

Determination of aliphatic aldehydes by liquid chromatography with pulsed amperometric detection

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Abstract

An electrochemical detection method for short-chain saturated and unsaturated aliphatic aldehydes separated by liquid chromatography in moderately acidic medium is described. A triple-step waveform of the potentials applied to the polycrystalline platinum electrode, is proposed for sensitive detection of aliphatic aldehydes in flowing streams avoiding tedious pre- or post-column derivatization and/or cleanup procedures. The influences of the perchloric acid concentration and dissolved oxygen in the mobile phase, on the amperometric and chromatographic performance were evaluated and considered in terms of sensitivity and selectivity. Under the optimised experimental conditions (i.e., deoxygenated 50 mM HClO₄) the proposed analytical method allowed detection limits between 0.2 μM for acrolein and 2.5 μM for valeraldehyde. Regression analysis of calibration data indicates that responses for all investigated compounds are linear over about 2 orders of magnitude above the LOD, with correlation coefficients >0.990. The method was successfully applied to the determination of formaldehyde, acetaldehyde, propionaldehyde and acrolein in real matrices such as spiked water and red wines with good mean recoveries (81–97%).

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1. Introduction

Low-molecular-weight aliphatic aldehydes such as formaldehyde, acetaldehyde and propionaldehyde have received much attention as hazardous and odorous substances in studies of air pollution. They often enter the environment from industrial plants, automobile engines, incinerators and several photochemical reactions or incomplete combustion of many organic substances. In addition, several aldehydes are mostly minor compounds in foodstuffs and their occurrence can be an indication of quality deterioration, overheating, microbial fermentation and/or off-flavour. Many aldehydes have been shown to be highly cytotoxic and genotoxic due to the nucleophilic attack to amine and sulphhydryl groups of proteins, nucleic acids or related amino acids [1,2]. Consequently, these compounds cause several biological complications such as cardiovascular diseases, carcinogenesis,

mammographic dysplasia, atherosclerosis, etc. Therefore, because of the environmental, biological and industrial importance of these compounds, sensitive and selective analytical methods for their determination in air and biota are needed.

Most analytical methods for aldehyde determination are based on gas chromatography (GC) and reversed-phase LC where the investigated compounds are determined as their 2,4-dinitrophenylhydrazine derivatives [3–6]. Other GC and LC methods involve the use of thiazolidine [7], oxime [8,9] or fluorescent derivatives [10–12].

In order to improve the procedures for routine applications, it is desirable to develop a simple and direct detection method for the determination of aldehydes in complex matrices. In general, derivatization procedures increase the analysis time and risk of errors in quantification caused by partial derivatization processes and/or accidental contamination. Thus, direct determinations without time-consuming clean-up or derivatization procedures, when available with acceptable sensitivity should be preferred. In this respect,

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aldehyde biosensors based on the determination of NADH generated by the enzymatic activity of immobilized aldehyde dehydrogenase were characterized for the selective detection of several aliphatic and aromatic aldehydes without extensive sample pre-treatment and derivatisation procedures [13–15]. On the other hand, electrochemical detection following LC is a very attractive analytical method in terms of sensitivity and selectivity for the determination of many important aliphatic organic compounds characterized by the absence of pronounced chromophore and/or fluorophore groups. Interesting results regarding aldehyde determination have been published on the pulsed amperometric detection (PAD) at a gold electrode in alkaline medium [16] or under constant applied potentials at carbon modified electrodes [17,18]. In addition, pulsed electrochemical detection applied at platinum electrode substrates has been used for the determination of alcohols in acidic medium [19,20]. Recently we have demonstrated PAD at platinum electrode substrates can be useful for the sensitive detection of electroactive compounds such as aliphatic organic acids after chromatographic separations [21].

In this respect, we developed a new electroanalytical procedure for the determination of several low-molecular-weight aliphatic aldehydes without the use of any derivatisation procedure. In particular, we show that the PAD mode applied to the platinum electrode can successfully be used for the determination of saturated and unsaturated aliphatic aldehydes in acidic medium. LC was employed in order to evaluate the analytical performance of the amperometric detector for the determination of saturated and unsaturated aliphatic aldehydes in aqueous matrices.

