An Executive Summary

Implementation of Dual-Channel Chromatography to Improve Productivity in the Analysis of Pesticides Residues in Food

Łukasz Rajski, PhD
Senior Chemist, European Union Reference Laboratory for Pesticide Residues in Fruit & Vegetables
University of Almería, Almería, Spain

A new approach to boosting sample throughput without sacrificing data quality.

Introduction

The productivity of a pesticide residues laboratory is often limited by the chromatographic timespan. In a typical liquid chromatography-tandem mass spectrometry (LC-MS/MS) multi-residue method, the run time is a compromise between the number of target analytes, an acceptable number of coelutions, the desired chromatographic peak width, and the minimum dwell time of the MS detector needed to achieve acceptable data quality. A fast-chromatographic run that produces very narrow peaks can have a negative impact on sensitivity, selectivity, and data quality. An effective solution is the use of a novel dual-channel ultrahigh performance liquid chromatography (UHPLC) system with two independent flow paths capable of operating two analytical columns simultaneously. Sample extracts are injected alternately on the two columns using optimized gradient elution conditions to either increase sample throughput or reduce the coelution of compounds to improve data quality. The chromatographic separation of the two columns is synchronized so the flow is directed to the mass spectrometer only during the method data window that includes the elution of first analyte up to the elution of the last analyte of interest. By overlapping the chromatography, the mass spectrometer is continuously acquiring data, so the utilization of the MS is increased substantially, thus enabling either more samples to be analyzed in a defined period or improved chromatographic resolution without increasing the time required for analysis.

Dual-Channel LC-MS/MS

Today, a typical LC-MS/MS method for pesticides analysis will use a column that is 100 mm in length, containing particles of 2–3 µm in diameter, and with gradient elution at a flow rate of 300–400 µL/min. The total chromatographic analysis time is around 15–20 min and the number of target pesticides is 150–400. For compliance with the EC guidelines (SANTE/12682/2019) for identification, at least two transitions should be monitored for each pesticide, meaning a total of 300–800 precursor to product ion transitions should be included in the acquisition method. The question for laboratories is how to decrease the analysis time in order to improve productivity without compromising the quality of the data? The simple answer to achieve a shorter method is to use a shorter column, a steeper gradient, or a higher flow rate, but the number of co-elutions will increase, and the data quality will be compromised.

Implementing a faster shorter method on a short column and with a triple quadrupole mass spectrometer has several considerations and drawbacks. If the duty cycle is maintained,

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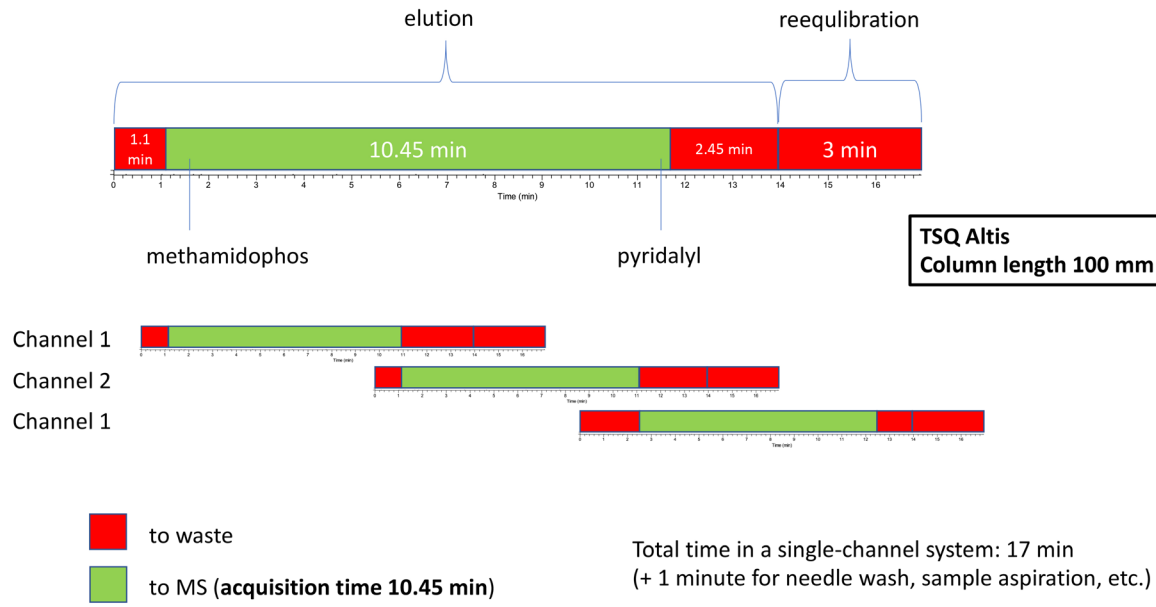
Figure 1: Time segments in dual-channel chromatography.



