



Analysis of Polyphenols in Fruit Juices Using ACQUITY UPLC H-Class with UV and MS Detection

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Abstract

In this application note, a rapid method for the detection and confirmation of polyphenolic compounds in fruit juices using the ACQUITY UPLC H-Class System is described. With the selectivity of the MS from the ACQUITY SQ Detector, analysis times can be further reduced for quantification of key components.

Benefits

- Rapidly separate, detect, identify, and quantify polyphenolic compounds in fruit juices.
- Reduces analysis time from over an hour with HPLC methods to under 10 minutes using ACQUITY UPLC, increasing sample throughput for juice QC labs along with improved chromatographic performance.
- Allows easy access to UV and mass spectral information for analytical labs performing quantification, data interpretation, and compound identification.

Introduction

Polyphenols are widely recognized as functional components of food and beverage products, such as tea, wine, and fruits. Polyphenols are reported to play a potential role in protecting human health from degenerative and cardiovascular diseases.^{1,2,3}

Over the years, the determination of natural phenolic compounds has proved an extremely difficult analytical challenge, which has been hindered by the complexity of the numerous chemical classes and species, combined with inherent limitations of conventional HPLC separation methods.⁴ Typical HPLC methods that are used to separate and identify polyphenolic compounds by reversed phase chromatography often take more than an hour for a single run.⁵

More recently, analysis methods of polyphenols using Waters ACQUITY UPLC System have been developed, which provide increased resolution and shortened analysis times.^{3,6,7} The reduction in run time enables an increase in sample throughput, which is crucial for juice QC laboratories to deliver their products to store shelves more quickly.

Waters ACQUITY UPLC H-Class System enables routine HPLC users to move to UPLC Technology easily by combining the speed and performance of UPLC with the ability to run HPLC separations. The use of the

ACQUITY H-Class with UV and mass spectrometry provides a robust solution for the QC analysis of juice samples.

In this application note, a rapid method for the detection and confirmation of polyphenolic compounds in fruit juices using the ACQUITY UPLC H-Class System is described. With the selectivity of the MS from the ACQUITY SQ Detector, analysis times can be further reduced for quantification of key components.

Experimental

Materials

Seven commercially available fruit juice samples, together with 11 polyphenol standards were analyzed.

Sample preparation

Each juice sample was filtered through a 0.45 µm filter and diluted with an equal amount of water.

LC conditions

| | |
|-----------------|---------------------------------|
| LC system: | ACQUITY UPLC H-Class |
| Mobile phase A: | Water + 0.1% acetic acid |
| Mobile phase B: | Acetonitrile + 0.1% acetic acid |

MS conditions

| | |
|--------------------|---------------------|
| MS System: | ACQUITY SQ Detector |
| Ionization: | Electrospray |
| Capillary voltage: | 3.0 kV |
| Sampling cone: | 30 V |

| | |
|--------------------|----------------------|
| Extraction cone: | 3.0 V |
| Source temp.: | 150 °C |
| Desolvation temp.: | 500 °C |
| Desolvation gas: | 1000 L/hr |
| Cone gas: | 20 L/hr |
| Scan mass range: | 50 to 650 <i>m/z</i> |

ACQUITY UPLC PDA conditions

| | |
|-----------------------|--|
| UV range: | 210 to 400 nm (Extracted at 280 and 305 nm) |
| Sampling rate: | 20 pts/sec |
| Filter time constant: | Fast |

All data were acquired and processed using Empower 2 Software.

Acquisition and processing methods

Two methods were developed for the analysis of polyphenols on the ACQUITY UPLC H-Class System. Method 1 is ideal for chromatographic fingerprinting, offering increased separation resolution resulting from a longer column. For QC laboratories performing routine quantification of the key phenolic compounds present, Method 2 would be the preferred method, utilizing a shorter run time, thereby improving lab productivity.

