METHOD 145A

COLLABORATIVE TRIAL 145 OF A METHOD FOR THE DETECTION AND DETERMINATION OF SUDAN I IN CHILLI PRODUCTS BY HPLC

Note: This procedure will be validated in the FSA collaborative trial programme to assess methods of analysis of interest or of particular concern. It was developed in the Lincolne Sutton and Wood Norwich Laboratory as a relatively "simple" method for the determination of Sudan I in chilli products. The method is applicable to other products, e.g. chutneys.

THE DETECTION AND DETERMINATION OF SUDAN I IN CHILLI PRODUCTS BY HPLC

1. SCOPE AND FIELD OF APPLICATION

1.1 The method describes the determination of Sudan I in chilli products.

1.2 Sudan I is an oil-soluble, mono azo dyestuff which is carcinogenic and is not permitted in food.

2. DEFINITION

The Sudan I content means the content of Sudan I as extracted and determined by this method.

3. PRINCIPLE

Sudan I is extracted from the prepared sample with Industrial methylated spirit (IMS). After centrifuging and filtering, any Sudan I is detected and determined by HPLC using UV detection.

4. REAGENTS

4.1 GPR and AR grade reagents are suitable unless otherwise stated. Water should be deionised, distilled or of similar quality.

4.2 Sudan I: That obtained from Acros Organics has been found suitable.

4.2.1 Sudan I stock solution: Weigh 0.1000g Sudan I (4.2) and transfer to a 100mL volumetric flask with Industrial methylated spirit (4.3). Dissolve and make to volume with IMS. Mix well. This solution has a concentration of 1000mg/L.

4.2.2 Sudan I working solutions: Prepare as appropriate, the following working solutions by transferring the stated volumes of Sudan I stock solution (4.2.1) to a 100mL volumetric flask. Dilute to volume with IMS. Mix well.

Volume (4.2.1)	concentration
50 µL	0.5 mg/L
100 µL	1.0 mg/L
200 µL	2.0 mg/L
0.5 mL	5.0 mg/L
1.0 mL	10.0 mg/L
1.5 mL	15.0 mg/L
2.0 mL	20.0 mg/L

Once prepared the solutions should be protected from the light as far as is practicable e.g., by wrapping in aluminium type foil.

4.3 Industrial Methylated Spirit 99 (IMS): That obtained from Tennants has been found suitable.

4.4 Chloroform: HPLC grade.

4.5 Acetonitrile: HPLC grade.

4.6 Ammonium acetate

4.7 Triethylamine

4.8 Methanol: HPLC grade.

4.9 0.05mol/L ammonium acetate in methanol: Dissolve 3.850g ammonium acetate (4.6) in methanol (4.8), dilute to volume with methanol in a 1 litre volumetric flask. Mix well.

4.10 Mobile phase for HPLC: Mix in the following proportions:

4 part of Acetonitrile (4.5), and
1 part of 0.05mol/L ammonium acetate in methanol (4.8)

Add 1mL triethylamine (4.7) per litre prepared.

5. APPARATUS

5.1 Normal laboratory glassware and apparatus.

5.2 Ultra Turrax mixer fitted with 18N shaft or suitable equivalent.

5.3 Centrifuge: the Jouan G412 operating at a setting of 3000 ± 100 has been found suitable.

5.4 Centrifuge tubes for (5.3) 50mL capacity.

5.5 Filter papers: GF/A 70mm diameter.

5.6 HPLC with UV detector (see Appendix 1).

5.7 Laboratory homogeniser.

5.8 Screw cap glass vials: 9mL capacity.

5.9 ‘Tall form’ glass beakers 150mL capacity.

5.10 Timing device.

6. PROCEDURE

6.1 Preparation of test sample

6.1.1 Dry chilli products (e.g. chilli powder or crushed chilli) need only to be mixed thoroughly. No attempt should be made to macerate chilli powder in high speed blenders due to the potential formation of irritating aerosols.

6.1.2 Wet chilli-containing food products (e.g. relishes, chutneys) should be rendered as homogenous as possible in a suitable laboratory homogeniser (5.7)

6.1.3 Keep the prepared sample in an airtight, opaque plastic container and store it in such a way that deterioration and change in composition are avoided.

6.1.4 Analyse the prepared sample as soon as possible after homogenisation. Immediately prior to analysis the prepared sample should be mixed to ensure homogeneity.

