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PII: S0026-265X(16)30199-0
DOI: doi: 10.1016/j.microc.2016.07.018
Reference: MICROC 2538

To appear in: *Microchemical Journal*

Received date: 22 March 2016
Revised date: 18 July 2016
Accepted date: 19 July 2016

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Abstract

A rapid, selective and sensitive ultra-high performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) method was developed to determine carnitine in human urine. Solid phase extraction approaches based on the use of polymeric and weak cationic exchange cartridges were evaluated and applied to the treatment of urine samples. After optimizing the various stages of SPE, a satisfactory set up for retaining substances interfering on carnitine's response was achieved for both type of cartridges. The UPLC separation was carried out on a reversed phase column. The detection was performed on a triple quadrupole tandem mass spectrometer by multiple reaction monitoring (MRM) mode via electrospray ionization (ESI) source. Residual matrix components were specific to urine samples and interfered on the carnitine signal (a response suppression of 50% was observed). It was then demonstrated that sample treatment by SPE could reduce the effect of the above mentioned interferents, without needing a preliminary derivatization step. The recovery percentage of carnitine obtained after the application of SPE was of approximately 83±7% and, consequently, the matrix effect was minimized. Thus, a sensitive, precise and reliable methodology was developed to determine traces of carnitine in biological fluids.

Keywords: Carnitine; SPE; UPLC-(+)ESI-MS/MS; Urine.

1. Introduction

Carnitine (*β*-hydroxy-*γ*-(trimethylammonio) butanoate) is a low molecular weight trimethylammonio carboxylate found in the human diet that is also biosynthesized from the essential amino acids lysine and methionine [1, 2]. Within a biological system, the carnitine content consists of non-acylated carnitine ("free carnitine") along with endogenously formed acylcarnitines, generated by reversible enzymatic transacylation between acyl-CoAs and free carnitine. The sum of the free carnitine and the acylcarnitine content is designated as the "total carnitine" level in the sample [2].

As well known, carnitine plays a key role in fatty acid oxidation [3]. It can be conjugated to fatty acids to form acylcarnitines, which facilitates fatty acid transport into the mitochondrial matrix where oxidation takes place. Acylcarnitines have become important biomarkers for various types of diseases [4]. The carnitine/acylcarnitine pool is reflective of metabolic homeostasis. Therefore, quantification of free carnitine is of diagnostic value for the characterization of conditions associated with altered carnitine metabolism, including disorders of fatty acid oxidation [4-10].

Urinary acylcarnitine analysis can also be useful since the distribution pattern of these species or the excretion of particular acylcarnitines provides some information about metabolic disease [11]. To separate, identify, and quantify acylcarnitine species, chromatographic or capillary electrophoresis techniques have been combined with mass spectrometry [12].

Electrospray is currently the most widely used ionization source for carnitine and carnitine derivatives analysis [13]. HPLC–MS systems using an electrospray ion source coupled with tandem mass analyzers (HPLC–ESI–MS/MS) have been applied to a wide variety of

studies in pharmaceutical analysis and life sciences. Additionally, HPLC– ESI–MS/MS is now considered the benchmark for measurement of drugs and their metabolites in biological matrices [14]. Many biochemical markers, included carnitine and related compounds, have been monitored by this analytical technique [15-17].

While HPLC (UPLC)–ESI–MS/MS offers much promise for clinical laboratories, one issue that must be addressed in method development, validation, and routine use is the matrix effect. Matrix effects are produced by undetected coeluting substances which alter the ionization efficiency of the electrospray interface [18]. Recent papers have highlighted the importance of understanding matrix effects in clinical mass spectrometry applications; and although this is critical to the success of an HPLC–ESI–MS/MS analytical method, few published methods address this problem adequately [19]. It has been found that the most polar compounds experience the largest ion suppression and the least polar one show less ion suppression [20, 21]. To this point, it is important to note that carnitine is the most polar analyte within the acylcarnitines group.

The importance of assessing the matrix effects on the reliability of HPLC–ESI–MS/MS has been shown in terms of accuracy and precision. When ion suppression occurs, the sensitivity and the quantification limit of a method may be adversely affected [13, 22]. Thus, to develop a reliable HPLC–ESI–MS/MS method, experiments should be performed to understand these matrix effects and, if they occur, minimize/eliminate them.