2. Materials and methods

2.1. Chemicals

Solutions were prepared daily from analytical reagent-grade chemicals (Aldrich-Chemie) without further purification and by using distilled and deionized water. Unless otherwise specified, experiments were performed by using a 10 or 50 mM perchloric acid (HClO₄) solution as mobile phase. The mobile phase was deoxygenated, when necessary, by purging the reservoir bottle with high-purity nitrogen and successively, immediately before the introduction of the mobile phase onto the pump by an on-line degasser system Series 1050 (Hewlett Packard, Avondale, PA, USA). Air-saturated distilled and deionized water (by purging the reservoir bottle with air) was used for the preparation of not-deoxygenated mobile phase and the on-line degasser system was disconnected during the experiments.

2.2. 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 1.0 mm-diameter platinum working electrode, an Ag/AgCl combined reference electrode and a stainless steel auxiliary counter electrode were also purchased from Dionex. The flow channel is formed by the insertion of a Teflon gasket of 0.25 mm thickness between the auxiliary and working electrodes. The polycrystalline platinum working electrode, was weekly polished with 0.05 μm of alumina oxide powder on microcloth using water as the lubricant. The reference electrode was daily washed with distilled water and, when not used, stored in saturated KCl solution. The experiments were performed using an analytical pump Mod. PU-1580i (Jasco Corporation, Tokyo, Japan) equipped with a rotary injection valve Mod. 7125i (Rheodyne, Cotati, CA) with a 20 μL sample loop. A personal computer equipped with a Kontron PC Integration Pack Software (Milan, Italy) allowed acquisition and processing of chromatograms. Unless stated otherwise, the pulsed amperometric detector settings were as follows: $E_{\text{det}} = 0.40 \text{ V}$ ($t_{\text{det}} = 340 \text{ ms}$, $t_{\text{int}} = 60 \text{ ms}$), $E_{\text{ox}} = 1.40 \text{ V}$ ($t_{\text{ox}} = 120 \text{ ms}$), and $E_{\text{red}} = -0.40 \text{ V}$ ($t_{\text{red}} = 520 \text{ ms}$). Currents are measured and integrated with respect to time (t_{int}) to give a faradaic charge (coulombs) for the detection cycle.

Chromatographic separations of aliphatic aldehydes have been conducted with an Aminex HPX-87H BioRad column. The HPX-87H column (300 mm × 7.8 mm i.d.) is packed with 9-μm spherical sulphonated polystyrene-divinylbenzene co-polymer beads with 8% cross-linking, providing an ion-exchange capacity of 1.7 mmol/g.

All experiments were carried out at ambient temperature (20 ± 2 °C).

2.3. Sample preparation

The mineral water “Lilia” with low CO₂ concentration, the “Sveva” with dissolved CO₂ (Sorg. Traficante, Rionero in Vulture, Italy) and the red wine “Barbera” (Gruppo Coltiva, 2001, Modena, Italy) were purchased from a local store. Standard solutions were prepared by dissolving the filtered original samples in double-distilled water and directly injected into the column. The samples were filtered by using nitrocellulose membrane 0.45 μm pores (Millipore, Bedford, MA, USA). The peak identification of the analysed compounds was based on the retention time and was confirmed by adding authentic standard to the diluted extract of the analysed sample. The quantitative analysis of the considered analytes was performed using the area of the chromatographic peaks and the concentrations were calculated by a linear-square regression procedure using the method of standard addition. The relevant recoveries were evaluated for each analysed compound by spiking the considered real sample with stock solutions of analyte at approximate level of 40–60% of the measured content and performed duplicate assays after each addition of standard.

3. Results and discussion

3.1. Amperometric measurements and chromatographic separations

A triple-step pulsed waveform (PAD), can be used for sensitive detection of saturated and unsaturated aliphatic oxygen-containing compounds such as alcohols, acids, aldehydes, etc. in flowing stream [19–21]. In order to obtain a better optimization of the PAD waveform, the detection potential (E_{det}) and the integration time (t_{int}) were varied in consecutive runs by injections of 0.1 mM each considered aldehyde, leaving unchanged both the oxidation (E_{ox}) and reduction (E_{red}) potential values. Fig. 1 shows some relevant hydrodynamic voltammograms. It shows that all aldehydes investigated are very well detected in the range of 0.2–0.5 V versus Ag/AgCl. The detection potential of 0.4 V represents the best compromise between maximum analytical signal, minimum background contribute and good selectivity. In fact, under the same experimental conditions, the hydrodynamic voltammograms of alcohols [19–21] show a maximum of sensitivity at 0.2 V, while exhibiting for higher detection potentials a marked diminution of the amperometric signals. Thus, using a detection potential of 0.4 V the saturated and unsaturated aldehydes species can be selectively determined with acceptable sensitivity in presence of alcoholic compounds. Table 1 summarize the effect of the integration time on the background currents and charge signal for the formaldehyde, propionaldehyde and acrolein. As expected, the integration time induces a considerable increase in the charge signal of all analysed aldehydes. Nevertheless, the background signal

