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Analytical Methods

Determination of *para*-synephrine and *meta*-synephrine positional isomers in bitter orange-containing dietary supplements by LC/UV and LC/MS/MS

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Abstract

Dietary supplements that contain bitter orange (*Citrus aurantium*) fruit as an integrated component have rapidly replaced ephedra-containing dietary supplements for use as weight loss products. However, the safety of bitter orange-containing supplements has been questioned because synephrine, an adrenergic alkaloid and a key component of bitter orange fruit, has potential adverse health effects. Conflicting reports have stated that synephrine exists as the *para* (*p*) and/or *meta* (*m*) positional isomers in some bitter orange-containing supplements and this is problematic because the *p*- and *m*-isomers have distinctly different pharmacological and metabolic activities. Two liquid chromatographic (LC) methods have been developed for the baseline separation and quantitation of *p*- and *m*-synephrine in bitter orange-containing supplements. An isocratic LC method that utilizes ultraviolet (UV) absorbance detection and a gradient LC method that utilizes tandem mass spectrometry (MS/MS) detection were optimized for separation of the isomers within a run time of 25 min. Terbutaline was utilized as an internal standard compound in both LC methods. The LC/UV and LC/MS/MS methods demonstrated limits of quantitation (LOQs) for synephrine of ≈30 ng (on-column) and ≈0.02 ng (on-column), respectively, and each method exhibited analytical linearity over three orders of magnitude. Both LC methods were used to evaluate the synephrine levels in a limited selection of commercially available bitter orange-containing supplements. Significantly, *m*-synephrine was not detected in any of the tested dietary supplements.
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Keywords: Bitter orange-containing dietary supplements; Liquid chromatography/tandem mass spectrometry; *meta*-Synephrine; *para*-Synephrine; Quantitation

1. Introduction

Synephrine is a sympathomimetic amine and it is also the predominant and most active adrenergic alkaloid found in bitter orange (*Citrus aurantium*) fruit, as well as in other *Citrus* species (Wheaton & Stewart, 1970). Synephrine, because of its purported thermogenic and lipolytic properties (Carpene et al., 1999), has been intensely investigated as a weight loss agent. In fact, bitter orange-containing dietary supplements, nominally containing bitter orange extract and other herbal and botanical extracts, are marketed as weight loss products (Fugh-Berman & Myers, 2004). Bitter orange-containing supplements have largely replaced ephedra (*Ephedra sinica*)-containing supplements as weight loss products due to demonstrated health and safety problems with ephedra (Haller & Benowitz, 2000).

Synephrine can potentially exist in three different structural or positional isomeric forms (*para* – *p*, *meta* – *m* or *ortho* – *o*, Fig. 1) which have nonequivalent pharmacology and metabolism (James, Midgley, & Williams, 1983). Both *p*-synephrine (Fig. 1A) and *m*-synephrine/phenylephrine

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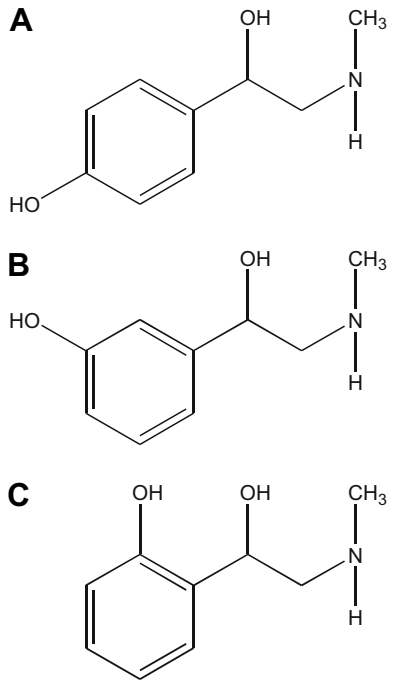

Fig. 1. Chemical structures for synephrine positional isomers. The isomeric form is based on the location of the ring hydroxyl group. (A) *p*-Synephrine; (B) *m*-synephrine; (C) *o*-synephrine.

