Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1081 (2005) 60–64
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Short communication

Capillary electrophoresis determination of carnitine in food supplements

Vendula Prokorátová, František Kvasnička *, Rudolf Ševčík, Michal Voldřich

Institute of Chemical Technology, Department of Food Preservation and Meat Technology, Technická 5, 166 28 Prague 6, Czech Republic

Available online 1 April 2005

Abstract

L-Carnitine is a substance natural for human body which transfers fatty acids to the place of burning—mitochondria and aids the transformation of fats into energy and this way supports overweight reduction and immediate physical performance, increases resistance from physical load and protect heart from overload. In this study are described newly developed electrophoretic methods (ITP, CZE with direct and/or indirect UV detection) for carnitine determination in various samples. The results were compared with results obtained by validated HPLC method. All of these methods gave comparable results. The detection limits of the electrophoretic methods were between 2.4 and 4.7 μg/ml, reproducibility (relative standard deviation, RSD%) was between 1.2 and 4.4% and recoveries were between 91 and 113% in different samples. The shorter analysis and low running cost are the main advantages of CE methods.

Keywords: Carnitine; Electrophoresis; ITP; CZE; Food supplements

1. Introduction

L-Carnitine ((*R*)-3-carboxy-2-hydroxy-*N*,*N*,*N*-trimethyl-1-propaminium hydroxide inner salt) is a critical substance needed for the maintenance of health, but is not a required nutrient in the diet. L-Carnitine is used as a carrier to transport long-chain fatty acids into the mitochondria of a cell for beta-oxidation to produce energy. L-Carnitine also participates in the control of the mitochondrial acyl-CoA/CoA ratio, peroxisomal oxidation of fatty acids, and the production of ketone bodies. Due to their intrinsic interaction with the bioenergetic processes, they play an important role in diseases associated with metabolic compromise, especially mitochondrial-related disorders. A deficiency of carnitine is known to have major deleterious effects on the CNS. Several syndromes of secondary carnitine deficiency have been described that may result from defects in intermediary metabolism and alterations principally involving mitochondrial oxidative pathways [1]. The absence of L-carnitine affects the loss of energy and toxic accumulations of free fatty acids. L-Carnitine is a naturally occurring quaternary ammonium compound endogenous in all mammalian species [1]. Adult people can synthesise carnitine in the liver and kidney (from the amino acids L-lysine and L-methionine) or taken up in food. Plasma carnitine concentration positively correlates with the dietary intake of carnitine. Exogenous supply of this conditionally essential nutriment is mainly supplied by meat, milk and vegetables but also by human milk or by infant formulae with low-carnitine supplementation [2,3]. Generally, only very small amounts of it are found in plants, with few exceptions, such as avocado and some fermented soy products, e.g. tempeh. L-Carnitine is a chiral molecule. Its stereoisomer D-carnitine has been found to have a considerable toxic influence on biochemical processes due to inhibition effects on the carnitine acetyltransferase, leading to a depletion of the body's L-carnitine stock [4,5]. Several methods have been described for the determination of carnitine in biological tissues, body fluid, infant formulae and drugs. Thus, enzymatic flow injection [2], radio-enzymatic assay, gas chromatography, gas chromatography–mass spectrometry (GC–MS) and fast atom bombardment mass spectrometry (FAB–MS) among other methods [6–10]. Nevertheless, the most common separation technique has been liquid chromatography (LC) with UV detection and electrophoresis. Capillary electrophoresis (CE) has proved to be a highly efficient separation technique for different molecules. Its detection system includes direct and indirect UV or fluorescence detection, electrochemical and

* Corresponding author. Tel.: +420 224 355 117; fax: +420 233 337 337.
E-mail address: frantisek.kvasnicka@vscht.cz (F. Kvasnička).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.03.033

other detectors [11]. As carnitine has no specific UV absorption, it has to be derivatized for direct detection. Different chromophores have been described in the literature, reacting either with the carboxyl or the hydroxyl group of carnitine [12]. CE also may have several advantages in comparison to HPLC for the separation of carnitine. The preparation of sample is generally easier and the amount of solvent is smaller. The analysis is faster and less expensive. Accordingly, CE procedures for the separation of carnitine standard solutions have been described [5,11,13–16]. Recently, it is produce a lot of food supplements with L-carnitine, optionally with other additives (e.g. chromium), but the product control necessary for the producers made by HPLC method is demanding and time-consuming. Consequently, this paper describes three different electrophoretic methods (ITP-ITP, CZE with direct/indirect UV detection) for the determination of L-carnitine. All of these methods were compared each other and correlated with HPLC results on model and real samples.

