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Determination of carnitine and acylcarnitines in urine by high-performance liquid chromatography–electrospray ionization ion trap tandem mass spectrometry

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Abstract

A high-performance liquid chromatography–mass spectrometry method has been developed for the simultaneous determination of native carnitine and eight acylcarnitines in urine. The procedure uses a solid-phase extraction on a cation-exchange column and the separation is performed without derivatization within 17 min on a reversed-phase C_8 column in the presence of a volatile ion-pairing reagent. The detector was an ion trap mass spectrometer and quantification was carried out in the MS–MS mode. Validation was done for aqueous standards at ranges between 0.75 and 200 μmol/l, depending on the compound. Carnitine was quantified in urine and comparison with a radioenzymatic assay gave a satisfactory correlation (R^2=0.981). The assay could be successfully applied to the diagnostic of pathological acylcarnitines profile of metabolic disorders in urines of patients suffering from different organic acidurias.

Keywords: Validation; Carnitine; Acylcarnitines

1. Introduction

Carnitine is an endogenous compound present in most mammalian tissues, reaching particularly high concentrations in skeletal muscle and heart [1,2]. The most important role of carnitine is the transport of fatty acids into the mitochondrial matrix, where they are metabolized via β-oxidation to produce energy [1,3]. For this purpose carnitine can be esterified on the hydroxyl group in position 3 (see R^1 in Fig. 1), generating various acylcarnitines with different chain lengths. A second function of carnitine is the maintenance of the cellular pool of free (unesterified) coenzyme A (CoA) by the transfer of acyl groups from acyl-CoAs to carnitine [1,4]. While free carnitine and acetylcarnitine represent the major constituents of the carnitine pool in normal subjects [1,2], analysis of the different acylcarnitines can provide important information about inherited or acquired metabolic disorders such as organic acidurias [5].

Carnitine determination has been performed with numerous methods. A radioenzymatic assay [6] allows the specific determination of L-carnitine, whereas high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) have

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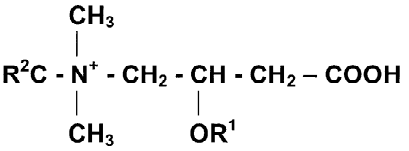

R^2	R^1	Name	Transitions (m/z)
H_3	H	Carnitine	162.2 → 103.0 + 60.1
D_3	H	Carnitine-d_3	165.2 → 103.0 + 63.1
H_3	$COCH_3$	Acetylcarnitine	204.2 → 144.9 + 85.1
H_3	$COCH_2CH_3$	Propionylcarnitine	218.2 → 158.8 + 85.1
H_3	$COC(CH_3)_3$	Isovalerylcarnitine	246.2 → 186.9 + 85.1
H_3	$CO(CH_2)_4CH_3$	Hexanoylcarnitine	260.2 → 200.9 + 85.1
H_3	$CO(CH_2)_6CH_3$	Octanoylcarnitine	288.2 → 229.0 + 85.1
D_3	$CO(CH_2)_6CH_3$	Octanoylcarnitine-d_3	291.2 → 229.0 + 85.1
H_3	$CO(CH_2)_{12}CH_3$	Myristoylcarnitine	372.3 → 313.0 + 211.0
H_3	$CO(CH_2)_{14}CH_3$	Palmitoylcarnitine	400.4 → 341.1 + 239.1
H_3	$CO(CH_2)_{16}CH_3$	Stearoylcarnitine	428.4 → 369.1 + 267.2

Fig. 1. Chemical structures of carnitine and the acylcarnitines analyzed and MS–MS transitions used for quantification.

been used to get enantiomeric separation of D- and L-carnitine [7–10] and to obtain the acylcarnitine profile [5,11–14]. The main drawback of these methods is the need of a derivatization step to introduce a lacking chromophoric group on carnitine and its esters to gain UV or fluorescent absorption. Different chromophores have been used, reacting either on the hydroxyl [7,9,10] or on the carboxyl groups [5,8,11–14] of carnitine. Concerning capillary electrophoresis, another approach is to use indirect UV detection [15]. The acylcarnitine profile can also be obtained with gas chromatography (GC) [16].

