Determination of Ephedra Alkaloids & Synephrine by Strong Cation-Exchange SPE and LC-MS/MS Detection Using a Pentafluorophenylpropyl HPLC Column

UCT Part Numbers:

- **CUBCX1HL56:** high-load benzenesulfonic acid sorbent , 500mg / 6mL SPE column
- **Selectra® PFPP HPLC column** (pentafluorophenylpropyl phase bonded to a high purity silica, (USP L43))
  - **SLPFPP100ID21-3UM:** 100 x 2.1mm, 3µm analytical column
  - **SLPFPPGDC20-3UM:** 10 x 2.0mm, 3 µm guard column

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Introduction

Ephedra alkaloids are phenethylamines that occur naturally in plants, including the herb *Ma Huang* used in traditional Chinese medicine. Ephedra alkaloids are potent CNS stimulants and also have a sympathomimetic effect on the peripheral nervous system. Some active ingredients of these plants are used as ingredients in cold remedies (e.g. pseudoephedrine). The ephedra alkaloids are also incorporated into dietary supplements to promote weight loss or to increase alertness and physical activity (e.g. body building). However, severe contraindications have been reported for individuals with hypertension or other cardiovascular diseases, particularly when used in combination with caffeine [1]. Products containing ephedrine were popular dietary supplements until the FDA banned their use in 2004 [2]. Since then the active ingredient in dietary supplements has largely been replaced by synephrine, a naturally occurring alkaloid found in plants such *Citrus* fruits; it is similar in structure to ephedrine.

The ephedra alkaloids are small, hydrophilic, basic analytes that are difficult to retain and separate on traditional HPLC columns using alkyl-bonded stationary phases. They are capable of strongly interacting with free silanols on the surface, which leads to peak tailing of the analytes and affects the resolution and quantification. Current methodologies used for the separation of ephedra alkaloids use reversed- phase columns with ion-pairing reagents, time-consuming derivatization procedures, or use strong cation-exchange phases. However, these approaches are not very amenable to LC-MS/MS analysis. An alternative approach to traditional alkyl phases is the use of a fluorinated stationary phase. In addition to dispersive interactions available on traditional alkyl phases, pentafluorophenylpropyl phases can undergo dipole–dipole, and pi–pi interactions. This imparts unique selectivity to the column that can sufficiently resolve the ephedra alkaloids.

The aim of this study was to develop a multi-analyte procedure for the extraction, cleanup, and quantification of the ephedra alkaloids in functional foods and natural products. High capacity strong cation-exchange SPE cartridges were used for the isolation of the phenethylamines from dietary supplements. HPLC separation, including separation of the stereoisomers, was carried out using a UCT Selectra® PFPP column prior to detection by LC-MS/MS.

Sample Preparation Procedure

1. Sample Extraction

a) Weigh 1 ± 0.1g of sample into a 15 mL polypropylene centrifuge tube.
   - For this study a dietary supplement (1 g tablet) for weight loss was used.

b) Add 10 mL of 1% formic acid to each sample.

c) Shake or vortex sample for 15 minutes to fully extract the ephedra alkaloids. Ensure tablet samples are fully dissolved.
   - For this study a SPEX® SamplePrep® GenoGrinder® was used.

d) Centrifuge the sample for 10 min at ≥3000 × g and 4°C.

2. Condition Cartridge

a) Add 2 × 4 mL of methanol to **CUBCX1HL56** SPE cartridge.

b) Add 4 mL of ultrapure water.

c) Add 4 mL of 1% formic acid

Note: Do not let the cartridge go dry otherwise repeat steps a) through c).

3. SPE Extraction

a) Load supernatant from step 1d).

b) Allow sample to percolate through the cartridge or apply a vacuum if necessary (adjust vacuum for flow of 1–3 mL per minute).

4. Second extraction (Optional)

a) Add 5 mL of 1% formic acid to each sample.

b) Shake or vortex sample for 5 minutes.

c) Centrifuge the sample for 10 min at ≥3000 × g and 4°C.

d) Apply supernatant to the SPE cartridge.

