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Structure elucidation of sildenafil analogues in herbal products

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Abstract

The structure of unknown compounds present in herbal products was elucidated using liquid chromatography-electrospray ionization-mass spectrometry, direct-infusion electrospray ionization-mass spectrometry, and nuclear magnetic resonance. Compounds ***1***-***3*** were identified as sildenafil analogues, ***1*** bearing an N-ethylpiperazine moiety instead of an N-methylpiperazine, and an acetyl group instead of the sulfonyl group, named acetildenafil, ***2*** bearing an N-ethylpiperazine moiety instead of an N-methylpiperazine (homosildenafil), and ***3*** bearing an N-hydroxylethylpiperazine moiety instead of an N-methylpiperazine, named hydroxyhomosildenafil. When analysing products marketed for penile erectile dysfunction or marketed as aphrodisiacs, attention should be given to the possible presence of these components.

Introduction

Sildenafil is the active compound in Viagra®, a prescription medicine for penile erectile dysfunction. However, it is also found in aphrodisiacs, mainly advertised as natural products. As aphrodisiacs and natural products increase in popularity, especially via the World Wide Web, this will be a global problem: consumers are not aware of taking a prescription drug that has contraindications.

Recently, the present authors identified three sildenafil analogues in three different herbal products. A completely new analogue **1**, named acetildenafil, was identified in product **A**, oral capsules, advertised as 'a firm erection of Mother Nature', based on traditional Chinese medicine. Homosildenafil (**2**) was identified in product **B**, Chinese oral tablets, labelled as a herbal preparation containing *Juglans regia* (walnut), to be used as an anti-fatigue agent according to the package leaflet. However, it was sold as herbal Viagra. Shin *et al*. (2003) published the structure of this analogue using nuclear magnetic resonance (NMR) spectroscopy. The compound was discovered in a beverage marketed for erectile dysfunction. Hydroxyhomosildenafil (**3**) was identified in product **C**, Chinese oral capsules, advertised as an herbal alternative for Viagra.

Based on the structural analogy of these three compounds with sildenafil, similar biological activity is to be expected. Pfizer (unpublished data) reported limited pharmacological data for hydroxyhomosildenafil, demonstrating biological activity comparable with sildenafil. In Japan, one case of liver function impairment was reported that might be due to the use of a product containing hydroxyhomosildenafil (Japan, Pharmaceutical and Food Safety Bureau - Health, Labor, and Welfare Ministry 2004). Beside this limited information, no data on toxicology and efficacy are described in the public (medical) literature for these three analogues. It seems there is a tendency towards the development of designer drugs based on sildenafil that might present a risk for human health.

This paper describes the elucidation of these three compounds using liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS^n), direct-infusion ESI-MS^n, and ^1H- and ^{13}C-NMR.

Materials and methods

Samples

Product A and C were submitted by the Dutch Food and Consumer Product Safety Authority for analysis on phosphodiesterase type 5 enzyme (PDE5) inhibitors. Product B was submitted by the Dutch Health Care Inspectorate for analysis on sildenafil.

Materials for LC-ESI-MS^n and direct-infusion ESI-MS^n

Sildenafil citrate (pharmaceutical quality) was obtained from Pfizer, Inc. (Groton, USA). Tablets of Cialis®, containing 20 mg tadalafil per tablet, were obtained from Eli Lilly Nederland B.V. (Houten, The Netherlands). Tablets of Levitra®, containing 20 mg vardenafil per tablet, were obtained from Bayer AG (Leverkussen, Germany). Methanol (high-performance liquid chromatography grade; MeOH) was obtained from Promochem (Wesel, Germany). Formic acid (p.A.) was obtained from Merck (Darmstadt, Germany). Ammonium hydroxide (NH_4OH) was obtained from two suppliers: Merck (32%, extra pure) and Acros Organics (Geel, Belgium) (reagent ACS), being of comparable quality. Water was demineralized and filtered using a Millipak® 200 0.22-µm filter from Millipore B.V. (Amsterdam, The Netherlands). Acidified water was prepared by addition of 2 ml formic acid to 1000 ml water and adjusting the pH to 4.0 using NH_4OH.

Materials for NMR

Solvents and chemicals for NMR analysis - acetone, acetone-d_6, acetonitrile (ACN), chloroform ($CHCl_3$), deuterated chloroform ($CDCl_3$), deuterium oxide (D_2O), dimethylsulfoxide (DMSO), MeOH, hydrochloric acid (HCl), C_{18} material for reversed-phase liquid chromatography, sodium hydroxide (NaOH), sodium sulphate (Na_2SO_4), trimethylsilyl-d_4-propanoic acid, sodium salt (TMSP) - were obtained from commercial sources in the best possible quality.

Materials for (IR)

Potassium bromide (KBr) powder, spectroscopic grade, used for IR analysis was supplied by Anadis Instruments B.V. (Malden, The Netherlands).

Instrumentation

Direct-infusion ESI-MS^n experiments were carried out using an LCQ Advantage ion-trap mass spectrometer equipped with an ESI interface, operated by Xcalibur software version 3.1, from Thermo Finnigan B.V. (Breda, The Netherlands). For LC-ESI-MS^n experiments, an LC system, consisting of a Surveyor autosampler, LC pump and photodiode array (PDA) detector (all Thermo Finnigan B.V.), was connected to the mass spectrometer.

^1H-, ^{13}C-, and two-dimensional NMR data were recorded on a JEOL Eclipse 400 spectrometer.

IR spectroscopy was performed on a Bruker IFS55 FT-IR spectrometer with a DTGS detector and OPUS software version 4.0.

Methods

Preparation of standard and sample solutions

A standard stock solution was prepared for LC-MS^n analysis on sildenafil by dissolving sildenafil citrate in MeOH at a concentration of about 450 µg ml^{-1} equivalent to approximately 325 µg sildenafil ml^{-1}. The working solution was prepared by diluting the stock solution with mobile phase to about 6.5 µg ml^{-1} sildenafil. Tablets of Cialis® were ground, tablet powder was dissolved in MeOH and diluted with mobile phase to about 2 µg ml^{-1} tadalafil. Similarly, a solution of about 2 µg ml^{-1} vardenafil was prepared from tablets of Levitra®.

