Journal of Food and Drug Analysis, Vol. 15, No. 1, 2007, Pages 20-24 藥物食品分析 第十五卷 第一期

Isolation and Identification of a Sibutramine Analogue in a Healthy Food for Weight Loss

KUO-CHIH LAI[1], YI-CHU LIU[1], MU-CHANG TSENG[1], YUN-LIAN LIN[2] AND JER-HUEI LIN[1]*

[1.] *Bureau of Food and Drug Analysis, Department of Health, Executive Yuan, 161-2, Kuen Yang St., Nangang, Taipei City 115, Taiwan, R.O.C.*
[2.] *National Research Institute of Chinese Medicine, 155-1, Sec. 2, Li Nung St., Beitou, Taipei City 112, Taiwan, R.O.C.*

(Received: July 21, 2006; Accepted: October 10, 2006)

ABSTRACT

A sibutramine analogue was detected and added illegally in known as the healthy food that can reduce weight. The structure was determined as N-1-[1-(4-chlorophenyl) cyclobutyl]-3-methylbutyl-*N*-methylamine with the molecular formula $C_{16}H_{24}NCl$ and molecular weight 265 Da. The sample was desalted and extracted with water, further purified by a liquid-liquid partition method. The structure was identified with a series of 1-D and 2-D NMR techniques, IR and FAB mass. Comparing with the structure of sibutramine, a tertiary amine was substituted as a secondary amine. This is one of the active metabolite of sibutramine. Basic on the law, foods with any illegal additive such as synthetic chemical compound shall not be sold, manufactured, imported, processed, used, prepared, stored or transported. Therefore, this sibutramine analogue has been included in the inspection list of illegal adulterants in Taiwan.

Key word: sibutramine analogue, adulterants, NMR, FAB mass

INTRODUCTION

The prevalence of obesity has reached epidemic dimension in industrialized countries and it is known that obesity is associated with increased risk of cardiovascular morbidity and mortality[(1)]. Sibutramine (Figure 1) was the currently approved medication for long-term management of obesity. Although the benefit-risk profiles of sibutramine appear positive, sibutramine continues to be monitored because of long-term safety concerns[(2-3)].

Studies in healthy subjects suggested that sibutramine might have opposing effects on peripheral and central sympathetic activity, an increase in blood pressure has been claimed[(4-5)]. Other reports suggesting arrhythmias associated with sibutramine were assessed[(6)].

Since January 2006, the list of prohibited substances established by the World Anti-Doping Agency includes the antidepressant / anti-obesity drug sibutramine. Due to its rapid degradation to its active metabolites N-desmethyl and N-bisdesmethyl sibutramine, reference compounds had been synthesized and monitored by liquid chromatography/tandem mass spectrometry[(7)]. Other documents of organic synthesis, called this N-desmethyl sibutramine as desmethylsibutramine[(8-10)].

In our laboratory we have found sibutramine adulterated with many dietary supplements. A sample from mainland China was detained and sent to our laboratory by the Customs Bureau of Treasury Department. An unknown compound was detected by thin layer chromatography (TLC). Although its molecular weight was different from that of sibutramine, the data of NMR indicated a sibutramine analogue was inferred.

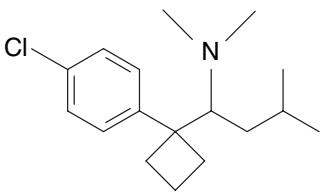

Figure 1. Structure of sibutramine.

MATERIALS AND METHODS

I. *Equipments*

The NMR spectra were recorded on a BRUKER AVANCE-500MHz FT-NMR (500MH_Z for ^1H, 125MH_Z for ^{13}C) with chloroform-*d* as solvent. The FAB mass was acquired on a JEOL JMS-700. The UV spectrum was determined on a Varian CARY 50 Conc UV-Visible spectrophotometer. The infrared (IR) spectrum was recorded in the 400 ~ 4000 cm^{-1} range using a Jasco FT-IR-480 plus spectrometer and KBr pellets. All chemicals used were of analytical grade.

