Application Note

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Using Self-Aware Agilent InfinityLab LC/MSD iQ to Measure Trace-Level Impurities in a Brand Versus Generic Medication

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Abstract

This study presents a method for comparison of related impurities in a brand and a generic over-the-counter (OTC) acetaminophen drug. The workflow comprised an Agilent InfinityLab liquid chromatography/mass selective detector (LC/MSD iQ) and an Agilent 1290 Infinity II LC. The study identified impurities at concentrations lower than can be reliably detected by UV.

Introduction

Most OTC pharmaceutical drugs are available as a generic form developed from a brand name product, and do not require a prescription for purchase. These generic products are sold by different manufacturers to help spur competition, and potentially lower prices making the drug more affordable. Generic OTC drugs manufacturers must meet the same quality attributes such as strength, purity, stability, and the limits for related impurities as determined by the USFDA for approved brand name drugs.[1] Generic drug manufacturers must be able to characterize their product and its impurities, typically with HPLC and UV detection. Adding a mass selective detector can significantly reduce the analysis time of typical USP or EP methods[2] since coeluting components can be unambiguously determined by the mass of target compounds.

This study shows the comparison of related impurities in a brand versus generic OTC drug of acetaminophen. Impurities were screened using an InfinityLab LC/MSD iQ and, 1290 Infinity II LC. The LC/MSD iQ, based on single quadrupole technology, is a robust detection system designed to be easy to use and reliable. It is an intuitive instrument that requires minimal user training. It can handle high LC flow rates, and automatically optimizes MS parameters to provide optimal results. The use of mass determination ensures unambiguous detection of active pharmaceutical ingredients (APIs), their related impurities, and, more importantly, any unknown or unexpected impurities. Chromatographers can easily incorporate the LC/MSD iQ as a new module in their existing LC stack. It is specifically designed to be easily integrated into the Agilent 1260 Infinity II LC, 1290 Infinity II LC, or even older LCs with a space-design that plugs into the same power outlets as the LC modules. The HPLC stack, including the MS and vacuum pump, can be stored on the Agilent InfinityLab Flex Bench MS, freeing bench space, and allowing the entire system to be relocated swiftly and safely.

Experimental

Standards and chemicals

All reagents and solvents were HPLC or LC/MS grade. Methanol was purchased from Honeywell (Morristown, NJ, USA). Standards of the acetaminophen API, its related impurities, and acetic acid were purchased from Millipore-Sigma (Merck, Darmstadt, Germany). Ultrapure water was produced using a Milli-Q Integral system equipped with a LC-Pak Polisher and a 0.22 µm point-of-use membrane filter cartridge (EMD Millipore, Billerica, MA, USA).

OTC drug samples were purchased from local drug stores, and designated as drug 1 for a brand product and drug 2 for a generic product.

Instrumentation

The LC/MSD iQ system consists of the following modules:

- Agilent 1290 Infinity II High Speed Pump (G7120A)
- Agilent 1290 Infinity II Vialsampler (G7129B)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent 1290 Infinity II Diode Array Detector (G7117B)
- Agilent LC/MSD iQ (G6160AA)

The DAD was used with an Agilent Max-Light cartridge cell, 60 mm (G4212-60007) to use the maximum sensitivity of the UV detector with the LC/MSD iQ.

Sample preparation

Selected drug impurity standards were weighed out into 15 mL Falcon tubes, and diluted to a working concentration of 1 mg/mL with MeOH then sonicated for approximately 15 minutes. A 100 µL amount of each standard was added to a 2 mL screw top vial, and diluted to 1 mL with 80:20 MeOH/H_2O. Serial dilutions with 80:20 MeOH/H_2O were performed to make up the following concentrations for an external calibration curve: 10, 5, 2.5, 1, 0.5, and 0.1 µg/mL. OTC drug samples came in tablet form with 500 mg per dose of the acetaminophen (API). A simple liquid extraction of the API and its impurities was performed using MeOH. A drug tablet was placed in a 15 mL Falcon tube with 10 mL of MeOH, shaken for 30 minutes, sonicated for approximately 30 minutes, and spun down in a centrifuge at 4,500 rpm for approximately 15 minutes. Then, 1 mL of the final solution was pipetted from the Falcon tube into a 2 mL screw top vial for analysis with a final API concentration of 50 mg/mL.

