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Liquid chromatography–tandem mass spectrometry for the determination of low-molecular mass aldehydes in human urine

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ABSTRACT

A simple and sensitive method is proposed for the determination of seven low-molecular mass aldehydes in human urine samples using liquid chromatography with tandem mass spectrometric detection. Urine samples diluted twofold with 0.3 M hydrochloric acid are aspirated into a LiChrolut EN solid-phase extraction column impregnated with 2,4-dinitrophenylhydrazine for cleanup, derivatization and preconcentration of the aldehydes. After elution of the hydrazones with acetonitrile, an aliquot is injected directly into the chromatograph. Identification and quantification of aldehydes was performed with electrospray in negative ion mode by selected reaction monitoring. By using synthetic urine samples, linearity is established over the concentration range 0.1–30 μg/l and limits of detection from 15 to 65 ng/l. The intra- and inter-day precision (RSD, %) of the aldehydes ranged from 2.9% to 6.4% and 3.6% to 9.3%, respectively, and specific uncertainties were ca. 5.0 ± 0.3 ng for all aldehydes. Average recoveries performed on two levels by enriching synthetic urine samples ranged between 92% and 100%. The method was also validated in terms of study sample stability including long-term and short-term analyte stability, freeze–thaw and extract stability. In summary, the method proposed surpasses other recent chromatographic alternatives in terms of the limit of detection and sample requirements for analysis.

1. Introduction

Aldehydes are organic compounds that are widespread in nature. As a result, there are many possible aldehyde sources for humans, such as aldehydes of environmental or occupational concerns, dietary aldehydes, aldehydes formed endogenously by intermediary metabolism, and drugs that are aldehydes or that form reactive aldehyde metabolites that cause side-effect toxicity, among other things [1]. Analysis of aldehydes in human urine is a non-invasive and simple assay extensively used to evaluate possible adverse health effects due to these carbonyl compounds. Thus, urinary aldehydes have mainly been used to study lipid peroxidation (LPO)-induced DNA damage in cancer diseases [2–4] and for the determination of acrolein, the metabolite of the anticancer drugs cyclophosphamide and ifosfamide, which causes hemorrhagic cystitis [5–7]. Additionally, these aldehydes have also been detected in urine as indicators of their presence [8], as biomarkers in bladder and prostate cancer [9,10] and as metabolites formed in semicarbazide-sensitive amine-mediated deamination in diabetic complications [11].

Low-molecular mass aldehydes (LMMAs) have usually been determined in human urine by gas chromatography (GC). Owing to their volatility and activity, derivatization is often required although direct analysis has also been used, as in the monitoring of urinary acrolein concentration by headspace (HS) [5] or by HS/solid-phase microextraction (SPME) [6,7] coupled to GC with mass spectrometric (MS) detection. Via derivatization, hydrazine reagents such as pentafluoro- [12] and 2,4,6-trichlorophenylhydrazine [3,13] or O-(2,3,4,5,6-pentafluobenzyl) hydroxylamine [8,14] have been used as labels for the determination of the aldehydes in urine as biomarkers of LPO in polyunsaturated lipids [3,12–14] or as indicators of exposure to these chemicals [8]. An additional liquid–liquid extraction (LLE) step with *n*-hexane is usually required for the cleanup and preconcentration of derivatization products prior to their separation by GC [3,12,14]. Liquid chromatography (LC) with UV detection has also been used for the determination of LMMAs in urine samples, although these methods have some drawbacks with regard to specificity and sensitivity due to the nature of UV measurements [15–17]. However, recent LC–MS methods seem to be valuable choices with respect to the GC–MS analysis of carbonyl compounds in biological matrices including urine [18–22]. As in GC determinations, the high polarity and reactivity of aldehydes impose the need for their derivatization prior to their detection, 2,4-dinitrophenylhydrazine (DNPH) being the most widely used derivatization agent [18–21]. Few references have been reported about the determination of LMMAs in urine by LC–MS; to our knowledge, one is related to the determination of succinic semialdehyde after its derivatization with

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DNPH [21] and the other to the evaluation of a novel derivatization agent for improving the efficiency of electrospary ionization (ESI) in the LC–ESI-MS/MS analysis of aldehydes in pooled healthy human urine samples [22].