The most commonly used biological samples in clinical analysis are urine, serum, plasma, and whole blood, which contain high concentrations of proteins [23]. The signal intensity can be suppressed by high mass flows and co-elution of specific compounds - e.g., high concentrations of sugars, proteins, lipids, salts, amines, glycopeptides, phosphocholines or

metabolites of these targets-. Alternatively, the signal can be enhanced by the accumulation of positively charged ions or by neutralizing the charge of the target molecules [24-25].

Today, LC-MS has evolved into a technique characterized by sensitivity, selectivity, and specificity, allowing for the analysis of trace amounts of target analytes in complex mixtures. Based on these characteristics, one would expect that sample preparation prior to analysis could be minimized or even eliminated [10, 26]. However, as mentioned, co-elution of similar compounds is an important issue when complex samples are analyzed.

A prospective solution to the problems derived from the matrix effects could be the application of separation techniques - as a pretreatment - which offer sample clean-up. This eliminates the detrimental effect of the sample matrix and improves the detection sensitivity of the whole analytical system, which can be achieved using conventional extraction techniques, such as liquid-liquid extraction [27] and solid-phase extraction [28-35]. In previous studies by our group, a carnitine signal decrease of approximately 50% for the analysis of urine samples was observed. Thus quantification was performed using the standard addition method [30]. At present, many efforts have been dedicated to the clean-up and the interference elimination (via diverse SPE strategies) in order to achieve a confident detection and quantification of carnitine and its derivatives in biological fluids. Although satisfactory recoveries were obtained, many of these methodologies are based on a previous derivatization step, which make the sample processing time consuming and expensive (**Table 1**).

This paper describes the ESI-MS/MS quantification of the carnitine levels present in urine samples after applying a solid-phase extraction procedure based on the use of cartridges of different nature, including weak cation exchange (CBA) and neutral-basic acid (ABN). Thus SPE was used for the rapid and selective extraction of the interferents of the carnitine

analytical response from urine samples, without needing a previous analyte's derivatization procedure. After optimizing the various stages of the SPE method, a satisfactory retention of the interfering substances present in urine samples was observed. Finally, a sensitive, reliable and precise methodology for the determination of carnitine present in human urine samples of clinical interest in the μg L^{-1} levels was achieved.

2. Material and methods

2.1 Chemicals and reagents

L-Carnitine inner salt, 98% was purchased from Sigma-Aldrich (St. Louis, USA). Water, methanol and acetonitrile Optima® LC-MS grade were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Formic acid, 98%, was obtained from Fisher Scientific (Loughborough, UK). Ammonium hydroxide was obtained from Cicarelli Laboratories (Santa Fe, Argentina). Ultrapure water (18 MΩ cm) was obtained from EASY pure (RF Barnstead, IA, USA).

Methanolic standard solutions of carnitine were daily prepared by appropriate dilutions of a 1 mg L^{-1} aqueous stock solution. Recovery studies were achieved by preparing spiked urine samples with proper amounts of the analyte. The solutions were maintained at 4°C.

2.2 Sample collection

In this study human urine samples were analyzed. These samples were part of a study related to the evaluation of carnitine variations with some thyroid gland disorders. As mentioned earlier, carnitine has been used as a biomarker for metabolic disorders, including those related to the thyroid gland [5]. In the case of hypothyroid patients, the trend observed demonstrated a lower carnitine content compared with healthy individuals. Earlier

studies indicated that carnitine levels in skeletal are down-regulated when the hypothyroidism condition is present, this decline may contribute to certain myopathies of skeletal and cardiac muscle.

After obtaining written informed consent, urine samples (n=20) were collected from healthy female and male adults, ranging 40 to 60 years old. The whole procedure was made anonymous and both the study and informed consent were approved by the Ethics Committee of the National University of Rosario, Argentina.

2.2.1 Urine samples

Urine samples were collected in sterile specimen collection bottles. The samples were then centrifuged for 10 min at 5,000 rpm. An aliquot of the supernatant was filtered through a 0.22 μm syringe filter (Osmonics®), diluted with ultrapure water up to 1 mL final volume.

