MORPHINE AND ITS GLUCURONIDE METABOLITE IN RAT PLASMA BY REVERSE-PHASE SPE

HPLC METHOD

COLUMN:	XTerra MS C_{18}, 2.1 x 30 mm, 3.5 µm
PART NUMBER:	186000398
MOBILE PHASE A:	Acetonitrile + 0.1 M NH_4HCO_3 pH 9.7
MOBILE PHASE B:	Water + 0.1 M NH_4HCO_3 pH 9.7
GRADIENT:	Time Profile

(min)	%A	%B
0	95	5
1	5	95

FLOW RATE:	0.2 mL/min
TEMPERATURE:	Ambient
LC:	Alliance® 2795
MS:	Micromass Quattro Altima™
ION SOURCE:	Electrospray positive
SOURCE TEMP:	150 °C
GAS CELL:	2.0 e-3 bar Argon
DESOLVATION TEMP:	350 °C
CAPILLARY VOLTAGE:	3.5 KV
DRYING GAS FLOW:	500 L/hr
CONE GAS FLOW:	50 L/hr
CONE VOLTAGE:	35 V

MRM TRANSITIONS

MORPHINE:	m/z 285.8, 152.8 (40 collision)
MORPHINE-GLU.:	m/z 462.0, 286.0 (25 collision)
MORPHINE-3D (I.S.):	m/z 288.9, 152.8 (40 collision)

OASIS® HLB GENERIC EXTRACTION METHOD

Conditions for Oasis® HLB µElution Plate
Part Number 186001828BA

CONDITION: 200 mL MeOH

EQUILIBRATE: 200 mL Water

LOAD: 35 mL spiked rat plasma

LOAD: 35 mL IS (25 pg/mL) in water

WASH: 200 mL 5% MeOH in water

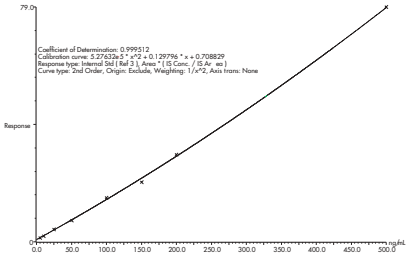
ELUTE: 25 mL ACN:IPA 40:60 + 2% FA

EVAPORATE:

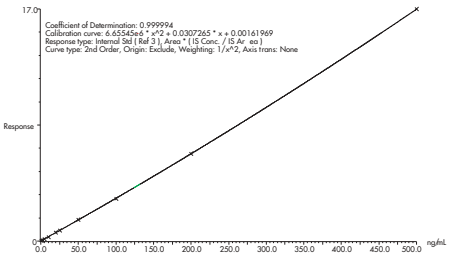
RECONSTITUTE: 75 mL water

INJECT: 40 mL

Morphine- glucoronide calibration curve

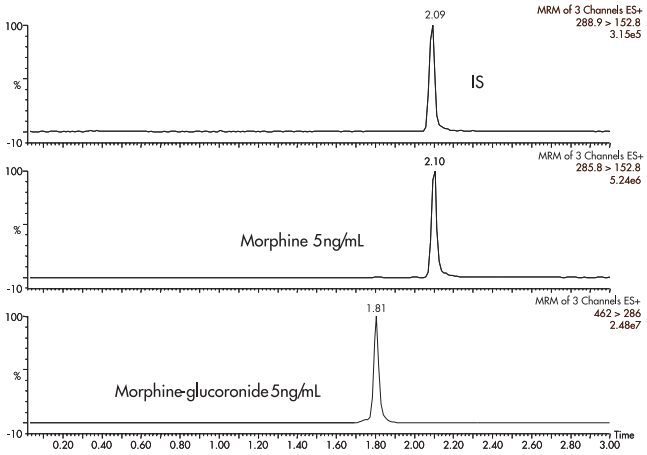


Morphine calibration curve



MORPHINE Nominal conc. (ng/mL)	Calculated conc.	MORPHINE-GLUCORONIDE Nominal conc. (ng/mL)	Calculated conc.
1.0	1.00	5.0	5.09
2.0	2.02	10.0	9.42
5.0	4.97	20.0	na
10.0	9.93	25.0	26.64
20.0	20.05	50.0	48.73
25.0	25.05	100.0	103.79
50.0	50.49	150.0	141.30
100.0	99.07	200.0	203.43
200.0	199.84	250.0	na
500.0	500.51	500.0	501.26

HLB



Oasis, Quattro, Ultima, XTerra and Waters are trademarks of Waters Corporation. © 2003 Waters Corporation