Ephedrine-Type Alkaloid Content of Nutritional Supplements Containing *Ephedra sinica* (Ma-huang) As Determined by High Performance Liquid Chromatography

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Abstract ☐ Nutritional supplements containing *Ephedra sinica* (ma-huang), a botanical source of ephedrine-type alkaloids, have been linked to numerous episodes of ephedrine (EPH) toxicity. With passage of the 1994 Dietary Supplement Health and Education Act, nutritional supplements are no longer subject to the same FDA preapproval requirements as food additives, prescription, or nonprescription medications. As a consequence, EPH content is not a label requirement for *Ephedra*-containing supplements. Less stringent labeling requirements, therefore, may contribute to toxicity associated with these products. A validated HPLC method for the determination of ephedrine-type alkaloids, commonly found in *Ephedra* supplements, is presented. Nine commercially available supplements exhibited considerable variability in alkaloid content (EPH range: 1.08–13.54 mg). Only three products listed EPH content on the label while one exhibited lot to lot variations in EPH of 137%.

Introduction

The plant genus *Ephedra*, known also by its Chinese name "ma-huang," is a botanical source of ephedrine alkaloids commonly found in products marketed as "natural stimulants" or thermogenic diet aids. As part of the rapidly growing market in "herbal medicines"—a market projected to exceed $2 billion in annual sales for 1998—nutritional supplements containing ma-huang are especially popular among consumers.[1] Much of this popularity stems from the stimulant effects associated with *Ephedra* alkaloids. These alkaloids include ephedrine (EPH) and pseudoephedrine (PSE) which are the most abundant in *Ephedra sinica*, as well as methylephedrine (MEPH), norpseudoephedrine (NPSE), and norephedrine (NEPH).[2]

Ephedrine alkaloids are sympathetic agonists which when ingested in low to moderate doses produce tachycardia, vasoconstriction, transient hypertension, bronchodilation, nervousness, insomnia, appetite suppression, and headache. While these effects are usually harmless, they can be severe in persons with underlying heart disease, hypertension, diabetes, and hyperthyroidism, as well as those individuals taking prescription medications or exhibiting a sensitivity to EPH. Nutritional supplements containing *Ephedra* have been linked to several deaths and over 800 adverse events including heart attacks, hepatitis, psychosis, seizure, and stroke.[3–7]

In 1994 Congress passed the Dietary Supplement Health and Education Act (DSHEA) which defined dietary supplements as being distinct from drugs or food additives.[8] Along with the new definition came other reforms which dealt with supplement labeling issues, claims of nutritional support, and a shift in the burden of proof from manufacturer to the Food and Drug Administration (FDA) with respect to product safety.[9] In short, the DSHEA cleared the way for companies "to market products that do not meet the strict, established definitions of a food or drug" and, in so doing, shifted the FDA's procedure for regulation "from one of preclearance to one of policing".[10] Botanical EPH supplements, therefore, are not required to the meet same FDA standards for premarket approval as prescription or over-the-counter products containing EPH. As a result, manufacturers of *Ephedra*-containing supplements are not required to make a label claim of EPH content. Nevertheless, without knowledge of the presence and/or quantity of EPH in each supplement, consumers may unknowingly face a greater risk of potential overdose.

Surprisingly, only two groups have reported on the quantity of ephedrine-type alkaloids found in commercially available dietary supplements containing ma-huang in the United States. Betz et al.[11] recently determined the alkaloid content of nine ma-huang supplements using a chiral gas chromatographic method, while Flurer et al.[12] examined three *Ephedra*-containing supplements via capillary electrophoresis. One drawback, however, to both these methods was that elution[11] and migration[12] times were greater than 40 min. Other groups have described capillary electrophoretic[13,14] as well as liquid[15,16] and gas chromatographic[17,18] methods for quantitating *Ephedra* alkaloids with shorter elution times, but these have been restricted to the analysis of crude herb preparations.

In this report we describe a liquid chromatographic method for the determination of EPH, MEPH, NPSE, NEPH, and PSE in nutritional supplements containing ma-huang. Our method differs from those reported earlier with regard to column type, mobile phase composition, column temperature, and application. Nine nutritional supplements labeled to contain *E. sinica* in combination with other botanicals and vitamins were examined. To our knowledge, this is the first study of its kind to quantify the five principal ephedrine alkaloids in commercially available ma-huang dietary supplements using high performance liquid chromatography.

