Journal of Pharmaceutical and Biomedical Analysis 57 (2012) 104–108

Contents lists available at SciVerse ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Isolation and structural elucidation of flibanserin as an adulterant in a health supplement used for female sexual performance enhancement

Min-Yong Low[a,b], Lin Li[a,b], Xiaowei Ge[b], Chee-Leong Kee[b], Hwee-Ling Koh[a,*]

[a] Department of Pharmacy, Faculty of Science, National University of Singapore, 18 Science Drive 4, Singapore 117543, Singapore
[b] Pharmaceutical Division, Applied Sciences Group, Health Sciences Authority, 11 Outram Road, Singapore 169078, Singapore

ARTICLE INFO

Article history:
Received 5 May 2011
Received in revised form 12 August 2011
Accepted 13 August 2011
Available online 30 August 2011

Keywords:
Health supplement
Flibanserin
LC-LTQ Orbitrap XL FTMS
NMR

ABSTRACT

A health supplement used for female sexual performance enhancement was sent to Health Sciences Authority of Singapore for testing. An unknown compound was detected and isolated from the health supplement and its structure was elucidated using LC-DAD, LC-FTMS, NMR and IR. The detected compound was identified to be flibanserin, a non-hormonal treatment developed for pre-menopausal woman with hypoactive sexual desire disorder (HSDD).

1. Introduction

Sexual dysfunction is more prevalent for women (43%) than men (31%) and is associated with various demographic characteristics, including age and educational attainment [1]. Hypoactive sexual desire disorder (HSDD) is a form of Female Sexual Dysfunction (FSD). HSDD is defined as a persistent or recurrent deficiency (or absence) of sexual fantasies/thoughts and desire for sexual activity causing marked distress or interpersonal difficulty [2]. Up to 10% of women in the USA suffer from FSD of which HSDD is the major subindication [3]. Sexual Desire Disorders are generally underdiagnosed as many women are reluctant to discuss sexual issues with their physicians and have low expectations concerning the prospects for help [1,4]. Currently, no pharmacologic therapy is approved by US FDA for the treatment of HSDD. With no approved HSDD therapies available to women, women with reservations to seek help from their physicians may turn to natural health products for help. As a result, health supplements for treating FSD have become increasingly popular in recent years as they are believed to be natural with no side effects. Many of these products claim to be libido enhancer that stimulates blood flow, circulation and hormone activity which result in a more enjoyable orgasm experience for women.

In this paper, we report the detection and isolation of flibanserin, a compound developed to treat HSDD [5–7], from a natural health product used for female sexual performance enhancement and its structural elucidation using LC-DAD, LC-FTMS, IR and NMR.

2. Materials and methods

2.1. Sample and chemicals

A health supplement, named MMP, was sent from an overseas company to the Health Sciences Authority (HSA) of Singapore for testing. It is presented as transparent capsules with white powder. Methanol (AR grade) and acetonitrile (HPLC grade) were supplied by Lab-Scan Analytical Sciences (Patumwan, Bangkok, Thailand). Methanol (HPLC grade) was supplied by RCI Labscan Limited (Thailand). Dimethylsulfoxide (DMSO) was supplied by Sigma–Aldrich (Steinheim, Germany). 0.45 μm nylon membrane filters were supplied by Whatman International Ltd. (Maidstone, UK). Ultrapure water was obtained using a Elga Purelab Water Purification System. Sodium dihydrogen phosphate was supplied by Sigma–Aldrich (Steinheim, Germany). Deuterated formic acid and deuterated chloroform used for NMR analysis were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Extraction of sample

For LC-DAD, 1 g of the capsular contents (white powder) were ultrasonically extracted in 10 ml methanol (AR grade) for 30 min. 1 ml extract was further diluted to 10 ml with methanol (AR grade) and filtered through the 0.45 μm PTFE membrane filter. For LC-LTQ Orbitrap XL FTMS, NMR and IR analysis, 3.3 g of the

* Corresponding author. Tel.: +65 6516 7962; fax: +65 6779 1554.
E-mail address: phakohhl@nus.edu.sg (H.-L. Koh).

