

Application Note

A Non-Targeted Metabolomic Study of Pomegranate Juice to Investigate the Nutritional and Quality Characteristics Using Novel SONAR DIA Acquisition and Vion Ion Mobility QToF MS

Sara Stead, Joanne Connolly

Waters Corporation

Abstract

In this application note, we report the potential of a new data independent acquisition (DIA) mode (SONAR) on a QToF instrument in combination with a scanning quadrupole mass filter and ultra-fast detection system. This methodology along with Vion ion mobility enabled QToF-MS (IM-QToF-MS) were used as tools to improve analytical selectivity and facilitate the process of marker identification in complex juice samples following a simple sample preparation step.

Benefits

Benefits of SONAR for the application of fruit juice metabolomic profiling for quality control and authenticity purposes include:

- Cleaner product ion spectra compared to other DIA methods
- High spectra/second scan rate compatible with HRMS
- Increased confidence in identification through selectivity
- Cataloging of a complex food sample within a single experiment
- Unbiased data acquisition within a targeted mass range

Introduction

Comprehensive identification of phytoactive compounds is a critical starting point for assessing the biological and technological properties in food matrices. Due to the complexity of plant secondary metabolism the full characterization of phytochemicals in fruits and vegetables is recognized as a significant analytical challenge and requires sensitive and accurate techniques to be employed. Pomegranate fruit (*Punica granatum* L.) is commonly reported as a rich dietary source of phenolic compounds with regular consumption being linked to a wide range of associated health benefits. Phenolic compounds are also known to play an important role in the quality and sensorial performance of fruit juice products and as such of value to the food industry.¹

In this application note, we report the potential of a new Data Independent Acquisition (DIA) mode (SONAR) on a QToF instrument in combination with a scanning quadrupole mass filter and ultra-fast detection system.²⁻³ This methodology along with Vion ion mobility enabled QToF-MS (IM-QToF-MS) were used as tools to improve analytical selectivity and facilitate the process of marker identification in complex juice samples following a simple sample preparation step, as illustrated in Figure 1. The resulting information was further subjected to database searching which indicates the presence of several significant polyphenolic compounds and processing additives in a selection of commercially available processed juice products in the UK.