2.3 UPLC instrumentation and conditions

An Acquity™ Ultra High Performance LC system (Waters, Milford) equipped with autosampler injection and pump systems (Waters, Milford) was used. The autosampler vial tray was maintained at 15 °C. The needle was washed with proper mixtures of acetonitrile and water. The separation was performed by injecting a 10 μL sample onto an ACQUITY UPLC® BEH C_{18} (Waters, Milford, USA) analytical column with 2.1 mm internal diameter, 50 mm length, and 1.7 μm particle size. The binary mobile phases consisted of water with formic acid 0.1% (v/v) (A) and acetonitrile with formic acid 0.1% (v/v) (B) delivered at 0.25 mL min^{-1}. The C_{18} gradient was started at an initial composition of 90% A and 10% B, then 1 min linear gradient to 80% A, held for 1 min. A return to the initial conditions was accomplished by a 0.5 min gradient to 90% A, where it was held for 1 min.

Thus, the total chromatographic run time was 3.5 min. The column was held at a temperature of 30 °C. Under these conditions, no sample contamination or sample-to-sample carryover was observed.

2.4 Mass spectrometry instrumentation and MS/MS conditions

Mass spectrometry analyses were performed on a Quattro Premier™ XE Micromass MS Technologies triple quadrupole mass spectrometer with a ZSpray™ Electrospray ionization source (Waters, Milford, USA). The source was operated in the positive electrospray mode ((+) ESI) at 345 °C with N_2 as the nebulizer and the source temperature was kept at 150 °C. The capillary voltage was maintained at 3.75 kV and the extractor voltage was set at 5.0 V. Ultrapure nitrogen was used as desolvation gas with a flow of 800 L h^{-1}. Argon was used as the collision gas at a flow of 0.18 mL min^{-1}, achieving and analyzer pressure of ca. 3×10^{-5} Torr. After optimization, detection was performed in multiple reaction monitoring (MRM) mode of selected ions at the first (Q_1) and third quadrupole (Q_3). To choose the fragmentation patterns of m/z (Q_1) → m/z (Q_3) for the analyte in MRM mode, direct infusion (via syringe pump) into the MS of carnitine standard solutions in methanol was performed and the product ion scan mass spectra was recorded. Quantification of carnitine was done by measuring the area under the specific peak using MassLynx Mass Spectrometry Software (Waters, Milford, USA). Carnitine fragments are shown in **Table 2**.

2.5 Solid Phase Extraction

The sample cleanup was as follows: urine samples were diluted and 100 µg L^{-1} of carnitine standard were added into 1 mL of methanol.

2.5.1 CBA cartridges

ISOLUTE CBA, a carboxy propyl phase, is normally used for the extraction of cations that exhibit a positive charge. Solid phase extraction with a carboxypropyl-bonded phase weak cation exchanger (CBA) has been previously used to extract quaternary amines compounds, other than carnitine, in biological fluids [36]. This previous experiments show the feasibility of applying these cartridges for diminishing carnitine interferences in urine samples.

Solid phase extraction of carnitine was performed using weak cation exchange (CBA) (3 mL, 100 mg, ISOLUTE, Biotage). Spiked urine samples were loaded onto CBA cartridges pre-conditioned with 1 mL of water and 1 mL of 2% formic acid in water. From, our experiments it was observed that the carnitine present in the sample was partially retained in the SPE cartridges, while the interferences were completely retained. Thus, the SPE procedure consisted in a first stage in which after loading the urine, the eluent was saved. After that, a second step was applied, it was based on passing 1 mL of 2% formic acid and 1 mL of 5% ammonium hydroxide through the SPE column. The obtained eluent was also collected and mixed together with the first one. The whole volume was dried under a stream of nitrogen gas and was reconstituted with 1 mL of methanol.

2.5.2 ABN cartridges

EVOLUTE ABN has been used for the extraction of acidic, neutral or basic compounds from a variety of matrices, including biological fluids. The polystyrene is modified with defined hydroxyl-functional oligomers. An optimized combination of non-polar and polar interactions allows efficient extraction of wide ranging polarities. These facts made SPE extraction of carnitine interferents from biological fluids an interesting alternative.