From it has been already mentioned, the objective of this work was to develop an accurate, precise, sensitive and easy-to-use LC–MS/MS analytical method to extend the scope of LMMAs detectable in human urine at levels below microgram-per-litre. In a previous paper [23], a novel DNPH derivatization procedure for LMMAs was successfully developed and coupled to LC–ESI-MS/MS for the analysis of these aldehydes in water samples at nanogram-per-litre levels. The current study describes the development of a sample work-up using continuous solid-phase extraction (SPE) for the cleanup, in situ derivatization and preconcentration of aldehydes from urine, followed by LC–ESI-MS/MS. The method proposed was validated according to ICH guidelines for bioanalytical method validation [24,25] in terms of study sample stability (long-term and short-term analyte stability, freeze–thaw and extract stability) linear range, limits of detection and quantification, precision, uncertainty, accuracy and selectivity, and proved to be appropriate for the determination of LMMAs in human urine samples. Finally, it is noteworthy that the method proposed is the first contribution to the quantification of these LMMAs in urine samples using LC–ESI-MS/MS and DNPH as derivatizing agent, being more rapid and simpler and providing higher sensitivity than other reported chromatographic alternatives based on the use of other derivatization agents.

2. Experimental

2.1. Chemicals and reagent preparation

Formaldehyde (FA), acetaldehyde (AA) and hexaldehyde (HA) were supplied by Sigma (Sigma–Aldrich Química, Madrid, Spain), whereas acrolein (AC), propionaldehyde (PA), crotonaldehyde (CA), butyraldehyde (BA), valeraldehyde (VA) and triphenyl phosphate, as an internal standard, were acquired from Fluka (Sigma–Aldrich Química). A 60-mM DNPH (Fluka) stock solution was made by dissolving 594.4 mg of the chemical in 50 ml of concentrated HCl:water:acetonitrile (ACN) solution (2:5:1) and then storing it in a freezer. A 0.25-mM DNPH solution was prepared by appropriate dilution of the stock solution with purified water using a Milli-Q system (Millipore, Bedford, MA, USA). LiChrolut EN (particle size 40–120 μm, surface area ~1200 m^2/g) was provided by Merck (Darmstadt, Germany). All reagents were of analytical grade and the solvents of LC grade, which were obtained through commercial sources.

2.2. Preparation of standards and calibration curves

Individual stock solutions of aldehydes (1 mg/ml) and internal standard (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 0.05 and 6.00 μg/ml. Aldehydes were quantified by means of calibration curves formed from known concentrations of mixtures of analyte standards with a constant level of the internal standard. These standards were spiked into synthetic urine and subjected to the normal sample preparation procedure. Ten calibration levels were used (0, 0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10, 20 and 30 μg/l). The concentration of internal standard in urine was 1 μg/l. Quantification was based on peak area ratios related to the internal standard.

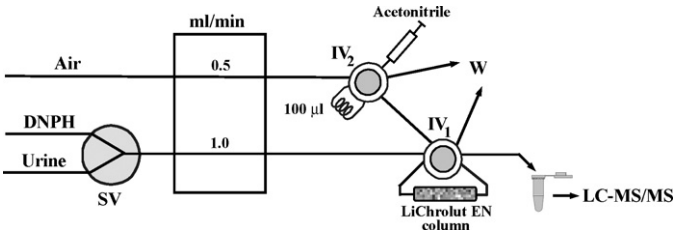

Fig. 1. SPE manifold for the in situ derivatization/preconcentration of LMMAs and the determination by liquid chromatography with a tandem mass spectrometric detector [23]. *Abbreviations*: IV, injection valve; W, waste; SV, selecting valve.

2.3. Urine samples

The composition of the synthetic urine was the typical one for normal urines according to conventional urological research [26–28] and it was prepared by dissolving $Ca_2Cl{\cdot}2H_2O$ (0.65 g/l), $Mg_2Cl{\cdot}6H_2O$ (0.65 g/l), NaCl (4.6 g/l), Na_2SO_4 (2.3 g/l), sodium citrate (0.65 g/l), sodium oxalate (0.02 g/l), KH_2PO_4 (2.8 g/l), KCl (1.6 g/l), NH_4Cl (1.0 g/l), $NaHCO_3$ (0.47 g/l), ascorbic acid (0.34 g/l), urea (25.0 g/l), uric acid (0.2 g/l) and creatinine (1.1 g/l) in purified water.

