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Determination of fumaric acid in apple juice by on-line coupled capillary isotachophoresis–capillary zone electrophoresis with UV detection

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Abstract

An on-line coupled capillary isotachophoresis–capillary zone electrophoresis (cITP–CZE) method for the determination of the fumaric acid content in apple juice is presented. A clear separation of fumaric acid in real samples is achieved within 20 min. The leading, terminating and background electrolyte of the employed system consist of 10 m*M* HCl+β-alanine+5 m*M* β-cyclodextrin+0.05% hydroxypropylmethylcelullose (HPMC), pH 3, 10 m*M* citric acid and 20 m*M* citric acid+β-alanine+5 m*M* β-cyclodextrin+0.1% HPMC, pH 3.3, respectively. The linearity, recovery, repeatability and detection limit of the developed method are 25–1000 ng/ml, 1.07%, 95.4±3.5 (±*s*)% and 10 ng/ml, respectively. Low laboriousness (no sample pretreatment), sufficient sensitivity and low running cost are the important attributes of the cITP–CZE method which was successfully applied to analyses of real samples of apple juices. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Isotachophoresis–capillary zone electrophoresis; Fruit juices; Food analysis; Fumaric acid; Malic acid; Tartaric acid; Organic acids

1. Introduction

Apple juices can contain various concentrations of fumaric acid, (*trans*-but-2-enedioic acid). The natural content of fumaric acid in freshly prepared clarified apple juices (8–14.8 °Brix) without heat treatment varied from 0 to 1.7 mg/l [1]. During the processing of apple juices, when heat treatment (evaporation, pasteurisation, and sterilisation) is used the content of fumaric acid slightly increases due to malic acid dehydration. The levels of fumaric acid of well-prepared (authentic and not decayed) apple juice usually do not exceed 3 mg/l [2]. A higher content of fumaric acid in apple juices indicates microbial spoilage of juices or of an intermediate, or the processing of decayed fruits. The other source of fumaric acid in apple juice can be the addition of synthetic malic acid, which contains fumaric acid as a minor contaminant [3]. Fumaric acid is an important indicator of microbial spoilage as well as the authenticity of juices. Comparing the other criteria for the evaluation of degree of microbial spoilage of fruit juices or processed fruits and fruit juices intermediate, fumaric acid seems to be a better parameter than other constituents such as lactic acid, acetic acid and ethanol. An increase of lactic acid content in juices is specific for lactic acid bacteria

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spoilage. An evaporation step and other appropriate technological processes can easily remove ethanol as the main metabolite of yeast alteration. Acetic acid is to some extend a natural component of fruits, it can be produced by majority of microbial changes in fruit juices, but similarly as for ethanol the volatility of acetic acid reduces its importance as an index of microbial quality. The evidence of a higher content of fumaric acid, together with levels of other metabolites which have limits[1], indicates the addition of synthetic malic acid to the analysed sample. The recent Guidelines of the AIJN[2] do not give the limits for fumaric acid; microbial spoilage is defined using the above mentioned parameters and the addition of synthetic malic acid is detected by the determination of D-malic acid, which should not be present in any apple juice sample. The AIJN guideline is to consider 5 mg/kg as a limiting concentration for fumaric acid content in apple juices [4].

Several methods are used for the determination of fumaric acid in real samples. HPLC methods using different separation principles (ion chromatography, ion exclusion or reversed-phase) and detection methods (UV absorption or conductimeter) are very commonly used [5–9]. Fumaric acid in different food samples was determined by capillary GC [10,11] after derivatization (methyl- or butylesters). Capillary isotachophoresis [12–16] was used for the determination of fumaric acid in fruit juices, chemicals and animal feed additives. The above-mentioned methods have some drawbacks. The HPLC technique usually needs sample pretreatment (e.g., solid-phase extraction) and GC needs a derivatization step prior to analysis. Capillary isotachophoresis enables direct (without any pretreatment) analysis of fumaric acid, however quantification of low levels of fumaric acid (from UV detector record) is rather problematic (see below).

An on-line capillary isotachophoresis–capillary zone electrophoresis (cITP–CZE) method for the determination of fumaric acid is presented as an alternative to the above-mentioned techniques. cITP–CZE is a simple (no sample pretreatment), quick, sufficiently sensitive and low running cost method and therefore well suited for routine analysis.

[1] According to the recommendation of the AIJN the limits are: 0.5 g/kg of lactic acid, 0.4 g/kg of acetic acid and 3 g/kg of ethanol.

