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**Analysis of adulterated herbal medicines and dietary supplements
marketed for weight loss by DOSY ^1H NMR**

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synephrine

Abstract

Twenty herbal medicines or dietary supplements marketed as natural slimming products were analyzed by Diffusion Ordered Spectroscopy (DOSY) ^1H NMR and DOSY-COSY ^1H NMR. The method allows analysis of the whole sample with detection of both active and inactive ingredients in these complex matrices. Among the 20 formulations analyzed, 2 were strictly herbal and 4 had a composition corresponding to declared ingredients on the packaging or the leaflet. The others were all adulterated. Eight formulations contain sibutramine alone at doses ranging from 4.4 to 30.5 mg/capsule. Five formulations contain sibutramine (from 5.0 to 19.6 mg/capsule or tablet) in combination with phenolphthalein (from 4.4 to 66.1 mg/capsule), and the last formulation was adulterated with synephrine (19.5 mg/capsule). Quantification of the actives was carried out with ^1H NMR. Several other compounds were also characterized including methylsynephrine, vitaberin, sugars, vitamins etc. DOSY NMR is thus proposed as a useful tool for detection of unexpected adulteration.

Introduction

According to WHO (<http://www.who.int/mediacentre/factsheets/fs311/en/>), approximately 1.6 billion adults were overweight and at least 400 million were obese in 2005. WHO further projects that by 2015, approximately 2.3 billion adults will be overweight and more than 700 million will be obese. Overweight and obesity are associated with premature mortality, chronic morbidity, diabetes, heart disease, osteoarthritis and cancer. Given (i) the medical and social impact of being overweight or obese, (ii) the few drugs marketed for these indications, and (iii) the safety concerns of these medications, more and more patients turn to weight-loss natural herbal medicines (HM) or dietary supplements (DS). Indeed, in contrast to conventional pharmaceuticals, these products are regarded by many as being harmless because of their natural origin for HM or of their safe composition (vitamins, minerals, herbs or other botanicals, amino acids, and substances such as enzymes, organ tissues, glandular, and metabolites) for DS.

The WHO's definition indicates that medicines containing plant material combined with chemically defined active substances, including chemically defined, isolated constituents of plants, are not considered to be HM (<http://www.who.int/medicines/areas/traditional/definitions/en/index.html>). Moreover, FDA regulations require that a complete list of ingredients and the net contents of the product appear on DS labels (<http://www.fda.gov/food/DietarySupplements/default.htm>). Despite these clear definition and regulation and as it is very easy for the consumer to purchase these formulations over the Internet, common problems affecting the safety of these preparations include fraudulent adulteration with synthetic actives used in conventional pharmaceutical drugs which can lead to severe side-effects.

Reported methods for screening illegal adulterations in HM or DS are mainly HPLC-DAD, GC-MS and LC/MS/MS (Liu et al. 2001; Lau et al. 2003; Bogusz et al. 2006; Liang et

al. 2006; Chen et al. 2009). Recently, Zhu et al. (2009) developed an algorithm, termed local straight-line screening (LSLS), to resolve complex IR spectra of possibly adulterated HM. The analytical methods reported concerning more specifically the adulteration of herbal formulations marketed for weight loss are chromatographic methods coupled with various detectors (Tseng et al. 2000; Jung et al. 2006; Yuen et al. 2007; Zou et al. 2007; Date et al. 2008; Huang et al. 2008; Wang et al. 2008), capillary electrophoresis (CE) (Cianchino et al. 2008) and IR-LSLS (Lu et al. 2007). None of these methods is ideal as HM often contain mixture of herbs and other natural products, and the formulas of those products are very complex. Chromatographic methods do not provide global information as they are mainly targeted screening methods and thus sometimes too selective. Moreover, false-positive results might occur with both chromatographic and IR-LSLS methods because it is in fact an analogue of a known active compound with a very close chemical structure which is detected instead of the target compound.

In this study we show an application of 2D Diffusion Ordered Spectroscopy ^1H Nuclear Magnetic Resonance (2D DOSY ^1H NMR) spectroscopy as an untargeted screening method for the detection and unequivocal identification of adulterants in “herbal” slimming products. The main advantage of NMR is to provide global information as it allows for considering the drug preparation as a whole. Moreover, the method is non-selective and requires no prior knowledge of the structures of the various components present in the mixture, which is a major advantage for screening of adulteration.

Experimental

Samples

Twenty commercial formulations of HM or DS marketed for weight loss were analyzed. Formulations **1**, **5**, and **7** were bought in China and formulations **16-18** in Syria. Other formulations were purchased over the internet. The list of the tablets or capsules analyzed is

given in Table 1. All samples, as received, were stored in the dark at ambient temperature and humidity. They were all analyzed within their expiration dates (when indicated).

¹H and 2D DOSY ¹H NMR

The ¹H NMR experiments were performed on a Bruker Avance 500 spectrometer equipped with a 5 mm cryoprobe at 298K. Typical acquisition parameters were as follows: acquisition time 2.72 s, spectral width 6000 Hz, pulse width 3.0 μs (flip angle ≈35°), 32 K data points, relaxation delay 1 s and number of scans 16. All chemical shifts (δ) were referred to an internal δ and quantification sodium 2,2,3,3-tetradeutero-3-trimethylsilylpropionate (TSP) reference. Authentic standards (sibutramine, phenolphthalein, synephrine, vitaberine, caffeine, carnitine, linoleate, stearate, citrate, vitamin C, dehydroascorbic acid, vitamins B1, B2, B3, B5, B6, and E, lipoic acid, glucose, fructose and sucrose) were added into the samples to assign resonances in the ¹H NMR spectra. Moreover, 1D ¹H NMR spectra extracted from the 2D DOSY ¹H NMR spectrum also helped to substantiate compound identification.

