DRUGS, COSMETICS, FORENSIC SCIENCES

Chiral Gas Chromatographic Determination of Ephedrine-Type Alkaloids in Dietary Supplements Containing *Má Huáng*

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***Má Huáng* is a traditional Chinese medicine derived from the aerial parts of several *Ephedra* species (Ephedraceae). These plants produce (–)-ephedrine, (+)-pseudoephedrine, (–)-norephedrine, (+)-norpseudoephedrine, (–)-*N*-methylephedrine, and (+)-*N*-methylpseudoephedrine. Racemic and (–)-ephedrine, (+)-pseudoephedrine, and (±)-norephedrine (phenylpropanolamine) are used clinically in the United States and are largely synthetic in origin. Current interest in *Má Huáng* is spurred by reports describing a "thermogenic" (calorie burning) effect provided by mixtures of ephedrine, caffeine, and aspirin. Products providing the key thermogenic compounds from natural sources are available as dietary supplements in retail outlets. Reports of potentially unsafe levels of the alkaloids, as well as possible fortification of *Má Huáng*-containing products with synthetic *Ephedra* alkaloids, prompted the development of a chiral gas chromatographic (GC) method that allows determination of alkaloid patterns and identification of isomerically impure synthetic alkaloids. Nine products were analyzed on a γ-cyclodextrin capillary GC column. Identity of the alkaloids was verified by GC/mass spectrometry (MS) and GC/matrix isolation/Fourier transform infrared spectroscopy. No synthetic isomers were found in the dietary supplements analyzed. Three products contained only one of the ephedrine-type alkaloids. One product that listed *Má Huáng* as an ingredient contained no detectable ephedrine-type alkaloid. In products containing measurable quantities of these compounds, total alkaloid levels ranged from 0.3 to 56 mg/g.**

Má Huáng is a traditional Chinese medicine (TCM) derived from the aerial parts of *Ephedra sinica* Stapf, *E. equisetina* Bunge, *E. intermedia* var. *tibetica* Stapf, and *E. distachya* L. (Ephedraceae) (1–8). This TCM has been used medicinally as a diaphoretic, stimulant, and antiasthmatic (1, 9). The medicinal properties of these *Ephedra* species are due to their content of (–)-ephedrine and 5 structurally related alkaloids (Figure 1): (+)-pseudoephedrine, (–)-*N*-methylephedrine, (+)-*N*-methylpseudoephedrine, (+)-norpseudoephedrine, and (–)-norephedrine. Ephedrine is the main active principle in most *Ephedra* species and comprises 30–90% of the total alkaloids in the plant (10). *Ephedra* species are found in temperate and subtropical regions of Europe, Asia, and the Americas (11). The North American species, such as *Ephedra nevadensis* S. Wats, *E. antisyphilitica* C.A. Meyer, and *E. trifurca* Torr, are generally considered to be alkaloid free (12).

Racemic and (–)-ephedrine, (+)-pseudoephedrine and (±)-norephedrine (phenylpropanolamine, PPA) are used clinically in the United States (13). Ephedrine promotes bronchodilation by activating β-adrenergic receptors in the lungs, and it is used for this purpose in patients with asthma (13, 14). Pseudoephedrine and PPA have similar, but weaker, actions. The alkaloids readily cross the blood–brain barrier and possess weak amphetaminelike central nervous system (CNS) stimulant activity (13, 14). Pseudoephedrine and PPA are generally preferred for use as oral decongestants because they are less potent and less likely to cause CNS stimulation or hypertension than ephedrine (13). Adverse reactions to these compounds include anxiety and restlessness, toxic psychosis, irregular heartbeat, tachycardia, hypertension, and skin eruptions. They are contraindicated in pregnant and lactating women and in patients with preexisting cardiac conditions (13, 14–26).

Ephedra herb was once recognized as an official drug in the United States (11), but widespread availability of synthetic ephedrine-type alkaloids virtually eliminated its clinical use (11, 13). Although most pharmaceutical ephedrine is produced by a biosynthetic procedure that yields optically enriched (–)-ephedrine, other synthetic methods yield racemic alkaloid mixtures (Table 1; 27–29).

Resurgence in interest in this TCM has been spurred by reports that certain combinations of ephedrine, caffeine, and aspirin raise the rate at which calories are expended in mammalian metabolic energy production. This "thermogenic" effect has been evaluated in a number of clinical trials (30–49), but a review of these studies by Bray (50) concluded the data were

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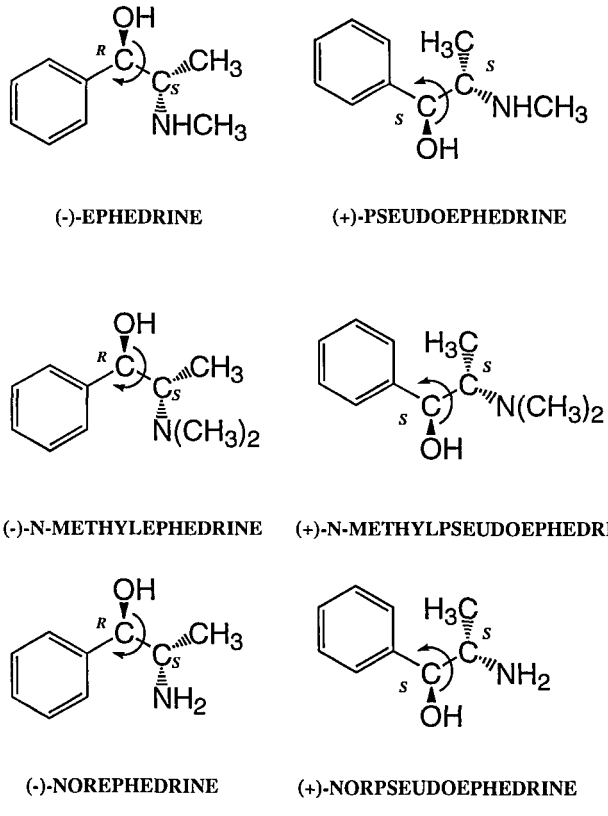

Figure 1. Naturally occurring ephedrine-type alkaloids of *Ephedra* spp.

insufficient to establish safety and efficacy of these combinations.

Products containing *Má Huáng* or *Má Huáng* extract, alone or in combination with other ingredients, are marketed as dietary supplements in the United States. In these products, kola nut (*Cola nitida* Schott et Endlicher, Fam. Sterculiaceae), guarana paste (*Paullinia cupana* Kunth, Fam. Sapindaceae), and other botanicals are used as natural caffeine sources. Willow bark (*Salix* spp., Salicaceae) is used as a natural source of salicylates.

