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2D DOSY ^{1}H NMR for the detection of active pharmaceutical ingredients in herbal dietary supplements

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Introduction

In the last few decades, worldwide traditional herbal medicines are gaining popularity as a source of complementary and alternative remedies. In contrast to conventional pharmaceuticals, these products are regarded by many as being harmless because of their natural origin and helpful to the treatment of some chronic diseases and the maintenance of physical fitness. Obesity and overweight affect over 1.6 billion individuals worldwide and are associated with premature mortality, diabetes, heart disease, chronic morbidity and cancer. Given the medical and social impact of being overweight, more and more patients often turn to over-the-counter propriety weight-loss products. The dietary ingredients in these products may include vitamins, minerals, herbs or other botanicals, amino acids such as enzymes, organs tissues, and metabolites. However, common problems affecting the safety of herbal medicines include adulteration with conventional pharmaceuticals which can lead to severe side effects.
In this study we show an application of 2D Diffusion Ordered SpectroscopY ^{1}H Nuclear Magnetic Resonance (2D DOSY ^{1}H NMR) for the analysis of twenty herbal dietary supplements marketed as natural slimming products.

Experimental part

Formulations

Twenty herbal dietary supplements marketed as natural slimming products were analyzed. Formulations Garslim (16), Alattar Psyllium (17), Alattar slimming mixture (18) were bought in Syria, Perfect slim cherry (1), Perfect slim grape (5), Cider Vinegar dieting (7) in China. The other formulations were purchased over the internet : 9 rapide (2, China), Unknown (3, China), Unknown (4, China), Honey Life (6, China), Botanical Slimming Herbs (8, China), Botanical Slimming Herbs (9, China), Oringinal (10, Japan), Lipro diet pills (11, China), Lida (12, China), onting quianweisu slimming herbs (13, China), Meekeyes (14, China), Perfect slim (15, China), Hyper Drive 3.0 (19, USA), Elan-Sil (20, UK).

Sample preparations

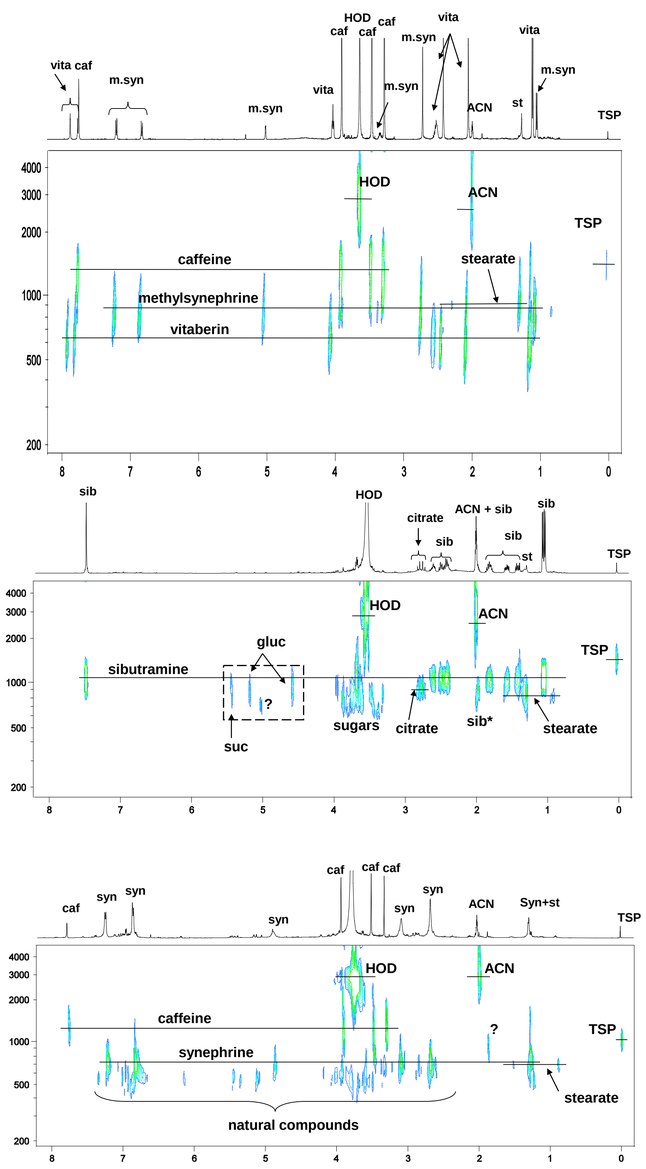
The tablets were powdered (or capsule emptied) and the powder dissolved in 4 mL of CD_3CN:D_2O (80:20 v/v) under magnetic stirring during 10 min and then sonicated for 10 min. The suspension was centrifuged and the supernatant analyzed.

