ON-LINE PROTOCOL FOR BASIC DRUGS IN CELL CULTURE LYSATE USING LC/MS/MS

HPLC METHOD

COLUMN: XTerra® MS C_{18}, 2.1 x 30 mm, 3.5 µm
PART NUMBER: 186000398
MOBILE PHASE A: Acetonitrile + 0.5% Formic Acid
MOBILE PHASE B: Water + 0.5% Formic Acid
GRADIENT:

Time (min)	Profile %A	%B
0.0	5	95
0.5	5	95
1.5	95	5
4.40	95	5
4.50	5	95
6.0	5	95

FLOW RATE: 1.0mL

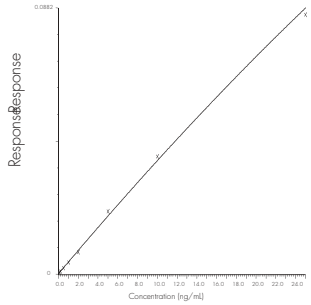
LC_1: Alliance® 2690 - 0.4 mL/min
LC_2: Waters 515 - 4.0 mL/min
LOADING MOBILE PHASE: 100 % water
ELUTING MOBILE PHASE: 1 minute gradient 5% ACN to 95% ACN
ELUTING MOBILE PHASE ADDITIVE: 0.5 % Formic acid
EXTRACTION COLUMN TEMP.: 40 °C
SWITCHING VALVE: Rheodyne LabPro 10 ports, 2 position

MS: Waters Micromass Quattro™ Ultima
SOURCE: Electrospray positive
SOURCE TEMPERATURE: 150 °C
DESOLVATION GAS: 600 L/hr
GAS CELL: 1.5e-3 mbar
CONE VOLTAGE: 20 volts
COLLISION ENERGY: 20

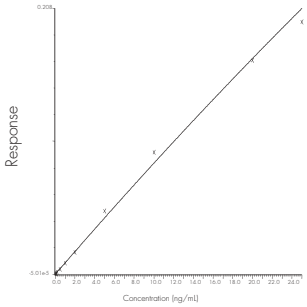
Compound 2 name: propranolol
Coefficient of Determination: 0.998650
Calibration curve: -1.52393e-5 * x^2 + 0.00390583 * x + 0.000102086
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: 2nd Order, Origin: Exclude, Weighting: 1/x^2, Axis trans: None

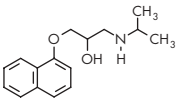
Compound 3 name: naloxone
Coefficient of Determination: 0.995263
Calibration curve: -2.47393e-5 * x^2 + 0.00892751 * x + -5.00665e-5
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: 2nd Order, Origin: Exclude, Weighting: 1/x^2, Axis trans: None

PROPRANOLOL

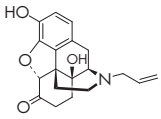


NALOXONE





PROPRANOLOL



NALOXONE

ON-LINE PROTOCOL

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

10^6 Jurkat cells were grown in RPMI 1640 culture medium

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Cells were centrifuged at 1500 g for 5 min

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Cells were washed twice with cold PBS

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Cells were centrifuged at 1500 g and supernatant was removed

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Cell pellets were spiked at various level

↓

Lyse with 1 mL 50/50 MeOH/ACN

↓

Dilute with 4 mL H_2O
Inject 400 mL

Conc. ng/mL n=6	Average	Standard Deviation	RSD%
PROPRANOLOL CALIBRATION CURVE			
0.1	0.09	0.05	4.8
0.5	0.49	0.03	5.9
1.0	1.03	0.04	3.9
5.0	4.93	0.26	5.3
10.0	9.76	0.34	3.5
20.0	20.21	0.89	4.4
25.0	25.21	0.69	2.7
NALOXONE CALIBRATION CURVE			
0.1	0.10	0.04	4.2
0.5	0.48	0.02	5.0
1.0	0.99	0.06	6.8
5.0	5.25	0.25	4.8
10.0	10.43	0.41	3.9
20.0	19.98	0.54	2.7
25.0	24.44	1.12	4.6

