HPLC Determination of the Main Organic Acids in Teeth Bleaching Gels Prepared with the Natural Fruit Juices

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An efficient high performance liquid chromatographic (HPLC) method was developed and validated for fast and simultaneous determination of oxalic, citric, tartaric, malic and succinic acids in the teeth bleaching gels prepared with natural fruit juices and to establish the organic acids profile of natural fruit juices. Organic acids were separated through a Carbosep Coregel 87H3 column at 35°C using ultraviolet (UV) absorbance detector at 214 nm. The mobile phase was the sulphuric acid 0.005 M solution at a flow rate of 1 mL min^{-1}. Validation of the method was performed to verify the linearity, limits of detection and quantification, intra- and inter-day precision and accuracy. The method showed excellent linearity (R^2>0.9993) for target compounds. Limits of detection (LOD) were between 0.15 and 2.47 µg mL^{-1} and quantification (LOQ) were between 0.49 and 8.15 µg mL^{-1}. The accuracy at three concentration levels ranged between 96 and 102 %. The all relative standard deviations (RSD) values of the precision and accuracy were lower than 4%. Finally, the proposed methodology was successfully applied to the analysis of organic acids in sixteen teeth bleaching gels samples and to find the organic acids profile of eleven natural fruit juices used for preparing teeth bleaching gels.

Keywords: organic acids, HPLC-UV, method validation, teeth bleaching gel, fruit juice

Many people are concerned about their tooth when appear discoloration. The importance of tooth whitening for patients and consumers has seen a dramatic rise in the number of tooth whitening products and procedures. At the same time, there has been a rapid increase of published *in vivo* and *in vitro* tooth whitening studies. Optimization of the factors controlling the tooth whitening process will continue to expand. This will give further improvements to the tooth whitening products and procedures, and give significant benefits to the field of aesthetic dentistry [1]. Searching for natural remedies without the use of chemicals is of interest to the field. Thus, the fruits like strawberries, containing natural organic acids can be used in this purpose. The malic acid from fruits is considered natural teeth whitening agent. The lemon juices and the juice of apples, especially green apples, also contains malic acid but it should be used sparingly because can easily damage the teeth enamel [2].

Organic acids are naturally found in fruits and vegetables. The nature and concentration of the organic acids in the fruits presents the influence on the organoleptic properties and stability of fruit juices. The organic acid profile and concentration in fruits and vegetables depends on factors such as species, soil and stress conditions to which the fruit was submitted. The organic acid composition from fruits has an impact on the sensory properties. The most common organic acids present in fruits and juices are citric, tartaric and malic acids as acidulants and ascorbic acid as an antioxidant [3]. The citrus fruits contain as main the citric and malic acids and the trace amounts of tartaric, benzoic, oxalic and succinic acids [4, 5].

Several analytical techniques have been used to determine organic acids in fruit juices as non-enzymatic spectrophotometric, enzymatic, chromatographic and electrophoretic methods [6]. Capillary electrophoresis technique has a lot of advantages like high resolution, simplicity and automation, short analysis times, low consumption of reagents and samples, and minimum preparation of sample even in complex matrices [7-9]. High performance liquid chromatography (HPLC) is one of the more promising and more used techniques for simultaneous analysis of the organic acids from fruits (apricot, mulberry, blackberry), fruit juices (orange, grapefruit, tangerine, kiwi, pineapples, blackcurrant, redcurrant, sweet cherry) and vegetables (green and red peppers, tomato, lettuce.). Most of HPLC procedures used reverse phase partition chromatography [10-17] or ion exchange chromatography [18-22] with UV, photo-diode array and mass spectrometry detectors. Recently, the link between HPLC and isotope ratio mass spectrometry (IRMS) was achieved through an interface insuring a chemical oxidation (co). HPLC-co-IRMS link is a powerful tool for food authentication as a system that combines HPLC separation of product constituents and their determination with carbon 13 isotope ratios. This technique was applied to lemon juices to determine organic acids and sugars authenticity [23]. Gas chromatography technique with flame ionization detector was used for determination of organic acids as their trimethylsilyl derivatives from alpine bearberry [24].Also, visible and near infrared spectroscopy technique was used for rapid determination of citric and tartaric acids in orange juices [25]. The near-infrared transmittance spectroscopy was used to predict titratable acidity, malic and citric acids in bayberry fruit [26] and Fourier transform mid-infrared spectroscopy was developed for simultaneously determining sugar and organic acid contents in apricot fruit slurries using the attenuated total reflectance [27].

