Identification of L-carnitine and its impurities in food supplement formulations by online column-switching liquid chromatography coupled with linear ion trap mass spectrometry

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Running title: Identification of impurities in L-carnitine by column-switching HPLC-MS.

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Abbreviations: 3-chloro-2-hydroxy-*N*,*N*,*N*-trimethylpropan-1-aminium (CLTA), 3-cyano-2-hydroxy-*N*,*N*,*N*-trimethylpropan-1-aminium (CNTA), 3-carboxy-*N*,*N*,*N*-trimethylprop-2-en-1-aminium (CTEA),

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4-chloro-2,3,4-trihydroxy-*N*,*N*,*N*-trimethylbutan-1-aminium (CTTA), active pharmaceutical ingredient (API).

Keywords: L-carnitine; column-switching; impurities; high-performance liquid chromatography; mass spectrometry.

Abstract

The identification of impurities in L-carnitine by mass spectrometry is difficult because derivative reagents or ion pair reagents are usually used to separate and increase the retention of L-carnitine on the reversed-phase column. In this study, four impurities including 3-chloro-2-hydroxy-*N*,*N*,*N*-trimethylpropan-1-aminium, 3-cyano-2-hydroxy-*N*,*N*,*N*-trimethylpropan-1-aminium, 3-carboxy-*N*,*N*,*N*-trimethylprop-2-en-1-aminium and 4-chloro-2,3,4-trihydroxy-*N*,*N*,*N*-trimethylbutan-1-aminium were identified in L-carnitine and its tablets by using two-dimensional column-switching high-performance liquid chromatography coupled with linear ion trap mass spectrometry. The first column was a C_8 column at a flow rate of 0.15 mL min^{-1}; the detection wavelength was 220 nm. The second column was an Acclaim Q1 column using a gradient elution program with aqueous 30 mM ammonium acetate (pH 5.0) and acetonitrile as the mobile phase at a flow rate of 0.5 mL min^{-1}. The mass fragmentation patterns and structural assignments of impurities were

studied, and the quantitative validation of three impurities was further investigated. The linearity (r^2) was found to be > 0.99, with ranges from 0.2 to 50 ng mL^{-1} and 0.1 to 10 ng mL^{-1}. The method was used successfully for determination of impurities in five samples of L-carnitine and tablets.

1. Introduction

L-Carnitine is a natural substance used by humans for fatty acid oxidation and energy production. L-Carnitine has been reported in Pharmacopoeias and is used for the treatment of carnitine deficiency or as a dietary supplement for various chronic diseases [1]. Several oral formulations including tablets, capsules and solutions are commercially available from various manufacturers [2]. L-Carnitine can be chemically synthesized from 2-(chloromethyl)oxirane, 4-ethyl 4-chloroacetoacetate, L-malic acid, hexose or ascorbic acid. Figure 1 shows the synthesis routine of L-carnitine from 2-(chloromethyl) oxirane and 3-chloro-2-hydroxy-*N*,*N*,*N*-trimethylpropan-1-aminium (CLTA) [3]. HPLC–MS has been used for the analysis of L-carnitine and its analogues [4], and derivative reagents including butanol [5, 6], (*S*)-naproxen [7], pentafluorophenacyl trifluoromethanesulfonate [8], 1-aminoanthracene and [9] and 4-bromomethylbiphenyl [10] have been used to increase the retention of L-carnitine on the reversed-phase column. Ion pair reagents have also been used

for separation of carnitine, butyrobetaine and acylcarnitines in biological samples but could not be coupled with MS online [11–13]. In the United States Pharmacopeia [14], a heptanesulfonate ion-pairing mobile phase (containing phosphate buffer) with methanol is used in HPLC for quantitative determination of L-carnitine in oral solutions and tablet formulations. Other columns, such as HILIC columns, have also been used to determine carnitine and acylcarnitines in milk, human urine, serum and plasma [15–19]. A CN-analytical column was used for analysis of piracetam and L-carnitine in human plasma [20, 21].

