



On-Line Protocol for Basic Drugs in Cell Culture Lysate Using LC-MS/MS

Waters Corporation



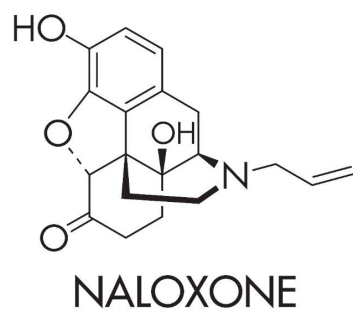
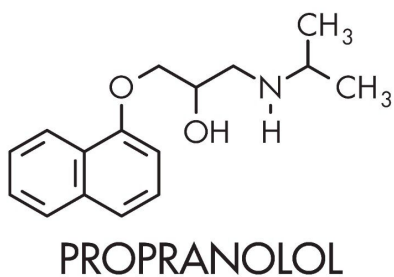
This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief highlights the analysis of basic drugs in cell culture lysate using Oasis HLB on-line protocol.

Introduction

Basic drugs has been analyzed in cell culture lysate using LC-MS/MS in this application brief.



Experimental

HPLC Method

Column:

XTerra MS C₁₈, 2.1 x 30 mm, 3.5 μm (p/n:
186000398)

Mobile phase A:	Acetonitrile + 0.5% Formic Acid
Mobile phase B:	Water + 0.5% Formic Acid
Flow rate:	1.0 mL
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
Sorce temp.:	150 °C
Desolvation gas:	600 L/hr
Gas cell:	1.5e ⁻³ mbar
Cone voltage:	20 volts
Collision energy:	20

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

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

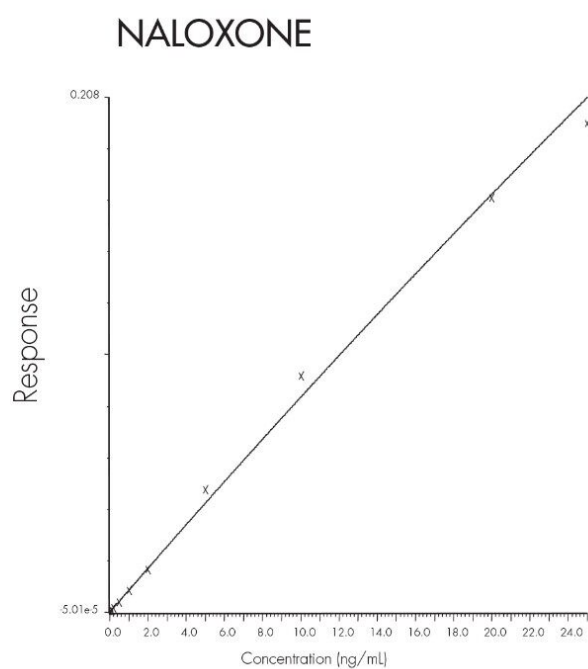
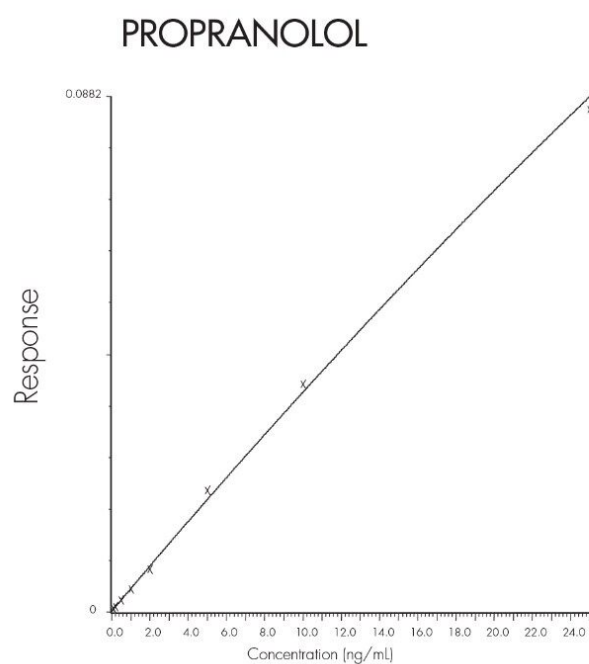
Compound 3 name: Naloxone

