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High-performance liquid chromatographic separation and quantification of citric, lactic, malic, oxalic and tartaric acids using a post-column photochemical reaction and chemiluminescence detection

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Abstract

An HPLC method was developed for the determination of citric, lactic, malic, oxalic and tartaric acids by chemiluminescent detection following online irradiation with visible light. The organic acids were irradiated with visible light in the presence of Fe^{3+} and UO_2^{2+} to generate Fe^{2+}, which was determined by measuring the chemiluminescence intensity in a luminol system in the absence of added oxidant. Factors affecting the photochemical and chemiluminescence reactions were optimised so that their contribution to the total band-broadening was negligible. The chromatographic separation was performed on a C_{18} column under isocratic reversed-phase conditions using 0.005 M H_2SO_4 mobile phase. The optimised method was validated with respect to linearity, precision, limits of detection and quantification, accuracy specificity and robustness. The applicability of the assay was demonstrated by analysing these compounds in real samples such as milk, fruit juices, soft drinks, wine and beer.

Keywords: Fruit juices; Beer; Soft drinks; Food analysis; Chemiluminescence detection; Detection, LC; Photochemically induced chemiluminescence detection; Milk; Wine; Organic acids

1. Introduction

The application of high-performance liquid chromatography (HPLC) to the determination of trace levels of analytes in complex matrices has been limited by the inadequate selectivity and sensitivity provided by conventional liquid chromatographic detectors. Commonly employed detection systems include those based on ultraviolet–visible absorbance, fluorescence and electrochemistry. Both the selectivity and sensitivity of these detectors can be further enhanced by using suitable derivatization techniques. This has led to the use of procedures which render the substance more readily detectable by chemical reaction either before (pre-column) or after chromatography (post-column). The main advantages of the post-column approach include separation of the analytes in their original form without the need for a complete derivatization reaction (assuming reproducibility) and the fact that the reaction products need not be stable.

Chemiluminescence (CL) detection is very sensitive because the absence of a light source reduces noise and eliminates Rayleigh and Raman scattering, allowing photon detectors to be operated at high gains to improve the signal to noise ratio. This often leads to detection limits that rival lasers and mass spectrometers at a fraction of the cost, and has made HPLC–CL an attractive alternative for sensitive detection [1,2].

Photochemical reactions have long been used to enable the determination of photoactive analytes [3–6]. In some cases, irradiation alone is sufficient to produce the detectable compound, although reagents are often added in order to stabilise or aid in the generation of the photoproduct. For analytical purposes, photochemical reactions are extremely useful because of their selectivity and sensitivity and many of them have been adapted as post-column detection schemes in liquid chromatography [1].

This paper presents a novel post-column-reaction detection system based on the coupling of photochemical and CL

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reactions. The feasibility of this approach was evaluated by using a model mixture composed of the organic acids, citric, lactic, malic, oxalic and tartaric, because the absence of strong chromophores or fluorophores in these analytes has precluded their sensitive detection by common HPLC detectors.

The photochemical−CL principle used in this work took advantage of the oxidation which these acids undergo when they are irradiated with visible or UV light in the presence of Fe^{3+} or UO_2^{2+}. The photochemical process consists of the reduction of Fe^{3+} to Fe^{2+} or UO_2^{2+} to U^{4+}, the emission of CO_2 and the formation of other oxidation products [3,7–9]. The Fe^{2+} produced is then quantified by measuring the CL intensity in a luminol system in the absence of added oxidant [10,11]. In this study, we have successfully combined the photochemical–CL detection system with HPLC for the analysis of the above listed organic acids in different real samples, including wines, beer, milk, fruit and soft drinks.

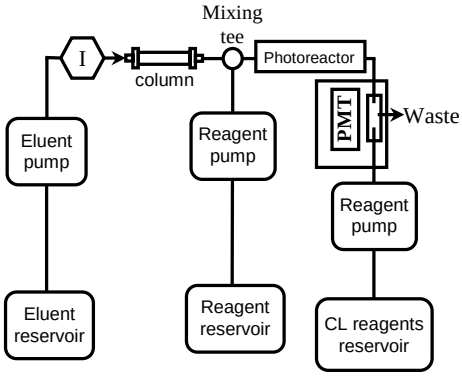

Fig. 1. Instrumental diagram of an HPLC post-column photochemical reaction system for the CL detection of organic acids. Column: Ultrasphere ODS. Mobile phase: 0.005 sulphuric acid at a flow-rate of 0.30 ml/min. Photochemical reagent: Fe^{3+} 5×10^{-3} M, UO_2^{2+} 1×10^{-3} M, H_2SO_4 0.01 M, PVA 0.02%. Photochemical reagent flow-rate: 0.70 ml/min. CL reagent: luminol 5×10^{-4} M in 0.1 M borate buffer (pH 10.8). CL reagent flow-rate: 2 ml/min. A 3.0 m PTFE reactor was used for photochemical reaction. The remaining conditions are described in Section 2.

