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A novel clean-up method for urine analysis of low-molecular mass aldehydes by capillary electrophoresis with laser-induced fluorescence detection☆

Clara Eugenia Baños, Manuel Silva*

Department of Analytical Chemistry, Marie-Curie Building (Annex), Campus of Rabanales University of Cordoba, E–14071 Cordoba, Spain

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ABSTRACT

A rapid clean-up method using ultra-filtration was developed to remove sample matrix in the determination of low-molecular mass aldehydes in human urine. The ensuing filtrate was derivatized with fluorescein 5-thiosemicarbazide and the labelled aldehydes determined by capillary zone electrophoresis with laser-induced fluorescence detection. Practical aspects related to the effect of the urine sample matrix on the label chemistry and the electrophoretic separation showed that the urine samples must be diluted 20-fold after their ultra-filtration. By using synthetic urine, linear ranges were established in the range of 15–5000 μg/l with limits of detection between 4.5 and 9 μg/l. The intra- and inter-assay precision (relative standard deviation, %) of the aldehydes ranged from 4.1% to 8.4% and 6.1%–9.6%, respectively, and the average specific uncertainty was 149 ± 12 ng. The average recoveries performed on two levels by enriching synthetic urine samples ranged between 94% and 100%. Finally, the proposed method was applied to check low-molecular mass aldehydes in the human urine of a female volunteer to obtain information about the risk in her exposure to these chemicals in the workplace.

1. Introduction

In the last decade, capillary electrophoresis (CE) has become increasingly important in the analysis of diverse compounds in a variety of matrices. Because of its highly efficient peak separation and short analysis time, CE methods can be regarded as an interesting complement or even as an alternative to the chromatographic techniques commonly employed in separation problems [1–4]. However, it is commonly accepted that the sensitivity and selectivity of the detection are relatively weak points of CE [5,6]. One way to overcome these limitations is the conversion (derivatization) of the analytes into product(s) with more favourable detection characteristics. Thus, in most cases, analytical derivatization in CE is detection-oriented, and therefore associated with the incorporation of chromophore or fluorescent groups into the analytes to obtain better sensitivity and selectivity. In addition, analytical derivatization is also used to give the analyte a more suitable mass-to-charge ratio, to increase the hydrophobicity necessary for micellar electrokinetic chromatography (MEKC) separations or to provide better properties for mass spectrometric detection [7–9], among others. Although, in principle, almost any detection mode can be combined with an analytical derivatization procedure, in practice, laser-induced fluorescence (LIF) monitoring is favoured in many cases because it affords a remarkable increase in detection sensitivity [4]. From the foregoing, it can be asserted that, as occurs in chromatographic techniques, analytical derivatization has played an important role in the CE determination of analytes such as amino acids, carbohydrates, aldehydes, etc. This is because their direct determination is troublesome due to the one or other reason of the lack of strong chromophoric moiety, high polarity, reactivity or volatility of these compounds. In this context, a great number of CE procedures have been reported for the determination of amino acids and carbohydrates based on their derivatization with different probes, whereas not many CE methods for low-molecular mass aldehydes (LMMAs) have been published.

CE analysis of LMMAs is usually performed after their derivatization with an acidic solution of 2,4-dinitrophenylhydrazine (DNPH) to form their corresponding hydrazones, followed by MEKC separation and UV detection [10–14]. Other hydrazine-based reagents such as dansylhydrazine [15,16], 4-hydrazinobenzoic acid [17,18], hydrazino benzene sulfonic acid [19] and 3-methyl-2-benzothiazoline hydrazone [20] have also been used for the analytical derivatization of LMMAs. CE analysis involving use of these hydrazine-based derivatizing agents is relatively sensitive because linearity is established at μg/ml level; however, by using a pre-concentration step, the determination of these aldehydes is feasible at μg/l level, such as in air samples [15,18,20]. On the other hand, the majority of these electrophoretic methods have

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* Corresponding author. Tel.: +34 957 212099; fax: +34 957 218614.
E-mail address: qa1sirom@uco.es (M. Silva).

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been applied to the determination of LMMAs in such simple matrices as air [10,12,15,18,20] or water [16,19] samples, and therefore very little work has been focused on their analysis in more complex matrices; to our knowledge, only human saliva [11] and food samples [14,17] have been assayed. For these reasons, it is clear that the determination of LMMAs through CE demands more sensitive approaches that do not cause detriment to the resolution and also that will extend their analysis to more complex analytical samples. The determination of the LMMAs is an important area of research in clinical chemistry because the analysis of aldehydes in human urine is a non-invasive and simple assay to evaluate possible adverse health effects due to these carbonyl compounds. Aldehydes are organic compounds that are widespread in nature, and as a result there are many possible aldehyde sources for humans: significant exogenous sources include environmental, dietary and drug aldehydes, whereas reactive aldehyde species formed in the organism are the main endogenous source [21]. Urinary malondialdehyde has been widely used to monitor oxidative stress [22,23], the control of acrolein in urine (the metabolite of the anticancer drugs cyclophosphamide and ifosfamide) can be used to prevent severe hemorrhagic cystitis [24,25] and the quantification of formaldehyde in urine may serve as a non-invasive marker of bladder and prostate cancer [26,27].

