JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

An Isotopically Labeled Internal Standard Liquid Chromatography−Tandem Mass Spectrometry Method for Determination of Ephedrine Alkaloids and Synephrine in Dietary Supplements

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We report a simplified analytical procedure for determination of ephedrine alkaloids and synephrine in dietary supplements. Cleanup by simple filtration, when combined with tandem mass spectrometry (MS/MS) detection, provided results comparable to our published method with solid phase extraction (SPE) cleanup and single-stage MS detection with in-source fragmentation. We also compared three mass spectrometric experimental configurations: electrospray (ESI) and atmospheric pressure chemical ionization (APCI) with MS/MS and APCI-MS with fragmentation provided by increasing cone voltage. Because these methods used one isotopically labeled internal standard to determine several different analytes, quantitation errors may arise from susceptibility to ionization suppression caused by the matrix. We therefore compared the results obtained by ESI and APCI ionization.

KEYWORDS: Ephedrine; synephrine; ephedra alkaloid; dietary supplement; determination; analysis; isotope dilution; mass spectrometry; MS/MS

INTRODUCTION

Naturally occurring ephedrine alkaloids consist of a mixture of three diasteriomeric pairs of compounds. Botanical sources of the alkaloids include ma huang, a traditional Chinese medicine derived from aerial parts of *Ephedra sinica* Stapf, *Ephedra equisetina* Bunge, and others of the family Ephedraceae, as well as *Ephedra gerardiana* Wall. (used primarily in India) and *Sida cordifolia*. In addition, small amounts of ephedrine alkaloids have been reported in *Pinellia ternate*. Several traditional Chinese or Japanese medicines—including zhi shi [containing the fruit of *Citrus aurantium* (bitter orange)] (*1*, *2*), Pericarpium Citri Reticulatae (*Citrus reticulata* Blanco) (*3*, *4*), and Aurantii fructus Immaturus (*4*)—have been found to contain synephrine, an alkaloid closely related to ephedrine. Structures of these compounds are shown in **Figure 1**. Synephrine has also been found in other citrus varieties (*1*, *3*) as well as foods containing citrus (*5*). Ganzera and co-workers recently reported simultaneous determination of *E. sinica* and *C. aurantium* alkaloids using ion pairing and a pH gradient (*6*). As a result of their alleged thermogenic properties and their consequent use to enhance energy and promote weight loss, products containing ma huang (or another botanical source of ephedrine) were among the most popular dietary supplements on the market, until their sale was banned by the U.S. Food and Drug Administration (FDA) in 2004. (Methods listed do not replace current official methods used for enforcement purposes.) Synephrine-containing products have largely replaced them.

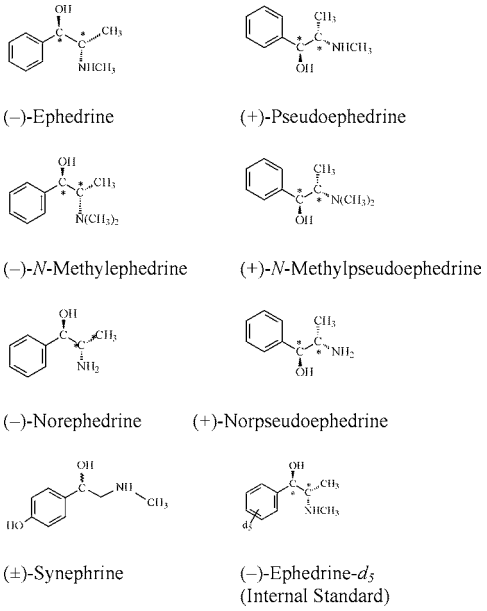

Figure 1. Structures of the natural ephedrine alkaloids and synephrine.

Adverse health events attributed to consumption of products containing ephedrine alkaloids led to the development of many analytical methods for their determination. The underivatized

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10.1021/jf051735w
Published on Web 12/24/2005