(Fig. 1B) are α-adrenergic agonists and vasoconstrictive agents that have been shown to increase blood pressure in humans (Fugh-Berman & Myers, 2004; Haaz et al., 2006). However, *m*-synephrine has been reported to be a much more potent α-adrenergic agonist compared to *p*-synephrine (Brown et al., 1988). In addition, *m*-synephrine is used as a mydriatic agent and it is also used as a nasal decongestant (Neo-synephrine®). *p*- and *m*-Synephrine are present at trace levels in the body and both compounds have been synthesized chemically. The presence of *o*-synephrine (Fig. 1C) in the body or in nature has not been documented, however, *o*-synephrine has been synthesized chemically (Midgley, Couch, Crowley, & Williams, 1980). *o*-Synephrine is not commercially available.

Conflicting information exists in the literature regarding the status of specific synephrine isomers in bitter orange-containing supplements. There is presently no knowledge regarding the presence or absence of *o*-synephrine in supplements and research in this area is warranted (Allison, Cutter, Poehlman, Moore, & Barnes, 2005; Haaz et al., 2006). Most researchers have reported synephrine levels in supplements without regard to isomeric designation (Li, Huang, & Huang, 2006; Pellati, Benvenuti, Melegari, & Firenzuoli, 2002; Schaneberg & Khan, 2004), while other researchers have reported that supplements contain only *m*-synephrine (Bent, Padula, & Neuhaus, 2004; Penzak et al., 2001) or only *p*-synephrine (Avula, Upparapalli, & Navarrete, 2005; Tang et al., 2006) or perhaps both *p*- and *m*-synephrine (Allison et al., 2005; Haaz et al., 2006). Due to the differences in the pharmacological activities and uses of *p*- and *m*-synephrine, it is important to correctly distinguish between the isomers in supplements. The detection of *m*-synephrine in some bitter orange-containing supplements could be a valid observation, or it could be due to adulteration or to incorrect identification. Because synephrine can increase blood pressure, it has been speculated that synephrine could pose cardiovascular health risks (Fugh-Berman & Myers, 2004; Haaz et al., 2006). Additionally, synephrine may contribute to the pathophysiology of migraines and primary headaches (D'Andrea et al., 2004, 2006). Therefore, it is evident that validated analytical procedures are needed that can definitively determine which and how much of each relevant synephrine isomer is present in bitter orange-containing supplements.

Synephrine has been extensively determined in bitter orange-containing supplements and other *Citrus* plant formulated supplements/materials on the basis of liquid chromatography (LC) coupled with UV absorbance (LC/UV) (Avula, Joshi, Weerasooriya, & Khan, 2005; Avula et al., 2005; Li et al., 2006; Putzbach, Sharpless, & Sander, 2007a; Roman, Betz, & Hildreth, 2007; Tang et al., 2006; Vieira, Theodoro, & Gloria, 2007), electrochemical (LC/EC) (Kusu, Matsumoto, & Takamura, 1995), fluorescence (LC/FL) (Niemann & Gay, 2003; Putzbach et al., 2007a), single stage mass spectrometry (LC/MS) (Putzbach, Sharpless, & Sander, 2007b) and tandem mass spectrometry (LC/MS/MS) (Allison et al., 2005; Barnes, Prasain, Wang, & Moore, 2006; Gay, Niemann, & Musser, 2006; Mattoli et al., 2005; Nelson, Putzbach, Sharpless, & Sander, 2007; Sander et al., 2005) detection methods. Out of all of the previously reported methods, only one recently developed LC/UV method (Roman et al., 2007) presents a validated analytical procedure for the simultaneous and quantitative determination of *p*- and *m*-synephrine in bitter orange-containing supplements. The LC/UV method demonstrates an excellent baseline separation of *p*- and *m*-synephrine under ion-pairing mobile phase conditions, but the performance and accuracy of the LC/UV method were not assessed in relation to alternative analytical procedures. Other LC-based methods for determining *p*- and *m*-synephrine in bitter orange-containing supplement or *Citrus* plant extracts have been much less successful and have only resulted in poor chromatographic separations (Kusu et al., 1995) or minimal or missing validation data for the *m*-synephrine isomer (Avula et al., 2005). However, a recent qualitative LC/MS/MS analysis of *p*- and *m*-synephrine standards (Allison et al., 2005; Barnes et al., 2006) exhibited promising features for further development of a quantitative MS-based method for the determination of the isomers in supplement extracts. We now report the development of two analytical methods based on reversed-phase LC/UV detection and positive-ion mode LC/MS/MS detection for the determination of *p*- and *m*-synephrine in bitter orange-containing supplements. The utility and performance of the methods were tested and validated through the determination of the synephrine levels in NIST Standard Reference Material (SRM) 3260 bitter orange-containing solid oral dosage form and in six