Sample *A*

For direct-infusion ESI-MS^n, the content of four capsules was homogenized and approximately 380 mg were dissolved in 100 ml MeOH by sonification. After filtration over a 0.45-µm filter, the solution was diluted 1:10 with mobile phase. This solution was also diluted 1:10 with mobile phase for LC-MS^n analysis. For NMR analysis, the compound of interest was isolated by dissolving the content of one capsule, approximately 100 mg, in 1 ml DMSO : water (20:80 v/v). The product was isolated by reversed-phase liquid chromatography on C_{18} material, washing with 10 ml water, followed by 10-ml mixtures of methanol and water with increasing amounts of methanol (v/v 10, 40, 60 and 90%). The compound was eluted from the column with 10 ml ACN:0.5 M HCl (10:90 v/v). The fractions containing the product were pooled and evaporated to dryness under a stream of nitrogen. The residue was dissolved in 2 ml water, basified using 1 M NaOH solution and extracted with $CHCl_3$. The organic layer was washed with water, dried over Na_2SO_4 and evaporated yielding a white solid. The solid was dissolved in $CDCl_3$ for NMR analysis. For IR analysis, the solvent was evaporated and the residue prepared as for the KBr tablet.

Sample *B*

For LC-MS^n analysis, one tablet was ground and about 50 mg were dissolved in 25 ml MeOH by sonification. After filtration over a 0.45-µm filter, the solution was diluted 1:200 with mobile phase. For NMR analysis, the compound of interest was isolated by extracting one ground tablet three times with 5 ml acetone followed by three times with 5 ml water. The water layer was basified using 5 M NaOH solution and extracted three times with 10 ml $CHCl_3$. The organic layers were combined, dried with Na_2SO_4, filtered and evaporated to dryness under a stream of nitrogen. The residue was dissolved in approximately 0.8 ml $CDCl_3$.

Sample *C*

For direct-infusion ESI-MS^n, the content of three capsules was homogenized and approximately 210 mg were dissolved in 50 ml MeOH by sonification. After filtration over a 0.45-µm filter, the solution was diluted 1:10 with mobile phase. This solution was also diluted 1:10 with mobile phase for LC-MS^n analysis. For qualitative NMR analysis, the compound of interest was isolated by extraction of 500 mg sample using acetone and water, and acid/base separation as described for sample **A**. For quantitative NMR analysis, 25 mg were dissolved in 0.7 ml D_2O containing 0.075% TMSP as internal standard and 0.7 ml acetone-d_6.

LC-ESI-MS^n and direct-infusion ESI-MS^n

For chromatographic separation and ultraviolet light detection, the following conditions were used: XTerra MS® C_{18} guard column (20 × 2.1 mm, 3.5 µm) and XTerra MS® C_{18} analytical column (100 × 2.1 mm, 3.5 µm; Waters Chromatography B.V., Etten-Leur, The Netherlands): isocratic elution using MeOH : acidified water (50:50 v/v); flow rate at 0.25 ml min^{-1}; column temperature of 25°C; injection volume of 20 µl; ultraviolet light detection from 200 to 350 nm. Mass spectrometry was carried out in positive-ion mode using the ESI interface. Nitrogen was used as sheath gas (20 arbitrary units) and as auxiliary gas (10 arbitrary units). Source settings used: ion spray voltage 5.0 kV, capillary temperature 300°C, capillary voltage 31 V,

tube lens offset 55 V. MS^1: mass range *m/z* = 80-1000. MS^n: relevant ions selected and fragmented using a collision energy of 40.00 arbitrary units. Instrument conditions were checked using sildenafil citrate standard working solution. Samples **A** and **C** were analysed for PDE5 inhibitors; sample **B** was analysed for sildenafil only, using a standard analytical method (Bakker *et al*. 2004).

NMR spectra were recorded using standard single and multipulse sequences for one- and two-dimensional NMR spectra. FIDs were zero-filled, apodized using exponential (single pulse) or sinebell (multipulse) functions, phase and baseline corrected, and scaled against the residual $CHCl_3$ signal at 7.26 (^1H) or 77.0 ppm (^{13}C).

IR measurements were carried out over the spectral range 4000-400 cm^{-1} with an optical resolution of 2 cm^{-1}, and 32 scans were co-added.

Results and discussion

Sample *A*, compound *1*

Based on LC-ESI-MS^n, direct-infusion ESI-MS^n, ^1H- and ^{13}C-NMR, and IR data, the compound was identified as a sildenafil analogue bearing an *N*-ethylpiperazine moiety in stead of an *N*-methylpiperazine, and an acetyl group instead of the sulfonyl group (figure 1).

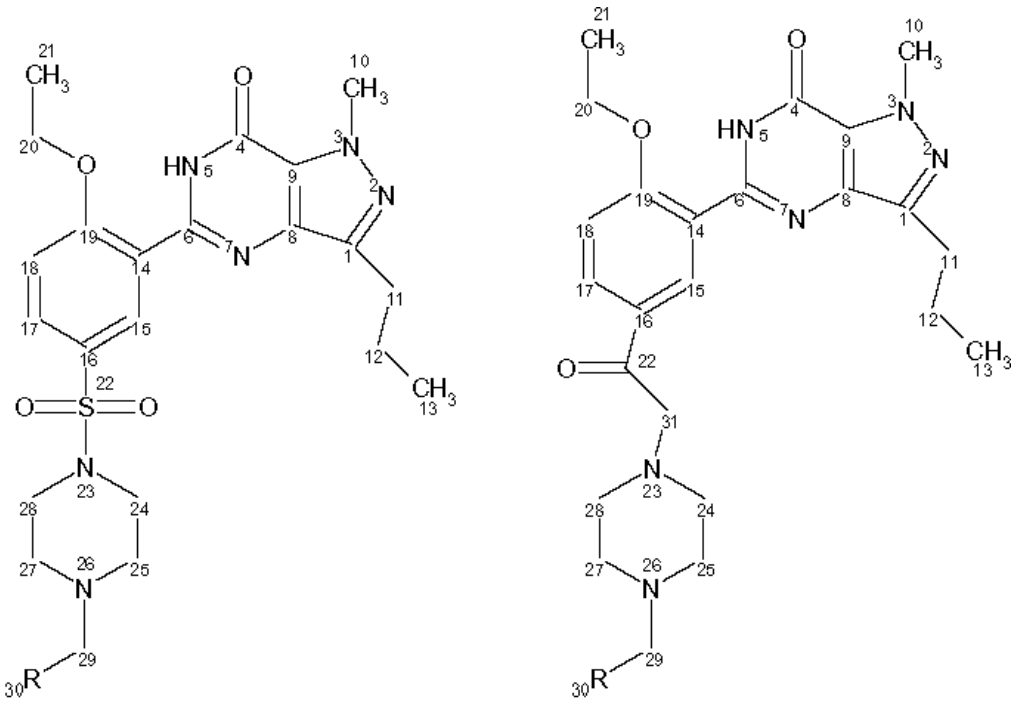

*Figure 1. Structures of sildenafil (**1**) and analogues homosildenafil (**2**) and hydroxyhomosildenafil (**3**). Structures: sildenafil (**1**), (1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]-sulfonyl]-4-methylpiperazine) R=H; **2**, (1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]-sulfonyl]-4-ethylpiperazine) R=CH_3; **3**, (1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo [4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]-sulfonyl]-4-(2-hydroxyethyl)-piperazine) R=CH_2OH; acetildenafil (**1**), (1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]-acetyl]-4-ethylpiperazine) R=CH_3.*