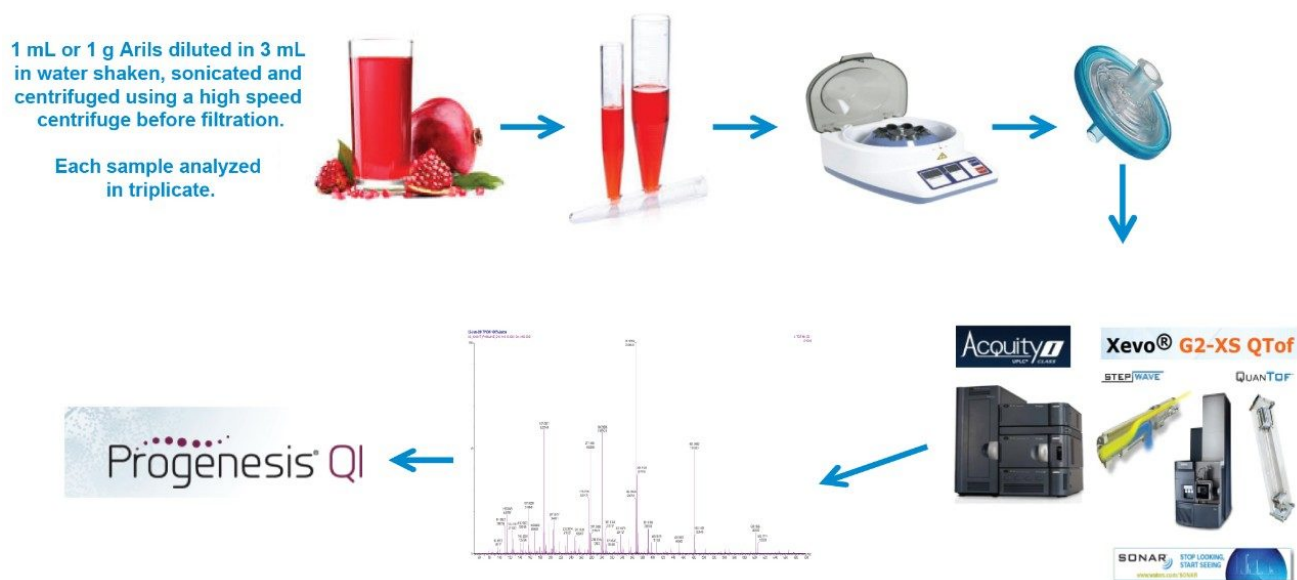


Figure 1. Overall workflow schematic.

Experimental

UPLC Conditions

UPLC system:	ACQUITY UPLC I-Class
Column:	ACQUITY UPLC HSS T3, 100Å, 1.8 µm, 2.1 mm × 100 mm (p/n: 186003539)
Column temp.:	45 °C
Injection volume:	3 µL
Flow rate:	0.4 mL/min

Mobile phase A: 10 mM Ammonium acetate with water

Mobile phase B: Acetonitrile

	Time (min)	Flow rate (mL/min)	%A	%B
1.	0.0	0.40	99	1
2.	0.75	0.40	99	1
3.	2.0	0.40	95	5
4.	3.0	0.40	95	5
5.	6.5	0.40	45	55
6.	8.5	0.40	10	90
7.	9.0	0.40	10	90
8.	9.1	0.40	99	1

Table 1. Elution gradient.

MS Conditions

MS system: Xevo G2-XS QTof, SONAR-enabled

Analyzer mode: Sensitivity

Ionization mode: ESI

Capillary voltage: 2.1 kV

Sampling cone: 90 eV

Desolvation temp.: 600 °C

Desolvation gas: 800 L/hr

Source temp.:	150 °C
MS ^E Low energy CE:	4eV
MS ^E High energy CE:	10–30 eV
Acquisition range:	50–1200 <i>m/z</i>
Scan time:	0.1 sec
SONAR start mass:	100.0 DA
SONAR stop mass:	500.0 DA
SONAR quad peak width:	20



As for MS^E, MS scans alternate between precursor (low energy) and CID product ions (high energy).

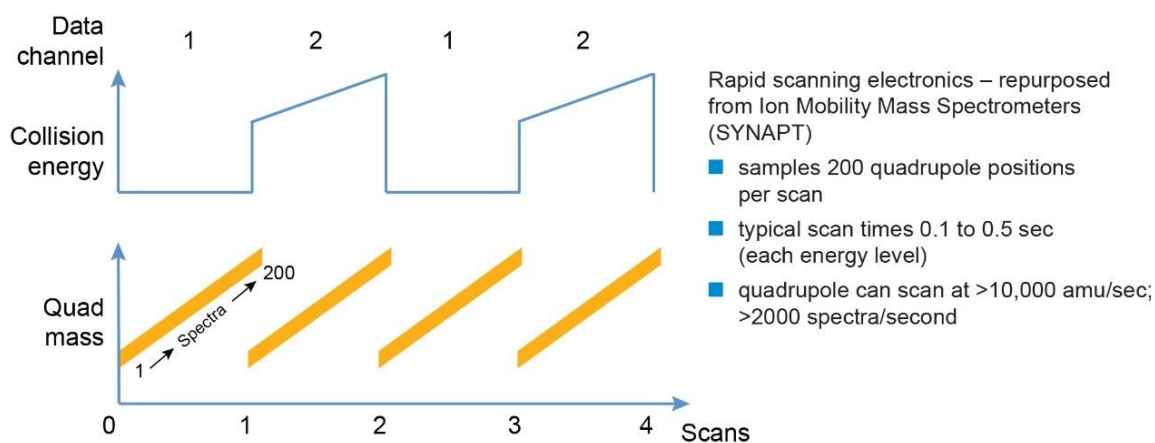


Figure 2 SONAR DIA method and acquisition parameters CE ramped as a function of *m/z*.