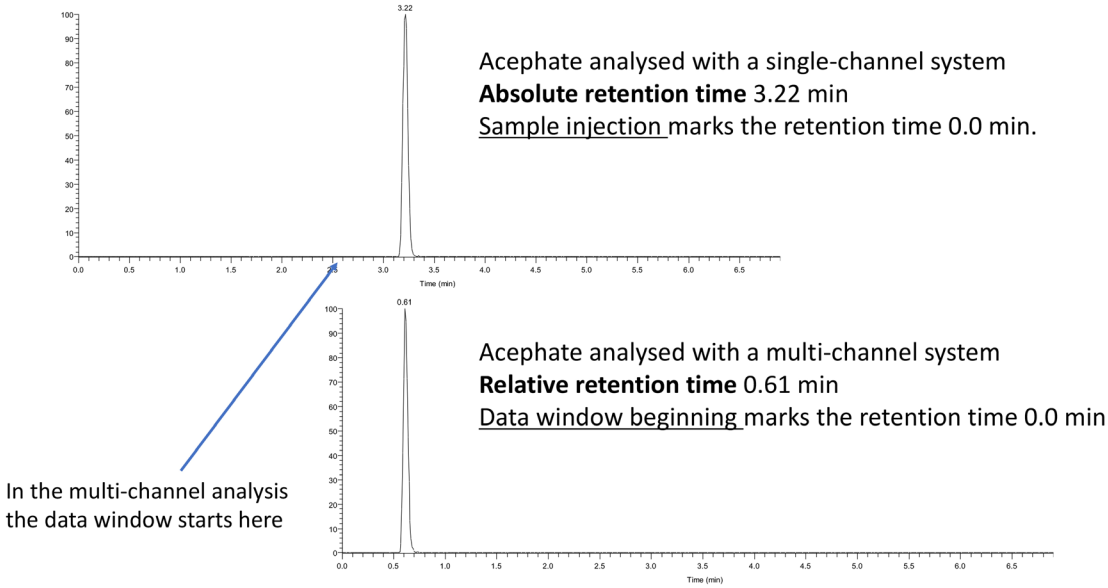
dwell times become shorter and sensitivity is reduced. If dwell times are maintained, the duty cycle is longer, which leads to fewer data points per peak and reduced peak area precision. In addition, the coelution of pesticides with common transitions makes quantitation impossible while coeluting matrix may cause ion suppression and lower sensitivity. Since the elution of matrix compounds and pesticides is not evenly distributed, the most crowded portions of a chromatogram are particularly affected by speeding up the chromatography with an increased probability of interferences from coeluting matrix with the same transitions as the analytes. Thus, more time is spent optimizing different transitions, which are more selective and therefore less affected, but generally less sensitive.

A different approach for decreasing the analysis time and increasing throughput, without further compromise to the separation, is the application of dual-channel chromatography. With this methodology, the total analysis time is decreased by minimizing unused mass spectrometer time while maintaining efficient chromatographic separations. Designed to enhance throughput, the Thermo Scientific™ Transcend™ Duo LX-2 System is configured with two pumps, and an autosampler with two independent injection units and a column oven. The combination of a pump and its dedicated column is referred to as a channel. Two columns are operated in parallel and sample run times are overlapped, as shown in **Figure 1**, so the mass spectrometer is not sitting idle during re-equilibration of the column, autosampler needle wash, and other time-consuming steps. Consecutive injections are synchronized such that the first analyte from the second column elutes just after the elution of the last analyte from the first column. The mass spectrometer collects data during the elution portion— "the data window"—for each sample, highlighted green in **Figure 1.** The mass spectrometer acquisition switches between channels to maximize efficiency. When the data window ends in channel 1, the data window starts in channel 2. Then at the end in channel 2, the channel 1 data window starts again, and so on.

Thermo Scientific™ Aria™ MX software schedules and controls the staggered but synchronized parallel operations by control of selector and bypass valves. As with a single-channel LC system, the typical method parameters are entered in the software. The dual-channel technique requires only two new parameters to be loaded, namely, the start time for the data collection window and its duration. With this information, the software will synchronize the injections on the two channels. The duration of the mass spectrometer data collection is also edited in the MS instrument method.

