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アプリケーションノート

Increasing Throughput by Run Time Reduction Leads to Greater Efficiency in Juice Laboratory

Antonietta Gledhill

日本ウォーターズ株式会社

Abstract

This application note demonstrates to provide rapid analysis for the detection and confirmation of polyphenolic compounds in fruit juices, and increase productivity through the QC use of UPLC-MS analysis.

Introduction

Phenolic compounds widely exist in many fruits and vegetables and are reported to have a diverse range of health benefits.[1,2,3,4] Their presence in food and beverages help contribute to the flavor, stability, nutrition, acceptability, and quality — all important aspects for successful products in a competitive marketplace.

Today, consumers expect manufacturers and retailers to supply wholesome and authentic fruit juices. Manufacturers must satisfy both consumer and regulatory requirements by providing products that are diverse in their flavor offerings and also meet any relevant compliance standards for quality and safety. Ultimately this allows the manufacturer to protect and strengthen their brand image in a highly competitive market place.

These factors highlight the need for reliable, robust techniques that can authenticate the purity of fruit juices and guarantee product quality. Often, a variety of fruits can have a similar physical appearance, for example apples and pears. This may result in the incorrect mixture of juices during processing. By monitoring key compounds with analytical techniques, the presence of unlabeled fruits in a given product can be detected.

For decades, reversed phase chromatography has been used to separate and identify polyphenolic compounds in a variety of sample matrices. While methods have been developed to examine polyphenolic content, the run times for these analyses are often long (60 - 100 minutes[1,2,3,4,5]) and they create bottlenecks within the QC laboratory.

For a QC lab, the demands to improve productivity and reduce costs are two key areas that must are assessed by lab managers.

This application note describes a rapid method for the chromatographic fingerprinting of fruit juices and the quantification by MS of key polyphenolic compounds in commercial fruit juices.

Experimental

Two methods were developed for the analysis of polyphenols in the QC laboratory. The first (Method 1) is

suitable for chromatographic fingerprinting. This has the advantage of increased resolution, which is suitable for either UV (qualification/chromatographic fingerprinting, and quantification) or MS detection (quantification and confirmation of unknowns).

The second method (Method 2) is dedicated to the quantification of the key compounds that have already been identified as markers for adulteration in fresh fruit beverages. This method uses MS detection to quantify these components utilizing its increased selectivity compared with UV single wavelength monitoring.

Sample Preparation

Each sample was diluted with water:methanol (75:25) and filtered through a 0.45 µm filter.

ACQUITY UPLC Conditions

Solvent Name A:	Water + 0.1% acetic acid
Solvent Name B:	Acetonitrile + 0.1% acetic acid

Method 1

Pre-column:	VanGuard Pre-column, BEH C_{18}, 2.1 x 5 mm, 1.8 µm
Column:	ACQUITY UPLC HSS T3, 2.1 x 100 mm 1.8 µm
Column temp:	45 °C

LC Gradient Table

Time (min)	Flow rate	%A	%B	Curve
Initial	0.650	99.0	1.0	
1.00	0.650	99.0	1.0	6
17.00	0.650	60.0	40.0	6
21.00	0.650	5.0	95.0	6
22.00	0.650	99.0	1.0	6
25.00	0.650	99.0	1.0	6

Method 2

Pre-column:	VanGuard Pre-column, BEH C$_{18}$, 2.1 x 5 mm, 1.8 µm
Column:	HSS T3, 2.1 x 50 mm, 1.8 µm
Column temp:	45 °C

LC Gradient Table

Time (min)	Flow	%A	%B	Curve
Initial	0.800	99.0	1.0	
0.50	0.800	99.0	1.0	6
5.00	0.800	60.0	40.0	6
7.00	0.800	5.0	95.0	6
7.10	0.800	99.0	1.0	6
10.00	0.800	99.0	1.0	6

ACQUITY TUV Conditions

The UV chromatogram was used to obtain a chromatographic fingerprint for each of the samples analyzed.

