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Quantitative profiling of bile acids in rat bile using ultrahigh-performance liquid chromatography–orbitrap mass spectrometry: Alteration of the bile acid composition with aging

Gakyung Lee[a,b], Hyunbeom Lee[a], Jongki Hong[b], Soo Hyun Lee[d], Byung Hwa Jung[a,c,*]

[a] Molecular Recognition Research Center, Korea Institute of Science and Technology, Seoul 02792, Republic of Korea
[b] Department of Basic Pharmaceutical Science, Kyung Hee University, Seoul 02447, Republic of Korea
[c] Department of Biological Chemistry, Korea University of Science and Technology, Daejeon 34113, Republic of Korea
[d] Department of Medical Records and Health Information Management, College of Nursing and Health, Kongju National University, Kongju 32588, Republic of Korea

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ABSTRACT

Bile acids (BAs) play important roles in physiological functions, including the homeostasis of cholesterol and lipids and as ligands for G protein-coupled receptors (GPCRs). With the increasing importance of BAs, analytical methods for their quantification and screening have been developed. However, due to the diverse range and variety of BAs with different activation potency, a simple, effective, and sensitive method is required to screen BAs for accurate quantification and identification. This paper presents an application of ultrahigh-performance liquid chromatography-orbitrap mass spectrometry (UHPLC-LTQ–Orbitrap MS) for profiling BAs in bile. Using this method, along with the accurate quantification of 19 targeted BAs, 22 unknown BAs were detected and characterized by their fragmentation patterns. The method is beneficial for screening most of the BAs (quantitatively and qualitatively) in rat bile with simple preparation in a single run. The sample dilution ranges of each BA were optimized depending on the concentration of BAs in the bile to obtain good peak separation and accurate data. The method validation was performed successfully using charcoal-treated bile and the intra and inter-day coefficients of variation were less than 20% for all BAs while the recovery were above 88.5% except for the lithocholic acid. The method was applied to profile the age-dependent changes in the contents of rat BAs. Through statistical analysis, we found that as the rats aged, unconjugated BAs and glycine-conjugated BAs decreased or were unaffected, while taurine-conjugated BAs were increased in general. Among the unknown BAs, 5 of the taurine-conjugated BAs increased, while a glycine-conjugated BA decreased, in agreement with the trends of the targeted BAs.

1. Introduction

Bile acids (BAs), the major constituents of bile [1], are well known for their role in the regulation of cholesterol homeostasis and lipid absorption. They also have functions in the ligands of nuclear receptors, such as the farnesoid X receptor (FXR) [2–4], G-protein-coupled bile acid receptor (GPBAR1, also known as TGR5) [5], vitamin D receptor (VDR) [6], and pregnane X receptor (PXR) [7], which are related to liver disease. BAs are also known to be cytotoxic [8] and cancer promoters [9]. With the increasing importance of BAs, the development of analytical methods for screening BAs in various biological fluids and tissues has been investigated by many researchers [10–16].

However, due to the BA structural similarity (Fig. 1), screening requires an analytical method with high sensitivity and specificity for efficient analysis. Because BAs exist in various amounts in biofluids and tissues, it is difficult to select a specific BA for quantification. In addition, a wide range of unknown BAs has been detected in various biofluids and tissues [17].

For the detection of bile acids, gas chromatography–mass spectrometry (GC–MS) has been used conventionally, providing high sensitivity and specificity [10]. Nevertheless because of the complicated and time-consuming preparation steps associated with GC–MS, including hydrolysis or derivatization techniques, liquid

* Corresponding author at: Molecular Recognition Research Center, Korea Institute of Science and Technology, 5, Hwarang-ro 14-gil, Seongbuk-gu, Seoul 02792, Republic of Korea.
E-mail address: jbhluck@kist.re.kr (B.H. Jung).

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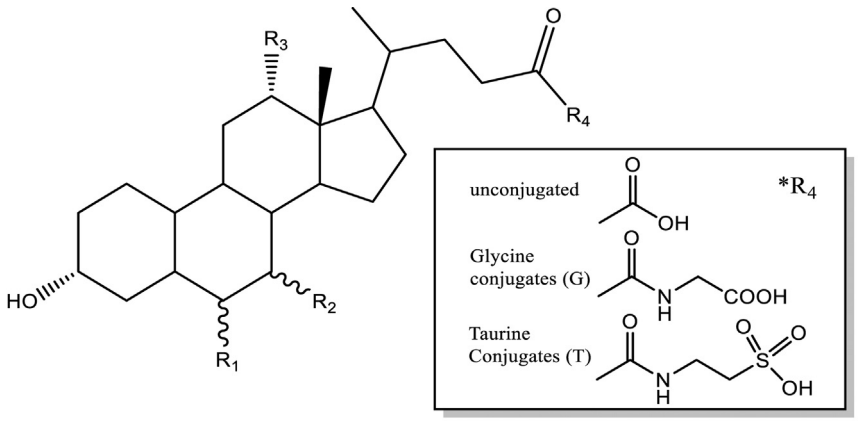