Experimental Section

Materials—Ephedrine hydrochloride, pseudoephedrine hydrochloride, norephedrine hydrochloride, methylephedrine, and sodium lauryl sulfate were purchased from Sigma (St. Louis, MO). Norpseudoephedrine hydrochloride and *d*-amphetamine sulfate (AMP) were purchased from Research Biochemicals International (Natick, MA). The following ma-huang-containing products were

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Table 1—Additional Ingredients Contained in *Ephedra* (ma-huang) Supplements

botanicals	*Camellia sinensis*, *Capsicum frutescens*, *Centella asiatica*, *Cola acuminata*, *Cola nitida*, Ginko biloba, *Gymnema sylvestra*, *Hypercium perforatum*, *Myristica fragrans*, *Panax gensing*, *Paullinia cupana*, *Polygonum multiflorum*, *Spirulina pratensis*, *Triticum aestivum*, bromelian, dandelion, fennel, ginger, kelp, passion flower, pullulan, schizandra, tumeric
vitamins	cyancobalamin, *d*-calcium pantothenate, niacinamide, pyridoxine
miscellaneous	acetyl L-carnitine, arginate/chelidamate, calcium succinate, choline bitartrate, chromium picolinate, chromiun polynicotinate, croscarmellose sodium, dicalcium phosphate, inositol, L-phenylalanine, magnesium stearate, magnesium succinate, microcrystalline cellulose, potassium chloride, stearic acid, silica

purchased either from local retailers or via the Internet: "Product A" (Diet Pep, Pep Products, Inc., Castle Rock, CO); "Product B" (Diet Phen, Source Naturals, Inc., Scott's Valley, CA); "Product C" (Energel, General Nutrition Corp., Pittsburgh, PA); "Product D" (Ephedra, Solaray, Inc., Ogden, UT); "Product E" (Escalation, Enzymatic Therapy, Green Bay, WS, USA); "Product F" (Excel, Excel Corp., Salt Lake City, UT); "Product G" (Herbal Ecstacy, Global World Media Corp., Venice, CA); "Product H" (Herbal Phen-Fen, HPF L. L. C., Horsham, PA); "Product I" (Up Your Gas, National Health Products, Orlando, FL). HPLC grade acetonitrile and tetrahydrofuran were purchased from Burdick and Jackson (Muskegon, MI). Deionized water was obtained from a Milli-Q Plus ultrapure water system purchased from Millipore (Bedford, MA). Unprocessed *Ephedra lepidosperma* was the kind gift of Dr. Chen Hu-biao at Beijing Medical University's School of Pharmacy (Beijing, China).

Instrumentation and Chromatographic Conditions—A component HPLC system (Shimadzu Scientific Instruments, Columbia, MD) consisted of a LC-600 solvent delivery system, a Model SIL-9A autoinjector, and a Model SPD-6A UV absorbance detector operated at 208 nm. A prepacked, 25 cm × 4.6 mm (5 μm particle size) base-deactivated C-18 HPLC column and guard column (Alltima, Alltech Associates, Inc., Deerfield, IL) were operated with a mobile phase consisting of acetonitrile, tetrahydrofuran, and water (38:5:57, v/v/v). Sodium lauryl sulfate, an ion-pairing agent, was added to the mobile phase to achieve a final concentration of 5 mM. The mobile phase was continuously sparged with helium and delivered at a flow-rate of 0.7 mL/min. Column temperature was maintained at 37 °C with a Model CTO-6A column oven (Shimadzu). Detector output was recorded, and chromatograms were analyzed by a CR5-A Chromatopac recorder/integrator (Shimadzu).

Standard Solutions and Sample Preparation—The hydrochloride salts of EPH, PSE, NEPH, and NPSE as well as MEPH (free base) were dissolved in methanol to yield a 1 mg mL^{-1} stock solution of the free bases. An AMP (internal standard) spiking solution of 5 μg mL^{-1} was also prepared in methanol. Methanolic stock solutions were stored at −70 °C and were stable for over 6 months. Standard solutions covering the concentration range of 400, 200, 100, 50, 25, 12.5, and 6.25 μg mL^{-1} were prepared daily in clean borosilicate glass conical tubes by serial dilution with mobile phase.

Fifteen samples from two separate lots of each commercial product were analyzed. Individual dosage forms of each product (tablets, hard gelatin capsules, or softgel capsules) were weighed. Each tablet was then pulverized in a mortar and pestle, the contents scrupulously recovered, and the material weighed again. Hard gelatin capsules were emptied and the contents weighed. Softgel capsules were left intact. The extraction of EPH alkaloids from each dosage form was accomplished using a modified version of the method of Sagara et al.[11] Materials recovered from single tablets and hard gelatin capsules or individual softgel capsules were placed in separate round-bottomed reflux flasks. Thirty milliliters of mobile phase were added to each flask and the contents refluxed at 80 °C for 30 min in a circulating water bath. This mixture was transferred to glass conical centrifuge tubes and centrifuged for 10 min at 36 g. Each supernate was decanted into 50 mL volumetric flasks. The residue remaining in the centrifuge tube was washed with two additional 10 mL volumes of mobile phase, and the contents were vortex-mixed for 2 min. These washings were centrifuged and the supernates added to their respective volumetric flasks. Each flask was then diluted to 50 mL with mobile phase.