Method 1

| | |
|---------|--|
| Column: | ACQUITY UPLC HSS T3 2.1 x 100 mm, 1.8 μ m |
|---------|--|

Column temp: 45 °C

Flow rate: 0.65 mL/min

Total run time: 15.0 min

Gradient

| Time (min) | %A | %B |
|------------|----|----|
| Initial | 99 | 1 |
| 1.0 | 99 | 1 |
| 10.0 | 70 | 30 |
| 12.0 | 5 | 95 |
| 12.1 | 99 | 1 |
| 15.0 | 99 | 1 |

Method 2

Column: ACQUITY UPLC HSS T3 2.1 x
50 mm, 1.8 µm

Column temp.: 45 °C

Flow rate: 0.80 mL/min

Total run time: 7.0 min

Gradient

| Time (min) | %A | %B |
|------------|----|----|
| Initial | 99 | 1 |
| 0.5 | 99 | 1 |
| 4.0 | 70 | 30 |
| 5.5 | 5 | 95 |
| 5.6 | 99 | 1 |
| 7.0 | 99 | 1 |

IntelliStart Software was used to automatically develop SIR acquisition methods for the 11 phenolic compounds targeted in this analysis. IntelliStart requires only the entry of basic compound information; then it automatically locates the precursor ion and optimizes the cone voltage, as shown in Table 1. The dwell times were optimized to give a minimum of 15 points across each chromatographic peak for reproducible quantitation. Quantitation of the juice samples was performed by calculating against a calibration curve for each of the polyphenol analytes in solution.

| Compound | <i>m/z</i> | Polarity | Cone voltage (V) |
|-------------------------------------|------------|----------|------------------|
| Arbutin | 271.2 | - | 30 |
| Gallic acid | 169.1 | - | 30 |
| 5-hydroxymethyl-2-furaldehyde (HMF) | 127.1 | + | 20 |
| Chlorogenic acid | 353.3 | - | 25 |
| Catechin | 289.3 | - | 40 |
| Caffeic acid | 179.1 | - | 30 |
| Epicatechin | 289.2 | - | 35 |
| p-Coumaric acid | 163.1 | - | 25 |
| Ferulic acid | 193.1 | - | 30 |
| Phloridzin | 435.5 | - | 40 |
| t-Cinnamic acid | 147.1 | - | 25 |

Table 1. MS analysis parameters.

Figure 1 shows a typical MS method setup in Empower Software.

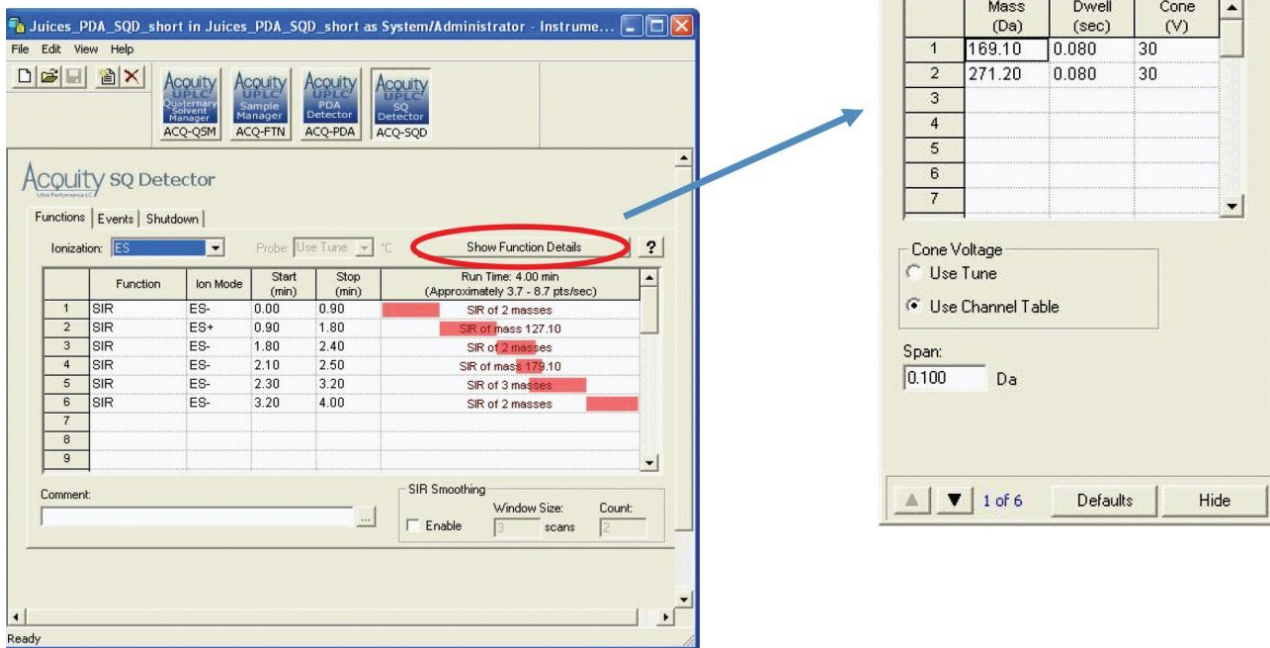


Figure 1. MS method setup in Empower Software.

