Poster Reprint

ASMS 2022
Poster number MP334

Simultaneous Identification, Confirmation and Quantitation of Impurity in Antibiotics Formulations Using Mixed Scan Mode of Ultivo LC/TQ

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Introduction

It is a vital requirement of pharma- and agro-chemical as well as other related bulk manufacturers to identify and to quantify impurities[1]. The challenging task of assessing non-chromophores in various formulations can be achieved using a combination of Mixed Scan Mode on the Agilent Ultivo LC/TQ (Fig 1) in addition to UV based detection in a single injection.

Figure 1. Agilent Ultivo LC/TQ Mass Spectrometer.

Sulbactam is a β–lactamase inhibitor which is given in combination with β–lactam antibiotics to inhibit β–lactamase, an enzyme produced by bacteria that destroys the antibiotics[2]. Here, an LC/TQ-based method is used to achieve ≤50 times the allowed limit (0.01%) for Impurity A (CAS 23315-18-6) in Sulbactam formulations. Data obtained using both UV detection and Mixed Scan Mode on an Agilent Ultivo LC/TQ provide identification and confirmation in a single injection, allowing this method to be used for purity and impurity workflows in both R&D and QA/QC labs.

Experimental

Sample Preparation:

All formulations and standards were prepared using water as a diluent. The dilution protocol for Impurity A standards is seen in Table 1. A spike study was performed at a concentration of 450 ppb in a 5mg/ml solution of formulation for recovery experiments.

S. No	Stock Conc	Stock vol	Diluent	Final Conc
1	3 mg/ml	10 ul	990 ul	30ppm
2	30 ppm	100 ul	900 ul	3 ppm
3	3 ppm	300 ul	700 ul	900ppb
4	900ppb	500 ul	500ul	450 ppb
5	450 ppb	500 ul	500 ul	225 ppb
6	225 ppb	400 ul	600 ul	90 ppb
7	90 ppb	500 ul	500 ul	45 ppb
8	45 ppb	500 ul	500 ul	22.5 ppb
9	22.5 ppb	400 ul	600 ul	9 ppb

Table 1. Dilution protocol for Sulbactam Impurity A

Instrumentation:

Chromatographic separations were performed on an Agilent 1260 Prime LC system equipped with an Agilent C18 column using a water/methanol gradient. Mass detection was done on an Ultivo LC/TQ running MassHunter software for LC-UV-MS acquisition and analysis. The mass spectrometer was operated in Mixed Scan mode [3]; scan parameters are in Figure 2.

Acquisition Parameters

Scan type	Polarity	Compound/Segment name	Precursor (m/z)	MS1 res	Mass (m/z)	Product (m/z)	MS2 range start (m/z)	MS2 range end (m/z)	MS2 res	Scan/Dwell time (ms)	Frag (V)	ISTD?	CE (V)	Threshold
Product Ion	Positive		182.1	Unit			20	200		100	75		16	0
Product Ion	Negative		180.1	Unit			20	200		100	75		24	0
Scan	Positive						140	200		50	75			0
Scan	Negative						140	200		50	75			0
MRM	Negative	MRM_N	180.1	Unit		116.1			Unit	20	75		12	
MRM	Negative	MRM_N	180.1	Unit		64.9			Unit	20	75		16	
MRM	Positive	MRM_P	182.1	Unit		91			Unit	20	75		8	
MRM	Positive	MRM_P	182.1	Unit		81.8			Unit	20	75		24	
SIM	Negative	SIM_N			180.1				Unit	20	75			
SIM	Positive	SIM_P			182.1				Unit	20	75			

Figure 2. Screenshot of Acquisition Method Showcasing Full Scan, Product Ion, SIM and MRM Scan in +/-ve mode.

LC-UV-MS Data:

LC-UV analysis of a 450-ppb standard of Impurity A furnished no identified peaks, whereas multiple high intensity peaks were seen for the 5mg/ml Sulbactam formulations indicating that Impurity A is not a detectable chromophore (Fig 3a).