In typical applications, the Aria MX software will alternate sequence injections between the two channels and the resultant data, if viewed in aggregate, will generate cross-channel results. If desired, users may override the alternating channel selection to perform consecutive runs on the same channel from the same sample plate with the other channel dedicated to a different sample plate and all data generated will be within the channel. The graphical interface is intuitive, making conversion of a single-

Figure 2: Retention time in multi-channel chromatography.



channel chromatographic method into a dual-channel method rapid and simple.

> Immediately before that, the MS system was busy detecting compounds in the previous sample.

Although separations are not impacted by switching to a dual-channel method, retention times are altered. Retention times in single-channel systems denote the absolute retention time, reflecting the entire chromatographic run, including the start and end time segments when the mass spectrometer is idle. On sample injection, the autosampler sends a signal to the MS instrument to initiate the start of the retention time measurement. In this traditional mode, the retention time for a compound is a measurement of the time it spends on the column, from injection to elution.

By contrast, data collection in a dual-channel system does not begin on injection, but rather just before elution of the first peak of interest as determined by the data window start time in the LC method, and as depicted in **Figure 2**. Immediately before that, the MS system was busy detecting compounds in the previous sample. In this mode, the start signal for the MS system to begin acquisition occurs at the data window start time configured in the method and typically close to the elution of the last peak of interest in the previous sample. The beginning of the data window marks the 0.0 retention time, resulting in a relative, rather than absolute, retention time. The sample spends the same amount of time on the column, but the MS detection window is shorter in duration to create a narrower data window that is focused only on the elution times of the analytes of interest. This technique optimizes the use of the MS detector and shortens overall run time without sacrificing the quality of the chromatography.

Applications

A Transcend DUO LX-2 System was interfaced with a Thermo Scientific™ TSQ Altis™ Triple Quadrupole Mass Spectrometer system or a Thermo Scientific™ Q Exactive™ Focus Hybrid Quadrupole-Orbitrap™ Mass Spectrometer system to evaluate the dual-channel approach for the multi-residue analysis of pesticides using columns of different lengths. Using a Thermo Scientific™ Accucore™ HPLC column C18, 2.1 x 100 mm, 2.6 µm column with a TSQ Altis, the chromatography method allowed 14 minutes for the elution stage, three minutes for column re-equilibration, and approximately one minute for all the autosampler operations. **Figure 1** illustrates that of the 18-minute total run time, the pesticides eluted in

Figure 3: Retention time stability (methamidophos).

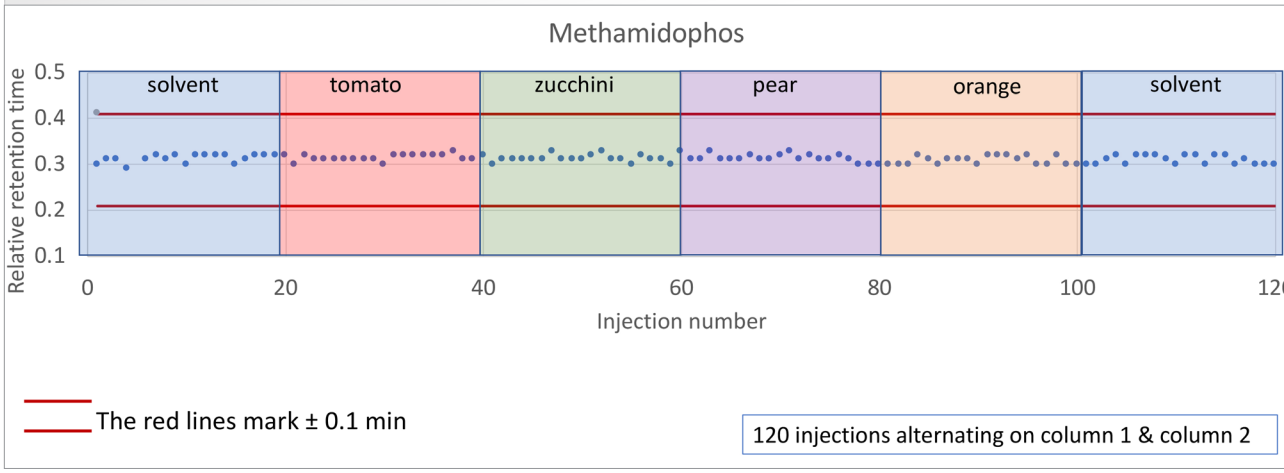
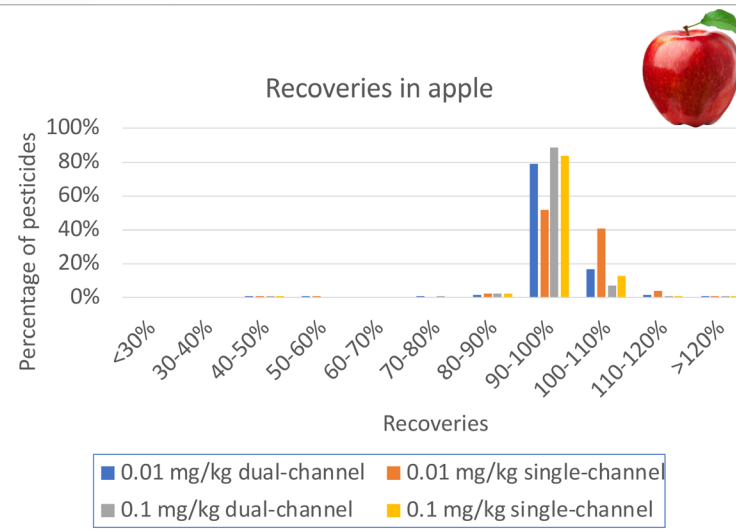