Agilent OpenLab CDS

Agilent OpenLab CDS software was used for data acquisition, processing, and reporting. OpenLab CDS provides compliance features that support data integrity in accordance with US FDA 21 CFR Part 11, EU Annex 11, and other similar regulations. The 1290 Infinity II LC and LC/MSD iQ are designed to ensure reliable and robust LC/MS for routine applications in GxP laboratories.

Results and discussion

Determining the linear calibration range using OpenLab CDS 2.4

Calibration curves were generated using Agilent OpenLab CDS 2.4 software. In Data Analysis within OpenLab CDS, the user can easily view the calibration curves (Figure 1), and this data is stored for automatic and quick quantification of test samples. External calibration curves were generated for the standard impurities at six levels: 0.1, 0.5, 1.0, 2.5, 5.0, and 10.0 µg/mL with three replicates. These concentrations correspond to 2, 10, 20, 50, 100, and 200 ppm in relation to API in the sample. This range was chosen to bracket the expected concentration of impurities in the drug samples. Figure 2 shows the calibration curves, and all target compounds had calibration curves with an $R^2 > 0.99$.

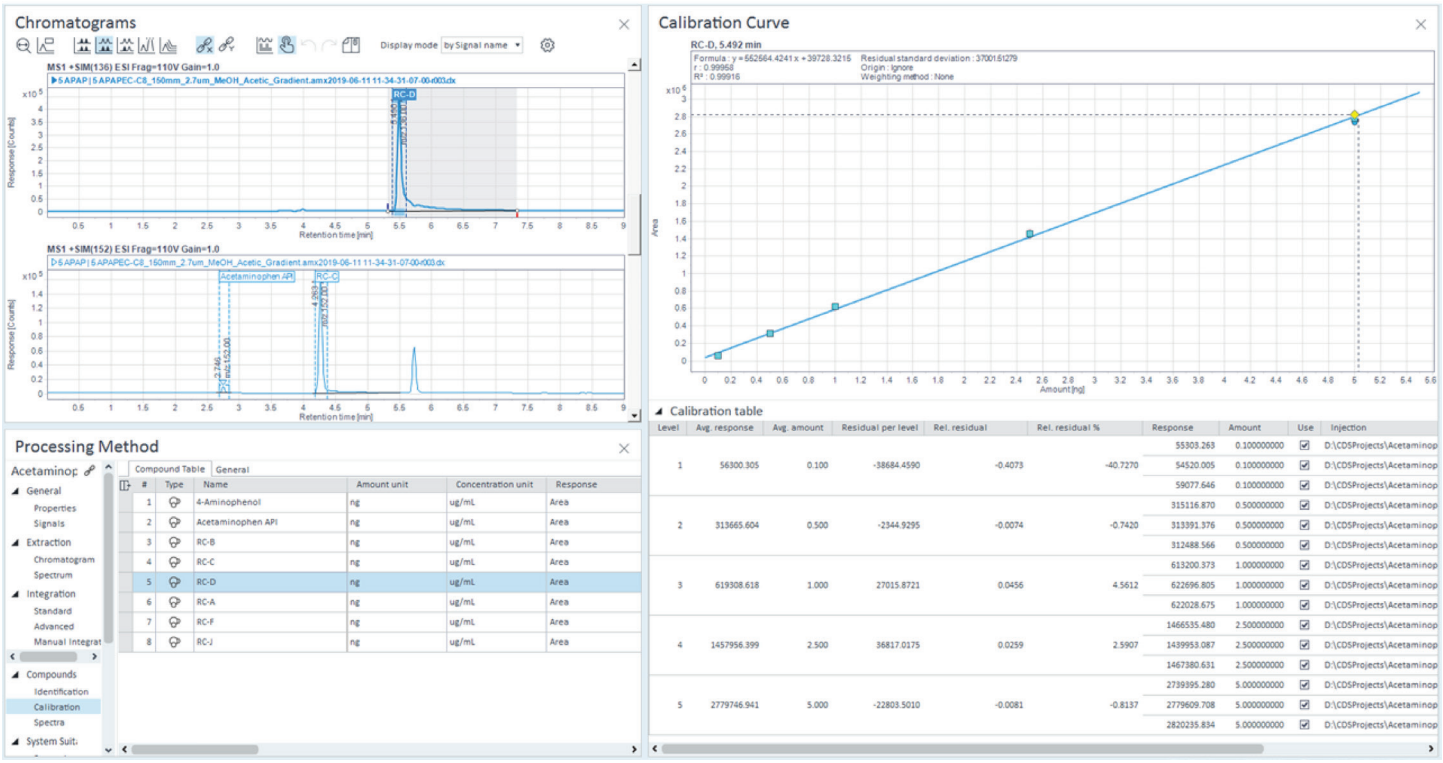


Figure 1. The Data Analysis section in Agilent OpenLab CDS with the calibration curve tab open. Users can easily view the chromatograms and calibration curves together, and select the calibration curve with real-time updates to the calculations.

