CHAPTER 8

Tadalafil

Alaa A.-M. Abdel-Aziz,[*] Yousif A. Asiri,[#] Adel S. El-Azab,[*] Mohamed A. Al-Omar,[*] and **Takehisa Kunieda[†]**

Contents

[*] Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia
[†] Faculty of Pharmaceutical Sciences, Sojo University, Kumamoto, Japan
[#] Department of Clinical Pharmacy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

Profiles of Drug Substances, Excipients, and Related Methodology, Volume 36
ISSN 1871-5125, DOI: 10.1016/B978-0-12-387667-6.00008-7

1. USES AND APPLICATIONS

Erectile dysfunction (ED), a common and widespread health problem that affects approximately 30 million men in the United States [1], is suggested to represent an early clinical manifestation of a diffuse vascular disease [2,3].

Tadalafil (Cialis®) [4–11], which is a cyclic guanosine monophosphate (cGMP) specific Type V phosphodiesterase (PDE5) inhibitor similar to sildenafil (Viagra®) [12] and vardenafil (Levitra®) [13], has an improved PDE5/PDE6 selectivity compared to sildenafil [14–16]. Tadalafil (Cialis®) is a newly approved oral selective PDE5 inhibitor indicated for the treatment of ED.

2. DESCRIPTION

2.1. Nomenclature [17,18]

2.1.1. Chemical name

- (6*R*,12a*R*)-6-(1,3-Benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-methylpyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione.
- (6*R*,12a*R*)-2,3,6,7,12,12a-Hexahydro-2-methyl-6-(3,4-methylenedioxyphenyl)pyrazino[2′,1′:6,1]pyrido[3,4-*b*]indole-1,4-dione.

2.1.2. Generic name [17]

Tadalafil

2.1.3. Trade name

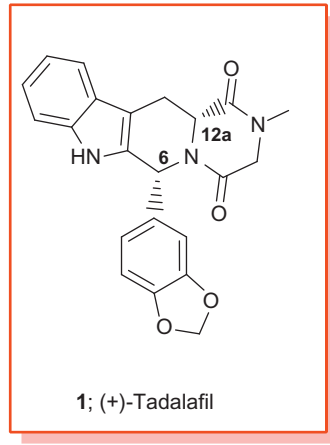
Cialis.

2.2. Formulae [17,18]

2.2.1. Empirical

$C_{22}H_{19}N_3O_4$

2.2.2. Structural



1; (+)-Tadalafil

2.3. Molecular weight, HRMS and CAS registry number [17,18]

- MW = 389.40
- (6*R*,12a*R*)-(+)-Tadalafil (**1**); HRMS calcd M^+ for $C_{22}H_{19}N_3O_4$ 389.1376; found 389.1386
- (6*S*,12a*R*)-(+)-Tadalafil (**8**); HRMS calcd M^+ for $C_{22}H_{19}N_3O_4$ 389.1376, found 389.1387 (**6-*epi*-tadalafil**)
- CAS = 171596-29-5

2.4. Optical rotation [17,18]

- (6*R*,12a*R*)-(+)-Tadalafil (**1**); $[\alpha]_D^{20} = +71.0$ ($CHCl_3$, $c = 1.0$)
- (6*S*,12a*R*)-(+)-Tadalafil (**8**); $[\alpha]_D^{20} = +250.0$ ($CHCl_3$, $c = 1.0$) (**6-*epi*-tadalafil**)
- (6*R*,12a*S*)-(−)-Tadalafil (**11**); $[\alpha]_D^{20} = -303.1$ ($CHCl_3$, $c = 1.2$) (**12a-*epi*-tadalafil**)

2.5. Elemental composition [18]

C = 67.86%, H = 4.92%, N = 10.79%, O = 16.43%.

3. PHYSICAL PROPERTIES [17,18]

3.1. Melting point

- (6*R*,12a*R*)-(+)-Tadalafil (**1**); 302–303 °C
- (6*S*,12a*R*)-(+)-Tadalafil (**8**); 286–288 °C (**6-*epi*-tadalafil**)
- (6*R*,12a*S*)-(−)-Tadalafil (**11**); 295–296 °C (**12a-*epi*-tadalafil**)

3.2. Solubility

Practically insoluble in water; very slightly soluble in ethanol.

3.3. Appearance

A white crystalline powder.

3.4. Peak plasma concentration

378 ng/mL occurs 2 h postdose

3.5. Apparent volume of distribution (Vd/F)

62.6 L

3.6. Apparent oral clearance (CL/F)

2.48 L/h.

3.7. The mean elimination half-life

17.5 h.

3.8. Duration of action

36 h

4. METHOD OF PREPARATION

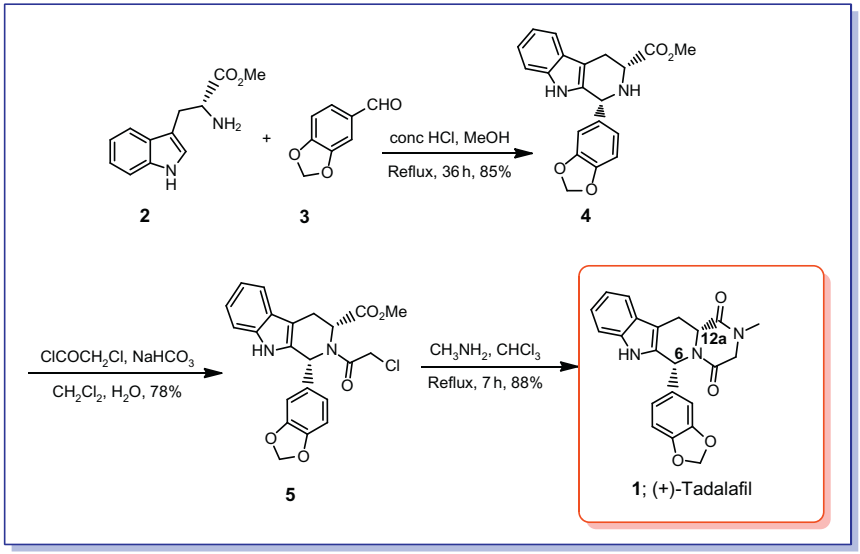
(+)-(6*R*,12a*R*)-2,3,6,7,12,12a-Hexahydro-2-methyl-6-(3,4-methylenedioxyphenyl)-pyrazino-[2′,1′:6,1]pyrido[3,4-*b*]indole-1,4-dione; Cialis; **1** [(+)-Tadalafil] is one of the well-known PDE5 inhibitors indicated for the treatment of ED [7–9].

4.1. Diastereoselective synthesis of (+)-tadalafil (**1**) [19,20]

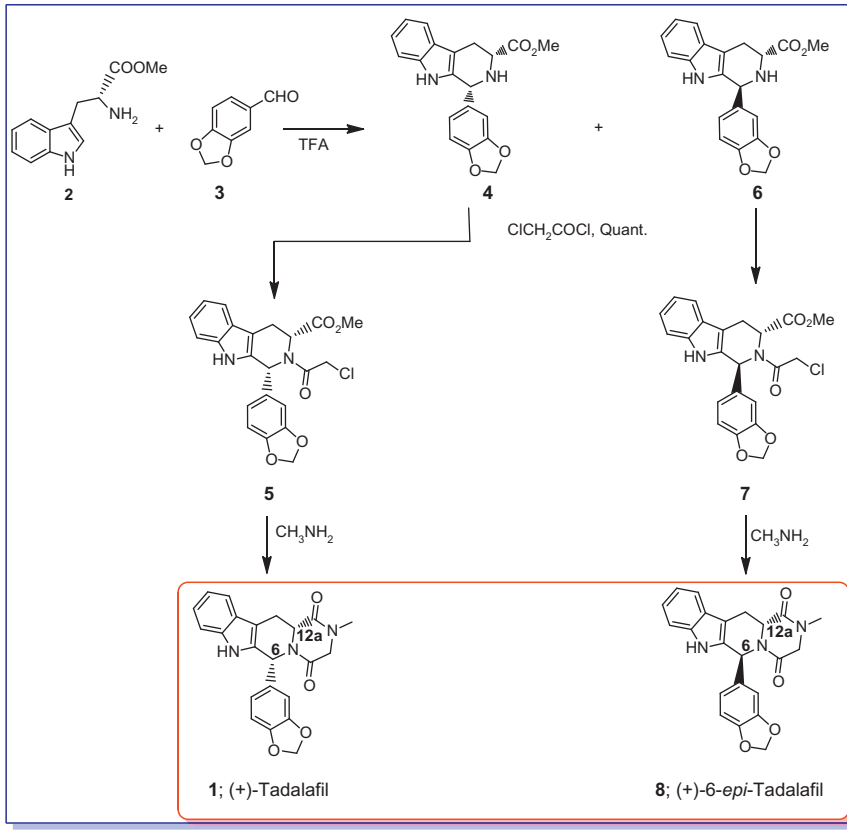
Scheme 8.1 describes a process for the synthesis of tadalafil (**1**) and its intermediate of formula **5** which involves reacting *D*-tryptophan methyl ester **2** with a piperonal **3** in the presence of methanol and conc. HCl to give compound **4**. The later compound is then reacted with chloroacetyl chloride in the presence of $NaHCO_3$ to afford the intermediate **5**, which is reacted with methylamine in chloroform to give tadalafil in 88% yield.

4.2. Stereoselective synthesis of (+)-tadalafil (**1**) and (+)-6-*epi*-tadalafil (**8**) [20]

The target isomeric tadalafil molecule is shown in Scheme 8.2. Thus, *D*-tryptophan methyl ester reacted with piperonal **3** under Pictet–Spengler reaction condition (TFA/CH_2Cl_2/MeOH) to furnish two diastereomers **4** and **6** in 25% and 24% yields, respectively. Condensation of **4** or **6** with chloroacetyl chloride provided acylated intermediate **5** or **7** in almost quantitative yield. Subsequent cyclization of **5** with *N*-methyl amine in methanol at 50 °C for 16 h provided diastereomers tadalafil (**1**) in 54% yield. Compound **1** is in full accordance with the literature data {$[\alpha]_D^{20} = +71.4$ (c 1.00, $CHCl_3$); lit. $[\alpha]_D^{20} = +71.2$ (c 1.00, $CHCl_3$)} [17,18]. Thus, under the elongated reaction time, 48 h, compound **8** was obtained from precursor **7** with decreased yield of 21%.



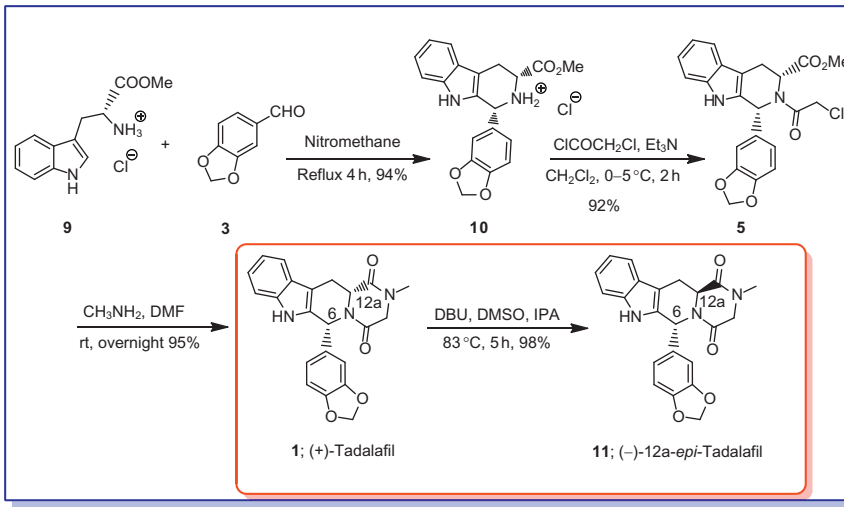
SCHEME 8.1 Diastereoselective route for the synthesis of (+)-tadalafil (**1**).



SCHEME 8.2 Diastereoselective synthesis of (+)-tadalafil (**1**) and (+)-6-*epi*-tadalafil (**8**).

4.3. Stereoselective synthesis of (+)-tadalafil (**1**) and (−)-12a-*epi*-tadalafil (**11**) [21]

Scheme 8.3 depicts an efficient and stereospecific synthesis of tadalafil (**1**) as well as 12a-*epi*-tadalafil (**11**). Pictet–Spengler reaction of *D*-tryptophan methyl ester hydrochloride **9** with equal molar piperonal by refluxing for 4 h in nitromethane afforded *cis*-**10**-HCl in 98% ee and 94% yield. The hydrochloride salt of *cis* tetrahydro-β-carboline derivative *cis*-**10**-HCl was directly treated with 1.5 equiv of chloroacetyl chloride in dichloromethane at 0°C in the presence of 3 equiv of triethylamine to form *N*-chloroacetyl tetrahydro-β-carboline derivative **5** in 92% yield. Then compound **5** reacted with 5 equiv of methylamine overnight in DMF at room temperature to furnish tadalafil **1** in 95% yields.



SCHEME 8.3 Stereoselective synthesis of (+)-tadalafil (**1**) and (−)-12a-*epi*-tadalafil (**11**).

TABLE 8.1 The base-catalyzed epimerization of tadalafil (**1**) at the C-12a position to form 12a-*epi*-tadalafil (**11**)

Entry	Solvent (ratio)	Base (equiv)	Condition	Yield (%)
1	DMSO	KOH (2)	rt, 4 h	82
2	DMSO	*t*-BuOK (3)	0 °C, 0.5 h	83
3	DMSO	DBU[a] (3)	70 °C, 10 h	91
4	DMSO (1) THF (5)	DBU (2)	70 °C, 10 h	96
5	DMSO (4) H_2O (1)	KCO_3 (2)	65 °C, 15 h	95
6	DMSO (1) DME[b] (9)	DBU (3)	85 °C, 9 h	97
7	DMSO (1) *i*-PrOH (5)	DBU (2)	83 °C, 5 h	98

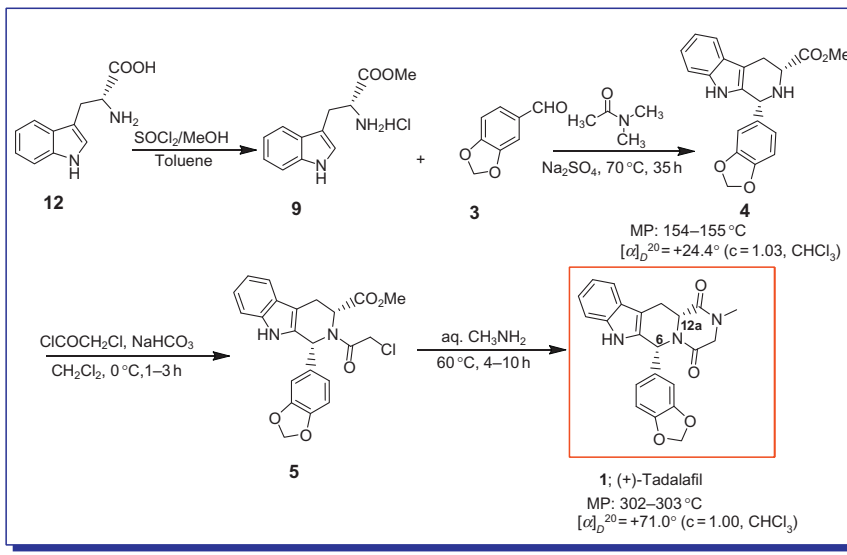
[a] 1,8-Diazabicyclo [5,4,0]undec-7-ene.
[b] 1,2-Dimethoxyethane.