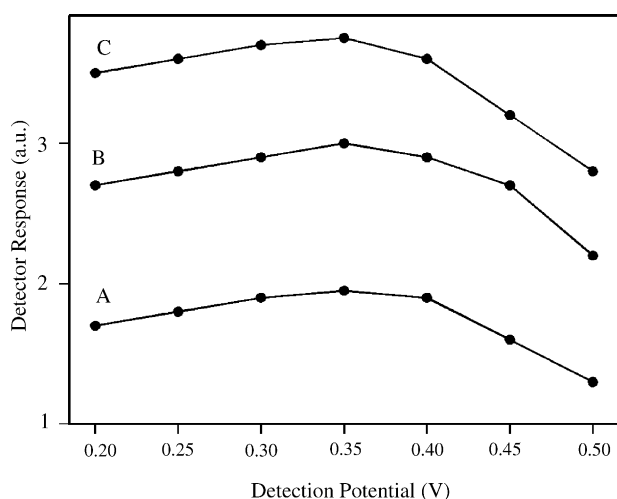


Fig. 1. Hydrodynamic voltammograms of (A) 0.1 mM formaldehyde, (B) 0.1 mM valeraldehyde and (C) 0.1 mM propionaldehyde at platinum electrode using PAD. Experimental conditions: Aminex HPX-87H column, deoxygenated 50 mM HClO_4 eluent at 0.8 mL/min. E_{det} (varying), $t_{\text{det}} = 340$ ms, $E_{\text{ox}} = 1.40$ V, $t_{\text{ox}} = 120$ ms, $E_{\text{red}} = -0.40$ V, $t_{\text{red}} = 520$ ms. The potential values are given relative to the Ag/AgCl. Integration time, 60 ms. The detector response represents the area of the chromatographic peak and is expressed as arbitrary units.

Table 1

Effect of the integration time (t_{int}) on the background (nC) and charge signal (nC) of 0.1 mM acrolein, 0.1 mM formaldehyde and 0.1 mM propionaldehyde

t_{int} (ms)	Background	Acrolein	Formaldehyde	Propionaldehyde
10	68	0.2	0.04	0.1
40	170	1.4	0.4	0.8
60	245	2.3	0.6	1.1
100	352	5.0	0.9	1.7
160	576	7.6	2.0	3.1

Experimental conditions: column, Aminex HPX-87H; mobile phase, 50 mM HClO_4 ; flow rate, 0.8 mL/min; sample loop, 20 μL ; applied waveform, $E_{\text{det}} = 0.40$ V ($t_{\text{det}} = 340$ ms), $E_{\text{ox}} = 1.40$ V ($t_{\text{ox}} = 120$ ms), and $E_{\text{red}} = -0.40$ V ($t_{\text{red}} = 520$ ms). The mobile phase was deoxygenated by purging the reservoir bottle with high-purity nitrogen and using an on-line degasser system.

increases drastically with increasing integration time. Generally, large background currents are responsible for baseline instability, poor precision and high detection limits. Thus, the selected triple-step PAD waveform with the integration time of 60 ms, was considered as optimal in terms of charge signal, background currents and acceptable baseline stability.