LC-MS analysis of the same Impurity A standard using an Agilent Jet Stream ionization source shows signal in both positive and negative modes: negative ion SIM at m/z 180.1 (Figure 3b) gives a high intensity peak (RT 4.94 min) in the standard and a lower intensity peak (RT 5.36 min) in the formulations while positive ion SIM at m/z 182.1 (Figure 3c) shows an intense peak (RT 4.95 min) in the standard and in formulations (RT 5.36 min).

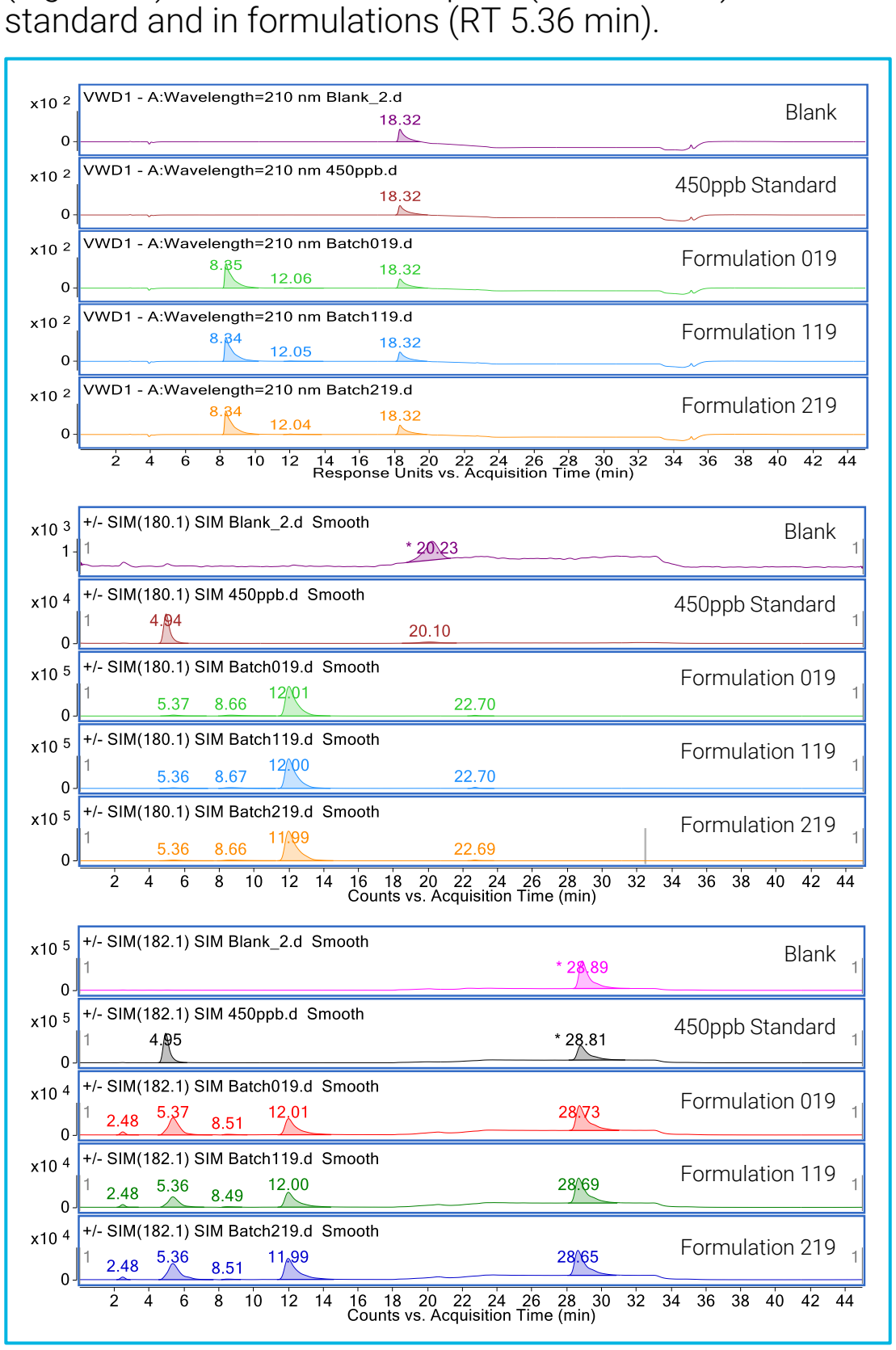

Figure 3. Data obtained in LC-UV (top), LC-MS SIM -ve ion (middle) and LC-MS SIM +ve ion (bottom) modes.