Results and Discussion

Using the ACQUITY UPLC H-Class System coupled with ACQUITY UPLC PDA and ACQUITY SQ detectors, 11 polyphenol standards were separated and analyzed in 10 minutes, as shown in Figure 2. The PDA result was extracted at wavelengths 280 nm, shown in Figure 2, and 305 nm (data not shown), and SIR chromatograms for the 11 analytes were overlaid, as shown in Figure 2.

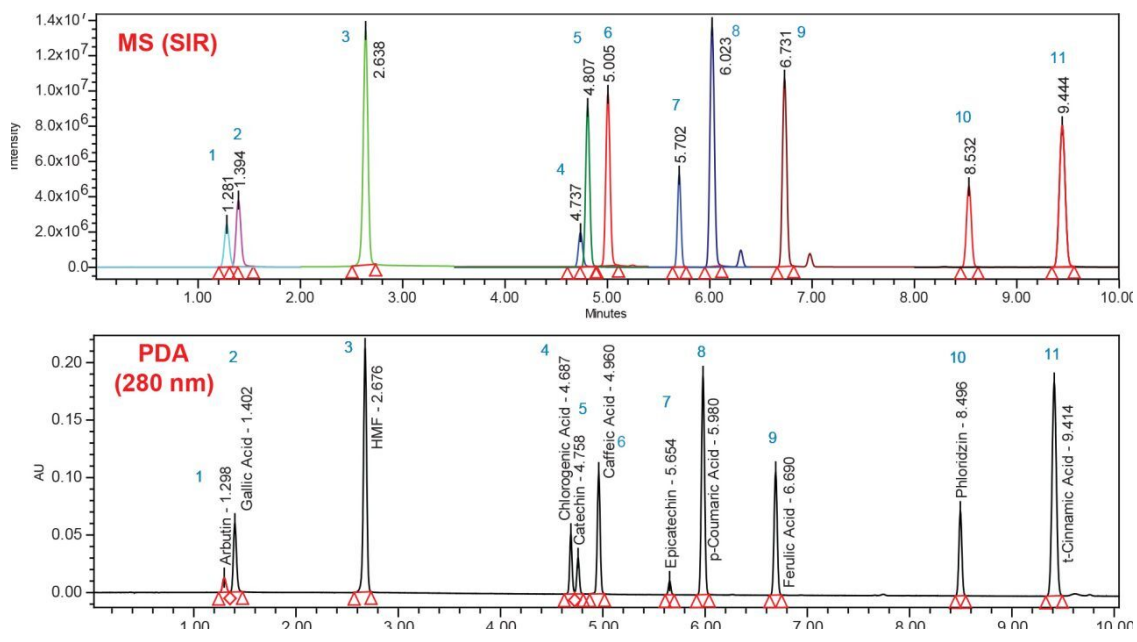


Figure 2. Simultaneous acquisition of PDA and MS (SIR) data for a 10 ppm standard mix in a single analysis.

The retention times (Method 1 and Method 2) of the polyphenolic compound standards, along with their retention times and peak area %RSDs (based on six replicate injections) for MS detection using Method 1 are shown in Table 2.