Acid medium is required for obtaining reproducible retention times and good column efficiency for the separation of hydroxyl-containing compounds typically accomplished via Donnan separation mechanism (ion/steric exclusion) and adsorption/partitioning schemes. In addition, in acid solutions the platinum electrodes show considerable catalytic activity towards the electrochemical oxidation of several electroactive organic molecules [16,19–23]. Therefore, the influence of the HClO_4 concentration on the retention time and electrode performance was evaluated. Thus, a standard mixture of aliphatic aldehydes eluted isocratically with mobile phases containing various concentrations of HClO_4 ranging from 5–100 mM was studied for this purpose. Table 2 summarize the relevant results. As can be seen, the retention times of the investigated aldehydes are nearly independent of the HClO_4 concentration. In addition, the peak areas of the saturated aldehydes decreased markedly with decreasing HClO_4 concentration. On the contrary, the peak area relevant to the unsaturated aldehydes such as acrolein (1-propenal) and crotonaldehyde

Table 2

Effect of the perchloric acid concentration on the peak area (PA, arbitrary units) and the retention time (RT, min), of the investigated aliphatic aldehydes separated by an HPX-87H Analytical column

	HClO_4 Concentration							
	5 mM		10 mM		50 mM		100 mM	
	PA	RT	PA	RT	PA	RT	PA	RT
Formaldehyde	0.1	9.9	0.4	9.9	1.7	9.9	2.9	9.9
Acetaldehyde	–	–	0.3	12.3	1.6	12.3	1.8	12.4
Propionaldehyde	–	–	1.4	15.1	3.7	15.2	4.4	15.2
Acrolein	15.9	20.2	18.1	20.3	24.5	20.3	26.2	20.3
Butyraldehyde	–	–	0.6	21.0	4.1	21.0	5.4	21.1
Crotonaldehyde	11.2	31.1	14.9	31.1	18.3	31.1	19.1	31.1
Valeraldehyde	–	–	0.5	33.3	3.1	33.3	4.6	33.4

Experimental conditions: as in Table 1.

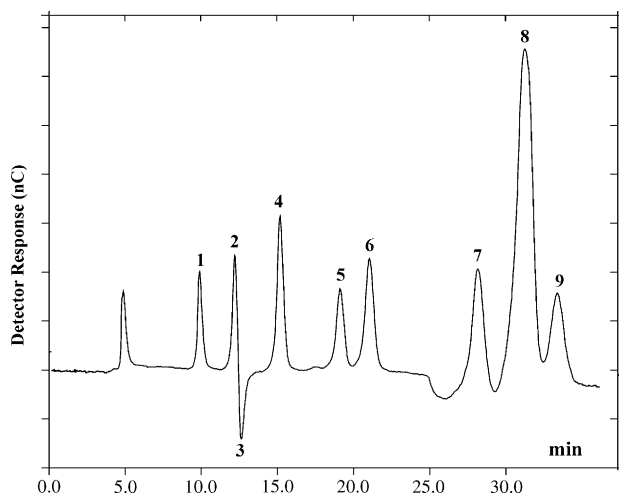


Fig. 2. Typical LC separation with pulsed amperometric detection of a mixed standard solution (100 μM each compound) of aliphatic aldehydes: (1) formaldehyde, (2) acetaldehyde, (3) oxygen, (4) propionaldehyde, (5) isobutyraldehyde, (6) butyraldehyde, (7) isovaleraldehyde, (8) crotonaldehyde and (9) valeraldehyde. Experimental conditions: column, Aminex HPX-87H; mobile phase, deoxygenated 50 mM HClO_4 ; flow rate, 0.8 mL/min; sample loop, 20 μL ; waveform, $E_{\text{det}} = 0.4$ V, $t_{\text{det}} = 340$ ms, $t_{\text{int}} = 60$ ms, $E_{\text{ox}} = 1.40$ V, $t_{\text{ox}} = 120$ ms, $E_{\text{red}} = -0.40$ V, $t_{\text{red}} = 520$ ms.

(1-butenal) tend to decrease slightly with the diminution of the HClO_4 concentration.

Fig. 2 shows a chromatogram of a standard mixture of some representative aldehydes, separated with an HPX-87H column with 50 mM HClO_4 as mobile phase at 0.8 mL/min of flow rate. A satisfactory separation of the aldehydes was completed in 35–40 min. The negative peak is due to the imbalance in the oxygen concentration between the mobile phase and the injected sample [19]. The limits of detection (LOD), dynamic linear ranges and repeatability are summarized in Table 3. The LODs, determined at a signal-to-noise ratio equal to 3, are between 0.2 μM for acrolein and 2.5 μM for valeraldehyde. These LODs for the short-chain aliphatic aldehydes are similar or slightly higher than those obtained with other LC methods based on derivatization procedures [11,18,24–26]. Nevertheless, it is interesting to underline that in this case the determination of aliphatic aldehydes in complex matrices is possible without time-consuming sample pre-treatment like distillation, derivatization or solid-phase extraction procedures.

