

Waters® Alliance® LC/MS System



Experiment Conditions
MS & UV
Chromatograms
Quantitation

Key Word:

ESI LC/MS
OASIS HLB Plate
Quantitation
Pharmaceutical
Human Urine

LC/MS Coupled with 96-well OASIS® HLB Plate for Drug Mixture Analysis in Human Urine

Kate Yu, Waters Corporation, Milford, MA

As a detector, the mass spectrometer is well recognized for its superb sensitivity and selectivity. The combination of the HPLC/MS offers a wide scope of applications. For example, assays in biological fluid have become a relative easy task with a single quadrupole LC/MS, especially with an SPE sample prep step.

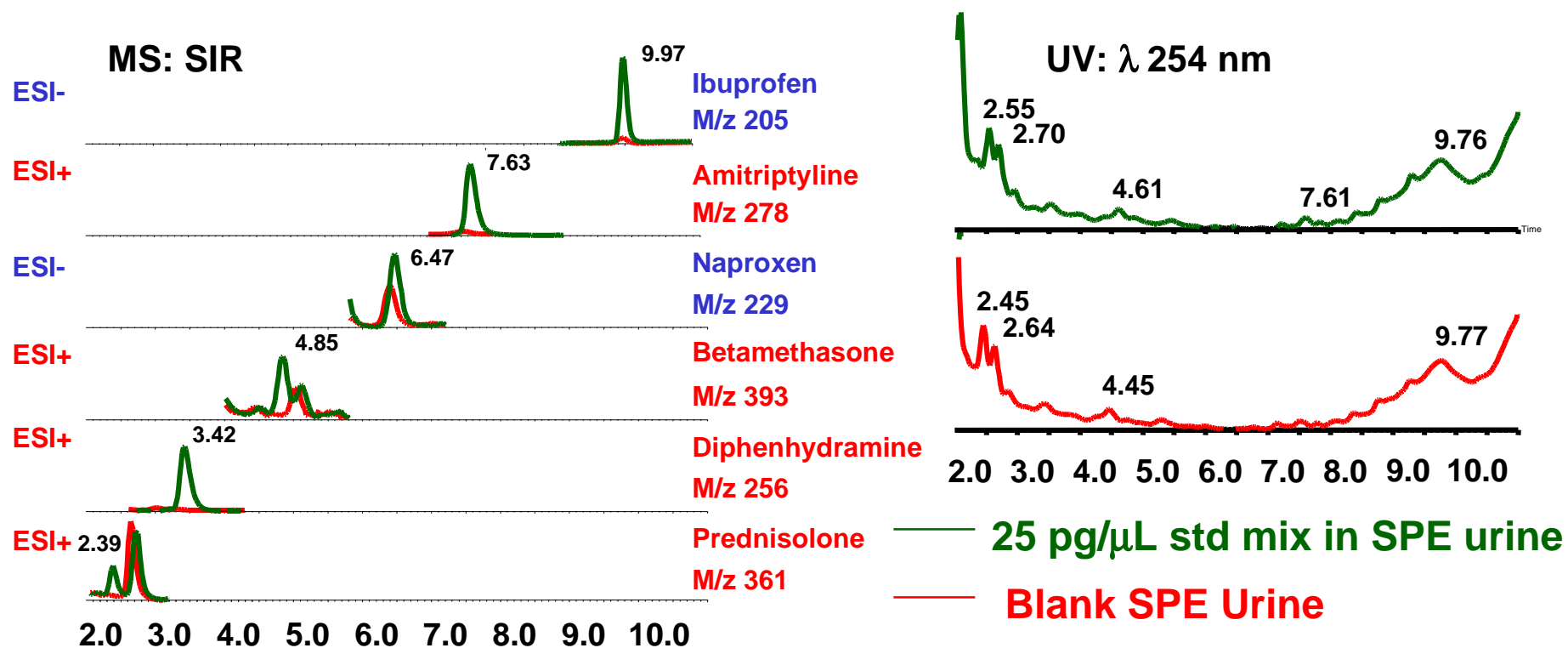
The Waters OASIS HLB extraction plate utilizes a polymeric packing contains both hydrophobic monomer units and hydrophilic monomer units. This unique packing feature allows a simple and general method be used to a wide range of compounds including acids, neutrals and bases in biological fluid.

The purpose of this work is to demonstrate the analysis of a drug mixture exhibiting widely diverse properties from a biological matrix, utilizing the Waters OASIS HLB extraction plate for sample preparation and Waters Alliance ZMD LC/MS for separation and detection. The model used is a mixture of six drugs including acids, neutrals and bases. With the unique feature of OASIS HLB sorbent, and the ZMD's user-friendly Masslynx software (which allows polarity switching within a run), all six analytes with different chemical properties in human urine can be analyzed simultaneously with a single injection.

Experiment Conditions

- **SPE: Waters OASIS HLB Extraction Plate (10 mg)**
 - Sample loading: 1.6 mL
 - Wash: 5% MeOH 200 μ L + 600 μ L
 - Elute: MeOH 200 μ L + Water 600 μ L
- **HPLC: Waters Alliance[®] 2690 Separation Module**
 - Column: Waters Symmetry[®] C₁₈ 2.1 x 50 mm
 - Mobile Phase: A: 10 mM NH₄OAc in Water pH 5.0
B: 10 mM NH₄OAc in AcN pH 5.0
 - Gradient: 75%A to 25%A in 8 minutes
 - Flow Rate: 0.3 mL/min
 - Injection Volume: 10 μ L
- **PDA: Waters 996 PDA Detector**
 - Range: 210 - 320 nm
 - Resolution: 1.2 nm
- **MS: Waters ZMD 2000 MS Detector**
 - Ionization Mode: Electrospray
 - Data Collection: Single Ion Recording (SIR)

MS & UV Chromatograms



Compared with the MS, UV lacks the selectivity and sensitivity at the level shown above. The two graphics are from the same injections. The LC flow goes into PDA, then MS in sequence.

Quantitation by MS in SPE Human Urine

	Concentration (pg/uL)	Recovery (%)	RSD (%) n = 5	Linearity	LOD (pg/uL)	LOQ (pg/uL)
Prednisolone	43.5	111.0	3.13	0.999	30.4	101.0
Diphenhydramine	47.0	90.2	3.30	0.998	13.8	45.8
Betamethasone	32.5	102.0	8.16	0.999	16.6	55.1
Naproxen	44.0	150.0*	20.90	0.999	16.8	55.8
Amitriptyline	46.0	90.4	2.08	0.997	14.8	23.9
Ibuprofen	49.0	123.0	11.00	0.999	17.2	23.9

* The relative high recovery and RSD of Naproxen may be due to the background interference from the matrix as shown in MS SIR chromatogram on previous page.