A 500 μL aliquot from each flask and each standard curve sample was transferred to a 1.5 mL disposable polypropylene microcentrifuge tube (Brinkman, Westbury, NY), spiked with 25 μL of AMP, and vortex-mixed for 20 s. Using an Eppendorf Model 5414 microcentrifuge (Brinkman, Westbury, NY), tubes were centrifuged at 12000 rpm for 5 min. Aliquots (250 μL) were then transferred to autoinjector vials, and a 25 μL volume was injected onto the column.

Method Validation—Plots of peak area ratio (i.e. EPH/AMP) versus spiked concentration were used for quantitative computations. Calibration curves were calculated by weighted (1/concentrated) least squares linear regression analysis using a commercial software package (DeltaGraph, Monterey, CA). Accuracy and precision of the method were determined by replicate analysis of four known concentrations equally divided over the calibration curve. Interday and intraday accuracy were expressed as percentage deviation from the spiked value using the following equation:

$$\%\ \text{error} = \frac{(C_{\text{mean obs}} - C_{\text{spiked}})}{C_{\text{spiked}}} \times 100$$

where $C_{\text{mean obs}}$ is the mean observed concentration for each standard and C_{spiked} is the theoretical spiked concentration. The lower limit of quantitation was defined as the concentration of the lowest standard which was quantitated with a definite level of certainty (precision <10%) at a recorder attenuation of 8 (0.64 AUFS, absorbance units full scale).

The amount of EPH, PSE, NEPH, NPSE, and MEPH in each dosage form was determined as follows:

$$\text{concentration}_{\mu\text{g mL}} \times 50\ \text{mL} = \text{total}_{\mu\text{g}}\ \text{amount per dosage form}$$

To confirm that the chromatographic peaks of interest were due only to ephedrine-type alkaloids, 500 mg of powdered *E. lepidosperma*, a species reported to be devoid of alkaloids, was extracted and analyzed as described above. The effect of extraction reflux temperature (80 °C) on alkaloid stability was determined by comparing peak areas of spiked samples subjected to reflux and those prepared at room temperature. The percentage recovery was determined from the analysis of *E. lepidosperma* samples (500 mg) spiked with known amounts (10 and 2 mg) of EPH, PSE, NEPH, and MEPH. To further establish that ephedrine-type alkaloids were sufficiently recovered by this procedure, known amounts (5 mg) of AMP, a compound structurally similar to the *Ephedra* alkaloids and not present in ma-huang, were added to the contents of each product and extracted. Amphetamine recovery was determined from standard curves of known AMP concentrations (200, 100, 50, 25, and 12.5 μg/mL), and plots of AMP peak area versus spiked concentration were used for quantitative computations.

Except for product D, all products contained ma-huang in combination with other ingredients (amino acids, botanicals, excipients, vitamins, etc.). Samples of these additional ingredients (listed in Table 1) were extracted separately and evaluated for chromatographic interference with the five alkaloids of interest.

Results

Chromatograms for *E. lepidosperma* and *E. lepidosperma* spiked with AMP, EPH, MEPH, NPSE, NEPH, and PSE are shown in Figure 1. No interfering peaks were noted for *E. lepidosperma*, and good resolution was achieved among all the alkaloids. Retention times for NPSE, NEPH, PSE, EPH, MEPH, and AMP were approximately 14.9, 15.5, 16.3, 17.3, 18.5, and 20.9 min, respectively. With respect to the internal standard, relative retention times

Table 2—Linearity Data (mean ± sd, $n = 10$) for Standard Curves of Each *Ephedra* Alkaloid

	EPH	MEPH	NPSE	NEPH	PSE
slope	0.01102 ± 0.0002	0.01250 ± 0.0002	0.01228 ± 0.0002	0.01152 ± 0.0002	0.01109 ± 0.0002
intercept	−0.00146 ± 0.0041	−0.0027 ± 0.0049	−0.00081 ± 0.0011	−0.00012 ± 0.0038	−0.00087 ± 0.0040
R^2	0.9992 ± 0.0005	0.9992 ± 0.0005	0.9993 ± 0.0004	0.9990 ± 0.0007	0.9991 ± 0.0006
R^2 range	(0.9985–0.9998)	(0.9984–0.9997)	(0.9989–0.9998)	(0.9981–0.9997)	(0.9983–0.9998)