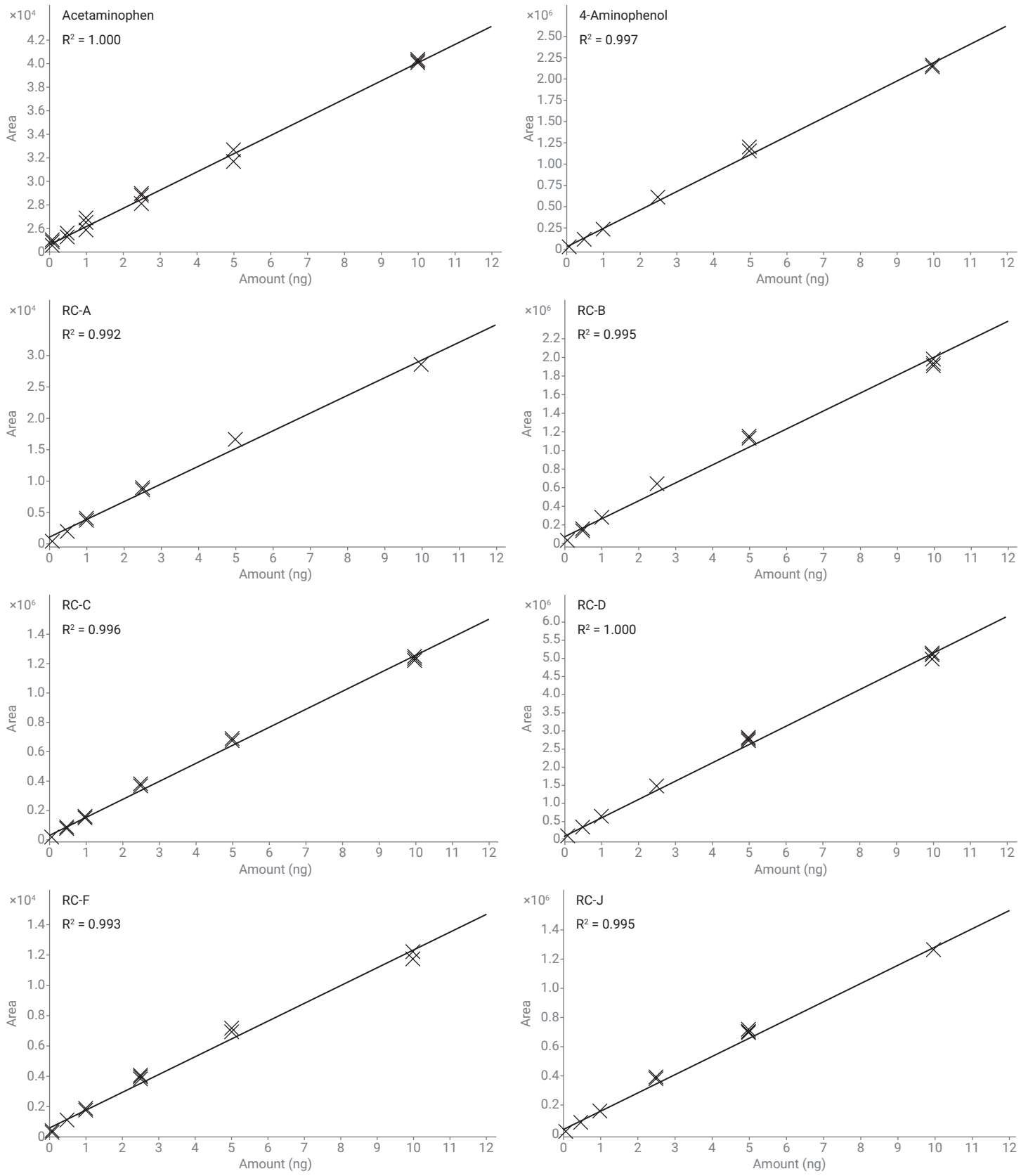


Figure 2. External standard calibration curves for the API and impurities listed in Table 1. All target compounds were measured in triplicate and have an $R^2 > 0.99$.

Diverting the drug API is essential to protect the MS detector

The LC/MSD iQ is designed with a built-in divert valve to facilitate the analysis of impurities in the presence of high-concentration sample, and ensure MS detector longevity and robustness. To avoid oversaturating the MS detector, due to the high concentration of the API in the sample (50 mg/mL), the flow is diverted to waste when the API elutes after the DAD. First, the sample is measured without the MS in the flow path to determine the time window in which the API should be diverted (Figure 3).

The built-in LC/MSD iQ divert valve was set to divert flow to waste from 2.5 to 3.5 minutes. As shown in Figure 3, this diverts only the API, and switches the flow back to the mass spectrometer before the first impurities begin to elute.