0731-7085/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2011.08.027

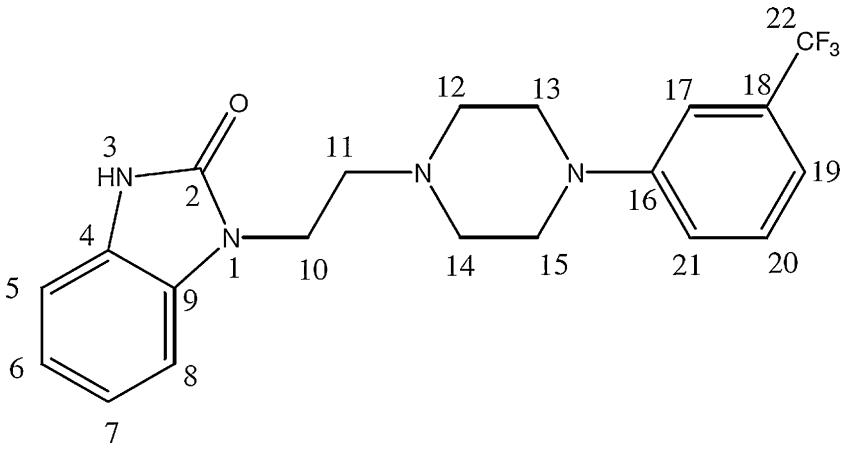

Fig. 1. Chemical structure of flibanserin.

capsular contents were ultrasonically extracted in 40 ml methanol (AR grade) for 30 min. The extract was filtered and the solvent was evaporated using rotary evaporator. The residue was reconstituted in 1.5 ml DMSO and filtered through 0.45 μm PTFE membrane filter. The filtered sample was further purified using preparative HPLC and the purified compound of interest was analyzed by LC-LTQ Orbitrap XL FTMS, NMR and IR.

2.3. Preparative HPLC

A Shimadzu HPLC system with two preparative pumps (LC-8A, Kyoto, Japan) and an automatic fraction collector (FRC-10A, Kyoto, Japan) were used. An Agilent ZORBAX SB-C18 reversed phase semi-preparative column (250 mm × 9.4 mm i.d., 5 μm) was used for the sample separation. The mobile phases were water and acetonitrile (HPLC grade). The gradient elution profile was as follows: acetonitrile was increased from 20% to 90% in 15 min and maintained for 3 min. The flow rate of mobile phase was 4 ml/min and injection volume was 50 μl. Column oven temperature was set at 40 °C. The UV and visible spectra from 190 to 600 nm were recorded on-line during the chromatographic run. Fractions containing the target compound were collected by the automatic fraction collector based on UV detection (254 nm). The solvent was removed using a rotary evaporator and the residue was freeze dried using Christ Alpha 2-4 LD Plus Freeze Dryer (Osterode, Germany).

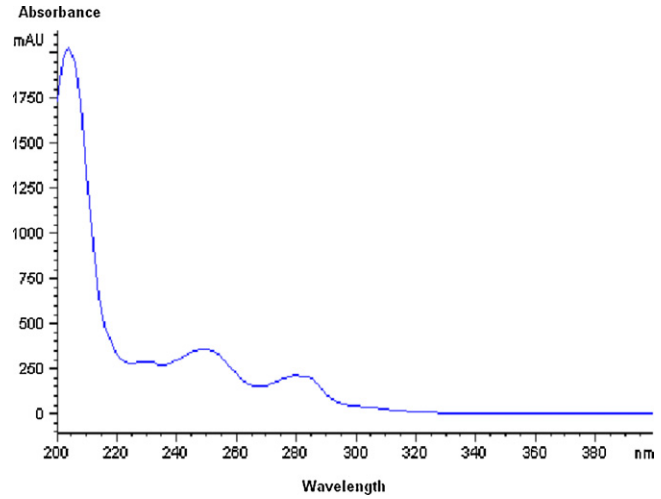

Fig. 2. UV–vis spectrum of flibanserin in methanol, scanned from 200 nm to 400 nm, showing the maximal absorbances at 210 nm, 230 nm, 250 nm and 280 nm.

