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Adulteration in Fruit Juices: A Solution to a Common Problem

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Abstract

This application note describes a solution to easily identify any key differences between authentic and adulterated pineapple juices in a single run.

Benefits

- Obtain reliable and highly detailed information about food adulteration in a single run using ACQUITY UPLC in combination with Xevo G2 QTof MS.
- Confident structural elucidation through the use of exact mass precursor and fragment ion data.

Introduction

Adulteration of food and beverages is a significant problem that involves many different edible products. The high cost of the fruit, and the possibility of poor harvests conflicting with high consumer demand, makes the fruit juice industry, as well as many other relatively high commodity products susceptible.

Detection and prevention of fruit juice adulteration is a very complex task due to the natural variation in the cultivars, as well as differences that occur with different growing regions, storage conditions, and processing techniques. Analytical methods that have been used to identify adulteration have been comprehensively

reviewed.[1,2,3,4,5] The most common forms of adulteration that occur within the fruit juice industry usually take the form of juice dilution, the addition of high fructose corn syrup (HFCS),[6] or the addition of other fruit juices.[7]

In this application note, pineapple juice authenticity was investigated. Pineapples are the only members of the *bromeliad* family that produce edible fruit. The sweet fruit is usually consumed either in its fresh fruit form, or as a processed solid. Pineapple juice, which is primarily made from re-diluted concentrates, ranks fourth in the volume of fruit juice consumed globally.[8] There are also many reports about the health benefits from the consumption of pineapple juice. It is claimed to aid in the prevention of cancer, reduce joint pain due to its anti-inflammatory properties, increase bone strength, and aid digestion.[9] Its popularity and the cost of the fruit make it a target for adulteration.

Currently within the industry, the determination of juice authenticity involves many different analytical procedures, which can often be time-consuming. For this application note, pineapple juice samples were analyzed using UltraPerformance Liquid Chromatography (UPLC) for high resolution separations, photo diode array (PDA) detection, and accurate mass MS and MS/MS. Data interpretation involved the use of multi-variate analysis (MVA) and database searching in order to easily identify any key differences between authentic and adulterated pineapple juices.

Experimental

Sample preparation

Three pineapple juice concentrate samples were obtained from a collaborator. One sample was known to be authentic, and the two others were known to be adulterated. Additional pineapple juice samples were purchased from local grocery stores.

All samples were centrifuged, filtered, and diluted before analysis using Waters ACQUITY UPLC System with PDA detection, coupled with a Xevo G2 Quadrupole Time-of-flight Mass Spectrometer (QTof MS).

A description of the pineapple samples is provided in Table 1.

Name	Sample description
S10	Study sample – Adulterated
S11	Study sample – Adulterated
S12	Study sample – Authentic
KJ	Bought – Unknown authenticity
LJ	Bought – Unknown authenticity

Table 1. Description of the pineapple juice samples.

UPLC conditions

LC system:	ACQUITY UPLC
Column:	ACQUITY UPLC HSS T3, 2.1 x 100 mm, 1.8 µm
Column temp.:	45 °C
Injection volume:	3 µL
Flow rate:	0.4 mL/ min
Mobile phase A:	10 mM ammonium acetate in water
Mobile phase B:	Acetonitrile

Gradient

Time (min)	%A	%B
0.00	99	1
0.75	99	1
2.00	95	5

Time (min)	%A	%B
3.00	95	5
6.50	45	55
8.50	10	90
9.00	10	90
9.10	99	1

UV conditions

UV system:	ACQUITY PDA Detector
Range:	210 to 500 nm
Sampling rate:	20 pts/s

QTof MS conditions

MS system:	Xevo G2 QTof MS
Ionization mode:	ESI Negative (ESI-)
Analyzer mode:	Resolution
Capillary voltage:	2.0 kV
Cone voltage:	25 V
Desolvation temp.:	450 °C

Desolvation gas:	900 L/Hr
Source temp.:	130 °C
MS^E	
Low energy collision:	4 eV
High energy collision:	15 to 45 eV
Acquisition range:	50 to 1200 *m/z*
Scan time:	0.1 sec
Lock mass reference:	Leucine enkephalin

Data analysis

Data analysis and trending were performed using MarkerLynx XS Software. This software solution performs multivariate analysis (MVA) on mass spectral data sets. Markers identified by MarkerLynx were then automatically transferred to EleComp, ChemSpider, and MassFragment, in order to obtain the elemental composition and potential structures for the key markers.

Results and Discussion

All samples were analyzed using ACQUITY UPLC, ACQUITY PDA, and Xevo G2 QTof MS. Replicate injections of the pineapple samples were used in random sequence order to ensure that any experimental trends observed were directly associated with the sample.

Once the samples were analyzed, the data were interpreted using a chemometric approach with MarkerLynx XS Software. MarkerLynx XS integrates and aligns MS data converting them into exact mass retention time pairs (EMRT). These EMRT pairs can then be used for multivariate statistical analysis to visualize and interpret complex MS data sets.[10] The complete workflow used in the experiment is shown in Figure 1.

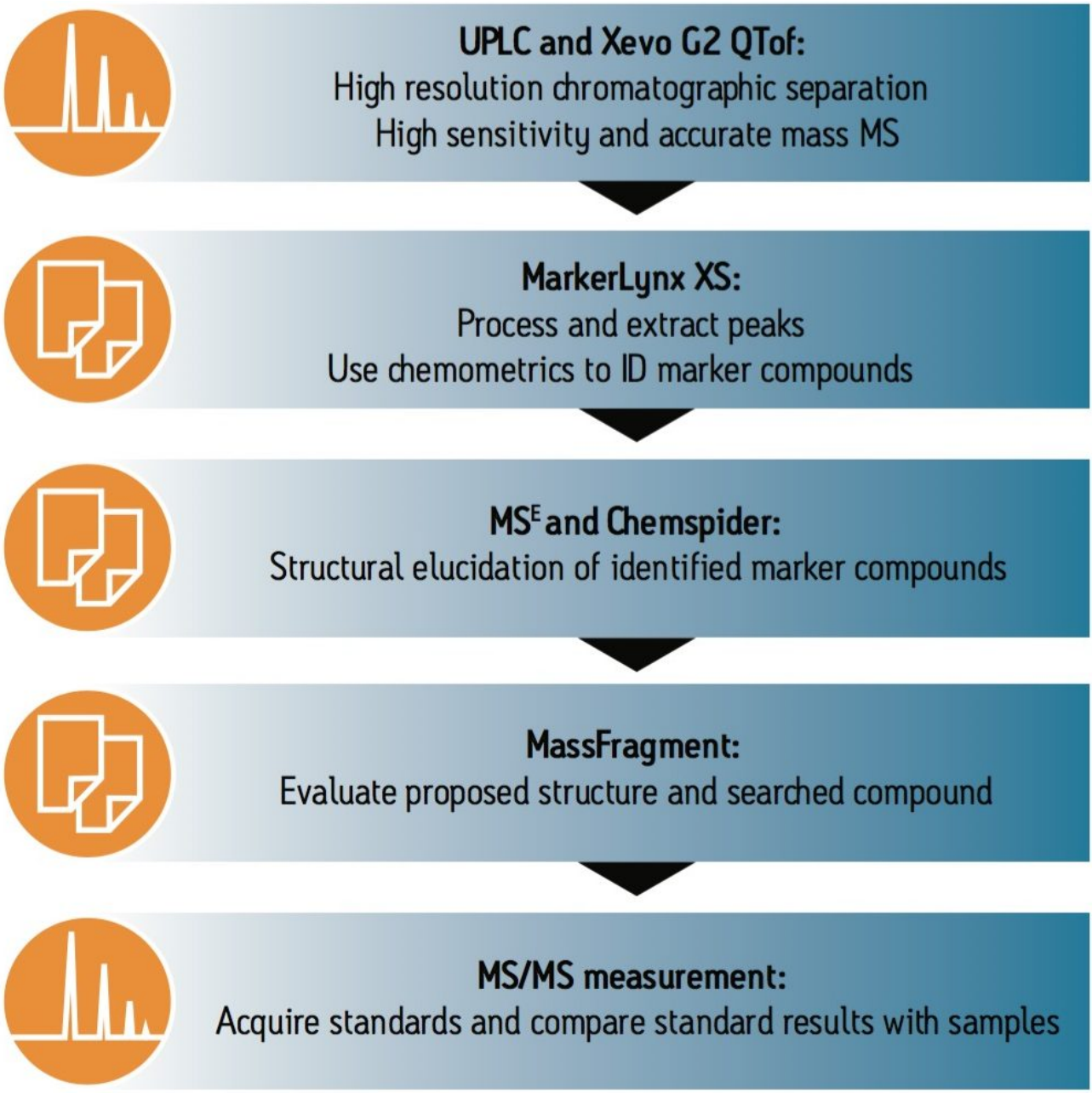


Figure 1. Pineapple juice profiling experimental workflow.