enantiomers were resolved by capillary gas chromatography using a chiral liquid phase (*5*, *7*). Alternatively, chiral derivatives were separated on a conventional, symmetrical liquid phase such as polyphenylmethylsiloxane (*8*, *9*) or capillary electrophoresis on a chiral column (*10*). Isocratic high-performance liquid chromatography (HPLC) with UV detection has been used by several groups to determine ephedrine type alkaloids in a variety of products (*11*−*14*). Recently, flow injection-electrospray ionization (ESI)-high field asymmetric waveform ion mobility spectrometry-mass spectrometry (FAI-MS) was shown to successfully separate the diasteriomers for determination in supplement tablets (*15*). Capillary electrophoresis with flow injection has also been used to determine ephedrine and pseudoephedrine in Chinese medicinal preparations (*16*). Synephrine has been determined in herbal products by HPLC with UV (*2*, *4*), tandem UV and native fluorescence (*17*), ESI-MS (*1*), and electrochemical detection (*5*). Recently, Chen (*3*) analyzed hesperidin and synephrine in Paricarpium Citri Reticulatae by capillary electrophoresis with electrochemical detection. In support of the FDA's regulatory effort, analytical methods are desired that not only determine the amounts of the various alkaloids but also provide unambiguous identification of them. This led us to adapt HPLC methods to mass spectrometry using an isotopically labeled internal standard (IS) to correct for recovery as well as matrix effect on ionization efficiency. This was particularly important in analyzing finished products, because matrices were highly variable and chemically complex. So that the method would be usable in single-analyzer instruments, an in-source fragmentation method was developed for the mass spectrometer (*18*). Sullivan et al. (*19*) recently reported the use of tandem mass spectrometry (MS/MS) for the analysis of ephedrine alkaloids. This method was the subject of a successful collaborative study (*20*). Sander recently reported the determination of ephedrine alkaloids in dietary supplement standard reference materials (*21*). Jacob and co-workers used HPLC with atmospheric pressure chemical ionization (APCI) MS/MS to determine ephedra alkaloid and caffeine concentrations in dietary supplements and biological fluids (*22*). We developed an independent (nonmass spectrometric) highly selective method for synephrine and ephedrine alkaloids that used column-switching cation exchange HPLC to enrich and separate synephrine and the ephedrine alkaloids from product extract. Qualitative identification was provided by computer matching of run-time UV spectra to those of the standards (*17*).

We now report a liquid chromatography (LC)-MS/MS method that uses selective reaction monitoring to obtain product ion spectra. This mass spectrometric configuration is often more robust than full-scan MS with in-source fragmentation. Synephrine and ephedrine alkaloids extracted from finished products were measured by both techniques. Determination of synephrine, ephedrine, and pseudoephedrine by each method is compared. Some results for the minor components are also reported. This is an achiral method, which does not separate enantiomers.

MATERIALS AND METHODS

Reagents. Acetonitrile (ACN), methanol, and water were HPLC grade (Honeywell Burdick and Jackson, Muskegon, MI). Extraction solvent (80% methanol) was prepared by adding water (100 mL) to a 500 mL volumetric flask and diluting to the mark with methanol. The solid phase extraction (SPE) elution buffer was prepared in two steps. A 30% ACN solution was prepared by adding ACN (75 mL) to a 250 mL volumetric flask and diluting to the mark with water. Immediately prior to use, sufficient ammonium acetate was added to produce a 150 mM solution. For example, 577 mg of ammonium acetate was added to 50 mL of the ACN solution. Strong cation exchange (propylsulfonic acid) SPE columns (Isolute SCX-2, 500 mg in 6 mL) were purchased from Argonaut Technologies, Inc. (Redwood City, CA). Mobile phase solvent A (2% acetic acid in aqueous 50 mM ammonium acetate) was prepared by dissolving 1.9 g of ammonium acetate in water in a 500 mL volume flask, adding 10 mL of glacial acetic acid, and diluting to the mark with water. Mobile phase solvent B (2% acetic acid in 25% ACN) was made up in a 500 mL volume flask from 10 mL of glacial acetic acid and 125 mL of ACN diluted to the mark with water.

Table 1. Quantities of Each Analyte (mg/mL) in Each Level of the Standards Used to Generate the Calibration Curve

	level					
	1	2	3	4	5	6
Syn	11.4	28.5	57	142.5	228	285
NE	3.3	8.25	16.5	41.25	66	82.5
NPE	0	2.5	5	12.5	20	25
E	12.8	32	64	160	256	320
PE	10.4	26	52	130	208	260
ME	4.9	12.25	24.5	61.25	98	122.5
MPE	7.1	17.75	35.5	88.75	142	177.5

Chemicals and Standard Solutions. (−)-(1*R*,2*S*)-norephedrine, (+)-1*S*,2*S*)-norpseudoephedrine·HCl (cathine hydrochloride, a controlled substance), (−)-(1*R*,2*S*)-ephedrine, (+)-(1*S*,2*S*)-pseudoephedrine, racemic synephrine [4-hydroxy-α-(methylaminomethyl)benzyl alcohol], (−)-(1*R*,2*S*)-*N*-methylephedrine, and (+)-(1*S*,2*S*)-*N*-methylpseudoephedrine were obtained from Sigma-Aldrich Chemical (St. Louis, MO). (−)-(1*R*,2*S*)-Ephedrine-d_5·HCl was obtained from Isotec, Inc. (Miamisburg, OH). A working IS solution was prepared by accurately weighing ∼12 mg of (−)-(1*R*,2*S*)-ephedrine-d_5·HCl into a 10 mL volume flask and adding extraction solvent to make up the volume. An analyte working standard solution was prepared by accurately weighing ∼10 mg of synephrine, ephedrine, and pseudoephedrine; ∼5 mg of *N*-methylephedrine and *N*-methylpseudoephedrine; and ∼1 mg of norephedrine into a 10 mL volume flask and dissolving them in the SPE elution buffer solvent. Norpseudoephedrine·HCl standard solution was prepared separately by placing 3.2 mg of the hydrochloride, which corresponded to 2.59 mg of the free base, in a vial. The elution buffer solvent (2.59 mL) was added to the vial to produce a 1 mg/mL standard. Appropriate amounts (10−250 μL) of analyte solutions were placed in autosampler vials with 50 μL of IS solution. Sufficient mobile phase was added to bring the total volume in the vial to 1 mL.