II. *Extraction and Isolation*

The powder sample (521.2 mg) was dissolved in

* Author for correspondence. Tel: +886-2-26531239;
Fax: +886-2-26531244; E-mail: linjerhuei@nlfd.gov.tw

Journal of Food and Drug Analysis, Vol. 15, No. 1, 2007

Table 1. NMR correlation of sibutramine analogue (**A**)

No.	^{13}C (δ_C)	^{1}H (δ_H)	DEPT	COSY	HMBC
1, 2	22.0, 24.0	0.83 (3H, *d*, *J* = 6.6 H_Z) 0.87 (3H, *d*, *J* = 6.5 H_Z)	3,3	H-3	H-4
3	25.4	1.60 (1H, *m*)	1	H-1, 2 / H-4	H-1, 2 / H-4 / H-5
4	41.4	0.66 (1H, *m*), 1.06 (1H, *m*)	2	H-5	H-1, 2 / H-5
5	65.6	2.64 (1H, *m*)	1	H-4	H-4 / H-7 / H-9 / H-10
6	-	1.38 (1H, *br*.)	-	-	-
7	37.4	2.50 (3H, *s*)	3	-	H-5
8	51.8	-	0	-	H-5 / H-9 / H-10 / H-11/ H-13, 14
9	33.7	2.27 (2H, *m*), 2.40 (1H, *m*)	2	H-11	H-5 / H-10 / H-11
10	32.3	2.17 (1H, *m*), 2.27 (2H, *m*)	2	H-11	H-9 / H-11
11	16.3	1.75 (1H, *m*), 1.87 (1H, *m*)	2	H-9 / H-10	H-9 / H-10
12	144.8	-	0	-	H-5 / H-9 / H-10 / H-15,16
13, 14	129.1	7.17 (2H, *d*, *J* = 6.8 H_Z)	1,1	H-15, 16	H-14, 13
15, 16	127.5	7.25 (2H, *d*, *J* = 6.5 H_Z)	1,1	H-13, 14	H-16, 15
17	131.3	-	0	-	H-13, 14 / H-15, 16

δ ppm in $CDCl_3$, *J* in H_Z, 125 MH_Z for ^{13}C, 500 MH_Z for ^{1}H.
DEPT is the number of attached protons.
The δ_H 2.27 (2H, *m*) signal belongs to the 9th and the 10th.

water (100 mL) and alkalized with sodium hydroxide to pH 8~9. The filtrate was extracted with dichloromethane (100 mL) twice. The dichloromethane layers were combined, washed with water (300 mL) two times and dehydrated by anhydrous magnesium sulfate. The solution was evaporated to dryness in vacuum oven and yielded the pale yellow liquid compound **A** (366.0 mg).

III. *NMR Correlation Data of Compound A*

The purified compound **A** was identified with a series of 1-D and 2-D NMR spectroscopic techniques, including ^{1}H, ^{13}C, DEPT, COSY, HMQC and HMBC. The data are showed in Table 1.

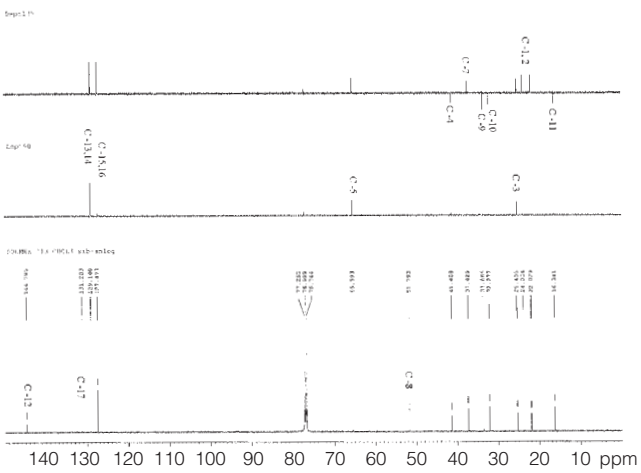

Figure 2. DEPT spectrum of sibutramine analogue (**A**).