commercially available bitter orange-containing dietary supplements products.

2. Materials and methods[1]

2.1. Materials

p-Synephrine (CAS #97-07-5, d/l racemic mixture, 99.9% purity) and *m*-synephrine (CAS #59-42-7, 99.7% purity) were obtained from ChromaDex (Santa Ana, CA). Terbutaline (CAS # 23031-32-5, hemisulfate salt) and ammonium acetate were obtained from Sigma Chemical Company (St. Louis, MO). HPLC-grade methanol was obtained from J.T. Baker (Phillipsburg, NJ). All other chemical reagents and solvents were ACS reagent-grade unless stated otherwise. SRM 3260 Bitter Orange-Containing Solid Oral Dosage Form was obtained from the Standard Reference Materials Group at NIST. SRM 3260 was prepared by grinding and blending a select number of commercially available bitter orange-containing dietary supplements (both tablets and capsules). A limited selection of six different bitter orange-containing dietary supplement products (A, B, C, D, E, F) were purchased from local health food stores or directly from commercial vendors for use in the present study; selected products were formulated as either capsules or tablets.

Reagent concentrations given in terms of percent (%) are to be considered as mass fractions (g/g) in all listed procedures. Preparation of analyte stocks/standards, samples and calibrants was performed gravimetrically in all procedures, except where noted otherwise. Additionally, all procedures were conducted under subdued lighting conditions to prevent light-induced degradation of the analytes.

2.2. Development and optimization of synephrine separation

Aqueous standards containing *p*- and *m*-synephrine (500 ng/μL each) in combination and as individual standards were utilized to develop and optimize LC separation conditions. Terbutaline was added to the standards as needed. The ionic strength, pH and aqueous/organic composition of the mobile phase and the temperature of the LC column were systematically optimized.

2.3. Preparation of synephrine calibration/linearity testing solutions

For the LC/UV method, 1 l of terbutaline internal standard solution (50 ng/μL) was prepared in water. A stock solution containing 1000 ng/μL of each synephrine isomer (*para* and *meta*) was prepared using the terbutaline solution as diluent. A set of 20 volumetric serial dilutions was prepared starting from the stock solution using the terbutaline solution as diluent. The serial dilutions were used both as calibration solutions and as linearity testing solutions to estimate the method's linear range, limit of detection (LOD), and limit of quantification (LOQ) for each synephrine isomer. The working calibration range (≈18 ng/μL synephrine to ≈1100 ng/μL synephrine) bracketed the synephrine levels in the test supplements. The synephrine calibration/linearity solutions were stable up to 1 week when stored at −20 °C.

For the LC/MS/MS method, the calibration and linearity testing solutions were prepared identically to the LC/UV solutions, except that the concentration of the terbutaline internal standard solution was 0.1 ng/μL instead of 50 ng/μL and the concentrations of *p*- and *m*-synephrine in the stock solution were 5 ng/μL each instead of 1000 ng/μL each. The working calibration range (≈0.02 ng/μL synephrine to ≈1.3 ng/μL synephrine) bracketed the synephrine levels in the test supplements.

