Waters™

应用纪要

Reducing Risk in Cleaning Validation and Cleaning Verification Workflows by Implementation of Mass Detection

George Wilson, Marian Twohig, Stephanie Harden, Cristian Cojocariu

Waters Corporation, United States

Published on September 09, 2026

Abstract

Cleaning validation and cleaning verification are critical activities that prevent cross-contamination of different products which are manufactured using shared equipment. Effective cleaning validation and verification strategies minimize the risk to patient/consumer safety while maximizing the up-time of manufacturing equipment, ultimately maximizing batch output. In this study, the benefits of mass detection in cleaning verification workflows are presented using a range of active pharmaceutical ingredients (APIs) that span highly potent APIs, compounds with poor chromophores and generics.

Benefits

- Mass detection is extremely useful where analytes do not possess a strong chromophore for UV detection
- Mass detection provides increased sensitivity compared to UV, useful when cleaning limits are low (e.g. ppb level) or when high baseline drift is observed using optical detection

- Selectivity of selected ion recording mode reduces the burden on the chromatographic method to separate all sample components
- Empower™ 3 Chromatography Data System (CDS) can be used to automatically flag out of specification cleaning results in customizable reports

Introduction

Cleaning validation is the process of developing and justifying an effective cleaning strategy that prevents cross-contamination. During cleaning method development, different solvents and procedures are evaluated at laboratory scale, with analytical data collected to support selection of the most effective process. During cleaning validation, the cleaning procedure is implemented at manufacturing scale with further analytical data collected across multiple production batches to demonstrate effectiveness and reproducibility. Cleaning verification is then required to ensure continued effectiveness during routine manufacturing.

An important aspect of cleaning validation is establishing an acceptable level of product carryover. European Medicines Agency (EMA) guidance recommends calculating permitted daily exposures (PDEs) using toxicological data.[1] The PDE is then used to derive cleaning limits. Two of the most common tests for monitoring residual product/degradant/by-product are the rinse test and the swab test. In rinse testing, the solvents used to clean manufacturing equipment are analyzed for product residues. Swab testing involves directly swabbing different locations within the equipment and subsequently extracting and analyzing the swabs for residues. For biotherapeutics, alternative approaches may be appropriate, as demonstrating API degradation is often considered sufficient to prove cleaning effectiveness. It is expected that analytical data be collected to support this strategy, however.

UV spectroscopy or LC-UV methods often provide sufficient sensitivity to demonstrate compliance with cleaning limits. However, these techniques lack the excellent selectivity and sensitivity offered by mass detection. With standalone UV detection, any sample component that absorbs at the quantification wavelength can interfere with the assay. Similarly, co-eluting compounds in LC-UV analyses may not always be readily apparent. In contrast, selected ion recording (SIR) mass detection enables highly selective monitoring of target analytes, reducing dependence on complete chromatographic separation and increasing confidence in analyte identification and quantification. This can also shorten method

development time.

This application note demonstrates how the ACQUITY™ QDa II Mass Detector can support practical cleaning verification workflows for a diverse set of APIs, including challenging compounds with weak UV response and highly potent APIs with low cleaning limits. By providing greater confidence in residue detection, traceable data management, and automated exception reporting, mass detection helps manufacturers maintain audit-ready cleaning verification processes while reducing compliance risk.

Experimental

Ten APIs were chosen to demonstrate the utility of mass detection for cleaning verification workflows. This range of compounds was selected to cover analytes with weak/no chromophore (as is the case for azithromycin, gabapentin, and bempedoic acid), and highly potent compounds which typically require lower cleaning limits (for example, budesonide and decitabine). Based on solubility of each API, a suitable class two or three solvent as described in ICH Q3c[2] was selected to be used as a cleaning solvent. The full list of compounds and cleaning solvents is given in Table 1.