Relaxation delay was lengthened to 3 s and 128 scans were recorded for the quantitative analysis of sibutramine, phenolphthalein, synephrine, methylsynephrine, caffeine and vitaberine. The concentrations of adulterant compounds were measured by comparing the expanded areas of their respective NMR signals with that of the internal standard for quantification TSP. The areas were determined by manual integration using TopSpin software. Each data is the mean of at least five integrations. In order to check that the NMR conditions used allowed an accurate quantitation of adulterants, a longer interval between pulses (10 s) was used in some experiments. The same concentration values were obtained, thus demonstrating that a pulse interval of 3 s for a flip angle of 35° is sufficient to record the spectra under conditions of full T₁ relaxation. Sibutramine was assayed from the singlet at 7.47 ppm, phenolphthalein from the two doublets at 7.17 and 6.84 ppm, synephrine from the

two doublets at 7.23 and 6.83 ppm, methylsynephrine from the two doublets at 7.20 and 6.83 ppm, caffeine from the singlet at 7.77 ppm in formulation **12** and from the two singlets at 3.93 and 3.50 ppm in formulation **19**, and vitaberine from the triplet at 4.04 ppm. The limit of quantification of the NMR assay in the recording conditions used in this study is 5 μM .

Stimulated echo bipolar gradient pulse experiments were used for 2D DOSY ^1H NMR with a pulse field gradient length between 1.4 and 1.8 ms, a delay of 3 ms after each gradient and a diffusion delay between 50 and 100 ms. Sequence parameters were optimized in order to have the intensity of the main signals of the spectrum in the aromatic region strongly decreased (at least divided by 50) at 95% of the full gradient strength. The longitudinal eddy current delay was 20 ms, spoiler gradients were 1 ms long with a field strength of -7.92 and -6.09 $\text{G}\cdot\text{cm}^{-1}$ and other acquisition parameters were the same as those reported above for 1D ^1H NMR except for the relaxation delay fixed to 2 s. Forty experiments were recorded with gradient intensity linearly sampled from 5 to 95%. The gradient system had been calibrated to 46.25 $\text{G}\cdot\text{cm}^{-1}$ at maximum intensity.

All data were processed using the Gifa 5.2 software with the inverse Laplace transform method using the Maximum Entropy (MaxEnt) algorithm. The processing parameters were 2048 points along the Laplace spectrum diffusion axis and 20000 MaxEnt iterations. The inverse Laplace transform was computed only on the columns presenting a signal 32 times greater than the noise level of the experiment. DOSY spectra are presented with chemical shifts on the horizontal axis and diffusion coefficients expressed in $\mu\text{m}^2\cdot\text{s}^{-1}$ on the vertical axis.

The 3D DQF-COSY iDOSY experiment was recorded on formulation **7**. In the diffusion dimension, 28 gradient steps were used with 50 ms for the diffusion delay, 1.9 ms for the gradient pulse length and 3 ms for the recovery delay. In the COSY dimension, 8192×104 data points were used, corresponding to 0.74 s for acquisition time and 8.6 ms for the other

dimension, with a spectral window of 12 ppm. The sineshaped gradients for DQF selection, of 4.62 and 9.25 G.cm⁻¹ strength, were applied for 1 ms. The relaxation delay was 1.3 s and the number of scans was 8 (half-day experiment). The spectrum was processed using the NPK software (Tramesel et al. 2007) with Fourier transform in magnitude mode in the COSY dimension and with the inverse Laplace Transform method (MaxEnt algorithm) in the DOSY dimension. The processing parameters were 192 points along the diffusion axis and 20000 MaxEnt iterations. The algorithm was computed only on the columns with a signal-to-noise ratio above 32. The corresponding 2D COSY and DOSY experiments were acquired with the 3D DQF-COSY iDOSY parameters. These experiments were analyzed with the NMRnotebook package (NMRtec 2007).

MS-MS analysis

An Applied System QTRAP triple-quadrupole mass spectrometer, equipped with a Turbo Ion Spray (TIS) interface and controlled by the Agilent Analyst software (version 1.4), was used for analysis. The mass spectrometer was operated in positive ionization mode. Nitrogen served both as auxiliary, collision gas and nebulizer gas. The operating conditions for TIS interface were as follows: (i) in MS mode: mass range 100–600 u (1 s), step size 0.1 u; Q1 TIS MS spectra were recorded in profile mode, IS 5000 V, DP 40 V; (ii) in MS–MS mode: precursor mass 280 u; mass range 30–300 u; step size 0.1 u; MS–MS spectra were recorded in profile mode, IS 5000 V, DP 40 V, CE 20 V. All samples were dissolved in MeOH and analyzed after direct introduction.

Preparation of samples for NMR analysis

The tablets were powdered (or capsule emptied) and dissolved in 5 mL of CD₃CN:D₂O 80:20 v/v (CD₃CN for formulation **8** and D₂O for formulation **16**) under magnetic stirring

during 10 min and then sonicated for 10 min. The suspension was then centrifuged (5 min, 3000 rpm) and the supernatant (550 μL) analyzed.

For the quantitative analysis of sibutramine, phenolphthalein, synephrine, methylsynephrine, vitaberine and caffeine found in some formulations, the tablet was powdered (or capsule emptied), and 10% of the powder was dissolved in 10 mL of methanol under magnetic stirring during 15 min and then sonicated for 10 min. An aliquot of 1 mL was evaporated to dryness and the residue dissolved in 1 mL of MeOH-d_4 . TSP was added at a final concentration of 0.2 mM before the NMR analysis. The experiments were done in triplicate.