| Peak | Compound | Retention times (min) | | % RSD for Method 1 (MS) | |
|------|-------------------------------------|-----------------------|----------|-------------------------|-----------|
| | | Method 1 | Method 2 | Retention time | Peak area |
| 1 | Arbutin | 1.30 | 0.57 | 0.10 | 0.26 |
| 2 | Gallic acid | 1.40 | 0.64 | 0.06 | 0.20 |
| 3 | 5-hydroxymethyl-2-furaldehyde (HMF) | 2.68 | 1.23 | 0.19 | 0.26 |
| 4 | Chlorogenic acid | 4.69 | 2.12 | 0.29 | 0.37 |
| 5 | Catechin | 4.76 | 2.13 | 0.29 | 0.37 |
| 6 | Caffeic acid | 4.96 | 2.22 | 0.27 | 0.34 |
| 7 | Epicatechin | 5.65 | 2.45 | 0.22 | 0.31 |
| 8 | p-Coumaric acid | 5.98 | 2.59 | 0.20 | 0.27 |
| 9 | Ferulic acid | 6.69 | 2.84 | 0.17 | 0.24 |
| 10 | Phloridzin | 8.50 | 3.39 | 0.13 | 0.18 |
| 11 | t-Cinnamic acid | 9.41 | 3.78 | 0.11 | 0.75 |

Table 2. Retention times and repeatability results.

A variety of commercially available fruit juices, namely apple, orange, berry, guava, mango, and lime were analyzed for their polyphenolic content. Both UV and MS data were simultaneously acquired.

An example of the results from an apple juice sample analyzed using Method 1 is shown in Figure 3.

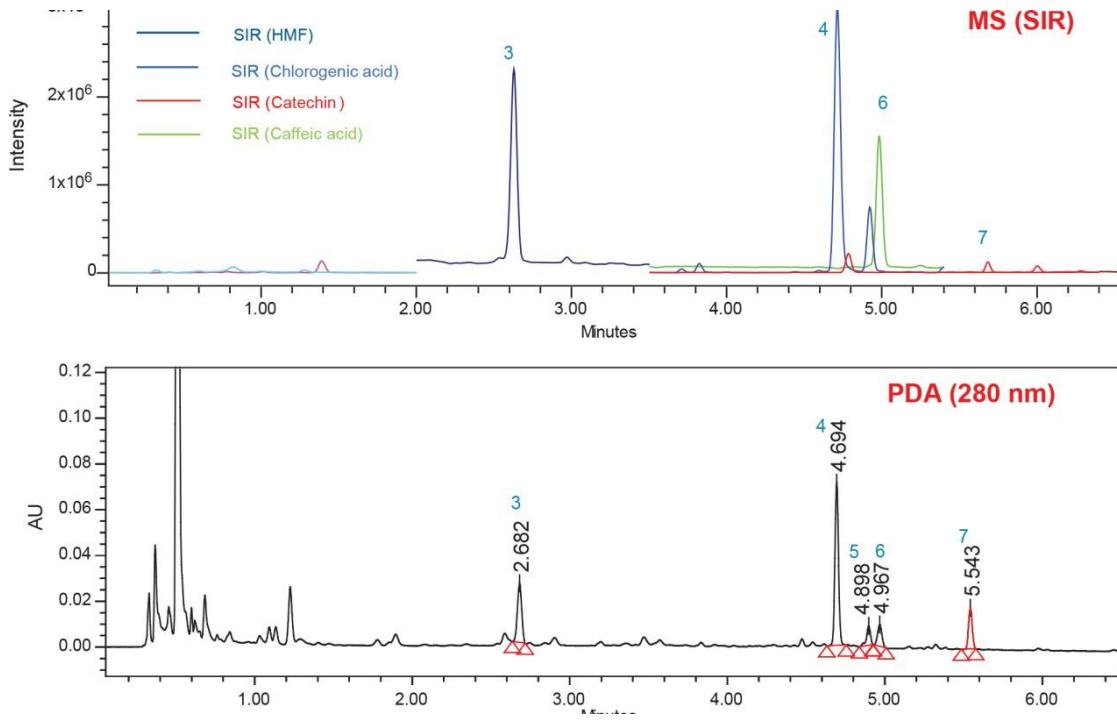


Figure 3. Overlay of UV and MS chromatograms of polyphenol analysis in apple juice.

By examining the PDA chromatogram in Figure 3, the polyphenol compounds present in apple juice can be easily assigned and identified by Empower Software.

To increase confidence in component identification beyond simple retention time matching, UV spectral library functions with PDA and Empower Software can be employed. Using HMF (Peak 3) to illustrate, Figure 4 shows the spectrum index plot for HMF standard, and the peak detected at the same retention time in apple juice. The spectral match between the HMF standard and Peak 3 in apple juice confirms the identity of HMF in the apple juice.

