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Improved high-performance liquid chromatographic method for analysis of L-carnitine in pharmaceutical formulations

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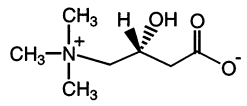
Abstract

A reversed-phase high performance liquid chromatographic method for analysis of L-carnitine is described. The improved method is able to provide a high resolution between L-carnitine and crotonoylbetaine, a major impurity and degradation product, and suitable for quantitative analysis of L-carnitine in pharmaceutical formulations, such as solution, tablets, and capsules. The resolution, linearity, accuracy and reproducibility of the method are discussed.

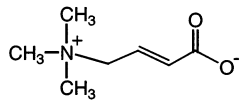
Keywords: L-Carnitine; Quantitative analysis; Reversed-phase high performance liquid chromatography; Pharmaceutical formulations

1. Introduction

L-Carnitine, (*R*)-3-carboxy-2-hydroxy-*N*,*N*,*N*-trimethyl-1-propanaminium hydroxide, inner salt, is a naturally occurring substance, essential for fatty acid oxidation and energy production in the human body [1–3]. Without L-carnitine, long-chain fatty acids cannot be transported from the cellular cytoplasm into the mitochondria, resulting in loss of energy and toxic accumulations of free fatty acids. Currently, L-carnitine is used for the treatment of carnitine deficiency or as a dietary supplement for various chronic diseases [4–6]. Several oral formulations, including solutions, tablets, and capsules, are available from different manufacturers.



L-Carnitine



Crotonoylbetaine

Due to its high polarity and lack of chromophore, quantitative analysis of L-carnitine using reversed-phase high performance liquid chromatography (HPLC) is challenging. Many HPLC methods based on pre-derivatization of L-carnitine have been developed to analyze trace amounts of L-carnitine in biological samples [7–9]. However, for quantitative analysis of L-carnitine in pharmaceutical formulations, only a few methods are available.

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The US Pharmacopeia (USP 23) lists two HPLC-based methods suitable for the quantitative analysis of L-carnitine in an oral solution formulation [10] and a tablet formulation [11], respectively. The USP method for solution formulation involves HPLC analysis under ion-pairing conditions. However, this method cannot separate crotonoylbetaine, a major impurity and degradation product, from L-carnitine. The USP method for tablet formulation involves HPLC analysis using a column filled with aminopropylsilane-bonded silica gel. This method is not suitable for the analysis of an oral formulation containing a high concentration of sugars, since reducing sugars can rapidly degrade the column. In addition, the method is very time consuming when the formulation contains an organic acid due to the long retention time of the acid under the specified HPLC conditions.

In this paper, we would like to report an improved HPLC method for the quantitative analysis of L-carnitine in pharmaceutical formulations. The improved method is able to provide a high resolution between L-carnitine and crotonoylbetaine and is suitable for both solution and tablet formulations of L-carnitine as well as capsule formulations containing L-carnitine L-tartrate [12].

2. Experimental

2.1. Equipment

The HPLC analysis was conducted on a chromatographic system consisting of a solvent degasser (SCM 400), pump (P4000), autosampler (AS3000), UV detector (UV1000, Thermo Separation Products, Inc., Riviera Beach, FL) as well as a data acquisition system (PeakPro, Beckman Instruments, Allendale, NJ).

2.2. Materials

Carnitor® tablets containing 330 mg of L-carnitine free base and Carnitor® solution containing 100 mg/ml of L-carnitine free base were purchased from Sigma–Tau Pharmaceuticals, Inc. (Gaithersburg, MD). Capsules containing 500 mg equivalent of free base L-carnitine as L-carnitine L-tartrate were purchased from vendor A and vendor B. USP reference standard L-carnitine (lot No. F-1) and crotonoylbetaine hydrochloride (lot No. F) were purchased from the US Pharmacopeia (Rockville, MD). HPLC grade acetonitrile, methanol, and methylene chloride were obtained from Burdick and Jackson (Muskegon, MI). Phosphoric acid (85%) and sodium hydroxide used to prepare buffers were analytical grade obtained from Mallinckrodt (Paris, KY). Deionized water purified through a water purification system (Millipore Milli-Q, Millipore, Bedford, MA) was used. Sodium 1-heptanesulfonate monohydrate and sodium 1-octanesulfonate monohydrate were purchased from Fluka (Milwaukee, WI). All chemicals were used without further purification.