2.4. Preparation and extraction of dietary supplements

Development, optimization and validation of the alkaloid extraction procedure for bitter orange-containing dietary supplements (NIST SRM 3260) has been completely described in a separate publication (Putzbach et al., 2007a); however, a brief description of the modified procedure follows. For each test supplement, an appropriate number of tablets or capsules, in excess of the recommended single serving size, were homogenized using an automatic mortar grinder. From the homogenized supplement, a 500-mg sample was weighed into a 50-mL plastic centrifuge tube and spiked with 1000-μL of terbutaline solution (4000 ng/μL). The sample was diluted with 40-mL of water and vortex mixed, and the tube was sonicated at room temperature for 30 min. The sample was then centrifuged at $3000g_n$ for 10 min and the supernatant was transferred into a clean 50-mL plastic centrifuge tube and stored on ice. The remaining solid material was re-extracted via sonication (30 min, room temperature) using a fresh 40-mL portion of water. Following centrifugation, 5-mL of the supernatant from the second extraction was combined with 5-mL of the supernatant from the first extraction. The combined extracts were vortex mixed and a 5-mL aliquot was filtered through a 0.45 μm pore polyvinylidene fluoride (PVDF) membrane filter. Three independent sample preparations of each supplement were performed and all extract filtrates were stored at 4 °C until analysis. For the LC/UV method, the extract filtrates were analyzed without further dilution. For the LC/MS/MS method, the extract filtrates were analyzed after diluting an aliquot 1 to 1000 (volume ratios, 10-μL extract + 10-mL water) with water.

[1] Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology (NIST) nor does it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose.

2.5. Analysis of sample extracts, calibration solutions and linearity test solutions

Sample extracts and calibration/linearity test solutions were analyzed using both the LC/UV method and the LC/MS/MS method (single injections for sample extracts and duplicate injections for calibration/linearity test solutions, 2-μL injections). Analyte/internal standard peak area ratios and concentration ratios were subjected to linear least squares regression analysis to produce calibration curves and calibration equations from the calibration solutions. Synephrine levels in the sample extracts were quantified on the basis of the relevant calibration equation and the alkaloid/terbutaline peak area response ratio detected in the sample extract.

2.6. LC/UV instrumentation

LC/UV experiments were conducted on a modular LC system coupled to a UV absorbance detector. The LC system consisted of a Varian 9012 ternary LC pump and a Dionex ASI-100 auto sampler. The detector was an ABI Analytical Spectroflow 783 single wavelength UV absorbance detector set at 220 nm. Samples were analyzed using a Supelco Discovery HS-F5 (pentafluorophenyl) analytical column (4.6 mm × 150 mm, 5 μm particle diameter) with an attached HS-F5 guard column (3 mm × 20 mm, 5 μm particle diameter) held at 35 °C ± 1°C. The isocratic LC elution conditions were as follows (solvent percentages are volume fractions): mobile phase A = 10 mmol/L ammonium acetate in water; mobile phase B = 10 mmol/L ammonium acetate in methanol; A/B = 70/30; flow rate = 1000 μL/min; total run time = 25 min.

2.7. LC/MS/MS instrumentation

Positive-ion mode LC/MS/MS experiments were conducted on a Waters 2795 Separations Module coupled to a Micromass Quattro Ultima triple quadrupole MS/MS system. The LC system was outfitted with a quaternary pump, a temperature controlled (10 °C) autosampler and an in-line mobile phase vacuum degasser. Samples were analyzed using the same type of pentafluorophenyl LC column and guard column as described for the LC/UV method, however, the mobile phase elution conditions involved the use of an LC gradient. The gradient LC elution conditions were as follows (solvent percentages are volume fractions): mobile phase A = 10 mmol/L ammonium acetate in water; mobile phase B = 10 mmol/L ammonium acetate in methanol; time program = 0 min, 75% A/25% B; 12.0 min, 75% A/25% B; 20.0 min, 25% A/75% B; 20.1 min, 75% A/25% B; 25.0 min, 75% A/25% B; flow rate = 500 μL/min; total run time = 25 min. The *p*- and *m*-synephrine isomers and terbutaline were detected and quantified on the basis of electrospray-ionization multiple-reaction-monitoring (ESI-MRM) MS/MS using protonated analyte molecules $[M+H]^+$. MS/MS operating parameters are summarized in Table 1S (see Supplement Data).