The purpose of this work was to develop a rapid and useful clean-up method based on ultra-filtration (UF) for the sensible and selective determination of LMMAs in human urine, namely formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, acrolein and malondialdehyde. Because the aldehydes have no native fluorescence, the UF urine samples were derivatized with fluorescein 5-thiosemicarbazide (FTSC) and the derivatives separated by CE and detected by LIF using an air-cooled argon-ion laser as the excitation source. In a previous work [28], we reported a FTSC derivatization procedure for some of these LMMAs and their analysis in drinking waters at μg/l levels by CE-LIF. The current study describes the development of a sample work-up procedure for the application of the FTSC-CE-LIF method to a more complex matrix like human urine samples. UF and solid-phase extraction (SFE) have been evaluated as clean-up methods and their performance compared in terms of simplicity and degree of purification. The method was validated in terms of selectivity, linearity, precision and accuracy, and proved to be appropriate for its purpose. The method proposed is the first contribution to the quantification of LMMAs in human urine by CE. It introduces FTSC as fluorescence probe for urine analysis of LMMAs. This class of analyte polar and reactive is not amenable to isolation by SPE. These characteristics required the development of a novel sample clean-up method for removing urine sample matrix based on UF.

2. Experimental

2.1. Reagents and solutions

All reagents and solvents used were of analytical grade. Water was purified using an Elix-3 electrodeionization station coupled with a Milli-Q Simplicity water purification system (Millipore Ibérica S.A., Madrid, Spain). Formaldehyde (37% aqueous solution) and acetaldehyde (99.5%) were supplied by Sigma (Sigma–Aldrich Química, Madrid, Spain). Propionaldehyde (96%), butyraldehyde (99%) and acrolein (99%) were obtained from Fluka (Sigma–Aldrich Química). Malondialdehyde tetrabutylammonium salt (96%) was purchased by Aldrich (Sigma–Aldrich Química). The standards were used without further purification. FTSC (80%, FW 421.43) was obtained from Fluka. The derivatizing agent (ca. 0.5 mM) was prepared by dissolving 21.1 mg of FTSC in 100 ml of chromatographic grade dimethylformamide (Merck, Darmstadt, Germany) and storing it in a freezer at −20 °C. Polyethylene glycol tert-octylphenyl ether (Triton X-100) was acquired from Sigma. Surine™ Negative Control, a simulated urine matrix, was purchased from Dyna-Tek Inc. (Lenexa, KS). Surine™ is rugged non-biological urine with constituents that mimic human urine without many of their disadvantages such as odour, foam, and biohazard disposal requirements. Certificate of analysis: pH, 7.0; specific gravity, 1.008; and appearance, consistent.

2.2. CE-LIF instrumentation and electrophoretic conditions

All analyses were carried out on a P/ACE™ MDQ CE system (Beckman, Fullerton, CA, USA) fitted with a Laser Module 520 nm LIF detector (Beckman) using an air-cooled argon-ion laser (excitation wavelength of 488 nm and emission wavelength filter of 520 nm). The acquisition and the processing of data were performed with a PS/2 computer (IBM, Greenwock, UK) running 32 Karat™ software (version 8.0). The samples were systematically injected in hydrodynamic mode (injection pressure: 0.5 p.s.i. or 3.4 kPa) for 5 s and their analysis was achieved on a fused-silica capillary of 57 cm (50 cm effective length to detection window) × 50 μm I.D. Injections were performed on the anodic end of this capillary and electrophoresis was carried out at 25.0 ± 0.1 °C and a voltage of 20 kV. To assure good reproducibility, at the beginning of each experimental session or sample injection the capillary was rinsed with 0.1 M hydrochloric acid for 2 min, 1.0 M sodium hydroxide for 5 min followed by 5 min with water and then equilibrated with the running buffer (60 mM sodium borate adjusted to pH 10 and 10 μM of Triton X-100) for 2 min. In addition, the peak with a migration time of ca. 14.5 min in electropherograms from the FTSC background emission can be used as internal standard in order to correct possible variations between runs in the migration time of the labelled analytes and also differences in the peak areas due to potential significant injection variability. Using this peak as internal standard, relative standard deviations (RSDs) for migration times ranged from 0.7% to 1.8%.

2.3. Preparation of standards

Individual stock solutions of aldehydes (1 mg/ml) were prepared in methanol (Romil Chemicals, Cambridge, UK) and stored at 4 °C. The stock solutions were diluted daily with purified water to prepare working standard solutions between 0.3 and 100 μg/ml. Ten working standard spiking solutions containing all six aldehydes were generated by serial dilution of the initial stock solutions with synthetic urine (Surine™ Negative Control) diluted 20 times with water. These standards covered concentration ranges of 15–5000 μg/l. Quantification was based on peak area.