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Sample **A** was analysed for PDE5 inhibitors. Although sildenafil, vardenafil and tadalafil are absent, an abundant peak at a retention time of 0.8 relative to sildenafil is observed as well in the total scan chromatogram of the PDA as in the total ion current (TIC) chromatogram of the MS detector. The UV spectrum is very different from sildenafil, vardenafil and tadalafil (table 1); the most abundant peak in the MS^1 spectrum at that retention time is at m/z = 467, accompanied with a peak at m/z = 489, indicating the ions $[M + H]^+$ and $[M + Na]^+$ for a compound with a molecular mass of 466. Direct-infusion ESI-MS^2 showed that the fragmentation pattern of m/z 467 is very different from the patterns of $[M + H]^+$ of sildenafil, vardenafil or tadalafil (Bakker *et al*. 2004). The ions m/z = 311 and 313 are observed in the mass spectrum of $[M + H]^+$ of both **1** and sildenafil, but the relative intensities are less than 5% for both ions in **1** compared with 100 respectively 60% in sildenafil (figure 2a, b).

Table 1. Retention time (RRT, based on the photo diode array signal) and ultraviolet light (UV) maxima of sildenafil, vardenafil, tadalafil and compounds 1-3.

Compound (product)	RRT (min)	UV_{max} (nm)
Sildenafil (Viagra®)	1.0	230, 292
Vardenafil (Levitra®)	1.2	230†
Tadalafil (Cialis®)	1.5	230, 283
1 Product A	0.8	234, 279
2 Product B	1.0	230, 292
3 Product C	1.0	230, 292

†Maximum at 292 nm is absent; curving at 246 nm.

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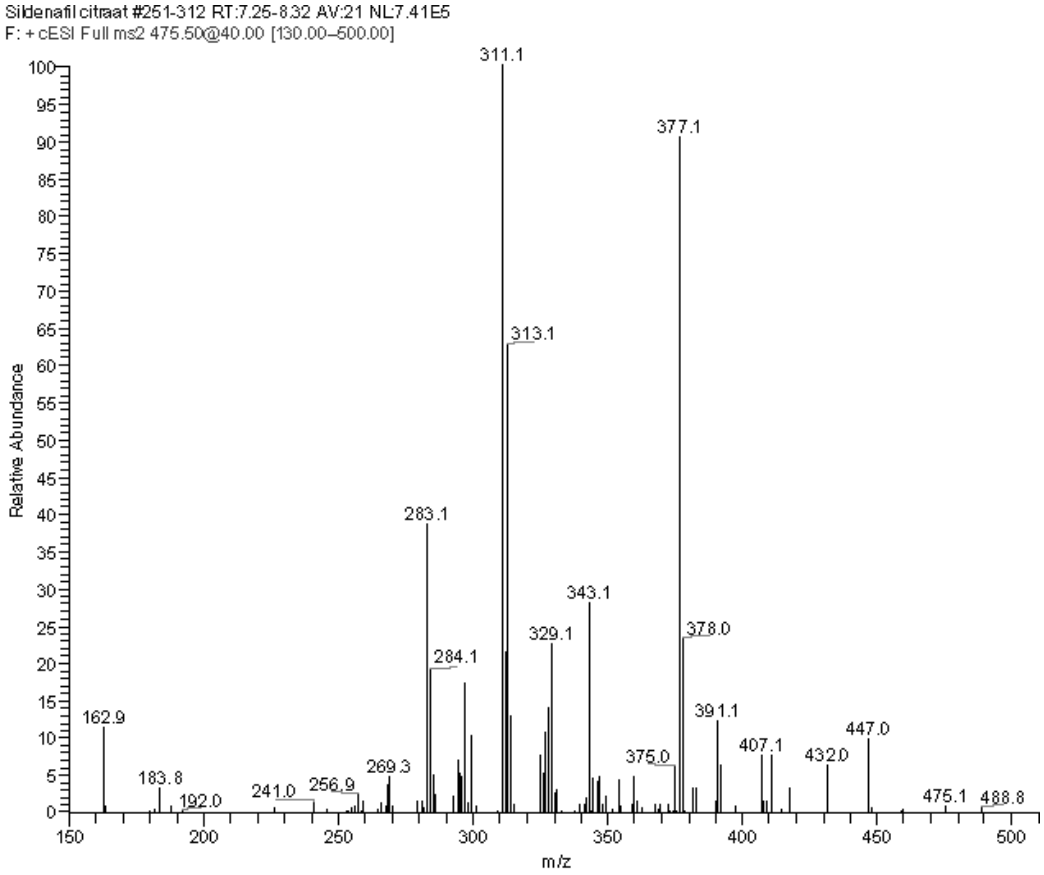
(a)



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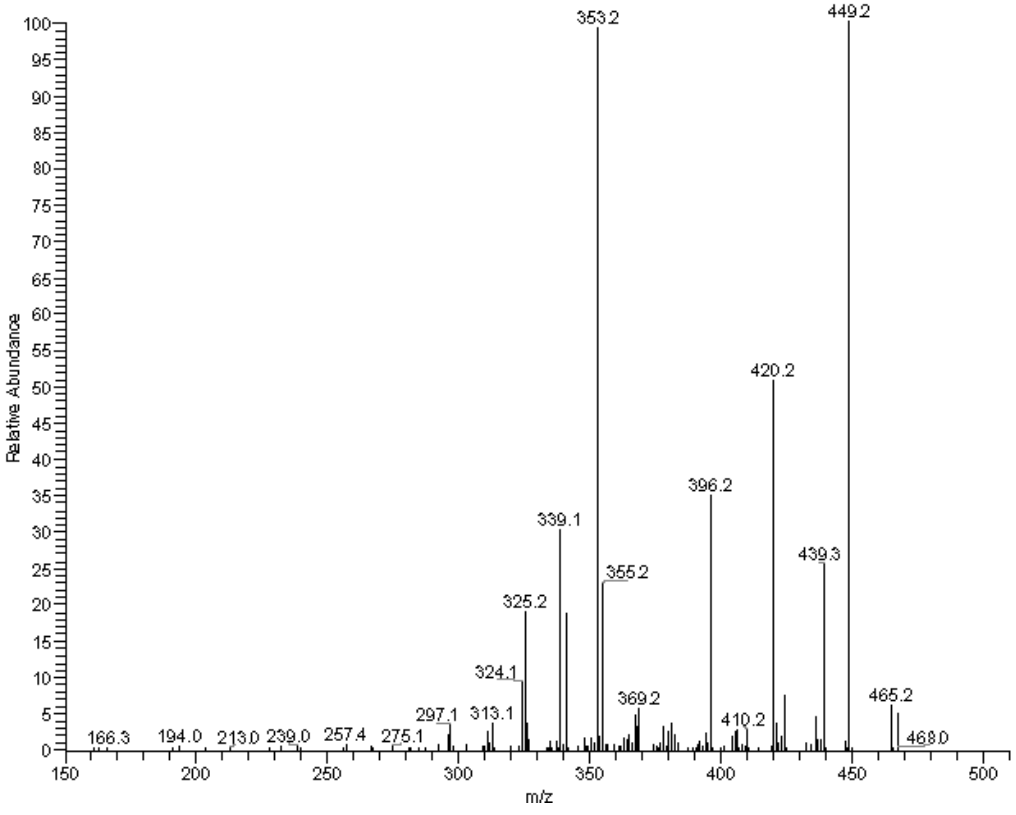