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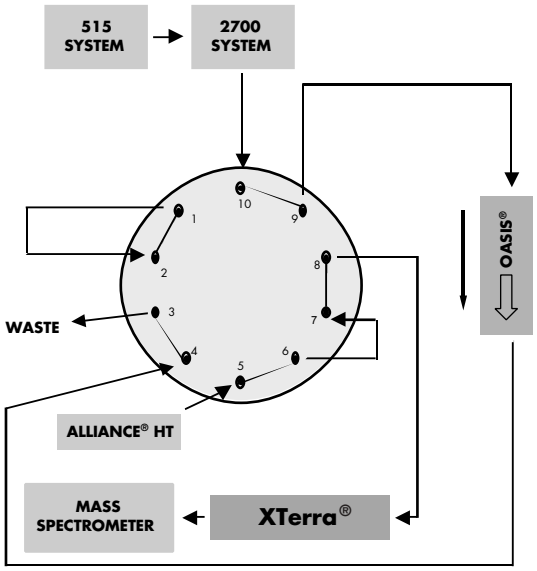
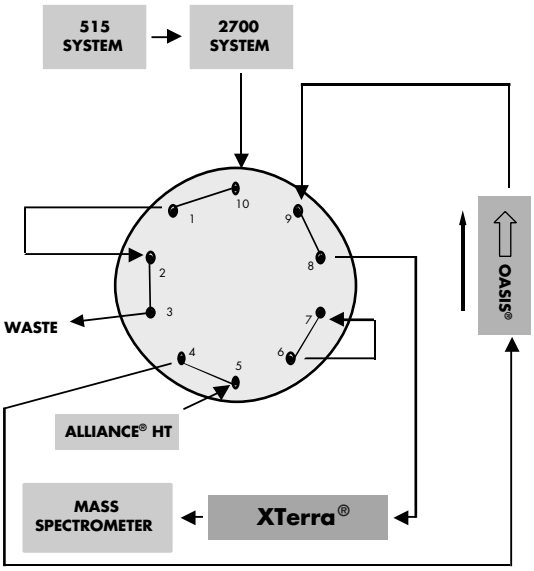
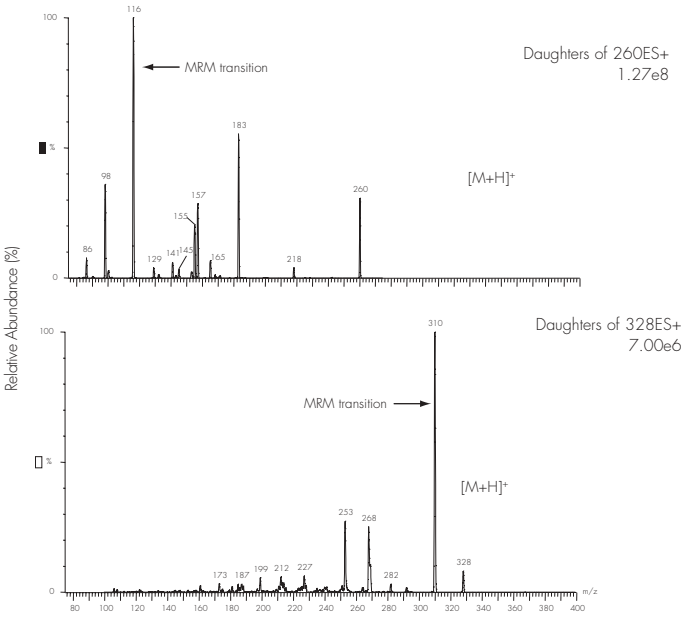
LOAD POSITION
(BACKFLUSH CONFIGURATION)



ELUTION POSITION
(BACKFLUSH CONFIGURATION)

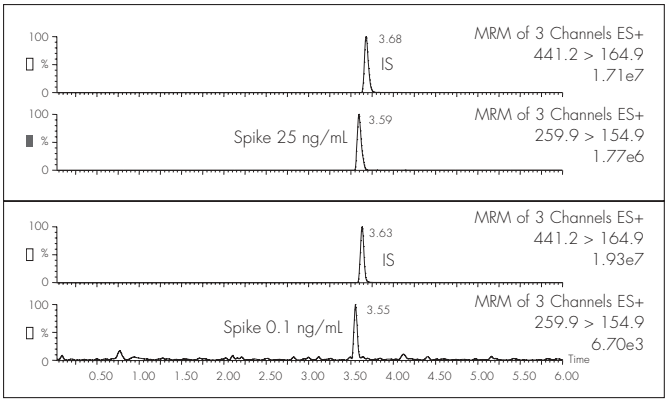


CID MASS SPECTRA

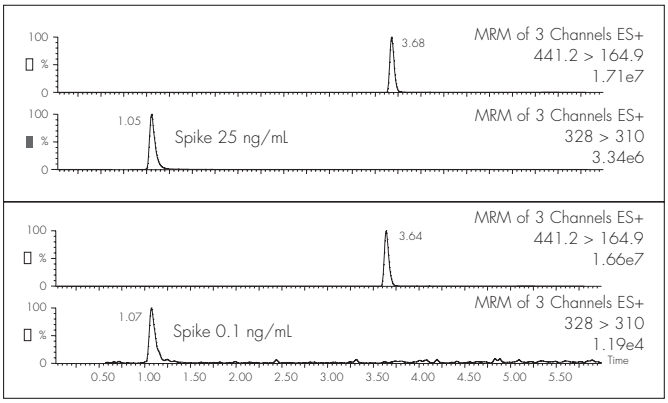


50/50 MEOH/ACETONITRILE CELL LYSING

PROPRANOLOL AT 0.1 ng/mL AND 25 ng/mL



NALOXONE AT 0.1 ng/mL AND 25 ng/mL



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