The urine samples were loaded onto neutral-basic-acid (ABN) (1mL, 25 mg, EVOLUTE, Biotage) cartridges previously conditioned with 1 mL of water and 1 mL of methanol. After that, carnitine was eluted with 1 mL of water: methanol (95:5) and 1 mL of methanol. The sample collected from the eluting step was transferred to UPLC vials for further analysis by LC-MS/MS by separate injections.

2.6 Recovery study

Standard addition experiments, in which different amounts of carnitine standard solutions were added to diluted urine samples prior SPE extraction and the resulting concentrations determined, were used to assess the method recovery. The biological samples containing carnitine at different concentration levels were prepared in triplicate. Carnitine stock solution was used to spike 1 mL of urine sample previously diluted. The carnitine levels varied from 0 to 150 µg L^{-1}. Then the obtained samples were processed using the proposed SPE procedure. The recovery was then determined.

Linearity

The calibration plots were measured under optimal experimental conditions for urine samples. Six points calibration curve (ten technical replicates at each level of concentration) were determined. The calibration equations were calculated by the least-squares linear regression method. Thus, linearity considering the SPE procedure was evaluated from values closer to the LoQ value up to approximately 150 µg L^{-1} of carnitine. The linearity of the calibration curves for spiked urine samples was satisfactory with determination coefficients (R^2) of about of 0.9987. The F-test demonstrated that linear

regression was statistically acceptable in the working range and this model showed goodness of fit.

In order to estimate the accuracy, intra-day repeatability, and inter-day reproducibility, spiked urine samples were analyzed. For urine samples, 5 blank samples, 10 replicate measurements at 0, 5; 25, 50, 75, 100, and 150 μg L^{-1} carnitine concentrations were prepared.

3. Results & Discussion

This study focused on the sample pre-treatment required for the confident quantification of carnitine in human urine by UPLC–MS/MS. The intended method has been thought in order to use carnitine as a biomarker of the thyroid gland related diseases. This type of studies requires the processing of large amount of samples (which should involve minimum sample treatment), low detection limits, and reliable results.

3.1 Evaluation of matrix effect

The matrix effect under study was evaluated by comparing: i) the signal of carnitine in pure solvent (methanol) with ii) the signal in the sample matrix (urine). The percentage of the quotient of the slope (b) in the spiked and solvent samples was used as an indicator of the extent of the ion suppression or signal enhancement, which was calculated as shown in the equation (1):

$$100-(b_{spiked}/b_{solvent}\times 100) \qquad (1)$$

Therefore, the calibration curves from spiked matrix and spiked pure solvent samples were created. For the pure solvent and urine samples, mean responses were: y = 895.97x +

222.60, y = 498.89x + 4021.15; respectively. The slope values were used as described as indicators of the magnitude of ion suppression or enhancement of the signal. From our results, no signal enhancement, but the fact that urine matrix diminished the analytes response ~50 percent was observed (**Fig. 1**). As a consequence, carnitine quantification was carried out following the standard addition method. More details regarding this study are described in [30].

3.2 Sample preparation optimization

Although technology related to chromatographic separations and mass spectrometry techniques progresses, sample clean-up remains one of the major bottlenecks in the analytical process. The effective sample preparation is essential for achieving good analytical results because matrix related compounds may also co-extract and interfere in the analysis. The selection of the sample clean-up conditions in the analysis of carnitine in biological material is a difficult task considering the nature of analyte. For this reason, the optimization of a SPE stage for cleaning urine samples with special attention to the recovery of the analyte was performed. The accuracy of the proposed method was evaluated by recovery experiments with spiking diluted sample solutions as mentioned earlier.

3.3 Evaluation and optimization of cleaning methods

For matrix interferents' removal, SPE was considered an essential step to obtain a more sensitive and reliable analytical method for carnitine determination in biological fluids. In this work, SPE was directed to clean-up the sample rather than to enrich the analyte. Thus ABN and CBA sorbents were tested. As mentioned above and due to their physical-

chemical properties by the combination of hydrophobic interaction and ion-exchange, both sorbents were expected to properly retain the interferences of carnitine present in urine sample when a two-steps elution procedure was performed.