Typical examples of the high-resolution UPLC PDA and QTof MS chromatograms obtained from pineapple juice are shown in Figure 2.

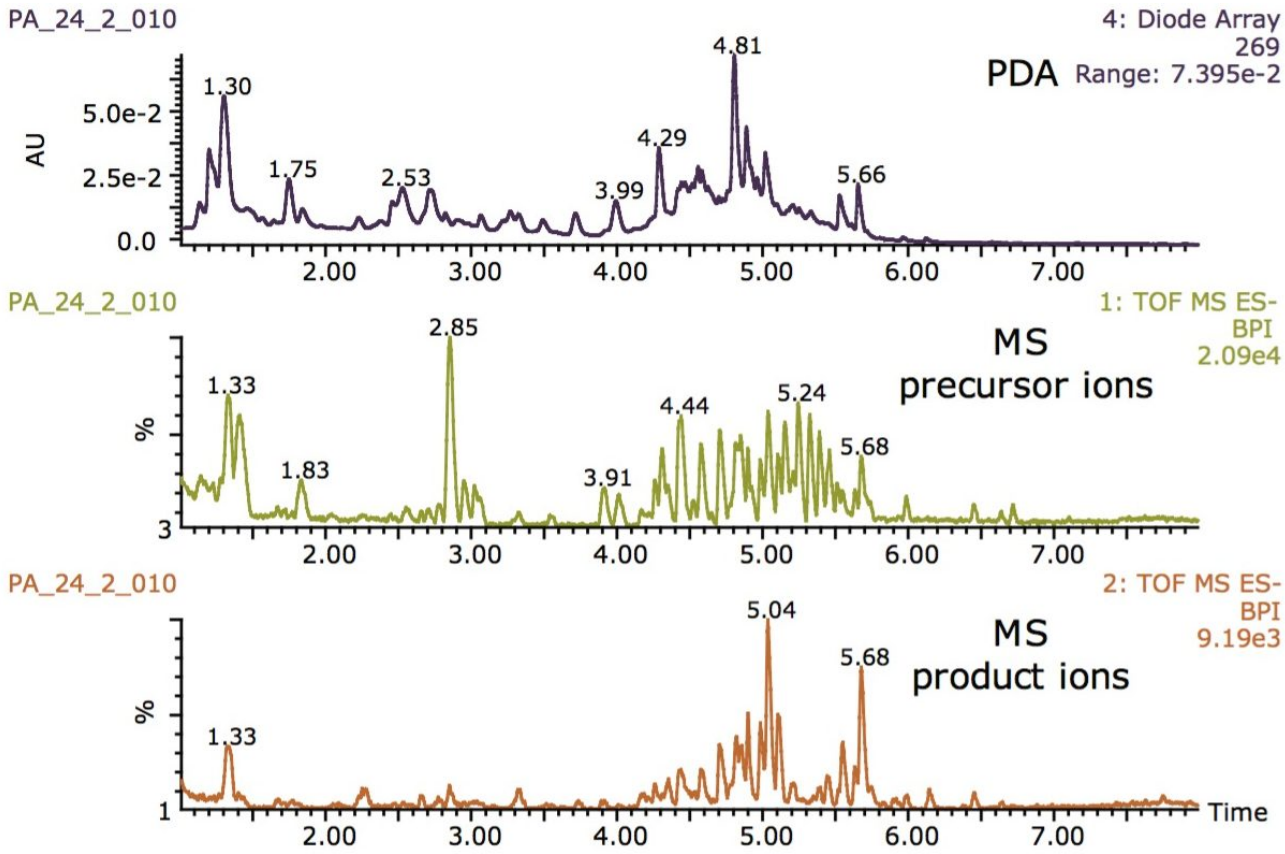


Figure 2. ACQUITY UPLC, ACQUITY PDA, and Xevo G2 QTof-MS chromatograms of pineapple juice.

Data from the PDA Detector provided a useful chromatographic profile and chemical information that indicated the types of chemical structures for the compounds of interest. Data from the QTof MS detector provided simultaneous accurate mass information, for both the precursor and fragment ions in a single injection. For the Xevo G2 QTof MS, two separate functions from the MS data were produced. The low collision energy (5 eV) chromatogram provided the exact mass precursor ion information, while the high collision energy (an energy ramp of 15 to 45 eV), provided the exact mass fragment ion data. This unique capability is called MS^E and it is this spectral information that enables potential structural identification of unknown marker compounds.

When either the PDA or the MS chromatograms are manually compared some subtle differences between the different samples can be observed. Figure 3 illustrates the visual differences observed with UV data. However, it is very difficult to ascertain using this manual approach, the significance of those differences without having prior extensive experience of the samples. Visual inspection of the data was also found to be extremely time consuming and inconclusive with respect to identifying the authenticity of the samples.

