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Evaluation of a Benchtop Cryogen-Free Low-Field ^{1}H NMR Spectrometer for the Analysis of Sexual Enhancement and Weight Loss Dietary Supplements Adulterated with Pharmaceutical Substances

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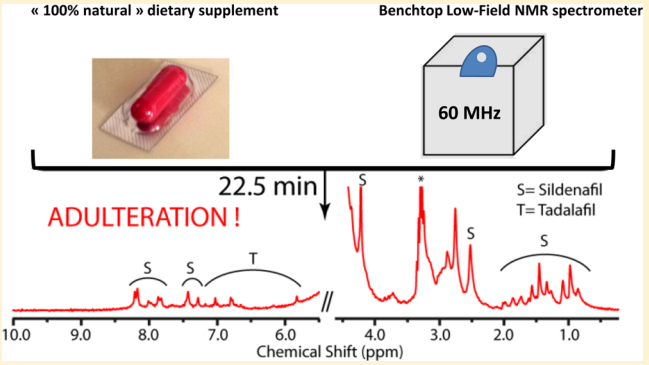
Supporting Information

ABSTRACT: Nuclear magnetic resonance (NMR) spectroscopy is a unique tool for detection, structural characterization, and quantification of compounds in complex mixtures. However, due to cost constraints, NMR is rarely used in routine quality control (QC) analysis. The recent release of benchtop cryogen-free low-field NMR spectrometers represents a technological break in the NMR field. In this paper, we evaluated the potential of a benchtop cryogen-free 60 MHz spectrometer for uncovering adulteration of "100% natural" sexual enhancement and weight loss dietary supplements. We demonstrated that the adulterant(s) can readily be detected in ≈20 min of recording after a very simple and rapid sample preparation. We also showed that the quantification by the internal standard method can be done on the low-field NMR spectrometer and leads to results similar to those obtained with high-field NMR. Considering the cost and space efficiency of these spectrometers, we anticipate their introduction in QC laboratories as well as in governmental agencies, especially in the field of fraud detection.



While high-resolution nuclear magnetic resonance (NMR) spectrometers are used daily in several research fields, their use as a routine tool in quality control (QC) laboratories or industries is limited due to both the expensive maintenance cost and the need for highly skilled scientists to operate the spectrometer.[1] Permanent low-field magnets are commercially available with a field up to 1.4 T (corresponding to 60 MHz for ^{1}H). The main advantages of such equipment deal with (i) the cheap maintenance cost due to the absence of cryogenic fluids, (ii) the small size of the instruments, and (iii) the easy operation and troubleshooting of the spectrometers. Most of the studies have focused on relaxometry (T_1 and/or T_2) and diffusion-type experiments on bulk liquid or soft-solid samples.[2,3] Applications have been developed in a wide range of industrial sectors: from food industries[4−6] to construction materials[7−10] through petroleum production.[11,12]

Until recently, the magnetic field homogeneity for benchtop low-field NMR spectrometers did not reach the subparts per million needed to record spectra with sufficient resolution. Manufacturers now propose benchtop cryogen-free magnets working at either 42.5, 60, or 82 MHz for ^{1}H for educational or industrial applications that overcome this issue. To the best of our knowledge, the routine analytical power of such instruments has been established in the sole paper of Parker et al.[13] who compared low-field NMR and Fourier transform infrared (FT-IR) methods as screening tools to detect adulteration of olive oil by hazelnut oil. Indeed, the authors demonstrated that NMR combined with chemometric analysis of olive oil samples containing varying amounts of hazelnut oil to simulate adulteration has comparable sensitivity and better specificity than FT-IR.

Dietary supplements (DS) are products between medicines and conventional foods whose consumption is rising steeply.[14] A significant number of unscrupulous manufacturers add pharmaceutical compounds to DS to improve their effects. This represents an alarming emerging risk to public health. The QC of DS is therefore of paramount importance to ensure their safety and to protect consumers. Among the 237 DS recalled by the FDA from 2004 through 2012 because they contained hidden ingredients that could be harmful, sexual enhancement products were the most numerous (40%), followed by body-

Received: October 2, 2014
Accepted: October 22, 2014
Published: October 22, 2014

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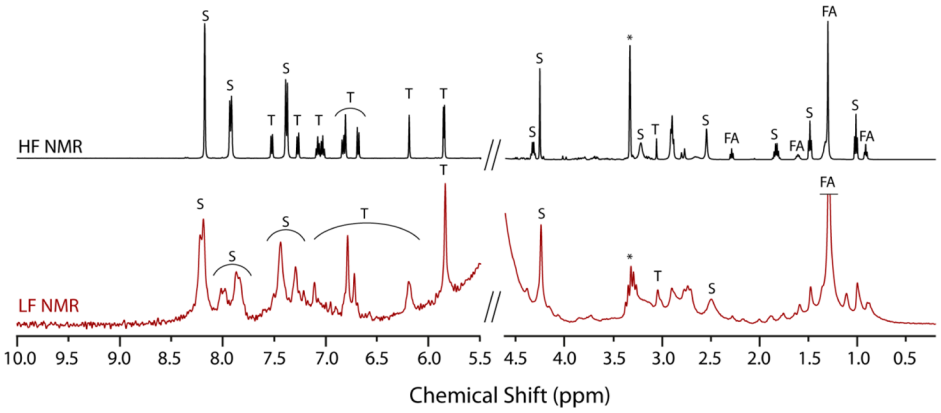


Figure 1. Comparison of the ^{1}H NMR spectra of DS 9 recorded in CD_3OD on the high-field (500 MHz; 1 ppm = 500 Hz) and low-field (60 MHz; 1 ppm = 60 Hz) NMR spectrometers. S: sildenafil; T: tadalafil; FA: fatty acids; ∗: CD_2HOD.