Results

Conventional ^1H NMR and 2D DOSY ^1H NMR analyses

All formulations were analyzed with 2D DOSY ^1H NMR. 2D DOSY spectra of formulations **3**, **12** and **19** along with their corresponding 1D spectrum are presented in Figure 1. All the peaks of a same compound are lined up. The peaks at 3.62 and 1.99 ppm correspond to the signals of residual HOD in D_2O and CHD_2CN in acetonitrile- d_3 , respectively. As shown in Figure 1, the separation according to diffusion coefficients (D) in the diffusion dimension allows an easier identification of the components of a mixture. Indeed, in formulation **3** (Figure 1A), the active pharmaceutical ingredient (API) adulterant is sibutramine ($D = 1107 \pm 9 \mu\text{m}^2.\text{s}^{-1}$) citrate ($D = 959 \mu\text{m}^2.\text{s}^{-1}$) and low amounts of natural compounds including glucose, sucrose, and fatty acids ($D = 931 \pm 29$, 1006 ± 29 , and 872 ± 29 , respectively) are also detected. In formulation **12** (Figure 1B), the main signals are those of caffeine ($D = 1156 \pm 39 \mu\text{m}^2.\text{s}^{-1}$) and synephrine ($D = 734 \pm 20 \mu\text{m}^2.\text{s}^{-1}$), whereas formulation **19** (Figure 1C) contains caffeine, methylsynephrine, vitaberine (thiamine-O-isobutyryl disulphide) and

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3 stearate with diffusion coefficients of 1288 ± 93 , 851 ± 41 , 624 ± 9 and $918 \mu\text{m}^2.\text{s}^{-1}$,
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5 respectively.
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8 All the ingredients detected in the herbal drugs studied are reported in Table 2, which
9 shows the comparison between the indications provided by the manufacturer and the
10 compounds found in each formulation. Several unexpected actives, sibutramine (formulations
11 **1-5, 7-11, 13-15**), phenolphthalein (formulations **1, 5, 7, 9, 15**) synephrine and caffeine
12 (formulation **12**), were found. The unambiguous identification of the components in the
13 herbal formulations analyzed was achieved by comparison of 1D ^1H NMR data of standards,
14 addition of authentic standards, and comparison of 1D NMR spectra extracted from the 2D
15 DOSY ^1H NMR spectrum with those of authentic standards (Figure 1D).
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27 The NMR spectral data of sibutramine measured in $\text{CD}_3\text{CN}:\text{D}_2\text{O}$ (80:20) are reported in
28 Table 3. The ^1H NMR resonances were assigned by 2D NMR (gCOSY, gHSQC, gHMBC,
29 gNOESY) and 1D selective NOE experiments. The chemical shifts and coupling constants of
30 all the other ingredients are reported in Table 4.
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36 Even if sensitivity is not an issue with respect to adulteration, we determined that in the
37 experimental conditions used in this study (1h of recording), 2D DOSY ^1H NMR allows an
38 accurate determination of diffusion coefficients for compounds at a concentration of $50 \mu\text{M}$.
39 The limit of detection corresponds to $10 \mu\text{M}$ with only CH_2 and CH_3 signals being detected.
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48 ***3D DOSY-COSY ^1H NMR analysis***

49 Figure 2 illustrates the different NMR spectra obtained in the 3D DOSY-COSY ^1H
50 NMR experiment applied to formulation **7**. First, the classical 2D DOSY (Figure 2A) and
51 COSY-DQF (Figure 2B) spectra are shown. In the COSY spectrum, diagonal signals and off-
52 diagonal cross peaks of sibutramine, phenolphthalein, glucose and sucrose are observed.
53 Other spectra (2C and 2D) are those of the projections from the 3D DOSY-COSY
54 experiment. The main interest of this 3D experiment is to extract the COSY spectrum of each
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component of the mixture from a selected line in the DOSY spectrum. For example, Figure 2C shows the COSY projection of phenolphthalein at $D = 990 \mu\text{m}^2.\text{s}^{-1}$; typical cross peaks of aromatic signals are detected for this compound while the on-diagonal signal from aromatic protons of sibutramine has disappeared. The COSY projection corresponding to $D = 711 \mu\text{m}^2.\text{s}^{-1}$ (Figure 2D) shows exclusively the signals of sucrose without any signal from phenolphthalein or anomeric protons from α - or β -glucose. These spectra highlight the interest of 3D experiment as virtual separation provided by DOSY acquisition can lead to structural determination by extraction of COSY spectra.

Quantitative analysis

The contents of sibutramine and phenolphthalein in the herbal drugs analyzed were measured by NMR (Table 5). Eight formulations (**2, 3, 4, 8, 10, 11, 13, 14**) contain sibutramine alone at doses ranging from 4.4 to 30.5 mg/capsule. Five formulations (**1, 5, 7, 9, 15**) contain sibutramine (from 5.0 to 19.6 mg/capsule or tablet) and phenolphthalein (from 4.4 to 66.1 mg/capsule). Formulation **12** contains 19.5 ± 0.5 and 10.0 ± 0.5 mg/capsule of synephrine and caffeine, respectively. Formulation **19** contains 38.3 ± 0.9 , 184.9 ± 5.6 and 75.4 ± 1.6 mg/capsule of methylsynephrine, caffeine and vitaberine, respectively.

Mass spectrometry

The aim of mass spectrometry experiments was to cross-validate NMR data. MS/MS mass spectrometry with ESI ionization was carried out in order to confirm the presence of sibutramine and to investigate the presence of its previously described mono- or di-desmethylated analogs (Wang et al. 2008). Molecular $[\text{M}+\text{H}]^+$ ions corresponding to sibutramine, N-mono-desmethylsibutramine and N-di-desmethylsibutramine were searched at m/z 280, 266 and 252, respectively (Wang et al. 2008). Only sibutramine was found. MS/MS was then carried out on the m/z 280 ion giving characteristic fragments at m/z 153, 139, and

125. The presence of sibutramine was thus confirmed by MS/MS in thirteen formulations (**1-5, 7-11, and 13-15**). MS was also used to confirm the presence of methysynephrine, vitaberine and caffeine ($[MH]^+$ ions at m/z 182, 703, and 195 respectively) in formulation **19** and synephrine ($[MH]^+$ ion at m/z 168) in formulation **12**.

Discussion

To date, identification of synthetic drugs adulterating HM to inhibit appetite and achieve weight loss using NMR and more specifically 2D DOSY 1H NMR has not been reported. The objective of this study was to apply this method to analyze the content of 20 different HM or DS.