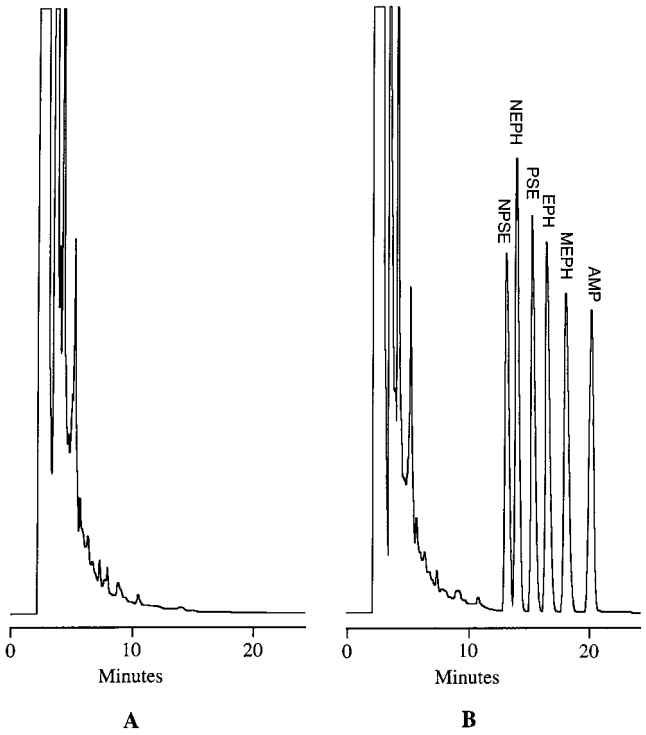

Figure 1—(A) Chromatogram for *E. lepidosperma* extract. (*E. lepidosperma* is a species devoid of ephedrine alkaloids.) (B) Chromatogram for *E. lepidosperma* extract spiked with norpseudoephedrine (NPSE), norephedrine (NEPH), pseudoephedrine (PSE), ephedrine (EPH), and methylephedrine (MEPH) to yield a concentration of 50 μg/mL (0.64 AUFS). Internal standard was *d*-amphetamine (AMP). AUFS = absorbance units full scale.

Table 3—Percentage Recovery of *Ephedra* Alkaloids

amt added (mg)		amt determined (mg)			
		NEPH	PSE	EPH	MEPH
2	mean	1.84	1.95	1.99	1.93
	(sd)	(0.17)	(0.05)	(0.04)	(0.10)
	% recovery	92	97.5	99.5	96.5
10	mean	9.98	9.91	9.80	9.71
	(sd)	(0.10)	(0.14)	(0.13)	(0.20)
	% recovery	99.8	99.1	98.0	97.1

for each alkaloid were 0.71, 0.74, 0.78, 0.83, and 0.89, respectively. When extracted separately, no chromatographic interference was noted for any of the additional ingredients listed in Table 1.

Standard curves were linear over the concentration range of 6.25 to 400 μg mL^{-1} and weighted linear regression analysis of peak area ratios vs spiked concentrations resulted in correlation coefficients greater than 0.999 (see Table 2). The percentage recovery of *E. lepidosperma* samples spiked with 2 and 10 mg of EPH, MEPH, NEPH, and PSE are presented in Table 3. The mean recovery for all alkaloids exceeded 97% (range = 92−99.8%). Because of its status as a controlled substance (class IV), recovery for NPSE was not determined because of the difficulty and expense in obtaining sufficient quantities for such determinations; however, based on results for the other alkaloids, its recovery would, in all likelihood, have exceeded 95%.

Standard curves based on AMP peak areas were linear over the concentration range of 12.5 to 200 μg mL^{-1} and weighted linear regression analysis of peak area vs spiked concentrations resulted in correlation coefficients greater than 0.999. Mean recovery of AMP from each product exceeded 93% (range = 93.2−97.8%, Table 4), indicating that various formulation matrices had little effect on the extraction of *Ephedra* alkaloids.

Intraday and interday accuracy and precision of the method is presented in Tables 5 and 6. Data from both tables indicate that the method was accurate and precise. On the basis of results presented in Tables 5 and 6, 6.25 μg mL^{-1} was taken as the limit of quantitation. Concentrations below 6.25 μg mL^{-1} were easily measured using lower attentuation settings, but an attenuation of 8 (AUFS = 0.64) proved to be adequate for quantitating EPH alkaloids in the nine supplements.

Representative chromatograms for each product tested are shown in Figure 2. *Ephedra* alkaloid content for each product is presented in Table 7. All supplements contained EPH; however, the quantity varied greatly, with product C containing the least amount (1.08 mg) and product E the most (13.54 mg). Pseudoephedrine, the second most abundant alkaloid, was present in seven of the products and ranged in content from 0.52 mg (product B) to 9.46 mg (product F). Norephedrine was the least prevalent with quantities consistently below 0.25 mg. Four products (D−G) contained measurable quantities of all five alkaloids.

Except for product G, quantities of *Ephedra* alkaloids in each supplement were consistent among the two lots examined. Separate lots of product G (designated "a" and "b"), however, differed significantly in both type and quantity of *Ephedra* alkaloids (see Figure 3 and Table 7). "Lot a" contained EPH, MEPH, NPSE, NEPH, and PSE while "lot b" contained only EPH and PSE. Furthermore, the EPH content of "lot b" exceeded that found in "lot a" by 137%. Except for lot numbers on the label, there was no discernible difference in the appearance of tablets from either lot of product G.