It is important to notice that, to the best of our knowledge, a method applying CBA and/or ABN cartridges to eliminate carnitine's interferences in urine samples has not been reported in the literature yet. As a consequence, this is the first time that the use of these cartridges for the mentioned aim is reported. Both cartridges were compared each other. The SPE procedures were tested using working standard solutions containing known carnitine concentrations. The recoveries obtained for both cartridges were similar and varied from 83±7% (**Table 3**). Chromatograms of carnitine before and after SPE are shown in **Fig. 2**.

One of the benefits observed in the use of CBA and ABN cartridges was the reduction in the matrix effect in the electrospray interface when biological samples are analyzed by UPLC–ESI-MS/MS. It can be assumed that this reduction in the matrix effect resulted from the selective retention of the interfering substances, such as glycopeptides, phosphocholines, high concentrations of sugars, lipids, salts, and amines, into the sorbent and these compounds were not released under the elution conditions.

3.4 Validation studies

In order to evaluate the methodology, recovery, precision and trueness were calculated. Precision of the whole method was evaluated in terms of repeatability (intraday precision) and reproducibility (inter-day precision). Also, it is acceptable to study the trueness – expressed as bias (%)– of the measurements through recovery of additions of known amounts of the analyte to a blank matrix.

In this context, a Certified Reference Material of the studied matrix with an informed value for carnitine do not exist. However, it is acceptable to assess the trueness of the measurements through recovery of additions of known amounts of the analyte to a blank matrix. Thus, spiked urine samples were analyzed, the concentrations of the carnitine standard at the respective points on the calibration curves were 0, 5; 25, 50, 75, 100 and 150 µg L^{-1}.

The same experiment was repeated on three other independent occasions with at least 1 week interval. The recovery studies were reasonable, leading recoveries higher than 76.0% and lower than 90.0%. Repeatability as intra-day variability was determined by calculating the relative standard deviation (RSD (%)) for the replicated measurements. The obtained values were better than 0.4% for the retention times and 4.1% for the peak areas for all the concentrations evaluated. The overall within-laboratory reproducibility ranged from 1.6 to 7.5% at the tested concentration levels. The obtained results are shown in Table 3.

In summary and taking into account the matrix complexity, the reported values for the method assessment parameters could be considered highly satisfactory. The values obtained for, limit of detection (LoD) and limit of quantification (LoQ) were 12±0.5 nmol L^{-1} and 37±1.5 nmol L^{-1}; respectively. LoD and LoQ were calculated by the equations (2) and (3) respectively:

$$LoD = \frac{3.3S_y}{b}\sqrt{\frac{1}{m}+\frac{1}{n}+\frac{\bar{x}^2}{\sum_{i=1}^{n}(x_i-\bar{x})^2}} \qquad (2)$$

$$LoQ = \frac{10S_y}{b}\sqrt{\frac{1}{m}+\frac{1}{n}+\frac{\bar{x}^2}{\sum_{i=1}^{n}(x_i-\bar{x})^2}} \qquad (3)$$

Where $\bar{x}$ the mean concentration, S_y the standard error of the estimate, b slop of calibration curve, m the number of the replicates per concentration level of the spiked samples, and n the

number of concentration levels for the spiked samples: $i = 1, 2 \ldots I$. The values calculated for LoD and LoQ were compatible and much lower than the normal range established for the carnitine concentration in urine samples.

3.5 Sample analysis

Urine has become a popular medium for biomarker discovery in various metabolic disorders due to its noninvasive nature, and patients often prefer to give urine samples instead of blood.

Once optimal conditions were established, the developed methodology was applied to the analysis of real samples (n=20, 95% confidence level). Carnitine concentrations were found in the normal range as reported in the literature [4, 37], ranging between 81 and 290 µM.