2. Experimental

2.1. Chemical and solutions

All chemicals were from analytical reagent grade and were used without further purification. Ultrapure water (Milli-Q water purification system, Millipore-Ibérica, Madrid, Spain) was used to prepare all solutions. Citric, malic, lactic, oxalic and tartaric acids were purchased from Sigma (St. Louis, MO, USA). Iron (III) sulphate and uranyl acetate were obtained from Merck (Darmstadt, Germany) and luminol from Fluka (Buchs, Switzerland).

Stock solutions of the organic acids were prepared at a concentration of 1×10^{-2} M by dissolving the compounds in ultrapure water; these solutions remained stable for several weeks if kept refrigerated at 4 °C. Standard solutions were prepared by further dilutions of the stock solution with ultrapure water. The luminol solution (1×10^{-3} M) was made in 0.1 M borate buffer at pH 11. Iron(III) (1×10^{-2} M) and uranium(VI) (1×10^{-2} M) solutions were prepared in 0.01 M sulphuric acid.

Unless otherwise specified, the mobile phase was 0.005 M sulphuric acid solution, usually at a flow rate of 0.3 ml/min.

2.2. Apparatus

The instrumental setup used in this study (Fig. 1) consisted of an HPLC Beckman Coulter (Fullerton, CA, USA) instrument, composed of a System Gold 125 nm Solvent Module, a Rheodyne 7725i manual injection valve and a System Gold 168 diode array detector. The chemiluminescence detector was a Camspec CL-2 (Cambridge, UK) luminometer equipped with a three-port flow cell (two inlets and one exit). The CL detector was connected to the HPLC equipment through a SS420x interface (Beckman). The reagent solution (containing Fe^{3+} and UO_2^{2+}) was added to the column effluent through a T-connector, transported through the photoreactor (see below) and finally directed to one of the flow cell ports. The buffered luminol solution was pumped to the other inlet of the flow cell. Thus, the mixing took place into the flow cell. An IBM Pentium personal computer using a 32 Karat software (Beckman) was used for data acquisition and treatment.

Chromatography was performed using an Ultrasphere C_{18} column packed with 5 μm particle size and dimensions of 250 mm × 4.6 mm (Beckman).

2.2.1. Photochemical reactor

The photoreactor consisted of PTFE tubing (0.5 mm i.d., length 300 cm) coiled around a glass tube 0.5 cm diameter placed inside a Pyrex cylinder with a double-walled well, through which cooling water continuously flowed (Fig. 2). The lamp (Eurolight tungsten-halogen lamp, 500 W, 250 V) was placed 10 cm above the reactor. This assembly was housed in a fan-ventilated metal box covered with aluminium foil to enhance the reflection of the light from the lamp.

2.3. Sample treatment

The sample volume injected was always 20 μl.

2.3.1. Beer and soft drinks

The carbonated beverages (50 ml) were degassed for 10 min in an ultrasonic bath, filtered through a 0.45 μm Millipore membrane and diluted 1:5 with ultrapure water before injection into the column.

2.3.2. Milk

Milk samples (10 ml) were treated with trichloroacetic acid (3 ml, 0.5 M) and centrifuged for 5 min at 3000 × *g*.

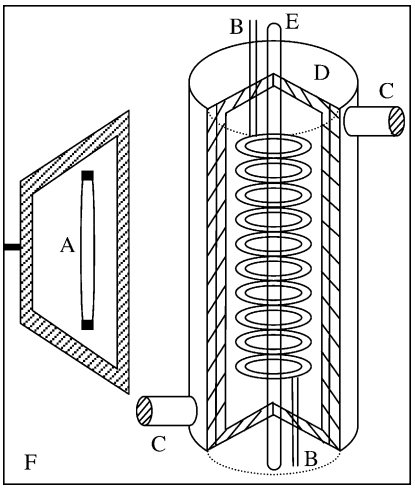

Fig. 2. Sectional drawing of the photochemical reactor for HPLC. (A) Lamp; (B) PTFE tubing; (C) coolant connections; (D) Pyrex cylinder; (E) glass tube; (F) metal box covered with aluminium foil.

The liquid supernatant was filtered through a 0.45 μm filter and diluted to 100 ml with ultrapure water.