Table 4—Percentage Recovery of AMP from *Ephedra* Supplements ($n = 5$)

amt added (mg)		amt determined (mg)						
		product A	product B	product C	product E	product G	product H	product I
5.0	mean	4.74	4.73	4.94	4.80	4.66	4.70	4.89
	(sd)	(0.12)	(0.07)	(0.24)	(0.07)	(0.08)	(0.09)	(0.20)
	% recovery	94.8	94.6	98.8	96	93.2	94	97.8

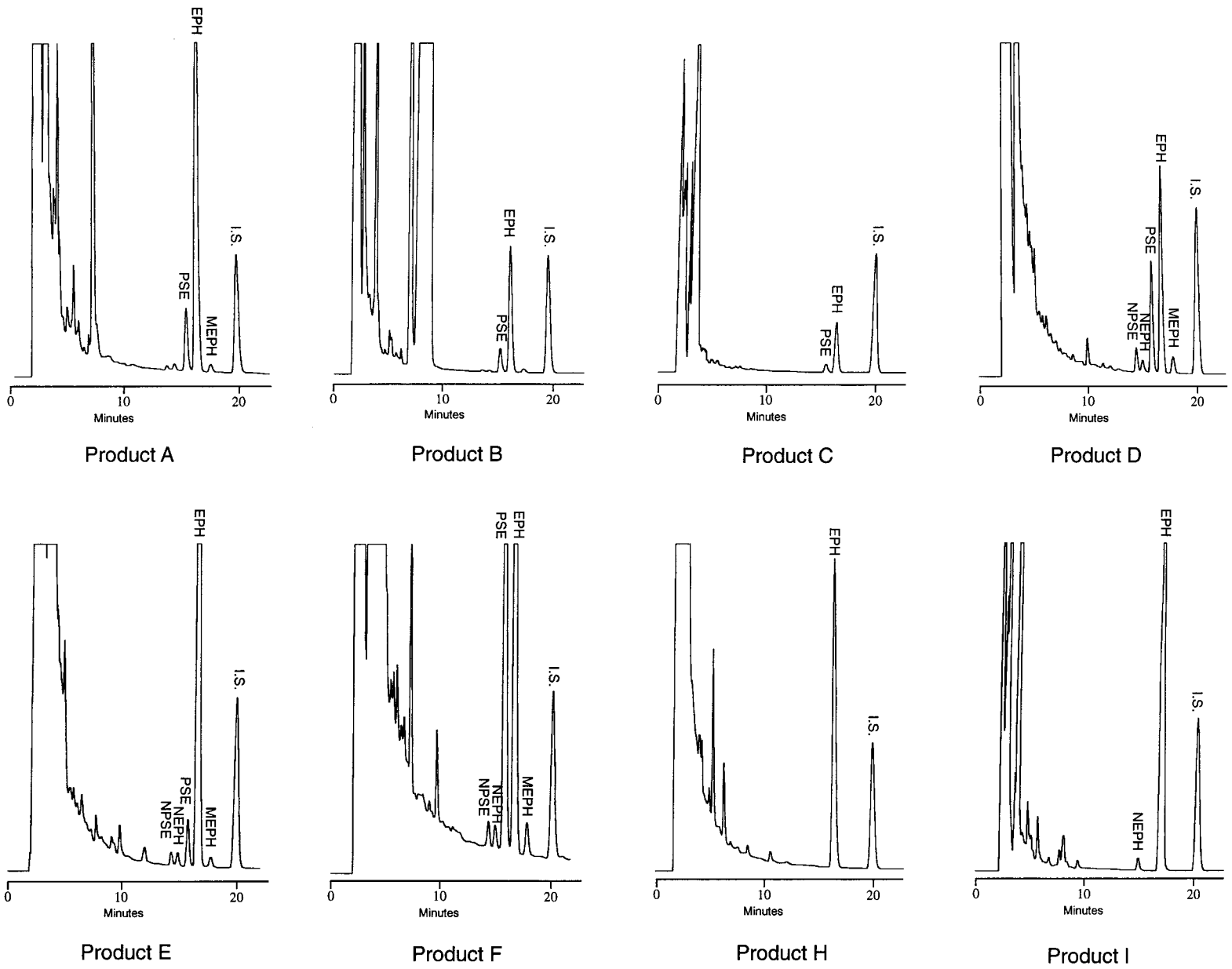

Figure 2—Representative chromatograms of extracts from several commercially available nutritional supplements containing ma-huang (AUFS = 0.64). EPH = ephedrine, I.S. = internal standard (*d*-amphetamine), MEPH = methylephedrine, NPSE = norpseudoephedrine, NEPH = norephedrine, PSE = pseudoephedrine, AUFS = absorbance units full scale.