Mass spectrometry approaches have been introduced first using fast atom bombardment tandem mass spectrometry (FAB–MS–MS) [17] and then electrospray tandem mass spectrometry (ESI–MS–MS). ESI–MS–MS is frequently used for screening purposes in newborn blood spots [18–21], where acylcarnitines are usually determined after formation of butyl [18–20] or methyl [21] esters. This technique allows the determination of amino acids within the same experiment. Quantification is performed with help of a triple stage quadrupole using either multiple reaction monitoring or precursor ion scans of the common acylcarnitines product m/z 85. Mass spectrometry was also used as a detection method after prior separation of carnitine and acylcarnitines by HPLC [22–24], CE [25,26] or GC [27,28]. Very recently, a method for the analysis by HPLC–MS–MS of biosynthetic carnitine precursors has been published [24]. The method uses derivatization with methyl chloroformate for the extraction and an ion pair reagent for the HPLC separation. So far, quantification of carnitine and acylcarnitines was performed with quadrupole mass spectrometers, single or triple, except in one study in which an ion trap method was used [23].

Since separation and quantification of carnitine and acylcarnitines in urine has currently not been described for ion trap systems, we decided to develop such a method. We describe a novel reversed-phase HPLC separation of underivatized carnitine and eight biological relevant acylcarnitines using an electrospray ion trap mass spectrometer detector. We will discuss the application of this system to urine samples of volunteers as well as of patients suffering from organic acidurias and describe the problems we were facing for quantification with an ion trap system.

2. Materials and methods

2.1. Chemicals

L-Carnitine was from Fluka (Buchs, Switzerland), and acetylcarnitine, hexanoylcarnitine, octanoylcarnitine, myristoylcarnitine, palmitoylcarnitine and stearoylcarnitine were from Sigma (St. Louis, MO, USA). Propionylcarnitine and isovalerylcarnitine were a gift from Sigma-Tau (Zofingen, Switzerland). The deuterated internal standards [$^2H_3$]carnitine (carnitine-d_3) and [$^2H_3$]octanoylcarnitine (octanoylcarnitine-d_3) were purchased from Cambridge Isotope Labs (Andover, MA, USA). Methanol and water were of HPLC grade from Merck. Other reagents were of analytical grade and purchased either from Fluka or from Merck.

2.2. Preparation of internal standards and standard solutions

Internal standard stock solutions of 1 m*M* carnitine-d_3 and 1 m*M* octanoylcarnitine-d_3 were prepared in methanol. A mixed internal standard spiking solution was prepared by diluting these stock solutions to have respective concentrations of 100 and 20 μmol/l.

Standard stock solutions of carnitine and acylcarnitines for calibration were prepared in methanol in concentrations between 5 and 20 m*M*. A mix standard stock solution was prepared by diluting the respective stock solutions to obtain a solution containing all acylcarnitines of concentrations ranging from 80 to 800 μmol/l. This solution was further diluted in methanol to obtain the calibration standard solutions. The carnitine concentration was standardized using a spectrophotometric method [29]. Carnitine contamination due to spontaneous hydrolysis was estimated for each acylcarnitine by injecting the individual standard stock solution and measuring the respective peaks area. The carnitine content was not found to be relevant in any of them.

2.3. Urine sample collection

Urine samples were obtained from the chemical laboratory of the University Hospital of Basel. One of these urines was used as a quality control sample and to establish calibration curves. Patient urines were obtained from the Children Hospital in Basel.

2.4. Urine sample preparation

Urine pretreatment included dilution, acidification and solid-phase extraction. Urine (200 μl) was mixed with 50 μl internal standard spiking solution and 50 μl of 1 *M* HCl. For spiked urine samples, 50 μl of the appropriate standard stock solution was also added. The volume was adjusted to 1 ml with water. The solid-phase extraction was performed as described by Kamimori et al. [13], after some modifications, using disposable cation-exchange columns (SCX 100 mg, Varian, Harbor City, CA, USA) and a Vac-Elut system (Analytichem, Harbor City, CA, USA). After preconditioning with 1 ml methanol, 1 ml water and 1 ml of 10 m*M* aqueous HCl, samples (1 ml) were loaded, washed with 3 ml of 10 m*M* aqueous HCl and eluted with 2 ml of 75 m*M* pyridine in methanol–water (1:1, v/v). The eluate was collected in silanised glass tubes an evaporated to dryness at 40 °C using a TurboVap evaporator (Zymark, Hopkinton, MA, USA). The residue was dissolved in 1 ml methanol and reevaporated. The residue was reconstituted in 200 μl methanol–water (1:1, v/v) containing both 10 m*M* heptafluorobutyric acid and 10 m*M* ammonium acetate.