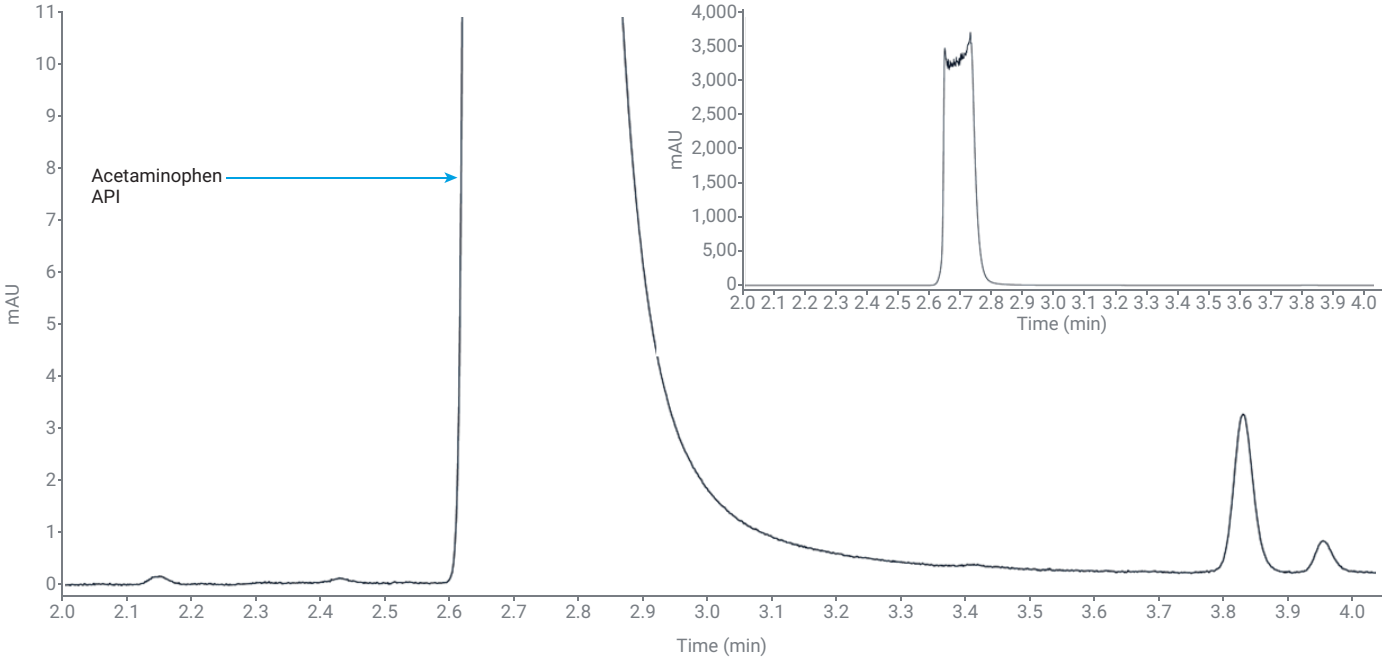


Figure 3. Zoomed in UV chromatogram of a 1 µL injection of a drug sample containing 50 mg/mL of the drug API, acetaminophen. The inset image shows the full signal scale of the same injection.

Identifying impurities in the OTC samples

To optimize the LC method for adequate separation, the impurities listed in Table 1 were measured individually from standard samples. Then, a 1 µL injection of a mixed sample containing the API and impurity standards at a concentration of 10 µg/mL was measured to compare with the drug samples for determining the identity of impurities present in the samples (Figure 4).

A 1 µL injection of the drug samples was measured for all the impurities listed in Table 1 being monitored in selected ion monitoring (SIM) mode. Figure 5 shows that not all the related impurities were detected in the drug samples. Only four of the impurities were confirmed in drug samples: 4-aminophenol, RC-A, RC-B, and RC-D. For this study, only SIM of the related compound profiles (Figure 6) were compared, and other components that are part of the formulation were excluded.

Table 1. List of standard API and impurities.

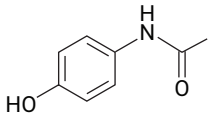
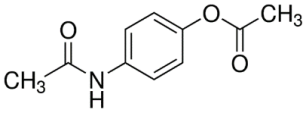