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/x², Axis trans: None



On-Line Protocol

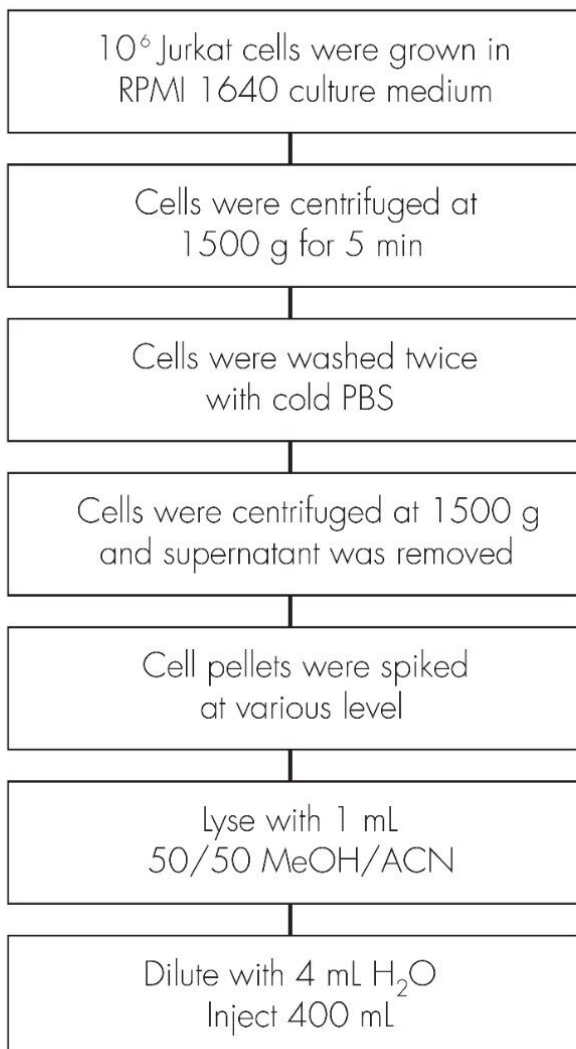
Oasis HLB 2.1 x 20 mm, 25 µm Extraction Column Part Number [186000706](#) <

<https://www.waters.com/nextgen/xg/en/shop/sample-preparation--filtration/186000706-oasis-hlb-online-column-80a-25--m-21-mm-x-20-mm-1-pk.html>>

