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Qualitative and Quantitative Analyses of Water-Soluble Vitamins and Flavonoids in Pomegranate Aril Juice, Skin, and Commercially Available Fruit Juice Using the ACQUITY UPLC H-Class with PDA Detector

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APPLICATION BENEFITS

- Ease-of-use and familiar operation of the ACQUITY UPLC® H-Class System compared to a traditional HPLC system.
- The ACQUITY UPLC H-Class SmartStart Technology automatically manages gradient start time and pre-injection steps in parallel. By overlapping these typically serial processes, cycle time is minimized and this helps to develop shorter run times.
- These studies suggest pomegranate skin extracts have both antioxidant properties and may be exploited as biopreservatives in food applications and as nutraceuticals.

WATERS SOLUTIONS

ACQUITY UPLC H-CLASS System

ACQUITY UPLC HSS T3 Column

ACQUITY UPLC PDA Detector

KEY WORDS

Vitamins, flavonoids, fruit juice, UPLC, UV

INTRODUCTION

The pomegranate (*Punica granatum L.*; *inhmn* in ancient Egyptian) grows wild in southwest Asia and is cultivated in Mediterranean countries. It is a deciduous shrub or tree with scarlet, scented flowers, and later, a hard yellowish to reddish fruit which contains bright-red seeds.

At least 14 different varieties of pomegranate are cultivated and available in different parts of the Indian Subcontinent, some of which include Arakta, Dholka, G 137, Ganesh, Jyoti, Kabul, Kandhari, Mrudula, Muskat, P23, P26, and Ruby.[1]

It has been reported that the vitamin content for this fruit is "simply remarkable"[2] and hence it tops in the list of fruits high in vitamins.[2]

In this application note, a comparative and quantitative study of water-soluble vitamins like ascorbic acid, thiamine, riboflavin, cyanocobalamin, and quercetin flavonoid was carried out in aril juice, skin, and commercially available fruit juice of pomegranate. The ACQUITY UPLC H-Class System with PDA Detector was used to perform the method development, and the rapid routine analyses thereafter.

Compound of interest	Class	Health benefits
Ascorbic acid (Vitamin C)	Vitamins	Daily intake of Vitamin C can help to prevent wrinkling of skin; strengthens immune system.
Thiamine (Vitamin B_1)	Vitamins	Required for normal functioning of the body, ensure proper metabolic rate in the body, as well as promote cell growth.
Riboflavin (Vitamin B_2)	Vitamins	Required for normal functioning of the body, ensure proper metabolic rate in the body, as well as promote cell growth.
Cyanocobalamin (Vitamin B_{12})	Vitamins	Important for the normal functioning of the brain and nervous system and for the formation of blood.
Quercetin	Flavonoids	Effective in preventing cancer .

Table 1. Health benefits of various vitamins and flavonoids.[3]

EXPERIMENTAL

Sample preparation

A commercially available juice and freshly-squeezed fruit juice were centrifuged at 13,000 rpm for 10 minutes, and the supernatant filtered using a 0.22 µm filter. The resulting solution was used for direct injection.

The pomegranate skin was removed, sun dried, and powdered. The powdered skin was then weighed and soaked to about five to seven times the amount of the weighed powdered skin in methanol for 48 hours. This solution was filtered through Whatman filter paper no. 41, centrifuged at 13, 000 rpm for 10 minutes, and the supernatant filtered using a 0.22 µm filter was used for direct injection.

UPLC conditions

UPLC system:	ACQUITY UPLC H-CLASS
Column:	ACQUITY UPLC HSS T3 2.1 x 100 mm, 1.8 µm
Mobile phase A:	0.1% Formic acid in water
Mobile phase B:	0.1% Formic acid in methanol
Flow rate:	0.4 mL/min
Column temp.:	35 °C
Sample temp.:	4 °C
Needle wash:	5:1:1 ACN/Isopropanol/ Methanol (500 µL)
Cycle time:	12 min
Injection volume:	2 µL
Syringe draw rate:	Standards – automatic sample – 10 µL/min

Gradient:

Time (min)	%A	%B	Curves
0.00	98	02	Initial
0.99	95	05	6
2.39	80	20	6
4.89	45	55	6
4.99	02	98	6
6.89	98	02	6
12.00	98	02	6

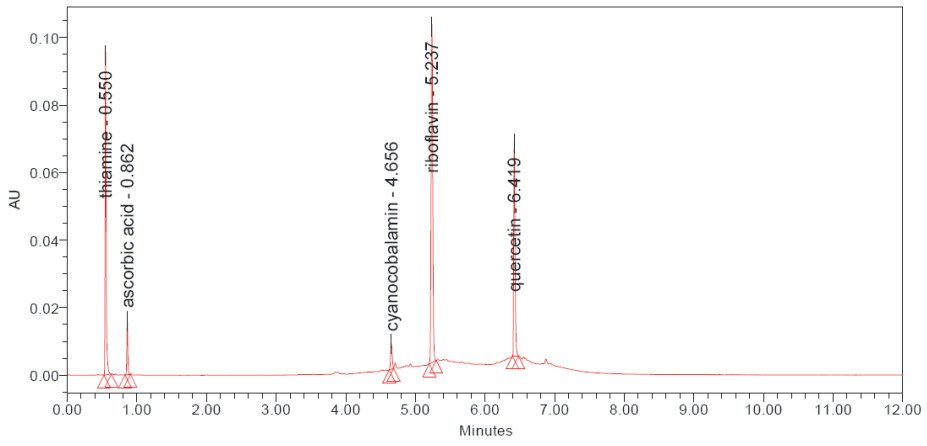
Detector:	ACQUITY UPLC PDA
Wavelength range:	210 to 400 nm

Extraction

Wavelengths:	270 and 360 nm
Resolution:	1.2 nm
Filter response:	0.1 s
Sampling rate:	20 points/s
Exposure time:	Auto

RESULTS AND DISCUSSION

All the data were acquired between 210 to 400 nm wavelength range and processed at wavelength 270 nm (360 nm for cyanocobalamin).



Name	Area	Height	USP tailing	USP resolution
Thiamine	92,271	95,566	1.54	
Ascorbic acid	15,338	16,030	1.13	12.63
Cyanocobalamin	11,308	7,483	1.05	114.76
Riboflavin	165,289	100,267	1.10	13.68
Quercetin	83,282	63,289	1.55	29.46

Figure 1. Reference mix of thiamine, ascorbic acid, cyanocobalamin, riboflavin, and quercetin standards.

Using the UPLC® Technology, a gradient method was developed to obtain chromatographic resolution for the standards used for these analyses. The ACQUITY UPLC H-Class System delivers high resolution chromatography, enabling much sharper peaks as a result.

The spectrum of each standard was recorded using the ACQUITY UPLC PDA Detector. A reference mix of ascorbic acid, thiamine, riboflavin, cyanocobalamin, and quercetin standards were made and injected to check the reproducibility.

Based on the qualitative analysis, it was observed that only two components – ascorbic acid and quercetin were present in the skin and commercially available pomegranate fruit juice.

Compound name	Content (pomegranate fruit)*		Linearity range (in ppm)
	mg/mL	ppm	
Ascorbic acid	0.102	102	12.5,25.0,50.0,100.0,200.0,400.0
Quercetin	0.002-0.01	2-10	1.0,2.0,4.0,8.0, 16.0,32.0
Riboflavin	165,289	100,267	1.10
Quercetin	83,282	63,289	1.55

**(Based on U,S, FDA Nutritional Database 10, 2010).*

Table 2. Concentration of standards used for linearity graph.

For quantitative analysis, linearity was prepared based on a literature survey based on the expected concentrations of ascorbic acid[4] and quercetin.[5]

To quantity the compounds ascorbic acid and quercetin identified to be present in the analytical samples, a linearity plot was prepared.