Compound	Cleaning Solvent	Molecular Weight (g/mol)	Classification
Azithromycin	Isopropanol	748.98	Macrolide antibiotic
Cefalexin	Methanol	347.39	First-generation cephalosporin antibiotic
Gabapentin	Methanol	171.24	Anticonvulsant / neuropathic pain agent
Artemisinin	Ethanol	282.33	Antimalarial
Budesonide	Ethanol	430.54	Corticosteroid (glucocorticoid anti-inflammatory)
Bempedoic Acid	Ethanol	344.49	Lipid-lowering agent (ATP-citrate lyase inhibitor)
Decitabine	Dimethylsulfoxide	228.21	Antineoplastic agent; DNA methyltransferase inhibitor
Metformin	Methanol	129.16	Antidiabetic (biguanide)
Carboprost	Ethanol	368.51	Prostaglandin analogue / uterotonic agent
Sitagliptin	Isopropanol	407.31	Antidiabetic (DPP-4 inhibitor)

Table 1. Details of APIs chosen for this study. These analytes were chosen as they belong to a wide variety of therapeutic classifications, provide examples of compounds with weak/no chromophore, while some are considered highly potent and therefore typically require lower cleaning limits.

Chromatographic Method Development

An initial generic chromatographic method screening strategy was applied using a single column and two mobile phase pH (low and high). Chromatographic conditions and gradient are in Table 2. The gradient timetable was designed to deliver 0.05% v/v formic acid *or* ammonium hydroxide in the mobile phase.

LC Conditions

System:	ACQUITY ARC™ HPLC System with PDA Detector and ACQUITY QDa II Mass Detector
Column:	XBridge™ C_{18} Column, 3.5 µm, 4.6 x 50 mm *(p/n: 186003031)*
Column temperature:	40 °C

Flow rate:	1.0 mL/min
Mobile phase A:	Water
Mobile phase B:	Acetonitrile
Mobile phase C:	1% Formic acid in water (Low pH method)
Mobile phase D:	1% Ammonium hydroxide in water (High pH method)
Injection volume:	Compound dependent

Time (min)	A%	B%	C or D%
0	90	5	5
0.5	90	5	5
2.5	0	95	5
3.5	0	95	5
3.6	90	5	5
6.0	90	5	5

Table 2. Generic Reversed Phase Chromatographic conditions for screening purposes.

Unacceptable tailing was observed for Gabapentin when analyzed under the low pH condition whilst poor retention was observed at the high pH condition. A common strategy to reduce tailing at low pH with UV detection is to use ion pairing additives such as trifluoroacetic acid (TFA). Whilst TFA meets the volatility requirements for mass detection, its use often results in increased background and suppression of analyte ionization , especially where negative ion mode is used. TFA is also known to be persistent and difficult to remove from surfaces within both the LC and the MS. Difluoroacetic acid (DFA)

is an alternative to TFA that is less of an ionization suppressant and less persistent in the MS source. Figure 1 shows the chromatography obtained for Gabapentin when formic acid, TFA, and DFA were used as mobile phase modifiers. 0.1% v/v DFA was selected as the additive for the cleaning verification SIR method. Waters offers an LCMS certified grade DFA known as IonHance™ (p/n:186009201 < https://www.waters.com/nextgen/global/shop/standards--reagents/186009201-ionhance-difluoroacetic-acid-1-vial.html>).