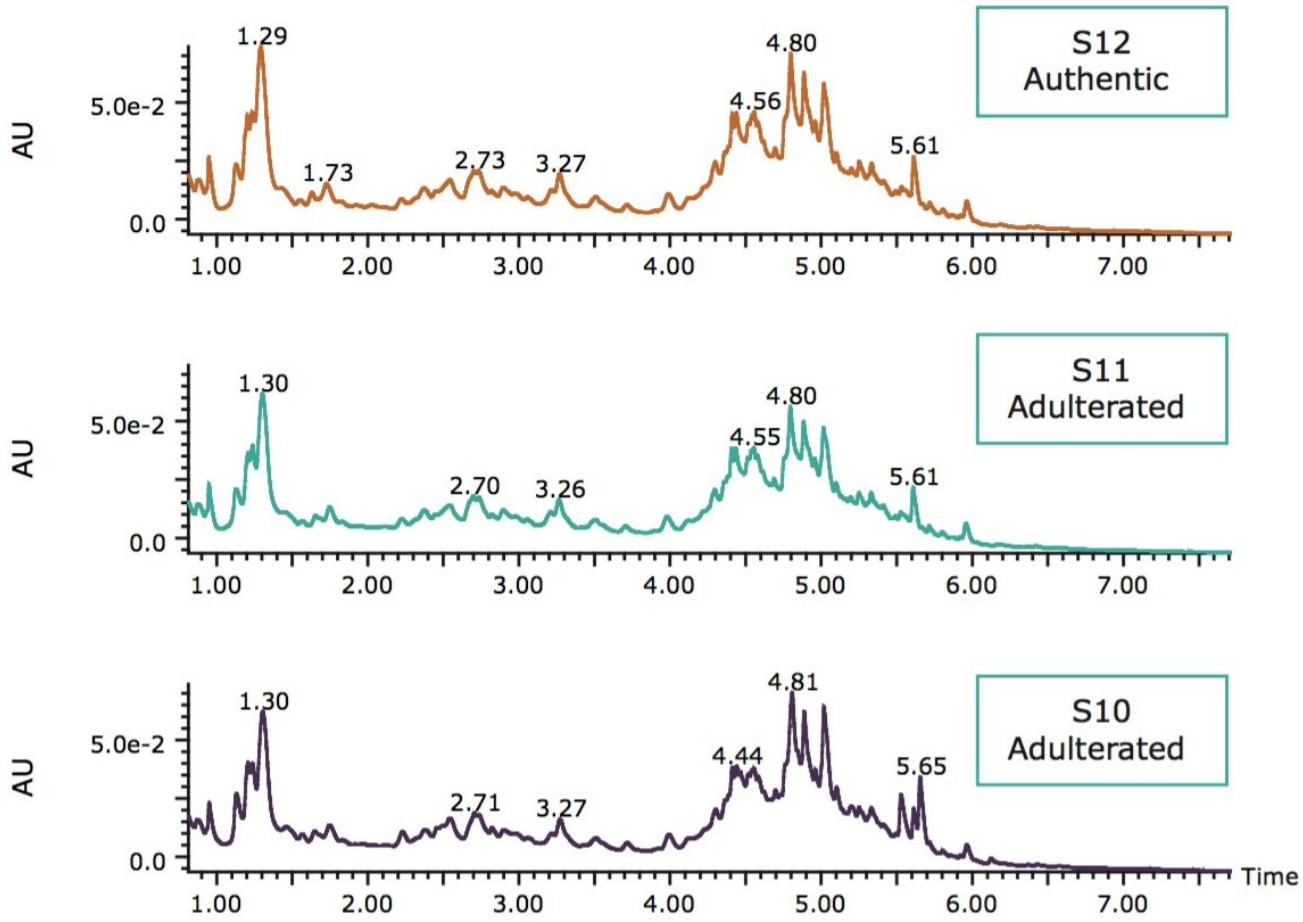


Figure 3. UV comparison (extracted wavelength – 283 nm) of three pineapple juices: S10, S11, and S12 (authentic sample).

To address this issue a chemometric approach was used to intelligently mine the MS data. The first approach used principal component analysis (PCA). Five distinct pineapple juice sample groups are apparent in the resulting scores plot, as shown in Figure 4. A scores plot explains the relationships between the observations in the data. Each point in the scores plot represents a single injection.

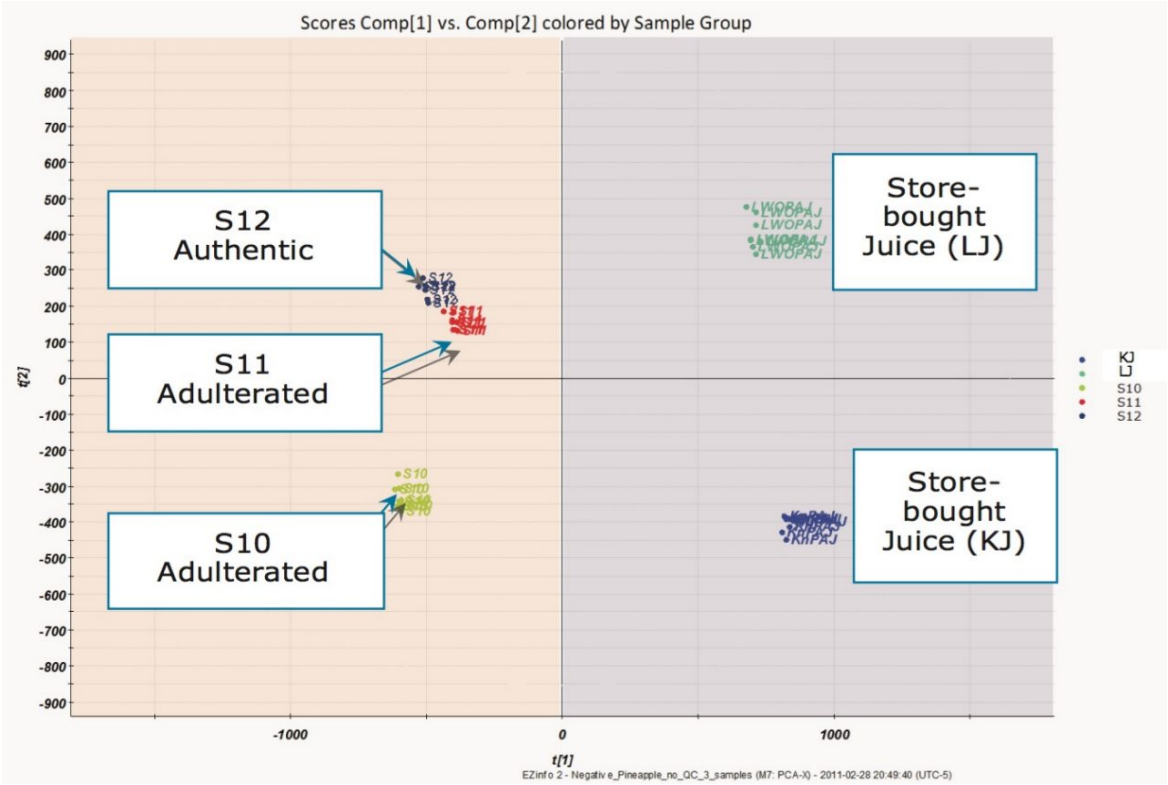


Figure 4. The PCA scores plot obtained from all the pineapple juices analyzed.

In order to rationalize the observed differences, additional information can be obtained using another more predictive multivariate model, such as orthogonal partial least squares data analysis (OPLS-DA)[10]. OPLS-DA provides a deeper understanding of inter-class variations and allows a relationship to be drawn between the classes and the potential marker compounds in each sample group.

Figure 5 shows an S-Plot from the OPLS-DA model of the authentic (S12) and one of the adulterated juice samples (S10). The upper right hand corner of the S-plot show EMRT pairs that are significant and representative of the adulterated juice (S10) and the lower left are those in the authentic pineapple juice (S12). The S-plot identified the EMRT pairs contributing to the most significant differences between S12 and S10. Four markers were highlighted and a trend plot of these markers was produced for all of the analyzed samples, shown in Figure 6.

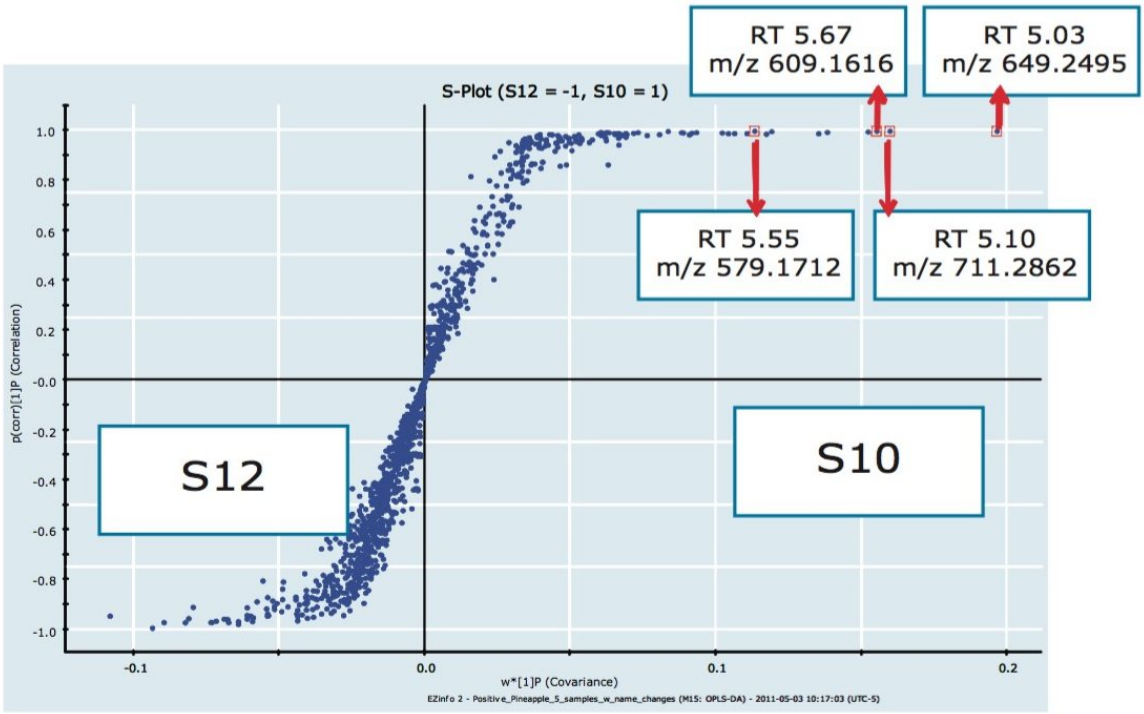


Figure 5. S-plot of S12 (authentic) versus S10 (adulterated): Each dot is an exact mass/retention time pair (EMRT) and the dots on the top right and bottom left are all potential marker compounds for S12 (left) and S10 (right).

