Application Note

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Trace Level Quantification of Potential Mutagenic Impurities in Pharmaceuticals

Using an Agilent Ultivo LC/TQ with mixed mode data acquisition

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Abstract

This Application Note highlights an analytical method for the precise quantitation of two potential mutagenic impurities, 3-aminopyridine and isonicotinamide, that can be formed from the production of 4-aminopyridine, the active pharmaceutical ingredient (API) in dalfampridine. The Agilent Ultivo LC/TQ features mixed-mode acquisition, which enables simultaneous acquisition of quantitative (SIM and MRM), and qualitative information (full scan and product ion scan) from the sample. Mixed-mode works by incorporating a scanning function into the same time segment as the SIM or MRMs for the analytes of interest. Exceptional sensitivity and precision were observed for this method, as the potential mutagenic impurities had quantitation limits far below regulatory requirements.

Introduction

In the manufacturing process of an API, mutagenic starting material or side-reaction products may remain in the production batch. These mutagenic impurities may be introduced into the final product, and manufactured batches must be screened for these potential mutagenic impurities (PMIs). In this method, 4-aminopyridine, the API of dalfampridine, is analyzed for two targeted PMIs: 3-aminopyridine and isonicotinamide. A standard dose of dalfampridine is 20 mg/day, which means that total PMIs need to be accurately detected below 75 ppm (threshold of toxicological concern for PMI = 1.5 µg/day).[1,2] This Application Note demonstrates the quantitation of two PMIs, 3-aminopyridine and isonicotinamide, in a high concentration of API using the Ultivo LC/TQ with the mixed-mode acquisition feature.

Typically, this type of analysis would be carried out using LC/UV or single quadrupole LC/MS. However, using an LC/TQ allows for more accurate quantitation due to improved specificity when searching for known impurities and better sensitivity due to reduced chemical noise. The mixed-mode acquisition feature with Ultivo allows simultaneous collection of MRM, SIM, product ion scan, precursor ion scan, and neutral loss scan data in the same time segment. This mixed-mode feature allows the user to collect more information in addition to the precise quantitation of target compounds in one injection. Based on regulatory sensitivity requirements, a standard ESI source is an appropriate and economic choice for this application.

A challenge in this method is the relatively high concentration of API that needs to be sampled to detect the PMI compounds. Separation is important in this method due to the high concentration of the API that elutes after the impurities. Because of the hydrophilic nature of the analytes in this method, the analytes will not retain well on a typical reversed-phase column. Therefore, an exceptionally robust hydrophilic interaction liquid chromatography column (Agilent InfinityLab Poroshell 120 HILIC-Z column) was used for analyte separation. Lastly, while the API elutes from the column, the LC stream is diverted away from the MS, avoiding potential contamination of the ion source and saturation of the MS detector.

Experimental

Reagents and chemicals

All reagents used in this Application Note were HPLC or LC/MS grade. Acetonitrile was purchased from Honeywell (Morristown, NJ, USA). Ultrapure water was sourced from a Milli-Q Integral System with a LC-Pak Polisher and a 0.22 µm point-of-use membrane filter cartridge (Merck, Darmstadt, Germany). Formic acid was purchased from Fisher Scientific (Fair Lawn, NJ, USA), and 5 M ammonium formate solution was purchased from Agilent Technologies, Santa Clara, CA, USA (part number G1946-85021). Pharmaceutical and impurity standards were purchased from Millipore-Sigma (Merck, Darmstadt, Germany).

Sample preparation

A solution of 4-aminopyridine at 20 mg/mL acted as a matrix for the impurities. 4-Aminopyridine was dissolved in acetonitrile at 20 mg/mL. 3-Aminopyridine and isonicotinamide were dissolved in acetonitrile and diluted to a stock concentration of 10 µg/mL. Calibration curves of 3-aminopyridine and isonicotinamide were made by serial dilution with the 4-aminopyridine stock at 20 mg/mL.