Sample preparation

A number of retail pomegranate juices were purchased and analysed for the purpose of the study. The sample set included four different samples representing each of the following categories of fruit juice:

- Not from concentrate, 100% juice (NFC)
- From concentrate (CONC)
- Crushed arils – freshly squeezed juice (Arils)

Pooled samples were prepared from each individual sample type (NFC, CONC, and Arils) and a global QC Pool was prepared from all sample types

Identifier	Anonymized supplier code	Description
1	A	NFC
2	A	NFC
3	A	NFC
4	B	NFC
5	C	CONC
6	D	CONC
7	A	CONC
8	B	CONC
9	C	Arils
10	D	Arils
11	E	Arils
12	F	Arils

Table 2. Small scale retail survey samples.

1 mL of neat pomegranate juice (or 1g Arils crushed with pestle and mortar) were pipetted or weighed into a 15 mL falcon tube. 3 mL of LCMS grade water was added to the juice. The extract was centrifuged at 6000 rpm for 6 mins at room temperature. In each case, 3 mL of supernatant was removed and filtered using a Waters Acrodisc Syringe Filter, PVDF, 25 mm, 0.45 µm, Aqueous, p/n: WAT200510.

Results and Discussion

The different phytochemical fingerprints of pomegranate juice types were determined using UPLC-ESI-QToF operated in negative ionization mode with SONAR enabled acquisition.

Figure 3 shows the scores and loadings plot of multivariate statistical analysis in Progenesis Q1 Software (PCA following univariate scaling ANOVA $p \leq 0.05$; fold change ≥ 2). The analysis revealed three distinct populations grouped according to the juice preparation type. These were visualized following unbiased non-targeted analysis; not from concentrate (NFC); juice/drink and fresh fruit (Arils) only with the QC pool clustering centrally.

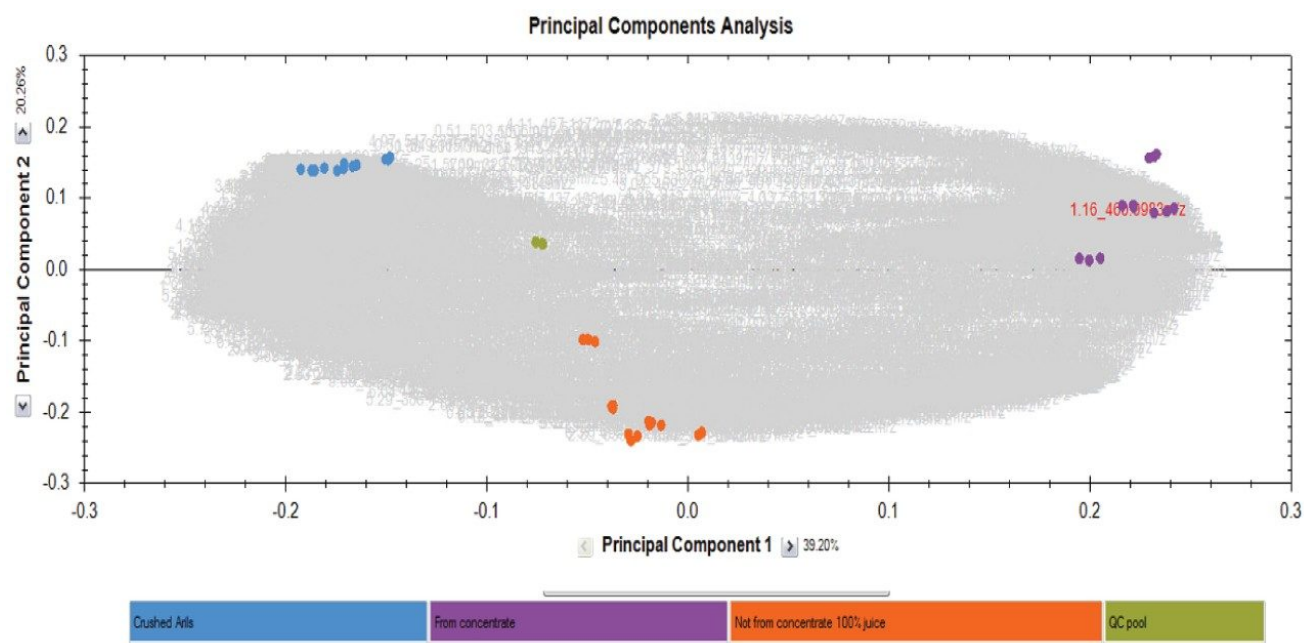


Figure 3. PCA scores and loadings plot from Progenesis Q1 following univariate scaling ANOVA $p \leq 0.05$; fold change ≥ 2 Blue = Arils; purple = CONC; orange = NFC; and green = pool.

