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HRMS QTOF-PRM (Parallel reaction Monitoring) for ultra-trace level quantification of N-Nitroso Moxifloxacin in API and Formulations.

Uday Agarwal[1], Ashish Pargaonkar[2], Saikat Banerjee[3], Vivek Dhyani[4], Ju-Hwan Lim[5], Aimei Zou[6]

[1]Agilent technologies Inc, Gurgaon, India

[2]Agilent Technologies Inc, Bangalore, India

[3]Agilent technologies Inc, Hyderabad, India

[4]Agilent Technologies Inc, Mumbai, India

[5]Agilent Technologies Korea Ltd, Seoul, South Korea

[6]Agilent Singapore, Singapore

Introduction

Moxifloxacin is a widely used fluoroquinolone antibiotic and is listed by the WHO as an essential medicine. Its nitrosamine-related impurity, N-Nitrosomoxifloxacin (N-MOX), is classified under EMA CPCA category 4 with an acceptable intake limit of 1500 ng/day, reflecting its potential genotoxic and carcinogenic hazard. Accurate quantitation of N-MOX in Active Pharmaceutical Ingredient (API), placebo, and final formulations is therefore critical to ensure patient safety and regulatory compliance. (1,2)

In this study, we present a high-resolution mass spectrometry workflow employing PRM (Parallel Reaction Monitoring) on the Agilent Revident LC/Q-TOF, which leverages accurate-mass full MS/MS acquisition. The method utilizes end-to-end optimization from triple quadrupole MRM, including averaged collision energies and fragment voltages, to seamlessly transfer the method to Q-TOF PRM, achieving precise quantitation without false positives. This workflow is broadly adaptable and can be extended to other NDSRIs, subject to technical considerations..

Experimental

Sample Prep

- Tablet Samples: Five 400 mg moxifloxacin tablets were weighed to determine the average tablet weight (729.88 mg). The tablets were crushed and homogenized to obtain a uniform powder.
- API & Placebo: Accurate amounts of API and placebo were used to prepare spiked test solutions to simulate finished product matrices.
- Test Solution: A target concentration of 1.5 mg/mL API was prepared. For 1 mL of solution, 2.74 mg of tablet powder (including excipients) was dissolved to achieve 1.5 mg/mL of API. (2)

N-MOX Threshold Concentration

The N-Nitrosomoxifloxacin (N-MOX) concentration corresponding to the CPCA Cat 4 AI (1500 ng/day per 400 mg tablet) was calculated to be ~5.63 ng/mL in the test solution, representing the regulatory decision threshold for quantitation. (3,4)

