ppm	m/z 300.1 → m/z 221.0 ± 1	4.26	S/N=90.7±10.8% → (N=3)
1 ppm			S/N=228±21.6% → (N=3)

Comments / Additional Information

Blank (0.03M acetic acid), 1 ppm STX and 0.1 ppm STX solutions evaporated to dryness in centrifugal evaporator and redissolved in appropriate volume of mobile phase B to give the same concentrations of STX. Original 0.03M acetic acid solutions are not compatible with analysis using HILIC conditions.
Instrument tune for STX was not optimised under HILIC conditions. Optimisation would be expected to improve sensitivity.
Noise level was difficult to measure owing to almost complete lack of baseline noise, even on expanded baseline. Small differences in noise lead to large differences in S/N.

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STX- Identification: LC/SRM (1 Transition)

Linear Ion Trap

Thermo Fisher Scientific - LCQ Fleet

Sample	SRM transition	t_R [min]	S/N (mean value ± relSD[1])
0.1 ppm	Q1: m/z 300 Q3: m/z 204	0.7	S/N=33895±3.6% (N=3)
1 ppm			S/N=68617±8.6% (N=3)

Comments / Additional Information

Analysis was carried out with 1 SRM transition as described in the instructions section. The analytical technique was reproducible.

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STX- Identification: LC/SRM (1 Transition)

MS	LC	Q1 m/z	Q3 m/z	S/N Mean±relSD	
				0.1ppm	1ppm
Ion Trap *QTrap*	C18	300.1	204.1	43±14%	291±4%
Triple Quad *Xevo TQ*		-	-	-	-
Ion Trap *LCQ Deca XP PLus*	C18	300.1	221.0±1	253±0.23%	2352±10.2%
	HILIC			90.7±10.8%	228±21.5%
Ion Trap *LCQ Fleet*	C18	300	204	33895±3.6%	68617±8.6%

Totally individual S/N-values. Difficult to calculate. All S/N-values>>5 and rel SD<21.5%
No specific structure information, except for transition m/z values.

LC/MS: Proposal for identification criteria

•**LC**: Report blank, reference and sample SRM-chromatograms according to *Work Instruction for the Evaluation of the Results of OPCW Proficiency Tests, QDOC/LAB/WI/PT03, Attachment 1, §3*

•**MS**: S/N-values>5 OK.

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STX- Identification: LC/MRM (2 Transitions)

7. LC/MRM

Experimental Details	
Chemicals	STX: "CRM-STX-e", LOT-#: 20060419 National Research Council of Canada NRC Communications & Corporate Relations 1200 Montreal Road, Bldg. M-58 Ottawa, Ontario Canada K1A 0R6 Phone: (613) 993-9101 or toll-free 1-877-NRC-CNRC (1-877-672-2672) TTY number: (613) 949-3042 Fax: (613) 952-9907 E-mail: info@nrc-cnrc.gc.ca http://www.nrc-cnrc.gc.ca/eng/programs/imb/cmp/shellfish/crm/stx-e.html
Stock solution	1 ppm in 0.03 M acetic acid Dilute 0.45mL of the 65 µmol/L certified NRC-solution with 8.30 mL acetic acid (c=0.03 mol/L)
Dilution solution	Acetic acid (c=0.03 mol/L)
Samples	- 0.1 ppm in 0.03 M acetic acid - 1 ppm in 0.03 M acetic acid
Sample Preparation	Dilution
Measurement	- 2 MRM transitions: Choose two m/z-pairs for Q1 and Q3, respectively, with sufficiently high intensities. Precursor ion can be the same for both transitions. Care must be taken not to choose the ammonia or water loss peaks for the MRM experiment because that would result in a less than unique transition. - Use the same two transitions for all samples - Optimize MRM-transition parameters - At least 3 measurements per sample
Data treatment	No data treatment
Analysis	1. For each measurement calculate the ratio R_i (using peak intensities) and R_A (using peak areas): $R_i = \frac{Intensity_1}{Intensity_2}$ $R_A = \frac{Area_1}{Area_2}$ $Intensity_{1,2}$: Signal intensity of MRM transition 1 and 2, respectively $Area_{1,2}$: Area of MRM signal for transition 1 and 2, respectively $R_{i,A}$: Ratio of peak intensities or areas, respectively 2. Calculate the mean ratios R_i and R_A and the relative standard deviations.

Method B / Method A	LC/ESI-MS	LC/ESI/MS/MS 1 MRM-Transition	LC/ESI-MS/MS	LC/ESI/MS/MS 2 MRM-Transitions
Lateral Flow Assay	A: Positive B: MS full scan: $[M+H]^+$ 1 structure specific ion, Δm<0.2 Da, no intensity restriction	A: Positive B: Ret. time ±0.2 min 1 MRM-Transition S/N ≥ 5	A: Positive B: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	A: Positive B: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value
ELISA	A: Positive B: MS full scan: $[M+H]^+$ plus 1 structure specific ion, Δm<0.2 Da, no intensity restriction	A: Positive B: Ret. time ±0.2 min 1 MRM-transition S/N ≥ 5	A: Positive B: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	A: Positive B: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value
LC/Fluorescence	A: Ret. time ±0.2 min, S/N ≥ 5 B: MS full scan: $[M+H]^+$ plus 1 structure specific ion, Δm<0.2 Da, no intensity restriction	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min 1 MRM-transition S/N ≥ 5	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value

MRM-Transitions	t_R [min]
1. Transition m/z →	
2. Transition m/z →	

Sample	Signals and Ratios R_i and R_A	Ratio (mean±relSD)
0.1ppm	1. Measurement: Intensity$_1$ / Intensity$_2$ = R_i; Area$_1$ / Area$_2$ = R_A 2. Measurement: Intensity$_1$ / Intensity$_2$ = R_i; Area$_1$ / Area$_2$ = R_A 3. Measurement: Intensity$_1$ / Intensity$_2$ = R_i; Area$_1$ / Area$_2$ = R_A add additional measurements if you wish	Intensity ratio R_i = ± % (N=) Area ratio R_A = ± % (N=)
1 ppm	1. Measurement: Intensity$_1$ / Intensity$_2$ = R_i; Area$_1$ / Area$_2$ = R_A 2. Measurement: Intensity$_1$ / Intensity$_2$ = R_i; Area$_1$ / Area$_2$ = R_A 3. Measurement: Intensity$_1$ / Intensity$_2$ = R_i; Area$_1$ / Area$_2$ = R_A add additional measurements if you wish	Intensity ratio R_i = ± % (N=) Area ratio R_A = ± % (N=)

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STX- Identification: LC/SRM (1 Transition)

Ion Trap - *ThermoFinnigan LCQ Deca XP Plus*

C18

Sample	SRM/MRM transition	t_R [min]	S/N (mean value ± relSD[1])
0.1 ppm	m/z 300.1→ m/z 221.0±1	11.05	S/N=253±0.23% → (N=3)
1 ppm			S/N=2352±10.2% → (N=3)

Comments / Additional Information
Noise level was difficult to measure owing to almost complete lack of baseline noise, even on expanded baseline. Small differences in noise lead to large differences in S/N.

HILIC

Sample	SRM/MRM transition	t_R [min]	S/N (mean value ± relSD[1])
0.1 ppm	m/z 300.1→ m/z 221.0±1	4.26	S/N=90.7±10.8% → (N=3)
1 ppm			S/N=228±21.6% → (N=3)

Comments / Additional Information
Blank (0.03M acetic acid), 1 ppm STX and 0.1 ppm STX solutions evaporated to dryness in centrifugal evaporator and redissolved in appropriate volume of mobile phase B to give the same concentrations of STX. Original 0.03M acetic acid solutions are not compatible with analysis using HILIC conditions. Instrument tune for STX was not optimised under HILIC conditions. Optimisation would be expected to improve sensitivity. Noise level was difficult to measure owing to almost complete lack of baseline noise, even on expanded baseline. Small differences in noise lead to large differences in S/N.

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STX- Identification: LC/MRM (2 Transitions)

Linear Ion Trap *AB 3200QTrap*

MRM-Transitions		t_R [min]
1. Transition	m/z 300→204	1.0
2. Transition	m/z 300→138	

Sample	Signals and Ratios R_I and R_A	Ratio mean±relSD
0.1 ppm	**1. Measurement:** Intensity$_1$: 2137 Intensity$_2$: 770 R_I: 2.78; Area$_1$: 9456 Area$_2$: 3439 R_A: 2.75 **2. Measurement:** Intensity$_1$: 2189 Intensity$_2$: 872 R_I: 2.51; Area$_1$: 10482 Area$_2$: 3430 R_A: 3.05 **3. Measurement:** Intensity$_1$: 2205 Intensity$_2$: 783 R_I: 2.82; Area$_1$: 9860 Area$_2$: 3625 R_A: 2.72 ...add additional measurements if you wish	Intensity ratio: R_I=2.70±6% (N=3) Area ratio: R_A=2.84±6% (N=3)
1 ppm	**1. Measurement:** Intensity$_1$: 2.25e4 Intensity$_2$: 8.31e3 R_I: 2.70; Area$_1$: 9.45e4 Area$_2$: 3.54e4 R_A: 2.67 **2. Measurement:** Intensity$_1$: 2.28e4 Intensity$_2$: 8.54e3 R_I: 2.67; Area$_1$: 9.63e4 Area$_2$: 3.61e4 R_A: 2.67 **3. Measurement:** Intensity$_1$: 2.2e4 Intensity$_2$: 8.62e3 R_I: 2.56; Area$_1$: 9.5e4e4 Area$_2$: 3.50e4 R_A: 2.65 ...add additional measurements if you wish	Intensity ratio: R_I=2.64±3% (N=3) Area ratio: R_A=2.66±1% (N=3)

Triple Quad *WATERS Xevo TQ*

MRM-Transitions		t_R [min]
1. Transition	m/z 300.2→204.2	4.60
2. Transition	m/z 300.2→138.2	

Sample	Signals and Ratios R_I and R_A	Ratio (mean±relSD)
0.1 ppm	**1. Measurement:** Intensity$_1$: 12528 Intensity$_2$: 5930 R_I: 2.06; Area$_1$: 1019 Area$_2$: 494 R_A: 2.11 **2. Measurement:** Intensity$_1$: 12519 Intensity$_2$: 6340 R_I: 1.99; Area$_1$: 1015 Area$_2$: 511 R_A: 1.97 **3. Measurement:** Intensity$_1$: 14025 Intensity$_2$: 7232 R_I: 2.01; Area$_1$: 1139 Area$_2$: 567 R_A: 1.94 **4. Measurement:** Intensity$_1$: 13603 Intensity$_2$: 7585 R_I: 1.81; Area$_1$: 1097 Area$_2$: 605 R_A: 1.79	Intensity ratio: R_I=1.97±2.7% (N=4) Area ratio: R_A=1.95±3.4% (N=4)
1 ppm	**1. Measurement:** Intensity$_1$: 101972 Intensity$_2$: 51218 R_I: 2.00; Area$_1$: 8469 Area$_2$: 4229 R_A: 1.99 **2. Measurement:** Intensity$_1$: 97068 Intensity$_2$: 50162 R_I: 1.94; Area$_1$: 8127 Area$_2$: 4190 R_A: 1.94 **3. Measurement:** Intensity$_1$: 98095 Intensity$_2$: 50657 R_I: 1.96; Area$_1$: 8189 Area$_2$: 4174 R_A: 1.94 **4. Measurement:** Intensity$_1$: 106273 Intensity$_2$: 49529 R_I: 2.10; Area$_1$: 8736 Area$_2$: 4154 R_A: 2.15	Intensity ratio: R_I=2.00±1.8% (N=4) Area ratio: R_A=2.00±2.5% (N=4)

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STX- Identification: LC/MRM (2 Transitions)

Ion Trap - *ThermoFinnigan LCQ Deca XP Plus*

C18

MRM-Transitions		t_R [min]
1. Transition	m/z 300.1→204.1 ±1	11.06
2. Transition	m/z 300.1→221.0 ±1	

Sample	Signals and Ratios R_I and R_A	Ratio (mean±relSD)
0.1 ppm	**1. Measurement:** Intensity$_1$: 8.84E4 Intensity$_2$: 1.48E5 R_I: 1.67 Area$_1$: 1837659 Area$_2$: 3887648 R_A: 2.12 **2. Measurement:** Intensity$_1$: 7.60E4 Intensity$_2$: 1.52E5 R_I: 2.00 Area$_1$: 151/165 Area$_2$: 35930023 R_A: 2.37 **3. Measurement:** Intensity$_1$: 8.87E4 Intensity$_2$: 2.05E5 R_I: 2.31 Area$_1$: 1668417 Area$_2$: 4284888 R_A: 2.57 ...add additional measurements if you wish	Intensity ratio: R_I=1.99±16.1% (N=3) Area ratio: R_A=2.35±9.8% (N=3)
1 ppm	**1. Measurement:** Intensity$_1$: 1.07E6 Intensity$_2$: 2.54E6 R_I: 2.37 Area$_1$: 16985890 Area$_2$: 38716367 R_A: 2.28 **2. Measurement:** Intensity$_1$: 1.06E6 Intensity$_2$: 2.45E6 R_I: 2.31 Area$_1$: 16679415 Area$_2$: 39269914 R_A: 2.35 **3. Measurement:** Intensity$_1$: 1.05E6 Intensity$_2$: 2.39E6 R_I: 2.28 Area$_1$: 16564449 Area$_2$: 37675738 R_A: 2.27 ...add additional measurements if you wish	Intensity ratio: R_I=2.32±2.0% (N=3) Area ratio: R_A=2.30±1.9% (N=3)

HILIC

MRM-Transitions		t_R [min]
1. Transition	m/z 300.1→204.1 ±1	4.25
2. Transition	m/z 300.1→221.0 ±1	

Sample	Signals and Ratios R_I and R_A	Ratio (mean±relSD)
0.1 ppm	**1. Measurement:** Intensity$_1$: 9.14E3 Intensity$_2$: 2.15E4 R_I: 2.35 Area$_1$: 208385 Area$_2$: 439197 R_A: 2.11 **2. Measurement:** Intensity$_1$: 6.42E3 Intensity$_2$: 2.23E4 R_I: 3.47 Area$_1$: 159594 Area$_2$: 495255 R_A: 3.10 **3. Measurement:** Intensity$_1$: 6.93E3 Intensity$_2$: 2.05E4 R_I: 2.96 Area$_1$: 180332 Area$_2$: 482695 R_A: 2.68 ...add additional measurements if you wish	Intensity ratio: R_I=2.93±19.1% (N=3) Area ratio: R_A=2.63±19.0% (N=3)
1 ppm	**1. Measurement:** Intensity$_1$: 1.31E5 Intensity$_2$: 3.40E5 R_I: 2.60 Area$_1$: 2644826 Area$_2$: 6626726 R_A: 2.51 **2. Measurement:** Intensity$_1$: 1.62E5 Intensity$_2$: 3.38E5 R_I: 2.22 Area$_1$: 2930834 Area$_2$: 6716345 R_A: 2.29 **3. Measurement:** Intensity$_1$: 1.30E5 Intensity$_2$: 3.35E5 R_I: 2.58 Area$_1$: 2701683 Area$_2$: 6688353 R_A: 2.48 ...add additional measurements if you wish	Intensity ratio: R_I=2.47±8.5% (N=3) Area ratio: R_A=2.43±4.9% (N=3)

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STX- Identification: LC/MRM (2 Transitions)