2.5. HPLC–MS–MS analysis

The HPLC separation was performed on a HP 1100 system (Agilent Technologies, Palo Alto, CA, USA) equipped with a heated column compartment. The analytical column was a reversed-phase LUNA, C_8, 150×2.0 mm I.D. (5 μm particles) (Phenomenex, Torrance, CA, USA), equipped with a corresponding pre-column MOS, C_8, 4.0×2.0 mm (Phenomenex). The column was maintained at 30 °C. A gradient elution with two mobile phases was used: 10 m*M* heptafluorobutyric acid and 10 m*M* ammonium acetate in water (A) or in methanol (B). The gradient started with 20% B, went up to 90% B between 0.1 min and 4 min and was followed by a plateau at 90% B for 14 min. The column was then reequilibrated for 10 min with 20% B. The flow was

set at 200 μl/min and the injected sample volume was 2 μl.

Mass spectrometry detection was performed on a Finnigan LCQ^{DECA} ion trap instrument (Finnigan MAT, San Jose, CA, USA) equipped with an electrospray ionization source that was run in the positive ion mode (4.0 kV). Sheath gas (N_2) pressure was set at 70 arbitrary units. The temperature of the heated capillary was kept at 300 °C. Quantitative analysis of carnitine and acylcarnitines was monitored in the MS–MS mode. For this purpose each of the nine peaks chromatographed was associated with a segment. During the analysis time of each segment, one precursor ion was stored in the trap and further fragmented with a collision energy that allowed a sufficient fragmentation of the selected ion. In the case of carnitine and octanoylcarnitine, as their respective deuterated derivative used as internal standards are co-eluting, two ions were attributed to their respective segment. The relative collision energy was set up at 35% for each compound, the isolation width fixed at 1.8 and the product ions were scanned. Automatic gain control was employed using one microscan and a maximum injection time of 200 ms. Carnitine-d_3 was used as internal standard at a concentration of 20 μmol/l for carnitine, acetylcarnitine and propionylcarnitine, whereas octanoylcarnitine-d_3 was used as internal standard at a concentration of 5 μmol/l for all other acylcarnitines. Areas obtained for the two most intense product ions (a common and a specific one) were corrected with the respective areas of the internal standard and were used for quantification. The observed transitions are given in Fig. 1. The HPLC and MS system were computer controlled using Xcalibur 1.2 software (Finnigan). The same software was also used for all quantitative calculations. Linear regressions were calculated with a weighting factor of 1/X.

2.6. Method validation

Linearity of the developed HPLC–MS–MS method was investigated for carnitine and five short and medium chain acylcarnitines on aqueous standards. Calibrations (six points) were prepared in concentration ranges of 5–200 μmol/l for carnitine, 2.5–100 μmol/l for acetylcarnitine and 0.75–30 μmol/l for propionylcarnitine, isovalerylcarnitine, hexanoylcarnitine and octanoylcarnitine. Precision and accuracy were determined for carnitine, acetyl-, propionyl-, isovaleryl-, hexanoyl- and octanoylcarnitine by running aqueous quality controls at three different concentrations covering the calibration range, on the same (intra-day) and on different days (inter-day variability). Urine quality controls were run simultaneously at two different concentrations, namely a blank (unspiked) urine and the same urine after spiking with carnitine, acetyl-, propionyl-, isovaleryl-, hexanoyl- and octanoylcarnitine.

2.7. Urine

Blank and spiked urines were extracted as described above. Carnitine quantitation results of quality control urine obtained with an aqueous standard calibration were compared with the one obtained with a calibration done with spiked urine. Free carnitine was determined in 15 different urine samples and the results were compared with radioenzymatic assay. The radioenzymatic assay used has been described by Hoppel [6].

3. Results and discussion

Carnitine and acylcarnitines belong to a family of compounds with a large span of polarities, from the polar carnitine to the quite apolar long-chain acylcarnitines, such as for instance stearoylcarnitine. From these characteristics one can easily presume that their simultaneous extraction from a biological matrix and their chromatographic separation will be a rather delicate task. For instance, it is not possible to use the common C_8 or C_{18} reversed-phase material, because carnitine and short-chain acylcarnitines are not properly chromatographied under these conditions. So far, the use of reversed-phase HPLC was used after a derivatization step that does not only introduce a chromophore group for UV or fluorescence detection, but also decreases the polarity of carnitine, enabling thereby its retention on such material. To develop our method we used the zwitterionic feature of these analytes for their extraction and exploited the formation of ion-pair for their separation on reversed-phase material.

