Talanta 96 (2012) 223–229

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Determination of lipoic acid by flow-injection and high-performance liquid chromatography with chemiluminescence detection

E. Wołyniec*, J. Karpińska, S. Łosiewska, M. Turkowicz, J. Klimczuk, A. Kojło

Institute of Chemistry, University of Białystok, Białystok, Poland

ARTICLE INFO

Article history:
Available online 15 March 2012

Keywords:
Flow-injection
Chemiluminescence
Lipoic acid
Pharmaceutical preparation
Food products

ABSTRACT

A new flow-injection (FI) and high performance liquid chromatography (HPLC) with chemiluminescence detection method has been proposed for the determination of α-lipoic acid (LA). The assay is based on the measurement of chemiluminescence (CL) produced during the reaction of α-lipoic acid with potassium permanganate in a sodium hexametaphosphate medium (pH 3). This reaction is accompanied by a weak CL, which is greatly increased in the presence of a formaldehyde solution. The proposed FI method allows the determination of LA over the range: 0.5–20 μg mL^{-1} with LOD 4 × 10^{-3} μg mL^{-1}. An introduction of HPLC into the flow manifold improves selectivity of the method and allows the determination of LA in a complex sample. The chromatographic linear range is 2.5–30 μg mL^{-1} with LOD 1.774 μg mL^{-1}. Chromatographic separation was achieved by isocratic elution (acetonitrile/potassium dihydrogen phosphate, pH 3, adjusted with phosphoric acid): 30/70 using a Cosmosil 5C_{18}-MS-II (4.6 mm × 150 mm I.D.) column at a flow rate of 1.0 mL min^{-1}. The presented methods were utilized to determine the α-lipoic acid content in "*Alfa-lipoic acid*" capsules and in food products.

1. Introduction

Lipoic acid (LA, 1,2-dithiolane-3-pentanoic acid or 6,8-thioctic acid, Fig. 1) is a natural ubiquitous compound which is distributed in every cell of all eukaryotic and prokaryotic organisms, microorganisms, plants, animals and humans [1]. Being soluble in water and fats, it is widely distributed both in cellular membranes and the cytosol. Due to this property, it acts as the connector between lipophylic (tocopherol, coenzyme Q10) and hydrophilic antioxidants (vitamin C, glutathione) [1,2]. It is essential for many processes such as oxidative decarboxylation of pyruvate to acetyl-CoA [1,2]. LA is reduced in mitochondria to its thiol form, dihydrolipoic acid (DHLA). It retains powerful antioxidant properties in both forms: reduced (dihydrolipoic acid) and oxidised (lipoic acid). The redox potential of the DHLA/LA couple is −320 mV [3]. It is involved in the regeneration of exogenous and endogenous antioxidants, such as vitamin C, vitamin E and glutathione, the chelation of metal ions and the repair of oxidized proteins [2,4]. LA is known as a scavenger of hydroxyl radicals, singlet oxygen, hydrogen peroxide, hypochlorous acid, peroxynitrite and nitric acid. DHLA quenches peroxyl and superoxide radicals [4]. Lipoic acid exists in biological systems in three forms: free α-lipoic acid, weakly protein bound LA by hydrogen bonds, and strongly protein bound LA (lipoyllysine) via covalent bounds [1]. There are no recommended daily consumption level of LA [5], but clinical data points to the fact that supplementation with LA supports the therapy of arteriosclerosis, diabetes, cataracts, liver diseases and AIDS [1,2]. LA has been successfully used as a therapeutic agent for the treatment of diabetes, neurodegenerative disorders, mitochondrial cytopathies, radiation damage [1,2], in cases of poisoning with mercury, arsenic and cadmium [1,2] and human immunodeficiency virus (HIV) infection [6]. Moreover, lipoic acid has been widely reported to induce apoptosis in various cancer cell lines [7]. Studies on the application of LA in geriatrics seem to be very promising [1,2]. LA content in food varies over a wide range. Animal products, especially red meat, contain 0.25–2.36 μg LA/g [1]. Plant food is characterized by a higher level of LA, e.g. the LA level in potatoes varies from 1.5 μg LA/g up to 4.2 μg LA/g [8].

Numerous chromatographic methods with electrochemical, MS or fluorescent detections have been reported for the determination of lipoic acid in pharmaceutical preparations and dietary supplements [9–13], biological fluids [3,14–21] and food samples [8,21]. The assay of lipoic acid in food or biological samples is not an easy analytical task. As α-lipoic acid mainly exists in biological samples in bound forms [8], an analyst should apply an isolation procedure which is efficient for the extraction free fraction or total content of the analyte. Durrani et al. [8] proposed a chromatographic determination method of free α-lipoic acid in different food matrices: eggs, mayonnaise, green peas and potatoes. The authors obtained the best isolation after ultrasonification of the

* Corresponding author. Tel.: +48 85 745 78 17; fax: +48 85 747 01 13.
E-mail address: elaw@uwb.edu.pl (E. Wołyniec).

