

## Isolation and Identification of N-Mono-Desmethyilsibutramine in a Slimming Herbal Product

### Zayıflama Bazlı Bitkisel Bir Üründen N-Mono-Desmetilsibutramin İzolasyonu ve Tayini

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#### Abstract

N-Mono-desmethyilsibutramine which has been added illegally as adulterant in a Chinese herbal product for weight loss; was isolated and identified. The adulterant was purified by preparative tlc from the methanol extract of herbal product. The structure of the isolated compound was identified using 1D- and 2D- NMR and ESI-MS techniques.

**Keywords:**Herbal product; N-mono-desmethyilsibutramine; adulterant; isolation; NMR.

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#### Introduction

The use of herbal medicines as a source of complementary and alternative remedies is growing worldwide. Medicinal plants are also used not only as food, but also functional food, nutritional and dietary supplements in several countries.

Physicochemical and biological contaminants, toxicity, adulteration, lack of standardization, incorrect labelling, largely unregulation, lack of effective quality control system and loose distribution channels (including mail order and internet sales ) can be concluded as the main problems of herbal medicine and herbal products(WHO guidelines 2007).

The presence of the potentially hazardous contaminants, residues as well as synthetic adulterants in herbal products, can lead to acute or chronic toxicity, severe adverse effects and drug interactions.

Sibutramine and its metabolites as well as homosibutramine as adulterants were detected in some herbal products prepared for weight loss (Jung et al.,2006; Zou et al.,2007; Lai et al.,2007). In addition to this sibutramine and an acetylated sibutramine metabolite were identified in patients' urine after intake of a herbal drug for slimming product (Jung et al., 2006; Vidal et al., 2006 ).There are many reports on adulterations of herbal products by synthetic drugs (Sakar, 2005; Ku et al.,2003; McConkey, 2003; Miller et al.,2007).

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Sibutramine, which has been used as an antiobesity drug, decreases the bodyweight by reducing food intake and by increasing energy expenditure (Scholze et al., 2007). However sibutramine has several disorders such as insomnia, constipation (Bray 2001), memory impairment (Clark et al., 2004), panic attacks (Binkley et al., 2002). It also increases the blood pressure and heart rate (Luque et al., 2002). Therefore since January 2006, sibutramine is in the list of prohibited substances established by the World Anti-Doping Agency.

According to a Turkish daily newspaper (Radikal 2007), a 19 years old boy had used a slimming herbal product which has no permission to be sold in pharmacy, was dead. Primary and secondary amine metabolites of sibutramine are more active than sibutramine HCl as inhibitors of the uptake of noradrenaline, dopamine and 5-hydroxytryptamine in vitro (Luscombe et al., 1989).

The aim of this study is to isolate and identify of unknown synthetic adulterant in Chinese herbal product named "LiDa Dai Dai Hua capsules".

## Material and Methods

### Material

Lida Dai Dai Hua capsules (Chinese herbal slimming product) were received from Istanbul Governorship, Province of Agricultural Department.

### Equipments

The NMR spectra were recorded on a Varian-Mercury 400 MHz (400 MHz for  $^1\text{H}$ , 100MHz for  $^{13}\text{C}$ ) with methanol- $d_4$  as solvent. The ESI-MS spectrum was recorded on a Micromass ZQ (Waters).

### Extraction and isolation

The powdered sample ("LiDa Dai Dai Hua" capsules) was dissolved in methanol and applied to four silica gel 60F<sub>254</sub> pre-coated TLC plates, (20x20 cm, Merck, Art No; 1.05554) and developed with mobile phase toluene-ethylacetate-formic acid (25:20:5 (v/v)). The TLC plates were observed under UV<sub>254</sub> lamp and the N-mono-desmethylsibutramine (1) spot was marked (Rf:0.26) and a part of plate was sprayed with Dragendorff reagent for the detection. The spots were scraped and dissolved in methanol and then evaporated. The residue (32mg) was used for structure determination by NMR and MS methods.

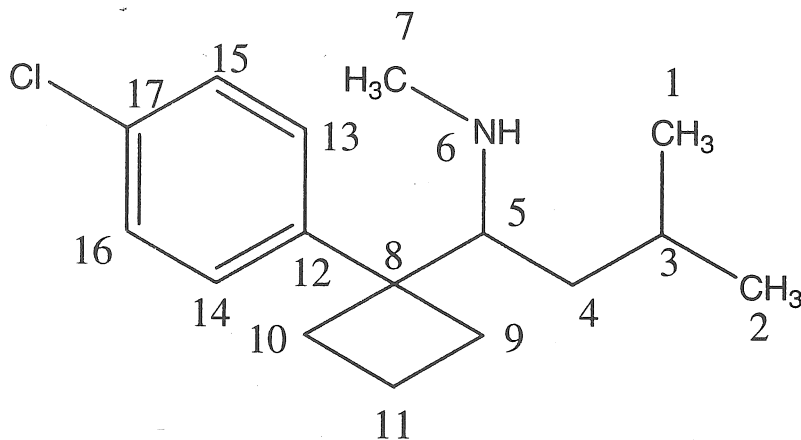
Table 1.  $^1\text{H}$  and  $^{13}\text{C}$  NMR data for N-mono-desmethylsibutramine ( $\text{CD}_3\text{OD}$ , J in Hz)