Regression analysis of calibration data indicates that responses are linear over about two orders of magnitude above the LOD, with correlation coefficients >0.990 .

In order to choose the best experimental conditions, the effect of the oxygen concentration in the mobile phase was evaluated. Fig. 3 compares the LC traces of the standard mixture of aldehydes obtained in a nitrogen-purged mobile phase (A) and with an eluent containing dissolved oxygen (B). Fig. 3B clearly demonstrates that the not-deoxygenated mobile phase markedly inhibits the detection of saturated aliphatic aldehydes. Similar inhibition effects were observed when saturated aliphatic alcohols such as methanol, ethanol or propanol or

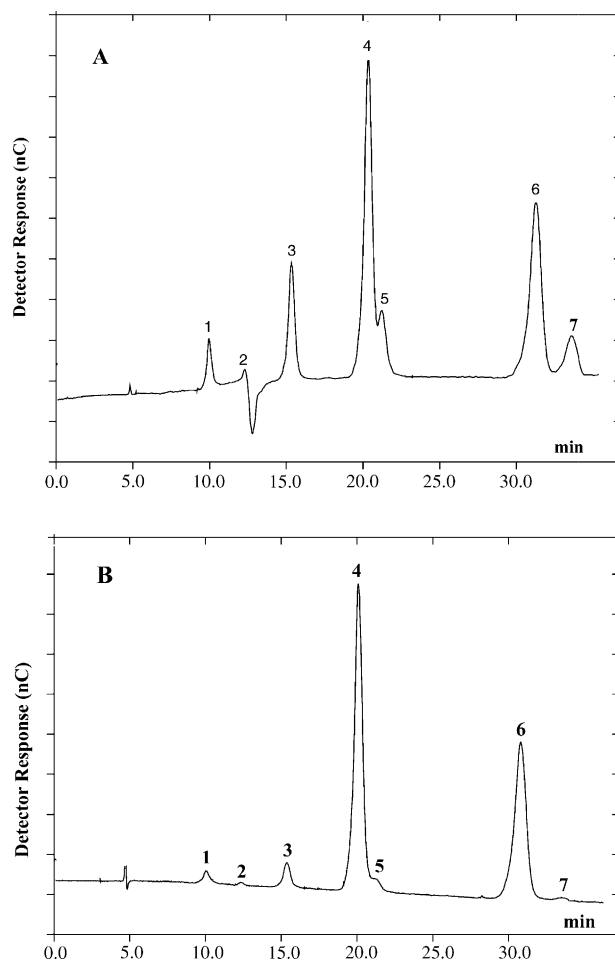


Fig. 3. Effect of the dissolved oxygen in the mobile phase. (A) Mobile phase, deoxygenated 50 mM HClO_4 solution and (B) mobile phase, not-deoxygenated 50 mM HClO_4 solution: (1) formaldehyde, (2) acetaldehyde, (3) propionaldehyde, (4) acrolein, (5) butyraldehyde, (6) crotonaldehyde and (7) valeraldehyde. Other experimental conditions as in Fig. 2.

organic acids such as citric, formic or acetic acid were analysed. On the contrary, the amperometric signal of the unsaturated aldehydes (i.e., acrolein and crotonaldehyde) is nearly independent of the oxygen concentration level in the mobile phase. This behaviour may well be due to the exclusion of the saturated aldehydes from the electrode surface caused by a favourable and strong adsorption of dissolved oxygen or, more probably, its reduction products (i.e., adsorbed hydroxyl species). In this respect, when using a mobile phase containing dissolved oxygen and a HClO_4 concentration ≤ 10 mM, saturated low-molecular-weight oxygen-containing analytes such as aldehydes, alcohols and several acids (i.e., citric, tartaric, acetic, etc.) at relatively high concentrations (about 30 mM of each compound) did not produce any significant amperometric signal in the LC run. Thus, the marked inhibition effect of the dissolved oxygen on the electrochemical signal of the saturated hydroxyl-containing compounds can be considered as an analytical strategy for the determination of unsaturated aldehydes in the presence of other saturated oxygen-containing compounds with enhanced selectivity.