building (31%) and weight loss products (27%).[15] To analyze these products, the gold-standard method is HPLC hyphenated to MS.[16−18] While its sensitivity is excellent, its specificity for detecting unknown adulteration can be problematic. The spectral fingerprint recorded by ^{1}H NMR makes the technique a unique and powerful tool for identification of molecules in general and in the adulteration field in particular.[19] Currently, there is a need for an ideal screening method to characterize adulterated DS. This method should be of low cost, rapid, sensitive, specific, and accurate and would require minimum sample preparation.[18]

The aim of this paper is to evaluate the capabilities of a benchtop low-field (60 MHz) ^{1}H NMR spectrometer to detect and identify pharmaceutical adulterants in "100% natural" sex enhancement and slimming DS. Our approach was based on real samples bought on the Internet with an easy sample preparation to limit the equipment needed. Moreover, the quantification of sildenafil in standard solutions as well as in an adulterated DS is reported.

MATERIALS AND METHODS

Chemicals. Standards of sildenafil citrate and tadalafil were purchased from Sigma-Aldrich (Saint Louis, MO, USA) and TLC Pharmachem, Inc. (Vaughan, Ontario, Canada), respectively. Vardenafil was extracted from the pharmaceutical formulation Levitra. Thiohydroxyhomosildenafil (THHS), desmethylcarbodenafil (DMC), and dithiodesmethylcarbodenafil (DTDMC) were previously purified by HPLC from adulterated DS.

Sample Preparation. Ten adulterated and one nonadulterated sexual enhancement DS as well as four adulterated and one nonadulterated slimming DS were analyzed (Table S-1 in the Supporting Information). All these products claimed as "100% natural" were bought on Internet Web sites. They were previously analyzed in our laboratory and were chosen because they contain a variety of adulterants. The sample preparation was kept as simple as possible. To the powdered tablet or the whole content of the capsule (weight range of 188−802 mg; see Table S-1 in the Supporting Information), 1 mL of deuterated methanol (CD_3OD) containing 0.03% of tetramethylsilane (TMS) as chemical shift reference was added. The mixture was vortexed for 15 s and, after sedimentation, ≈600 μL of the supernatant were transferred into an Aldrich ColorSpec disposable 5 mm × 8 in. NMR tube. However, for the four DS, 1 and 12 (both nonadulterated) and 11 and 7 (both adulterated), adding 1 mL of CD_3OD to the powder did not allow recovery of a sufficient volume. The respective addition of 1.5, 1.5, 2.5, and 3.0 mL of CD_3OD was required to obtain 600 μL of supernatant.

Comparative ^{1}H NMR quantification studies with high-field (HF) and low-field (LF) spectrometers were performed on solutions of standard sildenafil and of DS 2 that was adulterated with this compound. First, from a stock solution of authentic sildenafil citrate (sample 1), a series of 3 diluted samples (dilution by a factor of 1.33, 2, and 4) were prepared. Second, around 10, 20, 30, and 40 mg exact weights of the DS 2 capsule content were vortexed for approximately 15 s with 1.0 mL of CD_3OD. The mixture was then sonicated for 5 min and finally magnetically stirred for 20 min. After centrifugation, 800 μL of the supernatant was introduced in an NMR tube. Thirty μL of a 5 mM solution of sodium 2,2,3,3-tetradeutero-3-(trimethylsilyl)propanoate (TSP) was added as an internal quantification reference before NMR analysis (concentration of 0.181 mM in the NMR tube). Moreover, to evaluate our extraction protocol of tablet or capsule contents, HF ^{1}H NMR quantification of the adulterants in all the DS analyzed was achieved.

High-Field NMR Analysis. Samples were recorded on a Bruker AVANCE 500 MHz spectrometer equipped with a 5 mm cryoprobe. The temperature was set at 298 K. After a 90° radio frequency (RF) pulse, the free induction decay was recorded using a spectral width of 6500 Hz and 32K complex points leading to an acquisition time of 2.52 s. After 4 dummy scans, 8 transients were recorded with a relaxation delay of 3 s. Quantification measurements were performed with a 30° RF pulse, 64K complex points, and 64 transients. To ensure a sufficient time for relaxation, a relaxation delay of 7.5 s was used allowing recovery of 98.8% of the signal intensity of the TSP methyl protons that have the longest T1. Repeatability was estimated from three distinct recordings of the same tube. Data processing consisted in an exponential weighting of the FIDs of 0.3 Hz before Fourier transform. Zero-filling was applied to the data.

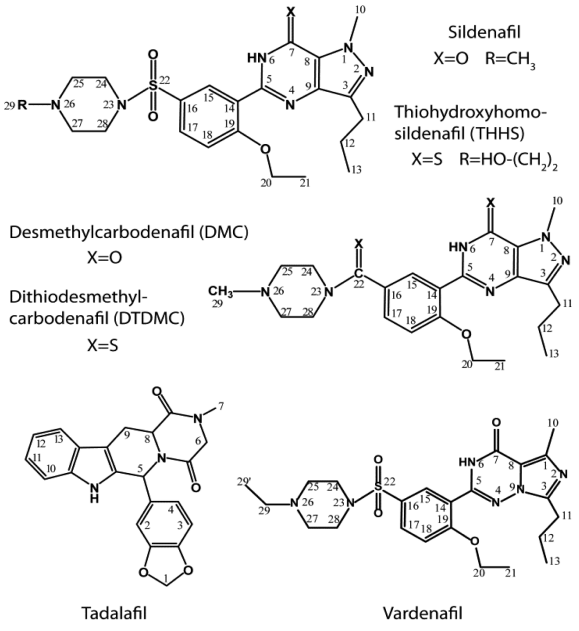
Low-Field NMR Analysis. Spectra were acquired on an Oxford Instruments PULSAR benchtop spectrometer operating at a frequency of 60 MHz for ^{1}H. The temperature inside the spectrometer was 310 K. The acquisition was performed by using SpinFlow 1.2.0.1 (Oxford Instruments) while the processing was done in MNova 9.0 (MestReNova). For the qualitative analysis of standard compounds and DS samples, the