Wavelengths: 280 nm and 305 nm.

ACQUITY SQD Conditions

Capillary (kV):	2
Source temp (°C):	140
Desolvation temp (°C):	420
Desolvation gas (L/Hr):	950
Cone gas flow (L/Hr):	50

For Method 1, full scan was selected for the mass spectrometer so that the information from the detector

could be used to identify unknown peaks of interest.

Method 1	
Retention window (mins):	0.00 - 25.00
Scan mass range:	50 - 550
Function 1:	ES+
Function 2:	ES-

For Method 2, SIR mode was selected for the mass spectrometer and the conditions were optimized for each compound using Waters IntelliStart Software.

Method 2

Selected Ion Recording (SIR) Parameters

Function 1 - ES-

Retention window (mins): 0.00-1.20

Chan mass	Dwell (secs)	Cone volt.	Delay (secs)
1 : 125.00	0.005	48.0	0.10
2 : 169.00	0.005	29.0	0.20
3 : 271.00	0.005	27.0	0.20

Function 2 - ES+

Retention window (mins): 0.70-2.00

Chan mass	Dwell (secs)	Cone volt.	Delay (secs)
1 : 109.00	0.300	37.0	0.10
2 : 127.00	0.300	21.0	0.20

Function 3 - ES-

Retention window (mins): 1.50-2.20

Chan mass	Dwell (secs)	Cone Volt.	Delay (secs)
1 : 135.00	0.005	49.0	0.10
2 : 179.00	0.005	31.0	0.20
3 : 191.00	0.005	53.0	0.20
4 : 245.00	0.005	50.0	0.20
5 : 289.00	0.005	35.0	0.20
6 : 353.00	0.005	23.0	0.20

Function 4 - ES-

Retention window (mins): 2.10-2.60

Chan mas	Dwell (secs)	Cone volt.	Delay (secs)
1 : 163.00	0.080	26.0	0.10
2 : 245.00	0.080	54.0	0.20
3 : 289.00	0.080	35.0	0.20

Function 5 - ES-

Retention window (mins): 2.30-2.90

Chan mass	Dwell (secs)	Cone volt.	Delay (secs)
1 : 134.00	0.350	50.0	0.10
2 : 193.00	0.350	27.0	0.20

Function 6 - ES-

Retention window (mins): 3.00-4.50

Chan mass	Dwell (secs)	Cone volt.	Delay (secs)
1 : 147.00	0.300	29.0	0.10
2 : 273.00	0.010	58.0	0.20
3 : 435.00	0.100	31.0	0.20

long run times that often are greater than 60 minutes per sample. The aim of this experiment was to transfer an existing HPLC method requiring 90 minutes per sample, to the ACQUITY UPLC System to improve productivity in the lab.

Various fruit juices were chosen for the analysis of polyphenolic content: apple, pear, peach, orange, and tangerine. For both methods 11 compounds listed in Table 1 were monitored and quantified during the analysis. During QC analysis, the presence or absence of these compounds was monitored along with the amounts at which they are present.

	Compound	m/z	RT (Method 1)	RT (Method 2)
A	Arbutin	271	1.04	0.63
B	Gallic acid	169	1.15	0.72
C	5-hydroxymethyl-2-furaldehyde (HMF)	127	2.23	1.16
D	Chlorogenic acid	353	4.27	1.88
E	Catechin	289	4.38	1.94
F	Caffeic acid	179	4.59	2.04
G	Epicatechin	245	5.59	2.28
H	p-Coumaric acid	163	5.87	2.47
I	Ferulic acid	193	6.83	2.72
J	Phloridzin dihydrate	435	9.48	3.38
K	trans-Cinnamic acid	147	10.64	3.82

Table 1. Compound retention times.

Figure 1 shows a typical chromatogram of a tangerine sample using HPLC and UPLC.