Table 3
Quantitative analytical results of some investigated aliphatic aldehydes by liquid chromatography with electrochemical detection

	LOD (μM)	Linear range (μM)	$S = a + xC$ (mM)		R.S.D.(%)
			a	x	
Mobile phase: deoxygenated 50 mM HClO_4					
Formaldehyde	0.5	1.0–200	0.016 ± 0.001	1.96 ± 0.08	4.4
Acetaldehyde	0.9	1.4–200	0.022 ± 0.001	1.7 ± 0.1	6.5
Propionaldehyde	0.6	1.2–150	0.027 ± 0.002	3.65 ± 0.05	3.2
Acrolein	0.2	0.7–250	0.013 ± 0.002	24.7 ± 0.03	3.9
Isobutyraldehyde	0.9	1.4–200	0.022 ± 0.004	3.9 ± 0.04	4.7
Butyraldehyde	0.8	1.7–200	0.025 ± 0.004	4.2 ± 0.03	4.5
Isovaleraldehyde	1.5	2.1–200	0.042 ± 0.003	3.4 ± 0.04	5.2
Crotonaldehyde	0.6	0.9–250	0.015 ± 0.002	18.3 ± 0.03	4.2
Valeraldehyde	2.5	2.9–200	0.047 ± 0.004	3.2 ± 0.03	4.7
Mobile phase: not deoxygenated 10 mM HClO_4					
Acrolein	0.3	0.6–250	0.015 ± 0.004	22.1 ± 0.05	4.2
Crotonaldehyde	0.6	1.1–250	0.019 ± 0.005	16.5 ± 0.04	4.5

Experimental conditions: HPX-87H analytical column (300 mm \times 7.8 mm i.d.). LOD determined for $S/N = 3$ from the lowest injected concentration of analyte. The repeatability was expressed as percent relative standard deviation (R.S.D.%) and was obtained from four replicate consecutive chromatographic injections of about 0.1 mM each considered analyte. The correlation coefficients determined in the linear range were always >0.990 . Other experimental conditions as in Table 2.

3.2. Analysis of real samples

The real samples were chosen as example for the determination of saturated aldehydes and unsaturated aldehydes, using deoxygenated 50 mM HClO_4 and not-deoxygenated 10 mM HClO_4 as mobile phases, respectively.

The major sources of short-chain aliphatic aldehydes found in drinking water are from the discharge of industrial wastes, oxidative water treatment and bottles used for storing mineral water [27–29]. A representative chromatogram of a spiked sample of mineral water with formaldehyde, acetaldehyde and propionaldehyde, is shown in Fig. 4. The examined aldehydes are well resolved and detected with appreciable sensitivity. Good reproducibility was obtained for chromatographic separations on repetitive injections and no deterioration of the electrochemical signal was apparent.

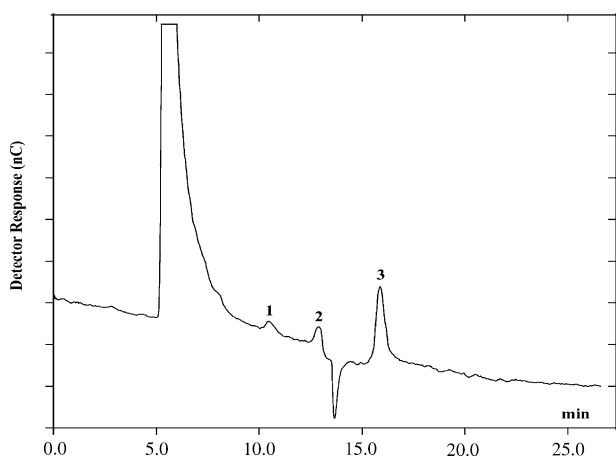


Fig. 4. Chromatogram of a mineral water (Lilia) spiked with 7.0, 10.4 and 14.7 $\mu\text{mol/L}$ of formaldehyde, acetaldehyde and propionaldehyde, respectively. Other experimental conditions as in Fig. 2.

The relevant analytical results are summarised in Table 4. Analyte recoveries ranged from 81% for acetaldehyde to 92% for propionaldehyde.