Figure 4: Recovery data (apple): single-channel vs dual-channel.

	<70%	70-120%	>120%
Single channel 0.01 mg/kg	2	269	2
Dual channel 0.01 mg/kg	2	270	1
Single channel 0.1 mg/kg	2	270	1
Dual channel 0.1 mg/kg	2	270	1



Results obtained using TSQ Altis

Number of compounds: 273

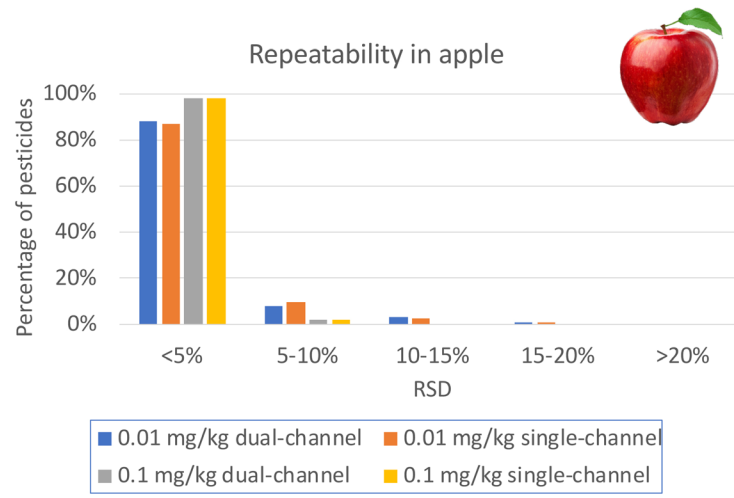
the green range, which spanned less than 11 minutes. By employing the dual-channel method, in which the run time consists of the elution time only, the time of analysis was reduced from 18 minutes to 10:45 minutes.

Compared with the single-channel system, the shortened run times of the dual-channel system amounted to significantly higher throughput. In one hour, three samples could be analyzed using conventional analysis, versus five samples on the new set-up. Accordingly, over a 24-hour period, the single-channel could analyze 80 samples, whereas the dual-channel could run 135 samples, almost a 70% increase in productivity. Thus, the cumulative effect of the productivity enhancement that can be gained with the dual-channel system could substantially improve throughput for laboratories striving to meet demanding analytical turnaround times.

Retention time stability for the dual-channel system was assessed over 120 injections that alternated between column 1 and column 2. Pesticides spiked into samples of varying complexity (e.g., tomato, zucchini, pear, and orange) were analyzed and their retention times plotted as a function of injection number. In the graph for methamidophos, shown in **Figure 3**, the two red horizontal lines denote the +/-0.1 retention time tolerance specified in the EU SANTE guidance documents. The retention times for all of the pesticides in every matrix in

Figure 5: Repeatability data (apple): single-channel vs dual-channel.