FID was recorded with a 90° RF pulse, a spectral width of 5000 Hz, and 16K complex points (acquisition time of 3.4 s). The relaxation delay was set at 2 s, and 256 transients were recorded leading to a total acquisition time of 22.5 min. For the quantification experiments, a 30° RF pulse was used to optimize the signal per time unit and the number of transients was increased to 512, doubling the experimental time (45 min). To ensure that the protons were fully relaxed, we recorded the ^{1}H NMR spectrum of a solution of standard sildenafil (sample 1) with a relaxation delay of 60 s which did not lead to a significant change in the signal intensities. Quantification experiments were repeated three times for each sample in a random order. For the data processing, the FIDs were apodized with either an exponential/Gaussian (−1/1 Hz) or an exponential (0.5 Hz) filter for detection and quantification, respectively. The number of points was increased to 64K in the Fourier transform spectra.

RESULTS AND DISCUSSION

On the basis of our previous ^{1}H HF NMR studies on adulterated DS,[20−23] we decided to analyze one of them with a benchtop 60 MHz spectrometer. Figure 1 compares the spectra of the same DS solution (DS 9) recorded at both 500 and 60 MHz. LF NMR shows detection of the two adulterants sildenafil and tadalafil previously identified by HF NMR. Decreasing the spectrometer magnetic field decreases the signal-to-noise ratio per unit time by a factor of 60−120 in our recording and processing conditions. As the coupling constants are independent of the magnetic field, the signals appeared much more spread in the spectrum at LF where 1 ppm = 60 Hz than at HF where 1 ppm corresponds to 500 Hz. For instance, the two doublets at 8.19 and 7.43 ppm and the doublet of doublet at 7.92 ppm of the aromatic protons H15, H18, and H17 of sildenafil (Chart 1) are obvious at 60 MHz but cannot be as readily observed in the spectrum recorded at 500 MHz (Figure 1). Also, the characteristic signals of fatty acids (FA) at 500 MHz (≈0.9 ppm (terminal CH_3 protons), ≈1.3 ppm ($-(CH_2)_n-$ protons), ≈1.5 ppm (CH_2 protons β to the carboxyl group), and ≈2.2 ppm (CH_2 protons α to the carboxyl group)) are clearly resolved from those of the adulterant sildenafil, which is not the case at 60 MHz. The region from ≈0 to 2 ppm cannot therefore be used to identify adulterants when high amounts of FA are detected. This encouraging result led us to continue the evaluation of several DS at LF.

Chart 1. Chemical Structures of Sildenafil and Its Analogues, Tadalafil, and Vardenafil



NMR Spectra of Reference Compounds Recorded at Low-Field. The chemical structures of the adulterants found in the sexual enhancement DS are reported in Chart 1. The ^{1}H NMR spectra recorded on the benchtop 60 MHz NMR spectrometer for these reference compounds are shown in Figure 2, and the chemical shifts of each ^{1}H observable at 60 MHz are listed in Table 1.

The visual observation of the spectra is sufficient to point out a specific fingerprint for each of these compounds. The pattern of the aromatic protons of sildenafil, its analogues, and vardenafil (a doublet of doublet for H17 located between a deshielded doublet (apparent singlet for vardenafil) for H15 and a shielded doublet for H18) is clearly different from that of tadalafil (a multiplet between 6.5 and 7.8 ppm for the seven protons H2−H4 and H10−H13 which cannot be assigned individually due to the complexity of the nonfirst order coupling system). However, tadalafil can be identified unambiguously by its characteristic aromatic proton signals at 6.79 and 6.73 ppm as well as its H5 and $CH_2$1 singlets at 6.19 and 5.85 ppm, respectively. Sildenafil and its analogues can be discriminated from vardenafil by their N−$CH_3$10 singlet resonance at 4.2−4.5 ppm while the C−$CH_3$10 singlet of vardenafil is at 2.6 ppm. Moreover, the chemical shift of the H15 doublet which is shifted 0.1−0.2 ppm downfield in C_7═S sildenafil derivatives with respect to C_7═O ones (8.4 ppm in THHS versus 8.2 ppm in sildenafil and 8.1 ppm in DTDMC versus 8.0 ppm in DMC) can be used to differentiate the two series. However, the $CH_3$10 singlet is more convenient to distinguish sildenafil derivatives including DMC from thio-sildenafil derivatives as, in the two families, it appears, respectively, at ≈4.2 and ≈4.5 ppm, although this last signal can be nonvisible if the signal of water is too large.