The aim of the study was to develop and validate a HPLC method for the simultaneous determination of organic acids from the teeth bleaching gels prepared with natural juices and to establish the organic acids profile of fruit juices used. The procedure was applied to analysis of five organic acids oxalic, citric, tartaric, malic and succinic, in sixteen samples of teeth bleaching gels and seven fruit juices samples. Given the above, we used for the first time the natural organic acids from fruit juices in the composition of the teeth bleaching gels. The natural organic acids were

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used as active agent with action in tooth discoloration and stain removal. It was determined the total quantity of organic acids of these gel samples that are an important parameter for establish the whitening degree of teeth.

Experimental part

Materials and methods

Chemicals

All reagents were of analytical grade. Tartaric, citric and succinic acids were purchased from Merck (Darmstadt, Germany), oxalic acid was purchased from Chimopar (Bucharest, Romania) and malic acid was purchased from Polynt (Italy). Analytical grade water was obtained from Milli-Q Ultrapure water purification system (Millipore, USA). The sulphuric acid was obtained from Merck (Darmstadt, Germany).

Sample preparation

Some fruits were use from this study: strawberries, quinces, grapes (red and white), pears, dogwood, sea-buckthorn. The fruits were purchased from local stores and were selected on the basis of a similar degree of ripeness and apparent fruit quality (size, colour and absence of physical damages). The organic acids were analyzed from the whole edible part of fruit. The fruits were crushed and homogenized with a blender. Mashed (10 g) fruit was dissolved with 50 mL of twice distilled water for 30 min at room temperature. The organic acids are water soluble materials. The extracted sample was centrifuged (Eppendorf 5804 R centrifuge, Hamburg, Germany) at 4500 rot min^{-1} for 10 min at 20°C. The supernatant juice was partially decolorized by addition of animal charcoal and heated at a temperature of 40°C and then is vacuum filtration through a 0.45μm nylon membrane filter (Teknokroma) and used for HPLC analyses.

Teeth bleaching gels samples were prepared with natural fruit juices, individual or in mixture, and concentrated using freeze-drying method together with other components of the studied gels. The extraction of organic acids from teeth bleaching gels samples was made by dissolved in 10 mL water of 1 g of gel samples. The obtained solution was ultrasonication for 15 min and then filtered under vacuum on a 0.45 mm filter and injected into the HPLC.

Equipment and method

The analyses were carried out on a Jasco Chromatograph (Japan) equipped with an intelligent HPLC pump (PU-980), a ternary gradient unit (LG-980-02), an intelligent column thermostat (CO-2060 Plus), an intelligent UV/VIS detector (UV-975) and an injection valve equipped with a 20mL sample loop (Rheodyne). The system was controlled and the experimental data analyzed were performed with the ChromPass software. Separation was carried out on a Carbosep Coregel 87H3 column (300 x 7.8 mm), Carbosep 87H guard column and Carbosep coregel 87H Cartridge at 35°C column temperature. Mobile phase was the sulphuric acid 0.005M solution. The flow rate was 1 mL min^{-1} and UV detection was 214 nm.

Validation parameters

A validated analytical method was developed for the organic acids in teeth bleaching gels samples, considering the parameters described by ICH [28]. The validation parameters consisted at selectivity, linearity range, precision, accuracy and limits of detection and quantification.