LC-MSMS Data:

Ultivo LC/TQ Mixed Scan Mode enables simultaneous acquisition of data in Full Scan, SIM, Product Ion and MRM mode in a single run. Mixed Scan Mode data obtained for a 450-ppb standard (0.01%) of Impurity A are shown in Figure 4.

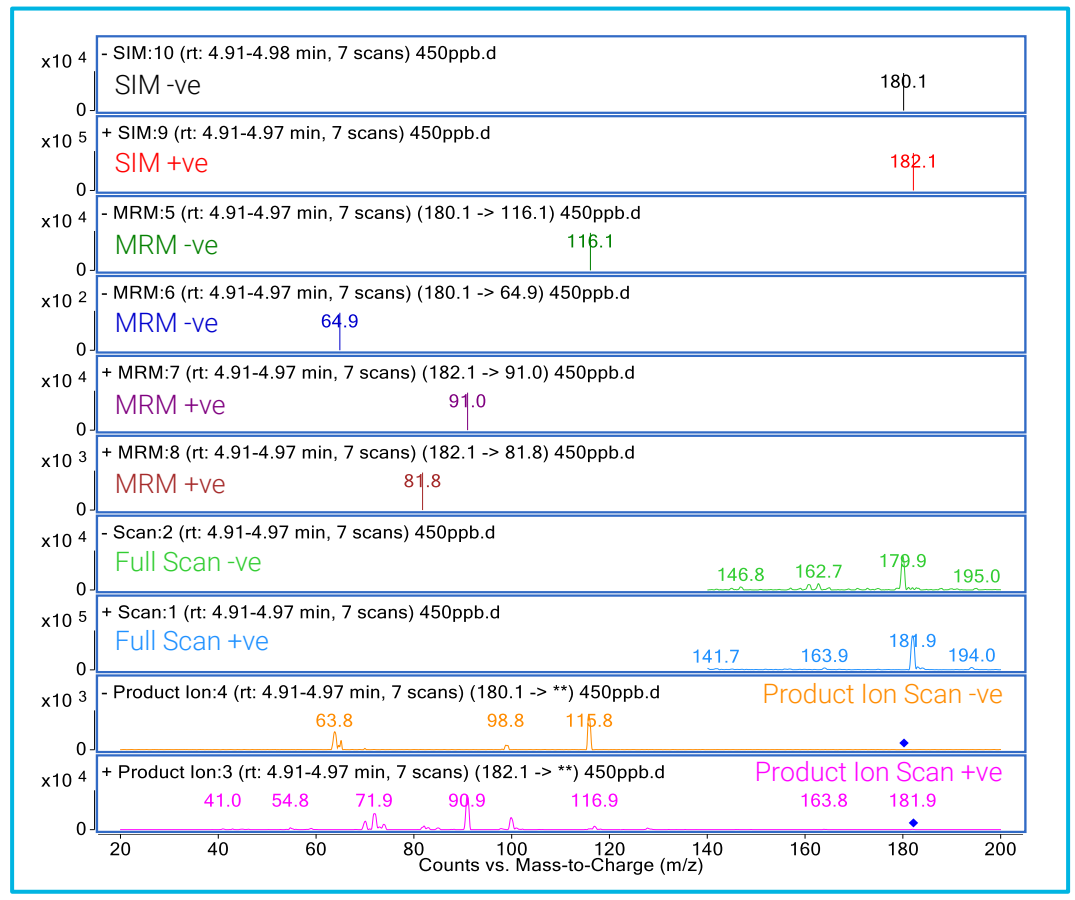

Figure 4. Mixed Scan Mode (Full Scan, Product Ion, SIM and MRM Scan in +/-ve modes).