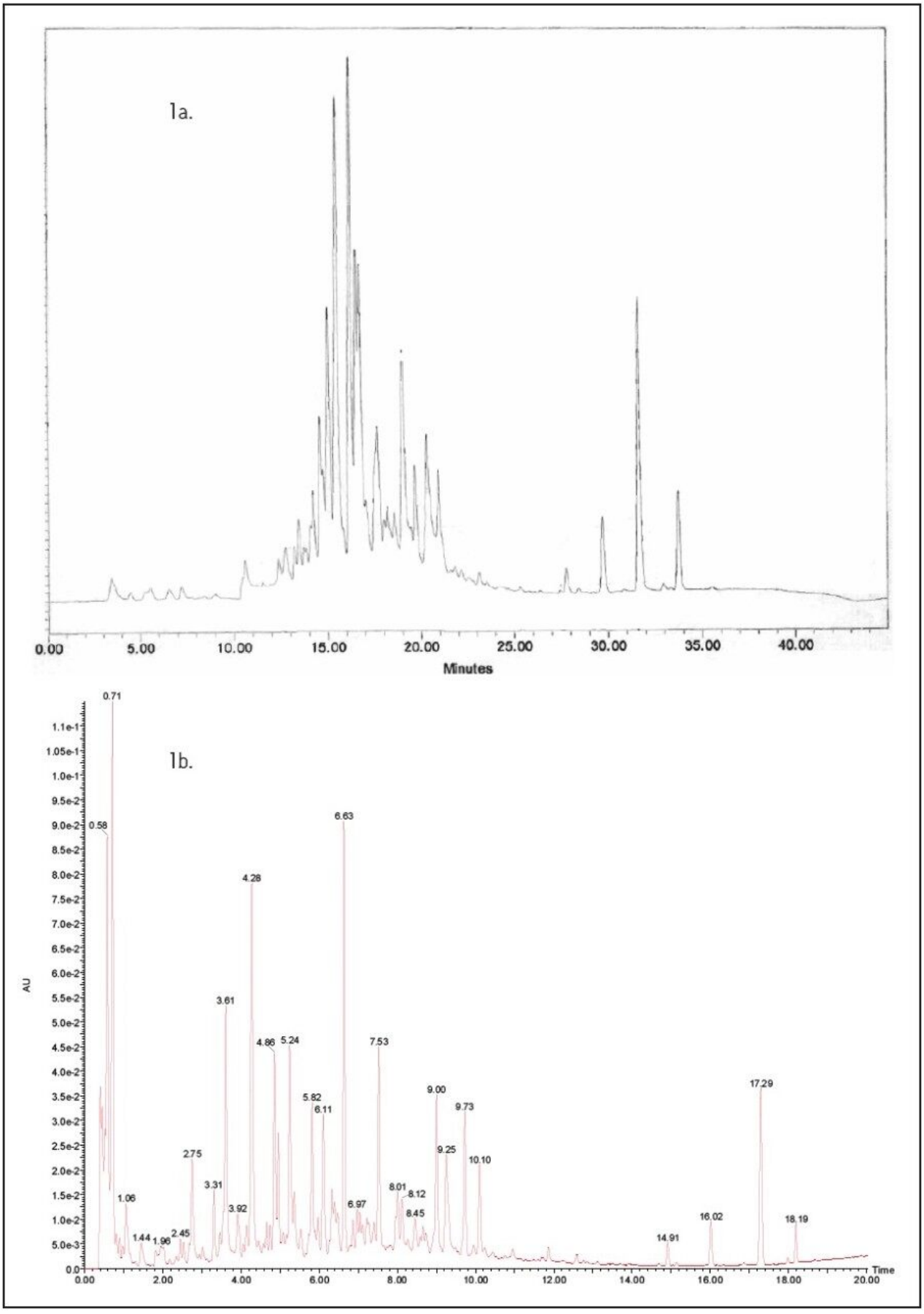

Figure 1. Tangerine sample using HPLC (1a) and UPLC (Method (1b) .

The ACQUITY UPLC System, in combination with 1.8 µm particle

size columns were used to achieve increased resolution (as seen in Figure 1b) and rapid run times — the 100 minute run time was reduced to less than 25 minutes.

