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Screening for multiple weight loss and related drugs in dietary supplement materials by flow injection tandem mass spectrometry and their confirmation by liquid chromatography tandem mass spectrometry

Fenhong Song[a,*], Douglas Monroe[a], Aref El-Demerdash[a], Cynthia Palmer[b]

[a] Food and Drug Administration, Method Development and Total Diet Research Center, 11630 W. 80th Street, Lenexa, KS 66214, United States
[b] Food and Drug Administration, Philadelphia Lab, 200 Chestnut Street, Philadelphia, PA 19106, United States

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ABSTRACT

A new method has been developed using flow injection tandem mass spectrometry to semi-quantitatively screen for weight loss drugs, including sibutramine, N-desmethylsibutramine, N-didesmethylsibutramine, and phenolphthalein in dietary supplements. Positive identification of these drugs in samples was further confirmed and quantified by liquid chromatography tandem mass spectrometry. The degradation products of sibutramine were observed and identified by LC–MS/MS which include N-desmethylsibutramine, N-didesmethylsibutramine, N-formyldesmethylsibutramine, and N-formyldidesmethylsibutramine.

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1. Introduction

A dietary supplement in the U.S. is defined under the Dietary Supplement Health and Education Act (DSHEA) of 1994 as a product that is intended to supplement the diet which may include vitamins, minerals, herbs or other botanicals, amino acids, or concentrates, and extracts of these. However, prescription drugs, such as erectile dysfunction, weight loss, and also other drugs are being detected in dietary supplement products at the FDA and other laboratories [1,2]. These illegal products can cause serious problems when consumers take them without knowing the presence of drugs [3]. Recent adverse event reports were received by the FDA from taking "Reumofan Plus" and "Reumofan Plus Premium" products, which were marketed as a natural remedy from Mexico for arthritis muscle pain, osteoporosis, bone cancer, and other conditions, but they cause side effects such as liver injury, severe bleeding, sudden worsening of glucose (sugar) control, weight gain, swelling, leg cramps, withdrawal syndrome, adrenal suppression, stroke and death. Lab testing results showed that some of the products contain dexamethasone (a corticosteroid), diclofenac sodium (an anti-inflammatory drug), and methocarbamol (a muscle relaxant). There are many dietary supplement products coming into US markets every year, promoted and available on the Internet. To prevent adulterated products from coming into the hands of U.S. consumers, portable fast screening instruments such as ion mobility spectrometry (IMS) have been used to screen these dietary supplement products at a few FDA Labs and by trained FDA inspectors at Ports of Entry [4]. To meet with this challenge of testing many products, we also have developed methods in our lab to use flow inject tandem mass spectrometry (FI-MS/MS) [5] to screen for adulterated drugs such as lovastatin and sildenafil in these products [6,7] In comparison to ion mobility spectrometry as a screening method to detect adulterants, FI-MS/MS offers improved sensitivity, selectivity, and wider detection ranges.

Sibutramine is a drug approved by the FDA in 1997 (as Abbott's *Meridia*) to treat obesity and was withdrawn in 2010 due to associated cardiovascular risks. Sibutramine is one of the common adulterants in dietary supplements sold for weight loss which has been detected by LC-PDA [8] GC/MS [9] LC–MS/MS [10–13] NMR [14] X-ray powder diffractometry [15] and IMS [4]. Here we report our results using FI-MS/MS for the screening of sibutamine and other weight loss drugs in dietary supplements and our use of LC–MS/MS for confirmation and quantification of these adulterated drugs.

2. Materials and methods

The mass spectral measurements were performed on an Agilent 6490 LC–MS/MS system (Santa Clara, CA) with an electrospray

* Corresponding author. Tel.: +1 913 752 2125; fax: +1 913 752 2122.
E-mail address: fenhong.song@fda.hhs.gov (F. Song).

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source equipped with an Agilent 1260 LC system. The LC system consists of a binary pump, a vacuum degasser, a temperature-controlled column compartment, and a refrigerated autosampler component. An isocratic chromatographic condition was used using 10% Mobile Phase A (2 mM of ammonium formate in water with 0.05% formic acid) and 90% Mobile Phase B (2 mM of ammonium formate in methanol with 0.05% formic acid) at a flow rate of 0.3 ml per minute with a runtime of 0.50 min. The source parameters were set as follows: dry gas temperature = 200 °C, drying gas flow = 11 l/min, nebulizer pressure = 45 psi, sheath gas temperature = 350 °C, sheath gas flow = 11 l/min, capillary potential = 3500 V, nozzle voltage = 500 V. The collision energies were optimized with standard references. The spectra were obtained in the positive ion mode.

For the LC–MS/MS method, a Zorbax C-18 column (50 mm × 2.1 mm and 1.8 μm particle size) was used. Gradient elution was performed using the same mobile phase components as above starting with 30% Mobile Phase B, increasing to 90% B at 7 min and holding for 2 min, then changing to 30% Mobile Phase B at 10 min. The flow rate was set at 0.3 ml/min and the runtime of the method was 12 min.