0039-9140/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2012.02.064

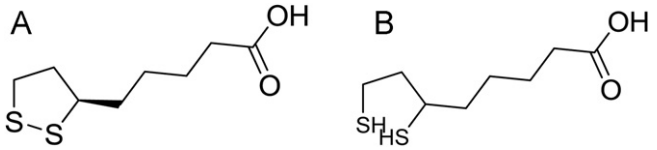

Fig. 1. Structures of lipoic (A) and dihydrolipoic acids (B).

samples for 1 h in 0.5% of glacial acetic acid in methanol at room temperature. Because the extracts of the samples were transparent, they had only to be centrifuged before injection into the HPLC system. Separation of the total contents of α-lipoic acid requires the use of a hydrolisation step of the samples. For this purpose, acidic hydrolysis with the use of inorganic or organic acids was proposed [21]. Mattulat and Baltes [21] have released protein-bound lipoic acid by enzymatic hydrolysis with ε-lipoyllysine prior to its assay using HPLC and GC/MS methods. Nowadays, lipoic acid is used in the therapy of some diseases as well as dietary supplements [1,2], thus, there are some methods devoted to its determination in pharmaceuticals. Corduneanu et al. described the procedure of the determination of lipoic acid in dietary supplements using cyclic, differential pulse and square wave voltammetry with a glass carbon electrode [22]. Abbas and Radwan have proposed a selective sensor, based on a mercuric lipoate ion-pair as a membrane carrier for direct potentiometric and flow-injection determination of LA in pharmaceutical preparations and urine [23]. Ziyatdinova et al. have developed procedures for the determination of lipoic acid by voltammetry and coulometric titration with electrogenerated halogens and a biamperometric indication of the titration end-point in model solutions [24]. Electrodes modified with carbon nanotubes were used for the electrochemical determination of lipoic acid in pharmaceuticals [25].

Chemiluminescence detection has become a useful tool in food analysis due to its low cost, high sensitivity and simplicity [26]. Many chemiluminescence reagents have been investigated but only a limited number: luminol [27], diaryloxalates, tris(2,2′-bipyridyl)ruthenium(III) and potassium permanganate have been widely used in analytical chemistry. Chemiluminescence produced during reduction of acidic potassium permanganate has been used to determine a variety of compounds such as: pharmaceuticals, antioxidants, pesticides, environmental pollutants, food and clinical samples [28]. Different applications of this reagent in chemical analysis have also been reviewed by Hindson and Barnett [29]. As chemiluminescent reactions proceed very quickly and the emission of luminescence lasts very briefly, it requires detection at the moment of mixing reagents. The application of flow methods allows fast and reproducible merging of the reagents and the sample in the mixing cell, which is mounted close to the detector. The main disadvantage of chemiluminescent methods is their low selectivity [26]. This drawback can be eliminated by the implementation of a separation unit into the flow system which allows an increase in the selectivity of determination [30]. Fluorescent or electrochemical detectors are used as conventional detectors for liquid chromatographic assays, but chemiluminescence detectors surpass them. They allow to obtain lower detection limits by a $10–10^2$ order of magnitude than the conventional detectors [26]. As luminescence detectors do not require external sources of light, they are characterized by the lack of background. The only source of background is the dark current of the photomultiplier. These features allow to reach a high relation of signal to background and this leads to a significant increase in the sensitivity of determination and in the lowering of the limit of detection.

In the current study, a flow injection chemiluminescence (FI-CL) method for the determination of lipoic acid is proposed. The oxidation of lipoic acid in the reaction with potassium permanganate in sodium hexametaphosphate medium (pH 3) is accompanied by a weak chemiluminescence, which is greatly enhanced by formaldehyde. Next, the elaborated FI-CL method has been successfully implemented into the HPLC system and applied to the determination of free α-lipoic acid in some food stuffs.

2. Experimental

2.1. Apparatus

The flow-injection set-up shown schematically in Fig. 2, consists of a Gilson Miniplus 2 peristaltic pump, a Model 5021 rotary injection valve (Rheodyne, Cotati, CA) and a flow luminometer (KSP, Poland) with a coiled PTFE tube of 1 mm I.D. (length of 25.0 cm in six windings). The photomultiplier was operated at 1100 V, and the detector response was recorded on a 386-series personal computer with KSP software. The flow system was made of PTFE tubing of 0.8-mm I.D.

2.1.1. Utilized chromatographic system

A Varian 920-LC set consisting of a quaternary gradient pump, an autosampler with a 50 μL sample loop, a UV–Vis detector and Galaxy chromatographic software were used for extracts analysis. Chromatographic separations were performed with a Cosmosil $5C_{18}$-MS-II (4.6 mm × 150 mm I.D.) column (150 mm × 4.6 mm I.D.). The flow rate of the mobile phase (acetonitrile: 0.05 M KH_2PO_4 (pH 3) = 30:70, v/v) was set at 1 mL min^{-1}. The UV–Vis detector was set at 210 nm.

Chemiluminescence spectra were performed using a Hitachi F-7000 fluorescence spectrophotometer.

2.2. Chemicals

α-Lipoic acid (LA) and sodium hexametaphosphate (SMP) were obtained from Sigma–Aldrich (Steinheim, Germany), potassium permanganate, formaldehyde and ortho-phosphoric acid from POCh (Gliwice, Poland). Acetonitrile was purchased from J.T. Baker (Germany). All the reagents used were of an analytical or a chromatographic grade.