Experimental

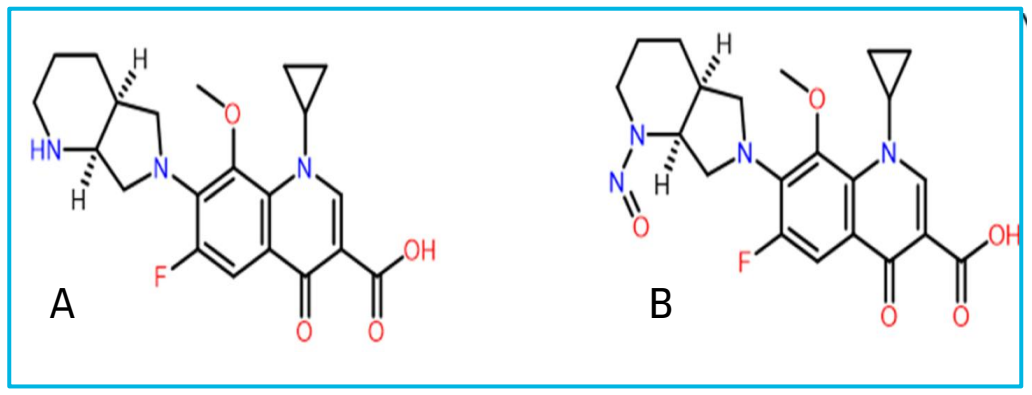

Figure 1. (A) Moxifloxacin (B) N-nitroso moxifloxacin

Figure 2. Agilent 1290 infinity III and Revident LC/Q-TOF

LC and MS Parameters	
Column	Zorbax RRHD Diphenylx 2.1*100mm 1.8u
Gradient Run	16 mins
Mobile Phase	A: 5mM Ammonium formate, 0.5mM NH4F, 0.1%FA in Water B: Methanol
Injection Vol	5 ul
Polarity	Positive
Acquisition mode	PRM
instrument Mode	750 Stable
Capillary Voltage	3000 V
Precursor Ion	431.172525
Product Ions	301.1, 386.1, 231.9, 319

Table 1: LC-QTOF Revident parameters.

Results and Discussion

PRM Workflow

MRM transitions for N-MOX were optimized on a triple quadrupole, including collision energy (CE) and fragmentor voltage (FragV).

Optimized MRM parameters were transferred to Agilent Revident Q-TOF PRM, averaging CE and FragV values across transitions to ensure optimal fragmentation.

High-resolution full MS/MS spectra were acquired for each precursor, allowing accurate-mass quantitation and confirmatory fragment verification across API, placebo, and tablet matrices.

