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Short communication

A fast and simple solid phase microextraction coupled with gas chromatography-triple quadrupole mass spectrometry method for the assay of urinary markers of glutaric acidemias

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ABSTRACT

The analysis of characteristic urinary acidic markers such as glutaric, 3-hydroxyglutaric, 2-hydroxyglutaric, adipic, suberic, sebacic, ethylmalonic, 3-hydroxyisovaleric and isobutyric acid constitutes the recommended follow-up testing procedure for glutaric acidemia type 1 (GA-1) and type 2 (GA-2). The goal of the work herein presented is the development of a fast and simple method for the quantification of these biomarkers in human urine. The proposed analytical approach is based on the use of solid phase microextraction (SPME) combined with gas chromatography-triple quadrupole mass spectrometry (GC-QqQ-MS) afterward a rapid derivatization of acidic moieties by propyl chloroformate, propanol and pyridine. Trueness and precision of the proposed protocol, tested at 5, 30 and 80 mg l^{-1}, provided satisfactory values: recoveries were in the range between 72% and 116% and the relative standard deviations (RSD%) were between 0.9% and 18% (except for isobutyric acid at 5 mg l^{-1}). The LOD values achieved by the proposed method ranged between 1.0 and 473 μg l^{-1}.

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1. Introduction

Glutaric acidemia type 1 (GA-1) is an autosomal recessive inherited metabolic disorder caused by a deficiency of glutaryl-CoA dehydrogenase (GCDH). The deficiency of this enzyme induces an increase of glutaric acid, 3-hydroxygluraric acid, and glutaconic acid in the free and conjugated forms in tissue and body fluids [1–3]. Glutaric acidemia type 2 (GA-2), also called multiple acyl-CoA dehydrogenase deficiency (MADD), is another disorder characterized by an increased excretion of glutaric acid. Presymptomatic treatment of GA-1 and GA-2 significantly reduces the risk of neurological damages and therefore these disorders are included in many newborn screening (NBS) programs and detected based on elevated glutarylcarnitine in dried blood spot [4]. Newborns with elevated glutarylcarnitine values undergo follow-up testing for urinary excretion of glutarate metabolites. Guidelines describing in detail a well-functioning and designed NBS and Follow-Up Program were published by National Academy of Clinical Biochemistry (NACB) [5]. The recommended follow-up testing procedure for GA-1 and GA-2 is the analysis of urinary follow-up markers such as glutaric and 3-hydroxyglutaric acid for GA-1 and glutaric, 2-hydroxyglutaric, adipic, suberic, sebacic, ethylmalonic, 3-hydroxyisovaleric and isobutyric acid for GA-2 [5].

The most common approaches to determine urinary organic acids are based on gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) [6–13]. Other studies also used high performance liquid chromatography (HPLC) [14,15] and capillary electrophoresis (CE) [16]. For GC approaches, a derivatization reaction is needed before analysis to increase volatility and decrease polarity of organic acids. Although some protocols are based on in-situ derivatization [9], the most used method for derivatization is trimethylsilylation with commercially available reagents [17]. These derivatization reactions require an organic reaction medium, thus urinary organic acids are conventionally isolated by liquid–liquid extraction (LLE) prior to derivatization [6].

Generally speaking, the esterification of acidic moiety can be performed directly in the aqueous phase using alkyl chloroformates in the presence of the corresponding alcohols and pyridine [18]. Moreover, this approach is compatible with the use of solid phase microextraction (SPME) that allows the extraction of analytes directly in the aqueous phase [19–24].