Calibration Data in SIM and MRM modes:

A 7-point calibration curve for 9ppb to 900ppb Impurity A was constructed for positive and negative ionization mode SIM data. An accuracy of ±20% and r2 ≥0.995 was obtained for both ionization modes (Figure 5).

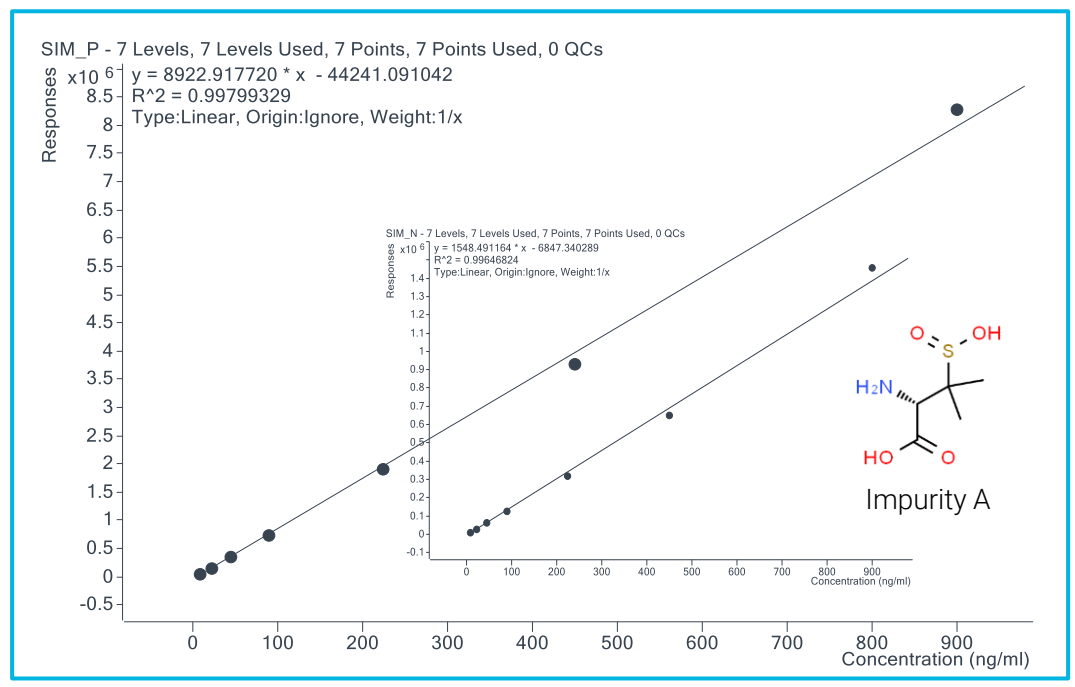

Figure 5. Calibration plot in SIM +/- ve modes.

Results and Discussion

A 7-point calibration curve for 9ppb to 900ppb Impurity A was also constructed for positive and negative ionization mode MRM data. An accuracy of ±20% and r2 of ≥0.995 was obtained for both ionization modes (Figure 6).

