



APPLICATION NOTE

Mass Spectrometry

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Rapid Screening of Adulteration in Pomegranate Juice with Apple Juice Using DSA/TOF with Minimal Sample Preparation

Introduction

Pomegranate juice is in high demand due to its well documented health benefits. Studies based on a significant body of scientific research conducted on authentic pomegranate juice have established that pomegranate juice has superior antioxidant activity compared to other popular fruit juices such as grape, orange and apple^{1,2,3}. These superior health benefits have led to a meteoric rise in the popularity of pomegranate juice over the last 10 years. This has resulted in pomegranate juice having a significantly higher cost

than other juices, especially in comparison to apple, orange and grape juice. This high cost has provided an economic incentive for the adulteration of pomegranate juice⁴. There are a number of other reasons for adulteration including: customers demanding their suppliers reduce costs; there comes a point when a supplier may adulterate the product to lower the cost and maintain a workable margin. Another reason for pomegranate juice adulteration is that it is in high demand and short supply, leading to incentives to extend limited supplies by the addition of other cheaper fruit juices.

The adulteration of pomegranate juice is accomplished by using cheap ingredients. One of the common adulterants of pomegranate juice is a cheaper so called filler fruit juice such as apple juice. The advantage of adulterating pomegranate juice with apple juice is that it is clear and provides no noticeable change to the sugar content. Organic acid analysis plays a fundamental role in testing the authenticity of pomegranate juice. One organic acid of interest is malic acid. Although it is present in both pomegranate and apple juice, the amount in apple juice is significantly higher than in pomegranate juice, 4.5 g/L and 0.57 g/L respectively^{5,6}. Therefore, the adulteration of pomegranate juice with apple juice can be detected by the elevated levels of malic acid present in the juice. Analytical methods used routinely for

for organic acid analysis in fruit juices are based on liquid chromatography (reverse phase or ion exchange) coupled to UV detection⁷ or mass spectrometry^{8,9}. These measurement techniques are either expensive or time consuming or both, and require extensive method development and sample preparation. In this work, we demonstrated that the AxION[®] Direct Sample Analysis[™] (DSA[™]) system integrated with the AxION 2 time-of-Flight (TOF) mass spectrometer, with stable isotope dilution, can be used for rapid measurement of adulteration in pomegranate juice with apple juice with minimal sample preparation.

Experimental

Pomegranate and apple juices were purchased from a local supermarket. Both juices were diluted by a factor of 200 in water and spiked with an internal standard of deuterated malic acid (malic acid-d₃) at a concentration of 0.005 g/L. The juices were mixed in different proportions to simulate the adulteration of pomegranate juice with grape juice at levels of 5, 10, 20, 30 and 50 percent apple juice. In this analysis, the deuterated internal standard of malic acid was added to quantitate the amount of malic acid in both juices (pomegranate and apple) and their mixtures. 10 µl of each sample was pipetted directly onto the stainless mesh of the AxION DSA system and the position of this was optimized, prior to ionization and analysis.

The DSA/TOF experimental parameters were as follows:

Corona current of 5 µA, heater temperature of 300 °C, auxiliary gas (N₂) pressure of 80 psi, drying gas (N₂) flow of 3 l/min and drying gas (N₂) temperature of 25 °C. The AxION 2 TOF MS was run in negative ionization mode with a flight voltage of 8000 V. The capillary exit voltage was set to -80 V for the analysis. Mass spectra were acquired in a range of m/z 50-700 at an acquisition rate of 5 spectra/s. All samples were analyzed within 15 sec. To obtain higher mass accuracy, the AxION 2 TOF instrument was calibrated before each analysis by infusing a calibrant solution into the DSA source at 10 µl/min.

Results

The diluted fruit juice samples of pomegranate and apple and their mixtures spiked with an internal standard (malic acid-d₃) were directly analyzed by DSA/TOF with no further sample preparation. Figure 1 and Figure 2 show the mass spectra for pomegranate and apple juice diluted by a factor of 200 respectively, and spiked with an internal standard (malic acid-d₃) at a level of 0.005 g/L in negative ion mode using DSA/TOF.

The data in Figure 1 and Figure 2 shows that the response ratio for malic acid with respect to malic acid-d₃ in pomegranate and apple juice was 0.575 and 3.453, respectively. In this analysis, we have used the stable isotope labeled analog of the analyte (malic acid) as an internal standard, which has identical chemical and structural properties to that of the analyte¹⁰. Therefore, it can be assumed that both the analyte and its internal standard (malic acid-d₃) will have similar ionization efficiencies. Based on

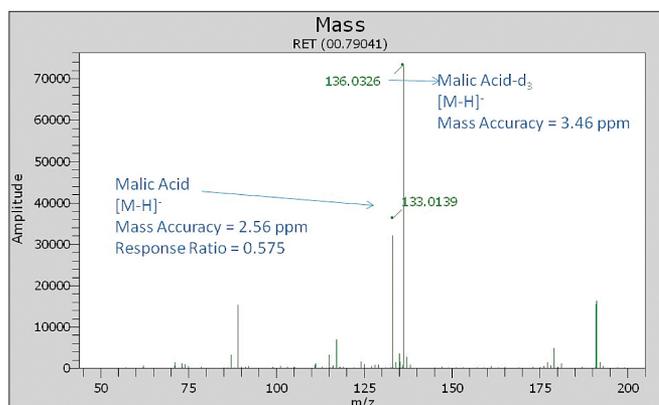


Figure 1. Mass spectra of pomegranate juice diluted by a factor of 200 and spiked with 0.005 g/L of internal standard.