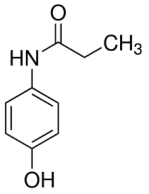
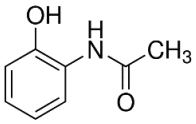
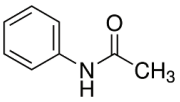
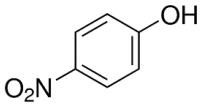
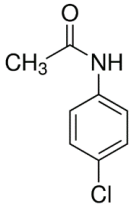
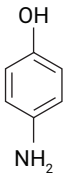
Compound	g/mol	Structure
Acetaminophen	151.2	
Related compound A	193.2	
Related compound B	165.2	
Related compound C	151.2	
Related compound D	135.2	
Related compound F	139.1	
Related compound J	169.6	
4-Aminophenol	109.1	

Table 2. Agilent 1290 Infinity II LC method parameters.

Parameter	HPLC Set Value
Column	Agilent InfinityLab Poroshell 120 EC-C8, 3.0 × 150 mm, 2.7 µm at 40 °C (p/n 693975-306)
Mobile Phase A	0.1% acetic acid in H_2O
Mobile Phase B	0.1% acetic acid in MeOH
Gradient	Time %B 0 10 7 50 8 80 8.5 80 9.0 10
Post Run	2.5 minutes
Flow Rate	0.6 mL/min
Injection Volume	1 µL
Detection UV	(265, 5/ref. 360, 80), (318, 5/no ref.)

Table 3. Agilent InfinityLab LC/MSD iQ parameters.

Parameter	Single Quadrupole Set Value
Ion Source	ESI+
Peak Filter	0.02 minutes
Scan/Dwell Time	SCAN 100 ms
	SIM 50 ms
Drying Gas Temperature	325 °C
Gas Flow	10 L/min
Nebulizer Pressure	35 psi
Capillary Voltage	3.5 kV
Fragmentor Voltage	90 V
Scan Range	*m/z* 100 to 300
SIM Ions	*m/z* 110, 152, 194, 166, 136, 140, 170

