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ARTICLES

Determination of Aliphatic Organic Acids by High-Performance Liquid Chromatography with Pulsed Electrochemical Detection

INNOCENZO G. CASELLA* AND MARIA GATTA

Dipartimento di Chimica, Università degli Studi della Basilicata, Via N. Sauro 85, 85100 Potenza, Italy

A new ion exclusion HPLC procedure accomplished with a pulsed electrochemical detection for the determination of several common aliphatic acids is described. A triple-step waveform of the applied potentials, based on the formation/inhibition of PtOH species on the electrode surface, is successfully used for sensitive detection of several aliphatic acids in flowing systems avoiding pre- or postcolumn derivatization and/or cleanup procedures. Under optimal chromatographic conditions (i.e., 50 mM $HClO_4$) the proposed method allowed detection limits between 0.5 and 7 μM for all investigated acids, and the dynamic linear range spanned generally over 1 or 2 orders of magnitude. Determination of citric, malic, tartaric, lactic, formic, and acetic acids in several foods and beverages was performed, in ∼15 min, without the necessity of any sample pretreatment.

KEYWORDS: Electrochemical detection; chromatography; aliphatic acids; food analysis

INTRODUCTION

Organic acids are commonly present in beverages, foods, medicine, and a variety of real samples of biological and industrial interest. In addition, these compounds occur widely in soils and are intermediate in the metabolism of larger molecular weight compounds, such as carbohydrates, lipids, and proteins. Thus, many scientific disciplines are in need of analytical methods for the accurate, sensitive, and fast determination of acids and their derivatives in various types of samples. In foods, various organic acids are present and the content of any acid has a significant effect on the taste and aroma (*1*−*4*). In this respect, acid assay of foods and beverages is important for quality control during their production, transformation, storage, and distribution.

A commonly used method for determining the acid content is alkali titration using an appropriate visual indicator. However, the titration methods are generally not selective and not sufficiently sensitive and precise to detect a small acid content. To avoid the problems of conventional titration, gas chromatography, liquid chromatography, and capillary electrophoretic methods have gained importance in organic acid analysis (*5*−*8*). Ion exclusion chromatography has been recognized as a useful technique for the separation of various weak acids, and these molecules are separated on cation-exchange resin depending on their first dissociation constant and their relevant hydrophobicity. The Donnan exclusion effect, caused by the ionic repulsion between the negatively charged resin and the partially negatively charged analytes, is the main separation mechanism. Further influences on the separation of organic acids are derived from size exclusion effects, reversed-phase interactions, and van der Waals forces. Thus, in ion exclusion chromatography, the combination of a column packed with a high-capacity sulfonated styrene−divinylbenzene (PS-DVB) copolymer resin in the proton form and an acidic eluent has been commonly adopted for the analytical separation of several important weak acids (*9*, *10*). Different detection methods have been combined with these separation procedures to determine the organic acids in various real samples. A major problem with aliphatic organic acid detection in HPLC is that these compounds generally do not exhibit any pronounced chromophore and/or fluorophore groups, thus eliminating the use of direct UV−vis and fluorescence detectors. Because the analytes (i.e., aliphatic acids) do not exhibit sufficiently high UV absorptivity, the indirect absorbance method has been extensively used for the quantification of these compounds (*4*, *5*, *7*). In addition, suppressed conductivity detectors have been also used for the determination of aliphatic acids under flow-stream conditions (*3* ,*8*). However, because of the relatively high concentration of ions and aromatic molecules in real samples, the previous techniques often require, in order to improve sensitivity and precision, extraction, derivatization, and/or other sample cleanup procedures. In addition, in indirect photometric detection, the analytical signal is generally accomplished with a high background contribution.