2.4. LC-DAD analysis

An Agilent 1100 series HPLC chromatograph with diode-array detector (Palo Alto, CA, USA) was employed. A BDS Hypersil C18 column (200 mm × 4.6 mm i.d., 5 μm) from Thermo Scientific (USA) was used. Mobile phase A consisted of 25 mM sodium dihydrogen phosphate in water, adjusted to pH 3.2 ± 0.1 with phosphoric acid. Mobile phase B was acetonitrile (HPLC grade). The gradient elution profile was as follows: 0–30 min, acetonitrile rose from 10% to 70% (v/v), and maintained for 5 min. The flow rate of mobile phase was 1 ml/min. The injection volume was 10 μl. The UV spectra from 200 to 400 nm were recorded on-line during the chromatographic run. The chromatograms of both original methanol extract and purified compound were recorded at the wavelength 254 nm.

2.5. LTQ Orbitrap XL FTMS analysis

The isolated compound was dissolved in methanol (HPLC grade) at a concentration of 0.5 μg/ml and was injected to Thermo Fischer Scientific LTQ Orbitrap XL™ hybrid FTMS System (Bremen, Germany) controlled by Xcalibur (Version 2.0.7) software. The ESI ionization source was operated in the positive ion mode with spray voltage set at 3 kV, sheath gas flow rate at 40 arb, auxiliary gas flow rate at 10 arb, capillary voltage and temperature at 39 V and 250 °C respectively. The tube lens were set at 120 V, mass range was set to 200–500 Da with a resolution of 30,000. The high-resolution MS spectrum was acquired by direct infusion with a flow rate of 5 μl/min. The $[M+H]^+$ was selected as a precursor ion and the MS^2 spectrum was acquired with Collision energy (CE) set at 45 V under HCD (High energy Collision Dissolution) mode.

2.6. NMR and IR analyses

The isolated compound was dissolved in deuterated chloroform ($CDCl_3$) for NMR analysis. ^{13}C and DEPT spectra were recorded on a Bruker AMX 500 spectrometer (Bruker BioSpin, Rheinstetten, Germany). ^{1}H, COSY, HMQC and HMBC spectra were recorded on a Bruker DRX 500 spectrometer (Bruker BioSpin, Rheinstetten, Germany). Chemical shifts were reported in ppm using the solvent peak as an internal standard. IR spectrum in KBr disc was recorded on a Nicolet 6700 FTIR spectrometer (Madison, WI, USA) and recorded over the spectral range 4000–400 cm^{-1}.

3. Results and discussion

Approximately 10 mg of white amorphous powder was isolated from 3.3 g of MMP powder. Fig. 1 shows its proposed chemical