2.3.3. Fresh fruit juices

The freshly pressed fruit juice samples were filtered through a 0.45 μm filter. An amount of 10 ml of the filtrate were diluted to 200 ml with ultrapure water.

2.3.4. Wines

An amount of 10 ml of the wine samples (red, white and rosé) were diluted to 200 ml with ultrapure water and filtered through a Millipore filter of 0.45 μm pore size.

3. Results and discussion

The irradiation of solutions containing citric, lactic, malic, oxalic or tartaric acids and Fe^{3+} or UO_2^{2+} involves the reduction of Fe^{3+} to Fe^{2+} or UO_2^{2+} to U^{4+} and the formation of oxidation products (mainly CO_2) from the organic acid [7,12]. Some of this reactions have been used as chemical actinometers [1,13,14]. The primary photochemical reaction is assumed to be an intramolecular oxidation–reduction caused by an electron transfer from the organic acid to Fe^{3+} and UO_2^{2+} [15]. The mechanism proposed for these systems is:

$$\text{–CO–O}\,Fe^{3+} \xrightarrow{h\nu} Fe^{2+} + CO_2$$

$$\text{–CO–O}\,UO_2^{2+} \xrightarrow{h\nu} UO_2^{2+} + CO_2$$

The photooxidation rate of the organic acids in the presence of UO_2^{2+} is higher than with Fe^{3+}. Therefore, when a solution of some of these acids is irradiated with visible light in the presence of both UO_2^{2+} and Fe^{3+}, the amount of Fe^{2+} produced was greater than that obtained when using only Fe^{3+}. The predominant reaction path is the photolysis of the uranyl complex, and the U^{4+} produced reacts with Fe^{3+} to yield Fe^{2+} and UO_2^{2+}. The results obtained in the photolysis of lactic acid in batch experiments are listed in Table 1.

Table 1
Fe^{2+} produced in the photochemical decomposition of lactic acid[a]

Fe^{3+} added (μmol)	UO_2^{2+} added (μmol)	Fe^{2+} produced (μmol)
8	–	0.011
20	–	0.032
50	–	0.090
75	–	0.095
100	–	0.100
50	60	0.135
50	80	0.175
50	100	0.265
50	150	0.275
50	200	0.280

[a] All samples contain lactic acid (1 μmol) and sulphuric acid (1000 μmol). Final volume: 100 ml; irradiation time: 30 s.

For normal carboxylic acids, the presence of hydroxyl groups(s) on a polycarboxylic acid provides an easier oxidation route because the OH group can be transformed into an aldehyde or ketone through a two-electron oxidation step. Thus, the quantum yield for the photoreduction of Fe(III) complexed by α-hydroxy carboxylic acids is higher than that obtained with the structurally analogous non-hydroxylated carboxylic acids [16]. The non-hydroxylated carboxylic acids probably participate in one-electron radical chemistry upon oxidation, and the C–C bonds in these compounds are harder to oxidize. This is probably due to the unfavourable energetics of producing a methyl radical during one-electron oxidative decarboxylation. The structural features that lead to one-electron reduction make photochemistry more efficient. This means that ligands like oxalate can give higher quantum yields because they undergo a one-electron oxidation easily [17].

The photochemical determination of these organic acids involves measuring the Fe^{2+} formed in the photochemical process, and so the sensitivity will be greater when the photolysis is carried out in the presence of both UO_2^{2+} and Fe^{3+}. In addition, the sensitivity can be improved by the use of an extremely sensitive procedure to detect the Fe^{2+} produced in the photochemical reactor. Thus, luminol CL was used to quantify Fe^{2+} in the absence of H_2O_2 as long as molecular oxygen was present. Seitz and Hercules [18] reported an Fe^{2+} detection limit of approximately 5×10^{-10} mol/l using such a system. The use of oxygen as the primary oxidant in the luminol CL reaction allows the selective quantification of Fe^{2+} in the presence of Fe^{3+}, since Fe^{3+} is not a catalyst for this system in the absence of H_2O_2.

The variables affecting the photochemical and CL reactions were studied with an FI system in which the separation column was removed from the HPLC system.

3.1. Optimisation of photochemistry

The effects of composition and pH of the carrier, concentration of the reagents, Fe^{3+} and UO_2^{2+}, and irradiation time on the CL signal were studied.