[2] Association of the Industry of Juices and Nectars from Fruits and Vegetables of the European Union.

2. Experimental

2.1. Chemicals

Standard of fumaric acid, citric acid monohydrate, hydrochloric acid (all analytical grade) were purchased from Lachema (Czech Republic), β-cyclodextrin (β-CD) and hydroxypropylmethylcellulose (HPMC) from Sigma–Aldrich, (Czech Republic) and β-alanine from Janssen (Belgium). Hydrochloric acid and β-alanine were purified by conventional method prior to use for the electrolyte preparation. Deionized water (specific conductivity lower than 1 μS/cm) was used for electrolyte, standard solutions, and sample preparation.

Samples of apple juice, concentrate of orange juice and red wine were obtained from a local market.

2.2. Apparatus

The electrophoretic analyser used was an EA 100 (Labeco-Villa, Slovak Republic) with column coupling. The separation was performed in a FEP (fluorinated ethylene–propylene copolymer) preseparation capillary (90 mm×0.8 mm I.D.) which was coupled with a FEP analytical capillary (90×0.3 mm I.D.). Detection was carried out with contact conductivity detectors (both capillaries) and with UV absorbance detector (254 nm, analytical capillary). The samples were injected via sample valve of 35 μl fixed volume or with the help of a 10-μl Hamilton syringe. The electropherograms were evaluated with the help of a personal computer software package ITPWIN ver. 2.31 (KasComp, Slovak Republic).

2.3. Conditions of analysis

The electrolyte systems are described in Table 1. The time required for one analysis is 20 min.

Table 1
Condition of analysis

Parameters	System		
	I	II	III
Solvent	Water	Water	Water
Leading electrolyte (LE)	10 m*M* HCl + β-alanine + 0.05% HPMC, pH 3.00	10 m*M* HCl + β-alanine + 0.05% HPMC, pH 3.00	10 m*M* HCl + β-alanine + 5 m*M* β-CD + 0.05% HPMC, pH 3.00
Terminating electrolyte (TE)	10 m*M* citric acid	10 m*M* citric acid	10 m*M* citric acid
Background electrolyte (BGE)	–	20 m*M* citric acid + β-Alanine + 0.1% HPMC, pH 3.30	20 m*M* citric acid + β-alanine + 5 m*M* β-CD + 0.1% HPMC, pH 3.30
Driving current (μA)			
Preseparation capillary	250	250	250
Analytical capillary	50	150	150

2.4. Calibration

The external standard technique was used. Calibration standard solutions (six concentration levels from 25 to 1000 ng/ml) of fumaric were prepared from the 1000 mg/l stock solution and injected into analyser by the use of sampling valve with fixed volume (35 μl).

2.5. Sample treatment

Samples were injected directly (5 μl) using a 10-μl Hamilton syringe (without any treatment) or after dilution (10 times) via internal valve with fixed volume.

3. Results and discussion

The column-coupling configuration of the separation unit enables several separation modes, i.e., coupled capillary isotachophoresis (cITP–cITP, one- or two-dimensional) or cITP–CZE. In the case of cITP–cITP mode both preseparation and analytical capillary are filled with the same leading electrolyte (LE, one-dimensional cITP) or different LEs (two-dimensional cITP). The cITP–CZE mode is when the preseparation capillary is filled with the LE and analytical capillary with the terminating electrolyte (TE) or some other background electrolyte (BGE). It has been recently shown [17,18] that on-line combination of cITP with CZE is suitable for the analysis of trace ionogenic constituents present in a large excess of matrix ions. The cITP–CZE mode utilises the advantages of both methods. The cITP stage enables injection of large amounts of a sample and thus permits analysis of ionic constituents present at below nmol/ml levels. The sample constituents are separated into a stack of the zones with the minor constituents focused in narrow bands. Bulk ionogenic components are forced to migrate out of the separation compartment at the end of the preseparation capillary. The minor analytes concentrated and cleaned up from the bulk component in the cITP step are transferred into the analytical capillary as a narrow sample pulse in the CZE step. The removal of bulk component is well defined and reproducible when it is based on the signal from the conductimeter of the preseparation capillary. The CZE step offers high resolution and aids in the identification of minor components using migration times.