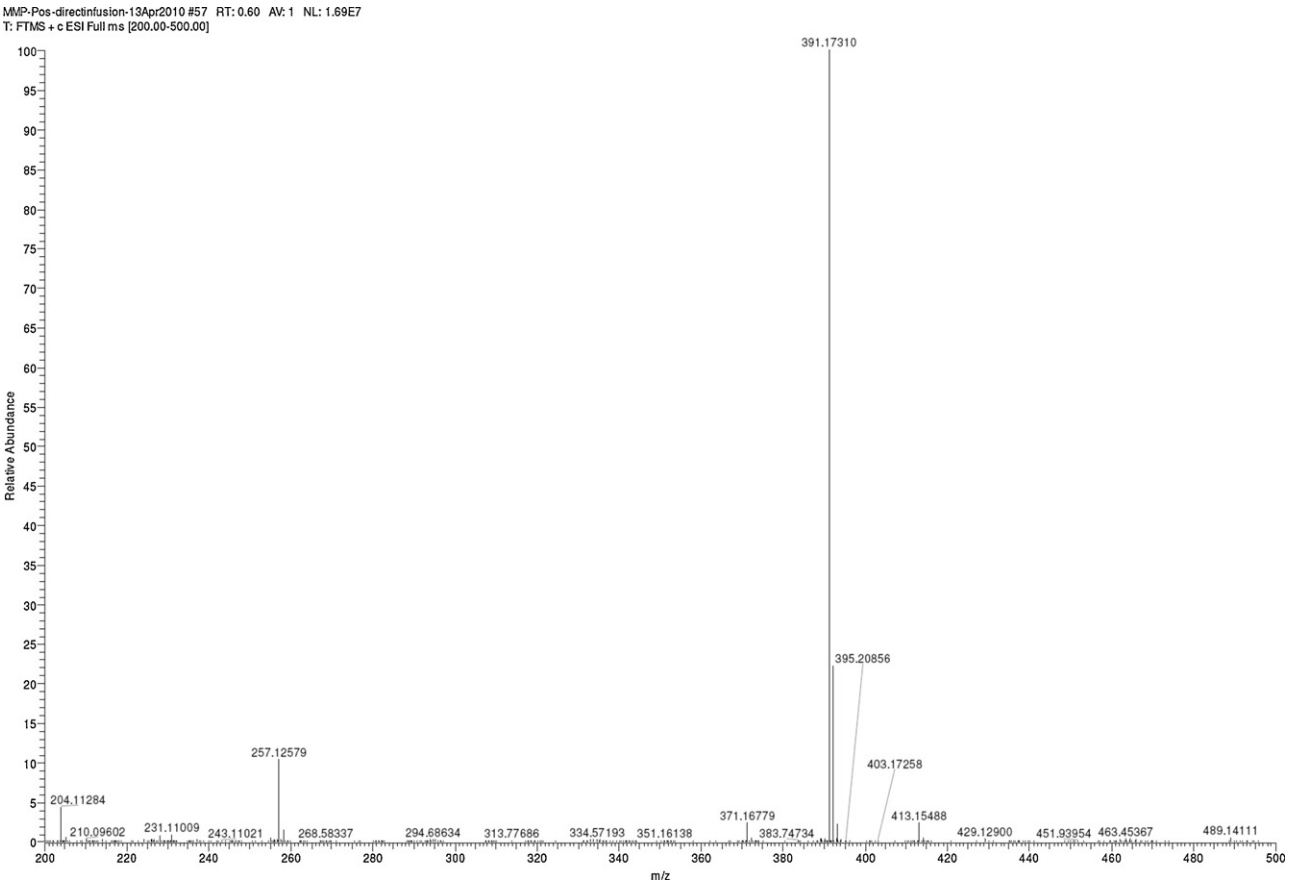

Fig. 3. High resolution MS spectrum of flibanserin.

structure. As shown in Fig. 2, the UV spectrum of isolated compound in methanol showed maximal absorbances at 210 nm, 230 nm, 250 nm and 280 nm. The retention times for the compound in both the extract and purified sample were 17.2 min and 17.4 min respectively under the LC-DAD chromatographic conditions.

High-resolution ESI-MS spectrum of the compound in Fig. 3 revealed $[M+H]^+$ at m/z 391.17310, suggesting a molecular formula of $C_{20}H_{21}F_3N_4O$. The error between observed mass and theoretical mass of $[M+H]^+$ was −2.352 ppm. This molecular formula was further supported by ^1H and ^{13}C NMR data which indicated the presence of 20 carbon atoms and 21 protons. 1D and 2D NMR (Table 1) data were used to assign protons and carbons.

^1H NMR data and their HMBC correlations were used to assign the protons. ^{13}C and DEPT NMR data showed signals of six

Table 1
NMR data for flibanserin.