2.4. Pre-treatment and derivatization of urine sample

Urine samples were obtained from a female volunteer. Samples were collected in sterilized polyethylene bottles of 250 ml without headspace to prevent the formation of air bubbles and stored at 4 °C up to 72 h. When the time between urine collection and analysis exceeded 72 h, samples were stored at −20 °C up to 30 days to avoid storage losses. The frozen urine samples were left in a refrigerator until completely thawed. If required, the thawed urine samples can be stored for 4 h prior to their analysis in a refrigerator. Before derivatization, 2-ml aliquots of the urine sample were UF using Vivaspin 6 cartridges (cut-off = 5 kDa, Sigma) by centrifugation at 1850 × *g* for ca. 15 min at room temperature. The UF urine sample was diluted 20 times with water and a 400-μl aliquot was derivatized in a 1.5-ml PTFE vial by adding 100 μl of the derivatization buffer (100 mM sodium dihydrogen phosphate adjusted to pH 7.0 with 0.1 M sodium hydroxide) and 500 μl of 0.5 mM FTSC

reagent solution. The derivatization was carried out at 60 °C in the dark for 3 h. All the derivatives were kept stored in a freezer at 4 °C and analyzed within 8 h after completing the reaction. When the time between derivatization and analysis exceeded 8 h, derivatives could be stored at −20 °C up to 7 days to avoid storage losses. The blank electropherogram corresponding to the background emission of FTSC was obtained using the same experimental procedure but with synthetic urine instead of human urine.

3. Results and discussion

3.1. Development of the method

The determination of aldehydes in biological matrices can be a complex task because sample purification and pre-concentration procedures are mainly required to improve selectivity and facilitate finding these analytes in the samples. This difficulty is even more complex in the analysis of LMMAs since the high polarity and reactivity of these compounds preclude the use of sample pretreatment techniques such as SPE, solid-phase microextraction and liquid-phase microextraction because it is difficult to achieve the quantitative separation of a mixture of LMMAs from other components of the sample matrix. Therefore, further developments in clean-up methods are needed to ensure efficient separation of these aldehydes from the sample matrix. On the other hand, we have recently reported a novel sensitive derivatization scheme for LMMAs with FTSC that obviates the need for off-line or on-line pre-concentration and allows the CE-LIF quantification of aldehydes at nanogram-per-litre levels in drinking water samples [28]. As a result of the good features of this method, it is used in this work for the analysis of LMMAs in human urine. In the present research, not only saturated mono aldehydes (C_1 to C_4) are studied, but also an unsaturated one like acrolein and a dialdehyde such as malondialdehyde, because they are generally analyzed in urine as biomarkers of certain diseases, namely hemorrhagic cystitis [24,25] and lipid peroxidation induced by oxidative stress [22,23], respectively.

FSTC is a fluorescent molecular probe used in biochemical and clinical analysis that reacts with a wide variety of biomolecules such as proteins (immunoglobulins, glycoproteins, etc.) among others [28]. As a result, to analyze LMMAs in human urine a fraction of aldehydes free from urine proteins is needed and therefore they should be removed or significantly decreased in the urine matrix. For this purpose, dilution of the urine sample with water and the use of the SPE or the UF approaches have been assayed in this work as clean-up methods. This study was carried out in the absence of the analytes and using synthetic urine as the sample matrix with the aim to obtain the cleanest background electropherogram (blank). After the treatment, a 400-μl aliquot of the resulting urine sample was subjected to derivatization with FTSC and analyzed by CE-LIF under the experimental conditions stated in Section 2.

The effect of urine dilution was evaluated over the range 1:1 to 1:20, and the highest dilution factor tested was selected for further studies despite the many peaks that were observed in the electropherogram (see Fig. 1A). Dilution factors higher than 1:20 were rejected to avoid a dramatic decrease in aldehyde sensitivity in the original urine sample. From these results, a SPE system was also assayed to decrease, moreover, the urine matrix effect. Thus, a volume of 5 ml of the synthetic urine sample was passed through the continuous SPE system at 1.0 ml/min. Several sorbent materials: silica RP-C_{18} (100 mg), LiChrolut EN (50 mg) and graphitized carbon black (50 mg) were used for packing the SPE mini-columns. A volume of 1.0 ml of the eluate was diluted 1:20 with water just before the derivatization of the aldehydes. From the experimental data it can be concluded that although this clean-up method reduced the effect of the urine matrix appreciably (see Fig. 1B and C for RP-C_{18} and LiChrolut EN, respectively) many peaks were still observed in the electropherograms. The poorest results were obtained using graphitized carbon black as the sorbent. Next, UF was tested as alternative to SPE systems. It was selected instead of the classical protein precipitation approach because it is a very mild method for removing proteins with little risk of producing artifacts or introducing impurities. Thus, synthetic urine samples were transferred into disposable filtration device containing a polyethersulfone membrane filter with a 5 kDa cut-off. After centrifugation for 15 min at 1850 × *g*, the filtrate was diluted 1:20 with water and derivatized