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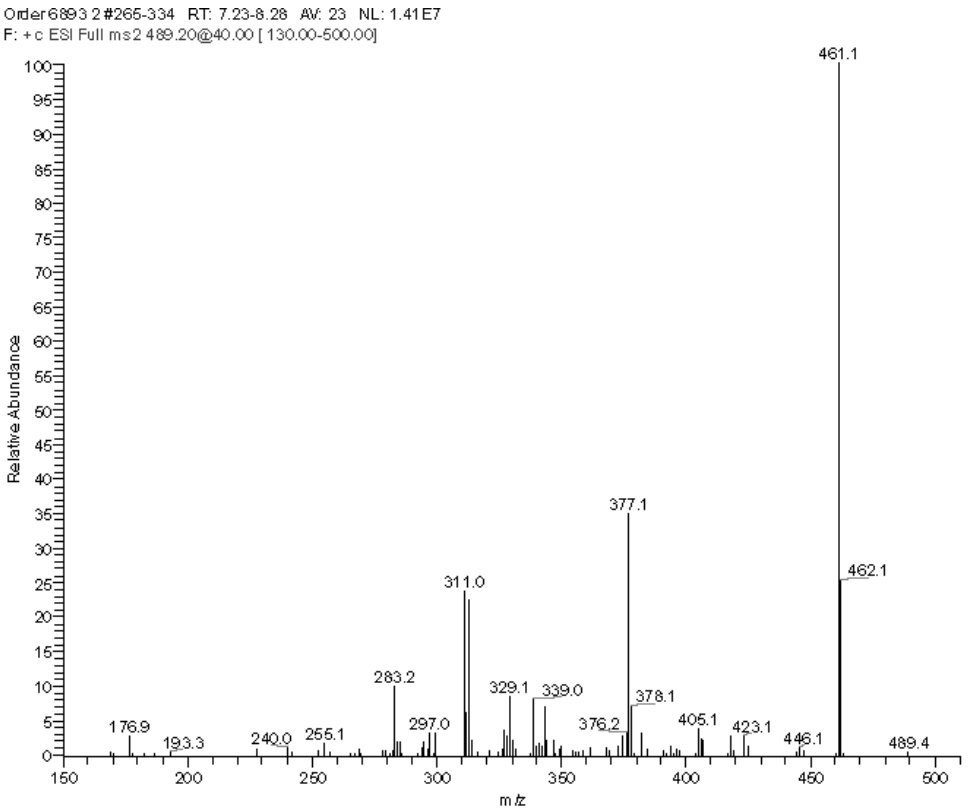
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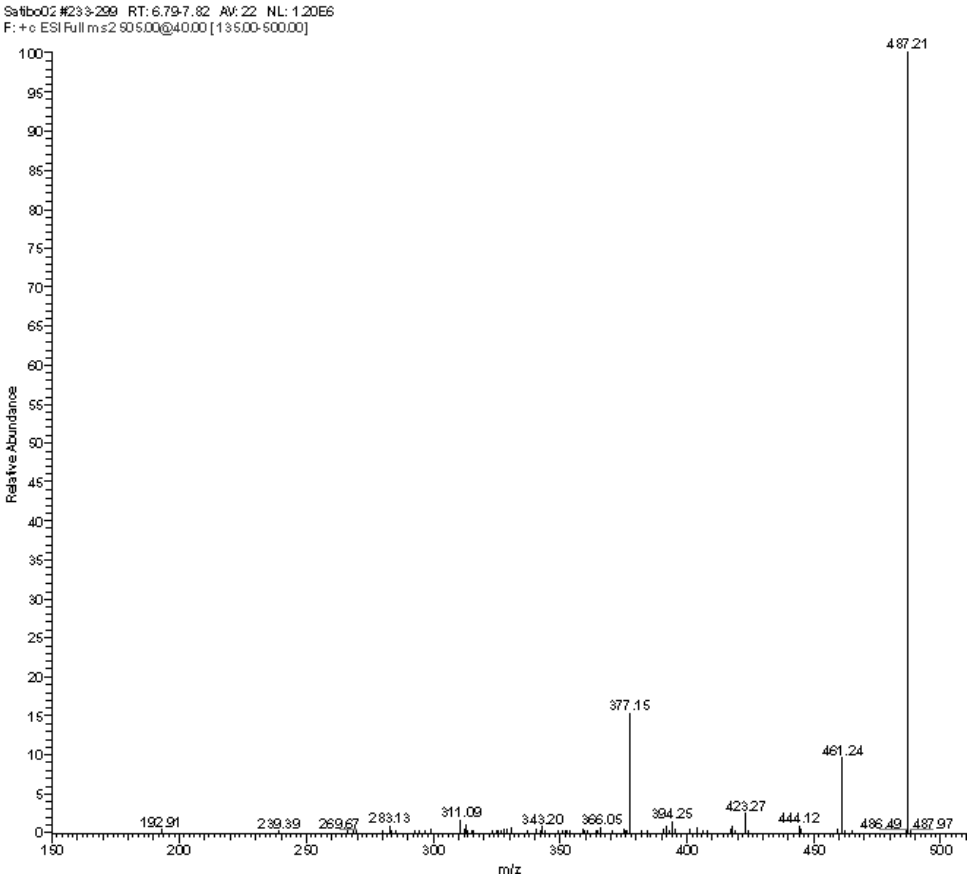
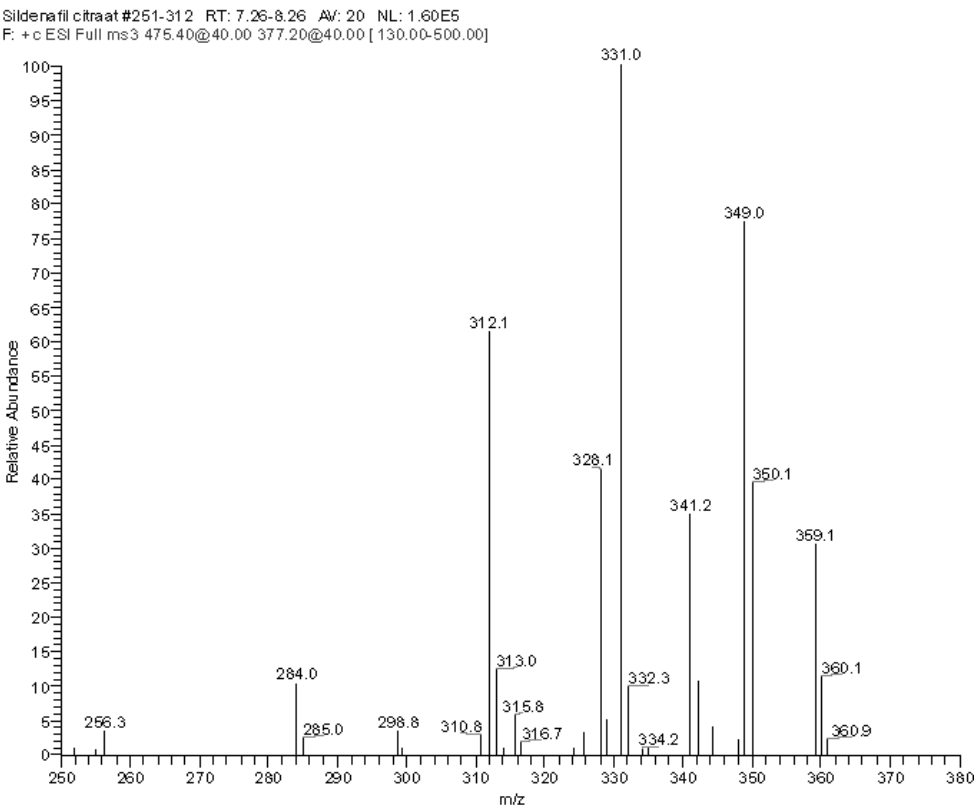
F: + c ESI Full ms2 489.20@40.00 [130.00-500.00]
Relative Abundance
m/z
461.1
377.1
462.1
311.0
283.2
329.1
339.0
378.1
405.1
423.1
446.1
489.4
176.9
193.3
240.0
255.1
297.0
376.2