Instrumentation and experimental parameters

Agilent 1260 Infinity II prime LC system

- 1260 Infinity II flexible pump (G7104C)
- 1260 Infinity II vialsampler with integrated column compartment (G7129A)
- 1260 Infinity II diode array detector HS (G7117C)

Agilent Ultivo triple quadrupole LC/MS system

- Electrospray ionization source (G1948B)

Software

Agilent MassHunter Quantitative Analysis software B.09 with the Quant-My-Way feature

Samples were analyzed with a sampler pretreatment sequence, which washed the needle and injector loop with water and methanol, then flushed with starting mobile phase conditions before injection. This injection sequence was easily programmed in Agilent MassHunter Acquisition to minimize carryover between injections due to the high concentration of 4-aminopyridine in each sample. The LC method was adapted from the Agilent Application Note 5994-0864EN[3] with slight modifications. Table 1 lists the HPLC gradient condition, and Table 2 shows the ESI source and Ultivo LC/TQ parameters.

MRM and scan method setup using mixed-mode acquisition

Figure 1 shows scan parameters for unknown compounds and MRM parameters for targeted PMIs. Ultivo LC/TQ provides the capability of simultaneously collecting MRM and scan data.

Table 1. Agilent 1260 Infinity II Prime LC parameters.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 HILIC-Z, 2.1 × 150 mm, 2.7 µm, PEEK-lined (p/n 673775-924)
Column Temperature	35 °C
Injection Volume	1 µL
Mobile Phase	A) 10 mM ammonium formate + 0.02% formic acid in H_2O B) 0.1% formic acid in acetonitrile
Flow Rate	0.5 mL/min
Gradient	Time (min) %B 0 95 2 90 4 60 8 60
Stop Time	8.0 minutes
Post Time	6.0 minutes
Detection UV	[265, 10/Ref 360, 80] nm

Table 2. ESI ion source parameters.

Parameter	Value
Gas Temperature	350 °C
Gas Flow	10 L/min
Nebulizer Pressure	40 psi
Capillary Voltage	4,000 V(+)
Divert LC to Waste	3 minutes

Acquisition Parameters

Scan type	Polarity	Compound/Segment name	Precursor (m/z)	MS1 res	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
Scan	Positive					70	400		300	110			0
MRM	Positive	3-aminopyridine	95.1	Unit	68.1			Unit	100	110		20	
MRM	Positive	3-aminopyridine	95.1	Unit	52			Unit	100	110		44	
MRM	Positive	3-aminopyridine	95.1	Unit	41.1			Unit	100	110		28	
MRM	Positive	Isonicotinamide	123.1	Unit	80			Unit	100	110		20	
MRM	Positive	Isonicotinamide	123.1	Unit	53			Unit	100	110		32	
MRM	Positive	Isonicotinamide	123.1	Unit	52			Unit	100	110		52	

Figure 1. Setting up mixed mode scanning is as simple as adding a scan row to the MRM list.

Results and discussion

Method linearity, sensitivity, and precision

The Ultivo LC/TQ with standard ESI source exhibited exceptional sensitivity for the two impurities measured. 3-Aminopyridine can accurately be quantified at 5 ng/mL, and isonicotinamide could be quantified as low as 0.5 ng/mL in 4-aminopyridine matrix (20 mg/mL). Precision for this analytical method for each analyte was outstanding, with a RSD% of 4.25% for 3-aminopyridine and 0.23% for isonicotinamide for six replicate injections at the quantitation limit (Figure 3). Excellent linearity was observed, with both impurities having R^2 >0.99 for a six-point calibration curve (Figure 2).