An analysis of apple juice in both cITP–cITP (Fig. 1a and b) and cITP–CZE (Fig. 1c and d) modes using different operational system (see Table 1) is shown. The trace from the conductimeter of the preseparation capillary is depicted on Fig. 1a. Minor components (inside the dashed rectangle) including fumaric acid are separated from bulk component (sulphate, phosphate, malate[3], citrate) and transferred into analytical capillary. A record from the UV detector is shown in Fig. 1b. The short zone of fumarate sandwiched by unknowns is difficult to quantify in the cITP–cITP mode. Carrying out the analysis in the cITP–CZE mode enables easy quantification of fumaric acid from the UV trace as shown

[3]Malate migrates in the zone of citrate (terminating anion).

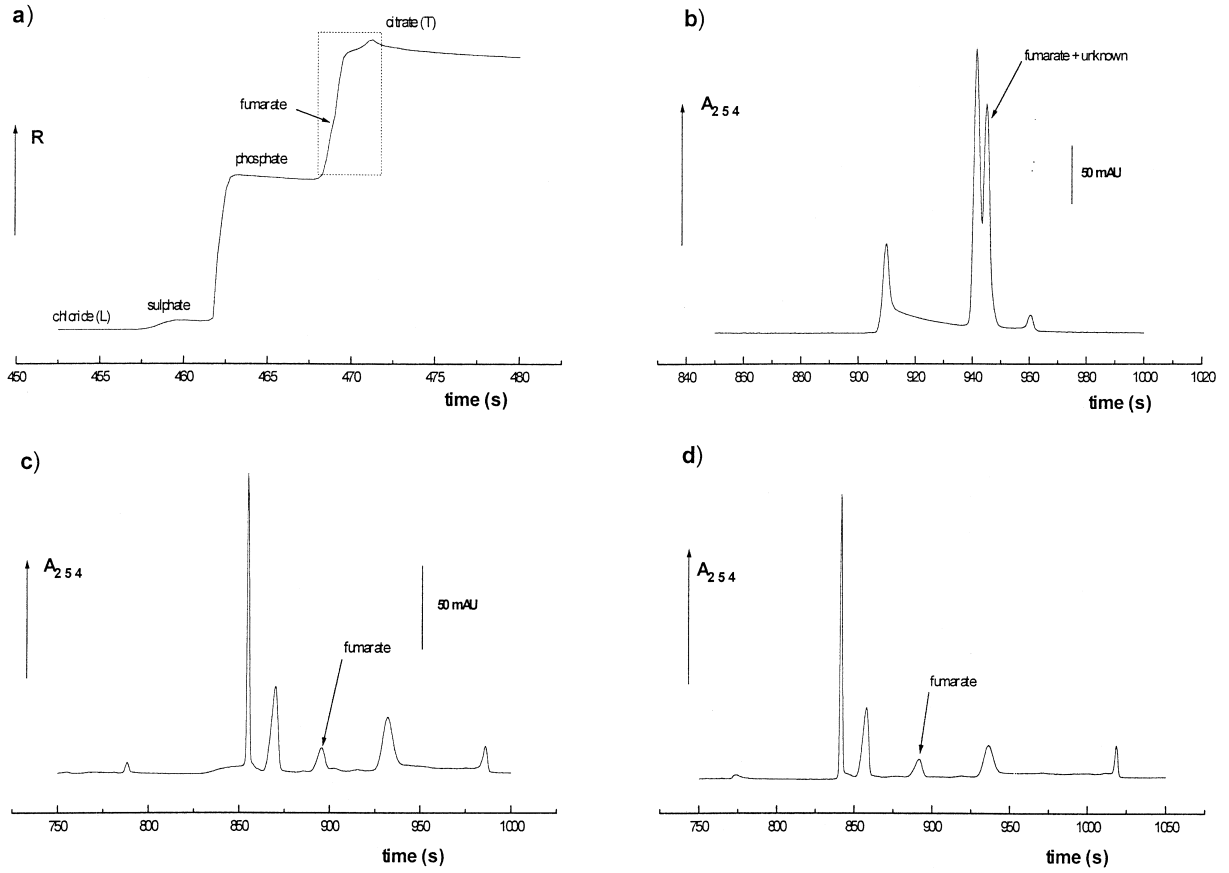

Fig. 1. Analysis of apple juice (10 times diluted) by different separation modes. (a) cITP–cITP (operational system I, Table 1). Trace from conductimeter of preseparation capillary; zones inside the dashed rectangle are transferred into analytical capillary (for all figures); cITP step; R = response of conductimeter. (b) cITP–cITP (operational system I, Table 1). Trace from UV detector of analytical capillary; cITP step. (c) cITP–CZE mode (operational system II, Table 1). Trace from UV detector of analytical capillary; CZE step. (d) cITP–CZE mode (operational system III, Table 1). Trace from UV detector of analytical capillary; CZE step.