6.2 Weigh 5 ± 0.1g prepared sample into a 150mL 'Tall form' glass beaker (5.9).

6.3 Add 50.0mL Industrial Methylated Spirit (IMS) (4.3) to the test portion.

6.4 Blend using the Ultra Turrax mixer (5.2) for 3 minutes (yellow/green setting).

6.5 Transfer to balanced centrifuge tubes (5.4) and centrifuge (5.3) for 10 minutes.

6.6 Filter the supernatant through a filter paper (5.5) into a glass vial (5.8).

6.7 Secure the screw top of the vial and protect from the light as far as practicable e.g. by wrapping in aluminium.

6.8 Set up the HPLC system (Appendix 1) and allow to stabilize for at least 1 hour.

Calibration

NB. Linearity has been previously established over the range of working standard solutions (4.2.2) and it is therefore not necessary to perform a full calibration on each occasion. A full linearity check, however, must be performed when new working standard solutions are prepared.

6.9 Sequentially inject an appropriate volume (15µL) of test portion extract (6.7) and the same appropriate volume of suitable working standard solutions (4.2.2). Ensure that duplicate injections of the extract are bracketed by standard injections.

6.10 Identify the compound of interest in the extract chromatogram by virtue of its retention time with reference to the working standard solution chromatogram (see Appendix 2 for a typical chromatogram).

6.11 Record the areas of any identified peaks from the test portion extract chromatograms (6.9).

6.12 Record the areas of the peak of interest from the working standard solution chromatograms (6.9).

6.13 If the concentration of Sudan I in the test portion extract exceeds the highest working standard solution it is necessary to dilute a suitable aliquot to a known volume with IMS.

7. CALCULATION

For 5g 50

Concentration (mg/kg):

$$\frac{A}{B} \times Y \times 10 \times \frac{100}{WRm} \times D$$

Where

A = mean area of any identified peak in the test portion extract.

B = mean area of peak of interest in the working standard solution.

Y = concentration (mg/L) of the suitable working standard solution

WRm = working recovery mean (9.1.2)

D = the dilution factor (if any) (6.13)

8. EXPRESSION OF RESULTS

Record the result to the nearest 1 mg/kg.

9. ANALYTICAL QUALITY ASSURANCE

9.1 Performance Characteristics (Typical)

9.1.1 Limit of detection: to be assessed in the light of the collaborative trial.

9.1.2 Bias

WRm(n=12): 105

WRs: 8.3

9.1.3 Precision

Wp: to be determined

Wp(relative): to be determined

APPENDIX I: EXAMPLE OF CHROMATOGRAPHIC AND EXPERIMENTAL CONDITIONS

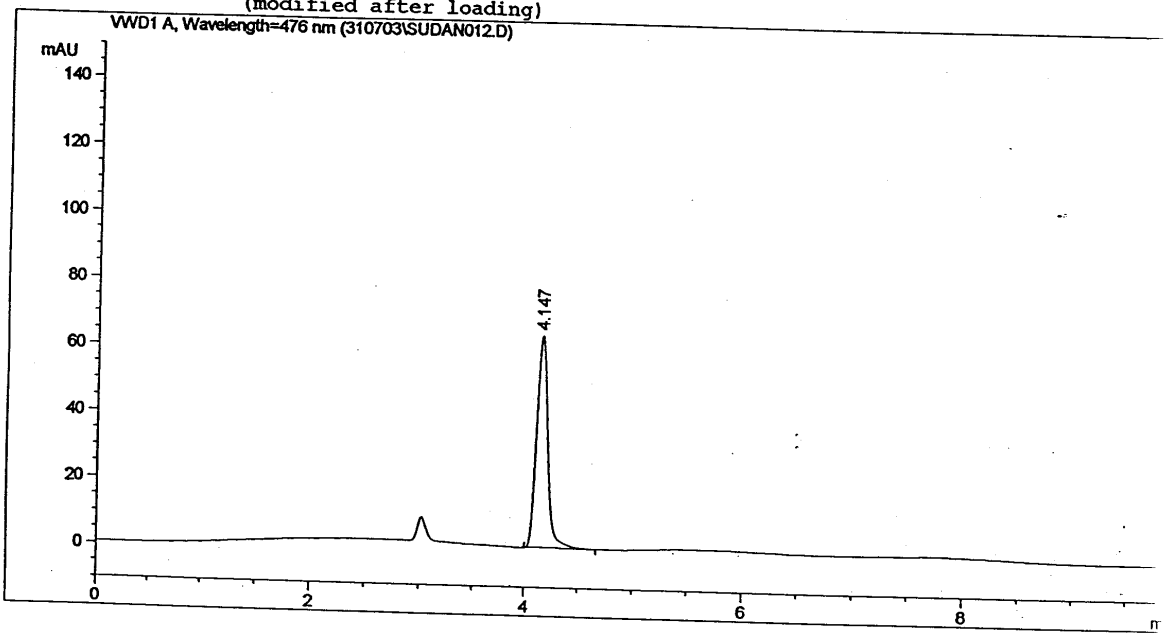
Instrument:	Isocratic HPLC system including variable wavelength UV/VIS detector, injector and electronic data handling system.
Column:	Vydac 201 TP54 (250mm x 4.6mm) with suitable guard column.
Injection Volume:	15µL
Mobile Phase:	4 parts of Acetonitrile(4.5), and 1 part of 0.05mol/L ammonium acetate in methanol (4.9). Add 1mL triethylamine (4.7) per litre prepared.
Mobile phase flow rate:	1mL min^{-1}
Detector:	Variable Wavelength Detector set at 476nm.
Data Collection:	Suitable integrator or PC based data collection system.
Other Details:	After use the system must be flushed by pumping degassed acetonitrile for at least 20 minutes.