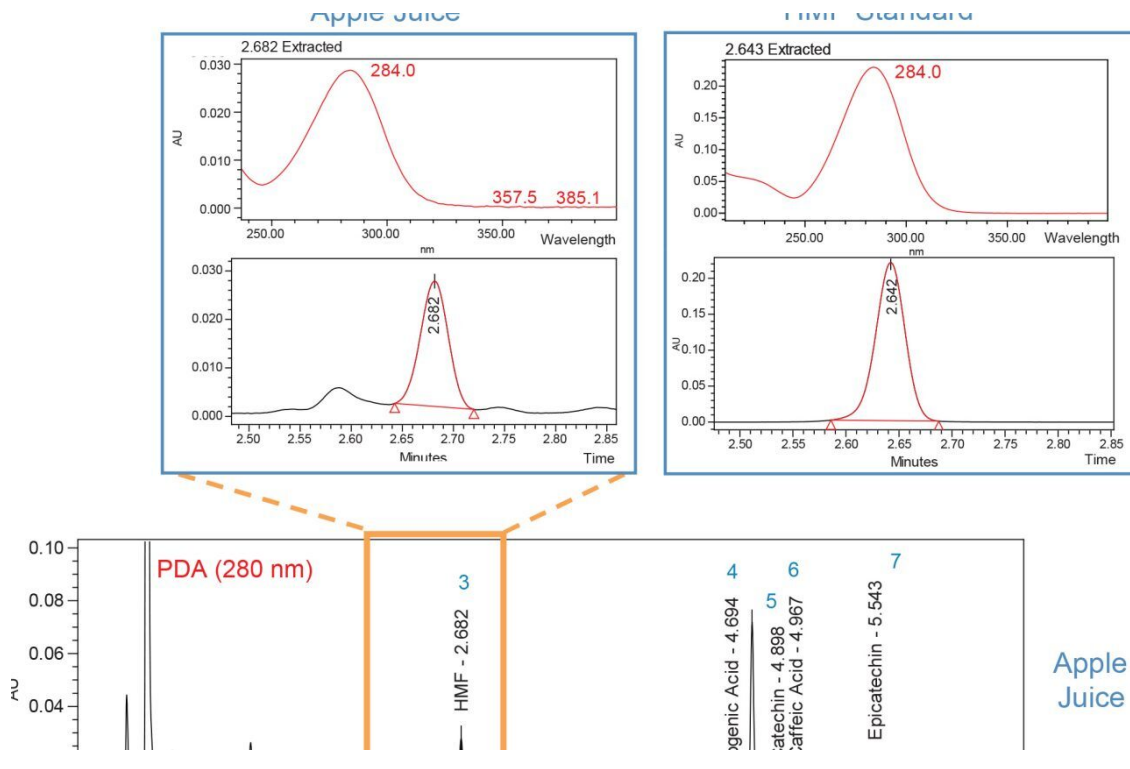


Figure 4. Spectrum index plot confirming the identity of HMF in apple juice.

With the additional coupling of a more selective detector, such as the ACQUITY SQ Detector, more information can be obtained in a single sample injection. This provides QC analysts with increased confidence in data interpretation and compound confirmation. From Figure 5, the HMF peak (Peak 3) detected with MS further confirms the polyphenol identity in the juice.

A closer look at the apple juice results in Figure 5 revealed differences in the UV and MS chromatograms.