Electrochemical detection following liquid chromatography (LC-ED) represents a very attractive analytical scheme in terms of sensitivity, selectivity, and wide linear dynamic range for the determination of many important analytes in biological and

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foods matrices. In this respect, recently, we have proposed some anion-exchange chromatography methods with electrochemical detection for the determination of electroactive organic acids (*11*, *12*). However, direct amperometric detection based on solid electrodes is generally restricted to active redox species. Over recent years, new amperometric detectors based on the repetitive doping−undoping polymer films (*13*−*17*), redox properties of quinone species in unbuffered protic solvents (*18*), or alteration of the rate of surface oxide formation following pulsed potential waveforms (*19*) have been characterized and proposed for the determination of electroinactive ions in flow injection and chromatographic systems. In particular, adsorption/desorption of organic molecules at a platinum surface will affect the rate of oxide formation during the positive potential step. Hence, pulsed amperometric detection (PAD) at platinum electrodes can be useful for the sensitive detection of electroinactive adsorbates.

On the basis of these facts, we developed a new electroanalytical procedure for the determination of several aliphatic acids without using any derivatization procedure. In particular, here we show that the PAD mode applied to the platinum substrate electrode can be used successfully for the determination of acids in liquid chromatographic conditions. We determine the sensitivity, the linear dynamic range, the temporal reproducibility of response and the detection limit of several aliphatic electroinactive acids. Examples of possible applications including the chromatographic separation and direct electrochemical determination of several common aliphatic acids in foods and beverages are given.

MATERIALS AND METHODS

Chemicals. Solutions were prepared daily from analytical reagent grade chemicals (Aldrich-Chemie) without further purification and by using doubly distilled deionized water. Unless otherwise specified, experiments were performed by using 50 mM perchloric acid ($HClO_4$) as mobile phase. All experiments were carried out at ambient temperature.

Apparatus. Amperometric measurements in flowing streams were performed by using a pulsed amperometric detector model ED 40 (Dionex, Sunnyvale, CA). A thin-layer electrochemical cell consisting of a 1.0-mm-diameter platinum working electrode, an Ag/AgCl reference electrode, and a stainless steel body of the cell serving as the counter electrode was also purchased from Dionex. All experiments were performed using a metal-free pump model PU-1580i (Jasco Corp., Tokyo, Japan) equipped with a programmable gradient module model LG-1580-04 (Jasco) and a metal-free rotary injection valve model 7125i (Rheodyne, Cotati, CA) with a 20 μL sample loop. A personal computer equipped with Kontron PC Integration Pack software (Milan, Italy) allowed acquisition and processing of chromatograms. Unless stated otherwise, the pulsed amperometric detector settings were the following: $E_{det} = 0.30$ V ($t_{det} = 280$ ms, $t_{int} = 20$ ms), $E_{ox} = 1.40$ V ($t_{ox} = 130$ ms), and $E_{red} = -0.40$ V ($t_{red} = 420$ ms). Currents are measured and integrated with respect to time (t_{int}) to give a faradic charge (coulombs) for the detection cycle.

Ion exclusion chromatographic separations of aliphatic acids have been conducted with an Aminex HPX-87H Bio-Rad column. The HPX-87H column (300 × 7.8 mm i.d.) is packed with 9 μm spherical sulfonated PS-DVB copolymer beads with 8% cross-linking, providing an ion-exchange capacity of 1.7 mmol/g.

The mobile phase was protected from oxygen by purging the reservoir bottle with high-purity nitrogen and in addition, immediately before the introduction onto the pump was degassed by an on-line degasser system series 1050 (Hewlett-Packard, Avondale, PA)

Sample Treatment. *Apple and Orange Juices.* Samples of apple juice from Derby (Salfa SpA, Bologna, Italy) and orange juices from Santal (Felegara, Parma, Italy) were passed through filter paper, diluted (1:200) with distilled water, and injected into the column.