Detection and Identification of Adulteration in Dietary Supplements. Comparison of the ^{1}H spectra of the sexual enhancement DS shows that all the formulations analyzed except DS 1 are adulterated (Figure 3). The ^{1}H NMR profile of DS 6 indicates the presence of tadalafil identified from its characteristic signals: aromatic protons at 6.79 and 6.73 ppm, H5 (singlet at 6.20 ppm), H1 (singlet at 5.85 ppm), and N−$CH_3$7 (singlet at 3.05 ppm). As most of tadalafil analogues are structurally modified on the N−$CH_3$7 position,[24] this last resonance allows differentiating tadalafil from its analogues. The chemical shifts of the aromatic H15 and H17 and that of the C−$CH_3$10 at 2.60 ppm in DS 8 are characteristic of vardenafil but also of vardenafil analogues. The pattern of the aromatic H15, H17, and H18 and the chemical shifts of the H15 doublet and of the N−$CH_3$10 singlet permit to identify the adulterant: (i) in DS 2 and 3, as sildenafil (previously identified by HR NMR) or one sildenafil analogue with a *N*-methyl piperazine entity characterized by the singlet at ≈2.5 ppm, and (ii) in DS 4 and 5, as a thiosildenafil analogue (THHS as determined by HR NMR), the absence of the singlet characteristic of the N−CH_3 piperazine group (at 2.5−3.0 ppm) excluding the presence of thiosildenafil itself. It should be noted that the resonances of the counterions citrate and mesylate appear in this region (at 2.78 and 2.69 ppm,

dx.doi.org/10.1021/ac503699u | Anal. Chem. 2014, 86, 11897−11904

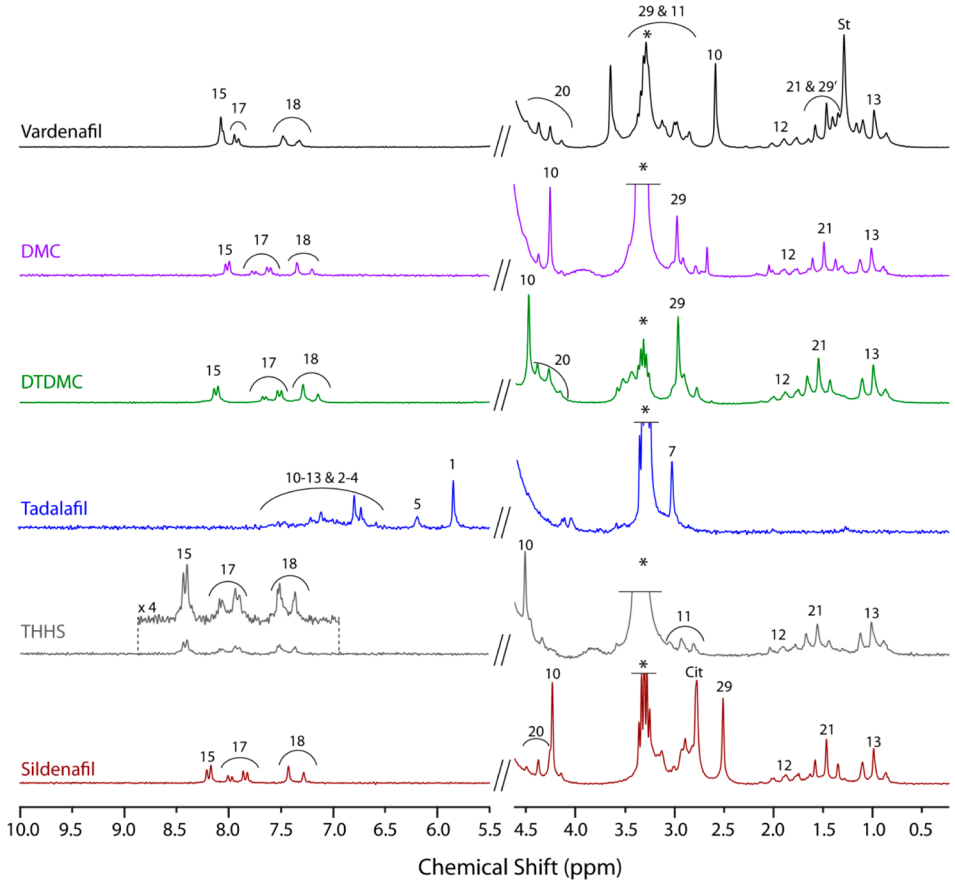


Figure 2. ^{1}H NMR spectra at 60 MHz of standard sildenafil, three of its analogues, tadalafil, and vardenafil recorded in CD_3OD at 37 °C. DMC: desmethylcarbodenafil; DTDMC: dithiodesmethylcarbodenafil; THHS: thiohydroxyhomosildenafil; Cit: citrate; St: residual stearate coming from the formulation of the medicine Levitra; ∗: CD_2HOD. For proton numbering, see Chart 1.

Table 1. ^{1}H NMR Characteristics of Standard Sildenafil and Three Analogues, Vardenafil, and Tadalafil[a]

	chemical shifts (δ, ppm), multiplicity[b], coupling constants (J, Hz)						
position[c]	sildenafil	THHS[d]	DMC[d]	DTDMC[d]	vardenafil	position[c]	tadalafil
10	4.22, s	4.50, s	4.24, s	4.47, s	2.58, s	1	5.85, s
11	2.88, t, 7.2	2.92, t, 7.2	2.89, t, 7.2	2.90, t, 7.2	2.97, t, 7.2	2–4	6.5–7.8, m
12[e]	1.80, sext, 7.2	1.83, sext, 7.8	1.81, sext, 7.2	1.81, sext, 7.2	1.82, sext, 7.8	5	6.19, s
13	0.98, t, 7.2	1.00, t, 7.2	1.00, t, 7.2	0.99, t, 7.2	0.98, t, 7.2	6	N.D.
15	8.19, d, 2.4	8.41, d, 1.8	8.01, d, 2.4	8.12, d, 2.4	8.07, app[b] s	7	3.04, s
17	7.92, dd, 2.4, 9.0	8.00, dd, 1.8, 9.6	7.68, dd, 2.4, 8.4	7.58, dd, 2.4, 9.0	7.99, dd, 2.4, 9.0	8	N.D.
18	7.43, d, 9.0	7.43, d, 8.4	7.27, d, 8.4	7.21, d, 9.0	7.40, d, 9.1	9	N.D.
20	4.31, q, 7.2	N.D.	N.D.	4.32, q, 6.6	4.31, q, 7.2	10–13	6.5–7.8, m
21	1.46, t, 7.2	1.55, t, 7.2	1.47, t, 7.2	1.55, t, 6.6	1.46, t, 7.2		
24/28, 25/27	N.D.	N.D.	3.6–4.1, br[b]	N.D.	N.D.		
29	2.50, s	N.D.	2.96, s	2.96, s	3.06, q, 7.2		
29′		3.80, br[b]			1.28, t, 7.4		

