Poster Reprint

ASMS 2019
WP788

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

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Introduction

Dalfampridine may contain impurities from the manufacturing process of the active pharmaceutical ingredient (API), which are potential mutagenic impurities (PMIs). Specifically, two PMIs were targeted in this work: isonicotinamide and an isomer of the API itself, 3-aminopyridine, whose structures are shown in Figure 1.

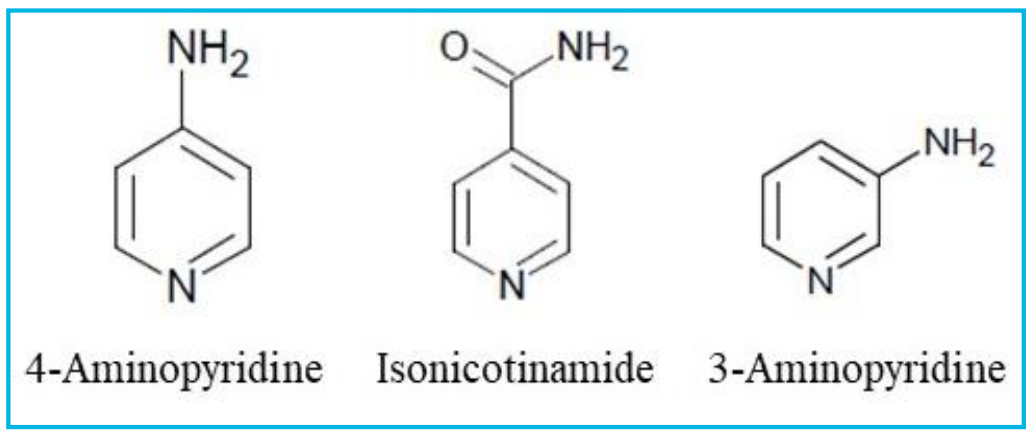

Figure 1. API and two potential mutagenic impurities

With a threshold of toxicological concern for PMIs at 1.5 µg/day, a standard daily dose of 20 mg dalfampridine corresponds to no more than 75 ppm of PMIs in relation to the API[1,2]. Therefore, detection of these PMIs requires a sensitive, precise, and high-throughput methodology and instrumentation that can meet the requirements.

The Agilent single quadrupole LC/MSD XT can be easily incorporated into an HPLC stack and is the perfect choice for impurity detection with mass confirmation alongside UV detection. The API and PMIs are hydrophilic, and therefore do not retain well on a typical reverse-phase liquid chromatography column.The new InfinityLab Poroshell 120 HILIC-Z (zwitterionic) column offers the perfect solution for hydrophilic compound separation and detection. It is designed to retain polar compounds with superior robustness.

Experimental

Sample preparation

API samples were prepared by dissolving 20 mg of 4-aminopyridine into 1 mL of ACN in a 2 mL screw top sample vial (20,000 µg/mL). Stock solutions of the impurity standards were prepared by dissolving 10 mg of each into 100 mL of ACN in a volumetric flask (100 µg/mL). A series of serial dilutions was performed to make up: 10, 1.0, 0.1, and 0.01 µg/mL solutions of the impurity standards. Separate samples of 20,000 µg/mL API standard were spiked with 150 µL of the previous impurity standards, respectively, to make ~1150, 115, 11.5, 1.15 ng/mL of impurity spiked API samples.

Experimental

Instrumentation

The LC/MSD XT with the 1260 Infinity II Prime LC System consists of the following modules:

- Agilent 1260 Infinity II Flexible Pump (G7104C)
- Agilent 1260 Infinity II Vialsampler (G7129A)
- Agilent 1260 Infinity II Multicolumn Thermostat (G7116A)
- Agilent 1260 Infinity II Diode Array Detector HS (DAD) (G7117C)
- Agilent LC/MSD XT system (G6135CA)

Figure 2. The LC/MSD XT with the 1260 Infinity II LC system

Parameter	HPLC Set Value	
Column	Agilent InfinityLab Poroshell 120 HILIC-Z 2.1 × 150 mm, 2.7 µm, PEEK lined at 35 °C	
Mobile phase A	10 mM ammonium formate + 0.02% formic acid (FA) (pH 5.0)	
Mobile phase B	0.1% formic acid in acetonitrile (ACN)	
Gradient	Time	%B
	0	95
	2	90
	4	60
	5	60
Postrun	5 minutes	
Flow rate	0.5 mL/min	
Injection volume	1 µL	
Detection UV	[265, 10 / ref. 360, 80] nm	