Chromatographic methods are preferred by most analysts for mixture analysis as they are very common, easy to handle, quantitative, sensitive, relatively cheap and do not require highly trained personnel. Nevertheless, NMR has also several advantages; it is non-selective, quantitative, highly powerful for structural elucidation and it requires no prior knowledge of the structures of the various components present in a mixture. 2D DOSY 1H NMR allows the virtual separation of several components of a mixture in a single run based on the difference in their translational self-diffusion coefficients (Figure 1). Moreover, DOSY experiments do not need complicated setup procedures or separation steps and the method can be used routinely. This technique has been used successfully for analyzing several kinds of counterfeit drugs (Trefi et al. 2008; Nyadong et al. 2009). Table 6 shows a comparison on information obtained with various analytical methods on slimming herbal drug composition and highlights the interest of DOSY NMR for a global analysis of these formulations.

Among the 20 formulations analyzed, 2 were strictly herbal (formulations **17** and **18**), and 4 had a composition corresponding to declared ingredients on the packaging or the leaflet (formulations **6, 16, 19, 20**). The others were all adulterated. Thirteen formulations (**1-5, 7-11,**

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3 **13-15**) contained sibutramine alone or in combination with phenolphthalein (**1**, **5**, **7**, **9**, and
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5 **15**). The two metabolites of sibutramine, N-mono- and N-di-desmethylsibutramine,
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7 previously described by some authors (Blachut et al. 2007; Lai et al. 2007; Yuen et al. 2007;
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9 Huang et al. 2008) as possible adulterants in slimming dietary supplements were not detected
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11 in our samples neither with NMR nor with MS and MS/MS analyses. The amount of
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13 sibutramine found in adulterated formulations ranged from 4.4 (formulation **2**) to 30.5
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15 mg/capsule (formulation **11**). The recommended daily dose is 5 to 15 mg, 15 mg being the
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17 maximum daily dose (de Simone and D'Addeo 2008). Three formulations (**3**, **5** and **11**) were
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19 thus overdosed, which poses a serious health risk to consumers and particularly a
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21 cardiovascular risk as an increase in systolic blood pressure has been noticed in patients
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23 receiving sibutramine at the non recommended doses of 20 or 30 mg (Narkiewicz 2002).
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25 Several cases of mild to severe poisoning due to herbal slimming medicines adulterated with
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27 sibutramine (Lida (Jung et al. 2006; Müller et al. 2009), Ever Youth and unknown (Yuen et
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29 al. 2007), Meizitanc (Sein Anand and Chodorowski, 2007)) have been recently reported. It is
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31 interesting to point out that during the reviewing process of this article, the European
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33 Medicines Agency's Committee for Medicinal Products for Human Use has concluded that
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35 the benefits of sibutramine do not outweigh its risks, and that all marketing authorizations for
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37 medicines containing sibutramine should be suspended throughout Europe
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39 (<http://www.ema.europa.eu/pdfs/human/referral/sibutramine/3940810en.pdf>).
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48 Other actives found may also cause serious adverse effects. Phenolphthalein is a laxative
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50 that was withdrawn as a medicine several years ago after concerns about carcinogenicity (No
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52 authors listed 1999). Moreover, interaction profiles are unknown for the combination
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54 sibutramine/phenolphthalein. After the ban on ephedrines in 2004, manufacturers changed to
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56 using the weaker stimulant synephrine that has purported thermogenic and lipolytic properties
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58 due to its α -adrenergic agonist property (Haaz et al. 2006). The safety of synephrine is
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controversial but it could pose cardiovascular health risks (Haaz et al. 2006), especially in combination with caffeine (Haller et al. 2005). Formulation **12**, claimed as purely herbal, contains both compounds and formulation **19** contains methylsynephrine (para-hydroxyephedrine) in combination with a high dose of caffeine, a potentially hazardous combination.

Conclusion

This study presents a new application of 2D DOSY ^1H NMR for the analysis of HM and DS, which are undeniable complex mixtures. Among the twenty formulations marketed for weight loss studied thirteen were adulterated with sibutramine alone or with a combination of sibutramine and phenolphthalein. Adulteration of “natural herbal medicines” with undeclared synthetic drugs is a common and dangerous phenomenon and all the analytical methods that ensure the quality and safety of these products have thus to be developed. In this context, 2D DOSY ^1H NMR spectroscopy is a powerful method for providing a multivariate fingerprint of a very complex mixture especially in situations where the identity of the components is not known beforehand as it permits to consider the drug preparation as a whole. The technique should be now considered as a useful and complementary tool among a standard 2D NMR analytical package. Moreover, its evolution towards 3D DOSY-COSY experiments noticeably increases its interest as structural data are easily obtained.

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References

- Blachut D, Siwinska-Ziolkowska A, Szukalski B, Wojtasiewicz K, Czarnocki Z, Kobylecka A, Bykas-Strekowska M. 2007. Identification of N-desmethylsibutramine as a new ingredient in Chinese herbal dietary supplements. *Probl Forensic Sci.* 70:225-235.
- Bogusz MJ, Hassan H, Al-Enazi E, Ibrahim Z, Al-Tufail M. 2006 Application of LC-ESI-MS-MS for detection of synthetic adulterants in herbal remedies. *J Pharm Biomed Anal.* 41:554-564.
- Chen Y, Zhao L, Lu F, Yu Y, Chai Y, Wu Y. 2009. Determination of synthetic drugs used to adulterate botanical dietary supplements using QTRAP LC-MS/MS. *Food Add Cont.* 26:595-603.
- Cianchino V, Acosta G, Ortega C, Martinez LD, Gomez MR. 2008. Analysis of potential adulteration in herbal medicines and dietary supplements for the weight control by capillary electrophoresis. *Food Chem.* 108:1075-1081.
- Date H, Toyota A, Terauchi M, Sugimura M, Matsuo T, Mochiike C. 2008. Rapid determination of medical components found in the health food for weight loss by liquid chromatography/tandem mass spectrometry (LC/MS/MS). *Yakugaku Zasshi.* 128:811-817.
- de Simone G, D'Addeo G. 2008. Sibutramine: balancing weight loss benefit and possible cardiovascular risk. *Nutr Metab Cardiovasc Dis.* 18:337-341.
- Haaz S, Fontaine KR, Cutter G, Limdi N, Perumean-Chaney S, Allison DB. 2006. Citrus aurantium and synephrine alkaloids in the treatment of overweight and obesity: an update. *Obes Rev.* 7:79-88.
- Haller CA, Benowitz NL, Jacob P. 2005. Hemodynamic effects of ephedra-free weight-loss supplements in humans. *Am J Med.* 118:998-1003.
- Huang Z, Xiao S, Luo D, Chen B, Yao S. 2008. Simultaneous determination of sibutramine and N-di-desmethylsibutramine in dietary supplements for weight control by HPLC-ESI-MS. *J Chromatogr Sci.* 46:707-711.
- Jung J, Hermanns-Clausen M, Weinmann W. 2006. Anorectic sibutramine detected in a Chinese herbal drug for weight loss. *Forensic Sci Int.* 161:221-222.
- Lai KC, Liu YC, Tseng MC, Lin YL, Lin JH. 2007 Isolation and identification of a sibutramine analogue in a healthy food for weight loss. *J Food Drug Anal.* 15:20-24.
- Lau AJ, Holmes MJ, Woo SO, Koh HL. 2003. Analysis of adulterants in a traditional herbal medicinal product using liquid chromatography-mass spectrometry-mass spectrometry. *J Pharm Biomed Anal.* 31:401-406.
- Liang Q, Qu J, Luo G, Wang Y. 2006. Rapid and reliable determination of illegal adulterant in herbal medicines and dietary supplements by LC/MS/MS. *J Pharm Biomed Anal.* 40:305-311.
- Liu SY, Woo SO, Koh HL. 2001. HPLC and GC-MS screening of Chinese proprietary medicine for undeclared therapeutic substances. *J Pharm Biomed Anal.* 24:983-992.