Compound	Abbreviation	R_1	R_2	R_3	Conjugates (R_4)*	
Cholic acid	CA	H	α-OH	OH	TCA	GCA
α-muricholic acid	αMCA	β-OH	α-OH	H	TαMCA	-
β-muricholic acid	βMCA	β-OH	β-OH	H	TβMCA	-
ω-muricholic acid	ωMCA	α-OH	β-OH	H	TωMCA	-
Ursodeoxycholic acid	UDCA	H	β-OH	H	TUDCA	GUDCA
Chenodeoxy cholic acid	CDCA	H	α-OH	H	TCDCA	GCDCA
Deoxycholic acid	DCA	H	H	OH	TDCA	GDCA
Lithocholic acid	LCA	H	H	H	TLCA	GLCA

Fig. 1. Chemical structures of bile acids.

chromatography (LC)-based methods were developed in the last decade. Although several detector-based (UV, fluorescence) assays coupled with LC have been developed, they still have disadvantages, such as limited sensitivity and specificity, and limited coverage of detectable complex biological matrices [11–13]. Recently, high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) [18–21] and ultrahigh-performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) methods [14,16] were applied for BAs analysis. However, these methods have limitations in the separation of isomers, such as α-, β-, ω-muricholic acid [14]. In particular, when using multiple reaction monitoring (MRM) mode, a wide range of unknown BAs in bile cannot be characterized, and small amounts of BAs cannot be detected due to the low sensitivity. For this purpose, high-resolution mass spectrometers (HRMS), such as Orbitrap or time-of-flight (ToF), have been increasingly used for quantification, identification, and characterization of various compounds [22]. These types of MS offer high resolution (>60,000 fwhm), accurate mass measurement (<5 ppm), excellent sensitivity and complete MS/MS fragment information. Nicholson et al. reported a comprehensive study on BA profiling and quantification method using UPLC coupled with Q-ToF mass spectrometer [16]. In addition, very few studies have been performed for the identification of unknown BAs and the quantification of major BAs at the same time [16,17]. Generally the quantitative and qualitative analysis of BAs were performed using two different instruments, comparing the two data sets became difficult. A simultaneous, simple, and rapid method that could analyze BAs in one run in a single instrument would be helpful.

In this study, a method that can perform quantification and identification of BAs in bile simultaneously was developed using UPLC-LTQ–Orbitrap MS. BAs were extracted by solid phase extraction (SPE), and the concentration range for the quantification was optimized according to those of the individual BAs. The MS scan mode was used to quantify the targeted BAs and to detect unknown BAs, while MS/MS mode was used to acquire the information of unknown BAs.

We applied this method to study the age-dependent alteration of the bile composition in rats. Aging is highly correlated with the incidence of various diseases related to the liver and gastrointestinal tract [23]. BAs are correlated with the progression of liver gastrointestinal disease [24–26]. Therefore, investigation of the composition of BAs in bile is a useful way to study the metabolism related to many physiological conditions and to predict the potential therapeutic markers for longevity [27]. Furthermore, detected unknown BAs showing significant changes during aging are attractive targets for further studies to elucidate their biological functions in aging.

2. Materials and methods

2.1. Materials

Compounds, α-muricholic acid (αMCA), β-muricholic acid (βMCA), ω-muricholic acid (ωMCA), tauro-α-muricholic acid (TαMCA), and tauro-β-muricholic acid (TβMCA) were purchased from Steraloids, Inc. (Newport, RI, USA). Cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), lithocholic acid (LCA), ursodeoxycholic acid (UDCA), tauro-cholic acid (TCA), tauro-chenodeoxycholic acid (TCDCA), tauro-deoxycholic acid (TDCA), tauro-lithocholic acid (TLCA), tauro-ursodeoxycholic acid (TUDCA), glyco-cholic acid (GCA), glyco-chenodeoxycholic acid (GCDCA), glyco-deoxycholic acid (GDCA), glyco-lithocholic acid (GLCA), glycol-ursodeoxycholic acid (GUDCA), d_4-cholic acid (d_4-CA), formic acid, and activated charcoal were purchased from Sigma-Aldrich (St. Louis, MO, USA). d_4-Chenodeoxycholic acid (d_4-CDCA) and d_4-lithocholic acid (d_4-LCA) were purchased from Toronto Research Chemicals Inc. (North York, Ontario, Canada). HPLC-grade acetonitrile and MS-grade methanol were obtained from SK chemicals (Ulsan, Republic of Korea). Ultrapure water (18.2 MΩ cm) was obtained from a Milli-Q apparatus from Milli-