Results and discussions

Optimization of the chromatographic conditions

Preliminary experiments were carried out in order to optimize the chromatographic conditions (i.e. concentration and flow rate of mobile phase, temperature of column) in terms of peak shape, chromatographic analysis time and resolution. The resolution of investigated compounds was tested at a concentration of mobile phase of 0.005 M, 0.025 M, and 0.05 M sulphuric acid solutions. The results show that there are no significant differences, thus the mobile phase was set to a 0.05 M sulphuric acid solution. Different isocratic flow rates and column temperatures were also checked. Decreasing flow rate from 1.0 to 0.6 mL min^{-1} resulted in time analysis amounting to 16 min. The used polymeric column Carbosep Coregel 87H3 is specific for organic acids separation and the column temperature was tested at 25, 35 and 55°C for separation and determination. Based on symmetry peak and the peak area, the 35°C was optimal temperature for the good separation of organic acids and used in all our further experiments. The representative chromatograms for the standard mixture of organic acids (oxalic, citric, tartaric, malic and succinic) and some studied sample (Gel 11 and fruits juices of quince and strawberry) were shown in figure 1.

Validation of HPLC assay

The results of validation data, such as regression equations, correlation coefficients of calibration curves, detection and quantification limits (LOD, S/N > 3; LOQ, S/N > 10) for the analytes under optimized conditions are summarized in table 1.

As seen there, good correlations were found between the peak area (Y) and concentration of the tested compounds (X) (R^2 > 0.9993) within test ranges. The LODs and LOQs were in the range of 0.15 - 2.47μg mL^{-1} and 0.49 - 8.15 μg mL^{-1}, respectively, showing the excellent sensitivity of the method. The analytical precision from the data of the intra-day (three times of three concentration level per day) and inter-day (twice a day for four consecutive days) determinations were performed on the standard solutions. Table 2 shows that the RSD (%) values obtained are smaller than 4% which is in accordance to acceptable values lower than 5%, which provided to the present method the acceptable intermediate precision [28].

To demonstrate the accuracy of the method, a sample of teeth bleaching gel was analyzed before and after the addition of known amounts of studied organic acids. Each set of samples was analyzed three times. The results are presented in table 3. The RSD values were in the range of 1.18–3.58% and the average recoveries obtained are in the range 90.6–102.5% and demonstrated that the method has an acceptable accuracy [28].

The results of our experiments confirmed that the HPLC-UV method definitely possesses the advantages of high precision, good reproducibility and accuracy for the analysis of the samples of teeth bleaching gel.

Analysis of the studied organic compounds from the teeth bleaching gel and the fruit juices samples

The analytical methodology proposed by this work was applied to analysis of sixteen samples of the teeth bleaching gel samples prepared with natural fruit juices and the seven juices samples of different types of fruit. The results are presented in figure 2 and figure 3. The determination of the organic acids quantities in teeth bleaching gel samples has great importance for determination of the total quantity of organic acids in these samples as an important factor