NMR experiments

^{1}H NMR experiments were performed on a Bruker Avance 500 spectrometer operating at 500.13 MHz equipped with a 5 mm cryoprobe at 298K.

Stimulated echo bipolar gradient pulse experiments were used for 2D DOSY experiments (gradient pulse length from 1.4 to 1.75 ms and diffusion delay from 50 to 100 ms) with 40 gradient steps for the diffusion dimension. 3D DQF-COSY DOSY including water presaturation pulse was recorded (gradient pulse length of 1.9 ms and diffusion delay of 50 ms) with 28 gradient steps and 104 data points for the diffusion and COSY dimensions, respectively. These experiments were processed using the Gifa 5.2 and NPK softwares and analyzed with the NMRnotebook package.

2D DOSY ^{1}H NMR spectra of formulations



Hyperdrive (USA), capsule
Formulation **19**

Unknown (China), capsule
Formulation **???**

Lida (China), capsule
Formulation **12**

Figure 1. 2D DOSY ^{1}H NMR spectra of three formulations

Caf : caffeine, syn : synephrine, st : stearate, suc : sucrose, gluc : glucose, sib : sibutramine, vita : vitaberin, msyn : methylsynephrine, ? : unknown products, sib* : processing artefact due to superimposition with signal of solvent (ACN)

Compounds detected by NMR

Table 1. Ingredients detected by ^{1}H NMR and 2D DOSY ^{1}H NMR in the 20 herbal products analyzed in CD_3CN:D_2O (80:20).

Formulations	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
sibutramine	+	+	+	+	+		+	+	+	+	+		+	+	+					
phenolphthalein	+				+		+		+						+					
synephrine												+							+	+
methylsynephrine																			+	+
vitaberin																			+	+
yohimbine																				
caffeine												+						+	+	
carnitine						+														+
stearate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
citrate			+																	
vitamin C																				+
vitamin C[a]	+														+					+
vitamin B_1																				+
vitamin B_3						+														+
vitamin B_5																				+
vitamin B_6																				+
vitamin E																				+
glucose	+		+	+	+		+		+	+	+		+		+					
fructose	+		+	+									+							
sucrose			+	+	+		+		+			+	+	+	+					

All formulations were analyzed with 2D DOSY ^{1}H NMR. 2D DOSY spectra of formulations **3**, **12** and **19** along with their corresponding 1D spectrum are presented in **Figure 1**.

All ingredients detected by NMR investigations in the herbal drugs studied are reported in **Table 1**.

Several active pharmaceutical ingredients (API) were detected : sibutramine in formulations **1-5**, **7-11**, **13-15**, phenolphthalein in formulations **1**, **5**, **7**, **9**, synephrine and methylsynephrine, two adrenergic amines, in formulations **12** and **19**, respectively. Some publications reported that sibutramine can present a possible cardiovascular risk[1] and that phenol-phatalein[2] has carcinogenic effects. Synephrine has also possible cardiovascular side-effects[3][4].

Various other components were detected in tablets or capsules, mainly sugars (lactose, glucose, fructose and sucrose) and vitamins (B3, B1, B5, B6, E, C)…