The stock standard solution of lipoic acid was prepared by dissolving 0.100 g of the substance in methanol. The oxidizing solution of potassium permanganate was prepared daily by dissolving 0.0395 g of the substance in a 250 mL 1% solution of sodium hexametaphosphate. The solution of 1% sodium hexametaphosphate was prepared by dissolving 10 g of the substance in 1 L bidistilled deionised water and adjusted to pH 3 with phosphoric acid. The solution of 3×10^{-1} mol L^{-1} formaldehyde was prepared by diluting 5.7 mL of the concentrated solution in a 250 mL 1% solution of sodium hexametaphosphate. Working standard solutions containing lipoic acid were prepared by diluting a concentrated standard solution of LA (1000 μg mL^{-1}) with 1% of a sodium hexametaphosphate solution.

2.2.1. Sample preparation for pharmaceuticals

"Alpha-lipoic acid" (A-Z Medica, Poland) capsules with a certified amount of 100 mg of LA were used for flow analysis. Each capsule contains maltodextrin. The contents of the capsule were transferred into a beaker and diluted with 20 mL of methanol. Next, the obtained solution was filtrated and the clean filtrate was transferred to a 100-mL calibrated flask and made up to the mark with methanol. After that, an appropriate volume of this solution was diluted with MilliQ water to adjust the concentration of analyte to the linear calibration range. The obtained working solution of the capsule was then directly injected into the FI system.

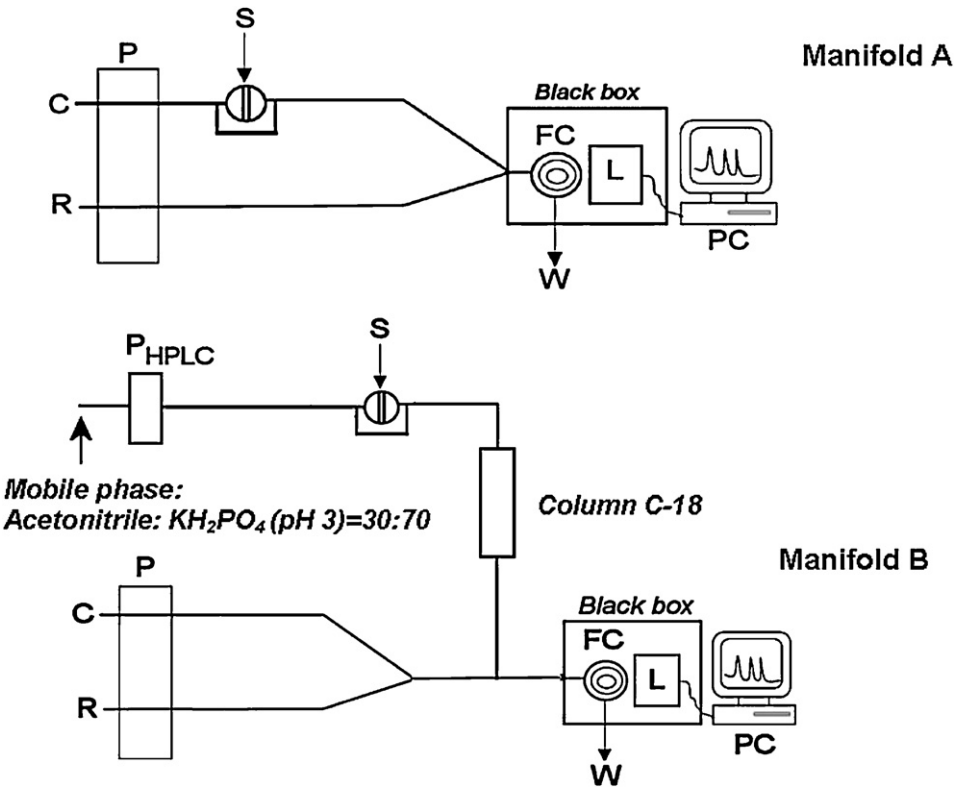

Fig. 2. Schematic diagram of the optimized flow-injection system used for the determination of lipoic acid: C, carrier stream, formaldehyde in sodium hexametaphosphate medium (pH 3) solution; R, reagent stream, potassium permanganate solution; P, peristaltic pump; S, sample injection point; FC, flow cell; L, flow-luminometer; PC, computer; W, waste.

2.2.2. Food samples

Different samples of potatoes, tomatoes and broccoli were obtained from a local market. The fresh potatoes were peeled, cut into cubes, crushed and homogenised. The other food samples were washed, dried under folded tissue paper, crushed and homogenised. Next, 0.5 g of homogenisate was weighed into 12-mL test tubes, dissolved in 3 mL of acetonitrile and shaken for 5 min at room temperature. The mixture was then centrifuged at 4000 rpm for 10 min. The supernatant solution was transferred into 5-mL vials. Next, its volume was reduced to 1-mL under a gently applied stream of nitrogen. The prepared extracts were then transferred into autosampler vials and 50 μL was injected into the HPLC-system. The extraction procedure was based on work of Chng et al. [20], but conditions for extraction were modified. Average recovery of lipoic acid was calculated as 107.18% ± 17.01.