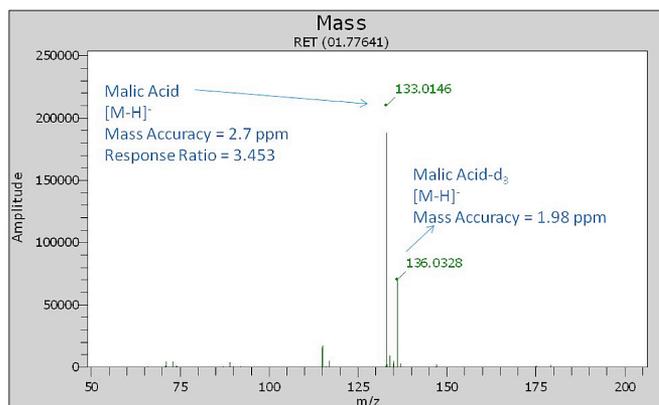


Figure 2. Mass spectra of apple juice diluted by a factor of 200 and spiked with 0.005 g/L of internal standard.

Type of Juice	Malic Acid Reported in Literature	Malic Acid Measured by DSA/TOF
Pomegranate	0.53 g/L ⁵	0.575 g/L
Apple Juice	4.5 g/L ⁶	3.453 g/L

Table 1. Measured versus literature values for amount of malic acid in pomegranate and apple juice.

this assumption and after accounting for the dilution, the amount of malic acid in pomegranate and apple juice was determined to be 0.575 and 3.453 g/L, respectively. The relative standard deviations of these measurements were 2% with and 20% without the internal standard. This demonstrates that the addition of an internal standard improves the accuracy of the measurement with DSA/TOF. Table 1 shows that there is a good agreement between the values for malic acid measured by DSA/TOF and those reported in the literature for pomegranate and apple juice. Figure 3 shows that the amount of malic acid in pomegranate juice increases linearly with the increase in apple juice adulteration with an excellent correlation coefficient value of R² equal to 0.9997. Using DSA/TOF, we can detect adulteration of pomegranate juice with 5% or higher of apple juice. Table 2 shows good correlation between the expected and measured values for malic acid in pomegranate juice adulterated with different levels of apple juice.

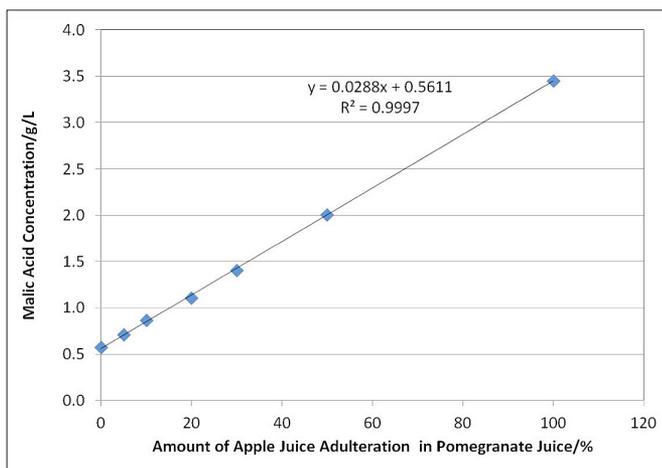


Figure 3. Malic acid concentration as a function of amount of adulteration of apple juice in pomegranate juice.

Apple Juice % in Pomegranate Juice	Expected Amount of Malic Acid	Measured Amount of Malic Acid	RSD
5	0.719 g/L	0.711 g/L	1.45%
10	0.863 g/L	0.865 g/L	2.12%
20	1.151 g/L	1.110 g/L	0.99%
30	1.438 g/L	1.408 g/L	1.80%
50	2.013 g/L	2.008 g/L	1.69%

Table 2. Expected versus measured amount of malic acid in pomegranate juice adulterated with different levels of apple juice.

Conclusion

This study shows the first work for the rapid measurement of adulteration of pomegranate juice with apple juice using DSA/TOF by quantitating the amount of malic acid present. The data showed that the level of malic acid in pomegranate juice increased linearly with the increase in apple juice adulteration.

The best internal standard is an isotopically labeled version of the molecule you want to quantify because an isotopically labeled internal standard will compensate for possible variations in sample preparation, sample injection and ionization efficiency in different matrices and improve accuracy of quantitation. This was demonstrated in this experiment, as the average relative standard deviation of the measurements without internal standard was 10 times higher in comparison to measurements done with internal standard. The average relative standard deviation of all measurements with DSA/TOF with stable isotope dilution was less than 2%. The mass accuracy of all measurements was less than 5 ppm with external calibration. All samples were screened, with minimal sample preparation, in 15 sec. per sample. In comparison to other established techniques such as LC/MS and LC/UV, DSA/TOF will improve laboratory productivity and decrease costs and analysis time.

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