When the reaction of compound **5** operated with methylamine in DMSO, it found that the reaction produced both **1** and its epimer 12a-*epi*-tadalafil (**11**). The amount of **11** increased as the temperature elevated. To understand the epimerization of **1** at the C-12a position more clearly, the epimerization of **1** in different solvents using various bases as the catalyst was established. Table 8.1 summarizes the outcomes of epimerization of **1** into **11**. It should be pointed out that DMSO was

crucial for the epimerization, the reaction took place smoothly in DMSO or a mixed solvent containing DMSO, while it was very slow in other solvents. When a strong base such as potassium hydroxide or potassium *tert*-butoxide was used, the reaction was fast, but the yield was not high (Table 8.1, entries 1 and 2). A weak base such as DBU or potassium carbonate turned out to be a suitable catalyst, and the yield was high (Table 8.1, entries 3–7). Tadalafil (**1**) was almost quantitatively transformed into 12a-*epi*-tadalafil (**11**) in a mixed solvent (DMSO–*i*-PrOH = 1:5) in the presence of 2 equiv of DBU after refluxing for 5 h.

4.4. US patent for stereoselective synthesis of (+)-tadalafil (**1**) [22,23]

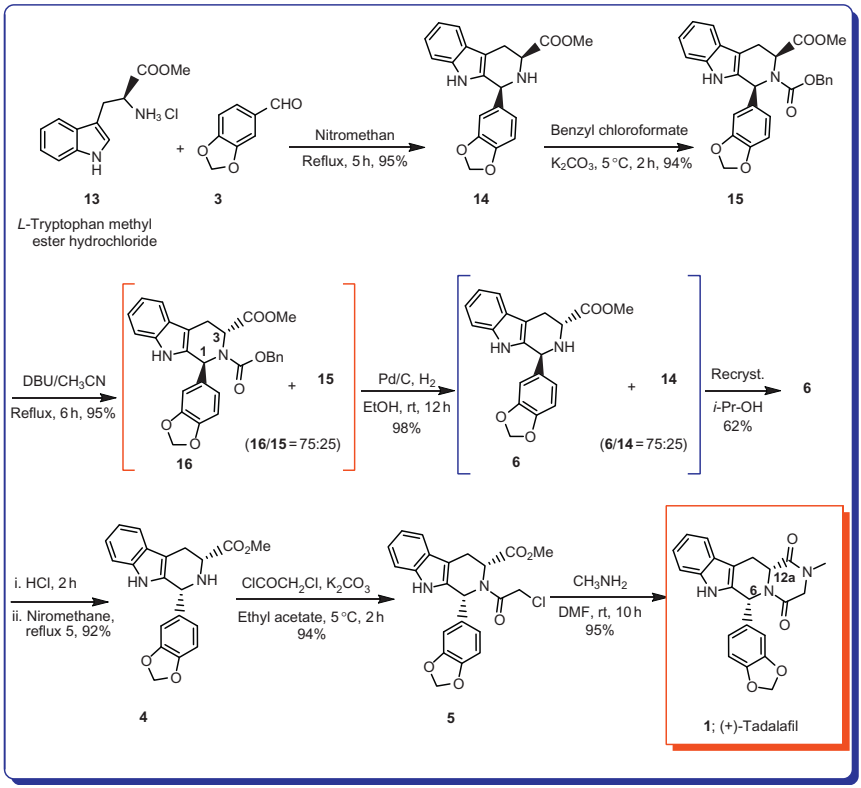
The present patent invention provided process of preparation of tadalafil (**1**) through the reaction of *D*-tryptophan methyl ester hydrochloride with piperonal in the presence of high boiling point dimethylacetamide and Na_2SO_4 as dehydrating agents to give compound **4** as mixture of *cis* and *trans* isomers which is reacted further without isolating and separating isomers with an aqueous HCl to provide hydrochloride salt of *cis* isomer of compound **4** as major isomer (Scheme 8.4). Reacting the compound **4** with chloroacetyl chloride in the presence of $NaHCO_3$ and CH_2Cl_2 as solvent to get the intermediate **5** which is further reacted with aqueous methylamine solution to obtain crude tadalafil which is purified by crystallization form IPA to get (+)-tadalafil (**1**) [22,23].



SCHEME 8.4 Synthesis of (+)-tadalafil (US patent).

4.5. The first synthesis of tadalafil (**1**) (Cialis) from *L*-tryptophan [24]

As depicted in Scheme 8.5, *L*-tryptophan methyl ester hydrochloride (**13**) was first treated with 1.1 equiv of piperonal in nitromethane at reflux temperature. Similar to *D*-tryptophan methyl ester hydrochloride, the highly stereoselective Pictet–Spengler reaction of *L*-tryptophan methyl ester hydrochloride with piperonal produced the hydrochloride salt of (1*S*,3*S*)-1,3-disubstituted-tetrahydro-β-carboline **14**-HCl [17,25]. After neutralization of **14**-HCl, compound **14** was obtained in 95% yield and with 99% ee. Compound **14** was then treated with 1.2 equiv of benzyl chloroformate in ethyl acetate at around 5 °C in the presence of 3 equiv of potassium carbonate powder to afford (1*S*,3*S*)-1,2,3-trisubstituted-tetrahydro-β-carboline (**15**) in 94% yield. The base-catalyzed epimerization of



SCHEME 8.5 Synthesis of tadalafil (**1**) (Cialis) from *L*-tryptophan.

compound **15** at the C-3 position to form (1*S*,3*R*)-1,2,3-trisubstitutedtetrahydro-β-carboline (**16**) was carried out. The first tried epimerization of compound **15** in methanol at reflux in the presence of 0.5 equiv of sodium methoxide, monitoring by TLC showed that compound **16** was gradually formed during the reaction, and the ratio of **16** and **15** increased meanwhile. Reflux was continued for more than 5 h, with the ratio of **16** and **15** becoming constant, meaning that the reaction was in equilibrium. Purification by flash chromatography gave pure compounds **16** and **15** in 89% combined yield and with a ratio of 75:25. Compound **16** is thermodynamically more stable than compound **15**; hence the base-catalyzed reversible epimerization would produce mixtures in which the more stable compounds **16** were major products.

The less stability of compound **15** when compared with compound **16** is probably due to the repulsion between the indole ring and the axial COOMe group. Comparison between the ^{1}H NMR spectra of compounds **15** and **16** supports this assumption, the axial COOMe group of compound **15** exhibits a chemical shift (3.16 ppm) at a relatively upper field due to the closeness of indole ring and COOMe, while equatorial COOMe group of compound **16** exhibits a normal chemical shift (3.48 ppm). When the mixture of **16** and **15** (75:25) was treated with catalytic amounts of Pd/C in ethanol at room temperature for 12 h under an atmosphere of hydrogen gas, the benzyloxycarbonyl group at the N-2 position in both compounds **16** and **15** was successfully removed, and a mixture of compounds **6** and **14** was formed in 98% yield [26–28]. After recrystallization of the crude product (**6**/**14** = 75:25) in isopropanol, compound **6** could be obtained in 62% yield and with more than 99% purity. The transformation of (1*S*,3*R*)-1,3-disubstituted-tetrahydro-β-carboline (**6**) to (1*R*,3*R*)-1,3-disubstituted-tetrahydro-β-carboline (**4**) could be carried out by first converting **6** into its hydrochloride salt **6**-HCl, and then performing a CIAT process [17,25] to afford the hydrochloride salt **4**-HCl. After neutralization of **4**-HCl, compound **4** was obtained in 92% yield. Herein, the (*S*)-configuration of C-1 of compound **6** was inversed to the (*R*)-configuration of C-1 of compound **4** during the CIAT process. This acid-catalyzed epimerization at C-1 position was very clean, and the (*R*)-configuration of C-3 of compound **6** remained intact. As shown in Fig. 8.1, high-performance liquid chromatographic (HPLC) analysis showed that enantiomerical purity of compound **4** is 98.68% (er is 99.34:0.66). Compound **4** was then treated with 1.3 equiv of chloroacetyl chloride in ethyl acetate in the presence of 3 equiv of the powder of K_2CO_3 to furnish compound **5** in 94% yield. Finally, compound **5** was converted into title compound **1** in 95% yield according to a known procedure [17], and the analytical data showed that the compound **1** obtained from this synthesis is identical with authentic sample of tadalafil.

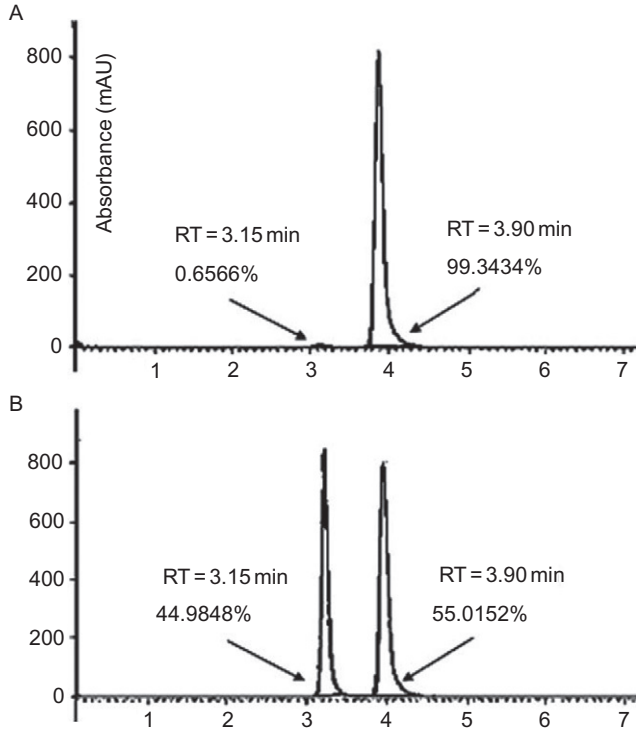


FIGURE 8.1 HPLC analysis of compound **4** by a chiral column (spectra A for compound **4**, and spectra B for a racemic mixture of compounds **4** and **14**). Conditions: Column: AS-H; Mobile phase: methanol (0.1% DEA); flow rate: 0.6 mL/min; wavelength: 214 nm.

5. SPECTRAL PROPERTIES [17–25]

5.1. Infrared spectrum

- The FT-IR spectrum of (+)-tadalafil (**1**) as KBr disc is presented in Fig. 8.2. Principal peaks at wave numbers IR (KBr film) 3328, 2904, 1677, 1649, 1489, 1438, 1401, 1323, 1269, 1242, 1152, 1097, 1041, 939, 922, 746 cm^{-1}.
- The IR spectrum of (−)-12a-*epi*-tadalafil (**11**) as KBr film was 3326, 2902, 1676, 1649, 1489, 1437, 1400, 1323, 1269, 1241, 1150, 939, 922, 746 cm^{-1}.

5.2. Nuclear magnetic resonance spectra

^{1}H, ^{13}C NMR, and other 2D spectra were recorded in DMSO-d_6 at 500MHz, Brucker instrument (Brucker Company, USA); chemical shifts are expressed in δ ppm with reference to TMS (Figs. 8.3–8.8).

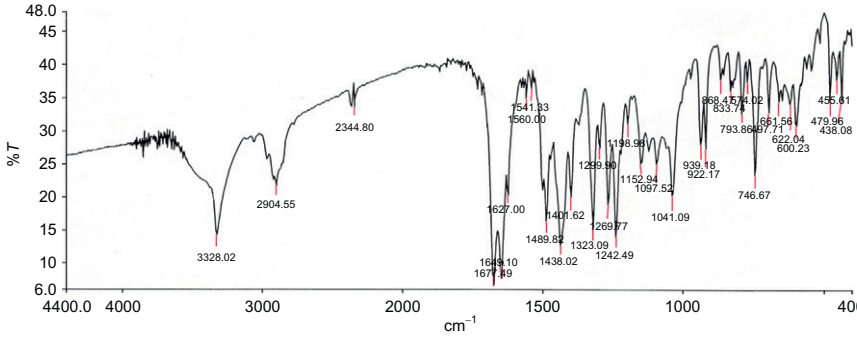


FIGURE 8.2 Infrared spectrum of (+)-tadalafil (**1**) (KBr disc).

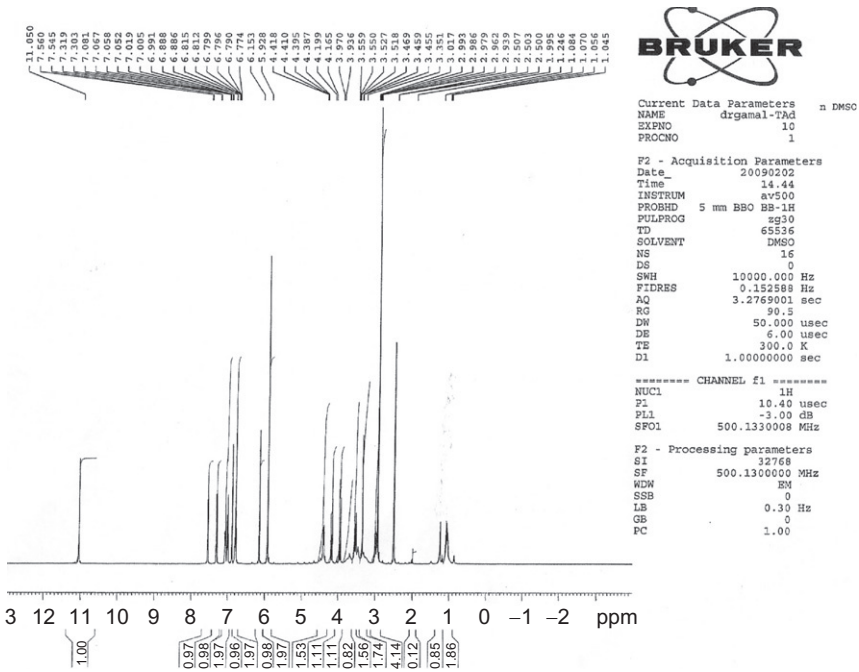


FIGURE 8.3 ^{1}H NMR spectrum of (+)-tadalafil (**1**) in DMSO-d_6.