3. Results and discussion

3.1. Flow method

3.1.1. Selection of oxidant

Preliminary experiments were designed to examine a range of oxidants for the possible generation of CL emission with lipoic acid. Various oxidizing systems were tested in the assembly of Fig. 2A: 1×10^{-3} mol L^{-1} solutions $KMnO_4$, $K_2Cr_2O_7$, $KBrO_3$, 2×10^{-2} mol L^{-1} $Ce(SO_4)_2$ (all oxidants were dissolved in 1 mol L^{-1} H_2SO_4) and 1×10^{-2} mol L^{-1} solutions NBS (N-bromosuccinimide), H_2O_2, 1×10^{-3} mol L^{-1} $K_3[Fe(CN)_6]$ in 1×10^{-1} mol L^{-1} NaOH. It was found that a very weak CL emission was only produced from the reaction between lipoic acid and acidic $KMnO_4$, but in the case of when an oxidant was dissolved in 1% of the sodium hexametaphosphate solution. Therefore, it was decided to investigate the effect of a few potential sensitizers on the CL emission produced during the oxidation of lipoic acid. The sensitizers, including 2,7-dichlorofluoresceine, fluoresceine, rhodamine B and rhodamine 6G, calcein and formaldehyde, were tested. Formaldehyde gave the greatest CL enhancement intensity and it was selected as a sensitizer for further investigation.

In order to explain the possible mechanism of the chemiluminescence reaction, the chemiluminescence spectra of the reactions of lipoic acid and $KMnO_4$ products generated with and without the addition of formaldehyde and reaction of $KMnO_4$ with HCHO were also recorded. All spectra shown in Fig. 3 have a maximum emission at 620 ± 4 nm, but their intensities are different. The emission peak appeared in a mixed system: $KMnO_4$–LA–HCHO and is

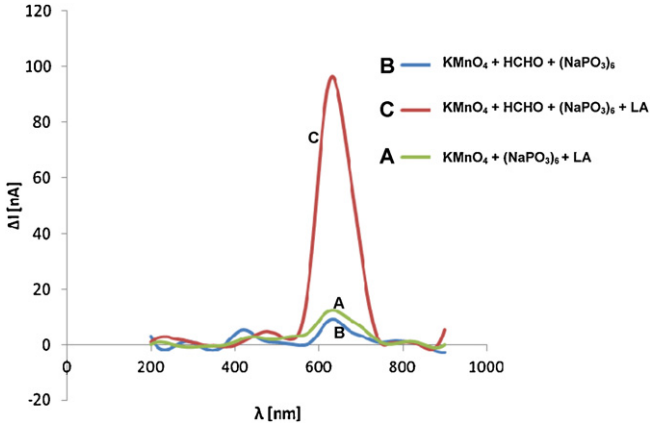

Fig. 3. Chemiluminescence spectra from the reactions of potassium permanganate (5×10^{-4} mol L^{-1}) in sodium hexametaphosphate (1%, m/v; adjusted to pH 3 with phosphoric acid) with lipoic acid (10 μg mL^{-1}) (A); formaldehyde (0.3 mol L^{-1}) (B); lipoic acid (10 μg mL^{-1}) and formaldehyde (0.3 mol L^{-1}) (C).

Table 1
Parameters of optimized FI system for determination of lipoic acid.

Optimized parameter	Studied range	Optimal value
Concentration of $KMnO_4$ [10^{-3} mol L^{-1}]	0.1–2	1
Concentration of $(NaPO_3)_6$ [%]	0.25–1	1
Concentration of HCHO [mol L^{-1}]	0.2–0.6	0.3
Flow rate [mL min^{-1}]	2.4–6.4	5.2
Sample volume [μL]	200–900	700
Photomultiplier voltage [V]	700–1100	1100

about 10-times greater than the peak for the system $KMnO_4$–LA. On the other hand, formaldehyde also reacts with $KMnO_4$, but this reaction is accompanied by a weak chemiluminescence. The emitter of chemiluminescence was probably an excited manganese(II) species of unknown constitution [31]. When the reaction occurs in the presence of formaldehyde, energy is transferred from excited manganese(II) to formaldehyde to form HCHO in an electronically excited state, which emits more light, because this compound has a higher quantum efficiency. All reactions were observed only in cases of when the reagents were dissolved in sodium hexametaphosphate adjusted to pH 3 with phosphoric acid. Sodium hexametaphosphate plays the essential role in these reactions, and is often used in a chemiluminescence reaction of potassium permanganate [32].