Severe adverse events associated with consumption of dietary supplements that contain *Ephedra* herb and similar to those described above recently have been reported (51–54). This study was undertaken because adverse reactions to these alkaloids are often due to overdose and because questions have been raised regarding the addition of synthetic ephedrine-type alkaloids to some products.

Nonchromatographic (colorimetric, titrimetric, and gravimetric) methods for determining *Ephedra* alkaloids have been published (27, 55–57). Official methods for determining (–)-ephedrine used by AOAC INTERNATIONAL and the United States Pharmacopeia (USP) are nonchromatographic and thus unsuitable for alkaloid mixtures or plant matrixes because they yield quantitative results that fail to distinguish between the naturally occurring alkaloids (58, 59). The AOAC method for alkaloids in *Ephedra* (a titrimetric method; 60) also fails to distinguish among the several alkaloids and was relegated to surplus status in 1965 (58). There is no USP monograph for *Ephedra* (59).

Because current AOAC and USP methods are subject to limits of applicability, additional methods for determining these compounds have been published. The 2 biggest problems in analysis of *Ephedra* products are cleanup and resolution of naturally occurring diastereomeric pairs. Adequate resolution of the 3 ephedrine-type alkaloids used clinically or in illicit drugs has been achieved by liquid chromatography (LC) of raw materials, dosage forms (61–65), or biological fluids (66–72). Methods for separating the alkaloids in plant material have been published (73–77). Ephedrine has been determined in both liquid (78) and tablet (79) dosage forms of TCM by thin-layer chromatography (TLC)/spectrodensitometry. Ephedrine, pseudoephedrine, and strychnine were simultaneously determined in a TCM by LC, but the other ephedrine-type alkaloids were not reported (80). A reversed-phase LC method was used to determine ephedrine, pseudoephedrine, norephedrine, and methylephedrine in 4 *Ephedra* species, but norpseudoephedrine and methylpseudoephedrine were not reported (81). A cyano column with an ion-pair reagent in the mobile phase was used for LC determination of the alkaloids in 12 species of *Ephedra* (82). Pre- or postcolumn derivatization enhances sensitivity of methods for urine (83, 84) and may allow chiral LC separation of enantiomeric mixtures in forensic materials (85) or urine (86). Derivatization efficiencies for alkaloid mixtures have not been reported. Addition of chiral modifiers to the mobile phase (87–89) or use of a specialized detector (90, 91) have permitted resolution of enantiomeric pairs in dosage forms, but separation of complex mixtures of diastereomers and enantiomers has not been reported.

Capillary electrophoresis (CE) was used to determine ephedrine and norephedrine in urine (92) and ephedrine in a pharmaceutical formulation (93). Chiral CE separations of en-

Table 1. Ephedrine-type alkaloids

(1*R*,2*S*)-2-(methylamino)-1-phenylpropan-1-ol or (–)-ephedrine[a] (E)
(1*S*,2*S*)-2-(methylamino)-1-phenylpropan-1-ol or (+)-pseudoephedrine[a] (ψ)
(1*R*,2*S*)-2-(dimethylamino)-1-phenylpropan-1-ol or (–)-*N*-methylephedrine[a] (ME)
(1*S*,2*S*)-2-(dimethylamino)-1-phenylpropan-1-ol or (+)-*N*-methylpseudoephedrine[a] (Me ψ)
(1*R*,2*S*)-2-amino-1-phenyl-1-propanol or (–)-norephedrine[a] (Nor E)
(1*S*,2*S*)-2-amino-1-phenyl-1-propanol or (+)-norpseudoephedrine[a] (Nor ψ)
(1*S*,2*R*)-2-(methylamino)-1-phenylpropan-1-ol or (+)-ephedrine
(1*R*,2*R*)-2-(methylamino)-1-phenylpropan-1-ol or (–)-pseudoephedrine
(1*S*,2*R*)-2-(dimethylamino)-1-phenylpropan-1-ol or (+)-*N*-methylephedrine
(1*R*,2*R*)-2-(dimethylamino)-1-phenylpropan-1-ol or (–)-*N*-methylpseudoephedrine
(1*S*,2*R*)-2-amino-1-phenyl-1-propanol or (+)-norephedrine
(1*R*,2*R*)-2-amino-1-phenyl-1-propanol or (–)-norpseudoephedrine

[a] Naturally occurring.