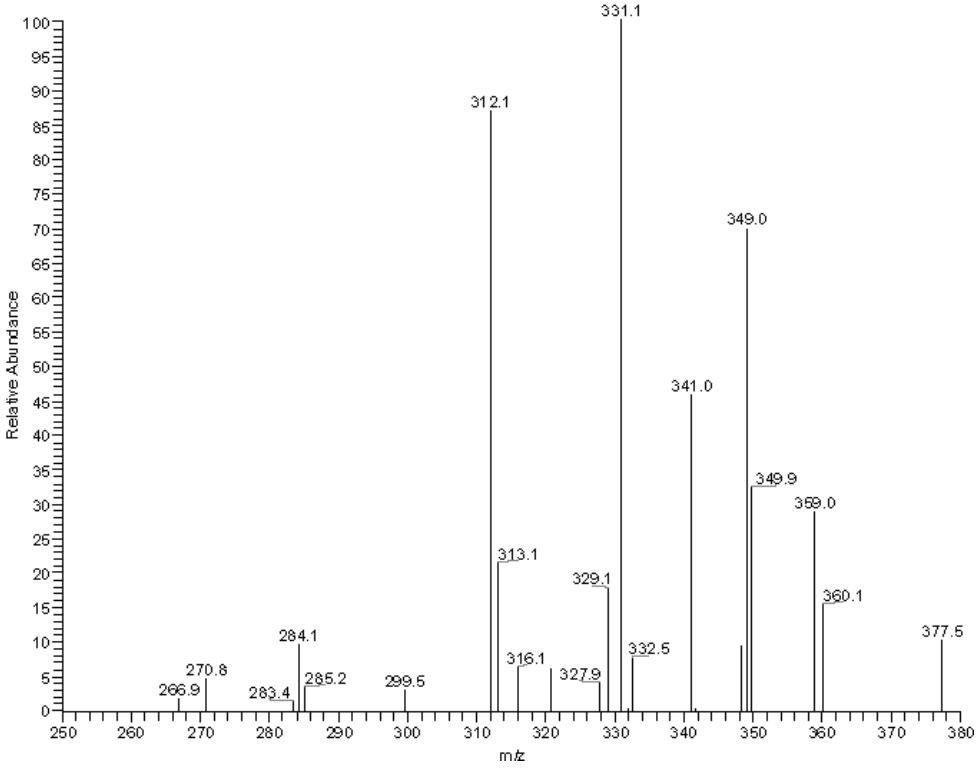
(d)


Satibo02 #233-299 RT: 6.79-7.82 AV: 22 NL: 1.20E6
F: + c ESI Full ms2 505.00@40.00 [135.00-500.00]
Relative Abundance
m/z
487.21
377.15
461.24
192.91
239.39
269.67
283.13
311.09
343.20
366.05
394.25
423.27
444.12
486.49
487.97

(e)


Sildenafil citraat #251-312 RT: 7.26-8.26 AV: 20 NL: 1.60E5
F: + c ESI Full ms3 475.40@40.00 377.20@40.00 [130.00-500.00]
Relative Abundance
m/z
331.0
349.0
312.1
328.1
350.1
341.2
359.1
313.0
284.0
332.3
360.1
315.8
256.3
285.0
298.8
310.8
316.7
334.2
360.9

(f)


Order6893 2 #265-334 RT: 7.24-8.28 AV: 23 NL: 1.14E6
F: + c ESI Full ms3 489.20@40.00 377.20@40.00 [130.00-500.00]
Relative Abundance
m/z
331.1
312.1
349.0
341.0
349.9
359.0
313.1
329.1
360.1
284.1
377.5
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316.1
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285.2
299.5
266.9
283.4