[a]Spectra were recorded in CD_3OD at 37 °C on a 60 MHz Benchtop NMR Spectrometer. [b]s, singlet; d, doublet; dd, doublet of doublet; t, triplet; q, quadruplet; sext, sextuplet; m, multiplet; br, broad signal; app, apparent; N.D., not determined. [c]Proton numbering is shown in Chart 1. [d]THHS: thiohydroxyhomosildenafil; DMC: desmethylcarbodenafil; DTDMC: dithiodesmethylcarbodenafil. [e]Usually only three to four signals of the sextuplet were observed.

respectively). Despite the poor quality of the DS 7 spectrum, the aromatic proton profile and the singlet resonance at 2.72 ppm (N−CH_3) suggest the presence of an adulterant of the sildenafil family with a N−CH_3 piperazine group but the large signal of residual water precludes detection of a resonance at ≈4.5 ppm characteristic of a C_7═S derivative. These findings do not allow unambiguous identification of the adulterant but are compatible with the chemical structure of DTDMC which is the actual adulterant determined by HR NMR.

The profiles of the ^{1}H NMR spectra of DS 9 and 10 are similar and illustrate the presence of two adulterants. Indeed, the resonances of aromatic protons at ≈8.2 (d), 7.9 (dd), and 7.4 (d) ppm as well as the N−CH_3 singlets at ≈4.2 and 2.5 ppm are characteristic of sildenafil itself (the compound