The entire instrument system and data acquisition were controlled by Agilent's Mass Hunter software. The methanol and acetonitrile were high purity grade (Burdick and Jackson, Morristown, NJ, USA). Ammonium formate was purchased from Fisher Scientific (Waltham, MA, USA). Sibutramine hydrochloride was 99% pure (Sigma–Aldrich, St. Louis, MO, US). N-Desmethylsibutramine hydrochloride (98% purity) and N-didesmethylsibutramine (98% purity) were obtained from Toronto Research Chemicals (Toronto, Ontario, Canada). Phenolphthalein was obtained from U.S. Pharmacopeia (Rockville, MD, USA). The standard solutions were prepared by proper dilution in acetonitrile of stock solutions of sibutramine, N-desmethylsibutramine, N-didesmethylsibutramine, and phenolphthalein to 2, 5, 25, 100, and 250 ng/ml. The calibration curves for both flow injection and liquid chromatograph tandem mass spectrometry were obtained by injecting the same standards and fitting the data by linear regression.

Dietary supplement materials were purchased from Internet websites and also collected at the import ports. Five capsules or tablets were typically used to make sample composites. The tablet samples were ground into fine powder; capsule samples were emptied and mixed. About 100 mg of one tablet or capsule composite was weighed for each product and then added to 20 ml of acetonitrile. To determine the spike recoveries of the analytes, approximately 0.5 and 5 mg of sibutramine; 1 and 10 mg of phenolphthalein reference standards were added to portions of the sample composites before addition of the extraction solvent. The sample was sonicated for 30 min, centrifuged at 4000 rpm at 22 °C for 5 min, supernatant separated, and then diluted 2000- or 5000-fold with acetonitrile. After filtration with 0.2 μm polytetrafluoroethylene membranes, a 1 μl sample volume was injected onto the column and to the mass spectrometer.

3. Results and discussion

In an effort to improve the throughput for screening adulterated drugs in dietary supplements, we have developed FI-MS/MS methods by using multiple reaction monitoring (MRM) as applied in fast screening of lovastatin in red yeast rice products and erectile dysfunction drugs (PDE-5 inhibitors) in male sexual performance products [6,7]. In this study, we extended the methods to detect weight loss drugs in dietary supplement products.

It is known that sibutramine is rapidly metabolized in humans to the pharmacologically active N-desmethylsibutramine and N-didesmethylsibutramine [16,17]. These metabolites have also been added as hidden drugs in dietary supplements [9–12]. These three sibutramines were selected as the targeted analytes in this study. In addition, phenolphthalein had been used as an over-the-counter laxative until it was reclassified as "not generally recognized as safe and effective" in 1997 due to its potential carcinogenicity [18]. Phenolphthalein is often found together with sibutramine in adultered weight loss supplement products, so it was also included as one of the analytes.

The mass spectrometer parameters for product ions of the standards were optimized by flow injection using the Agilent auto-optimization software as described [6,7]. The mass spectrometer parameters used for FI-MS/MS are listed in Table 1. To understand the fragmentation pathways, the full product ion spectra of the sibutramine standards were also acquired. The proposed fragmentation pathways of sibutramine and its analogs are shown in Fig. 1. Their product ion spectra show common ions at *m*/*z* 235, 179, 153, 139 and 125, probably due to the common structural moiety. We propose that the intermediate at *m*/*z* 235, with a four-member ring intermediate, could rearrange to give an intermediate with the more stable five-member ring, and also the open ring form. The open ring intermediate could result in product ions observed at *m*/*z* 153, 139, and 125 after neutral loss of C_6H_{10}, C_7H_{12}, and C_8H_{14}, respectively. These three daughter ions were used for monitoring MRM transitions by flow injection measurement and then followed by LC–MS/MS analysis for confirmation. The product ion at *m*/*z* 125 is used as the quantifier ion, and the other two masses at *m*/*z* 139 and 153 were used as qualifier ions. The identification of the analytes was based on the ion ratio of the qualifier ion over the quantifier ion. The ion ratios of the analytes found in the samples should be within 20% deviation from those of the standards.

The five levels of standard solutions at concentrations of 2, 5, 25, 100, and 250 ng/ml of each analyte were used to obtain the calibration curves by flow injection, which were found

Table 1
Mass spectrometer parameters for sibutramine, N-desmethylsibutramine, N-didesmethylsibutramine, and phenolphthalein product ions.

Compound name	Precursor ion	Product ion	Dwell (ms)	Collision energy (V)	Polarity
Sibutramine	280.2	153	20	13	Positive
Sibutramine	280.2	138.9	20	13	Positive
Sibutramine	280.2	125	20	29	Positive
Demethylsibutramine	266.2	153	20	9	Positive
Demethylsibutramine	266.2	139	20	5	Positive
Demethylsibutramine	266.2	125.1	20	33	Positive
Didemethylsibutramine	252.2	179.1	20	9	Positive
Didemethylsibutramine	252.2	153	20	5	Positive
Didemethylsibutramine	252.2	139	20	5	Positive
Phenolphthalein	319.3	225	20	21	Positive
Phenolphthalein	319.3	141	20	50	Positive
Phenolphthalein	319.3	115	20	49	Positive