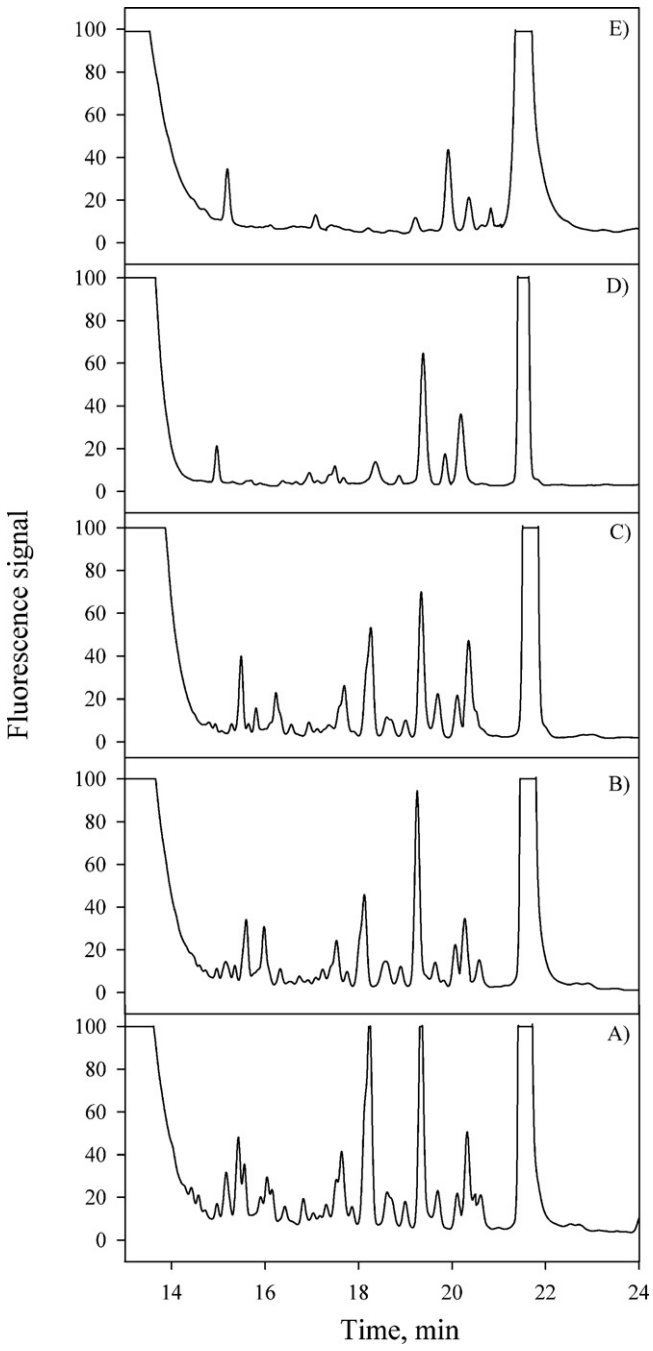


Fig. 1. Electropherograms of the effect of the urine matrix on FTSC background emission. In all cases the urine was diluted to 1:20 with water. (A) Untreated urine; (B) urine purified by SPE with RP-C_{18}; (C) urine purified by SPE with LiChrolut EN; and (D) UF urine. (E) Electropherogram of the FTSC background emission using purified water as sample matrix. Derivation conditions as in Section 2.

with FTSC. As can be seen in Fig. 1D, UF was the best choice to remove the urine matrix effect because only some peaks were observed in the electropherogram and these were similar to those obtained using water as sample matrix. Therefore, these peaks can be assigned to fluorescent hydrolysis products and/or impurities of the FTSC and not to possible fluorescent derivatives from the urine matrix (see Fig. 1E and D).

The derivatization conditions for LMMAs were essentially the same ones used in a previous work [28] in spite of the fact that two different aldehydes, acrolein and malondialdehyde, were included in this study. Only one variable was re-adjusted: the pH of the 100 mM phosphate buffer, which was fixed at 7.0 instead of 6.5 in order to increase accuracy when measuring formaldehyde and acetaldehyde peak areas. In this point, it can be interesting to discuss why the formation of thiosemicarbazones requires slightly acidic conditions or even neutral pH (as in this work) while hydrazine-based reagents (the most widespread choice for labelling carbonyl compounds) react at low pH. The formation of imine compounds is based on a sequence of reactions involving protonation of carbonyl oxygen, nucleophilic attack of amine group on the electron deficient carbon atom, and elimination of water molecule from the intermediate as shown in Scheme 1. In the case of the formation of hydrazones, high acidity in the reaction medium favours the formation of the carbocation and the subsequent nucleophilic attack of hydrazine group [29], which is the rate-limiting step. However, when thiosemicarbazide reagents are used, a change from rate-limiting addition of the nucleophile at low pH to rate-limiting dehydration at pH higher than ca. 4.5 is observed [30,31]. Thus, although high acidity in the reaction medium favours the formation of the carbocation and the subsequent nucleophilic attack of hydrazine group; however when using thiosemicarbazide reagents, the sulfur atom of the C=S bond can be protonated at low pH values, which reduces the electronic density of nitrogen in the amine group and subsequently the nucleophilic attack.

Fig. 2A shows a typical electropherogram showing the elution profile of LMMA derivatives and unreacted FTSC obtained using a running buffer containing 60 mM sodium borate adjusted to pH 10 and 10 μM of Triton X-100 [28]. At this point, some comments related to the CE separation process are needed: (1) an additional rinsing step of the capillary was required between runs with 0.1 M hydrochloric acid for 2 min in order to achieve a good reproducibility in the migration times. In fact, in the absence of this step, the migration time of the FTSC derivatives increased progressively from ca. 20 min (see Fig. 1) to 60 min, after five repeated injections. This effect can be assigned to a possible adsorption of the urine matrix in the capillary wall, even when the urine was diluted 1:20 with water. (2) Based on its chemical formula, malondialdehyde forms two derivatives with FTSC, which were eluted according to their respective charge/mass ratio. The formation of these derivatives has been corroborated when constructing the calibration curves for this aldehyde. In fact, at lower concentrations (greater excess of FTSC) the di-FTSC derivative was formed and eluted at higher migration times than the mono-derivative, which is formed at higher concentrations of malondialdehyde (lower excess of FTSC). Only one peak was observed in both conditions, whereas two peaks appeared at intermediate concentrations. Although one would have also expected the di-FTSC derivative to yield a comparatively stronger fluorescence signal than the mono-derivatized malondialdehyde, this was not the case presumably because of lower derivatization yields due to steric hindrance.