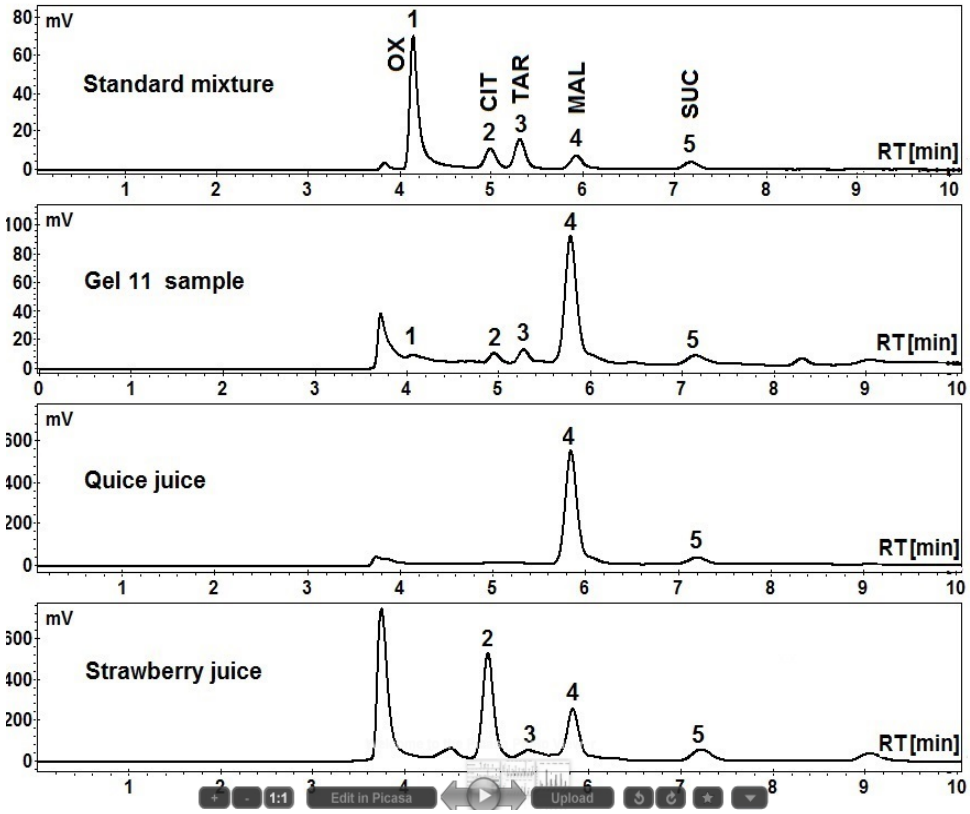

Fig. 1. HPLC chromatogram of organic acids standard mixture of oxalic, citric, tartaric, malic and succinic acids (each of 100 µg mL^{-1}), a sample of teeth bleaching gel (Gel 11) and two fruit juices samples (quince, strawberry) under optimized conditions.

Table 1
LINEAR REGRESSION DATA, LOD AND LOQ OF THE ORGANIC ACIDS COMPOUNDS

Organic acid	RT [a] (min)	Calibration curve [b]	Regression coefficient [c] R^2	Linear range (µg mL^{-1})	LOD [d] (µg mL^{-1})	LOQ [d] (µg mL^{-1})
Oxalic (OX)	4.145	Y = 0.09622 X - 0.47523	0.9998	25-1000	0.15	0.49
Citric (CIT)	4.987	Y = 0.01411 X - 0.00740	0.9993	25-1000	0.89	2.96
Tartaric (TAR)	5.310	Y = 0,02113 X - 0.05142	0.9996	25-1000	0.63	2.07
Malic (MAL)	5.925	Y = 0,01098 X - 0.01258	0.9999	25-1000	1.46	4.81
Succinic (SUC)	7.350	Y = 0,07328 X - 0.03471	0.9996	25-1000	2.47	8.15

[a] RT, the retention time; [b] Y, the peak area; X, the concentration of reference compound (µg/mL); [c] R^2 regression coefficient of calibration curve (n=7, seven points); [d] LOD, the limit of detection (S/N = 3); LOQ, the limit of quantification (S/N = 10)

Table 2
INTRA AND INTER-DAY PRECISION OF ORGANIC ACIDS

Organic acid	Concentration (µg mL^{-1})	Intra-Day Precision		Inter-Day Precision	
		Measured concentration Mean [a] ± SD [b], (µg mL^{-1})	RSD [c] (%)	Measured concentration Mean [a] ± SD [b], (µg mL^{-1})	RSD [c] (%)
Oxalic	25	24.85±0.68	2.74	25.32±0.85	3.46
	100	99.93±1.31	1.31	100.57±1.88	3.80
	250	250.33±2.56	1.02	250.94±3.31	3.02
Citric	25	24.42±0.52	2.11	25.72±0.75	2.78
	100	98.32±1.91	1.95	99.04±2.57	3.21
	250	251.25±2.64	1.05	251.32±3.07	2.04
Tartaric	25	25.46±0.69	2.74	24.66±0.64	3.12
	100	97.31±2.19	2.25	99.64±2.89	2.58
	250	251.41±2.86	1.14	251.04±3.66	2.62
Malic	25	24.64±0.43	1.76	25.23±0,78	3.98
	100	101.34±2.47	2.44	100.98±2.77	2.69
	250	250.81±3.31	1.32	251.61±4.75	2.73
Succinic	25	25.08±0.54	2.14	26.2±0.72	2.87
	100	97.63±1.82	1.86	100.48±3.19	2.93
	250	251.24±2.97	1.18	249.18±3.46	2.97