2.3. Chromatographic conditions

In the improved method, the chromatographic results were obtained on the HPLC system using a C_8 column (Zorbax Rx-C_8, 4.6 × 75 mm, 3.5 μ) purchased from MAC-MOD Analytical, Inc. (Chadds Ford, PA). The column was eluted at a flow rate of 1.0 ml/min with a 98:2 (v/v) mixture of pH 2.0 sodium phosphate buffer (50 mM) and acetonitrile containing 2.5 mM of sodium 1-octanesulfonate. The injection volume was 20 μl for both standards and samples. The detection wavelength was 215 nm and the column was maintained at ambient temperature. The mobile phase was prepared by mixing 980 ml of pH 2.0 sodium phosphate buffer (50 mM) with 20 ml of acetonitrile and then dissolving 586 mg of sodium 1-octanesulfonate monohydrate into the solvent mixture followed by filtering and degassing. The pH 2.0 sodium phosphate buffer (50 mM) was prepared by dissolving 11.5 ml of 85% phosphoric acid into 1950 ml of water and then adding about 50 ml of 1 N aqueous sodium hydroxide solution to adjust a pH value of 2.0.

The USP method for L-carnitine solution formulation was performed on the HPLC system using a C_{18} column (Inertsil ODS-2, 4.6 × 150 mm, 5 μ) purchased from Keystone Scientific (Bellefonte, PA). A 98:2 (v/v) mixture of pH 2.4

sodium phosphate buffer (50 mM) and methanol containing 2.5 mM of sodium 1-heptanesulfonate was used as the mobile phase and the flow rate was 1.5 ml/min. The UV detection wavelength was 225 nm. Other conditions and the sample preparation procedure were the same as those described in USP 23 [10].

The USP method for tablet formulation was performed on the HPLC system using an NH_2 column (μBondapak™ NH_2, 3.9 × 300 mm, 10 μ) purchased from Waters (Milford, MA). A 35:65 (v/v) mixture of potassium phosphate buffer (50 mM) and acetonitrile was used as the mobile phase. The apparent pH was adjusted to 5.3 for the mobile phase. The flow rate was 1.0 ml/min. The UV detection wavelength was 205 nm. Other conditions and the sample preparation procedure were the same as those described in USP 23 [11].

2.4. Sample preparation

2.4.1. Standard sample

L-Carnitine, USP reference standard, was weighed and dissolved in water to prepare a standard solution. For the standard solutions used to determine the concentration of L-carnitine in commercial products, the concentration of the L-carnitine standard solution was corrected based on the water content in the reference standard. The water content in the reference standard was determined with the Karl Fischer titration on a coulometer (Metrohm 684 KF, Herisau, Switzerland) on the same day when the standard solution was prepared.

2.4.2. Solution formulation

Five milliliters of Carnitor® 100 mg/ml solution were transferred with a 5-ml pipette to a 50-ml volumetric flask. Water was added to the volume mark and the solution was mixed. The diluted solution, 5 ml, was loaded onto a pre-packed column (6-ml BAKEBOND spe™, J. T. Baker, Phillipsburg, NJ) containing 1 g of C_{18}-bonded silica gel. The column was previously conditioned by washing successively with 2 × 5 ml of methylene chloride, 2 × 5 ml of methanol, and 3 × 5 ml of water. Then, the column was rinsed with 2 × 5 ml of water. The eluate was collected in a 25-ml volumetric flask and diluted to the volume mark with water to give a sample solution containing about 2.0 mg/ml of L-carnitine. Five samples were prepared from 5 different bottles (two lots) of Carnitor® 100 mg/ml solution. The sample solution was filtered through a 0.45 μm filter (Acrodisc®, 25 mm diameter, Gelman Sciences, Ann Arbor, MI) and injected into the HPLC system. The injection was repeated twice for each sample.