While several studies have investigated methods for the analysis of L-carnitine, none of the current methods are suitable for identification and quantification of impurities in L-carnitine from pharmaceutical or food supplement formulations. For instance, ion pair and ion exchange reagents are incompatible with MS. Moreover, impurities or the excipient could co-elute with major pharmaceutical ingredients, especially when the peak area of the major ingredient is high and peak tailing, or the retention times are close. Thus, identification and quantification of impurities in L-carnitine are difficult, and the co-eluted major ingredients or other excipients may contaminate the MS results and suppress the ionization efficiency, leading to poor detection sensitivity.

Online 2D separation, heart-cutting or column-switching LC shows better separation selectivity and resolving power than the conventional HPLC analysis due to the inclusion of

different separation modes. 2D HPLC can significantly increase the resolving power and achieve separations that cannot be obtained by 1D separation. 2D HPLC has been reported extensively in the analysis of biological samples [22–24], proteomics [25, 26] and natural products [27–32]. We have also developed an off-line 2D-HPLC MS method for proteomics analysis [33]. The use of 2D-HPLC in pharmaceutical analysis has also been getting more attention recently [34–38].

In this study, 2D column-switching HPLC coupled with linear ion trap MS (HPLC–LIT MS) was developed for identification of impurities in L-carnitine active pharmaceutical ingredient (API) and its tablet formulations. Four impurities including 3-chloro-2-hydroxy-*N*,*N*,*N*-trimethylpropan-1-aminium (CLTA), 3-cyano-2-hydroxy-*N*,*N*,*N*-trimethylpropan-1-aminium (CNTA), dehydrogenation product 3-carboxy-*N*,*N*,*N*-trimethylprop-2-en-1-aminium (CTEA) and 4-chloro-2,3,4-trihydroxy-*N*, *N*, *N*-trimethylbutan-1-aminium (CTTA) were identified. The mass fragmentation patterns and structural assignments of these impurities were studied, and CLTA, CNTA and CTEA in L-carnitine were further quantified. The method afforded satisfactory results in terms of sensitivity, specificity, precision and accuracy of the analytes. The proposed 2D column-switching HPLC–LIT MS method is a powerful tool for QC of L-carnitine in manufacturing.

2. Materials and Methods

2.1. Chemicals and reagents

Standards of CLTA, CNTA and CTEA (purity > 99.9%), and three batches of L-carnitine API samples (Serial Nos.: 1603001, 1603002 and 1603003, respectively) were provided by ChengDa Pharmaceuticals (Jiashan, China). The deionized water used in this study was obtained using a Milli-Q water purification system (Millipore, Bedford, MA, USA). Acetonitrile and methanol (LC–MS grade) were purchased from Anpel Laboratory Technologies (Shanghai, China). Food supplement formulations: Two batches of L-carnitine polyphenolic tablets used for losing weight were purchased at a local pharmacy (0.8 g/tablet; Serial Nos.: 160102 and 151204; expiration dates: 2018.01.03 and 2017.12.05, respectively, declared content: 16% L-carnitine, 5% tea polyphenols and magnesium stearate). The L-carnitine content in the API and tablets was also determined by HPLC–MS by comparing the peak area of *m/z* 162 in the API, tablet and standard. The chromatogram and chromatographic conditions of L-carnitine for the tablets and standard are listed in Supporting Information Figures S1 and S2. Other supplementary ingredients in the tablets include sodium carboxymethyl cellulose, magnesium stearate, coating powder and lactose.

2.2. Preparation of stock solutions

A stock solution of L-carnitine API was prepared by dissolving 5 mg in 1 mL of water for impurity identification, and then diluted to 100 μg mL^{-1} for determining the location of the main peak. Stock solutions of CLTA, CNTA and CTEA standards were prepared by dissolving 1 mg in 1 mL of water. From this solution, standard solutions at various concentrations (0.1–50 ng mL^{-1}) were prepared in water. The stock solutions were stored at 4°C in the dark, and working dilutions were freshly prepared on the day of use.