dx.doi.org/10.1021/ac503699u | Anal. Chem. 2014, 86, 11897−11904

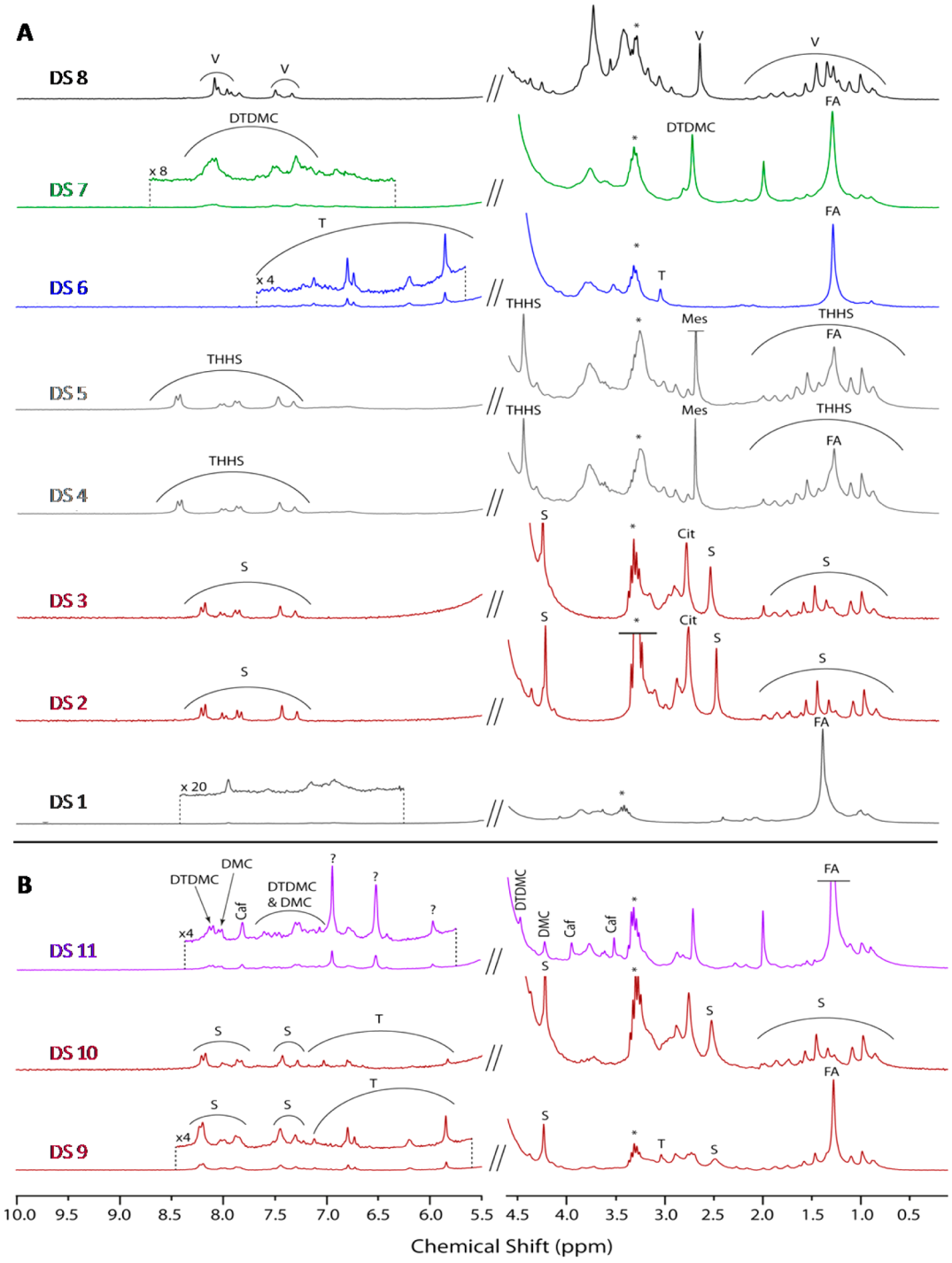


Figure 3. ^{1}H NMR spectra at 60 MHz of the sexual enhancement dietary supplements (DS) analyzed in this study and recorded in CD_3OD at 37 °C. (A) DS containing one adulterant; (B) DS containing two adulterants. The color code for the monoadulterated samples is the same as the one used in Figure 2. DMC: desmethylcarbodenafil; DTDMC: dithiodesmethylcarbodenafil; S: sildenafil; T: tadalafil; THHS: thiohydroxyhomosildenafil; V: vardenafil; Caf: caffeine; Cit: citrate; FA: fatty acids; Mes: mesylate; ∗: CD_2HOD; ?: unknown. As mentioned in the text, the unambiguous identification of most adulterants indicated on the figure was previously done by HF NMR.

identified by HR NMR) or a sildenafil analogue with a *N*-methyl piperazine substituent while the resonances at 6.79, 6.73, 6.20, and 5.85 ppm can be attributed to tadalafil (identified by HR NMR) or a tadalafil analogue. However, in DS 9, the structural identification of tadalafil is validated by the singlet resonance at 3.05 ppm ($N-CH_3$7). In the spectrum of DS 11, the pattern of the signals at 8.14 and 8.06 ppm (2 doublets), 7.57 and 7.43 ppm (2 doublets of doublets), 7.27 and 7.22 ppm (2 doublets), and 4.45 and 4.22 ppm (2 singlets) indicates the presence of two sildenafil analogues, one with a C_7═S moiety and the other with a C_7═O group. Moreover, the fact that the chemical shifts of the aromatic protons are similar to those of standard DMC and DTDMC (Table 1) suggests the presence of carbodenafil and thiocarbodenafil derivatives (DMC and DTDMC were previously identified by HR NMR). The three singlets at 7.78, 3.92, and 3.50 ppm

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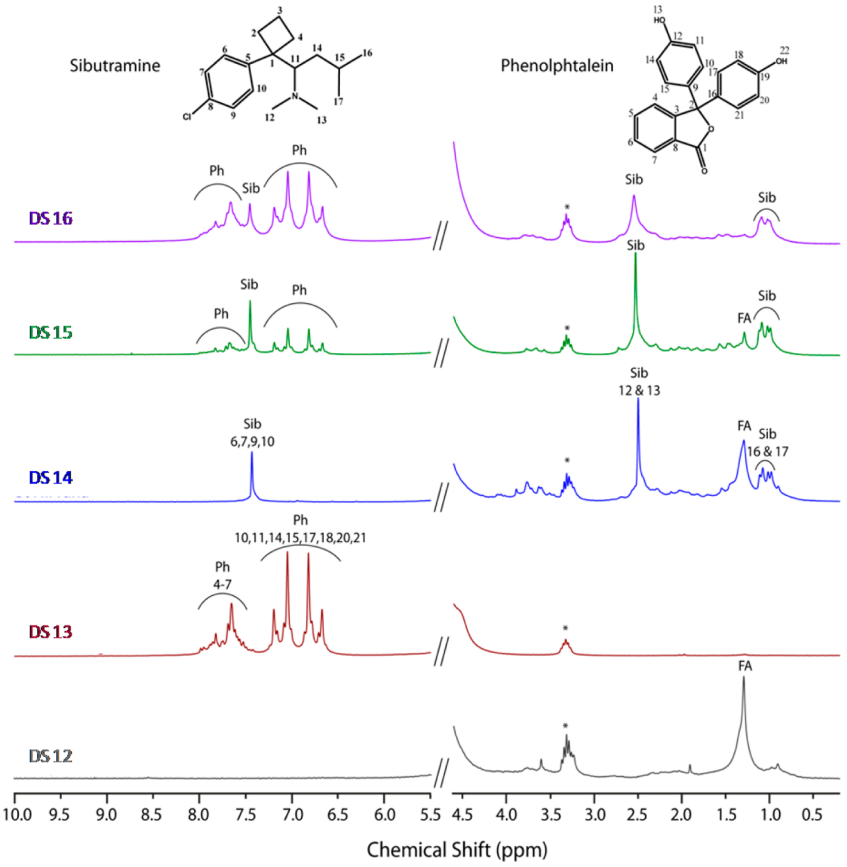

Figure 4. ^{1}H NMR spectra at 60 MHz of the weight loss dietary supplements (DS) analyzed in this study and recorded in CD_3OD at 37 °C. Ph: phenolphtalein; Sib: sibutramine; FA: fatty acids; ∗: CD_2HOD.

belong to caffeine while those at 6.94, 6.52, and 5.97 ppm are not attributed.

The ^{1}H NMR spectra of the weight loss DS analyzed (one nonadulterated and four containing sibutramine or/and phenolphtaleine as identified by HR NMR) are shown in Figure 4. Phenolphtaleine and sibutramine, present alone in DS 13 and 14, respectively, are characterized by a multiplet between 7.4 and 8.0 ppm and an "AB-like" system centered at 6.94 ppm for the former and two singlets at 7.43 and 2.50 ppm and two doublets at 1.03 and 1.06 ppm for the latter. Hence, the presence of both sibutramine and phenolphtaleine is easily detected in DS 15 and 16.