Natural urine samples were collected from volunteers. All subjects gave informed written consent. Samples were collected in sterilized polyethylene bottles of 250 ml (with hermetical close) without headspace to prevent the formation of air bubbles and stored at 4 °C up to 72 h. Urine samples supplied by volunteers outside the laboratory were transported to it in a portable freezer. When the time between sample 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 thaw urine samples can be stored for 4 h prior their analysis in a refrigerator.

2.4. Continuous SPE derivatization/preconcentration system

The SPE system used for the in situ derivatization/preconcentration of LMMAs in urine samples is depicted in Fig. 1 [23]. It consisted of a Minipuls-3 peristaltic pump from Gilson (Middleton, WI, USA) fitted with poly(vinylchloride) tubes, two model 5041 injection valves from Rheodyne (Cotati, CA, USA) and a laboratory-made sorption column (PTFE, 3 mm ID, 1.2 cm long) packed with 50 mg of LiChrolut EN. Columns prepared sequentially exhibited similar analytical signals which confirm their high reproducibility.

An aliquot of 10 ml of urine sample spiked with 20 μl of the internal standard solution and adjusted to pH 1.5 with 10 ml of 0.3 M HCl was transferred to a 25-ml glass vial that was tightly sealed and immersed in an ice bath in order to prevent possible evaporative losses. The LiChrolut EN column of the SPE system (Fig. 1) was conditioned with 0.5 ml of acetonitrile and 1.0 ml of purified water, and afterward impregnated with 2.0 ml of a 0.25-mM DNPH solution (0.1 mg of DNPH). The urine sample was loaded onto the sorbent column with a flow-rate of 1.0 ml/min for the cleanup, in situ derivatization and preconcentration of aldehydes. Simultaneously, the loop of IV_2 was filled with the eluent (ACN). Prior to elution, by switching IV_1, residual aqueous solution inside the column and the connectors were flushed by passing an air stream through the carrier line of IV_2 at 0.5 ml/min for 2 min. The hydrazones formed were eluted with 100 μl of ACN carried by an air stream at a rate of 0.5 ml/min, and the collected in an eppendorf vial and 10-μl aliquot injected into the LC. If it is required, this ACN extract can be stored at −20 °C up to 30 days or in a refrigerator for 1 week prior to LC–MS/MS analysis. In the case of freezing the ACN extract, it should be thawed in a refrigerator and as stated

above, it can be stored in these conditions up to 7 days. Under these conditions, the sorbent column was serviceable for about 6 months.

2.5. LC–ESI-MS/MS instrumentation and chromatographic conditions

The LC–ESI-MS/MS system was from Varian (Palo Alto, CA, USA) and comprised a ProStar 410 autosampler, two ProStar 210 pumps, and a 1200 L triple quadrupole mass spectrometer equipped with an ESI source. Varian MS workstation version 6.3 software was used for data acquisition and processing.

The mass spectrometer was operated in the negative ion mode. Nitrogen was used as both nebulizing (50 psi) and drying (25 psi) gas, and argon as the collision gas at 1.8 mTorr. The capillary temperature was 300 °C and the capillary voltage 5000 V. Automated MS/MS optimization for each DNPH-derivative was accomplished using MS/MS Breakdown of the Varian workstation and the collision energy ranged from 5 to 20 V. Quantification was performed using selected reaction monitoring (SRM) with the following transitions: m/z 209→151 for FA, m/z 223→178 for AA, m/z 235→235 for AC, m/z 237→163 for PA, m/z 249→172 for CA, m/z 251→162 for BA, m/z 265→163 for VA, m/z 279→152 for HA and m/z 327→233 for triphenyl phosphate (internal standard). The optimized collision energy was 5 V for FA and AC, 7 V for AA, 10 V for PA, BA and VA, 12 V for CA and 20 V for HA and internal standard. The detection voltage was set to 2000 V.

Chromatographic separation was performed on a Polaris C_{18} column (150 mm × 2.0 mm, 3 μm particle size, Varian) maintained at 35 °C using a gradient elution with ACN:methanol, 75:25 v/v (A) and 0.1% formic acid (B) as follows: an initial concentration of 45% solvent A was held for 1 min, and then it was raised linearly (5 min) to 100% and immediately afterwards, held constant for 5 min and operated at a flow-rate of 0.3 ml/min. The injection volume was 10 μl, and the analysis time was 10 min per sample. Retention times were 5.618 ± 0.034, 6.235 ± 0.028, 6.872 ± 0.032, 7.138 ± 0.043, 7.619 ± 0.040, 7.807 ± 0.042, 8.427 ± 0.038 and 9.351 ± 0.051 for hydrazones of FA, AA, AC, PA, CA, BA, VA and HA, respectively.