Table 1. Effect of the Detection Potential (E_{det}) on the Charge Signal (nC) of Various Acids[a]

E_{det} (mV)	citric	tartaric	malic	maleic	lactic	acetic
50 (190 nC)	3.6	4.2	3.2	11.7	1.0	3.9
100 (172 nC)	3.5	4.3	3.3	11.3	1.5	3.8
150 (161 nC)	5.4	6.0	4.8	14.3	1.6	3.6
200 (133 nC)	5.0	5.4	4.2	13.5	1.5	3.6
250 (118 nC)	4.4	4.8	3.8	12.1	1.4	3.4
300 (101 nC)	3.6	4.3	2.8	9.0	0.8	3.4
350 (83 nC)	2.4	2.8	2.4	8.1	0.7	3.0
400 (62 nC)	1.8	2.2	1.8	5.9	0.8	1.9
450 (44 nC)	1.0	1.4	1.3	4.2	0.8	1.4

[a] Experimental conditions: column, HPX-87H; flow rate, 0.7 mL/min; sample loop, 20 μL; mobile phase, 50 mM $HClO_4$; waveform, $E_{red} = -0.40$ V, $t_{red} = 240$ ms, $E_{ox} = 1.4$ V, $t_{ox} = 130$ ms, $t_{det} = 300$ ms, $t_{int} = 20$ ms; analyte composition, 80 μM each component. The background charge is reported in parentheses.

Cheese Samples. A suspension of 10 g of finely ground cheese from Edam (product of Holland, 1999) or Asiago cheese samples from Sapori d'Italia (Consorzio per la tutela dell'Asiago, Italy) in 100 mL of 50 mM ammonia solution (or distilled water) was sonicated for 20 min at 70 °C. Then, the suspension was filtered thorough filter paper (0.45 μm) and diluted 1:200 with distilled water. The resulting solution was injected into the column.

Red and White Wines. Red wines (Aglianico, 1995, 2000, Italy) and white wine (Tavernello, Caviro S.C. arl, 1999, Italy) were passed through filter paper (0.45 μm), diluted (1:900) with distilled water, and injected into the column.

Yogurt Sample. Ten grams of yogurt samples from Parmalat (Parma, Italy) was sonicated for 20 min at 70 °C in 200 mL of 50 mM ammonia solution and then filtered with a 0.45 μm nitrocellulose membrane (Millipore, Bedford, MA) and diluted 1:200 with distilled water.

All analyzed samples were purchased from a local retailer, and the relevant packagings were opened just before the analytical determinations. The determinations were performed in triplicate.

RESULTS AND DISCUSSION

Optimization of the PAD Parameters. It was observed by cyclic voltammetry that adsorbed species at a reduced platinum electrode surface will affect the $i-t$ curves relevant to the formation of the surface oxide following a positive potential step. Here, a triple-step waveform, based on the formation/inhibition of PtOH species on the electrode surface, a consequence of the absence/presence of adsorbing species, is used for sensitive detection of aliphatic acids. Depending upon the choice of detection potential (E_{det}) and integration time (t_{int}), both "positive" and "negative" peak currents can be observed when adsorbing species are injected into a flowing stream of the acid medium. To obtain a better optimization of the PAD waveform in liquid chromatography, the detection potential (E_{det}) and the integration time (t_{int}) were varied in consecutive runs by injections of 80 μM of each considered acid, leaving unchanged both the oxidation (E_{ox}) and reduction (E_{red}) potential values. The relevant results, considering the effects of E_{det} and t_{int} on the charge signal, are summarized in Tables 1 and 2, respectively. As can be seen, all examined acids exhibited almost the same behavior as a function of the detection potential except the acetic acid. The integration time induces a sensible increase in the charge signal of all analyzed acids. The maximum charge signals were observed at ~0.15 V versus Ag/AgCl, corresponding to the presence of a clean surface of Pt^0. Thus, in this region of the potentials, a favorable adsorption process between analytes and the platinum active surface is responsible of the PAD response. These data also support the hypothesis that upon stripping of the platinum oxide and adsorbed hydrogen (i.e.,

Table 2. Effect of the Integration Time (t_{int}) on the Charge Signal (nC) of Various Acids[a]

t_{int} (ms)	citric	tartaric	malic	maleic	lactic	acetic
20 (105 nC)	3.6	4.3	2.8	9.0	0.8	3.4
40 (218 nC)	5.2	5.9	4.7	21.1	2.4	4.3
60 (316 nC)	10.8	12.0	9.2	32.0	4.0	5.6
120 (430 nC)	24.0	26.4	20.8	65.2	8.0	11.2
160 (790 nC)	34.1	38.0	30.5	86.0	14.0	16.1

[a] Experimental conditions as in Table 1. The detection potential E_{det} was 0.3 V. The background charge is reported in parentheses.

potential region between 0.05 and 0.25 V), platinum sites are immediately active for direct adsorption of aliphatic acids.