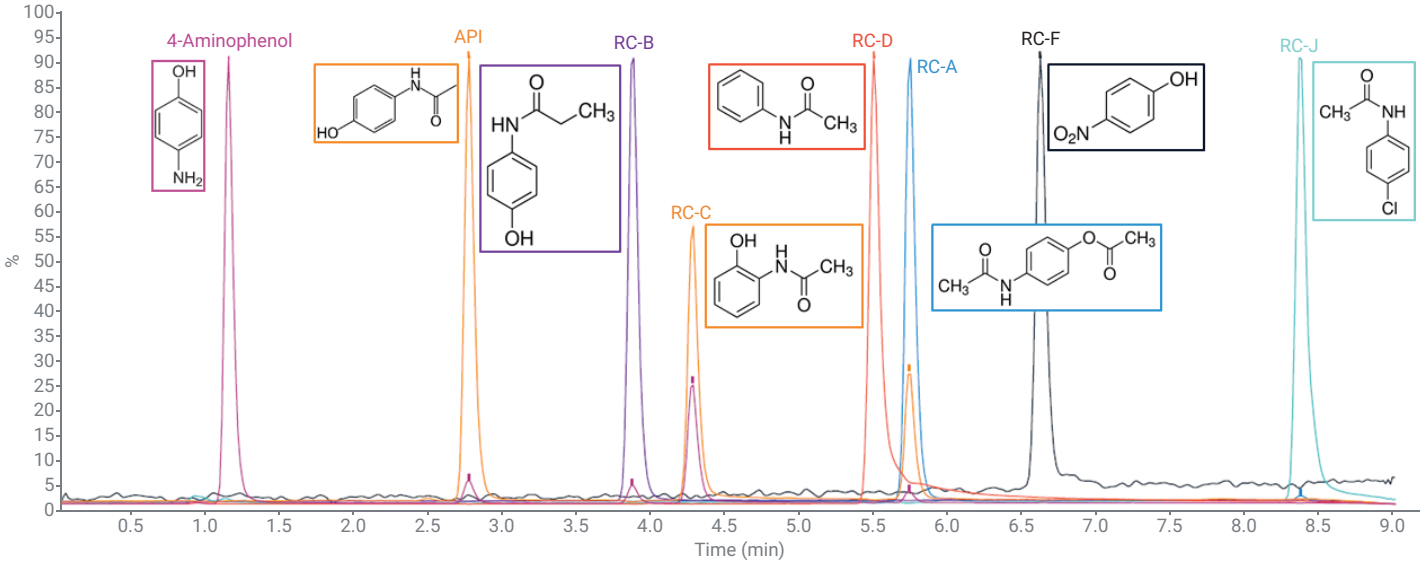


Figure 4. SIM mass chromatogram of a 1 µL injection of the API and impurity standards (10 µg/mL). Each peak is labeled with a color-coded structure. The signal scale is set to relative abundance so that each peak can be clearly observed.