3.1. Optimization of solid-phase extraction

In acidic conditions the carboxylic group of the analytes (see Fig. 1) is protonated, resulting in the presence of permanently positively charged quaternary amines. Under these conditions, the choice of a cation-exchange column for the extraction is particularly well suited. According to the literature, ammonia [16] and pyridine [13] were evaluated to displace carnitine and its esters from the column. The entire extraction process was investigated using [^{14}C]-carnitine and [^{14}C]-palmitoylcarnitine, a hydrophilic and a lipophilic representant of the analyte family. As we were facing hydrolysis of acylcarnitines during the extraction process when using ammonia, what was absolutely not desired, we changed to pyridine. The minimal effective concentration able to displace in a satisfactory way both analytes was found to be at 75 m*M* pyridine in a water–methanol (1:1, v/v) mixture. Recovery for [^{14}C]-carnitine was 75 and 73% for [^{14}C]-palmitoylcarnitine. Silanised glass tubes were used to collect the eluate in order to prevent interactions of positively charged carnitines with the hydroxyl groups of glass. It was impossible to resolubilize the dried eluate using the starting HPLC mobile phase, since with 20% methanol the long-chain acylcarnitines could not be brought into solution. We, therefore, used a water–methanol (1:1, v/v) mixture, containing 10 m*M* heptafluorobutyric acid and 10 m*M* ammonium acetate.

3.2. Analysis of carnitine and acylcarnitines by HPLC–MS–MS

For the HPLC separation we investigated different volatile ion-pairing agents, in order to mask the positive charge on the nitrogen atom and to increase the interactions of the polar compounds with reversed-phase material. While the addition of trifluoroacetic acid was not successful (carnitine was still not retained by the C_8 column), heptafluorobutyric acid at an optimal concentration of 10 m*M* showed enough ion-pairing interactions in order to get retention of carnitine and acetylcarnitine. The pH of the eluant was fixed to 3.2 with 10 m*M* ammonium acetate, as a more acidic mobile phase was not required to increase the number of positive charges and in order to protect the column material. With this composition of the mobile phase and using a gradient, from 20 to 90% organic solvent, to increase the elution strength, we could elute stearoylcarnitine within 17 min. A typical chromatogram obtained under the developed conditions, recorded in MS mode, shows a good baseline separation of carnitine and eight acylcarnitines (see Fig. 2). With higher concentrations of heptafluorobutyric acid, as described in the literature for the determination of acetylcholine and related quaternary amines such as carnitine and acetylcarnitine [23] or, most recently, for the determination of carnitine and carnitine precursors [24], the retention times of long-chain acylcarnitines increases without any further benefit for the retention of carnitine and acetylcarnitine. As samples had to be solubilized in a water–methanol (1:1, v/v) mixture, their elution strengths were higher than the one of the mobile phase and we were facing peak broadening, especially for carnitine and acetylcarnitine. By reducing the injection volume from 10 to 2 μl, the resulting peak sharpening compensated for the water–methanol (1:1, v/v) mixture, and we did not lose too much sensitivity, since the number of scans through the peaks was still sufficient.

The electrospray ionization process shows a predominant protonated molecular ion of $[M+H]^+$ for all analyzed compounds (data not shown). The formation of these positively charged molecular ions may also be supported by the presence of heptafluorobutyric acid in the mobile phase. These peaks were selected as precursor ions to undergo collision induced dissociation in the ion trap and produce the MS–MS data. The fragmentation pattern of these ions follows a similar process, namely loss of the trimethylamine moiety (see Fig. 3). While the corresponding m/z 60 ion is seen for carnitine and acetylcarnitine, only the complementary ions $[M+H–N(CH_3)_3]^+$, that are compound-specific, are seen for all the other acylcarnitines. In addition for short- and medium-chain acylcarnitines, a common fragment at m/z 85 is present, corresponding to a rearrangement of the butyric acid chain ($^+CH_2$–CH=CH–COOH), as suggested in the literature [22,26]. This common fragment ion is the most intense for the short-chain acylcarnitines, whereas the specific fragment ion $[M+H–N(CH_3)_3]^+$ is the