The photochemical decomposition of the organic acids in the presence of Fe^{3+} and UO_2^{2+} must be carried out in an acid medium in order to obtain reproducible results during the generation of Fe^{2+}. A sulphuric acid concentration over the range 0.1–0.01 M was sufficient to avoid the deleterious action of oxygen. In these conditions, the rate of oxidation of Fe^{2+} by oxygen is insignificant. The sulphuric acid concentration in the solution used as carrier should be the lowest of the recommended range (0.01 M) so that after mixing the photolysed solution with the streams of the CL reagent, the pH remains as close as possible to the optimum value for the CL reaction.

The influence of the concentration of Fe^{3+} on the photochemical reaction of these organic acids was studied in the range 5×10^{-5}–3×10^{-3} M. The CL signal increased up to 5×10^{-4} M, but levelled off at higher concentrations (Fig. 3A). The photochemical reaction is also affected by the concentration of UO_2^{2+}. Keeping the Fe^{3+} constant (5×10^{-4} M), the CL signal increased with increasing UO_2^{2+} concentrations up to 1×10^{-3} M, above which it remained virtually constant (Fig. 3B).

The solution emerging from photoreactor contained small amounts of Fe^{2+} in the presence of large amounts Fe^{3+} and UO_2^{2+}; when this solution was used in the CL reaction of luminol, which always takes place in basic media, the precipitation of iron(III) and uranium(VI) hydrated oxides led to instability of the CL signal caused the flow cell to block. This problem was prevented by the addition of surfactants. In addition, to overcoming the solubility problem, their presence also alters the pathway of the chemical and photophysical processes and is an effective means of enhancing the photochemical and CL reactions [19]. Hence, the effects of micellar systems of different charge type were studied. The surfactants employed were sodium dodecylsulphate, hexadecyltrimethylammonium bromide, poly(vinyl alcohol) (PVA), Triton X-100 and Brij-35. PVA was chosen because it provided the best results as regards sensitivity and reproducibility. Fig. 3C shows that a 0.02% (m/v) PVA concentration is sufficient to obtain maximum CL signals for all the organic acids.

Summarizing the above results, it can be stated that the optimised composition of the photochemical reagent solution is Fe^{3+}, 5×10^{-4} M, UO_2^{2+}, 1×10^{-3} M, H_2SO_4, 0.01 M and PVA, 0.02% (m/v).

The residence time of the sample in the photoreactor had a great influence on the amount of Fe^{2+} produced. Different irradiation times were achieved by varying the length of the reaction coil and/or the flow rate of the photochemical reagent stream. The amount of Fe^{2+} produced increased with the length of the irradiation time. A residence time of 30/40 s was sufficient to obtain a significant CL signal in all cases.

3.2. Optimisation of CL reaction

The efficiency of the light emission was measured at different pH values. CL intensity increased with increasing pH up to 10.4, remained constant up to 10.9 and then decreased at higher pH values. Of the several buffers tested, borate exhibited the best properties. The CL signal increased with increasing luminol concentration until a plateau was reached between 4×10^{-4} and 5×10^{-4} M, and then decreased steadily at higher concentrations (Fig. 3D). The buffered 5×10^{-4} M luminol solution containing 0.1 M borate buffer pH 10.8 was pumped to the flow cell.

Under the above conditions, the effects of flow-rates of the photolysed solution emerging from photoreactor and the CL reagent on CL intensity were examined. The flow-rate of the photolysed solution was varied over the range of