From the PCA scores plot, it is possible to see that S12 (authentic pineapple juice) and S11 (deliberately adulterated) are grouped closely together. Sample S10 (deliberately adulterated) is well separated, indicating something about this sample is distinctly different. The supermarket samples KJ and LJ are also very different, grouping on the far right of the PCA model. These differences may be attributed either directly to the fruit (harvest, geography, growing conditions, manufacturing), or they could be due to another type of adulteration.

The trend plot shows that S10 and one of the store bought samples (LJ) are the only samples to contain the marker with *m/z* 609.1816 at a retention time of 5.66 min. The QC sample, also shown in Figure 6, is a combination of all samples analyzed in this experiment – hence also contains the marker *m/z* 609.1816. In addition, S10 also contained markers with *m/z* of 579.1712, 649.2495, and 711.2862 (and retention times (Rt) of 5.54, 5.03, and 5.10, respectively).

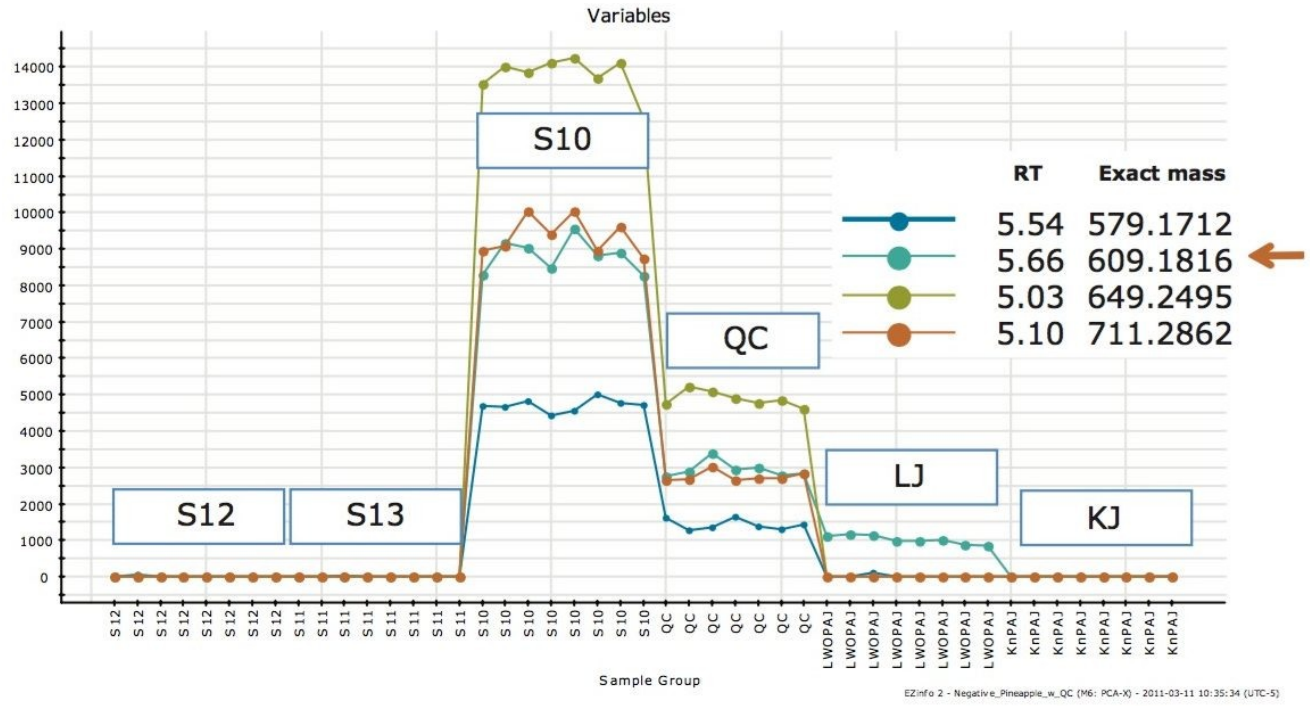


Figure 6. Trend plots of four potential markers extracted from the S-plot in Figure 5. The four different colored plots show the variation in intensity of each of the EMRT markers in the samples.

Marker identification

To further investigate the potential marker at 5.66 min the UV data was interrogated. A component at this retention time showed an absorption maximum of 283 nm. It can be seen from the extracted wavelength chromatogram, shown in Figure 7, that even when an extracted wavelength is used the chromatograms are extremely complex, making compound identifications a challenge.

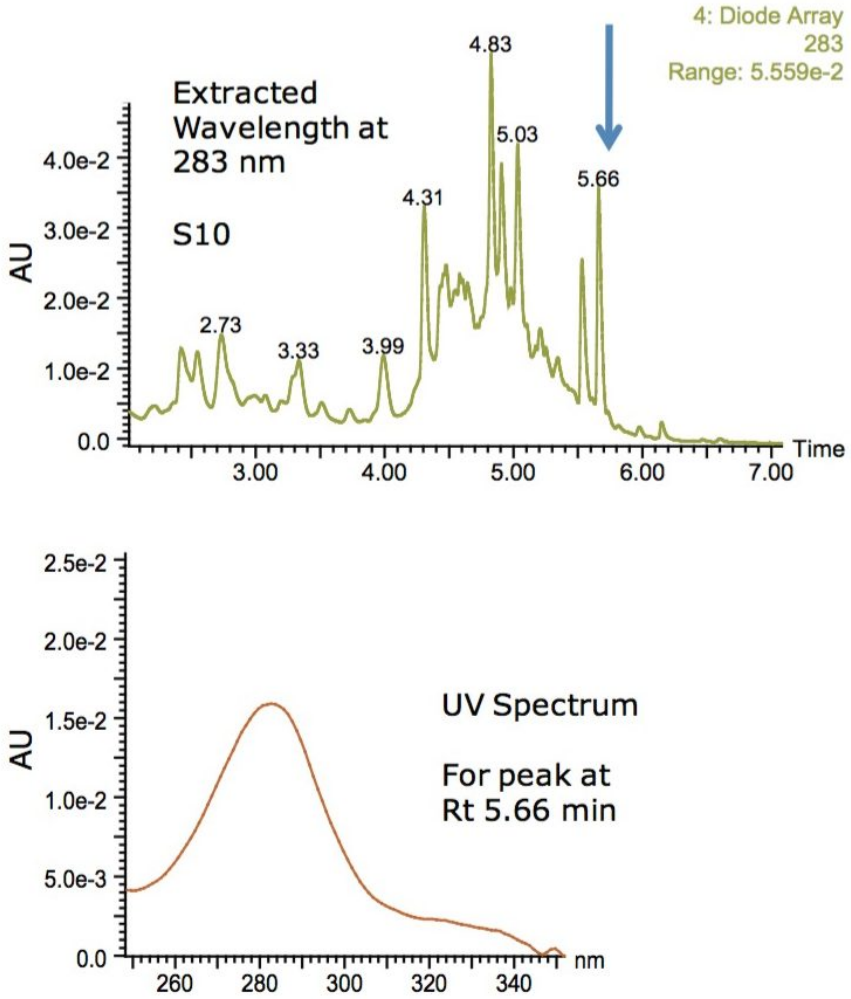


Figure 7. Extracted wavelength chromatogram and UV spectrum for the peak at a retention time of 5.66 min.

As the Xevo G2 Qtof MS has excellent mass accuracy, it is possible to use narrow window extracted ion chromatograms (XIC) to extract *m/z* 609.1816 (+/- 1.5 mDa), as shown in Figure 8. With this approach it can be seen that this marker at Rt 5.66 is present in the adulterated pineapple juice (S10), but it is absent in the authentic juice (S12) and the second adulterated sample (S11). The selectivity benefit obtained by combining MS detection with the more traditional UV detection method is clear from the contrast between the two chromatograms for S10 shown in Figure 7 and Figure 8.

