



# Simultaneous quantification of glycine- and taurine-conjugated bile acids, total bile acids, and choline-containing phospholipids in human bile using $^1\text{H}$ NMR spectroscopy

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## ABSTRACT

Bile acids, phospholipids, and cholesterol are the major lipid components in human bile. The composition of bile is altered in various cholestatic diseases, and determining such alterations will be of great clinical importance in understanding the pathophysiology of these diseases. A robust method for the simultaneous quantification of major biliary lipids – glycine-conjugated bile acids (GCBA), taurine-conjugated bile acids (TCBA), total bile acids (TBAs) and choline-containing phospholipids (choline-PLs) has been devised using  $^1\text{H}$  NMR spectroscopy. Bile samples were obtained from patients with various hepatopancreatobiliary diseases ( $n=10$ ) during an endoscopic retrograde cholangiopancreatography (ERCP) examination. Peak areas of metabolite-signals of interest were obtained simultaneously by deconvoluting the experimental spectrum, making the present method robust. GCBA and TCBA have been quantified using the peak areas of their characteristic methylene ( $\text{CH}_2$ ) signals resonating at 3.73 and 3.07 ppm, whereas TBA and choline-PLs were quantified using their methyl ( $\text{CH}_3$ ) and trimethylammonium ( $-\text{N}^+(\text{CH}_3)_3$ ) signals resonating at 0.65 and 3.22 ppm respectively. The present method was compared with an NMR-based literature method (which involves dissolving bile in DMSO), and a good correlation was observed between the two methods with regression coefficients – 0.97, 0.99, 0.98 and 0.93 for GCBA, TCBA, TBAs, and choline-PLs respectively. This method has the potential to be extended to *in vivo* applications for the simultaneous quantification of various biliary lipids non-invasively.

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## 1. Introduction

Bile acids, phospholipids, and cholesterol are the major lipid components in human bile. Bile acids account for ~72% of the total lipid pool whereas phospholipids and cholesterol contribute ~24% and ~4% respectively [1]. The composition of bile is altered in various chronic cholestatic diseases [2–8]. The ratio of taurine- to glycine-conjugated bile acids is especially altered and it has been reported that the taurine-conjugates are elevated in cholestatic conditions [9]. Analysis of bile for

the quantification of biliary lipids and for the determination of the conjugation pattern of bile acids (with glycine and/or taurine) would be of value in understanding the pathophysiology of cholestatic diseases. The biliary lipid components are generally quantified by conventional techniques such as enzymatic methods, gas chromatography (GC), high performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC–MS)/liquid chromatography–mass spectrometry (LC–MS) techniques [10–13]. These methods are tedious, involve multiple steps, and result in longer experimental times [14]. Due to the unique advantage of  $^1\text{H}$  NMR spectroscopy in the simultaneous analysis of multiple metabolites (generally in a single experiment), the analysis of biliary lipid components using  $^1\text{H}$  NMR has become relatively easy [15–17]. Previously, we have identified amide proton signals (NH) of glycine- and taurine-conjugated bile acids resonating in the region 7.8–8.1 ppm as marker signals for the quantification of these bile acids [14]. However, these amide protons are in dynamic exchange with biliary water under physiologic conditions, and their signal intensity was not 100%. To minimize this effect, we adjusted the pH of the bile samples to slightly lower

**Abbreviations:** CBD, common bile duct; ERCP, endoscopic retrograde cholangiopancreatography; GC, gas chromatography; GCBA, glycine-conjugated bile acids; GC–MS, gas chromatography–mass spectrometry; GDCA, glycodeoxycholic acid; HPLC, high performance liquid chromatography; LC–MS, liquid chromatography–mass spectrometry; MRS, magnetic resonance spectroscopy; PLs, phospholipids; TBAs, total bile acids; TCBA, taurine-conjugated bile acids; TSP, 3-(trimethylsilyl)propionic-2,2,3,3- $d_4$  acid sodium salt.