RESULTS AND DISCUSSION

Table 1 showed the ^{1}H-NMR, ^{13}C-NMR, DEPT, ^{1}H-^{1}H COSY and HMBC spectral data of compound **A**, which were similar to that of sibutramine, except the two methyl groups connected on the nitrogen was replaced with one methyl group. The DEPT, ^{1}H-^{1}H COSY, HMQC and HMBC spectra of sibutramine analogue (**A**) were shown in Figures 2, 3, 4, 5, 6, 7 and 8, respectively. The spectroscopic numbering used is given in Figure 9. All signals were assigned unequivocally according to the various NMR spectroscopic data.

The ^{1}H-NMR spectrum showed characteristics of an amine at δ_H 1.38 (1H, *br*.) was obviously different from sibutramine (Figure 1). Four aromatic protons at δ_H 7.17 (2H, *d*, *J* = 6.8 H_Z), 7.25 (2H, *d*, *J* = 6.5 H_Z). One singlet peak at δ_H 2.50 was assigned as the methyl group attended to nitrogen for H_3-7. Two doublet peaks at δ_H 0.83 and δ_H 0.87 were assigned as the methyl group for H_3-1 and H_3-2. Five multiple peaks at δ_H 2.40, δ_H 2.27, δ_H 2.17, δ_H 1.75 and δ_H 1.87 were assigned as the methylene for H_2-9, H_2-10 and H_2-11, respectively. Two multiple peaks at δ_H 0.66 (1H, *m*) and δ_H 1.06 (1H, *m*), further proved with 2D NMR data, was assigned as the methylene for H_2-4. One methine proton for H-5 was shown at δ_H 2.64 (1H, *m*).

The ^{13}C-NMR and DEPT (Figure 2) spectra indicated three primary carbons, which was one less than sibutramine, together with four secondary carbons,

six tertiary carbons and three quaternary carbons, the same as that of sibutramine. Three methylene signals of cyclobutyl were shown at δ_C 16.3, δ_C 33.7 and δ_C 32.3. Two peaks at δ_C 129.1 and δ_C 127.5 were assigned as the aromatic carbons for C-13,14 and C-15,16, respectively.

In the HMQC (Figure 5) and HMBC (Figures 6, 7, 8) spectra, the correlation between H-1,2 (δ_H 0.83,0.87) and C-3 (δ_C 25.4) (Figure 6) suggested that one methyl group linked to the butyl group. The correlation of H-13, 14/C-8 (Figure 7) and H-9, H-10/C-12 (Figure 8) indicated that

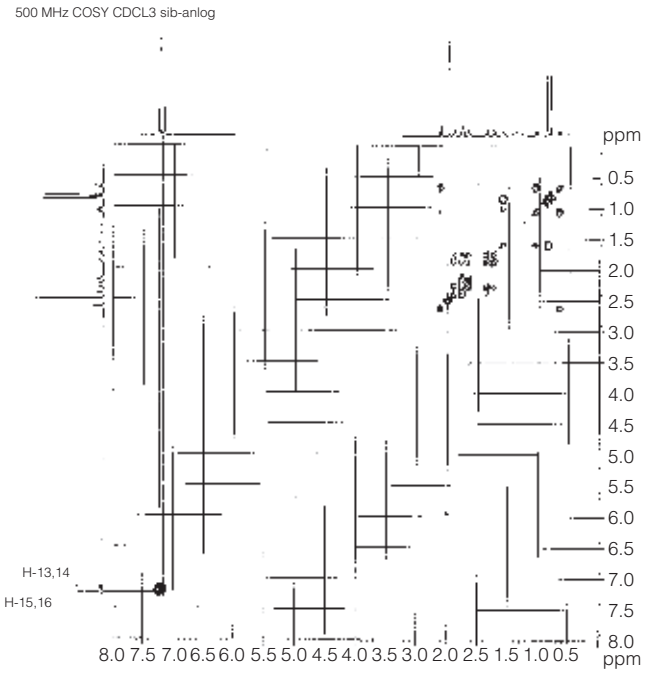

Figure 3. ^{1}H-^{1}H COSY spectrum of sibutramine analogue (**A**).