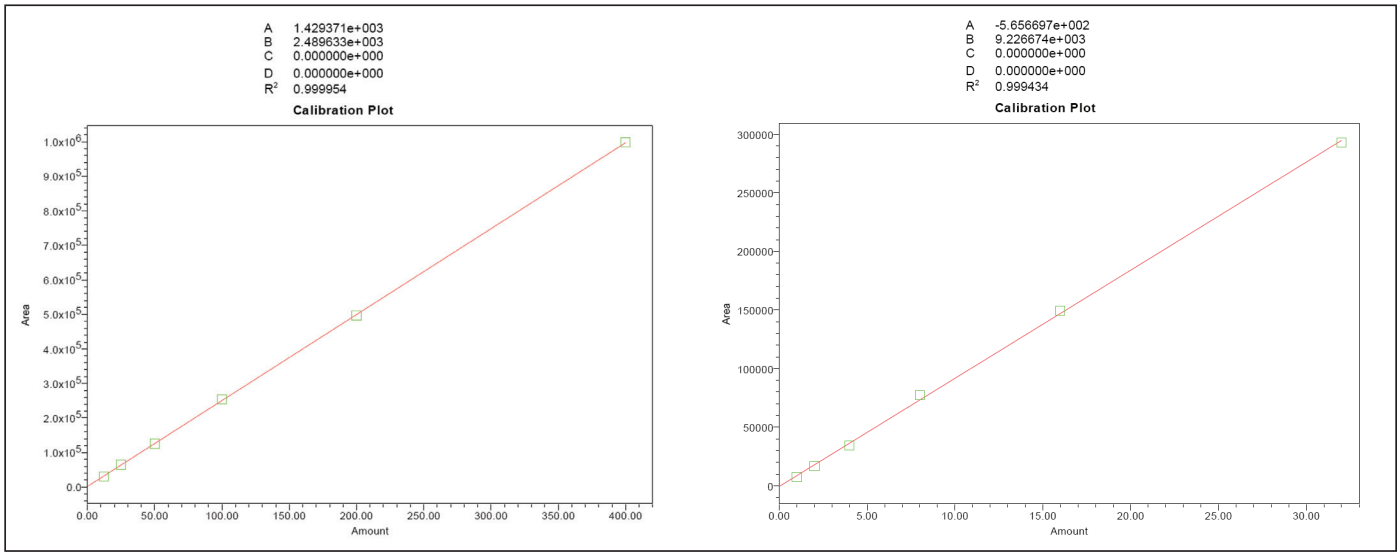


Figure 2. Calibration curves of the two major compounds ascorbic acid and quercetin found in this study.

Using these linearity graphs, the unknown amount in the samples were quantified in the three different sample types and the results can be shown in Table 3.

Sample type	Thiamine	Ascorbic acid	Cyanocobalamin	Riboflavin	Quercetin
Commercially available fruit juice	–	103.53 µg/mL	–	–	0.510 µg/mL
Fruit skin	–	58.23 µg/mL	–	–	16.58 µg/mL
Freshly squeezed juice (from fruit)	–	–	–	–	–

Table 3. Amount of vitamins and polyphenols found in three samples.