Compound level interrogation of the data highlighted in Figure 4, shows the abundance profiles in the juice samples. Phytochemicals including members of the flavonoid-o-glycosides were found to predominate in the Aril and 100% juice samples compared to the NFC juices, whereas various permitted food dyes, flavorings and preservatives were detected in the juice/drink samples. The abundance plots reveal that the flavonoid compound, myricetin-3-galactoside to be elevated in both the freshly squeezed (Arils) and 100% juice sample

sets (a) and the synthetic dye compound, C.I. Pigment red 149, to be elevated in the with CONC samples (b).



Figure 4. Progenesis QI compound level abundance plots selected compounds identified in the analysis: a) shows the compound myricetin 3 galactoside to be elevated in both Arils and 100% juice; b) shows the compound C.I. Pigment red 149 to be elevated in the from CONC samples.

Figure 5 depicts the S-plot generated following OPLS-DA analysis of the Arils vs. CONC samples. The further statistical analysis was performed using EZinfo to identify the significant marker ions indicative of the different processed juice types. A subset of selected markers were further subject to database searches for compound identification.

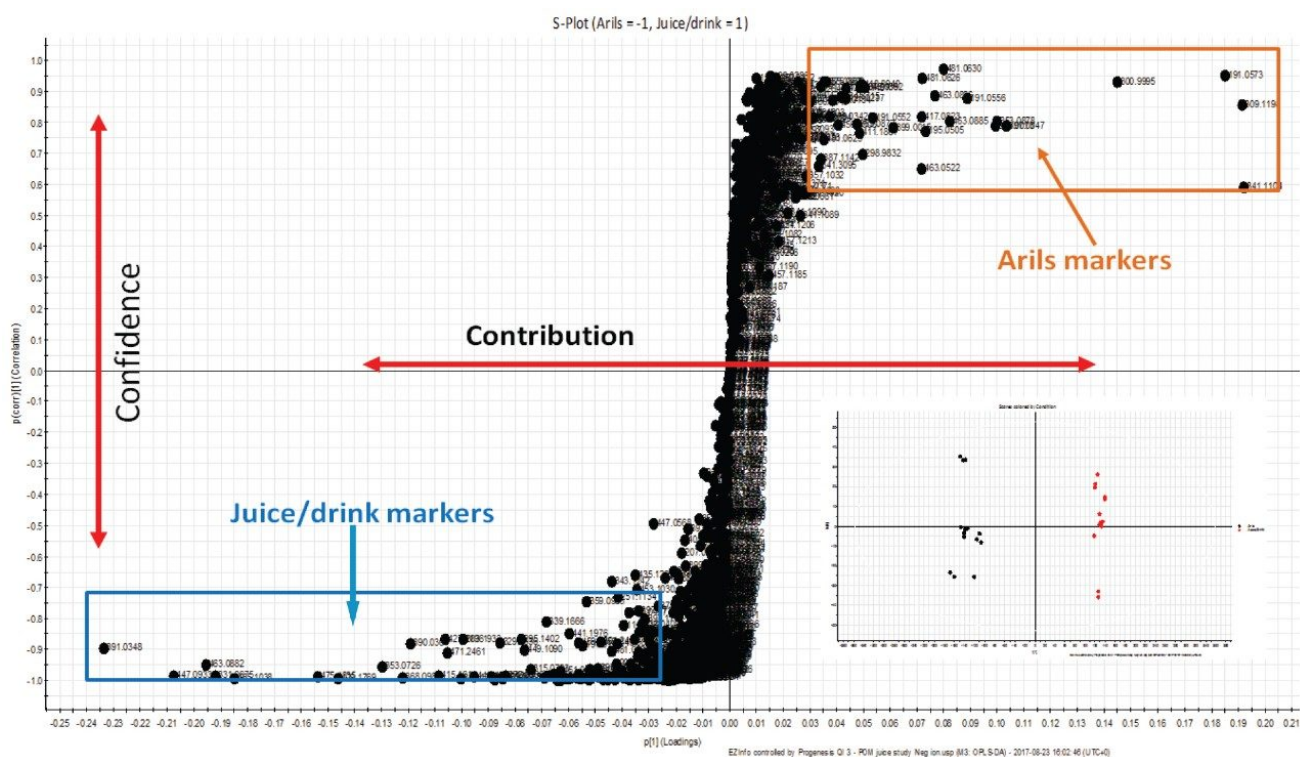


Figure 5. S-plot generated following OPLS-DA analysis in EZinfo software showing features with high confidence and contribution to the Aril and CONC samples.

Table 3 shows the tentative chemical assignments based on precursor and fragment ion accurate mass and isotopic pattern matching against published databases (Phenol Explorer and HMDB) for 12 representative ions from the “Aril marker” subset. The assignment was conducted using the Metascope search engine in Progenesis Q1.

Compound name and formula	Compound class	Retention time (min)	[M-H] ⁻ m/z	Mass error (ppm)	PQI fragmentation score	Number of matched fragments and exact mass	Isotope similarity (%)