3.1.2. Optimization of chemical and instrumental variables

Optimization of chemical (concentration of reagents: $KMnO_4$, HCHO, $(NaPO_3)_6$) and instrumental variables (the volume of injected sample, flow-rate, photomultiplier voltage) was performed changing one variable in every turn and keeping the others at their optimum values. All of them were optimized mainly with respect to the sensitivity, on the basis of the maximum of the CL emission intensity. The ranges of the various experimental variables tested and the optimum values finally chosen are summarized in Table 1. Fig. 4 shows data obtained during optimisation process of chemical and instrumental variables. The optimum potassium permanganate and formaldehyde concentrations were found to be 1.1×10^{-3} and 3×10^{-1} mol L^{-1}, respectively. It was observed that at concentrations higher and lower than the optimal ones, there was a sharp decrease in the CL (Fig. 4A and B). Potassium permanganate reacts with lipoic acid to produce light emission in the sodium hexametaphosphate medium (pH 3). Therefore, the influence of the concentration of sodium hexametaphosphate on the CL reaction was examined. As can be seen (Fig. 4C), the CL signal increased with raising the sodium hexametaphosphate concentration up to 1%. Greater concentrations of sodium hexametaphosphate were difficult to prepare because its solubility in water is limited, so a 1% concentration of $(NaPO_3)_6$ was selected.

The flow rate is a critical parameter in the CL-FIA system. If it is too high or too low, the maximum emission from excited molecules can appear before or after the flow cell. The CL signal markedly increased (Fig. 4D) with the increase of a flow rate up to 2.6 mL min^{-1} per channel (5.2 mL min^{-1} total final flow rate), so this flow rate was selected.

3.1.3. The analytical performance of the developed FIA-CL system

The performed method has been evaluated under optimal detection conditions by the determination of the linearity, detection limit, precision, reproducibility and sample throughput. The calibration curve CL intensity (I, nA) versus LA concentration (C, μg mL^{-1}) shows good linearity in the concentration range: 0.05–20 μg mL^{-1}. The linear correlation coefficient is 0.9998, the limit of detection (defined as a signal-to-noise ratio of 3:1) is 0.004 μg mL^{-1}. The precision of method has been evaluated by the analysis of 20 replicates of the sample containing 10 μg mL^{-1} (R.S.D. = 0.50%). The sampling rate is 95 samples h^{-1}. The method is characterised by good day-to-day reproducibility expressed as relative standard deviation of calibration graphs recorded over three different days (R.S.D. = 3.34%). The results obtained under the optimum experimental conditions described above are listed in Table 2.

Table 2
Analytical characteristics of the determination of lipoic acid by the proposed FI-CL method.

Linear range (μg mL^{-1})	0.05–20
Calibration equation[a] $I = (a \pm SD)C + (b \pm SD)$	$I = (287.1 \pm 2.9)C + (750.6 \pm 87.5)$
Correlation coefficient	0.9998
Detection limit[b] (μg mL^{-1})	0.004
Repeatability R.S.D. (%), $n = 20$	0.5
The day-to-day reproducibility R.S.D. (%)	3.3
Sample throughput (samples/h)	95

[a] I: CL intensity (nA); C: concentration of lipoic acid (μg mL^{-1}).
[b] Concentration of lipoic acid that generates signal equal to the blank plus three times standard deviation.

3.1.4. Application to real samples

To demonstrate the applicability of the developed FIA-CL system for the determination of lipoic acid, it was applied to the determination of lipoic acid in a commercially available pharmaceutical product "*Alfa-lipoic acid*". The obtained results are in agreement with the declared amount (100 mg in a capsule). The relative error in comparison with the declared amount is 1.25%. Recovery of lipoic acid from the pharmaceutical samples was satisfactory (Table 3).

3.2. HPLC method with chemiluminescence detection

The chemiluminescence detection of LA described above is characterised by great sensitivity, which allows us to use it for LA determination in food samples. As the chemiluminescence measurement is characterised by low selectivity, an intense separation step of analyte is necessary before analysis. The sample preparation procedure can be greatly simplified if the FI-CL system will be used as a detection technique after LC separation. The manifold B (Fig. 2) was employed for chromatographic separation and determination of the analyte. It is known that introduction of organic solvents into the reaction system suppress emission of chemiluminescence but a reduction of their amount in the mobile phase resulted in a longer retention time of the analyte, so a combination of chromatographic separation with chemiluminescence detection required a compromise between an acceptable decrease in chemiluminescence intensity and an extension of retention time. For this purpose, many chromatographic systems were examined. Among them, the modified method of Teichert et al. [14] appeared to be compatible with post column reaction. The retention time of lipoic acid recorded in these conditions was 16.7 ± 0.1 min. It was found that an introduction into the flow system of a stream composed of 30% of acetonitrile and 70% of KH_2PO_4 adjusted to pH 3 with phosphoric acid, causes an increase of selectivity and an acceptable decrease in the intensity of emitted chemiluminescence. A 50 μL sample loop was used for HPLC-CL determination of LA.