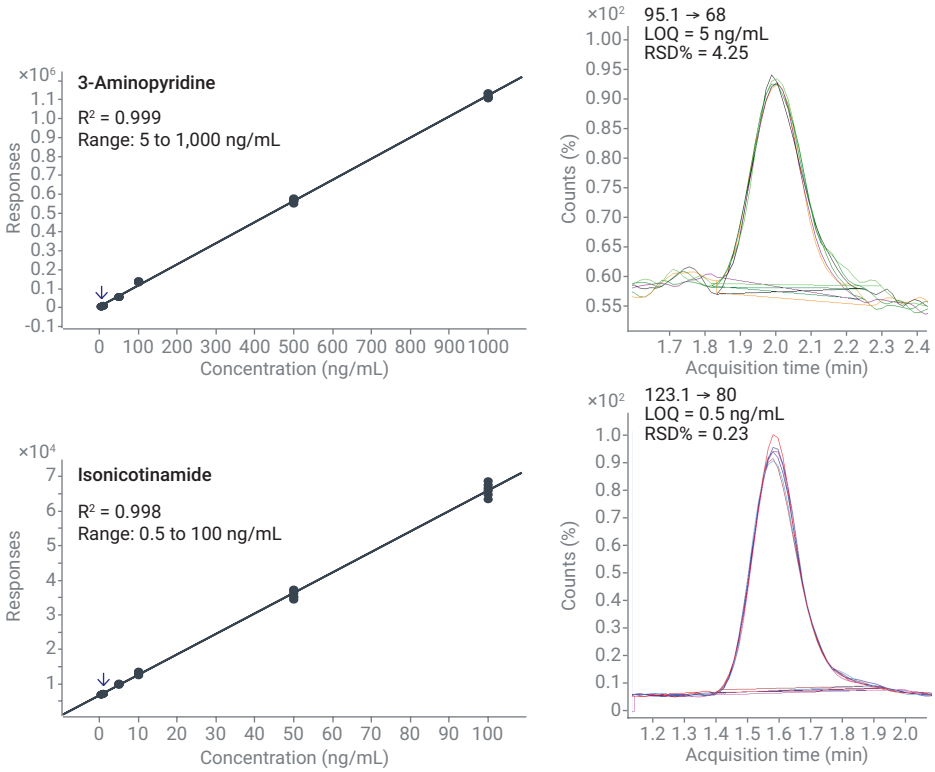

Figure 2. A six-point standard curve for 3-aminopyridine and isonicotinamide measured in API matrix, with an overlay of six replicate injections of the quantifier transition at the quantitation limit.

Using mixed-mode acquisition to get more information for your analysis

Mixed-mode acquisition allows the MS user to simultaneously collect scan data and MRM data, where the operator could potentially find additional eluting compounds in the same sample. MRMs for target compounds provide more selective, sensitive, and accurate quantitation over scan data. Scan data can provide information on unexpected analytes existing in the sample. A DAD module can also easily be incorporated into the LC stack, bringing the added benefit of detecting the high concentration of API while the LC flow to the mass spectrometer has been diverted to waste (Figure 3). Using mixed-mode scanning does not alter the peak shape or area of the MRM transition (Figure 4).

