ß-Blockers Methodology

Sample Preparation

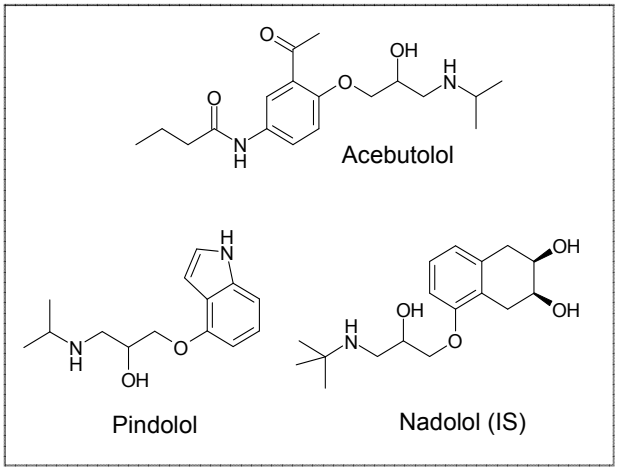
Protein Precipitation Method:

- 200µL plasma was spiked with
  - 50µL Nadolol IS (1.0µg/mL in water)
  - 50µL spike solution (from 0.8ng/mL – 600ng/mL in water)
  - Where the IS and/or spike solution was not required, the appropriate volume of water was added
- 600µL acetonitrile was added to crash proteins
- Centrifuged at 13,000rpm for 5 minutes
- 200µL of supernatant diluted with 800µL water prior to injection

Table 1: Spike Concentrations and their equivalent concentration in plasma

Spike Conc. (ng/mL)	Actual Conc. in Plasma (ng/mL)	Sample Type
0.8	0.2	Standard
2	0.5	
4	1	
20	5	
40	10	
200	50	
320	80	
400	100	
600	150	
0.8	0.2	QC
3	0.75	
80	20	
300	75	
360	90	
600	150	

Standard curves and QC samples were prepared as described above and in Table 1.



UPLC™ Conditions

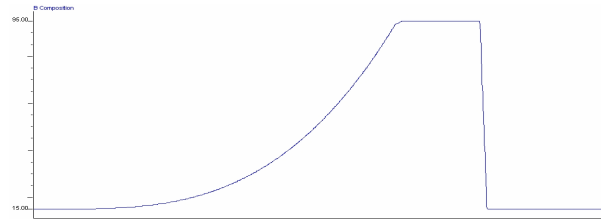
LC System:	Waters ACQUITY UPLC™ System
Column:	Waters ACQUITY UPLC™ BEH C_{18} 2.1x50mm, 1.7µm particles
Eluents:	A: 2mM ammonium acetate +0.1% formic acid in water B: 0.1% formic acid in acetonitrile
Column Temp:	40ºC
Sample Temp:	4ºC
Flow Rate:	0.6 ml/min

Gradient:

Time	%A	%B	Curve
0.0	85	15	-
0.8	5	95	8
1.0	85	15	11

Run Time:	1.6 minutes
Injection Volume:	20µL

Curve 8 Gradient Profile



MS Conditions

MS System:	Waters Micromass® Quattro Premier™
Ion Mode:	Electrospray +ve
Capillary Voltage:	3.00kV
Source Temp:	120ºC
Desolvation Temp:	380ºC
Desolvation Gas Flow:	1000L/hour
Cone Gas Flow:	50L/hour
Detection Mode:	MRM (see below)

Compound	Transition	Cone (V)	Collision Energy (eV)
Acebutolol	337.25>116.00	35	22
Pindolol	249.15>116.00	35	18
Nadolol (IS)	310.30>201.20	25	20

Dwell Time:	0.02 seconds
Inter-scan Delay	0.01 seconds
Collision Gas:	Argon ($3.45x10^{-3}$mbar)