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Fast and sensitive quantification of human liver cytosolic lithocholic acid sulfation using ultra-high performance liquid chromatography–tandem mass spectrometry

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ABSTRACT

Detoxification of lithocholic acid (LCA) to lithocholic acid sulfate (LCA-S) is catalyzed by sulfotransferases, mainly SULT2A1. We developed and validated an ultra-high performance liquid chromatography–tandem mass spectrometric (UPLC–MS/MS) method to quantify human liver cytosolic-dependent LCA sulfation. Chromatographic separation was achieved on an UPLC C_{18} column (2.1 × 50 mm, 1.7 μm) and a gradient elution of 0.1% formic acid in water and acetonitrile. Negative electrospray ionization with multiple reaction monitoring (MRM) mode was used to quantify LCA-S (455.3 → 97.0) and cholic acid (407.2 → 343.3; internal standard). The retention time was 3.51 min for LCA-S and 3.08 min for cholic acid. The lower limit of quantification of LCA-S was 0.5 nM (or 0.23 ng/ml in 400 μl total volume) and the assay was linear from 0.2 to 200 pmol. Intra-day and inter-day accuracy and precision were <14%. The quality control samples were stable at room temperature for 4 h, 4 °C for 24 h, −20 °C for 14 days, and after three freeze–thaw cycles. The matrix (20–100 μg cytosolic protein) did not affect LCA-S quantification. This is the first UPLC–MS/MS method applied to optimization of the human liver cytosolic LCA sulfation assay. The optimal levels of $MgCl_2$ and 3′-phosphoadenosine 5′-phosphosulfate (PAPS) cofactor were 2.5 mM and 20 μM, respectively. Addition of reducing agents (2-mercaptoethanol and DL-dithiothreitol) did not affect LCA-S formation. Human liver cytosolic LCA sulfation was linear with 20–100 μg of cytosolic protein and 5–30 min incubation time. This UPLC–MS/MS approach offers a specific, sensitive, fast, and direct approach for quantifying human liver cytosolic LCA sulfation.

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1. Introduction

Bile acids are steroidal products synthesized from cholesterol in the liver. These amphipathic molecules act as lipid surfactants and play essential physiological roles, including intestinal absorption of dietary fats/cholesterol, fat-soluble vitamins, and drugs [1]. Upon synthesis in hepatocytes, primary bile acids are conjugated to amino acids (*e.g.* glycine and taurine) and secreted into bile and intestines. In the intestines, primary bile acids (*e.g.* cholic acid and chenodeoxycholic acid) are deconjugated and metabolized to secondary bile acids [*e.g.* deoxycholic acid and lithocholic acid (LCA)] by 7α-dehydroxylase enzymes in intestinal microorganisms. Some of the bile acids are then reabsorbed back into the liver in a process known as enterohepatic circulation [1]. Among the various bile acids, lithocholic acid is the most toxic bile acid [2,3]. It leads to cytotoxicity [4], cellular necrosis [5], apoptosis [5], and DNA damage [6]. Administration of LCA to rodents leads to hepatotoxicity [2,4,7,8] and promotes carcinogenesis [9]. LCA is sulfated by cytosolic sulfotransferases to yield lithocholic acid sulfate (LCA-S) in the presence of the 3′-phosphoadenosine 5′-phosphosulfate (PAPS) cofactor, which is an universal donor of sulfate moiety (Fig. 1). LCA is detoxified by sulfation [10] in an enzymatic reaction catalyzed by sulfotransferase 2A1 (SULT2A1; also known as hydroxysteroid sulfotransferase or dehydroepiandrosterone sulfotransferase) [11,12], and, to a lesser extent, by SULT1C3 [13].

Abbreviations: DMSO, Dimethyl sulfoxide; DTT, DL-dithiothreitol; HPLC-UV, High-performance liquid chromatography-ultraviolet spectroscopy; LC-MS/MS, Liquid chromatography-tandem mass spectrometry; LCA, Lithocholic acid; LCA-S, Lithocholic acid sulphate; $MgCl_2$, Magnesium chloride; MRM, Multiple reaction monitoring; PAP, 3′-Phosphoadenosine 5′-phosphate; PAPS, 3′-Phosphoadenosine 5′-phosphosulfate; SULT, sulfotransferase; SULT2A1, Sulfotransferase 2A1; UPLC–MS/MS, Ultra-high performance liquid chromatography-tandem mass spectrometry.

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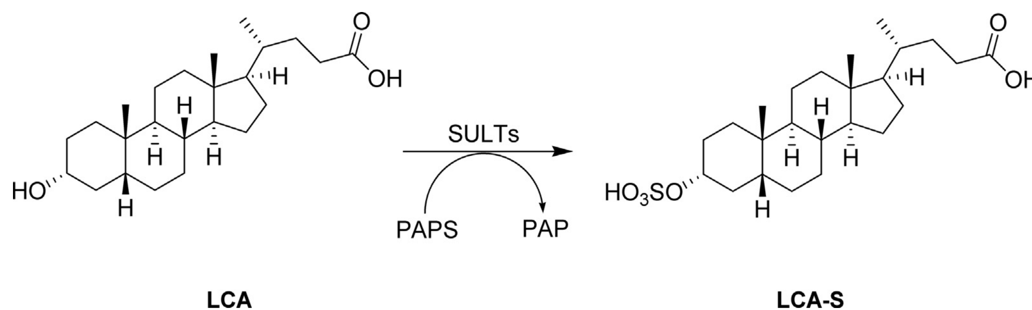

Fig. 1. LCA sulfation in the presence of PAPS cofactor.