5.2.1. ^{1}H NMR spectrum (Fig. 8.3)

- (+)-Tadalafil (**1**): (*6R*,12a*R*)-2,3,6,7,12,12a-Hexahydro-2-methyl-6-(3,4-methylenedioxyphenyl)-pyrazino-[2′,1′:6,1]pyrido[3,4-b]indole-1,4-dione

^{1}H NMR (DMSO-d_6) δ 11.05 (s, NH on the indole ring), 7.54 (d, J = 7.5 Hz, 1H), 7.30 (d, J = 8.0 Hz, 1H), 7.07 (d, J = 7.0 Hz, 1H), 7.00 (d, J = 7.0 Hz, 1H), 6.86 (s, 1H), 6.77 (s, 2H), 6.15 (s, 1H), 5.92 (s, 2H), 4.39

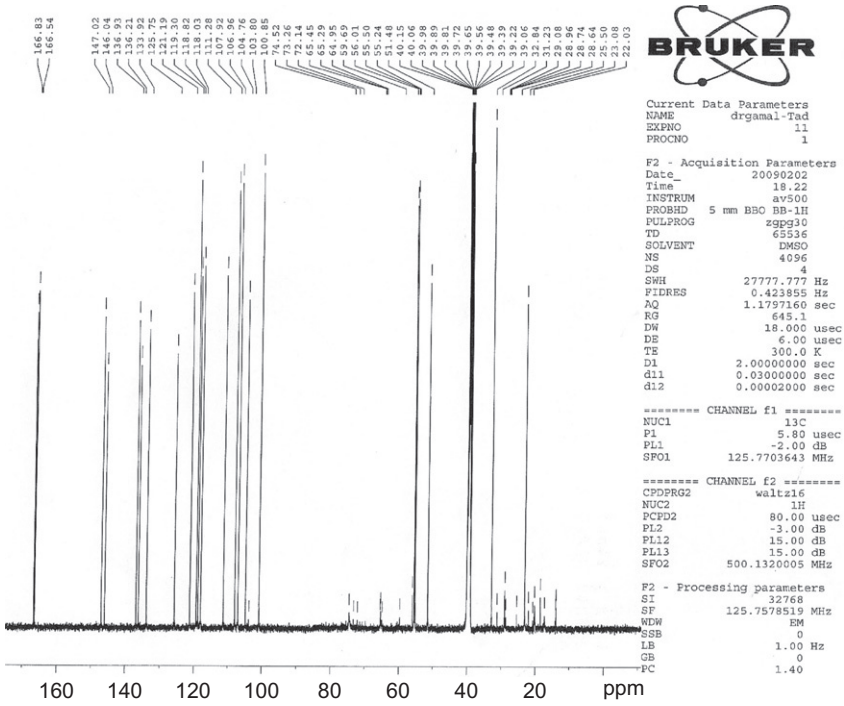


FIGURE 8.4 ^{13}C NMR spectrum of (+)-tadalafil (**1**) in DMSO-d_6.

(dd, $J = 4.0$ Hz; 11.5 Hz, 1H), 4.17 (d, $J = 17.0$ Hz, 1H), 3.94 (d, $J = 17.0$ Hz, 1H), 3.52 (dd, $J = 4.5$ Hz; 16.0 Hz, 1H), 2.96 (dd, $J = 12.0$ Hz; 15.5 Hz, 1H), 2.94 (s, 3H).

- (+)-Tadalafil (**1**): (*6R*,12a*R*)-2,3,6,7,12,12a-Hexahydro-2-methyl-6-(3,4-methylenedioxyphenyl)-pyrazino-[2′,1′:6,1]pyrido[3,4-b]indole-1,4-dione

^{1}H NMR ($CDCl_3$) δ 3.08 (s, 3H), 3.19 (m, 1H), 3.79 (m, 1H), 4.05 (quartet, 2H, $J = 61.8$, 18.5 Hz), 4.31 (m, 1H), 5.92 (d, 2H, $J = 7.7$ Hz), 6.18 (s, 1H), 6.70–7.82 (m, 7H).

- (−)-12a-*epi*-Tadalafil (**11**): (6*R*,12a*S*)-2,3,6,7,12,12a-Hexahydro-2-methyl-6-(3,4-methylenedioxyphenyl)-pyrazino-[20,10:6,1]pyrido[3,4-b]indole-1,4-dione

^{1}H NMR (DMSO-d_6) d 11.06 (s, NH on the indole ring), 7.49 (d, $J = 7.7$ Hz, 1H), 7.31 (d, $J = 8.1$ Hz, 1H), 7.10 (dd, $J = 7.2$ Hz; 7.9 Hz, 1H), 7.01 (dd, $J = 7.7$ Hz; 7.2 Hz, 1H), 6.86 (d, $J = 8.0$ Hz, 1H), 6.82 (s, 1H), 6.75 (d, $J = 1.4$ Hz, 1H), 6.60 (dd, $J = 1.1$ Hz; 8.0 Hz, 1H), 5.99 (d, $J = 6.5$ Hz, 2H), 4.24 (d, $J = 17.6$ Hz, 1H), 4.07 (dd, $J = 4.1$ Hz; 11.8 Hz, 1H), 4.03 (d, $J = 17.7$ Hz, 1H), 3.25 (dd, $J = 4.2$ Hz; 15.4 Hz, 1H), 2.95 (dd, $J = 12.1$ Hz; 14.8 Hz, 1H), 2.84 (s, 3H).

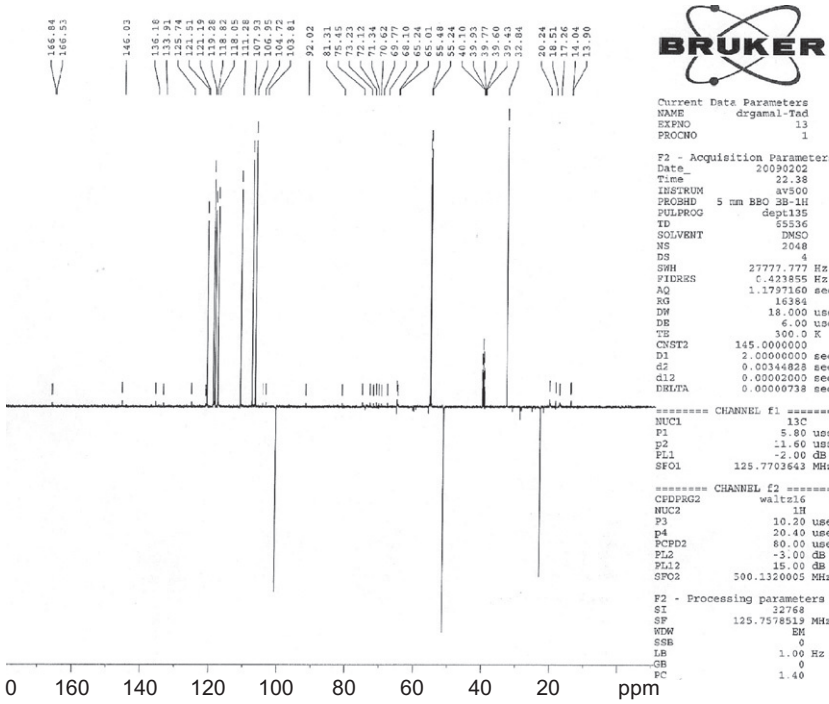


FIGURE 8.5 Dept ^{13}C spectra of (+)-tadalafil (**1**).

- (+)-6-*epi*-Tadalafil (**8**): (6*S*,12a*R*)-2,3,6,7,12,12a-Hexahydro-2-methyl-6-(3,4-methylenedioxyphenyl)-pyrazino-[2′,1′:6,1]pyrido[3,4-b]indole-1,4-dione

^{1}H NMR (CDCl3) d 2.95 (m, 1H), 3.02 (s, 3H), 3.56 (m, 1H), 4.10 (quartet, 2H, $J = 55.6$, 14.0 Hz), 4.39 (m, 1H), 5.96 (s, 2H), 6.72–7.88 (m, 8H).

5.2.2. ^{13}C NMR and Dept ^{13}C spectra (Figs. 8.4 and 8.5)

- (+)-Tadalafil (**1**):

^{13}C NMR (DMSO-d_6) δ: 166.83, 166.54, 147.02, 146.04, 136.93, 136.21, 133.92, 125.75, 121.19, 119.30, 118.82, 118.03, 111.28, 107.92, 106.96, 104.76, 100.85, 55.50, 55.24, 51.48, 32.84, 23.08.

- (−)-12a-*epi*-Tadalafil (**11**):

^{13}C NMR (DMSO-d_6) δ: 164.78, 162.37, 147.69, 147.33, 136.20, 132.92, 130.25, 125.94, 121.78, 118.99, 118.16, 111.36, 108.42, 108.21, 107.55, 101.32, 52.09, 50.94, 50.73, 32.69, 26.74.

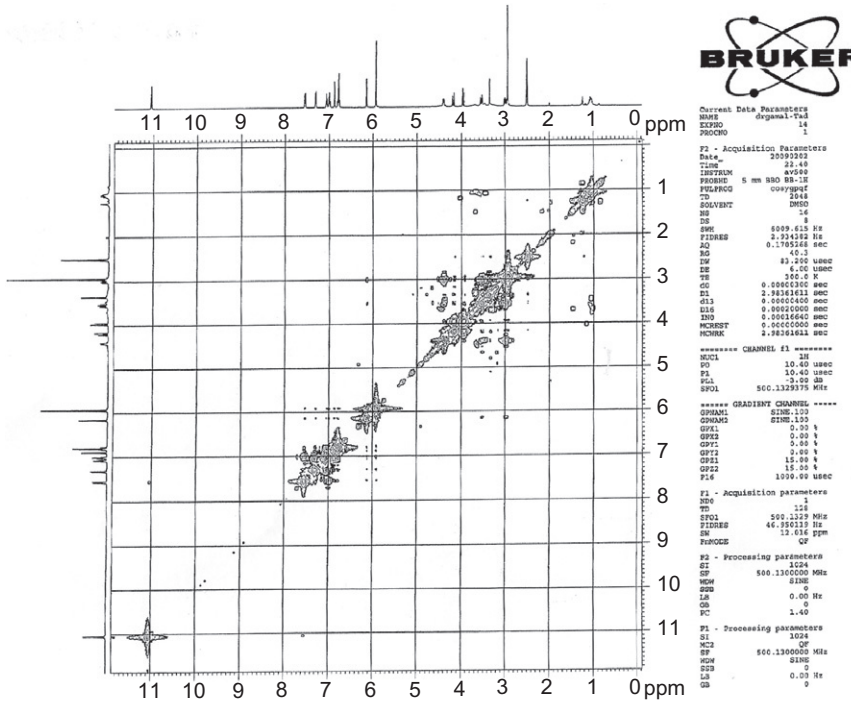
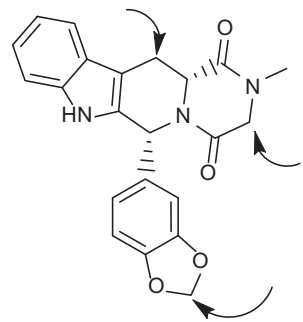


FIGURE 8.6 ^{1}H–^{1}H-cosy of 135 of (+)-tadalafil (**1**).

- Dept ^{13}C spectra of (+)-tadalafil (**1**) (Fig. 8.5) showed three CH_2 fragments as illustrated in tadalafil skeleton



1; (+)-Tadalafil

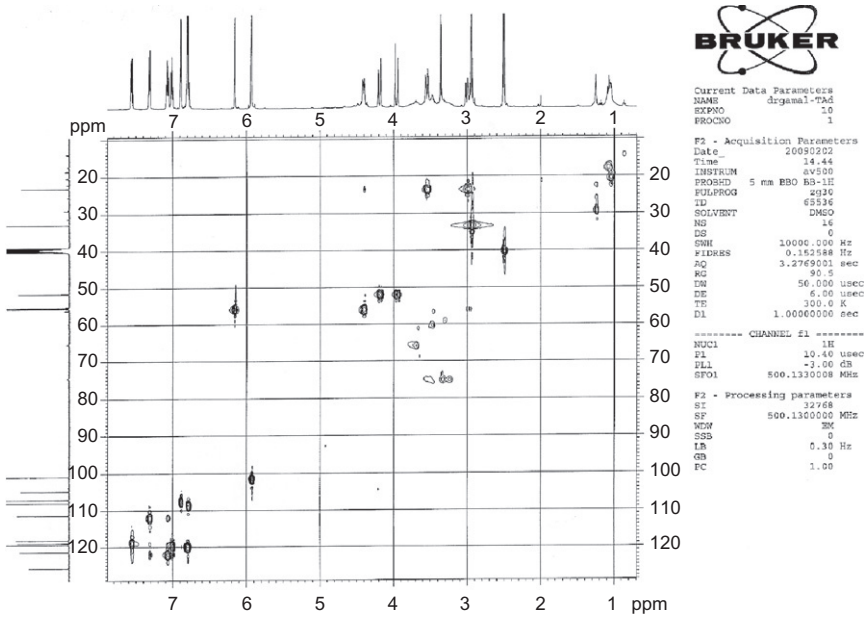


FIGURE 8.7 $^{1}H–^{13}C$ HETCOR (hsqc) Maps of (+)-tadalafil (**1**).

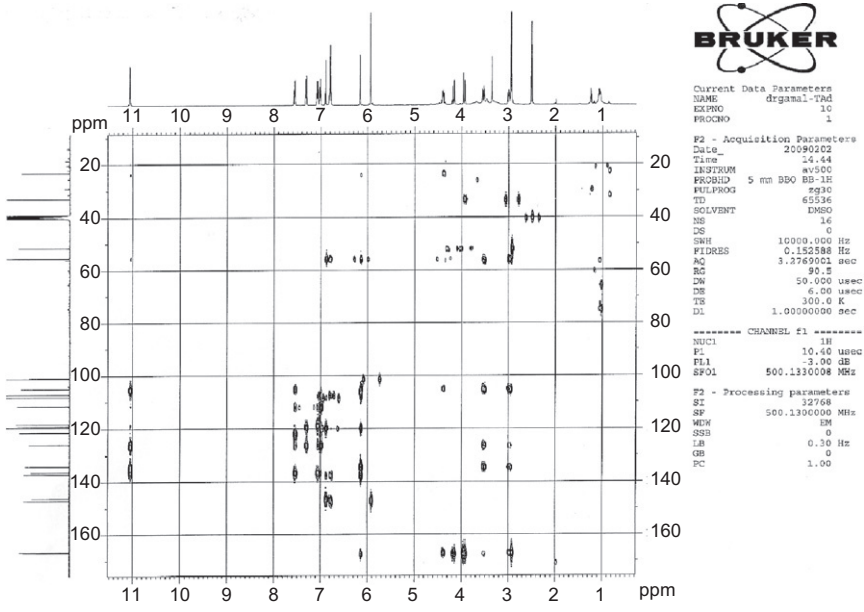
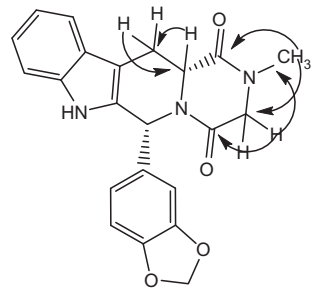


FIGURE 8.8 $^{1}H–^{13}C$ HETCOR (hmbc) Maps of (+)-tadalafil (**1**).

5.2.3. 2D NMR (^{1}H–^{1}H cosy and ^{1}H–^{13}C HETCOR maps)



1; (+)-Tadalafil

The assignment and interpretation of the ^{1}H–^{1}H and ^{1}H–^{13}C HETCOR spectra of (+)-tadalafil (**1**) has been carried out (Figs. 8.6–8.8). The resulting ^{1}H–^{1}H cosy assignments are shown in Fig. 8.6, indicating the coupling of each adjacent proton. ^{1}H–^{13}C HETCOR (HSQC and HMBC) assignments are shown in Figs. 8.7 and 8.8.

5.3. Mass spectrum

Mass spectra of (+)-tadalafil (**1**), carried out with electron impact method, were registered at 70 eV using a ION TRAP GCQ FINNIGAN mass spectrometer (Fig. 8.9). EI (MeOH): m/z 391.3 (M+2). Principal ions are presented in (Table 8.2).