At a detection potential of 0.3 V, the charge signals, for all investigated acids, were found to be directly related to integration time (see Table 2). As can be seen, the background signal increases drastically with increasing integration time. Generally, large background currents are responsible for baseline instability with subsequent poor precision and high detection limits. Thus, the triple-step PAD waveform described under Materials and Methods, with $E_{det} = 0.3$ V and $t_{int} = 20$ ms, was considered as optimal in terms of charge signal, low background signal, sensitivity, and temporal reproducibility. In addition, the described PAD waveform adopted here shows a rapid establishment of baseline stability accompanied by constant analytical signals during repetitive injections.

Optimization of Separation Conditions: Effect of Perchloric Acid Concentration on the Charge Signal and Chromatographic Performance. In contrast with other transition metals (i.e., Au, Cu, Ni, etc.), platinum electrodes show interesting electrochemical properties in terms of amperometric sensors for the determination of several organic molecules in acid medium. In addition, it is well-known that the proton concentration in the eluent medium is a deciding factor in the retention behavior of organic acids in ion exclusion chromatography. Therefore, the influence of the $HClO_4$ concentration on the retention time and electrode performance in terms of charge signal was studied extensively. Thus, a standard mixture of aliphatic acids was eluted isocratically with mobile phases containing various concentrations of $HClO_4$ ranging from 20 to 200 mM using an HPX-87H analytical column. Capacity factors related to the separation system were calculated on the basis of the retention times and void times. The retention times, capacity factors, and peak area evaluated from each chromatographic component are summarized in Table 3. As can be seen, all examined acids exhibited the same behavior in terms of chromatographic performance and electrode sensitivity. In general, the maximum PAD responses were observed at an $HClO_4$ concentration of 50 mM. The retention times and the capacity factors tend to increase slightly with an elevation of the $HClO_4$ concentration from 20 to 200 mM. Mobile phases with acid concentration <50 mM (i.e., 20 mM) were not recommended because the peaks were large and accompanied by a significant tailing. In addition, an $HClO_4$ concentration <50 mM led to poor resolution of the peaks and produces a partial overlapping of the malic−maleic peaks. Therefore, for the analytical determinations of aliphatic acids present in complex real matrices, which form the focus of this work, an acid eluent with a concentration of 50 mM $HClO_4$ was chosen as being a good compromise between reasonable peak resolution, magnitude of the PAD signal, and minimum background charge noise.

Calibration Graphs, Detection Limits, and Reproducibility. Figure 1 shows a typical chromatographic separation of a sample mixture of aliphatic acids using an Aminex HPX-87H ion exclusion column with PAD accomplished under isocratic conditions using a mobile phase composed of 50 mM $HClO_4$ at a flow rate of 0.7 mL/min. As can be seen, good chromatographic separations of various aliphatic acids were obtained under the optimized experimental conditions, and the elution of all examined compounds was completed in ~13 min of chromatographic run. Several common electroactive molecules such as carbohydrates and alditols (i.e., glucose, sucrose, fructose, lactose, xylitol, mannitol, etc.) are completely eluted in <6 min, whereas alcohols species (i.e., methanol, ethanol, 1-propanol, etc.) are eluted after 13 min. Thus, the presence of a relatively high concentration of other common electroactive compounds did not produce visible effects on the signals of the aliphatic acids investigated here. Under these experimental conditions, any imbalance in the oxygen level between the

