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Review

Analytical approaches to determination of carnitine in biological materials, foods and dietary supplements

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ABSTRACT

L-Carnitine is a vitamin-like amino acid derivative, which is an essential factor in fatty acid metabolism as acyltransferase cofactor and in energy production processes, such as interconversion in the mechanisms of regulation of cetogenesis and termogenesis, and it is also used in the therapy of primary and secondary deficiency, and in other diseases. The determination of carnitine and acylcarnitines can provide important information about inherited or acquired metabolic disorders, and for monitoring the biochemical effect of carnitine therapy. The endogenous carnitine pool in humans is maintained by biosynthesis and absorption of carnitine from the diet. Carnitine has one asymmetric carbon giving two stereoisomers D and L, but only the L form has a biological positive effect, thus chiral recognition of L-carnitine enantiomers is extremely important in biological, chemical and pharmaceutical sciences. In order to get more insight into carnitine metabolism and synthesis, a sensitive analysis for the determination of the concentration of free carnitine, carnitine esters and the carnitine precursors is required. Carnitine has been investigated in many biochemical, pharmacokinetic, metabolic and toxicokinetic studies and thus many analytical methods have been developed and published for the determination of carnitine in foods, dietary supplements, pharmaceutical formulations, biological tissues and body fluid. The analytical procedures presented in this review have been validated in terms of basic parameters (linearity, limit of detection, limit of quantitation, sensitivity, accuracy, and precision). This article presented the impact of different analytical techniques, and provides an overview of applications that address a diverse array of pharmaceutical and biological questions and samples.

Contents

Abbreviations: 1AA, 1-aminoanthracene; CAT, carnitine acetyltransferase; CE, capillary electrophoresis; CSP, chiral stationary phase; CV, coefficient of variation; CZE, capillary zone electrophoresis; DM, dry matter; DTNB, 5,5′-dithiobis-2-nitrobenzoate; EDAC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; ESI, electrospray ionisation; FIA, flow injection analysis; (+)-FLEC, (+)-[1-(9-fluorenyl)-ethyl]-chloroformate; FMOC, fluorenylmethyloxycarbonyl; GC, gas chromatography; HPLC, high performance liquid chromatography; HP-β-CD, [2-hydroxypropyl]-β-cyclodextrin; HRP, horseradish peroxidase; IC, ion pair chromatography; INT, 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl tetrazolium chloride; ITP, capillary isotachophoresis; LC, liquid chromatography; LOD, limit of detection; LOQ, limit of quantification; MS, mass spectrometry; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide; NAD^+, nicotinamide adenine dinucleotide; NAM, *N*-(9-acridinyl)-malemide; NP, normal phase; p-BPB, p-bromophenacyl bromide; PFT, partial-filling technique; *r*, correlation coefficient; RP, reversed phase; Rs, peak resolution; RSD, relative standard deviation; SIA, sequential injection analysis; TNB, 5-thio-2-nitrobenzoate; UV, ultraviolet; VIS, visible; wet wt., wet weight; α, separation factor; λ_{em}, emission wavelength; λ_{exc}, excitation wavelength.

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1. Introduction

Carnitine (Fig. 1) and acylcarnitines belong to a family of the non-protein amino acid with a large span of polarities, from the polar carnitine to the quite apolar long-chain acylcarnitines. 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, and is used as a carrier to transport long-chain fatty acids into the mitochondria of a cell for betaoxidation to produce energy (Kumaran, Deepak, Naveen, & Panneerselvam, 2003). 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 its intrinsic interaction with the bioenergetic processes, it plays an important role in diseases associated with metabolic compromise, especially mitochondrial related disorders (Zammit, Ramsay, Bonomini, & Arduini, 2009).