3.2. Method validation

The validation of the method was based on the recommendations published on-line by the Food and Drug Administration [32] as well as on the ICH guidelines for bioanalytical method validation

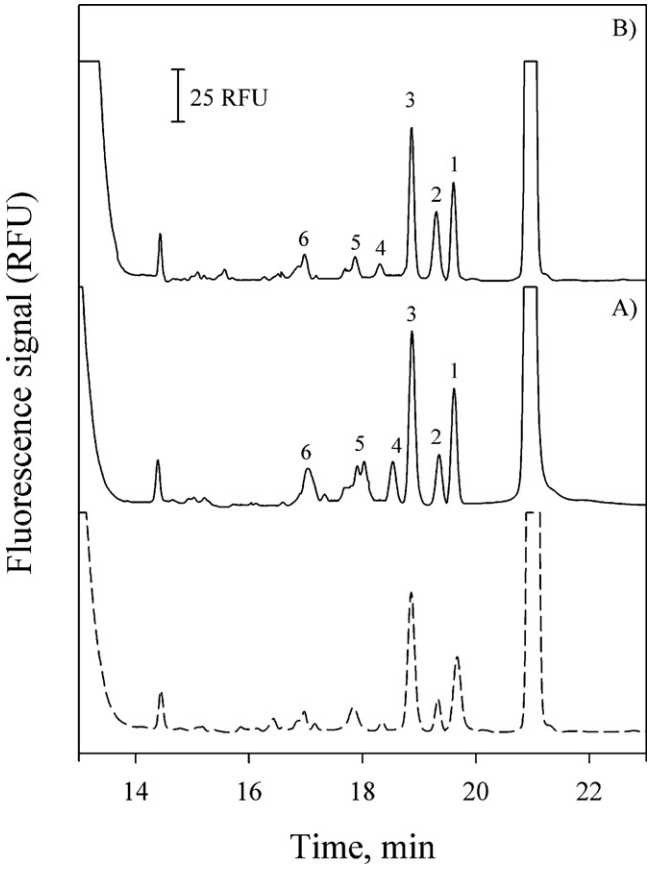
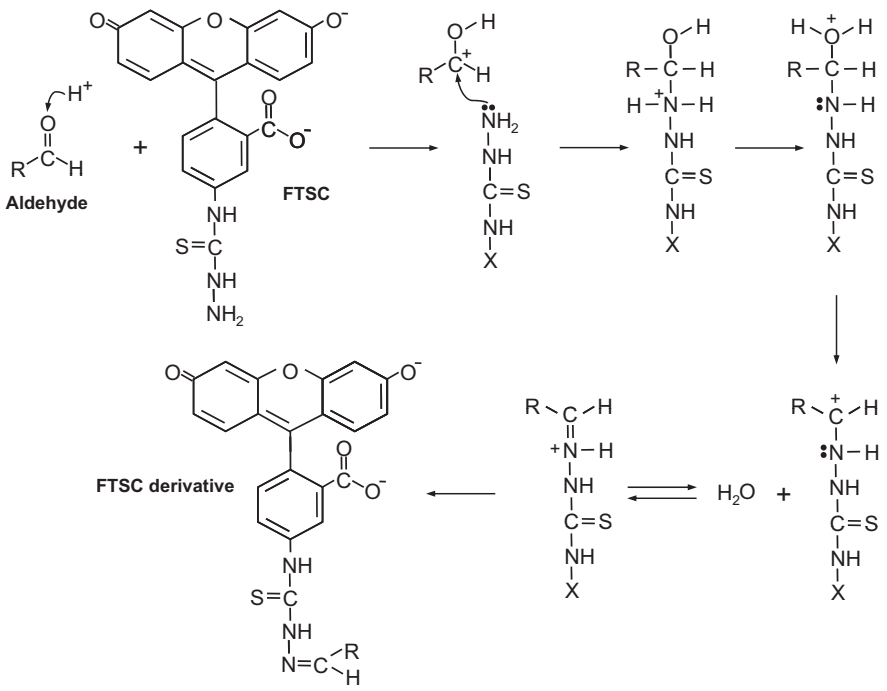


Fig. 2. Electropherograms of (A) a standard mixture of aldehydes in synthetic urine at 250 μg/l (formaldehyde and acetaldehyde at 100 μg/l) derivatized with FTSC and (B) FTSC derivatives of the aldehydes detected in the human urine sample collected within 15 min after exposure. Blank electropherogram is plotted in dotted line and corresponds to the FTSC background emission in synthetic urine. Peak identification: 1, formaldehyde; 2, acetaldehyde; 3, propionaldehyde; 4, acrolein; 5, malondialdehyde; 6, butyraldehyde. For experimental conditions see Section 2.

[33]. The validation parameters studied were linearity and range, limit of detection (LOD) and quantification (LOQ), precision, specific uncertainty, accuracy and stability assays. This study was carried out in optimized conditions by using synthetic urine as the sample matrix. The calibration curves and corresponding regressive equations were obtained with ten concentration levels of mixed standard solutions of LMMAs in synthetic urine. Table 1 gives the linear range, the FTSC background emission, the analytical sensitivity, expressed as the slope of the calibration plot, and the correlation coefficient of the regression equation for each aldehyde obtained by plotting peak area *versus* analyte concentration. The results indicated that a high degree of linearity between the two variables was attainable over the concentration range studied. The inter-day variation of calibration slopes (three consecutive days) measured as RSD was less than 4.3%.