The reduction in run time also enables an increase in throughput for this analysis. The potential throughput increase for this method can be seen in Table 2.

Increased Productivity in the Lab	HPLC	UPLC
Chromatography Conditions		
Flow Rate ml/min	1.00	0.65
Run time	100 min	25 min
Max. no of Injections / Month	101	403
Sample throughput		**300%**

Table 2. The potential increase in productivity using UPLC compared with HPLC.

Table 3 shows, the ACQUITY UPLC shows the potential cost savings that a laboratory can achieve by transferring from HPLC to UPLC technology.

Cost Savings in the Lab Per Month	HPLC	UPLC
No of Samples / Month	**85**	**85**
Chromatography Conditions		
No of Injections / Sample	1	1
No of Injections / Month	85	85
Flow Rate ml/min	1.00	0.65
Runtime	100 min	25 min
Solvent used	8.5 L	1.4 L
Operating Cost		
Solvent Purchase & Disposal / Month	US$ 272	US$ 44
Savings / Month		**84 %**

Table 3: Cost savings of method transfer from HPLC to UPLC.

In order to achieve the increased analysis speed and improved peak resolution, both methods were run using optimal UPLC flow rates for a 1.8 µm column (between 0.45-1.05 mL/min: as specified by the Van Deemter curve[1]). By running the ACQUITY UPLC System between these flow rates (which in turn will produce elevated pressures due to the small particle size), maximum results for the system will be returned.

For Method 1, the throughput tripled. It can be seen that the chromatographic resolution was improved by using Method 1 as shown in Figure 1. Figure 1a shows that when using the 100 minute HPLC method, there were many co-eluting peaks occurring between 12 and 22 minutes, while in Figure 1b, these peaks were better resolved using the UPLC technique.

The advantage of the improved resolution of Method 1 means that it is possible to use the UPLC method to monitor for known adulterations. As discussed in the Aim section, some common issues such as mistaken identity of fruits often occur. Figure 2 shows how easy it is to detect pear in apple juices, since there are several unique components associated with each fruit using UV detection. However, identification alone with UV in this example might lead to an incorrect assignment of the peak eluting at t_R 1.08 min in the apple sample as arbutin. Using the MS data, this was proven not to be the case and Table 4 shows that there is a high presence of arbutin in pear that is absent in the apple sample.