2. Experimental

2.1. Chemicals and samples

All used chemicals were of analytical grade. Only demineralized distilled water was added to standard solutions and buffers. L-Carnitine ([−]-β-hydroxy-γ-[trimethylammonio] butyrate; Vitamin B_T) was purchased from Sigma (Prague, Czech Republic) and carnitine-tartrate was provided by Lonza Biotec Ltd (Kouřim, Czech Republic). Acetic acid, phosphoric acid, nitric acid, NH_4OH, NaOH and $NaHCO_3$ were obtained from Lach—Ner s.r.o. (Neratovice, Czech Republic), FMOC (9-fluorenyl-methylchlorformate) and TRIS (α,α,α-TRIS-(hydroxymethyl)-methylamin) were from Sigma–Aldrich (Prague, Czech Republic). Acetonitrile was obtained from Merck (Darmstadt, Germany), Quinine anhydrous was from Fluka (Buchs, Switzerland). Series of six manufactory samples LC1–LC6 with different concentration of L-carnitine were supplied from Lonza Biotec Ltd (Kouřim, Czech Republic). Four samples of food supplements (*Karnitin + chrom*, Naturvita a.s., Czech Republic; *Chroma Slim Ultra*, GSN, USA; *Neo carnitargin*, Nutrend D. S., a.s., Czech Republic; *Viaredin*, Walmark a.s., Czech Republic) were obtained from the Czech market.

2.2. Standard solutions, sample preparation and derivatization

A stock 1 mg/ml L-carnitine solution was prepared in water, stored at 4 °C and diluted with water to the desired concentration just before use. Fresh stock 100 mM solution of FMOC was prepared in acetonitrile before derivatization, 50 mM carbonate buffer (pH 10.4) and 1 M acetic acid were prepared in water and stored at ambient temperature.

Liquid manufactory samples LC1–LC6 were only diluted according to the expected concentration in water and samples from the market were homogenized and diluted for CZE with indirect UV and ITP-ITP analyses. All samples were derivatized with FMOC for CZE with direct UV. The procedure was following: 1 ml of the sample was mixed with 1 ml of carbonate buffer, after the addition of 2 ml of FMOC solution the sample was derivatized for 1 h at 50 °C. To complete the reaction, 2 ml of acetic acid buffer and 4 ml of water were added to a final volume of 10 ml. The solution was then filtered and injected. No further sample pre-treatment was performed.

2.3. Capillary isotachophoresis

The electrophoretic analyser EA 100 (LABECO-VILLA, Ltd., Slovakia) with column coupling was used. The separation was performed in a PTFE pre-separation capillary (90 mm × 0.8 mm i.d.), which was coupled with a PTFE analytical capillary (90 mm × 0.3 mm i.d.). Zones were detected by a conductivity detector. The isotachopherograms were evaluated by the help of PC software package supplied with analyser (KasComp Ltd., Slovakia). The samples and/or standard solutions were injected either by a valve with fixed internal sample loop (30 μl) or by a 10 μl Hamilton syringe. The terminating electrolyte was 10 mM acetic acid and leading electrolyte was 40 mM acetic acid and 20 mM NH_4OH. The separation was carried out with cationic mode and constant driving current was of 250 and 25 μA on the pre-separation capillary and the analytical capillary, respectively. The analysis took 25 min.

2.4. Capillary zone electrophoresis

Electrophoretic analyzer EA 101 (Villa-Labeco, Slovakia) with FEP capillary – 90 mm × 0.3 mm i.d. and ^{3D}CE (Hewlett-Packard, USA) with fused silica capillary – effective length 255 mm, 50 μm i.d. were used for CZE with indirect UV detection. The wavelengths for detection were 254 nm (EA 101) and 200 nm (^{3D}CE). The background electrolyte (5 mM TRIS + 7 mM H_3PO_4 + 0.5 mM quinine) was the same for both instruments. The analysis time was 6 min.