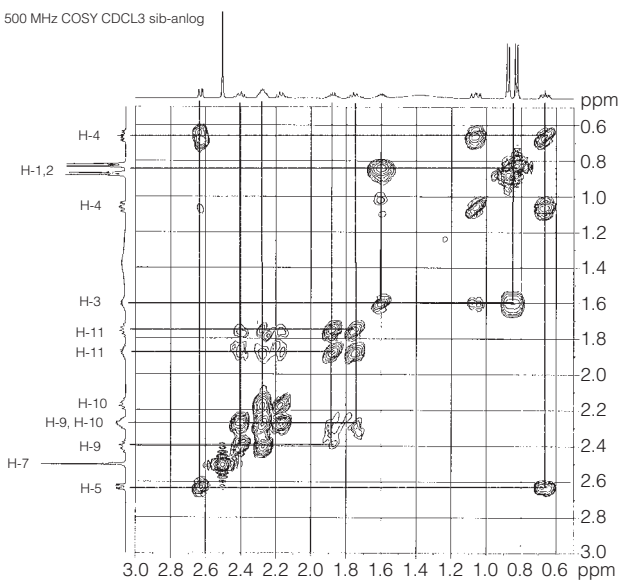

Figure 4. Partial ^{1}H-^{1}H COSY spectrum of sibutramine analogue (**A**).

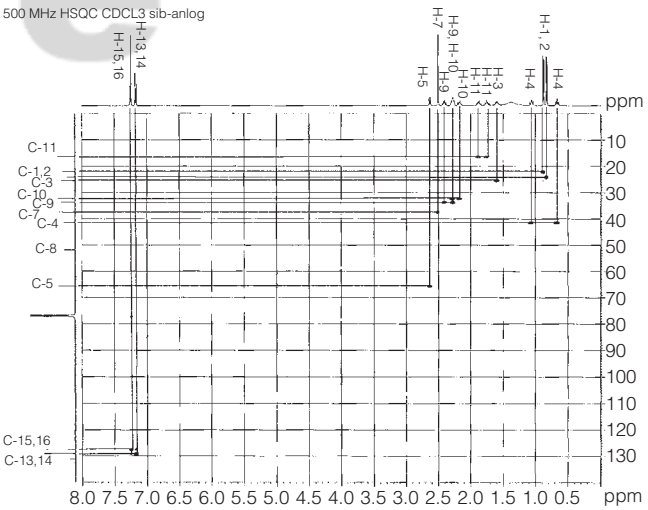

Figure 5. HMQC spectrum of sibutramine analogue (**A**) .

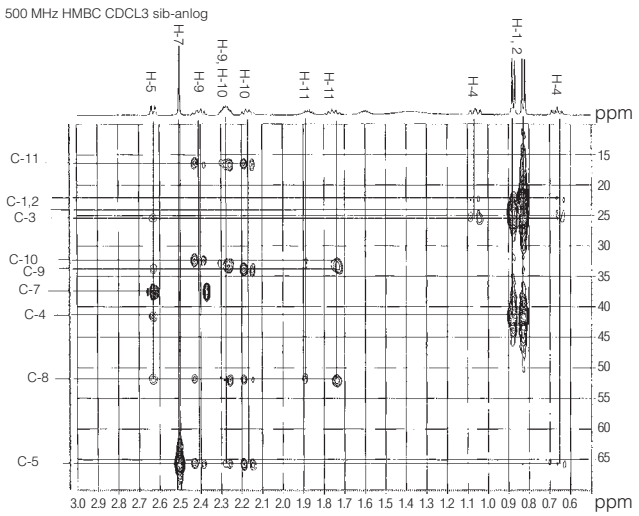

Figure 6. Partial HMBC spectrum 1 of sibutramine analogue (**A**).