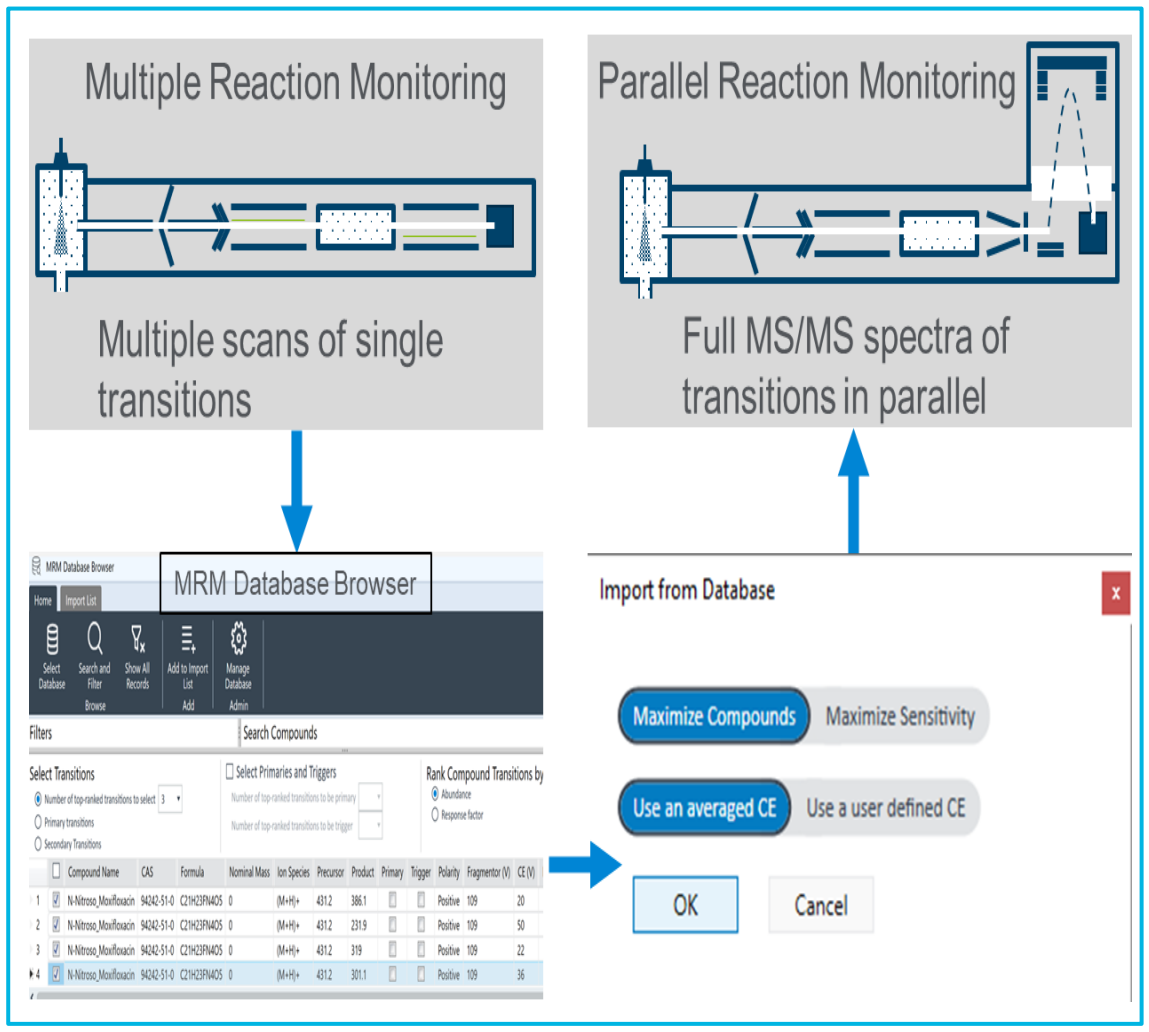

Figure 3. PRM workflow chart for the method development

LLOQ, ULOQ and Linearity of N-nitroso moxifloxacin

PRM-based analysis of N-nitroso moxifloxacin showed excellent linearity with R^2 = 0.9997 over a wide dynamic range. Based on CPCA Category 4 (1500 ng/400 mg MDD), the calculated specification limit for a 1.5 mg/mL test solution was 5.63 ng/mL. The method achieved a highly sensitive LLOQ of 12 pg/mL and a ULOQ of 25 ng/mL, ensuring quantification well below regulatory requirements. The monitored PRM transition m/z 431.1725 → 301.1 provided high selectivity with no interference from API or matrix components. Clear chromatographic separation of moxifloxacin and N-nitroso moxifloxacin confirmed absence of crosstalk and false positives under high-resolution accurate-mass conditions.