5.4. Ultraviolet spectra

The structure of tadalafil contains conjugated configuration and exhibit intensive UV absorption, thus it can be measured by UV detection with quite a low LOQ [29]. Also noted is that the structure of tadalafil possesses

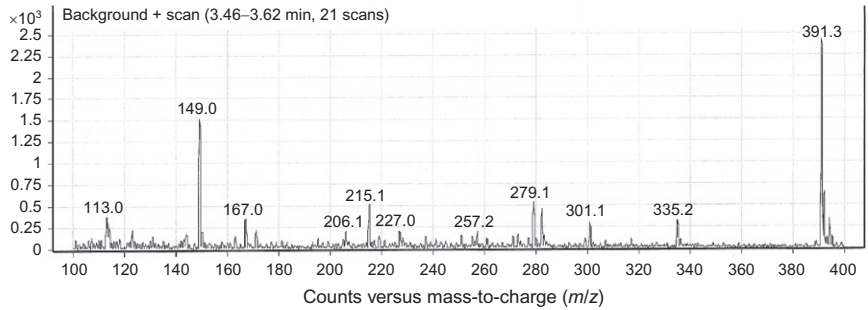
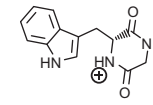
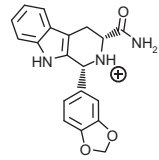
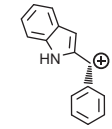
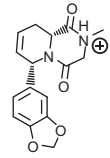
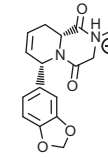
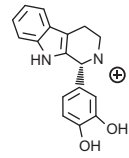
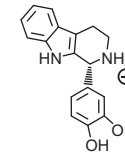


FIGURE 8.9 Mass spectrum of (+)-tadalafil (**1**) in methanol.

TABLE 8.2 Mass fragmentation pattern of (+)-tadalafil (**1**)

m/z	Ions	*m/z*	Ions
391.3	M + 2	257.2	
335.2		206.1	
301.1		301.1	
279.1		279.1	

three amine groups, which might be ionized under acidic conditions. Based on the migration behavior in capillary zone electrophoresis (CZE), Hassan and Imran reported that tadalafil existed as a cation at pH 3 and migrated towards cathode [30]. On the contrary, Flores *et al.* concluded that tadalafil remained neutral in the running buffer with pH 2.2–13, as tadalafil migrated with the electro-osmotic flow under these pH conditions [31]. Because the degree of ionization could potentially affect the UV absorbance of a compound, it is worthy to evaluate the effect of pH value on the UV spectrum of tadalafil. As displayed in Fig. 8.10, tadalafil has similar absorption spectrum in acidic, neutral, and basic medium, with high molar absorbance around 220, 280, and 290 nm. Because higher sensitivity can be attained at low UV wavelength,

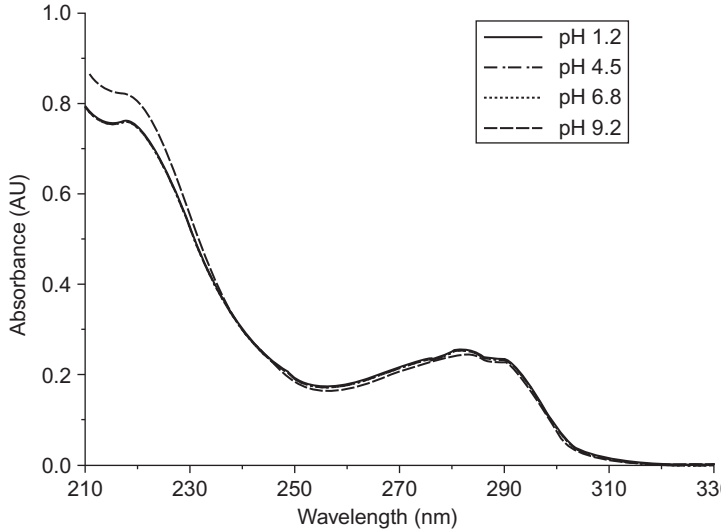


FIGURE 8.10 The UV spectra of tadalafil (5 g/mL) in aqueous solutions with various pH values.

irrespective to the pH of the mobile phase or running buffer, the reported HPLC and CE methods applied UV detection at the region of 220–254 nm to determine tadalafil in pharmaceutical and nutraceutical preparations [31,32].

5.4.1. Ultraviolet spectra in aqueous solutions

The *UV* spectra of tadalafil (5 μg/mL) in aqueous solutions with various pH values were recorded with 10-mm quartz cell using a Hitachi U2010 spectrophotometer (Tokyo, Japan). The aqueous solutions used were 0.1 N HCl (pH 1.2) and 50 mM phosphate buffers (pH 4.5, 6.8, and 9.2). Tadalafil exhibited similar absorption spectrum in acidic, neutral, and basic medium, with high molar absorbance around 220, 280, and 290 nm [33].

5.4.2. Ultraviolet spectra in ethanolic solution

UV spectra of (+)-tadalafil (**1**) in ethanol (5 mg %) was scanned from 200–350 nm, using UV/VIS spectrophotometer. Tadalafil exhibited the maximum absorption at 215 nm (Fig. 8.11).

5.4.3. Ultraviolet spectra in aqueous solution of cyclodextrin

Figure 8.12 showed the effect of CDs concentrations on the absorption spectra of tadalafil in aqueous solutions. Increasing the concentration of all CDs from 5 to 15 mmole/L resulted in an increase in the absorbance of

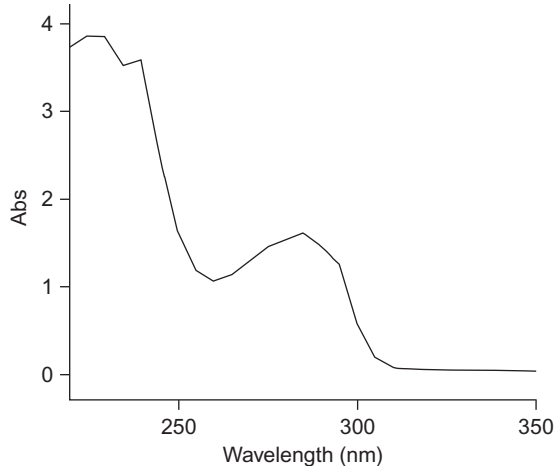


FIGURE 8.11 UV spectrum of tadalafil (**1**) in ethanol.

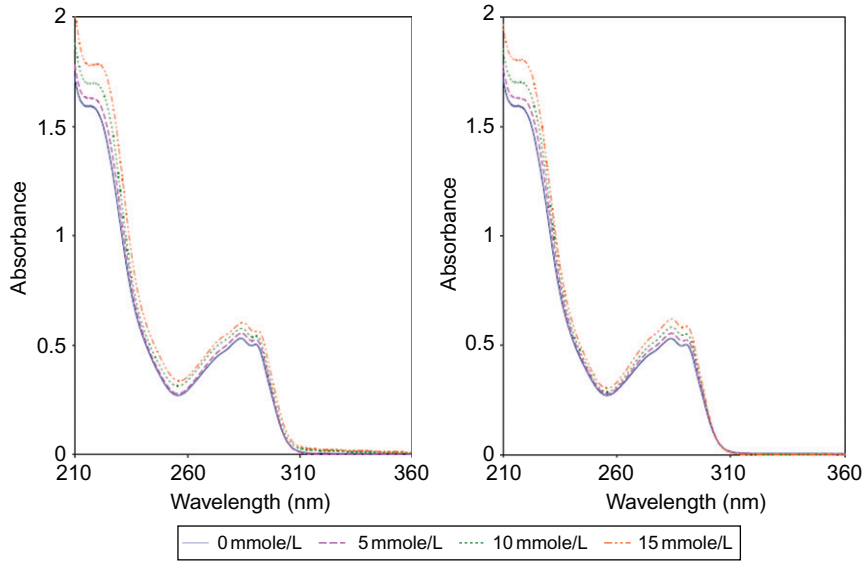


FIGURE 8.12 Differential ultraviolet absorbance spectra of tadalafil in presence of β-CD (left panel) and HP-β-CD (right panel).

tadalafil without any shifts of λ_{max} [34]. The observed hyperchromic shift might be due to the perturbation of the chromophore electrons of the drug due to the inclusion into the cyclodextrin cavity [35]. It could be indicative of cyclodextrin guest–host complex formation [35,36].

6. X-RAY POWDER DIFFRACTOMETRY

6.1. X-ray powder diffractometry of tadalafil with poloxamer 407

The XRPD pattern of tadalafil displayed intense and sharp peaks (Fig. 8.13A), indicating its crystalline nature. Relative decrease in crystallinity (RDC value) was determined by comparing some representative

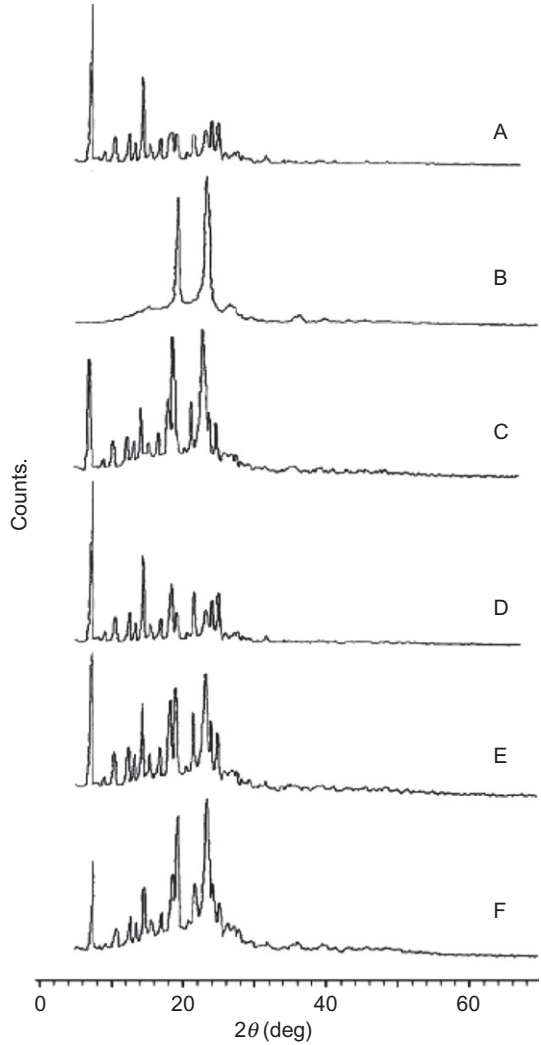


FIGURE 8.13 XRPD patterns of: (A) tadalafil, (B) poloxamer 407, (C) physical mixture, (D) 1:0.5 solid dispersion, (E) 1:1.5 solid dispersion, (F) 1:2.5 solid dispersion.

peak heights in the diffraction patterns of the binary systems with those of a reference (pure tadalafil). Tadalafil (Fig. 8.13A) showed sharp peaks at 7.33, 12.62, 14.47, 14.56, 18.49, and 21.14° (2θ) with peak intensities of 2798, 243, 1043, 1282, 353, and 246, respectively. The crystalline nature of poloxamer 407 is displayed in Fig. 8.13B. The peak height at 7.335° (2θ) was used to calculate the RDC of tadalafil in all binary systems (Fig. 8.13C–F). The XRPD pattern of the physical mixture (Fig. 8.13C) displayed tadalafil and polymer peaks with a small decrease in the tadalafil peak intensity, indicating reduction in crystallinity (RDC = 0.144). Crystallinity of tadalafil was significantly reduced in the physical mixture, which might be due to higher proportion of the polymer in it (1:2.5). In the diffraction patterns of solid dispersion systems, a gradual decrease in crystallinity was observed with an increase in polymer concentration (Fig. 8.13D–F). The RDC values for 1:0.5, 1:1.5, and 1:2.5 solid dispersions were 0.278, 0.130, and 0.110, respectively. The peaks of tadalafil at 14.56° and 21.14° disappeared in all solid dispersion systems. The absence of intense peaks in solid dispersions suggested that the drug had lost its crystalline nature and possibly might have been transferred into amorphous form [37].

The XRPD patterns of pure tadalafil, poloxamer 407 and solid dispersions were recorded using a Philips Analytic X-Ray PW3710 (Philips, The Netherlands) diffractometer with tube anode Cu over the 5–70°/2θ interval at a scanning speed of 2°min^{-1}. The generator tension (voltage) and generator current were kept at 40 kV and 30 mA, respectively.

6.2. X-ray powder diffractometry of tadalafil with cyclodextrin [34]

Figure 8.14A–E showed the XRD patterns for pure components and their binary systems prepared by different techniques at molar ratio of 1:5 (drug to CD). The diffraction pattern of tadalafil powder revealed several sharp high intensity peaks at diffraction angles (2θ) of 7.8°, 10.2°, 12.2°, 14.5°, 18.2°, 22.2°, and 24.5° suggesting that the drug existed as crystalline material. Pure β-CD showed a crystalline diffractogram, while a diffuse halo-pattern was recorded for HP-β-CD demonstrating its amorphous nature. Similar observations have been reported by other authors [38,39]. The diffraction patterns of the investigated PMs correspond to the superposition of those of the pure components. However, lower intensities of the diffraction peaks were observed due to particle size reduction during mixing and dilution of the pure crystalline components [40]. Overlapping of some tadalafil diffraction peaks with those of β-CD was evident. The diffractograms of the KN systems showed almost similar diffraction behavior to the PMs. The crystallinity of tadalafil was higher in the kneaded tadalafil-β-CD than the corresponding PM. Similar