- Lu F, Li S, Le J, Chen G, Cao Y, Qi Y, Chai Y, Wu Y. 2007. A new method for testing synthetic drugs adulterated in herbal medicines based on infrared spectroscopy. *Anal Chim Acta*. 589:200-207.
- Müller D, Weinmann W, Hermanns-Clausen M. 2009. Chinese slimming capsules containing sibutramine sold over the Internet: a case series. *Dtsch Arztebl Int*. 106:218-222.
- Narkiewicz K. 2002. Sibutramine and its cardiovascular profile. *Int J Obes*. 26:S38-S41.
- Nyadong L, Harris GA, Balayssac S, Galhena AS, Malet-Martino M, Martino R, Parry RM, Wang MD, Fernandez FM, Gilard V. 2009. Combining two-dimensional diffusion-ordered nuclear magnetic resonance spectroscopy, imaging desorption electrospray ionization mass spectrometry and direct analysis in real time mass spectrometry for the integral investigation of counterfeit pharmaceuticals. *Anal Chem*. 81:4803-4812.
- NMRtec, Nmrnotebook software. Available from: <<http://www.NMRnotebook.com>>
- No authors. 1999. Laxative drug products for over-the-counter human use. Food and Drug Administration, HHS. Final rule. *Fed Regist*. 64:4535-4540.
- Sein Anand J, Chodorowski Z. 2007. Side effects after the usage of Chinese dieting product Meizitanc. *Przegl Lek*. 64:346-347.
- Tramesel D, Catherinot V, Delsuc MA. 2007. Modeling of NMR processing, toward efficient unattended processing of NMR experiments. *J Magn Reson* 188:56-67.
- Trefi S, Routaboul C, Hamieh S, Gilard V, Malet-Martino M, Martino R. 2008. Analysis of illegally manufactured formulations of tadalafil (Cialis®) by ^1H NMR, 2D DOSY ^1H NMR and Raman spectroscopy. *J Pharm Biomed Anal*. 47:103-113.
- Tseng MC, Tsai MJ, Lin JH, Wen KC. 2000. GC/MS analysis on anorectics adulterated in traditional Chinese medicines. *J Food Drug Anal*. 8:315-330.
- Wang J, Chen B, Yao S. 2008. Analysis of six synthetic adulterants in herbal weight-reducing dietary supplements by LC electrospray ionization-MS. *Food Addit Contam*. 25:822-830.
- Yuen YP, Lai CK, Poon WT, Ng SW, Chan AYW, Mak TWL. 2007. Adulteration of over-the-counter slimming products with pharmaceutical analogue-an emerging threat. *Hong Kong Med J*. 13:216-220.
- Zhu X, Zhang Z, Lu F, Wu Y, Qi Y. 2009. Modified local straight-line screening to detect synthetic drugs in adulterated herbal medicines. *Appl Spectrosc*. 63:471-476
- Zou P, Oh SS-Y, Kiang K-H, Low M-Y, Bloodworth BC. 2007. Detection of sibutramine, its two metabolites and one analogue in a herbal product for weight loss by liquid chromatography triple quadrupole mass spectrometry and time-of-flight mass spectrometry. *Rapid Commun Mass Spectrom*. 21:614-618.

Captions for figures

Figure 1. 2D DOSY ^1H NMR spectra recorded in $\text{CD}_3\text{CN}:\text{D}_2\text{O}$ (80/20) of formulations 3 (A), 12 (B) and 19 (C). (D) Comparison of 1D ^1H NMR spectra of caffeine (D1) and vitaberine (D2) extracted from the 2D DOSY ^1H NMR spectrum of formulation 19 with those of authentic standards.

sib, sibutramine; caf, caffeine; syn, synephrine; msyn, methysynephrine; vita: vitaberine; fa, fatty acids; st, stearate; TSP, sodium 2,2,3,3-tetradeutero-3-trimethylsilylpropionate (internal standard); ?, unknown; sib* means that the diffusion coefficient of this sibutramine signal is lower due to its superimposition with CHD_2CN , which affects DOSY processing. A deeper section of some signals is shown in box with dotted lines.