Table 1
Intra-day and inter-day accuracy and precision for the quantification of LCA-S in the QC samples.

Sample	Nominal amount (pmol)	Intra-day (n = 6)		Inter-day (n = 3)	
		% Deviation	% RSD	% Deviation	% RSD
Low QC	1	6.63	6.35	8.31	8.92
Mid QC	10	8.33	5.71	4.66	6.16
High QC	100	1.9	13.87	1.35	10.37

LCA-S has been quantified by various analytical methods. An old method involves desulfation of LCA-S using enzymatic or chemical methods prior to quantification of LCA in urine samples using GC–MS [14]. The limitations of this approach include incomplete cleavage of sulfate moiety and potential degradation of LCA. To improve speed and sensitivity, a radioimmunoassay was used to determine LCA-S in human serum [15]. Subsequently, HPLC-UV assays were used for the direct separation and quantification of a mixture of sulfated bile acids [16], and LC–MS assays were developed for quantification of LCA-S in urine, serum, and liver samples [17,18]. These methods were not as selective or sensitive as compared to LC–MS/MS or UPLC–MS/MS assays of LCA-S in human urine and serum [19–21] or mouse liver, plasma, bile, and urine [22]. To quantify LCA sulfation formed by various recombinant sulfotransferase enzymes, an indirect method involves measurement of 3′-phosphoadenosine 5′-phosphate (PAP), which is a desulfated product of PAPS, by HPLC-UV [13]. Another approach is the use of radiolabeled substrate and determination of radioactivity by liquid scintillation [11]. Despite the high sensitivity of radioactivity assays, these assays involve tedious sample preparation, separation of radioactive substrate and metabolites, expensive radiolabeled reagents, radioactivity hazards, and strict regulations associated with handling of radioactive chemicals/materials. To date, only one study used ultra-high performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) for the determination of LCA sulfation [12]. However, the conditions of the method were not described [12]. Human liver cytosol is an important *in vitro* model for investigating LCA sulfation and toxicity. However, cytosol is a more challenging matrix because it contains many endogenous chemicals, which may interfere with the detection of the analyte. Currently, there is a lack of a detailed, validated, highly sensitive, and rapid analytical method for quantifying human liver cytosolic LCA sulfation.

In the present study, the specific aims were to: (1) develop and validate a fast and sensitive UPLC–MS/MS method for the quantification of human liver cytosolic LCA sulfation; (2) apply the analytical method to determine the optimal concentration of magnesium, reducing agents (2-mercaptoethanol and DL-dithiothreitol), and PAPS required for the assay; and (3) characterize the linearity of the assay with respect to the amount of human liver cytosol and incubation time. Our UPLC–MS/MS method offers a specific, sensitive, fast, and direct approach for quantifying human liver cytosolic LCA sulfation.

2. Materials and methods

2.1. Enzymes, chemicals, and reagents

Human liver cytosol (pooled from 150 individual donors; catalog #452115; lot #38290) was purchased from Corning Gen-

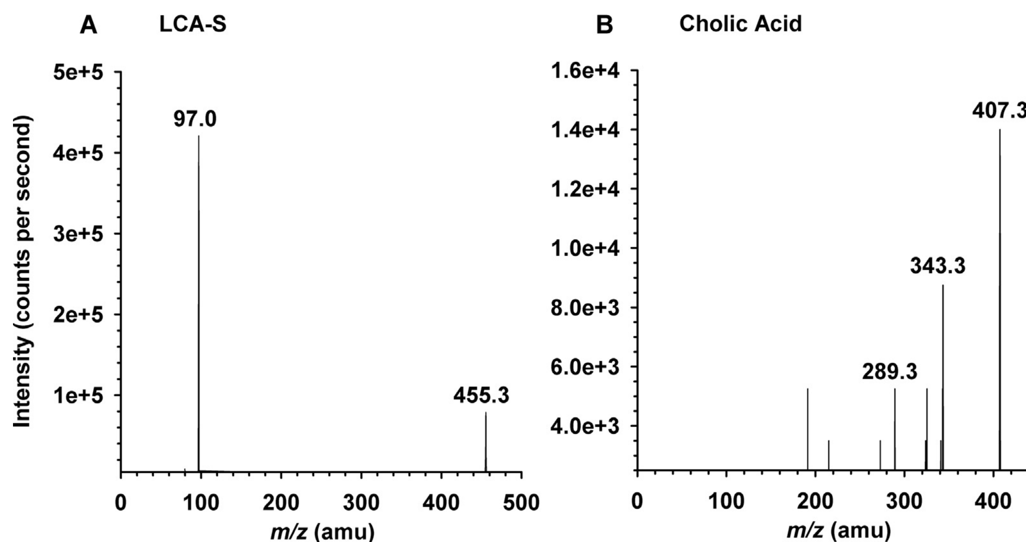

Fig. 2. Product ion mass spectra of (A) LCA-S and (B) cholic acid (internal standard).

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