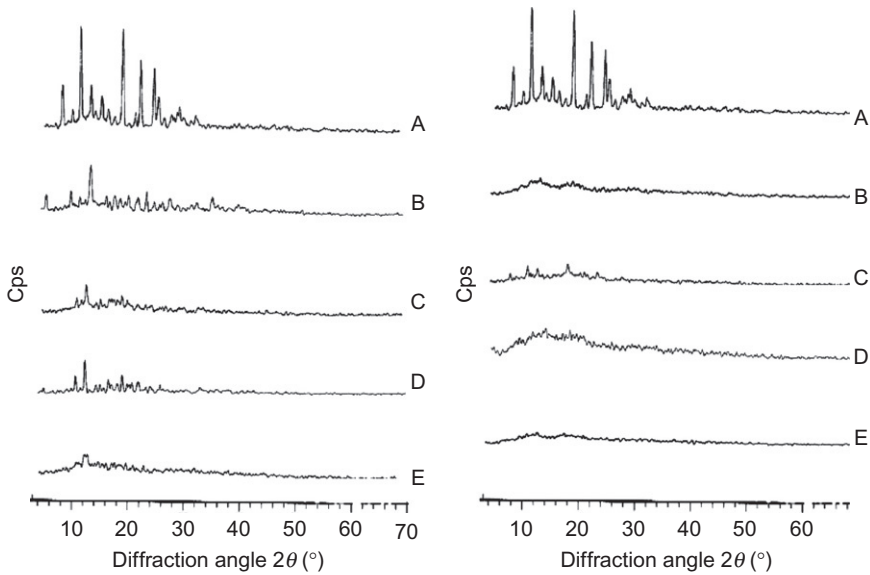


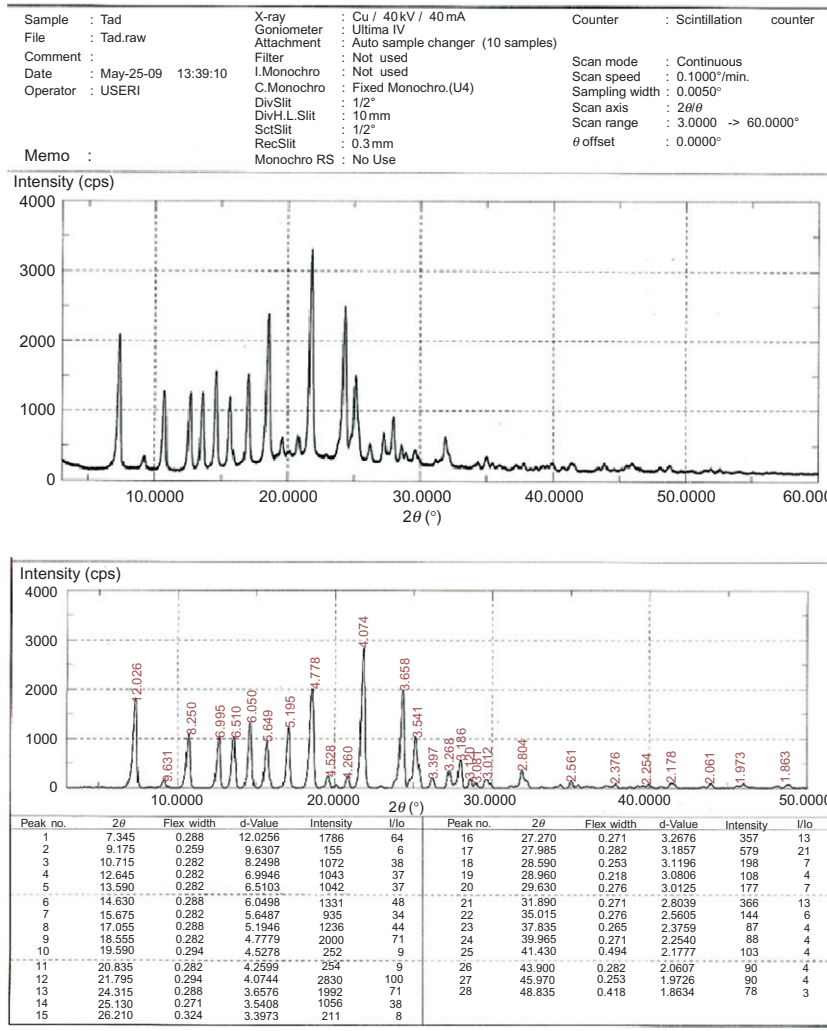
FIGURE 8.14 Left panel showed X-ray diffraction patterns of tadalafil-β-CD systems: (A) pure tadalafil; (B) pure β-CD; (C) PM 1:5; (D) KN 1:5; (E) FD 1:5. Right panel showed X-ray diffraction patterns of tadalafil–HP-β-CD systems: (A) pure tadalafil; (B) pure HP-β-CD; (C) PM 1:5; (D) KN 1:5; (E) FD 1:5.

increment in crystallinity was observed for Griseofulvin-2-HP-β-CD kneaded systems, and was attributed to the formation of mixed crystalline particles during the desiccation process [41]. The presence of tadalafil peaks in the diffractogram of tadalafil-β-CD, freeze-dried system could suggest the presence of the free crystalline drug, although reduction in number and intensities were observed. On the other hand, the diffractograms of FD systems prepared using HP-β-CD showed a typical diffuse pattern indicating the entirely amorphous nature of tadalafil in both systems. According to Robert *et al.* [42], lack of crystallinity is an added evidence for the formation of inclusion complex. However, since the amorphization of the drug can be a sequence of the lyophilization process, it is possible that the X-ray data cannot discriminate whether the drug–CD lyophilized systems obtained are true inclusion complexes or homogenous dispersed mixtures of the amorphous components [43]. Nevertheless, having in account the results of the DSC analysis, one can assume the formation of new solid phases that might be credit to the formation of inclusion complexes.

The X-ray diffraction patterns were recorded at room temperature using a Scintag diffractometer (XGEN-4000, Scintag Corp., USA). The samples were irradiated with Ni-filtered Cu-Kα radiation, at 45 kV voltage and 40 mA current. The scanning rate employed was 2°/min over a diffraction angle of 2θ and range of 3°–70°.

6.3. X-ray powder diffractometry of pure (+)-tadalafil

The X-ray diffraction patterns were recorded at room temperature using a Rigaku diffractometer with graphite monochromated Cu-Kα rotating anode generator. The samples were irradiated with Ni-filtered Cu-Kα radiation, at 40 kV voltage and 40 mA current. The scanning rate employed was 1°/min over a diffraction angle of 2θ and range of 3°–60°. Figure 8.15 showed the X-ray powder diffractometry of pure (+)-tadalafil and its peak location.



Peak no.	2θ	Flex width	d-Value	Intensity	I/Io	Peak no.	2θ	Flex width	d-Value	Intensity	I/Io
1	7.345	0.288	12.0256	1786	64	16	27.270	0.271	3.2676	357	13
2	9.175	0.259	9.6307	155	6	17	27.985	0.282	3.1857	579	21
3	10.715	0.282	8.2498	1072	38	18	28.590	0.253	3.1196	198	7
4	12.645	0.282	6.9946	1043	37	19	28.960	0.218	3.0806	108	4
5	13.590	0.282	6.5103	1042	37	20	29.630	0.276	3.0125	177	7
6	14.630	0.288	6.0498	1331	48	21	31.890	0.271	2.8039	366	13
7	15.675	0.282	5.6487	935	34	22	35.015	0.276	2.5605	144	6
8	17.055	0.288	5.1946	1236	44	23	37.835	0.265	2.3759	87	4
9	18.555	0.282	4.7779	2000	71	24	39.965	0.271	2.2540	88	4
10	19.590	0.294	4.5278	252	9	25	41.430	0.494	2.1777	103	4
11	20.835	0.282	4.2599	254	9	26	43.900	0.282	2.0607	90	4
12	21.795	0.294	4.0744	2830	100	27	45.970	0.253	1.9726	90	4
13	24.315	0.288	3.6576	1992	71	28	48.835	0.418	1.8634	78	3
14	25.130	0.271	3.5408	1056	38						
15	26.210	0.324	3.3973	211	8						

FIGURE 8.15 Upper panel showed X-ray diffraction patterns of (+)-tadalafil and the Lower panel showed its Peak location.

7. METHOD OF DETERMINATION

7.1. Chromatographic methods

7.1.1. HPLC/UV

A simple and sensitive high-performance liquid chromatographic (HPLC) method for the determination of tadalafil in 50 μL of rat plasma was described [33]. Tadalafil and the internal standard lamotrigine were extracted with 0.5 mL of *tert*-butyl methyl ether, after the samples alkalinized with 20 μL of sodium hydroxide solution (1 N). Chromatographic separation was achieved on a C18 column with the mobile phase of acetonitrile: water containing 20 mM phosphate buffer (pH 7) (35/65, v/v), at a flow rate of 1 mL/min. The eluant was detected at 290 nm. The retention time was about 4.5 min for lamotrigine and 15 min for tadalafil. No endogenous substances were found to interfere. Calibration curves were linear from 10 to 2000 ng/mL. The recovery of tadalafil from plasma was greater than 77%. The limit of quantitation was 10 ng/mL. The intra- and inter-day imprecision (expressed as coefficient of variation, C.V.) did not exceed 10.7%, and the accuracy was within 5.9% deviation of the nominal concentration. The method is suitable in pharmacokinetic investigation and monitoring tadalafil concentration.

On the same time, the simple, reliable and reproducible HPLC and extraction methods were developed for the analysis of tadalafil in pharmaceutical preparation [30]. The column used was monolithic silica column, Chromolith Performance RP-18e (100 mm × 4.6 mm, i.d.). The mobile phase used was phosphate buffer (100 mM, pH 3.0)–acetonitrile (80:20, v/v) at the flow rate of 5 mL/ min with UV detection at 230 nm at ambient temperature. Extraction of tadalafil from tablet was carried out using methanol. Linearity was observed in the concentration range from 100 to 5000 ng/mL for tadalafil with a correlation coefficient (R2) 0.9999 and 100 ng/mL as the limit of detection. The values of linearity range, correlation coefficient (R2) and limit of detection were 50–5000 ng/mL, 0.9999–50 ng/mL, respectively for sildenafil. Parameters of validation prove the precision of the method and its applicability for the determination of tadalafil in pharmaceutical tablet formulation. The method is suitable for high throughput analysis of the drug.

More recently, a highly selective, sensitive, and rapid HPLC method has been developed and validated to quantify tadalafil in human plasma [44]. The tadalafil and internal standard (loratadine, I.S.) were extracted by liquid–liquid extraction technique followed by an aqueous back-extraction allowing injection of an aqueous solvent in the HPLC system. The chromatographic separation was performed on a reverse phase BDS Hypersil C18 column (250 mm × 4.6 mm, 5 μm, Thermo Separation Co., USA) with a mobile phase of acetonitrile and aqueous solution containing 0.012 M

triethylamine: 0.020 M orthophosphoric acid (50/50, v/v). The analytes were detected at 225 nm. The assay exhibited a linear range of 5–600 ng/mL for tadalafil in human plasma. The lower limit of quantitation (LLOQ) was 5 ng/mL. The within- and between batch precision (expressed as coefficient of variation, C.V.) did not exceed 10.3% and the accuracy was within 7.6% deviation of the nominal concentration. The recovery of tadalafil from plasma was greater than 66.1%. Stability of tadalafil in plasma was excellent with no evidence of degradation during sample processing (auto-sampler) and 30 days storage in a freezer. This validated method is applied for the clinical study of the tadalafil in human volunteers.

7.1.2. HPLC–DAD and ESITM spectrometry

A high performance liquid chromatography/diode array detection (HPLC–DAD) method and a liquid chromatography/electrospray ionization tandem mass spectrometry (LC–ESI–MS/MS) method were developed to screen for the presence of synthetic PDE5 inhibitors such as tadalafil, sildenafil, and vardenafil [45]. The methods were applied to premarket samples submitted to the Health Sciences Authority of Singapore (HSA) for testing. One sample was in the form of capsules while six other samples were premixed bulk powder samples for dietary supplements to be repackaged or formulated into the final dosage forms (usually capsules). Identification of PDE5 inhibitors and their analogues was achieved by comparing individual peak retention times, UV spectra and mass spectra with those of reference standards (Fig. 8.16). The seven samples were found to contain at least one of the following compounds: sildenafil, vardenafil, hydroxyhomosildenafil, homosildenafil, and acetildenafil. The five compounds were simultaneously determined by LC–ESI–MS/MS in multiple reactions monitoring (MRM) scan mode. The method has been validated for accuracy, precision, linearity, and sensitivity.

Moreover, a high performance liquid chromatographic method coupled with ultraviolet detection and electrospray ionization mass spectrometry (HPLC/UV/ESI/MS) was developed for simultaneous determination of banned additives: sildenafil, vardenafil, and tadalafil in dietary supplements for male sexual potency [29]. The separation was achieved on a C18 column with acetonitrile and aqueous solution (20 mmol ammonium acetate, 0.2% formic acid) as mobile phase at a flow rate of 1 mL/min with a linear gradient program. UV detection was at 292 nm. Identification of drugs was accomplished using ESI/MS. Good linearity between response (peak area) and concentration was found over a concentration range of 0.8–80 μg/mL for sildenafil; 2.25–225 μg/mL for vardenafil; and 1.1–110 μg/mL for tadalafil, with regression coefficient is better than 0.999. The recovery of the method ranged from 93.3% to 106.1%, and the relative standard deviation varied

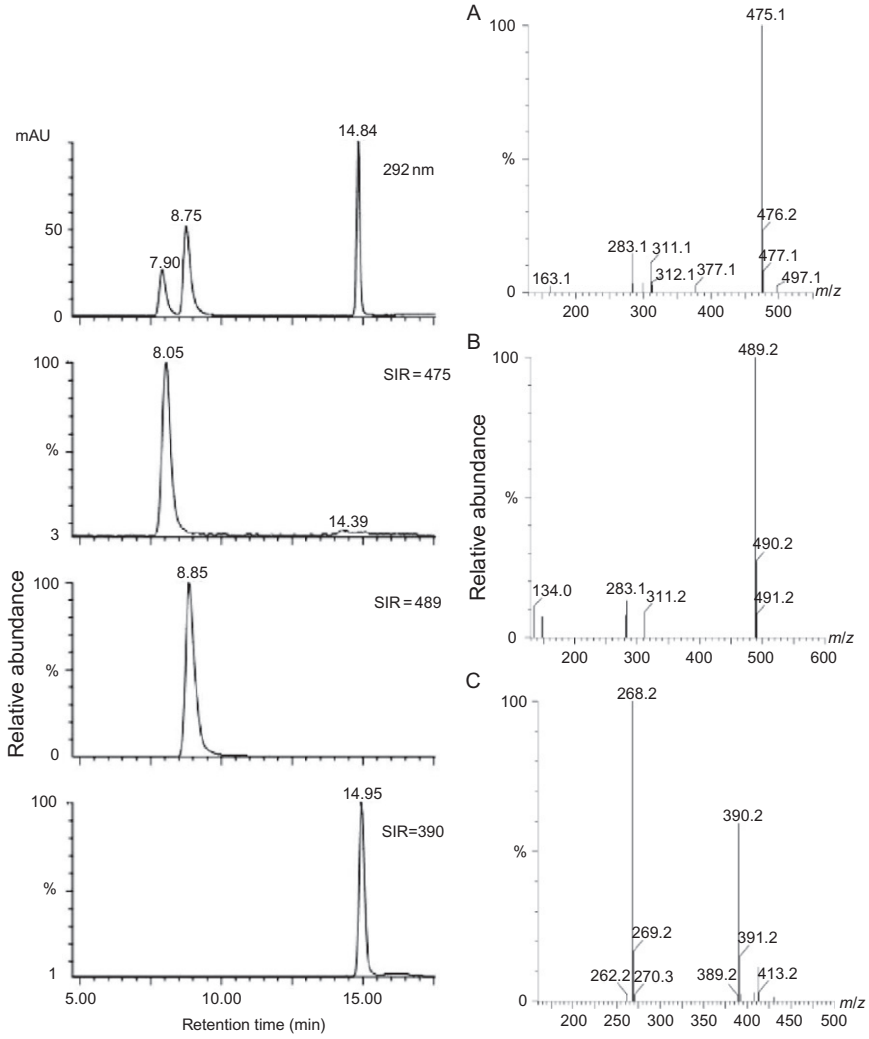


FIGURE 8.16 Left panel showed the chromatogram of mixed standards. Peak identification: sildenafil (*t*R = 7.9), vardenafil (*t*R = 8.8) and tadalafil (*t*R = 14.8). The concentration of the three compounds in the mixture was 16, 45, and 22 g/mL, respectively. Right panel showed the mass spectrum of examined analytes. (A) Sildenafil, (B) vardenafil, and (C) tadalafil.

from 2.0% to 5.6% ($n = 6$). The method has been successfully applied to the analysis of practical samples of natural dietary supplements.