Table 5—Intraday Accuracy and Precision of *Ephedra* Alkaloids ($n = 6$)

concn added (μg/mL)		concn determined (μg/mL)				
		NPSE	NEPH	PSE	EPH	MEPH
400	mean ± sd	398 ± 7.0	396 ± 6.5	396 ± 6.5	397 ± 6.6	399 ± 6.5
	RSD (%)	1.8	1.6	1.6	1.7	1.6
	% error	–0.5	–1.0	–1.0	–0.8	–0.3
100	mean ± sd	104 ± 3.9	107 ± 4.4	107 ± 4.2	107 ± 4.5	107 ± 4.3
	RSD (%)	3.8	4.1	3.9	4.5	4.3
	% error	+4.0	+7.0	+7.0	+7.0	+7.0
25	mean ± sd	25.7 ± 1.2	27.5 ± 1.0	26.9 ± 0.8	26.8 ± 0.8	26.8 ± 1.8
	RSD (%)	4.7	3.8	3.1	2.9	6.7
	% error	+2.8	+10.0	+7.6	+7.2	+7.2
6.25	mean ± sd	6.18 ± 0.4	6.0 ± 0.2	6.23 ± 0.3	6.34 ± 0.3	6.55 ± 0.2
	RSD (%)	6.5	3.0	4.5	4.4	3.7
	% error	–1.1	–4.2	–0.3	+1.4	+4.8

Table 6—Interday Accuracy and Precision of *Ephedra* Alkaloids ($n = 6$)

concn added (μg/mL)		concn determined (μg/mL)				
		NPSE	NEPH	PSE	EPH	MEPH
400	mean ± sd	396.1 ± 1.22	394.6 ± 1.15	394.4 ± 1.08	394.9 ± 1.26	395.6 ± 1.15
	RSD (%)	0.31	0.29	0.27	0.32	0.29
	% error	–0.98	–1.25	–1.5	–1.28	–1.10
100	mean ± sd	99.0 ± 0.63	102.3 ± 0.40	102.3 ± 0.49	102.1 ± 0.52	101.8 ± 0.48
	RSD (%)	0.64	0.39	0.48	0.51	0.47
	% error	–1.0	+2.3	+2.3	+2.1	+1.8
25	mean ± sd	24.7 ± 0.27	25.7 ± 0.24	25.7 ± 0.15	25.6 ± 0.16	25.6 ± 0.18
	RSD (%)	1.09	0.93	0.58	0.63	0.70
	% error	–1.2	+2.8	+2.8	+2.4	+2.4
6.25	mean ± sd	6.40 ± 0.18	6.06 ± 0.17	6.11 ± 0.09	6.12 ± 0.09	6.13 ± 0.09
	RSD (%)	2.81	2.81	1.47	1.47	3.7
	% error	+2.4	–3.0	–2.2	–2.1	–1.9

Table 7—*Ephedra* Alkaloid Content for Several Commercially Available Ma-huang Supplements ($n = 30$)

product	*Ephedra* alkaloids				
	NPSE (mg)	NEPH (mg)	PSE (mg)	EPH (mg)	MEPH (mg)
A (Diet Pep)	–	–	1.39 ± 0.06	11.26 ± 0.44	–
B (Diet Phen)	–	–	0.52 ± 0.05	3.02 ± 0.25	–
C (Energel)	–	–	0.15 ± 0.02	1.08 ± 0.08	–
D (Ephedra)	0.30 ± 0.03	0.15 ± 0.02	1.47 ± 0.10	2.85 ± 0.42	0.27 ± 0.02
E (Escalation)	0.22 ± 0.03	0.20 ± 0.04	0.81 ± 0.17	13.54 ± 1.01	0.21 ± 0.06
F (Excel)	0.37 ± 0.07	0.24 ± 0.10	9.46 ± 0.67	12.80 ± 0.77	0.61 ± 0.05
G, lot a (Herbal Ecstacy)[a]	0.25 ± 0.07	0.14 ± 0.04	7.52 ± 0.27	2.63 ± 0.09	2.71 ± 0.11
G, lot b (Herbal Ecstacy)[a]	–	–	8.44 ± 0.69	6.25 ± 0.50	–
H (Herbal Phen-Fen)	–	–	–	8.07 ± 0.73	–
I (Up Your Gas)	–	–	–	11.59 ± 1.16	–

[a] Results of 30 samples.

Table 8—Comparison of HPLC Results to Information Provided on Ma-huang Product Labels

product	dosage form[a]	*Ephedra*[a] (mg/unit)	EPH[b] (mg)	total alkaloids[b] (mg/unit)	EPH label claim[a]	directions for use[a]
A	tablet	–	11.26	12.65	none	do not exceed 2 tablets/day
B	tablet	150	3.02	3.54	4.0	do not exceed 3 tablets/day
C	softgel capsule	125	1.08	1.23	none	1–2 capsules with meals
D	capsule	375	2.85	5.04	none	2–8 capsules daily
E	capsule	250	13.5	15.0	15.0	2 capsules every 3–4 hours
F	capsule	–	12.8	23.5	24.0	2–3 capsules daily
G (lot a)	tablet	–	2.63	13.25	none	1 tablet every 72 hours
G (lot b)	tablet	–	6.25	14.69	none	1 tablet every 72 hours
H	tablet	–	8.07	8.07	none	2 tablets twice daily
I	tablet	285	11.59	11.59	none	do not exceed 2 tablets/day

[a] Information taken from product labels. [b] Results of HPLC analysis.

