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New method for the determination of bile acids in human plasma by liquid-phase microextraction using liquid chromatography-ion-trap-time-of-flight mass spectrometry☆

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ABSTRACT

Bile acids (BAs) are derived from cholesterol and produced in the liver. The most abundant bile acids in humans are usually conjugated with glycine and taurine and are divided into primary BAs such as cholic acid (CA) and chenodeoxycholic acid (CDCA) and secondary BAs like deoxycholic acid (DCA), lithocholic acid (LCA) and ursodeoxycholic acid (UDCA).

The differences amongst individual bile acids (BAs) are significant in order to distinguish different pathological processes and exposure to chemical compounds. Hollow fiber based liquid-phase microextraction (HF-LPME) is a technique that combines sample cleansing, extraction and the concentration of analytes, where a hydrophobic porous capillary membrane is impregnated with an organic extraction solvent and the lumen is filled with microliters of a phase acceptor both organic by nature. The aim of this study was to develop a new method to extract bile acids from plasma through HF-LPME of two phases (octanol as the acceptor phase) using LCMS-IT-TOF. The optimized two-phased LPME procedure for the extraction of bile acids showed limits of detection 1.0 $\mu g\,L^{-1}$ and limits of quantification of 5.0 $\mu g\,L^{-1}$. The intra-assay precision ranged from 2.1 to 11.9%. The method developed was linear over the range of 5.0–200.0 $\mu g\,L^{-1}$ for all analytes. The hollow-fiber liquid-phase microextraction method was applied to human plasma from workers exposed to organic and halogenated solvents and also to unexposed volunteers. The method is simple, low cost and it does not require large amounts of organic solvents, therefore it is quite suitable for the analysis of bile acids exposed to hepatotoxic compounds.

1. Introduction

Human primary bile acids (BAs) are produced from cholesterol in the liver. They possess a carboxylic acid moiety usually conjugated with either glycine and/or taurine. Secondary BAs are produced by microbial flora from primary BAs [1–5]. Sample preparation for bile acid analysis include conventional techniques such as liquid-liquid extraction (LLE) [26] and solid phase extraction (SPE) [13]. The use of chromatographic methods enables the separation, identification and quantification of all bile acids individually [1,2,6,7]. Liquid chromatography (LC) using a UV-Vis detector, evaporative light scattering detection (ELSD) or fluorescence detector has limited sensitivity and various interferences of biological components [8,9]. Mass spectrometry detectors are a good alternative to BAs. Gas chromatography coupled with mass spectrometry shows higher sensitivity and resolution, however, the required derivatization of BAs analysis can be a limiting factor to the extraction step [10–14]. Alternatively, analyses of BAs by LC–MS have been routinely used as they do not require prior derivatization and extraction procedures [7,15,16].

Extractions by membranes are among the main kinds of extraction such as supported liquid extraction (SLE), microporous membrane liquid-liquid extraction (MMLLE) [17–20], and hollow fiber liquid-phase microextraction (HF-LPME) [17]. HF-LPME

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combines the concept of extraction of membranes in a small volume of organic solvent or acceptor phase and aqueous matrix or donor phase favoring the analytes transference from aqueous to the organic phase [20–24].

Due to the pore's small diameter and the viscosity of the solvent – typically 0.2 μm – plasma proteins present in the complex biological matrices are not extracted, which is extremely beneficial in the analysis of complex biological matrices. Furthermore, the low cost of each extraction unit can only be used once avoiding issues such as the carry-over effect typically observed in other membrane extraction techniques.

The HF-LPME extraction can be carried out using a system of two phases to extract analytes of neutral or low polarity or three phases to extract moderately hydrophobic analytes with ionizable groups (weak acids or bases) [25].

Several methods have been used to analyze bile acids in biological fluids and other matrices such sewage. Usually, these methods require sample pretreatment due to their complexity. The aim of the present study was the development of a new and fast method of liquid phase microextraction with hollow polypropylene fiber for the determination of bile acids through liquid chromatography coupled to a high-resolution ion trap and time-of-flight mass spectrometry (LCMS-IT-TOF). The method developed was used to analyze BAs in plasma samples from exposed and unexposed subjects to solvents.

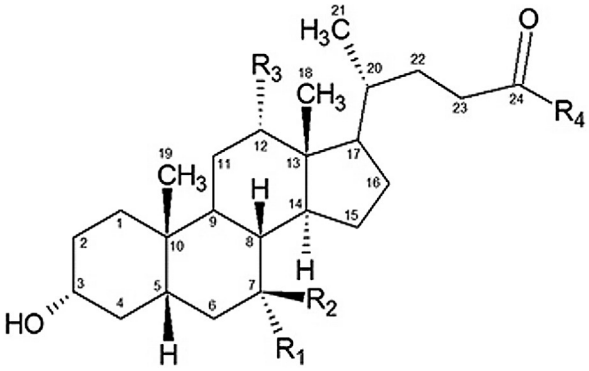
2. Experimental

2.1. Reagents and standard solutions

The bile acids studied were obtained from Sigma–Aldrich (St. Louis, MO, USA): cholic acid (CA) 98% (w/w), litocholic acid (LCA) 98% (w/w), hiodeoxycholic 98% (w/w), ursodeoxycholic acid 99% (w/w) (UDCA), glycochenodeoxycholic acid (GCDCA), glycocholic acid (GCA), taurodeoxycholic acid (TDCA), taurochenodeoxycholic acid (TCDCA), taurocholic (TCA) and taurolitocholic acid (TLCA) 98% (w/w). Fig. 1 shows the structures of these bile acids included in the study.

The other compounds used were: acetic acid from Fluka (Buch, Switzerland), formic acid from J.T. Baker (Philipsburg, USA), and hydrochloric acid ACS reagent, 37% (w/w) from Merck KGaA (Darmstadt, Germany), ammonium hydroxide 28% (w/w) from Sigma–Aldrich (St. Louis, MO, USA).

Analytical-grade solvents were used, including *n*-octanol and ethyl octanoate from Sigma–Aldrich (St. Louis, MO, USA). Ultrapure water was provided by a Milli-Q water purification system from Elga (Buckinghamshire, United Kingdom). HPLC-grade methanol was acquired from Merck KGaA (Darmstadt, Germany) and acetonitrile was purchased from J. T. Baker (Philipsburg, MO, EUA). Sodium sulfate heptahydratade was obtained from Merck KGaA (Darmstadt, Germany).



Bile Acid	Abbreviation	R_1	R_2	R_3	R_4
Cholic	CA	OH	H	OH	OH
Hiodeoxycholic	HDC	H	H	OH	OH
Ursodeoxycholic	UDCA	H	OH	H	OH
Litocholic	LCA	H	H	H	OH
Glycoholic	GCA	OH	H	OH	Glycine
Taurocholic	TCA	OH	H	OH	Taurine
Taurodeoxycholic	TDCA	H	H	OH	Taurine
Glycochenodeoxycholic	GCDCA	OH	H	H	Glycine
Taurochenodeoxycholic	TCDCA	OH	H	H	Taurine
Taurolitocholic	TLCA	H	H	H	Taurine

Fig. 1. Backbone and side chain structures of the bile acids free and conjugated.