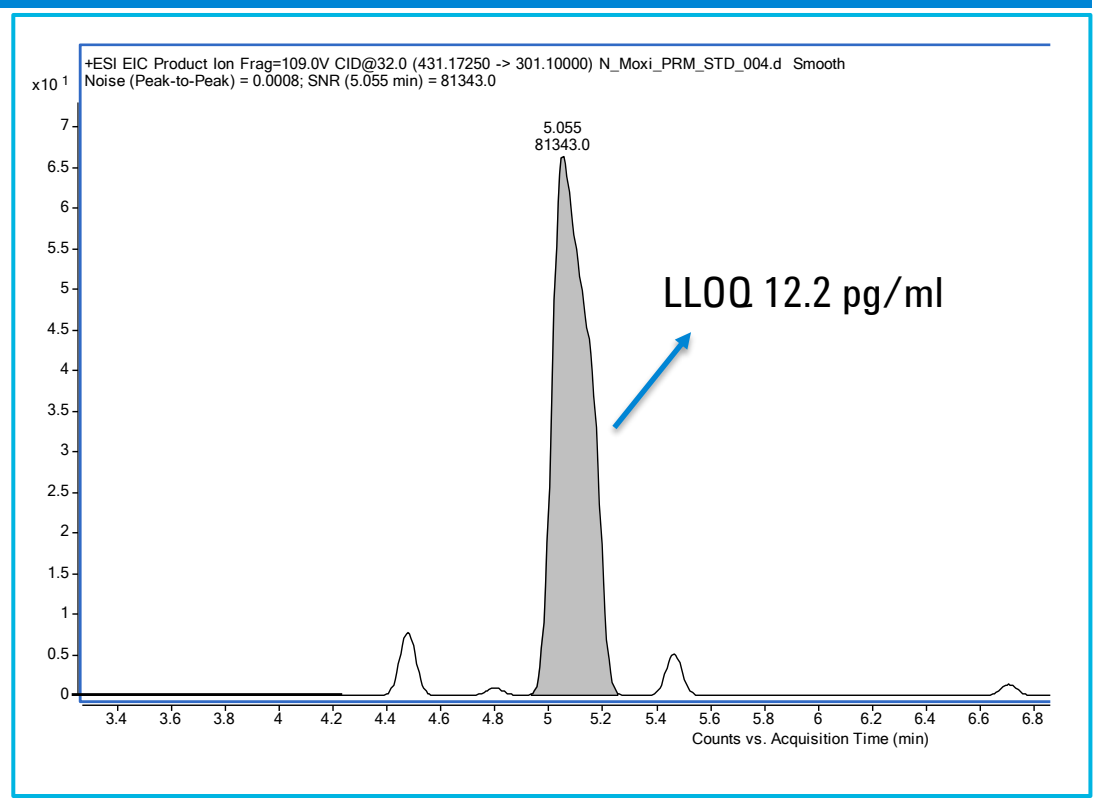

Figure 4. PRM EIC LLOQ peak

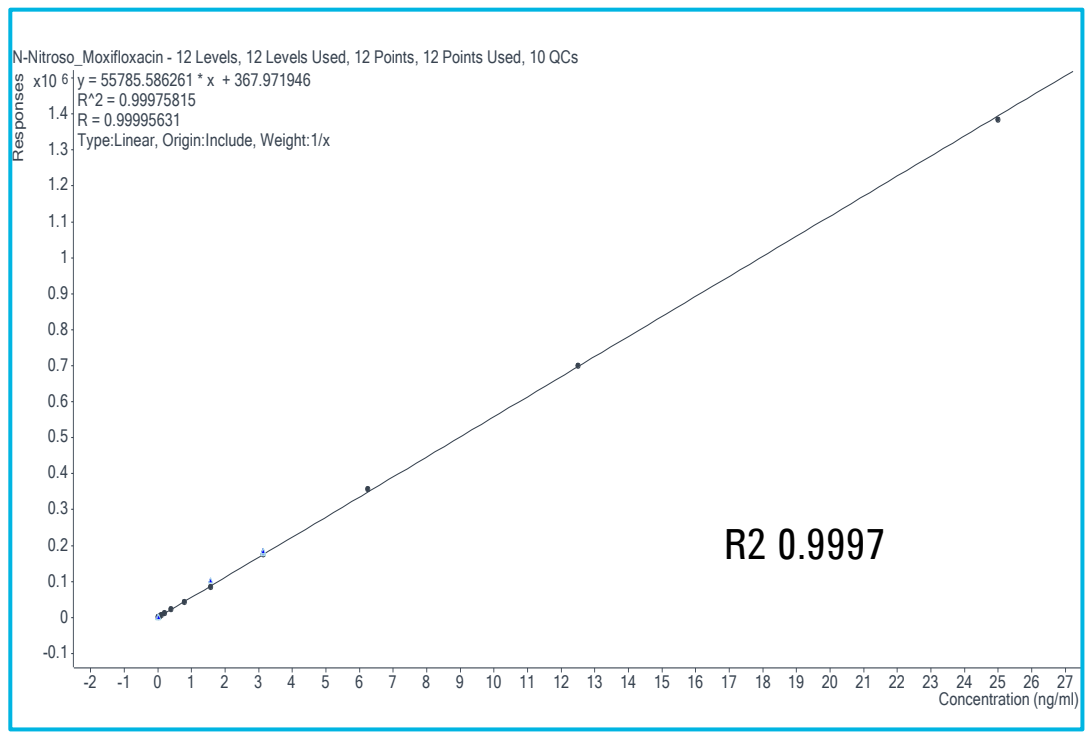

Figure 5. Linearity of N-MOX, cal range 12.2pg/ml - 25ng/ml

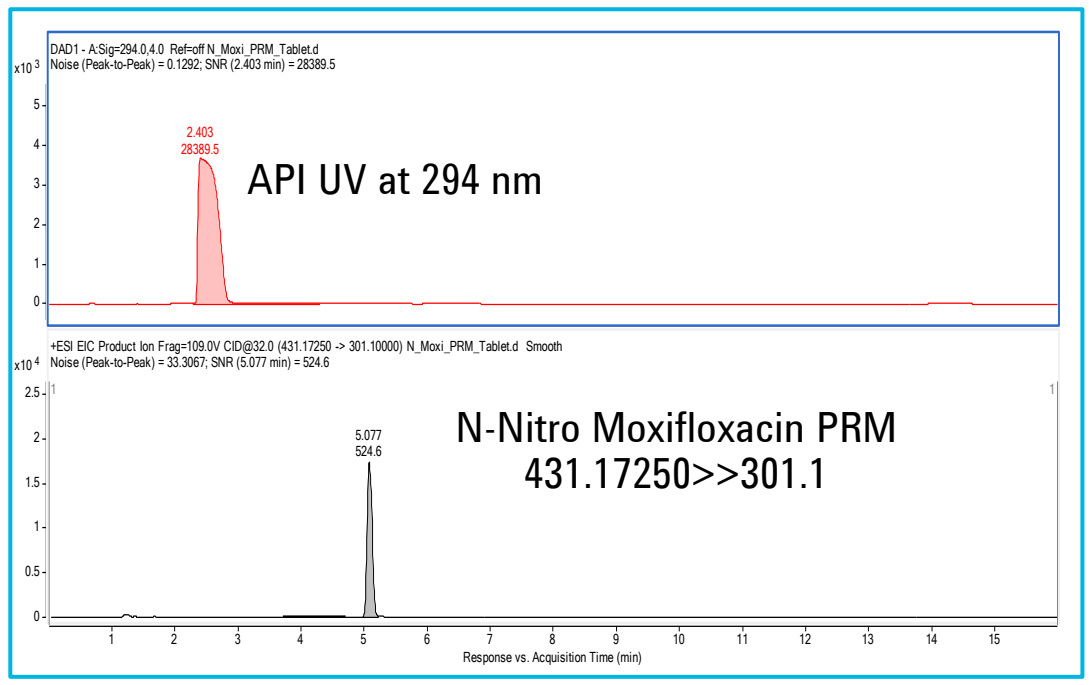

Figure 6. Chromatographic separation with DAD spectra of API and PRM EIS of N-MOX

Results and Discussion

N-nitroso moxifloxacin API, Placebo & Formulation: PRM mode based LOQ, ULOQ, Linearity, Accuracy, and Recovery data.

Data File	Type	Final Conc.	Accuracy	RT
PRM_Blank_001.d	Blank			
PRM_Blank_002.d	Blank			
PRM_Blank_003.d	Blank			
N_MOX_STD_0.0122ngml.d	Cal	0.0103	84.1	5.061
N_MOX_STD_0.0244ngml.d	Cal	0.0221	90.5	5.093
N_MOX_STD_0.0488ngml.d	Cal	0.0544	111.3	5.081
N_MOX_STD_0.0975ngml.d	Cal	0.1003	102.7	5.076
N_MOX_STD_0.1953ngml.d	Cal	0.2118	108.4	5.076
N_MOX_STD_0.3906ngml.d	Cal	0.4128	105.7	5.08
N_MOX_STD_0.781ngml.d	Cal	0.765	97.9	5.083
