Accurate determination of adulterants in dietary supplements using shotgun high-resolution tandem mass spectrometry

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Introduction

Sales of dietary supplements (DS) increase each year, particularly in developed countries, where they remain considered by many consumers and physicians as harmless because of their natural origin or presumed safe composition. Nowadays, a growing trend consists in the intentional adulteration of DS with synthetic drugs and represents an alarming emerging risk to public health. DS for treating sexual dysfunction are among the most sold and the most affected by adulteration. Indeed, manufacturers add synthetic actives like phosphodiesterase type 5 (PDE-5) inhibitors (sildenafil, tadalafil, vardenafil) to the natural matrix in order to intensify the pharmacological effect. Furthermore, in an attempt to evade regulatory inspection, unscrupulous manufacturers use not only approved active pharmaceutical ingredients but also unapproved analogs in which minor chemical modifications are brought to the parent structure. These occurrences enhance the need to propose a performant analytical procedure for detecting multiple adulterations.
In this poster, we focus on how NMR and MS were used to study two complex formulations called “Maximen Pills International” and “Herbal Stud”. Both contained a mixture of PDE-5 analogs.

Experimental part

Sample preparation

For each DS, the tablets were powdered (or capsules emptied) and the powder was analyzed by MS and NMR.

MS analysis

The powder was dissolved in CH_3CN:H_2O (80:20 v/v) and analyzed directly using a Waters XEVO G2 QTOF mass spectrometer under positive and negative ionization modes.

NMR analysis

The powder was dissolved in 1 mL of CD_3CN:D_2O (80:20 v/v). ^{1}H NMR experiments were run on a Bruker Avance 500 spectrometer operating at 500.13 MHz, equipped with a 5 mm cryoprobe at 298K.

^{1}H NMR spectra of dietary supplements

^{1}H NMR was used for rapid profiling of DS content. NMR detects the presence of adulteration, particularly by screening the area of the aromatic protons (7-9 ppm). In the two cases presented below, due to the presence of several adulterants with a close chemical structure, ^{1}H NMR did not allow the identification of each adulterant it was thus necessary to perform a complementary method.

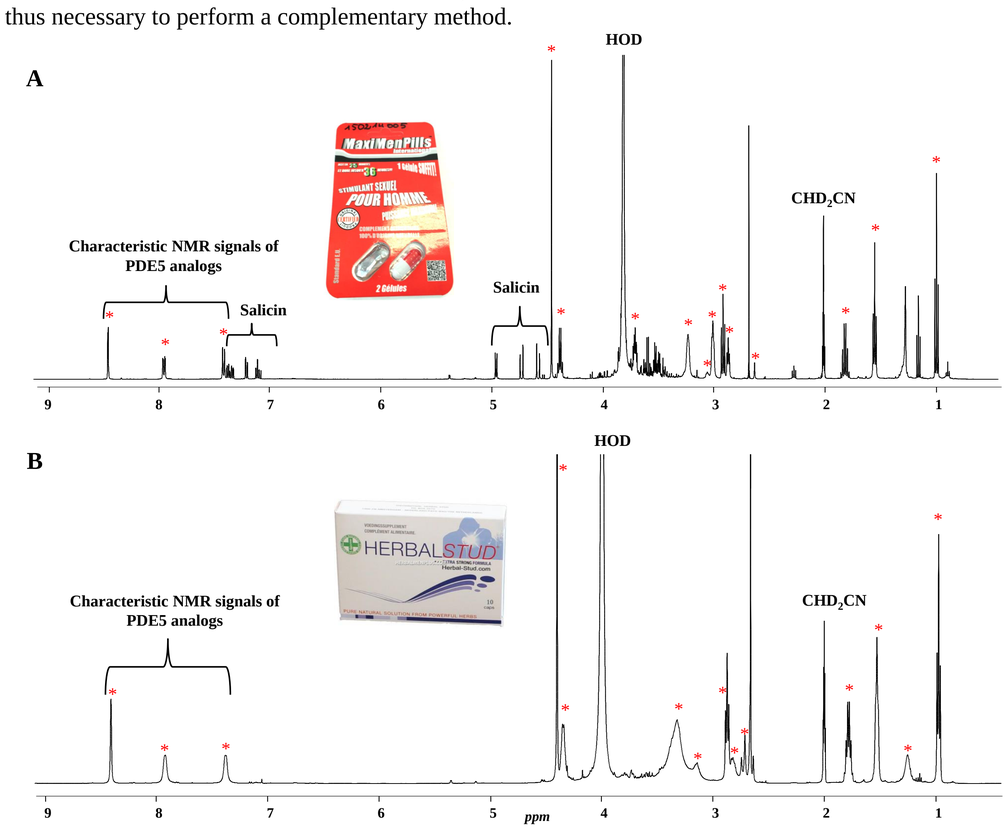


Figure 1. ^{1}H NMR spectra of two formulations, (A) Maximen Pill International and (B) Herbal Stud. *main detectable signals of adulterants

Results

135 DS were analyzed using both ^{1}H NMR and MS. NMR was used for a rapid screening of these preparations and for the detection of adulterants mainly based on the characteristic signals of their aromatic protons. Then high resolution MS/MS with direct infusion was performed as an orthogonal analytical method for the determination of their accurate molecular masses and elucidation of their structures from the analysis of their fragmentations. Because the chemical structures of some PDE-5 analogs are very similar, HR-MS is not sufficient for the non-ambiguous determination of some analogs, and the exact structure can be only assigned after analysis of the MS/MS fragmentation pattern.

