

**Liquid Chromatography/
Mass Spectrometry****Author:**

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Rapid Analysis of Apple Juice Adulteration with Pear Juice Using LC/TOF

Introduction

Adulteration of fruit juices is quite common in the market place and is often done through juice substitution.

Pear juice has a slightly lower market value than apple juice yet both have similarities in major chemical composition, making pear juice an ideal adulterant for apple juice. Both juices have similar carbohydrate, organic acid and amino acid levels, therefore the detection of apple juice adulteration with pear juice, based on these compounds, has limited applicability. According to various references¹⁻⁴, the phenolic profiles in pear and apple juice are slightly different. One of the phenolic compounds, arbutin, can be used as a marker compound to detect adulteration. The structure of arbutin is shown in Figure 1. In this application note, we present a rapid method for detecting apple juice adulteration with pear juice using LC/TOF, which requires minimal sample preparation.

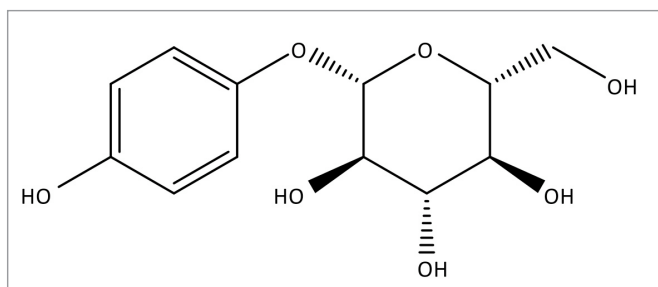


Figure 1. Chemical structure of arbutin.

Method

Pear and apple juices were purchased from a local supermarket. Both juices were diluted by a factor of 5 in water and filtered through a 0.2 μm PTFE syringe filter. The juices were mixed in different proportions to simulate the adulteration of apple juice with pear juice at levels of 10, 20, 30, 40 and 50 percent. The diluted and filtered juices and their mixtures were analyzed with LC/TOF without any further sample preparation.

Table 1. LC conditions.

Pump:	PerkinElmer Flexar™ FX-15 UHPLC pump		
Flow:	0.3 mL/min		
Mobile phase A:	100 % water with 0.1 % formic acid		
Mobile phase B:	100 % acetonitrile with 0.1 % formic acid		
Gradient conditions:			
Time (min)	%A	%B	Curve
0.5	95	5	
7.0	60	40	1
7.5	60	40	1
Injection volume:	10 μL Full Loop Mode		
Column:	PerkinElmer Brownlee™ SPP RP-Amide, 2.1x100 mm, 2.7 μm (part number N9308501)		

Table 2. MC conditions.

Mass spectrometer:	PerkinElmer AxION® 2 TOF MS
Ionization source:	PerkinElmer Ultraspray™ 2 (Dual ESI source)
Ionization mode:	Negative
Capillary exit voltage:	-90 V
ESI source temperature:	350 °C
Drying gas flow rate:	10 L/min
Internal calibration:	Performed using m/z 112.9856 and 601.9790 as lock mass ions

Results and Discussion

A 7.5 minute gradient LC method coupled with TOF MS was developed to determine the presence of arbutin in apple and pear juices. Figures 2-4 show the extracted ion chromatograms (EICs) for the formate adduct of arbutin in a sample containing 10 $\mu\text{g/ml}$ arbutin, pear juice diluted by a factor of 5 and apple juice diluted by a factor of 5, respectively. The data in Figures 2-4 show that arbutin is present in pear juice, whereas, it is either absent or present below the TOF MS detection limit of arbutin in apple juice. Figure 5 shows that the presence of arbutin in pear juice was further confirmed by experimental accurate mass measurement of the deprotonated molecular ion and the formate adduct of arbutin in pear juice with TOF MS. The data demonstrates that arbutin can be used as a marker compound to determine the adulteration of apple juice with pear juice using LC/TOF. This is supported further by data in Figure 6, which shows that the response for arbutin increases linearly with the increase in adulteration of apple juice with pear juice from 10 to 50%. It confirmed that the adulteration of apple juice with pear juice can be detected by the presence of arbutin. Figure 7 shows overlay of arbutin response in apple juice diluted by a factor of 5 in water and spiked with 10 $\mu\text{g/ml}$ arbutin and apple juice mixed with 10% pear juice and diluted by a factor

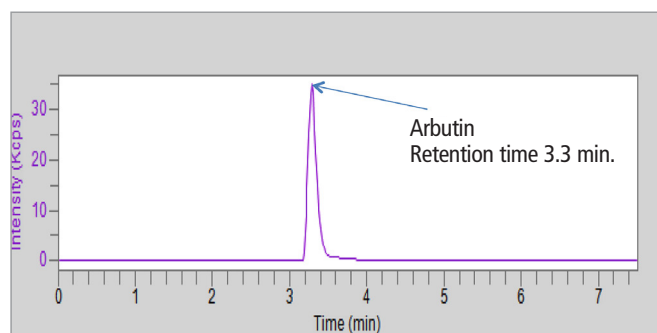


Figure 2. EIC for arbutin in a standard containing 10 $\mu\text{g/ml}$ arbutin with LC/TOF.

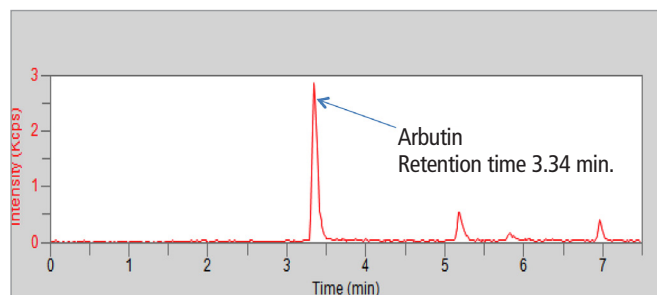


Figure 3. EIC for arbutin in pear juice, diluted in water by a factor of 5 with LC/TOF.

