EPHEDRA ALKALOIDS IN FUNCTIONAL FOODS

HPLC METHOD

COLUMN: XTerra® Phenyl, 2.1 x 150 mm, 3.5 µm

PART NUMBER: 186001181

MOBILE PHASE A: 10 mM NH_4HCO_3 , pH 9.5

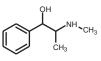
MOBILE PHASE B: ACN FLOW RATE: 0.23 mL/min

ISOCRATIC MOBILE

PHASE COMPOSITION: 90% A; 10% B

INJECTION VOLUME: 5 μL **TEMPERATURE:** 40 °C **DETECTION:** UV @ 254 nm

INSTRUMENT: Alliance® 2695, 2996 PDA



EPHEDRINE

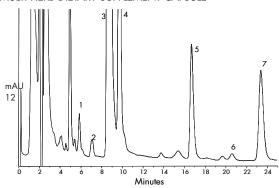
PREPARE SAMPLE

 Accurately weigh 1 gram of ephedra sample into a 100 mL volumetric flask, add 20 mL water and mix. Add 50 mL of MeOH and 1 ml of Internal Standard.

-Use 0.5 g sample for ephedra extracts

- Use 10 g sample weight for High Protein Powdered Drinks and other functional foods.
- Sonicate for 1 hour at ambient temperature
- $\operatorname{\mathsf{Cool}}$ and $\operatorname{\mathsf{bring}}$ to $\operatorname{\mathsf{volume}}$ with $\operatorname{\mathsf{MeOH}}$
- Allow the suspended solids to settle, preferably certrifuge.
- Filter a 3 mL aliqout through a $0.45~\mathrm{mm}$ filter before a sample preperation
 - This step is critical to good SPE recovery.
- For SPE, dilute 2 mL of filtered sample extract to 10 mL with 0.1% formic acid (aqueous)

MULTI-HERB DIETARY SUPPLEMENT CAPSULE



OASIS® MCX EXTRACTION METHOD

Oasis® MCX Extraction Cartridge, 3 cc/60 mg

CONDITION:				
1 mL methanol/1 mL water				
THE MICHARIOY THE WAIG				
LOAD:				
10 mL sample				
WASH 1:				
2 ml 0.1 N HCl				
Z IIIL O. I IN I ICI				
WASH 2:				
1 mL methanol				
RE-EQUILIBRATE:				
1 mL water				
WASH 3:				
1 mL $75:25$ 5% NH ₄ OH/methanol				
FIUTF				
1.5 mL 95% MeOH/5% NH ₄ OH				
1.6 1.12 7 5.75 1.15 61 17 676 1 11 14 61 1				
ADJUST TO E O ALL FINIAL VOLUME				
ADJUST TO 5.0 ML FINAL VOLUME:				
10 mM NH ₄ HCO ₃ , pH 9.45				

	50% Level	100% Level	150% Level
	% Recovery	% Recovery	% Recovery
	% RSD	% RSD	% RSD
1 NE	81.9 ± 6.7	74.7 ± 5.9	66.7 ± 3.7
	8.12%	7.95%	4.46%
2 NPE	77.6 ± 1.8	66.2 ± 0.8	63.9 ± 0.73
1 occasion	2.25%	1.26%	1.15%
3 E	101.9 ± 2.5	102.3 ± 3.7	98.0 ± 5.5
	8.12%	3.60%	5.60%
4 PE	89.0 ± 1.2	93.2 ± 3.4	92.8 ± 5.3
	8.12%	3.61%	5.70%
5 ME	94.7 ± 4.2	98.7 ± 3.3	81.9 ± 1.8
	4.46%	3.34%	1.88%
6 MPE	99.8 ± 16.3	85.4 ± 13.3	88.0 ± 7.7
	16.4%	15.6%	8.73%

100% Level

- 1. norephedrine (NE) 0.24mg/g
- 2. norpseudoephedrine (NPE) 0.40mg/g
- 3. ephedrine (E) 20.0mg/g
- 4. pseudoephedrine (PE) 5.0mg/g
- 5. methylephedrine (ME) 0.70mg/g
- 6. methylpseudoephedrine (MPE) $0.17 \, \mathrm{mg/g}$