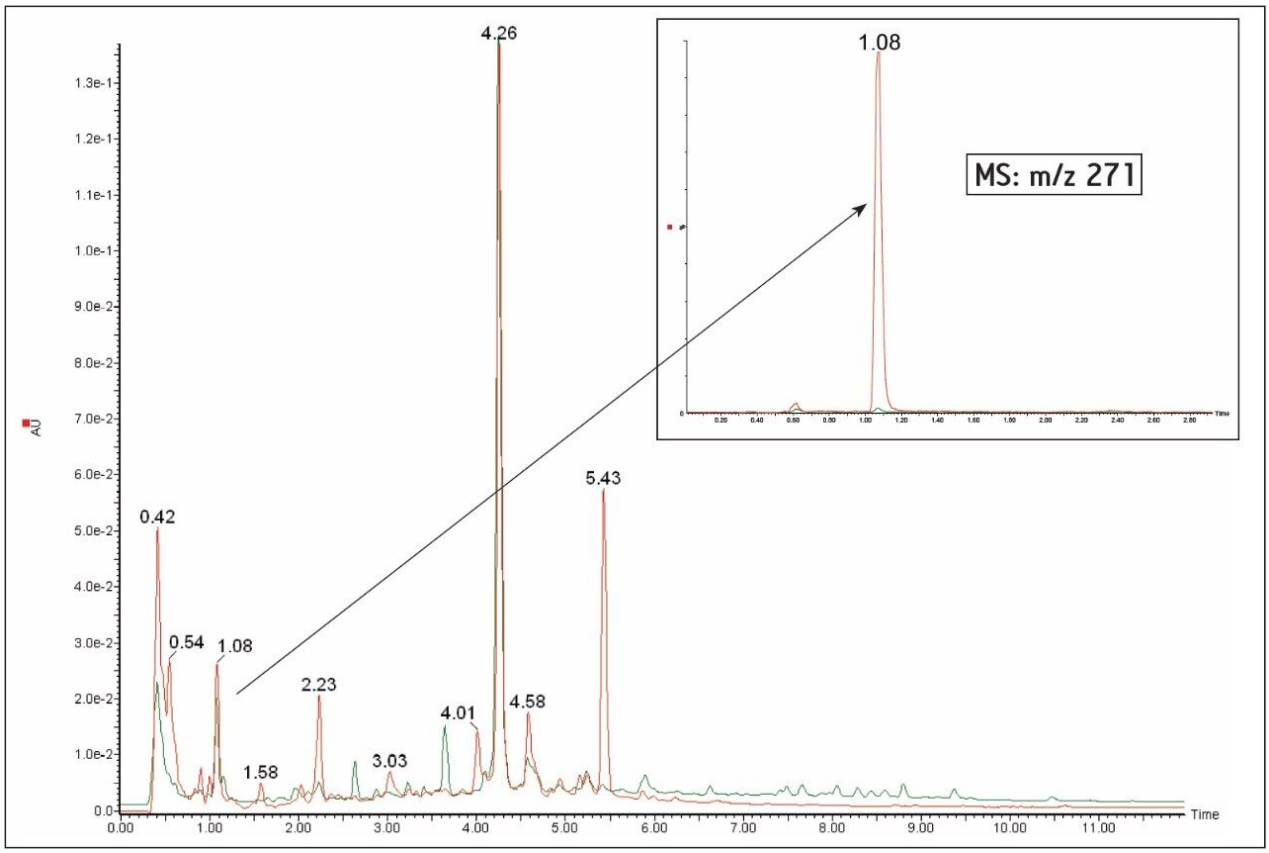

Figure 2. Comparison of pear (green) and apple (red) using UV, and MS (SIR) of arbutin.

	Tangerine	Lime	Lemon	Orange	Apple	Pear	Apple	Peach
Arbutin						36.74		0.98
Gallic acid	1.5	1.53	1.59	1.48	1.52	1.47	1.48	2.61
HMF					3.11	1.65	0.6	
Chlorogenic acid			14.1	54.5		8.52	53.74	
Catechin			0.08		0.08	0.27	0.29	2.61
Caffeic acid						2.18	1.83	3.43
Epicatechin	0.04	0.03	0.03	0.05	0.02	2.01	1.84	0.12
p-Coumaric acid					0.7		0.03	
Ferulic acid				0.1				
Phloridzin					1.68			
t-Cinnamic acid								

Table 4. List of compounds identified by MS in the fruit juice samples analyzed.

Figure 3 shows chlorogenic acid in another apple sample. The top chromatogram shows the data for UV and the bottom shows the same sample using MS. For both, the chromatograms the peak at t_R 4.63 min appears to be one peak.