[a] Mean = Average of three determination; [b] SD = Standard deviation; [c] RSD = Relative standard deviation.

for controlling the tooth whitening process. In addition, establish the organic acids profile of fruit juices sample is important for determination of the type and the quantity of each organic acid present in fruit juices. As seen from these results, the citric acid was found as predominant compound in high quantity in Gel 1-7 samples, followed by malic, succinic and oxalic acids in all studied sample except Gel 1 sample. For these samples the total quantities of organic acids (mg acids / 100 g gel) were between 1.664 and 3.922.

Table 3

RECOVERIES OF THE STUDIED ORGANIC ACIDS IN THE TEETH BLEACHING GEL SAMPLE (Gel 11)

Organic acid	Initial amount ($\mu g\ mL^{-1}$)	Spiked amount ($\mu g\ mL^{-1}$)	Found amount ($\mu g\ mL^{-1}$)	Recovery (%)	Recovery Mean [a] ±SD [b]	RSD [c] (%)
Oxalic	2.55	2.4	5.10	106.25	101.38±3.24	3.20
			4.98	101.25		
			4.87	96.66		
		3.0	5.48	97.60	98.75±2.53	2.57
			5.60	101.66		
			5.46	97.00		
		3.6	6.02	96.38	96.57±2.23	2.31
			6.11	98.89		
			5.95	94.44		
Citric	41.55	32	72.83	97.75	98.04±3.22	3.29
			74.00	101,4		
			73.92	94.97		
		40	80.51	97.40	100.37±2.68	2.67
			82.60	102.62		
			81.98	101.07		
		48	89.50	99.89	97.91±1.74	1.77
			88.21	97.20		
			87.94	96.64		
Tartaric	32.25	24	56.00	98.85	100.18±2.77	2.77
			57.06	103.37		
			55.85	98.33		
		30	61.58	97.76	100.84±2.72	2.69
			62.81	101.86		
			63.12	102.90		
		36	68.25	100.00	100.35±2.15	2.14
			69.21	102.66		
			67.68	98.41		
Malic	865	680	1530.00	97.79	99.27±1.3	1.32
			1547.69	100.29		
			1543.14	99.72		
		850	1698.89	98.10	98.22±2.41	2.45
			1720.91	100.69		
			1680.00	95.88		
		1000	1840.00	97.50	98.61±1.167	1.18
			1863.25	99.82		
			1850.00	98.50		
Succinic	112.5	80	190.00	96.87	96.43±2.32	2.40
			187.64	93.92		
			191.30	98.50		
		100	215.00	102.50	98.72±3.53	3.58
			208.00	95.50		
			210.67	98.17		
		120	230.00	97.91	98.75±3.00	3.04
			235.00	102.08		
			228.00	96.25		