3. Results and discussion

3.1. Development of LC/UV quantitation method

Previous researchers have demonstrated the feasibility of separating *p*- and *m*-synephrine under reversed-phase LC conditions, however, only minimal baseline separation of the isomers was achieved (Allison et al., 2005; Barnes et al., 2006). We have now optimized specific LC conditions, such as mobile phase ionic strength, mobile phase pH, column temperature and percentage of organic eluent (%B) in order to achieve complete baseline separation of *p*- and *m*-synephrine under isocratic elution conditions. Beginning with a mobile phase aqueous/organic composition (A/B = 10% water/90% methanol) based on previously developed LC conditions for separating the five predominant adrenergic alkaloids in bitter orange-containing supplements (Nelson et al., 2007), the ionic strength of the mobile phase was incrementally changed (in nominal steps of 5 mmol/L) to contain an increasing concentration of ammonium acetate modifier (from 0 mmol/L to 25 mmol/L) in order to determine the effect of ionic strength on separating a standard mixture of *p*- and *m*-synephrine. As the concentration of ammonium acetate increased from 0 mmol/L to 25 mmol/L, separation of the isomers was not observed; however, slight peak splitting was evident at 10 mmol/L and 15 mmol/L, but not at 20 mmol/L or higher. Based on these observations, 10 mmol/L ammonium acetate was selected as the optimal modifier concentration. Subsequent testing of the mobile phase pH (pH 4 to pH 8) and LC column temperature (10–75 °C) did not elicit any further separation of the isomers. The unadjusted apparent pH of the 10/90 mobile phase containing 10 mmol/L ammonium acetate was pH 8 and all mobile phase pH values less than pH 7 resulted in complete loss of analyte retention. Interestingly, column temperature had negligible effect on analyte retention. However, the aqueous/organic composition of the mobile phase had an extraordinary effect on separation of the isomers. The %B was incrementally changed (in steps of 10%) from 100% B to 0% B. No separation of the isomers was observed from 100% B to 80% B, but at 70% B, partial separation of the isomers was observed. Baseline separation (1 min) of the isomers was evident from 50% B onward and at 20% B the separation was greater than 10 min. On the basis of these results, an organic mobile phase composition of 30% B (3 min separation of isomers) was selected as the optimal mobile phase for the LC/UV method. Addition of the terbutaline internal standard compound to the synephrine isomer standard mixture resulted in a suitable isocratic separation of all three compounds (Fig. 2) using the optimized mobile phase conditions.

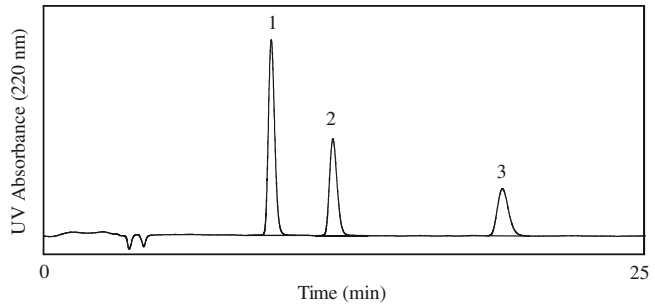

Fig. 2. Representative chromatogram from the LC/UV absorbance analysis of a mixed standard containing (1) *p*-synephrine; (2) *m*-synephrine; and (3) terbutaline. Each peak represents 500 ng analyte on-column. The LC/UV chromatogram was produced using the conditions described in Section 2.