2.3. Sample preparation

Ten tablets (0.8 g/tablet) were smashed using a pestle and mortal, and 5 g of the smashed tablets were weighed accurately and placed in a 200 mL volumetric flask. Water (100 mL) was added to the volume mark. The mixture was sonicated at room temperature for 30 min until the tablets had disintegrated completely. Then, 5 mL of the resulting solution was transferred to a 25 mL volumetric flask and diluted with water to give a sample solution containing approximately 2 mg mL^{-1} L-carnitine tablet power. The sample solution was filtered through a 0.45-mm filter and injected into the HPLC system.

2.4. Instrumentation

Supporting Information Figure S3 shows the setup of the 2D-HPLC system and illustrates the valve configuration. The first dimension includes a ternary pump, an auto sampler, a thermostatic column compartment, and a UV detector (Infinity 1260 HPLC,

Agilent Technologies, Santa Clara, CA, USA). The second dimension includes a ternary pump, an auto sampler, and a thermostatic column compartment with two six-column-switching features, diode array detector (Surveyor, Thermo Fisher Scientific, San Jose, CA, USA), and a linear ion trap mass spectrometer (LTQ XL, Thermo Fisher Scientific) equipped with an ESI source. The interface between the first and second dimensions is a six-loop valve trapping system with a ZORBAX C_{18} trap column (12.5 mm × 4.6 mm, 5 μm) from Agilent Technologies (between port 6 and port 3 of valve 1). The interface between the chromatography system and mass spectrometer is a second six-loop valve. Data acquisition, handling, and instrument control were performed using Xcalibur 2.3.1 software (Thermo Fisher Scientific).

2.5. Chromatographic conditions

The first dimension utilized a BEH C8 column (2.1 mm × 50 mm, 1.7 μm; Waters, Milford, MA, USA) at a temperature of 35°C. The mobile phases A (water) and B (acetonitrile) were used, and the gradient program was: 0–5 min 5% B, 5.1–11 min 5%–100% B, a flow rate of 0.15 mL min^{-1}, a wavelength of 220 nm, and an injection volume of 20 μL. For the second dimension of the chromatography, an Acclaim Trinity Q1 column (2.1 mm × 50 mm, 3 μm; Thermo Fisher Scientific) was used. The column temperature was maintained at 35°C. The mobile phases A (30 mM ammonium acetate, adjusted to pH 5.0 with acetic acid) and B (acetonitrile) were used, and the gradient program was: 0–5 min

95–70% B, 5.5–8 min 95% B, and a flow rate of 0.5 mL min^{-1}. The wavelength was also set at 220 nm. As shown in the Supporting Information Figure S3, products eluted from the first dimension were split three ways, for a split-flow ratio of 10:1 to the first and the second valve, respectively. The second valve was diverted at the 1–2 position, and the products eluted from the first and second columns were diverted to the mass spectrometer and waste, respectively. In the first dimension, the first valve was diverted from the 1–2 position (Supporting Information Figure S3A) to the 1–6 position (Supporting Information Figure S3B) at 1.9 min, which means the first six-loop valve switch from the trap column to the analytical column was set at 0.2 min after the retention time of the peak observed in the first dimension. Thus, the products eluted at 1.5–1.8 min were trapped and transferred to the analytical column in the second dimension. Meanwhile, the second six-loop valve was diverted from the 1–2 position to the 1–6 position at 1.9 min (Supporting Information Figure S3B). Thus, the analytes in the trap column were eluted to the Q1 analytical column in the second dimension and finally eluted to the ESI-MS/MS system. After 0.8 min, the first valve was switched to the original position, and the column in the first dimension was eluted with the mobile phase for the next injection (Supporting Information Figure S3A), while the products eluted from the first column were diverted to waste. The vials at port 3 of the second valve were of no use, and the vials at port 4 of the second valve were used for collecting the waste from the

first column. The UV detector, which has a flow cell, is connected to the valve with a stainless-steel pipe (inner diameter [ID]: 0.17 mm).