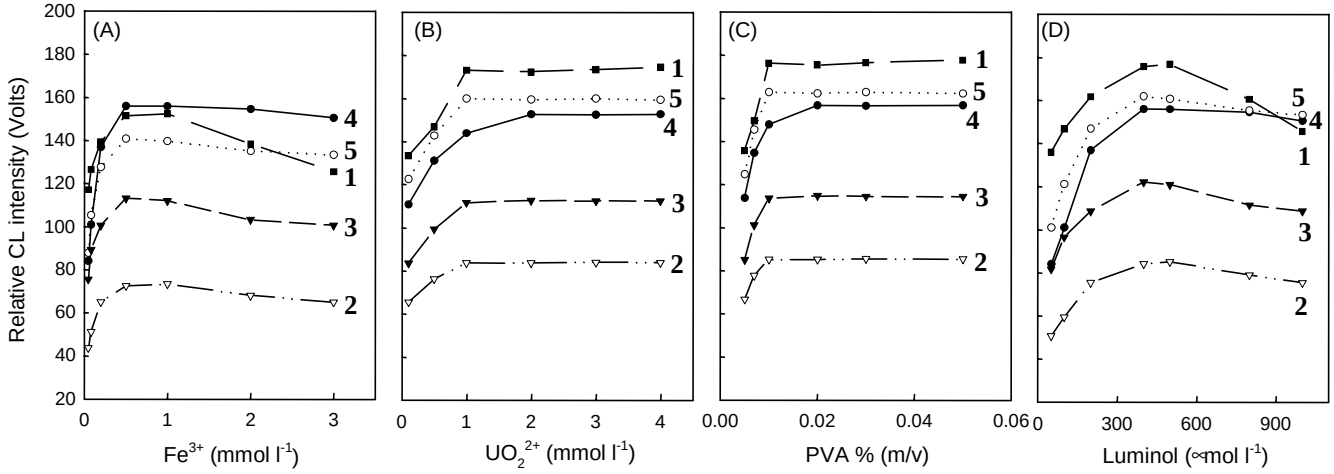

Fig. 3. Effect of the concentrations of (A) Fe^{3+}; (B) UO_2^{2+}; (C) PVA; (D) luminol on the CL detection response. (1) Citric acid; (2) lactic acid; (3) malic acid; (4) oxalic acid; (5) tartaric acid.

0.5–1.5 ml/min while keeping the flow-rate of the CL reagent constant at 2 ml/min. The maximum detector responses were observed at 1 ml/min for each organic acid. The flow-rate of buffered luminol solution provided the strongest CL signal at 2 ml/min (examined range 1.5–3.0 ml/min) for each analyte when the flow-rate of the photolysed solution was set at 1 ml/min.

3.3. Chromatography

An HPLC system equipped with an ODS column was used to separate the organic acids. The choice of chromatographic conditions that ensure resolved peaks for the analytes was based on previous works [20–23]. Good separation was achieved using an isocratic mobile phase composed of 0.005 M sulphuric acid. The flow-rate of the mobile phase was set at 0.3 ml/min in order to obtain a reasonable analysis time (<30 min) with a window between 8 and 35 min. A chromatogram of a mixture of citric, lactic, malic, oxalic and tartaric acids with photometric detection at 210 nm obtained under these conditions is shown in Fig. 4A. An example of the post-column photochemical reaction and CL detection of these organic acids is depicted in Fig. 4B. From a comparison of the two chromatograms, it may be concluded that the proposed approach can indeed be employed for the sensitive detection of the analytes in question.

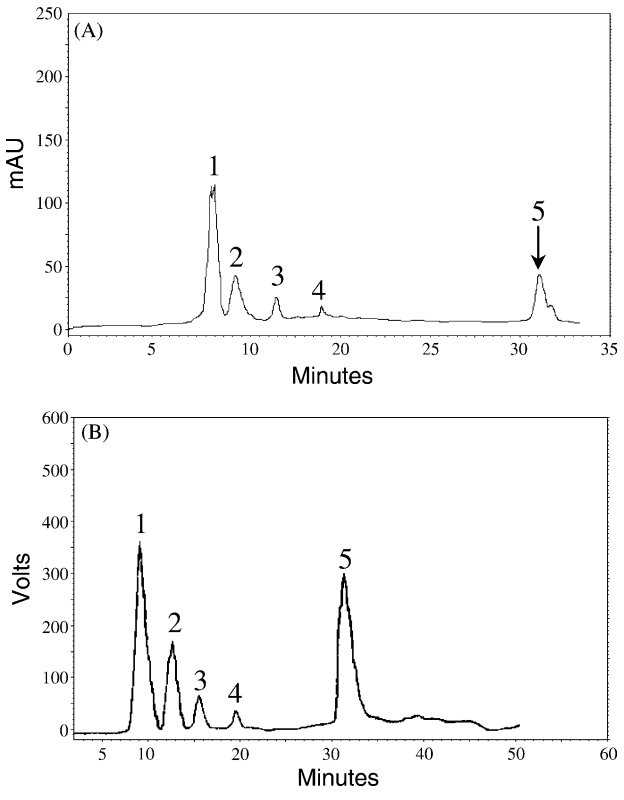

Fig. 4. Typical chromatograms of a standard mixture of the organic acids using (A) absorbance detection at 210 nm; (B) post-column photochemical reaction and CL detection. Mobile phase 0.005 M sulphuric acid; mobile phase flow-rate 0.3 ml/min. Analyte concentration: 250 μM of each injected compound. Peak identification: (1) oxalic acid; (2) tartaric acid; (3) malic acid; (4) lactic acid; (5) citric acid.

The photochemical reagent solution was pumped at 0.7 ml/min in order to attain the optimum flow-rate of 1 ml/min at the inlet of the photoreactor. Because the irradiation time must be in the range 30–40 s, a reaction coil of 3 m was selected to obtain a residence time of the sample in the photoreactor of about 35 s.