Figure 2. NMR spectra of formulation 7 in $\text{CD}_3\text{CN}:\text{D}_2\text{O}$ (80/20). (A) 2D DOSY ^1H spectrum; (B) COSY-DQF spectrum; COSY extractions from 3D DOSY-COSY experiment at (C) $D = 990 \mu\text{m}^2.\text{s}^{-1}$, (D) $D = 711 \mu\text{m}^2.\text{s}^{-1}$.

sib, sibutramine; phth, phenolphthalein; suc, sucrose; gluc, glucose; st, stearate; S, satellite signals from CHD_2CN ; TSP, sodium 2,2,3,3-tetradeutero-3-trimethylsilylpropionate (internal standard); sib* means that the diffusion coefficient of this sibutramine signal is lower due to its superimposition with CHD_2CN , which affects DOSY processing.

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Fig 2
254x190mm (96 x 96 DPI)

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Table 1. Herbal commercial formulations analyzed in this study.

	Formulation name	Batch number	Expiration date	Manufacturer name	Country of manufacturing	Product form	Color
1	Perfect slim cherry	2007070183	07/2009	Chun Tian	Japan	Capsule	Pink
2	9 rapide 9	Q/CL5-2004n0487	05/2009	Zhengda health food	China	Capsule	Red
3	Unknown ^a	Unknown	Unknown	Unknown	China	Capsule	Red and white
4	Unknown ^a	Unknown	Unknown	Menova	China	Capsule	Blue
5	Perfect slim grape	2007080601	08/2009	Chun Tian	Japan	Capsule	Green and white
6	Honey life	20080302	03/2010	B&H (USA)	China	Capsule	Mauve and white
7	Cider vinegar dieting	20080216	02/2010	Qinghai Oifield	China	Tablet	Yellow (with green spots)
8	Botanical slimming soft gel	20081006	10/2010	Meizitang	China	Capsule with gel	Green
9	Botanical slimming Herbs	0991-8280809	08/2010	Meizitang	China	Capsule	Orange and brown
10	2 Day Diet Japan Lingzhi slimming formula	Unknown	12/2012	Unknown	Japan	Capsule	Purple
11	Lipro diet pills	20081020	10/2010	China Karyhen International Co.	China	Capsule	Green

12	Lida	20080924	09/2010	Kunming Dali Industry and Trade Co.	China	Capsule	Green
13	Onting quianweisu slimming herbs ^a	Unknown	03/2010	Menova	China	Capsule	Blue
14	Meekeyes ^a	95882108	Unknown	Marlian	China	Capsule	Green and white
15	Perfect slim ^a	Unknown	Unknown	Unknown	China	Capsule	Pink
16	Garslim	22	05/2011	Balsam	Syria	Tablet	Beige
17	Alattar psyllium	4	06/2009	Alattar Medical Co.	Syria	Capsule	Green
18	Alattar slimming mixture	255161M2	06/2009	Alattar Medical Co.	Syria	Powder	-
19	Hyper drive 3.0 +	110802	10/2013	ALR industries	USA	Capsule	Red
20	Elan-Sil	9.026.6	01/2001	Vitaline Ltd	UK	Capsule	White

^aThis formulation has been seized by French Customs.

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Table 2. Comparison of formulation composition with indications provided by the manufacturer.

	Formulation name	Indications provided by the manufacturer	Compounds found	
1	Perfect slim cherry	Only natural compounds	Sibutramine Phenolphthaleine Vitamin C ^a Very low amounts of natural compounds including glucose, fructose, fatty acids	Identical to formulation 15
2	9 rapide 9	Only natural compounds	Sibutramine Very low amounts of natural compounds including fatty acids	
3	Unknown	No indication ^b	Sibutramine citrate Low amounts of natural compounds including glucose, fructose, fatty acids	
4	Unknown	No indication ^b	Sibutramine Low amounts of natural compounds including glucose, fructose, fatty acids	Identical to formulation 13
5	Perfect slim grape	Only natural compounds	Sibutramine Phenolphthaleine Very low amounts of natural compounds including glucose, fructose, fatty acids	Very similar to formulation 1 except that it contains no vitamin C
6	Honey life	L-carnitine L-tartrate Natural compounds (Konjac flour, Cassia seed)	Carnitine tartrate Low amounts of natural compounds including sugars, fatty acids and vitamin B3	
7	Cider vinegar dieting	Carnitine Flavone glycoside Oligosaccharide	Sibutramine Phenolphthaleine Stearate Sucrose Very low amounts of natural sugars	No carnitine

8	Botanical slimming soft gel	Only natural compounds	Sibutramine Soybean oil	Soybean oil is used to prepare the gel
9	Botanical slimming herbs	Only natural compounds	Sibutramine Phenolphthaleine Very low amounts of natural compounds including glucose, sucrose, fatty acids	
10	2 Day Diet Japan Lingzhi slimming formula	Only natural compounds	Sibutramine Very low amounts of natural compounds including glucose, fatty acids	
11	Lipro diet pills	Only natural compounds (Chinese herbs)	Sibutramine Stearate Very low amounts of natural sugars including glucose	
12	Lida	Only natural compounds (Yunnan herbs)	Caffeine Synephrine Low amount of natural compounds	
13	Onting quianweisu slimming herbs	No indication ^b	Sibutramine Low amounts of natural compounds including glucose, fructose, fatty acids	Identical to formulation 4
14	Meekeyes	No indication ^b	Sibutramine Sucrose Fatty acid High amount of sinapine and very low amounts of other natural compounds	
15	Perfect slim	No indication ^b	Sibutramine Phenolphthaleine Vitamin C ^a Very low amounts of natural compounds including glucose, fructose, fatty acids	Identical to formulation 1
16	Garslim	Fruit extracts with 50% hydroxycitric acid and citric acid	Hydroxycitric acid Citric acid	

17	Alattar psyllium	Mucilages, fatty oil, iridoide monoterpenes	Fatty acids including stearic and linoleic acids	
18	Alattar slimming mixture	Only natural compounds	Only natural compounds including caffeine, polyphenols, sugars, fatty acids	
19	Hyper drive 3.0	Methylsynephrine Xanthines Vitaberine Lipoic acid Tyrosine ethyl ester Propionylcarnitine ethyl ester ^c Natural compounds	Methylsynephrine Caffeine Vitaberine Stearate Very low amounts of natural compounds	The amounts of lipoic acid and tyrosine ethyl ester are probably too low to be detected. Both compounds were undetected with ¹ H NMR or MS.
20	Elan-Sil	L-carnitine L-tartrate Vitamin C Vitamin E acetate Vitamin B1 Vitamin B2 Vitamin B3 Vitamin B5 (Ca pantothenate) Vitamin B6 Vitamin B7 Vitamin B9 Vitamin B12 Mg salts of fatty acids	Carnitine Vitamin C ^a Vitamin E acetate Vitamin B1 Vitamin B3 Vitamin B5 Vitamin B6 Stearate	Tartrate was not detected. The amounts of vitamins B2, B7, B9 and B12 are too low to be detected.