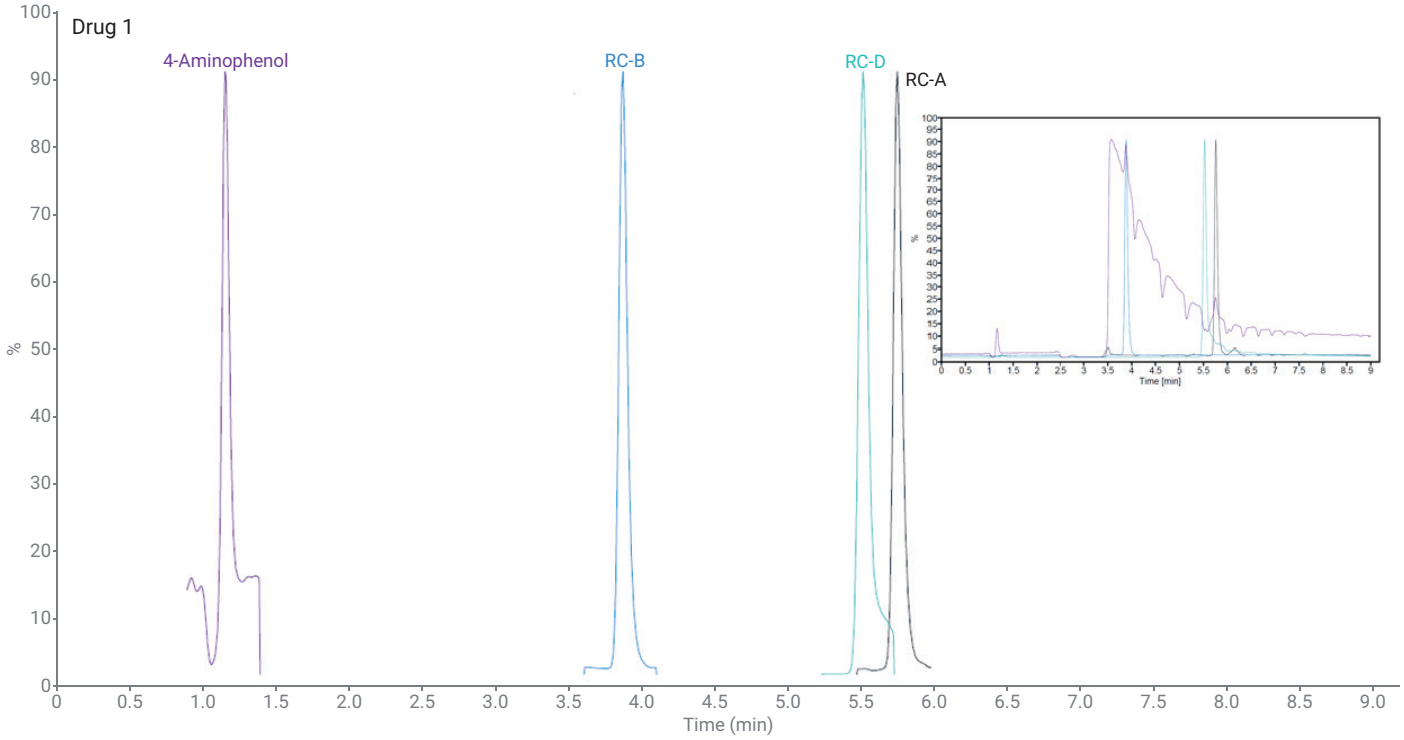


Figure 5. Selected SIM extracted ion chromatogram (EIC) mass chromatogram of a 1 µL injection of the drug 1 sample, which contains 50 mg/mL of the API. The SIM signals are bracketed at ± 0.5 minute for clarity, with the inset image showing the full SIM TIC chromatogram. The broad peak in the inset image is a fragment of the leftover API being detected at the same *m/z* as 4-aminophenol (*m/z* 110).