No.	^1H (δ_H)	^{13}C (δ_C)	DEPT[b]	COSY	HMBC
2	–	155.6	0	–	–
3	10.00 (1H, s)	–	–	–	C-2, C-4, C-9
4	–	128.1	0	–	–
5	7.06 (1H, m)	108.0	1	–	C-4
6[a]	7.10 (1H, m)	121.3	1	–	[c]
7[a]	7.10 (1H, m)	121.6	1	–	[c]
8	7.11 (1H, dd, J = 6.3, 1.6)	110.0	1	–	C-6, C-7, C-9
9	–	130.3	0	–	–
10	4.08 (2H, t, J = 6.8)	38.5	2	H-11	C-2, C-9, C-11
11	2.80 (2H, t, J = 6.8)	55.7	2	H-10	C-10, C-12, C-14
12, 14	2.74 (4H, t, J = 4.5)	53.1	2	H-13, H-15	C-11, C-13, C-15, C-12/C-14,
13, 15	3.22 (4H, brs)	48.6	2	H-12, H-14	C-12, C-14, C-13/C-15, C-16
16	–	151.3	0	–	–
17	7.09 (1H, m)	112.2	1	–	[c]
18[a]	–	123.2	0	–	–
19	7.01 (1H, dd, J = 8.0, 2.0)	115.8	1	H-20	C-17
20	7.31 (1H, t, J = 8.0)	129.5	1	H-21/H-19	C-16, C-19, C-21
21	7.07 (1H, m)	118.7	1	H-20	[c]
22[a]	–	125.4	0	–	–

δppm in $CDCl_3$, J in Hz.
[a] Overlapping signals.
[b] Number of attached protons.
[c] Correlation cannot be accurately assigned due to overlapping signals.