Parameter	Single Quadrupole Set Value
Ion source	ESI+
Peak filter	0.02 minutes
Scan/dwell time	Scan 250 ms, SIM 50 ms
Drying gas temperature	350 °C
Gas flow	10 L/min
Nebulizer pressure	40 psi
Capillary voltage	3 kV
Fragmentor voltage	135 V
Scan range	m/z 80 to 300
SIM ions	m/z 95.2, 123.1
Divert to waste	3.5 to 5 min

Table 1. LC and MS parameters

Integrated divert valve ensures system robustness

MS spectral information facilitates the detection and confirmation of impurities. However, repeated sample injection of high API concentrations may slowly cause contamination of the MS ionization source over time. This challenge can be minimized by using the built-in diverter valve included in Agilent mass spectrometers. A 1 µL injection of 20,000 µg/mL API standard without impurities was performed with only UV detection. Figure 3 shows the UV profile of the API to determine the time window to divert the LC flow to waste and protect the MS detector. This same sample is injected again, shown in blue in Figure 4, diverting the API to waste from 3.5 to 5.0 minutes.

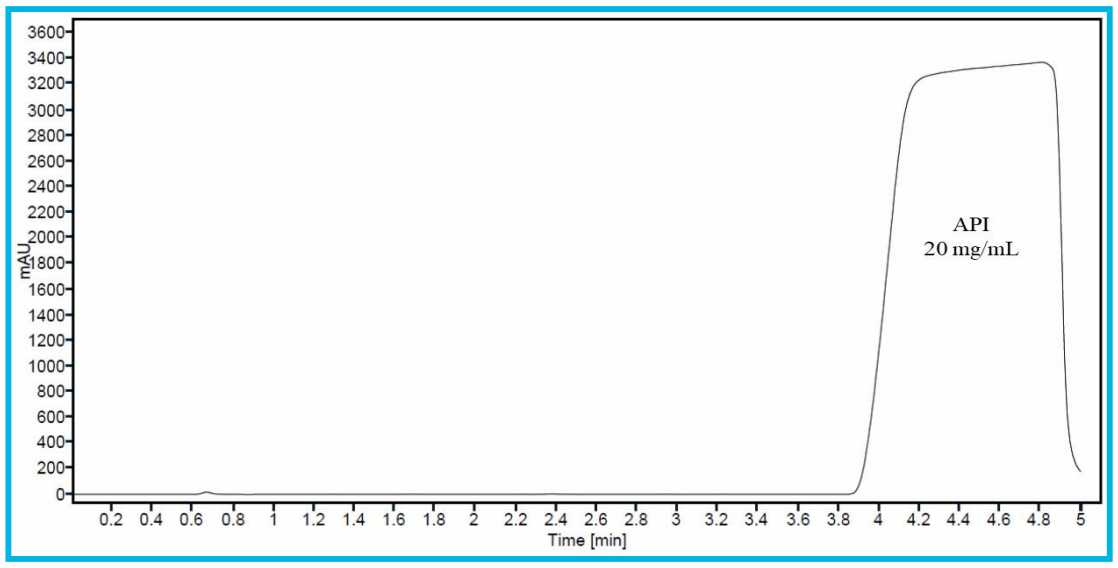

Figure 3. UV chromatogram of the API (20 mg/mL).

