SPE Procedure

Oasis® HLB 3cc /60mg

CONDITION:
A. 3 mL methanol
B. 3 mL water

LOAD:
2.5 mL sample

WASH 1:
3 mL 1% sodium bicarbonate (1g/100mL)

WASH 2:
1 mL 0.1% acetic acid

Dry Cartridge using vacuum manifold

ELUTE:
2 x 1.5 mL 10% ethyl acetate in methyl t-butyl ether (MTBE)

RECONSTITUTE:
500 µL water

LC Conditions

Instrument:	Waters ACQUITY UPLC® System
Column:	ACQUITY UPLC BEH Shield RP18, 2.1 x 100 mm, 1.7 µm
Flow rate:	600 µL/min
Mobile phase:	A. 0.1% aqueous ammonium hydroxide B. 0.1% ammonium hydroxide in acetonitrile

Gradient:

Time (min)	A%	B%
0	99	1
1.8	99	1
2.3	10	90
2.8	10	90
2.81	99	1

Injection volume:	20 µL, Full loop injection
Column temperature:	40 °C
Sample temperature:	4 °C
Detector:	ACQUITY UPLC PDA
Detection:	276 nm

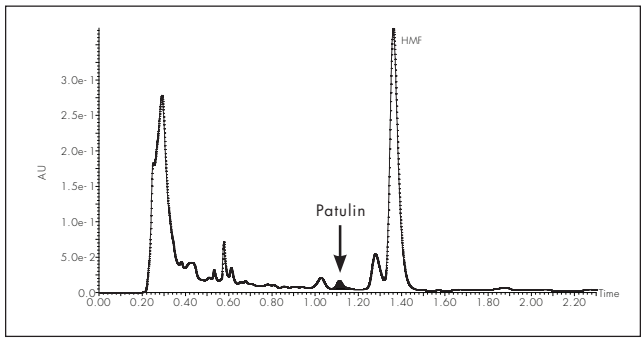
MS Conditions

Instrument:	Waters ACQUITY® TQ Detector
Ionization mode:	Negative electrospray (ESI^-) Multiple reaction monitoring

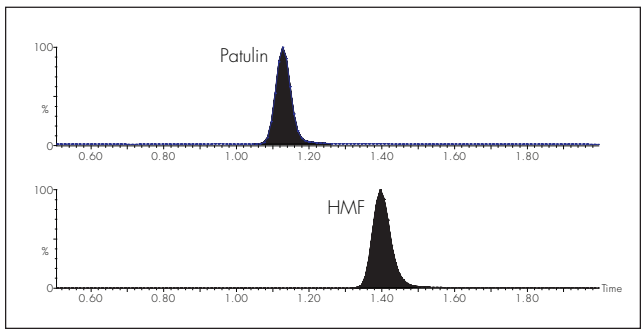
Analytes	MRM Transition
Patulin	153 → 109
	153 → 81
5-hydroxymethylfurfural (HMF)	125 → 95

MRM method parameters.

Results



Apple juice extract at 50 µg/kg containing patulin and 5-hydroxymethylfurfural (HMF) at 276 nm.



Apple juice extract at 50 µg/kg containing patulin and 5-hydroxymethylfurfural in negative electrospray mode.

Concentration	Average Recovery (%RSD)
5 µg/kg	86.1% (13.6)
50 µg/kg	95.4% (5.9)
500 µg/kg	89.9% (17.5)

Recovery data obtained from Oasis HLB extraction of patulin in apple juice. Four data points were measured at each level.

Ordering Information

Description	Part Number
Oasis HLB, 3cc /60mg, 100/box	WAT094226
ACQUITY UPLC BEH Shield RP18, 2.1 x 100 mm, 1.7 µm	186002854
LCMS Certified Vials	600000749CV

Ref: Developed by Vural Gökmen, Food Engineering Department, Hacettepe University, Ankara, Turkey and John Martin, Waters Corporation