Instrumentation and Apparatus. LC-MS and LC-MS/MS procedures, including comparison of APCI and ESI interfaces, were carried out on a Waters Micromass Quattro Micro triple quadrupole mass spectrometer with interchangeable ESI and APCI probes, interfaced to a model 1100 HPLC (Agilent Technologies, Inc., Palo Alto, CA) with an autoinjector (injected 5 μL test solutions) and solvent vacuum degasser. MassLynx and QuanLynx (ver. 4.0 SP1) Windows-based software from Waters Micromass (Manchester, United Kingdom) provided instrument control and data acquisition and reduction.

Calibration. A six-point linear regression calibration (five point in the case of norpseudoephedrine) of relative peak area vs concentration of each analyte was obtained at intervals as the samples were analyzed. Quantities of each analyte in each level are presented in **Table 1**.

Samples. Dietary supplement finished products in capsule or tablet form were purchased from retail outlets. The contents of 10−20 capsules were emptied into a scintillation vial, which was shaken and rolled to provide thorough mixing. A sample of a similar number of tablets was prepared by grinding.

Analysis. When sample label information indicated >2.5 mg of ephedrine alkaloids/g, a 100 mg test portion was accurately weighed into a 50 mL polypropylene centrifuge tube with a screw cap; otherwise, a 1 g test portion was used. The IS working solution (0.400 mL) and extraction solvent (19.6 mL) were added, and a batch of capped tubes was extracted at room temperature for 20 min in a sonicator, followed by 20 min of centrifugation at 7000 rpm. Each supernatant was decanted into a labeled scintillation vial. When analytical results were outside the linear range of the method, the extraction was repeated using an appropriate test portion size.

SPE. Columns were conditioned with 2.0 mL of methanol, then reverse osmosis water, and ending with dilute (1:20 aqueous) mobile phase. The extract (10 mL) was pipetted onto the column and allowed to drain through. The column was washed twice with dilute mobile phase (3.0 mL) and dried under vacuum. Before elution, the column was wetted with methanol (2.0 mL), and the effluent was discarded. The target compounds were collected in the SPE elution buffer (4.0 mL) for instrumental analysis.

Filtration. Crude extract (~2.0 mL) in a disposable syringe was filtered through a 0.2 μm filter with a built-in prefilter (Anotop 25 Plus, Whatman Inc., Florham Park, NJ).

LC Conditions. The mobile phase (2% acetic acid, 44 mM ammonium acetate, and 3% ACN) was generated from phases A (2% acetic acid and 50 mM ammonium acetate) and B (2% acetic acid and 25% ACN) by pumping 88:12 A/B at 0.230 mL/min through a 5 μm YMC phenyl column (250 mm × 2 mm i.d., Waters Corp., Milford, MA) held at 40 °C.

MS Conditions: MS/MS with Selected Ion Monitoring. For each analyte, three reactions of the protonated molecule were monitored. Precursor and product ions for each analyte are shown in **Table 2**.

ESI Conditions. Source temperature, 150 °C; desolvation temperature, 250 °C; cone gas flow, 200 L/h; desolvation gas flow, 500 L/h; collision gas cell pressure, 2.07×10^{-3} mbar argon; and cone voltage, 14 V. Collision energies varied by analyte and are given in **Table 3**.

APCI Conditions. Source temperature, 130 °C; desolvation temperature, 350 °C; cone gas flow, 100 L/h; desolvation gas flow, 300 L/h; and collision gas cell pressure, 2.07×10^{-3} mbar. Collision energies are given in **Table 3**. In both ESI and APCI, quantitation was based on the total ion current generated by the three reactions. In our previous work, quantitation was based on the most abundant ion for each analyte.

RESULTS AND DISCUSSION

Synephrine Chromatography. Our earlier work (*18*) described selection of a robust phenyl column that consistently baseline-separated the ephedrine alkaloids. Under those conditions, synephrine tended to elute as a distorted peak near the void volume, increasing the probability of encountering interfering compounds. When this occurred, injecting a smaller volume improved the peak shape. As discussed below, interference can usually be eliminated by use of MS/MS.

