Determination of Carboxylic Acids in Apple Juice by RP HPLC

A.Hakan Aktaş*[+]***; Songül Şen, Mustafa Yılmazer and Ebru Cubuk***

Department of Chemistry, Art & Science Faculty, Suleyman Demirel University, 32260 Isparta, TURKEY

ABSTRACT: *Low molecular weight organic acids are most predominant in apple juice among these components fumaric acid was not exceed more than 5 ppm. It was thought that the HPLC is the best method of the determination of organic acids in the apple juice. In this study, chromatographic separation of organic acids of apple juice was obtained by preparing a sample and by applying them to acid phase for extraction and various organic acids content of apple juice, fumaric acid, oxalic acid, tartaric acid and shikimic acids were commented as qualitative and quantitative, too.*

The organic acids, of which the chromatographic separations were examined, were the acids of fumaric acid, oxalic acid, tartaric acid, ascorbic acid, lactic acid, malic acid, succinic acid and shikimic acid. For this separation, the suitable value are determined by regulating the pH of mobile phase with phosphoric acid in range of pKa ±1.5 (This is the space in which the capacity factors are effectively changed). The concentration of juice was distilled to 11.2 Brix and same pH was applied to cartridge by adjusting with the phosphate buffer at 8.00, and the cartridge was washed with the same buffer and the phase was combined with the first eluant. A suitable distilled percentage of samples were injected and injection volume were determined. In this study, the cartridges of Supelco C_{18} and Waters C_{18} were used, datas obtained by both types of cartridges were compared. In this study, the methods of external calibration and added method of standards were applied and slopes were compared.

KEY WORDS: *Organic acids, Apple juice, RP HPLC, Selectivity factor, Separation.*

INTRODUCTION

RC HPLC is a most efficient quantitative analytical tool for determination of organic amino acids [1-12]. In liquid chromatographic separation of organic acids, generally, the solutions, whose pH have been prepared in a way that prevent ionisation of organic acids are used. The separation of organic acids with liquid chromatography and their quantitative determinations are extremely difficult because there is no clear difference between their structural similarities and spectral characteristics. Besides, pKa values of most of the organic acids are rather similar and this situation limits the usage of pH for chromatographic separation.

* *To whom correspondence should be addressed.*

+E-mail: ahakan@fef.sdu.edu.tr

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Organic acids are usually of weak acid and their capacity factors changing with pH is in sigmoidal shape. Capacity factors of molecular form are larger than those belonging to anionic forms. The interval, at which capacity factors of organic acids change efficiently, is pKa±1.5 [12-16].

During the sample analysis stage in liquid chromatography when UV determination is used, the types of different organic acids that make absorbance in the same absorbance wave length cause deteriorating effect. Because of this, by applying liquid-liquid or solid-liquid extractions, analits in the sample are separated from the deteriorating types. That's why C_{18} type of cartridge is the one used clinging extensively. As it is stated above in RP HPLC, column occurs more in an unionised form, in anionic form they cling less than cartridge. This situation makes pH extremely important in solid phase extraction.

In this study the organic acids, which were separated are fumaric acid, oxalic acid, tartaric acid, ascorbic acid, lactic acid, malic acid, succinic acid and shikimic acids (Table 1).

In liquid chromatography studies, the studies, which are conducted through careful and detailed examination and, by this way prevent the decrease in the number of column plate in experimental study, are gradually more preferred. In this study, the proper condition for separation has been determined by examining the pKa±1.5 intervals, in which capacity factors underwent a change efficiently for separation of organic acids (Table 2).

For a limit below, column's usage, pH has been taken into consideration. The pH of Mobil phase adjusted with phosphoric acid. IUPAC rules were obeyed in calibration. Also while using the solid phase extraction cartridges in preparing samples, defined cases have been taken into consideration.

Organic acids are widely distributed in fruits and vegetables. They are also used extensively as food acidulates in the manufacturing of juices. The principal acids used to en change beverage flavours are tartaric, oxalic and fumaric acids. The content of organic acids in fruit juice not only influences their flavour but also their stability, nutrition, acceptability and quality keeping. Therefore, it is important to be able to precisely determine food acids that will be used for quality control

Table 1: Formulas and private nouns of organic acids studied.