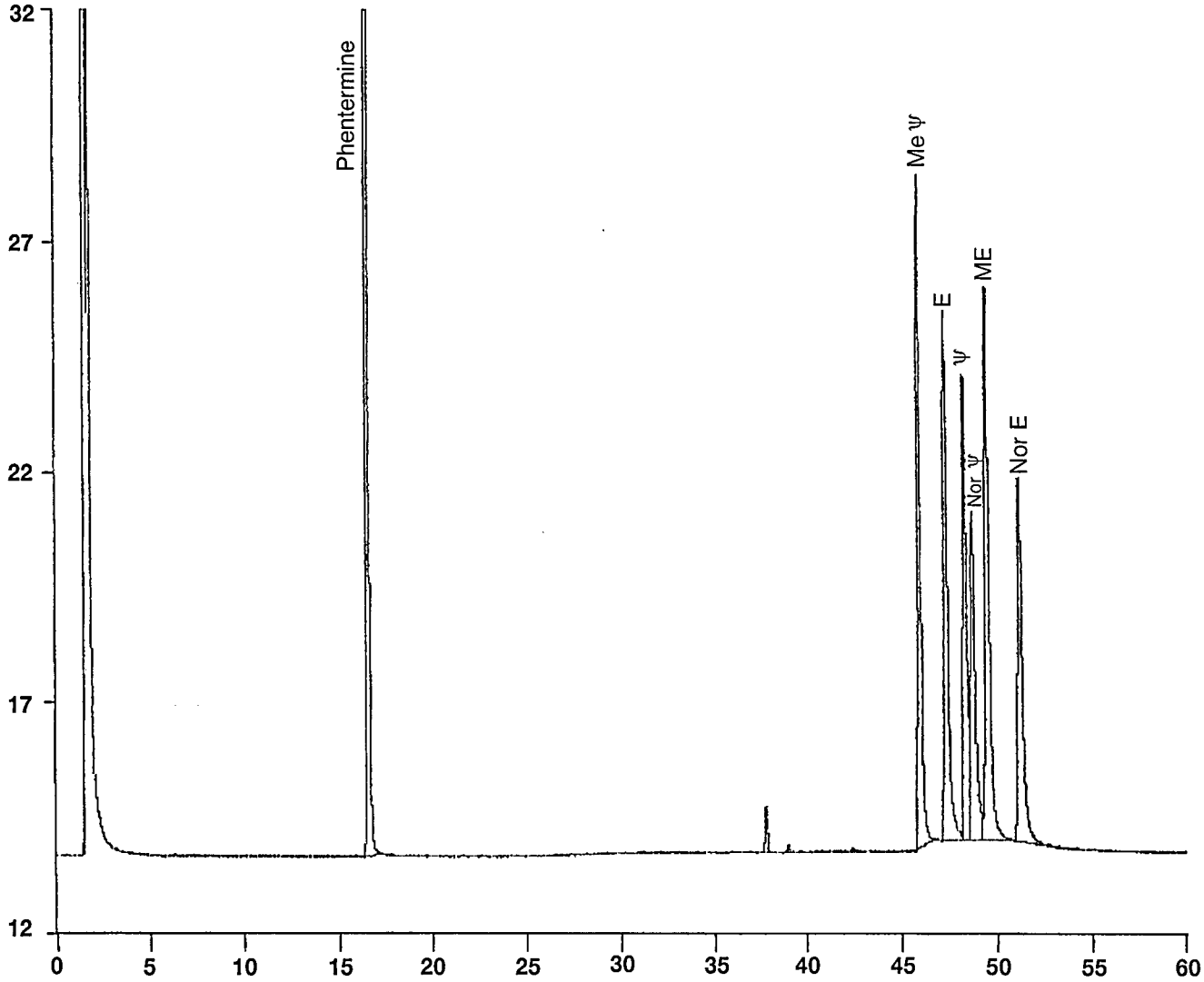

Figure 2. Representative gas chromatogram of mixed ephedrine-type alkaloid standards and phentermine internal standard: (–)-ephedrine (E), (+)-pseudoephedrine (ψ), (–)-*N*-methylephedrine (ME), (–)-norephedrine (Nor E), phentermine = 0.1 mg/mL; (+)-norpseudoephedrine (Nor ψ), (+)-*N*-methylpseudoephedrine (Me ψ) = 0.08 mg/mL. Chromatographic conditions: γ-cyclodextrin capillary column (30 m, 0.25 mm id, 0.25 μm film thickness), injector temperatures = 120°C, FID detector temperature = 220°C, oven temperature program = 100°C (25 min), then 5°C/min to 130°C (25 min); helium carrier flow = 30 cm/s; 3 μL split injection; split ratio of 1:40.

antiomeric mixtures of *Ephedra* alkaloids was achieved by using cyclodextrins as chiral selectors (94–97). CE also was used to determine ephedrine and pseudoephedrine (98) and the 6 natural ephedrine alkaloids in Herba Ephedrae, commercial *Ephedra*-containing products, and in various plant parts (99, 100). A cyclodextrin-modified capillary was used to separate all 12 of the ephedrine-type alkaloids, but a dietary supplement product with a *Má Huáng* extract contained only the natural stereoisomers (101).

GC methods for determining underivatized (102–107) and derivatized (108–112) ephedrine-type alkaloids in biological fluids have been published. Yamasaki et al. (113) used packed-column GC of oxazolidine derivatives to determine ephedrine, pseudoephedrine, and methylephedrine in several Himalayan *Ephedra* species. Cui et al. (114) determined underivatized natural *Ephedra* alkaloids in 12 *Ephedra* species with a 25 m 5% phenylmethylsilicone phase, but we could not reproduce these results in our laboratory. GC methods for chiral separation of enantiomers also have appeared recently (115–117). Methods using derivatized alkaloids allow much better separation of diastereomers, but Wu et al. (118) and Hornbeck et al. (119) found that derivatization of ephedrine with 4-carbethoxyhexafluorobutyryl chloride, heptafluorobutyric anhydride, or *N*-trifluoroacetyl-l-prolyl chloride produce methamphetamine as an artifact. Monoacetate derivatives made with sodium bicarbonate and acetic anhydride do not yield artifacts (120), but derivatization efficiency for mixtures of the alkaloids was not reported.