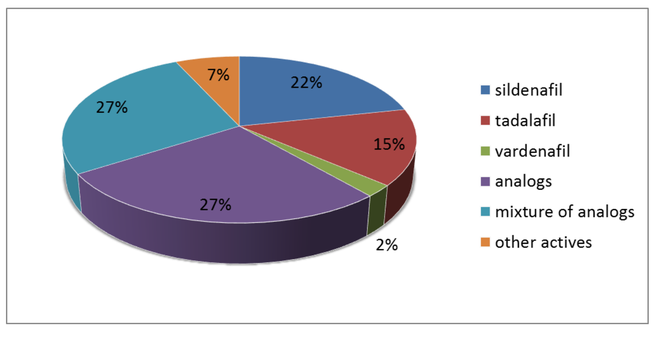


Figure 4. Pie-chart representing the nature of the adulteration for the 88 adulterated formulations

Among the DS analyzed, 65% were adulterated. Figure 4 shows that the adulterants were either registered PDE-5 inhibitors (sildenafil, tadalafil, vardenafil) or unregistered analogs. Formulations containing analogs alone or as mixtures represented 55% of the adulterated formulations. Other actives detected were DHEA, testosterone, flibanserine and phentholamine.

MS and MS/MS analyses for accurate determination of adulterants

The MS spectrum of “Maximen Pills International” (not shown) presents three main ions at m/z 491, 505 and 521 corresponding to pseudomolecular ions of three adulterants. The MS/MS spectra are presented below (figure 2). MS/MS allowed the identification of thiosildenafil, thiomethisosildenafil and hydroxythiohomosidenafil each presenting a characteristic fragmentation pattern.

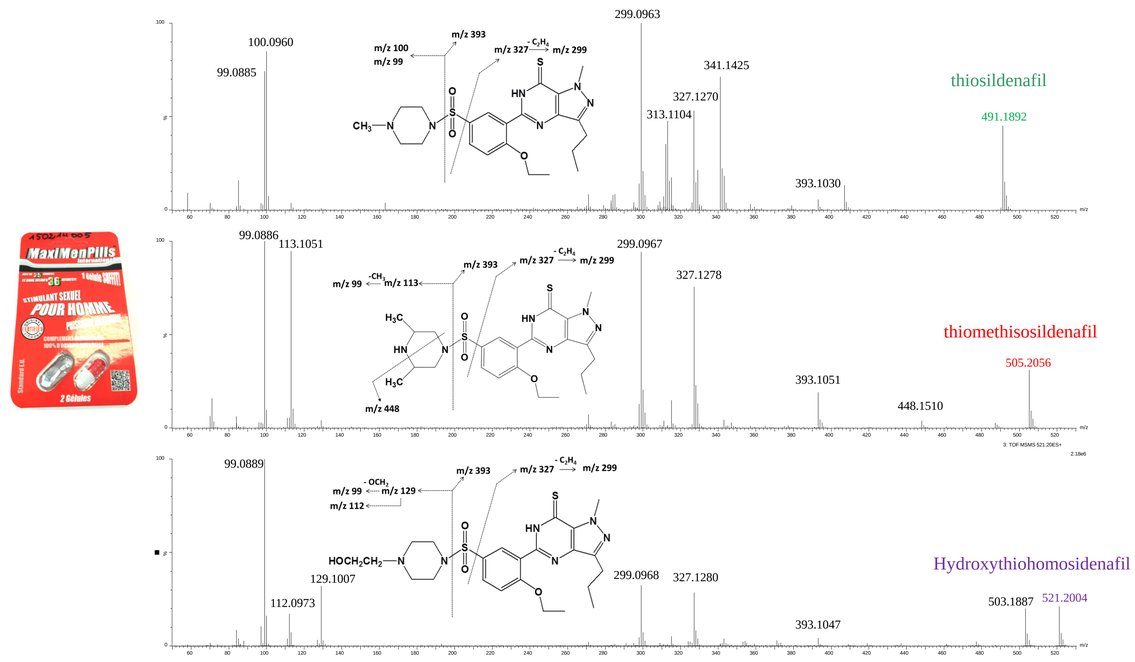
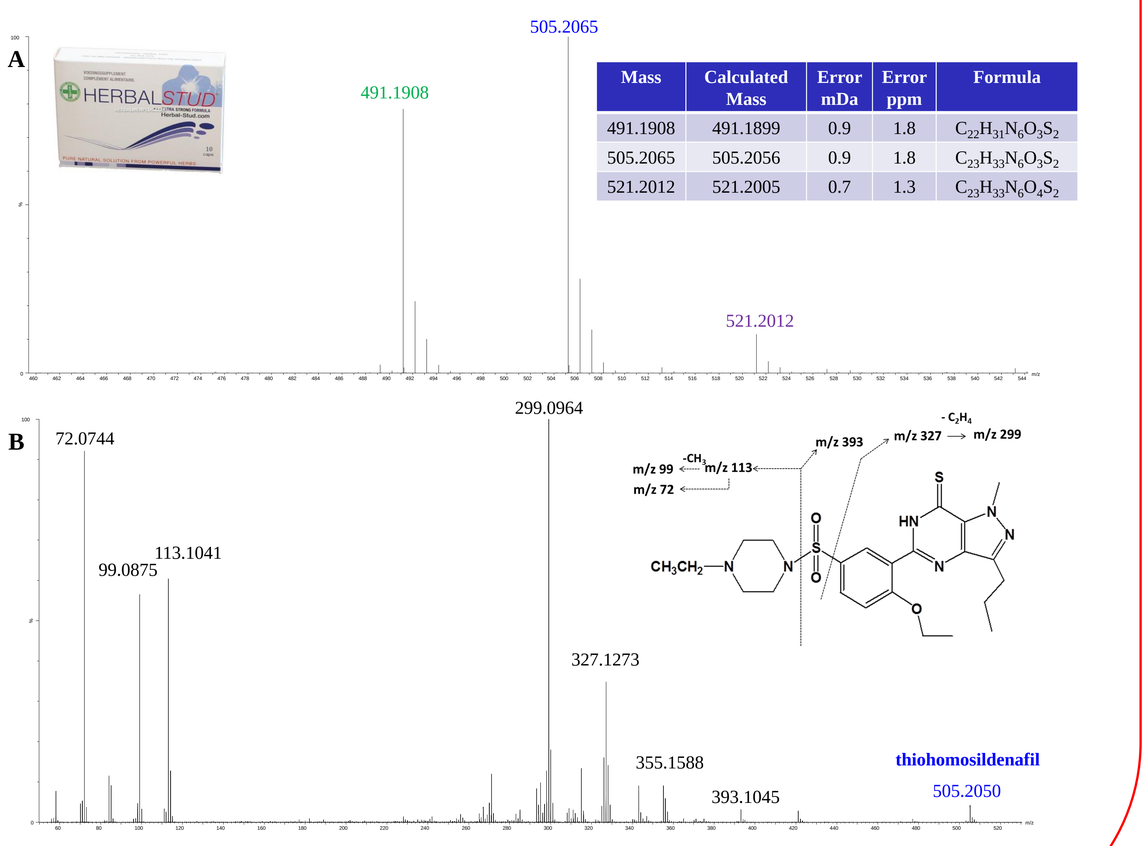