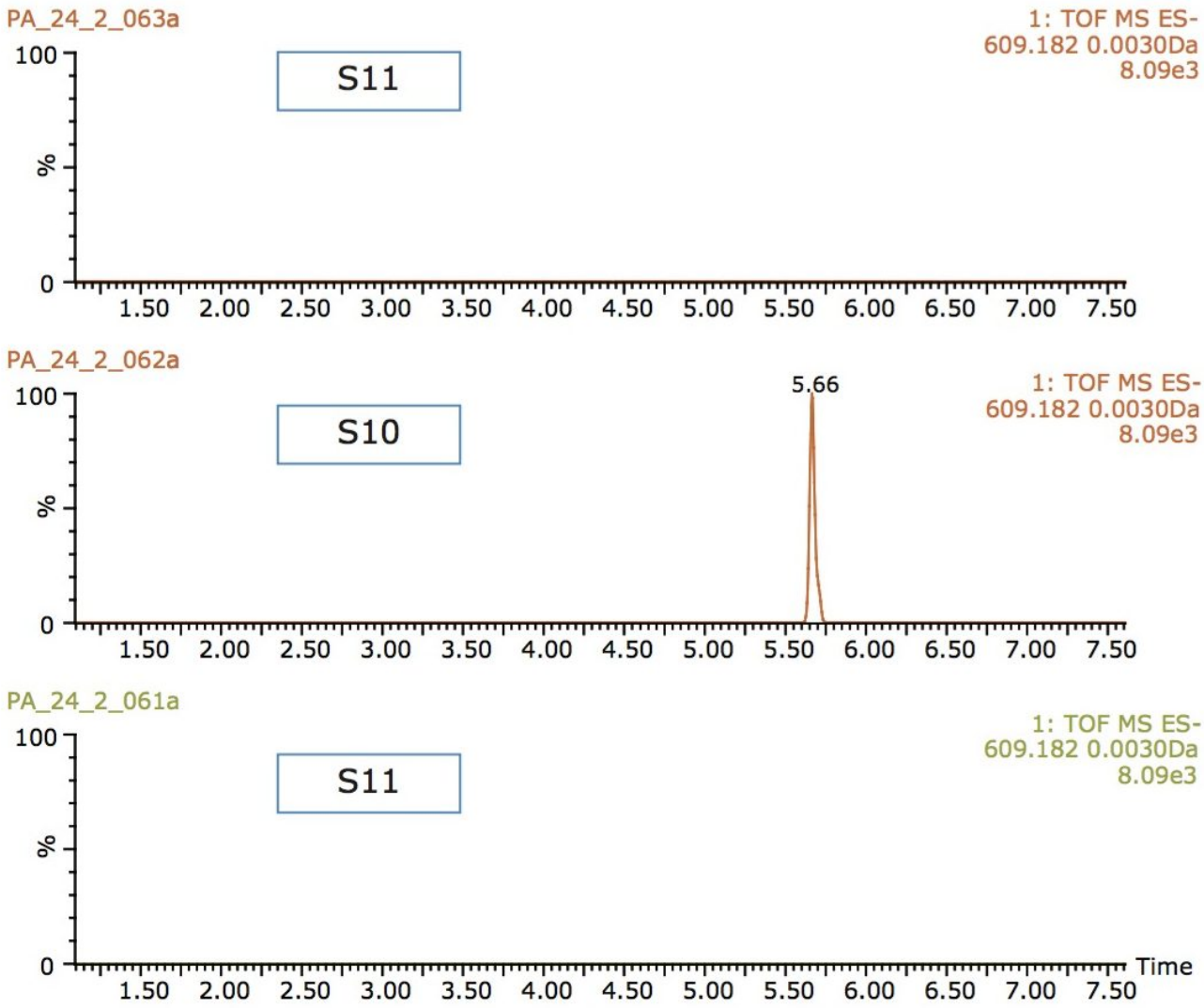
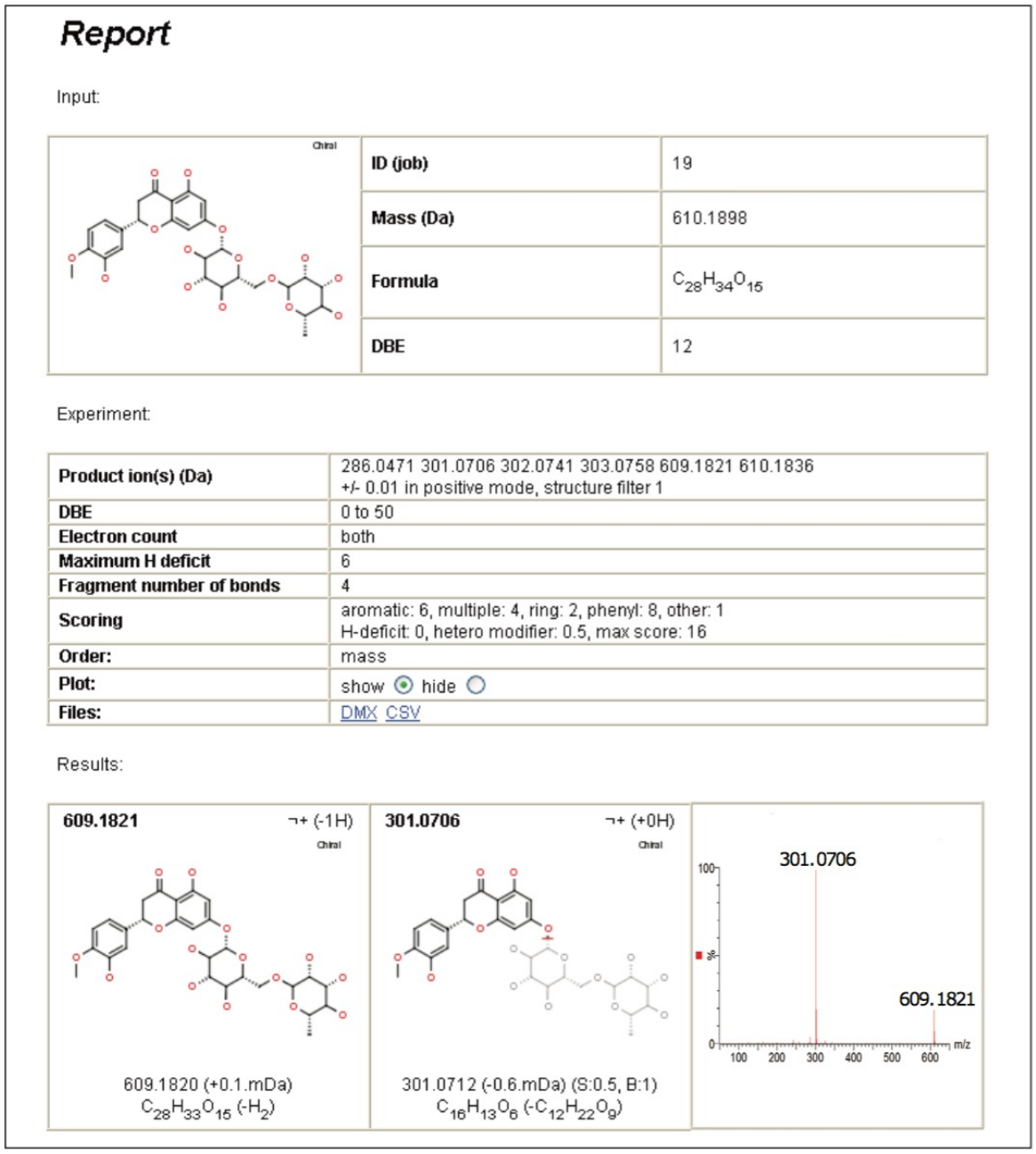


Figure 8. Extracted ion chromatograms (XIC) for m/z 609.1816 in S10, S11, and S12.

Using EleComp software, the formula $C_{28}H_{34}O_{15}$ was proposed for the ion with *m/z* 609.1819. Entering this information into ChemSpider provided a possible identification as hesperidin. Research indicated some possible sources of hesperidin to be citrus juices (grapefruit, lemon, lime, orange, and tangerine). Hesperidin has not been reported in pineapple, suggesting it could be an adulteration marker for this study.