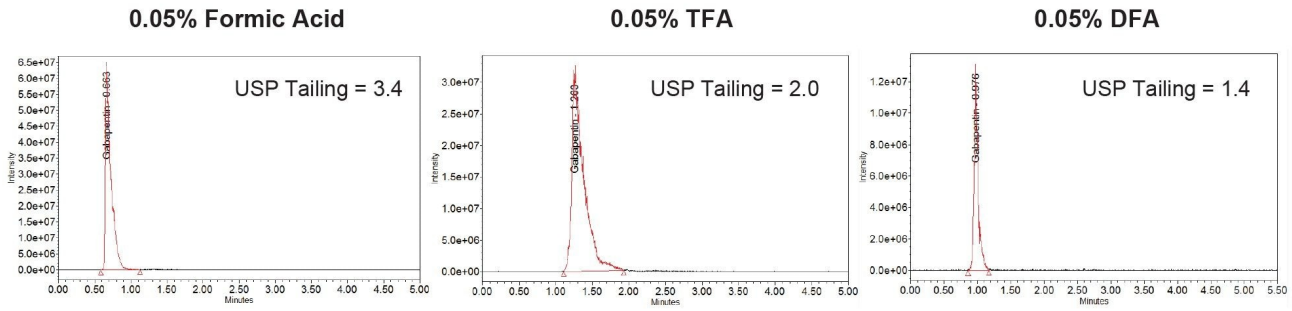

Figure 1. Tailing for Gabapentin was unacceptable with formic acid as acidic modifier. TFA is commonly used with UV detection to improve tailing but often results in unacceptable ion suppression in mass spectrometry. DFA is a less persistent and suppressing alternative for mass detection. USP Tailing was automatically calculated in Empower with the system suitability option installed.

Due to their highly polar nature, an alternative retention mechanism was required for metformin and decitabine. Hydrophilic interaction chromatography (HILIC) was selected with the chromatographic conditions in Table 3 found to give suitable chromatography.

Chromatography Conditions

System:	ACQUITY ARC System with PDA Detector and ACQUITY QDa II Mass Detector
Column:	Atlantis™ Premier BEH™ Z-HILIC Column, 2.5 µm, 2.1 mm x 50 mm *(p/n: 186009985)*

Column temperature:	30 °C
Flow rate:	1.0 mL/min
Mobile phase A:	90/10 v/v acetonitrile/water + 10 mM ammonium formate + 0.1% v/v Formic acid
Gradient:	Isocratic
Run time:	3 minutes

Table 3: HILIC Chromatographic conditions for Metformin and Decitabine.

Developing an SIR method for cleaning verification is a straightforward process. If the MS base peak for the analyte is unknown, it is recommended to prepare a standard of reasonable concentration (e.g. 0.01-0.1 mg/mL) and acquire both positive and negative MS scans whilst applying a generic chromatographic gradient. Additional detectors (e.g. UV, ELSD, CAD) may be used to aid identification of the retention time. The best chromatographic conditions (e.g. column and mobile phases) are then chosen to suit the analyte based on retention, peak shape etc. When acquiring MS scan data for method development, it is recommended to limit the scan range to that of the expected *m/z* (e.g. for a typical small molecule analyte with exact mass of 350, a suitable scan range would be 100-450 *m/z* to ensure collection of any multiply charged species and/or common adducts).

Once the base peak is identified, an SIR method can be written for quantification of residual analyte in cleaning samples. Figure 2 shows how the MS scan data channels were used to define the SIR method for sitagliptin. A similar process was followed to define SIR methods for all the APIs in this study. In most cases, the recommended capillary voltages and cone voltages were selected as described in the ACQUITY QDa II Mass Detector – starting point settings and optimization guidelines.[4] Further optimization of MS parameters was not required except for decitabine where a higher cone voltage was selected to deliberately generate the in-source fragment ion, *m/z* = 113.0. The fragment ion was used for quantitation because multiple adduct species were observed, with apparent differences in their distribution across the calibration range. These differences were hypothesized to result in non-linear

behavior at higher decitabine concentrations. Using the in-source fragment ion gave a linear calibration curve. All other MS parameters (e.g. desolvation temperature) were left as default for all compounds. A summary of MS parameters and chromatographic methods can be found in Table 4.