in Fig. 1c. The unknowns are well separated from fumaric acid. Since real samples could contain tartaric acid, whose effective mobility is very close to that of fumaric acid, β-cyclodextrin (β-CD) was added to both the LE and the BGE [16]. The effect of β-CD addition into the electrolytes is illustrated in Fig. 2. Tartrate and fumarate create a stable mixed zone using electrolytes (LE and BGE) without β-CD (see Fig. 2a and b). After addition of β-CD into the LE and BGE clear separation of fumaric acid from tartrate is achieved (Fig. 2c and d). Fumaric acid creates stronger host–guest association with β-CD than tartaric acid due to its higher hydrophobicity at low pH resulting in lower effective mobility and thus clear separation of both acids. From β-CD comes an unknown anion, which is marked as 'impurity' in Fig. 2d, and Fig. 3c and d. This impurity is well-separated from fumaric acid and does not influence the analysis. Fig. 1d demonstrates that the addition of β-CD to the electrolytes does not influence the results of the analysis of apple juice, which normally do not contain tartaric acid. We recommend the use of electrolytes incorporating β-CD.

A linear relationship between the fumaric acid concentration and the peak area was found [peak area = 0.2329·concentration (ng/ml) – 0.851, r^2 = 0.9981]. The method characteristics, i.e., linearity, precision, accuracy (recovery) and detection limits