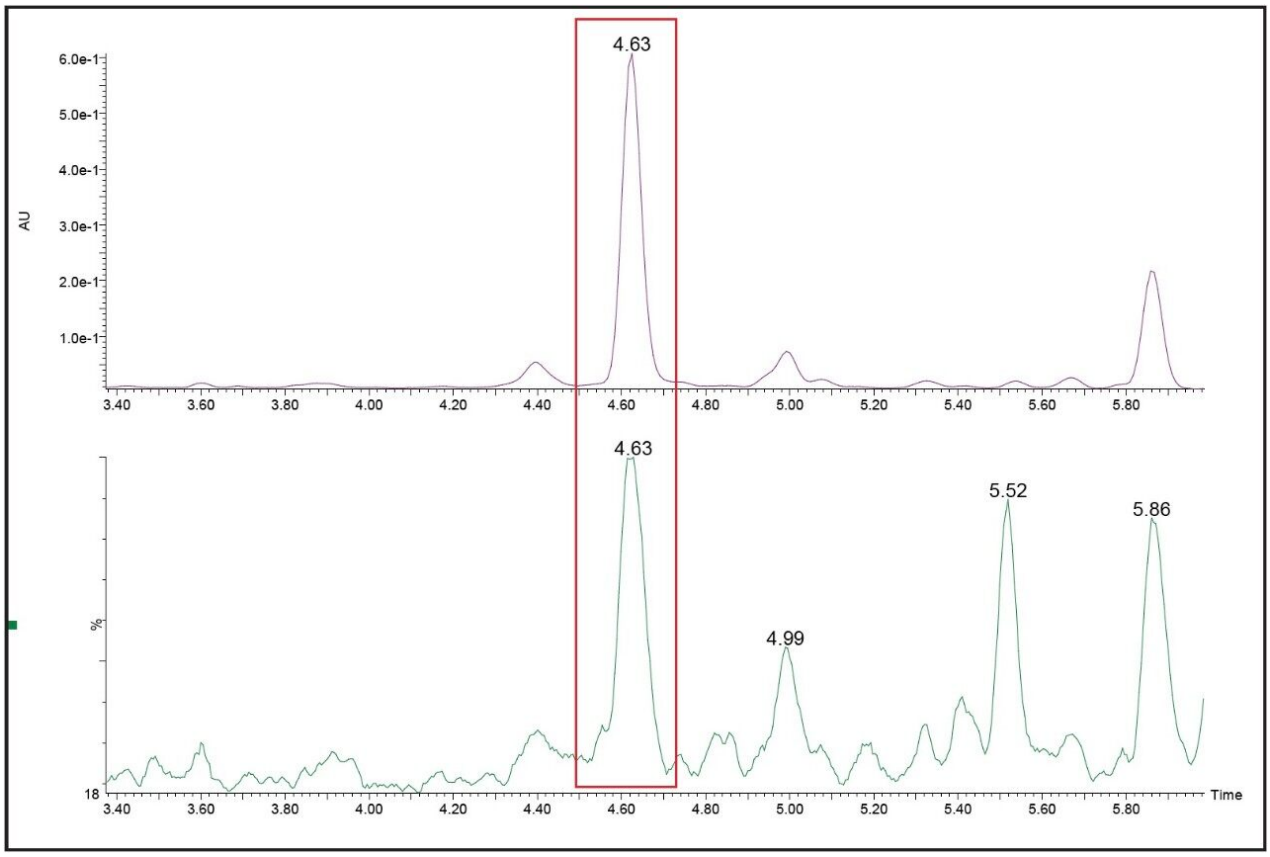

Figure 3. UV and MS chromatograms of an apple sample.

However, the mass spectrum indicates the presence of two compounds, as shown in Figure 4. By using the MS data to quantify for chlorogenic acid, the result was more accurate than the result from the UV data. The use of the Waters ACQUITY SQD allows the analyst to perform a more comprehensive search for more subtle adulterations that may occur.