3. Results and discussion

3.1. Development of the method

As mentioned in the Introduction of this work, LC determinations of aldehydes in urine samples involve a pre-column derivatization of aldehydes, mostly based on the reaction with DNPH. The derivatization requires relatively harsh conditions, e.g. high temperature and acidity and long reaction times, higher than 1 h in many cases. In addition, the hydrazones are extracted by LLE or SPE and in some cases the concentration of the extract down to a small volume for improving sensitivity [15,17,20,21]. These protocols provide good results but they involve an extensive work-up and consume materials and solvents. Recently, we have demonstrated that LMMAs can be readily quantified at nanogram-per-litre levels in water by LC–ESI-MS/MS after their in situ DNPH derivatization/extraction using a continuous SPE system [23]. As a result of the good features of this method, it was used in the present study to improve the determination of LMMAs in human urine samples.

The optimization of the continuous SPE system used in this work for the cleanup, derivatization and preconcentration of aldehydes is not necessary because it has been reported elsewhere [23], although some variables must be re-studied in order to evaluate the possible matrix effect of the urine samples on the analytical signal. So, the influence of three key variables, namely sample pH, the DNPH amount and the breakthrough volume of the sample were reassessed by using different dilution factors per urine sample. From the experimental data, accuracy results (recoveries over 90%) can be achieved when the urine sample is diluted at least twice with 0.3 M HCl (final pH 1.5). Using this dilution factor, none of the above mentioned variables showed significant changes in their dependency with respect to what was observed in the method reported elsewhere for the determination of aldehydes in water samples [23], including the derivatization/extraction efficiency which ranged from 75% to 82%. However, it is note worthy that, although the breakthrough volume of the sample subjected to the SPE protocol was also 20 ml, it is actually half of that for the urine sample due to the dilution factor used. By way of summary, the optimum value of each variable in the continuous SPE system is listed in Table 1. On the other hand and regarding chromatographic conditions, the amount of formic acid in solvent B of the mobile phase was fixed at 0.1% as a compromise between chromatographic resolution and ESI ionization efficiency for the aldehydes-DNPH. Higher concentrations caused a decrease in the sensitivity and the precision of the analytical signals probably due to the formation of adducts in the ionization process. The optimized values of the parameters in ESI–MS/MS have also been reported elsewhere [23]. The product ions at m/z 152 or 163 (similar to those reported in previously published works [29,30]) were used for the quantification of DNPH derivatives of FA, PA, BA, VA and HA, whereas fragment ions at m/z 172, 178 and 235 were selected for the hydrazones of CA, AA and AC, respectively, because of their higher signal intensity.

Table 1
Chemical and flow variables selected for the proposed continuous SPE method.

Variable	Optimum range (selected value)
Sample pH	1.0–3.5 (1.5)
DNPH amount (mg)	0.05–0.3 (0.1)
Sorbent amount (mg)	25–200 (50)
Sample flow-rate (ml/min)	0.5–2.5 (1.0)
DNPH flow-rate (ml/min)	0.5–2.5 (1.0)
Eluent volume (μl)	50–200 (100)
Air flow-rate (ml/min)	0.5–2.5 (0.5)
Sample breakthrough volume (ml)	5.0–30 (20)

3.2. Method performance

The method was validated according to ICH guidelines for bioanalytical method validation [24,25] by using synthetic urine since all human urine samples tested contained appreciable levels of at least some of the aldehydes of interest. Calibration curves were obtained from aldehydes standard spiked in synthetic urine plus the internal standard (1.0 μg/l) and by plotting the analyte to the internal standard peak area against the analyte concentration. Table 2 gives the linear ranges and the least-squares parameters of the working curves. Calibration curves showed a wider linear range (from 0.15 to 30 μg/l) with an adequate linearity (correlation coefficients greater than 0.999). Inter-day variation of calibration slopes (3 consecutive days), measured as the RDS, was less than 2%. It is noteworthy that formaldehyde cannot be determined quantitatively due to the high degree of uncertainty associated with the MS/MS signals measured, around 35% expressed as RSD, and therefore it can only be detected qualitatively in urine samples. This behaviour has already been described in previous works on the determination of LMMAs in water samples by DNPH derivatization coupled to LC–ESI-MS/MS [23,29], and can be ascribed to irreproducibility in the fragmentation process, which avoids obtaining an accurate calibration plot and also to a possible loss of the DNPH-formaldehyde derivative due to the increasing of the temperature of the drying gas for removing all the ions of the front of the solvent, especially the excess of DNPH.

