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Application News

SSI-LCMS-141

Liquid Chromatography Mass Spectrometry

Using a Highly Sensitive and Selective Single-Quad LCMS-2050 for Cleaning Validation

■ Summary

This application news demonstrates the advantage of coupling a single quadrupole mass spectrometer, LCMS-2050, to a HPLC-UV system by providing highly sensitive (pg level) and selective quantitative analysis in cleaning validation. A single quadrupole mass spectrometer is especially crucial for compounds that cannot be sufficiently detected by UV detector due to poor UV absorbance.

■ Background

Quality control (QC) and safety assurance are critical in the manufacturing of pharmaceutical products. Cleaning validation is an evaluation process to prevent contamination of products from cross-contamination, residues of formulations, and cleaning agents used during the cleaning process in manufacturing facilities.

Cleaning validation is an essential part of the QC workflow in the pharmaceutical industry. cGMP and ICH Q7 Chapter 12 provide the basic concepts in the cleaning process of pharmaceutical manufacturing facilities. Documented evidence ensuring that manufacturing spaces or equipment can be reliably and reproducibly cleaned according to predefined tolerances is required. An acceptable residue limit (ARL) must be determined based on scientific principles, depending on the safety and efficacy of the product. The testing methodology must be highly specific with sufficient sensitivity to achieve low level residue detection.

Depending on the product category and ARL, TOC (Total Organic Carbon) analyzers and HPLC-UV are commonly used for cleaning validation. TOC analyzers can detect all organic carbon sources at once and are superior in terms of simplicity and speed of operation. However, they cannot provide quantitative data on individual compounds. HPLC-UV has the advantage of selective detection and quantitation of target compounds by chromatographic separation, but detection is limited to compounds with UV absorbance. In addition, UV detection may have difficulty reaching the required low ng to pg level for certain residual APIs in cleaning validation.

LC-MS is an analytical technique with superior qualitative and quantitative performance. Together with HPLC, LC-MS can provide highly sensitive, selective, and specific data for both qualitative and quantitative analysis. This application news shows an example of a highly sensitive and selective quantitative analysis using a single quadrupole mass spectrometer (LCMS-2050) for macrolides with poor UV absorbance.

Nexera™ High Performance Liquid Chromatograph and LCMS™-2050 Single Quadrupole Mass Spectrometer

■ Materials and Methods

Four macrolides (Spiramycin, Azithromycin, Erythromycin, Clarithromycin) were selected as test compounds. Stock standard solutions (1000 µg/mL) of each compound were mixed and diluted with a water-acetonitrile solution appropriately to prepare standard mixtures ranging from 0.02-100,000 ng/mL.

The analytical conditions are shown in Table 1. A Nexera XR UHPLC system with a photodiode array detector (PDA) was coupled to the single quadrupole mass spectrometer (LCMS-2050). The compact size of the LCMS-2050 is comparable to other LC modules and can be integrated into an existing LC system, such as the Nexera™ Series or i-Series. It is also equipped with a heated DUIS™ ion source as a standard ionization interface. The DUIS™ ion source can detect a wider range of compounds than ESI or APCI alone.

Table 1: Analytical conditions

HPLC Conditions	[Nexera XR with PDA M40]
Mobile phase A	: Water containing 0.1% formic acid and 2.5mM ammonium formate
Mobile phase B	: Acetonitrile containing 0.1% formic acid
Gradient program	: 5% B (0 min.) -> 95% B (2 - 4 min.) -> 5% B (4.01-5 min.)
Column	: Shim-pack Velox SP-C18 (2.1 x 50 mm, 1.8 µm)
Flow rate	: 0.5 mL/min
Column Oven temperature	: 40 ℃
Injection volume	: 5 uL
Detection	: PDA 190-500 nm
MS Conditions	[LCMS-2050)
Ionization	: DUIS (ESI/APCI), Positive
Mode	: Scan (*m/z* 150-1000) SIM (*m/z* 734, 748, 749, 843)
Interface voltage	: +0.5 kV
Corona Needle voltage	: +0.5 kV
DL/Qarray voltage	: 20 kV
Nebulizing gas	: 2 L
Dry gas flow	: 3 L
Heating gas flow	: 7 L
Desolvation temperature	: 300℃
DL temperature	: 250℃

■ Results and Discussion

The cleaning validation procedure includes sample collection from reaction vessels, production facilities, and subsequent extraction processes. This application will discuss the analytical process after sample collection and extraction.