Cleanup. The SPE cleanup of our original method produced a cleaner and more concentrated extract but became tedious for high volume work. To determine whether this step was necessary, we re-extracted 17 of the original products and compared results obtained from simple filtration with those obtained with SPE cleanup of the same extracts. Each extract was injected several times under varying chromatographic conditions. Results for ephedrine and pseudoephedrine are plotted in **Figures 2** and **3**. The small deviation of the regression coefficients from zero intercept and unity slope indicates bias, which could be due to less than complete elution of analytes or IS from the SPE column. In fact, further elution from the SPE column did result in recovery of small amounts of analytes and IS. We therefore increased the elution volume to 5 mL in the subsequent recovery experiments.

Recoveries. As shown in **Table 4**, excellent recoveries, corrected for control levels, were obtained when the product containing no synephrine was spiked ($N = 4$) at two levels. Data for both SPE cleanup (5 mL elution) and filtration only cleanup are presented.

APCI vs ESI. When constructing calibration curves using an isotopically labeled IS, the IS area may be suppressed by the amount of native analyte present (*23*, *24*). In addition, at high concentrations, the response for an analyte may become nonlinear due to self-suppression. This signal suppression has been found to be more evident in electrospray. Additionally, the use of one IS for multiple analytes can create problems if care is not taken (*24*). In the work reported here, only ephedrine out of seven analytes had an isotopically labeled analogue, which also served as a surrogate for the other analytes. In addition,

Table 2. Relative Abundances of Ions Used to Identify and Quantitate Synephrine and the Ephedrine Alkaloids

Synephrine

	standards (5 levels)		products (5): SPE		products (5): filter	
reaction monitored	average (%)	SD (%)	average (%)	SD (%)	average (%)	SD (%)
168→150	81.3	0.22	80.9	0.63	79.2	2.24
168→135	8.2	0.14	8.3	0.32	9.7	1.35
168→119	10.4	0.23	10.8	0.47	11.1	1.07

Norephedrine

	standards (5 levels)		products (4): SPE		products (1): filter	
reaction monitored	average (%)	SD (%)	average (%)	SD (%)	average (%)	SD (%)
152→152	19.0	0.5	21.1	3.07	20.3	
152→134	77.9	0.6	74.9	3.52	74.9	
152→117	3.1	0.2	3.9	1.25	4.7	

Norpseudoephedrine

	standards (5 levels)		products (4): SPE		products (1): filter	
reaction monitored	average (%)	SD (%)	average (%)	SD (%)	average (%)	SD (%)
152→152	10.0	2.41	10.1	1.68	10.2	
152→134	86.6	2.65	86.4	2.01	86.1	
152→117	3.4	0.36	3.5	0.48	3.6	

Ephedrine

	standards (5 levels)		products (5): SPE		products (5): filter	
reaction monitored	average (%)	SD (%)	average (%)	SD (%)	average (%)	SD (%)
166→148	54.0	2.03	55.0	0.50	55.0	0.45
166→133	21.4	3.09	19.9	0.19	20.0	0.47
166→117	24.6	1.11	25.1	0.35	25.0	0.57

Pseudoephedrine

	standards (5 levels)		products (5): SPE		products (5): filter	
reaction monitored	average (%)	SD (%)	average (%)	SD (%)	average (%)	SD (%)
166→148	54.8	0.53	53.0	2.10	53.9	1.97
166→133	20.7	0.39	22.3	1.01	22.2	1.09
166→117	24.5	0.49	24.7	1.49	23.8	1.47

Methylephedrine

	standards (5 levels)		products (4): SPE		products (1): filter	
reaction monitored	average (%)	SD (%)	average (%)	SD (%)	average (%)	SD (%)
180→162	74.4	1.55	73.7	1.7	77.2	
180→147	10.7	1.17	12.5	1.3	9.5	
180→135	14.9	0.71	13.7	1.4	13.3	

Methylpseudoephedrine

	standards (5 levels)		products (5): SPE		products (5): filter	
reaction monitored	average (%)	SD (%)	average (%)	SD (%)	average (%)	SD (%)
180→162	85.5	0.49				
180→147	13.7	0.46	none found			
180→135	0.8	0.09				

Table 3. Collision Energies (eV) Required for Fragmentation of Analyte Ions in ESI and APCI

	collision energy	
analyte	ESI	APCI
synephrine	13	15 (168 > 119), 13
NE, NPE	7	7.5
E, PE, IS	17	17
ME, MPE	14	14.5

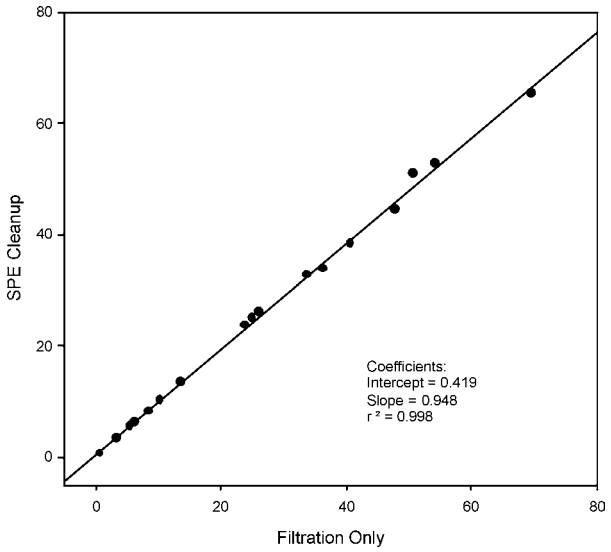

Figure 2. Determination of ephedrine (mg/g) in 17 products using SPE cleanup as compared to simple filtration.