The limits of detection (LODs) and quantification (LOQs) were also determined in synthetic urine and calculated as the lowest

Table 2
The linear range, calibration curve parameters (slope with Std. error and regression coefficient), LODs and LOQs of LMMAs.

Aldehyde	Linear range (μg/l)	Slope ± Std. error	*R*	LOD (ng/l)	LOQ (ng/l)
Acetaldehyde	0.15–30	0.419 ± 0.003	0.9992	40	150
Acrolein	0.05–10	1.273 ± 0.003	0.9996	15	50
Propionaldehyde	0.10–20	0.646 ± 0.004	0.9994	30	100
Crotonaldehyde	0.20–30	0.387 ± 0.002	0.9995	65	200
Butyraldehyde	0.15–30	0.454 ± 0.003	0.9994	35	150
Valeraldehyde	0.15–30	0.464 ± 0.003	0.9991	35	150
Hexaldehyde	0.20–30	0.309 ± 0.002	0.9985	60	200

absolute concentration of the analyte in a sample that provides a chromatographic signal 3 or 10 times, respectively higher than background noise [31]. As can be seen in Table 2, the method allows the determination of these aldehydes at very low levels in human urine samples: the calculated LODs ranged from 15 to 65 ng/l, and the estimated LOQs were in the range of 50–200 ng/l. These values reflect the very good sensitivity provided by the method.

The precision of the method was determined by calculating the intra- and inter-day precision for aldehydes in synthetic urine samples from 30 repeated measurements of quality control low (QCL) and quality control high (QCH) materials over a period of 5 consecutive days (Table 3). Thus, six samples were prepared each day by spiking aldehydes at two concentration levels (0.5 and 10.0 μg/l each) plus the internal standard (1.0 μg/l) to synthetic urine samples. The intra-day precision was calculated based on the measurements ($n = 6$) accomplished on the first day, and the inter-day precision was calculated based on the results of the analyses carried out on 5 consecutive days ($n = 30$). As can be seen in Table 3, the RSDs, which reflect the intra- and inter-day variability of the method, ranged from 2.9% to 9.3% and demonstrate good precision for all of the analytes.

The specific uncertainty of each LMMAs for the whole procedure has also been calculated. This parameter includes several individual standard uncertainties associated with different sources such as precision, preparation of the standards, instrumental calibration (e.g. autosampler stability) and other random error sources associated with the analytical method. In order to calculate it, 11 synthetic urine samples containing 5 ng of each LMMA were subjected to all the process: preparation of calibration curves, storage the urine samples at −20 °C for 3 days and then after its complete thaw in a refrigerator for 2 h, continuous SPE in situ derivatization/preconcentration of LMMAs and analysis by LC–MS/MS. 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 the uncertainty, t is a statistical parameter and n is the number of measures). The specific uncertainty can be easily calculated from: $U = KS$, where $K = 2$ for a probability imposed at the 95% confidence levels [32,33]. Table 3 also summarizes the estimated specific uncertainty of each LMMA for the whole procedure, which represents a mean relative uncertainty of 6.9%.

Method accuracy was assessed by six replicate analyses of synthetic urine spiked at two different concentrations and was expressed as the percentage of expected levels (Table 3). The quality control (QC) urine samples used in this study were obtained by spiking LMMAs standards at low (0.5 μg/l, QCL) and high (10 μg/l, QCH) concentrations and the internal standard at 1.0 μg/l in synthetic urine. The spiked recovery, reflected in method accuracy, ranged from 92% to 100% for all seven analytes at the two spike levels. These results revealed that no matrix effect was observed in the determination of the aldehydes in synthetic urine samples under these experimental conditions.