Figure 1 shows MS chromatograms of a standard mixture (100 ng/mL) of four macrolides. For all compounds, protonated ions $[M+H]^+$ were successfully detected in scan mode (Spiramicin *m/z* 843, Azithromycin *m/z* 749, Erythromycin *m/z* 734, Clarithromycin *m/z* 748).

The dynamic range of the calibration curves, linearity, lower limit of quantitation (LOQ), reproducibility (%RSD), S/N at the lower limit of quantitation, and accuracy for each compound are summarized in Table 2.

Figures 2 and 3 show the calibration curves and MS chromatograms at the lower limit of quantitation for each compound, respectively.

The lower limit of quantitation ranged from 0.02-0.5 ng/mL (0.1-2.5 pg on column), and calibration curves with a dynamic range of more than 2000-fold were obtained for all compounds. Although spiramycin and azithromycin co-eluted on the chromatogram, detection at different *m/z* values ensured accurate quantitation of each compound. Clarithromycin's isotope *m/z* 749 was observed in the trace of azithromycin, but accurate quantitation of both compounds was achieved because they were chromatographically separated.

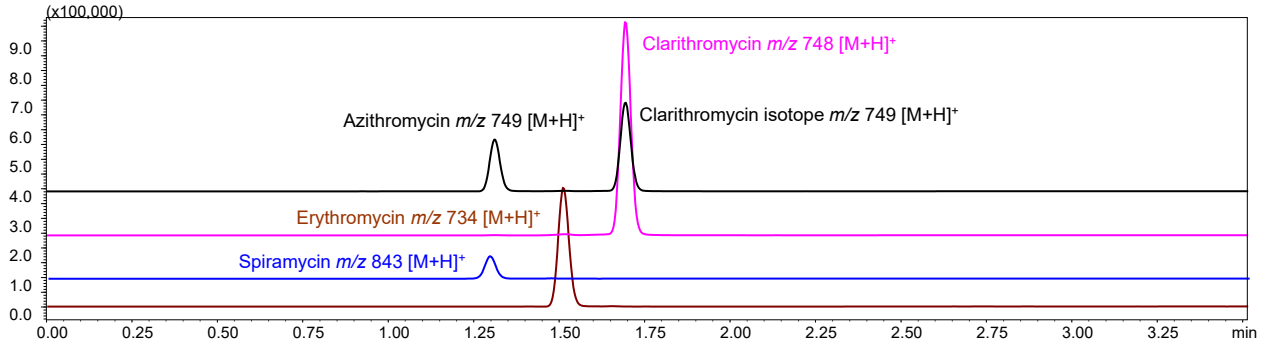
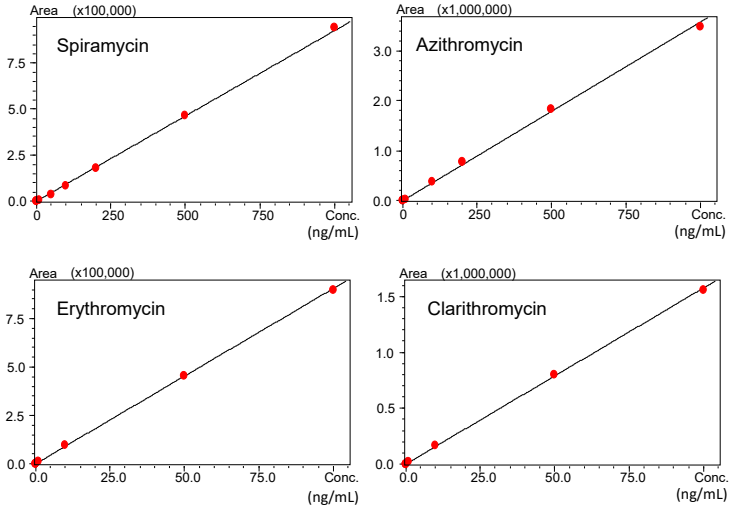