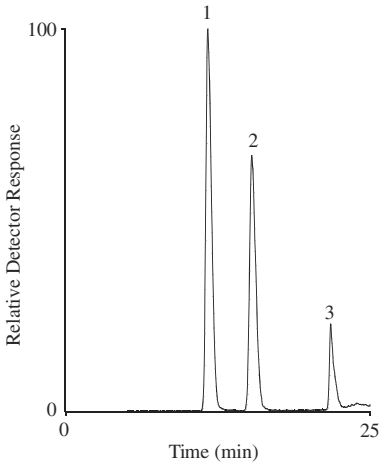

Fig. 3. Representative total ion current (TIC) chromatogram from the LC/MS/MS analysis of a mixed standard containing (1) *p*-synephrine, 5 ng on-column; (2) *m*-synephrine, 5 ng on-column; and (3) terbutaline, 0.2 ng on-column. The TIC chromatogram was produced using the conditions described in Section 2.

3.2. Development of LC/MS/MS quantitation method

The LC/MS/MS quantitation method was developed based on the LC mobile phase conditions previously optimized for the LC/UV quantitation method. Due to the lower LC flow rate requirement for efficient ESI MS, the LC flow rate was reduced from 1000 μL/min to 500 μL/min; this change necessitated a slight change in the mobile phase composition (A/B composition = 75% water/25% methanol in 10 mmol/L ammonium acetate) and the addition of an LC gradient to keep the overall analysis run time from doubling (see Section 2). Following the LC changes, the optimal MS/MS MRMs for each synephrine isomer and terbutaline were determined based on direct infusion collision-activated-dissociation (CAD) studies. Because *p*- and *m*-synephrine differ only in the location of a single ring hydroxyl group (Fig. 1), the isomers produced identical ESI fragmentation patterns. A representative spectrum from direct infusion analysis of *p*-synephrine is shown in Fig 1S (see Supplementary Data). The most intense fragment ions (*m*/*z* 135) were formed due to the combined loss of a molecule of water and a methyl group from the protonated precursor molecules (*m*/*z* 168). The MRM transitions for each synephrine isomer (*m*/*z* 168→*m*/*z* 135) and terbutaline (*m*/*z* 226→*m*/*z* 125) were established and the appropriate LC/MS/MS instrument conditions were optimized via analysis of analyte standard mixtures. A representative LC/MS/MS total ion current (TIC) chromatogram of a standard mixture is shown in Fig. 3. The optimized gradient LC separation and MS/MS detection conditions allow the analysis to be conducted with good sensitivity within a total run time of 25 min.

3.3. Comparison of method performance characteristics

The LC/UV and LC/MS/MS methods were evaluated and compared in terms of linear dynamic range and overall analytical sensitivity for the quantitation of both synephrine isomers (Table 1). As expected, the analytical metrics for *p*- and *m*-synephrine were essentially equivalent within a given method. The LC/UV and LC/MS/MS methods were highly comparable in terms of overall linear range capabilities; the linear range was at least three orders of magnitude for both isomers. However, the linear range for the LC/UV method extended from nanogram to microgram amounts of the isomers on-column while the LC/MS/MS method's linear range extended from picogram to nanogram amounts of the isomers on-column. The methods were not comparable in terms of detection or quantitation sensitivities. The LC/MS/MS method was 2000 times more sensitive (expressed by the LOD and LOQ) than the LC/UV method for both isomers.

Table 1
Comparison of method performance characteristics for the detection of synephrine isoforms

Analytical parameter	LC/UV		LC/MS/MS	
	p-Synephrine	*m*-Synephrine	*p*-Synephrine	*m*-Synephrine
Linear dynamic range[a]	11–11318[b]	10–10696[c]	0.005–10[d]	0.005–10[e]
LOD[a,f]	11	10	0.005	0.005
LOQ[a,g]	33	30	0.015	0.015

Values in () signify the standard error (SE).

[a] Determined via analysis of serially diluted calibrants as described in Section 2. All synephrine values are masses (ng) of analyte injected on-column.

[b] Calculated $r^2 = 1.00$, slope = 0.0413 (0.0001), *y*-intercept = −0.1055 (0.0589), SE estimate for regression line = 0.1668.

[c] Calculated $r^2 = 1.00$, slope = 0.0184 (0.0001), *y*-intercept = −0.0597 (0.0303), SE estimate for regression line = 0.0858.