^a Vitamin C is slightly unstable in the extraction conditions used. It was thus detected as a mixture of ascorbic and dehydroascorbic acids.

^b This formulation was provided by French Customs with no packaging.

^c Incorrect chemical nomenclature as propionylcarnitine is the propionic ester of carnitine.

Table 3. NMR characteristics of sibutramine detected in the herbal formulations analyzed. Spectra were recorded in CD₃CN:D₂O (80:20).

Sibutramine

Atom number	δ ¹ H (ppm) ^a	Multiplicity ^b (J in Hz)	δ ¹³ C (ppm)
1			49.2
2 or 4	2.60 (1H syn aromatic ring) 2.49 (1H anti aromatic ring)	m m	32.5
3	1.98 (1H) 1.78 (1H)	m m	15.5
4 or 2	2.37 (1H syn aromatic ring) 2.41 (1H anti aromatic ring)	m m	33.7
5	-	-	140.2
6,10	7.47	s	128.7
7,9			129.9
8			132.9
11	3.67	t (6.3)	71.7
12 or 13	2.19	broad s	39.6 ^c
13 or 12	2.81	broad s	47.0 ^c
14	1.53 (1H) 1.39 (1H)	m m	33.5
15	1.78	m	25.4
16 or 17	1.01	d (6.5)	22.0
17 or 16	1.04	d (6.5)	21.2

^a The value given is the chemical shift of the centre of the signal.

^b s: singlet; d: doublet; t: triplet; m: multiplet.

^c The chemical shift of this carbon was measured in a spectrum recorded in CD₃CN.

Table 4. ^1H NMR characteristics of the main signals of ingredients found in herbal formulations (except sibutramine given in Table 3).

Compound	^1H NMR δ (ppm) ^a and multiplicity ^b (J, Hz)
phenolphthalein	7.93 d (7.5; H8), 7.79 t (7.5; H6), 7.63 t (7.0; H7), 7.63 d (7.5; H6), 7.17 d (8.5; 4 H11), 6.84 d (8.5; 4 H12)
synephrine	7.23 d (8.5; Ar), 6.83 d (8.5; Ar), 4.70 dd (4.3, 8.8; CHOH), 2.70 ABd system (12.3, 8.8, 4.3; CH_2), 2.36 s (CH_3)
methylsynephrine ^c	7.20 d (8.5; Ar), 6.83 d (8.5; Ar), 5.00 d (3.1; CHOH), 3.34 qd (3.1, 6.7; CHNH), 2.71 s (CH_3NH), 1.04 d (6.7; CH_3CH)
vitaberine (sulbutiamine)	7.86 s (H pyrim), 7.77 s (CHO), 4.45 centre of a very broad s (CH_2N), 4.04 t (6.3; CH_2O), 2.55 sept (7.0; $\text{CH}(\text{CH}_3)_2$), 2.52 broad t (6.3; $\text{CH}_2\text{CH}_2\text{O}$), 2.39 s (CH_3 pyrim), 2.05 s ($\text{CH}_3\text{C}=\text{C}-$), 1.12 d (7.0; $\text{CH}(\text{CH}_3)_2$)
riboflavine	8.00 s (Ar), 7.99 s (Ar), 4.93 m (CH_2N), 4.37 m (CHOH), 3.87 m (CHOH), 3.79 m (CH_2OH), 3.66 m (CHOH), 2.58 s ($\text{CH}_3\text{-Ar}$), 2.48 s ($\text{CH}_3\text{-Ar}$)
caffeine	7.77 s (H), 3.93 s (CH_3), 3.50 s (CH_3), 3.32 s (CH_3)
carnitine	4.56 app q (7.0; CHOH), 3.45-3.31 m (CH_2N^+), 3.19 s ($(\text{CH}_3)_3\text{N}^+$), 2.62-2.53 m (CH_2COOH)
lipoic acid	3.62 m (H3), 3.20 m (H5 α), 3.13 m (H5 β), 2.46 sext (6.2; H4 α), 2.30 t (7.4; H9), 1.91 sext (6.7; H4 β), 1.72 m (H6 α), 1.61 m (H6 β , H8), 1.44 m (H7)
stearate	2.21 t (7.5; CH_2CO), 1.53 quin (6.9; $\text{CH}_2\text{CH}_2\text{CO}$), 1.27 broad s (14 CH_2), 0.98 t (6.9; CH_3)
linoleic acid	5.36 m (4 =CH), 2.78 t (6.6; =CH- CH_2 -CH=), 2.26 t (7.5; - CH_2 -COOH), 2.06 q (6.8; 2 - CH_2 - CH_2 -CH=), 1.55 quin (6.9; - CH_2 - CH_2 -COOH), 1.32 m (7 CH_2), 0.89 t (6.9; CH_3)
sinapine ^d	7.65 d (15.9; Ar-CH=), 6.96 s (Ar), 6.48 d (15.9; =CH-COO-), 4.57 m (O- CH_2), 3.87 s (OCH $_3$), 3.68 m (- CH_2 -N(CH_3) $_3^+$), 3.17 s (N(CH_3) $_3^+$)
citrate	2.75 AB system (17.3; CH_2)
vitamin C	4.78 d (2.0; CH-O-), 3.89 td (X part of the ABX system) (2.0, 6.7; CHOH), AB part of the ABX system: $\delta_A = 3.62$, $\delta_B = 3.60$ ($J_{AB} = 11.2$, $J_{AX} = J_{BX} = 6.7$; CH_2OH)
dehydroascorbic acid	4.58 s (CHO), 4.43 dd (5.6, 3.1; CHOH), 4.21 dd (5.6, 10.2; 1H of CH_2OH), 4.05 dd (3.1, 10.2; 1H of CH_2OH)
vitamin B1 (thiamine)	9.56 s (H thiazole ring), 7.97 s (H pyrimidine ring), 5.47 s (CH_2N^+), 3.82 t (5.6; CH_2OH), 3.13 t (5.6; $\text{CH}_2\text{CH}_2\text{OH}$), 2.61 s (CH_3), 2.50 s (CH_3)
vitamin B3 (niacin)	8.98 d (2.1; H2), 8.70 dd (4.9, 1.7; H6), 8.23 td (8.0, 1.7; H4), 7.52 dd (4.9, 8.0; H5)
vitamin B5 (pantothenic acid)	3.90 s (CHOH), 3.46-3.34 m (CH_2OH and CH_2NHCO), 2.33 t (6.7; CH_2COOH), 0.89 s (CH_3), 0.88 s (CH_3)
vitamin B6 (pyridoxine)	8.08 s (H), 5.04 s (CH_2OH), 4.67 s (CH_2OH), 2.59 s (CH_3)
vitamin E (tocopherol acetate)	2.62 t (6.8; =C- CH_2 -), 2.31 s ($\text{CH}_3\text{-COO}^-$), 2.08 s ($\text{CH}_3\text{-Ar}$), 1.99 s ($\text{CH}_3\text{-Ar}$), 1.96 s ($\text{CH}_3\text{-Ar}$), 1.82 m (O-C- CH_2), 1.53 m (HC-CH_3), 1.41 m (HC-CH_3), 1.27 m (CH_2), 1.25 s ($\text{CH}_3\text{-C-O}$), 1.16 m (CH_2), 1.09 m (CH_2), 0.88 d (6.6; HC-(CH_3), 0.86 d (6.6; HC- CH_3)