An examination of Table 8 reveals the disparate nature of information contained on ma-huang supplement labels. Five of the nine products listed the quantity of *Ephedra* herb in each dosage form (range = 125–375 mg), but only three made a label claim for the actual EPH content. Moreover, the quantity of either raw herb or "*Ephedra* extract" did not appear to correlate with EPH, since the product containing the greatest quantity of ma-huang

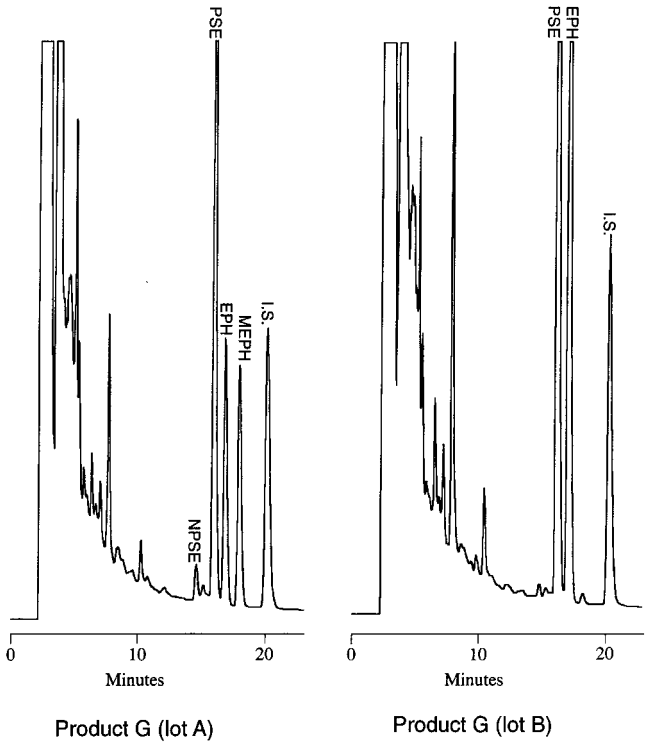

Figure 3—Chromatograms of extracts from two separate lots of the *Ephedra*-containing supplement Herbal Ecstacy (AUFS = 0.64). EPH = ephedrine, I.S. = internal standard (*d*-amphetamine), MEPH = methylephedrine, NPSE = norpseudoephedrine, PSE = pseudoephedrine, AUFS = absorbance units full scale.

(product D, 375 mg) contained one of the lowest amounts of EPH (2.85 mg). Finally, label claims appeared to be more indicative of total alkaloids instead of EPH alone.

Discussion

The assay described here proved to be an accurate and precise method for the determination of EPH, MEPH, NPSE, NEPH, and PSE in nutritional supplements containing ma-huang. Although liquid chromatographic methods for quantitating EPH alkaloids in crude *Ephedra* herb have been previously described,[15,16] this is the first HPLC method applied to the analysis of commercially available ma-huang supplements on the U.S. market. Unlike the crude plant material, ma-huang supplements are a blend of *Ephedra*, or "*Ephedra* extract," and other botanicals, vitamins, minerals, excipients, etc., in either capsule or tablet form.

Our method differed significantly from those of Sagara et al.[15] and Zhang et al.[16] with regard to column type, mobile phase composition, and column temperature. Like Sagara et al., we incorporated sodium lauryl sulfate in the mobile phase as an ion-pairing agent to increase retention and facilitate resolution of the five alkaloids and internal standard. To improve peak symmetry and optimize resolution, we utilized a base-deactivated C-18 column with smaller particle size (5 μm), added tetrahydrofuran to the mobile phase, and operated the column at 37 °C. These improvements precluded the need for amine modifiers and gradient elution called for by Zhang et al. and, unlike the method of Sagara et al., resulted in sharp, symmetrical peaks that were all baseline-resolved.

Of the six alkaloids reported to be in ma-huang, EPH, MEPH, NPSE, NEPH, and PSE are the most common. The remaining alkaloid, methylpseudoephedrine, appears infrequently and only in trace amounts;[11,15,16] therefore, we chose not to evaluate it in the present study. Previous chromatographic analyses of unprocessed *Ephedra* herb revealed that total alkaloid content varies greatly among species, and can range from 0.4 to 25 mg/g depending on the location and conditions under which the plant is grown and harvested.[15–17] This variability was also evident among the products analyzed in the present study suggesting that either several *Ephedra* species were represented, or a single variety was obtained from diverse sources.

Ephedrine and pseudoephedrine were the most abundant alkaloids found in commercial grade ma-huang with quantities ranging from 1.6 to 22.8 mg/g and 0.6–12 mg/g, respectively.[11,12,15,16] These two alkaloids were also the most prevalent among the products examined in our study with product E containing the most EPH (13.54 mg/capsule) and product F having the most PSE (9.46 mg/capsule). Our findings and those of Betz et al.[11] suggest that the quantity of EPH encountered in most nutritional supplements lies well below that found in prescription and over-the-counter EPH products (24–30 mg). For self-medication, the recommended adult dose of EPH is 25–50 mg every 4 to 6 h, not to exceed 150 mg/day, and that for PSE is 30–60 mg every 6 h, not to exceed 240 mg/day.[19] If directions and warnings on most of the supplement labels studied here were followed, cumulative EPH and PSE doses would not exceed daily recommendations. The one exception, however, was product E. Ingesting "2 capsules every 3 hours" of product E would lead to a daily EPH dose of 216 mg—well above the recommended safe dose.