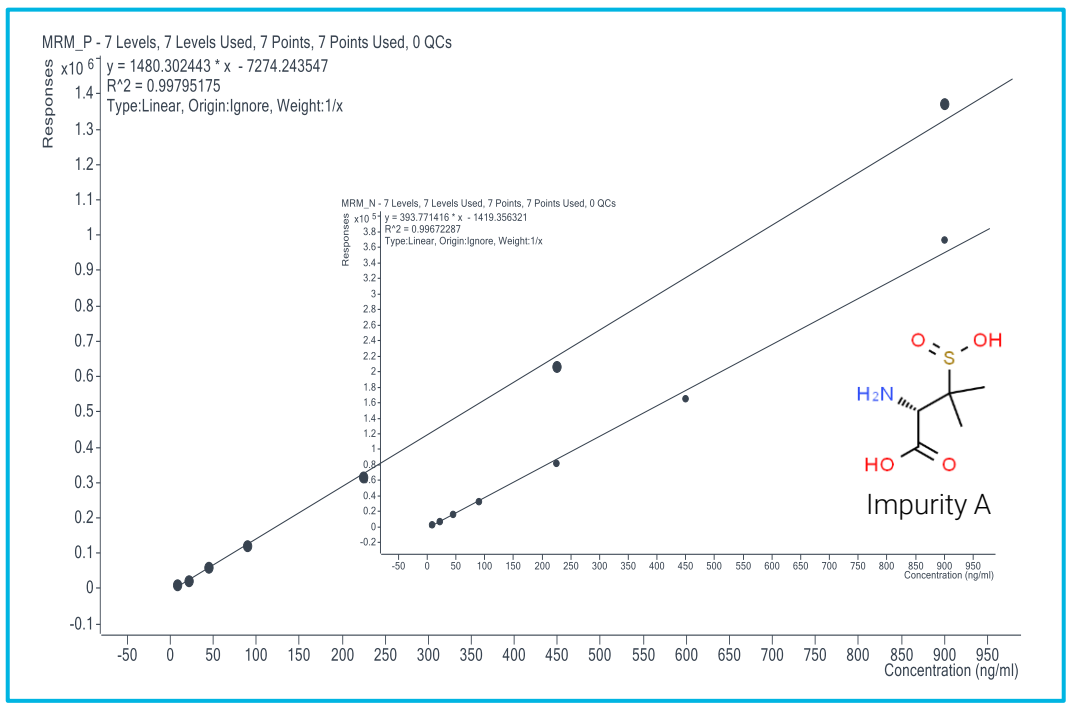

Figure 6. Calibration plot in MRM +/- ve modes.

Quantitation and Recovery Experiment:

Sulbactam-related compound A (Impurity A) is present at 150-250 ppb in 5mg/ml formulations; this range is less than the allowed limit of 0.01%.

Batch No	Calc Conc in SIM –ve	Recovery %	Calc Conc in SIM +ve	Recovery %
019	223.5	100 (659.7-223.5)/450 = 96.93 %	241.2	100 (699.3-241.2)/450 = 101.8 %
119	146.7	100 (617.8-146.7)/450 = 104.69 %	166.0	100 (674.5-166.0)/450 = 113 %
219	233.4	100 (648-233.4)/450 = 92.13 %	267.3	100 (716.5-267.3)/450 = 99.82 %

Table 2. Recovery Calculation for Sulbactam Impurity A in formulations at 0.01% in SIM mode

A recovery experiment, conducted by spiking 450 ng of Impurity A in 5mg/ml formulations, showed recoveries between -10% to +15%. A comparative analysis can be seen for data acquired in SIM mode (Table 2) versus data acquired in MRM mode (Table 3).

Batch No	Calc Conc in MRM –ve	Recovery %	Calc Conc in MRM +ve	Recovery %
019	227.8	100 (654.4-227.8)/450 = 94.8 %	240.4	100 (678.2-240.4)/450 = 97.29 %
119	145.5	100 (614.6-145.5)/450 = 104.24 %	156.2	100 (650.7-156.2)/450 = 109.89 %
219	229.5	100 (648.4-229.5)/450 = 93.09 %	254.2	100 (697.4-254.2)/450 = 98.49 %

Table 3. Recovery Calculation for Sulbactam Impurity A in formulations at 0.01% in MRM mode

Conclusions

- Simultaneous identification, confirmation and quantitation are established in single injection.
- UV, SIM and MRM scan modes in a single method are useful in impurity profiling workflows.
- Quantitative analysis is enabled by fast polarity switching on an Ultivo LC/TQ without loss of sensitivity.
- Ultivo LC/TQ is the suitable solution for purity and impurity analysis for agricultural and pharma industry.

References

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2. https://pubchem.ncbi.nlm.nih.gov/compound/Sulbactam
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https://explore.agilent.com/asms

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DE33863895

Published in USA, May 20, 2022

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