3.4. Validation

Validation of this method included assessment of stability of the solutions, linearity, precision, detection and quantification limits, specificity, accuracy and robustness.

3.4.1. Stability of the solutions

Although this test is often considered as a part of ruggedness of the procedure, it should be carried out at the beginning of the validation procedure because it conditions the validity of the data of the other tests.

The response factors of standard solutions were found to be unchanged for up to 20 days. Less than a 0.3% concentration difference was found between the solutions freshly prepared and those aged for 20 days. The solutions can therefore be used within this period without affecting the results.

3.4.2. Linearity and precision

The results obtained with the proposed method are summarized in Table 2. The calibration curves were prepared over the concentration range 5.0×10^{-6} to 5.0×10^{-3} M (at least 10 samples covering the whole range were used). Each point of the calibration graph corresponded to the mean value from three independent peak measurements. The linearity between peak area and concentration was good for all the analytes, as shown by the fact that the regression coefficients (r) were greater than 0.999 for all the curves.

The intra-day precision was tested with 11 repeated injections of two sample solutions containing the analytes at two concentration levels, 1.0×10^{-5} and 4.0×10^{-4} M for citric, oxalic and tartaric acids and 2.0×10^{-4} and 8.0×10^{-4} M for lactic and malic acids. The relative standard deviations (R.S.D.s) were always less than 1.4%. The inter-day precision of the method was studied by analysing three identical samples (all analytes at 2.0×10^{-4} M level), injected six times every day, on five consecutive days. The R.S.D.s for the peak area were less than 2.5%.

3.4.3. Limit of detection

The limits of detection (fmol per 20 μl injection volume) at a signal-to-noise ratio of three were 50, 56, 78, 400, 540 fmol for oxalic, citric, tartaric, malic and lactic acids, respectively. Fig. 5 depicts the chromatogram of the analytes at the concentration level corresponding to their detection limit.

Table 2
Parameters of calibration graphs and precision values

Compound	$y = a + bx$			Precision		
	a	b	r	Intra-day		Inter-day
Citric acid	−466131 ± 5751	133400 ± 2445	0.9990	1.0[a]	0.5[b]	2.5[c]
Lactic acid	−679606 ± 1674	8760 ± 11	0.9990	0.7[a]	0.5[b]	2.4[c]
Malic acid	−1548258 ± 3340	20314 ± 218	0.9996	0.8[a]	0.6[b]	2.1[c]
Oxalic acid	−601699 ± 8506	183400 ± 3616	0.9990	1.1[a]	0.6[b]	2.3[c]
Tartaric acid	−257815 ± 2836	65200 ± 1014	0.9991	1.4[a]	0.6[b]	1.9[c]

x: analyte concentration in μmol/l; a: intercept of the regression lines fitted to the calibration data set ± standard deviation; b: slope of the regression lines fitted to the calibration data set ± standard deviation. Precision data are presented as the relative standard deviation (see the text). Concentration of the analytes.
[a] 1×10^{-5} M.
[b] 4×10^{-4} M.
[c] 2×10^{-4} M.

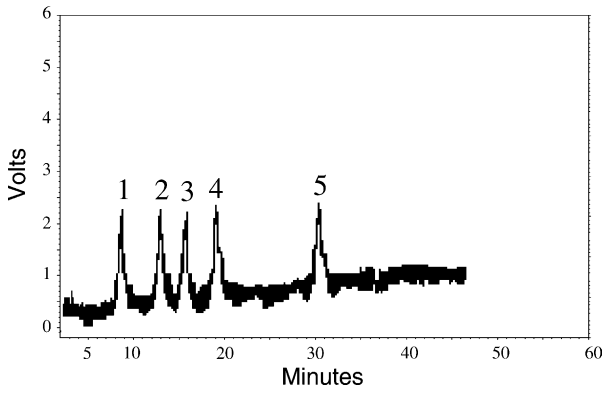

Fig. 5. HPLC–CL response of (1) oxalic (50 fmol); (2) tartaric (78 fmol); (3) malic (400 fmol); (4) lactic (540 fmol); (5) citric (56 fmol) acids. Concentration levels corresponding to their detection limit. Conditions are similar to those of Fig. 4B, except that sensitivity is 100 times greater.

3.4.4. Accuracy

The accuracy of the proposed method was tested with several synthetic mixtures containing the analytes in different proportions. Ratios higher than 10:1 or 1:10 corresponding to the nearest peaks were not analysed. The results obtained were excellent because the recoveries ranged between 98.8 and 101.6%.