5. Wash Cartridge

a) Add 2 × 4 mL of 0.1% formic acid and slowly draw through.

b) Add 2 × 4 mL methanol and slowly draw through.

c) Dry under vacuum for ≈30 sec to remove excess solvent.

6. Elute Cartridge

a) Elute the ephedra alkaloids using 8 mL of methanol containing 2% ammonium hydroxide.

b) Evaporate off the methanol solvent at 40°C under a gentle stream of nitrogen until it reaches a volume of ≈1 mL.

c) Add 1 mL of aqueous mobile phase (10mM ammonium acetate).

d) Evaporate off any remaining methanol.
   - Ephedra alkaloids are similar to amphetamines, which are known to be volatile compounds. Therefore, extra care was taken during the evaporation step to avoid any potential loss in recovery that may occur during this step.

e) Vortex the samples for 1 min and filter through a 0.2 µM syringe filter directly into a HPLC vial.

LC-MS/MS Conditions:

HPLC Conditions	
Instrumentation	Thermo Scientific™ Dionex™ Ultimate™ 3000 LC system
HPLC column	UCT Selectra® PFPP, 100 × 2.1 mm, 3 µm (p/n: SLPFPP100ID21-3UM)
Guard column	UCT Selectra® PFPP, 10 × 2.1 mm, 3 µm, (p/n: SLPFPPGDC20-3UM)
Guard column holder	p/n: SLDGRDHLDR
Mobile phase A	10 mM ammonium acetate
Mobile phase B	methanol
Isocratic elution	85:15 (A:B, v:v)
Flow rate	500 µL/min
Column temp.	50°C
Run time	20 min
Injection volume	5 µL
Autosampler temp.	10°C
Wash solvent	methanol: water (1:1, v/v)
Divert valve	mobile phase was sent to waste for 1.5 min to reduce ion source contamination

MS Conditions	
Instrumentation	Thermo Scientific™ TSQ Vantage™ tandem mass spectrometer
Ionization mode	ESI^+
Spray voltage	4500 V
Vaporizer temperature	400°C
Capillary temperature	300°C
Sheath gas pressure	60 arbitrary units
Auxiliary gas pressure	55 arbitrary units
Ion sweep gas	0 arbitrary units
Declustering potential	2 V
Q1 and Q3 peak width	0.2 and 0.7 Da
Collision gas	argon
Collision gas pressure	1.8 mTorr
Acquisition method	EZ method (scheduled SRM)
Cycle time	2 sec
Software for data processing	TraceFinderTM version 3.0
Weighting factor applied to calibration curves	1/X

SRM Transitions							
Analyte	**t_R (min)**	**Precursor ion**	**Product ion 1**	**CE 1**	**Product ion 2**	**CE 2**	**S-lens (V)**
Ephedrine	9.9	166.17	91.01	29	115.02	22	46
Pseudoephedrine	11.4	166.17	91.01	29	115.02	22	46
Norephedrine	5.6	152.141	91.02	31	115.02	22	38
Norpseudoephedrine	6.7	152.141	91.02	31	115.02	22	38
Methylephedrine	14.7	180.17	90.99	31	147.04	15	52
Synephrine	2.1	168.10	90.97	19	106.97	29	37
Ephedrine-d_3 (IS)	9.9	169.17	90.99	31	115.00	24	49
Pseudoephedrine-d_3 (IS)	11.4	169.17	90.99	31	115.00	24	49

Results

Accuracy & precision Data for 6 the Ephedra Alkaloids at 100 ppb (n=5)						
	Ephedrine	Pseudoephedrine	Norephedrine	Norpseudoephedrine	Methylephedrin	Synephrine*
Sample 1	94.13	93.07	80.22	108.30	79.8	47.35
Sample 2	91.62	94.28	48.49	88.11	79.9	38.14
Sample 3	92.62	92.74	63.24	89.02	89.7	52.41
Sample 4	92.60	93.56	61.63	75.05	79.4	45.43
Sample 5	93.39	93.56	49.86	70.75	83.2	51.85
Mean	**92.87**	**93.44**	**60.69**	**86.25**	**82.44**	**47.04**
RSD	**1.02**	**0.62**	**21.08**	**17.03**	**5.27**	**12.30**

***Note:** Synephrine is more polar than the ephedra alkaloids and is not as well retained on the sorbent. It is recommended to include an isotopically labeled internal standard in order to achieve the best results.