A new calibration graph was recorded using integrated HPLC-FIA-CL system. For this purpose chromatograms were recorded and a new graph of CL intensity of LA peaks versus concentration of the standards was plotted. In order to minimise the influence of the natural matrix and errors connected with the sample preparation step, each standard solution was prepared by the addition of an appropriate volume of working solution of lipoic acid to 0.5 g of

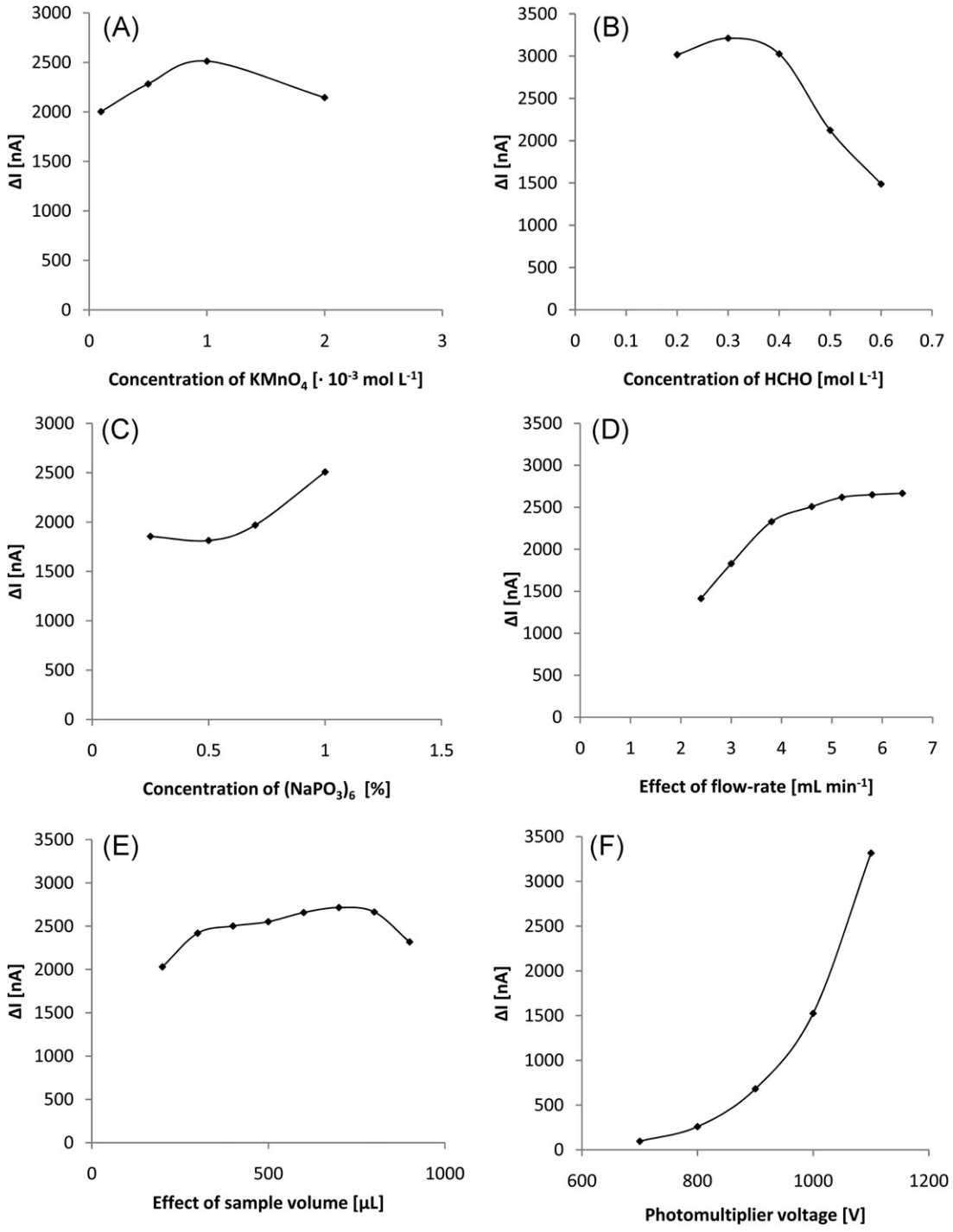

Fig. 4. Influence of chemical, hydrodynamic and instrumental parameters on relative CL intensity of lipoic acid. (A) Effect of concentration of $KMnO_4$; (B) effect of concentration of HCHO; (C) effect of concentration of $(NaPO_3)_6$; (D) effect of flow-rate; (E) effect of sample volume; (F) effect of photomultiplier voltage (concentration of LA 10 μg mL^{-1}).

Table 3
Results of lipoic acid determination in pharmaceutical preparation samples by FI-CL method.

Concentration of lipoic acid [μg mL^{-1}]	Determined x [μg mL^{-1}]	$\bar{X} \pm$ SD	Concentration of added standard [μg mL^{-1}]	Determined x [μg mL^{-1}]	$\bar{X} \pm$ SD	Recovery [%]
5.0	4.8	4.9 ± 0.1	10.0	15.3	15.3 ± 0.1	104
5.0	4.8		10.0	15.3		
5.0	5.0		10.0	15.4		
5.0	4.9	4.9 ± 0.1	15.0	19.6	19.6 ± 0.0	98.0
5.0	4.8		15.0	19.6		
5.0	5.0		15.0	19.6		