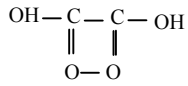
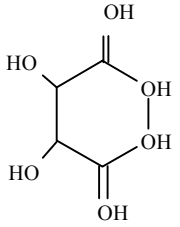
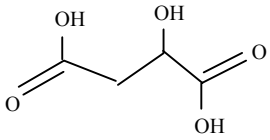
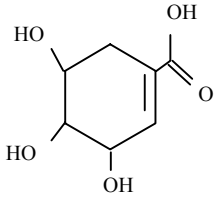
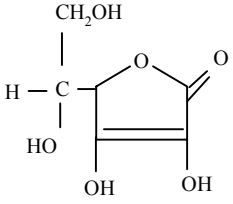
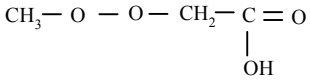
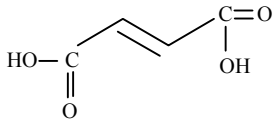
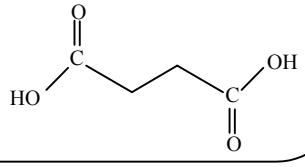
Private Nouns	Formulas
Oxalic acid	
Tartaric acid	
Malic acid	
Shikimic acid	
Ascorbic acid	
Lactic acid	
Fumaric acid	
Succinic acid	

Table 2: pKa_1 and pKa_2 values of carboxylic acids in water, whose separations were examined with RP HPLC technique (25 °C).

Organic acid	pKa_1	pKa_2
Tartaric acid	3.03[17]	4.45
Malic acid	3.40[18]	5.2
Oxalic acid	1.23[19]	4.19
Ascorbic acid	4.30	11.82
Lactic acid	3.86	—
Fumaric acid	3.03[18]	4.54
Succinic acid	4.19[19]	5.48

purposes, as well as to meet various laws and regulations and labelling purposes [20].

Apple juice contains a variety of organic acids compounds such as oxalic acid, tartaric acid, lactic acid and fumaric acids. Measurements of organic acids are useful for labelling purpose as well as for the determination of the authenticity of the juice. For example, the levels of fumaric acid in apple juice could be important indicators of microbial spoilage of juices such as fumaric acid produced by moulds [21], processing of decayed fruits, or addition of synthetic malic acid, which contains fumaric acid or a minor contaminant [22,23].

Addition of a relatively low concentration of either ionic or non-ionic surfactant to mobile phase gives a dramatic improvement in the separation of aromatic compounds on a standard C_{18} silica column [24,25]. Retention times were shorter and the peaks were sharper due to the interaction between analytes and surfactant in the mobile phase. The presence of organized micelles was doubtful because of the high acetonitrile content of the mobile phase.

The difficulty arises from low resolution among the organic acids compounds. In addition, large differences in the levels of phenolic compounds in a juice create complication to simultaneous analysis of different classes of phenolic compounds. The purpose of this study is to separate, identify and quantify common organic acids in apple juice using HPLC with photodiode array detection (DAD), which will identify compounds not only with their retention times but also with their individual spectra.

EXPERIMENTAL

Chemicals and standards

Concentrated phosphoric acid analytical-reagent grade was obtained from Fluka. Fumaric acid, oxalic acid, tartaric acid, ascorbic acid, lactic acid, malic acid, shikimic acid and succinic acid were purchased from Merck.

Samples

Fruit juices have been purchased from convenient stores and local markets. Apple juices are 100 % fresh apple juice without any of preservative and sugar.

Apparatus

The HPLC analyses were carried out using Shimadzu class LC-vp HPLC system with class SCL-10A vp software, a pump (LC-10 Advp), an auto sampler (SIL-10Advp, 70 vial Model Rack 7), diode-array detector (SPD-M 10Avp), column oven (CT0 10Avp) and gas removing unit (DGU 14A). The separation carried out on an YMC-Pack ODS-AM (250x4.6 mm I.D.).

In this study, pH of aqueous water was adjusted to 3.0 with H_3PO_4 and used as mobile phase. The pH of the HPLC mobile phase was measured by pH/ion analizier (Mettler Toledo MA 325) and combined glass electrode (Hanna HI 1332 Ag/AgCl) [26,27]. pH was measured in thermostated externally 25±0.1°C.