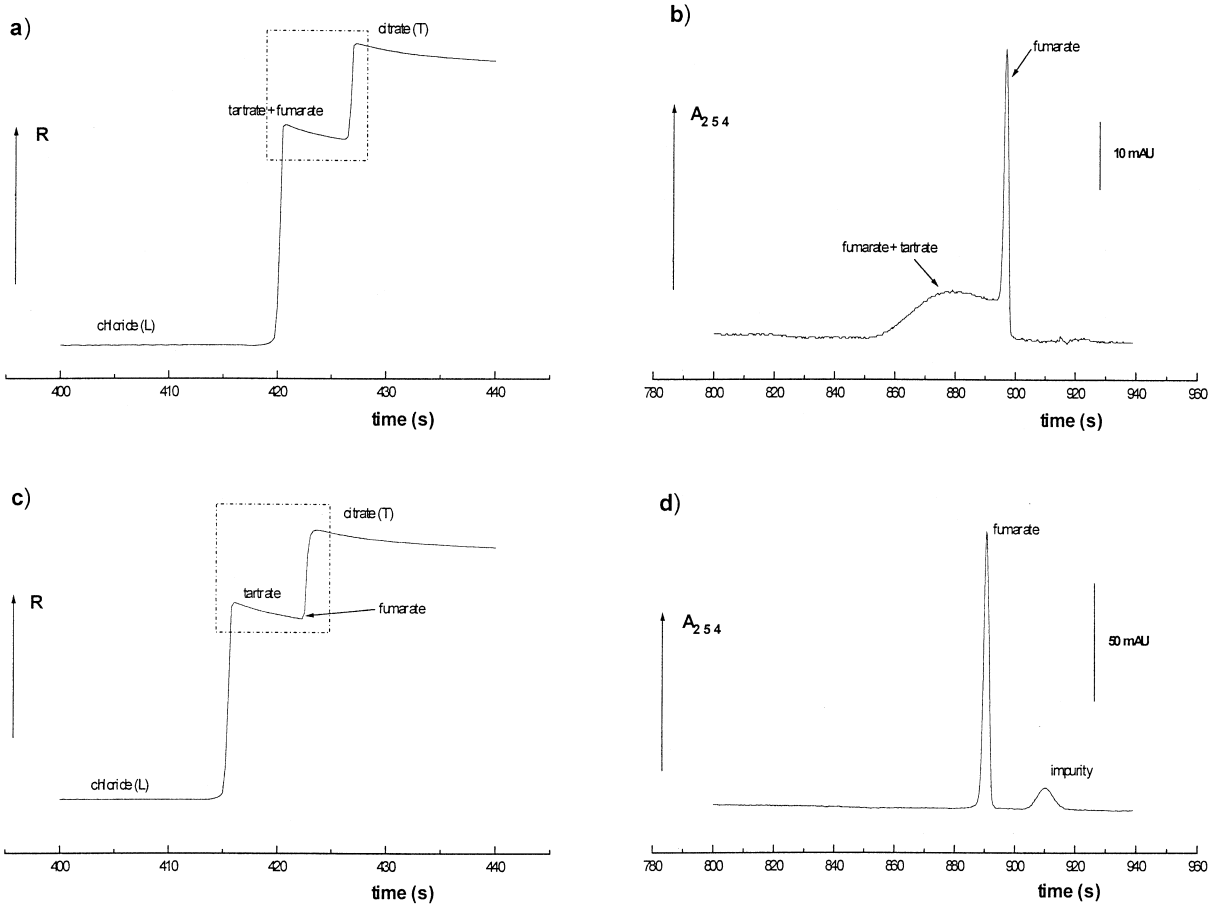

Fig. 2. Effect of β-CD on separation of 30 μg/ml tartrate and 1 μg/ml fumarate (cITP–CZE mode). (a) Trace from conductimeter of preseparation capillary (operation system II, see Table 1); cITP step. (b) Trace from UV detector of analytical capillary; (operation system II, see Table 1); CZE step. (c) Trace from conductimeter of preseparation capillary; (operation system III, see Table 1); cITP step. (d) Trace from UV detector of analytical capillary (operation system III, see Table 1); CZE step.

are summarised in Table 2. It is clear that the developed method is suitable for intended purpose, i.e., determination of fumaric acid in apple juice at ppm levels (see above). The cITP–CZE method is 50 times more sensitive than HPLC[4] or GC techniques.

The fumaric acid content (see Table 3) was calculated from peak area rather than from the peak height. Due to possible uncompleted destacking of fumaric acid from adjacent zones in CZE step,

Table 2
Characteristics of the method

Parameter	Value	
	From height	From area
Precision[a] (RSD, %, $n=6$)	0.87	1.07
Accuracy (recovery, %)[b]	Not tested	95.4±3.5
Linearity (ng/ml)	0–200	0–1000
Detection limit[c] (ng/ml)	10	10

[a] Repeated injection of the same sample (apple juice V, see Table 3).

[b] Three different real samples of apple juice (I, II and V, see Table 3) spiked at a concentration of 5 mg/l.

[c] Smallest detectable peak (signal/noise = 3; noise = 0.2 mAU).

[4] Limit of detection, 500 ng/ml [8].

Table 3
Results of sample analyses (average from three analyses)

Sample	Content of fumaric acid (mg/l)
Apple juice I, 100%	3.40
Apple juice II, 100%	2.35
Apple juice III, 100%	2.80
Apple juice IV, 100%	2.50
Apple juice V, 100%	8.50
Apple juice VI, 50%	0.80
Apple juice VII, 12.5%	0.35
Concentrate of orange juice (100%)[a]	7.10[b]
Red wine	2.30
D,L-Malic acid (purity 99%)	6340[b]
D,L-Tartaric acid (purity 99%)	1660[b]

[a] Recommended dilution 1:5.
[b] mg/kg.

variations in peak height may occur. The UV traces of analyses of orange juice concentrate (4 g/l) and the same juice spiked with 200 ng fumaric acid/ml are shown in Fig. 3a and b, respectively. It is clear that fumaric acid is separated from other UV absorbing constituents of juice. The trace from conductimeter of preseparation capillary of red wine (10 times diluted) is given in Fig. 3c and the UV trace of this analysis is shown in Fig. 3d. It is clear that the fumaric acid is well separated from wine UV absorbing constituents. These figures demonstrate that cITP–CZE can also be used for the determination of fumaric acid in samples other than apple juice.

Most of the results given in the Table 3 are under or slightly above the limit for authentic and undecayed apple juice (3 mg/l). In the case of the sample