Linear Ion Trap

Thermo Fisher Scientific - LCQ Fleet

Sample	Signals and Ratios R_I and R_A	Ratio (mean±relSD)
0.1 ppm	**1. Measurement:** Intensity$_1$: 6.55e5 Intensity$_2$: 4.02e5 R_I: 1.63 Area$_1$: 2220097 Area$_2$: 1342089 R_A: 1.65 **2. Measurement:** Intensity$_1$: 5.87e5 Intensity$_2$: 3.55e5 R_I: 1.65 Area$_1$: 2112400 Area$_2$: 1253176 R_A: 1.69 **3. Measurement:** Intensity$_1$: 5.82e5 Intensity$_2$: 3.42e5 R_I: 1.70 Area$_1$: 2071247 Area$_2$: 1237226 R_A: 1.67	Intensity ratio: R_I=1.66±2.2% (N=3) Area ratio: R_A=1.67±1.0% (N=3)
1 ppm	**1. Measurement:** Intensity$_1$: 2.31e6 Intensity$_2$: 1.38e6 R_I: 1.67 Area$_1$: 8103823 Area$_2$: 4654097 R_A: 1.74 **2. Measurement:** Intensity$_1$: 2.48e6 Intensity$_2$: 1.49e6 R_I: 1.66 Area$_1$: 8834277 Area$_2$: 4906726 R_A: 1.80 **3. Measurement:** Intensity$_1$: 2.51e6 Intensity$_2$: 1.5e6 R_I: 1.67 Area$_1$: 8936639 Area$_2$: 4827083 R_A: 1.85	Intensity ratio: R_I=1.67±0.4% (N=3) Area ratio: R_A=1.80±3.1% (N=3)

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STX- Identification: LC/MRM (2 Transitions)

Linear Ion Trap
Thermo Fisher Scientific - LXQ

SRM/MRM-Transitions		t_R [min]
1. Transition	m/z 300→282	6.4 min
2. Transition	m/z 300→204	

Sample	Signals and Ratios R_I and R_A	Ratio (mean±relSD)
1 ppm in eluent buffer	**1. Measurement:** $Intensity_1$: 318765 $Intensity_2$: 334820 R_I: 0.952 $Area_1$: 4230519 $Area_2$: 4703249 R_A: 0.899 **2. Measurement:** $Intensity_1$: 329204 $Intensity_2$: 352221 R_I: 0.935 $Area_1$: 4452906 $Area_2$: 4957871 R_A: 0.898 **3. Measurement:** $Intensity_2$: 342654 $Intensity_1$: 368622 R_I: 0.930 $Area_2$: 4582634 $Area_1$: 5055317 R_A: 0.906	Intensity ratio: R_I=0.939±1.2% (N=3) Area ratio: R_A=0.901±0.4% (N=3)

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STX- Identification: LC/MRM (2 Transitions)

MS	LC	1. Transition		2. Transition		Ratio Mean±relSD			
		Q1 m/z	Q3 m/z	Q1 m/z	Q3 m/z	0.1ppm		1ppm	
						Intensity	Area	Intensity	Area
Ion Trap	C18	300	204	300	138	2.70±6%	2.84±6%	2.64±3%	2.66±1%
Triple Quad	BEH AMIDE	300	204	300	138	1.97±2.7%	1.95±3.4%	2.00±1.8%	2.00±2.5%
Ion Trap	C18	300	204	300	221	1.99±16%	2.35±9.8%	2.32±2.0%	2.30±1.9%
	HILIC					2.93±19.1%	2.63±19%	2.47±8.5%	2.43±4.9%
Ion Trap	C18	300	204	300	138	1.66±2.2%	1.67±1.0%	1.67±0.4%	1.80±3.1%
Linear Ion Trap	HILIC	300	282	300	204	-	-	0.939±1.2%	0.901±0.4%

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STX- Identification: LC/MRM (2 Transitions)

- Transitions: m/z 300→204, m/z 300 →138 most often chosen.
- 0.1ppm sample relSD higher than for 1ppm, but still <20%
- 1ppm relSD <10% (HILIC), otherwise < 3.1%
- Intensity vs. area: No significant difference in accuracy.

LC/MS: Proposal for identification criteria

•**LC**: Measurement of blank, sample and reference according to *Work Instruction for the Evaluation of the Results of OPCW Proficiency Tests, QDOC/LAB/WI/PT03, Attachment 1, §3*

•**MS**: Area and intensity can both be used. Ratio of two freely selectable transitions (quantifier and qualifier). Relative Abundance >25% . Reference vs. sample ratio relSD <20%. Minimum unit resolution.

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STX- Identification: Conclusion

Method A \ Method B	LC/ESI-MS	LC/ESI/MS/MS 1 SRM-Transition	LC/ESI-MS/MS	LC/ESI/MS/MS 2 SRM-Transitions
Lateral Flow Assay	**A**: Positive **B**: MS full scan: $[M+H]^+$ 1 structure specific ion, $\Delta m<0.2$ Da, no intensity restriction	**A**: Positive **B**: Ret. time ±0.2 min 1 SRM-Transition S/N ≥ 5	**A**: Positive **B**: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	**A**: Positive **B**: Ret. time ±0.2 min Ratio of 2 SRM-transitions, must match within ±10% of the reference value
ELISA	**A**: Positive **B**: MS full scan: $[M+H]^+$ plus 1 structure specific ion, $\Delta m<0.2$ Da, no intensity restriction	**A**: Positive **B**: Ret. time ±0.2 min 1 SRM-transition S/N ≥ 5	**A**: Positive **B**: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	**A**: Positive **B**: Ret. time ±0.2 min Ratio of 2 SRM-transitions, must match within ±10% of the reference value
LC/Fluorescence	**A**: Ret. time ±0.2 min, S/N ≥ 5 **B**: MS full scan: $[M+H]^+$ plus 1 structure specific ion, $\Delta m<0.2$ Da, no intensity restriction	**A**: Ret. time ±0.2 min, S/N ≥ 5 **B**: Ret. time ±0.2 min 1 SRM-transition S/N ≥ 5	**A**: Ret. time ±0.2 min, S/N ≥ 5 **B**: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	**A**: Ret. time ±0.2 min, S/N ≥ 5 **B**: Ret. time ±0.2 min Ratio of 2 SRM-transitions, must match within ±10% of the reference value

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STX- Identification: Conclusion

Method B / Method A	LC/ESI-MS	LC/ESI/MS/MS 1 SRM-Transition	LC/ESI-MS/MS	LC/ESI/MS/MS 2 SRM-Transitions
Lateral Flow Assay	A: Positive B: MS full scan: [M+H]+ 1 structure specific ion, Δm<0.2 Da, no intensity restriction	A: Positive B: Ret. time ±0.2 min 1 SRM-Transition S/N ≥ 5	A: Positive B: Ret. time ±0.2 min,, QDOC/LAB/WI/PT03, Attachment 2, §4.3	A: Positive B: Ret. time ±0.2 min Ratio of 2 SRM-transitions (quantifier and qualifier), must match within ±20% of the reference value. Resolution≤ Unit
ELISA	A: Positive B: MS full scan: [M+H]+ plus 1 structure specific ion, Δm<0.2 Da, no intensity restriction	A: Positive B: Ret. time ±0.2 min 1 SRM-transition S/N ≥ 5	A: Positive B: Ret. time ±0.2 min, QDOC/LAB/WI/PT03, Attachment 2, §4.3	A: Positive B: Ret. time ±0.2 min Ratio of 2 SRM-transitions (quantifier and qualifier), must match within ±20% of the reference value. Resolution≤ Unit
LC/Fluorescence	A: Ret. time ±0.2 min, S/N ≥ 5 B: MS full scan: [M+H]+ plus 1 structure specific ion, Δm<0.2 Da, no intensity restriction	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min 1 SRM-transition S/N ≥ 5	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min, QDOC/LAB/WI/PT03, Attachment 2, §4.3	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min Ratio of 2 SRM-transitions (quantifier and qualifier), must match within ±20% of the reference value. Resolution≤ Unit

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STX- Identification: Conclusion

LC/MS: low significance. If a lab is equipped with LC/MS, it can perform LC/MS/MS experiments ⇨ **LC/MS can be omitted.**

Method B / Method A	LC/ESI/MS/MS 1 SRM-Transition	LC/ESI-MS/MS	LC/ESI/MS/MS 2 SRM-Transitions
Lateral Flow Assay	A: Positive B: Ret. time ±0.2 min 1 SRM-Transition S/N ≥ 5	A: Positive B: Ret. time ±0.2 min,, QDOC/LAB/WI/PT03, Attachment 2, §4.3	A: Positive B: Ret. time ±0.2 min Ratio of 2 SRM-transitions (quantifier and qualifier), must match within ±20% of the reference value. Resolution≤ Unit
ELISA	A: Positive B: Ret. time ±0.2 min 1 SRM-transition S/N ≥ 5	A: Positive B: Ret. time ±0.2 min, QDOC/LAB/WI/PT03, Attachment 2, §4.3	A: Positive B: Ret. time ±0.2 min Ratio of 2 SRM-transitions (quantifier and qualifier), must match within ±20% of the reference value. Resolution≤ Unit
LC/Fluorescence	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min 1 SRM-transition S/N ≥ 5	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min, QDOC/LAB/WI/PT03, Attachment 2, §4.3	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min Ratio of 2 SRM-transitions (quantifier and qualifier), must match within ±20% of the reference value. Resolution≤ Unit

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STX- Identification: Conclusion

If a lab is equipped with LC/MS, it can perform LC/MS/MS experiments. SRM experiments can be done using 1 or 2 transitions ⇨ **SRM (1Transition) can be omitted.**

Method A \ Method B	LC/ESI-MS/MS	LC/ESI/MS/MS 2 SRM-Transitions
Lateral Flow Assay	A: Positive B: Ret. time ±0.2 min, QDOC/LAB/WI/PT03, Attachment 2, §4.3	A: Positive B: Ret. time ±0.2 min Ratio of 2 SRM-transitions (quantifier and qualifier), must match within ±20% of the reference value. Resolution≤ Unit
ELISA	A: Positive B: Ret. time ±0.2 min, QDOC/LAB/WI/PT03, Attachment 2, §4.3	A: Positive B: Ret. time ±0.2 min Ratio of 2 SRM-transitions (quantifier and qualifier), must match within ±20% of the reference value. Resolution≤ Unit
LC/Fluorescence	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min, QDOC/LAB/WI/PT03, Attachment 2, §4.3	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min Ratio of 2 SRM-transitions (quantifier and qualifier), must match within ±20% of the reference value. Resolution≤ Unit

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STX- Identification: Conclusion

LC/MS/MS spectra must fulfill criteria in *Work Instruction for the Evaluation of the Results of OPCW Proficiency Tests, QDOC/LAB/WI/PT03, Attachment 2, §4.3*

Method A \ Method B	LC/ESI-MS/MS	LC/ESI/MS/MS 2 SRM-Transitions
Lateral Flow Assay	A: Positive B: Ret. time ±0.2 min,. QDOC/LAB/WI/PT03, Attachment 2, §4.3	A: Positive B: Ret. time ±0.2 min Ratio of 2 SRM-transitions (quantifier and qualifier), must match within ±20% of the reference value. Resolution≤ Unit
ELISA	A: Positive B: Ret. time ±0.2 min, QDOC/LAB/WI/PT03, Attachment 2, §4.3	A: Positive B: Ret. time ±0.2 min Ratio of 2 SRM-transitions (quantifier and qualifier), must match within ±20% of the reference value. Resolution≤ Unit
LC/Fluorescence	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min, QDOC/LAB/WI/PT03, Attachment 2, §4.3	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min Ratio of 2 SRM-transitions (quantifier and qualifier), must match within ±20% of the reference value. Resolution≤ Unit

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STX- Identification: Conclusion

Proposal for Identification Criteria:

New independent methods for identification:

1. Biochemical: LFA, ELISA, LC/Fluorescence(?)

2. LC/MS: Ratio of **2 SRM Transitions** (see e.g. WADA)

✓ **Current evaluation system** can be used (no new point system):

✓**LC-MS/MS spectrum**
Evaluation according to *Work Instruction for the Evaluation of the Results of OPCW Proficiency Tests, QDOC/LAB/WI/PT03, Attachment 2, §4.3*

✓***^{1}H-NMR** according to QDOC/LAB/WI/PT03, Attachment 4*

✓**LC/Fluorescence** instead of a biochemical method.
Evaluation according to *QDOC/LAB/WI/PT03, Attachment 1*

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STX- Identification: Conclusion

1. Method	Criteria
LFA	Positive/Negative, LOD
ELISA	Positive/Negative, LOD
LC/Fluorescence LC/Nitrogen Detector	QDOC/LAB/WI/PT03, Attachment 1. Use different LC-phase if LC/MS is second technique.

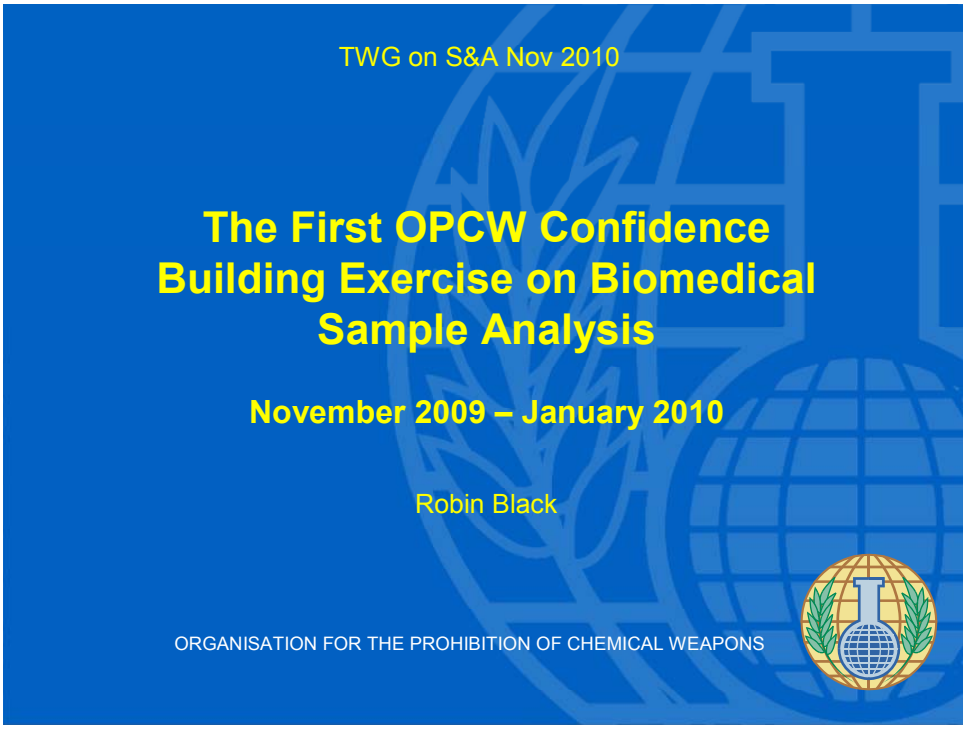
New

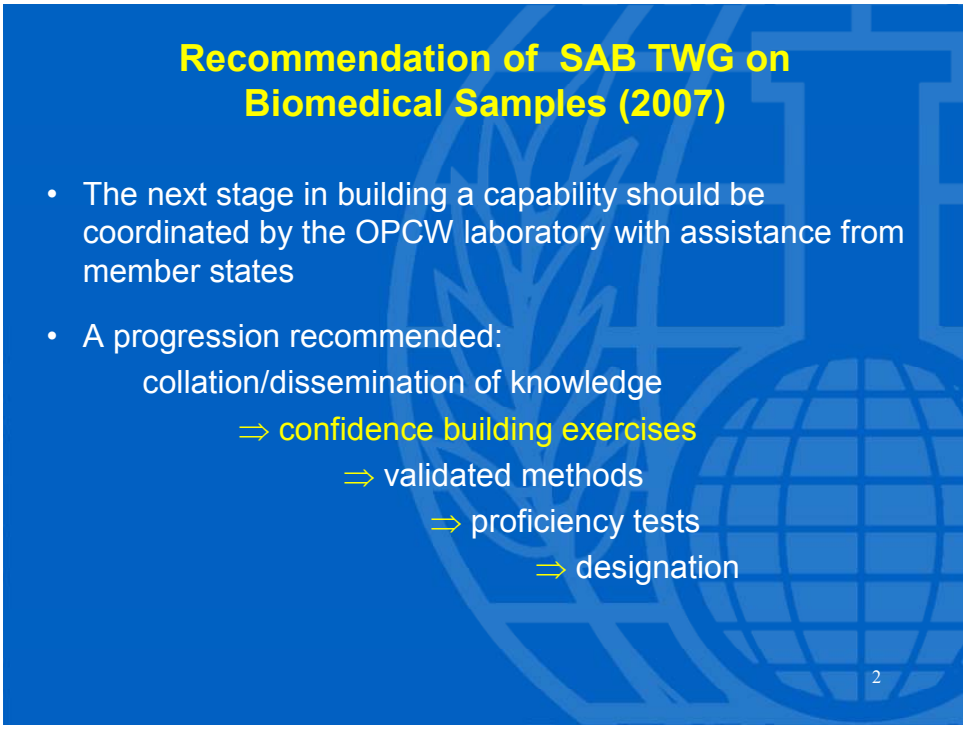
2. Method	Criteria
LC/MS: Ratio of 2 SRM Transitions	Ratios of 2 SRM-transitions of sample and reference must fall within ±20%. (see e.g. WADA). Resolution ≤ unit. Area or intensity.
LC-MS/MS spectrum	QDOC/LAB/WI/PT03, Attachment 2, §4.3
^{1}H-NMR	QDOC/LAB/WI/PT03, Attachment 4