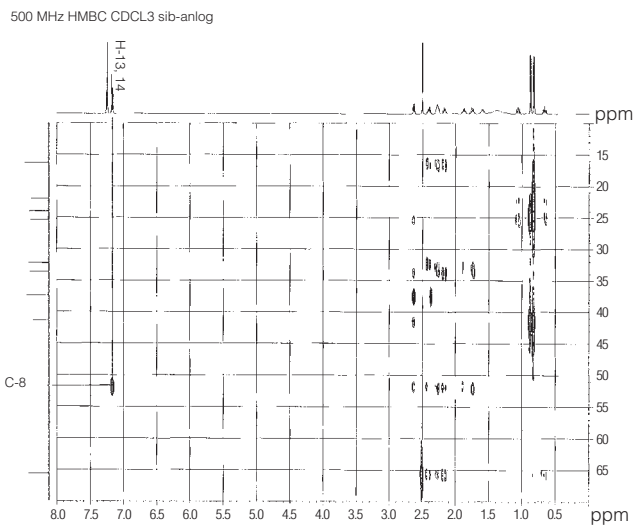

Figure 7. Partial HMBC spectrum 2 of sibutramine analogue (**A**).

the chlorophenyl is attached to a quaternary carbon (C-8) of the cyclobutyl. The correlation of H-5/C-8 (Figure 6) exhibited the attachment of the cyclobutyl carbon (δ_C 51.8) to the butyl group (δ_H 2.63). The correlation of H-7/C-5 (Figure 6) showed the linkage of the methylamine group (δ_H 2.50) to the butyl carbon (δ_C 65.6).

The FAB [M+H] of compound **A** found at m/z 266, corresponding to the molecular formula $C_{16}H_{24}NCl$, 14 a.m.u. less than sibutramine referred to CH_2 loss (Figure 10). Its UV spectrum shape (Figure 11) was similar to that of sibutramine, but was different in λ_{max} absorption at 224.9 nm.

The IR spectrum showed absorption bands with the characteristics of an amine at 2959 cm^{-1} and a *para*-

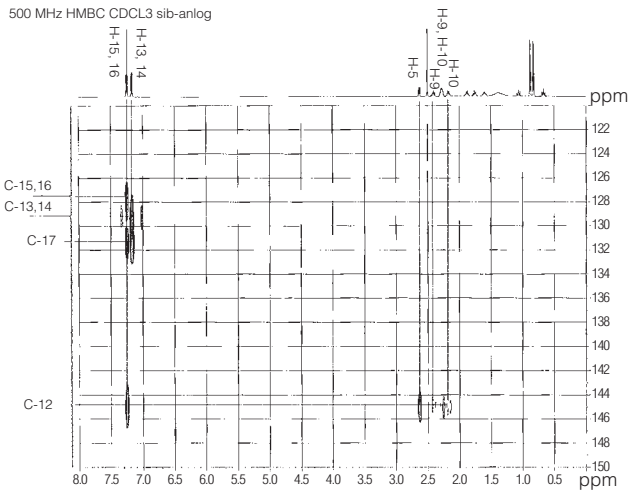

Figure 8. Partial HMBC spectrum 3 of sibutramine analogue (**A**).

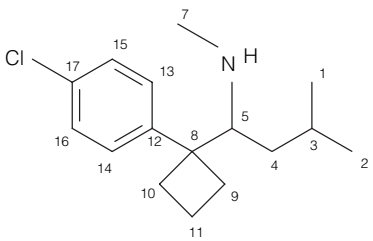

Figure 9. Structure of sibutramine analogue (**A**).