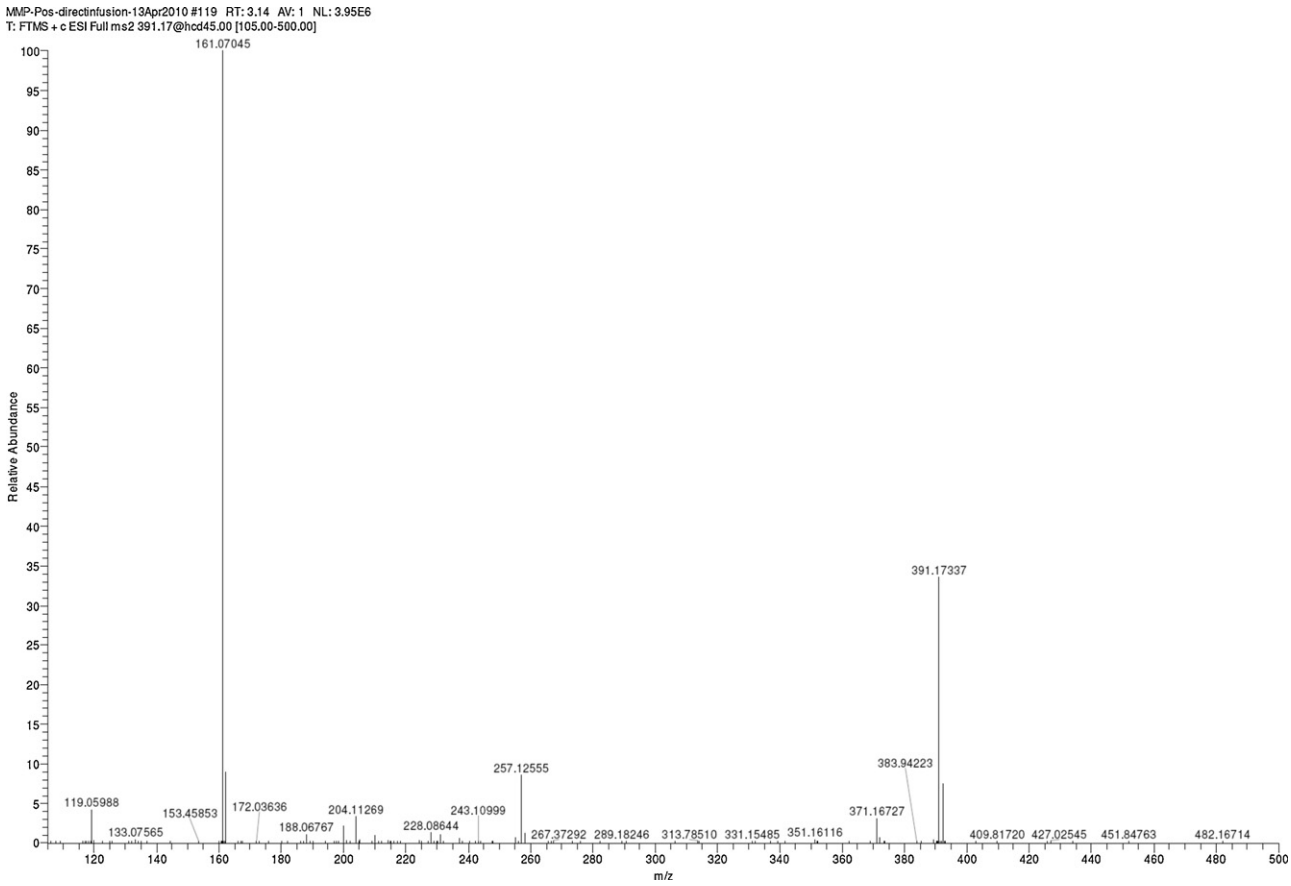

Fig. 4. High resolution ESI MS/MS spectrum of flibanserin.

methylene, eight methine and six quaternary carbon atoms. The DEPT signals at 53.1 ppm and 48.6 ppm were twice as intense as those from the rest of the methylene carbon signals, suggesting two methylene carbons each at signals 53.1 ppm and 48.6 ppm. HMQC correlated these methylene carbons to proton signals at 2.73 ppm and 4.08 ppm respectively, which corresponded to four protons each. A total of six methylene carbon atoms were hence confirmed with overlapping methylene carbon signals at 53.1 ppm and 48.6 ppm. The ^{13}C NMR data (Table 1) showed that there were two quaternary carbons at 155.6 ppm and 151.3 ppm. The carbon with chemical shift 155.6 ppm was C-2. In the structure of imidazolone, carbonyl carbon C-2 was attached to two nitrogen atoms which helped to withdraw electrons from oxygen to C-2. Hence, C-2 was less deshielded as compared to a normal carbonyl carbon which typically has chemical shift above 170 ppm. Eight methine carbons and two quaternary carbons with chemical shifts above 108 ppm suggested the presence of two aromatic rings. The quaternary carbon with chemical shift 125.4 ppm was C-22 which was attached to three fluorine atoms. Due to the strong electron withdrawing effect of the fluorine atoms, C-22 was highly deshielded and had a high chemical shift. The NMR results suggested the unknown compound to be 1-[2-[4-(3-trifluoromethyl phenyl) piperazin-1-yl] ethyl] benzimidazol-[1H]-2-one, which is also known as flibanserin.

This structure was further confirmed by high-resolution ESI-MS/MS. The high-resolution MS^2 spectrum in Fig. 4 showed the major fragments at *m*/*z* 161.07045, 204.11269, 243.10999, 257.12555 and 371.16727. The fragmentation process is indicated in Fig. 5, which is confirmed by Mass Frontier™ 5.0 (HighChem, Ltd., Slovak Republic). All proposed fragments have mass errors below 5 ppm.

The IR spectrum of the isolated compound showed absorption bands of amide ($\nu_{C=O}$ 1685 cm^{-1}, $\nu_{N-H\,(stretch)}$ 3180 cm^{-1}, $\nu_{N-H\,(bending)}$ 1610 cm^{-1}), alkyl fluoride (ν_{C-F} 1077 cm^{-1}, 1112 cm^{-1}, 1158 cm^{-1}), aromatic ring (ν_{Ar-H} 3028 cm^{-1}, 3078 cm^{-1} and $\nu_{C=C}$ 1401 cm^{-1}, 1446 cm^{-1}, 1453 cm^{-1}, 1468 cm^{-1}, 1487 cm^{-1}) and alkane (ν_{C-H} 2891 cm^{-1}, 2930 cm^{-1} 2948 cm^{-1}). The IR spectrum further supported the structure of flibanserin.