Table 3. Effect of Perchloric Acid Concentration on the Retention Time (t_r), Capacity Factor (k), and Peak Area (Pa) of Various Organic Acids Separated by an HPX-87H Analytical Column[a]

	citric	tartaric	malic	maleic	lactic
		20 mM $HClO_4$			
t_r (min)	7.4	7.9	8.6	8.7	11.0
k	0.28	0.37	0.48	0.49	0.84
Pa	16.8	11.4			6.6
		50 mM $HClO_4$			
t_r (min)	7.5	8.0	8.8	9.5	11.2
k	0.29	0.38	0.52	0.64	0.94
Pa	27.9	30.8	25.2	344	7.3
		100 mM $HClO_4$			
t_r (min)	7.6	8.2	8.9	10.1	11.3
k	0.30	0.40	0.53	0.73	0.94
Pa	23.8	25.9	19.9	203	1.5
		200 mM $HClO_4$			
t_r (min)	7.7	8.3	9.1	10.5	11.3
k	0.33	0.43	0.56	0.82	0.95
Pa	23.1	26.5	24.5	78	1.9

[a] Experimental conditions as in Table 2. $t_{int} = 20$ ms. The peak area (Pa) is expressed in arbitrary units.

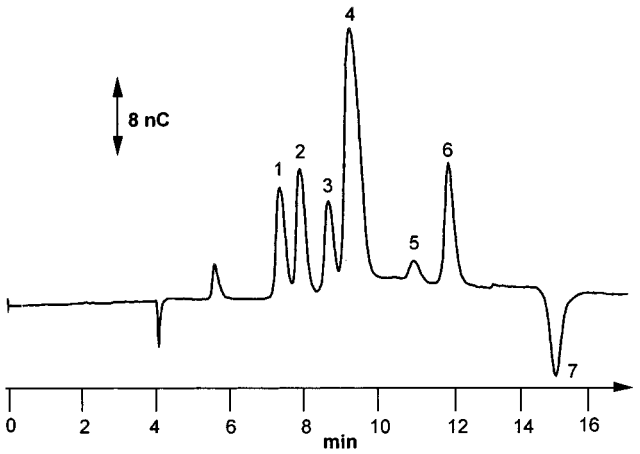

Figure 1. Typical isocratic separation in ion exclusion chromatography with pulsed electrochemical detection of a mixed standard solution of aliphatic acids: (1) citric acid; (2) tartaric acid; (3) malic acid; (4) maleic acid; (5) lactic acid; (6) formic acid; (7) oxygen imbalance. Conditions: column, HPX-87H (300 × 7.8 mm i.d.); mobile phase, 50 mM $HClO_4$; flow rate, 0.7 mL/min; sample loop, 20 μL; waveform of the potentials, $E_{det} = 0.3$ V, $t_{det} = 280$ ms, $t_{int} = 20$ ms, $E_{ox} = 1.4$ V, $t_{ox} = 130$ ms, $E_{red} = -0.4$ V, $t_{red} = 420$ ms; reference electrode, Ag/AgCl; analyte concentrations, ~250 μM of each injected compound.

Table 4. Quantitative Parameters of Some Aliphatic Acids Determined by Ion Exclusion Chromatography with PAD[a]

compound	LOD (μM)	linear range (μM)	repeatability (%)
citric	5	5–500	1.7
tartaric	7	10–500	1.9
malic	5	5–450	1.6
maleic	3	5–180	1.3
lactic	6	6–340	6.8
formic	0.5	1–2500	1.9

[a] Experimental conditions as in Table 2. t_{int} = 20 ms. The repeatability was expressed as relative standard deviation (percent) of 11 repetitive chromatographic experiments at 40 μM concentration of each analyte considered. The correlation coefficients were always >0.99.

mobile phase and injection matrix is readily detected with a negative or positive peak (see Figure 1, peak 7). As can be seen, the peaks of the organic acids considered here are sufficiently separated from the oxygen peak; a resulting large imbalance in oxygen level did not produce any variation in the quantification of the acids. Therefore, a woven Teflon mixing reactor, which connects the column to the electrochemical cell, serves to minimize the imbalance in the oxygen level and reduces the undesired peak (*20*).