APPENDIX 2

Typical chromatograms run under the chromatographic conditions given in Appendix 1.

10mg/L Standard chromatogram:-

```
Injection Date   : 31/07/2003 17:19:18 PM
Sample Name      : 10mg/L Std                    Location : Vial 1
Acq. Operator    : CS
Method           : C:\HPCHEM\1\METHODS\SUDAN1.M
Last changed     : 31/07/2003 10:35:23 PM by CS
                   (modified after loading)
```



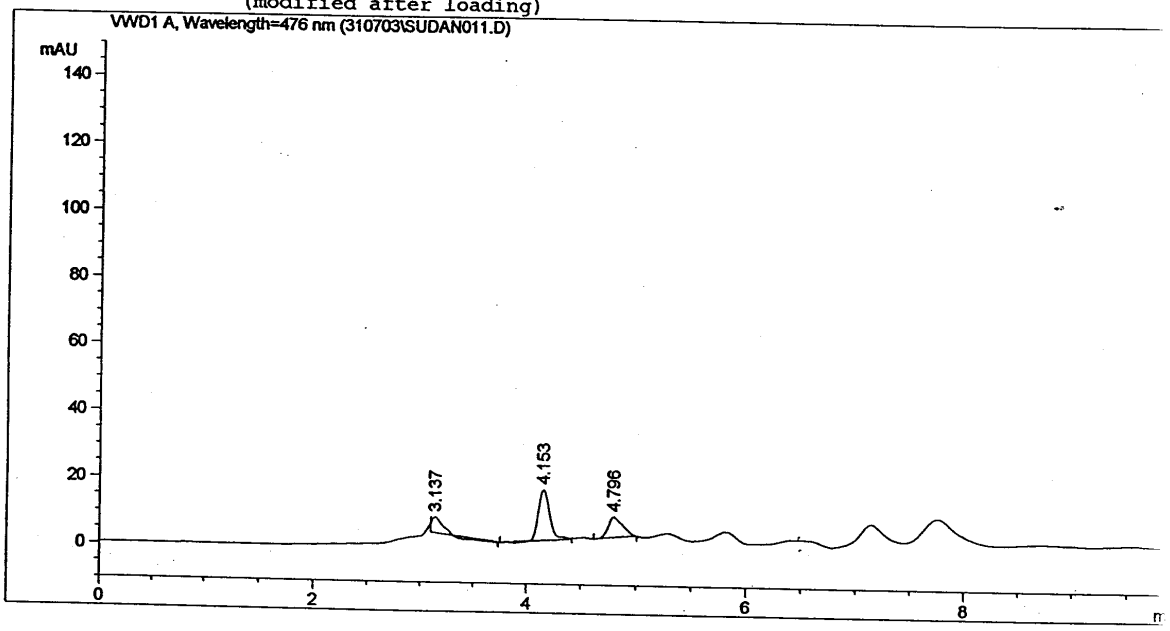
External Standard Report

```
Sorted By              :      Signal
Calib. Data Modified   :      31/07/2003 10:35:16 PM
Multiplier             :      1.0000
Dilution               :      1.0000
Sample Amount          :     15.00000  [mg/L]   (not used in calc.)

Signal 1: VWD1 A, Wavelength=476 nm

RetTime  Type      Area       Amt/Area     Amount    Grp   Name
 [min]          mAU   *s                   [mg/L]
-------|------|----------|----------|----------|--|------------------
  4.147 PP      464.26999 2.10904e-2    9.79163     SUDAN I

Totals :                                  9.79163
```