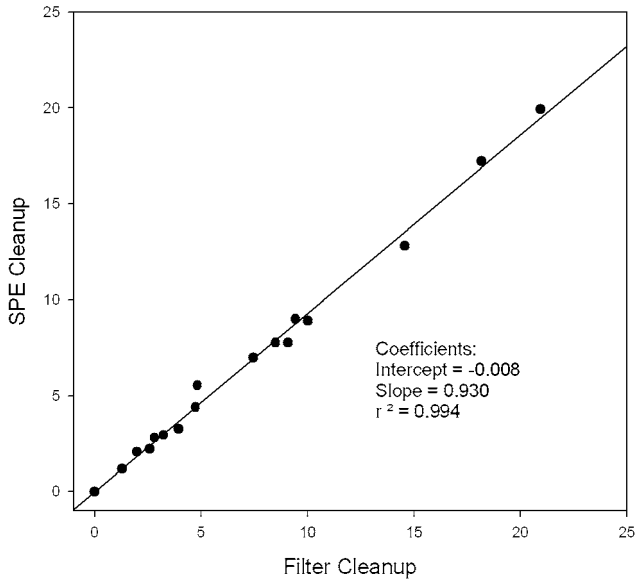

Figure 3. Determination of pseudoephedrine (mg/g) in 17 products using SPE cleanup as compared to simple filtration.

the concentration ratio of the other analytes to ephedrine (which coeluted with the IS) varied from product to product and was rarely, if ever, the same as the respective concentration ratio in prepared standard solutions. To obtain the desired dynamic range in the analysis, the native ephedrine concentration in the calibration standards was quite high whereas the labeled ephedrine concentration was kept constant at about the midrange value of the native. Suppression of the IS signal would be expected to distort the response factors of the other analytes, resulting in quantitation errors, especially for major constituents

Table 4. Average ($N = 4$) Recovery of Analytes at Two Spiking Levels[a]

	SPE (%)		filter (%)		amount spiked (mg/g)	
product/spike	low	high	low	high	low	high
synephrine	99.3	99.0	93.3	105	1.18	11.8
norephedrine	102	102	106	101	0.34	3.4
ephedrine	98.5	96.2	99.0	97.5	1.33	13.3
pseudoephedrine	104	103	91.1	96.0	1.08	10.8
methylephedrine	119	95.1	136	113	0.51	5.1
methylpseudoephedrine	89.8	96.8	104	99.1	0.74	7.2

[a] Analytes were spiked into a product, which contained no synephrine but did contain ephedrine alkaloids.

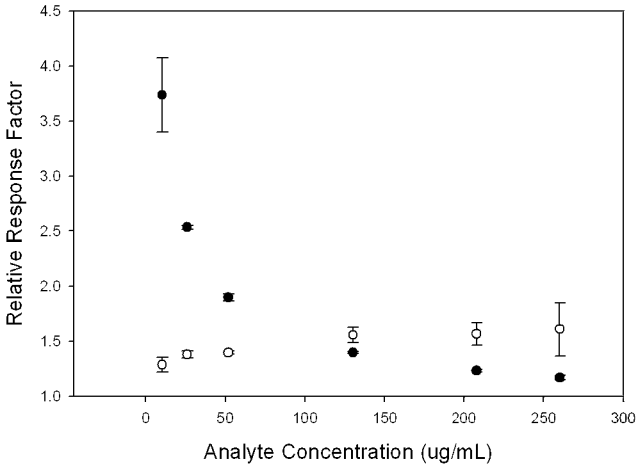

Figure 4. Dependence of pseudoephedrine response factors on amount in standard solutions analyzed under ESI and APCI conditions: ESI, ●; APCI, ○.

such as pseudoephedrine or synephrine (if present). To detect this effect and to develop analytical conditions that would minimize it, we compared the calibration curves and analytical results obtained under APCI and ESI conditions. Analyte relative response factors, defined as the reported relative response divided by the amount (μg/mL) of analyte injected, were calculated from calibration data and ideally should be independent of level. **Figure 4** shows pseudoephedrine relative response factors plotted as a function of amount injected. Particularly at lower amounts, the curve was quite steep for electrospray. In contrast, response factors obtained with APCI were approximately independent of amount, although precision seemed to worsen at higher amounts.