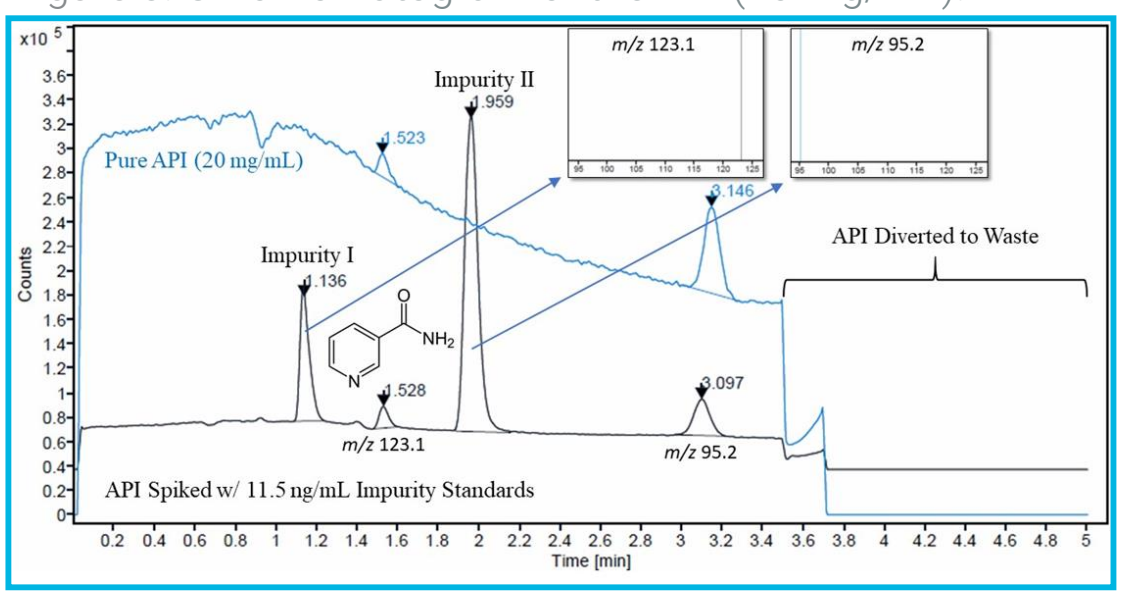

Figure 4. SIM chromatogram of the pure API (20 mg/mL) shown in blue and the same API spiked with 11.5 ng/mL of impurities, relative to the API, shown in black for comparison.

Enhanced sensitivity over UV detection alone

A 1 µL injection containing 11.5 ng/mL of impurities shows no discernable peaks for the impurities in the UV chromatogram as shown in Figure 5. Figure 6 features the SIM TIC, and the impurities are easily distinguished from the noise as peaks at 1.136 and 1.950 mins. SIM only allows single m/z ions to pass in the quadrupole, enabling greatly enhanced sensitivity versus scanning across an m/z range.

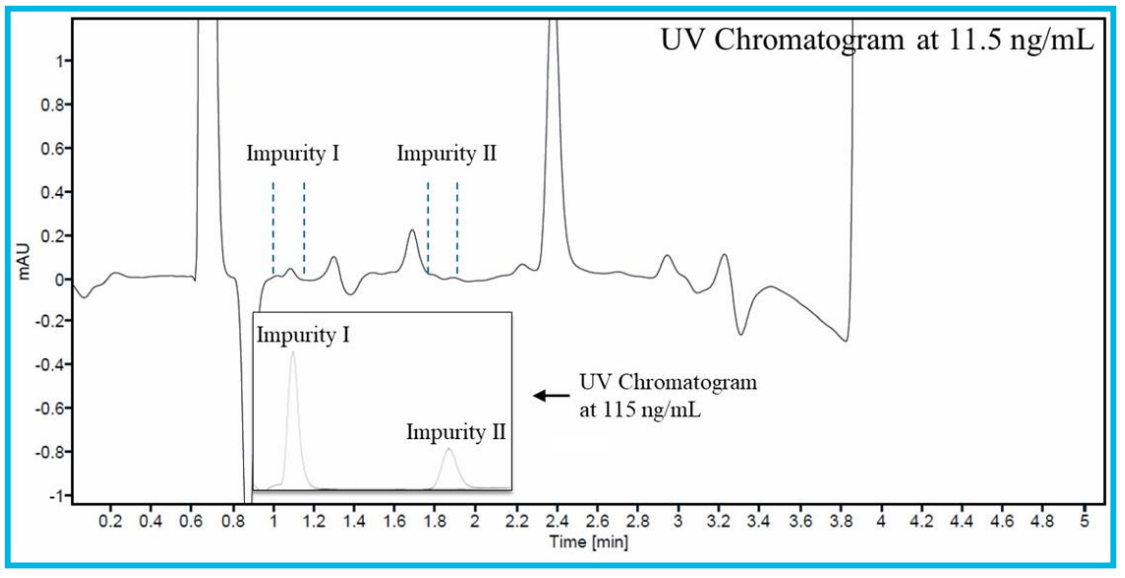

Figure 5. UV Chromatogram of the API spiked with 11.5 ng/mL of impurities. Zoomed-in region of UV chromatogram with the RT of the impurities highlighted. The insert is a UV chromatogram from a higher concentration of impurities