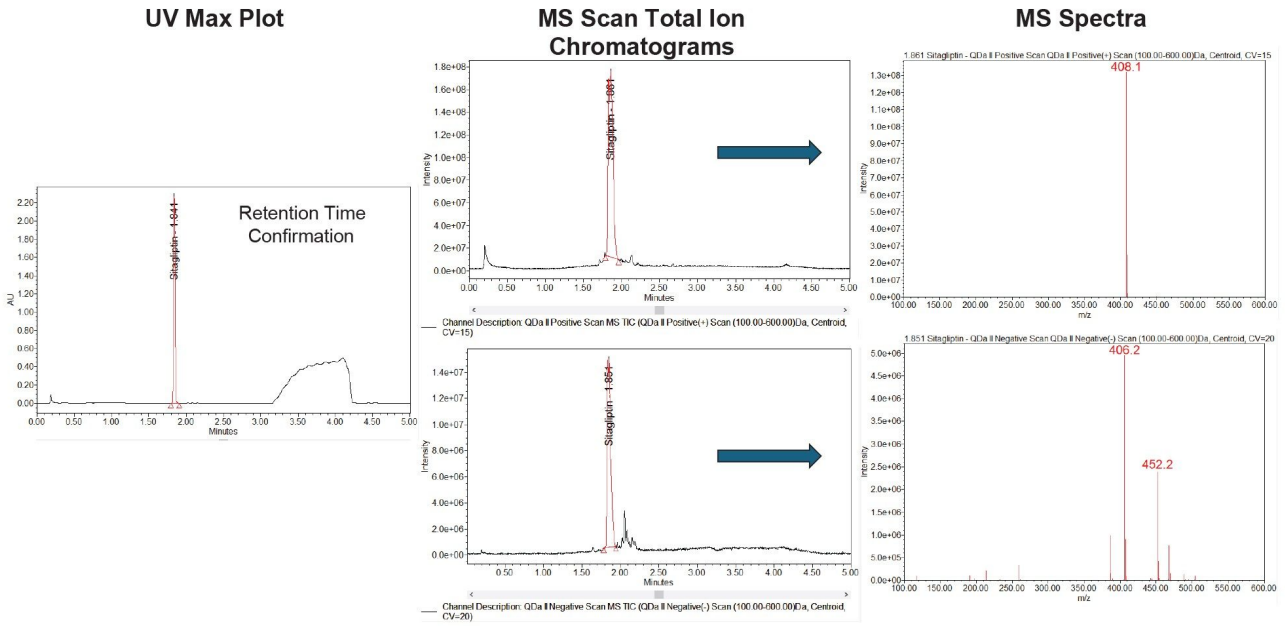

Figure 2. ESI+ and ESI- scans were collected to confirm the retention time and MS base peak for sitagliptin before writing the SIR method. In this case, UV Max Plot spectra were also used to aid confirmation of retention time, since sitagliptin has a strong chromophore. Based on the observed mass spectra, ESI+ m/z 408.1 was selected for the SIR method as this gave the most intense mass spectrum.

Compound	Method*	Monoisotopic Mass (g/mol)	Polarity for SIR	*m/z* for SIR	Cone Voltage (v)	Capillary voltage (kV)
Azithromycin	RP, high pH	748.51	ESI+	749.5 $[M+H]^+$	15	0.8
Cefalexin	RP, low pH	347.09	ESI+	348.1 $[M+H]^+$	15	0.8
Gabapentin	RP, low pH with DFA	171.13	ESI+	172.1 $[M+H]^+$	15	0.8
Artemisinin	RP, low pH	282.15	ESI+	283.1 $[M+H]^+$	15	0.8
Budesonide	RP, low pH	430.24	ESI+	431.2 $[M+H]^+$	15	0.3
Bempedoic Acid	RP, low pH	344.26	ESI-	343.3 $[M-H]^-$	20	0.8
Decitabine	HILIC	228.09	ESI+	113.0 (Fragment)	30	0.8
Metformin	HILIC	129.10	ESI+	130.1 $[M+H]^+$	15	0.8
Carboprost	RP, low pH	368.26	ESI-	367.3 $[M-H]^-$	20	0.8
Sitagliptin	RP, high pH	407.12	ESI+	408.1 $[M+H]^+$	15	0.8

RP = Reversed Phase

* High and low pH refers to the conditions as described in TABLE 2, HILIC refers to the conditions in TABLE 3.

Table 4. Details of SIR methods chosen for the APIs in this study. In all cases, the default cone voltage (15 V for ESI+, 20 V for ESI-) was used except for decitabine where a higher cone voltage was used to promote fragmentation. Sampling rate was chosen to ensure a minimum of 15 points across the SIR peak to ensure suitable peak definition for accurate quantitative measurements.