New

Appendix 6

THE FIRST OPCW CONFIDENCE-BUILDING EXERCISE ON BIOMEDICAL SAMPLE ANALYSIS





Objectives of the first confidence building exercise

- To broaden the capability for biomedical sample analysis across member states
- To assess advantages & disadvantages of different methods
- To commence a discussion on criteria for identification at trace levels
- Identification is the main requirement but laboratories encouraged to report quantitative results if obtained

3

Overview

- Samples and standards prepared by TNO Defence, Security & Safety, NLD
- Dispatched 6 November 2009 by OPCW laboratory
- Details of selected methods provided
  - but laboratories free to use any method
- Submission of reports by 15 January 2010
  - with some flexibility
- Results evaluated by Dstl, Porton Down, UK
  - but not on the lines of a proficiency test, no scoring
- Meeting to discuss results 25 March 2010

Samples

- Commercial synthetic urine selected as the matrix
  - to avoid problems of transport of biological materials
- Urinary metabolites of nerve agents and sulfur mustard as spiking chemicals
- Six spiked samples and one labelled blank
- Spiking levels:

s1 : blank synthetic urine	
s2 : ethyl methylphosphonic acid	100 ng/ml
s3 : isopropyl methylphosphonic acid	100 ng/ml
s4 : thiodiglycol	100 ng/ml
s5 : sulfur mustard β-lyase metabolite	100 ng/ml
s6 : isopropyl methylphosphonic acid	10 ng/ml
s7 : thiodiglycol	100 ng/ml
β-lyase metabolite	10 ng/ml

Overview of results

- 22 laboratories from 17 member states submitted reports (1 no results)
- 6 laboratories reported all spiking chemicals in all samples
- 5 laboratories did not analyse for thiodiglycol
  - 4 of these reported all other spiking chemicals
- 6 laboratories reported false positives
  - mostly alkyl methylphosphonic acids
- More than half the laboratories reported ‘system’ and/or urine blanks with traces of analyte or interferents

Instrumentation

	Number of labs			
	Triple quad	Ion trap	Single quad	Other
LC-MS/MS*	11			1 Orbitrap
LC-MS			1	1 Q-TOF (HR)
GC-MS/MS	5	3		1 linear ion trap
GC-MS			14	
GC/GC-MS				1 TOF
GC-FPD				2

* 4 labs used LC-MS/MS as the only technique

Negative controls (blanks)

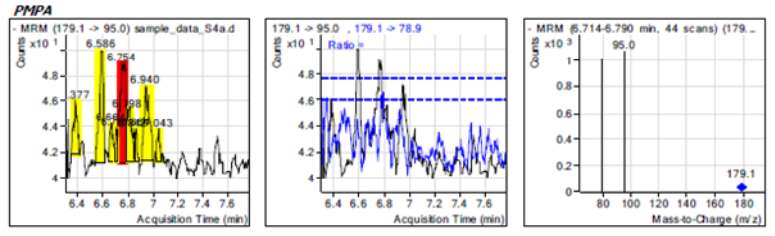
- Laboratories were asked to provide chromatograms for system blanks and urine blanks (sample s1)
- System blanks varied from simply injecting solvent, injecting derivatising mixture, to taking a sample of water through the entire procedure.
- If the GC injector is contaminated, e.g. with underivatised analyte, simply injecting solvent will not detect this contamination.
- Taking a sample of water through the entire procedure is recommended.
  - need to mandate procedure

System contamination

- Data presented by > half the laboratories showed evidence of system contamination
- The more selective the method the more likely that peaks in the retention window of an analyte represent contamination rather than interferents
- Problem much greater with GC-MS(MS) where derivatisation required
  - particularly very sensitive –ve CI methods, and silyl derivatives
- Common sources of contamination are underivatised agent in the GC injector (from incomplete derivatisation or thermal degradation), the SPE vacuum manifold, syringe in automated methods
- Very important that this problem is addressed

Need to define what is a significant peak

- This sample was reported as containing pinacolyl methylphosphonic acid
  - reflects either trace system contamination from calibrations or an interfering peak
  - in Proficiency Tests peaks with S/N < 5:1, or < 1% of analyte intensity are not deemed to be significant



Sample preparation

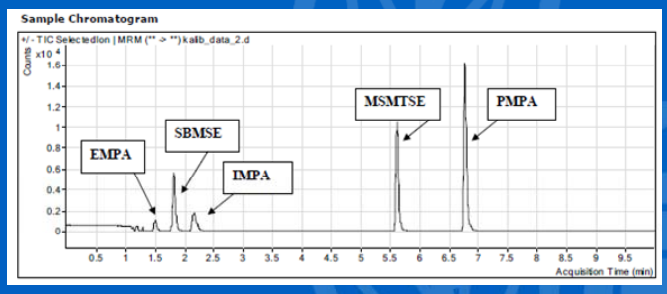
- Most labs followed literature procedures, sometimes with minor modifications
- Omitting sample clean-up, or simple lyophilisation, for LC-MS/MS OK for clean, high concentration samples but not recommended for real samples
- Some labs used liquid-liquid extraction for removing extraneous materials or for extracting analytes
  - solid phase extraction (SPE) would probably have been easier & more efficient
- SPE methods mostly based on polymeric materials (e.g. Oasis HLB, ENV+), SAX ion exchange, or silica

Methods: alkyl methylphosphonic acids

Technique	Derivative	Ionisation	Mode
LC-MS/MS	none	-ve ESI +ve ESI (1 lab)	MRM, full product ion scan
LC-MS	none	-ve ESI	SIM
LC-HRMS (1 lab)	none	-ve ESI	full scan, HR extracted ion
GC-MS/MS	PFB	-ve CI (CH_4, NH_3)	MRM
	TMS	EI	MRM
	TBDMS	EI, +ve CI	MRM
GC-MS	PFB	-ve CI (CH_4, iBu), EI	SIM, full scan
	TMS	EI, +ve CI	SIM, full scan
	TBDMS	EI	SIM
	Me ester	EI, +ve CI (CH_3CN)	full scan, extracted ion

Methods: combined

- One laboratory used LC-MS/MS as the sole technique and analysed alkyl methylphosphonic acids & β-lyase metabolites in a single run (TDG not analysed)



20 ng/ml calibration

Sample s2: ethyl methylphosphonic acid (EMPA) 100 ng/ml

- 20 laboratories reported EMPA in sample s2
  - 3 also reported EMPA as a false +ve in other samples
  - 4 laboratories reported other analytes (IMPA, PMPA, TDG) in s2
  - 2 laboratories reported MPA

Sample s2: EMPA by LC-MS/MS, -ve ESI

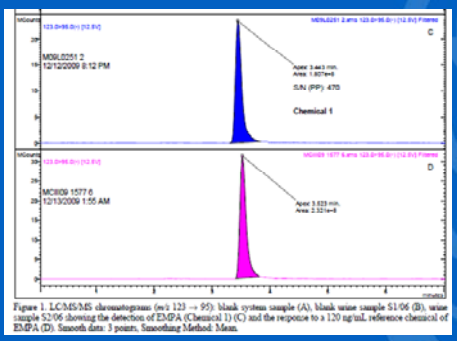


Figure 1. LC/MS/MS chromatograms (m/z 123 → 95): blank system sample (A), blank urine sample S1/06 (B), urine sample S2/06 showing the detection of EMPA (Chemical 1) (C) and the response to a 120 ng/mL reference chemical of EMPA (D). Smooth data: 3 points, Smoothing Method: Mean.

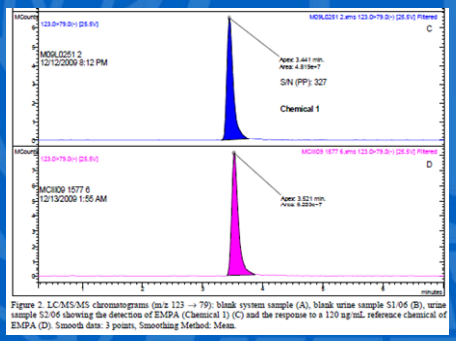


Figure 2. LC/MS/MS chromatograms (m/z 123 → 79): blank system sample (A), blank urine sample S1/06 (B), urine sample S2/06 showing the detection of EMPA (Chemical 1) (C) and the response to a 120 ng/mL reference chemical of EMPA (D). Smooth data: 3 points, Smoothing Method: Mean.

m/z 123 → 95

m/z 123 → 79

Sample s2: EMPA by GC-MS/MS, PFB deriv, -ve CI

S/N > LC-MS/MS

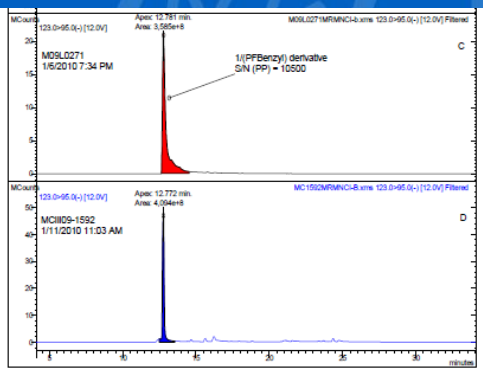


Figure 5. GC/MS/MS NCI chromatograms (m/z 123 → 95): Blank system (acetonitrile + pentafluorobenzyl bromide) (A), blank urine sample S1/06 derivatized with pentafluorobenzyl bromide (B), urine sample S2/06 derivatized with pentafluorobenzyl bromide, showing the detection of EMPA/(PFBenzyl) derivative (C) and the response to a 100 ng/mL reference chemical EMPA/(PFBenzyl) derivative (D). Smooth data: 3 points, smoothing method: Mean.

Sample s2: EMPA by GC-MS, TMS deriv, EI

S/N << LC-MS/MS or GC-MS/MS, PFB deriv, -ve CI

Selectivity lower as indicated by number of additional peaks across chromatogram

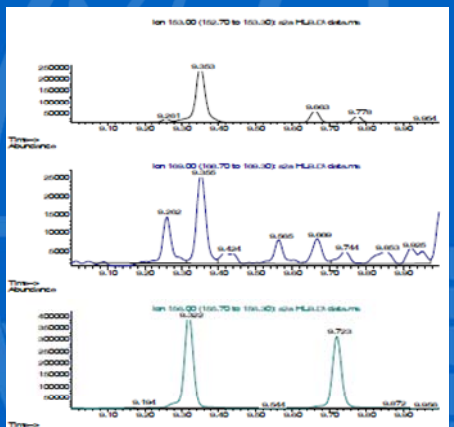


Figure 10. GC-MS SIM chromatograms of sample S2. Deuterated internal standards d_5-EMPA, d_5-IMPA and d_5-PMPA were added at 50 ng/ml. Upper and middle chromatograms m/z 153 and 169 show EMPA-TMS at the expected retention time 9.356 min. The ion ratio 153/169 is 10/1.

Sample s2: EMPA by GC-MS, TBDMS deriv, EI

S/N << LC-MS/MS, or GC-MS/MS, PFB deriv, -ve CI

Selectivity lower as indicated by number of additional peaks across chromatogram

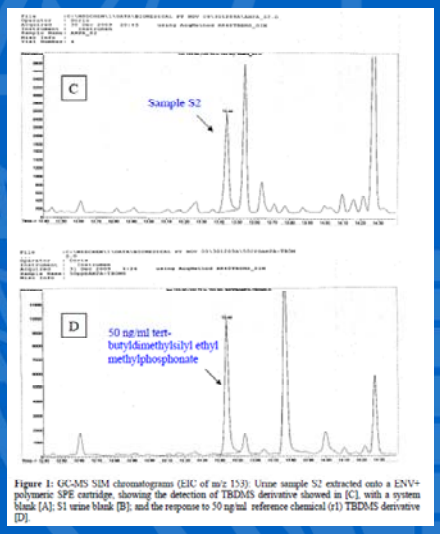


Figure 1: GC-MS SIM chromatograms (EIC of m/z 153). Urine sample S2 extracted onto a ENV+ polymeric SPE cartridge, showing the detection of TBDMS derivative showed in [C], with a system blank [A]; S1 urine blank [B]; and the response to 50 ng/ml reference chemical (r1) TBDMS derivative [D].

Samples s3 & s6: isopropyl methylphosphonic acid (IMPA) 100 & 10 ng/ml

- 21 laboratories reported IMPA in sample s3
  - 3 also reported IMPA as a false +ve in other samples
  - 3 laboratories reported other analytes in s3 (EMPA, PMPA)
  - 1 laboratory reported MPA
- 14 laboratories reported IMPA in sample s6
  - 2 laboratories reported other analytes in s6 (EMPA, PMPA)
- Of the 6 labs that detected IMPA in s3 but not s6, 2 used LC-MS/MS, 5 used GC-MS, 1 used GC-FPD
- All labs that used GC-MS/MS detected IMPA at 10 ng/ml

Sample s6: IMPA (10 ng/ml) by LC-MS/MS and GC-MS/MS

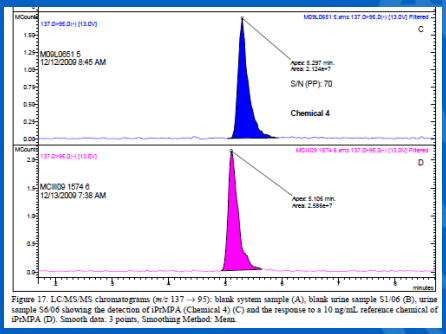


Figure 17. LC/MS/MS chromatograms (m/z 137 → 95): blank system sample (A), blank urine sample S1/06 (B), urine sample S6/06 showing the detection of iPrMPA (Chemical 4) (C) and the response to a 10 ng/mL reference chemical of iPrMPA (D). Smooth data: 3 points, Smoothing Method: Mean.

LC-MS/MS, -ve ESI

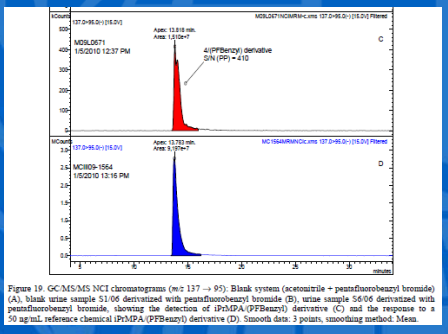


Figure 19. GC/MS/MS NCI chromatograms (m/z 137 → 95): Blank system (acetonitrile + pentafluorobenzyl bromide) (A), blank urine sample S1/06 derivatized with pentafluorobenzyl bromide (B), urine sample S6/06 derivatized with pentafluorobenzyl bromide, showing the detection of iPrMPA (PFBenzyl) derivative (C) and the response to a 50 ng/mL reference chemical iPrMPA (PFBenzyl) derivative (D). Smooth data: 3 points, smoothing method: Mean.