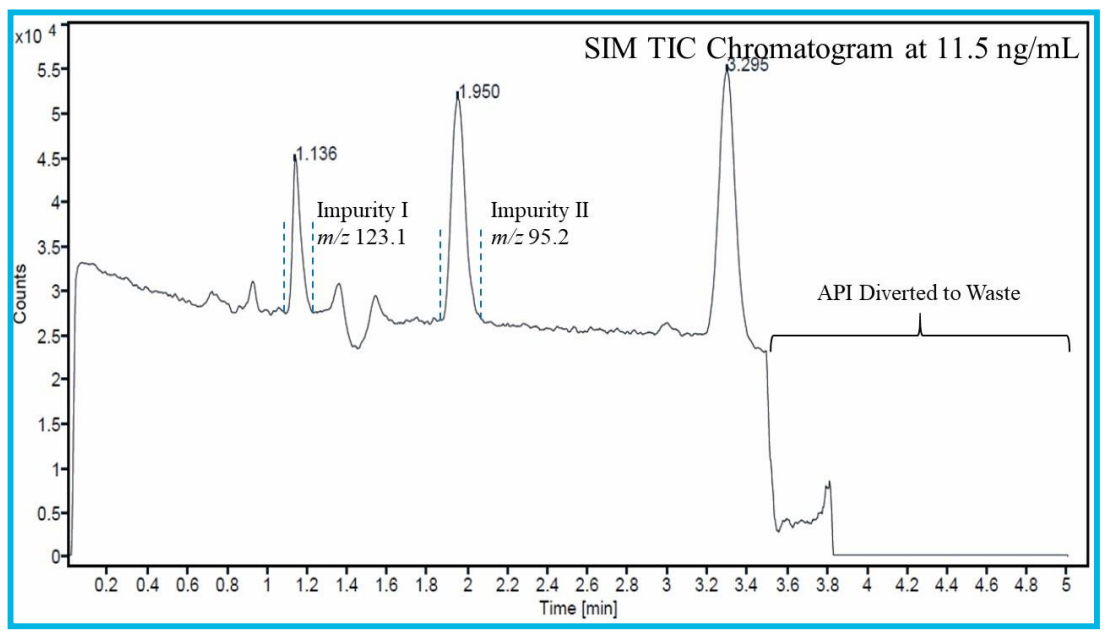

Figure 6. SIM TIC chromatogram, m/z 123.1 and 95.2, impurity I and II respectively at 11.5 ng/mL.

LC/MSD XT with improved sensitivity and higher precision for low-level impurities

The API was analyzed with a series of 1 µL injections where the impurities were spiked into the API at 115, 11.5, and 1.15 ng/mL concentrations and shown in Fig. 7. At the 115 ng/mL level, the impurities can be determined by UV at about the limit of detection and are easily distinguished by the LC/MSD XT in scan mode. At the 11.5 ng/mL level, the impurities cannot be seen in the UV chromatogram and are also not distinguishable in the scan mode of the mass spectrometer. However, the LC/MSD XT is easily able to detect the impurities in SIM mode.

The lowest concentration analyzed was 1.15 ng/mL for both impurities. The signal-to-noise ratio was 12 and 28 for impurity I and II, respectively, using SIM mode. This demonstrates that the LC/MSD XT in SIM mode has a detection limit 100x greater than UV detection for these compounds.