A more simple, rapid, sensitive, and specific liquid chromatography, tandem mass spectrometry method [46], was developed and validated for quantitation of tadalafil I in human plasma, a new selective, reversible

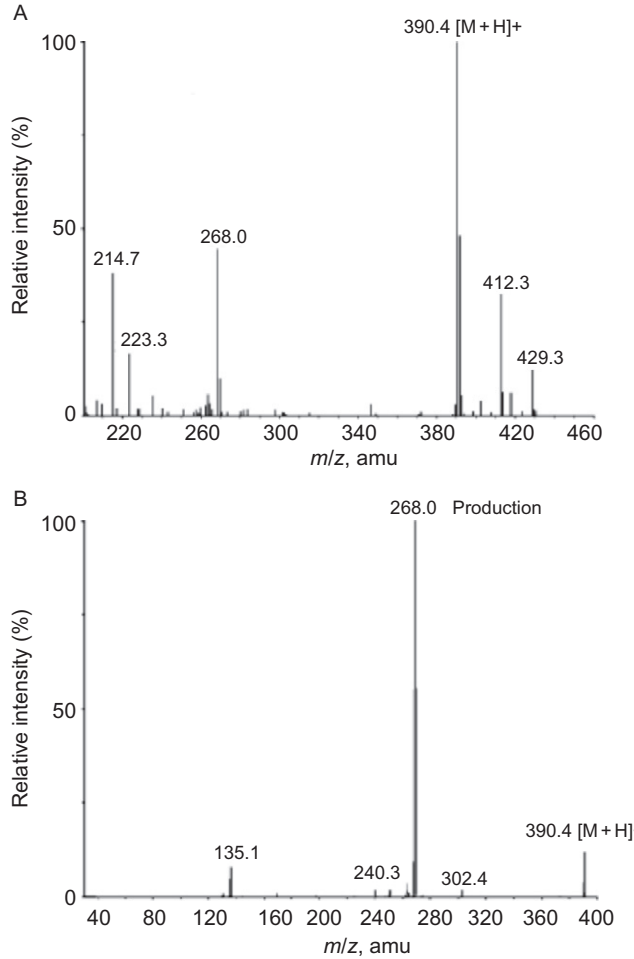


FIGURE 8.17 Full scan positive ion Turboionspray (A) Q1 mass spectra and (B) product ion mass spectra of tadalafil.

PDE5 inhibitor (Fig. 8.17). The analyte and internal standard (sildenafil, II) were extracted by liquid–liquid extraction with diethyl ether/dichloromethane (70/30, v/v) using a Glas-Col Multi-Pulse Vortexer. The chromatographic separation was performed on reverse phase Xterra MS C18 column with a mobile phase of 10 mM ammonium formate/acetonitrile (10/90, v/v, pH adjusted to 3.0 with formic acid). The protonate of analyte was quantitated in positive ionization by MRM with a mass spectrometer. The mass transitions m/z 390.4 > 268.0 and m/z 475.5 > 58.3 were used to measure I and II, respectively. The assay

exhibited a linear dynamic range of 10–1000 ng/mL for tadalafil in human plasma. The LLOQ was 10 ng/mL with a relative standard deviation of less than 15%. Acceptable precision and accuracy were obtained for concentrations over the standard curve ranges. Run time of 1.2 min for each sample made it possible to analyze a throughput of more than 400 human plasma samples per day. The validated method has been successfully used to analyze human plasma samples for application in pharmacokinetic, bioavailability, or bioequivalence.

7.1.3. HPLC-chiral

A high performance liquid chromatographic method was developed for the chiral separation of new selective PDE5 inhibitors, tadalafil and its three isomers [47]. The chiral separation was performed on a Chiralpak AD column. The mobile phase was hexane-isopropyl alcohol (1:1, v/v). UV detection was at 220 nm. Baseline chiral separation for the four isomers was obtained within 30 min. Each of the resolutions of the two pairs enantiomers were more than 2.0. The limits of quantitation were 0.60, 0.90, 1.20, and 1.80 ng for (6*R*,12a*S*), (6*R*,12a*R*), (6*S*, 12a*S*), and (6*S*,12a*R*) isomers, respectively. Relative standard deviation of the method was below 2% ($n = 5$). The method is suitable in quality control.

Moreover, a new simple isocratic chiral RP-LC method has been developed for the separation and quantification of the enantiomer of (*R*,*R*)-tadalafil in bulk drugs and dosage forms with an elution time of about 20 min [48]. Chromatographic separation of (*R*,*R*)-tadalafil and its enantiomer was achieved on a bonded macro cyclic glycopeptide stationary phase. The method resolves the (*R*,*R*)-tadalafil and its enantiomer with a resolution (R_s) greater than 2.4 in the developed chiral RP-LC. The mobile phase used for the separation and quantification of (*R*,*R*)-tadalafil and its enantiomer involves a simple mixture of reverse phase solvents and the cost of analysis was drastically decreased. The test concentration is 0.4 mg mL^{-1} in the mobile phase. This method is capable of detecting the enantiomer of (*R*,*R*)-tadalafil up to 0.0048 μg with respect to test concentration 400 μg mL^{-1} for a 20 μL injection volume. The drug was subjected to stress conditions of hydrolysis, oxidation, photolysis, and thermal degradation. There was no interference of degradants with (*R*,*R*)-tadalafil and its enantiomer in the developed method. The developed chiral RP-LC method was validated with respect to linearity, accuracy, precision, and robustness.

Recently, four blisters with suspect Cialis® (tadalafil) 20 mg tablets were screened for authenticity using near infrared spectroscopy (NIRS) and for the presence of PDE5 inhibitors using LC–DAD–MS (Figs. 8.18 and 8.19). All samples were identified as counterfeit Cialis® and contained sildenafil or a combination of tadalafil and sildenafil [49].

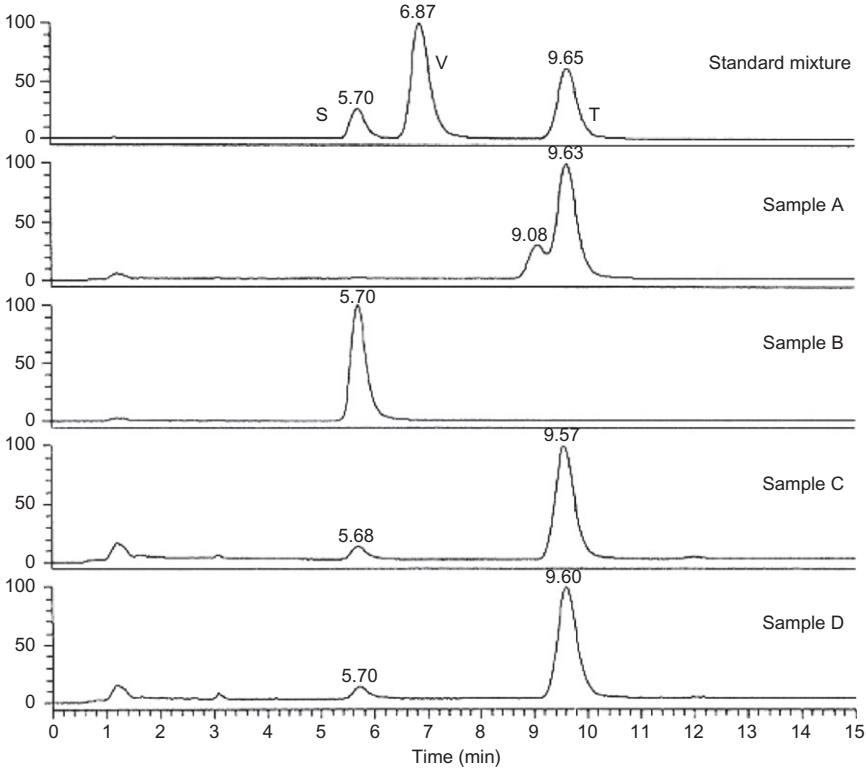


FIGURE 8.18 PDA traces of the standard mixture of sildenafil (S), vardenafil (V), tadalafil (T) and the suspect Cialis® 20 mg samples using an XTerra MS C18 column (100 mm × 2.1 mm, 3.5 μm).

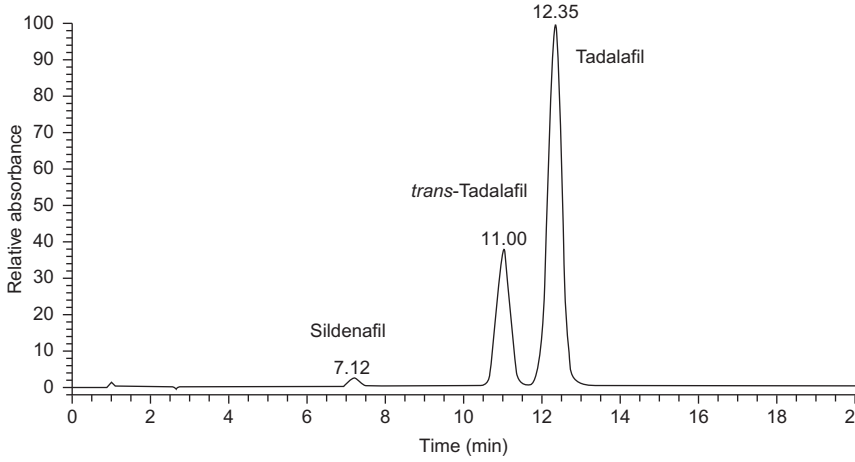


FIGURE 8.19 PDA trace for sample A using the LC–DAD–MS method using a symmetry C18 column (100 mm × 2.1 mm, 3.5 μm).

Although the tablets contained efficacious amounts of PDE5 inhibitors, neither the active ingredient nor the dosage corresponded to the description on the blister. This is the first reported case of a diastereomeric mixture of tadalafil and *trans*-tadalafil (3:1) being identified in a counterfeit medicine. The LC–DAD-CD revealed that both diastereomers had a high optical purity. The optical rotation of the diastereomeric mixture was measured indicating the presence of (−)-*trans*-tadalafil, which is the only other stereoisomer with some PDE5 inhibitory activity. As no safety profiles are known for the stereoisomers of tadalafil, there is a potential health risk. In addition, the optical purity of tadalafil needs to be taken into account when calculating the dosage in illegal medicines.

7.1.4. Capillary electrophoresis

A simple, rapid, and inexpensive capillary electrophoretic method [31] has been developed and validated for the determination of tadalafil in pharmaceutical preparations. The analysis was carried out using a fused silica capillary (60 cm × 75 mm, i.d.), phosphate buffer (50 mM, pH 3.0) as background electrolyte (BGE), 15 kV applied voltage with UV detection at 254 nm and at a working temperature of 23 ± 1 °C. Linearity was observed in the concentration range from 200–5000 μg/mL, with a correlation coefficient (R2) of 0.9998 and 200 μg/mL as the limit of detection. The percentage recovery of tadalafil from pharmaceutical preparations was 99.5. Validation parameters prove the precision of the method and its applicability for the determination of tadalafil in pharmaceutical tablet formulations. The method is fast and is suitable for high throughput analysis of the drug.

On the same time, a Micellar electrokinetic capillary chromatography method is proposed for the determination of sildenafil, vardenafil, and tadalafil [32], which are employed in oral therapy for ED. Optimum conditions for the separation were investigated. A background electrolyte solution consisting of 10 mM phosphate buffer adjusted to pH 12.0, sodium dodecyl sulfate (SDS) 25 mM, hydrodynamic injection, and 25 kV as separation voltage were used. Relative standard deviations (RSDs) were 1.0%, 1.0%, 0.4% and 2.9%, 2.9%, 1.9% for migration time and corrected peak area (CPA) ($n = 9$) for sildenafil, vardenafil, and tadalafil, respectively. Detection limits obtained for the three drugs ranged from 0.19 to 0.61 mg/L. A linear concentration range between 1 and 20 mg/L was obtained. A ruggedness test of this method was checked using the fractional factorial model of Plackett–Burman, in which the influence of six factors at three different levels was tested on different electrophoretic results: resolution and corrected peak area. The statistical evaluation of the electrophoretic results was achieved by Youden and Steiner method. The described method is rapid, sensitive, and

rugged and it was tested in the pharmaceutical formulations analysis obtaining recoveries between 98% and 107% respect to the nominal content.

7.2. NMR and Raman spectroscopy to analyze genuine Cialis

The reported method describe the use of Raman spectroscopy, 1H nuclear magnetic resonance (NMR) and 2D diffusion-ordered spectroscopy (DOSY) NMR to analyze genuine Cialis and seven illegally manufactured formulations of this drug purchased via the internet. Seven out of the eight commercial formulations of tadalafil contain the active ingredient, measured by HPLC, within 100 ± 5% of stated concentration. Vardenafil and homosildenafil instead of tadalafil were found in the Chinese imitation. 2D DOSY NMR spectra clearly showed similarities and differences in the composition of the pharmaceutical formulations of tadalafil, thus giving a precise and global ''signature'' of the manufacturer. Data show that the quality of the Cialis imitations manufactured in India and Syria is correct, whereas the Chinese formulation is adulterated with active pharmaceutical ingredients [50,51].

7.2.1. Analysis of the genuine formulation of Cialis® using Raman spectrum

The Raman spectrum of the whole tablet of Eli Lilly Cialis® (formulation 1) is shown in Fig. 8.20 and Table 8.3. The main signals observed are those of titanium dioxide (*) and talc (⅄) present in the coating of the tablet. Iron yellow and triacetin could not be detected. In order to obtain more information, the coating was eliminated and recorded a new spectrum (Fig. 8.20C and D). The unsaturated structures of tadalafil (T) appeared clearly between 1568 and 1676 cm^{-1} and around 3070 cm^{-1}. Tadalafil gives an intense response to the laser excitation at 632.8 nm, much stronger than that of the other components of the tablets, explaining why its bands are easily distinguished even if it represents only ≈5–10% of the tablet weight. In addition, most of the excipients used in the formulations do not contain aromatic, unsaturated or amide moieties. The peaks between 1550 and 1700 cm^{-1} can thus be attributed to tadalafil by comparing the observed wave numbers with those of the pure substance. On the other hand, the region below 1550 cm^{-1} was not considered for detecting tadalafil in the formulations as it is not specific since numerous bond vibrations (from aliphatic CH, CO, and CN groups) give Raman signals. The presence of the characteristic bands of magnesium stearate (□), lactose (○), and sodium lauryl sulfate (▲) were detected. Thus, it is able to detect the active substance and five excipients (titanium dioxide, talc, magnesium stearate, lactose, and sodium lauryl sulfate) by this technique.