2.6. Mass spectrometric conditions

For identification of impurities and major ingredients, MS was conducted using an ESI source in positive mode (mass range: *m/z* 50 to 800, source voltage: 4 kV, capillary temperature: 350°C, sheath gas flow: 22 arb, sweep gas flow: 0 arb, capillary voltage: 14 V). In MS^2 analysis, the fragmentation was performed through collision-induced dissociation at collision energies from 35–60 eV. For quantification of CLTA, CNTA and CTEA, MS was conducted in single ion monitoring (SIM) mode with *m/z* 152.0, *m/z* 144 and *m/z* 143. In the second dimension, eluted products were diverted to waste between 1.8 min and 3.1 min through a diverter valve on the inlet of the mass spectrometer when 5 mg mL^{-1} carnitine or 2 mg mL^{-1} tablet was injected.

3. Results and discussion

3.1. Identification of impurities in L-carnitine and its tablets

L-carnitine is a choline derivative with a hydrophilic group, so L-carnitine and its related impurities are not retained on the reversed-phase column. The retention times of major pharmaceutical ingredient L-carnitine or other impurities are short (within 2 min) and co-elute together using reversed-phase C_{18} or C_8 columns. Use of a C_8 column has been reported for

the analysis of L-carnitine in pharmaceutical formulations and urine sample [39–40], so a C_8 column was chosen in the first dimension in our experiment. Figures 2A and 2B show the chromatogram of L-carnitine API and its tablet acquired using the reversed-phase C_8 HPLC–UV method. L-carnitine eluted at 1.5–1.8 min with other ingredients. In the first dimension, L-carnitine at 1.5 min was split and eluted to the mass spectrometer directly, and $[M+H]^+$ ions with *m/z* 162 were detected. To identify the structures of impurities, the substance giving the peak for L-carnitine eluted at 1.5–1.8 min in the first dimension was trapped and transferred to an analytical column in the second dimension. The weak polar substances that eluted after 2.5 min were separated with L-carnitine and were removed. The analytes in the trap column were eluted to the Q1 analytical column in the second dimension and finally eluted to the ESI-MS/MS system.

Two different trap columns, a ZORBAX C_{18} column (12.5 mm × 4.6 mm, 5 μm) and a C_{18} column (12.5 mm × 2.1 mm, 5 μm) were compared. The 4.6 mm ID column had an internal volume of approximately 200 μL, and the 2.1 mm ID column had an internal volume of less than 40 μL. The flow rate in the first dimension was 0.15 mL min^{-1}. However, the peak width in the first dimension was 0.3 min, and hence the 2.1 mm ID column was unable to trap all of the eluate. The 4.6 mm ID column with an internal volume of approximately 200 μL was sufficient to trap all of the eluate at 0.2 min after the retention time of the corresponding peak observed in the first dimension.

In the second dimension, L-carnitine eluted at 2.55 min, and $[M+H]^+$ ions with *m/z* 162 were detected. Figures 3A and 3B show the extract ion current (EIC) and MS^1 spectrum of L-carnitine. The product ions from *m/z* 162 were acquired at a collision energy of 35 eV, and dominant fragment ion peaks were observed at *m/z* 144, 103 and 60 (Figure 3C). The product ion *m/z* 144 is produced from the loss of water, and *m/z* 103 and 60 are produced from the breakage of the C–N bond. To remove the major ingredient L-carnitine eluted from the trap column, products eluted from the Q1 analytical column were diverted to waste between 1.8 and 3.1 min, thereby reducing the ion suppression caused by L-carnitine being eluted to the mass spectrometer. MS signals in positive mode were obtained for these impurities, and structures were elucidated based on MS^2 data. The retention time of each peak in the second dimension, *m/z* in positive mode, MS^2 fragments, chemical name and the structures are listed in Table 1. The impurities were identified as the reactants, intermediate product and dehydrogenation product. The peaks of impurities in the HPLC–UV chromatogram of the second dimension were not observed because the peak intensities of impurities are small and can be masked by the baseline of the gradient elution. Thus, the UV detector in the second dimension is useless.