Chromatographic procedure

A flow rate of 1 mL/min was selected for all the chromatographic separations. The separation column was equilibrated with mobile phase until the baseline was stabilized. Sample injections were made at this point.

The dead-time, t_o, to be established for mobile phase and tested by injection of a potassium bromide solution in water monitoring the eluate at 210 nm [28]. These value changes in the experimental range of composition were studied.

Capacity factor, k, was calculated according to expression: $k=(t_r-t_o)/t_o$[29]. The system dead time, t_o, used to calculate capacity factor, k, was measured by injecting potassium bromide solution into the system. An average of at least three replicates was used to do all the calculations. Calculation of α value is the division of the k values of peaks following each other at chromatogram. For instance if tartaric acid and oxalic acid which are two

Table 3: Retention times, Capacity and selectivity factors for organic acids.

Organic acids	tr	k	α
Oxalic	5.057	0.01	
Tartaric	5.985	0.195	19.5
Malic	8.055	0.609	3.123
Shikimic	8.992	0.796	1.307
Ascorbic	9.513	0.900	1.131
Lactic	10.285	1.054	1.171
Fumaric	14.050	1.808	1.715
Succinic	18.250	2.645	1.463
KBr	$t_{o,mean}$:5.007		

Table 4: Statistical information regarding calibration curves for organic acids.

Name	Calibration function	r	S_{xo}*	CV**	Linear region,ppm	N
Oxalic acid	(204727.6±1023.11)C+(9288.9±1944.5)	0.9999	0.019	1.539	(0.040-4.358)	7
Tartaric acid	(147198±1046.2)C+(621.9±2875.8)	0.9999	0.034	1.794	0.151-5.763)	6
Malic acid	(96023.4±648.8)C+(328.7±2214.2)	0.9999	0.046	2.076	(0.111-7.287)	7
Shikimic acid	(116672.2±438.8)C+1851.7±1863.1)	0.9999	0.028	0.947	(0.267-8.988)	6
Ascorbic acid	(83988±926.8)C+(497.8±2256.9)	0.9999	0.041	2.276	(0.112-4.697)	5
Lactic acid	(111198.9±502.3)C+(2189±2365.8)	0.9999	0.037	1.129	(0.276-9.971)	6
Fumaric acid	(98964±1075.8)C+(5486.7±6347)	0.9997	0.129	3.366	(0.152-13.487)	7
Succinic acid	(46421.5±1845.4)C+(2893.3±4336.7)	0.9976	0.138	7.823	(0.253-4.465)	5

** Standard variation of method*

*** Variation coefficient of method*

substances succeeding each other is taken into consideration, k values for these are alternately 0.01 and 0.195. When the k value of tartaric acid is divided into the k value of oxalic acid, 0.195 / 0.01=19.5 is found, which the first value in Table 3 is. Also the others are found with the stated calculation.

RESULTS AND DISCUSSIONS

At the application of HPLC technique in the separation of organic acids, pH of the mobile phase and temperature are crucial parameters. The most suitable mobile phase used for separation of organic acids are aqueous water which its pH was adjusted 3.00 value with phosphoric acid, at, optimum temperature i.e. 30°C. Under these circumstances, the capacity factors (k) and selectivity factors (α) were calculated with help of retention times as presented in table 3.

H_3PO_4: water media; pH 3.00; flow rate: 0.5 mL/min.; column: YMC ODS AM;

Column temperature: 30°C; injection volume 10 μL; for organic acids λ=210 nm; for KBr λ=200 nm.

By chromatogram the concentration of various organic acid in standard mixture represented in Fig. 1, there was oxalic acid of 83.3 ppm, tartaric acid of 416.7 ppm, malic acid of 833.3 ppm, shikimic acid of 16.67 ppm, ascorbic acid of 75 ppm, lactic acid of 2083.3 ppm, fumaric acid of 8.3 ppm and succinic acid of 1750 ppm in the standard mixture. For the standards, calibration curves drew in the mixture, and coefficient of correlation was determined as > 0.999 and it was seen adequate (Table 4).

