Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

A High Throughput UPLC-MS Method in Support of Cleaning Validation Studies

Kenneth J. Fountain, Marleen van Wingerden, and Diane M. Diehl,
Waters Corporation

A UPLC-MS method employing a short, 30 mm long column and a fast-scanning single quadrupole MS detector was used for validation of cleaning procedures for eight active pharmaceutical ingredients (APIs). Total cycle time of the method is 1.2 min, and detection limits are in the 0.2–4 ng/mL range.

Equipment used in the production of drug products must be properly cleaned in order to avoid cross-contamination of subsequent batches. The safety acceptance criteria for API residues vary with drug substance. For example, more potent compounds will require lower safety acceptance limits. In general, most processes aim to have a lower acceptance limit in the 10 ppb–1 ppm range (10 ng/mL–1 µg/mL). In order to achieve these limits, sensitive analytical techniques are required.

Ultra Performance Liquid Chromatography (UPLC® technology) in conjunction with ultra-violet (UV) detection can provide a high degree of assurance that the API residue is below the safety acceptance limit in a relatively short period of time (less than 5 min analysis time). In cases where UV is not sensitive enough, mass spectrometry (MS) is a useful addition for detecting low levels of residual drug substances.

The current work highlights the use of UPLC-MS for the validation of cleaning procedures for eight APIs. A single UPLC analysis for all eight compounds with a cycle time of less than 2 min was desired. Previously, eight different HPLC methods were needed to measure drug residue levels after reactor vessel cleaning. This represents a significant reduction in quality control (QC) laboratory operating costs (i.e., less mobile phase preparation, less instrument down time), and an overall increase in productivity.

Experimental Conditions

UPLC Conditions

System:	ACQUITY UPLC® System with PDA detector
Column:	ACQUITY UPLC BEH C_8, 2.1 × 30 mm, 1.7 µm (Part # 186003910)
Mobile Phase:	A: 10 mM 10 mM NH_4HCO_3, pH 10.0 B: Acetonitrile (ACN)
Gradient:	35–100% B in 0.3 min, hold at 100% B for 0.45 min, reset (1.2 min total run time)
Flow Rate:	0.8 mL/min
Injection:	2 µL
Temperature:	50 °C
Detection:	UV @ 230 nm
Sampling rate:	40 Hz, no digital filter
Samples:	Compounds are proprietary drug candidates; molecular weight and dilution solvent information can be found in Table 1.

Mass Spectrometry

System:	ACQUITY™ SQ detector
Mode:	Electrospray Positive
Capillary:	3500 V

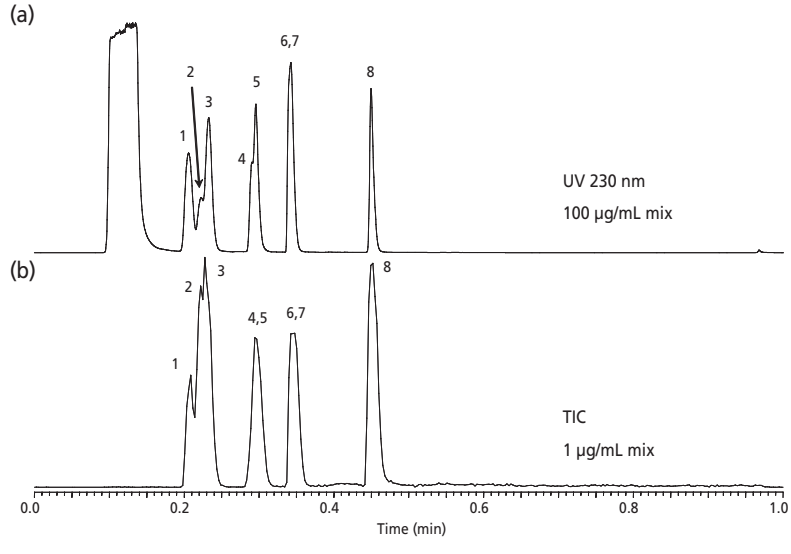

Figure 1: Separation of eight APIs in both UV (a) and MS (b). The large peak in the void (UV trace) is due to the presence of 10 % DMF in the sample.