In summary, the method proposed is accurate and precise between runs and within individual runs and allows the determination of aldehydes at low concentrations (below microgram-per-litre level) in human urine samples. Consequently, this non-invasive assay expands the possibilities to detect and quantify these carbonyl compounds in urine samples, which is of great relevance in clinical studies.

3.3. Analysis of urine samples

The proposed SPE–LC–ESI-MS/MS method was applied to the determination of seven LMMAs in four urine samples following the optimized method described in Section 2. The urine samples came from volunteers who presented the following characteristics: urine 1, adult male about 40 years old; urine 2, an old female about 75 years of age who is receiving a typical osteoporosis treatment for her age; urine 3, the female co-author of this paper; and urine 4, a male laboratory colleague. As can be seen in Table 4, the samples analyzed were found to contain residues of the aldehydes studied, except CA; FA was also detected in urine 3 and 4. By way of example, Fig. 2 shows the SRM chromatogram obtained in the analysis of urine 4, where six aldehydes were quantified. It is noteworthy that the higher levels of aldehyde were found in urine 3, which can be ascribed to the time the co-author of this paper has been slightly exposed to these chemicals on a daily basis in the laboratory. Her laboratory colleague carries out his work in an adjoining room and his levels, although superior to those of an average adult, are considerably lower. To assess the matrix effects, LMMAs at two concentration levels, depending on its value found in the human urine sample, were spiked to urine samples and the corresponding

Table 3
Inter- and intra-day precision[a], recovery[b] and uncertainty[c] of concentration of LMMAs measurements in spiked synthetic urine samples.

Aldehydes	QCL (0.5 μg/l)			QCH (10 μg/l)			Uncertainty (ng)
	Intra-day	Inter-day	Recovery	Intra-day	Inter-day	Recovery	
Acetaldehyde	6.4	9.3	93	5.8	7.5	95	5.1 ± 0.4
Acrolein	5.5	7.2	94	4.2	5.4	99	4.9 ± 0.3
Propionaldehyde	4.9	6.7	97	3.4	4.5	97	5.0 ± 0.3
Crotonaldehyde	5.3	7.5	97	4.5	5.7	100	5.1 ± 0.4
Butyraldehyde	4.3	6.8	92	3.6	4.8	97	4.8 ± 0.3
Valeraldehyde	5.8	7.7	96	4.3	5.5	98	5.0 ± 0.4
Hexaldehyde	3.6	5.9	96	2.9	3.6	97	5.1 ± 0.3

[a] Samples spiked at these two concentrations were analyzed on each day ($n = 6$ at each concentration) of the 5 consecutive days validation.
[b] Average recoveries were determined from intra-day data.
[c] Uncertainty of the whole procedure expressed as $R \pm U$ ($n = 11$, $K = 2$).

Table 4
Concentration of aldehydes in urine samples as determined by the proposed LC–ESI-MS/MS method.

Aldehyde	Concentration found in urine (μg/l; $n=3$)			
	Sample 1	Sample 2	Sample 3[a]	Sample 4
Acetaldehyde	12.3 ± 1.0	20.0 ± 1.4	24.2 ± 1.8	17.4 ± 1.3
Acrolein	2.9 ± 0.2	5.8 ± 0.4	56.9 ± 3.1	2.4 ± 0.2
Propionaldehyde	ND	2.1 ± 0.1	3.7 ± 0.2	1.3 ± 0.1
Crotonaldehyde	ND	ND	ND	ND
Butyraldehyde	ND	2.4 ± 0.1	7.1 ± 0.3	4.6 ± 0.2
Valeraldehyde	ND	3.1 ± 0.2	40.6 ± 2.2	11.6 ± 0.7
Hexaldehyde	ND	11.7 ± 0.5	20.4 ± 0.8	18.9 ± 0.8

ND: not detected.
[a] 5 ml urine was diluted to 20 ml.

extraction recoveries determined. No matrix effect was observed in the determination of aldehydes in human urine samples: the recoveries ranged from 91% to 99%.

Finally, it is noteworthy that the proposed method could also be a useful choice for the determination of these aldehydes in urine of diabetic people because no interferences from monosaccharides (glucose) have been detected even at ca. 3000 μg/ml (typical levels of glucose in urine from people with hyperglycemia). This behaviour can be ascribed to the great difference between the optimal experimental conditions required for the derivatization of monosaccharides with DNPH such as an acidic medium with a high content of organic solvent (50–95%, v/v) at 60 °C for 60–90 min [34,35] and those used in this work for the LMMAs.