2.4.3. Tablet formulation

Ten Carnitor® 330 mg tablets were weighed and placed in a 500-mL volumetric flask. Water was added to the volume mark. The mixture was sonicated at room temperature for 30 min or until the tablets disintegrated completely. Then, the resulting solution, 5 ml, was transferred to a 25-ml volumetric flask and diluted to the volume mark with water to give a sample solution containing about 1.3 mg/ml of L-carnitine. Five samples were prepared from two lots of Carnitor® 330 mg tablets. The sample solution was filtered through a 0.45 μm filter and injected into the HPLC system.

2.4.4. Capsule formulation

Ten L-carnitine 500 mg capsules were opened. The contents were poured out and weighed in a 500-ml volumetric flask. Water was added to the volume mark. The mixture was sonicated at room temperature for 30 min or until all solid particles disintegrated completely. The resulting solution, 5 ml, was transferred to a 25-ml volumetric flask and diluted to the volume mark with water to give a sample solution containing about 2.0 mg/ml of L-carnitine. Five samples were prepared from two lots of L-carnitine 500 mg capsules. The sample solution was then filtered through a 0.45 μm filter and injected into the HPLC system.

3. Results and discussion

3.1. Resolution

Fig. 1 shows the chromatograms for the L-carnitine solution formulation (A), the L-carnitine

tablet formulation (B), the L-carnitine L-tartrate capsule formulation (C), and the USP reference standard of L-carnitine (D) generated by the improved HPLC method. Under the conditions, retention times were 1.01 ± 0.01 min for tartaric acid, 2.16 ± 0.01 min for malic acid, 4.22 ± 0.09 min for L-carnitine, and 5.40 ± 0.09 min for crotonoylbetaine present in the three formulations analyzed in different days. All three formulations contain small amounts of crotonoylbetaine as an impurity. The identity of crotonoylbetaine was confirmed through coinjection with the USP reference standard. The improved HPLC method shows a high resolution between L-carnitine and crotonoylbetaine with $R > 2.0$ for all samples analyzed.

The chromatograms obtained with the USP methods for L-carnitine solution formulation (E) and tablet formulation (F) are also shown in Fig. 1. Retention times were 2.92 ± 0.01 min for malic acid, 4.04 ± 0.07 min for L-carnitine and crotonoylbetaine, and 13.31 ± 0.04 min for *p*-aminobenzoic acid analyzed with the USP method for L-carnitine solution formulation. Retention times were 7.32 ± 0.15 min for L-carnitine and 8.32 ± 0.17 min for crotonoylbetaine analyzed with the USP method for L-carnitine tablet formulation. The USP method for L-carnitine solu-