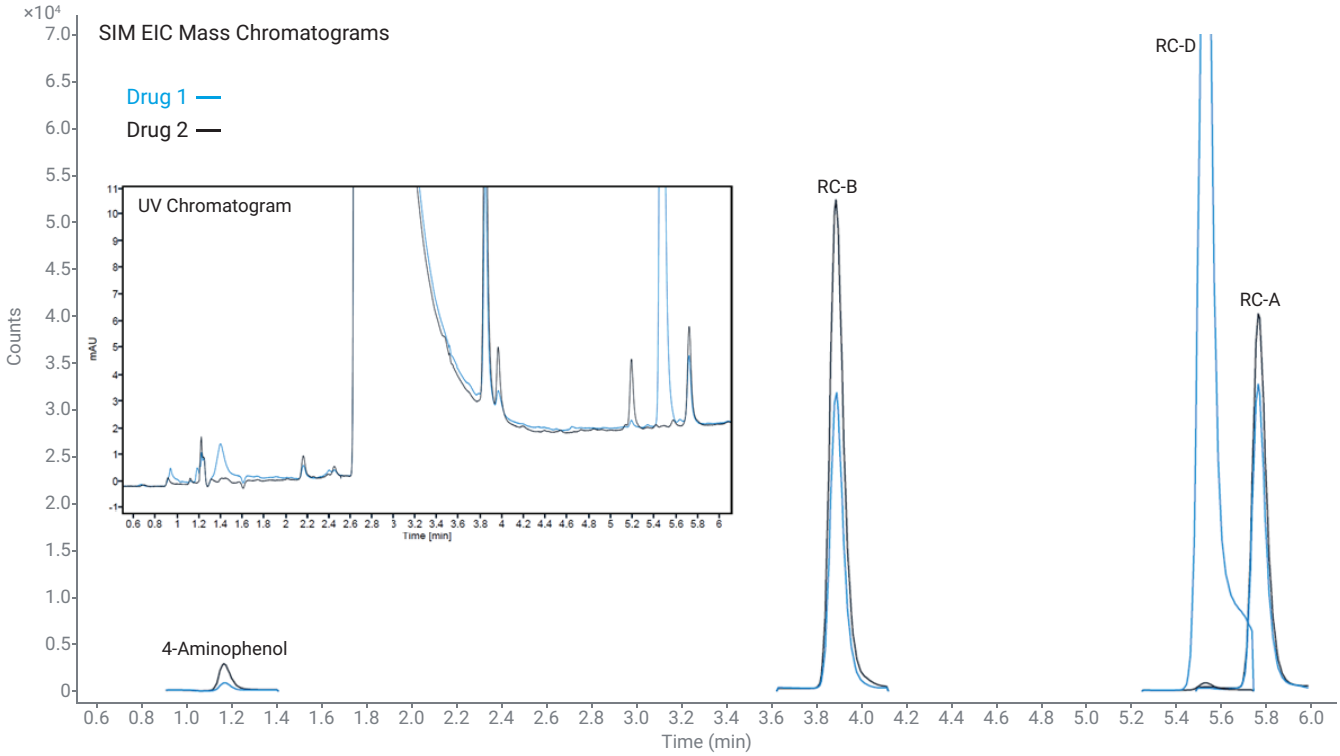


Figure 6. SIM EIC mass chromatograms of detected drug impurities in the OTC drug samples. The blue trace is for drug 1 and the black trace is for drug 2. The impurity EICs were bracketed around their retention times for clarity. The inset is the UV chromatogram of the same injections showing many other compounds not related to the impurities in Table 1.

Comparing a brand versus generic OTC drug sample

Figure 6 shows the comparison between drug samples 1 and 2 for the measured standard impurities: 4-aminophenol, RC-A, RC-B, and RC-D. Of most interest is RC-D, which has a much higher abundance in drug 1 than in drug 2. However, the overall abundance of impurities is larger in drug 2 for 4-aminophenol, RC-B, and RC-A. Table 4 shows the amount of detected impurities compared to the API (ppm) in each drug sample. While these amounts are well below the USFDA recommended guidelines,[3] it shows that there is a clear difference in the impurity compositions between a brand drug and generic drug.

Table 4. Comparison of the amount of impurities compared to the API (ppm) in the drug samples. The higher concentration is bolded.

Impurity	Drug 1 ppm to API	Drug 2 ppm to API
4-Aminophenol	0.440*	**1.586***
RC-A	3.010	**4.960**
RC-B	4.260	**14.72**
RC-D	**21.32**	Not detected
RC-C	Not detected	Not detected
RC-F	Not detected	Not detected
RC-J	Not detected	Not detected

* Below lowest calibration level, extrapolated

Conclusion

The Agilent LC/MSD iQ can easily detect impurities present in OTC drug products down to picogram on-column levels (ng/mL). Brand and generic OTC drug products were analyzed for impurities, and a comparison was made. The brand product contained four detectable impurities: 4-aminophenol, RC-A, RC-B, and RC-D, while the generic product contained three detectable impurities: 4-aminophenol, RC-A, and RC-B. Even though analysis of the brand name product showed the presence of more detected impurities, the generic product contained a higher concentration of these impurities. Both OTC products are manufactured under USFDA guidance[3] and the impurity concentrations are far below the levels specified in the guidance: 15 µg/mL or 0.03% of the API. At these concentrations (see Table 4), UV detectors cannot reliably quantitate these impurities. Furthermore, the difference in impurity profiles observed between two APIs can be attributed to the source of starting material and synthetic and purification processes used during manufacturing.

References

1. Generic Drugs. https://www.fda.gov/drugs/buying-using-medicine-safely/generic-drugs. USFDA, May 16, 2019. Retrieved July 22, 2019.
2. Acetaminophen USP Monograph. The United States Pharmacopeia Convention. May 1, 2014.
3. US Department of Health and Human Services; FDA; CDER; CBER. Guidance for Industry Q3B(R2) Impurities in New Drug Products. ICH, July 2006, Rev. 2.

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