Simultaneously quantitative profiling of 18 bile acids in human gastrointestinal fluid by a rapid UPLC-MS/MS assay

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Introduction

Bile acids (BAs) profiling is increasingly recognized as a useful diagnostic tool to characterize various forms of liver disease as well as genetic conditions that impact BAs metabolism. This BAs characterization has occurred primarily in serum but intraluminal human GI tract have not been well characterized.

We developed an ultra pressure liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) method to simultaneously quantify 18 BAs in human GI fluid from four different regions (stomach, duodenum, jejunum and ileum) to reveal the BAs homeostasis in human GI tract under physiological situations.

Methods

Sample collection and preparation

To obtain the human GI fluid, the customized multilumen GI catheter was orally inserted into the GI tract of the healthy subject at the Michigan Clinical Research Unit in the University of Michigan hospital. The catheter consisted of four independent aspiration ports. GI fluid samples were collected from available ports by aspiration from individual tubes within the GI catheter [1] (Fig.1). Then the collected GI fluid samples were centrifuged and the supernatant was collected.

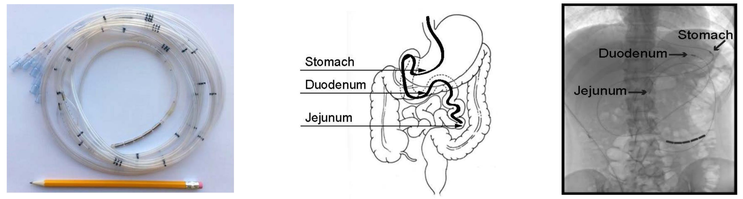

Fig.1. Customized multilumen GI catheter.

Reference: [1] Molecular pharmaceutics 14.12 (2017): 4295-4304

To prepare human GI fluid sample, 120 µL of methanol containing 5 ng/mL deuterium-labelled BAs were added to 30 µL of GI fluid sample. The extracts were centrifuged and the supernatant was transferred to the autosampler vials for LC-MS/MS analysis. The concentrations of the calibration standards were 1, 2.5, 5.0, 10, 25, 50, 100, 250, 500, 1000, 2500 and 5000 ng/mL.

Instruments

LC separation was achieved on a ACQUITY UPLC system with a CORTECS T3 column (2.1x30mm, 2.7 um) Gradient elution was used with mobile phase of 0.01% Formic Acid in water containing 0.2mM ammonium formate (solvent A)– 0.01% Formic Acid in isopropanol: ACN (50:50, v:v) containing 0.2mM Ammonium formate (solvent B). The detailed LC gradient was shown in Table 1.

Table 1. LC Gradient for the Bile Acids Separation

Time (min)	0	0.50	4.00	4.01	5.00
Solvent B %	10	10	90	10	10

MS/MS detection was carried out on a Waters TQD Tandem Quadrupole mass spectrometer. Optimized transitions for each bile acids were listed in Table 2.

Table 2. Parent and product ions of 18 bile acids

Compound Name	LCA	DCA	UDCA	CDCA	CA	GLCA	GUDCA	GCDCA	GDCA	GCA	TLCA	TDCA	TCDCA	TUDCA	TCA	HCA	HDCA	ω-MCA
Parent Ion (m/z)	375.3	391.3	391.3	391.3	407.2	432.2	448.2	448.2	448.2	464.2	482.2	498.2	498.2	498.2	514.3	407.2	391.3	407.2
Daughter Ion (m/z)	375.3	391.3	391.3	391.3	407.2	432.2	448.2	448.2	448.2	464.2	482.2	498.2	498.2	498.2	514.3	407.2	391.3	407.2
Collision Energy (V)	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Results

Separation of Bile Acids

All the 18 bile acids were separated well within 4 minutes (Fig.2).