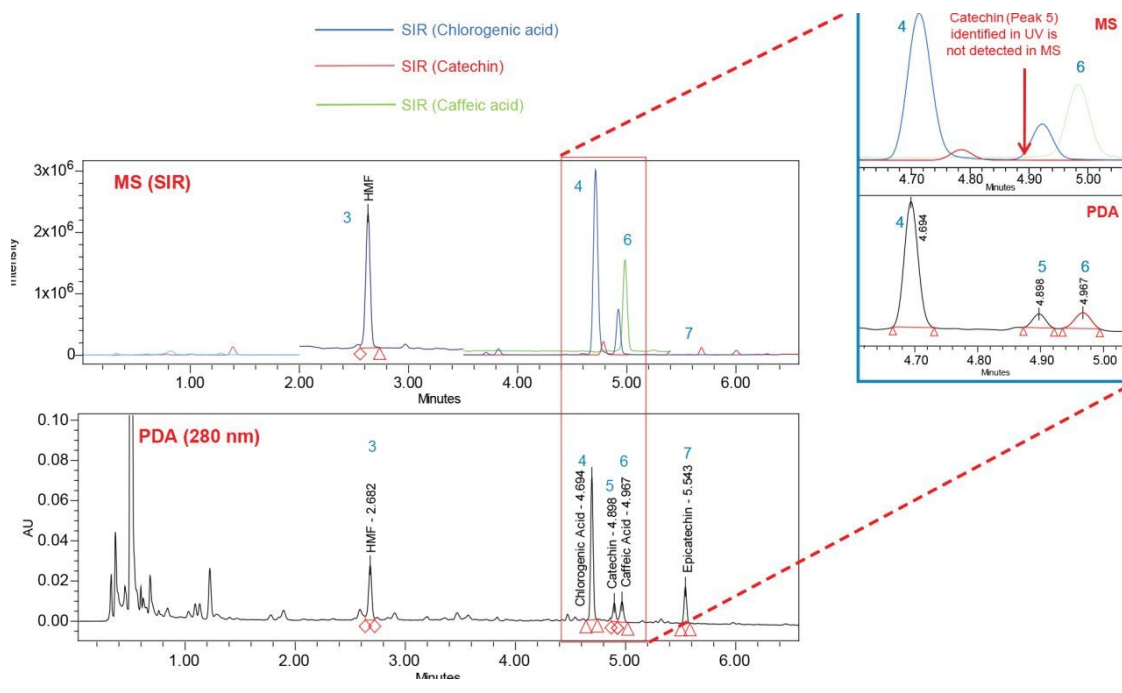


Figure 5. In this example, the ACQUITY SQ Detector provides greater specificity compared to the PDA.

Catechin (Peak 5), which was assigned and identified using UV retention times, was not detected and quantified by MS (as indicated by the red trace). Identification by UV alone might lead to an incorrect assignment of the polyphenol present, thereby causing mistaken identity of the fruit. The ACQUITY SQ Detector, with its greater specificity, can provide more confidence in data interpretation over a PDA detector alone.

The ACQUITY SQ Detector can be easily added to routine QC laboratories that already use the ACQUITY UPLC H-Class System with Empower Software. Empower users can thereby reap the benefits of MS without the need for additional training. IntelliStart (an MS software component in Empower) enables easy setup and system monitoring of the SQ Detector. It also can automate the method development of selected ion recording (SIR) channels for new compounds.

Another advantage of the MS system is the ability to monitor SIR channels for each of the compounds in the target analysis list. Using the ACQUITY SQ Detector in this mode of operation allows the detector to be selective, and for the user, it is very easy to see whether a specific peak is present or absent in each sample analyzed. Furthermore, an additional benefit that results from the ACQUITY SQ Detector's selectivity is that the runtime can be reduced, since it is less important to obtain chromatographic resolution (compared to the UV detector) for each of the targeted and matrix peaks. For quantification of key components only, the

analysis time was shortened from 10 min to 4 min, (Method 2), which improved sample throughput, as shown in Figure 6.