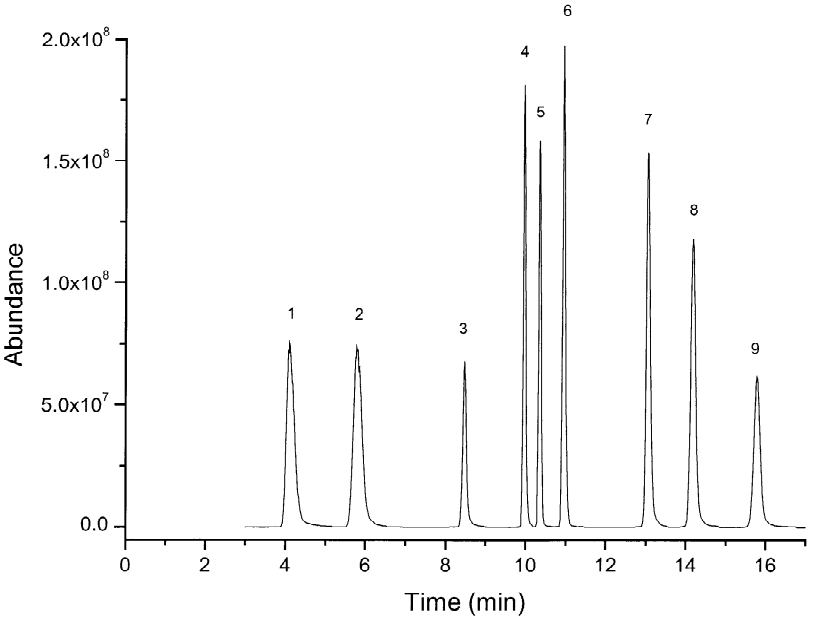

Fig. 2. Total ion current trace of a mixed standard containing: 1, carnitine (80 μmol/l; 160 pmol injected); 2, acetylcarnitine (40 μmol/l; 80 pmol injected); 3, propionylcarnitine (12 μmol/l; 24 pmol injected); 4, isovalerylcarnitine (12 μmol/l; 24 pmol injected); 5, hexanoylcarnitine (12 μmol/l; 24 pmol injected); 6, octanoylcarnitine (12 μmol/l; 24 pmol injected); 7, myristoylcarnitine (8 μmol/l; 16 pmol injected); 8, palmitoylcarnitine (8 μmol/l; 16 pmol injected); 9, stearoylcarnitine (8 μmol/l; 16 pmol injected).

most intense for the medium-chain acylcarnitines. With a chain length larger than six carbons, the fragmentation process produces the specific ion (due to the loss of trimethylamine), and a less intense ion corresponding to the fatty acid moiety itself (R^1 in Fig. 1).

3.3. Method validation

Quantification was carried out in the MS–MS mode using two product ions, a common and a specific one, as in the fragmentation patterns these two ions were of relative intensities between 40 and 90%. This way we could gain selectivity. Although carnitine exhibits a lower electrospray sensitivity than the acylcarnitines, the resulting increase in the limit of quantification does not disturb its determination in biological matrix, since the urine concentrations of carnitine are usually higher than that of acylcarnitines. We did not perform any quantification experiments on long-chain acylcarnitines because they are usually not present in urine [6].

The linearity of the method was evaluated for carnitine and five short- and medium-chain acylcarnitines on aqueous standards. Ranges were 5–200 μmol/l for carnitine, 2.5–100 μmol/l for acetylcarnitine and 0.75–30 μmol/l for propionylcarnitine, isovalerylcarnitine, hexanoylcarnitine and octanoylcarnitine. Limits of quantitation (LOQs) were determined as the lowest concentration with a relative deviation of replicate runs of less than 20%. They correspond to 5 μmol/l for carnitine, 2.5 μmol/l for acetylcarnitine and 0.75 μmol/l for propionylcarnitine, isovalerylcarnitine, hexanoylcarnitine and octanoylcarnitine, respectively, and were chosen as the lowest concentration of the respective calibration standards. For quantitation, areas of the two most intense product ions were used after division by the respective areas of the selected internal standard. The linearity of the method was validated by recalculating the aqueous calibration standard concentrations ($n=6$) with the obtained linear regression. Results for carnitine are given in Table 1. With precisions smaller than 8% and accuracies of 99.2±9.3% we found these results to be within acceptable limits. Precision and accuracy