3.4.5. Selectivity

The method is very selective because very few compounds can form photoactive complexes with Fe^{3+} and/or UO_2^{2+} that are capable of producing Fe^{2+} under irradiation with visible light. The chromatograms obtained with this photochemical–CL detector are very simple (only a few peaks) and the baseline is stable with a very low background.

3.4.6. Robustness studies

The robustness of a method is its ability to remain unaffected by small deliberate variations in the method parameters. The following changes in the optimum parameter value were examined: the flow rate of the mobile-phase (adjusted by ±0.05 ml/min), the pH of the mobile-phase (adjusted by ±0.5 units), the flow-rate of the photochemical and CL reagents (adjusted by ±0.05 ml/min), and the concentration of the reagents (adjusted by ±10% of the recommended value). The results obtained showed resolutions greater than 1.7 for the nearest peaks.

3.5. Analysis of real samples

The analytical usefulness of the proposed reaction detection system was tested by determining these analytes in milk, wine, beer, fruit juices and soft drinks.

The data of Tables 3–6 show that the content of the organic acids, as measured by the proposed method, was in excellent agreement with that obtained by HPLC with absorbance detection at 210 nm. It is worth noting that in the milk samples, the presence of co-eluting substances made

Table 3
Determination of citric, malic and oxalic acids in fruit juices and soft drinks[a]

Sample	Citric (g/l)		Malic (g/l)		Oxalic (mg/l)	
	CL	Abs	CL	Abs	CL	Abs
Fruit juice						
Orange	8.1 ± 0.2	7.9 ± 0.2	0.31 ± 0.01	0.33 ± 0.01	nd	nd
Apple	0.12 ± 0.01	0.11 ± 0.01	7.0 ± 0.2	6.9 ± 0.2	nd	nd
Soft drinks						
Aquarius	2.4 ± 0.1	2.3 ± 0.1	nd	nd	53 ± 1	56 ± 2

nd: not detected; Abs: absorbance detection at 210 nm; CL: post-column photochemical reaction and CL detection.
[a] Values are means for four determinations ± standard deviation.

Table 4
Determination of citric and lactic acids in milk[a]

Milk	Citric (mg/l)		Lactic (mg/l)	
	CL	Abs	CL	Abs
Hacendado	1423 ± 10	–	1050 ± 11	1047 ± 16
El prado	936 ± 9	–	1381 ± 12	1387 ± 16
Puleva	1624 ± 12	–	1497 ± 15	1493 ± 18

Abs: absorbance detection at 210 nm; CL: post-column photochemical reaction and CL detection.
[a] Values are means for four determinations ± standard deviation.

impossible the determination of citric acid using UV detection.

A chromatogram obtained with a sample of beer (San Miguel) is shown in Fig. 6A. The peaks corresponding to oxalic, tartaric, malic, lactic and citric acids were detected with good base line separation. In order to evaluate the selectivity and sensitivity of the proposed detection system, the same sample as in Fig. 6A was analysed using absorbance detection (Fig. 6B). It can be seen that the chromatogram was much more complex, due to the fact that other endogenous compounds were detected. In addition, the baseline was not stable and the peaks corresponding to some of these acids were poorly formed.

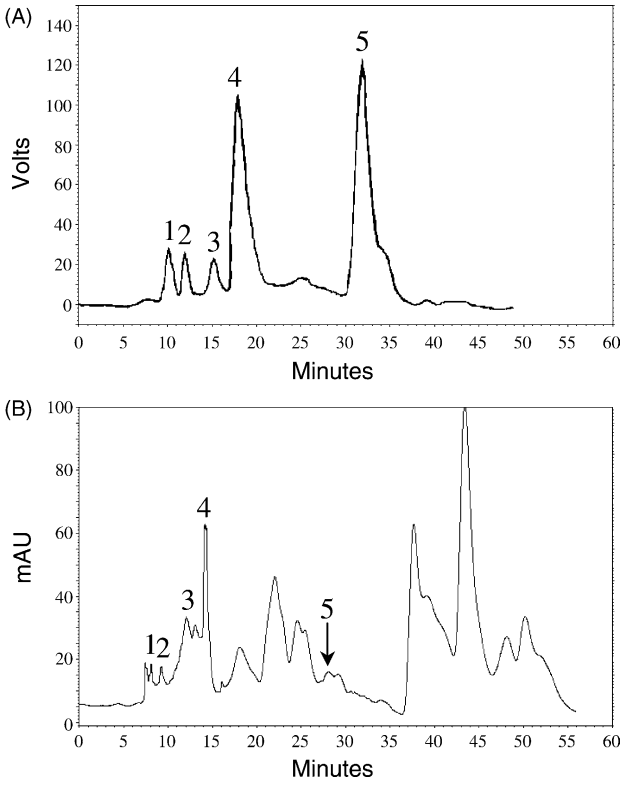

Fig. 6. Chromatograms of a beer (San Miguel) sample using (A) photochemical–CL detection; (B) detection at 210 nm. Peaks: (1) oxalic acid; (2) tartaric acid; (3) malic acid; (4) lactic acid; (5) citric acid.