[d] Calculated $r^2 = 0.998$, slope = 4.4248 (0.0618), *y*-intercept = 0.1780 (0.1030), SE estimate for regression line = 0.3091.

[e] Calculated $r^2 = 0.999$, slope = 3.4803 (0.0318), *y*-intercept = 0.0144 (0.0532), SE estimate for regression line = 0.1595.

[f] The LOD is the minimum detectable analyte signal that is at least three times the noise signal.

[g] The LOQ is calculated by multiplying the LOD by a factor of three. The S:N ratio at the listed LOQ is ⩾10 for all analytes.

3.4. Determination of synephrine isomers in dietary supplements

A selection of six commercially available bitter orange-containing dietary supplement products were analyzed for *p*- and *m*-synephrine levels using both LC methods. NIST SRM 3260 Bitter Orange-Containing Solid Oral Dosage Form was utilized as a control material throughout all of the analytical testing. Preliminary characterization of SRM 3260 extracts using both LC methods revealed no obvious problems with the optimized LC separations. The locations and identities of the synephrine isomer peaks and terbutaline in SRM 3260 extracts were confirmed based on analyte spiking studies and retention time matching using the LC/UV method. Additional analyte spiking studies confirmed that the synephrine isomer and terbutaline peaks were not affected by ion suppression during the LC/MS/MS procedure. Fig. 4 shows representative chromatograms from analysis of a typical bitter orange-containing dietary supplement (product A). Fig. 4A illustrates the analysis of the product A extract using the LC/UV method. *p*-Synephrine (peak 1) and terbutaline (peak 3) are cleanly separated from potentially interfering components and there exists no apparent signal for *m*-synephrine in the sample extract. Fig. 4B shows the same sample analyzed using the LC/MS/MS methodology. The MRM chromatogram shows only peaks for *p*-synephrine (peak 1) and terbutaline (peak 3), corroborating the results from the LC/UV analysis. Neither analytical approach detects *m*-synephrine in product A extracts, and in fact, this is a general finding for all of the tested dietary supplement products. Table 2 compares the data for the quantitative determination of *p*-synephrine levels (mg/kg) in all of the tested dietary supplement products using both LC methods. The *p*-synephrine levels (mean ± standard deviation) determined for the control sample by the LC/UV (19350 mg/kg ± 1054 mg/kg) and LC/MS/MS (21110 mg/kg ± 2995 mg/kg) methods were in agreement with the overall synephrine level (17786 mg/kg ± 582 mg/kg) generated by NIST interlaboratory certification measurements ($n = 6$ methods). The consistency and agreement of the control sample measurements combined with the agreement with the NIST interlaboratory certification measurements support the overall reliability and accuracy of both synephrine LC methods. In general, measured *p*-synephrine levels were in excellent agreement (within the 95% confidence interval) between both methods for products A, C and F and in good agreement (less than 15% difference) for products B, D and E. It was noted that the LC/UV *p*-synephrine levels for products B, D and E were all higher than the LC/MS/MS *p*-synephrine levels and this may be due, in part, to the presence of coeluting UV absorbing components within the LC/UV *p*-synephrine peaks. The specificity of UV absorbance detection is much lower than MS/MS detection, thus it is possible that unresolved dietary supplement components contributed to higher LC/UV *p*-synephrine levels for products B, D and E. The synephrine measurement imprecision of both LC methods were similar with the LC/UV method exhibiting a mean