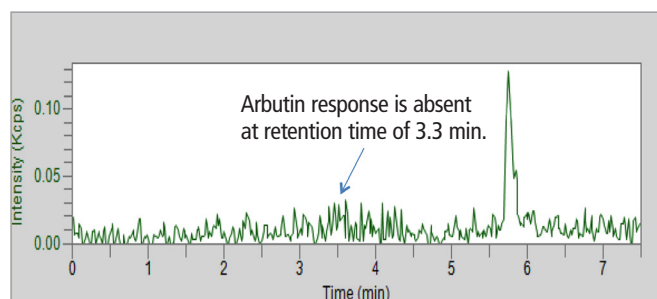


Figure 4. EIC for arbutin in apple juice, diluted in water by a factor of 5 with LC/TOF.

of 5. Based on the response for arbutin in diluted apple juice with 10 % pear juice relative to its response in diluted apple juice spiked with 10 µg/ml arbutin and after accounting for dilution factor, the amount of arbutin in pear juice was estimated to be about 66.3 µg/ml. In the references¹⁻⁴, it has been reported that the amount of arbutin in pear juice is in the range of 40.5-151.1 µg/ml with the mean value of 72.4 µg/ml. Therefore, the amount of arbutin measured in pear juice in this work with LC/TOF is consistent with the value for arbutin in pear juice as reported before in the literature.

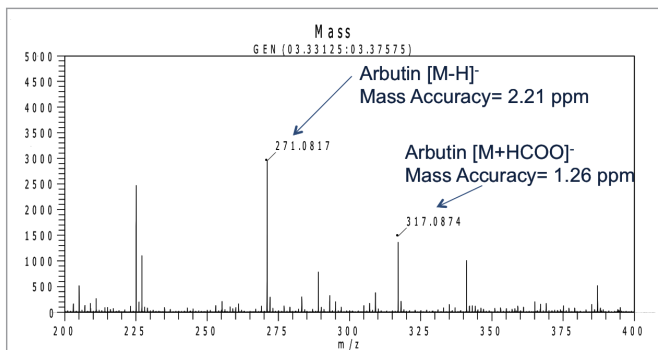


Figure 5. Mass spectrum of arbutin in pear juice, diluted by a factor of 5 in water with LC/TOF.

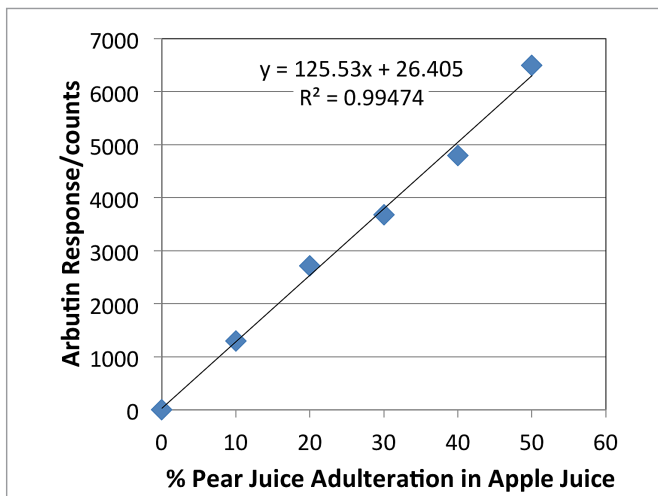


Figure 6. Arbutin response as a function of percentage of pear juice adulteration in apple juice.

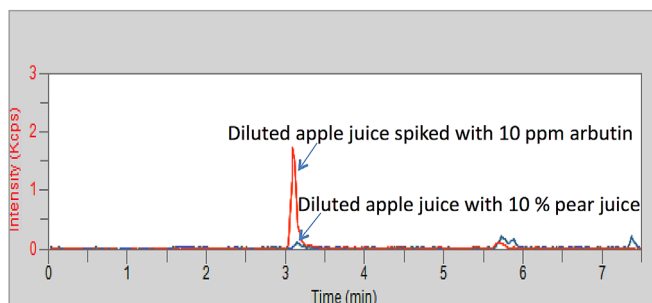


Figure 7. Overlay of EIC for arbutin in diluted apple juice spiked with 10 µg/ml arbutin and diluted apple juice with 10 % pear juice.

Conclusion

A dilute-and-shoot, rapid 7.5 minute LC/TOF method was developed for detecting 10 % or higher pear juice adulteration in apple juice. LC/TOF measurements showed the presence and absence of arbutin in pear and apple juice, respectively. The presence of arbutin in pear juice was confirmed unambiguously by retention time matching with arbutin standard and better than 3 ppm mass accuracy of arbutin's deprotonated and formate adduct ion. The arbutin response increased linearly as the amount of pear juice used to adulterate apple juice increased. This confirmed that arbutin can be used as a marker compound to detect the adulteration of apple juice in pear juice. The amount of arbutin determined in pear juice was 66.3 µg/ml.

References

1. L.Z. Lin, J.M. Harnly, Phenolic compounds and chromatographic profiles of pear skins, *J. Agric. Food Chem.*, 56, 9094-9101 (2008).
2. G.A. Spanos, R.E. Wrolstad, Influence of variety, maturity, processing, and storage on the phenolic composition of pear juice, *J. Agric. Food Chem.*, 38, 817-824 (1990).
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