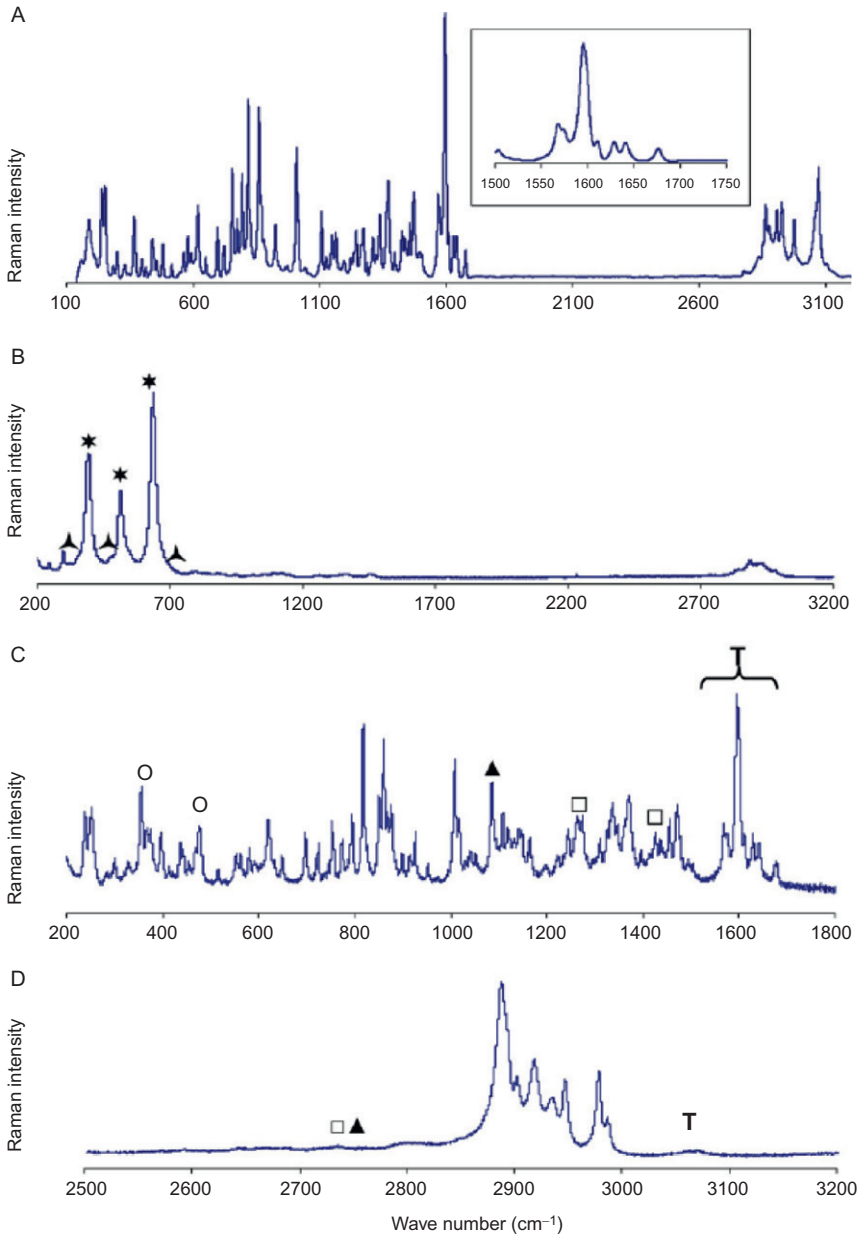


FIGURE 8.20 Raman spectra of pure tadalafil (A) and genuine Eli Lilly Cialis®: whole tablet (B), uncoated tablet from 200 to 1800 cm^{-1} (C), from 2500 to 3200 cm^{-1} (D). ⋆ TiO_2; ⅄ talc (as shoulders of TiO_2 bands); (○) lactose; (▲) sodium lauryl sulfate; (□) magnesium stearate; (T) tadalafil.

TABLE 8.3 Raman bands detected for aromatic C–C bond vibrations for pure tadalafil and the eight formulations analyzed

Pure tadalafil		
Wave number (cm^{-1})	Formulations 1–7 (mean ± S.D.)	Formulation 8
3071	3072 ± 2	3089
1676	1677 ± 3	1701
1641	1644 ± 3	1621
1629	1630 ± 1	1603
1611	1612 ± 3	1586
1594	1596 ± 2	
1575	1575 ± 2	
1567	1568 ± 1	

All bands were detected for formulations 1–3 and 5. For formulations 4, 6, and 7, two, three, and four bands, respectively, were not observed due to the poor resolution of the spectra.

7.2.2. Analysis of the genuine formulation of Cialis® using conventional and 2D DOSY ^{1}H NMR

All formulations of tadalafil were analyzed with 2D DOSY ^{1}H NMR. 2D DOSY spectra of genuine Cialis® and formulations 6 and 8 along with their corresponding 1D spectra are presented in Fig. 8.21. The peaks at 3.68 and 1.99 ppm correspond to the residual signals of water and acetonitrile, respectively. All the peaks of tadalafil are lined up and the value of the self diffusion coefficient was measured for each peak; an average self-diffusion coefficient was determined for each formulation. Several excipients could also be observed. All the formulations contain the lubricant magnesium stearate (□) that leads to four signals located at 0.89 (t), 1.28 (broad s), 1.52 (quin), and 2.13 (t) ppm. Lactose peaks (○) were identified in all formulations at 3.20 (t), 3.4–3.9 (m), 4.36 (d), 4.54 (d), and 5.12 (d) ppm. Except formulation 2, all the pharmaceutical preparations also contain a cellulose derivative as coating agent, giving three aligned signals at 1.11 (broad s), 3.37 (broad s), and 3.53 (broad s) ppm that is known to be hypromellose (●) (hydroxypropylmethyl cellulose) for the brand formulation 1 but is unknown for the other formulations. Another cellulose derivative with a higher molecular weight leads to broad signals at 1.1 ppm (s) and between 3.3 and 4.0 ppm; as for the first cellulose derivative, it is known to be hydroxypropylcellulose (■) for the brand formulation 1 but is unknown for the other formulations where it is observed (2 and 5). Signals of sodium lauryl sulfate (▲) (0.89 (t), 1.28 (broad s), 1.61 (quin), and 3.90 (t) ppm), a wetting agent, are present in the brand formulation 1 and in formulations 2, 3, and 5–7. The brand formulation 1 and the formulation 5 contain the hydrophilic plasticizer triacetin (◆)

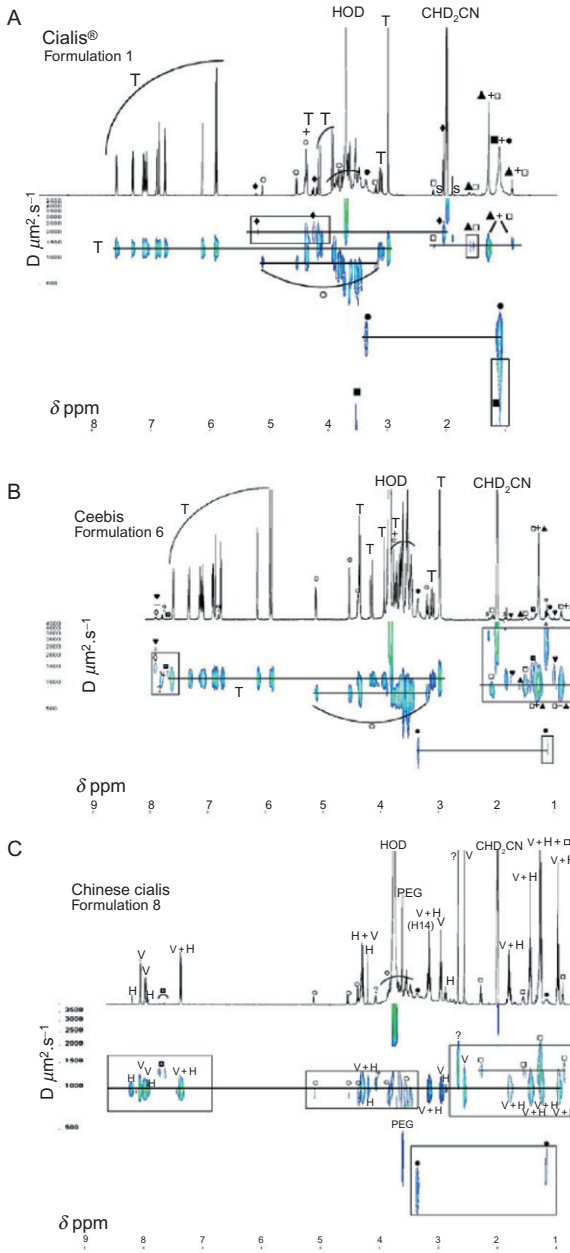


FIGURE 8.21 DOSY NMR spectra in CD_3CN:D_2O (80:20) of genuine Eli Lilly Cialis® (A), formulation 6 (B) and formulation 8 (C). (■) Hydroxypropylcellulose; (●) hypromellose; (○) lactose; (□) magnesium stearate; (▲) sodium lauryl sulfate; (◆) triacetin; (*) isopropanol; (□) diethylphthalate; (♥) propylparaben; (◊) methylparaben; (T) tadalafil; (V) vardenafil; (H) homosildenafil; (PEG) polyethylene glycol; s satellite; ? unknown.

(glyceryl triacetate; 5.22 (m), 4.23 (AB d), 2.06 (s), and 2.05 (s) ppm). The antimicrobial preservatives methylparaben (◆) (methyl-4-hydroxybenzoate, 3.83 (s), 7.88 (d) ppm) and propylparaben (♥) (propyl-4-hydroxybenzoate; 0.99 (t), 1.74 (app q), 4.20 (t), and 7.88 (d) ppm) were found in formulations 4, 6, and 7. Diethylphthalate (□) (1.32 (t), 4.33 (q), and 7.68 (m) ppm), a plasticizer, could be detected in formulations 6–8. Propylene glycol, an anticaking agent, was found in formulations 2–4 (1.07 (d), 3.39 (AB d), and 3.74 (m) ppm). The presence of isopropanol, a coating solvent, is detected only by the signal of its methyl group at 1.1 ppm (d); this excipient was detected in formulations 4, 6, and 7 but its amount was subjected to a great intertablet variability even from the same batch. Only three formulations (2, 7, and 8) include polyethylene glycol as a lubricant agent (3.61 ppm (s)). Formulation 2 contains signals of citrate giving a characteristic AB system pattern at 2.53 ppm. Classical ^{1}H NMR was then used to establish the structure of the active pharmaceutical ingredient(s) present in the Chinese fake formulation of Cialis (8) that had an atypical Raman spectrum. Figure 8.21C shows the ^{1}H NMR spectrum of the Chinese formulation, which is very different from that of the original Cialis® analyzed in the same conditions (Fig. 8.21A). Tadalafil is not present in the Chinese formulation. Indeed, only the signals of excipients were observed in the 2.4–0.8 ppm spectral region of the brand Cialis® formulation 1, whereas signals of alkyl groups from active(s) were observed in the Chinese formulation 8. Moreover, the intensity of the peaks located in the aromatic region of the spectrum demonstrates that formulation 8 contains a mixture of two active pharmaceutical ingredients. After chromatographic purification of these two active ingredients, their NMR spectra were recorded and the compounds were thus identified as vardenafil and homosildenafil.

8. PHARMACODYNAMICS [1–3,52–55]

8.1. An overview

The term pharmacodynamics covers all actions of a drug on the different body organs and in turn their functions (e.g., blood pressure, heart rate, vision). The pharmacodynamic interactions of any drug are influenced by the number of receptors available in the target organ and the affinity of the compound to the receptors in question. The most important parameters concerning the pharmacodynamic properties of a drug are its biochemical potency and its organ (PDE) selectivity [1–3,52–55].

Tadalafil is PDE5 inhibitor that has recently been approved for the treatment of ED. Despite the clear utility of this compound, one potential drawback is cross-reactivity with the closely related PDE6 and PDE11.

It is thought that this cross-reactivity is responsible for side effects such as blue-tinged vision and back and muscle pain that experienced by some patients that were treated with this drugs [52,53].

8.2. Mechanism of action

The mechanism of action of tadalafil is similar to the other PDE5 inhibitors, sildenafil and vardenafil. Through the inhibition on PDE5, tadalafil increases the concentrations of cGMP, producing smooth muscle relaxation and increased blood flow to the corpus cavernosum, thereby enhancing erectile response following appropriate sexual stimulation [52–55].

8.3. Efficacy and safety of tadalafil for the treatment of erectile dysfunction [56]

Recent integrated analyses of five 12-week randomized, double-blind, placebo-controlled trials demonstrated that tadalafil, taken as needed at maximum daily doses of 5–20 mg without specific instruction regarding food/alcohol intake, significantly enhanced erectile function. Over this dose range, tadalafil significantly increased the ability to attain and maintain erections among 1112 men with histories of mild to severe ED ascribed to various cause. Approximately 61% of men had organic ED, 9% psychogenic and 31% mixed. Nearly 60% of men had moderate or severe ED at baseline [56].

A total of 1112 men with a mean age of 59 years (range 22–82) and mild to severe ED of various etiologies were randomized to placebo or tadalafil, taken as needed without food or alcohol restrictions, at fixed daily doses of 2.5, 5, 10, or 20 mg in five randomized, double-blind, placebo controlled trials lasting 12 weeks. The three co-primary outcomes were changes from baseline in the erectile function domain of the International Index of Erectile Function and the proportion of ''yes'' responses to questions 2 and 3 of the Sexual Encounter Profile. Additional efficacy instruments included a Global Assessment Question. Compared with placebo, tadalafil significantly enhanced all efficacy outcomes. Patients receiving 20 mg tadalafil experienced a significant mean improvement of 7.9 in International Index of Erectile Function erectile function domain score from baseline ($p < 0.001$ vs. placebo), 75% of intercourse attempts (Sexual Encounter Profile question 3, a secondary efficacy outcome) were successfully completed ($p < 0.001$ vs. placebo) and 81% reported improved erections at end point compared with 35% in the control group ($p < 0.001$). Tadalafil was consistently efficacious across disease severities and etiologies, as well as in patients of all ages. Tadalafil was well tolerated, and headache and dyspepsia were the most frequent

adverse events. It was concluded that tadalafil was effective and well tolerated in this patient population.

8.4. Pharmacodynamic interactions between tadalafil and nitrates [57,58]

Because sildenafil, another PDE5 inhibitor, is contraindicated in men taking nitrates, studies were undertaken to examine potential interactions between tadalafil and nitrates. Two double-blind, randomized, 3-way crossover studies were conducted in patients with stable angina to determine: (1) Study A – response to sublingual nitroglycerin (SL NG) administered 2 h after tadalafil 5 or 10 mg or placebo ($n = 51$); and (2) Study B – response after tadalafil 5 or 10 mg or placebo administered during daily long-acting nitroglycerin (LA NG) therapy ($n = 45$). Results: The table shows the results for the primary endpoint, which was mean maximal change in standing systolic BP (MMCSBP). There were no statistically significant differences in either study between tadalafil 5 or 10 mg and placebo in the sitting position. Conclusions: Tadalafil had minimal effects, relative to placebo, on mean blood pressure changes induced by either SL NG or LANG. However, the frequency of outliers was higher in the tadalafil treatment groups, indicating that in a subset of patients, tadalafil augments the decrease in BP induced by nitrates. These results suggest that, as with sildenafil, tadalafil should not be used in combination with nitrates.

9. PHARMACOKINETICS [14,29,52,54,59–66]

9.1. An overview

Tadalafil is a potent and selective PDE5 inhibitor under regulatory review for the treatment of ED. In a clinical trial, tadalafil showed a clinical response in erectile function for up to 24 h postdosing.