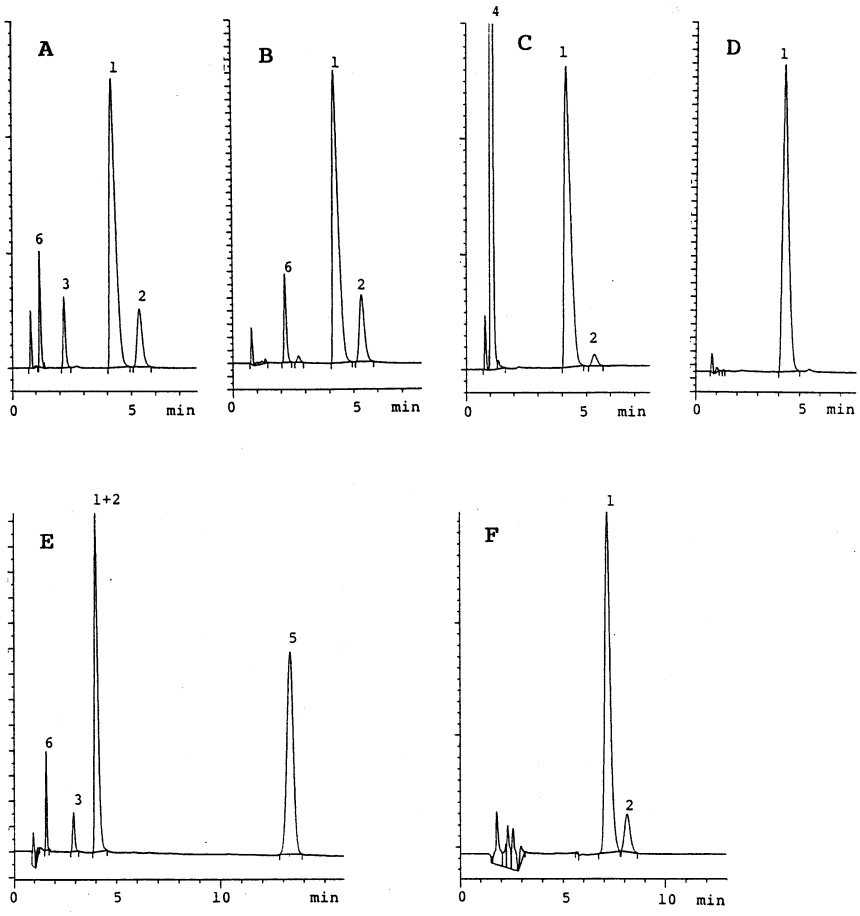

Fig. 1. HPLC chromatograms obtained with the improved HPLC method for Carnitor® solution (A); Carnitor® tablet (B); L-carnitine L-tartrate capsule (C) (vendor A); and USP reference standard of L-carnitine (D); and with the USP methods for Carnitor® solution (E); and Carnitor® tablet (F); 1, L-carnitine; 2, crotonoylbetaine; 3, malic acid (buffering agent); 4, tartaric acid; 5, *p*-aminobenzoic acid; 6, unknown excipient.

Table 1
Method accuracy and reproducibility

Label strength	Day	Assay concentration (mg/ml)[a]	% Label strength	Reproducibility (RSD)	
				Intra-day	Inter-day
1.00 mg/ml	1	0.99 ± 0.006	98.5	0.6	0.7
	2	0.99 ± 0.008			
	3	0.98 ± 0.004			
	4	0.98 ± 0.004			
2.00 mg/ml	1	1.98 ± 0.014	98.2	0.6	1.2
	2	1.97 ± 0.017			
	3	1.93 ± 0.013			
	4	1.97 ± 0.007			
3.00 mg/ml	1	2.97 ± 0.022	98.8	0.6	1.0
	2	2.98 ± 0.027			
	3	2.93 ± 0.013			
	4	2.98 ± 0.017			

[a] Presented as mean ± standard deviation ($n = 4$)

tion formulation cannot separate crotonoylbetaine from L-carnitine. In fact, the method uses *p*-aminobenzoic acid as an internal standard and requires that the resolution (R) between L-carnitine and *p*-aminobenzoic acid be not lower than 1.5. We found that the peaks of L-carnitine and crotonoylbetaine are completely co-eluted using the USP method, even when the resolution between L-carnitine and *p*-aminobenzoic acid is much higher than 1.5 (Fig. 1E). Crotonoylbetaine is a major impurity present in almost all L-carnitine formulations. Although its content is usually very low ($< 1\%$), it may introduce a significant error to the analytical result, since the UV absorption coefficient of crotonoylbetaine at 225 nm is about 80 times higher than that of L-carnitine.

3.2. Calibration linearity

Calibration curves were prepared by plotting the HPLC peak areas obtained with the improved method versus the L-carnitine concentrations of standard solutions. The curves were linear in the range of 0.4–4.0 mg/ml for L-carnitine with a correlation coefficient of 0.999 or greater. It was noticed that at a concentration higher than 2.5 mg/ml, the resolution between L-carnitine and crotonoylbetaine was slightly lower ($R > 1.5$), due to tailing of the L-carnitine peak.