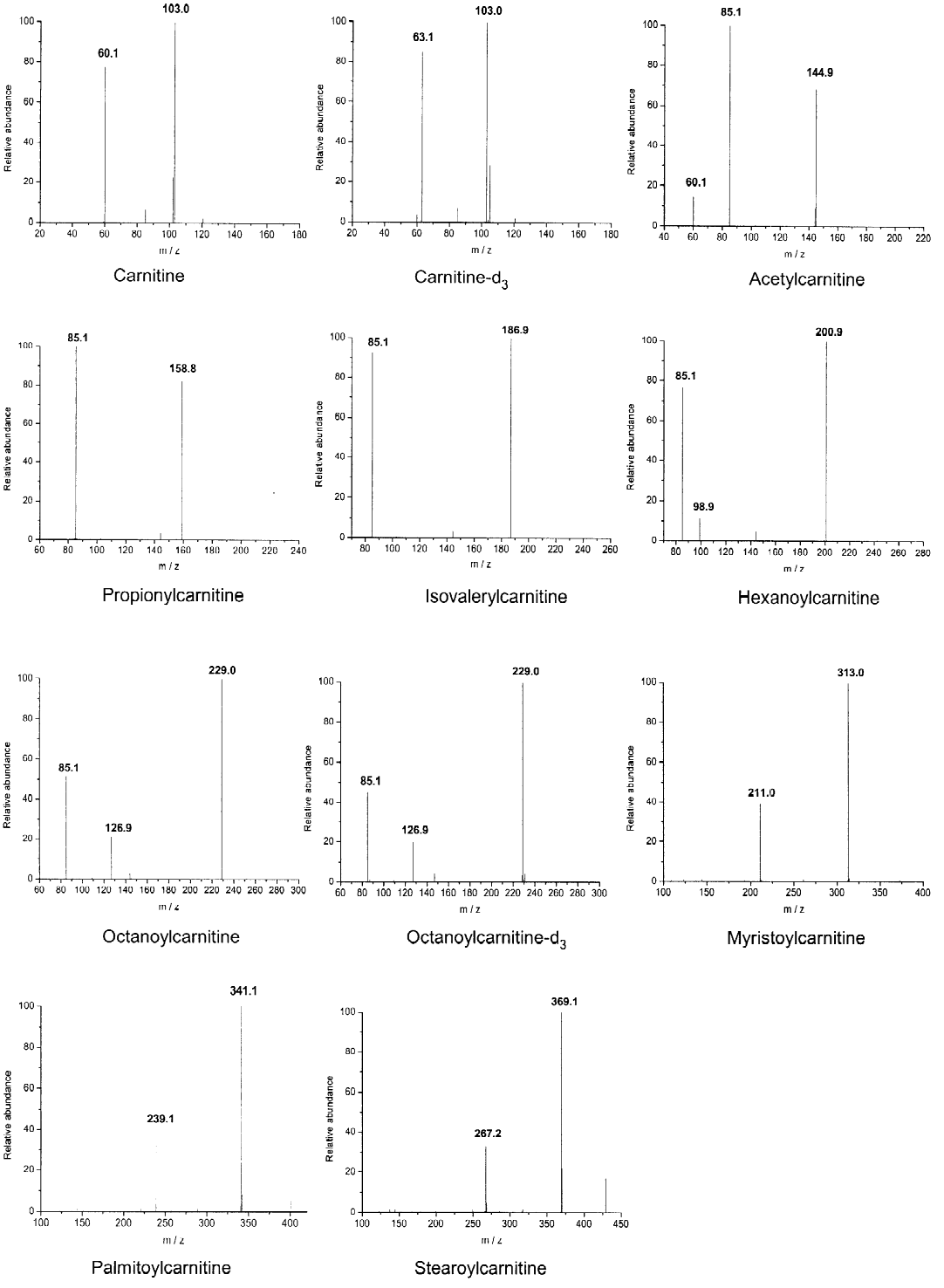

Fig. 3. Product ion spectra (MS–MS) of analyzed compounds with relative collision energy of 35% and an isolation width of 1.8.

Table 1
Accuracy and precision for the determination of carnitine in calibration standards

Added concentration (μmol/l)	Found concentration (μmol/l)	SD	Precision (%)	Accuracy (%)
5	5.8	0.4	7.2	116.5
10	9.8	0.6	5.7	98.0
20	17.8	1.3	7.1	89.2
50	46.9	3.5	7.4	93.7
100	100.4	4.5	4.5	99.6
200	204.2	3.5	1.7	97.9

$n=6$ determinations.

data for intra- and inter-day assays on aqueous quality controls were performed at three different concentrations, covering the concentration range of the calibration standards used. For intra-day analysis, the precision was between 1.2 and 8.0% for carnitine and acylcarnitines, whereas the values for inter-day precision were between 2.7 and 14.6%. Results for carnitine are presented in Table 2.

3.4. Application to urine

A chromatogram obtained for a urine sample is shown in Fig. 4A. We see a typical urine profile with predominant peaks of carnitine (peak 1) and acetylcarnitine (peak 2). Depending on the urine concentration, propionylcarnitine (peak 3) and isovalerylcarnitine (peak 4) can also occasionally been seen. In contrast to standards where the method could easily be validated with acceptable intra- and inter-day precisions and accuracies for all compounds, we were facing more difficulties using urine, where only the quantification of carnitine was validated. As already mentioned, carnitine and acylcarnitines are endogenous compounds, rendering it impossible to get carnitine-free urine. The presence of endogenous carnitine and acylcarnitines makes it difficult to use urine as a matrix for calibration standards. To facilitate the determination of carnitine in urine, we therefore investigated the possibility of using a calibration curve performed with aqueous calibration standards instead of spiked urine. As compared to calibration standards in urine, aqueous calibration standards yielded similar results, with deviations of 5.6 or 3.0% for non-spiked or spiked urine, respectively ($n=8$ determinations for each condition). These results indicated that urine could