Figure 2. MS/MS spectra in positive ionization mode of thiosildenafil (m/z 491), thiomethisosildenafil (m/z 505) and hydroxythiohomosidenafil (m/z 521) , obtained by direct infusion of the "Maximen Pill International " dietary supplement

The MS spectrum of “Herbal stud” (Figure 3A) presents three main ions at m/z 491, 505 and 521. The MS/MS spectra (not shown) of ions m/z 491 and 521 are similar to those presented in figure 2 and demonstrated the presence of thiosildenafil and hydroxythiohomosidenafil. However in this formulation, the MS/MS performed on the 505 ion allowed thiohomosildenafil to be identified. Indeed, thiohomosildenafil and thiomethisosildenafil are isomers, so they are impossible to distinguish on the basis of their pseudomolecular peak. A distinction can be made from the relative intensities of their product ions; in the spectrum of the thiohomosildenafil (Figure 3B) m/z 99 and m/z 113 peaks are markedly less intense compared to the spectrum of the thiomethisosildenafil , while peak at m/z 72 is considerably more intense. Other useful peaks to distinguish between these isomers are m/z 355 (alkyl transfer from the basic nitrogen to sulfur), which is only detectable in the spectrum of thiohomosildenafil or m/z 448, only detectable in the spectrum of thiomethisosildenafil.



Mass	Calculated Mass	Error mDa	Error ppm	Formula
491.1908	491.1899	0.9	1.8	$C_{22}H_{31}N_6O_3S_2$
505.2065	505.2056	0.9	1.8	$C_{23}H_{33}N_6O_3S_2$
521.2012	521.2005	0.7	1.3	$C_{23}H_{33}N_6O_4S_2$

Figure 3. A: MS spectrum in positive ionization mode of the " Herbal Stud " dietary supplement. B: MS/MS spectrum on the m/z 505 ion corresponding to thiohomosildenafil

Conclusion

The issue of adulteration with synthetic compounds is an increasing concern and all possible analytical tools should be used to ensure the quality and safety of products on the market. This study combined ^{1}H NMR profiling and HR-MS/MS analysis and allowed the detection of 3 PDE-5 inhibitors and 13 of their analogs among the 135 DS analyzed.
Despite the chemical complexity of DS, shotgun MS/MS analysis allowed a rapid and accurate determination of contaminants.

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