3.3. Accuracy and reproducibility

Three samples with label strengths of 1.00 mg/ml, 2.00 mg/ml, and 3.00 mg/ml were prepared from the USP reference standard of L-carnitine. Replicate injections ($n = 4$) were conducted for each sample in one day, and the same procedure was repeated on four different days. Assay concentrations were calculated by comparing the HPLC peak areas of samples to those of L-carnitine external standard solutions (2.00 mg/ml). As shown in Table 1, the mean recovery of L-carnitine is 98.5, 98.2 and 98.8% for the three samples. The slightly low results may be due to the error introduced when weighing and transferring very hygroscopic L-carnitine free base. In the table, the intra-day reproducibility is defined as the mean of the daily relative standard deviation (RSD) and the inter-day reproducibility is the RSD of the pooled four-day results. At the three concentrations, the intra-day reproducibility was 0.6%, while the inter-day reproducibility was 0.7–1.2%.

3.4. Analysis of L-carnitine oral formulations

Quantitative analysis of L-carnitine was conducted using the improved method for four commercially available oral formulations, including

one L-carnitine solution formulation (Carnitor® solution, 100 mg/ml), one L-carnitine tablet formulation (Carnitor® tablet, 330 mg, Sigma–Tau Pharmaceuticals, Inc.), and two L-carnitine L-tartrate capsule formulations (500 mg equivalent of L-carnitine free base, vendor A and vendor B). The solution formulation and the tablet formulation were also analyzed using the two USP methods for solution formulation and tablet formulation, respectively. The results are shown in Table 2.

The improved HPLC method provides accurate and reproducible results for all four oral formulations. For the tablet formulation, the result is very consistent with the result obtained with the USP method. For the solution formulation, however, the result obtained with the improved method is lower than that determined using the USP method. The difference can be explained by the interference from crotonoylbetaine, a major impurity present in the formulation. The improved method is able to separate L-carnitine from crotonoylbetaine, while the USP method is not.

The USP method for tablet formulation is able to separate L-carnitine from crotonoylbetaine with $R > 1$. However, the method uses an NH_2 column, which is not suitable for the analysis of formulations containing a high concentration of sugars, since reducing sugars can rapidly degrade the column. Therefore, the USP method for tablet formulation lacks the versatility and robustness required for the analysis of L-carnitine oral solution formulation. Moreover, the method is very time consuming when the formulation contains an organic acid, since the retention times of organic acids are relatively long under these HPLC conditions. For example, tartaric acid shows a broad peak at about 20 min. Therefore, the USP method is not suitable for the capsule formulation containing L-carnitine L-tartrate.

4. Conclusions

The improved HPLC method is suitable for quantitative analysis of L-carnitine in both solution and tablet formulations. Capsule formulations containing L-carnitine L-tartrate can also be analyzed with this method. The method provides quantitative results with an accuracy higher than or equal to that of the current USP methods. In addition, the improved method can be used for the analysis of a large number of samples, since all components present in these formulations are

Table 2
Results of five replicate analyses of commercially available L-carnitine products

L-Carnitine product	Day	% Label strength found by the improved method	% Label strength found by the USP method
L-Carnitine solution[a]	1	95.8 ± 0.64	101.3 ± 0.88
	2	96.0 ± 0.61	
L-Carnitine tablet[b]	1	98.1 ± 1.33	98.8 ± 0.55
	2	98.2 ± 0.48	
L-Carnitine capsule[c]	1	101.2 ± 1.19	–
	2	101.1 ± 0.97	–
L-Carnitine capsule[d]	1	100.2 ± 1.87	
	2	100.1 ± 1.89	–

[a] Carnitor® solution.
[b] Carnitor® tablet (Sigma–Tau Pharmaceuticals).
[c] Vendor A.
[d] Vendor B.

eluted in a short time period and repeated injections are possible at 6 min intervals while maintaining a high resolution.

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