Results and Discussion

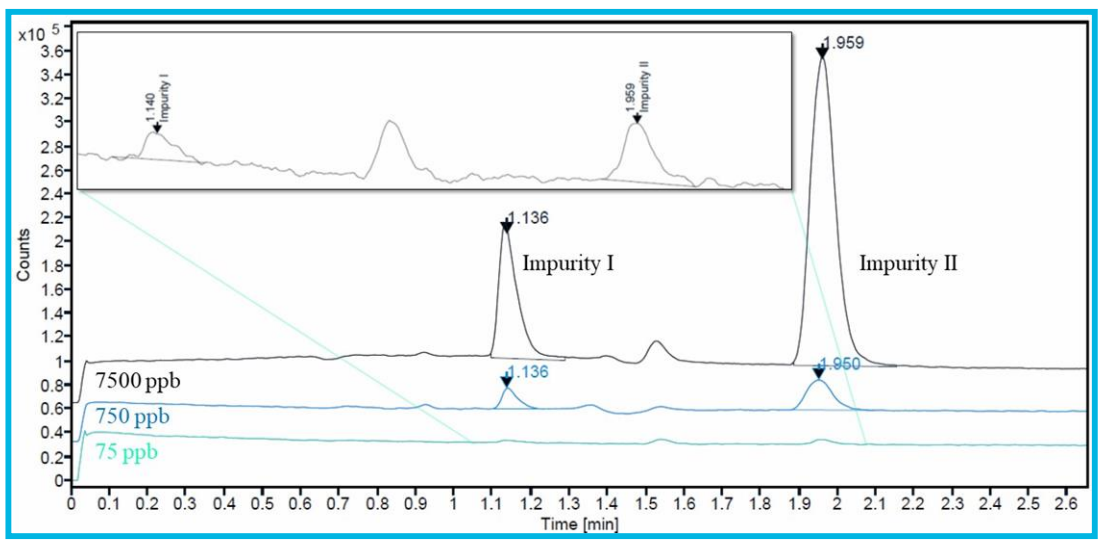

Figure 7. SIM chromatograms (stacked) of the impurities spiked into the API at 115, 11.5, and 1.15 ng/mL concentrations. The inlay is a zoomed portion of the SIM chromatogram at 1.15 ng/mL. (1.15 pg on column)

InfinityLab Poroshell 120 HILIC-Z and LC/MSD XT detection sensitivity (IDL < 16 pg)

A good measure of the overall sensitivity of a method can be established by calculating the instrument detection limit or IDL[3,4]. The IDL not only takes the detector of choice into account, but also the entire HPLC system from pump to column by comparing peak RSDs over multiple injections[3,4]. The IDL was determined for the LC/MSD XT operating in SIM mode for the impurity peaks. The IDL was determined to be 15.40 pg for impurity I and 5.65 pg for impurity II from 10 replicate 5 µL injections of a 20 ng/mL sample containing only the impurities as shown in Figure 8.

The reproducibility of the HILIC-Z column shows excellent performance, as shown in Figure 9, by performing three injections of the same sample over three days, approximately 24 hours apart. The %RSD of the peak areas were 6.7% for impurity I and 4.1% impurity II. Both impurities have a %RSD of the retention times of less than 1%.