N_MOX_STD_1.56ngml.d	Cal	1.5116	96.7	5.081
N_MOX_STD_3.125ngml.d	Cal	3.1382	100.4	5.081
PRM_Blank_004.d	Blank			
QC_1.56ngml_001.d	QC	3.2872	105.2	5.082
QC_1.56ngml_002.d	QC	3.2068	102.6	5.081
QC_1.56ngml_003.d	QC	3.2478	103.9	5.083
QC_1.56ngml_004.d	QC	3.2489	104	5.076
QC_1.56ngml_005.d	QC	3.3144	106.1	5.084
QC_1.56ngml_006.d	QC	3.2876	105.2	5.082
PRM_Blank_005.d	Blank			
N_MOX_STD_6.25ngml.d	Cal	6.4093	102.5	5.081
N_MOX_STD_12.5ngml.d	Cal	12.5551	100.4	5.081
N_MOX_STD_25ngml.d	Cal	24.797	99.2	5.078
PRM_Blank_006.d	Blank			
N_Moxi_PRM_Placebo.d	Sample			
PRM_Blank_007.d	Blank			
N_Moxi_PRM_Tablet.d	Sample	2.017		5.077
PRM_Blank_008.d	Blank			
N_Moxi_PRM_API.d	Sample	3.57		5.076
PRM_Blank_009.d	Blank			
N_Moxi_Placebo_SPK_15625ppb.d	QC	1.8379	117.6	5.077
PRM_Blank_010.d	Blank			
N_Moxi_Placebo_SPK_LLOQ_1.d	QC	0.0144	117.7	5.089
N_Moxi_Placebo_SPK_LLOQ_2.d	QC	0.0254	104	5.062