Tetra-packed juice

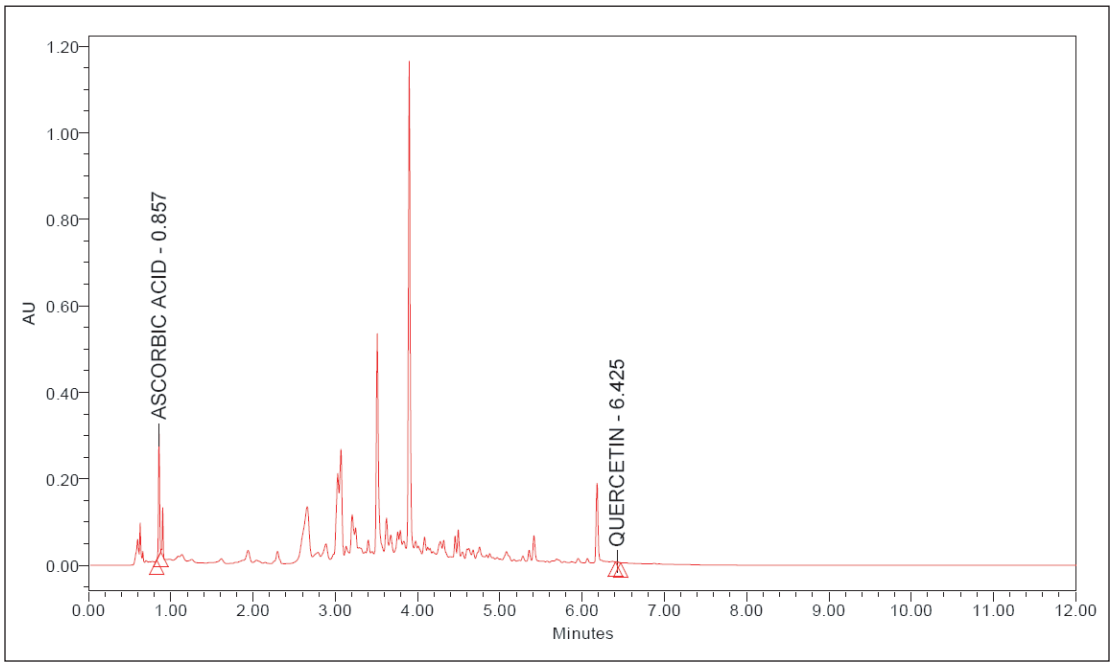


Figure 3. Chromatogram of tetra-packed juice.

Pomegranate skin

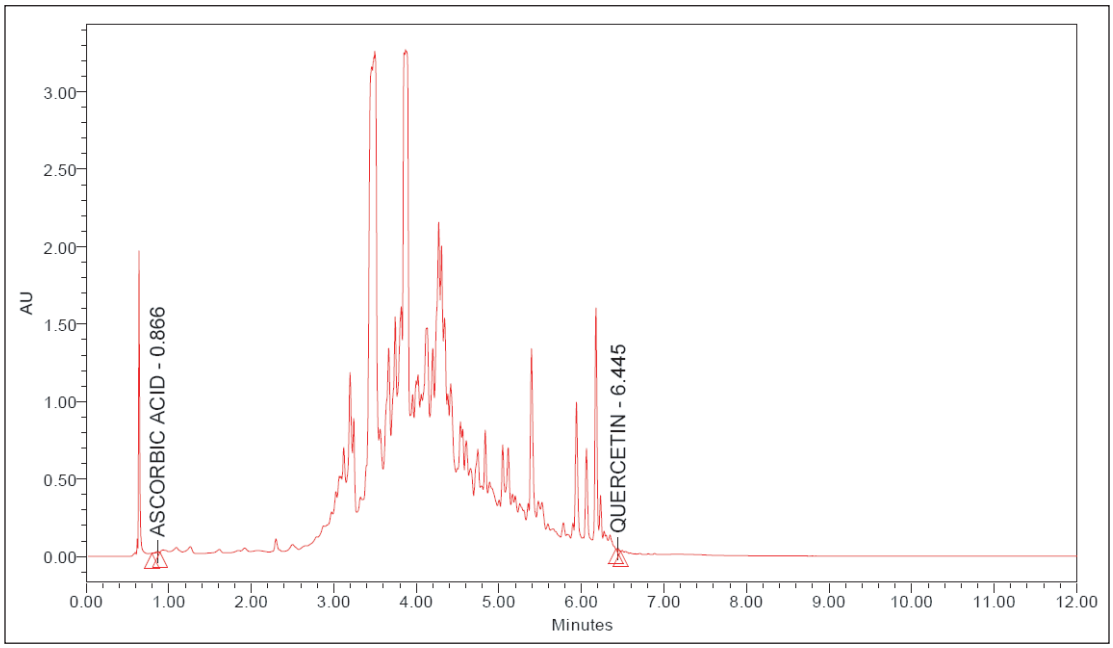


Figure 4. Chromatogram of pomegranate skin.

The amount of ascorbic acid and quercertin present in the pomegranate skin were 58.23 µg/mL and 16.58 µg/mL, respectively. The other compounds – thiamine, cyanocobalamin, and riboflavin were not detected in the sample using this methodology.

Freshly-squeezed fruit juice

It is observed that all the compounds thiamine, ascorbic acid, cyanocobalamin, riboflavin, and quercetin were absent in freshly-squeezed fruit juice – an example chromatogram is shown Figure 5. It was observed that ascorbic acid and quercetinwere present in the tetra-packed juice and pomegranate skin.