The LOD and LOQ for each aldehyde are also given in Table 1. The LOD was determined as the lowest concentration of the analyte that can be reliably differentiated from the background level (signal-to-noise ratio = 3) and the LOQ as the lowest concentration of the analyte that can be measured with a stated level of confidence (the lower limit of the linear range) [32,33]. The calculated LODs ranged from 4.5 μg/l to 9.0 μg/l and the estimated LOQs from 15 μg/l to 30 μg/l. Although areas of some FTSC background peaks are significant with respect to those provided by the corresponding FTSC derivatives (see formaldehyde, acetaldehyde and propionaldehyde in Fig. 2A), they are quite reproducible (RSD values from 2.5% to 4.8%) and thus, this enables to get lower values of



Scheme 1. Scheme of the reaction pathway for the formation of the FTSC derivatives with LMMAs.

LODs and LOQs. Despite the urine was diluted 20 times with water before its analysis, the method proposed provided smaller LODs for the aldehydes than those obtained by the CE methods described in the literature for the analysis of these carbonyl compounds in matrices other than air samples [11,14,16,17,19]. Furthermore, to our knowledge, the method proposed represents the first approach for the CE determination of some of the aldehydes assayed, such as malondialdehyde.

The precision of the method was determined by calculating intra- and inter-assay precision for aldehydes in synthetic urine samples. Intra-assay precision was estimated by analyzing six replicates containing LMMAs in two different quality control levels: 75 μg/l (quality control low, QCL) and 1500 μg/l (quality control high, QCH). The inter-assay precision was determined by analyzing six replicates of the QCL and QCH samples on three consecutive days ($n = 18$). The concentration in each sample was determined using calibration standards prepared on the same day and the precision was calculated as RSD. As can be seen in Table 2, RSD values ranged from 4.1% to 9.6%, which demonstrates the good precision obtained for all of the analytes.

The specific uncertainty for the determination of each aldehyde includes several individual standard uncertainties associated with different sources such as precision, preparation of the standards, the UF of the urine, instrumental calibration and other random error sources associated with the analytical method. To estimate specific uncertainty, 11 mixed standard solutions of LMMAs in synthetic urine containing 150 ng of each LMMA were subjected to the whole process: preparation of working curves, storage of the urine samples at −20 °C for 30 days, the thawing of the urine sample and its storage at −4 °C for 3 days in a refrigerator, the UF of the urine samples, derivatization of the aldehydes with FTSC, storage of the derivatives at −20 °C for 7 days and then, their complete thawing at 4 °C for 8 h in a refrigerator and analysis by CE-LIF. The specific uncertainty of a result is a symmetric interval around the result ($R \pm U$) and is calculated from the standard deviation (S) for a set of results: $U = tS/\sqrt{n}$ (where U is uncertainty, t is a statistical parameter and n is the number of measures) [34,35]. Table 2 summarizes the estimated specific uncertainty of each LMMA for the whole procedure, which represents a mean relative uncertainty of 8.1%.

Method accuracy was assessed by six replicate analyses of mixed standard solutions of LMMAs in synthetic urine at two different concentration levels for each aldehyde (the QCL and QCH samples had the same composition as those used for precision studies). Recovery of each aldehyde was calculated by comparing peak areas in synthetic urine samples with those in a corresponding standard solution, and was expressed as the percentage of expected levels. As can be seen in Table 2, the spiked recovery, which was reflected in method accuracy, ranged from 94% to 100% for all six analytes at the two spiked levels. The results were satisfactory and these data supported the suitability of the proposed CE-LIF method for its application to real samples like human urine.

Table 1
Analytical features obtained by CE-LIF for FTSC derivatives of aldehydes in synthetic urine samples.

Aldehyde	Linear range (μg/l)	Background emission[a] (RFU × min)	Analytical sensitivity[a] (RFU × min × l/μg)	Correlation coefficient	LOD (μg/l)	LOQ (μg/l)
Formaldehyde	20–2500	9.25 ± 0.23	0.1094 ± 0.0038	0.9984	6.0	20
Acetaldehyde	15–2500	3.12 ± 0.14	0.1996 ± 0.0041	0.9990	4.5	15
Propionaldehyde	30–2500	12.6 ± 0.31	0.0796 ± 0.0032	0.9987	9.0	30
Acrolein	25–5000	0.83 ± 0.04	0.0634 ± 0.0016	0.9994	7.5	25
Malondialdehyde	30–5000	2.57 ± 0.12	0.0557 ± 0.0015	0.9992	9.0	30
Butyraldehyde	25–5000	2.15 ± 0.10	0.0844 ± 0.0019	0.9995	7.5	25

[a] Values ± SD ($n = 10$).

Table 2
Inter- and intra-assay precision (RSD, %)[a], accuracy (average recovery, %)[b] and the uncertainty (ng)[c] of LMMAs in synthetic urine.