Table 2: LC-QTOF N-nitroso moxifloxacin Linearity and Recovery

Quality Control and Recovery data

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Sample ID	Area	Spike amount ng/ml	Standard area	Recovery %
Placebo	192	0.0122	941	103.93
Spike	1170			

Table 3: Placebo spike at LOQ level and recovery data

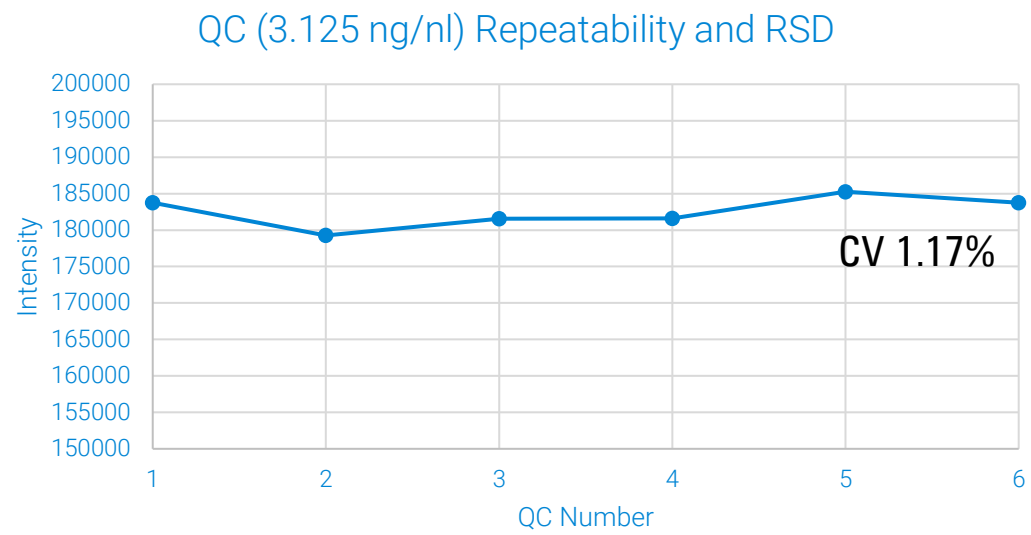

Figure 7. PRM Reproducibility of N-Mox quality control

Conclusions

PRM-based Q-TOF analysis enables confident ultra-trace quantification of N-nitroso moxifloxacin with excellent linearity and accuracy. The method achieves 12.2 pg/mL sensitivity, well below regulatory specification limits, ensuring robust impurity control. Accurate-mass PRM eliminates false positives and provides high selectivity in complex pharmaceutical matrices. This study establishes Q-TOF-PRM as a powerful, regulatory-ready platform for CPCA Cat 3/4/5 nitrosamine impurity analysis.

References

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4. EMA: Nitrosamine impurities: guidance for marketing authorization holders. https://www.ema.europa.eu/en/human-regulatory-overview/post-authorisation/pharmacovigilance-post-authorisation/referral-procedures-human-medicines/nitrosamine-impurities/nitrosamine-impurities-guidance-marketing-authorisation-holders.

https://www.agilent.com/en/promotions/asms

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