3D DOSY-COSY ^{1}H NMR

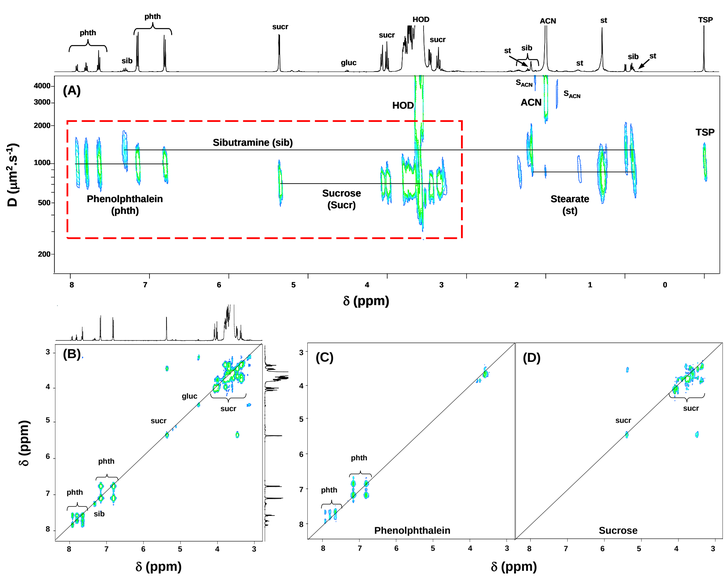
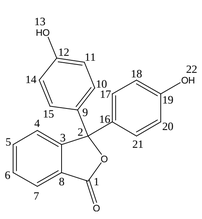


Figure 2. NMR spectra of formulation 7 in CD_3CN:D_2O (80:20)
(A) 2D DOSY ^{1}H spectrum; (B) COSY-DQF spectrum;
COSY extractions from 3D DOSY-COSY experiment at (C) D = 990 µm².s^{-1}, (D) D = 707 µm².s^{-1}.

Figure 2 illustrates the different NMR spectra obtained in the 3D DOSY-COSY ^{1}H NMR experiment applied to formulation **7**. The main interest of this 3D experiment is to extract the COSY spectrum of each component of the mixture from a selected line in the DOSY spectrum. These spectra highlight the interest of 3D experiment as virtual separation provided by DOSY acquisition can lead to real structural determination by extraction of COSY spectra.

Formulation **7**
Cider vinegar dieting (China), tablets

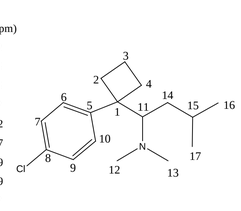
NMR assignments of phenolphtalein and sibutramine in CD_3CN:D_2O (80:20)



phenolphthalein

NMR characteristics of phenolphthalein

Atom number	δ ^{1}H (ppm)	Multiplicity (J in Hz)	COSY ^{1}H-^{1}H	δ ^{13}C (ppm)
1	-	-	-	170.7
2	-	-	-	92.4
3	-	-	-	124.7
4	7.64	d (7.6)	5	124.3
5	7.80	t (7.5)	4,6	134.9
6	7.64	t (7.2)	5,7	129.7
7	7.94	d (7.8)	6	125.7
8	-	-	-	152.9
9,16	-	-	-	157.2
10,15,17,21	7.18	d (8.7)	11,14,18,21	128.6
11,14,18,20	6.85	d (8.7)	10,15,17,22	115.3
12,19	-	-	-	132.0



sibutramine

NMR characteristics of sibutramine

Atom number	δ ^{1}H (ppm)	Multiplicity (J in Hz)	^{1}H-^{1}H COSY	^{1}H-^{1}H NOESY	δ ^{13}C (ppm)
1			-	-	49.2
2	2.59-2.48	m	3	6,3	32.5
3	1.98,1.79	m	2,4	2,4,6,11,14	15.5
4	2.43-2.37	m	3	3,11,14	33.7
5	-	-	-	-	140.2
6,10	7.47	AA'BB'	-	2,3	128.7
7,9		system	-		129.9
8			-	-	132.9
11	3.67	t (6.3)	14	3,4,12,13,14,15,16,17	71.7
12 or 13	2.19	broad s	-	11,13,14,15	39.6[a]
13 or 12	2.81	broad s	-	11,12,14,15	47.0[a]
14	1.53-1.39	m	11,15	3,4,11,12,13,15,16,17	33.5
15	1.79	m	14,16,17	11,12,13,14,16,17	25.4
16 or 17	1.01	d (6.5)	15,17	11,14,15	22.0
17 or 16	1.04	d (6.5)	15,16	11,14,15	21.2

[a]The chemical shifts of these signals were measured on a spectrum recorded in CD_3CN

s: singlet; d: doublet; t: triplet; m: multiplet

Conclusion

Adulteration of herbal medicines with undeclared synthetic drugs is a common and dangerous phenomenon. Methods that ensure the quality and safety of these products have thus to be developed. In this context, 2D DOSY ^{1}H NMR spectroscopy is a powerful method for providing a multivariate fingerprint of a complex mixture[5] especially when the identity of the components is not known beforehand. 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.

References

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