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than physiologic pH, to  $6.0 \pm 0.5$  [14]. With the advent of higher field strength magnets, we extended the above method to the quantification of individual conjugated bile acids in gallbladder bile [15]. Furthermore, we have also developed a robust method for the quantification of major lipid components (bile acids, phospholipids, and cholesterol) by dissolving bile in polar organic solvent such as dimethylsulfoxide- $d_6$  (DMSO- $d_6$ ) [16]. Duarte et al. have coupled high resolution NMR spectroscopy with HPLC–MS methods for the analysis of hepatic bile to detect metabolites other than lipid components [17]. However, all of the above methods are applicable to *in vitro* studies only. Since the conjugation pattern of bile acids (i.e., the ratio of taurine- to glycine-conjugates) is altered in cholestatic diseases, determining the levels of glycine- and taurine-conjugates, along with other biliary lipid components, using *in vivo* magnetic resonance spectroscopy (MRS) may have immense value in non-invasive diagnostics. Recently, the feasibility of *in vivo*  $^1\text{H}$  MRS of bile has been tested in humans [18] and cynomolgus monkeys [19]. Prescott et al. have been able to obtain a  $^1\text{H}$  MRS of human gallbladder bile using a clinical scanner (1.5 T), but the spectral quality was insufficient to quantify various lipid components except for phospholipids [18]. More recently, Kunnecke et al. also demonstrated the feasibility of *in vivo*  $^1\text{H}$  MRS of gallbladder bile in cynomolgus monkeys with a better spectral quality using a relatively higher magnetic field strength (4.7 T) [19]. They suggested the use of glycine methylene ( $\text{CH}_2$ ) signal for the quantification of glycine-conjugated bile acids (GCBAs) in monkey bile. However, no detailed study was undertaken to assess the utility of this signal for the accurate quantification of GCBAs. In this study, we present the details of quantifying GCBAs in human bile using its  $\text{CH}_2$  signal, and also propose a robust method for the simultaneous quantification of GCBAs, taurine-conjugated bile acids (TCBAs), total bile acids (TBAs) and choline-containing phospholipids (choline-PLs) in human bile using  $^1\text{H}$  NMR spectroscopy which can be extended to *in vivo* applications in the future.

## 2. Materials and methods

### 2.1. Bile collection

Bile samples were obtained from patients ( $n = 10$ ) undergoing endoscopic retrograde cholangiopancreatography (ERCP) examination for various cholestatic diseases at the Karolinska University Hospital, Huddinge, Sweden. The samples were collected by deep cannulation of the common bile duct (CBD) using a similar protocol as reported earlier [8]. The samples were transported by air to Canada on dry ice and stored in a  $-70^\circ\text{C}$  freezer until NMR experiments were performed. Informed consent was obtained from each patient and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the Institutions' human research ethics boards.

### 2.2. Chemicals

Deuterium oxide ( $\text{D}_2\text{O}$ ), dimethyl sulfoxide- $d_6$  (DMSO- $d_6$ ), glycodeoxycholic acid (GDCA) sodium salt, and 3-(trimethylsilyl)propionic-2,2,3,3- $d_4$  acid sodium salt (TSP) were purchased from Sigma–Aldrich (St. Louis, MO, USA).

### 2.3. $^1\text{H}$ NMR experiments

All NMR experiments were performed on a Bruker Avance 360 MHz NMR (8.5 T) spectrometer. Bile samples were thawed in the biological safety cabinet for approximately 10–15 min. After complete thawing, 500  $\mu\text{l}$  of the sample was taken into a 5-mm NMR tube along with a reusable co-axial capillary tube containing TSP in  $\text{D}_2\text{O}$  (0.0965 mg TSP/150  $\mu\text{l}$   $\text{D}_2\text{O}$ ). The TSP was used as

**Table 1**

Results of the recovery experiments for the GCBAs in bile (performed in duplicate).

GDCA sodium salt added (mg)	GDCA sodium salt recovered (mg)
0.43	0.43
	0.47
0.87	0.96
	0.84
1.30	1.32
	1.56
1.73	1.80
	1.76
2.16	2.31
	2.34