Evaluation of Mass Detection in Cleaning Verification

Cleaning limits are often calculated on a case-by-case basis considering inherent toxicity and risk of exposure of cross-contaminated API to the patient. However, for many APIs, cleaning limits often lie in the approximate range of 1-10 parts per million (ppm). Calibration curves for each API were prepared in cleaning solvent (Table 1) over the range 0.1-1.5 µg/mL (0.1-1.5 ppm) and injected. This range was selected to avoid saturation of the mass spectrometer ion source or detector. For APIs with a typical cleaning limit of 10 ppm, a 1:10 dilution would place samples within the calibration range, giving an effective reporting range of 1-15 ppm. For APIs with lower reporting limits (~1 ppm) such as highly potent APIs, rinse cleaning samples may be analyzed without dilution.

Linear fits were applied and the residuals in the calibration curves were assessed with appropriate weighting models selected using Empower 3 Software. Weighting ensures that the calibration is sufficiently accurate across the entire range as linear models can often suffer bias in either the high or low concentration ranges of the curve. The simplest weighting models were applied as a preference. Concentration deviations of ±20% at the limit of quantitation (LOQ) (set as the lowest point on the calibration curve) and ±15% at all other concentrations were targeted. The resulting calibration plots,

weighting methods, and coefficients of determination are displayed in Figure 3.

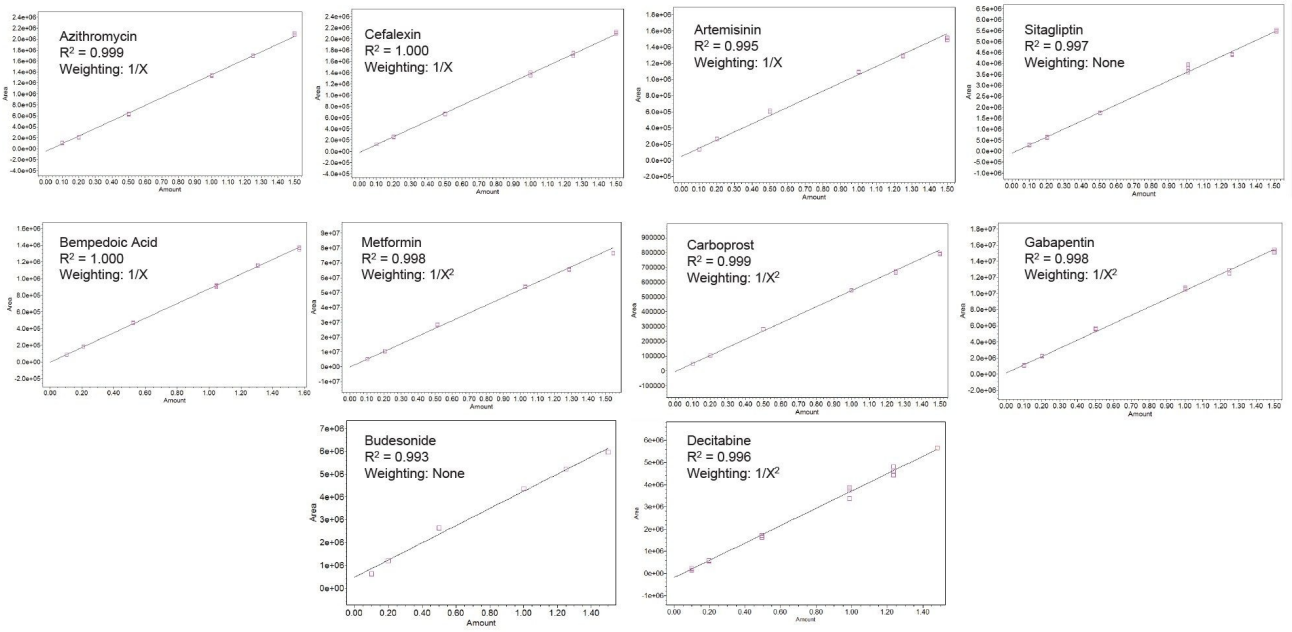

Figure 3. Calibration curves for all analytes with coefficient of determination and selected weighting models. All models demonstrated excellent linearity across the range (>0.99 coefficient of determination).