The goal of the work herein presented was to develop a rapid and easy method for the analysis of urinary organic acids which

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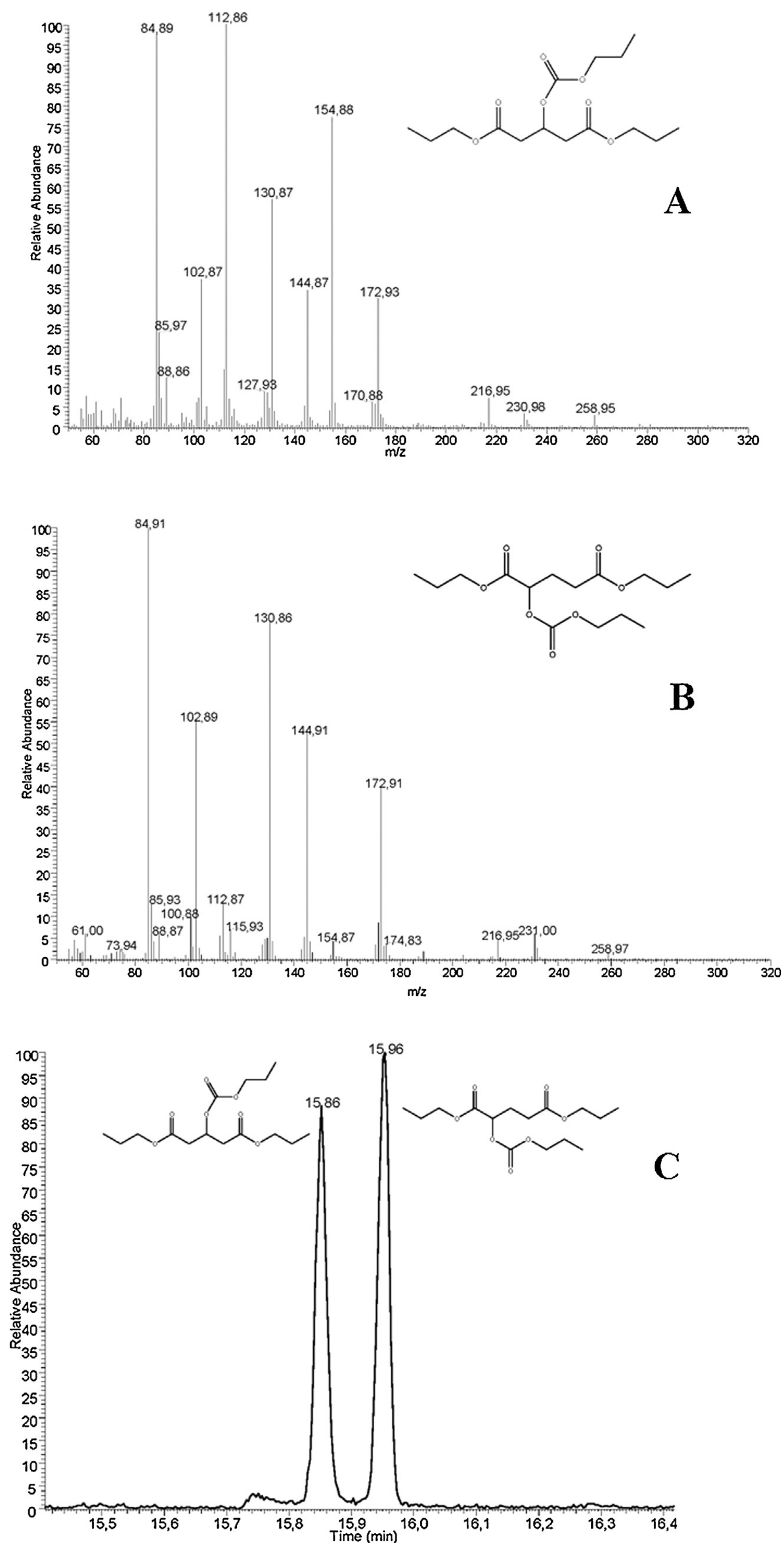

Fig. 1. Mass spectra and molecular structure of tri-derivatized 3-hydroxyglutaric acid (A) and 2-hydroxyglutaric acid (B) obtained with propyl chloroformate. (C) The chromatographic separation of tri-derivatized 3-hydroxy from tri-derivatized 2-hydroxyglutaric acid (full scan acquisition, extracted ion chromatogram at m/z 155) for a synthetic urine sample spiked at $10\,mg\,l^{-1}$.

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