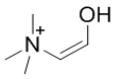
The mass fragmentation patterns and structural assignments of these impurities were studied. CLTA was identified in L-carnitine API and tablet samples by comparison of the retention times, as well as MS^1 and MS^2 spectra with those of the

standard. The EIC, MS^1 and MS^2 spectra are listed in Figures 4A–C. The product ion spectrum from the precursor ion *m/z* 152 and the retention time coincided with the standard of CLTA. Dominant fragment ion peaks were observed at *m/z* 134, 106 and 60. The proposed mechanism for the ion *m/z* 134 is the loss of water from the precursor ion *m/z* 152, that for ion *m/z* 106 is loss of two methyl groups from the precursor ion, and that for ion *m/z* 60 comes from the breakage of the C-N bond.

m/z 143 was detected in the API but not in the tablet samples and was identified as CNTA. Figure 5A shows the EIC of *m/z* 143 for the 5 mg mL^{-1} API sample with a retention time of 0.97 min. Figure 5B shows the MS^1 spectrum of CNTA. The product ions of *m/z* 143 were acquired at a collision energy of 50 eV, and dominant fragment ion peaks were observed at *m/z* 125, 114, 102, 100 and 99 (Figure 5C). The proposed mechanism for ion *m/z* 125 is the loss of water from the precursor ion *m/z* 143, those for the product ions *m/z* 100 and 99 are the loss of hydroxyl group and cyano group, and those for the product ions *m/z* 114 and 102 are the loss of two methyl groups and a CH_2CN group, respectively.

m/z 198 was detected in the 5 mg mL^{-1} L-carnitine API and tablet samples, and was identified as CTTA. Figure 6A shows the EIC of CTTA. Figures 6B and 6C show the MS^1 and MS^2 spectra of CTTA; the collision energy is 40 eV. The product ions *m/z* 139 and 60 are produced from the breakage of the C–N group, and *m/z* 121 is

from the further loss of a molecule of water. *m/z* 102 is produced from the breakage of C–C bond from *m/z* 198 and gives the fragment shown in Scheme 1.



Scheme 1

m/z 144 was also detected in the 5 mg mL^{-1} L-carnitine API and tablet samples, and was identified as the dehydrogenation product CTEA. Figures 7A and 7B show the MS^1 and MS^2 spectra of CTEA. The product ion *m/z* 100 is from the loss of the COOH group from precursor ion *m/z* 144 at a collision energy of 42 eV, and *m/z* 85 and 58 are from the breakage of the C–N bond.

3.2. Optimization of the method for separation of impurities from L-carnitine on the second column

In our experiment, an amide column was first used for separation of L-carnitine and its related impurities with gradient elution conditions, but the retention times of target impurities were sensitive to the mobile phase. The small amount of mobile phase A eluted from the first dimension greatly influenced the retention of impurities on the amide column, and all the impurities eluted within 1 min. The Acclaim Trinity Q1 column is a specialty column used for separating the herbicides diquat and paraquat. The Q1 column provides multiple retention mechanisms, including reversed-phase, anion-exchange, and cation-exchange. L-carnitine and impurities could be retained on the Q1 column under isocratic elution, but the peaks of the

main component and impurities are close. Other impurities can be masked by the main peak and can be diverted to the waste together. Thus, all the impurities could not be separated from each other. The Q1 column was further used with gradient elution conditions, but the broadening and tailing are still obvious, so ammonium acetate (30 mM, adjusted to pH 5 with acetate acid) was added to mobile phase A. Consequently, the peak shape was improved, and the peak width was within 0.5 min. Other gradient conditions were also investigated, but the retention times of major ingredients and impurities are close. When the second dimension separation was carried out, the first separation was not stopped and was eluted.

3.3 Validation of the analytical method

The linearity, sensitivity, specificity, precision and accuracy of the HPLC–LIT MS method was investigated by determining the contents of impurities CLTA, CNTA and CTEA in L-carnitine samples.

3.3.1 Linearity for the determination of impurities

To establish a linearity equation, different concentrations of CLTA standards (50, 10, 5, 2.5, 1, 0.5 and 0.2 ng mL^{-1}) and different concentrations of CNTA/CTEA standards (10, 2, 1, 0.5, 0.2 and 0.1 ng mL^{-1}) were prepared. Linear regression plots were obtained by plotting the peak area of the target standards versus the theoretical concentrations. As shown in Table 2, good linearity was obtained for CLTA standards in the range from 0.2 to 50 ng mL^{-1} and

CNTA/CTEA standards in the range from 0.1 to 10 ng mL^{-1}, with a correlation coefficient greater than 0.99.