After demonstrating linearity, five compounds (Metformin, Bempedoic acid, Sitagliptin, Decitabine, and Azithromycin) were selected to assess the accuracy when analyzing simulated cleaning samples. A highly accurate analytical cleaning method reduces the risk to patient safety and prevents unnecessary equipment downtime. To prepare representative cleaning samples, a 1 mg/mL stock solution of each API was prepared and serial-diluted in cleaning solvent (Table 1) to achieve a solution concentration of 11 ppm (11 ug/mL). These solutions are intended to mimic the solutions that would be supplied to the QC lab as part of rinse cleaning verification. A cleaning limit of 10 ppm was arbitrarily assigned to azithromycin, metformin, bempedoic acid, and sitagliptin; therefore, the solution concentration of 11 ppm represents a borderline “fail” scenario. For decitabine, a cleaning limit of 1 ppm was assigned to reflect its classification as a highly potent API, for which a lower cleaning limit typically applies. Sample solutions were diluted 1 in 10 with cleaning solvent prior to analysis and quantified against the calibration curves. Although a swab accuracy test was not conducted, the procedures employed after sample collection and swab extraction are expected to be equivalent to those described in this study.

The recovery data obtained can be found in Table 5. Excellent recoveries were obtained in the region of 94-108% across all compounds, giving the user confidence that the correct result would be obtained in routine cleaning verification assays.

Analyte	Actual Cleaning Sample Concentration (ppm)	Measured Cleaning Sample Concentration (ppm)	Recovery (%)
Metformin	11.8	11.7	100%
Bempedoic Acid	10.8	11.4	106%
Sitagliptin	10.8	10.6	98%
Decitabine	10.8	1.1	94%
Azithromycin	11.3	12.2	108%

Table 5. Recovery data for simulated cleaning samples. Recovery determined based on single measurement.

It is important that any samples failing to meet specification are promptly identified and appropriate action is taken. Empower Software supports this process by automatically flagging results that fall outside predefined acceptance criteria, displaying them in bold, red, italicized text within the review window. Additionally, custom report templates can be configured to clearly highlight results which exceed cleaning limits, as illustrated in Figure 4. This functionality improves result visibility and helps reduce the risk of errors, such as incorrect rounding when comparing results against specification limits.