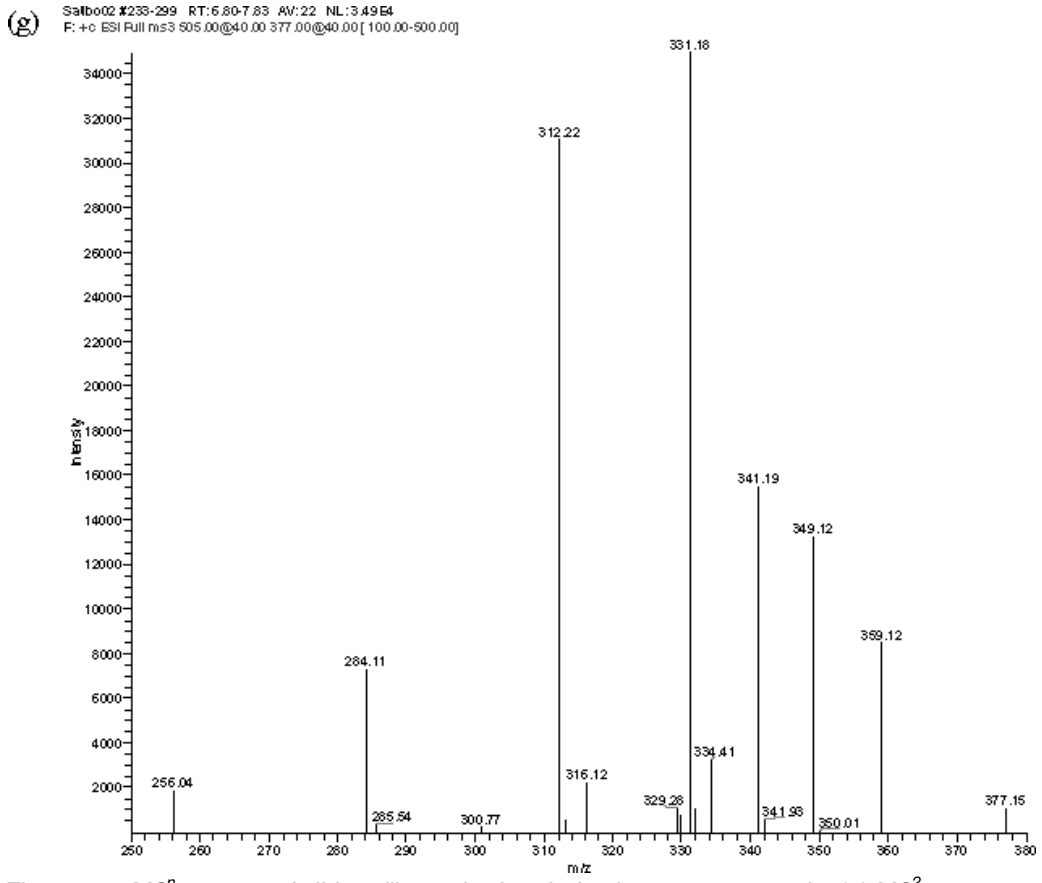

*Figure 2. MS^n spectra of sildenafil standard and of unknown compounds: (a) MS^2 spectrum of $[M + H]^+ = 475$ of sildenafil, (b) MS^2 spectrum of $[M + H]^+ = 467$ of **1** in Product A, (c) MS^2 spectrum of $[M + H]^+ = 489$ of **2** in Product B, (d) MS^2 spectrum of $[M + H]^+ = 505$ of **3** in Product C, (e) MS^3 spectrum of m/z = 475 → 377 of sildenafil, (f) MS^3 spectrum of m/z = 489 → 377 of **2** in Product B, (g) MS^4 spectrum of m/z = 505 → 489 → 377 of **3** in Product C.*

The difference in fragmentation behaviour can be explained by the results from the ^{1}H- and ^{13}C-NMR data (table 2). The ^{1}H-NMR spectrum (figure 3a) shows an amide proton at 10.8 ppm, three aromatic protons at 9.14, 8.15 and 7.09 ppm with a coupling pattern characteristic for a 1,2,4-substituted aromatic ring. Compared with sildenafil (table 3) (Badwan *et al*. 2001), the proton signals for H15 and H17 are shifted towards lower field consistent with a carbonyl group substituting the sulfonyl moiety. The *N*-methyl, *O*-ethyl and *C*-propyl groups give signals at the same field as sildenafil. The *N*-ethyl substituent shows signals at 1.05 and 2.41 ppm. The C23 methylene group gives a singlet at 3.82 ppm. Broad peaks at 2.54 and 2.68 could be assigned to the piperazinyl methylene resonances.

Table 2. Nuclear magnetic resonance data of compound 1.

C#	ppm	H#	ppm	Multiplicity
1	146.8			
4	153.7			
		5	10.8	1H, br, s
6	147.4			
8	138.5			
9	124.5			
10	38.2	10	4.27	3H, s
11	27.8	11	2.94	2H, t, *J* = 7.5 Hz
12	22.4	12	1.87	2H, m, *J* = 7.5 Hz
13	14.1	13	1.08	3H, t, *J* = 7.4 Hz
14	129.9			
15	132.3	15	9.14	1H, d, *J* = 2.2 Hz
16	120.0			
17	132.5	17	8.15	1H, dd, *J* = 8.8, 2.4 Hz
18	112.7	18	7.09	1H, d, *J* = 9.0 Hz
19	159.8			
20	65.8	20	4.36	2H, q, *J* = 7.0 Hz
21	14.6	21	1.63	3H, t, *J* = 7.0 Hz
22	194.9			
31	64.6	23	3.82	2H, s
24, 28	52.2		2.68	4H, br, m
25, 27	52.5		2.54	4H, br, m
29	53.3		2.41	2H, q, *J* = 7.3 Hz
30	11.8		1.05	3H, t, *J* = 7.0 Hz

Table 3. Nuclear magnetic resonance data of sildenafil citrate (Badwan et al. 2001).

C#	ppm	H#	ppm	Multiplicity
1	131.55			
4	153.56			
		5	10.83	1H, s
6	146.83			
8	146.36			
9	128.65			
10	45.57	10	4.15	3H, s
11	27.64	11	2.85	2H, t
12	22.15	12	1.78	2H, m
13	13.95	13	0.90	3H, t
14	124.39			
15	131.00	15	8.69	1H, d
16	121.01			
17	138.55	17	7.73	1H, d
18	112.90	18	7.07	1H, d
19	159.25			
20	65.97	20	4.29	2H, q
21	14.43	21	1.56	3H, t
24, 28	45.80	24, 28	3.03	4H, t
25, 27	53.91	25, 27	2.43	4H, t
29	76.69	29	2.24	3H, s