GC-MS/MS, PFB, -ve CI

Sample s6: IMPA (10 ng/ml) by LC-HRMS (Q-TOF)

- Full scan LC-MS using a Q-TOF instrument & HR (>10,000) extracted ion (m/z 137.0373) gave impressive results

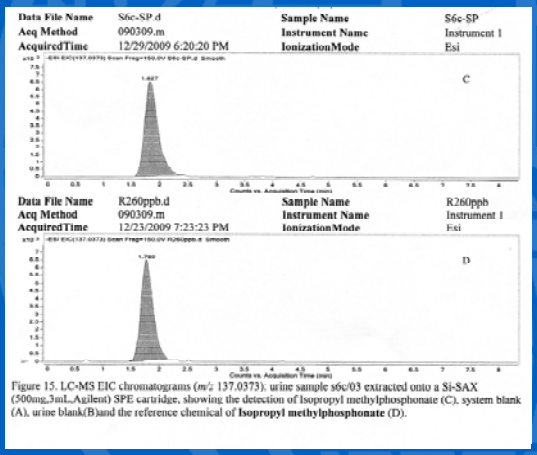


Figure 15. LC-MS EIC chromatograms (*m/z* 137.0373). urine sample s6c/03 extracted onto a Si-SAX (500mg,3mL,Agilent) SPE cartridge, showing the detection of Isopropyl methylphosphonate (C). system blank (A), urine blank(B)and the reference chemical of **Isopropyl methylphosphonate** (D).

Sample s4: TDG 100 ng/ml

- 13 laboratories reported TDG in sample s4
  - 5 laboratories did not analyse for TDG
  - 1 laboratory also reported TDG as a false +ve in 1 other sample
  - 5 laboratories reported other analytes in s4 (EMPA, PMPA, MPA, SBMSE)
- Only 2 labs used LC-MS/MS
  - I obtained poor S/N for s4
  - 1 detected TDG but only using ions for thiodiglycol sulfoxide, suggesting that in-source oxidation was occurring – only with Surine
- 1 lab obtained good results using LC-HRMS (Q-TOF)
- Good S/N with GC-MS/MS, $(PFBz)_2$ deriv, -ve CI, and GC-MS/MS $(HFB)_2$ deriv, +ve CI

Sample s4: TDG by LC-HRMS

- LC-HRMS using a Q-TOF instrument & HR (>10,000) extracted ion (m/z 145.0294) = $[M+Na]^+$

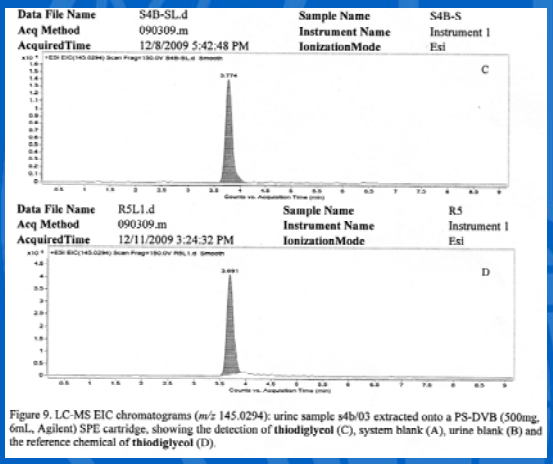
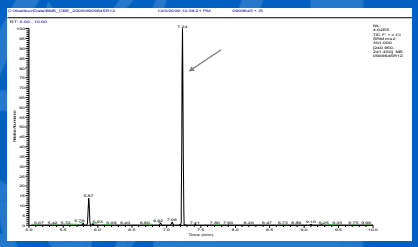


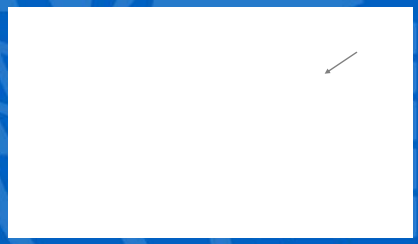
Figure 9. LC-MS EIC chromatograms (*m/z* 145.0294): urine sample s4b/03 extracted onto a PS-DVB (500mg, 6mL, Agilent) SPE cartridge, showing the detection of **thiodiglycol** (C), system blank (A), urine blank (B) and the reference chemical of **thiodiglycol** (D).

Sample s4: thiodiglycol by GC-MS/MS

GC-MS/MS, $(HFB)_2$ deriv, +ve CI (CH_4), m/z 301 →241

GC-MS/MS, $(PFBz)_2$ deriv, -ve CI (CH_4), m/z 501 →167





retention windows ~ 8 min

Sample s4: thiodiglycol by GC-MS, EI

GC-MS, $(TMS)_2$ deriv, EI, SIM, m/z 116, 176

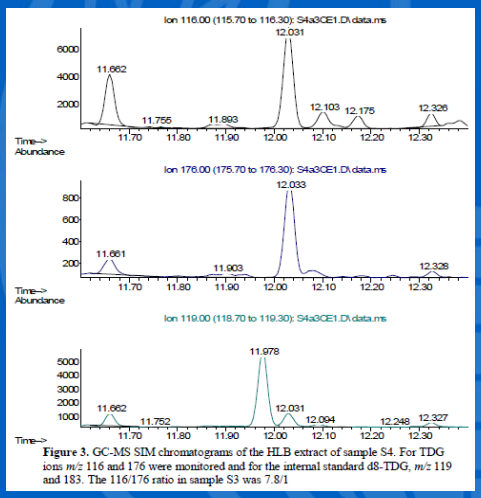


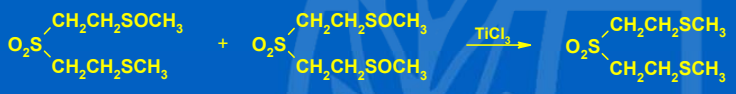
Figure 3. GC-MS SIM chromatograms of the HLB extract of sample S4. For TDG ions *m/z* 116 and 176 were monitored and for the internal standard d8-TDG, *m/z* 119 and 183. The 116/176 ratio in sample S3 was 7.8/1

Sample s5: SBMSE 100 ng/ml

- 18 laboratories reported SBMSE in sample s5
  - 1 laboratory reported SBMSE as a false +ve in 1 other sample
  - 4 laboratories reported other analytes in s5 (EMPA, IMPA, PMPA, TDG)
- LC-MS/MS (with one exception) proved to be the most reliable technique
- GC-MS, GC-MS/MS after reduction were successful in most cases
- Four laboratories experienced problems with $TiCl_3$ reduction not observed with real urine
  - appears to be a problem associated with synthetic urine

Sample s5: problems with $TiCl_3$ & Surine

- 2 β-lyase metabolites reduced to a single analyte, SBMTE



- Initially developed at Dstl, and has always been robust
  - used for confirming SM exposure in Iranian & Kurdish CW casualties
- 2 labs could not detect SBMTE by GC-MS/MS, one lab detected it only after diluting sample s5 with real urine.
- 1 lab could not detect the reduced analyte SBMTE by LC-MS/MS
  - nor the reduced internal std $^{13}C_4$-SBMTE, added before $TiCl_3$ reduction

Sample s5: SBMSE 100 ng/ml

- 18 laboratories reported SBMSE in sample s5
  - 1 laboratory reported SBMSE as a false +ve in 1 other sample
  - 4 laboratories reported other analytes in s5 (EMPA, IMPA, PMPA, TDG)
- LC-MS/MS (with one exception) proved to be the most reliable technique
- GC-MS, GC-MS/MS after reduction were successful in most cases
- Four laboratories experienced problems with $TiCl_3$ reduction not observed with real urine
  - appears to be a problem associated with synthetic urine

Sample s5: SBMSE by LC-MS/MS

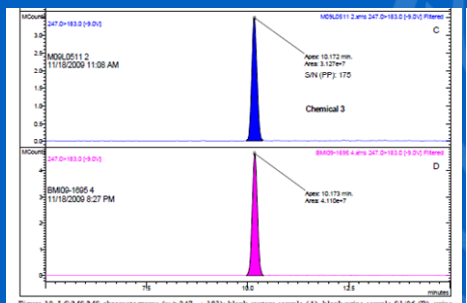


Figure 10. LC/MS/MS chromatograms (m/z 247 → 183): blank system sample (A), blank urine sample 51/06 (B), urine sample 55/06 showing the detection of SBMSE (Chemical 3) (C) and the response to a 120 ng/mL reference chemical of SBMSE (D). Smooth data: 3 points, Smoothing Method: Mean.

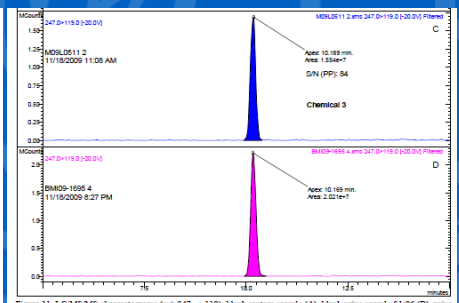


Figure 11. LC/MS/MS chromatograms (m/z 247 → 119): blank system sample (A), blank urine sample 51/06 (B), urine sample 55/06 showing the detection of SBMSE (Chemical 3) (C) and the response to a 120 ng/mL reference chemical of SBMSE (D). Smooth data: 3 points, Smoothing Method: Mean.

LC-MS/MS, +ve ESI, m/z 247 → 183, 119

Sample s5: SBMSE by GC-MS & GC-MS/MS

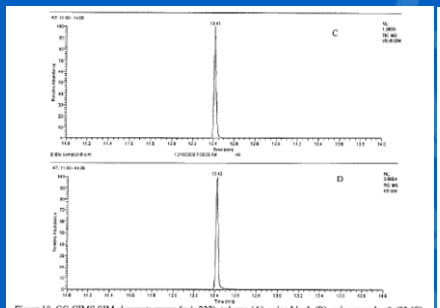


Figure 10. GC-CIMS SIM chromatograms (m/z 232): toluene (A), urine blank (B), urine sample s5c/03 (C), and the reference chemical of **1,1'-sulfonylbis[2-(methylthio)ethane]** (D).

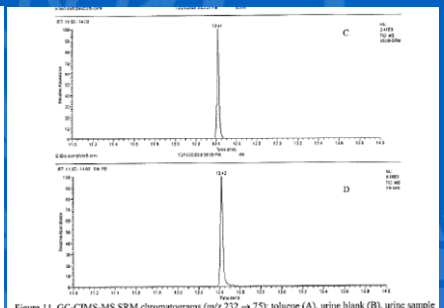


Figure 11. GC-CIMS-MS SRM chromatograms (m/z 232 → 75): toluene (A), urine blank (B), urine sample s5c/03 (C), and the reference chemical of **1,1'-sulfoaylbis[2-(methylthio)ethane]** (D).

GC-MS vs GC-MS/MS, SBMSE reduced to SBMTE

Sample s5: problems with $TiCl_3$ & Surine

- 2 β-lyase metabolites reduced to a single analyte, SBMTE

- Initially developed at Dstl, and has always been robust
  - used for confirming SM exposure in Iranian & Kurdish CW casualties
- 2 labs could not detect SBMTE by GC-MS/MS, one lab detected it only after diluting sample s5 with real urine.
- 1 lab could not detect the reduced analyte SBMTE by LC-MS/MS
  - nor the reduced internal std $^{13}C_4$-SBMTE, added before $TiCl_3$ reduction

Sample s7: TDG 100 ng/ml, SBMSE 10 ng/ml

- 11 laboratories reported TDG in sample s7
  - 5 did not analyse for TDG
  - 3 laboratories reported other analytes in s7 (EMPA, IMPA, PMPA)
- 14 laboratories reported SBMSE in sample s7
  - LC-MS/MS and GC-MS/MS (with exceptions) gave good results
  - single stage MS gave lower S/N

Sample s7: SBMSE by LC-MS/MS, +ve ESI

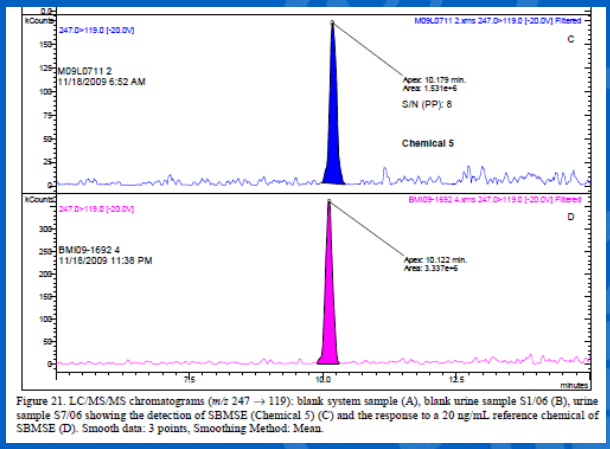
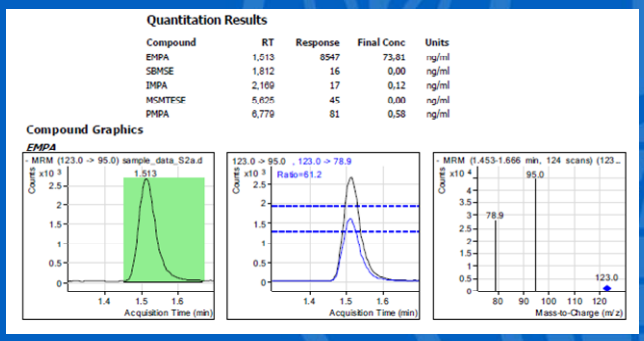


Figure 21. LC/MS/MS chromatograms (*m/z* 247 → 119): blank system sample (A), blank urine sample S1/06 (B), urine sample S7/06 showing the detection of SBMSE (Chemical 5) (C) and the response to a 20 ng/mL reference chemical of SBMSE (D). Smooth data: 3 points, Smoothing Method: Mean.

