Table 1. The relative retention times of internal standard compounds and authentic bile acids

Internal standard compounds	Retention time	Relative retention time			
(1) 7α,12β-Dihydroxy-5β-cholanoic acid	24.110	**1.000**	0.954	0.888	0.840
(2) 7α,12α-Dihydroxy-5β-cholanoic acid	25.279	1.048	**1.000**	0.931	0.880
(3) 7β,12α-Dihydroxy-5β-cholanoic acid	27.162	1.127	1.074	**1.000**	0.946
(4) 7β,12β-Dihydroxy-5β-cholanoic acid	28.713	1.191	1.136	1.057	**1.000**
Authentic compounds					
(5) Cholesterol	25.102	1.041	0.993	0.924	0.874
(6) Lithocholic acid	24.016	0.996	0.950	0.884	0.834
(7) 3β-Hydroxy-5-cholenoic acid	28.102	1.166	1.112	1.035	0.979
(8) 3α,12β-Dihydroxy-5β-cholanoic acid	29.915	1.241	1.183	1.101	1.042
(9) Deoxycholic acid	30.972	1.285	1.225	1.140	1.079
(10) Chenodeoxycholic acid	32.678	1.355	1.293	1.203	1.138
(11) Norcholic acid	33.513	1.390	1.326	1.234	1.167
(12) Hyodeoxycholic acid	33.877	1.405	1.340	1.247	1.180
(13) Ursodeoxycholic acid	34.810	1.444	1.377	1.282	1.212
(14) 3β,7β-Dihydroxy-5β-cholanoic acid	35.579	1.476	1.408	1.310	1.239
(15) 3α,7α,12β-Trihydroxy-5β-cholanoic acid	37.193	1.543	1.471	1.369	1.295
(16) Cholic acid	39.672	1.645	1.569	1.461	1.382
(17) 3α,7β,12α-Trihydroxy-5β-cholanoic acid	40.570	1.683	1.605	1.494	1.413
(18) Hyocholic acid	42.879	1.778	1.696	1.579	1.493
(19) 3α,7α-Dihydroxy-12-oxo-5β-cholanoic acid	43.790	1.816	1.732	1.612	1.525

Gas chromatographic condition was programmed at 250℃ for 5 min, from 250℃ to 270℃ at 0.2℃/min, at 270℃ for 10 min, from 270℃ to 280℃ at 0.5℃/min, and then at 280℃ for 30 min.
Capillary column, Hicap CBP-1 capillary column(25m x 0.25mm I.D).
Bile acids were derivatized to methyl ester DMES ether.

both peaks of αβ and lithocholic acid practically pile up in the same gas chromatogram (Fig. 5).

Calibration curves for the quantitative determination of several bile acids have a linear relationship going through the origin between the weight ratio and the peak area ratio of each bile acid to the internal standard compound(s).

By way of example, the linear calibration curves for several bile acids are shown in Fig. 6 when ββ is used as an internal standard compound. Then, the absolute amount of each bile acid in a biological sample can be calculated by the following formula (Arimoto et al., 1982).

$$W = A \times B$$

W: Absolute amount (weight) of each bile acid in the sample assayed.

A: Weight ratio of each bile acid to ββ, obtained by multiplying the slope value of the calibration curve by the peak area ratio of each bile acid peak to ββ peak from the gas chromatogram.

B: Amount (weight) of internal standard compound added in the biological sample.

Total bile acid amounts in an analytical sample can be calculated by adding the amounts of all kinds of bile acids analyzed.

Occasionally confirming the calibration curve is recommended.

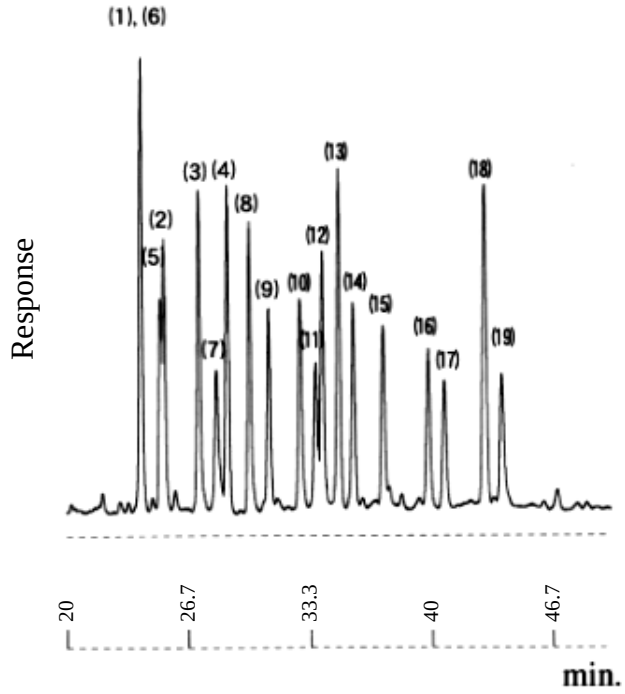

Fig. 5. Gas chromatogram of authentic bile acids and internal standard compounds (methyl ester DMES ether). (See Table 1 for the number of peaks and gas chromatographic condition.)

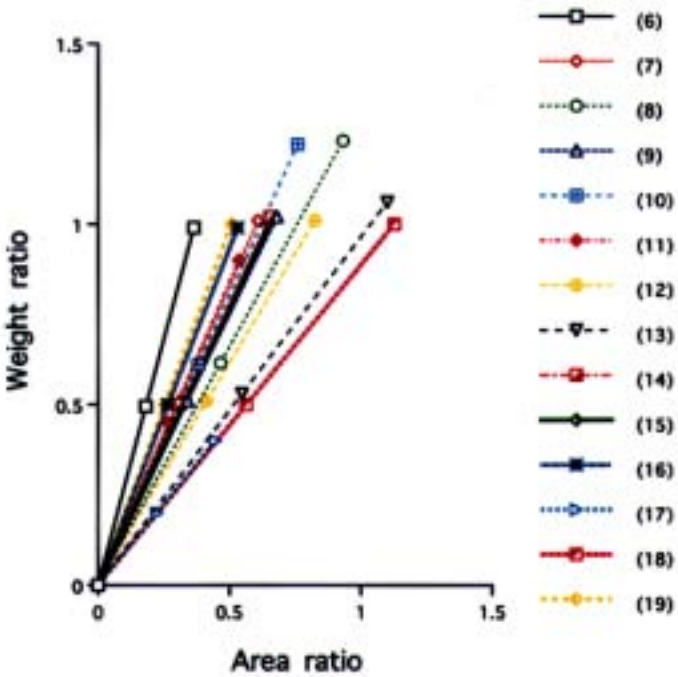

Fig. 6. Calibration curves of several natural bile acids to artificial ββ as internal standard compound for quantitative determination. (See Table 1 for the number of peaks.)

$$\text{Weight ratio: } \frac{\text{Weight of each bile acid}}{\text{Weight of } \beta\beta}$$

$$\text{Area ratio: } \frac{\text{Peak area of each bile acid}}{\text{Peak area of } \beta\beta}$$