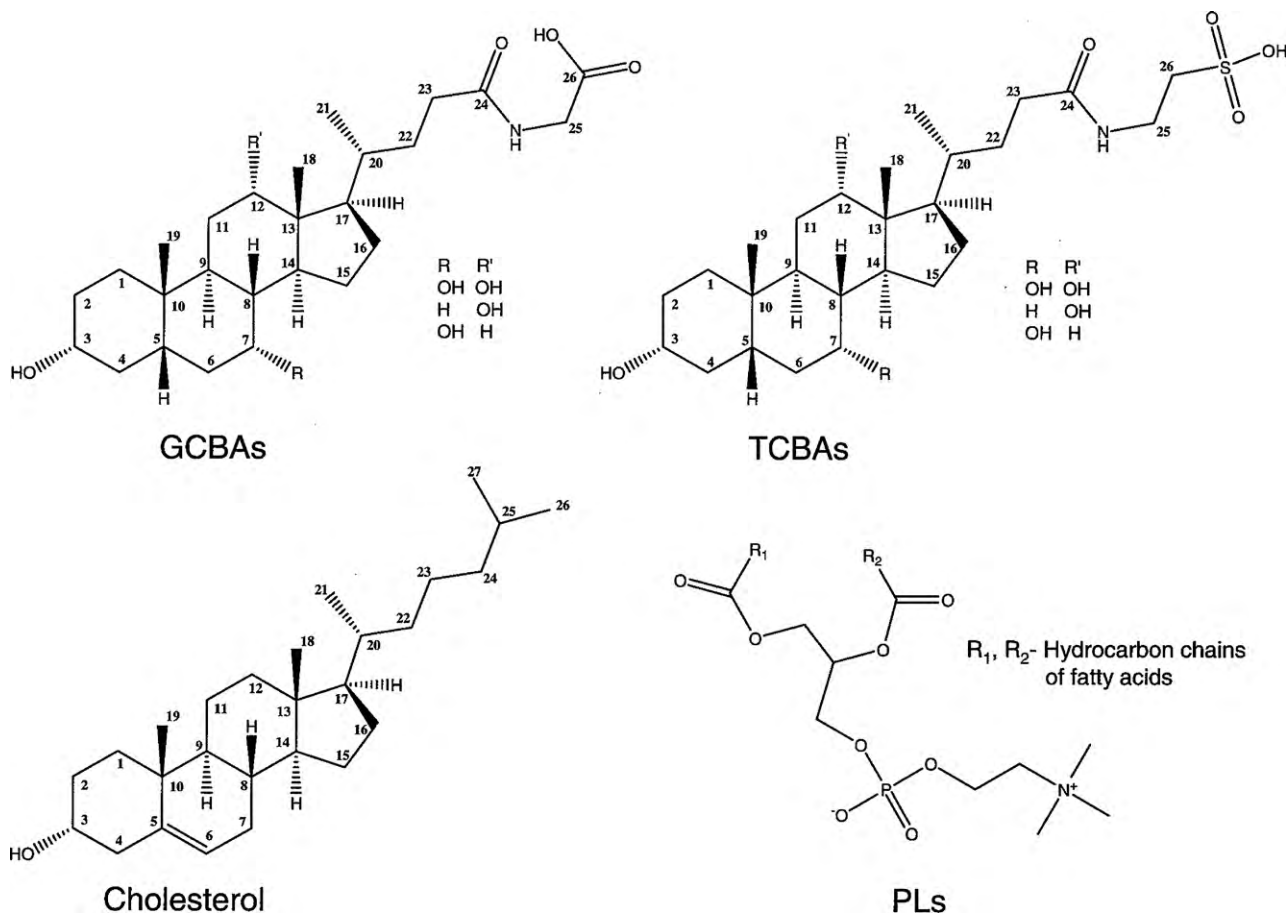
both quantitative and chemical shift reference (0 ppm), whereas  $\text{D}_2\text{O}$  served as a 'field-frequency lock'.  $^1\text{H}$  NMR spectra of all the samples were obtained with water suppression using presaturation technique with no spinning at a temperature of  $25^\circ\text{C}$ .  $^1\text{H}$  NMR spectra of bile dissolved in DMSO- $d_6$  (20  $\mu\text{l}$  bile in 500  $\mu\text{l}$  DMSO- $d_6$ ) were also obtained following the literature method [16]. The following acquisition parameters were employed in all 1D experiments: NS (number of scans)=32, P1 ( $90^\circ$  pulse)=5.93  $\mu\text{s}$ , PL9 (water presaturation power)=60 dB, TD (number of points in time domain)=32 k, D1 (interpulse delay)=5 s, SW (spectral width)=4310 Hz, AQ (acquisition time)=3.8 s.

### 2.4. Recovery experiments for GCBAs in bile

A series of test samples were prepared in duplicate by taking 100  $\mu\text{l}$  of bile, and adding the following amounts of 18.35 mM GDCA sodium salt solution to it: 0.0, 50, 100, 150, 200 and 250  $\mu\text{l}$ . The final volume was diluted to 500  $\mu\text{l}$  with the addition of Milli-Q water (Millipore Corporation, Billerica, MA). The set of samples containing no added GDCA sodium salt served as blank. The samples were transferred to 5-mm NMR tubes and  $^1\text{H}$  NMR spectra were obtained with presaturation of the water signal. A reusable co-axial capillary tube containing TSP dissolved in  $\text{D}_2\text{O}$  (0.0965 mg/150  $\mu\text{l}$ ) was inserted into the NMR tube to serve as a quantitative reference. The peak area of the conjugated-glycine  $\text{CH}_2$  signal at 3.73 ppm was measured by deconvolution relative to TSP using XWINNMR software version 3.0 (Bruker Biospin, Switzerland). The quantity of standard GDCA sodium salt recovered in bile through the  $^1\text{H}$  NMR experiment was calculated from the peak area of the  $\text{CH}_2$  signal of conjugated-glycine (obtained from the difference in the peak areas of the  $\text{CH}_2$  signals of conjugated-glycine in the test and blank samples) and compared with the actual quantity of GDCA sodium salt added to each test sample (results are shown in Table 1).