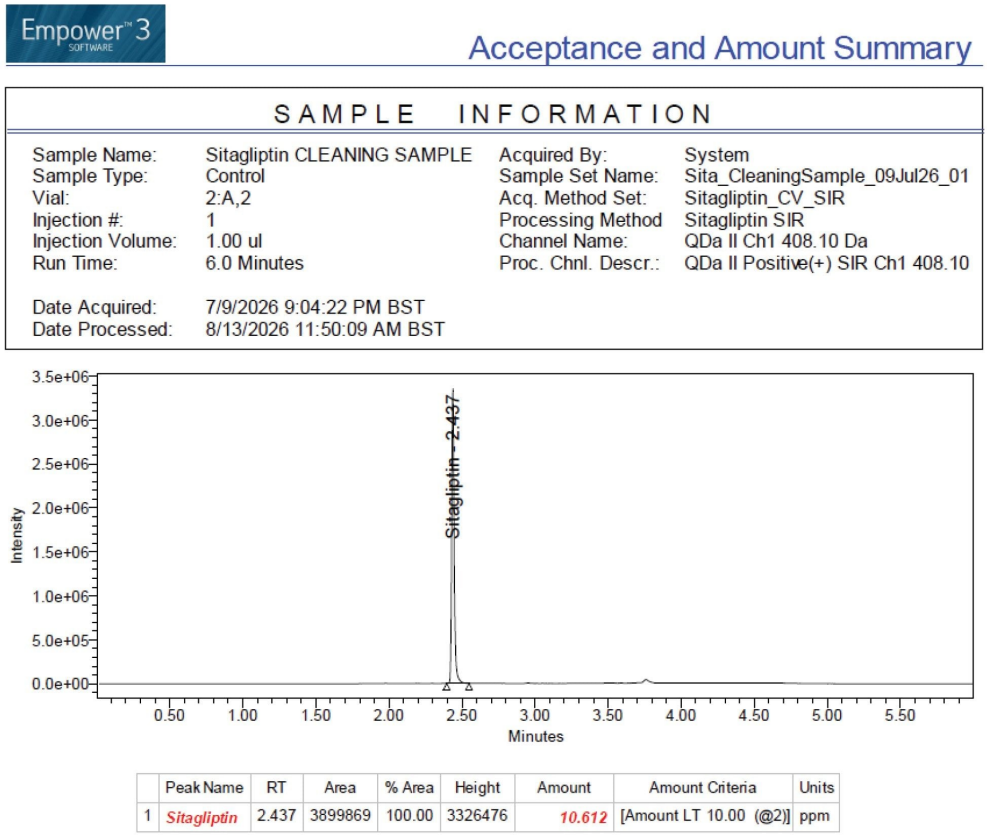

Figure 4. Empower reports can be user-configured to automatically flag results that exceed pre-defined acceptance limits. This improves result visibility and reduces the likelihood of rounding errors when comparing results against specifications.

Sitagliptin and azithromycin were selected to evaluate measurement repeatability with the ACQUITY QDa II Mass Detector. For both analytes, six-replicate cleaning sample preparations were made by dilution of a single simulated cleaning sample (~11 ug/mL) and injected. The results are displayed in Table 6. In both cases, good measurement precision was observed indicating a stable response from the ACQUITY QDa II Mass Detector.

Sitagliptin			
Replicate	**Theoretical Cleaning Sample Concentration (ppm)**	**Measured Sample Concentration (ppm)**	**Recovery (%)**
1	10.8	11.1	98%
2		11.4	101%
3		12.2	108%
4		11.7	104%
5		11.7	104%
6		11.7	103%
	Average	11.6	103%
	Standard Deviation	0.4	
	RSD (%)	*3.4*	

Azithromycin			
Replicate	**Theoretical Cleaning Sample Concentration (ppm)**	**Measured Sample Concentration (ppm)**	**Recovery (%)**
1	11.3	10.2	90%
2		10.3	91%
3		10.2	90%
4		10.4	92%
5		10.5	93%
6		10.0	89%
	Average	10.3	91%
	Standard Deviation	0.2	
	RSD (%)	*1.7*	

Table 6. Repeatability data for Sitagliptin and Azithromycin. Six replicate samples were prepared by dilution of a single cleaning sample and injected.

It should be noted that for many of the compounds in this experiment, assays based on UV detection would be much more challenging to develop and, in some cases, would likely incur higher risk of routine system suitability test (SST) failures. For example, Figure 5 shows the considerable increase in USP S/N

when switching from UV detection to SIR mass detection for azithromycin. While the development of a UV assay may be possible for azithromycin, the UV spectrum does not support robust quantitative analysis across the 200-400 nm range due to the absence of a distinct and reliable λ_{max}. Furthermore, Figure 5 demonstrates that the baseline drift commonly observed in gradient methods for UV based assays, which can challenge sensitivity, is typically far less significant in SIR mass detection. This is because UV detection measures the overall optical properties of the mobile phase, which vary throughout the gradient, whereas SIR mass detection is highly selective for the target *m/z* and is therefore much less influenced by changes in eluent composition during the gradient.