Flibanserin, a serotonin-1A (5-HT (1A)) receptor agonist and the serotonin-2A (5-HT (2A)) receptor antagonist is being developed as a novel, non-hormonal treatment for pre-menopausal women with HSDD [5–7]. However, the FDA Advisory Committee for Reproductive Health Drugs rejected the New Drug application as the efficacy was deemed not sufficiently robust to justify the risks and there were concerns over the safety signals and potential drug interactions involving flibanserin [8]. Data on the long term use of flibanserin and further documentation of efficacy will be needed for reconsideration. Women suffering from FSD and are deceived into taking herbal aphrodisiacs adulterated with flibanserin are being put at risk of experiencing side effects from this unapproved drug. The highest health risk is with consumers not expecting potentially serious drug–drug interactions.

As the sample was sent to the laboratory from an overseas company with the intention to market the product in Singapore, it is likely that the product is a pre-existing product in other countries, using the same or different product names. The problem can hence be a more widespread one and the finding will be useful to regulators, researchers and healthcare practitioners. They will be alerted on the possible adulteration of flibanserin, an unapproved drug in health products claimed for woman sexual performance enhancement. The methods used in this study also constitute a good tool in the detection of adulterant in health supplement and a combination

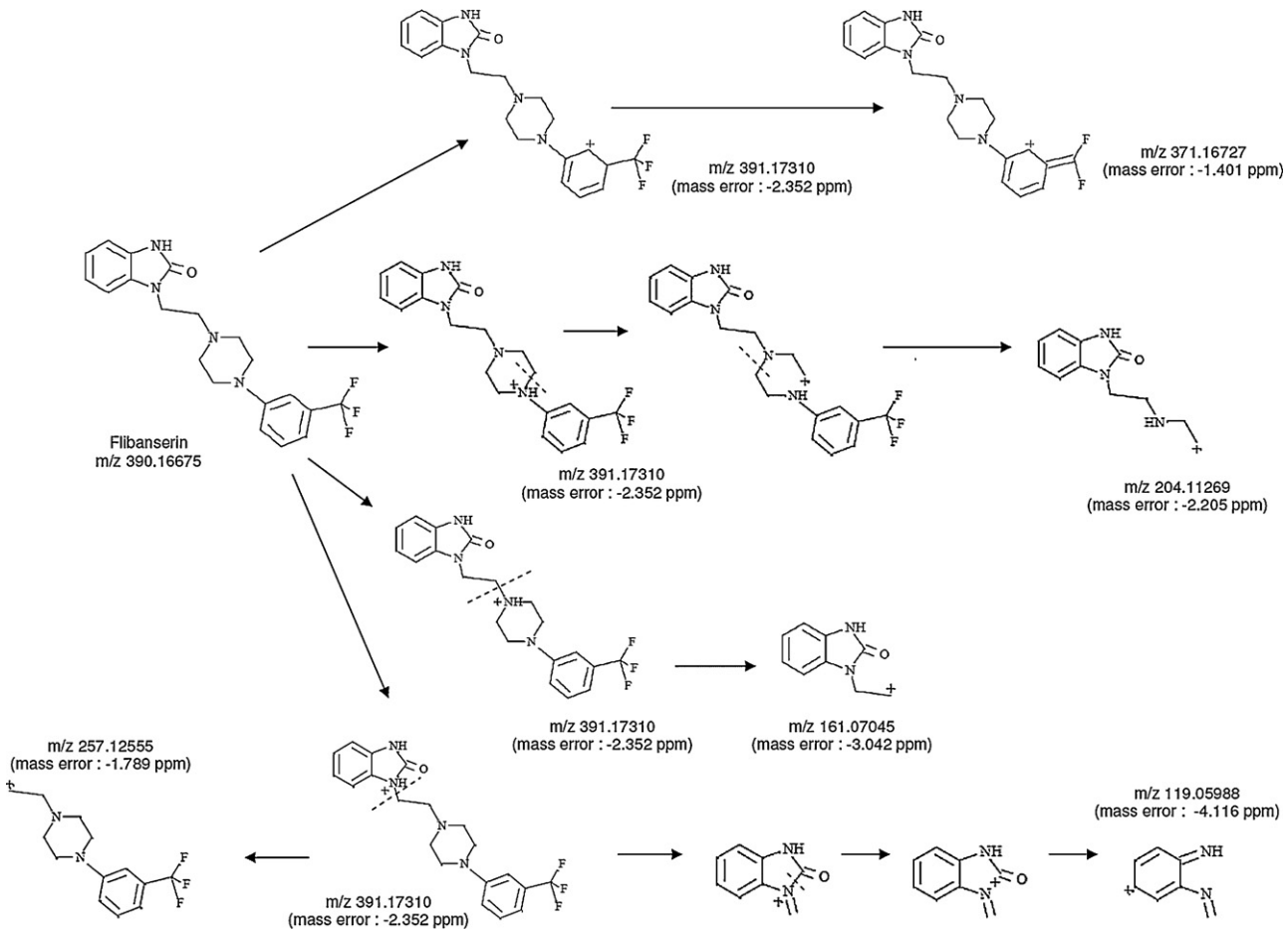

Fig. 5. Proposed ESI-MS/MS fragmentation of the protonated molecules of flibanserin ($[M+H]^+$ m/z 391.17310), further confirmed by Mass Frontier™ 5.0.

of up-to-date analytical procedures is nowadays highly necessary for such challenging tasks.

4. Conclusion