### 2.5. Quantification of lipid components

GCBAs, TCBAs, TBAs, and choline-PLs in bile samples were quantified by measuring the peak area of the respective marker signals resonating at 3.73, 3.07, 0.65, and 3.22 ppm. The peak areas of the above lipid signals and the TSP signal were obtained simultaneously by Lorentzian deconvolution (using the deconvolution software available with Bruker XWINNMR version 3.0.) which approximates the experimental spectrum into individual signals with Lorentzian lineshapes [20,21]. Briefly, the deconvolution procedure used was as follows: the experimental FID was Fourier transformed, phase corrected (both zero and first order), and the baseline was automatically corrected using Bruker XWINNMR software. The spectral region was defined in the range  $-0.5$  to 4.6 ppm in order to include all the lipid signals under investigation and the signal due to the quantitative reference, TSP. The minimum intensity was set at 0 cm, while the maximum was kept at 10,000 cm – the default value given in the software. The peak picking sensi-

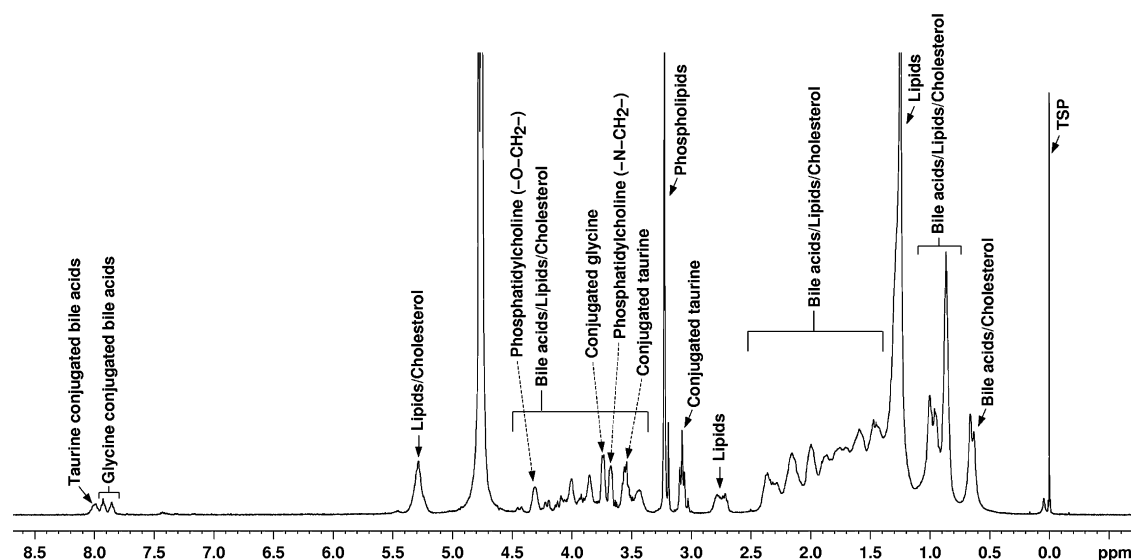


**Fig. 1.** Molecular structures of major biliary lipid components- glycine-conjugated bile acids (GCBA), taurine-conjugated bile acids (TCBA), cholesterol, and choline-containing phospholipids (choline-PLs) are shown with atom numbering.

tivity with respect to the noise was adjusted to provide the best fit. The minimum distance between peaks for independent integration was set at 0.1 ppm, meaning peaks that were more than 0.1 ppm apart were fitted/treated independently, while those that were less than 0.1 ppm apart were considered to be overlapping. After the Lorentzian deconvolution, the areas of the peaks were

obtained and were used for the quantification of the various lipid components using the following equation:

$$[\text{Lipid}](\text{mM}) = \frac{\text{wt.}(\text{TSP})\text{mg}}{\text{mol.wt.}(\text{TSP})} \times \frac{\text{Peak area}(\text{lipid})}{\text{Peak area}(\text{TSP})} \times \frac{\text{No. of protons}(\text{TSP})}{\text{No. of protons}(\text{lipid})} \times \frac{1000}{\text{volume}(\text{sample})\text{mL}} \quad (1)$$



**Fig. 2.**  $^1\text{H}$  NMR spectrum (360 MHz) of human bile from a control subject aspirated from common bile duct showing assignments of some important signals arising from major biliary lipid components.

