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Short Communication

Charge Reversal Derivatization Enhances Dicarboxylic Acid Quantification using Liquid Chromatography Tandem Mass Spectrometry

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ABSTRACT

Analysis of Dicarboxylic Acids (DCAs) in biofluids is important in biomarker discovery studies since DCAs play key roles in various physiologic functions. Low concentrations of some DCAs in body fluids require sensitive detection methods for quantification. Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) with chemical charge-reversal provides a sensitive means of quantifying low DCA levels.

Keywords: Liquid chromatography; Mass spectrometry; Cerebrospinal fluid; Ionization; Dicarboxylic acids

DESCRIPTION

LC-MS/MS has emerged as an important analytical technique for the analysis of various metabolites from biological matrices such as Cerebrospinal Fluid (CSF), plasma, urine samples in biomarker discovery studies. Due to their low abundance in the majority of biofluids and their poor ionization and fragmentation, DCAs are difficult to sensitively detect [1-3]. Derivatization of compounds using suitable reagents is an important strategy to enhance sensitivity. Dimethylaminophenacyl Bromide (DmPABr) has advantages over other derivatizing reagents due to its ability to derivatize a wide range of metabolites with carboxylic, amine, and sulfhydryl groups [4]. DmPABr reacts with carboxylic groups (R-COOH) of both ends of DCAs and reverses their polarity from negative to positive, thereby enhancing detection of the derivatized DCAs in the Multiple Reaction Monitoring (MRM) mode [5-8]. In addition to charge reversal, adding the bulky phenyl ring moiety on both ends of the DCA carboxylic groups facilitates better interaction and separation on the liquid chromatography column. The derivatized DCAs are also stable upon freeze-thaw and room temperature storage [3].

Strategies for the sample preparation

Removal of potential linking compounds is a key component of most metabolomic studies. The following steps are involved in sample preparation prior to DCA derivatization:

Protein removal *via* precipitation: The first step in the sample preparation is the removal of interfering proteins from the biofluids. Protein precipitation is accomplished using ice-cold acetonitrile (5X volume, -20°C) followed by centrifugation (14k RPM, 15 min, 4°C).

Extraction and purification of dicarboxylic acids: DCAs are effectively extracted from the deproteinated supernatant fluid using suitable solvents under acidic conditions that protonate and remove unwanted metabolites. Acidified ethyl acetate provides an efficient extraction of DCAs from biofluids [3]. The DCA-enriched extracts are then derivatized using base-catalyzed esterification described below.

Derivatization of dicarboxylic acids using DmPABr

DCAs are derivatized with DmPABr in a water bath using N,N-Diisopropylethylamine (DIPEA) as base catalyst for the reaction (Figure 1). The reactions are quenched by adding formic acid.

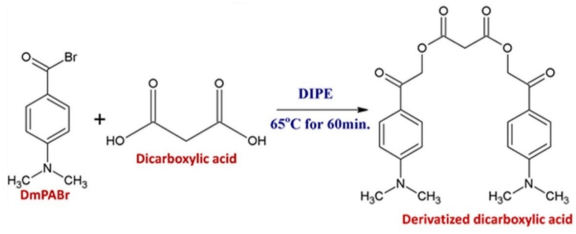

Figure 1: Derivatization of dicarboxylic acids.

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The derivatization strategy with DmPABr reduces the unwanted ionic interactions of the R-COOH groups with the stationary phase (LC column), decreases the polarity of the compounds and significantly improves DCA separation. Under positive ion Electrospary Ionization (ESI), derivatized DCAs produce unique precursors, quantifier, and identifier ions [3]. Thus, charge reversal combined with ESI increases the specificity, sensitivity and baseline resolution, and thus boosts the applicability for determining trace amounts of DCAs in various biofluids.

Quantification using isotope dilution

Prior to sample processing, deuterated DCAs are added to samples to monitor recovery and for quantification. A linear equation from synthetic standards is used to quantify DCAs in biofluids. Using this approach, the Limit of Detection (LOD) and Limit of Quantification (LOQ) are estimated to be in fg levels which is >1000 fold more sensitive than previous Gas Chromatography-Mass Spectrometry (GC/MS) methods using negative ion chemical ionization [3,9].

CONCLUSION

Derivatization enhanced the detection of DCAs using LC-MS/MS compared to previous GC/MS methods. This LC-MS/MS method can be applied to quantification of DCAs in biomarker discovery studies especially with trace amounts of biospecimen.

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