Using MassFragment Software, the MS^E exact mass fragment ions can be compared to the theoretical fragmentation of hesperidin, as shown in Figure 9. A positive fragmentation pathway was confirmed and excellent mass accuracy was obtained for the precursor (0.1 mDa, 0.4 PPM) and product ion (0.6 mDa, -2.0 PPM), which provided increased confidence in the compound identification.



Report

Input:

ID (job)	19
Mass (Da)	610.1898
Formula	$C_{28}H_{34}O_{15}$
DBE	12

Experiment:

Product ion(s) (Da)	286.0471 301.0706 302.0741 303.0758 609.1821 610.1836 +/- 0.01 in positive mode, structure filter 1
DBE	0 to 50
Electron count	both
Maximum H deficit	6
Fragment number of bonds	4
Scoring	aromatic: 6, multiple: 4, ring: 2, phenyl: 8, other: 1 H-deficit: 0, hetero modifier: 0.5, max score: 16
Order:	mass
Plot:	show ◉ hide ○
Files:	DMX CSV

Results:

Figure 9. MassFragment Software report showing the structural assignments for hesperidin: m/z 609.1819 and the major MS^E fragment ion m/z 301.0712.

To confirm the identification of the compound, a standard solution of hesperidin was analyzed and compared with the component in the sample. Figures 10A and 10C show that the component identified as hesperidin eluted at the same retention time as the standard. The MSE fragmentation data also correlated with the standard as shown in 10B and 10D.

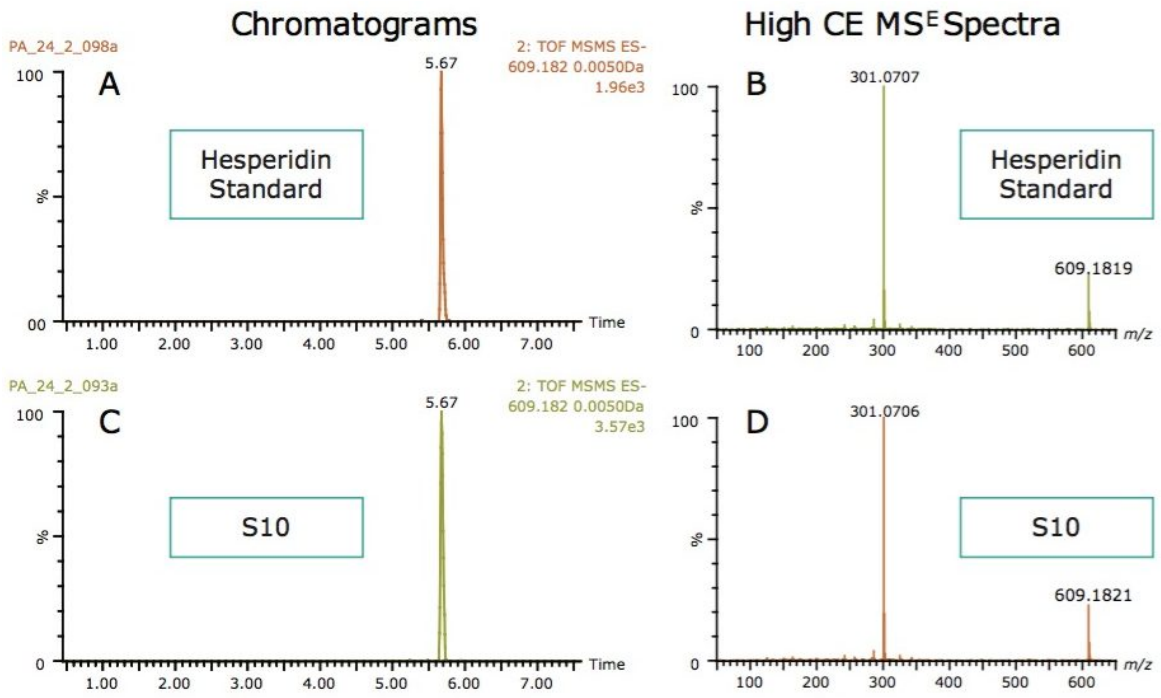


Figure 10. XIC of m/z 609.1820 from standard hesperidin (10A) and adulterated pineapple juice, S10 (10C): their respective high collision energy MS^E spectra are also shown (10B and 10D).

In addition to the MS data, confirmation was also provided by the UV chromatograms and spectra for standard hesperidin with those in S10. Retention time and UV spectral matches are shown in Figure 11.

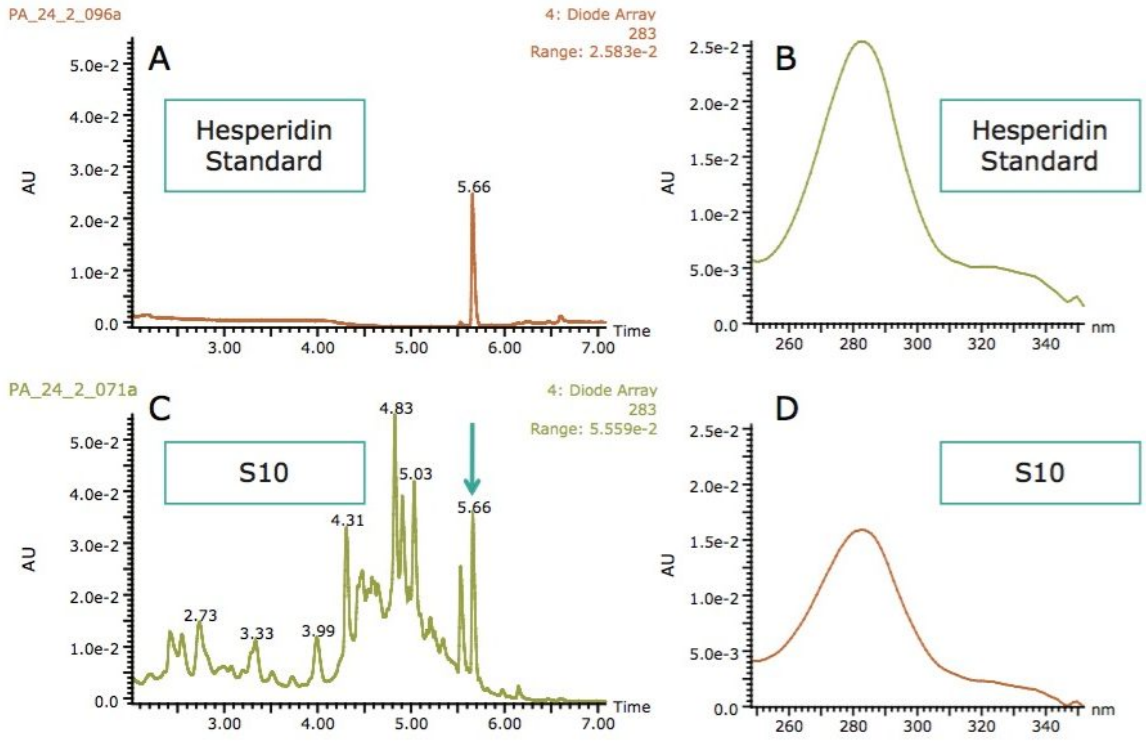


Figure 11. Extracted wavelength chromatograms (283 nm) for standard hesperidin (11A) and adulterated juice, S10 (11C). The corresponding UV spectra (11C) and (11D) are also shown.

The trend plot shown in Figure 6 also suggested that hesperidin was present in LJ (a sample purchased locally). Using the same workflow and data as before – Rt, elemental composition, and MS^E fragment – confirmed the presence of hesperidin. The concentration, however, was too low for UV detection, as shown in Figure 12.