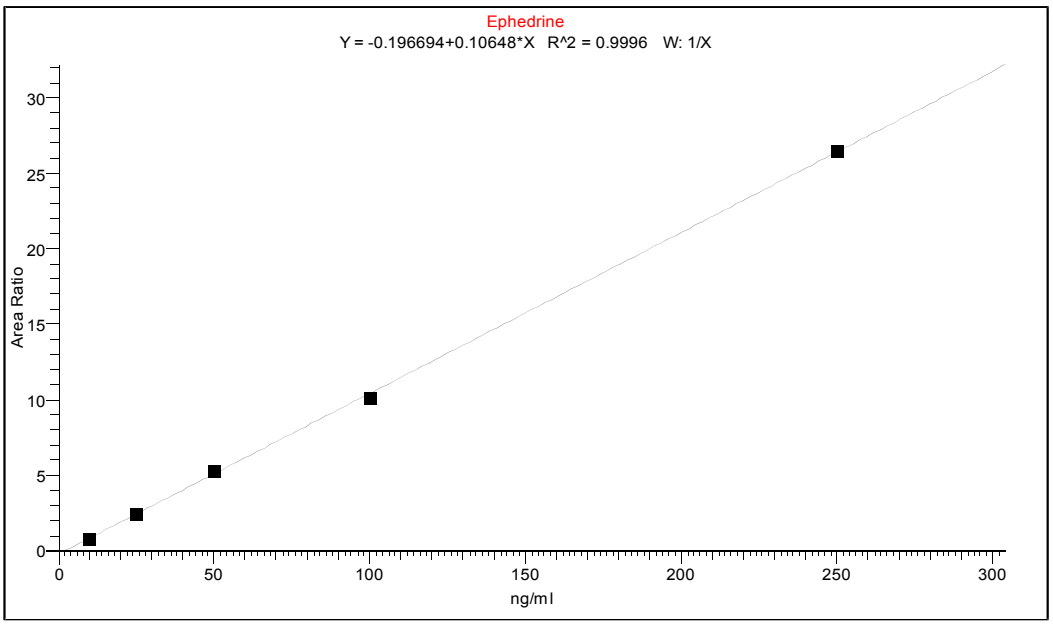

Figure 1. Example calibration curve (ephedrine) over a 10-250 ng/mL concentration range.