Table 5
Determination of citric, lactic, malic and tartaric acids in different wines[a]

Wine	Citric acid (mg/l)		Lactic acid (g/l)		Malic acid (g/l)		Tartaric acid (g/l)	
	CL	Abs	CL	Abs	CL	Abs	CL	Abs
Red wine								
Gran Viñedo	66 ± 1	65 ± 2	6.0 ± 0.1	6.0 ± 0.2	nd	nd	3.2 ± 0.1	3.0 ± 0.2
Burgo Viejo	54 ± 1	56 ± 2	5.6 ± 0.1	5.4 ± 0.2	nd	nd	2.2 ± 0.1	2.1 ± 0.3
White wine								
Conde Noble	195 ± 5	178 ± 6	2.8 ± 0.1	2.7 ± 0.2	1.5 ± 0.1	1.4 ± 0.2	2.6 ± 0.1	2.8 ± 0.2
Table wine	301 ± 6	286 ± 8	4.6 ± 0.1	4.7 ± 0.2	0.24 ± 0.01	0.23 ± 0.01	1.2 ± 0.1	1.2 ± 0.1
Rosé wine								
Tesoro de Bullas	183 ± 4	227 ± 6	3.9 ± 0.1	3.6 ± 0.2	0.52 ± 0.01	0.50 ± 0.02	1.5 ± 0.1	1.6 ± 0.2
Castillo de Velasco	207 ± 4	212 ± 5	5.1 ± 0.1	4.8 ± 0.3	0.24 ± 0.01	0.24 ± 0.01	2.8 ± 0.1	2.5 ± 0.1

nd: not detected; Abs: absorbance detection at 210 nm; CL: post-column photochemical reaction and CL detection.
[a] Values are means for four determinations ± standard deviation.

Table 6
Determination of citric, lactic, malic, oxalic and tartaric acids in four beers[a]

Beer	Citric acid (mg/l)		Lactic acid (mg/l)		Malic acid (mg/l)		Oxalic acid (mg/l)		Tartaric acid (mg/l)	
	CL	Abs	CL	Abs	CL	Abs	CL	Abs	CL	Abs
Estrella de Levante	202 ± 3	200 ± 7	592 ± 12	573 ± 18	68 ± 1	72 ± 6	25 ± 1	27 ± 4	nd	nd
Láger	109 ± 2	111 ± 5	559 ± 11	554 ± 19	43 ± 1	42 ± 8	13.5 ± 0.2	14 ± 5	nd	nd
Sol del Sur	74 ± 2	73 ± 7	631 ± 15	631 ± 17	40 ± 1	41 ± 5	12.0 ± 0.1	13 ± 2	nd	nd
San Miguel	144 ± 3	140 ± 7	616 ± 12	608 ± 25	41 ± 1	43 ± 5	13.5 ± 0.2	12 ± 1	24 ± 1	22 ± 6

nd: not detected; Abs: absorbance detection at 210 nm; CL: post-column photochemical reaction and CL detection.
[a] Values are means for four determinations ± standard deviation.

4. Conclusions

The above applications serve to demonstrate the potential of the detection system developed in this work. The usefulness of the HPLC method proposed for the determination of citric, lactic, malic, oxalic and tartaric acids is based on the selective photodecomposition of these analytes in the presence of Fe^{3+} and UO_2^{2+} combined with the sensitive determination of the Fe^{2+} produced by the CL luminol reaction in the absence of added oxidant.

Perhaps the high sensitivity of this method would also make it suitable for the determination of these organic acids in blood serum, if interferences can be overcome, because the lowest endogenous concentration level reported is 1 μg/ml for oxalic and malic acids [24]. It is worth noting that the presence of pyruvic acid is not a problem because its separation of the other acids is good.

The main characteristics of the chromatograms obtained with the proposed detection system are: (a) great stability and very low background of the baseline and (b) presence of very few peaks. This has permitted the sensitive and selective determination of citric, lactic, malic, oxalic and tartaric acids in real samples with minimum pretreatment.

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