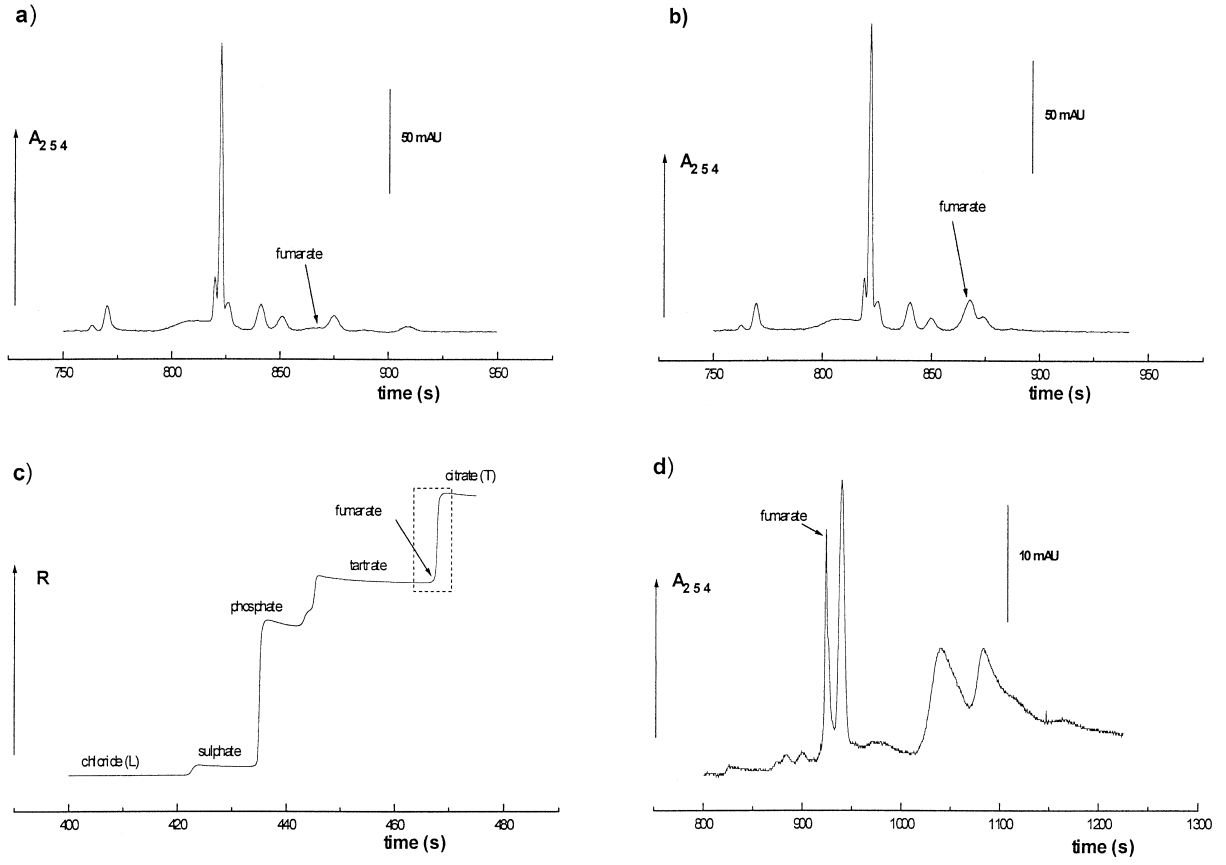

Fig. 3. (a) Concentrate of orange juice (4 g/l); trace from UV detector of analytical capillary; (operation system III, see Table 1); CZE step. (b) Concentrate of orange juice (4 g/l) spiked with fumaric acid (0.2 mg/1); trace from UV detector of analytical capillary; (operation system III, see Table 1); CZE step. (c) Red wine (10 times diluted); trace from conductimeter of preseparation capillary (operation system III, see Table 1); cITP step; R=response of conductimeter. (d) Trace from UV detector of analytical capillary; (operation system III, see Table 1); CZE step.

V the level of fumarate could indicate microbial spoilage and/or the addition of synthetic malic acid. For the confirmation of such a conclusion evaluation of the level of the other microbial metabolites or the determination of D-malic acid should be carried out. The found levels of fumaric acid in pure D,L-malic and tartaric acids can be used for the estimation of the amount of synthetic malic acid added to an apple juice sample (or addition of synthetic tartaric acid into grape juice), if the microbial decay of juices was not confirmed.

4. Conclusions

The presented results provide evidence that the developed cITP–CZE method for fumaric acid determination in apple juice is reliable and reproducible. cITP–CZE can easily be an alternative method to HPLC or GC. Low laboriousness (dilution only), sufficient sensitivity (0.1 mg fumaric acid/l of apple juice) and a low running cost (at least 10 times lower than HPLC) are the important attributes of presented method.

The described method is a good tool for the evaluation of the authenticity of apple and other fruit and vegetable juices and other products. The method allows us to check the quality and authenticity of the fruit and vegetables products in the Czech market within the project 'Detection of adulterated food products' which is now running at our department.

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