**Table 2**  
Levels of glycine-conjugated bile acids (GCBAs), taurine-conjugated bile acids (TCBAs), total bile acids (TBAs), and choline-containing phospholipids (choline-PLs) determined by the present method in bile samples collected from patients with various hepatopancreaticobiliary diseases (expressed in mM).

Bile no.	Medical diagnosis	GCBAs	TCBAs	(A) (GCBAs + TCBAs)	(B) (TBAs + cholesterol) <sup>a</sup>	(B) – (A)	Cholesterol <sup>b</sup>	PLs
1	Suspected CBD gallstones	27.98	8.33	36.31	36.96	0.65	3.4	11.82
2	CBD gallstones + duodenal ulcers	11.71	11.8	23.51	23.25	–0.26	1.45	7.28
3	Post liver Tx	19.01	5.96	24.97	26.25	1.28	2.44	6.25
4	Pancreatitis	37.41	25.19	62.6	67.29	4.69	5.69	16.85
5	Pancreatitis	42.73	13.64	56.37	60.47	4.1	4.54	17.05
6	Pancreatitis	18.26	5.69	23.95	24.65	0.7	1.82	5.78
7	Pancreatic cancer	8.99	4.56	13.55	13.41	–0.14	1.35	3.59
8	Pancreatic cancer	16.35	8.03	24.38	24.3	–0.08	1.90	7.39
9	Pancreatic cancer	19.72	18.36	38.08	38.54	0.46	3.45	11.42
10	Pancreatic cancer	16.75	9.05	25.8	24.83	–0.97	2.85	7.59

<sup>a</sup> Obtained from the signal at 0.65 ppm; (B) – (A): the difference of (TBAs + cholesterol) and (GCBAs + TCBAs).

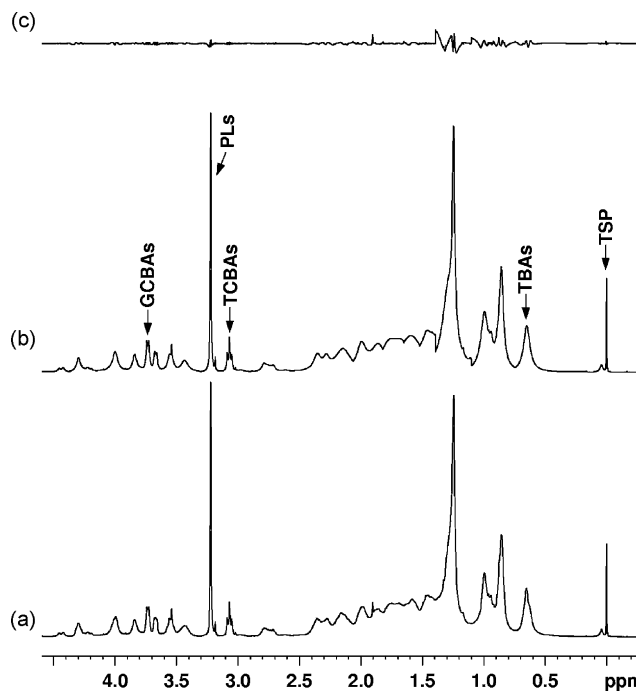
<sup>b</sup> Determined using the literature method [16].

where, number of protons contributing to the peak of interest are 9 for TSP; 2 for GCBAs and TCBAs; 3 for TBAs; and 9 for choline-PLs; molecular weight of TSP = 172.27; peak area of TSP was calibrated to unity. The results obtained by the present method were compared with an NMR-based literature method [16] and the regression coefficients ( $r^2$ ) for the analysis of all the four lipids were also calculated.