Table 2
Intra- and inter-day variability of free carnitine in aqueous standard and in urine

	Inter-day assay ($n=12$)			Intra-day assay ($n=7$)		
	Low	Medium	High	Low	Medium	High
Standard quality control						
Added concentration (μmol/l)	15	80	150	15	80	150
Found concentration (μmol/l)	13.6	78.3	150.9	14.7	86.0	150.3
Precision (%)	5.3	8.1	10.9	1.2	2.7	2.7
Accuracy (%)	91.0	97.8	100.6	98.0	107.5	100.2
	Inter-day assay ($n=4$)			Intra-day assay ($n=8$)		
	Unspiked	Spiked		Unspiked	Spiked	
Urine quality control						
Added concentration (μmol/l)	0	50		0	60	
Found concentration (μmol/l)	63.7	111.5		63.8	127.6	
Precision (%)	6.0	4.0		1.8	6.1	
Accuracy (%)	nd	95.7		nd	106.3	

nd: not determined.

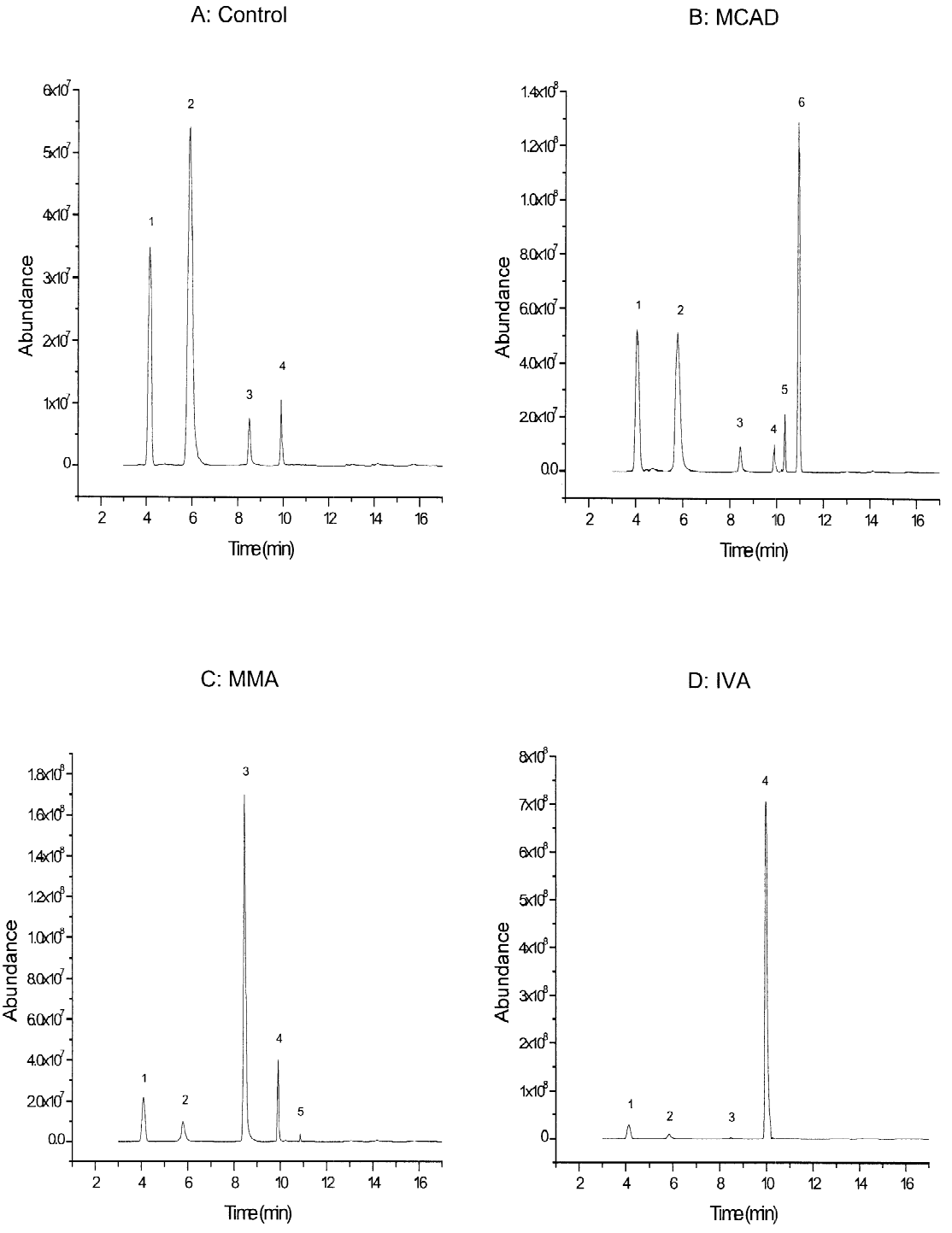

Fig. 4. Total ion current trace. (A) Control urine. (B) Medium chain acyl-CoA dehydrogenase deficiency (MCAD). (C) Methylmalonic aciduria (MMA). (D) Isovaleric acidemia (IVA). The samples were extracted and analyzed as described in the Materials and methods section. Peaks are: 1, carnitine; 2, acetylcarnitine; 3, propionylcarnitine; 4, isovalerylcarnitine; 5, hexanoylcarnitine; 6, octanoylcarnitine.

be substituted with water to prepare the calibration standards. Further experiments showed also that the calibration curves of standards undergoing extraction and HPLC–MS–MS were not different to curves of standards undergoing HPLC–MS–MS only (results not shown), as the use of an internal standard compensates for any extraction loss. Intra- and inter-day variabilities for unspiked and spiked urine quality controls are shown in Table 2 and are, with precisions smaller than 6.1%, as good as for standard quality controls.

The quantification of free carnitine in 15 urines was finally compared with the standard radioenzymatic method (Fig. 5) and a linear relationship is observed ($R^2=0.981$). However, the concentrations found with the HPLC–MS–MS method were approximately 10% lower than the ones obtained with the radioenzymatic assay and there was a positive intercept on the x-axis. Taking into consideration the quantification of carnitine in calibration standards (Table 1) and the recovery of carnitine added to urine (Table 2), the problem appears to be more likely due to the radioenzymatic than to the HPLC–MS–MS assay.