Quercetin 3'-glucoside C ₂₁ H ₂₀ O ₁₃	Polyphenol (flavonoid) Flavonoid-3-o-glycoside	2.78	479.0821	-2.02	95.0	1 163.0037	81.87
Kaempferol 3-O-arabinoside C ₂₀ H ₁₈ O ₁₀	Polyphenol (flavonoid) Flavonoid-3-o-glycoside	4.17	417.0820	-1.67	92.45	2 282.9874 172.0151	81.72
Apigenin-4'-O-glucoside C ₂₁ H ₂₀ O ₁₀	Polyphenol (flavonoid) Flavonoid-4-o-glycoside	4.89	431.0977	-1.60	98.4	1 268.0377	79.89
Myricetin-3-galactoside C ₂₁ H ₂₀ O ₁₃	Polyphenol (flavonoid) Flavonoid-3-o-glycoside	4.41	479.0837	1.19	93.0	2 287.0197 271.0248	86.01
Quercetin-3-galactoside (hyperoside) C ₂₁ H ₂₀ O ₁₂	Polyphenol (flavonoid) Flavonoid-3-o-glycoside	4.62	463.0885	0.56	93.4	4 300.0275 271.0248 255.0298 243.0299	89.86
Epicatechin/Catechin C ₁₅ H ₁₄ O ₆	Phenylpropanoid (flavonoid)	4.21	289.0717	-0.38	92.3	3 109.0287 123.0441 151.0383	93.27
Caryoptosidic acid C ₁₆ H ₂₄ O ₁₁	Irridoid-o-glycoside	4.21	373.1135	-1.38	94.3	1 124.0166	89.97
Dihydrozeatin-O-glucoside C ₁₈ H ₂₅ N ₅ O ₆	Fatty acyl glycoside	3.14	389.1729	-0.83	96.2	2 220.1203 202.1098	90.83
Chlorogenic acid C ₁₆ H ₁₈ O ₉	Quinic acid	2.77	353.0870	-0.04	98.1	2 191.0561 135.0451	92.3

Table 3. Tentative identifications for "AriI marker" subset.

Inspection of the SONAR DIA data revealed the presence of three chromatographically co-eluting features having a retention time of 2.77 mins with different precursor masses and common fragment ions. Figure 6 shows the heat map visualization of the data plotting the quadrupole scan window (y-axis) versus chromatographic retention time (x-axis). Using conventional DIA it was not possible to determine the relative contributions of the precursor and fragment ions in the low and high energy channels. The additional selectivity provided by the resolving quadrupole in the SONAR DIA is seen to facilitate spectral interpretation allowing precursor and fragment ions to be more confidently associated.