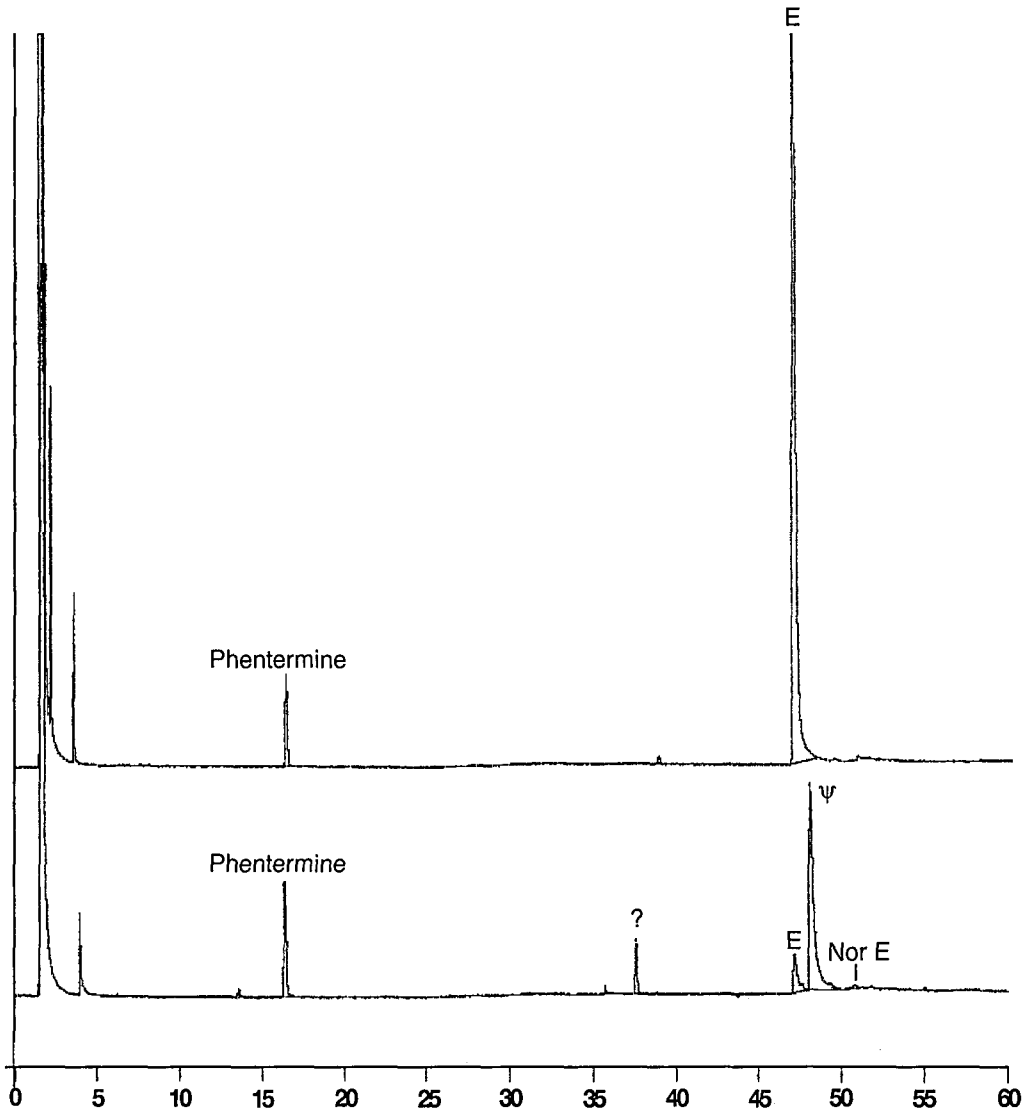

Figure 3. Representative chromatograms of dietary supplements containing *Ephedra* extract: liquid (product I, top) and capsule (product D). Chromatographic conditions are described in the legend to Figure 2.

In this paper, we report methods that allow extraction and determination of enantiomers and diastereomers of ephedrine-type alkaloids in dietary supplement products without derivatization.

Experimental

Materials

Dietary supplements containing *Ephedra* were purchased at retail health food outlets throughout the United States by U.S. Food and Drug Administration (FDA) field investigators during the summer of 1994. Optically pure ephedrine-type alkaloids and phentermine HCl were purchased from Sigma Chemical Co., St. Louis, MO. Phentermine HCl was converted to the free base for use as an internal standard. LC grade water and dichloromethane (CH_2Cl_2) were purchased from Baxter/Burdick and Jackson, Muskegon, MI. Reagent-grade ammonium hydroxide (NH_4OH) (28.0–30.0%) and anhydrous sodium sulfate (Na_2SO_4) were purchased from J.T. Baker, Inc., Phillipsburg, NJ.

Extraction

Approximately 2 g of capsule contents, product powder, or liquid product was weighed in a tared, glass-stoppered Erlenmeyer flask. Concentrated NH_4OH (5 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 150 mL). The organic extracts were combined, filtered through anhydrous Na_2SO_4, and concentrated by rotary evaporation at 35°C. Exactly 1.0 mL of a 1.0 mg/mL CH_2Cl_2 solution of phentermine base was added to the concentrated organic extracts, which were resuspended in 200 mL fresh CH_2Cl_2.

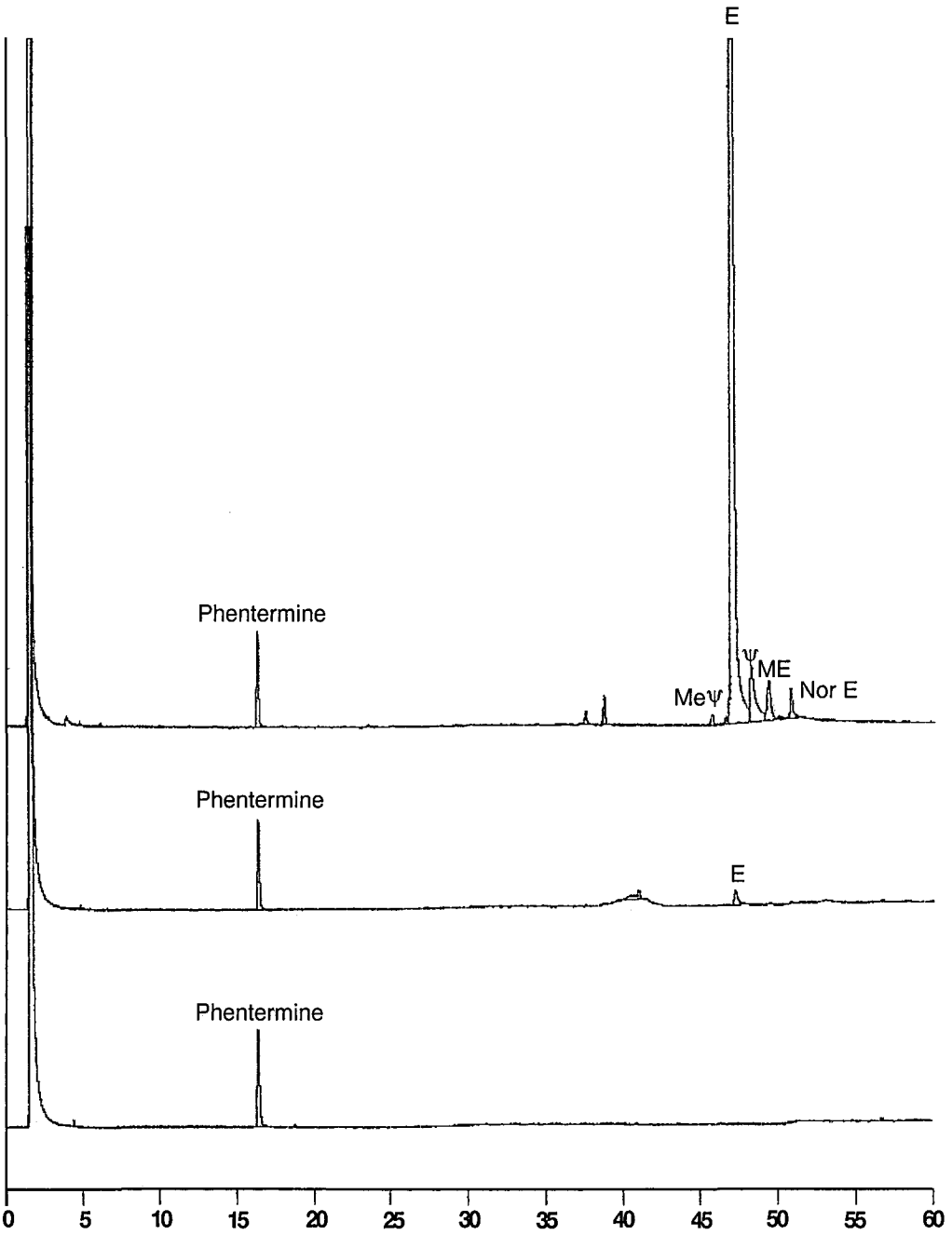

Figure 4. Representative chromatograms of dietary supplement capsule (product G, top) and beverage powder (product F, center) that contain *Ephedra* extract and a tea bag with no ephedrine-type alkaloid source (product C). Chromatographic conditions are described in the legend to Figure 2.