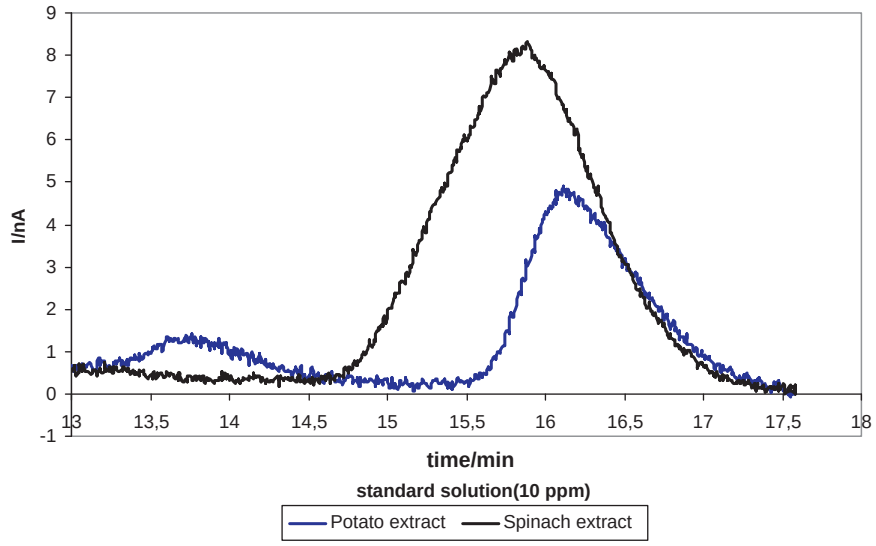

Fig. 5. Chromatogram of fortified food samples.

Table 4
Analytical characteristics of HPLC-FI-CL method.

Linear range ($\mu g\,mL^{-1}$)	2.5–30
Calibration equation	$y = 1.14C - 1.95$ (C-lipoic acid concentration)
r^2	0.988
LOD ($\mu g\,mL^{-1}$)	1.774
LOQ ($\mu g\,mL^{-1}$)	1.913
Intra-day R.S.D.	3.42% for 5 $\mu g\,mL^{-1}$ 1.67% for 15 $\mu g\,mL^{-1}$
Inter-day R.S.D.	6.91% for 10 $\mu g\,mL^{-1}$

homogenised potatoes and next isolated according to the procedures given in Section 2. The average recovery was calculated to be 107.18% ± 17.01. The recorded calibration curve has the regression equation of $y = 1.14C - 1.95$. The detection limit for LA was 1.77 $\mu g\,mL^{-1}$ per 50 μL injection volume at signal-to-noise ratio of 3, while the limit of quantification was 1.91 $\mu g\,mL^{-1}$ (at a signal-to-noise ratio of 10). To determine the intra-day precision of the analytical process, two levels of LA (5 and 15 $\mu g\,mL^{-1}$) were studied six times on the same day. The reproducibility was estimated by the determination of lipoic acid at 10 $\mu g\,mL^{-1}$ on three different days. The analytical performance of the new HPLC-FIA-CL method of LA determination is shown in Table 4.

The evaluated method was applied to the determination of the contents of free α-lipoic acid in some food samples. For this purpose, samples of fresh potatoes, tomatoes, spinach and broccoli were investigated by standard addition. Representative chromatograms obtained from the analysis of LA in potatoes and spinach samples are presented in Fig. 5.

Table 5
Results of LA determination in food products.

Product	Determined concentration of LA with standard addition method, $\mu g\,mL^{-1}$ ($n = 3$)
Fresh potatoes	5.37
Fresh tomatoes	1.66
Fresh spinach	2.23
Fresh broccoli	0.65
Fresh pig's liver	0.35

4. Conclusions

To the best of our knowledge, it is the first time, when the CL reaction of lipoic acid with potassium permanganate in sodium hexametaphosphate medium (pH 3) with the use of formaldehyde as a sensitizer has been reported for the FIA determination of lipoic acid.

Chemiluminescence as a detection technique used with HPLC system seems to be very attractive due to its high sensitivity and selectivity. An introduction of HPLC unit into the flow manifold improves selectivity of the method and allows the determination of LA in complex samples, such as food products. The integration of HPLC separation with CL-detection is not an easy analytical task. Utilized organic solvents depressed the intensity of chemiluminescence, so the composition of the chromatographic mobile phase should be adjusted to the CL-detector. In our case, a mixture of acetonitrile: KH_2PO_4 (pH 3) = 30:70 appeared to be the best. The proposed method is rapid, precise, accurate and economical. It is simpler than fluorescent methods. As molecule of lipoic acid does not possess any of fluorophore groups, the use of fluorescent detector demands chemical modification of LA molecule by introduction into it some fluorescent markers. HPLC methods with fluorescence detection allow the determination of lipoic acid in biological samples, but derivatization steps are time consuming [16,17] and create risk of analytical errors. Electrochemical detectors which sensitivity [18,19] is comparable with those of fluorescence detectors are very often characterized by narrow calibration range. Additionally many of them requires a laborious reconstitution of electrodes. MS detectors coupled with LC [20] or GC [21] methods of separation are suitable for the quantification of lipoic acid in complex matrices. They are characterized by high sensitivity and selectivity. The main drawback of such equipment is high price, so they are not still belonging to standard equipment of laboratories. Their utilization is very costly, so they rather are not appropriate for routine determination of countless amount of samples. The proposed new FIA-CL and HPLC-FIA-CL methods are free of mentioned above limitations of others detection techniques. The used systems are easy to construct using commercially available or home-made chemiluminescence detectors. So it could be easily implemented in every laboratory and used for routine analysis of LA (Table 5).