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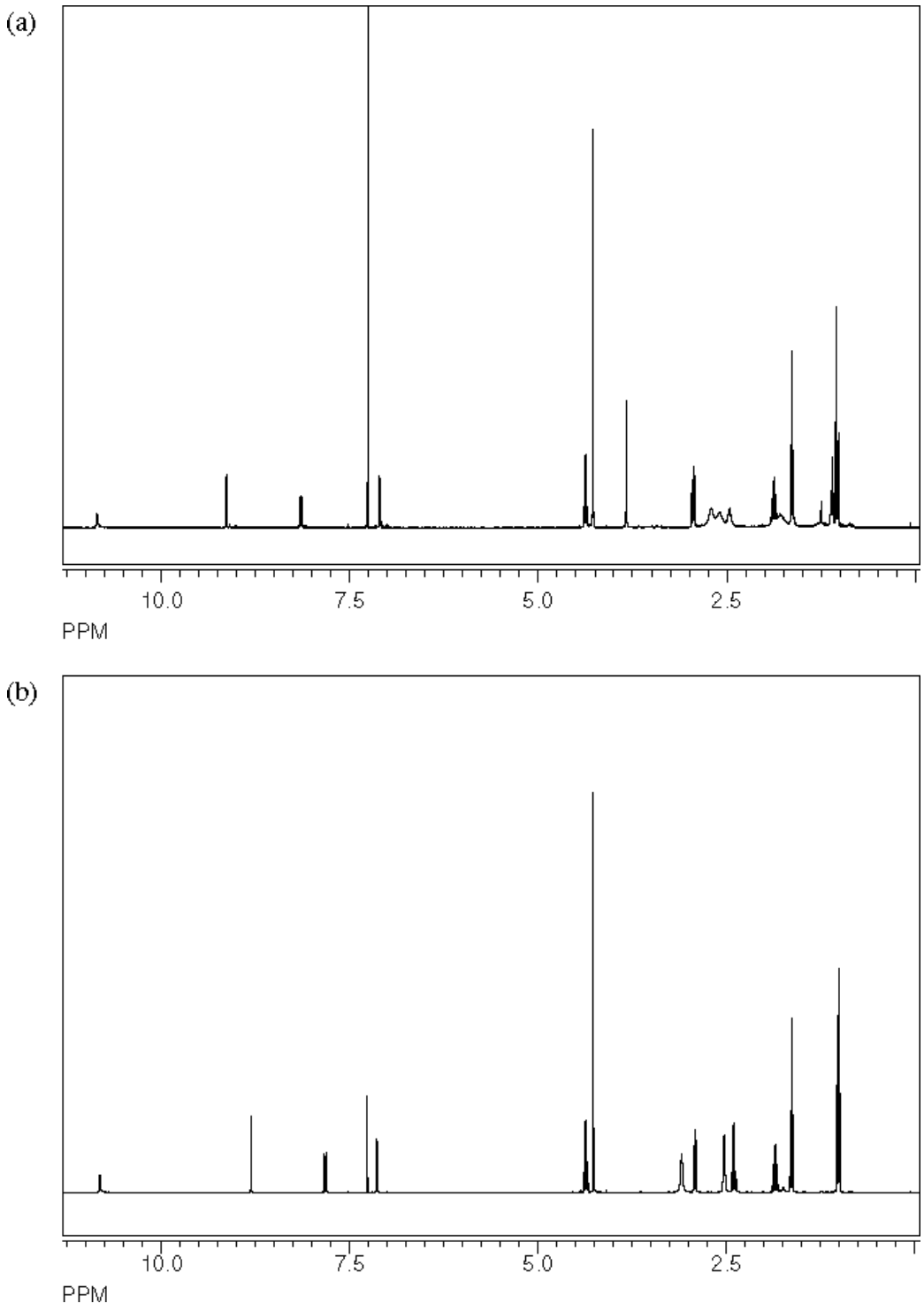

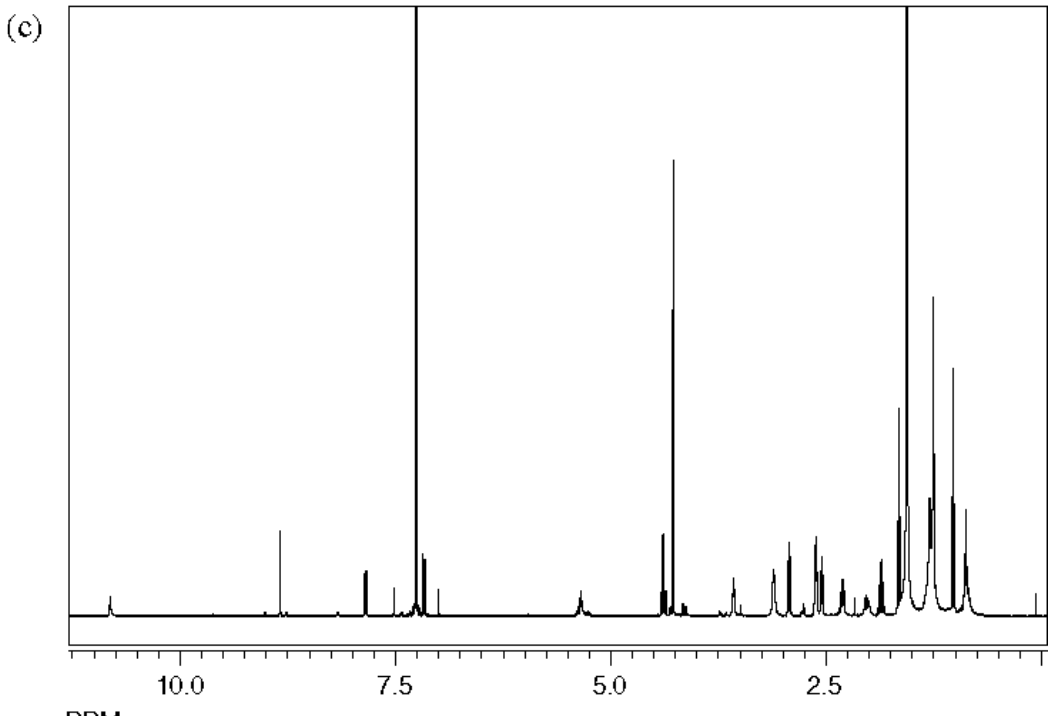

Figure 3. *^{1}H-NMR spectra of unknown compounds: (a) ^{1}H-NMR spectrum of **1** in Product A, (b) ^{1}H-NMR spectrum of **2** in Product B, (c) ^{1}H-NMR spectrum of **3** in Product C.*

The ^{13}C-NMR spectrum shows all the characteristic resonances for a 1-substituted [3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)-4-ethoxyphenyl structure element. Compared with sildenafil, the phenyl carbons show minor shifts, which is also indicative of a different substituent. The presence of a ketonic C=O is clear from the absorption at 194.9 ppm. Quantitative NMR analysis showed that sample **A** contained approximately 15% (w/w) of acetildenafil, which corresponds to 63 mg/capsule.