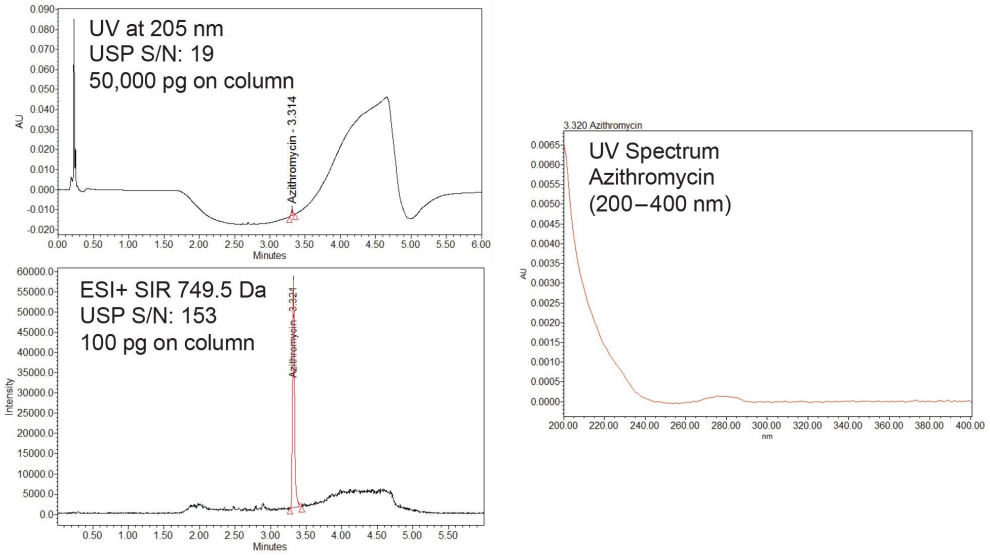

Figure 5. Comparison of UV sensitivity and MS sensitivity for Azithromycin. Despite a 500-fold lower on-column load, MS detection provided a USP S/N ratio that was 8-fold higher. Note that for Azithromycin, it is likely that the UV assay would be unreliable due to the absence of a UVmax In the range 200-400 nm. S/N was calculated using noise based on peak region in the blank injection.

Conclusion

In this application note, analytical cleaning methods were developed and applied for a diverse range of small molecule APIs using the ACQUITY QDa II Mass Detector operating in single ion recording mode.

Compared with UV-based approaches, mass detection offers enhanced selectivity and sensitivity, reducing reliance on complete chromatographic separation and helping to overcome challenges associated with baseline drift and limited UV response observed for poor chromophores. This was clearly demonstrated in the comparison of the UV and SIR chromatograms generated for azithromycin, where mass detection provided reduced baseline drift and superior USP S/N with much lower sample loading.

Excellent linearity, accuracy, and precision were achieved for all compounds investigated, supporting reliable quantification of cleaning residues at levels relevant to routine cleaning verification (~1-10 ppm). Such robust analytical performance enables confident assessment of cleaning effectiveness, helping manufacturers maintain patient safety by minimizing the risk of residue carryover while also reducing the risk of unnecessary investigations, re-cleaning activities, and production downtime.

By combining selective mass detection with Empower 3 CDS reporting, this workflow strengthens confidence in routine cleaning verification results and supports faster, more reliable decision-making in GMP manufacturing environments.

References

1. European Medicines Agency, Guideline on Setting Health-based Exposure Limits for Use in Risk Identification in the Manufacture of Different Medicinal Products in Shared Facilities, EMA/CHMP/CVMP/ SWP/169430/2012, 20 November 2014
2. International Council for Harmonisation (ICH), Guideline on Impurities: Guideline for Residual Solvents, Q3c (R9), Step 5 version, 5 April 2024, https://www.ich.org/page/quality-guidelines < https://www.ich.org/page/quality-guidelines>
3. García, M.C.; Hogenboom, A.C.; Zappey, H.; Irth, H. Effect of the Mobile Phase Composition on the Separation and Detection of Intact Proteins by Reversed-phase Liquid Chromatography–Electrospray Mass Spectrometry. *Journal of Chromatography A*. 2002;957(2):187-99.

https://www.sciencedirect.com/science/article/pii/S002196730200345X#cited-by <
https://www.sciencedirect.com/science/article/pii/S002196730200345X#cited-by>

4. ACQUITY QDa II Mass Detector – Starting Point Settings and Optimization Guidelines 715009274 < https://help.waters.com/help/en/support/library-details.html?documentid=715009274> Ver. 00, Waters Corporation, 2024

Featured Products

Arc HPLC System < https://www.waters.com/nextgen/global/products/chromatography/chromatography-systems/arc-hplc-system.html>

Empower Chromatography Data System (CDS) < https://www.waters.com/nextgen/global/products/informatics-and-software/chromatography-software/empower-software-solutions/empower-cds.html>

ACQUITY QDa II Mass Detector <https://www.waters.com/nextgen/global/products/mass-spectrometry/mass-spectrometry-systems/acquity-qda-ii-mass-detector.html>

720009596, September 2026