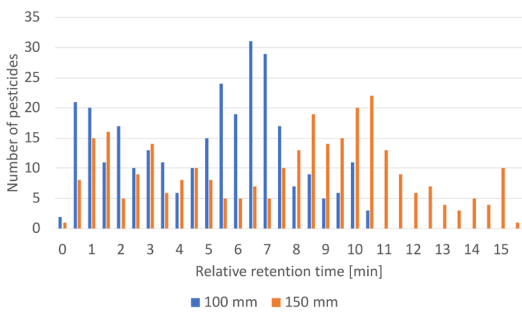
	<5%	5-20%	>20%
Single channel 0.01 mg/kg	87%	13%	-
Dual channel 0.01 mg/kg	88%	12%	-
Single channel 0.1 mg/kg	98%	2%	-
Dual channel 0.1 mg/kg	98%	2%	-



Results obtained using TSQ Altis

Number of compounds: 273

Figure 6: Distribution of pesticides.



Results obtained using QE Focus

the study were compliant with the EU SANTE guideline criteria. Two apparent outliers were not outliers at all, but simply the result of a software setup issue of labeling the retention time when closely eluting isomers of slightly varying responses were integrated together. This can be easily resolved by integrating the isomers separately.

Recoveries were studied in apple, bell pepper, and orange matrices using a QuEChERS extraction protocol with no clean-up applied. The extracts were diluted 5-fold with water prior to injection on the TSQ Altis (2.5 µL) or the Q Exactive Focus (10 µL). The recoveries of the dual-channel system were comparable with the single-channel results. **Figure 4** provides the recovery data for apple, which shows equivalence between the two channels. For apple, the recoveries for 269 and 270 of the total 273 pesticides at 0.01 mg/kg were within the 70–120%, in single- and dual-channel modes of operation, respectively. The associated %RSDs were all below 20%, with the majority below 5% (**Figure 5**). It is important to note that the results were equivalent for the single-channel and the dual-channel modes.

Cross-channel calibrations for pesticides in apples were performed by using calibration data from both alternating channels in a single calibration curve. Excellent linearity was observed for all calibration curves, in agreement with their single-channel counterparts. Coefficients of determination, R^2, of 0.9997+ were achieved in all cases.

Increasing Selectivity Without Reducing Throughput

The use of 100 mm columns for pesticide residue analysis is typically a compromise between the quality of separation and the speed of analysis. There are times when separations using a 100 mm column are insufficient, but laboratories would rather not use longer columns because of the increase in the run times. Dual-channel chromatography is especially useful if laboratories would prefer to improve the quality of the separation without incurring a time penalty. The reduced run time delivered by the dual-channel system can compensate for the extra time involved with substituting a 150 mm column for a 100 mm column. Thus, the separations can be improved without sacrificing productivity because dual-channel chromatography enables the use of longer columns for improved separations but with similar run times to a single-channel operating with a 100 mm column.

For instance, the same mixture of pesticide residues from **Figure 1** that took 18 minutes by the single-channel

Figure 7: Co-elution of analytes with a 100 mm column.