Instruments

A Hewlett-Packard (HP) Model 5890 capillary gas chromatograph equipped with a flame ionization detector (FID) and a Model 7673A autosampler were used for quantitative analysis of the naturally occurring diastereomers. Separations were performed on a Gamma-DEX-120 brand γ-cyclodextrin column (30 m, 0.25 mm id, 0.25 μm film; Supelco, Inc., Supelco Park, Bellefonte, PA). The injector and detector temperatures were 120° and 220°C, respectively. The oven temperature was held at 100°C for 25 min and raised to 130°C at 5°C/min. The final temperature was then held for 25 min. Helium was used as carrier gas at a linear flow velocity of 60 cm/s. Injection volume was 3 μL, with an injector split ratio of 1:40.

Enantiomers were separated by using the same injector and detector parameters. The column oven was held at 90°C for 60 min and then increased to 130°C at 1°C/min. Carrier velocity was 60 cm/s.

A 4-level calibration curve was developed for all standards by using 0.02 mg/mL phentermine free base at all concentrations. Recoveries were determined by spiking the weighed product with known amounts of phentermine, ephedrine, pseudoephedrine, methylephedrine, norephedrine, and methylpseudoephedrine prior to extraction and comparing the alkaloid levels with those of nonspiked product.

GC/MS determinations were performed on a Finnigan/MAT TSQ-46 with an INCOS data system interfaced to a HP 5890 Series II gas chromatograph. Splitless injections were made at an injector temperature of 120°C; the purge valve remained closed for the first 2 min. Chemical ionization (CI) data were acquired in the mass range of 50–350 Da in 1 s/scan. Methane was used as ionizing gas at 4.5×10^{-5} torr source pressure. Resolution was 1000. The carrier gas was helium with a linear velocity of 50 cm/s and vacuum compensation on. The oven temperature was held at 35°C for 2 min and then increased to 130°C at 10°C/min. The final temperature was held for 25 min. The interface temperature was 130°C.

GC/MI/FTIR determinations were performed on a HP Model 5890 gas chromatograph. Helium containing 1.5% argon (Matheson Gas Products, Secaucus, NJ) at ca 27 cm/s linear velocity was used as the carrier gas. The injector temperature was 250°C. The carrier gas mixture was purified with a Hydro-Purge II filter (Alltech Associates, Deerfield, IL) and a heated gas purifier filter (Supelco, Inc.). Split injections of ca 1 μL were used. The initial column oven temperature was 30°C with a 2 min hold and was followed by a 20°C/min increase to 130°C. The oven was held at this temperature until the analysis was complete.

A Sirius Model 100 FTIR spectrometer (Mattson Instruments, Inc., Madison, WI), equipped with an MI Cryolect interface operating at 12K under vacuum, was used to collect IR data. This system has been described in detail (121, 122). MI involved adding argon (1.5% by volume) to the GC carrier gas (helium) and trapping the effluent onto the outer rim of a slowly rotating gold disk (at ca 3 mm/min) held at cryogenic temperatures. During a run, helium was removed by the vacuum pumps, and the analyte molecules surrounded by an excess of argon atoms were frozen into a solid matrix on the gold disk. The infrared-transparent argon matrixes containing the isolated analytes were subsequently analyzed by IR spectroscopy. The position of each analyte peak on the Cryolect collection disk was indexed by its observed GC retention time. Procedures for optimizing the performance of the system and for reproducibly locating a peak maximum on the collection disk were previously described in detail (123). These procedures, which include optical alignment, can minimize the extent of postcolumn peak broadening.

Three hundred analyte interferograms were coadded (2 min 43 s at 4 cm^{-1} resolution), and the background (300 scans) was usually collected before or after the analyte peak.

Results

Figure 2 shows a chromatogram of a mixture of each of the 6 naturally occurring ephedrine-type alkaloid standards. Figures 3 and 4 display representative chromatograms of CH_2Cl_2 extracts of *Má Huáng* and *Ephedra*-containing dietary supplements. Results of the assay for ephedrine-type alkaloids are presented in Table 2. A list of ingredients and directions for use obtained from product labels are presented in Table 3. No ephedrine-type alkaloid sources were listed on the labels of products C and H, and these products appeared to lack the alkaloids. The label of product B lists *Sida cordifolia* L. (Malvaceae) as an ingredient. Ephedrine has been reported in roots and stems of Indian varieties of this plant (124, 125) but was absent in Sri Lankan specimens (126, 127). Ephedrine was not found in the product.

Detection limits (FID) were 0.08 mg/g product for pseudoephedrine and norpseudoephedrine and 0.03 mg/g product for all others. The lower limits of quantitation were 0.25 mg/g for pseudoephedrine and norpseudoephedrine and 0.1 mg/g for all others. Total alkaloid levels ranged from none detected to 55.6 mg/g. Ephedrine predominated in all of the products that contained more than one alkaloid. No norpseudoephedrine was detected in any product, and methylpseudoephedrine was found in only one product. The 4 lots of product B contained only pseudoephedrine, whereas products F and I contained only ephedrine.

Identities of the alkaloids in the diet supplements were established by spiking (GC/FID analysis), by direct comparison of their mass spectra with reference spectra obtained for standard compounds, and by comparison of their infrared spectra

Table 2. Results of quantitative analysis

Product	Alkaloids[a] found, mg/g						
	Me ψ	E	ψ	ME	Nor E	Nor ψ	Total
A_1	—[b]	9.1	6.0	0.76	+[c]	—	15.9
A_2	—	8.8	2.6	3.8	+	—	15.2
A_3	—	7.5	5.6	2.1	+	—	15.2
A_4	—	7.6	5.5	2.0	+	—	15.1
A_5	—	10.4	3.9	1.9	+	—	16.2
A_6	—	9.2	4.1	1.8	+	—	15.1
A_7	—	8.1	2.0	3.0	+	—	13.1
B_1	—	—	2.8	—	—	—	2.8
B_2	—	—	2.8	—	—	—	2.8
B_3	—	—	2.6	—	—	—	2.6
B_4	—	—	2.4	—	—	—	2.4
C	—	—	—	—	—	—	—
D	—	0.5	4.7	—	0.1	—	5.3
E	—	—	—	—	—	—	—
F	—	0.3	—	—	—	—	0.3
G	0.2	38.9	4.4	0.9	0.1	—	44.5
H	—	—	—	—	—	—	—
I	—	55.6	—	—	—	—	55.6

[a] Me ψ = (+)-methylpseudoephedrine; E = (–)-ephedrine; ψ = (+)-pseudoephedrine; ME = (–)-*N*-methylephedrine; Nor E = (–)-norephedrine; Nor ψ = (+)-norpseudoephedrine.

[b] —, not detected. Detection limits: 0.08 mg/g for ψ and Nor ψ; 0.03 mg/g for all others.

[c] +, not quantitated. Quantitation limits: 0.25 mg/g for ψ and Nor ψ; 0.1 mg/g for all others.