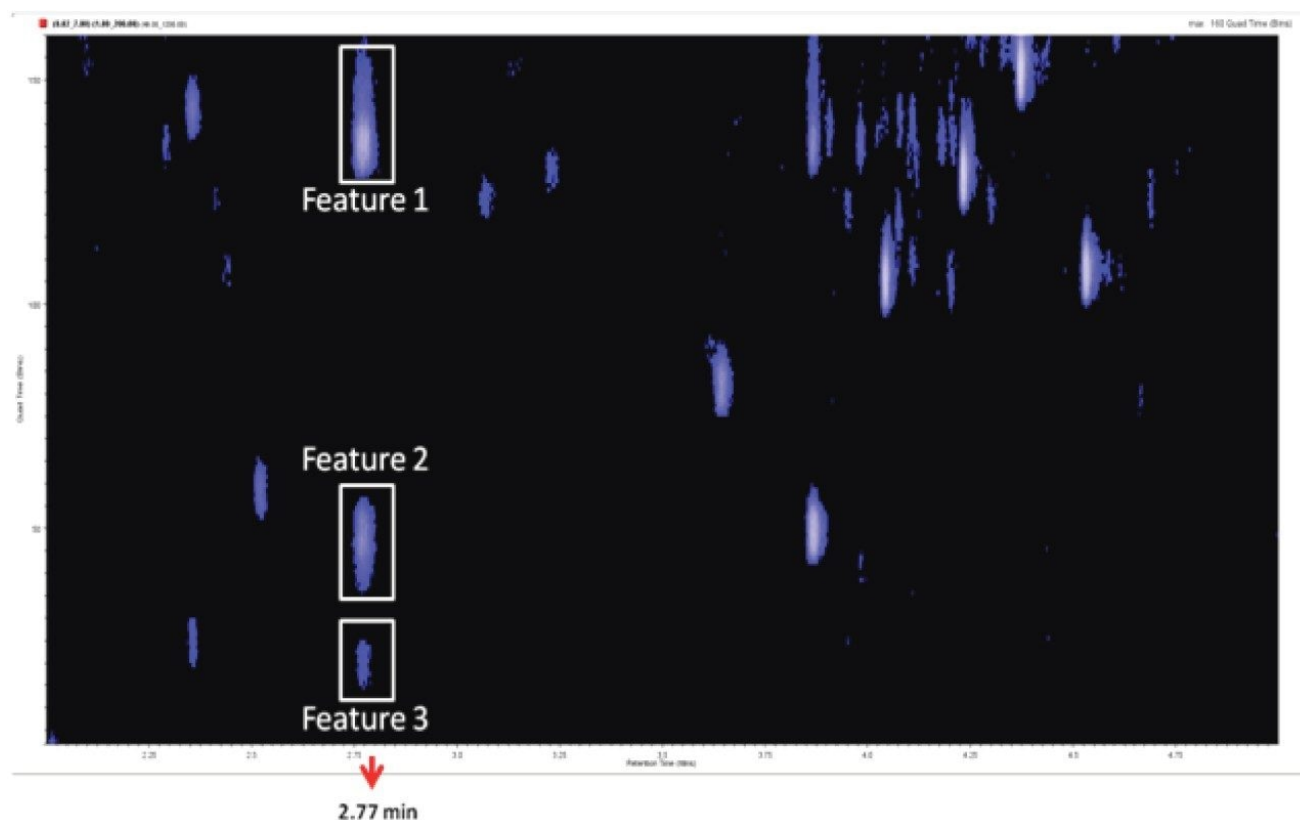


Figure 6. Heat map visualization and corresponding DIA spectra of DIA data showing 3 co-eluting precursors at 2.77 mins.

Compounds that are chromatographically co-eluting are now separated by SONAR and recorded individually (Figure 7) making the library searching more facile and thus improving the fragmentation scores obtained using Progenesis QI compared to conventional DIA techniques such as MS^E.