Identification

- Identification was based on retention time, & selected ions or MS/MS transitions, and in a few cases full scan spectra
- Approx one third of the labs reported ion ratios for MRM or SIM
  - and within 10-20% of ratios in reference chemical



LC-MS/MS MRM ion ratios for EMPA in sample s2

Quantitation

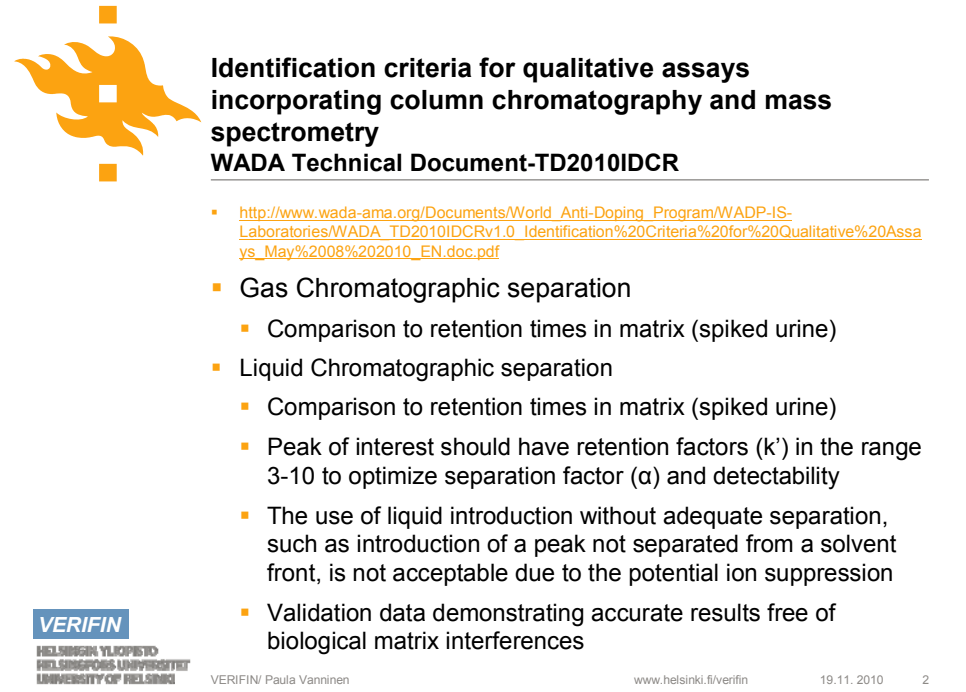
- Where reported was generally good
- Ranged from estimates by comparison of samples with one or two standard solutions to comparison with a multi-point calibration curve
- Most rigorous procedures compared peak areas of the analyte with isotopically labelled internal standard, against a multipoint calibration curve in Surine (sample s1)
- Use of internal standards aids quantitation and increases confidence in the performance of the method

Conclusions

- Broader capability for biomedical sample analysis demonstrated
- Levels of identification & limits of detection dependent on instrumentation
- Triple quadrupoles, other MS/MS instruments, and high resolution TOF provided best quality data
  - broader application of TOFs expected in the future?
- LC-MS/MS very sensitive for alkyl methylphosphonic acids & β-lyase metabolites
  - and generally less prone to system contamination than GC-MS(MS)
- Perfluorinated derivatives with –ve CI provided the most sensitive GC-MS(MS)
  - but number of ions for monitoring may be less than with silyl derivs
- System contamination was a significant problem

Appendix 7

CRITERIA FOR TRACE ANALYSIS IN INVESTIGATIONS OF ALLEGED USE



Retention factor k'

- If retention time is 5 min and t_0 is 0.5 min, k' is 9
- If retention time is 2 min and t_0 is 0.5 min, k' is 3
- If retention time is 1 min and t_0 is 0.5 min, k' is 1!!!

$$k = \frac{V_R - V_M}{V_M} = \frac{t_R - t_0}{t_0}$$

Criteria for Chromatographic Separation

Reference	GC, GC-MS		LC, LC-MS	
	RT	RRT	RT	RRT
OPCW Current	0.1 min	±20 units (OCAD)	0.2 min	
FDA	±2%		±5%	
AORC	±1% or 6 s	±1% or 6 s	±2% or 12 s	±2% or 12 s
EC	±0.5%	±0.5%	±2.5 %	±2.5 %
USDA	±0.05 min	±0.01 min	±0.5 min	±0.1 min
WADA	±2% or ±0.1 min	±1%	±2% or ±0.1 min	±1%
SOFT	±2%		≥±2%	
Baldwin	±2%		±2%	
FAO	±1.5-3% <1 sec for RT <500 sec 0.2% 500-5000 sec		±1.5-3%	

Mass spectrometric detection and Identification of Molecules with Mass Less than 800 Da

- ≥ ca. 100 ng/ml = 0.1 ppm in the matrix (urine) shall have a full scan acquired or shall have an accurate mass determined such as the elemental composition can be defined. Full scan is the preferred option.
- ≤ ca. 100 ng/ml = 0.1 ppm
  - SIM
  - Computing Ion Ratios from SIM data
  - Tandem mass spectrometry
    - Full scan
    - SRM

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Mass spectrometric detection and Identification of Molecules with Mass Less than 800 Da

- SIM
  - At least three diagnostic ions shall be acquired
  - The relative abundance of a diagnostic ion shall preferably be determined from the peak area or height of integrated selected ion chromatograms
  - Integration should be consistent
  - Ion ratios are then calculated by dividing area of each ion trace by the area obtained from the peak corresponding to the m/z of the base peak ion
  - The S/N ratio of the least intense diagnostic ion shall be greater than 3:1

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Mass spectrometric detection and Identification of Molecules with Mass Less than 800 Da

- SIM
  - Relative intensities of any of the ions shall not differ by more than the amount in Table 1 from the relative intensities of the same ions acquired from a spiked positive control urine, Reference Collection sample or Reference material
  - The concentration of prohibited substance, or its metabolite, or its marker should be comparable in the sample and the spiked urine, Reference Collection sample or reference material
  - For GC/MS, a full scan spectrum shall be acquired at the retention time of peak(s) of interest -> to show lack of presence of other substances that could contribute to the diagnostic ion intensity; to be reported

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Mass spectrometric detection and Identification of Molecules with Mass Less than 800 Da

- Computing Ion Ratios from SIM data
- If 3 diagnostic ions are not available, a second derivative (e.g. methylation, silylation) shall be prepared, or second ionization or fragmentation technique shall be used
- The second derivative should yield different diagnostic ions
- The second ionization technique shall be based on a different physical principle i.e. chemical ionization vs. electron ionization and again should provide different diagnostic ions
- It is not acceptable to utilize a techniques that changes only the relative abundances of the same mass ions
- In any case a minimum of 2 diagnostic ions shall be present in each spectrum

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Mass spectrometric detection and Identification of Molecules with Mass Less than 800 Da

- Tandem mass spectrometry
  - Mass selection, followed by CID and mass selection or scanning of the product ion -> increased sensitivity
  - In general, 2 precursor-product ion transitions should be monitored
  - If only one (1) precursor-product ion transition is used, validation data documenting the uniqueness of the transition
  - The mass resolution of the first mass analyzer shall be set to at least unity

Mass spectrometric detection and Identification of Molecules with Mass Less than 800 Da

- Tandem mass spectrometry
  - If more than one precursor-product ion transition is monitored, the relative abundance of a diagnostic ion shall preferably be determined from the peak area or height of integrated SRM chromatograms
    - Integration should be consistent
    - Ion ratios are calculated by dividing area of each ion trace by the area obtained from the peak corresponding to the m/z of the base peak ion
    - The S/N ratio of the least intense diagnostic ion shall be greater than 3:1

Mass spectrometric detection and Identification of Molecules with Mass Less than 800 Da

- Tandem mass spectrometry
  - Relative intensities of any of the ions shall not differ by more than the amount in Table 1 from the relative intensities of the same ions acquired from a spiked positive control urine, Reference Collection sample or reference material
  - The relative abundance of a diagnostic ion shall preferably be determined from the peak area or height of integrated selected ion chromatograms

Mass spectrometric detection and Identification of Molecules with Mass Less than 800 Da

- Tandem mass spectrometry
  - If 3 diagnostic ions are not available, a second derivative shall be prepared, or second ionization or fragmentation technique shall be used
    - The second derivative should yield different precursor and/or product ions
    - The second ionization technique may use a different chemical ionization reagent, but should provide different precursor or product ions
    - It is not acceptable to utilize a techniques that changes only the relative abundances of the same mass ions

Mass spectrometric detection and Identification of Molecules with Mass Less than 800 Da

- Estimation of concentration
  - Ratio of peak high (or area) to internal standard at the retention time for the analyte of interest and compared to a reference material (spiked or a positive control urine)
    - ^{2}H or ^{13}C is preferred but nor required
    - A single ion at the appropriate m/z ratio taken from an extracted ion chromatogram or from a selected ion monitoring chromatogram

Maximum tolerance Windows for Relative ion Intensities to Ensure Appropriate Confidence in Identification = Table 1

Relative Abundance	EI-GC/MS, CI-GC/MS, GC-MSn; LC/MS, LC/MSn
≥50%	±10% (absolute)
25%-50%	±20% (relative)
5% to <25%	±5% (absolute)
<5%	±50% (relative)

Mass spectrometric detection and Identification of Molecules with Mass Between 800 and 8000 Da

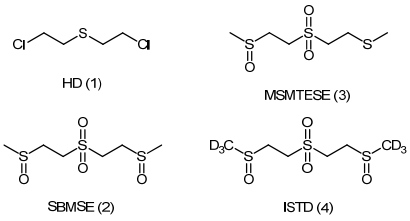
- Bottom up approach
  - Sequencing of proteolytic or chemically-produced fragments of intact protein
  - A minimum of 10 % of the amino acid sequence of the protein or peptide should be verified
  - During method validation, the sequence of amino acids should be compared to a sequence database (e.g. BLAST database)

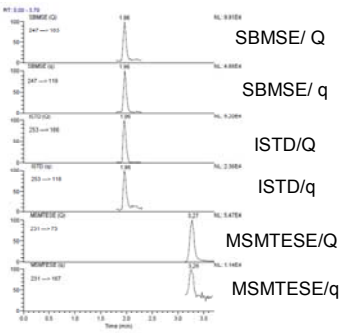
Mass spectrometric detection and Identification of Molecules with Mass Between 800 and 8000 Da

- Top Down approach
  - Determination of molecular mass, by deconvolution of multiply charged ion enveloped to calculate the mass (M) of the intact protein
    - Accurate mass/high resolution approaches
      - Mass shall be within 0.5 Da of the calculated from the reported amino acid sequence of the protein or peptide
    - Low resolution mass spectrometric approaches
      - Mass calculated from the multiple charged envelope shall be within 0.5 Da of the mass calculated from the reported amino acid sequence of the protein or peptide

Metabolites of Mustard Gas

- Verification analysis of biomarkers for CWAs in humans may be needed in cases of alleged use of chemical weapons or after low-level occupational exposure.





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Validation of the method for β-lyase metabolites

The calculated validation results for both β-lyase metabolites.

Chemical	Standard level	Average SD	Average RSD (%)	Variance within group	Variance between groups	Random error	Systematic error	Combined uncertainty
SBMSE	5	0.5	10.8 %	18.4 %	2.1 %	18.5 %	2.14 %	±18.6 %
	10	0.3	3.5 %	5.9 %	0.6 %	5.9 %	0.37 %	± 5.9 %
	25	0.9	3.7 %	4.8 %	2.4 %	5.4 %	0.16 %	± 5.4 %
	50	0.8	0.8 %	2.8 %	1.4 %	3.2 %	0.02 %	± 3.3 %
	100	0.7	0.8 %	4.0 %	2.2 %	4.5 %	0.01 %	± 4.9 %
	200	0.3	0.2 %	0.9 %	0.5 %	1.0 %	0 %	± 1.2 %
MSMTESE	5	1.1	22.9 %	26.3 %	17.2 %	31.4 %	4.88 %	± 31.8 %
	10	1.0	10.8 %	11.4 %	8.6 %	14.2 %	1.2 %	± 14.3 %
	25	0.5	2.2 %	6.9 %	3.3 %	7.6 %	0.09 %	± 7.6 %
	50	1.8	3.9 %	3.5 %	3.4 %	4.9 %	0.08 %	± 4.9 %
	100	2.2	2.4 %	5.3 %	1.9 %	5.6 %	0.03 %	± 5.8 %
	200	1.0	0.6 %	1.3 %	0.5 %	1.4 %	0 %	± 1.5 %

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Validation of the method for β-lyase metabolites

The recovery results for SBMSE and MSMTESE.

	Standard level (ng/ml)	Average Recovery (%)	Average SD	Average RSD (%)
SBMSE	5	124 %	5.1	4 %
	50	97 %	4.5	5 %
	200	94 %	1.7	2 %
MSMTESE	5	89 %	9.7	11 %
	50	117 %	7.2	6 %
	200	116 %	3.9	3 %

LOD and LLOQ

Formulas

LOD (y = mx + n)

If n > 0, LOD = 3x STDEV(n) / m (1)

If n < 0, LOD = (3x STDEV(n) - n) / m (2)

LLOQ (y = mx + n)

If n > 0, LOQ = 10x STDEV(n) / m (3)

If n < 0, LOQ = (10x STDEV(n) - n) / m (4)

Trial Proficiency Test organised by the OPCW
WADA's identification criteria

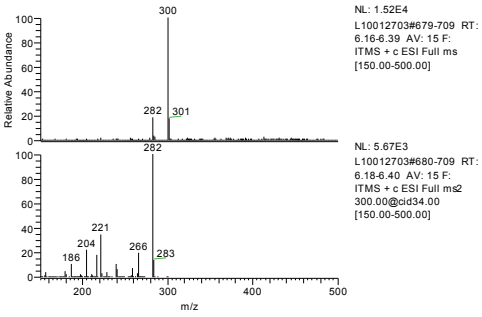
Chemical	Description	Rt (min)	Rt[1]		WADA		Relative Abundance	Relative Area[2]		WADA
			Absolute	Relative	Absolute	Relative	% of q/Q	Absolute	Relative	Relative
SBMSE 100 ng/ml	Ref 100 ng/ml	2.01			±0.1 min	2%	47.8%			± 20%
	Sample s5a/05	2.01	0.00	0.0%	✓	✓	44.2%	-3.6%	-7.5%	✓
	Sample s5b/05	2.02	+0.01	0.5%	✓	✓	46.8%	-1.0%	-2.1%	✓
	Sample s5c/05	2.02	+0.01	0.5%	✓	✓	45.2%	-2.6%	-5.4%	✓
SBMSE 10 ng/ml	Ref 10 ng/ml	2.01			±0.1 min	2%	47.0%			± 20%
	Sample s7a/05	2.00	-0.01	-0.5%	✓	✓	39.0%	-8.0%	-17.0%	✓
	Sample s7b/05	2.02	+0.01	0.5%	✓	✓	43.7%	-3.3%	-7.0%	✓
	Sample s7c/05	2.02	+0.01	0.5%	✓	✓	32.2%	-14.8%	-31.5%	✗

[1] Difference in retention time
[2] Difference in relative area (q/Q -ratio)

SBMSE 10 ng/ml LLOQ
MSMTESE 11 ng/ml LLOQ

STX

LXQ
1 µg/ml in eluent buffer



STX-guidelines from Spiez laboratory

- - 2 MRM transitions: Choose two m/z-pairs for Q1 and Q3, respectively, with sufficiently high intensities. Precursor ion can be the same for both transitions. Care must be taken not to choose the ammonia or water loss peaks for the MRM experiment because that would result in a less than unique transition.
- - Use the same two transitions for all samples
- - Optimize MRM-transition parameters
- - At least 3 measurements per sample

STX t_R 6.4 min

1. Transition m/z 300→282
2. Transition m/z 300→204

- **1. Measurement:**
- $Height_1$: 318765 $Height_2$: 334820 R_I: 0.952
- $Area_1$: 4230519 $Area_2$: 4703249 R_A: 0.899
- **2. Measurement:**
- $Height_1$: 329204 $Height_2$: 352221 R_I: 0.935
- $Area_1$: 4452906 $Area_2$: 4957871 R_A: 0.898
- **3. Measurement:**
- $Height_2$: 342654 $Height_1$: 368622 R_I: 0.930
- $Area_2$: 4582634 $Area_1$: 5055317 R_A: 0.906
- TSQ Quantum Ultra (Thermo Scientific)

STX: Quantum MRM 2 transitions

- Height ratio: R_I=0.939±1.2% (n=3)
  = 93.9% > 1.2% (absolute) WADA ±10% (absolute)

- Area ratio: R_A=0.901±0.4% (n=3)
  = 90.1% > 0.4% (absolute) WADA ±10% (absolute)

STX t_R 6.3 min

- LXQ (ThermoScientific)

Fragment ions

- 186 (q_3) 10
- 204 (q_2) 21
- 221(q_1) 36
- 282 (Q) 100
- **1. Transition** m/z 282→221 (q_1/Q)
- **2. Transition** m/z 282→204 (q_2/Q)
- **3. Transition** m/z 282→186 (q_3/Q)

STX

Run	Rt	S/N				Relative area (%)			Relative Intensity (%)		
		Q	q_1	q_2	q_3	q_1/Q	q_2/Q	q_3/Q	q_1/Q	q_2/Q	q_3/Q
L10012703	6,29	342	71	40	33	33,53	19,51	8,20	32,38	22,99	13,88
L10012704	6,28	387	72	39	25	33,08	18,28	7,79	34,03	23,12	10,01
L10012705	6,29	284	56	35	16	32,46	19,09	5,84	39,82	25,18	9,63
Average	6,29	337,7	66,3	38,0	24,7	33,0	19,0	7,3	35,4	23,8	11,2
SD	0,01	51,6	9,0	2,6	8,5	0,5	0,6	1,3	3,9	1,2	2,4
RSD (%)	0,09	15,3	13,5	7,0	34,5	1,6	3,3	17,3	11,0	5,2	21,0
2*SD	0,01	103,3	17,9	5,3	17,0	1,1	1,3	2,5	7,8	2,5	4,7
2*RSD (%)	0,18	30,6	27,0	13,9	69,0	3,3	6,6	34,7	22,1	10,3	42,1

1 ppm of STX in eluent buffer

- Height ratio:

  (q1/Q) RA=35.4 ± 11 % (n=3) WADA ± 20% (relative)

  (q2/Q) RA=23.8 ± 1.2 % (n=3) WADA ± 5% (absolute)

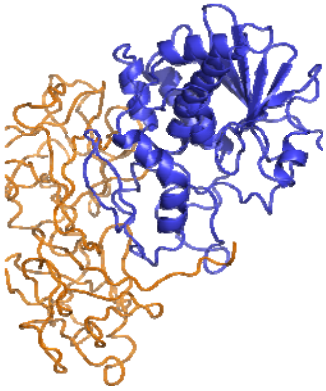
  (q3/Q) RA=11.2 ± 2.4 % (n=3) WADA ± 5% (absolute)
- Area ratio:

  (q1/Q) RI=33.0 ± 1,6 % (n=3) WADA ± 20% (relative)

  (q2/Q) RA=19.0 ± 0.6 % (n=3) WADA ± 5% (absolute)

  (q3/Q) RA=7.3 ± 1.3 % (n=3) WADA ± 5% (absolute)
- m/z 282 were chosen for quantifier ion, even if it is water loss of STX molecule. It is stable and main product ion in MS/MS spectra of STX.