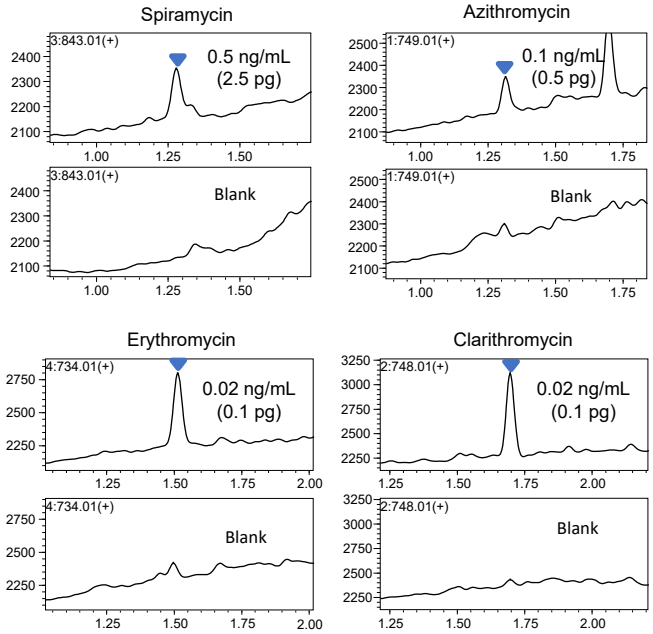
Figure 1: MS chromatograms (XIC) of macrolide standard mixture at 100 ng/mL

Table 2 Quantification summary of four macrolides (the mean of n=5 repetitions was used for %RSD and S/N)

Compounds	Dynamic range of calibration curves (ng/mL)	Linearity (R^2)	LOQ (ng/mL)	%RSD at LOQ	S/N at LOQ	Accuracy range in all calibrators (%)
Spiramycin	0.5-1000	0.999	0.5	9.1	13	83-116
Azithromycin	0.1-1000	0.999	0.1	6.2	13	82-114
Erythromycin	0.02-100	0.999	0.02	8.4	13	85-111
Clarithromycin	0.02-100	0.999	0.02	6.4	11	84-108



Figures 2: Calibration curves for four macrolides



Figures 3: LOQ chromatograms for four macrolides and corresponding blanks

Figure 4 shows a UV chromatogram of a four-macrolide standard mixture (10 µg/mL) measured with a PDA detector under the same conditions as the MS analysis. Due to their poor UV absorbance and background interferences, only spiramycin was detected at a concentration of 10 µg/mL. MS detection was much more sensitive than UV for macrolides, approximately 20,000 times more sensitive in the case of spiramycin.

Although only spiramycin was detected on the UV chromatogram (Figure 4), the MS-based information detected by the LCMS-2050 can be overlaid on the UV chromatogram to confirm the presence of the other macrolides.

Traditionally, it was necessary to display both UV and MS chromatograms to do side-by-side comparison for peak detection and identification. "Mass-it", a new LabSolutions function, allows users to intuitively display the mass information of the peaks detected by the mass spectrometer on the UV chromatogram. This Mass-it function not only allows the users to easily compare UV and MS data, but it can also help to check for compounds which were not detected by UV.