4. Conclusions

A sensitive, accurate, precise, reproducible, and specific methodology for carnitine determination in a complex biological sample as urine was proposed. The fast and effective sample clean-up process aided to remove the sample interferences, facilitating the analyte's quantification with unambiguous results. The method developed decreased notably the observed matrix effects on the carnitine response when urine samples were evaluated. In this context, two commercially available SPE cartridges demonstrated to be both quantitatively effective for the retention of interfering substances. The proposed

methodology can be used as a tool for the reliable analysis of carnitine as a biomarker in some metabolic disorders.

Acknowledgements

The authors wish to acknowledge the financial support received by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica and Instituto de Química de San Luis (INQUISAL, UNSL-CONICET).

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Figure Captions

Fig. 1. Calibration plots for carnitine standard solution (top) and carnitine-spiked urine (bottom); respectively. The chromatographic and ionization/mass spectrometric conditions were described in the *UHPLC instrumentation and conditions* and the *Mass spectrometry instrumentation and MS/MS conditions* sections.

Fig. 2. Relative response (%) vs Retention time (min). A) Carnitine's response in pure solvent. B) Carnitine's response after SPE cleaning. C) Carnitine's response in urine sample without prior SPE cleaning. Carnitine concentration assayed: 50 µg L^{-1}.

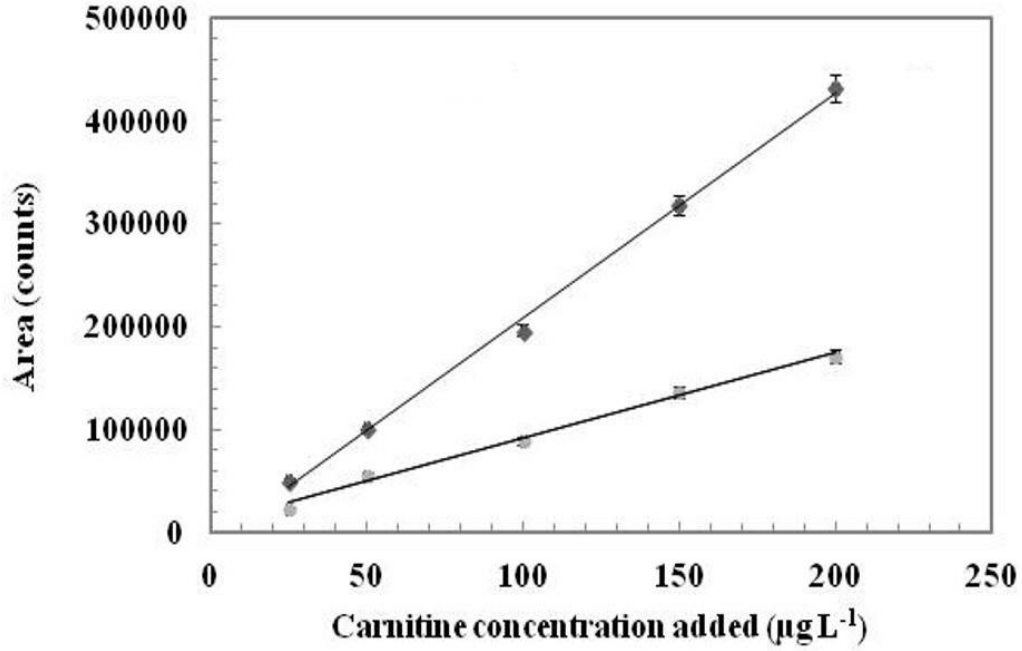


Figure 1

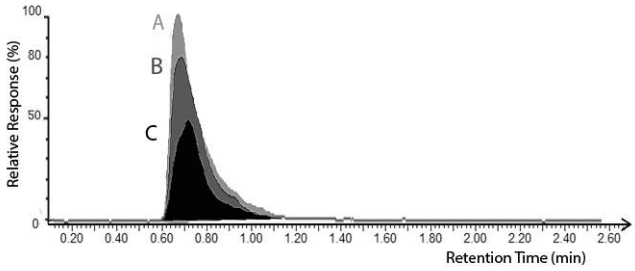


Figure 2

Table 1. State-of-the-art of the common SPE strategies used for sample clean-up and carnitine-and related compounds- extraction in biological fluids.