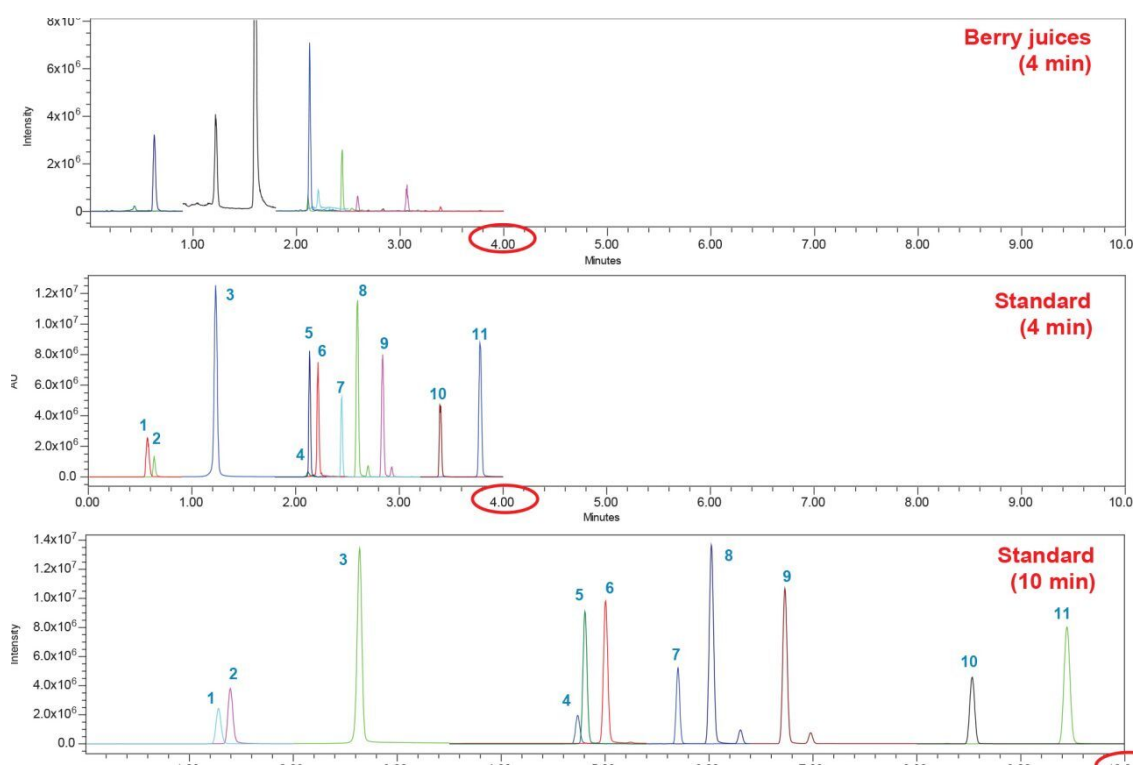


Figure 6. By utilizing the selectivity of the MS detector, the analysis run time can be shortened to increase sample throughput: 1.) arbutin, 2.) gallic acid, 3.) HMF, 4.) chlorogenic acid, 5.) catechin, 6.) caffeic acid, 7.) epicatechin, 8.) p-coumaric acid, 9.) ferulic acid, 10.) phloridzin, and 11.) t-cinnamic acid.

The polyphenol compounds identified and quantified by MS in the juice samples analyzed are listed in Table 3.

| Compound | Apple | Orange (Brand 1) | Orange (Brand 2) | Berries | Guava | Mango | Lime |
|-------------------------------------|-------|------------------|------------------|---------|-------|-------|------|
| Arbutin | | | 13.71 | | | | |
| Gallic acid | 0.31 | | | 10.46 | 0.54 | 11.39 | |
| 5-hydroxymethyl-2-furaldehyde (HMF) | 2.12 | | 0.48 | 2.10 | | 12.94 | 6.49 |
| Chlorogenic acid | 23.75 | 2.52 | 1.42 | 1.34 | | | |
| Catechin | | | | 7.47 | | | |
| Caffeic acid | 2.29 | | | 0.47 | | | |
| Epicatechin | 0.16 | 0.50 | 0.35 | 8.35 | | | |
| p-Coumaric acid | | | | 0.19 | | | |
| Ferulic acid | | | | | | | |
| Phloridzin | | | | | | | |
| t-Cinnamic acid | | | | | | | |

Table 3. List of compounds in the fruit juice samples identified and quantified by MS. Values stated as ppm or mg/L.

Conclusion

In this work, the ACQUITY UPLC H-Class System, coupled with ACQUITY UPLC PDA and ACQUITY SQ detectors, enabled the separation, detection, identification, and quantification of polyphenols in fruit juices.

- The use of the ACQUITY UPLC H-Class System with Empower Software provides the familiarity of HPLC operation along with improved chromatographic performance and throughput of UPLC.
- ACQUITY UPLC PDA with Empower Software increased the confidence in component identification beyond simple retention time matching with the UV spectra library functions. The ACQUITY SQ Detector with IntelliStart, provides the user with easy access to mass spectral information. This detector supports analytical labs performing quantification, data interpretation, and compound confirmation.

Overall, the use of both PDA and MS detectors brings complementary benefits for QC laboratories doing routine QC analyses.

References

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