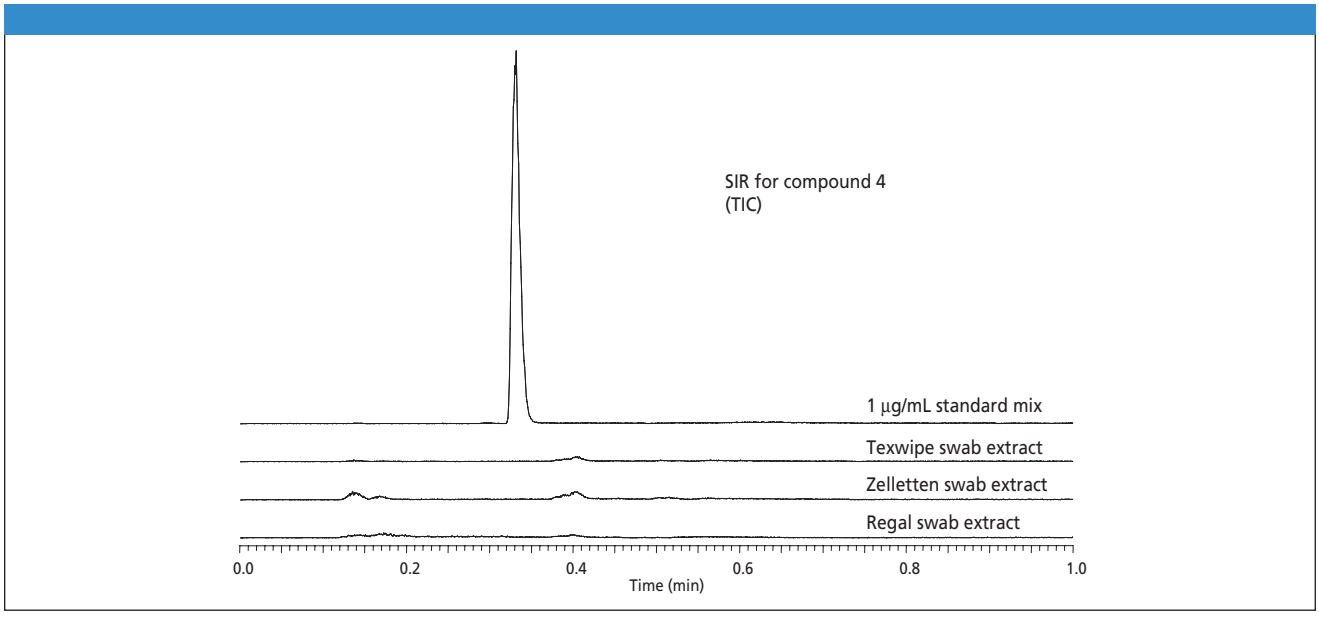

Figure 2: SIR traces of compound 4 for a 1 µg/mL standard mixture of all 8 APIs overlaid with three different swab extractions in methanol.

Cone:	30 V
Desolv. Temp:	500 °C
Desolv. Gas:	800 L/hr
Source Temp:	150 °C
Range:	150–750 *m/z*
Scan rates:	Full scan - 0.06 s scan
	SIR- 0.005 s dwell

Results and Discussion

The UPLC separation of the eight APIs can be seen in Figure 1. All compounds elute within the gradient window, and MS analysis using the SQ detector confirmed the elution order without the need for individual injections.

Repeatability of the method was determined by performing 100 injections of a 50 µg/mL mixture of compounds 1 (most hydrophilic), 4, and 8 (most hydrophobic) on two different columns. RSD values for retention times and peak areas (UV 230 nm) were 0.6% and 1.0%, respectively. The method was linear in the 0.05–50 µg/mL range for UV (R^2 = 0.9999–1.000). Detection limits for UV were in the 100–200 ng/mL range, while MS detection limits were 0.2–4 ng/mL.

Interferences eluting in the separation window can complicate accurate quantitation and identification of residual drug product after vessel cleaning. The UPLC-MS method was evaluated for the presence of interferences originating from cleaning solvents and swab extractions in both UV and MS. Figure 2 shows the single ion recording (SIR) traces of compound 4 for a 1 µg/mL standard mixture of all eight APIs, and for three different swab extractions in methanol. It is clear that all swab extracts are free from interferences, which allows for accurate detection of API residues in the ng/mL range.

Table I: Selected compounds with molecular weight and dilution solvent. Compounds are listed in order of elution.

Peak	Molecular Weight (g/mol)	Dilution Solvent
1	367.1663	water
2	204.0721	methanol
3	313.0863	N, N-dimethylformamide (DMF)
4	244.1212	methanol
5	379.1696	N, N-dimethylformamide (DMF)
6	661.8522	acetone
7	296.0483	2-propanol
8	368.2252	ethanol

Conclusions

A single UPLC-MS method was developed in order to support the validation of cleaning procedures for eight active pharmaceutical ingredients. Cycle time of the method is 1.2 min, which is suitable for high throughput analyses. This work demonstrates the utility of UPLC-MS for improving the efficiency and productivity of a pharmaceutical QC laboratory. Possessing the ability to separate many compounds in less than 0.5 minutes dramatically increases throughput and minimizes costs associated with mobile phase consumption and disposal. The presence of the SQ mass detector eliminates the error associated with peak identification.

Waters Corporation
34 Maple Street, Milford, MA 01757
tel. (508) 478-2000, fax (508) 478-1990
www.waters.com