Table 3. Product and ingredient key

Product	Label ingredients[a]	Directions for use
A_1	*E*, C, W, $Ch^{P,Pr}$	1–3 capsules 2×/day (500 mg ea)
A_2	*E*, C, W, $Ch^{P,Pr}$	1–3 capsules 2×/day (500 mg ea)
A_3	*E*, C, W, $Ch^{P,Pr}$	1–3 capsules 2×/day (500 mg ea)
A_4	*E*, C, W, $Ch^{P,Pr}$	1–3 capsules 2×/day (500 mg ea)
A_5	*E*, C, W, $Ch^{P,Pr}$	1–3 capsules 2×/day (500 mg ea)
A_6	*E*, C, W, $Ch^{P,Pr}$	1–3 capsules 2×/day (500 mg ea)
A_7	*E*, C, W, $Ch^{P,Pr}$	1–3 capsules 2×/day (500 mg ea)
B_1	Si, *E*, W, B, C, Ch^{N}, G	1–2 capsules 2×/day (500 mg ea)
B_2	Si, *E*, W, B, C, Ch^{N}, G	1–2 capsules 2×/day (500 mg ea)
B_3	Si, *E*, W, B, C, Ch^{N}, G	1–2 capsules 2×/day (500 mg ea)
B_4	Si, *E*, W, B, C, Ch^{N}, G	1–2 capsules 2×/day (500 mg ea)
C	S, M, G, L	Steep 1 tea bag (2 g) for 2–5 min; drink within 10 min of eating
D	*E*, C, W	1–3 D capsules (500 mg ea) with 1 H capsule (500 mg ea) 2×/day
E	*E*, C, W, $Ch^{P,Pr}$	3 tablets (580 mg ea) 2×/day; do not exceed 6/day
F	*E*, Ch^{A}	1 packet of mix (15 g) + 4 oz water 1–3×/day; do not exceed 3 servings/day
G	*E*, C, R	1–2 capsules (500 mg ea) 2×/day; do not exceed 4 capsules/day
H	R, L	1–3 D capsules (500 mg ea) with 1 H capsule (500 mg ea) 2×/day
I	*E*, C, W	7 drops (54 mg drop) 2×/day; do not exceed 50 drops/day

[a] *E* = Ephedra, Epitonin, *Má Huáng*; Si = *Sida cordifolia*; Ch = Chromium: ^{A} = aspartate, ^{P} = picolonate, ^{Pr} = proteinate, ^{N} = nicotionate, R = cascara (*Rhamnus purshiana*); S = senna (*Cassia* spp.); C = caffeine (Maté, kola nut, guarana, etc.); M = mallow (*Malva* spp.); W = willow (*Salix* spp.); B = barberry (*Berberis* spp.) i.e., berberine; G = ginseng; L = licorice.

(obtained by GC/MI/FTIR) with those of reference materials. The CI mass spectral data in Table 4 indicate the difficulty in distinguishing among naturally occurring diastereomers and reemphasizes the importance of achieving a chromatographic separation to positively identify them. MI spectra gave rise to sharp bands that allowed components to be identified with greater confidence, particularly for diastereomers. Differences in spectra of an illustrative diastereomeric pair may be observed in Figure 5, and the unique features of each MI/FTIR spectrum that permit confirmation of identity for each compound are illustrated in the infrared band assignments listed in Table 5. Work on GC/MI/FTIR of ephedrine-type alkaloids with the unnatural configuration is in progress.

Replicate extractions of products spiked with alkaloid standards were used to determine recoveries from the matrix. Recoveries for caplets and capsules were about 75% for ephedrine, 65% for pseudoephedrine, 54% for methylephedrine, 74% for norephedrine, and 82% for methylpseudoephedrine. Recoveries from the liquid product were not determined because matrix effects were not expected. Recovery of phentermine added to the matrix prior to extraction was both low (20–40%) and erratic, probably because the free base is a liquid at room temperature and therefore susceptible to loss during rotary evaporation. Attempts to use this compound as a measure of extraction efficiency were abandoned, and for quantitative analysis, a measured quantity of phentermine was added to the extracted alkaloid residue after the final rotary evaporation step.

The GC conditions developed for determining synthetic enantiomers did not provide sufficient resolution of these compounds from their natural counterparts to allow this method to be used for quantitative analysis. Resolution was sufficient to provide qualitative information about the presence or absence of the synthetic enantiomers in products. No unnatural ephedrine-type alkaloids were found in any of the products examined in this study.

Products were not examined microscopically because all purportedly contained mixed botanicals, *Má Huáng* extract, *Má Huáng* plant material, or both.

Discussion

The ephedrine-type alkaloid content of *Má Huáng* varies from 0.018% (128) to 3.4% (129), with ephedrine itself predominating (10, 130). A good grade of *Má Huáng* contains 1–2% total alkaloid (130, 131). As with many plant secondary metabolites, total alkaloid levels vary considerably, as does the ratio of alkaloids among different species (82, 113, 114, 128, 132–135) and varieties (130). *E. sinica* Stapf contains about 1.25% total alkaloid (about 0.8% ephedrine, 0.3% pseudoephedrine, 0.05% methylephedrine). These levels may be contrasted

Table 4. GC/MS data for natural ephedrine-type alkaloids in *Ephedra*-containing products

Compound	B^+, *m/z*	$M^+ + 1$, *m/z*
Phentermine	58	150
(+)-*N*-Methylpseudoephedrine	134	180
(–)-Ephedrine	58	166
(+)-Pseudoephedrine	58	166
(+)-Norpseudoephedrine	134	152
(–)-*N*-Methylephedrine	134	180
(–)-Norephedrine	134	152

to about 2.1% total alkaloids (1.25% ephedrine, 0.6% pseudoephedrine, 0.04% methylephedrine) in *E. equisetina* Bunge, 1.3% total (0.3% ephedrine, 0.9% pseudoephedrine, 0.01% methylephedrine) in *E. intermedia* Schr. et Mey., and 1.4% total (1.1% ephedrine, 0.1% pseudoephedrine, 0.2% methylephedrine) in *E. intermedia* v. *tibetica* Stapf (82, 134).

Other factors that affect alkaloid content are plant part (130–135), sex (134), season of harvest (132, 134), and geographical origin (82, 113). No ephedrine-type alkaloids have been reported in the roots, berries, or seeds of these plants (130–135).