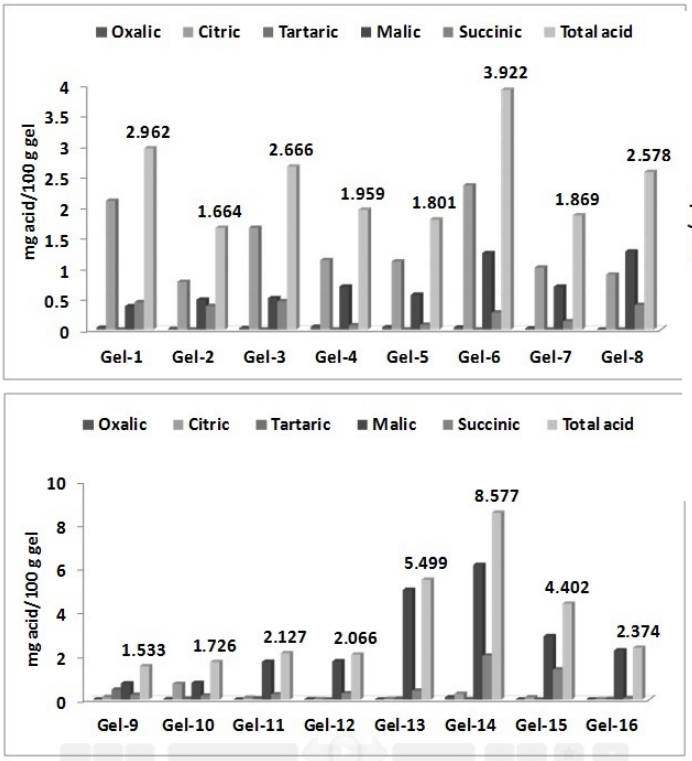

Fig. 2. The concentrations of organic acids in teeth bleaching gels samples

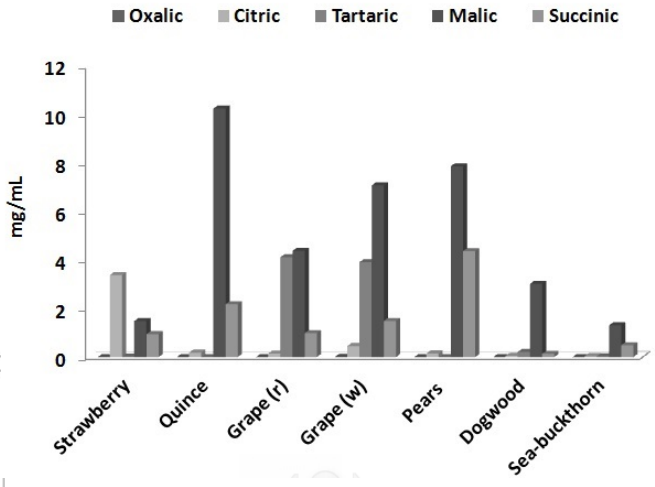

Fig 3. The concentration of organic acids in fruit juices samples

In the samples of Gel 8-16 were found the highest amounts (mg acid / 100 g gel) of malic acid with value range between 1.277 and 6.182 and the total quantities of organic acids (mg acids / 100 g gel) were between 1.533 and 8.577. The Gel 13, 14, 15 samples have the highest total quantities of organic acid (mg acids / 100 g gel) of 5.478, 8.577 and 4.402, respectively. Some of these results were used to evaluation of the effect of these studied teeth bleaching gels on restorative composites and were presented at the 6th International Conference *Biomaterials, Tissue Engineering & Medical Devices* BiomMedD'2014, Constanta, Romania, 17-20th September, 2014 (http://biommedd2014.srb.ro/).

Regarding fruit juices analysis, the content and the type of organic acids were important to establish the organic acids profile of these samples. In figure 3 are presented the organic acids quantities of seven samples of natural fruit juices which were used individual and / or in mixture to preparation of teeth bleaching gels. These fruit juice samples were concentrated using freeze-drying method together with other ingredients that constituted teeth bleaching gels. For these reason we can use the diluted solutions of fruit juices.

The results show that malic acid is a majority in all fruit juices studied, excepted strawberry juice which has citric acid in high amount of 3.385 mg mL^{-1}. The quince and pears juices contain malic acid in highest amounts of 10.253 and 7.871 mg mL^{-1}, respectively, comparatively with the other organic acids presents. Based on these results we can establish the quantity of fruit juice that can be used to prepare a gel sample with known total quantities of organic acids present.

Conclusions

A simple, accurate and efficient HPLC method for the simultaneous determination of oxalic, citric, tartaric, malic and succinic acids in teeth bleaching gels and fruit juices has been developed and validated. This method has been found to be reproducible and sensitive. The method has been successfully applied to qualitative and quantitative assay of sixteen teeth bleaching gels prepared with natural fruit juices in order to find the total quantity of organic acids in each of these samples. Also, the proposed analytical method was used to establish the organic acids profile of seven natural fruit juices studied. The present analytical work is the first report regarding determination of organic acids from teeth bleaching gels prepared with natural fruit juices using a validated HPLC method. The organic acids were used as active agent with action in tooth discoloration and stain removal.

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