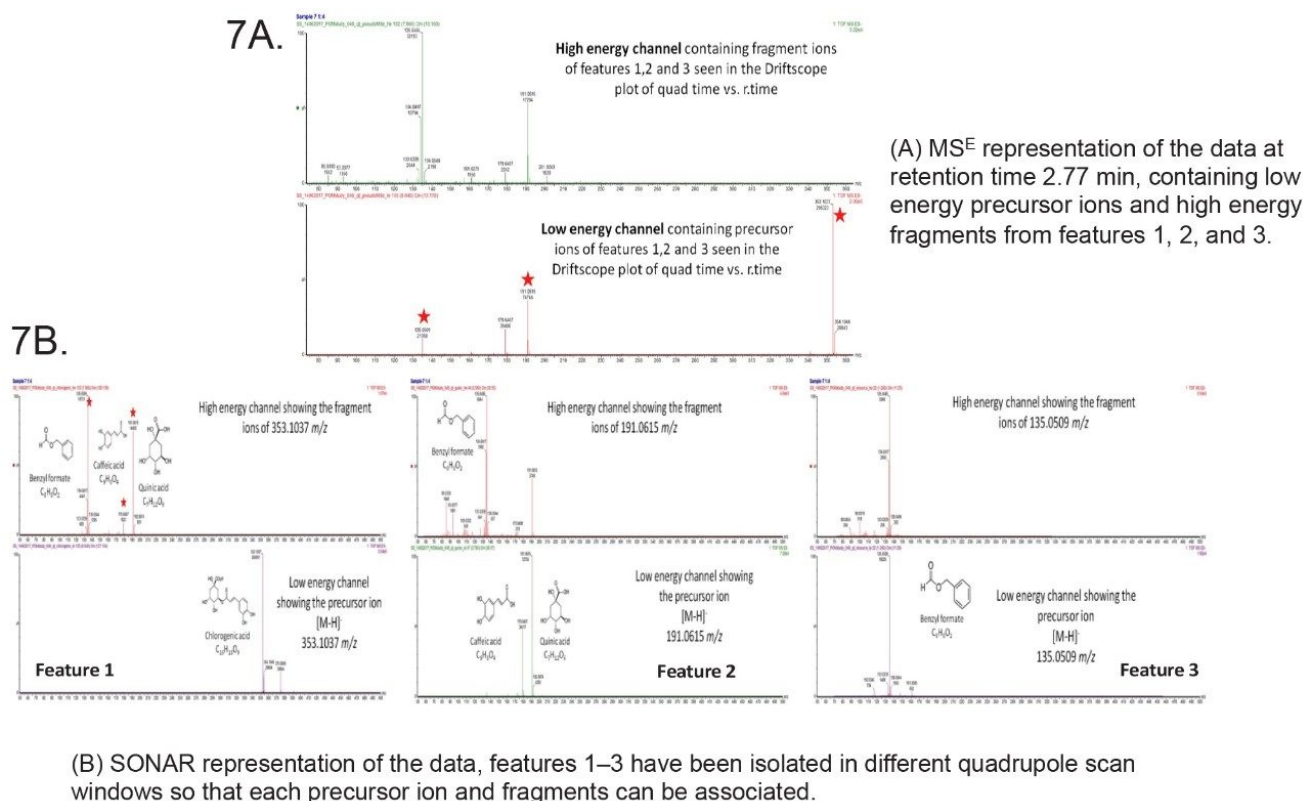
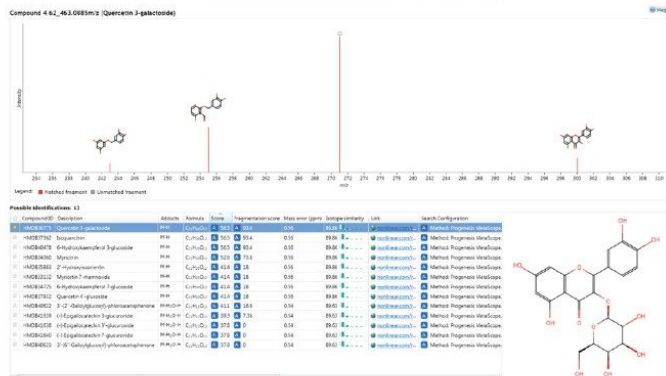
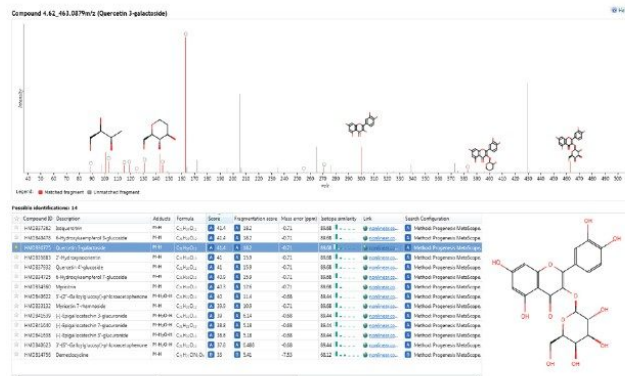