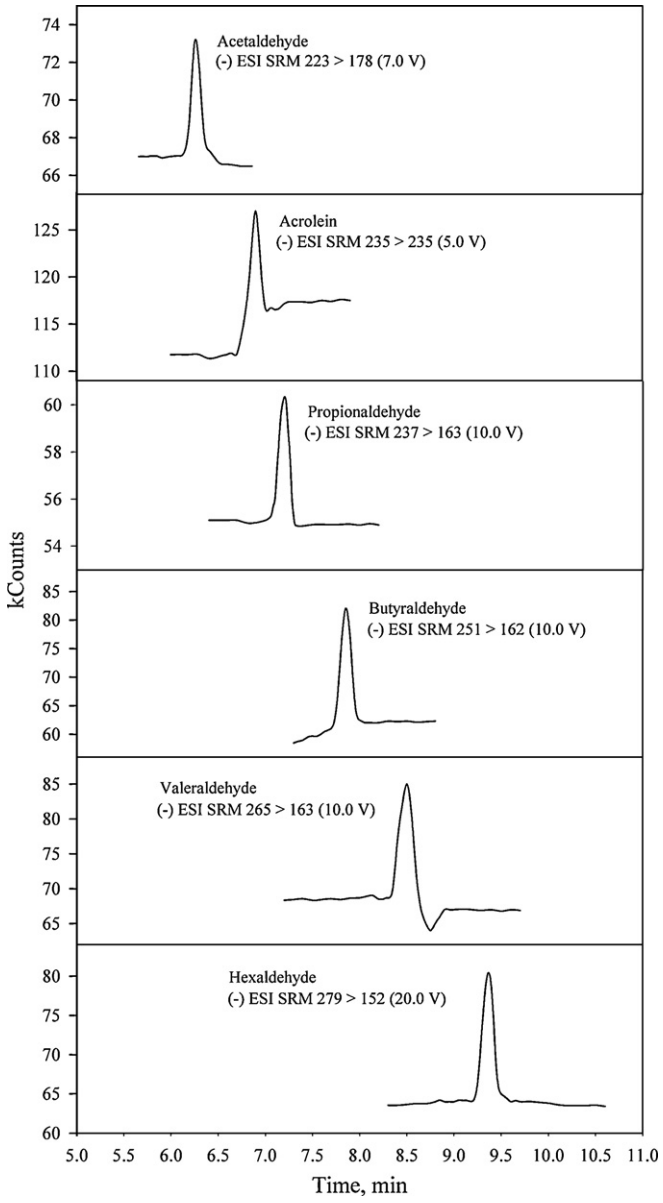

Fig. 2. SRM chromatograms for aldehydes detected in human urine sample 4 (see Table 4). For experimental conditions see Section 2.

4. Conclusions

A rapid and sensitive method has been developed for the direct determination of LMMAs in human urine samples based on their continuous SPE in situ derivatization/preconcentration with DNPH prior to LC–ESI-MS/MS analysis. The results obtained by the proposed method warrant the following comments: (i) the method extends the scope of MS/MS detection in the determination of LMMAs in urine samples by LC after their DNPH derivatization: to our knowledge, the small number of published papers on this topic has been focused on the determination of one/few aldehydes in other biological matrices [19,20] with only one in urine samples [21]; in any case a systematic quantitative analysis including recovery studies has been not carried out; (ii) the continuous SPE system proposed overcomes the main shortcomings associated with the use of DNPH [22] as derivatizing agent for the determination of aldehydes: high temperatures and in acidic medium, high reaction times, and the use of an additional LLE or SPE step required prior to LC separation. As a result, the whole analytical process is simple and subsequently the analysis time is shortened for the DNPH–LC–ESI-MS/MS method proposed; (iii) the LODs and LOQs afforded by combining the SPE step and MS/MS detection (15–65 ng/l and 50–200 ng/l) are lower than those provided by recent GC alternatives with MS [3,13,14] or electron capture [8] detection for the analysis of these carbonyl compounds in urine samples. In summary, the method is a powerful and robust alternative for quantification of LMMAs with an excellent LOD, accuracy and precision and is capable of detecting these aldehydes in human urine samples at levels as low as nanogram-per-litre.

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