Ricinus communis, Ricin and RCA



Identification Criteria for LC-MS/MS MRM (5 transitions)of five peptides

Parameter	Suggested	Sample 5 (10^6 µg/l)	Sample 3 (10^4 µg/l)
Retention time	≥10 min; ±0.2 min < 10 min: 2%	**a: 4** (-0.04–0.91%) **b: 5** (-0.26–1.00%)	**a: 5** (0.39–0.32%) **b: 5** (-0.19–0.91%)
Total range		-0.25 – 2.71 %	-0.19 – 0.91 %
Number of transitions	Min. 2	**a:** 3 for all **b:** 3 for all	**a:** 3 for 3; 2 for 1 **b:** 3 for 3
Intensity ratio	Diff. <10%	**a: 5** (-5.7 – 8.4%) **b: 5** (-1.9 – 5.3%)	**a: 4** (-5.7 – 8.4%) **b: 3** (-1.9 – 5.3%)
Total range		-5.7 – 8.4 %	-28.0 – 11.5 %
Peptides	Min. 3	**a: 4/5** **b: 5/5**	**a: 4/5** **b: 3/5**

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Summary of ricin PT test

- Retention index criteria
  - OK, also for UPLC
- Number of peptides
  - Achievable; have to be discussed
- MS/MS product ion spectrum
  - Evaluation very time consuming!
  - Valid for higher concentrations, problem for 10^4 µg/l
  - How many ions must match? All 10%?
  - Mass accuracy: ±0.2 Da→ ±0.5 Da or even ±0.7 Da
- MRM
  - OK

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Conclusions

- Currently: OPCW rule for unambiguous identification: chemical identified by two, if possible spectrometric, methods
- Criteria for trace analysis and biomedical sample analysis should be established
- New trial Proficiency test for trace analysis and biomedical samples should be organized
  - The data should be used for evaluation of identification criteria
  - Same criteria for trace and biomedical sample analysis
  - WADA's identification criteria tested
  - Both retention time and mass spectrometric criteria required

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Conclusions

- WADA's criteria
  - legally tested
  - global rules
  - applied widely
- Trace analysis to be decided

≤ ca. 100 ng/ml = 0.1 ppm OR ≤ ca. 1 µg/ml = 1 ppm

Acknowledgements

- Ministry for Foreign Affairs of Finland (funding)
- Defence Forces of Finland (funding)
- School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland
  - Professor Kirsi Vähäkangas
  - Professor Markku Pasanen
  - Dr. Maija Pesonen
- Coordinator Martin Söderström
- Doctoral student Mia Halme

Appendix 8

WADA TECHNICAL DOCUMENT – TD2010IDCR

WADA Technical Document – TD2010IDCR

Document Number: TD2010IDCR Version Number: 1.0
Written by: WADA Laboratory Committee Approved by: WADA Executive Committee
Approval Date: 08 May, 2010 Effective Date: 01 September, 2010

IDENTIFICATION CRITERIA FOR QUALITATIVE ASSAYS INCORPORATING COLUMN CHROMATOGRAPHY AND MASS SPECTROMETRY

The ability of a method to identify a compound is a function of the entire procedure:
sample preparation; chromatographic separation; mass analysis; and data assessment. Any description of the method for purposes of documentation should include all parts of the method. The appropriate analytical characteristics shall be
documented for a particular assay. The Laboratory shall establish criteria for
identification of a compound.

1.0 Sample Preparation

The purpose of the sample preparation and chromatographic separation is to
present a relatively pure chemical component from the sample to the mass
spectrometer. The sample purification step can significantly change both the
performance of the chromatographic system and the mass spectrometer. For
example, a change in extraction solvent can selectively remove interferences and
matrix components that might otherwise co-elute with the compound of interest.
In addition, selective preparation procedures such as immunoaffinity extraction or
fractions collected from high performance liquid chromatography separation can
provide a solution that is nearly devoid of any other compounds.

2.0 Chromatographic Separation

2.1 Gas Chromatography

• For capillary gas chromatography, the retention time (RT) of the analyte shall
not differ by more than two (2) percent or ±0.1 minutes (whichever is smaller) from that of the same substance in a spiked urine sample, Reference Collection sample, or Reference Material analyzed

contemporaneously;
• Alternatively, the laboratory may choose to use relative retention time (RRT)
as an acceptance criterion, where the retention time of the peak of interest is
measured relative to a chromatographic reference compound (CRC).
o The RRT shall not differ by more than ±1% from that of the same substance in a spiked urine sample, Reference Collection sample, or Reference Material analyzed contemporaneously;
o In general, the CRC is not a stable-isotope-labeled internal standard. If a stable isotope-labeled compound is used as the CRC for the same compound, the agreement of RRT between the *Sample* and the same substance in a spiked urine sample, Reference Collection sample, or Reference Material analyzed contemporaneously should be ±0.1%.

WADA Technical Document – TD2010IDCR

Document Number: TD2010IDCR Version Number: 1.0
Written by: WADA Laboratory Committee Approved by: WADA Executive Committee
Approval Date: 08 May, 2010 Effective Date: 01 September, 2010

2.2 Liquid chromatography

• For high performance liquid chromatography, the RT of the analyte shall not
differ by more than two (2) percent or ±0.1 minutes (whichever is smaller)
from that of the same substance in a spiked urine sample, Reference Collection sample, or Reference Material analyzed in the same analytical batch;
• Alternatively, the laboratory may choose to use relative retention time (RRT)
as an acceptance criterion, where the retention time of the peak of interest is
measured relative to a chromatographic reference compound (CRC);
o The RRT shall not differ by more than ±1% from that of the same substance in a spiked urine sample, Reference Collection sample, or Reference Material analyzed contemporaneously;
o In general, the CRC is not a stable-isotope-labeled internal standard. If a stable isotope-labeled compound is used as the CRC for the same compound, the agreement of RRT between the *Sample* and the same substance in a spiked urine sample, Reference Collection sample, or Reference Material analyzed contemporaneously should be ±0.1%.
• If so-called “dilute and shoot” methods are used with LC/MS or LC/MS/MS,
the use of a stable-isotope labeled internal standard is strongly advised due
to the potential for matrix ion suppression or enhancement;

• When the method relies on chromatographic retention times as part of the
identification process, the peak(s) of interest should preferably have retention factors (k') in the range of 3-10 to optimize separation factor (α) and detectability;
• The use of liquid introduction without adequate separation, such as introduction of a peak not separated from the solvent front, is not acceptable
due to the potential for ion suppression;
• If other purification techniques are used prior to mass spectrometric analysis,
the Laboratory shall have method validation documentation demonstrating
that the method provides accurate results free of biological matrix interferences.

3.0 Mass Spectrometric Detection and Identification of Molecules with Mass Less than 800 Da

All Prohibited Substances with a concentration greater than approximately 100
ng/mL in the urine shall have a full or partial scan acquired or shall have an
accurate mass determined such that the elemental composition can be defined.
Whenever possible, a full scan is the preferred option.

3.1 Full scan mode

• A full scan generally should begin at an m/z value of 50 daltons, avoiding the
inclusion of ions arising from permanent gases. A partial scan may begin at

an m/z value greater than any abundant ion due to the derivatizing agent (e.g., the m/z 73 ion arising from trimethylsilyl derivatives) or chemical ionization reagent;
• When a full or partial scan is acquired in GC/MS, all diagnostic ions with a
relative abundance greater than 10% in the reference spectrum obtained from a positive control urine, a Reference Collection sample, or a Reference
Material shall be present in the spectrum of the peak to be evaluated;
• For GC/MS_n and LC/MS_n techniques in Table 1, all diagnostic ions in the product ion scan with a relative abundance greater than 10% shall be

present;
• Ion abundances should be obtained from peak areas or heights from the integration of extracted ion chromatograms;
• The relative abundance of the diagnostic ions may be obtained from a single
spectrum at the peak apex or averaged spectra or integration of peak areas
of extracted ion profiles;
• The relative abundances of each of the diagnostic ions greater than 10% shall be within the limits specified in Table 1.

3.2 Background Subtraction, Averaging Spectra, Peak Deconvolution and Computer-Based Spectral Library Matching

• Background subtraction should be performed uniformly on all samples analyzed contemporaneously and used to make decisions regarding the presence of a *Prohibited Substance* or *Method*, its *Metabolite*, or *Marker*. Both an un-subtracted and a background-subtracted spectrum should be included
in any documentation;
• As a general practice, background subtraction involves averaging (or summing) five (5) or less spectra between the inflection points of the peak.
A similar number of spectra from the baseline, either before or after the peak, can be summed and subtracted from the peak spectra. Background subtracted spectra usually provide a more accurate representation of the mass spectrum of pure compounds available in spectral libraries;
• Recent advances in computer-assisted peak resolution using the mass spectral data have been established. One example of such a program is the
Automated Mass Spectral Deconvolution and Identification System (AMDIS).
The application of these freeware or commercial computer programs is permitted. The use of the program shall be validated as part of the written
procedure.

3.3 Accurate Mass Measurement

Accurate mass measurement provides the opportunity to determine the elemental
composition of an ion. While accurate mass measurement cannot distinguish
isomeric structures, it is often sufficient to determine the number of carbon,
oxygen, hydrogen, nitrogen and other atoms in the molecule.

Mass accuracy should be reported as parts per million (ppm), as calculated from
the equation:
When using an instrument for exact mass measurement, the method description
required under the Technical Document for Laboratory Documentation Packages
shall include:
• the mass spectrometer design (e.g., analyzer type and/or geometry);
• resolution;
• lock masses and lock mass reference materials, and;
• mass range.
If all reasonable alternative elemental compositions cannot be excluded on the
basis of exact mass, other considerations can be used to eliminate possible
compositions. For example, the presence of a chlorine or bromine atom (and other
elemental compositions) can be eliminated by examining the isotopomer pattern
around the mass of interest. In addition, other types of analyses or derivatization
schemes may also be used to eliminate uncertainty of composition.

3.4 Selected Ion Monitoring Mode

In cases where the concentration of the suspicious substance is less than
approximately 100 ng/mL, it may be necessary to acquire selected ions in order to
detect the substance.
• When selected ions are monitored, at least three diagnostic ions shall be
acquired. Recent research, in the absence of chromatographic retention time
data, has shown that the acquisition of more than three ions increases the
probability of correct identification[1];
• The relative abundance of a diagnostic ion shall preferably be determined
from the peak area or height of integrated selected ion chromatograms;
• The integration start and stop points for all of the chromatographic peaks for
each of the m/z values of each of the selected ions should be consistent. Ion
ratios are then calculated by dividing the area of the each ion trace by the
area obtained from the peak corresponding to the m/z of the base peak ion;

• The signal-to-noise ratio of the least intense diagnostic ion shall be greater
than three to one (3:1);
• The relative intensities of any of the ions shall not differ by more than the
amount in Table 1 from the relative intensities of the same ions acquired from a spiked positive control urine, Reference Collection sample, or Reference Material;

[1] Stein, S. E. and D. N. Heller (2006). "On the risk of false positive identification using multiple ion monitoring in qualitative mass spectrometry: Large-scale intercomparisons with a comprehensive mass spectral library." Journal of the American Society for Mass Spectrometry **17**(6): 823-835.

Mass accuracy (ppm) = ((Measured mass– calculated mass)/calculated mass) x 10^6

WADA Technical Document – TD2010IDCR

Document Number: TD2010IDCR Version Number: 1.0
Written by: WADA Laboratory Committee Approved by: WADA Executive Committee
Approval Date: 08 May, 2010 Effective Date: 01 September, 2010
Page 5 of 9

• The concentration of *Prohibited Substance*, or its *Metabolite*, or its *Marker* should be comparable in the *Sample* and the spiked urine, Reference Collection sample, or Reference Material.

If the Laboratory protocol requires three ions to be within a tolerance window to
identify a substance, it is not permissible to collect additional ions and select those
ion ratios that are within tolerance and ignore others that would not result in
meeting identification criteria without a valid explanation.

For GC/MS, in order to ensure that a large amount of a co-eluting substance could
not give rise to the observed diagnostic ions, a full scan spectrum shall be acquired
at the retention time of the peak(s) of interest. The purpose of this scan is **not**
identification, but rather to document the lack of presence of other substances that
could contribute to the diagnostic ion intensity. The acquisition of a full scan may
require analysis an additional aliquot of *Sample* to which the internal standards are
not added. This full scan spectrum shall be included in the documentation package.

3.5 Computing Ion Ratios from Selected Ion Monitoring Data

If three diagnostic ions are not available, a second derivative shall be prepared, or
a second ionization or fragmentation technique shall be used. The second derivative should yield different diagnostic ions.

The second ionization technique shall be based on a different physical principle, i.e.,
chemical ionization vs. electronic ionization and again should provide different
diagnostic ions. It is not acceptable to utilize a technique that changes only the
relative abundance of the same mass ions. In any case a minimum of two diagnostic ions shall be present in each mass spectrum.

3.6 Tandem mass spectrometric (MS_n) detection and identification

Tandem mass spectrometry data can be acquired in either the full scan or selected
reaction monitoring (SRM) mode. The combination of mass selection of the
precursor ion followed by a potentially unique collision-induced dissociation and
mass selection or scanning of the product ion gives tandem mass spectrometry
increased specificity. In general, two precursor-product ion transitions should be
monitored. In some cases, however, the combination of a single precursor-product
ion pair may be sufficiently unique to be definitive. If the Laboratory chooses to
use one precursor-product ion pair for identification, they shall have acquired
validation data documenting the uniqueness of the transition. The mass resolution
of the first mass analyzer shall be set to at least unity.

When more than one precursor-product ion pair is monitored, the relative abundance of a diagnostic ion shall preferably be determined from the peak area or
height of integrated selected reaction monitoring chromatograms.