The main aliphatic aldehydes found in wine are those that do not reduce to alcohols during natural aging processes and are generally formed during alcoholic fermentation [30,31]. Fig. 5 shows the chromatogram of a red wine, obtained, using non-deoxygenated 10 mM HClO_4 as mobile phase. As expected under these conditions, although the wine sample represents a complex matrix, acrolein can be determined with an excellent selectivity level. In addition, the good mean recoveries (94–97%) confirms the validity of this analytical strategy for the quantitative determination of unsaturated aldehydes such as acrolein in wines and alcoholic beverages.

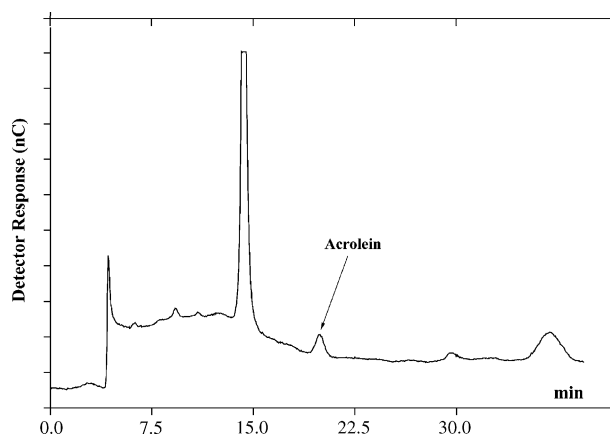


Fig. 5. Chromatogram of unspiked sample of red wine purchased from a local producer. The red wine was filtered by using nitrocellulose membrane 0.45 μm pores (Millipore, Bedford, MA, USA), diluted 1:50 with the mobile phase and injected into the column. Mobile phase, not-deoxygenated 10 mM HClO_4 solution. Other experimental conditions as in Fig. 2.

Table 4
Analytical determination of some aliphatic aldehydes in mineral water and red wines

Analyte	Added ($\mu\text{mol/L}$)	Found ($\mu\text{mol/L}$)	Recovery (%)
Sample: mineral water "Lilia" ^a			
Formaldehyde	–	nd	–
	7.0	6.3	90
Acetaldehyde	–	nd	–
	10.4	8.5	82
Propionaldehyde	–	nd	–
	14.7	13.5	92
Sample: mineral water "Sveva" ^a			
Formaldehyde	–	nd	–
	6.8	6.5	96
Acetaldehyde	–	nd	–
	9.1	7.7	85
Propionaldehyde	–	nd	–
	9.7	9.0	93
Sample: red wine "local product" ^b			
Acrolein	–	6.3	–
	8.5	13.5	91
Sample: red wine "Barbera" ^b			
Acrolein	–	nd	–
	16.8	15.7	93

Experimental conditions as in Table 2.

^a Chromatographic conditions: mobile phase, 50 Mm HClO₄ deoxygenated eluent.

^b Chromatographic conditions: mobile phase, 10 Mm HClO₄ not deoxygenated eluent.

4. Conclusions

A direct, sensitive and reproducible amperometric detection of numerous low-chain of saturated and unsaturated aliphatic aldehydes at platinum electrodes in acidic medium is made possible using an optimised multi-step potential–time waveform. An analytical method based on a mixed-mode of chromatographic separation with pulsed electrochemical detection of some aliphatic aldehydes, without any extraction, derivatization and sample cleanup procedures, was developed. The influence of the HClO₄ concentration on the retention time and electrode performance in terms of charge signal was evaluated. The peak areas of the saturated aldehydes decreased markedly with decreasing HClO₄ concentra-

tion, while the retention times are nearly independent of the acid concentration. In order to choose the best amperometric conditions, the effect of dissolved oxygen in the mobile phase was also evaluated. The presence of dissolved oxygen in the eluent markedly inhibits the detection of saturated aliphatic aldehydes, while the amperometric signal of the unsaturated aldehydes (i.e., acrolein and crotonaldehyde) remains practically unchanged. Thus, the level of oxygen concentration in the mobile phase can be considered as important experimental variables in order to improve sensitivity and selectivity for the routine determination of aldehydes in real matrices. Good separations of investigated aliphatic aldehydes were completed in 35–40 min of chromatographic run with detection limits and dynamic linear range comparable or slightly greater than those obtained with other chromatographic methods based on the derivatization procedures. This proposed method was tested for the determination of some aliphatic aldehydes in beverage samples such as mineral water and red wine. It can be applied to other real matrices. Work is in progress in order to evaluate the analytical potentiality of the proposed method for the determination of aldehydes in other real contexts such as environment, foods and biological samples.