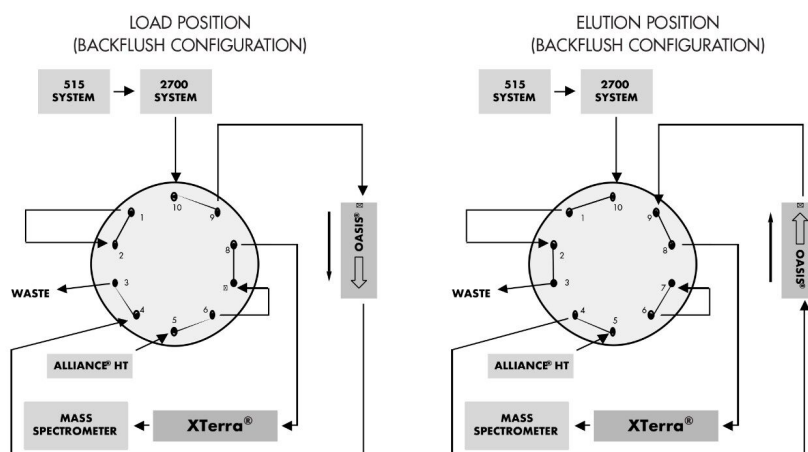
ON-LINE PROTOCOL

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

Part Number 186000706

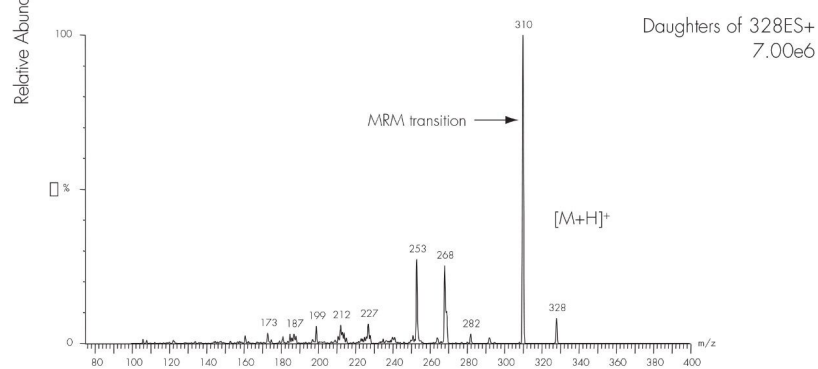
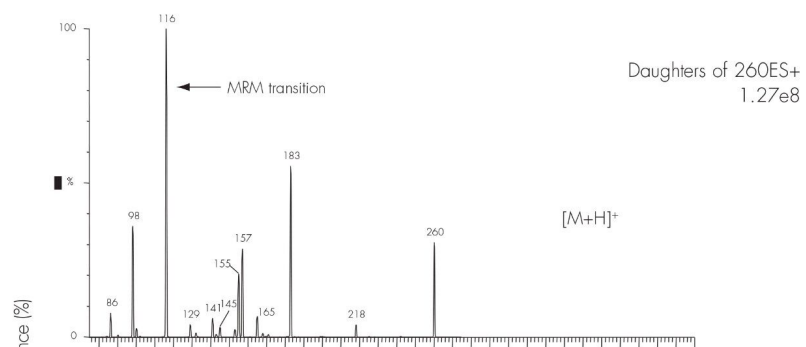


Conc. ng/mL n=6	Average	Standard deviation	RSD%
Propranolol calibration curve			
0.1	0.09	0.05	4.8
0.5	0.49	0.03	5.9
1.0	1.03	0.04	3.9
5.0	4.93	0.26	5.3
10.0	9.76	0.34	3.5
20.0	20.21	0.89	4.4
25.0	25.21	0.69	2.7
Naloxone calibration curve			
0.1	0.10	0.04	4.2
0.5	0.48	0.02	5.0
1.0	0.99	0.06	6.8
5.0	5.25	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