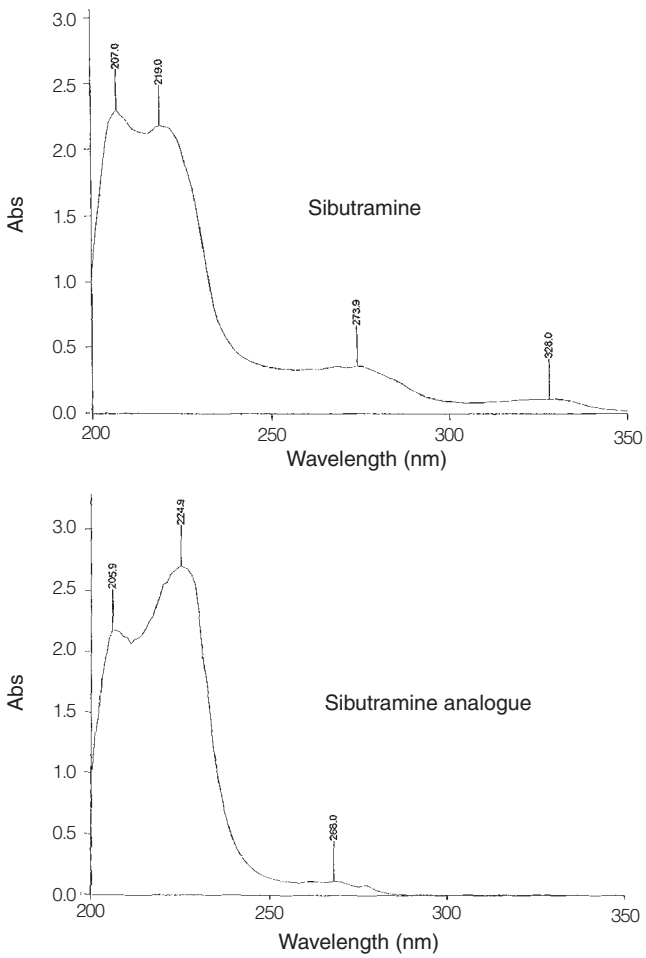

Figure 11. UV spectra of sibutramine and sibutramine analogue (**A**).