Figure 7. Individually recorded SONAR DIA spectra of three components co-eluting in Figure 6.

Figure 8 shows a specific example of spectral clarity on moving from traditional DIA acquisition to SONAR acquisition for the identified component quercetin-3-galactoside, a member of the flavonoid glucoside subclass.

Standard MS^E



Quercetin-3-galactoside C₂₁H₂₀O₁₂

Figure 8. MS^E and SONAR spectra from Progenesis QI of identified component quercetin-3-galactoside.

Table 4 highlights the comparative retail juice experiment performed using traditional DIA, against those which also included SONAR and IMS. Both IMS and SONAR are shown to increase the number of identified components and show increased fragmentation scores. The highest of these is shown to be as a result of SONAR analysis.

Parameter	VION IMS	Xevo G2 XS MS ^E	Xevo G2 XS SONAR
Mass <i>m/z</i>	50–1000	50–1200	50–1200
Gradient length (min)	7	7	7
Loading per inj (μL)	3 (1:4)	3 (1:20)	3 (1:4)
Progenesis QI features	2890	3435	1184
Total identified features			
HMDB 3.0 5/10 ppm	1006	592	1097
Frag score and [mass accuracy] for ellagic acid	53.2 [-2.57ppm]	38.1 [-3.85ppm]	92.8 [-1.85ppm]
Frag score and [mass accuracy] for chlorogenic acid	98.1 [-1.79ppm]	52.8 [-2.25ppm]	98.1 [-0.04ppm]
Frag score and [mass accuracy] for quercetin	84.1 [-1.30ppm]	20.7 [-1.10ppm]	92.1 [-0.32ppm]

Table 4. MS system acquisition method comparisons.

However, an additional feature of the IMS experiment is the ability to separate isobaric compounds. Isocitric/citric acid ratio is commonly used as a measure of pomegranate juice authenticity and was detected in the dataset. Additional analysis using ion mobility enabled QToF was performed and shown to be capable of separating this isomeric pair (data not shown).

Conclusion

Here we have shown benefits of SONAR for the application of fruit juice profiling for QC and authenticity purposes including:

- Cleaner spectra compared to other DIA methods
- High spectra/second scan rate compatible with HR-MS
- Increased confidence in identification through selectivity
- Cataloguing of a complex food sample within a single experiment
- Unbiased data acquisition within a targeted mass range

Ion mobility enabled QToF-MS analysis was also employed to facilitate isobaric marker identification and ratios in complex juice samples.

References

1. Mena et al. Rapid and Comprehensive Evaluation of (Poly)phenolic Compounds in Pomegranate (*Punica granatum L.*) Juice by UHPLC-MSn, *Molecules*. 2012; 17: 14821–14840.
 2. Gethings et al. Lipid Profiling of Complex Biological Mixtures by Liquid Chromatography/Mass Spectrometry Using a Novel Scanning Quadrupole Data Independent Acquisition Strategy, *Rapid Commun Mass Spectrom*., 2017; 31 (19): 1599–1606.
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Characterization, *J Proteome Res.*, 2018; 17 (2): 770–779.

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720006468, February 2019

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