The analytical results including linear range, detection limits (LOD), and precision are summarized in Table 4. As can be seen, the LOD, determined at the lowest injected concentration as a signal-to-noise ratio equal to 3, are generally between 0.5 μM for formic acid and 7 μM for tartaric acid. The precision, expressed as relative standard deviation (RSD%), of 11 repetitive chromatographic experiments (~3 h of operation time) was between 1.3% for maleic acid and 6.8% for lactic acid. The linearity extends over 1 or 2 orders of magnitude above the LOD, with correlation coefficients >0.996. Each data point (n = 7−8 experimental determinations in the linear range) was generated from at least two separate injections. Note, therefore, that the analytical quantitation of carboxylic acids was characterized by a rather restricted linear range, which is typical of PAD for strongly adsorbed molecules on the electrode surfaces (*21*).

Selected Applications. The aim of the present work was to apply the electrochemical detection in liquid chromatography to evaluate the concentration of major aliphatic acids in various foods and beverages. In this section, to demonstrate the analytical potentiality of the present method for practical applications, some selected examples of the determination of citric, tartaric, malic, lactic, formic, and acetic acids in commercially available samples are reported. Peak identification was based on the retention time of the considered acid and was confirmed by spiking authentic standard solutions to the sample extract. The identification of the considered molecules based on the retention time is not conclusive; further support was given both by spiking pure standard compounds to the sample extracts and by accomplishing chromatographic separations. Thus, the peak purity of the specific components was checked by measuring the degree of peak asymmetry factor (*22*) as a function of the added molecules. Generally, the peak asymmetry factors were between 0.9 ± 0.2 and 1.3 ± 0.3 (except for lactic acid, for which the asymmetry factor value was 0.8 ± 0.2) and are practically independent of the added compound in the extract solutions. Recoveries were evaluated for each constituent by spiking the extraction solutions with pure acids at the level of ~15−25% of the measured content and performing triplicate assays after each addition. The concentration of organic acids was calculated using the area of the peaks and was calculated by a linear-square regression approach using the method of standard addition. Good reproducibility was obtained for separations on repetitive injections, and no apparent chromatographic column and electrochemical detector deterioration are observed.

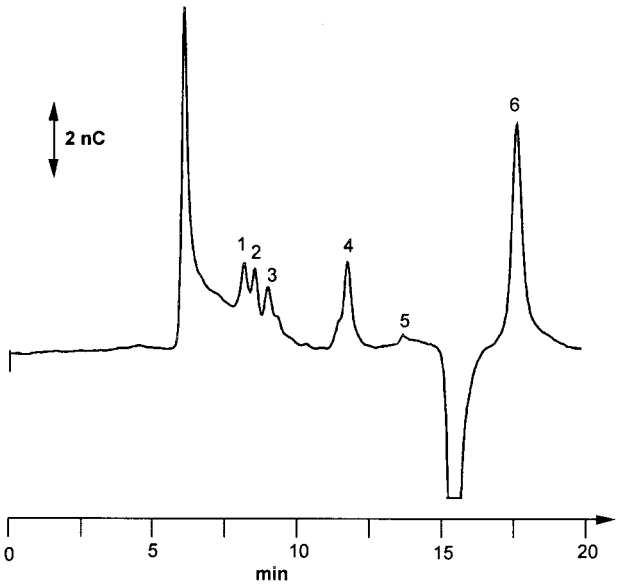

Figure 2. Chromatogram of a red wine (Aglianico, 2000) diluted 1:900 with distilled water: (1) citric acid; (2) tartaric acid; (3) malic acid; (4) lactic acid; (5) acetic acid; (6) ethanol. Experimental conditions were as in Figure 1.