No	$^{13}\text{C}$ ( $\delta_{\text{C}}$ )	$^1\text{H}$ ( $\delta_{\text{H}}$ )	COSY
1, 2	22.03, 22.13	0.97 (3H, d, J= 6.8) 0.98 (3H, d, J= 6.8)	H-1/2
3	25.65	1.69 (1H, m)	H-3
4	37.35	1.17 (2H, m)	H-5
5	65.55	3.53 (1H, dd, J= 5.2/4.8)	H-4/H-6
6	-	1.43 (1H, m)	H-5
7	32.82	2.70 (1H, s)	-
8	49.36	-	-
9	32.52	2.48 (2H, m)	H-11
10	32.23	2.48 (2H, m)	H-11
11	15.25	1.85 (1H, m) 2.03 (1H, m)	H-9/10
12	133.65	-	-
13, 14	128.60	7.43 (2H, d, J= 8.4)	H-15/16
15, 16	129.49	7.37 (2H, d, J= 8.8)	H-13/14
17	140.84	-	-

## Results and Discussion

The ESI-mass spectrum of the **1** showed a protonated molecule  $[M+1]^+$  at 266 suggesting the molecular formula  $C_{16}H_{24}NCl$ .  $^1H$ -NMR spectrum of this compound exhibited two doublet peaks at  $\delta_H$  0.97 and 0.98 assigned to the methyl group for  $CH_3$ -1 and  $CH_3$ -2, one singlet peak at  $\delta_H$  2.70 assigned to the methyl group attached to nitrogen for  $CH_3$ -7, and four aromatic protons for *p*-substituted ring at  $\delta_H$  7.37 (2H, d,  $J=8.8$ Hz), 7.43 (2H, d,  $J=8.4$ Hz). One methine proton for H-5 was shown at  $\delta_H$  3.53 (1H, dd,  $J=5.2/4.8$ ). Three multiplet signals at  $\delta_H$  2.48, 1.85 and 2.03 were readily attributed to the methylene protons of  $H_4$ -9/10 and  $H_2$ -11, respectively. In the  $^1H$ - $^1H$  COSY spectrum H-6 ( $\delta_H$  1.43) showed a coupling to H-5 ( $\delta_H$  3.53), which in turn were coupled to H-4 ( $\delta_H$  1.17). The  $^{13}C$  NMR spectrum showed 14 signals for 16 carbon atoms. Three methylene signals of the cyclobutyl fraction were shown at  $\delta_C$  15.25(C-11), 32.52(C-9) and 32.23(C-10). Three methyl signals were shown at  $\delta_C$  22.03(C-1), 22.13(C-2) and 32.82(N- $CH_3$ ). Two signals at  $\delta_C$  128.60 and 129.49 were assigned to the aromatic carbon atoms at for C-13,14 and C-15,16, respectively (s. Table 1).

Based on the mass and NMR spectroscopic data, the structure of the isolated compound **1** was determined as N-1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutyl-N-methylamine (N-mono-desmethylsibutramine) (s. Figure 1).



**Figure 1.** N-Mono-desmethylsibutramine (**1**)

N-mono-desmethylsibutramine is a metabolite of sibutramine, a synthetic noradrenaline, 5-hydroxytryptamine and serotonin reuptake inhibitor, was isolated and identified by spectroscopic methods in the Chinese herbal product of "Li Da Dai Hua" capsules, even though only in "Citrus aurantium var. amara flower extract, Cassia fruit extract, Citrus reticulata, Morus alba leaf extract" were declared the active ingredients.

Herbal products are required to show acceptable standards of quality, safety and efficacy.

Therefore for the protecting public health stronger regulation on the quality of herbal products are required, including authorization from Ministry of Health, labeling rules and quality control mechanisms to verify of all active ingredients. In addition a stronger market audit and a surveillance of herbal products is also required.

## Özet

İllegal olarak tahşiş edilmiş zayıflama bazlı bir Çin bitkisel ürününden N-mono-desmetilsibutramin izole edilip yapısı tayin edilmiştir. Tahşiş edilen bitkisel ürünün metanol ekstresi preparatif İTK ile saflaştırılmış, izole edilen bileşiğin yapısı 1D,2D-NMR ve ESI-MS teknikleri kullanılarak tayin edilmiştir.

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