Quantification of Sildenafil on the Benchtop Low-Field NMR Spectrometer. Quantification capabilities of the LF NMR spectrometer were evaluated on the basis of the internal standard method. The data were compared to those obtained on the same sample with HF NMR. First, we quantified four solutions of standard sildenafil at different concentrations ranging from 2.0 to 9.4 mM. The relationship between the concentrations measured at both NMR fields is very good as demonstrated by a correlation coefficient r of 0.997 and a slope of 1.029 (Figure 5) as well as differences between HF and LF NMR measurements <9%. Second, we determined the sildenafil content in a capsule of DS 2 after extraction of four different initial masses (10, 20, 30, and 40 mg).The differences between the values obtained with the HF and LF NMR spectrometers are in the range of 0.4−10.1% (Table 2). These results demonstrate that adulterants can be successfully quantified in DS using the benchtop 60 MHz NMR spectrometer. It can be noted that the duration of a quantatitative experiment on the LF NMR spectrometer is four times longer than that on the HF (45 versus 11 min).

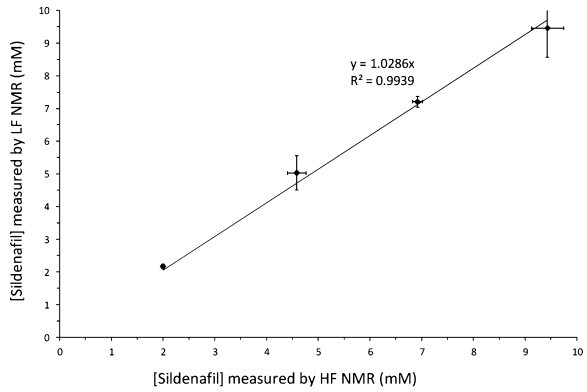

Figure 5. Sildenafil concentrations measured by HF and LF NMR spectroscopy. The error bars represent the standard deviation of the concentrations obtained from 3 measurements of the same sample.

The repeatability of the LF NMR measurements expressed as percentages of relative standard deviation (RSD) is better than 9.5% (range of 2.3−9.4%) except one value at 10.5%. Therefore, the precision of the quantification was considered to be satisfactory. For the HF NMR measurements, the repeatability is within 4% (range of 0.2−3.6%).

With the experimental conditions used (fully relaxed protons, 45 min recording time), the signal-to-noise ratio of sildenafil at a concentration of 2.0 mM is slightly higher than 10, the value most often considered as the limit of quantification (LOQ).

dx.doi.org/10.1021/ac503699u | Anal. Chem. 2014, 86, 11897−11904

Table 2. Comparison of Sildenafil Content in DS2 Measured by HF and LF NMR

mass of powder weighed (mg)	sildenafil amount per capsule (mg)[a] measured by HF NMR	sildenafil amount per capsule (mg)[a] measured by LF NMR	difference between HF and LF NMR values
10	32.4 ± 0.9 (2.8%)	32.6 ± 1.3 (4.1%)	0.6%
20	25.6 ± 0.8 (3.1%)	25.7 ± 2.4 (9.4%)	0.4%
30	24.5 ± 0.1 (0.2%)	27.1 ± 0.8 (2.9%)	10.1%
40	26.1 ± 0.9 (3.6%)	28.2 ± 0.9 (3.1%)	7.7%

[a]Average of 3 measurements ± standard deviation (SD) and relative SD (RSD) in %.

Similarly, the concentration of sildenafil in the supernatant resulting from the extraction of 10 mg of DS 2 is 2.4 mM, near the LOQ value.

Comments on the Experimental Methodology for the Detection of Adulterants. The sample preparation procedure chosen for this study is very simple and rapid as it consists of adding 1 mL of CD_3OD to the whole capsule content or to the whole tablet powdered and analyzing the supernatant. However, it does not allow the extraction of the total amount of adulterant(s). The recovery efficiency was determined by comparing the amounts of adulterants measured by HF NMR in each DS analyzed in this study to those measured in the same DS previously analyzed by HF NMR after an extraction protocol giving a recovery of >96%.[25] The recovery percentages were in the range of 4−71% for sexual enhancement DS and 51−90% for weight loss DS, depending on several factors such as the adulterant solubility, the amount of adulterant in the capsule or tablet analyzed (from 8 to 95 mg), the weight of capsule content or tablet (≈190 to 800 mg), and the matrix effect. The lowest concentration of adulterant detected with the LF NMR spectrometer was that of tadalafil in DS 10 (0.9 mM) as measured with HF NMR.

CONCLUSION

In this study, we demonstrated that the presence of adulterants in "100% natural" sexual enhancement and weight loss DS can readily be detected with a benchtop LF NMR spectrometer. After a very simple and rapid sample preparation, 22.5 min of recording is sufficient to detect a concentration of 0.9 mM. Even if HF NMR spectroscopy has to be employed for an unambiguous structural identification of the adulterants, LF NMR provides valuable clues on their chemical structure. We also showed that the quantification by the internal standard method can be done on the LF NMR spectrometer in a reasonable period of time (45 min) and leads to data similar to those obtained with HF NMR for concentrations as low as ≈2 mM.

Benchtop LF ^{1}H NMR spectroscopy obviously has the advantages and limitations of NMR. It requires minimal sample preparation although in a deuterated solvent. It is nonselective so all the compounds present in the solution, provided they contain the nucleus under investigation and are present at a sufficient concentration, are detected simultaneously in a single run. It gives structural information. NMR is intrinsically insensitive, which is not a real limitation in the case of DS adulteration as the concentration of the adulterant is usually substantial. Compared to HF ^{1}H NMR, both resolution and sensitivity are of course greatly impaired, thus limiting the easiness of detection and quantification, but the main advantage of the benchtop LF NMR equipment relies on its "saving capabilities" (saving purchase cost (≈8 times less expensive than the HF NMR spectrometer used in this study), saving maintenance and running costs, saving space, saving user experience). The requirement of no prior separation step and a much easier quantification procedure without resorting to reference substances constitute the major assets of LF NMR with respect to LC-MS, the most common analytical technique in laboratories controlling the quality of DS.[16−18] LF NMR is an invasive method in contrast to IR and Raman spectroscopies or X-ray powder diffractometry. However, these techniques, if they distinguish pure natural DS from adulterated ones, provide no or little information on the chemical identity of the adulterant(s) and are not quantitative.