Aldehydes	QCL (75 μg/l)			QCH (1500 μg/l)			Uncertainty
	Intra-assay	Inter-assay	Accuracy	Intra-assay	Inter-assay	Accuracy	
Formaldehyde	8.4	9.6	95	7.6	8.5	97	147 ± 14
Acetaldehyde	6.5	8.1	96	5.4	7.2	97	149 ± 12
Propionaldehyde	6.8	8.3	94	5.2	7.4	98	148 ± 13
Acrolein	5.7	7.4	99	4.4	6.2	100	151 ± 11
Malondialdehyde	6.1	7.8	97	4.7	6.5	99	149 ± 12
Butyraldehyde	5.2	7.2	98	4.1	6.1	100	150 ± 10

[a] Samples were analyzed on each day ($n = 6$ at each concentration) of the three consecutive day validation.
[b] Average recovery determined from intra-assay data.
[c] Uncertainty of the whole procedure expressed as $R \pm U$ ($n = 11$, $K = 2$ at a 95% confidence level).

3.3. Analysis of urine samples

The method proposed has been applied to the analysis of urine samples of a female volunteer who worked with the analytes included in this study to obtain information about the risk of her exposure to these chemicals in the workplace. In her daily laboratory work, the exposure to aldehydes was largely related to the preparation of the diluted solutions of LMMAs from stock ones of 1 mg/ml. It is noteworthy to point out that in most cases urinary metabolites reported in the literature are normalized to creatinine to correct urine dilution effects for single-spot sample collections among individuals. This practice is closely related to the determination of non-volatile analytes, and thus occupational and environmental health organisations like American Conference of Governmental Industrial Hygienists (ACGIH), National Institute for Occupational Safety and Health (NIOSH), Occupational Safety and Health Administration (OSHA), etc. establish biological exposure indexes in urine for metals and non-volatile organic compounds expressing them in mg/g creatinine. However, levels of volatile organic compounds in urine (LMMAs in this work) used as biomarkers of exposure must be expressed in concentrations, according to the literature [36–38] and the cited organisations. This is supported by the fact that at constant environmental concentrations, during exposure, equilibrium tends to be reached between partial pressure of volatile organic compounds in external air, alveolar air, arterial blood, body tissues and mixed venous blood. In the kidneys, the solvent is excreted in urine by a diffusive process determined by the equilibration of partial pressure in the urine and plasma; as a result, the ratio of concentrations in urine and blood tends to equal the urine/blood distribution coefficients. As the dissolution of gases and vapours in liquids is a fast process, a "steady state" can be reached rapidly; for this reason variations in environmental levels of volatile organic compounds induce relatively rapid changes of concentration in blood and, consequently, in urine; then, at any time, the concentration of volatile organic compounds in urine leaving the kidney can be considered to be representative of renal blood concentration and, ultimately, of environmental levels [39].

In this study, four urine samples were collected before exposure and at 0, 4 and 8 h after the termination of exposure, and analyzed. The sample collected within 15 min after exposure is considered to be the sample at time 0 and the rest follow this initial sample at determined intervals of time. To assess possible matrix effects, LMMAs at two concentration levels, depending on the value found, were spiked to urine samples and the corresponding recoveries were determined ($n = 6$) by comparing the aldehyde concentrations in spiked urine samples with those of synthetic urine standards subjected to the same procedure. From the experimental data the following conclusions can be drawn: (1) as expected, high levels of aldehydes were found in the urine taken within 15 min after exposure. Only the most volatile aldehydes were quantified: formaldehyde (54.3 ± 4.8 μg/l, recovery = 97%), acetaldehyde (248 ± 14 μg/l, recovery = 99%) and propionaldehyde (50.2 ± 3.6 μg/l, recovery = 98%), whereas acrolein, malondialdehyde and butyraldehyde were found at levels between their LOQ and LOD at concentrations around 10, 25 and 18 μg/l, respectively. Fig. 2B shows the electropherogram obtained in the analysis of this urine sample. (2) The aldehydes were not detected in the other urine samples analyzed, except in that taken 4 h after exposure, in which formaldehyde, acetaldehyde and propionaldehyde were found at levels between their LOQ and LOD. (3) These results showed that the most volatile aldehydes studied (formaldehyde, acetaldehyde and propionaldehyde) can be rapidly absorbed by inhalation and that only a small fraction of the aldehydes input was excreted with urine. This behaviour was in line with the results of other researchers, in which the proportion of compound excreted in urine with the amount absorbed was usually low and depends on the hydrophobicity of the chemical [40,41].

4. Conclusions

In this work, CZE-LIF was found to be a powerful analytical technique for the sensitive determination of LMMAs in human urine samples without the use of prior pre-concentration steps. The results obtained warrant the following comments: (i) a novel sample clean-up method for removing urine sample matrix based on UF has been developed to overcome the drawbacks associated to the use of other sample pre-treatment approaches such as SPE; (ii) to our knowledge, the method proposed is the first report on the analysis of these aldehydes by CZE in urine samples, which is possible owing to its high sensitivity and selectivity; and (iii) a derivatization scheme for labelling LMMAs with FTSC has been introduced in the urinary analysis of these aldehydes.