For acylcarnitines, the intra-day precisions were below 15% except for octanoylcarnitine, which had a variability of 40%. As for carnitine, the inter-day variabilities for acylcarnitines were higher than intra-day, with variabilities in the range of 20% for most of them. The use of only two internal standards for quantification of all analytes is probably associated with an increased variability, as observed also by others [24]. Better results might be observed with a deuterated derivative for each analyzed acylcarnitine, since the suppression effects would be the same for each compound and its internal standard, as they will enter the trap under the same conditions of ion trap gating and HPLC mobile phase composition.

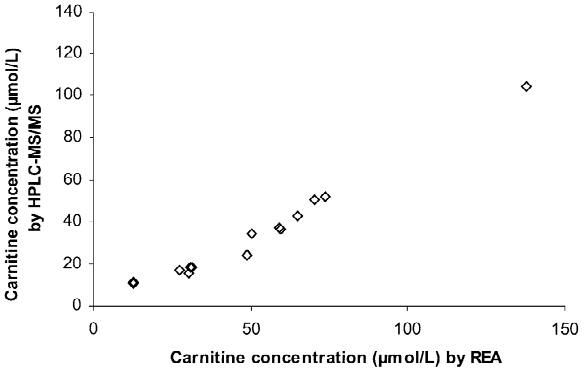

Fig. 5. Correlation between the quantification of free carnitine in urine, using a radioenzymatic method (REA) and HPLC–MS–MS. The relationship is characterized by the following equation $y = 0.909x - 7.32$ ($R^2=0.9806$).

Fig. 4B–D displays urine HPLC chromatograms from patients suffering from different metabolic disorders. Fig. 4B is from a patient with medium-chain acyl-CoA dehydrogenase (MCAD) deficiency. As expected with this metabolic disorder [18], the peaks for octanoylcarnitine and hexanoylcarnitine are increased (peaks 6 and 5, respectively) and the ratio octanoylcarnitine/acetylcarnitine is higher than 0.1. Fig. 4C depicts a chromatogram of a patient suffering from methylmalonic acidemia (MMA) where the propionylcarnitine peak (peak 3) is more than 8-fold increased when compared with a control urine [20]. Fig. 4D shows a urine of a patient with isovaleric acidemia (IVA). As expected, the isovalerylcarnitine peak (peak 4) is increased.

4. Conclusion

In this paper we presented a reversed-phase HPLC separation of underived carnitine and eight acylcarnitines with ion trap mass spectrometry detection. The method is based on the detection of common and specific daughter ions produced by collision-induced dissociation of the molecular cation. This method could be applied successfully to the identification and quantification of carnitine and acylcarnitines. The application to patient urines proved that the developed HPLC–MS–MS method is capable to identify metabolic disorders and can therefore be used for diagnostic purposes in a non-invasive way. The application of this method to plasma and tissue samples and the extension towards quantification of long-chain acylcarnitines is currently investigated.

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