3.3.2. Limits of detection and limits of quantitation

The LODs and LOQs for the method were estimated based on the analysis of impurity standards at S/Ns 3 and 10 times the average of the baseline. The LODs were 0.05 and 0.02 ng mL^{-1} for CLTA and CNTA/CTEA respectively, and the LOQs were 0.2 and 0.1 ng mL^{-1} for CLTA and CNTA/CTEA, respectively. Thus, the LOQs for CLTA and CNTA/CTEA can also be calculated as 0.04 and 0.02 ppm for 5 mg mL^{-1} L-carnitine API.

3.3.3. Accuracy and repeatability of the method

To check the accuracy and reproducibility of the developed method, a recovery study was performed at three different concentrations. Three replicate experiments were carried out for each spiked concentration. Experiments were performed for 5 mg mL^{-1} L-carnitine from tablets spiked with 0.5, 1 and 5 ng mg^{-1} of the impurity standards. The recovery of the compounds was calculated as the ratio between the experimentally observed concentration and the theoretical concentration. The achieved recoveries of CLTA, CNTA and CTEA were between 91.1 and 115.2% for the three different concentrations (Table 2). The repeatability, expressed as the RSD (RSD), is indicated in Table 2. The RSDs were within 13.1%. These results indicate that the proposed analytical method is reliable. Besides, the RSD values for

the added concentration (0.5 ng mg^{-1}) are >10% and higher than other concentrations, because the added concentration is low and is close to the LOQ value.

3.4 Quantitation of impurities in L-carnitine and tablets

The established analytical method was then applied to determine the contents of CLTA, CNTA and CTEA in five different batches of L-carnitine API and tablet samples. Of all the five samples, CLTA was found at a range of 0.06–0.52 ng mg^{-1} (as shown in Table 3) and CNTA was found at a range of 0.03–0.06 ng mg^{-1} in L-carnitine API but not detected in the tablets. For quantification, SIM mode with *m/z* 152.0 was also compared with selective reaction monitoring. Selective reaction monitoring with *m/z* 152→134 was used for quantification, but the LOD was higher than that for the SIM mode, with LOD at 1 ng mL^{-1} under the optimized conditions. Another impurity CTTA was not quantified because the standard is unavailable. Numerous reports on analytical methods for determination of L-carnitine in biological samples (plasma, serum and urine), food supplements and pharmaceutical formulations have been published [15–21, 41, 42], but there are few reports on determining the impurities in L-carnitine.

3-Carboxy-*N*,*N*,*N*-trimethylprop-2-en-1-aminium (crotonoylbetaine) was detected in pharmaceutical formulations of L-carnitine by ion pairs HPLC–UV, with the content at < 1% [39]. CE–MS/MS was used for determination of D-carnitine in pharmaceutical formulations of L-carnitine, with an LOD of 10 ng mL^{-1} [43, 44]. CE with UV detector was used for

determination of carnitine in food supplements, with an LOD of 0.5–4.4 μg mL^{-1} [45]. HPLC separations by chiral-achiral tandem column with UV detector has also been used, with an LOD of 640 ng mL^{-1} [46]. Supporting Information Table S1 summarizes the analytical parameters of the known (published) methods for determining the impurities in L-carnitine. The method established in our study has advantages regarding the detection limits and analytical speed, and is suitable for identification and quantification of impurities in various batches of L-carnitine samples during actual production.

4. Concluding remarks

We have developed 2D column-switching HPLC coupled with linear ion trap MS for identification of impurities in L-carnitine and its tablet formulations. The impurities include the reactants, intermediated product and dehydrogenation product. The method was successfully applied in the quantification of CLTA, CNTA and CTEA in L-carnitine API and tablets, with satisfactory results for sensitivity, specificity, precision, accuracy and recovery. With advantages regarding the detection limits and analytical speed, the proposed method is a powerful tool for the QC of L-carnitine and can be extended to other analogues in manufacturing.