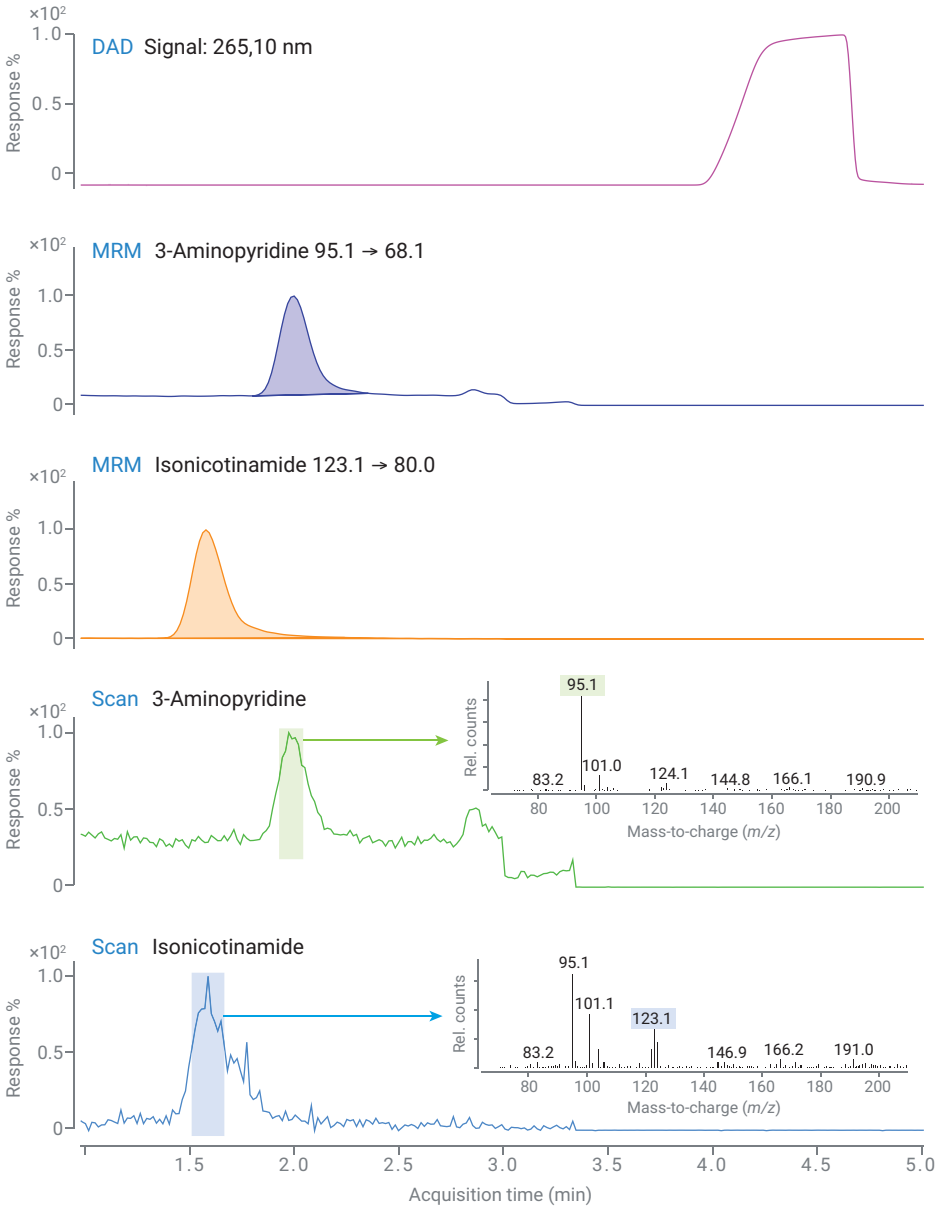

Figure 3. Mixed-mode acquisition allows the simultaneous use of scan and MRM. A DAD signal enables the detection of the API while the LC is diverted to waste, limiting MS contamination. The DAD signal does not detect the impurities found by the MS.

Conclusion

This Application Note demonstrates the sensitive and accurate quantitation of potential mutagenic impurities (3-aminopyridine and isonicotinamide) in 20 mg/mL of 4-aminopyridine, the API studied. Mixed-mode acquisition containing MRMs, a feature specific to the Agilent Ultivo, allows additional scan information to be collected from the sample while accurately quantifying for PMI compounds with MRMs in the same time segment. Because of the exceptional performance of Ultivo with a standard ESI source coupled to an Agilent 1260 Infinity II prime LC system, it is proposed that this economic combination of instruments allows detection of PMI with a smaller amount of the API to be sampled, while still maintaining accurate quantitation of the potential impurities.

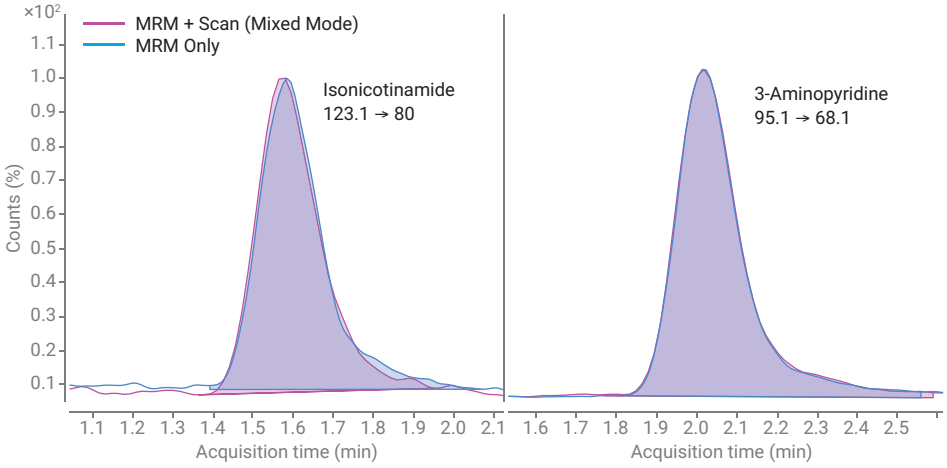

Figure 4. Nearly identical peak shape and peak area are observed when using mixed-mode and MRM-only scan types.

References

1. US Department of Health and Human Services; FDA; CDER; CBER. M7(R1) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk. ICH, March **2018**. 2.
2. Jain, M. *et al.* Determination of Five Potential Genotoxic Impurities in Dalfampridine Using Liquid Chromatography. *J. Pharm. Biomed. Anal.* **2016**, *133*, 27–31.
3. Covert, K. How to Catch a Potential Mutagenic Impurity Using an Agilent LC/MSD XT and Agilent InfinityLab Poroshell 120 HILIC-Z Column for Sensitive and Reliable Detection of Dalfampridine Impurities. *Agilent Technologies Application Note*, publication number 5994-0864EN, **2019**.

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