^{3D}CE-Hewlett-Packard capillary electrophoresis instrument (Hewlett-Packard, USA) equipped with a diode array detector operating at 200, 205 and 254 nm was used for L-carnitine determination with direct UV detection. The separation took place in a 360 mm (255 mm to detector) ×50 μm i.d. fused-silica capillary thermostated at 25 °C. A voltage of 15 kV was applied in a constant and cationic mode (current about 40 μA). The samples were hydrodynamically injected by pressure 25 mbar for 5 s. The carrier electrolyte was 15 mM TRIS and 25 mM H_3PO_4. The time of analysis was 10 min. The capillary was conditioned each morning during 5 min with 1 M HCl and 1 M and then during 10 min with the running buffer. Between runs, a 2 min wash with the running buffer was performed.

Table 1
Comparison of method characteristics for L-carnitine

Method	Concentration (μg/ml)	*r*	LOD (μg/ml)	LOQ (μg/ml)	Recovery (%)	RSD (%)
ITP-ITP	0.5–10	0.9999	0.5	2.0	91–113	1.6–7.8
CZE (indirect UV)[a]	10–200	0.9992	3.7	12.3	–	1.7
CZE (indirect UV)[b]	10–200	0.9991	4.2	14.0	104–106	1.2
CZE (direct UV)	5–50	0.9982	4.4	14.7	96–98	2.7–4.4

[a] Villa Labeco.
[b] Hewlett-Packard.

2.5. Liquid chromatography

The sample analysis was performed at Lonza Biotec on a Waters 515 HPLC pump with Rheodyne 7725i (20 μl) with conductivity detector. A Supelcosil LC-SCX, 250 mm × 4.6 mm, 5 μm, Cat. No. 5-89975 column and a Supelco Supelguard LC-SCX, 20 mm, 5 μm, Cat. No. 5-9519 as precolumn was used. The mobile phase was 5 mM HNO_3 in 5% acetonitrile and the flow rate was 1.5 ml/min. The run time took 25 min.

3. Results and discussion

All electrophoretic systems were compared in order to determine the best linear range, sensitivity and reproducibility for the L-carnitine determination in food supplements. The linearity was tested over a concentration range of 0.5–200 μg/ml for L-carnitine, and the peak area was used under the optimal operation conditions. An external calibration method was used and individual area/concentration sets were subjected to linear least-square regression. There was a good linearity ($r > 0.99$) for all applied methods. The limits of detection (LOD) and quantification (LOQ) were calculated as a signal-to-noise ratio of 3 and 10, respectively. The detection limit and quantification limit were slightly worse for capillary zone electrophoresis.

The relative standard deviation (RSD) was determined by eight measuring of three individual samples with different concentration levels of L-carnitine and the recoveries were obtained by measuring of samples and the same samples with addition of 100% of standard. The RSD was lower for all type of CZE then for 2D-CITP. The ranges of linearity, correlation factors and other characteristic parameters of all applied methods are shown in Table 1.

The comparison of characteristic parameters of the electrophoretic methods denoted that the results were very similar and consequently was necessary to notice difficulty of sample preparation, instrumentation and automatic injection possibilities. In this case CZE with indirect UV detection performed on Hewlett-Packard was seemed to be the best. Good linearity and sensitivity was obtained for capillary isotachophoresis. Disadvantage of this CITP method and of CZE with indirect performed on Electrophoretic analyzer EA 101 was impossibility of automatic injection. Derivatization with FMOC was necessary for electrophoresis with direct UV detection and this is not practical for series of samples. Additionally, the calibration curve had to be done always with new sample series. Capillary electrophoresis with direct UV has another advantages: the first one is possibility to separate

Table 2
Comparison of results obtained by different methods; carnitine content (g/100 g) in factory samples

Sample	HPLC	ITP-ITP	CZE[a] (indirect UV)	CZE[b] (indirect UV)	CZE (direct UV)
LC 1	1.4	1.6	1.1	1.6	1.3
LC 2	1.1	1.8	1.5	2.0	1.4
LC 3	38.9	38.2	36.2	35.5	35.9
LC 4	7.3	7.6	7.3	7.4	7.5
LC 5	36.8	33.8	37.3	37.3	35.8
LC 6	41.5	40.8	41.2	45.6	37.7

[a] Villa Labeco.
[b] Hewlett-Packard.