α/β -glucose	5.14 d (3.5; H1 α), 4.53 d (8.0; H1 β), 3.84-3.71 m, 3.71-3.60 m, 3.45-3.28 m, 3.15 t (8.0; H2 β)
fructose	4.06-3.99 m, 3.99-3.85 m, 3.85-3.68 m, 3.67-3.42 m
sucrose ^c	5.36 d (3.5; g1), 4.10 d (8.5; f3), 4.00 t (8.5; f4), 3.83-3.67 m (f5, g5, f6, g6, g3), 3.61 AB system (12.5; f1), 3.48 dd (3.5, 10.0; g2), 3.38 t (9.5; g4)

^a Spectral data were measured from authentic standards in CD₃CN:D₂O (80:20).

^b s: singlet; d: doublet; dd: doublet of doublet; t: triplet; td: doublet of triplet; q: quadruplet; quin: quintuplet; sext: sextuplet; sept: septuplet; m: multiplet; app: apparent.

^c The chemical shifts were measured in the spectrum of formulation **19**.

^d The structure of sinapine (choline ester of sinapic acid) was determined with 1D and 2D ¹H and ¹³C NMR experiments on formulation **14** and confirmed by MS ([M⁺] 310).

^e g: glucose; f: fructose.

Table 5. Amounts of adulterant sibutramine and phenolphthalein found in claimed herbal medicines analyzed in this study.

Formulation number	Sibutramine mg/capsule or tablet mean±SD (n = 3)	Phenolphthalein mg/capsule or tablet mean±SD (n = 3)
1	13.1 ± 0.3	47.0 ± 3.0
2	4.4 ± 0.3	-
3	23.3 ± 0.1	-
4	15.6 ± 0.5	-
5	19.6 ± 0.5	66.1 ± 1.4
7	5.0 ± 0.1	36.2 ± 0.5
8	12.7 ± 0.2	-
9	12.0 ± 0.2	4.4 ± 0.2
10	10.6 ± 0.3	-
11	30.5 ± 0.1	-
13	15.1 ± 0.3	-
14	14.0 ± 0.7	-
15	13.7 ± 0.6	40.8 ± 1.3

Table 6. Adulterants found in herbal products or dietary supplements marketed as slimming products reported in literature

References	Analytical methods for detection	Number of formulations analysed	Active Pharmaceutical Ingredients detected	Other compounds detected
Tseng et al. 2000	GC-MS	4	Mazindol Clobenzorex Diazepam Phentermine Caffeine	
Jung et al. 2006	GC-MS HPLC-DAD	1	Sibutramine	
Blachut et al. 2007	GC-MS	1 (Lida)	N-desmethylsibutramine	
Sein Anand and Chodorowski 2007	GC-MS	1 (Meizitanc)	Sibutramine	
Bogusz et al. 2006	LC-ESI-MS/MS	1	Fenfluramine Phentermine Caffeine	
Zou et al. 2007	LC-MS/MS LC-TOF-MS	1	Sibutramine N-desmethylsibutramine N-didesmethylsibutramine Homosibutramine	
Date et al. 2008	LC-ESI-MS/MS	10	Phenolphthalein Sibutramine Mazindol Sennosides A and B Bisacodyl Hydrochlorothiazide	
Huang et al. 2008	LC-ESI-MS	15	Sibutramine N-didesmethylsibutramine	
Wang et al. 2008	LC-ESI-MS	6	Sibutramine Phenolphthalein N-desmethylsibutramine	
Chen et al. 2009	QTRAP LC-MS/MS	18	Fenfluramine Sibutramine Phenolphthalein	
Cianchino et al. 2008	CE	4	Ephedrine Norephedrine Furosemide Caffeine	
This study	¹ H NMR and 2D DOSY ¹ H NMR	20	Sibutramine Phenolphthalein Synephrine Methylsynephrine Vitaberin Caffeine	Carnitine Vitamin C Dehydroascorbic acid Vitamins B1, B3, B5, B6, E Glucose Fructose Sucrose Stearate Linoleate Citrate Hydroxycitrate Tartrate Sinapine