• The integration start and stop points for all of the chromatographic peaks for
each of the m/z values should be consistent;

• Ion ratios are then calculated by dividing the area of the each ion trace by
the area obtained from the peak corresponding to the m/z of the base peak
ion;

• The relative intensities of any of the ions shall not differ by more than the
amount in Table 1 from the relative intensities of the same ions acquired from a urine spiked with Reference Material, Reference Collection sample, or
Reference Material analyzed contemporaneously;
• The signal-to-noise of the least intense diagnostic ion shall be greater than
three-to-one (3:1);
• The relative abundance of a diagnostic ion shall preferably be determined
from the peak area or height of integrated selected ion chromatograms.
If unique diagnostic precursor-product ion pair(s) are not available, a second
derivative shall be prepared, or a second ionization or fragmentation technique shall
be used.
• The second derivative should yield different precursor and/or product ions;
• The second ionization technique may use a different chemical ionization reagent, but should provide different precursor or product ions;
• It is not acceptable to utilize a technique that changes only the relative abundance of the same mass ions.
To ensure that the precursor and product ions are not arising from a co-eluting
compound in the chromatogram, a full scan spectrum at the retention time of the
peak(s) of interest shall be acquired. The purpose of this scan is **not** identification,
but rather to document the lack of presence of other substances that could
contribute to the precursor-product ion intensity. This may require analysis of an
additional aliquot in which the addition of a stable-labeled internal standard is
omitted. The scan shall be included in the documentation package.

Table 1
***Maximum Tolerance Windows for Relative Ion Intensities to Ensure Appropriate Confidence* in Identification**

Relative Abundance (% of base peak)	**EI-GC/MS; CI-GC/MS; GC/MS_n; LC/MS ; LC/MS_n**
> 50%	±10% (absolute)
25% to 50%	± 20% (relative)

5% to <25% ±5% (absolute)
<5% ± 50% (relative)

WADA Technical Document – TD2010IDCR

Document Number: TD2010IDCR Version Number: 1.0
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3.7 Estimation of concentration

The concentration may be estimated by any of the above techniques by taking the ratio of the peak height (or peak area) obtained at the retention time for the analyte of interest compared to that obtained from an internal standard.

• An internal standard that contains $_{2}H$ or $_{13}C$ in appropriate locations in the molecule is preferred but not required;

• The peak height (or peak area) ratio may then be compared to a reference material appropriately spiked or a positive control urine;

• The use of a single ion at the appropriate mass-to-charge ratio (e.g. m/z 405 for 19-norandrosterone di-TMS derivative) taken from an extracted ion chromatogram or from a selected ion monitoring chromatogram is sufficient for the estimation of concentration. Additional ions shall be used for meeting identification criteria.

4.0 Mass Spectrometric Detection and Identification of Molecules with Mass Between 800 and 8000 Da

In the last decade the advances in mass spectrometry of proteins and peptides has been a major contributor to the characterization of complex mixtures of proteins in proteomic research. In contrast, identification of proteins in anti-doping science is limited to specific compounds identified in the relevant sections of the Prohibited List. Thus, selective isolation of target proteins from the biological matrix is an integral part of applications in anti-doping. Numerous articles have been published on the “top down” and “bottom up” identification of proteins using mass spectrometry. The “top down” approach involves the mass measurement of the intact protein or peptide. The bottom up approach involves the sequencing of

proteolytic or chemically-produced fragments of the intact protein, and this section
applies to any such fragment with a molecular mass less than 8 kDa.

4.1 Top Down Approaches

• Determination of molecular mass: Unlike most small molecules, the ionization of proteins produces a number of multiply charged species (e.g.,
$M+5H^{5+}$, $M+6H^{6+}$, $M+7H^{7+}$, etc.). Deconvolution of these multiply charged ion envelopes allows the calculation of the mass (M) of the intact protein;

o Accurate mass/High resolution approaches: The mass shall be within 0.5 Da of the mass calculated from the reported amino acid sequence of the protein or peptide;

o Low resolution mass spectrometric approaches: The mass calculated from the multiply charged ion envelope shall be within 0.5 Da of the mass calculated from the reported amino acid sequence of the protein or peptide.

• Tandem mass spectrometric sequencing: It has been established that the
building blocks of proteins, amino acids, cause fragmentation of the protein
backbone in predictable ways. Thus, a multiply-charged peptide ion can be

selected and, after low energy collisionally-induced dissociation, a set of multiply charged ions can be analyzed to determine the sequence of amino
acids. Since the sequence of amino acids is frequently unique to the protein,
this can constitute an identification of the protein. During method validation,
amino acid sequences in the target protein should be checked against a sequence database (e.g. BLAST or similar) to ensure that the combination of
peptide sequences used for identification are unique to the protein in question or that other steps (e.g., immunoaffinity isolation or HPLC retention
time) in the analysis rule out other proteins with identical sequences.
The relative abundances of the sequence ions should be compared to a contemporaneously assayed protein standard. If a stable isotope-labeled protein (^{15}N and/or ^{13}C-labeling of the amino acids) is available, the characteristic sequence ions of the unknown can be compared directly to

those derived from the isotopically labeled protein. In either case, the relative abundance of the characteristic ions should agree within the range specified in Table 1 for MS/MS experiments.

During method validation, the sequence of amino acids should be compared to a sequence database (e.g. BLAST database). The uniqueness of the sequence ions should be described in the description of the method included in the documentation package.

4.2 Bottom-up approaches

If the protein has a mass-to-charge ratio that exceeds the range of the instrument, it is necessary to cleave the protein into pieces before mass spectrometric analysis.

The protein may be chemically-modified to increase the number of charges on the ions. It is also permissible to consider a single peptide dissociated from a multipeptide protein (e.g., cleavage of inter-chain disulfide bonds).

4.3 Identification of proteolytic or chemically-induced peptides

A minimum of 10% of the amino acid sequence of the protein or peptide should be verified. During method validation, the sequence of amino acids should be compared to a sequence database (e.g. BLAST database). The uniqueness of the sequence ions should be described in the description of the method included in the documentation package.

WADA Technical Document – TD2010IDCR

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5.0 Definitions

Accurate Mass Measurement*:* This technique usually requires high mass resolution and is frequently associated with the term high resolution mass spectrometry. The mass assignment is generally made to at least two decimal places.

Diagnostic ion(s): Molecular ion or fragment ions whose presence and abundance are characteristic of the substance and thereby may assist in its identification. A second ion belonging to the same isotopic cluster may also be used as diagnostic only when the peculiarity of the atomic composition of the fragment so justifies (e.g. presence of Cl, Br, or other elements with abundant isotopic ions).

High resolution mass spectrometry (HRMS): For the purposes of the *International Standards* for Laboratories, HRMS is defined as mass spectrometry at a resolving power equal to or greater than 10,000 at full-width half-height maximum.

Low resolution mass spectrometry (LRMS)*:* LRMS is defined as mass spectrometry at a resolving power less than 10,000 at full-width half-height maximum.

Relative abundance (mass spectrometry): The abundance of a particular ion relative to the most abundant ion monitored expressed as a percentage.

Maximum difference in relative abundance: The maximum permitted difference between the relative abundance of a particular ion obtained from the *Sample* and that obtained from the positive control urine. This may be expressed in ABSOLUTE or RELATIVE terms.

Absolute difference: Calculated by subtracting the stated percentage from the relative abundance obtained for the studied ion from the positive control urine or Reference Material. For example, if the relative abundance of an ion in the chromatographic peak of interest in the positive control urine or Reference Material is measured as 20%, then the observed relative abundance for the same ion in the peak of interest in the unknown urine sample would be required to be in the range of 15-25% (20% ± 5%) for the ion to meet the identification criteria.

Relative difference: Calculated by multiplying the stated percentage by the relative abundance obtained for the studied ion from the positive control urine or Reference Material. For example, if the relative abundance of an ion in the chromatographic peak of interest in the positive control urine or Reference Material appears as 30 % and the stated maximum permitted difference is 20 % (relative), then the observed relative abundance for the same ion in the peak of interest in the unknown urine sample would be required to be in the range of 24-36% (30% ± (30 x 20 %)) for the ion to meet the identification criteria.

Scan*:* Acquisition of ions of a continuous range of m/z values.

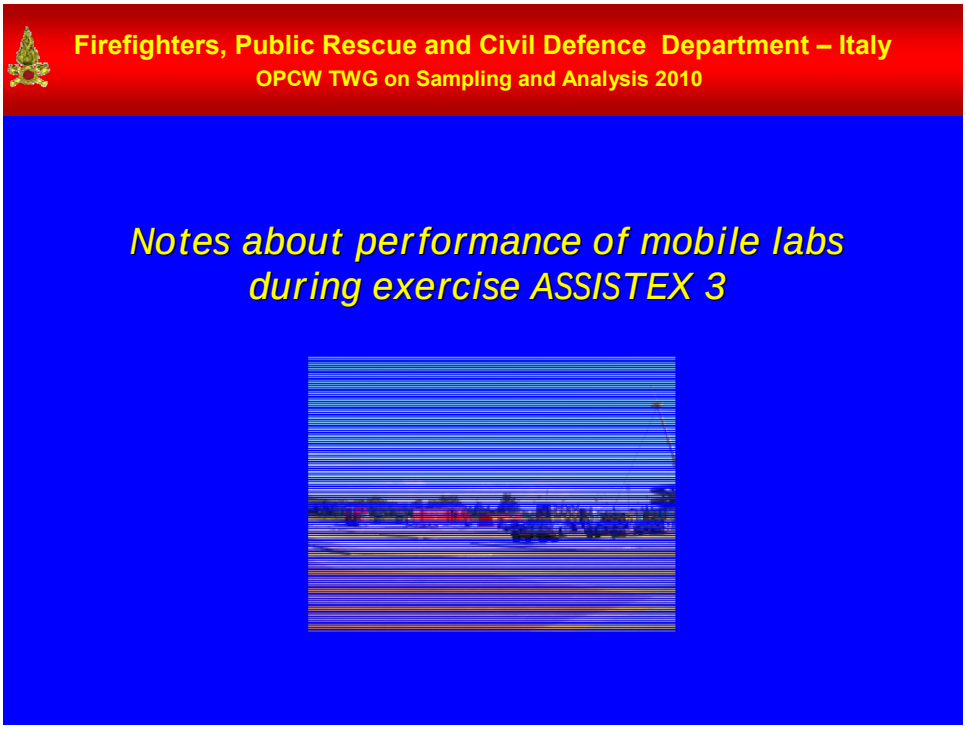
Selected ion monitoring (SIM)*:* Acquisition of ions of one or more pre-determined discrete m/z values for specified dwell times.

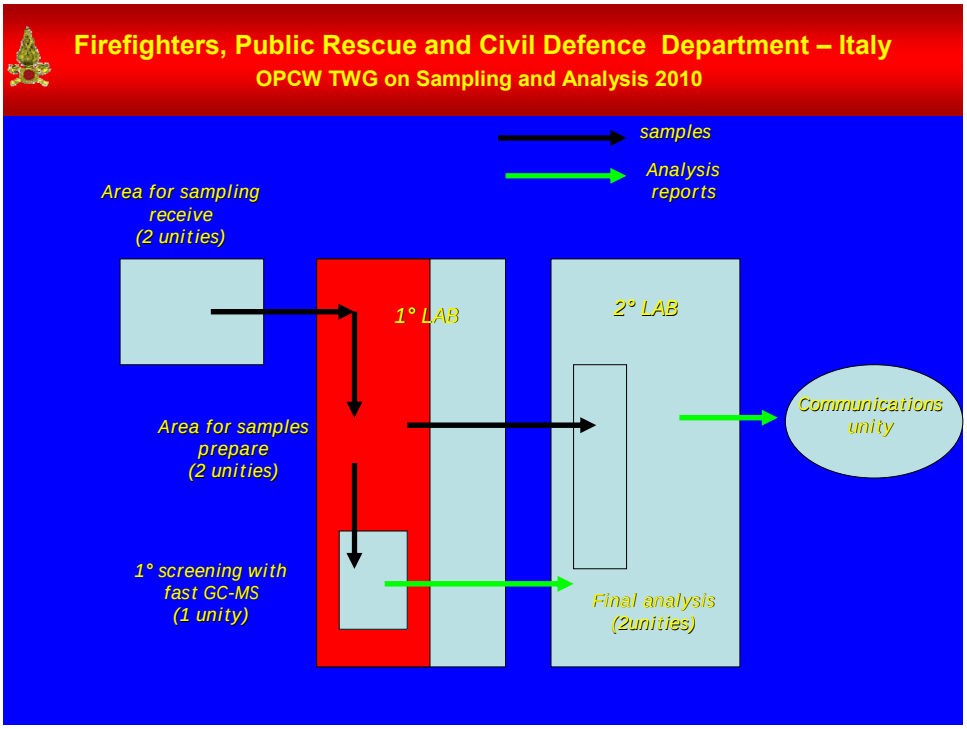
Selected Reaction Monitoring (SRM): Data acquired from specific product ions corresponding to m/z selected precursor ions recorded via two or more stages of mass spectrometry. Selected reaction monitoring can be performed as tandem mass spectrometry in time or tandem mass spectrometry in space.

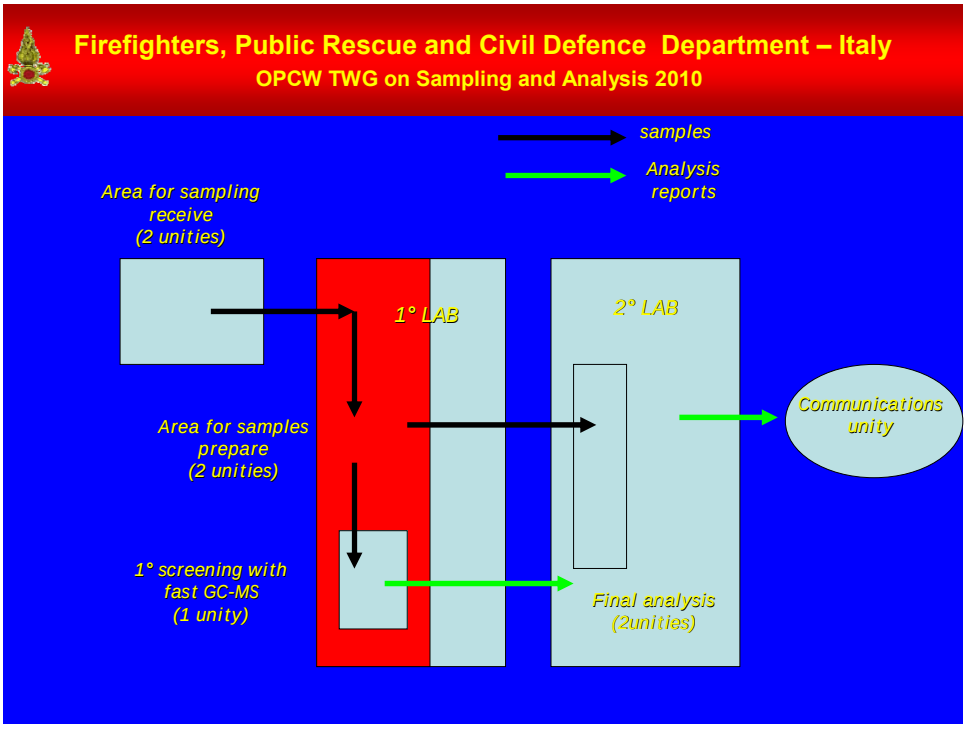
Signal-to-Noise Ratio*:* Magnitude of the instrument response to the analyte (signal) relative to the magnitude of the background (noise).