Table 3
Content of L-carnitine in some food supplements (g L-carnitine/100 g sample)

Food supplement	Declared by producer	ITP-ITP	CZE[a] (indirect UV)	CZE[b] (indirect UV)	CZE (direct UV)
"Karnitin + chrom" Naturvita a.s., Czech Republic	21.4	25.3	25.1	19.3	21.6
"Chroma Slim Ultra" GSN, USA	3.7	1.1	1.3	1.0	1.2
"Neo carnitargin" Nutrend D.S., a.s., Czech Republic	2.0	2.0	1.9	1.8	2.2
"Viaredin" Walmark, a.s., Czech Republic	0.4	0.3	0.2	–	0.3

[a] Villa Labeco.
[b] Hewlett-Packard.

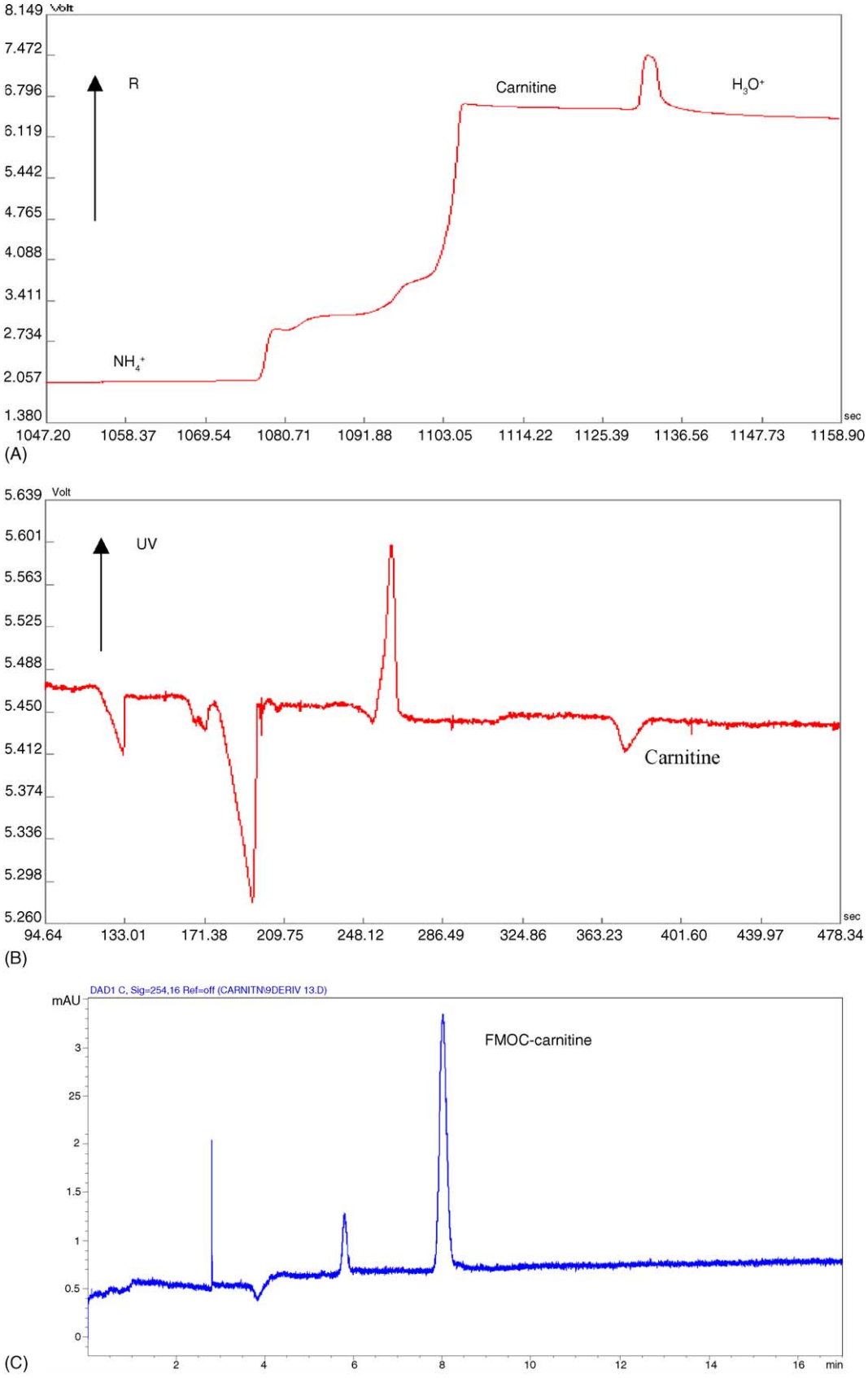

Fig. 1. Electropherograms of food supplement sample “Chroma Ultra Slim”; ITP mode (A); CZE mode with indirect UV detection (B), CZE mode with direct UV detection (C); R, response of conductimeter; UV, response of UV detector; for conditions see in text.