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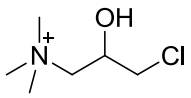
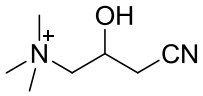
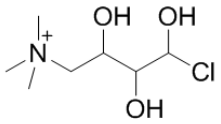
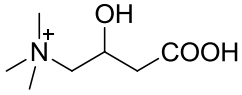
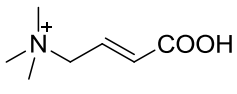
Table 1. Information on the identified compounds

Impurities No.	t_R in the 2nd dimension	Positive mode (*m*/*z*)	Chemical Name	MS^2 (+)	Chemical structure
1	0.84	152.0	3-chloro-2-hydroxy-*N*,*N*,*N*-trimethylpropan-1-aminium (CLTA)	134, 106, 60	
2	0.97	143.1	3-cyano-2-hydroxy-*N*,*N*,*N*-trimethylpropan-1-aminium (CNTA)	125, 114, 102, 100, 99	
3	1.04	198.1	4-chloro-2,3,4-trihydroxy-*N*,*N*,*N*-trimethylbutan-1-aminium (CTTA)	139, 121, 102, 60	
4 (main peak)	2.55	162.1	L-carnitine	144, 103, 60	
5	3.45	144.1	3-carboxy-*N*,*N*,*N*-trimethylprop-2-en-1-aminium	100,	

	(CTEA)	85, 58

Table 2. Regression data, correlation coefficients, accuracy and reproducibility, LODs, and LOQs for CLTA, CNTA and CTEA

Impurities	Regression (Y = aX + b)	R^2	Linear range (ng mL^{-1})	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	Added concentration (ng mg^{-1})					
						0.5		1		5	
						Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
CLTA	y=1121.4x+366.45	0.9931	0.2-50	0.05	0.2	115.2	12.6	114.5	13.1	91.1	9.77
CNTA	y=4640.8x+182.59	0.9979	0.1-10	0.02	0.1	108.4	10.4	97.2	8.75	101.3	6.87
CTEA	y=4159x + 82.788	0.9942	0.1-10	0.02	0.1	109.2	11.7	102.5	5.89	99.3	5.31

Table 3. Contents (ng per mg) of CLTA, CNTA and CTEA in five batches of L-carnitine

Sample Batch Number	Contents (ng per mg) ppm			L-carnitine (%)
	CLTA	CNTA	CTEA	

API 1603001	0.52	0.06	0.05	99.9
API 1603002	0.19	0.03	0.03	99.8
API 1603003	0.06	0.04	ND*	99.9
Tablet 160102	ND*	ND*	ND*	15.8
Tablet 151204	0.06	ND*	ND*	15.9

*Not detect.

Figure captions

Figure 1. The synthesis routine of L-carnitine.

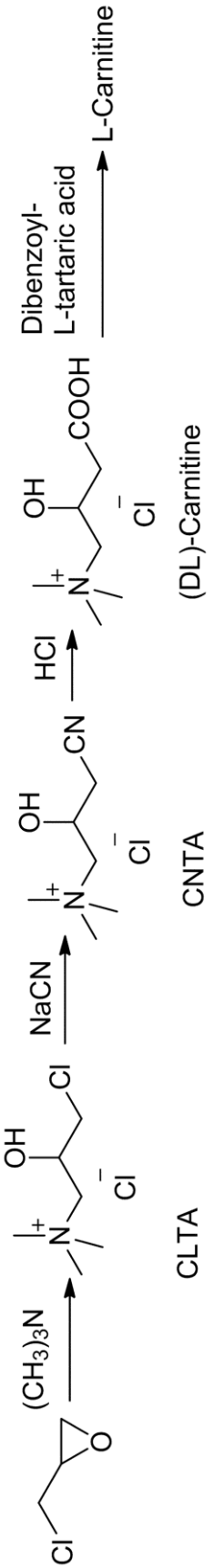


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Figure 2. The chromatogram of L-carnitine API (A) and tablet (B) acquired by the first dimension C_8 HPLC–UV.