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References

[1] K.D. Altria, D. Elder, J. Chromatogr. A 1023 (2004) 1.
[2] O. Salas-Solano, C. Felten, Sep. Sci. Technol. 9 (2008) 401.
[3] L. Geiser, J.L. Veuthey, Electrophoresis 30 (2009) 36.
[4] M. Silva, Electrophoresis 30 (2009) 50.
[5] M.C. Breadmore, J.R.E. Thabano, M. Dawod, A.A. Kazarian, J.P. Quirino, R.M. Guijt, Electrophoresis 30 (2009) 230.
[6] A. Varenne, S. Descroix, Anal. Chim. Acta 628 (2008) 9.
[7] J.C.M. Waterval, H. Lingeman, A. Bult, W.J.M. Underberg, Electrophoresis 21 (2000) 4029.
[8] T. Fukushima, N. Usui, T. Santa, K. Imai, J. Pharm. Biomed. Anal. 30 (2003) 1655.
[9] A.S. Ptolemy, P. Britz-McKibbin, J. Chromatogr. A 1106 (2006) 7.
[10] H. Sun, K.Y. Chan, Y.S. Fung, Electrophoresis 29 (2008) 3971.
[11] L. Annovazzi, V. Cattaneo, S. Viglio, E. Perani, C. Zanone, C. Rota, F. Pecora, G. Cetta, M. Silvestri, P. Iadarola, Electrophoresis 25 (2004) 1255.

[12] Y.S. Fung, Y.H. Long, J. AOAC Int. 82 (1999) 1571.
[13] S. Takeda, S.I. Wakida, M. Yamane, K. Higashi, Electrophoresis 15 (1994) 1332.
[14] N. Dossi, S. Susmel, R. Toniolo, A. Pizzariello, G. Bontempelli, J. Chromatogr. A 1207 (2008) 169.
[15] E.A. Pereira, E. Carrilho, M.F.M Tavares, J. Chromatogr. A 979 (2002) 409.
[16] A. Mainka, K. Baechmann, J. Chromatogr. A 767 (1997) 241.
[17] J. Ruiz-Jiménez, M.D. Luque de Castro, J. Chromatogr. A 1128 (2006) 251.
[18] E.A. Pereira, M.O.O. Rezende, M.F.M. Tavares, J. Sep. Sci. 27 (2004) 28.
[19] A. Asthana, D. Bose, S. Kulshrestha, S.P. Pathak, S.K. Sanghi, W.T. Kok, Chromatographia 48 (1998) 807.
[20] E.A. Pereira, A.A. Cardoso, M.F.M. Tavares, Electrophoresis 24 (2003) 700.
[21] P.J. O'Brien, A.G. Siraki, N. Shangari, Crit. Rev. Toxicol. 35 (2005) 609.
[22] O. Korchazhkina, C. Exley, S.A. Spencer, J. Chromatogr. B 794 (2003) 353.
[23] C.N. Konidari, T.S. Giannopoulos, C.G. Nanos, C.D. Stalikas, Anal. Biochem. 338 (2005) 62.
[24] S. Takamoto, N. Sakura, M. Yashiki, T. Kojima, J. Chromatogr. B 758 (2001) 123.
[25] S. Takamoto, N. Sakura, A. Namera, M. Yashiki, J. Chromatogr. B 806 (2004) 59.
[26] L.C. Short, T. Benter, Clin. Chem. Lab. Med. 43 (2005) 178.
[27] P. Spanel, D. Smith, T.A. Holland, W.A. Singary, J.B. Elder, Rapid Commun. Mass Spectrom. 13 (1999) 1354.
[28] C.E. Baños, M. Silva, Electrophoresis 31 (2010) 2028.
[29] A.K.K.V. Pillai, K. Gautam, A. Jain, K.K. Verma, Anal. Chim. Acta 632 (2009) 208.
[30] J.M. Sayer, W.P. Jencks, J. Am. Chem. Soc. 91 (1969) 6353.
[31] J.L. Palmer, W.P. Jencks, J. Am. Chem. Soc. 102 (1980) 6466.
[32] USFDA, http://www.fda.gov/cder/guidance/4252fnl.htm, 2001.
[33] V.P. Shah, K.K. Midha, S. Dighe, I.J. McGilveray, J.P. Skelly, A. Yacobi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D. McDowall, K.A. Pittman, S. Spector, Pharm. Res. 9 (1992) 588.
[34] R. Kellner, J.M. Mermet, M. Otto, M. Valcárcel, H.M. Widmer (Eds.), Analytical Chemistry, second edition, Wiley-VCH, Weinheim, 2004.
[35] R.G. Wilhelml, R. Hocken, H. Schwenke, CIRP Ann. – Manuf. Technol. 50 (2001) 553.
[36] H. Ukai, S. Okamoto, S. Takeda, S. Inui, T. Kawai, K. Higashikawa, M. Ikeda, Int. Arch. Occup. Environ. Health 71 (1998) 397.
[37] S. Fustinoni, R. Giampiccolo, S. Pulvirenti, M. Buratti, A. Colombi, J. Chromatogr. B 723 (1999) 105.
[38] D. Poli, P. Manini, R. Andreoli, I. Franchini, A. Mutti, J. Chromatogr. B 820 (2005) 95.
[39] M. Imbriani, S. Ghittori, Int. Arch. Occup. Environ. Health 78 (2005) 1.
[40] C.S. Moon, Z.W. Zhang, S. Shimbo, T. Watanabe, C.U. Lee, B.K. Lee, K.D. Ahn, S.H. Lee, M. Ikeda, Toxicol. Lett. 108 (1999) 173.
[41] J. Caro, M. Gallego, Environ. Sci. Technol. 41 (2007) 4793.