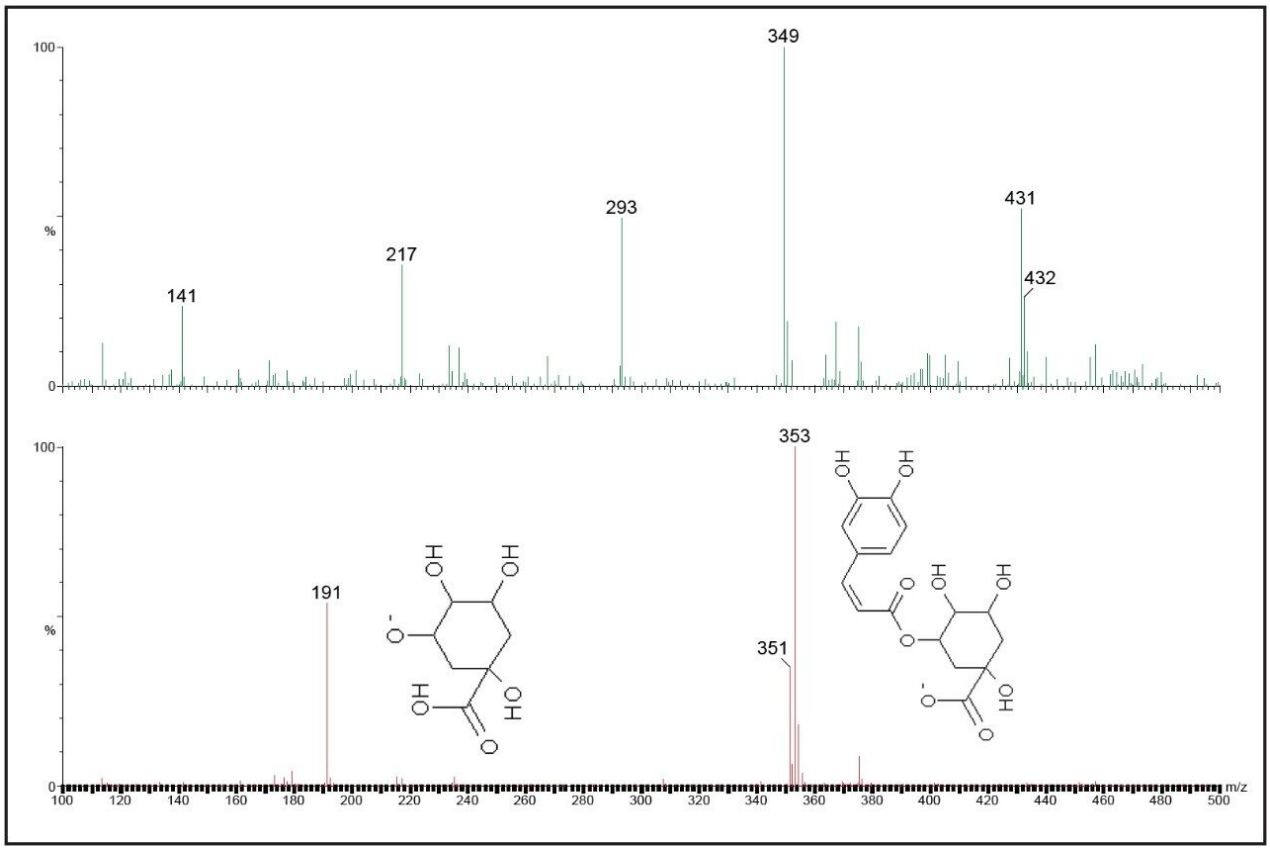

Figure 4: Spectra obtained for apple sample at retention time 4.63.

An additional advantage of the ACQUITY SQD is its ability to give fragmentation patterns associated to the compound, making it a very selective technique (see Figure 4). It is also possible to induce further fragmentation in-source, enabling the generation of more structurally significant ions that could assist with compound identification/elucidation.

The addition of the SQ Detector not only aids with correct quantification of peaks in the chromatogram (see Table 3), but also allows the throughput to be further increased. For quantification of the key compounds only, the run time was improved ten-fold using the ACQUITY UPLC System in Method 2. The mass selectivity of the ACQUITY SQD when compared with UV detection enables co-eluting peaks to be identified and quantified by their specific *m/z*.

Conclusion

Waters ACQUITY UPLC System with the SQ Detector affords a number of key benefits to the food testing QC

laboratory, including:

- HPLC methods can be readily transferred to the UPLC platform.
- The UPLC method provides superior resolution and speed as compared to traditional HPLC techniques. This results in improved laboratory efficiency (through the reduction of sample analysis bottlenecks) as well as lower operational costs.
- By employing the SQ Detector with the ACQUITY UPLC, it is possible to obtain more information in less time.
- The ACQUITY SQD provides additional security when quantifying known compounds in routine QC sample analyses.
- From an investigatory perspective, the mass spectral information allows for the recognition of sample non-conformance, such as adulteration.

References

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