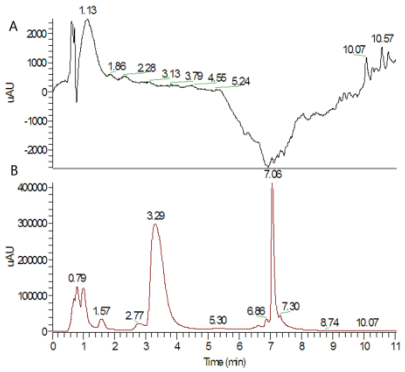


Figure 3. (A): EIC, (B) MS^1 spectrum, and (C) MS^2 spectrum of L-carnitine in tablet.

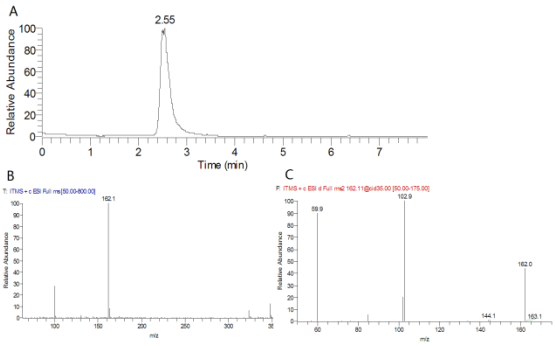


Figure 4. (A) EIC, (B) MS^1 spectrum and (C) MS^2 spectrum of CLTA in L-carnitine tablet.

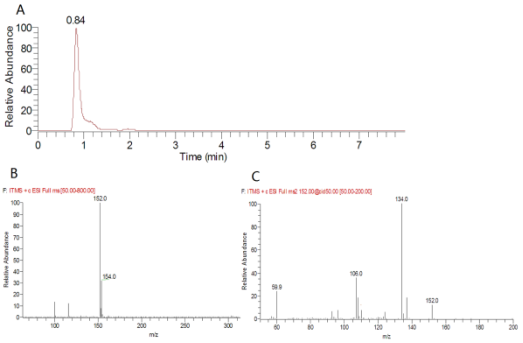


Figure 5. (A) EIC, (B) MS^1 spectrum and (C) MS^2 spectrum of CNTA in 5 mg mL^{-1} L-carnitine API.

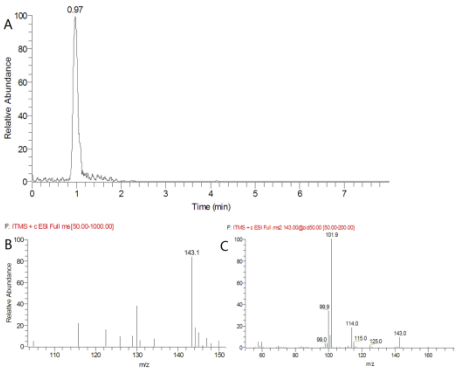


Figure 6. The EIC (A), MS^1 (B) and MS^2(C) spectra of CTTA in 5 mg mL^{-1} L-carnitine tablet.

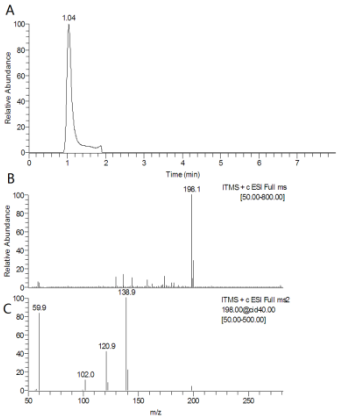
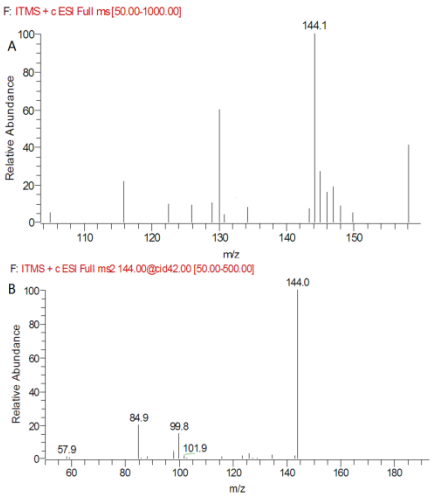


Figure 7. The MS^1 and MS^2 spectra of CTEA, *m/z* 144.



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