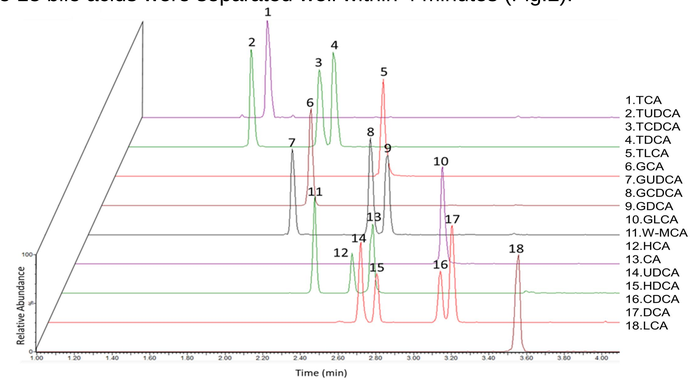

Fig.2. Chromatograms of 18 bile acids.

Calibration Curve and Linear Range

The representative calibration curve and the corresponding linear range were shown in Fig.3. All bile acids were found to be linear over the measured range.

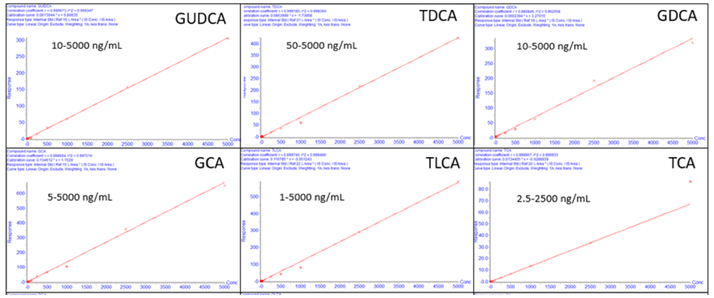

Fig.3. Representative calibration curves and linear range for bile acids quantitation.

Method Application

To show the utility of the method, an application to simultaneously quantify 18 bile acids in human GI fluids sample from four different regions (stomach, duodenum, jejunum and ileum) was performed. As shown in Fig.4, conjugated primary bile acids constituted more than 80% of total bile acids in human GI fluid, and taurocholate, glycocholate and glycochendeoxycholate were the top three bile acids in the stomach, duodenum and jejunum fluid.

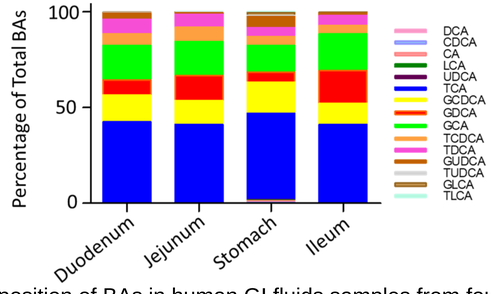

Fig.4. Composition of BAs in human GI fluids samples from four different regions.

As illustrated by the PCA score plots (Fig.5), there is a clear separation between stomach samples and other three location samples, whereas the samples of duodenum, jejunum and ileum overlap each other.

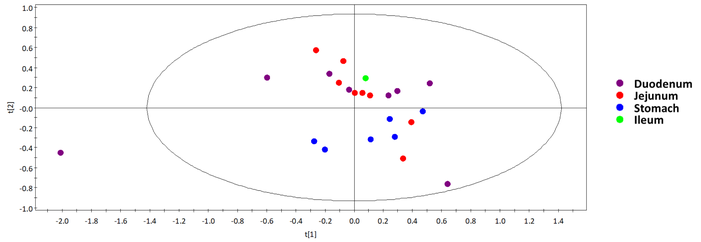

Fig.5. PCA score plots of human GI fluids samples from four different regions.

Conclusion

Conclusion: A sensitive UPLC-MS/MS method was developed for the simultaneous detection and quantification of the 18 bile acids in human GI fluids. Good linearity (R^2 > 0.99) was obtained for all 18 bile acids with the limits of quantification in range of 1 to 50 ng/mL.

The main improvements compared to related methods included reduced analysis time, enhanced sensitivity and reduced complexity of sample processing. The method has been applied to elucidate bile acids profiles in GI tract under physiology condition and provide a better understanding of BAs metabolism.