Acknowledgements

Spectroflurometer was funded by EU, as part of the Operational Programme Development of Eastern Poland 2007–2013, project Nr. POPW.01.03.00-20-034/09-00.

Part of this work was presented on 17th International Conference on Flow Injection Analysis, Kraków, 3–8 July 2011.

References

[1] F. Navari-Izzo, M.F. Quartacci, C. Sgherri, Plant Physiol. Biochem. 40 (2002) 463–470.
[2] L. Packer, K. Kraemer, G. Rimbach, Nutrition 17 (2001) 888–895.
[3] M.I. Khan, Z. Iqbal, L. Ahmad, Y. Shah, S. Nazir, A. Khan, F. Nasir, Chromatographia 73 (2011) 929–939.
[4] M. Kolgazi, N. Jahovic, M. Yuksel, F. Ercan, I. Alican, J. Gastroenterol. Hepatol. 22 (2007) 1859–1865.
[5] Recommended Dietary Allowances, 10th ed., National Academy of Sciences, Washington, 1989.
[6] L. Packer, H.J. Tritscher, K. Wessel, Free Radic. Biol. Med. 22 (1997) 359–378.
[7] S.J. Zhang, Q.F. Ge, D.W. Guo, W.X. Hu, H.Z. Liu, Bioorg. Med. Chem. Lett. 20 (2010) 3078–3083.
[8] A.I. Durrani, H. Schwarz, M. Nagl, G. Sontag, Food Chem. 120 (2010) 1143–1148.
[9] A.I. Durrani, H. Schwartz, W. Schmidt, G. Sontag, J. Pharm. Biomed. Anal. 45 (2007) 694–699.
[10] H.Y. Aboul-Enein, H. Hoenen, J. Liq. Chromatogr. 27 (2004) 3029–3038.
[11] T. Inoue, M. Sudo, H. Yoshida, K. Todoroki, H. Nohta, M. Yamaguchi, J. Chromatogr. A 1216 (2009) 7564–7569.
[12] W. Siangproh, P. Rattanarat, O. Chailapakul, J. Chromatogr. A 1217 (2010) 7699–7705.
[13] E.I. Kozlov, I.A. Solunina, M.L. Lyubareva, M.A. Nadtochii, Pharm. Chem. J. 38 (2004) 642–643.
[14] J. Teichert, R. Preiβ, J. Chromatogr. B 672 (1995) 277–281.
[15] S. Satoh, T. Toyoòka, T. Fukushima, S. Inagaki, J. Chromatogr. B 854 (2007) 109–115.
[16] W. Witt, B. Rüstow, J. Chromatogr. B 705 (1998) 127–131.
[17] A.I. Haj-Yehia, P. Assaf, T. Nassar, J. Katzhendler, J. Chromatogr. A 870 (2000) 381–388.
[18] M.I. Khan, Z. Iqbal, L. Ahmad, K. Shah, D.G. Watson, J. Chromatogr. B 878 (2010) 2782–2788.
[19] A. Khan, Z. Iqbal, D.G. Watson, A. Khan, I. Khan, N. Muhammad, S. Muhammad, H.A. Nasib, N. Iqbal, Faiz-ur-rehaman, M. Kashif, J. Chromatogr. B 879 (2011) 1725–1731.
[20] H.T. Chng, L.S. New, A.H. Neo, C.W. Goh, E.R. Browne, E.C.Y. Chan, J. Pharm. Biomed. Anal. 51 (2010) 754–757.
[21] A. Mattulat, W. Baltes, Z. Lebensm. Unters. Forsch. 194 (1992) 326–329.
[22] O. Corduneanu, M. Garnett, A.M.O. Brett, Anal. Lett. 40 (2007) 1763–1778.
[23] M.N. Abbas, A.A. Radwan, Talanta 74 (2008) 1113–1121.
[24] G.K. Ziyatdinova, G.K. Budnikov, V.I. Pogorel'tsev, J. Anal. Chem. 59 (2004) 324–326.
[25] G.K. Ziyatdinova, L.V. Grigorèva, G.K. Budnikov, J. Anal. Chem. 64 (2009) 185–188.
[26] M. Liu, Z. Lin, J.M. Lin, Anal. Chim. Acta 670 (2010) 1–10.
[27] C. Ming, W. Xiuhua, T. Yifeng, Talanta 85 (2011) 1304–1309.
[28] J.L. Adcock, P.S. Francis, N.W. Barnett, Anal. Chim. Acta 601 (2007) 36–67.
[29] B.J. Hindson, N.W. Barnett, Anal. Chim. Acta 445 (2001) 1–19.
[30] E. Nalewajko, A. Wiszowata, A. Kojło, J. Pharm. Biomed. Anal. 43 (2007) 1673–1681.
[31] N.W. Barnett, B.J. Hindson, P. Jones, T.A. Smith, Anal. Chim. Acta 451 (2002) 181–188.
[32] J.L. Adcock, P.S. Francis, N.W. Barnett, Anal. Chim. Acta 601 (2001) 36–67.