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References

- [1] H.D. Hoberman, R.C.S. George, J. Biochem. Toxicol. 3 (1988) 105.
- [2] V.L. Wilson, P.G. Foiles, F.L. Chung, A.C. Povey, A.A. Frank, C.C. Harris, Carcinogenesis 12 (1991) 1483.
- [3] M. Darlene, P. Persson, G. Skarping, J. Chromatogr. 626 (1992) 284.
- [4] D. Grosjean, K. Fung, Anal. Chem. 54 (1982) 1221.
- [5] H. Nishikawa, T. Sakai, J. Chromatogr. A 710 (1995) 159.
- [6] E. Koivusalmi, E. Haatainen, A. Root, Anal. Chem. 71 (1999) 86.
- [7] H. Kataoka, T. Kondo, A. Sumida, Anal. Chim. Acta 358 (1998) 269.
- [8] V. Jain, D. Thielen, J. Chromatogr. A 709 (1995) 387.
- [9] P. Vesely, L. Lusk, G. Basarova, J. Seabrooks, D. Ryder, J. Agric. Food Chem. 51 (2003) 6941.
- [10] B.E. Miller, N.D. Danielson, Anal. Chem. 60 (1988) 622.
- [11] A.N. Gachanja, S.W. Lewis, P.J. Worsfold, J. Chromatogr. A 704 (1995) 329.
- [12] R. Yang, K. Li, F. Liu, N. Li, F. Zhao, W. Chan, Anal. Chem. 75 (2003) 3908.
- [13] T. Noguier, J.L. Marty, Enzyme Microb. Technol. 17 (1995) 453.
- [14] F. Pariente, E. Lorenzo, F. Tobalina, H.D. Abruña, Anal. Chem. 67 (1995) 3936.
- [15] J. Schultheiss, D. Jensen, R. Galensa, J. Chromatogr. A 880 (2000) 233.
- [16] E. Le Fur, J.-M. Meunier, P.X. Etievant, J. Agric. Food Chem. 42 (1994) 2760.
- [17] K.E. Liu, H.D. Abruña, Anal. Chem. 61 (1989) 2599.
- [18] T.R.I. Cataldi, C. Campa, D. Centonze, Anal. Chem. 67 (1995) 3740.

- [19] W.R. LaCourse, D.C. Johnson, M.A. Rey, R.W. Slingsby, *Anal. Chem.* 63 (1991) 134.
- [20] E. Le Fur, P.X. Etievant, J.-M. Meunier, *J. Agric. Food Chem.* 42 (1994) 320.
- [21] I.G. Casella, M. Gatta, *J. Agric. Food Chem.* 50 (2002) 23.
- [22] D.C. Johnson, W.R. LaCourse, *Electroanalysis* 4 (1992) 367.
- [23] C. Lamy, E.M. Belgsir, J.-M. Lèger, *J. Appl. Electrochem.* 31 (2001) 799.
- [24] K. Takami, K. Kuwata, A. Sugimae, M. Nakamoto, *Anal. Chem.* 57 (1985) 243.
- [25] D.L. Du Val, M. Rogers, J.S. Fritz, *Anal. Chem.* 57 (1985) 1583.
- [26] R. Wu, L.B. White, *J. Chromatogr. A* 692 (1995) 1.
- [27] K.L. Froese, A. Wolanski, S.E. Hrudey, *Wat. Res.* 33 (1999) 1355.
- [28] B. Cancho, F. Ventura, M.T. Galceran, *J. Chromatogr. A* 943 (2001) 1.
- [29] J. Nawrocki, A. Dabrowska, A. Borcz, *Wat. Res.* 36 (2002) 4893.
- [30] I.D. Morton, A. MacCleod, *J. Food Flavours. Part B: The Flavour of Beverages*, Elsevier B.V., Amsterdam, 1986.
- [31] G. Sichei, *Industrie Agrarie: Enologica, Lattiero-Casearia-Olearia*, second ed., HOEPLI ed., 1994, Milano.