### 3. Results

Fig. 1 shows the molecular structures of major biliary lipid components with atom numbering. Fig. 2 shows the  $^1\text{H}$  NMR spectrum of human bile obtained from the common bile duct, with the assignment of important signals. From Fig. 2, we can see that the glycine- $\text{CH}_2$  protons of GCBAs, resonating at 3.73 ppm, partially overlap with signals from other biliary lipids such as choline-methylene ( $-\text{N}-\text{CH}_2-$ ) signals of phosphatidylcholine. As a result, it is difficult to measure the peak area of the conjugated-glycine signal by manual integration. However, the peak area of this signal can be measured by deconvolution and could be used for the quantification of GCBAs. Although Kunnecke et al. [19] used this signal for comparing total bile acid pool with the sum of glycine- and taurine-conjugated bile acids in monkey bile, they did not undertake a detailed study to assess the accuracy of this signal for the quantification of GCBAs. In this study, we have carried out experiments to assess the accuracy and precision of quantitative measurement of GCBAs using this signal. Recovery experiments were performed by adding known amounts of a glycine-conjugated bile acid, glycodeoxycholic acid (GDCA) sodium salt, to bile and the subsequent increase in the peak areas of glycine- $\text{CH}_2$  signal (3.73 ppm) after each addition of GDCA sodium salt were determined by deconvolution. The amounts of GDCA sodium salt recovered were compared with that of the actual amount of GDCA sodium salt added in each experiment (see Section 2.4 for details). From Table 1, we can see a good accuracy and precision of the measurement of GCBAs in bile using their  $\text{CH}_2$  signal. However, slight overestimation of GDCA in most cases of the recovery experiment could be attributed to the artefacts of the deconvolution.

Fig. 3 shows the  $^1\text{H}$  NMR spectrum of human bile along with its deconvoluted counterpart, showing the metabolite (marker) signals used for the quantification of various lipid components. Table 2 lists the levels of GCBAs, TCBAs, TBAs, and choline-PLs in bile samples from patients with various hepatopancreaticobiliary diseases, determined using the present method. We have compared the results of the present method with another NMR-based method which involves dissolving bile into an organic solvent such as DMSO [16]. We have obtained a good correlation between the results obtained using both methods with regression coefficients ( $r^2$ ) 0.97, 0.99, 0.98 and 0.93 for GCBAs, TCBAs, TBAs, and choline-PLs respectively (Fig. 4). It should be noted that the non-unity slope (1.178) observed in the case of choline-PLs could be attributed to



**Fig. 3.**  $^1\text{H}$  NMR spectrum (360 MHz) of human bile (a) along with its deconvoluted counterpart (b); Fig. 3(c) is the difference between experimental and deconvoluted spectra (i.e., a and b). It should be noted that there is a good fit of the experimental spectrum.

the fact that the comparison has been made between quantities of total choline-PLs (present method) and phosphatidylcholine, the predominant choline-PL in bile (literature method [16]).

### 4. Discussion

Although the signals used for the quantification of various lipid components in this study have been characterized earlier [14,19,22], there are no reports describing simultaneous quantification of these lipid molecules using neat human bile samples. In addition to the taurine-conjugated bile acids, we have also assessed the quantification of glycine-conjugated bile acids using glycine- $\text{CH}_2$  signal (3.73 ppm) by deconvolution method. It should also be stressed that we have devised this method keeping in mind of its future utility in the *in vivo* MRS applications at higher magnetic field strengths for the non-invasive assessment of biliary diseases.

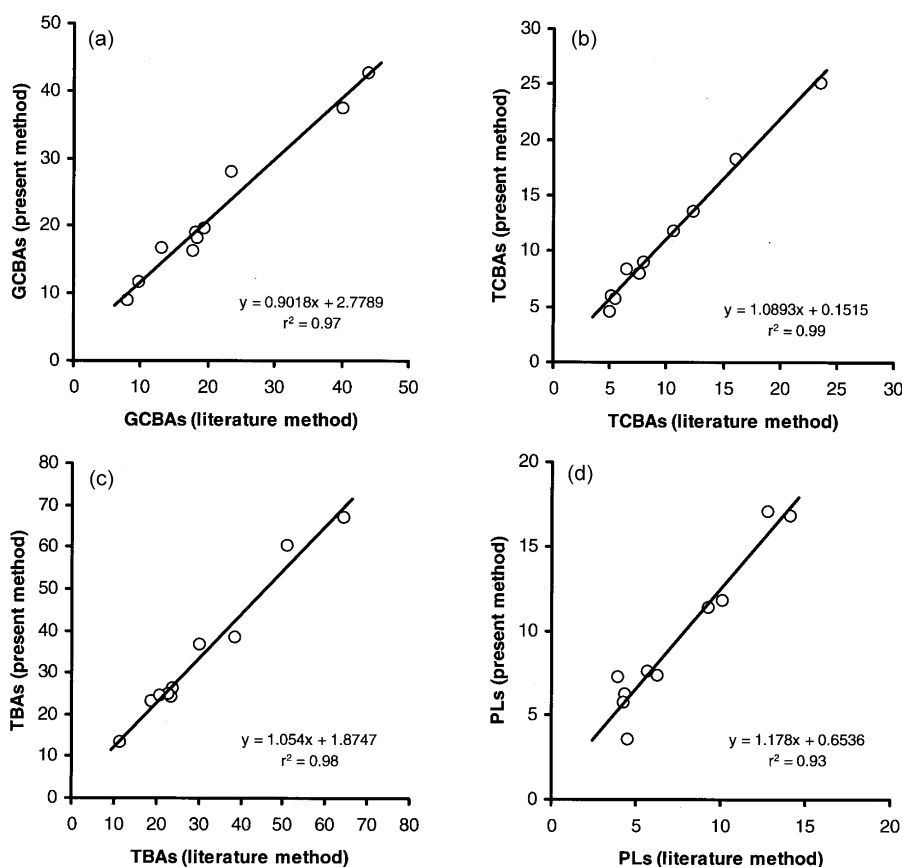
The levels of biliary lipids are modulated in various cholestatic conditions [1]. Recent studies on the analysis of bile samples obtained from various cholestatic patients have shown alterations