The IR spectrum shows peaks at 3315, 1697, 1604, 1494, 1393, 1261 and 1030 cm^{-1}. Bands, which could be attributed to a sulphonamide, are absent.

Sample *B*, compound *2*

Based on the LC-ESI-MS^{n} and ^{1}H- and ^{13}C-NMR data described below, the unknown compound was identified as a sildenafil analogue, in which the *N*-methylpiperazine moiety is substituted by *N*-ethylpiperazine (figure 1).

Sample **B** was analysed for sildenafil. Although a peak is observed showing retention time and ultraviolet light spectrum comparable with sildenafil (table 1), the MS^{1} spectrum does not show an ion at m/z = 475 representing $[M + H]^{+}$ of sildenafil; however, an ion at m/z = 489 is present. Although this corresponds to the molecular mass of vardenafil, the active substance of Levitra, a relatively new medicine for erectile dysfunction, its retention behaviour on this chromatographic system and ultraviolet light spectrum are different (Bakker *et al*. 2004).

MS^{2} spectra of $[M + H]^{+}$ of sildenafil (m/z = 475) and of the unknown compound (m/z = 489) were obtained (figure 2a, c). Both spectra show signals at m/z = 377, 313, 311 and 283. According to the fragmentation pathway proposed by Zhong *et al*. (2002), these ions lack the piperazine moiety. As the MS^{3} spectra of m/z = 377 of both compounds are comparable (figure 2e, f), this part of the molecule in both compounds is similar, suggesting that the difference of 14 amu (-CH_2-) should be in the piperazine moiety. The formation of m/z = 461

from 489 suggests the loss of C_2H_4, indicating the presence of an ethyl group. These findings are consistent with the ^{1}H- and ^{13}C-NMR data (figure 3b and table 4). These data are in agreement with the data of Shin *et al*. (2003).

Table 4. Nuclear magnetic resonance data of compound 2.

C#	ppm	H#	ppm	Multiplicity
Atoms in **bold** show correlation peaks.				
1	146.37			
4	153.56			
		5	10.81	1H, bs
6	146.86			
8	138.28			
9	124.39			
10	38.12	**10**	4.26	3H, s
11	27.65	**11**	2.92	2H, t, *J* = 7.5 Hz
12	22.16	**12**	1.85	2H, m, *J* = 7.5 Hz
13	13.95	**13**	1.02	3H, t, *J* = 7.3 Hz
14	128.57			
15	131.08	**15**	8.81	1H, d. *J* = 2.4 Hz
16	121.02			
17	131.63	**17**	7.82	1H, dd, *J* = 8.8, *J* = 2.5 Hz
18	112.94	**18**	7.13	1H, d, *J* = 8.8 Hz
19	159.23			
20	65.98	**20**	4.36	2H, q, *J* = 7 Hz
21	14.44	**21**	1.64	3H, t, *J* = 7 Hz
24	46.01	**24**	3.09	4H, bs
25	51.71	**25**	2.53	4H, bs
29	51.80	**29**	2.40	1H, q, *J* = 7.2 Hz
30	11.85	**30**	1.02	3H, t, *J* = 7.3 Hz

Based on the PDA total scan chromatogram and the assumption that the extinction coefficient of sildenafil and homosildenafil are comparable, the amount of homosildenafil in sample **B** was estimated at 142 mg/tablet.

Sample *C*, compound *3*

Based on LC-ESI-MS^n, direct-infusion ESI-MS^n, and ^{1}H- and ^{13}C-NMR data, the compound was identified as a sildenafil analogue bearing an *N*-hydroxylethylpiperazine moiety instead of an *N*-methylpiperazine (figure 1).

Sample **C** was analysed for PDE5 inhibitors. An abundant peak is observed with a retention time and ultraviolet light spectrum comparable with sildenafil. In de MS^1, spectrum peaks are present at m/z = 505, 527 and 543, indicating $[M + H]^+$, $[M + Na]^+$ and $[M + K]^+$ for a compound with a molecular mass of 504. The MS^2 spectrum of $[M + H]^+$ (figure 2d) shows peaks at m/z = 487 (loss of H_2O), 461 (loss of CH_2=CHOH), and 377. M/z = 461 is also observed in the MS^2 spectrum of m/z = 489 ($[M + H]^+$) of homosildenafil, indicating loss of CH_2=CH_2. M/z = 377 is observed in the MS^2 spectra of $[M + H]^+$ of sildenafil and homosildenafil, representing the remaining ion after loss of the piperazine moiety. The MS^3 spectrum of m/z = 505 $\rightarrow$377 from the unknown compound in sample **C** obtained by direct-infusion ESI-MS (figure 2g) shows resemblance to the MS^3 spectra of m/z = 377 of sildenafil and homosildenafil. Based on these results, the structure could be hydroxyhomosildenafil. This proposal is supported by the ^{1}H- and ^{13}C-NMR data, which are presented in figure 3(c) and table 5.

Table 5. Nuclear magnetic resonance data of compound 3.

C#	ppm	H#	ppm	Multiplicity
Atoms in **bold** show correlation peaks.				
1	146.39			
4	153.58			
		5	10.81	1H, bs
6	146.95			
8	138.33			
9	124.46			
10	38.22	**10**	4.28	3H, s
11	27.71	**11**	2.93	2H, t, *J* = 7.4 Hz
12	22.23	**12**	1.86	2H, h, *J* = 7 Hz
13	14.02	**13**	1.03	3H, t, *J* = 7.3 Hz
14	128.82			
15	131.13	**15**	8.84	1H, d, *J* = 2.4 Hz
16	121.12			
17	131.64	**17**	7.84	1H, dd, *J* = 8.8, *J* = 2.4 Hz
18	113.07	**18**	7.17	1H, d, *J* = 8.8 Hz
19	159.34			
20	66.09	**20**	4.39	2H, q, *J* = 7 Hz
21	14.52	**21**	1.66	3H, t, *J* = 7 Hz
24,28	46.11	**24,28**	3.1	4H, bs
25,27	51.93	**25,27**	2.62	4H, bt, *J* = 5.4 Hz
29	58.90	**29**	2.55	2H, t, *J* = 5.4 Hz
30	57.74	**30**	3.58	2H, t, *J* = 5.4 Hz
		OH	2.3	1H, bs

Quantitative NMR analysis showed that sample **C** contained approximately 10% (w/w) hydroxyhomosildenafil, which corresponds to 45 mg/capsule.

As three different sildenafil analogues were identified over a short period, it seems there is a tendency towards designer drugs for Viagra counterfeits and herbal aphrodisiacs being distributed on the market. When analysing these products, attention should be given to the possible presence of this type of components. Only screening for the active ingredients of prescription drugs such as sildenafil and vardenafil is not sufficient anymore.

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