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Clinica Chimica Acta 358 (2005) 104–112

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Rapid measurement of plasma acylcarnitines by liquid chromatography–tandem mass spectrometry without derivatization

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Received 7 December 2004; received in revised form 9 February 2005; accepted 10 February 2005
Available online 9 April 2005

Abstract

Background: Tandem mass spectrometry (MS/MS) is being increasingly used to identify and measure acylcarnitines in blood and urine of children suspected of having fatty oxidation disorders and other inborn errors of metabolism. Rapid MS/MS analysis requires simple and efficient sample preparation. We developed a LC-MS/MS method for the online extraction of acylcarnitines in plasma without derivatization that requires only precipitation of proteins by acetonitrile followed by centrifugation, thus increasing efficiency.
Methods: An API-3000 tandem mass spectrometer (SCIEX, Toronto, Canada) equipped with electrospray ionization (ESI), TurboIon Spray source, three Shimadzu LC10AD micropumps and autosampler (Shimadzu Scientific Instruments, Columbia, MD) was used to perform the analysis. Within-day and between-day imprecision was evaluated for 10 analytes in the MRM mode using 3 levels of controls. Accuracy was determined by comparing the method with another MS/MS procedure and by recovery experiments. Sensitivity and specificity were evaluated by identifying patient samples under a wide variety of clinical conditions.
Results: Within-day CVs was $<10\%$ for all analytes tested and between-day CVs ranged from 4.4% to 14.2%. The method was linear in the range between 1.0 and 100 μmol/l for C2 and 0.1 and 10 μmol/l for the other acylcarnitines. The results of the comparison study yielded r values ranging between 0.948 and 0.999. Recovery ranged from 84% to 112%. The method correctly identified patients with a variety of fatty acid oxidation disorders and organic acidemias.
Conclusions: Our method is a simple procedure for the analysis of acylcarnitines in plasma with minimal sample preparation. It is thus ideal in a routine clinical setting where efficient processing of clinical samples is necessary to reduce turnaround time under conditions of high-throughput.
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Keywords: Acylcarnitines; Fatty acid oxidation disorders; Organic acidemias; LC-MS/MS

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doi:10.1016/j.cccn.2005.02.011

1. Introduction

Fatty acid oxidation disorders encompass a wide spectrum of clinical phenotypes in children that include myopathy, cardiomyopathy and hepatic encephalopathy [1–3]. These disorders are characterized by enzyme deficiencies resulting in the accumulation of potentially toxic acyl-CoA esters in mitochondria. Acyl-CoA esters may also be produced in certain organic acidemias [4–6] and defects of branched chain amino acid metabolism [7].

Detoxification of the acyl-CoA species occurs by the formation of acylcarnitines and the release of free CoA. Consequently, there is an increased concentration of circulating acylcarnitines, increased excretion of acylcarnitines in urine and secondary carnitine deficiency. Therefore, the analysis of circulating free and total carnitine and acylcarnitines provides a powerful selective screening tool for these disorders. Tandem mass spectrometry (MS/MS) is being increasingly used to identify and measure acylcarnitines in blood and urine of children suspected of having fatty oxidation disorders and other inborn errors of metabolism [8–14]. Rapid MS/MS analysis requires simple and efficient sample preparation. Methods for the preparation of plasma samples prior to acylcarnitine measurement, however, require multiple extractions [15] or derivatization with acidified butanol [16], followed by reconstitution in a liquid matrix suitable for the type of MS/MS analysis.

2. Materials and methods

2.1. Chemicals

Acetylcarnitine hydrochloride (C2), hexanoylcarnitine chloride (C6), octanoylcarnitine chloride (C8), decanoylcarnitine chloride (C10), lauroylcarnitine chloride (C12), myristoylcarnitine chloride (C14) and palmitoylcarnitine chloride (C16) were purchased from Sigma (St. Louis, MO). Isovalerylcarnitine–HCl (C5), butyrylcarnitine–HCl (C4) and propionylcarnitine–HCl (C3) were purchased from VU Medical Center, Metabolic Laboratory (Amsterdam, The Netherlands). The internal standards (Set B) were obtained from Cambridge Isotope Labs (Andover, MA) and contained: [$^2H_9$]carnitine, [$^2H_3$]acetylcarnitine, [$^2H_3$]propionylcarnitine, [$^2H_3$]butyrylcarnitine, [$^2H_9$]isovalerylcarnitine, [$^2H_3$]octanoylcarnitine, [$^2H_9$]myristoylcarnitine and [$^2H_3$]palmitoylcarnitine. Methanol and acetonitrile (HPLC grade), and ammonium hydroxide were from Fisher Scientific (Fair Lawn, NJ). Formic acid was obtained form Riedel-de Haen (KG, Germany).

2.2. Preparation of internal standards, calibrators and controls

The stock solution containing the internal standards was prepared by dilution of Set B with 1.0 ml of methanol. This solution was further diluted 1:10 to produce the working internal standard solution in the following concentrations:15.2 μM [$^2H_9$]carnitine, 3.8 μM [$^2H_3$]acetylcarnitine, 1.52 μmol/l [$^2H_3$]palmitoylcarnitine and 0.76 μmol/l each of [$^2H_3$]propionylcarnitine, [$^2H_3$]butyrylcarnitine, [$^2H_9$]isovalerylcarnitine, [$^2H_3$]octanoylcarnitine and [$^2H_9$]myristoylcarnitine.

A stock solution of standards were prepared by mixing individual solutions of acylcarnitines in methanol (1.0 mg/ml) with pooled plasma obtained from Children's Medical Center to a concentration of 100 μmol/l for C2 and 10.0 μmol/l for the other acylcarnitines. This solution was further diluted in pooled plasma with known concentrations of acylcarnitines to obtain the calibration standard solutions. Quality controls were prepared by spiking pooled plasma specimens with the stock solutions of acylcarnitines in methanol.

2.3. Specimens

Plasma samples were obtained from patients from Children's National Medical Center, Washington, DC, and The Hospital for Sick Children, Toronto, ON.

2.4. Sample preparation

For sample preparation, 200 μl of serum, heparinized plasma or calibrator were placed into a 1.5 ml conical plastic Eppendorf test tubes containing 100 μl of internal standard solution and 300 μl acetonitrile. The tubes were capped, vortexed vigorously for 30 s and centrifuged at 14,000 rpm for 10 min. The supernatant was transferred into autosampler vials for injection into the LC-MS/MS system. Sample

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