Analyte	Biological Fluid	Sample Treatment	Recovery	LoD/LoQ	Reference
L-Carnitine, Butyrobetaine and Synthesized Acylcarnitines (C2, C3)	Rat Urine, Plasma and Skeletal Muscle.	Solid Phase Extraction with MCX (mixed cation Exchange) SPE was used. The analytes were derivatized with pentafluorphenacyl trifluoromethanelsulfonate after SPE.	68 - 105 %	LoQ: Carnitine 1.5 nmol L^{-1}, butyrobetaine 0.10 nmol L^{-1}, and acylcarnitines: 0.05- 0.5 nmol L^{-1}	8
L-Carnitine, acetylcarnitine and α-ketoglutaric acid (α-KG)	Human Urine	Solid Phase Extraction with Silica Gel and Quaternary Amine-based cartridges (without prior derivatization).	L-Carnitine and Acetylcarnitine: 86.64% - 98.96 % α-KG: 96.89%-106.49 %	LoQ: L-Carnitine and Acetylcarnitine 0.04-0.08 ng mL^{-1} and for α- KG acid 0.8 ng mL^{-1}	12
L-Carnitine, Acetylcarnitine, Octanoylcarnitine and Palmytoilcarnitine.	Human Plasma	On line solid-phase extraction with a MCX cartridge coupled to chromatographic separation, without prior derivatization.	98- 105 %	LoQ: L-Carnitine and Acetylcarnitine 250 nmol L^{-1} and Acylcarnitines 25- 50 nmol L^{-1}	31
L-Carnitine and Acylcarnitines (C_2, C_3, C_5, C_6, C_8, C_{14}, C_{16} and C_{18})	Human Urine	Cation Exchange (SCX), without prior derivatization	95.7 - 106.3 %	LoQ: L-carnitine 5 µmol L^{-1}, Acetylcarnitine 2.5 µmol L^{-1} and Acylcarnitines 0.75 µmol L^{-1}	32
Carnitine, Acetylcarnitine and other Acylcarnitines ($C_3 - C_{18}$)	Human Urine and Plasma	The samples were applied to the Silica Gel SPE, without prior derivatization.	Urine: Carnitine: 87.5 % and Acylcarnitines: 85.0- 96.5 % Plasma: Carnitine: 75.0 % and Acylcarnitines: 47.2 - 105 .9%	Urine LoD: Carnitine 200 ng mL^{-1} and Acylcarnitine 28 – 50 ng mL^{-1}. LoQ: Carnitine 400 ng mL^{-1} and Acylcarnitines 40 ng mL^{-1} Plasma LoD: Carnitine 222 ng mL^{-1} and Acylcarnitine 14.9 – 45.9 ng mL^{-1}. LoQ: Carnitine 160 ng mL^{-1} and Acylcarnitines 40 ng mL^{-1}	33
Acylcarnitines (C_2, C_3, C_4, C_6, C_8, C_{10}, C_{12}, C_{14},	Human Plasma	Cation Exchange SPE. After cleaning the sample the analytes	42- 67 %	LoD: C_2, C_3, C_4: 1 nmol L^{-1} and other acylcarnitines	34

$C_{16,}$ and C_{18}		were derivatized with diisopropylenethylamine		25 nmol L^{-1}.	
L-Carnitine, C_2, C_3, C_4-N, C_4-I, C_5-N, C_5-I, C_5-M, C_5-P, C_6, C_7, C_8-N, C_8-V, C_3DC, C_4DC-M, C_4DC-S and C_5DC	Human Serum and Urine	The analytes were extracted from samples using MCX SPE cartridges.	> 90 %	LoD: Methylmalonylcarnitine 0.1 µmol L^{-1} and other Acylcarnitines 0.05 µmol L^{-1}	35
L-Carnitine	Human Urine	The analyte was extracted from urine samples using ABN and CBA cartridges.	83±7 %	LoD: 12 nM LoQ: 37 nM	This work

Table 2. Mass spectrometric conditions for the analysis of carnitine.