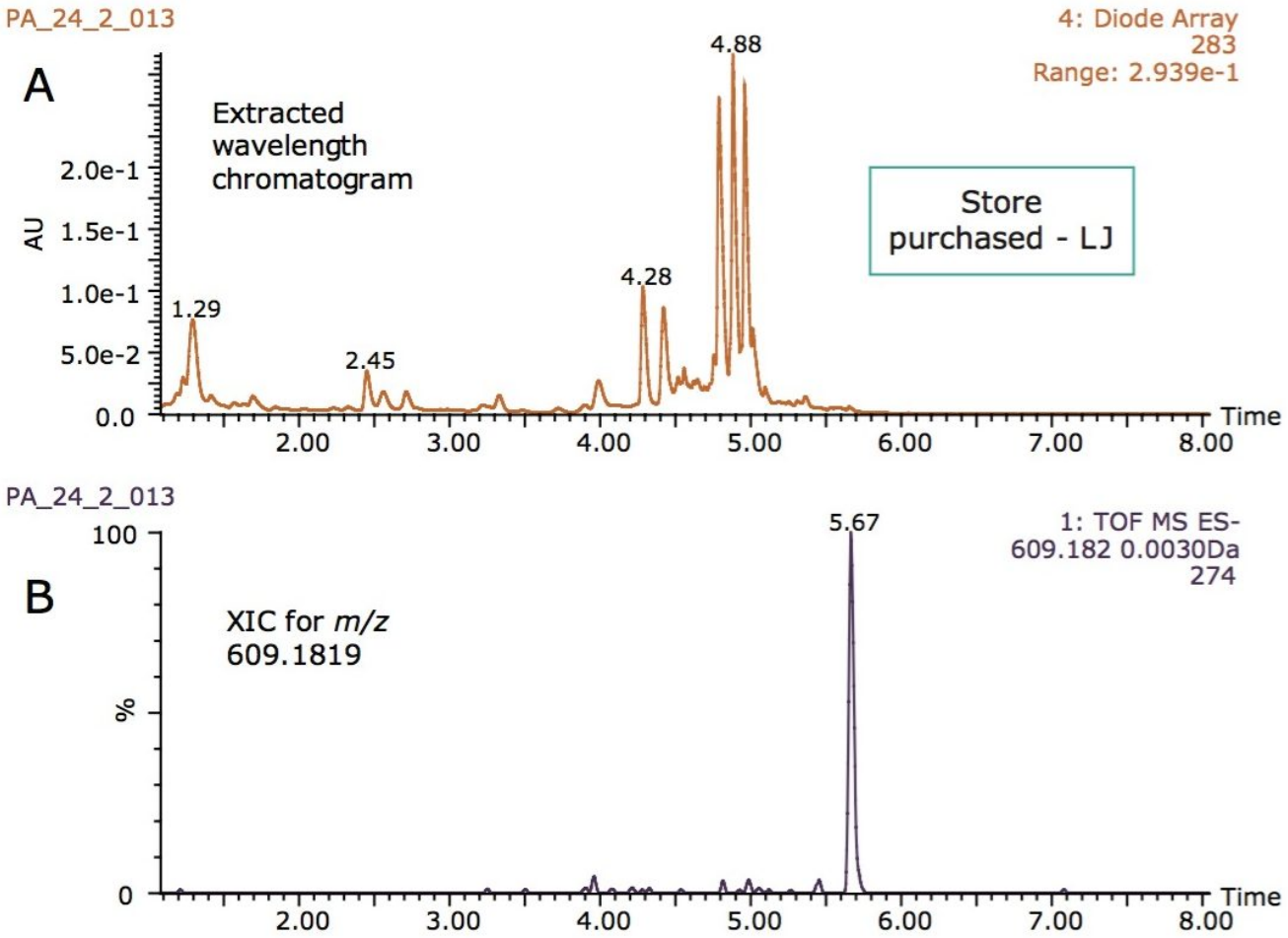


Figure 12. Hesperidin (extracted wavelength of 283 nm) (12A) is not detected in the UV data due to lack of sensitivity. Hesperidin (XIC for m/z 609.1819)(12B) is clearly detected at 5.67 min in the MS data.

The other EMRT pairs identified in the S-plot were investigated. For the component eluting at 5.55 min with *m/z* 579.1712, the elemental composition was determined to be $C_{27}H_{33}O_{14}$. In this instance ChemSpider proposed two potential hits: the isobaric compounds naringin and narirutin. These two compounds have identical molecular formulae and therefore are not resolved by high resolution MS alone.

A naringin standard was analyzed and the data were compared to the component in S10. The retention time of the naringin standard (Rt 5.59 min) was different to the component in the sample (Rt 5.54 min) as can be seen in Figure 13. The exact mass MS^E fragment ion data from the juice and the standard in Figure 13 show that although there are common fragments in both, there are other fragments present in the spectrum from the juice.

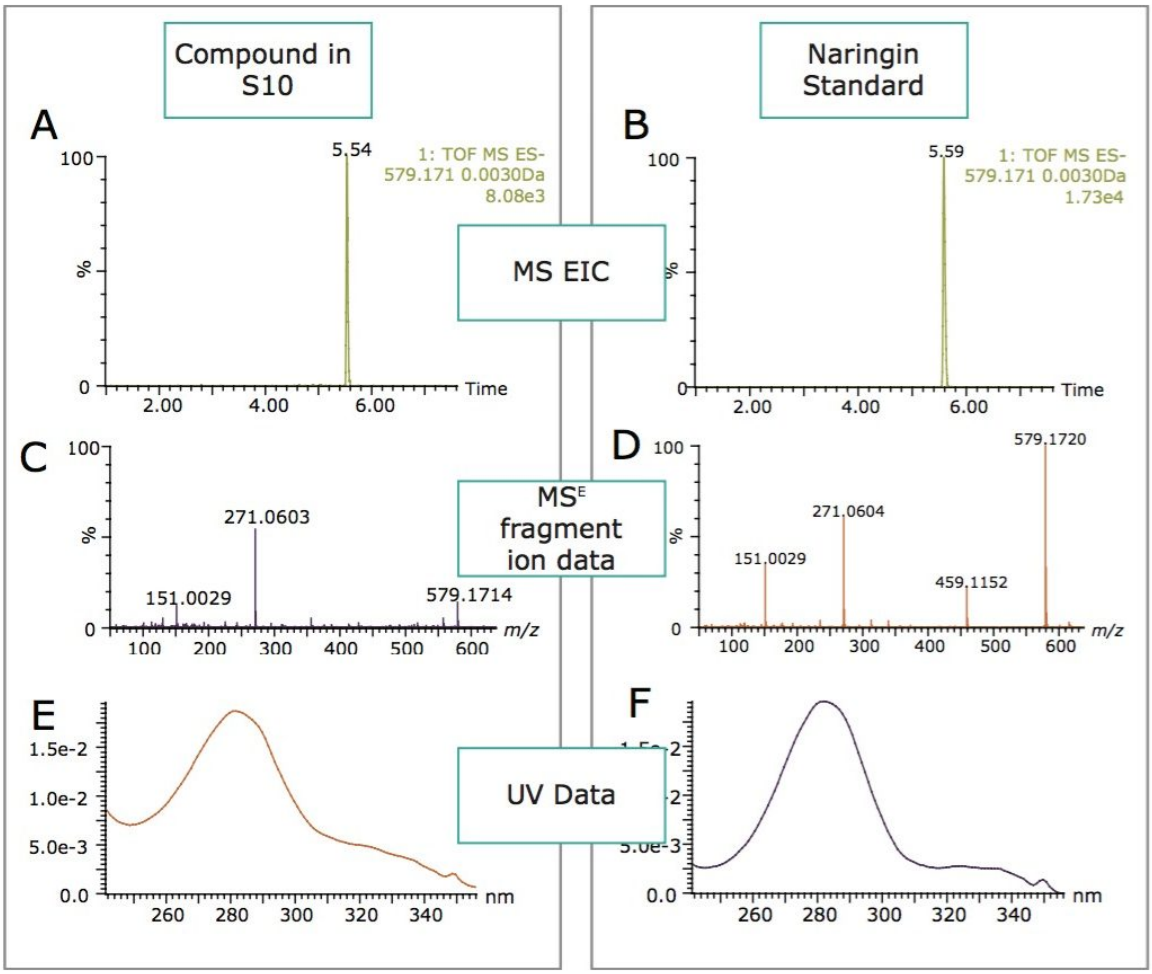


Figure 13. Extracted ion chromatogram of m/z 579.1713 in S10 (13A) and standard naringin (13B) with the MS^E fragment data for S10 (13C) and standard naringin (13D). UV spectrum for component in S10 (13E) and standard naringin (13F).