Sample chromatogram:-

Sudan 1 (Rf 4.153) found in sample equivalent to 20mg/kg

```
Injection Date  : 31/07/2003 16:26:17 PM
Sample Name     : SC4138                          Location : Vial 1
Acq. Operator   : CS
Method          : C:\HPCHEM\1\METHODS\SUDAN1.M
Last changed    : 31/07/2003 10:35:23 PM by CS
                  (modified after loading)
```

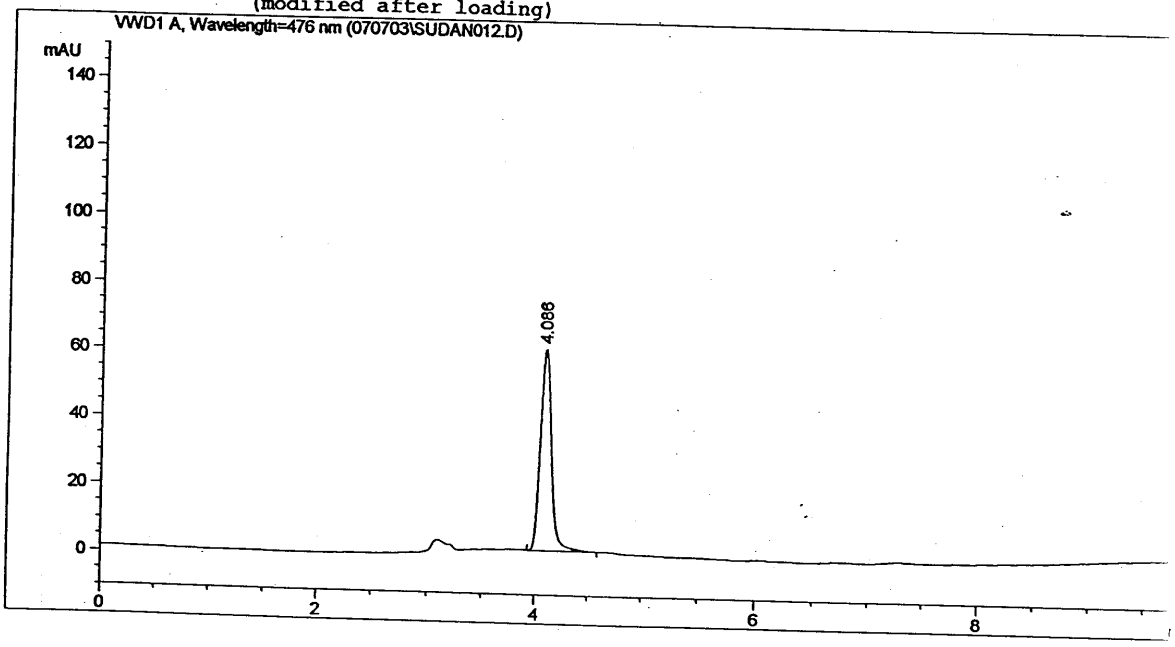



```
                    External Standard Report

Sorted By             :      Signal
Calib. Data Modified  :      31/07/2003 10:35:16 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount         :      15.00000  [mg/L]    (not used in calc.)

Signal 1: VWD1 A, Wavelength=476 nm

RetTime  Type      Area      Amt/Area     Amount    Grp    Name
 [min]          mAU   *s                 [mg/L]
-------|------|----------|----------|----------|--|------------------
  4.153 PV      112.38305 2.08900e-2    2.34768     SUDAN I

Totals :                                2.34768

 Results obtained with enhanced integrator!

                    *** End of Report ***
```

Sample chromatogram:-

Sudan 1 (Rf 4.086) found in sample equivalent to 2400mg/kg

```
Injection Date   : 07/08/2003 15:54:26 PM
Sample Name      : 5809/M A                          Location : Vial 1
Acq. Operator    : CS
Method           : C:\HPCHEM\1\METHODS\SUDAN1.M
Last changed     : 07/08/2003 10:33:13 PM by CS
                   (modified after loading)
```



External Standard Report

```
Sorted By             :      Signal
Calib. Data Modified  :      07/08/2003 10:33:05 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount         :      15.00000  [mg/L]   (not used in calc.)

Signal 1: VWD1 A, Wavelength=476 nm

RetTime  Type     Area        Amt/Area     Amount    Grp   Name
 [min]          mAU   *s                   [mg/L]
-------|------|----------|----------|----------|--|------------------
  4.086 BP      433.87668 2.15409e-2    9.34608     SUDAN I

Totals :                                9.34608
```