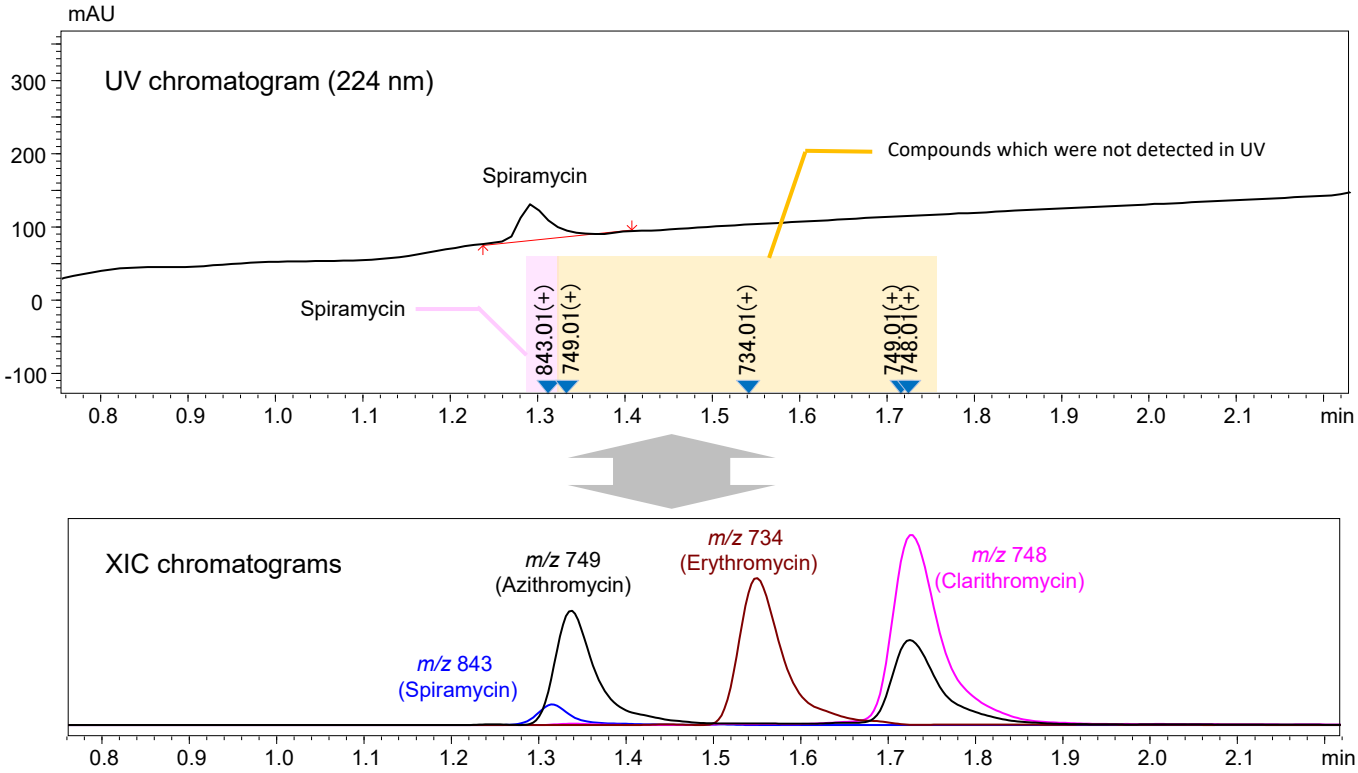


Figure 4: UV chromatogram at 224 nm of four macrolides (10 µg/mL, 50 ng on-column). XIC chromatograms for reference.

What is "Mass-it"?

MS information (*m/z* value and ionization polarity) of peaks is automatically extracted from MS data and overlaid on the UV chromatogram. Even mass information of analytes with no or poor UV absorbance, and consequently not detected by UV, can be displayed on the UV chromatogram. This feature can prevent compounds from being overlooked.

■ Conclusion

This application news demonstrated a highly sensitive and selective solution for cleaning validation using mass detection with an LCMS-2050. For UV detection, the lowest detection limit was 10 µg/mL (50 ng on-column) for the most sensitive component (Spiramycin), while for MS detection, the lowest detection limit was 0.02 ng/mL (0.1 pg on-column) for Erythromycin and Clarithromycin. Azithromycin and Spiramycin yielded lower limits of quantitation of 0.1 and 0.5 ng/mL (0.5 and 2.5 pg on-column), respectively. The calibration curves showed good linearity up to a dynamic range of 4 orders, confirming that accurate quantitation was possible over a wide concentration range.

We have also shown that the "Mass-it" function, which automatically adds MS information to UV chromatograms, allows the user to review information from UV and MS detection at once. In cleaning validation, UV and MS detectors can be used in combination effectively. By selecting the detector to be used for quantification according to the chemical properties of the analytes, the required level of detection, and the concentration range needed, a single system equipped with UV and MS detectors can handle samples with a wide range of compounds and concentration ranges.

ULTRA FAST MASS SPECTROMETRY


LCMS-8040


LCMS-8045


LCMS-8050


LCMS-8060NX


LCMS-2020


Q-TOF LCMS-9030

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