In this study, a serotonin-1A (5-HT (1A)) receptor agonist and the serotonin-2A (5-HT (2A)) receptor antagonist, flibanserin, was isolated from a health supplement and its chemical structure was elucidated using NMR, IR, high-resolution ESI-MS and MS/MS. The presence of flibanserin in the health supplement is dangerous for consumers as it is not an approved drug. This is the first report of flibanserin as an adulterant in a health supplement used for sexual performance enhancement.

References

[1] E.O. Laumann, A. Paik, R.C. Rosen, Sexual dysfunction in the United States: prevalence and predictors, JAMA 281 (1999) 537–544.
[2] American Psychiatric Association, Diagnostic and Statistical Manual of Mental Disorder, fourth ed., Washington DC, 2000, pp. 493–538.
[3] J.L. Shifren, B.U. Monz, P.A. Russo, A. Segreti, C.B. Johannes, Sexual problems and distress in United States women: prevalence and correlates, Obstet. Gynecol. 112 (2008) 968–969.
[4] C. Marwick, Survey says patients expect little physician help on sex, JAMA 281 (1999) 2173–2174.
[5] R.W. Invernizzi, G. Sacchetti, S. Parini, S. Acconcia, R. Samanin, Flibanserin, a potential antidepressant drug, lower 5-HT and raises dopamine and noradrenaline in the rat prefrontal cortex dialysate: role of 5-HT(1A) receptors, Br. J. Pharmacol. 139 (2003) 1281–1288.
[6] B. Ferger, M. Shimasaki, A. Ceci, C. Ittrich, K.A. Kelly, B. Sommer, Flibanserin, a drug intended for treatment of hypoactive sexual desire disorder in pre-menopausal women, affects spontaneous motor activity and brain neurochemistry in female rats, Naunyn-Schmied, Arch. Pharmacol. 381 (2010) 573–579.
[7] S. Stahl, B. Sommer, K. Allers, Multifunctional pharmacology of flibanserin: possible mechanism of therapeutic action in hypoactive sexual desire disorder, J. Sex Med. 8 (2011) 15–27.
[8] FDA, Summary Minutes of the Advisory Committee for Reproductive Health Drugs (18.06.10). Available at: http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/ReproductiveHealthDrugsAdvisoryCommittee/UCM248751.pdf (accessed 29.04.11).