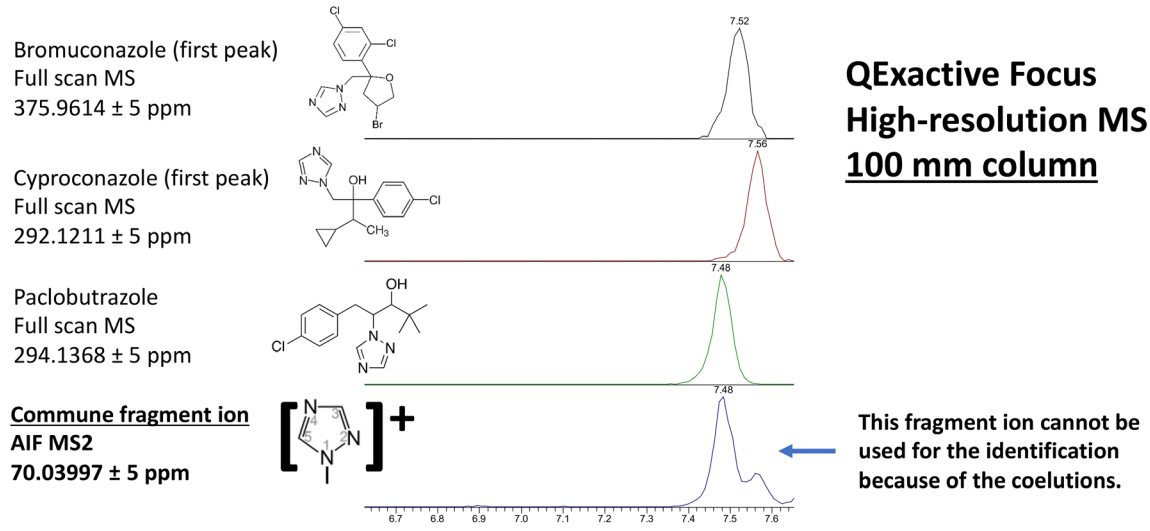
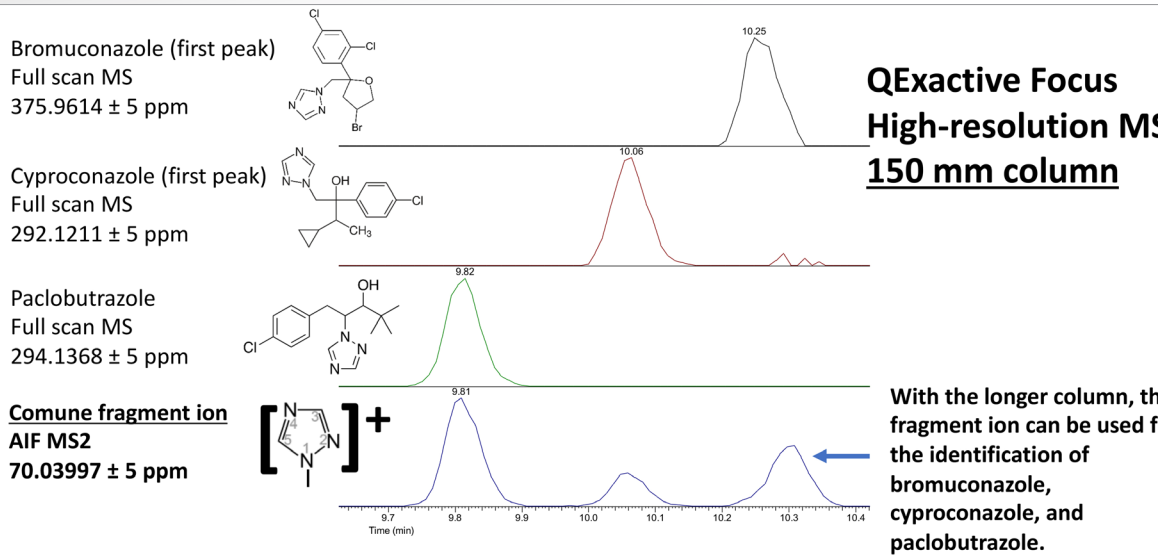


Figure 8: Using a longer column to overcome co-elution of analytes.



approach with a 100 mm column required a 26.5-minute run on a 150 mm column. However, the dual-channel system time of analysis only used a data window of 15.5 minutes, which is almost 14% faster than the traditional 100 mm column method, despite the increased column length.

Also, use of a longer column resulted in a fewer number of co-elution's as shown in **Figure 6**.

This is important because the coelution of common fragment ions can present serious problems with the analysis (see **Figure 6**). Using 100 mm columns, researchers must select different fragment ions, which are usually less intense, thereby negatively impacting identification and quantitation limits. Using a 150 mm column can resolve coelution for many compounds, thus providing higher quality data.

The co-elution of bromuconazole, cyproconazole, and paclobutrazole using a 100 mm column is shown in **Figure 7**. Using the high resolution accurate Mass of the Q Exactive Focus, the coeluting pesticides have a common fragment (*m/z* 70.03997) that cannot be mass resolved, but can be resolved chromatographically using a 150 mm column as shown in **Figure 8**. Thus, sensitivity is maintained with the 150 mm column, as the most intense fragment ion at *m/z* 70.03997 can be measured. Other benefits from the use of a longer LC

column are decreased matrix effects as well as increased dwell times, which are helpful with difficult matrices and compounds with low sensitivity. The dual-channel system facilitates the elimination of coelution and offers the other advantages of a longer LC column without adding to the analysis time.

Conclusion

The use of the Transcend Duo LX-2 system in dual-channel mode improves sample throughput by increasing the utilization of the mass spectrometer. The use of two 100 mm length columns in parallel can increase sample throughput by approximately 70%, while the use of two 150 mm length columns in parallel can enhance sensitivity and selectivity without any increase in analysis time.

The dual-channel mode produces very stable retention times and results, which are equivalent to the single-channel mode.

Conversion of a single-channel chromatographic method into a dual-channel method is fast and simple as the Aria MX software is user friendly and very easy to use.

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