Two products (H and I) contained measurable quantities of EPH only. Since most *Ephedra* species contain measurable quantities of EPH, MEPH, NPSE, NEPH, and PSE, and because none have been characterized by a solitary alkaloid present in large quantities,[15–17] ma-huang products of this type are suspect. Such findings bring into question the source of EPH for products such as H and I. Do single alkaloid products contain ma-huang or are they doped with synthetic EPH? In another report of *Ephedra* alkaloid content in dietary supplements, Betz et al.,[11] observed products with single alkaloid profiles, yet their chiral gas chromatographic analysis confirmed the presence of only naturally occurring enantiomers [(−)-EPH]. Still, like Betz et al., we question whether supplements distinguished by a single alkaloid were formulated with ma-huang or spiked with synthetic (−)-EPH.[11]

The resurgent interest in herbal medicine has spurred introduction of a host of dietary supplements into the marketplace incorporating botanical EPH. A marked increase in the consumption of these products among the general public has brought with it an increase in reported adverse events including 17 fatalities resulting from EPH overdose.[3,4,7,20] Considering that most ma-huang supplements contain only moderate amounts of EPH, why is it that these products are associated with such a high incidence of morbidity and mortality?

The answer to this question is possibly tied to the reformed guidelines set forth by the DSHEA in governing the manufacture and sale of dietary supplements, as well as the public's perception that natural products are inherently safe. Although *Ephedra* products contain active drug components (i.e. EPH, PSE, NPSE, etc.), they can be marketed as dietary supplements and therefore are not regulated by the Food and Drug Administration (FDA) to the same degree as prescription or nonprescription medications containing EPH or PSE. The DSHEA currently places the burden of proof on the FDA and not the manufacturer to provide evidence of safety prior to marketing an established supplement. The FDA, however, still

retains the authority to act against products that are deemed unsafe or adulterated.

Recently, the FDA has proposed several constraints regarding manufacture and sale of EPH supplements which may ease its "burden" in policing these products.[21] These proposals primarily focus on labeling issues that many supplement manufacturers have exploited up to now.

Ma-huang product labels usually indicate how much *Ephedra* herb is present, but few make a claim of EPH content. For consumers this can be misleading because *Ephedra* species vary widely in their alkaloid content as a consequence of where the plant is grown, the type of growing conditions, and the time of harvest.[15,16] This natural inconsistency gives rise to commercial products with significant interproduct and intraproduct variability as is evidenced by the results of the present study (see Figures 2 and 3 and Table 7). In short, the consumer is unaware how much EPH is being consumed.

The FDA has proposed to limit the quantity of total EPH alkaloids in supplements so that no more than 8 mg is consumed in a 6-h period and no more than 24 mg in a day.[21,22] Only three of the nine products presented here would meet both proposed conditions (products B, C, and G) while one, product E, would greatly exceed these recommendations. If limits are placed on EPH alkaloid content, current good manfacturing practices and in-house quality assurance must be adhered to if label claims are to be believed by the consumer. This would prevent exposing the public to products which exhibit lot to lot variations of EPH in excess of 130% (i.e. product G). This last example illustrates the dichotomy which currently exists between the nutraceutical and pharmaceutical industries regarding regulatory standards. Because of the good manufacturing practices that the pharmaceutical industry adheres to regarding quality control and assurance, a product exhibiting lot to lot variations in excess of 100% would never be released for public consumption.

Many supplement manufacturers also resort to off-label advertising claims which are based on insufficient scientific evidence. Promises that product usage will cause "euphoria and increase sexual sensation," or "melt pounds away in just days" are calling cards for potential overdose. Still, others like Herbal Ecstacy and Herbal Phen-Fen are portrayed as natural alternatives to the illegal street drug "ecstasy" (3,4-(methylenedioxy)-*N*-methylamphetamine) or the stimulant combination "Phen-Fen" (phentermine−fenfluramine), a weight-loss therapy recently removed from the market because of cardiovascular toxicities.[23] Proposed labeling changes would aid in curtailing exaggerated "off-label" marketing claims.

Finally, consumers are led to believe that "natural medicines" are inherently safe alternatives to conventional drug therapy. While many natural ingredients are generally regarded as safe, ma-huang does not fall into that category.[2,7,20]

Taken together, all the aforementioned elements appear to contribute to the growing problem of toxicity associated with ma-huang. Therefore, until proposed regulatory constraints regarding botanical EPH are enacted, caution and moderation should be stressed when using nutritional supplements containing ma-huang.

References and Notes

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