Table 6 summarizes reported variations in alkaloid content of a number of *Ephedra* species. Comparison of these values with those presented in Table 2 indicates that alkaloid levels for most of the products examined do not fall within ranges previously reported for *Ephedra* species containing ephedrine-type alkaloids. Product A (all lots) contained total alkaloid levels and ratios of individual alkaloids that fell within the range of natural variation to be expected of *E. sinica* or *E. equisetina*. The alkaloid pattern found in product D is consistent with that reported in *E. intermedia*, although total alkaloid level is well below that previously reported. Products B, F, and I contain only a single ephedrine-type alkaloid and most likely do not contain any *Ephedra*. Product G contains all of the expected alkaloids except norephedrine, but it contains a disproportionately high amount of ephedrine. It is possible that this compound has been added to the *Ephedra* extract used in the product. Labels of products C and H indicated that they did not contain any *Ephedra*, and this was confirmed by the absence of ephedrine-type alkaloids in the products. The labels of all of the products examined listed "Ephedra extract" (or some variant thereof) as the ephedrine-type alkaloid source. All the literature pertaining to alkaloid patterns and levels referred to plant material rather than extracts. Although the composition of a good-quality extract should approximate the composition of the plant in alkaloid pattern, some small variation would be expected. This appears to be the case with products A and D. Product G contained the expected *Ephedra* alkaloid pattern, but the proportion of ephedrine to the other alkaloids was not within the range previously reported for *Má Huáng*. As noted earlier, total alkaloid level in product D is lower than that reported for plant material. Because the product label indicated that the ephedrine-type alkaloid source was *Ephedra* extract, this discrepancy

Table 5. Observed matrix isolation Fourier transform infrared bands (cm^{-1})[a]

Proposed assignment	E	ψ	ME	Me ψ	Nor E	Nor ψ
O–H str./N–H str.	3468	3331	3446	3389	3504	3464
=C–H str. (aromatic)	3073	3070	3073	3074	3074	3074
	3036	3035	3034	3038	3038	3037
Asymmetric C–H str. (aliphatic)	2989		2995	2985		
	2970	2977	2971	2952	2975	2979
	2938	2940	2940		2939	2936
Symmetric C–H str. (aliphatic)	2859	2855	2877	2875	2884	2879
			2834	2839	2848	
	2807	2800	2792	2793		
Overtone bands for monosubstituted ring	1950	1951	1950	1948	1952	1950
	1880	1885	1882	1880	1881	1895
	1811	1814	1812	1810	1812	1811
	1759	1760	1759	1759	1760	1760
NH_2 def.					1620	1620
C=C str.	1608		1608			1608
	1497	1493	1497	1497	1497	1496
	1482	1481		1466		
	1455	1452	1455	1456	1455	1455
O–H def.	1405	1406	1407	1401	1406	1409
Symmetric CH_3 bend	1383	1381	1385	1376		
C–O str.	1123	1123	1120	1130	1120	1130
	1101	1095	1102	1096	1107	
Ring H rocking vibration	1071	1070	1068	1077	1084	1082
	1048	1049	1040	1061	1046	1054
	1030	1028	1003	1043	1026	1029
	994	992	964	954	993	997
NH_2 wag					807	813
Ring substitution: 5 adjacent H	752	745	745	750	743	756
	735					
	701	702	701	700	702	702

[a] E, (–)-ephedrine; ψ, (+)-pseudoephedrine; ME, (–)-*N*-methylephedrine; Me ψ, (+)-*N*-methylpseudoephedrine; Nor E, (–)-norephedrine; Nor ψ, (+)-norpseudoephedrine.

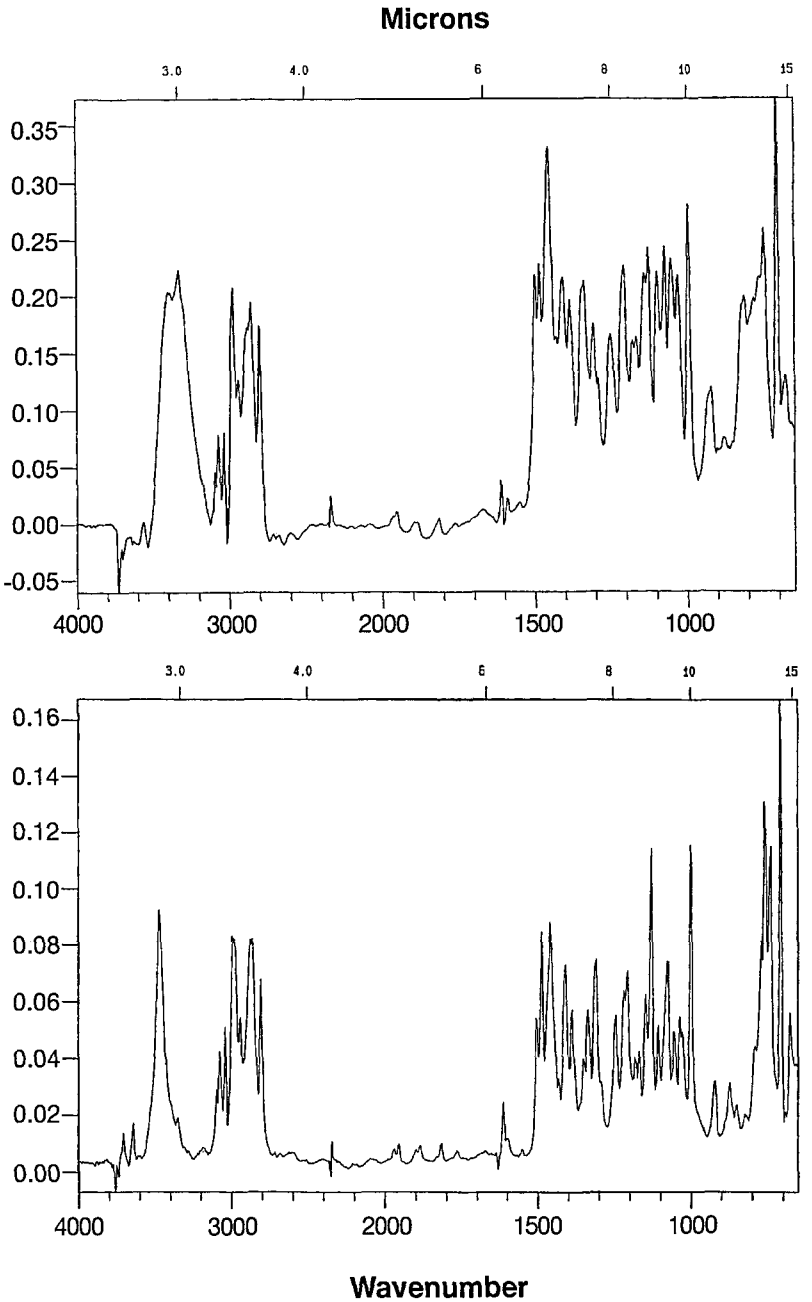

Figure 5. MI/FTIR spectra observed at 4 cm^{-1} resolution for pseudoephedrine (top) and ephedrine.

is probably intentional. Product A contains *Ephedra* extract at a total alkaloid level that approximates that found in the plant.