Subtle differences were also observed in the second absorption UV maxima. The UV and the MS^E fragment ion data, and the Rt difference supports that this compound is not naringin and suggests that it is more likely to be narirutin.

Like hesperidin, naringin and narirutin are flavanone-O-glycosides that can be found in citrus fruits including oranges, lemons, grapefruits, limes, and tangerines. Hesperidin is the most abundant flavonoid component found in sweet orange juices, with the next being narirutin.[11]

The other two EMRT pairs were each identified as Limonin 17-beta-D-glucopyranoside (LG) and Nomilinic acid 17-beta-D-glucopyranoside (NAG). LG and NAG are citrus limonoid glucosides found in citrus fruits and their related genera.[12]

In the scores plot, the authentic pineapple juice (S12) appeared in close proximity to S11 – an adulterated juice sample. Their appearance on the plot close together indicates the similarity between them. In this instance, the adulteration performed in this sample was later confirmed to be the addition of cane sugar syrup and ascorbic acid. The sugar syrup and ascorbic acid are not retained in the LC method selected for analysis and therefore these components did not contribute to the variation.

The difference between S11 and S12 merely resulted from the dilution effect and explains why S11 and 12 are spatially closer in the scores plot shown in Figure 4. There was subtle evidence of dilution effects when variable trend plots for compounds common to all juice samples were viewed. An example of two compounds exhibiting a dilution effect in the two juices is shown in Figure 14.

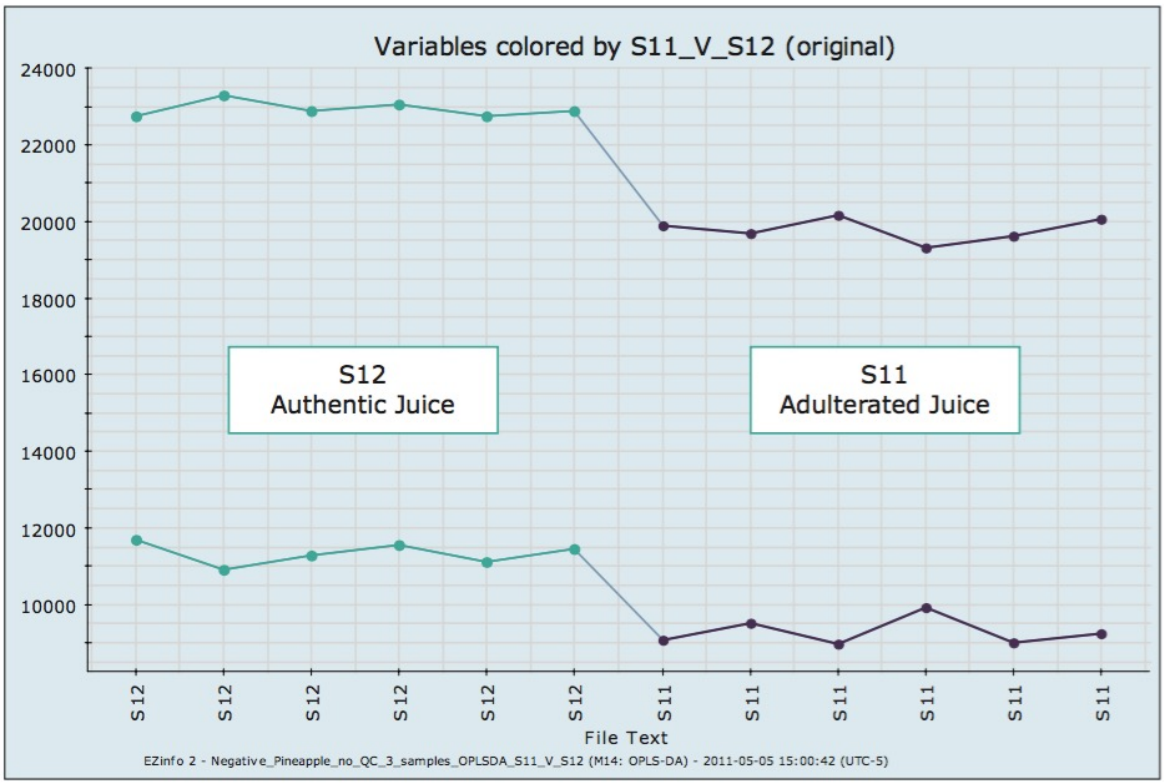


Figure 14. Variable trend plots for two compounds found in the authentic (S12) and adulterated (S11) juices. The lower levels found in S11, and the low spatial distance found in the PCA model indicate the adulteration is due to dilution. (This was confirmed by the collaborator).

In summary, a combination of high resolution ACQUITY UPLC, ACQUITY PDA, and the Xevo G2 QTof MS technology, combined with MarkerLynx XS Software enabled the identification of exogenous compounds in an adulterated pineapple juice sample. The compounds identified have all been reported in citrus juices. One of these compounds, hesperidin, was also detected in a locally purchased product claiming to be 100% pineapple juice. Although the level of hesperidin in this purchased sample was detected using ToF MS, it was not detected

by the less sensitive PDA detection. In addition, plots of components in the authentic juice versus another adulterated juice suggest that dilution of the sample had occurred.

Conclusion

Economic adulteration in the fruit juice industry is a problem that has increased in magnitude due to its lucrative outcome for unscrupulous members of the fruit juice manufacturing chain.

- Accurate mass detection enabled the determination of the elemental composition which can be used in the process of compound identification.
- The combination of ACQUITY UPLC, ACQUITY PDA, and Xevo G2 QTof MS provides reliable and highly detailed information about the samples that can help in the process of food authentication.
- The fragment data together with exact mass measurement provided added confidence and accuracy for structural elucidation. The MS^E functionality on the Xevo G2 QTof MS allowed the acquisition of exact mass low energy precursor (MS) and exact mass high energy product ions in a single run.

Without prior knowledge of the samples, this system solution combined with powerful data analysis tools allowed the identification of four citrus compounds in an adulterated pineapple juice sample.

PDA detection was able to assist in the confirmation of compound identity but PDA alone was insufficient for identification in a complex matrix like fruit juice.

The high sensitivity of the Xevo G2 QTof MS enabled the detection of hesperidin in a store-bought product that claimed to be authentic pineapple juice. PDA detection was not able to detect this low level, demonstrating the power of ultra-sensitive techniques in the search for potential adulterants.

References

1. WW Widmer, PF Cancalon, and S Nagy. *Trends in Food Sci and Technol.* 3,11: 278-286, 1992.
2. T Patel. *New Sci.* 142: 26-29, 1994.
3. A Yamamoto *et al*. *Determination of Adulteration in Apple Juice by HPLC.* J Agric Food Chem. 56, 16: 7302–7304, 2008.

4. D Castaldo *et al. Estimating Bergamot Juice Adulteration of Lemon Juice by High-Performance Liquid Chromatography (HPLC) Analysis of Flavanone Glycosides.* J Agric Food Chem. 56: 5407–5414, 2008.
5. D Heber D. *International Multidimensional Authenticity Specification (IMAS) Algorithm for Detection of Commercial Pomegranate Juice Adulteration. J. Agric. Food Chem*. 57, 6: 2550-2557, 2009.
6. W Simpkins and M Harrison. *The state of the art in authenticity testing., Trends. Food Sci. and Technol*. 10: 6, 1995.
7. PR Ashurst. *Chemistry and technology of soft drinks and fruit juices.*
8. C Grassin, P Fauquembergue. *Fruit Juices,* Industrial Enzymology, 2nd ed.; Godfrey, T. and West S., Eds.; MacMillan Press, London, U.K., pp 225-264, 1996
9. http://www.home-remedies-for-you.com/articles/Pineapple-Juice-Health-Benefits.html
10. L Eriksson. *Multi- and Mega-variate Data Analysis: Basic Principles and Applications. 2nd edition*, 2008.
11. G Gattuso, D Barreca, C Gargiulli, U Leuzzi, C Caristi. *Flavanoid composition of citrus juices. Molecules*. 12: 1641-1673, 2007.
12. S Hasegawa, M Miyake. *Food Rev Int.* 12: 413-435, 1996.

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720004173, February 2012