Carnitine Transitions (m/z)	Dwell (s)	Cone (V)	Collision (V)
162.2>103.1[a]			
162.2>85.1	0.08	15	17
162.2>60.3			

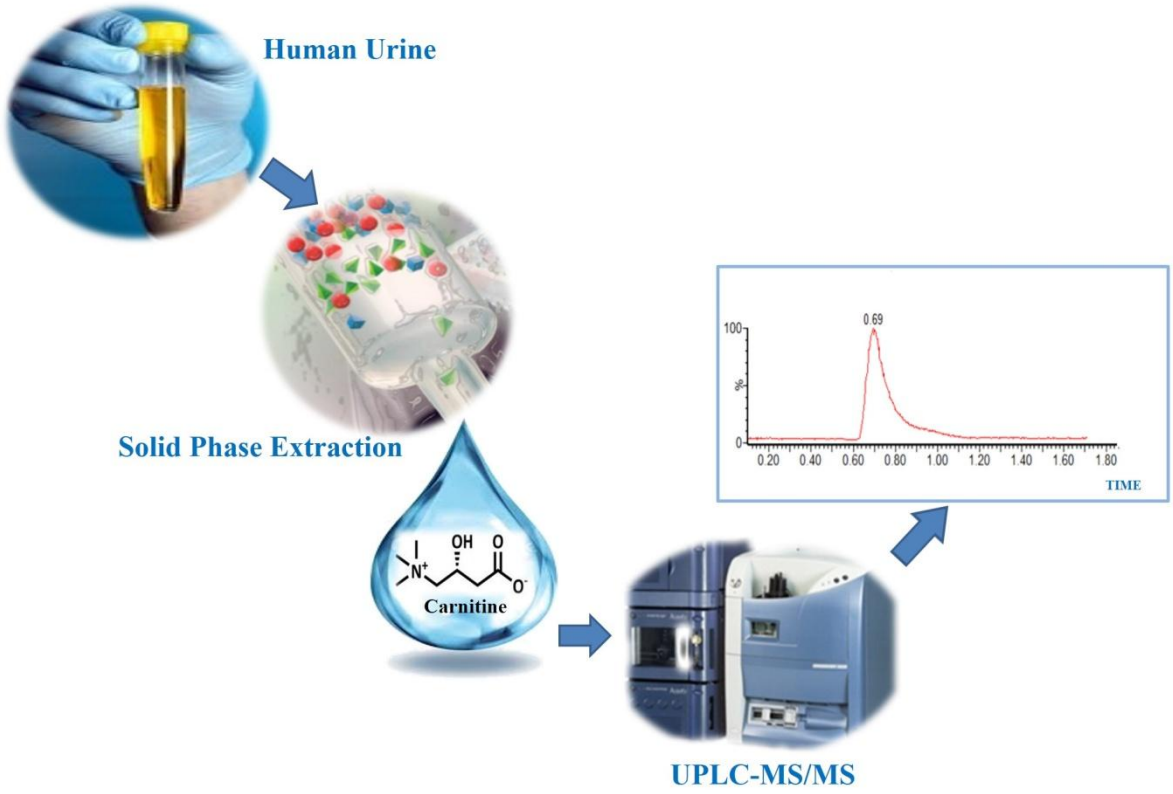
[a]Transition used for quantification

Table 3. Recovery and trueness studies on urine samples (95% confidence interval, n=10)

Aliquots	**Quantity of Carnitine added ($\mu g\ L^{-1}$)**	**Quantity of Carnitine found ($\mu g\ L^{-1}$)**	**Recovery (%)[a]**	**Bias (%)[c]**
1-5	0	15.3[b]	---	---
6	25	34.4	76.4	-23.6
7	50	57.7	84.8	-15.2
8	75	82.6	89.7	-10.3
9	100	105.3	90.0	-10.0
10	150	148.8	89.0	-11.0

[a]100 × [(Found-Base)/Added]; [b]Diluted urine diluted; [c]Bias (%) = [((Measured Content-Spiked Level)/ Spiked Level) × 100]

graphical abstract



Highlights

- Novel, sensitive, selective, rapid analytical method for carnitine analysis in urine.
- SPE approaches were evaluated and applied to the treatment of urine samples.
- SPE reduced the matrix effect.
- No need of sample derivatization before carnitine analysis.
- Applicable to the carnitine monitoring samples related to metabolic disorders.