In both chemical structure and PDE subtype selectivity profile, tadalafil differs markedly from sildenafil and vardenafil. These disparities are indicated by the respective suffixes: dalafil and denafil. Compared with sildenafil and vardenafil, tadalafil exhibits a prolonged plasma residence and window of therapeutic response. The $t_{1/2}$ of tadalafil is 17.5 h and the t_{max} is approximately 2.0 h (range, 0.5–12.0 h; normalized for a 20 mg dose) in healthy volunteers. In men with ED treated with tadalfil, a significantly higher percentage of attempts at sexual intercourse was successful as long as 24–36 h after dosing compared with placebo. The pharmacokinetics of tadalafil is also not clinically significantly influenced by extrinsic factors, such as food or alcohol intake; or intrinsic factors, such as diabetes or renal or hepatic impairment. The foregoing

advantages, particularly the reduced need to plan sexual activity around the time of either tadalafil dosing or meal/alcohol consumption, may translate in clinical practice into enhanced convenience and acceptability of tadalafil to the ED patient and/ or his partner [14,29,52,54,59–66].

9.2. Comparison of pharmacokinetic parameters between tadalafil and other PDE5 inhibitors

The pharmacokinetic properties of tadalafil comprise all the different steps from its entry into the body to its elimination out of the body. These steps include absorption rates with special regard to any food and alcohol interaction. In this regard of special interest is the speed of absorption, which can be seen by the T_{max} (time needed to reach the maximum plasma concentration, C_{max}). C_{max} (maximum plasma concentration of a drug) indicates the value of the highest drug concentrations reached in the plasma. According to the personal experiences in the clinical setting the T_{max} corresponds pretty well with the time needed to get a completely rigid erection and the emphasis is here on the word rigid. Of major importance for the patients and their partners is also the $T_{1/2}$ (half-life time), defined as that time it takes for the fall of the plasma concentrations of a drug to half of its C_{max} values. Generally speaking the $T_{1/2}$ corresponds very well with the duration of action of a drug. Tadalafil can be stated that the period of responsiveness, during which the majority of the responders to the drug are able to get a rigid erection after sexual stimulation, corresponds pretty well to the 2- to 3-fold half-life time. In this perspective, it has to be remembered that tadalafil is predominantly metabolized in the liver by the low-affinity cytochrome P450 enzyme 3A4 (CYP3A4) and secondarily by the high-affinity CYP2C9. Drugs with known inhibitory activities on CYP3A4 such as the H2 receptor antagonist cimetidine, the antibiotic erythromycin, the antimykotic drugs keto- and itraconazole or the protease inhibitors indinavir, ritonavir, and saquinavir can exert a major influence on the degradation rates of tadalafil and can increase considerably the C_{max} and the total exposure to a PDE5 inhibitor in question. In particular, the protease inhibitors mentioned before are increasing the plasma concentrations and prolonging the half-life time in a clinically meaningful way that the dosages of tadalafil have to be adjusted (reduced) when patients are on such medications. The same applies for grapefruit juice, a typical CYP3A4 inhibitor. Last but not least in terms of the pharmacokinetic profile of a drug the protein binding has to be considered. Both the pharmacokinetic and pharmacodynamic properties of a drug may be influenced by intrinsic factors such as age, kidney or liver function, respectively, and concomitant diseases or medication [14,52,54].

9.3. Tadalafil pharmacokinetics in patients with erectile dysfunction

Tadalafil pharmacokinetics in patients with ED showed linear relation with respect to dose and duration of treatment, and a one-compartment model adequately described the data. The absorption rate was rapid (1.86 h^{-1}), and the typical population estimates of the apparent oral clearance (CL/F) and apparent volume of distribution were 1.6 L/h and 63.8 L, respectively. Disposition parameters showed a moderate degree of interindividual variability (39–45%). The value of CL/F decreased slightly with increasing serum γ-glutamyl transferase (GGT) concentration, the only statistically significant covariate detected. Systemic exposure to tadalafil was not influenced by age, weight, smoking status, alcohol consumption, liver enzyme status, ED severity, cardiovascular condition, or diabetes mellitus.

Finally, pharmacokinetics in the efficacy/safety trial population are essentially similar to pharmacokinetics in healthy subjects, and no patient-specific factor warranting clinical consideration of dose regimen adjustment was identified in these analyses [65].

9.4. Pharmacokinetic interaction between tadalafil and bosentan in healthy male

Tadalafil, an oral PDE5 inhibitor, is being investigated as a treatment for pulmonary arterial hypertension. Bosentan is an oral endothelin receptor antagonist widely used in the treatment of pulmonary arterial hypertension. Tadalafil is mainly metabolized by cytochrome P450 (CYP) 3A4, and as bosentan induces CYP2C9 and CYP3A4, a pharmacokinetic interaction is possible between these agents. This open-label, randomized study investigated whether any pharmacokinetic interaction exists between tadalafil and bosentan. Healthy adult men ($n = 15$; 19–52 years of age) received 10 consecutive days of tadalafil 40 mg once daily, bosentan 125 mg twice daily, and a combination of both in a 3-period crossover design. Following 10 days of multiple-dose coadministration of bosentan and tadalafil, compared with tadalafil alone, tadalafil geometric mean ratios (90% confidence interval [CI]) for AUC_{tau} and C_{max} were 0.59 (0.55, 0.62) and 0.73 (0.68, 0.79), respectively, with no observed change in t_{max}. Following coadministration of bosentan with tadalafil, bosentan ratios (90% CI) for AUC_{tau} and C_{max} were 1.13 (1.02, 1.24) and 1.20 (1.05, 1.36), respectively. Tadalafil alone and combined with bosentan was generally well tolerated. In conclusion, after 10 days of coadministration, bosentan decreased tadalafil exposure by 41.5% with minimal and clinically irrelevant differences (<20%) in bosentan exposure [66].

REFERENCES

[1] T.F. Lue, N. Engl. J. Med. 342 (2000) 1802.
[2] C. Gazzaruso, S. Giordanetti, E. De Amici, G. Bertone, C. Falcone, D. Geroldi, P. Fratino, S.B. Solerte, A. Garzaniti, Circulation 110 (2004) 22.
[3] M. Bocchio, G. Desideri, P. Scarpelli, S. Necozione, G. Properzi, C. Spartera, F. Francavilla, C. Ferri, S. Francavilla, J. Urol. 171 (2004) 1601.
[4] P.J. Dunn, Org. Process Res. Dev. 9 (2005) 88.
[5] L.A. Sorbera, L. Martin, P.A. Leeson, J. Castaner, Drugs Future 26 (2001) 15.
[6] A.C.-M. Daugan, European Patent, EP 0740668.
[7] A. Daugan, P. Grondin, C. Ruault, A.-C. Le Monnier de Gouville, H. Coste, J. Kirilovsky, F. Hyafil, R. Labaudinière, J. Med. Chem. 46 (2003) 4525.
[8] A. Daugan, P. Grondin, C. Ruault, A.-C. Le Monnier de Gouville, H. Coste, J.M. Linget, J. Kirilovsky, F. Hyafil, R. Labaudinière, J. Med. Chem. 46 (2003) 4533.
[9] M.W. Orme, J.C. Sawyer, L.M. Schultze, World Patent WO 02/036593.
[10] J.D. Revell, N. Srinivasan, A. Ganesan, Synlett (2004) 1428.
[11] B.B. Lohray, V.B. Lohray, S.I. Patel, World Patent WO 05/068464.
[12] F. Montorsi, T.E.D. McDermott, R. Morgan, A. Olsson, A. Schultz, H.J. Kirkeby, I.H. Osterloh, Urology 53 (1999) 1011.
[13] S. Stark, R. Sachse, T. Liedl, J. Hensen, G. Rohde, G. Wensing, R. Horstmann, K.M. Schrott, et al., Eur. Urol. 40 (2001) 181.
[14] B. Patterson, A. Bedding, H. Jewell, C. Payne, M. Mitchell, Eur. Urol. Suppl. 1 (2002) 152.
[15] J.D. Corbin, S.H. Francis, Int. J. Clin. Pract. 56 (2002) 453.
[16] D.T. Manallack, R.A. Hughes, P.E. Thompson, J. Med. Chem. 48 (2005) 3449.
[17] S. Xiao-Xin, L. Shi-Ling, X. Wei, X. Yu-Lan, Tetrahedron Asymmetr. 19 (2008) 435.
[18] Merck index 2006, 14th edition pages 1550–1551.
[19] N.M. Graham, M.N.A. Charlotte, G. Eugene, A.M. William, Bioorg. Med. Chem. Lett. 13 (2003) 1425.
[20] Y. Zhang, Q. He, H. Ding, X. Wu, Y. Xie, Org. Prep. Proced. Int. 37 (2005) 99.
[21] W. Jiang, V.C. Alford, Y. Qiu, S. Bhattacharjee, T.M. John, D. Haynes-Johnson, P.J. Kraft, S.J. Lundeen, Z. Sui, Bioorg. Med. Chem. 12 (2004) 1505.
[22] D. Ben-Zion, D. Dov, United States Patent, US 2006/0276652 A1, 2006.
[23] B.D. Pandurang, B.B. Bharat, S.S. Sachin, P.S. Pranay, United States Patent, US 7, 223, 863 B2, 2007.
[24] X. Sen, S. Xiao-Xin, X. Jing, Y. Jing-Jing, L. Shi-Ling, L. Wei-Dong, Tetrahedron Asymmetr. 20 (2009) 2090.
[25] S. Xiao, X. Lu, X.-X. Shi, Y. Sun, L.-L. Liang, X.-H. Yu, J. Dong, Tetrahedron Asymmetr. 20 (2009) 430.
[26] H. Sajiki, Tetrahedron Lett. 36 (1995) 3465.
[27] J. Meienhofer, K. Kuromizu, Tetrahedron Lett. 15 (1974) 3259.
[28] J.-P. Mazaleyrat, J. Xie, M. Wakselman, Tetrahedron Lett. 33 (1992) 4301.
[29] Z. Xiaolan, X. Song, C. Bo, Z. Fei, Y. Shouzhuo, W. Zutian, Y. Dajin, H. Hongwei, J. Chromatogr. A 1066 (2005) 89.
[30] Y.A.-E. Hassan, A. Imran, Talanta 65 (2005) 276.
[31] A. Imran, Y.A.-E. Hassan, Chromatographia 60 (2004) 187.
[32] J. Rodríguez Flores, J.J. Berzas Nevado, G. Castañeda Peñalvo, N. Mora Diez, J. Chromatogr. B 811 (2004) 231.
[33] C. Ching-Ling, C. Chen-Hsi, J. Chromatogr. B 822 (2005) 278.
[34] M.B.-E. Shaimaa, A.E. Seham, M.G. Mahmoud, Eur. J. Pharm. Biopharm. 70 (2008) 819.
[35] A.V. Cinzia, G. Ignazio, M. Teresa, P. Rosario, R. Lorella, L. Carla, M. Claudio, P. Donatella, P. Giovanni, Eur. J. Med. Chem. 41 (2006) 233.

[36] V. Crupi, R. Ficarra, M. Guardo, D. Majolino, R. Stancanelli, V. Venuti, J. Pharm. Biomed. Anal. 4 (2007) 110.
[37] V. Vikrant, S. Pankajkumar, K. Poonam, S. Manali, P. Yogesh, Acta Pharm. 59 (2009) 453.
[38] A.B. Mohsen, A.A. Khalid, A.A.-A. Abdulaziz, Int. J. Pharm. 243 (2002) 107.
[39] M. Jiradej, G.A. Maria, F. Kuncoro, M. Aranya, Int. J. Pharm. 293 (2005) 235.
[40] S.S.R. Laura, C.F. Domingos, J.B.V. Francisco, Eur. J. Pharm. Sci. 20 (2003) 253.
[41] M.D. Veiga, P.J. Díaz, F. Ahsan, J. Pharm. Sci. 87 (1998) 891.
[42] O.W. Robert, M. Vorapann, S. Mongkol, Eur. J. Pharm. Biopharm. 46 (1998) 355.
[43] E. Redenti, T. Peveri, M. Zanol, P. Ventura, G. Gnappi, A. Montenero, Int. J. Pharm. 129 (1996) 289.
[44] A.K. Shakya, A.N. Abu-awwad, T.A. Arafat, M. Melhim, J. Chromatogr. B 852 (2007) 403.
[45] Z. Peng, S.-Y.O. Sharon, H. Peiling, L. Min-Yong, K. Hwee-Ling, J. Chromatogr. A 1104 (2006) 113.
[46] N.V. Ramakrishna, K.N. Vishwottam, S. Puran, M. Koteshwara, S. Manoj, M. Santosh, J. Chidambara, S. Wishu, B. Sumatha, J. Chromatogr. B 809 (2004) 243.
[47] W. Gao, Z. Zhang, Z. Li, G. Liang, J. Chromatogr. Sci. 45 (2007) 540.
[48] A. Madhavi, G.S. Reddy, M.V. Suryanarayana, A. Naidu, Chromatographia 67 (2008) 633.
[49] B.J. Venhuis, G. Zomer, M.J. Vredenbregt, D. de Kaste, J. Pharm. Biomed. Anal. 51 (2010) 723.
[50] S. Trefi, C. Routaboul, S. Hamieh, V. Gilard, M. Malet-Martino, R. Martino, J. Pharm. Biomed. Anal. 47 (2008) 103.
[51] B. Stéphane, T. Saleh, G. Véronique, M.-M. Myriam, M. Robert, D. Marc-André, J. Pharm. Biomed. Anal. 50 (2009) 602.
[52] P. Hartmut, EAU Update Series 2 (2004) 56.
[53] D.S. Allen, Clin. Cardiol. 27 (2004) I-14.
[54] F. Giuliano, L. Varanese, Eur. Heart J. Suppl. 4 (2002) H24.
[55] A.B. Mitsi, B. Alfreda, Z. Roya, R.S. Konjeti, P.B. Emmanuel, H.F. Sharron, D.C. Jackie, Mol. Pharmacol. 66 (2004) 144.
[56] G.B. Brock, C.G. McMahon, K.K. Chen, T. Costigan, W. Shen, V. Watkins, G. Anglin, S. Whitaker, J. Urol. 168 (2002) 1332.
[57] J. Emmick, M. Mitchell, A. Bedding, Eur. Urol. Suppl. 2 (2003) 95.
[58] K. Robert, E. Jeff, B. Alun, H. Dennis, J. Am. Colleg. Card. 39 (Suppl. 2) (2002) 291.
[59] H. Porst, Int. J. Impot. Res. 14 (Suppl. 1) (2002) S57.
[60] D.O. Sussman, J. Am. Osteopath. Assoc. 104 (2004) 11S.
[61] B.J. Ring, B.E. Patterson, M.I. Mitchell, M. Vandenbranden, J. Gillespie, A.W. Bedding, H. Jewell, C.D. Payne, S.T. Forgue, J. Eckstein, S.A. Wrighton, D.L. Phillips, Clin. Pharmacol. Ther. 77 (2005) 63.
[62] C.C. Carson, J. Rajfer, I. Eardley, S. Carrier, J.S. Denne, D.J. Walker, W. Shen, BJU Int. 93 (2004) 1276.
[63] S.T. Forgue, B.E. Patterson, A.W. Bedding, C.D. Payne, D.L. Phillips, R.E. Wrishko, et al., Br. J. Clin. Pharmacol. 61 (2006) 280.
[64] B. Gerald, Europ. Urology Supp. 1, 2002, 12.
[65] I.F. Trocóniz, C. Tillmann, A. Staab, J. Rapado, S.T. Forgue, Eur. J. Clin. Pharmacol. 63 (2007) 583.
[66] R.E. Wrishko, J. Dingemanse, A. Yu, C. Darstein, D.L. Phillips, M.I. Mitchell, J. Clin. Pharmacol. 48 (2008) 610.