Results for the determination of ephedrine and pseudoephedrine in 17 products by ESI and APCI are compared in **Figures 5** and **6**. Results for ephedrine compared quite well, but results for pseudoephedrine were dependent on ionization method. Typically, ESI gave erroneously high values for pseudoephedrine, except when relatively small amounts of ephedrine were present (products 6 and 16) in which ESI gave lower values vs APCI. Higher values indicate that suppression of the IS is the most important mechanism for these samples. Lower values indicate that self-suppression predominates.

Comparison of MS and MS/MS. Comparing single-stage vs MS/MS data demonstrated that sometimes SPE cleanup was needed with single stage. Previously, we showed that increasing the cone voltage provided sufficient fragmentation to confirm the identities of ephedrine alkaloid analytes. We have now tested the cone voltage method with filtered extracts without further

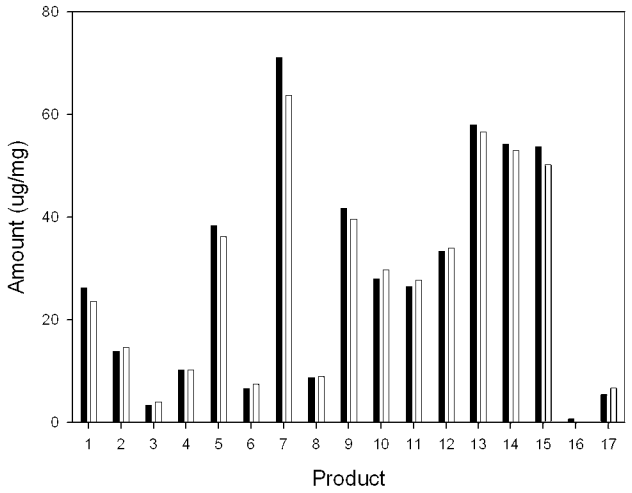

Figure 5. Determination of ephedrine in 17 products under ESI and APCI conditions: APCI, black; ESI, white.

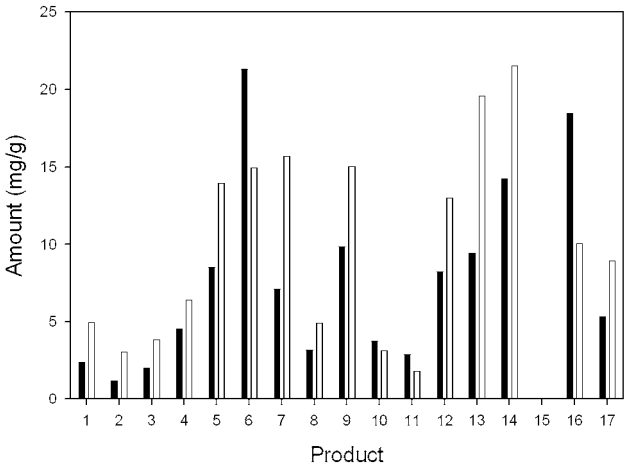

Figure 6. Determination of pseudoephedrine in 17 products under ESI and APCI conditions: APCI, black; ESI, white.

cleanup and for suitability in determination of synephrine. Careful examination of ion chromatograms of products revealed potential problems with both the IS and the synephrine. **Figure 7** shows plots for three ions characteristic of synephrine and the total ion current. Interference observed in the TIC plot clearly originated with the *m*/*z* 138 ion. This interference went unnoticed in previous work, because this ion was not used in quantitation.

The addition of synephrine to the suite of ephedrine alkaloids complicated both chromatography and mass spectrometry. The TIC plots in **Figure 8** show the chromatographic region of synephrine elution from analysis of a product that contained synephrine (**A**) and one that did not (**B**). In some products without synephrine, a peak nevertheless was found at the retention time of synephrine but was due almost entirely to response from the *m*/*z* 119 ion. Although the peak was not mistaken for synephrine (see below), had this material been present in a product that contained synephrine, then confirmation of synephrine identity would fail. SPE cleanup of the extract improved the chromatography but did not remove the interfering material. Only MS/MS provided a clean signal.

For both MS and MS/MS, excellent linearity and reproducibility were observed for all analytes across the tested range. Coefficients of determination r^2 were ≥ 0.98, except for norpseudoephedrine (0.940). Determination based on one ion, rather than the total ion current of three ions, produced similar results and coefficients of determination. Results for ephedrine and