L-/D-carnitine and the further is the removal of all interferon proteins and peptides takes place during the derivatization procedure, therefore not many interfering components have been observed during the separation. Some of sample electropherograms are shown in Fig. 1. Another available possibility to choose the accurate and the best of tested electrophoretic method was to compare them with HPLC results. Therefore six factory samples with different concentration of L-carnitine were measured by liquid chromatography and electrophoretic methods for this comparison (Table 2). There were not found statistical differences among electrophoretic methods and HPLC. Each correlation between the two methods was in an acceptable range ($r > 0.995$, confidence limits of intercepts and slopes cover zero and unity, respectively). Very good correlation was found for comparison of HPLC versus ITP or HPLC versus CZE with direct UV detection ($r = 0.999$).

To demonstrate the applicability of the proposed electrophoretic methods, they were applied to the determination of L-carnitine in food supplements containing different concentrations of it and also some other analytes. The obtained results from electrophoretic measurements were compared with the values declared by producer (Table 3). The data were very well comparable; only one producer declared for his product of about three times higher content of L-carnitine than it was found.

4. Conclusions

In this study three modes of capillary electrophoresis for L-carnitine determination in food supplements were tested. In comparison to HPLC method, the CE analysis is faster, consumes less solvents and total running costs are lower than HPLC. There is very simple sample preparation for capillary isotachophoresis and capillary electrophoresis using a quinine buffer with indirect photometric detection and both of methods allow direct determination of L-carnitine without the use of previous complex derivatization reactions.

Capillary zone electrophoresis with direct UV detection can be used for analysis of carnitine after derivatization. This analytical procedure is unfortunately not suitable for routine analysis in industrial laboratories.

Acknowledgements

The authors are grateful for financial support provided by Grant Agency of Czech Republic (grant no. 525/03/0351). Thanks are extended to Ing. Viktor Malaník from Lonza Biotec Ltd for factory samples of carnitine and HPLC method and results.

References

[1] A. Virmarni, Z. Binienda, Mol. Asp. Med. 25 (2004) 533.
[2] H.E. Indyk, D.C. Woolard, J. AOAC Int. 78 (1995) 69.
[3] A. Lee Carter, T.O. Abney, D.F. Lapp, J. Child Neurol. 10 (Suppl. 2) (1995) 253.
[4] H. Jung, K. Jung, H.P. Kleber, in: A. Fiechter (Ed.), Advances in Biochemical Engineering Biotechnology, Springer, Berlin, 1993.
[5] C. Vogt, S. Kiessig, J. Chromatogr. A 745 (1996) 53.
[6] S. Lowes, M. Rose, Analyst 115 (1990) 511.
[7] K. Matsumoto, Y. Ichitani, N. Ogasawara, H. Yuki, K. Imai, J. Chromatogr. A 678 (1994) 241.
[8] H. Kamimori, Y. Hamashima, M. Konishi, Anal. Biochem. 218 (1994) 417.
[9] J. Bounoure, J. Souppe, Analyst 113 (1988) 1143.
[10] K. Altria, Chromatographia 35 (1993) 177.
[11] C. Mardones, N. Vizioli, C. Carducci, A. Rios, M. Valcarcel, Anal. Chim. Acta 382 (1999) 23.
[12] L. Vernez, W. Thormann, S. Krähenbühl, J. Chromatogr. A 895 (2000) 309.
[13] P. De Witt, R. Deias, S. Muck, B. Galletti, D. Meloni, P. Celletti, A. Marzo, J. Chromatogr. B 657 (1994) 67.
[14] C. Vogt, A. Georgi, G. Werner, Chromatographia 40 (1995) 287.
[15] C. Mardones, A. Rios, M. Valcarcel, R. Cicciarelli, J. Chromatogr. A 849 (1999) 609.
[16] S. Kiessig, C. Vogt, J. Chromatogr. A 781 (1997) 475.