Nowadays the studies of carnitine for medical applications were becoming more and more popular. Carnitine status in humans is reported to vary according to body composition, gender, and diet. Plasma carnitine concentration positively correlates with the dietary intake of carnitine (Muthuswamy, Vedagiri, Ganesan, & Chinnakannu, 2006; Rani & Panneerselvam, 2001; Walter & Schaffhauser, 2000). The knowledge about carnitine concentrations in different biological samples is helpful when humans are suffering from carnitine deficiency and an exogenous supplementation is needed. The dual measurement of free and total carnitine is important in the clinical management of patients with abnormalities in carnitine metabolism. Carnitine deficiency is diagnosed from either a low free carnitine or a high proportion of esterified carnitine. Some papers reported that carnitine and its acetylated form have been studied as a treatment for erectile dysfunction in men and as a supplementation for the patients with asthenozoospermia to improve the quality and quantity of spermatozoa. Andrological clinicians have been paying increasing attention to the effect of carnitine on semen quality and its treatment and improved roles on infertility. The determination of free carnitine level in seminal plasma may prove useful as a potentially biochemical marker of fertility and this is a useful guidance for the clinic therapy and the mechanismic study on the male reproduction (Abd El-Baset, Abd El-Wahab, Mansour, & Mohamed, 2010; Matalliotakis et al., 2000). In addition, carnitine is an essential nutrient for the newborn, and its provision in the form of a supplement produces beneficial effects in patients infected with human immunodeficiency virus type 1 (Penn, Schmidt-Sommerfeld, & Wolf, 1982). It is used, as an alternative therapy in patients with Alzheimer's disease (Bianchetti, Rozzini, & Trabucchi, 2003), chronic fatigue syndrome (Werbach, 2000), end-stage renal disease patients undergoing dialysis (Bellinghieri, Santor, Calvani, Mallamace, & Savica, 2003), and as an agent capable of protecting the heart against ischaemia/perfusion injury leading to myocyte death (El-Beshlawy et al., 2004).

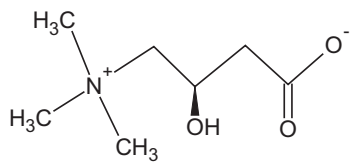

Fig. 1. The chemical structure of carnitine.

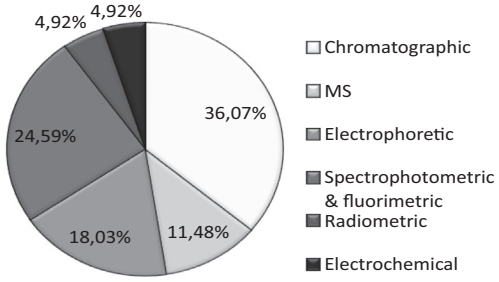

Fig. 2. Different methods employed for determination of L-carnitine.

Chiral recognition of enantiomers is extremely important in biological, chemical and pharmaceutical sciences. Enantiopurity tests of biologically active substances became increasingly important, particularly for chiral drugs, because in most cases only one of the enantiomers possesses the desired pharmacological effects whilst the other one can be toxic, less active, or can have unwanted side effects or even act as an antagonist to the pharmacologically active enantiomer. Pharmaceutical preparations contain the L-enantiomeric pure form or the racemic mixture of carnitine. Although D,L-carnitine can be obtained by chemical synthesis, an expensive resolution procedure is necessary to obtain pure L-carnitine. D-Carnitine is a by-product of the resolution process and so methods to transform D-carnitine into L-carnitine would be in industrial interest. The ability to determine L-carnitine and D-carnitine levels could be of use for determining the concentration of this compound during the biological or chemical production of L-carnitine, for ascertaining the racemate proportions, and for checking the purity of L-carnitine preparations. In medicine, it would enable the pharmacological effects and the pharmacokinetics of the enantiomers testing biological samples to be followed after administration of L,D-carnitine as a drug (Jung, Jung, & Kleber, 1993). L-Carnitine is highly therapeutically effective and, therefore, used worldwide for various nutritional and pharmaceutical applications, whilst D-carnitine displays serious side-effects, and hence the content of D-carnitine must be precisely determined and limited in pharmaceutical and nutritional formulations. The content of D-carnitine in the European Pharmacopoeia and United States Pharmacopoeia is limited to about 4%, as determined by optical rotation measurements (European Pharmacopoeia, 2008; The United States Pharmacopeia, 2006). Most popular techniques for discrimination of chiral drugs have been based on chromatography, capillary zone electrophoresis, mass spectrometry and electrochemistry.

To study the behaviour of L-carnitine in disease and therapy, investigators need a simple, rapid, accurate and specific procedure. Analytical methods for plasma free and total carnitine determination include enzymatic, spectrophotometric, chromatographic, and electrophoretic techniques (Fig. 2). Most important today, especially for organic pollutants, are separation techniques such as LC–MS and GC–MS. Other approaches include classic and miniaturised applications of spectroscopy, electrochemistry, radiochemistry. The latter are also important in effect-directed analysis, which aims to link biological effects to compound identification and quantitation. This review presents a comprehensive survey of recent progress on carnitine determination. Different schemes