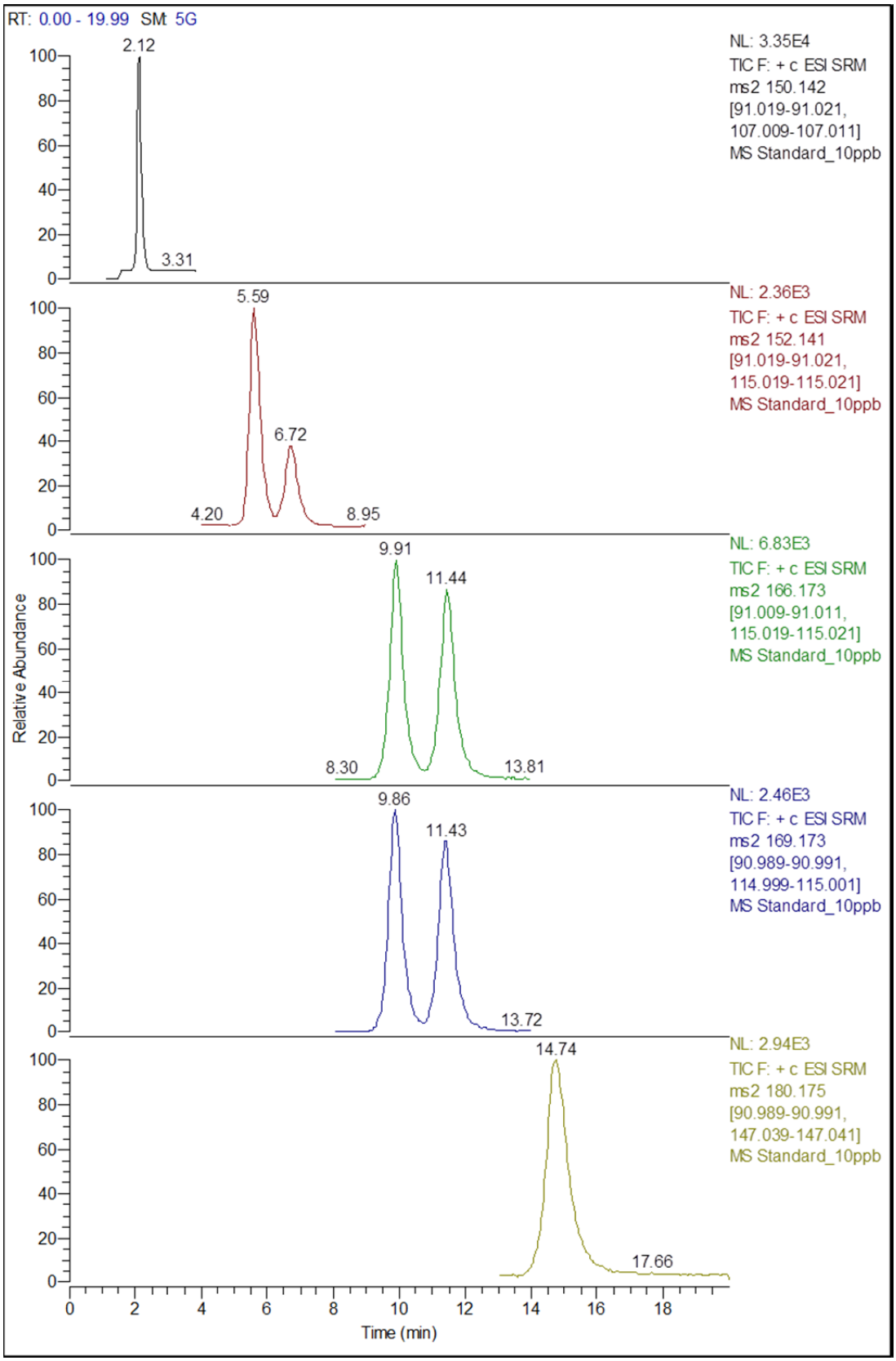

Figure 2. Chromatogram of a 10 ng/mL standard.

Conclusions:

- A method was successfully developed for the extraction, cleanup, and quantification of the ephedra alkaloids and synephrine in functional foods and natural products.
- Strong cation-exchange SPE was used to isolate the phenethylamines from the complex sample matrix, which consisted of 9 herbal extracts containing a high concentration of calcium, caffeine and additional excipients.
- A high capacity SPE sorbent was used as it offers better retention than standard SCX sorbent.
- It is recommended to include isotopically labeled internal standards into the method, particularly for the hydrophilic synephrine which is not as efficiently retained by SPE as the ephedra alkaloids.
- HPLC separation of the 5 ephedra alkaloids and synephrine was successfully conducted on UCT's Selectra® PFPP column, including baseline resolution of the 2 sets of stereoisomers included in the method (ephedrine / pseudoephedrine and norephedrine / norpseudoephedrine).
- Good LC-MS/MS sensitivity was observed for all compounds (< 10 ng/mL).

References

[1] Journal of Chromatography A, 1161 (2007) 71–88

[2] Food and Drug Administration, Federal Registry 69 (2004) 6787

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