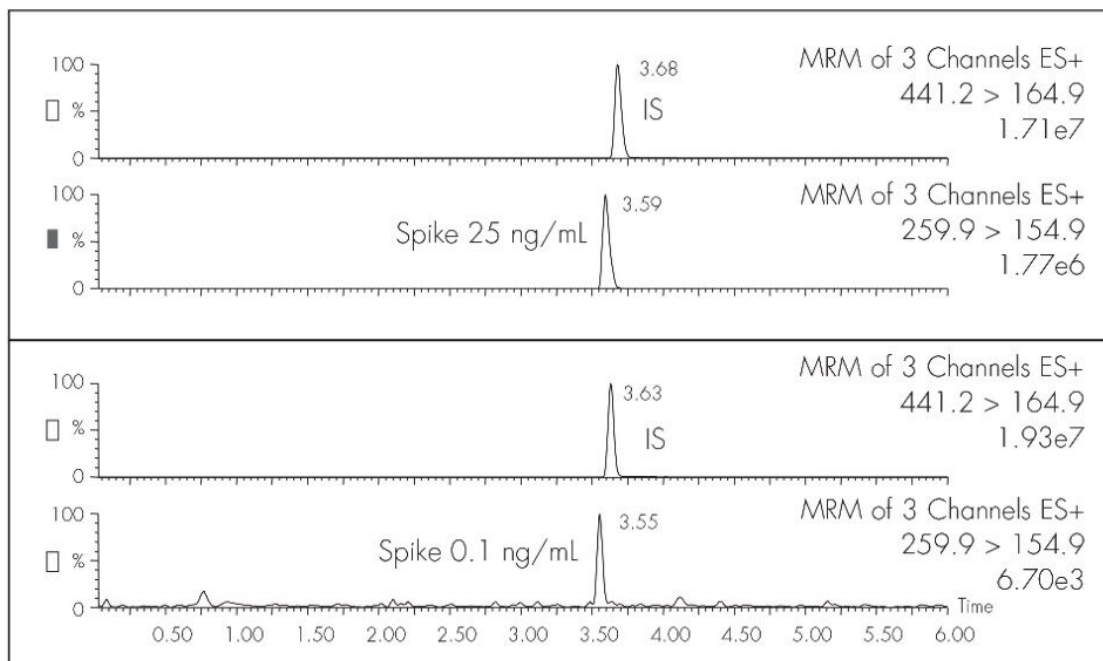
Results and Discussion

CID Mass Spectra

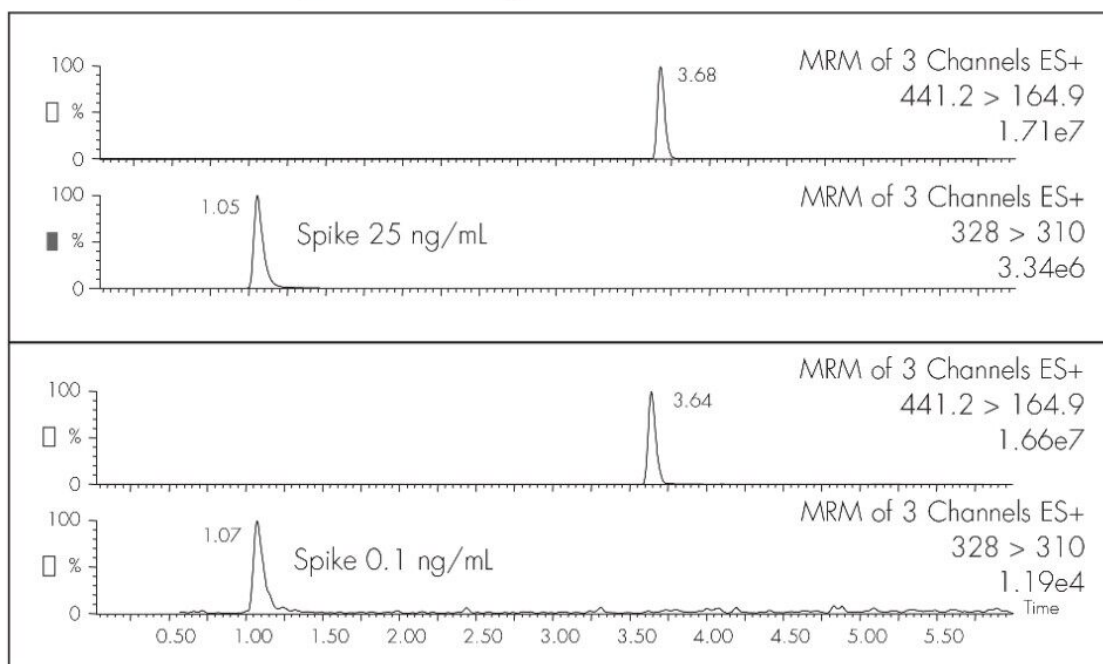


50/50 MEOH/Acetonitrile Cell Lysing

PROPRANOLOL AT 0.1 ng/mL AND 25 ng/mL



NALOXONE AT 0.1 ng/mL AND 25 ng/mL



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Alliance HPLC <<https://www.waters.com/514248>>

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