Red and White Wines. Aliphatic acids are interesting compounds in wine and its derivative products. Some of them are originally present in the matrices, but others may appear during alcoholic, acetic, or another kind of fermentation. Thus, the level and nature of the organic acids present in the wines may provide information concerning the origin of the raw material, microbiological growth, and even processing techniques. A typical chromatogram of a commercially available red wine (Aglianico 2000, southern Italy) diluted 1:900 with distilled water is shown in Figure 2. The relevant concentrations of citric, tartaric, malic, and acetic acids are reported in Table 5. As a comparison of this category of beverages, in Table 5 is also reported the analysis of acids in other wines with different natural aging degrees. As can be seen, tartaric and citric acids are the major acids in the investigated wines. Tartaric acid represents the fixed portion of acidity, and its presence is an indication of the origin of the wine. Citric acid is generally formed during alcoholic fermentation and may be used as a substrate by some organisms, producing acetic acid. In general, the aging process induces a significant increase in acid contents (i.e., tartaric and acetic acids). The white wine (Tavernello, 1999, middle Italy) shows an acidic profile similar to that of red wine (Aglianico, 1999, southern Italy) except for a high level of malic acid.

Orange and Apple Juices. The economical importance of identifying fraudulence related to juice adulteration is enormous, because of the large quantities consumed in the word. The identification and dosing of the various organic acids present in a fruit juice are of considerable interest, because they provide not only useful information regarding the authenticity of the products under examination but also information regarding any processes of microbiological alteration during production, transformation, and storage of the juices. The most frequent types of adulteration have been identified and classified (*23*, *24*). The simplest type of adulteration is likely to be the addition to the pure juice of a solution of sugars and citric acid. Thus,

Table 5. Analysis of Some Common Aliphatic Acids in Food and Beverage Samples by Ion Exclusion Chromatography with PAD[a]

	citric	tartaric	malic	lactic	formic	acetic
red wine						
Aglianico 1995						
found (g/L)	1.8	3.8	0.43			2.7
recovery (%)	96	95.5	97			95
Aglianico 2000						
found (g/L)	1.4	1.7	0.39			0.74
recovey (%)	97	96	95			96
white wine						
Tavernello 1999						
found (g/L)	1.0	2.4	1.3	4.0		0.75
recovery (%)	98	96	102	104		97
cheese						
Asiago						
found (mg/g)	2.3			39	0.85	5.1
recovery (%)	97			103	95	96
Edam						
found (mg/g)	8.5				1.1	9.0
recovery (%)	95				97	95
yogurt						
found (mg/g)		5.8		12.0	1.3	0.6
recovery (%)		96		104	98	95
juice						
orange						
found (g/L)	7.4		1.25			
recovery (%)	97		103			
apple						
found (g/L)	0.13		3.5			
recovery (%)	96		102			

[a] The real samples were treated as reported under Materials and Methods. Recoveries were evaluated for each analyte determined by spiking the extraction solutions with the relevant organic acid component at the level of ~15–25% of the measured content. The contents were evaluated by the standard addition method. The precision expressed as RSD (four injections), ranged between 5 and 11% for all determined compounds.

organic acid profiles can be used as fingerprints for establishing authenticity of the fruit juices. In this work, orange or apple juices were diluted 1:200 with distilled water and directly injected into the column. The analytical results in terms of concentration of citric and malic acids of the orange and apple juices are summarized in Table 5.

Cheese and Yogurt Samples. Aliphatic acids may be present in naturally and artificially produced milk derivatives such as mold cheese and yogurts and are important to indicate the presence of fungi and mycotoxins. In addition, these compounds impart a pleasant or unpleasant flavor depending upon the predominance of one or more of them. Cheese flavor develops as a consequence of the microbial metabolism and subsequent transformation of the degradation products of the protein, fat, and carbohydrate components present initially in the product. Lactic acid bacteria, for example, play a central role in the ripening process. These facts explain the need for a sensitive analytical procedure for the determination of organic acids in various foodstuffs and milk derivatives (*25*). To ascertain the potential analytical performance of the proposed method, the determination of some common carboxylic acids in two different milk derivatives, such as cheese and yogurt, was performed. Representative chromatograms of unspiked extracts of Asiago cheese and yogurt are shown in Figures 3 and 4, respectively. The relevant quantitative results, regarding the determination of the major assigned peaks such as citric, lactic, formic, and acetic acid, are listed in Table 5.