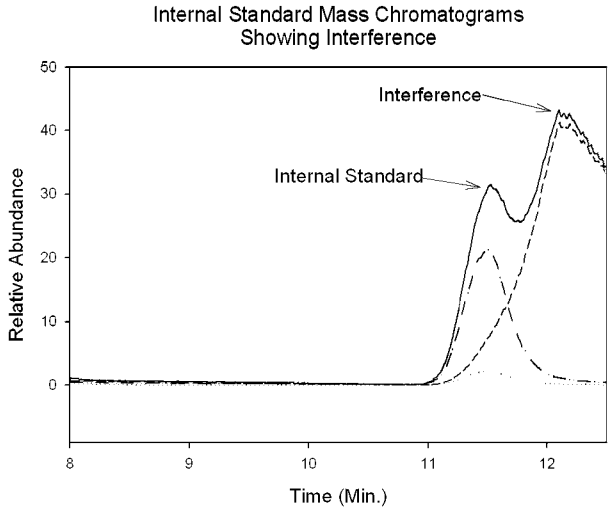

Figure 7. Interference observed in the ion chromatograms of the IS monitored reaction ions under MS conditions: TIC, —; *m*/*z* 121, • • •; *m*/*z* 138, – – –; *m*/*z* 153, – • •.

pseudoephedrine obtained by MS with fragmentation enhanced by increased cone voltage are included in **Table 5**. On the basis of quantitation using total ion current, Wilcoxon Signed Rank tests and paired *t*-tests indicated that the only pairwise comparison of three pairings that was not statistically significantly different at a 95% confidence level was filter vs SPE, Cone for pseudoephdrine. On the basis of using the most abundant ion for quantitation, paired *t*-tests indicated no significant difference at 95% confidence level for ephedrine for filter vs SPE (MS/MS) and for pseudoephedrine for SPE, Cone vs SPE (MS/MS). Although the sources of these small but statistically significant differences are not all known, it is clear that some result from use of different instrumental protocols and others are the result of differences in sample handling. This is in agreement with the results from the plots, which indicated the presence of a small amount of bias of unknown origin.

Because the U.S. banned the sale of ephedra-containing dietary supplements, most of these products are no longer available, and others have been reformulated. Therefore, product names have been omitted.

Limit of Quantitation. Limit of quantitation is dictated by the signal-to-noise ratio of the less abundant ions needed for confirmation of identity, as well as the range of linearity. Although ion abundances are much greater in single-stage (non-MS/MS) experiments, the range of linearity does not appear to be. On the basis of the least concentrated standard in the calibration sequence and a 1 g sample, the limits of quantitation would be 80 ppm for synephrine, ephedrine, and pseudoephedrine, 8 ppm for norephedrine and norpseudoephedrine, and 40 ppm for *N*-methyl-ephedrine and *N*-methylpseudoephedrine. Because products containing ephedrine alkaloids or synephrine usually contain large amounts of the major alkaloids, no attempt was made to optimize the limit of quantitation.

Confirmation of Identity. Three selected reactions were monitored for each analyte. **Table 2** presents relative abundance data for monitored reactions of all of the analytes in standards at five levels and in five products of varying concentration for both SPE cleanup and cleanup by filtration only. Minor constituents (norephedrine, norpseudoephedrine, methylephedrine, and methylpseudoephedrine) were confirmed in fewer than five products because too little analyte was present for a satisfactory signal-to-noise ratio for the least abundant ions of these analytes.