in the levels of biliary lipids [2–6,8,15,16]. The majority of the bile acid pool in human bile is conjugated to the amino acids glycine and/or taurine. As a result, distinct amide proton (NH) signals are seen in the downfield region (7.8–8.1 ppm) of  $^1\text{H}$  NMR spectrum of bile (Fig. 2). There are a few studies which utilized these amide signals for the *in vitro* analysis of bile acids in cholestatic diseases [3–5]. In our earlier studies on bile analysis in patients with cholestatic diseases, we have observed an absence or reduced levels of conjugated bile acids such as glycochenodeoxycholic acid and/or glycodeoxycholic acid [3]. Nagana Gowda et al. have also analyzed bile samples from various malignant and non-malignant liver disease patients and observed significant changes in the levels of glycine- and taurine-conjugated bile acids [4,5]. Conjugation pattern of bile acids in bile is altered during cholestasis, and determining this pattern in humans could be valuable in the diagnosis of various cholestatic diseases [4,5,9]. Since these amide signals are in dynamic exchange with the biliary water (at physiologic pH), the pH of the bile has to be adjusted to  $6 \pm 0.5$  for quantification purposes [14,15], which is not applicable to *in vivo* studies. Moreover, collection of bile (during ERCP or cholecystectomy) for *in vitro* studies is an invasive procedure. Thus, we have demonstrated here an alternative approach for the quantification of GCBAs and TCBA in human bile along with other lipid components such as TBAs and choline-PLs.

GCBAs have been quantified by measuring the peak area of glycine- $\text{CH}_2$  signal resonating at 3.73 ppm (see Section 2.4 for details). Taurine conjugated to bile acids has two  $\text{CH}_2$  signals, resonating at 3.07 and 3.56 ppm. The signal at 3.56 ppm overlaps with signal from other bile acids and is not accessible for quantification purposes, whereas the signal at 3.07 ppm is well-resolved and has

been utilized for the quantification of TCBA by us and others previously [14,22]. This signal has been utilized in the quantification of TCBA in the current study.

The H-18 methyl signals of bile acids and cholesterol resonate together around 0.65 ppm and their peak areas could be a measure of TBAs and cholesterol. There are contradicting reports on the levels of biliary cholesterol being detected by  $^1\text{H}$  NMR. Ellul et al. have reported that cholesterol in bile exists in both micellar and vesicular forms and that only the micellar cholesterol contributes to the  $^1\text{H}$  NMR of bile whereas the vesicular cholesterol could not be detected by  $^1\text{H}$  NMR [23,24]. But, the detection of micellar cholesterol was also contended by de Graaf et al. [25] suggesting that the levels of micellar cholesterol in bile are too low to be quantified by  $^1\text{H}$  NMR. In this study, we found that the sum of the total glycine- and taurine-conjugated bile acids (GCBAs and TCBA) determined using their  $\text{CH}_2$  signals was almost equal/comparable to the sum of the TBAs and cholesterol determined from the H-18 methyl signal in all the cases except in two (see Table 2). This observation indicates that the total bile acid pool in bile is almost completely conjugated to glycine and/or taurine, and the contribution of cholesterol to the H-18 methyl signal is minimal when the levels of cholesterol in bile are low. However, at higher concentrations its contribution to the H-18 methyl signal could be significant. The cholesterol in bile is mainly embedded in phospholipid bilayers, which imparts rigidity to the cholesterol-phospholipid vesicles, resulting in their molecular motion being reduced. As a result, the  $T_2$ -relaxation time of cholesterol is considerably reduced, leading to an increase in the linewidth of cholesterol signals. This broadening of cholesterol signals could result in a partial loss of peak area of the cholesterol signal contributing to the H-18 methyl signal [23].