The natural variability in alkaloid content makes it difficult to ascertain the exact botanical source of the alkaloids in these products, especially because the identity of products that contain *Ephedra* extracts (rather than plant material) cannot be verified microscopically. Although pure (–)-ephedrine, PPA, and (+)-pseudoephedrine are relatively inexpensive, other natural alkaloids are not. Products that contain a full (or almost full) complement of the alkaloids are thus more likely to be derived from the plant. The origin of products that contain only a single alkaloid (B_{1-4}, I), no alkaloid at all (C, H), or excessive levels of a single alkaloid may well be open to question. Synthetic ephedrine and pseudoephedrine enantiomers, which do not occur in nature, were not found in any of the products examined. However, this does not eliminate the possibility that pure natural isomers were added to such products. More sophisticated techniques, such as determination of isotope ratios may be required to discover such addition.

Results of this study did little to address the issue of the safety of the products examined. The usual oral adult doses for nasal decongestion and bronchodilation are 30–60 mg pseudoephedrine HCl every 6 h, not to exceed 240 mg/day; 25 mg PPA HCl every 4 h, not to exceed 150 mg/day; and 25–

50 mg ephedrine sulfate every 3–4 h, not to exceed 150 mg/day. Ephedrine HCl is used topically intranasally as a 0.5–3% solution (2–3 drops every 4 h) or a 0.6% jelly (a small amount every 4 h). Phenylpropanolamine is also used as an appetite suppressant at 25 mg 3 times a day, 1/2 h before meals (13). The most common cause of adverse reactions to these alkaloids is overdose, although a number of severe non-dose-dependent reactions have been reported (13, 14–26). Several products (B_{1-4}, C, D, E, F, H) probably do not contain enough ephedrine-type alkaloids to cause dose-dependent problems (when used as directed), except in susceptible or predisposed individuals. When used at the maximum directed levels, quantities of both ephedrine and total alkaloid in products A_{1-7} and I fall within the lower (over-the-counter or OTC) range of single recommended doses for the free base in pharmaceutical products (about 19–39 mg). Daily intake of both ephedrine and total ephedrine-type alkaloids in product A_{1-7} fall well below the recommended maximum daily dose for ephedrine free base (116 mg). If used as directed on the label (7 drops 2 times daily), the daily intake of product I would result in an intake of ephedrine that is well below the suggested maximum daily dose for the free base. Ingestion of the maximum recommended daily amount of I (50 drops) would deliver 149 mg ephedrine/day, which is above the maximum daily dose for pharmaceutical ephedrine products. Product G would deliver ephedrine itself at the high (i.e., prescription) end of the recommended single dose range, and total ephedrine-type alkaloids in the product would exceed this level. The recommended maximum daily intake of product G would provide a dose of ephedrine that is less than that for pharmaceutical products.

In addition to concerns over the safety of the ephedrine-type alkaloids, *Ephedra* species have been reported to contain *N*-methylbenzylamine (136), ephedralone (137), ephedroxane (138), and high levels of tannin (139). Stems of many *Ephedra* species also contain substantial amounts of 6-hydroxykynurenic acid (140, 141). Two glutamate analogs and the cyclopropane amino acid *cis*-3,4-methano-L-proline have been isolated from several of the alkaloid-producing European *Ephedra* species (141), and roots of *Ephedra* contain a number of spermine-type alkaloids (142). Although the pharmacology of most of the pure single ephedrine-type alkaloids has been well characterized, the effects of combinations of these other compounds are less well known. In addition, interactions between ephedrine-type alkaloids and xanthine alkaloids, as well as biologically active compounds in other plant species that are constituents of many dietary supplements have yet to be examined (27), although adverse reactions to ephedrine-type alkaloid/caffeine combinations have been reported (23, 24). Also lacking are data on the interactions between the ephedrine-type alkaloids and glutamate analogs and non-ephedrine-type alkaloids of *Ephedra*. This latter point is especially important because the products examined in the present study contained an undefined entity called *Ephedra* (or *Má Huáng*) extract rather than *Ephedra* itself. Because the nature of the extraction process is unknown, its effect on the levels of glutamate analogs, 6-hydroxykynurenic acid, and the non-ephedrine-type alkaloids in the diet supplements is also unknown.

Conclusions

Analytical methods to determine ephedrine-type alkaloids in pharmaceutical dosage forms are not adequate for resolution and quantitation of these compounds in botanical dietary supplements, because the presence of compounds in the botanical matrix interferes with the resolution of these compounds. Clinical methods (analysis of serum, urine) that require some sort of analyte isolation largely eliminate the problem of interfering substances, but the demands placed on the resolving power of these methods are lower because they have been developed for the purpose of separating a few relatively dissimilar compounds. Potential adulteration of *Ephedra* products with inexpensive (i.e., racemic) synthetic alkaloid(s) poses another challenge to the analysis of supplements containing botanical materials. Unfortunately, most of the chiral chromatographic

Table 6. Previously reported mean alkaloid levels in *Ephedra* species[a]

Ephedra species	Alkaloid level, mg/g						
	Me ψ	E	ψ	ME	Nor E	Nor ψ	Total
sinica	0.04	6.9	2.0	0.7	0.5	0.9	11.1
equisetina	tr	12.5	5.4	0.4	1.4	2.7	22.5
intermedia	0.03	2.7	9.0	0.1	0.5	1.1	13.5
intermedia v. *tibetica*	tr	11.1	0.8	1.6	0.4	0.3	14.1
likiangensis	0.05	7.0	6.5	0.3	0.6	1.6	16.1
monosperma	0.03	13.2	8.2	0.5	1.7	2.9	26.5
minuta	tr	5.6	2.3	0.4	0.6	0.5	9.4
gerardiana	0.08	7.3	1.2	0.4	0.8	0.8	10.6
saxatitis	tr	6.0	0.6	0.6	0.6	0.3	8.0
lomatolepis	tr	1.6	7.8	0.03	0.4	2.9	12.7
lepidosperma	tr	0.2	0.1	0.01	0.06	0.09	0.5
przewalskii	0.0	0.3	0.2	0.02	0.07	0.1	0.7

[a] Alkaloids are as defined in previous tables. Data are averages of values from Zhang et al. (82), Yamasaki et al. (113), and Cui et al. (114); tr = trace.

methods were designed to resolve enantiomers and will not resolve both diastereomers and enantiomers. The method used in the present study has addressed these problems and has proven its use in the analysis of these products.

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