CLEMASTINE IN RAT PLASMA - OASIS® ON-LINE 1 COLUMN APPROACH

HPLC GRADIENT AND WASH CONDITIONS

Time	HPLC gradient Flow 0.4 mL/min A	B	Valve position
0.0	5	95	
0.5			switch position 2 to 1
1.0	95	5	
2.60	95	5	
2.90			switch position 1 to 2
3.0	5	95	

A - Acetonitrile + 0.5% Formic Acid
B - Water + 0.5% Formic Acid

LC1:	Alliance® 2790 - 0.4 mL/min
LC2:	Waters 515 - 4.0 mL/min
LOADING MOBILE PHASE:	100% water
ELUTING MOBILE PHASE:	1 minute gradient 5% ACN to 95% ACN
ELUTING MOBILE PHASE ADDITIVE:	0.5% Formic acid
EXTRACTION COLUMN TEMP:	40 °C
SWITCHING VALVE:	Rheodyne LabPro 10 ports, 2 position
MS:	Quattro™ Ultima Triple Quadrupole
SOURCE:	Electrospray positive
SOURCE TEMP.:	150 °C
DESOLVATION GAS:	600 L/hr
GAS CELL:	1.5e-3 mbar
CONE VOLTAGE:	20 volts
COLLISION ENERGY:	20

Conc. ng/mL n=6	Average	Standard Deviation	RSD%
1.0	0.98	0.02	2.1
2.5	2.56	0.11	4.4
5.0	5.25	0.12	2.3
10.0	9.50	0.25	2.7
100.0	101.43	2.69	2.6
200.0	201.14	3.20	1.6
250.0	247.10	1.58	0.6

OASIS® HLB EXTRACTION METHOD

Oasis® HLB Extraction Column 2.1 x 20 mm, 25 µm
Part Number 186000706

SAMPLE PREPARATION:
centrifuge rat plasma

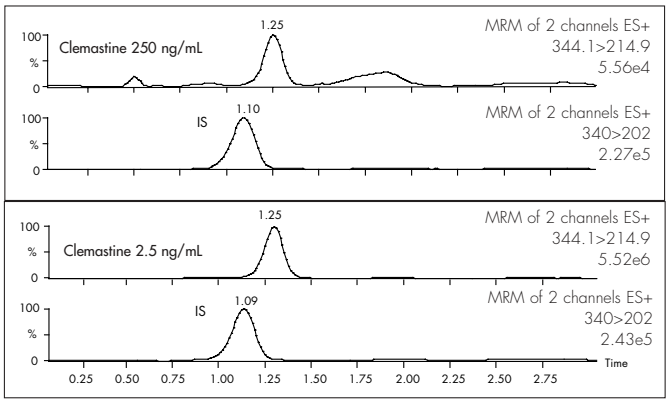
SAMPLE PREPARATION:
Spike 5 mL of rat plasma + 100 µL NH_4OH

SAMPLE PREPARATION:
500 µL of spiked rat plasma + 400 µL IS in water

LOADING:
200 µL at 4mL/min in 100% water

ELUTION:
0.4 mL/min gradient 5% ACN to 95% ACN in 1 minute

Oasis® HLB LC/MS/MS Analysis of Clemastine at 2.5 ng/mL and 250 ng/mL



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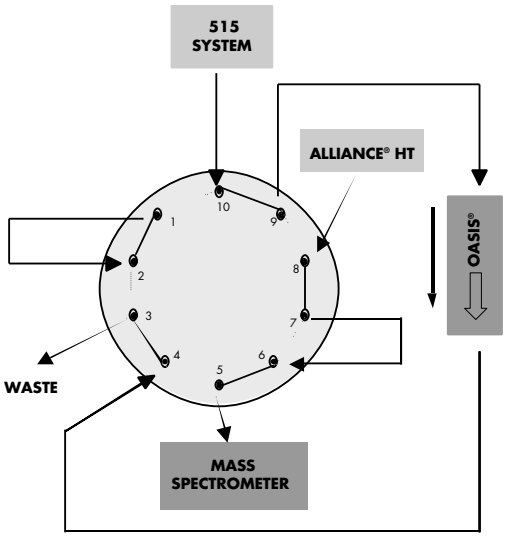
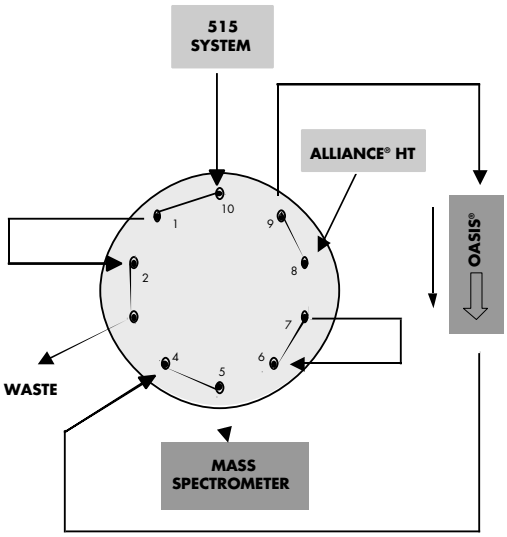
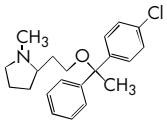
LOAD POSITION

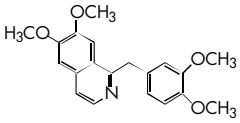


ELUTION POSITION



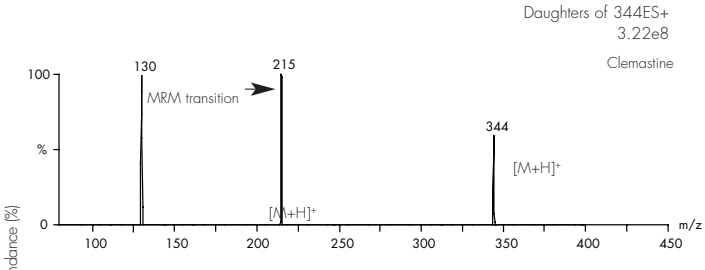


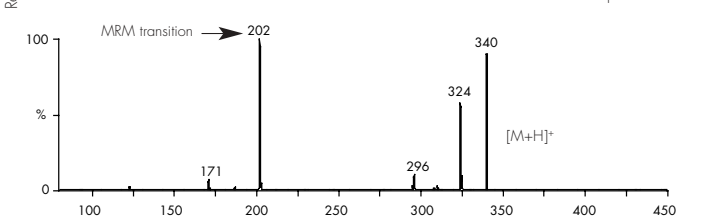
CLEMASTINE



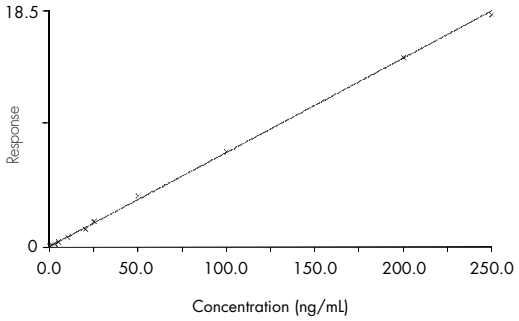
PAPAVERINE I.S.

CID MASS SPECTRA





Coefficient of Determination: 0.999397
Calibration curve: 1.59441e-6 * x^2 + 0.0733900* x + 0.00620068
Response type: Internal Std (Ref 1), Area* (IS Conc./IS Area)
Curve type: 2nd Order, Origin: Exclude, Weighting: 1/x^2, Axis trans: None



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