This work highlights that benchtop NMR spectroscopy is an excellent method to uncover DS adulteration. This "saving spectrometer" may become part of QC laboratories and governmental agencies (customs, fraud detection, health agencies, and so on) as an initial routine screening tool for detecting adulteration.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors wish to acknowledge the French National Agency for the Safety of Medicines and Health Products (Agence Nationale de Sécurité du Médicament et des produits de santé: ANSM) for financial support (project AAP-2012-082, convention ANSM/UPS No. 2012S071).

REFERENCES

(1) Colnago, L. A.; Andrade, F. D.; Souza, A. A.; Azeredo, R. B. V.; Lima, A. A.; Cerioni, L. M.; Osán, T. M.; Pusiol, D. J. *Chem. Eng. Technol.* **2014**, *37*, 191−203.

(2) Hills, B. P. In *Annual Reports on NMR Spectroscopy*; Webb, G. A., Ed.; Academic Press: Oxford, UK, 2006; pp 177−230.

(3) Mitchell, J.; Gladden, L. F.; Chandrasekera, T. C.; Fordham, E. J. *Prog. Nucl. Magn. Reson. Spectrosc.* **2014**, *76*, 1−60.

(4) Duval, F. P.; Cambert, M.; Mariette, F. *Appl. Magn. Reson.* **2005**, *28*, 29−40.

(5) Mortensen, M.; Thybo, A. K.; Bertram, H. C.; Andersen, H. J.; Engelsen, S. B. *J. Agric. Food Chem.* **2005**, *53*, 5976−5981.

(6) Hansen, C. L.; Thybo, A. K.; Bertram, H. C.; Viereck, N.; van den Berg, F.; Engelsen, S. B. *J. Agric. Food Chem.* **2010**, *58*, 10300−10304.

(7) van As, H.; Schaafsma, T. J. *Biophys. J.* **1984**, *45*, 469−472.

(8) Jaffel, H.; Korb, J.-P.; Ndobo-Epoy, J.-P.; Morin, V.; Guicquero, J.-P. *J. Phys. Chem. B* **2006**, *110*, 7385−7391.
(9) Muller, A. C. A.; Scrivener, K. L.; Gajewicz, A. M.; McDonald, P. J. *J. Phys. Chem. C* **2012**, *117*, 403−412.
(10) Simina, M.; Molnar, L.; Manea, D.; Ardelean, I. *Appl. Magn. Reson.* **2012**, *43*, 443−450.
(11) Borgia, G. C.; Bortolotti, V.; Brancolini, A.; Brown, R. J. S.; Fantazzini, P. *Magn. Reson. Imaging* **1996**, *14*, 751−760.
(12) van der Zwaag, C. H.; Stallmach, F.; Skjetne, T.; Veliyulin, E. *Magn. Reson. Imaging* **2001**, *19*, 543−545.
(13) Parker, T.; Limer, E.; Watson, A. D.; Defernez, M.; Williamson, D.; Kemsley, E. K. *TrAC, Trends Anal. Chem.* **2014**, *57*, 147−158.
(14) Harrison-Dunn, A.-R. *A global look at supplements on the rise*; http://www.nutraingredients.com/Industry/A-global-look-at-supplements-on-the-rise; Accessed: 19/10/2014.
(15) Harel, Z.; Harel, S.; Wald, R.; Mamdani, M.; Bell, C. M. *JAMA Int. Med.* **2013**, *173*, 926−928.
(16) Singh, S.; Prasad, B.; Savaliya, A. A.; Shah, R. P.; Gohil, V. M.; Kaur, A. *TrAC, Trends Anal. Chem.* **2009**, *28*, 13−28.
(17) Martino, R.; Malet-Martino, M.; Gilard, V.; Balayssac, S. *Anal. Bioanal. Chem.* **2010**, *398*, 77−92.
(18) Patel, D. N.; Li, L.; Kee, C.-L.; Ge, X.; Low, M.-Y.; Koh, H.-L. *J. Pharm. Biomed. Anal.* **2014**, *87*, 176−190.
(19) Holzgrabe, U.; Malet-Martino, M. *J. Pharm. Biomed. Anal.* **2011**, *55*, 679−687.
(20) Balayssac, S.; Trefi, S.; Gilard, V.; Malet-Martino, M.; Martino, R.; Delsuc, M.-A. *J. Pharm. Biomed. Anal.* **2009**, *50*, 602−612.
(21) Vaysse, J.; Balayssac, S.; Gilard, V.; Desoubdzanne, D.; Malet-Martino, M.; Martino, R. *Food Addit. Contam., Part A* **2010**, *27*, 903−916.
(22) Vaysse, J.; Gilard, V.; Balayssac, S.; Zedde, C.; Martino, R.; Malet-Martino, M. *J. Pharm. Biomed. Anal.* **2012**, *59*, 58−66.
(23) Balayssac, S.; Gilard, V.; Zedde, C.; Martino, R.; Malet-Martino, M. *J. Pharm. Biomed. Anal.* **2012**, *63*, 135−150.
(24) Venhuis, B. J.; de Kaste, D. *J. Pharm. Biomed. Anal.* **2012**, *69*, 196−208.
(25) Gilard, V.; Balayssac, S.; Tinaugus, A.; Martins, N.; Martino, R.; Malet-Martino, M. *J. Pharm. Biomed. Anal.* **2014**, DOI: 10.1016/j.jpba.2014.10.011.