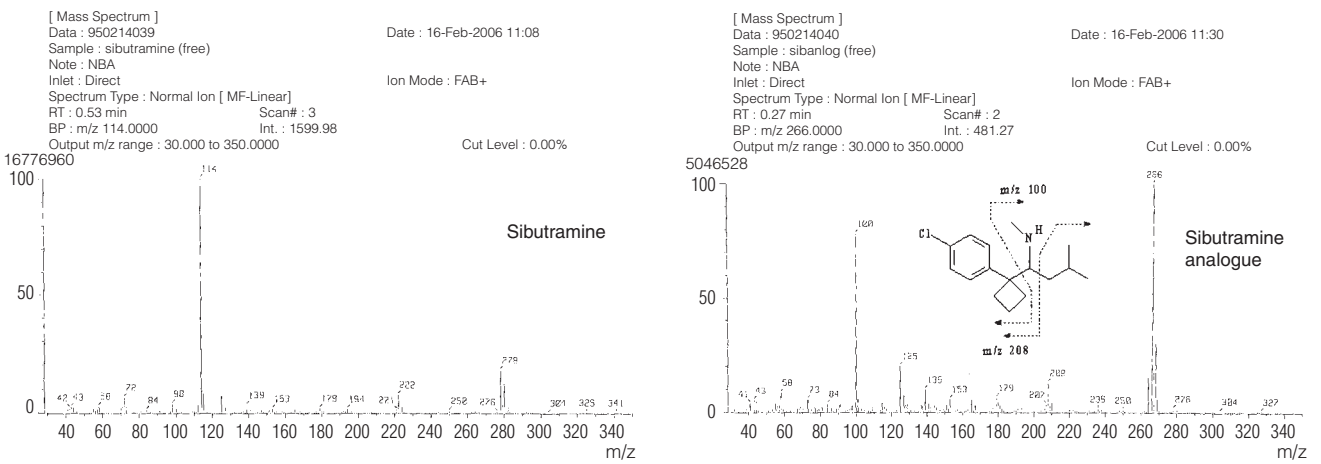

Figure 10. The FAB fragmentation of sibutramine and sibutramine analogue (**A**).

substituted phenyl at 1492, 1466, 826 cm^{-1}.

Based on the mass, infrared spectrum and NMR spectroscopic data, the structure of compound **A** was determined as N-1-[1-(4-chlorophenyl) cyclobutyl]-3-methylbutyl-*N*-methylamine, a sibutramine analogue (Figure 9), of which two methyl groups connected on the nitrogen in sibutramine was changed into one methyl group.

REFERENCES

1. Guagnano, M. T., Manigrasso, M. R., Capani, F. and Davi, G. 2005. The "problem obesity": the opinion of the internal medicine specialists. Ann. Ital. Chir. 76: 407-411.
2. Ioannides-Demos, L. L., Proietto, J., Tonkin, A. M. and McNeil, J. J. 2006. Safety of drug therapies used for weight loss and treatment of obesity. Drug Saf. 29: 277-302.
3. Mancini, M. C. and Halpern, A. 2006. Pharmacological treatment of obesity. Arq. Bras. Endocrinol. Metabol. 50: 377-389.
4. Yee, B. J., Phillips, C. L., Banerjee, D., Caterson, I., Hedner, J. A. and Grunstein, R. R. 2006. The effect of sibutramine-assisted weight loss in men with obstructive sleep apnoea. Int. J. Obes. (Lond). May 2.
5. Heusser, K., Tank, J., Diedrich, A., Engeli, S., Klaua, S., Kruger, N., Strauss, A., Stoffels, G., Luft, F. C. and Jordan, J. 2006. Influence of sibutramine treatment on sympathetic vasomotor tone in obese subjects. Clin. Pharmacol. Ther. 79: 500-508.
6. Harrison-Woolrych, M., Clark, D. W., Hill, G. R., Rees, M. I. and Skinner, J. R. 2006. QT interval prolongation associated with sibutramine treatment. Br. J. Clin. Pharmacol. 61: 464-469.
7. Thevis, M., Sigmund, G., Schiffer, A. K. and Schanzer, W. 2006. Determination of N-desmethyl- and N-bis-desmethyl metabolites of sibutramine in doping control analysis using liquid chromatography-tandem mass spectrometry. Eur. J. Mass. Spectrom. (Chichester, Eng). 12: 129-136.
8. Qun, K. F., Chris, H. S., Zhengxu, H., Cynthia, M., Paul, G., Robert, E. M., Hal, B., Stephen, A. W. and Stanley, T. C. 1999. First preparation of enantiomerically pure sibutramine and its major metabolite, and determination of their absolute configuration by single crystal X-ray analysis. Tetrahedron Asymmetry 10: 4477-4480.
9. Zhengxu, H., Dhileepkumar, K., Derek, P., Qun, K. F., Hal, B. T., Stanley, C., Stephen, A. W. and Chris, H. S. 2002. First practical synthesis of enantiomerically pure (*R*)- and (*S*)-desmethylsibutramine (DMS) and unambiguous determination of their absolute configuration by single-crystal X-ray analysis. Tetrahedron Asymmetry 13: 107-109.
10. Dhileepkumar, K., Zhengxu, H., Stephen, A. W. and Chris, H. S. 2002. First asymmetric synthesis of (*R*)-desmethylsibutramine. Tetrahedron Letters 43: 2331-2333.