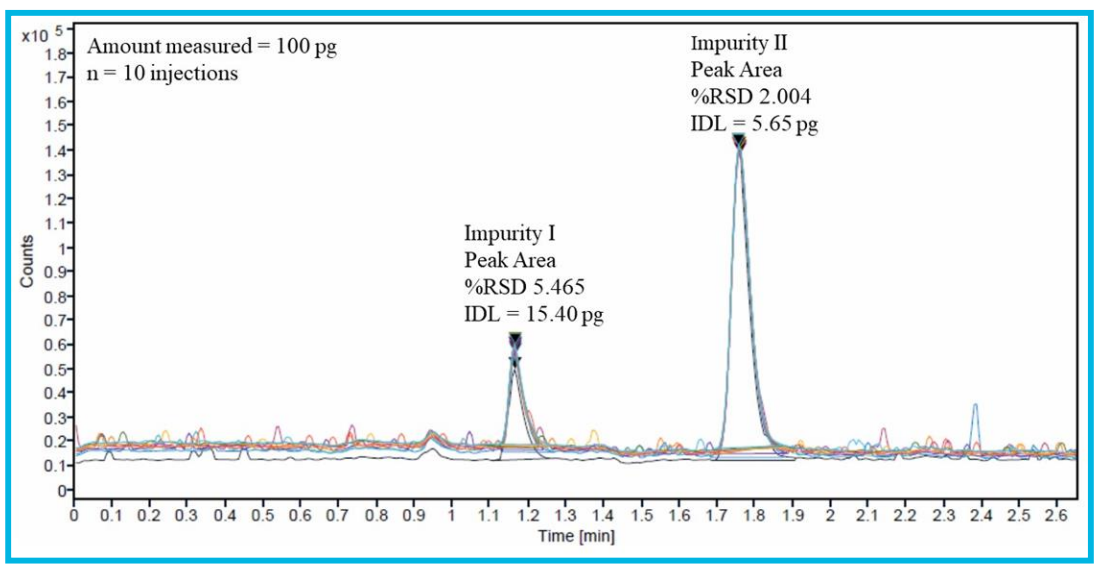

Figure 8. SIM chromatograms of 100 pg injections containing only the impurities. The IDL was determined to be 15.40 pg for impurity I and 5.65 pg for impurity II.

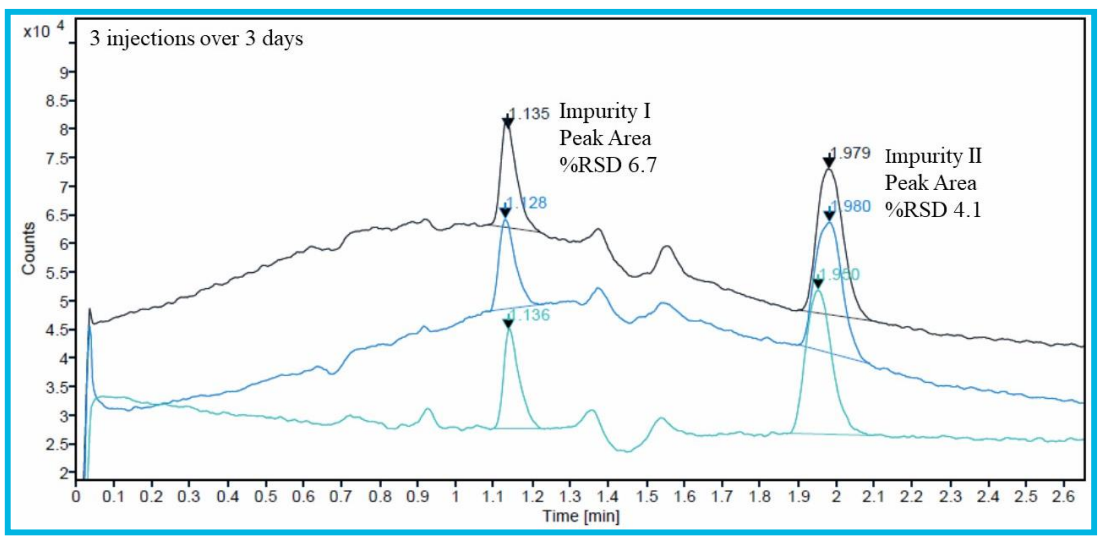

Figure 9. SIM chromatograms (stacked) of a 11.5 ng/mL spiked API sample injected over the course of 3 days. %RSDs of the peak areas are shown to display the reproducibility of the LC/MSD XT system with the Agilent InfinityLab Poroshell 120 HILIC-Z column.

Conclusions

- Dalfampridine and its impurities can be separated effectively and quickly using Agilent's HILIC-Z column.
- The detection limit of the DAD is >100 pg while the LC/MSD XT can go as low as 1 pg. Adding an LC/MSD XT to the HPLC stack improves sensitivity over 100x.
- In combination, the instrument detection limit for Agilent's 1260 Infinity II Prime LC and LC/MSD XT with a Poroshell HILIC-Z column is 15.40 pg for impurity I and 5.65 pg for impurity II

References

[1] US Department of Health and Human Services; FDA; CDER; CBER. ICH, March **2018**.

[2] Jain, M.; Srivastava, V.; Kumar, R.; Dangi, V.; Hiriyanna, S. G.; Kumar, A.; Kumar, P., *J. Pharm. Biomed. Anal.* **2016**, 133, 27–31.

[3] Parra, N. P.; Taylor, L., Agilent Technologies Technical Overview, **2014**.

[4] Sheehan, T. L.; Yost, R. A., *Spectroscopy* **2015**, 13(4), 16–22.

Published in USA, June 2, 2019

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