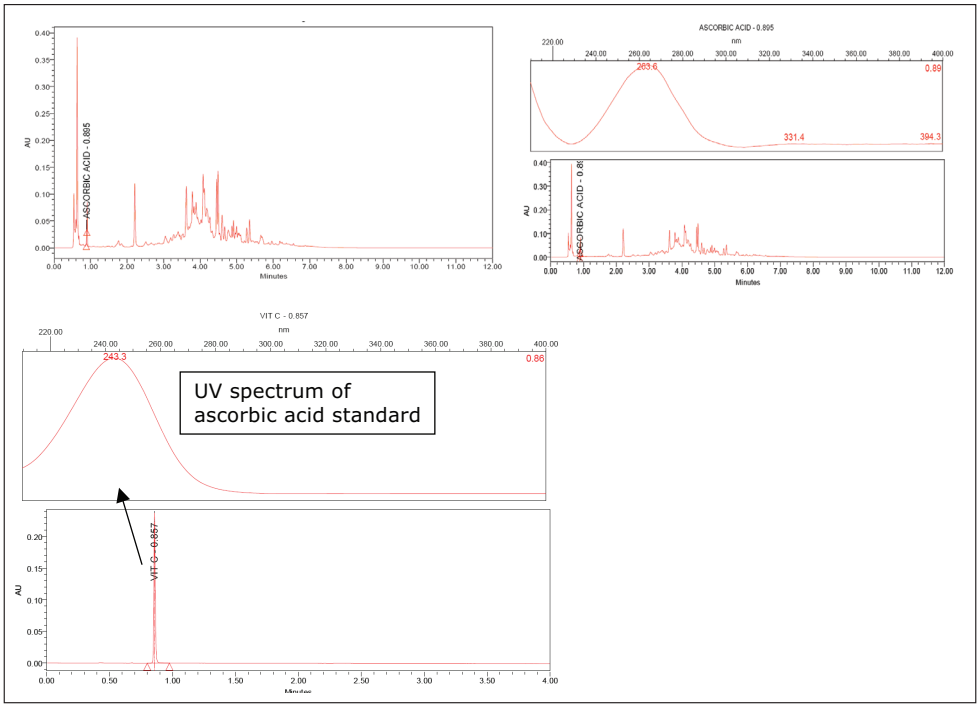


Figure 5. Chromatogram and UV spectrum of a peak eluting at the predicted retention time of ascorbic acid in pomegranate juice and UV spectrum of ascorbic acid standard. The UV spectrum shown confirms that this peak is not ascorbic acid.

The chromatogram of the freshly squeezed fruit juice, shown in Figure 5, exhibits a peak at the retention time of ascorbic acid. It is possible to utilize all of the wavelength data that has been collected by the PDA detector in order to confirm whether the peak highlighted is ascorbic acid. Upon review of the spectrum from the juice, it was apparent that it did not match the spectrum of the ascorbic acid standard. It was therefore concluded that this sample did not contain ascorbic acid.

The comparative study between freshly-squeezed fruit juice and commercially available pomegranate juice enriched with 10 mg ascorbic acid/100 mL juice revealed that the variation observed in ascorbic acid between the two samples. This study also indicates that fresh fruit is not always rich in essential nutrients such as vitamins and flavoinoids. They are likely to have natural variations (*e.g.* as a consequence of harvest time or season), or they may have been enriched in order to increase their nutritional value.

The quantification results obtained showed:

- The aril juice was not identified to contain ascorbic acid or quercetin inherently. But the skin contained ascorbic acid, as well as quercetin.
- The tetra-packed fruit juice contained both ascorbic acid and quercetin.

This was in agreement with the label claim from the tetra-packed manufacturers that the juice is enriched with ascorbic acid. But the quercetin content in the juice was unclaimed on the label.

CONCLUSIONS

Using UPLC Technology and the sub-2-µm particle size column chemistries, the studies carried out on the pomegranate Ganesh variety suggest that the pomegranate skin extracts have both antioxidant properties and may be exploited as biopreservatives in food applications and as nutraceuticals.

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