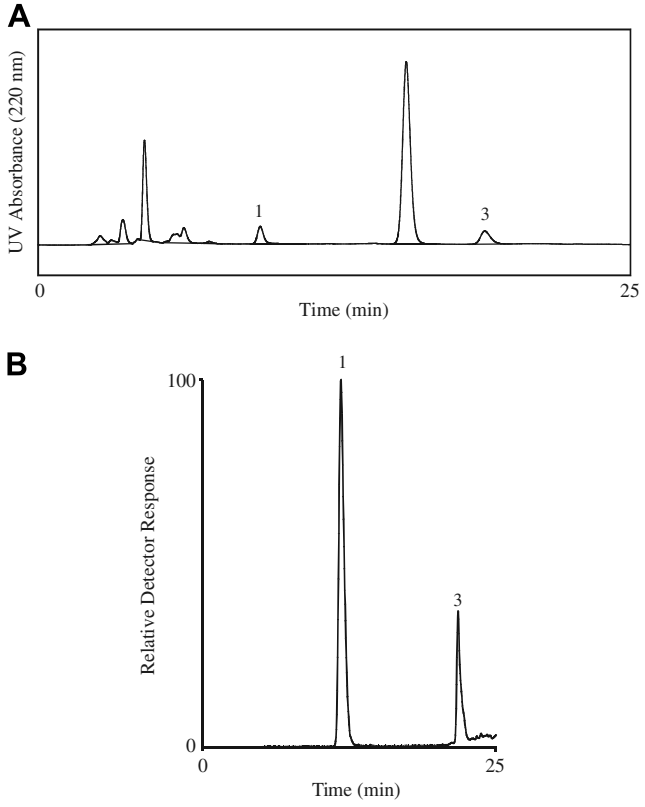

Fig. 4. Representative LC/UV absorbance and multiple reaction monitoring (MRM) LC/MS/MS chromatograms from the determination of synephrine isomers in product A dietary supplement (A) LC/UV analysis of product A extract; (B) MRM LC/MS/MS analysis of product A extract. The monitored MRM transitions were *m/z* 168 to *m/z* 135 for peak 1 and *m/z* 226 to *m/z* 125 for peak 3. For both chromatograms, peak 1 is *p*-synephrine and peak 3 is terbutaline. The chromatograms were collected using the LC/UV and LC/MS/MS conditions described in Section 2.

Table 2
Quantitation of synephrine (*p*) in bitter orange-containing dietary supplements[a]

Samples	LC/UV (mg/kg)	SD (mg/kg)	RSD (%)	LC/MS/MS (mg/kg)	SD (mg/kg)	RSD (%)
Control sample[b]	19350.1	1054.2	5	21110.0	2994.5	14
Product A	5421.1	394.3	7	5483.8	300.7	6
Product B	14111.6	685.7	5	12447.3	557.5	5
Product C	5739.6	659.2	12	4694.8	550.6	12
Product D	14297.6	197.4	1	12598.7	263.3	2
Product E	7133.4	192.9	3	6419.9	338.6	5
Product F	7208.7	346.0	5	6550.5	636.9	10

[a] *m*-Synephrine was not detected in any of the tested samples. *p*-Synephrine was detected in all of the tested samples. Reported *p*-synephrine levels are the means from $n = 3$ preparations of each sample.
[b] NIST SRM 3260 was utilized as a control material. The interlaboratory level of synephrine (*p*-) in SRM 3260 as determined by six different analytical methods is 17786 mg/kg ± 582 mg/kg (mean ± standard deviation).

percent relative standard deviation (%RSD) of 5% and the LC/MS/MS method exhibiting a slightly higher imprecision of 8% for all of the tested dietary supplements.

4. Conclusions

In conclusion, two LC methods have been developed for baseline separation and quantitative determination of *p*- and *m*-synephrine positional isomers in bitter orange-containing supplements. In the current work, *m*-synephrine was not detected in any of the tested dietary supplements with UV absorbance or with tandem mass spectrometry detection. The LC/MS/MS method (LOQ = 0.015 ng synephrine on-column, Table 1) is approximately 2000 times more sensitive than the LC/UV method (LOQ ≈ 30 ng synephrine on-column, Table 1) for the quantitation of synephrine isomers. The LC/MS/MS method provides more sensitivity than is realistically needed for the determination of the isomers in dietary supplements. Thus, it is more appropriate and probably easier to use the LC/UV method as a general screening method and the LC/MS/MS method as a confirmatory method for verifying the presence or absence of synephrine isomers in dietary supplements.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2007.12.076.

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