**Fig. 4.** Plots of the quantities of GCBAs, TCBA, TBAs and choline-PLs determined in human bile by the present method versus those obtained using a literature method (Ref. [16]). A good correlation was obtained between the results obtained using both methods with regression coefficients ( $r^2$ ) 0.97, 0.99, 0.98 and 0.93 for GCBAs, TCBA, TBAs, and choline-PLs respectively.

In this study, we found that at lower concentrations of cholesterol, its contribution to H-18 methyl signal is minimal. Therefore, H-18 methyl signals could in fact be used for the estimation of TBAs in neat/aqueous bile in most cases. However, when the levels of cholesterol are elevated in bile, the contribution of cholesterol would be reflected in the H-18 methyl signal (as is the case with bile specimens #4 & 5 in Table 2). Glucuronidation and sulfonation of bile acids are minor metabolic pathways and such conjugates are mostly eliminated from the body through urinary route, resulting in their minimal excretion into the bile [26,27]. As a result, the contribution of these bile acid conjugates to the H-18 methyl signal would be negligible. Given most of the bile acid pool in bile is conjugated to glycine and/or taurine [1], subtracting GCBA and TCBA from the sum of TBAs and cholesterol (quantified using the H-18 methyl signal), one could estimate the quantity of cholesterol contributing to the H-18 methyl signal.

In our earlier study, the median of the ratio of total taurine- to glycine-conjugated bile acids in bile from patients with chronic cholecystitis was 1:4.5 [15]. In the present study, this ratio (median value) was found to be 1:2, indicating the elevation in the levels of taurine-conjugates in patients with various hepatopancreaticobiliary diseases (Table 2). This information could augment routine liver function tests and would be useful in the early detection of chronic cholestatic diseases such as primary sclerosing cholangitis, primary biliary cirrhosis and other types of intrahepatic cholestatic conditions [2,3]. In this study, due to the small sample size, it is difficult to make a direct correlation between the ratio of TCBA to GCBA and the type of biliary disease. Further studies are necessary with a large patient-cohort to make a definitive diagnostic correlation between the levels of bile acid conjugates and the type of hepatopancreaticobiliary disease.

Phospholipids in human bile consist predominantly of phosphatidylcholine (~95%) [28]. In an earlier study, the phosphatidylcholine level calculated by stoichiometric conversion of phosphate ion, choline or fatty acid was found to be very close to the amount of total phospholipids determined in bile [28]. As a result, other forms of phospholipids such as phosphatidylethanolamine (PE) are almost negligible. It is worthwhile to mention that in our earlier studies, we have been able to detect low levels of PE in bile in patients with chronic pancreatitis and/or pancreatic cancer, but it was undetectable by <sup>1</sup>H MRS in healthy controls [29]. In the present study, the levels of choline-PLs in bile have been quantified using the peak area of their choline-N<sup>+</sup>((CH<sub>3</sub>)<sub>3</sub>) signal resonating at 3.22 ppm [24]. We have obtained the peak area of this signal along with GCBA, TCBA and TBA signals simultaneously by deconvolution. The ability to obtain the peak areas of all these lipid signals simultaneously by deconvolution makes the present method robust. Moreover, this methodology could be extended to *in vivo* applications for the quantification of lipid components in gallbladder bile non-invasively using clinical scanners.

## 5. Conclusions

The proposed method could be used for the simultaneous quantification of various lipid components such as GCBA, TCBA, TBA and choline-PLs in bile. The H-18 methyl signal contains contribution from cholesterol, albeit reduced due to line broadening effects. At lower concentrations of cholesterol, the TBAs in bile could be estimated from the H-18 methyl signal. However, when the cholesterol levels are present at elevated levels, one should exercise caution in interpreting the results. Under such circumstances, subtracting GCBA and TCBA from the sum of TBAs and cholesterol could provide an estimate of the quantity of cholesterol contributing to the H-18 methyl signal. Further *in vitro* studies (using model bile) are necessary to determine the exact contribu-

tion of cholesterol to the H-18 methyl signal. The present method facilitates simultaneous quantification of both glycine- and taurine-conjugated bile acids and hence in determining the conjugation pattern of bile acids. The conjugation pattern of bile acids could serve as an important measure of assessing cholestatic conditions. Such information could be valuable in non-invasive diagnostics if the analysis is performed in *in vivo* settings. *In vivo* <sup>1</sup>H MRS of gallbladder bile has been demonstrated in monkeys and humans, and the proposed methodology could be extended to *in vivo* applications using clinical scanners. Clinical scanners with higher magnetic field strengths (3 T and higher) are becoming increasingly common which will enhance the utility of *in vivo* MRS of bile for diagnostic purposes.

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