Conclusions. An optimized ion exclusion liquid chromatography with pulsed electrochemical detection method for the determination of some common aliphatic acids in various real samples was developed. The proposed method is rapid, inexpensive, sensible, and reproducible for the acid determination in routine analysis. To the best of our knowledge, this is the first example of the analytical determination of aliphatic organic acids on the platinum electrode in acid medium. A triple-step waveform, based on the formation/inhibition of PtOH species on the electrode surface, can be used for the sensitive detection of aliphatic acids in flowing systems. The good level of sensitivity and recoveries without any complicated extraction, chemical derivatization, and /or sample cleanup procedures represent important advantages for the determination of aliphatic

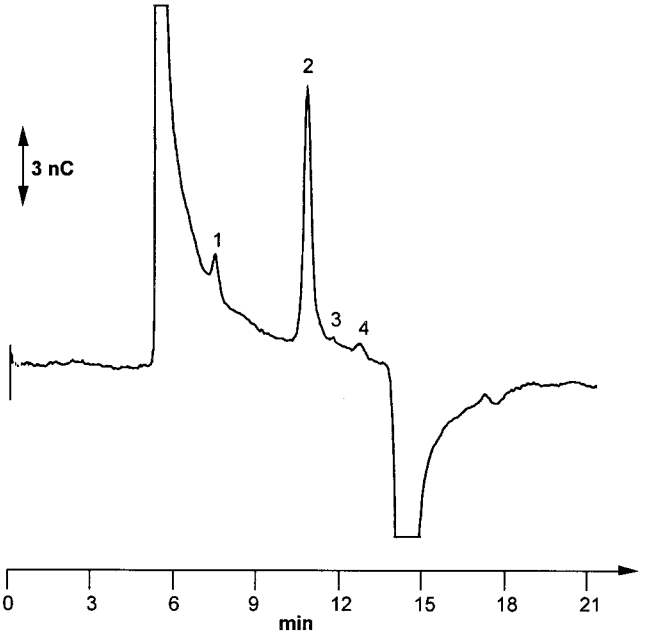

Figure 3. Chromatogram of a sample of Asiago cheese. Ten grams of finely ground sample was sonicated (20 min) in 100 mL of 50 mM ammonia and successively filtered with filter paper and diluted 1:200 with distilled water. Peaks: (1) citric acid; (2) lactic acid; (3) formic acid; (4) acetic acid. Experimental conditions were as in Figure 1.

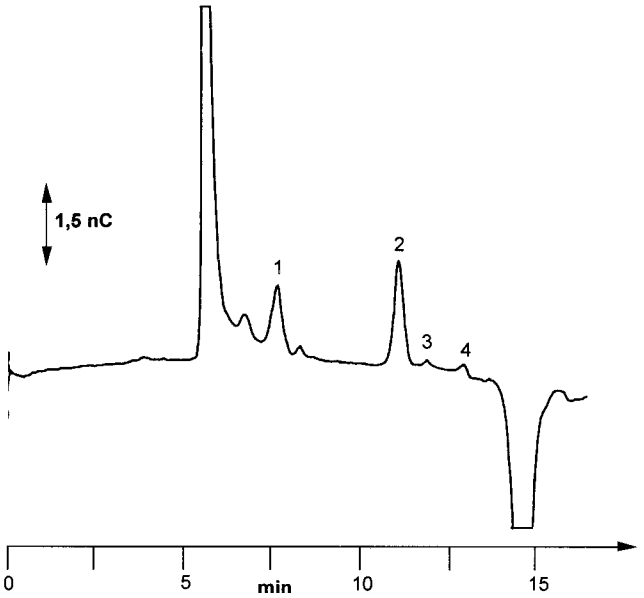

Figure 4. Chromatogram of a yogurt sample. Ten grams of the original sample was sonicated for 20 min at 70 °C with 200 mL of 50 mM ammonia solution, and then the suspension was filtered with filter paper and diluted 1:200 with distilled water. Peaks: (1) tartaric acid; (2) lactic acid; (3) formic acid; (4) acetic acid. Experimental conditions were as in Figure 1.

acids in complicated real matrices. The proposed method offers a valid alternative to indirect UV or suppressed conductivity detector schemes in analytical context.

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