Appendix 9

NOTES ABOUT PERFORMANCE OF MOBILE LABS DURING EXERCISE ASSISTEX 3




Firefighters, Public Rescue and Civil Defence Department – Italy
OPCW TWG on Sampling and Analysis 2010
samples
Analysis reports
Area for sampling receive (2 unities)
1° LAB
2° LAB
Communications unity
Area for samples prepare (2 unities)
1° screening with fast GC-MS (1 unity)
Final analysis (2unities)


Firefighters, Public Rescue and Civil Defence Department – Italy
OPCW TWG on Sampling and Analysis 2010
samples
Analysis reports
Area for sampling receive (2 unities)
1° LAB
2° LAB
Communications unity
Area for samples prepare (2 unities)
1° screening with fast GC-MS (1 unity)
Final analysis (2unities)

Sampling team

- *Danish team (3 unities)*
- *India team (10 unities)*
- *France Army (10 unities)*
- *France Air Force (6 unities)*

Firefighters, Public Rescue and Civil Defence Department – Italy
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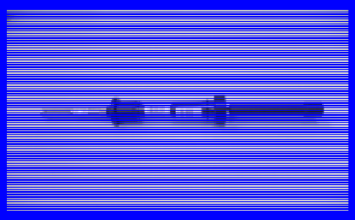
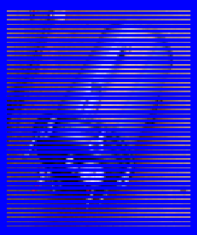
Sampling





CBRN Sampling Form — Assistex 3 – Tunisia 2010

CBRN Team: _____

Date & Time (International Time Code): ____ Z ____ DD HHH MMMM YYYY

Type: Solid ☐ Liquid ☐ Gas ☐ SPMT ☐

Sampling Area: ____

ID Sample 1	
ID Sample 2	
ID Sample 3	
ID Sample 4	

Personnel involved:

Radio Operator _____

Sampling Team names _____

Site /weather situation:
Indoor ☐ Outdoor ☐ | Wet ☐ Dry ☐ | Sunny ☐ Mostly Sunny ☐ Cloudy ☐ Overcast ☐

GPS Reading: _____

Sampling area brief description: _____
(ie. "Entrance", "near load machine", "under the smoke evacuator", "on the window" and so on.)

Concentration reading before sampling_____ PPM ppb/mg-m³

Instrument / Tube(s) used _____

Sampling tools: _____

Sample quantity / volume / exposure time: _____

Removed to the analysis team

Date of arrival: ___/___/___ Time ___/___/___ Seals are intact ? YES / NO

Delivered by_____ Received by _____

Date of opening: ___/___/___ Time ___/___/___ Operator _____

Remaining sample storage: _____

Remaining sample destroyed by: _____

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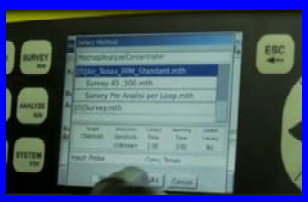
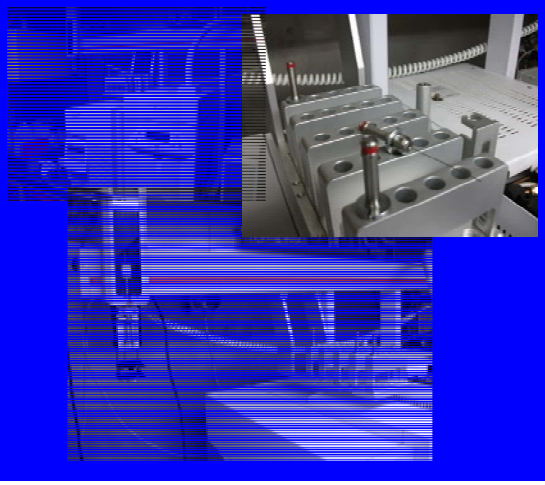
Analysis

FAST GC-MS, GC-MS, FT-IR, IONIC CROMATOGRAPHY





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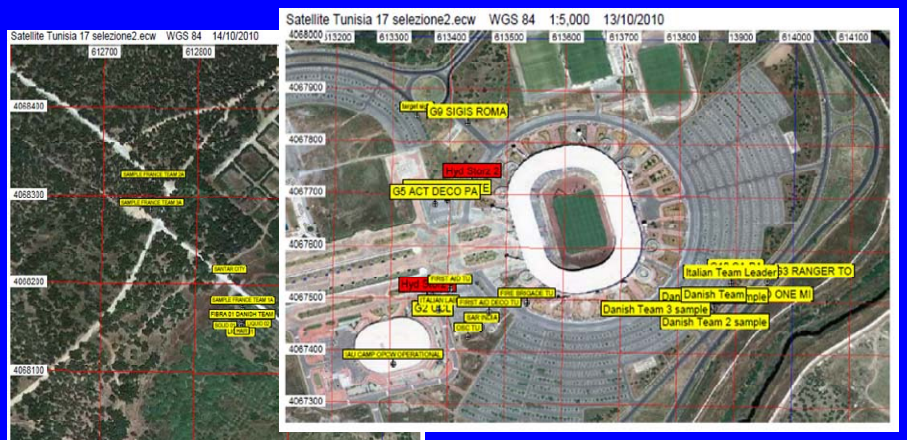

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SAMPLING REPORT

	AIR SAMPLE			LIQUID SAMPLE	SAMPLING TEAM
	DATE	13:10:10			
	fiber	holder	bags		
1	01C	RSSA 006			DANISH
2	16	RSSA 003			DANISH
3			8277-709-208		DANISH
4	01A	RSSA 001			INDIA
5	15	RSSA 002			INDIA
6			36982		INDIA
	DATE	14:10:10			
1				L001	DANISH
2			8277-709-208-A		DANISH
3				3A	FRANCE AF
4				1A	FRANCE AF
5				India1	INDIA

ANALYSIS REPORT

SAMPLE | **ANALYSIS**

			DATE: 13:10:10	INSTRUMENTS	SAMPLING TIME	SAMPLE TIME OF ARRIVE LAB	START ANALYSIS	final report issue	time of response from sampling time	lab time of response	alarm time
1	01C	RSSA 006		GC-MS traditional	16:20	17:00	17:18	18:00	01:40	01:00	14:30
2	16	RSSA 003		GC-MS traditional	16:15	16:50	16:56	18:00	01:45	01:10	14:30
3		8277-709-208		GC-MS portable	16:10	16:40	16:55	18:00	01:50	01:20	14:30
4	01A	RSSA 001		GC-MS traditional	14:10	15:10	15:20	16:05	01:55	00:55	11:30
5	15	RSSA 002		GC-MS traditional	14:20	15:05	15:15	16:10	01:50	01:05	11:30
6		36982		GC-MS portable	14:30	15:00	15:14	15:50	01:20	00:50	11:30
			DATE: 14:10:10								
1		L001		GC-MS traditional	12:20	13:00	13:05	14:20	02:00	01:20	11:00
1		L001		GC-MS portable	12:15	13:00	13:25	14:20	02:05	01:20	11:00
2		8277-709-208-A		GC-MS portable	12:15	13:00	15:30	15:50	03:35	02:50	11:00
1		3A		GC-MS traditional	15:02	15:30	15:41	17:05	02:03	01:35	11:00
3		3A		GC-MS portable							
4		1A		GC-MS portable	13:28	15:00	16:00	16:20	03:52	01:20	11:00
5		India1		GC-MS traditional	12:10	13:10	13:50	14:40	02:30	01:30	10:30
5		India1		GC-MS portable	12:15	13:00	13:00	14:20	02:05	01:20	10:30

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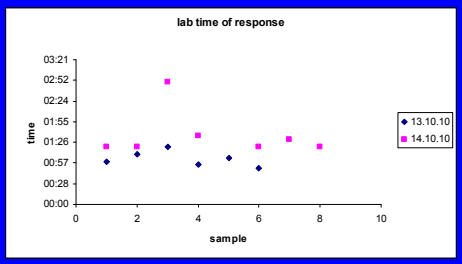
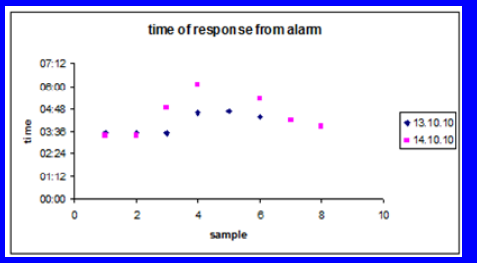
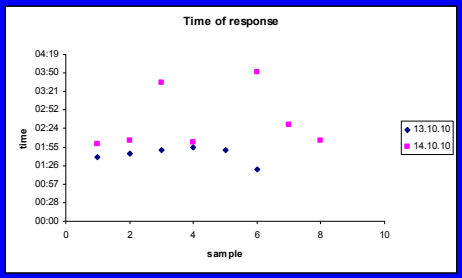
OPCW TWG on Sampling and Analysis 2010







Result:
Time to have a first qualitative report from alarm

Min: 3:30 h
Max: 6:00 h

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OPCW TWG on Sampling and Analysis 2010

VIGILI DEL FUOCO ITALY CHEMICAL LAB Analysis report

release time (time, data)	14.45 --- 14/10/2010
ID Sample	LØ01H
samplig time	12:15
analysis time	13.25- 14.45
sampling area	Top level water well (36°45'8,8" 10°15'51")
analysis instruments	GC/MS Hapsite

Results

no chemical compounds identified	
chemical compound identified	X

list

	name	cas number
1	Methilene chloride	75092
2	Ethene Chloro	75014
3	Acetic Acid, dichlore	79436
4	Heptane	142826

suspicios presens of	

list

	name	cas number
1		
2		
3		
4		

note	Compound n°2-3-4 traces.

VIGILI DEL FUOCO ITALY CHEMICAL LAB Analysis report

release time (time, data)	16.05 --- 14/10/2010
ID Sample	France 3a
samplig time	14.10.2010 / 15.02
analysis time	14.10.2010 / 15.41
sampling area	rocket surface
analysis instruments	GC/MS SPME fiber

Results

no chemical compounds identified	
chemical compound identified	X

list

	name	cas number
1	Dimethysulfoxide	67-68-5
2	Dichloromethane	75-09-2
3	Benzyl Alchool	100-51-6
4	Methyl Salicylate	119-36-8

suspicios presens of	

list

	name	cas number
1		
2		
3		
4		

note	Benzenepropanol 112-97-4

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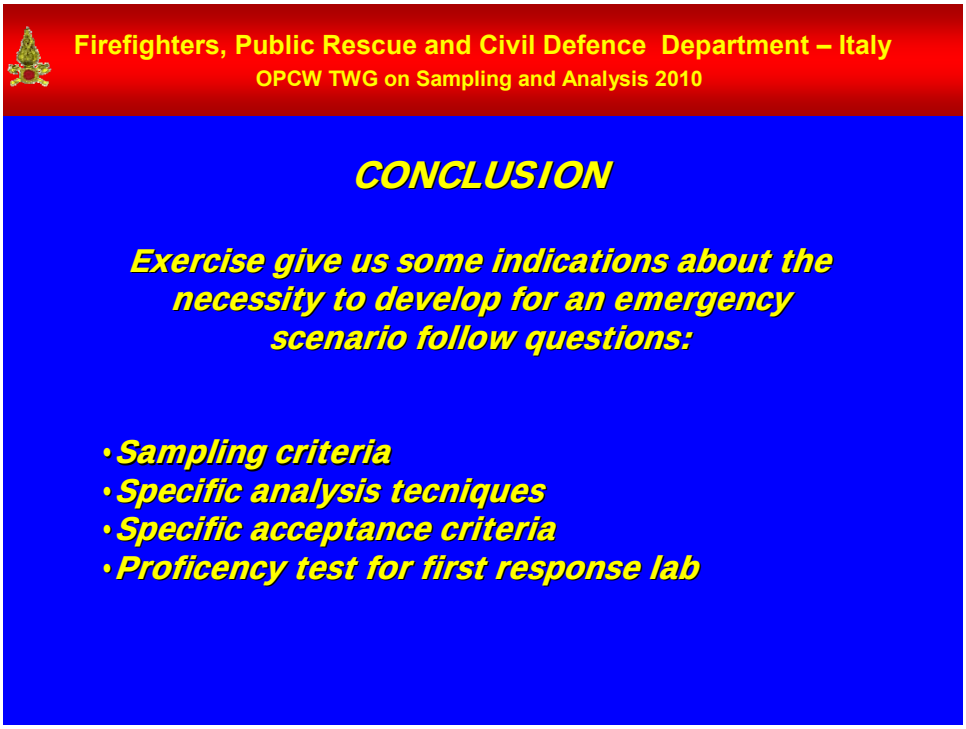
OPCW TWG on Sampling and Analysis 2010

COMMENTS

- ***Time to produce a first qualitative report 5-6 hours (without time to arrive mobile lab on scenario)***
- ***50 % of samples are completely unuseful (sampling error, problems during sample decontamination, lost of information,……)***
- ***No definition about acceptance criterias for results in first analysis tecnique (first information may determine consequences of scenario)***


Firefighters, Public Rescue and Civil Defence Department – Italy
OPCW TWG on Sampling and Analysis 2010
CONCLUSION
Exercise give us some indications about the necessity to develop for an emergency scenario follow questions:
• Sampling criteria
• Specific analysis tecniques
• Specific acceptance criteria
• Proficency test for first response lab

Appendix 10

UPDATING OF THE ROPs WITHIN INTERNATIONAL COOPERATION

https://wiki.helsinki.fi/display/ROPSite/Home

Development of the Blue Book

- Table of Contents
- Instructions for authors
- Model Chapters and Templates
- Contact Details
- 1994 ROP Book

Blue Book 2011: Table of Contents

Blue Book 2011: Table of Contents

Authors

- **AMMS;** China
  - **Jianwei Xie**
  - **LIU Qin**
  - **GUO Lei**
  - **LIN Ying**
- **DGA,** France
  - **Anne Bossée**
  - **Marie-Pierre Barrioulet**
  - **Yannick Juillet**
  - **Jacky Dissard**
  - **Sophie Gougeon**
  - **Christine Albaret**
- **DSO, Singapore**
  - **Chua Hoe Chee**
  - **Sng Mui Tiang**
  - **Nancy Lee Hoi Sim**
  - **Veronica Yeo Mui Huang**
  - **Jessica Woo Huizhen**
- **Dstl, United Kingdom**
  - **Robin Black**
  - **Colin Pottage**
  - **Robert Read**

VERIFIN
HELSINGIN YLIOPISTO
HELSINGFORS UNIVERSITET
UNIVERSITY OF HELSINKI

Authors

- **ECBC,**United States
  - **Joy M Ginter**
  - **John A. Tokarz III**
  - **Kevin J. Shefcheck**
  - **Lynn Hoffland**
  - **Eric Bukowski**
- **FOI,** Sweden
  - **Lars-Gunnar Hammarström**
- **LAVEMA,** Spain
  - **Esther Gómez Caballero**
  - **Juan Manuel Moreno Sobrino**
- **LLNL,** United States
  - **Hugh Gregg**
  - **Armando Alcaraz**
  - **Sarah Chinn**
  - **Carolyn Koester**
- **OPCW**
  - **Pingfeng Liu**
  - **Gary Mallard**
  - **Maciej Sliwakowski**
  - **Meehir Palit**

VERIFIN
HELSINGIN YLIOPISTO
HELSINGFORS UNIVERSITET
UNIVERSITY OF HELSINKI

Authors

- **Spiez Laboratory,** Switzerland
  - **Peter Siegenthaler**
  - **Urs Meier**
  - **Jean-Claude Dutoit**
  - **Stefan Mogl**
  - **Martin Schaer**
- **WIS, Germany**
  - **Damian Magiera**
- **VERIFIN,** Finland
  - **Ullastiina Hakala**
  - **Vesa Häkkinen**
  - **Karoliina Joutsiniemi**
  - **Harri Kiljunen**
  - **Marja-Leena Kuitunen**
  - **Harri Koskela**
  - **Olli Kostiainen**
  - **Martin Söderström**
  - **Paula Vanninen**

Blue Book 2011

- Next meeting on 9 February 2011 at the OPCW
- Workshop 16-17 November 2011 in Helsinki
  - Lectures on new ROPs
  - Other topics
    - Ricin toxin analysis
    - Biomedical sample analysis
    - Trace analysis