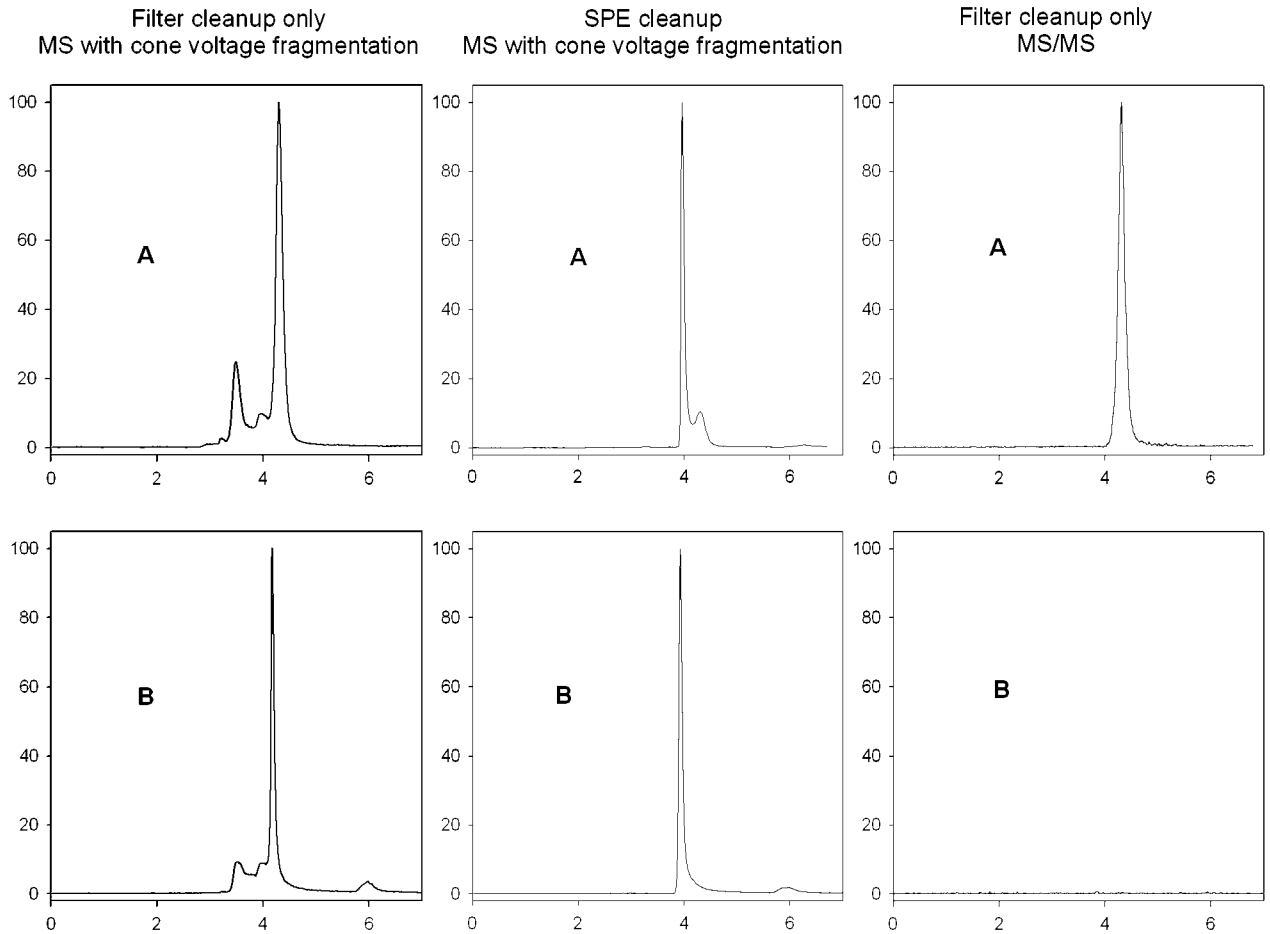

Figure 8. Interference in the determination of synephrine in dietary supplement products under MS conditions. The ingredient was declared in product A but was absent in B.

Table 5. Comparison of Determinative Results from Various Procedures for Synephrine, Ephedrine, and Pseudoephedrine in 17 Products

	synephrine (mg/g)		ephedrine (mg/g)			pseudoephedrine (mg/g)		
product	filter	SPE	filter	SPE	SPE, ConeV	filter	SPE	SPE, ConeV
1			25.0	24.9	25.9	2.6	2.2	2.8
2	1.8	2.5	13.6	13.4	14.1	1.3	1.2	1.4
3			3.4	3.4	3.1	2.0	2.1	2.3
4	2.2	3.6	10.3	10.2	10.7	4.7	4.4	5.3
5	4.9	5.9	36.4	33.9	36.6	9.1	7.8	8.3
6			6.3	6.2	6.7	20.9	19.9	21.8
7			69.7	65.4	70.3	7.4	7.0	8.1
8	2.3	2.4	8.6	8.3	8.8	3.2	2.9	3.4
9			40.7	38.2	40.8	10.0	8.9	8.9
10			26.1	26.1	27.5	3.9	3.3	3.6
11			24.0	23.8	25.4	2.8	2.8	2.9
12			33.8	32.7	34.6	8.5	7.8	9.2
13			54.4	52.7	56.1	9.4	9.0	10.6
14			50.9	50.9	54.8	14.6	12.8	13.7
15			47.9	44.5	47.3	0.0	0.0	0.0
16			0.7	0.7	0.5	18.2	17.2	16.6
17			5.5	5.5	6.0	4.8	5.6	6.6

Analyte identity was confirmed in a product when the relative abundances for all three reaction ions in analysis of the test portion fell within a ±10% window centered about the corresponding value found in analyzing standard solutions. These criteria were easily met for the major analytes of interest in the products that we analyzed. Minor alkaloids could also be confirmed when present in sufficient quantity to generate a signal for all three reactions that were monitored.

In summary, we have developed a method for the simultaneous determination of synephrine and ephedrine alkaloids in a variety of dietary supplement formulations. Both quantitation and qualitative identification of the analytes were provided by APCI mass spectrometric detection. The fragmentation necessary for identification was provided either by increasing the tube lens of the APCI source in a single-stage experiment or by collisional activation using a triple quadrupole instrument. Results obtained by these two methods were in good agreement, although quantitation of synephrine is more robust by MS/MS. The potential for incomplete elution from an SPE cleanup column, combined with the general disadvantages of multistep cleanup procedures, greatly favors the simple, filtration only approach and MS/MS.

ACKNOWLEDGMENT

We thank Martine Ferguson and Marc Boyer for statistical assistance.

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Received for review July 19, 2005. Revised manuscript received November 18, 2005. Accepted November 19, 2005.

JF051735W