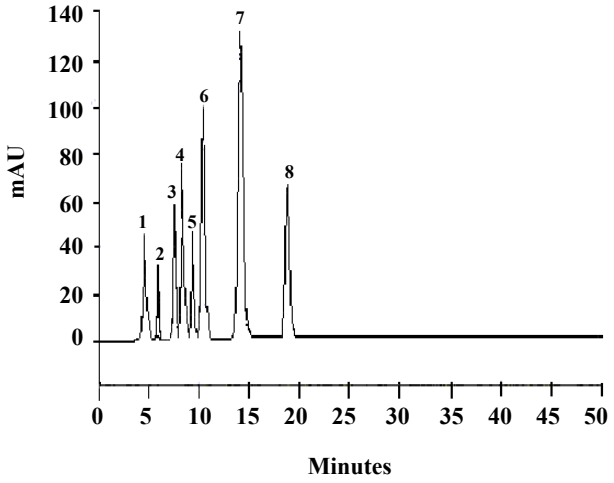


Fig.1: Chromatogram of standard organic acid mixture in YMC-Pack ODS-AM column (5 μm, 250 x4.6 mm I.D.). Peak identification: 1= oxalic acid, 2= tartaric acid, 3= malic acid, 4= shikimic acid, 5=ascorbic acid, 6= lactic acid, 7= fumaric acid, 8= succinic acid .

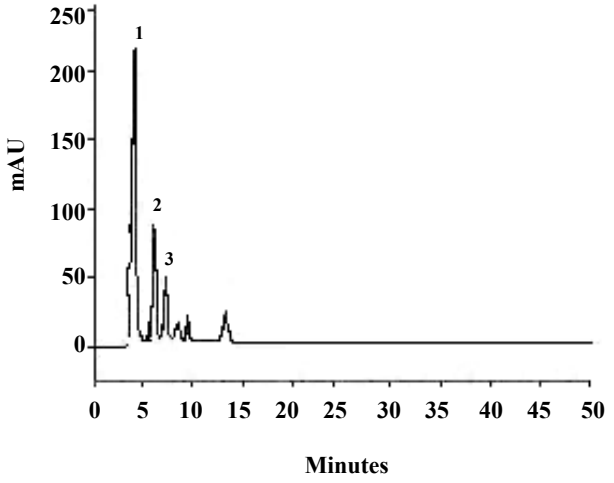


Fig. 2: Chromatographic separation of apple juice extracted by Supelco cartridge in YMC-Pack ODS-AM (5 μm, 250 x4.6 mm I.D.) Peak identification: 1= oxalic acid, 2= tartaric acid, 3= shikimic acid .

Table 5. Back gain and quantity of organic acids in samples.

Organic acids	Back gain %	Quantity in sample, ppm
Oxalic acid	110	(0.77 ± 0.07)
Tartaric acid	82	(0.83 ± 0.06)
Shikimic acid	76	(55.68 ± 4.3)
Fumaric acid	105	(4.10 ± 0.3)

The sample of the apple juice that was purchased from local market, the cartridges of that were prepare as solid phase, and oxalic acid, tartaric acid and shikimic acids were determined in this sample (Fig. 2).

It was observed that chromatographic separation in an apple juice, which was purchased from local market and extracted with Supelco C_{18} of cartridge, was much better than extraction position, done by water cartridge. In the extraction of concentration of an apple juice of Isparta region, it was determined that the cartridge of Supelco C_{18} is much better for the chromatographic separation of fumaric acid. From chromatographic seperation of apple juice one can observe that, in both samples which applied cartridge of Supelco C_{18}, the peak diffusions could not be separated completely.

By considering contribution from the organic acids, which have been detected to be present in samples and peaks which have been verified, by making use of sample quantities via peak field and added quantities, regaining have been calculated (Table 5). By evaluating these results, these methods are proved to be adequate in determination of organic acids in apple juice and that they can be used routinely.

CONCLUSIONS

A simple method was developed for determination of organic acids in apple juices by HPLC with photodiode array detector. The established method was successfully used to measure a variety of organic acids in fruit juices. This method could also be used to evaluate the authenticity, nutrient contents of juices.

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