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Simultaneous quantitation of fatty acids, sterols and bile acids in human stool by capillary gas–liquid chromatography

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Abstract

A simple method for the simultaneous gas–liquid chromatographic quantitation of fatty acids, sterols and bile acids from human fecal samples is described. The various compounds are directly converted into the *n*-butyl ester-trimethylsilyl ether derivatives, without prior isolation from the stool. Under these conditions, fecal bile acid derivatives are well resolved from each other and from those of fecal fatty acids and sterols without overlaps. The method was found to be reproducible and recoveries were similar to those obtained after exhaustive solvent extraction of fecal sterols, fatty acids and bile acids. Optimum derivatization conditions that allowed maximum recovery of fecal components with minimal destruction and application of the method for simultaneous bile acid, fatty acid and sterol analysis in human stool are described.

Keywords: Fatty acids; Sterols; Bile acids

1. Introduction

Fecal bile acids and sterols are routinely analyzed by capillary gas–liquid chromatography (GLC) as their methyl ester-trimethylsilyl (TMS) ether derivatives [1,2]. Analyzed in this way, bile acids show retention times of the same order as several naturally occurring sterols [2], and therefore, the two groups of compounds need to be completely separated from each other before quantitation. In an attempt to achieve group separation of sterols and bile acids, Child et al. [3] analyzed bile acids as the *n*-butyl ester-acetate derivatives and reported the separation of several fatty acids, sterols and bile acids in feces in the same GLC chromatogram. Tsaconas et al. [4] employed the isobutyl ester-TMS ethers, which increased sensitivity, and they reported separation of isobutyl ester-TMS ethers of bile acids from the TMS ethers of cholesterol and several other sterols. However, under these GLC conditions, although cholesterol is separated from bile acids, sitosterol, an important fecal sterol, still interferes [5]. Also, nor-bile acids that are routinely used as internal recovery

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standards, are also eluted in the same general region as the sterols. We have recently shown that the *n*-butyl ester-TMS ether derivatives of a number of common bile acids, including nor-deoxycholic acid and nor-cholic acid, showed significantly longer retention times than the TMS ethers of cholesterol as well as sitosterol. The method was used to determine serum and fecal bile acid composition in humans and no interference due to sterols was observed [5,6]. We have now extended the method to quantitate fecal bile acids, sterols and fatty acids in a single gas chromatogram. Since, mineral acid-catalyzed esterification of bile acids is reported to cause partial dehydration [7,8], we determined optimum extraction/derivatization conditions for the fecal lipids that result in minimum destruction and maximum derivatization of the fecal components. Application of the method for simultaneous quantitation of sterols, fatty acids and bile acids in fecal samples from healthy individuals is described. We believe that this method is highly suitable for routine screening of fecal lipid profiles in humans, e.g., in patients with hepatobiliary diseases and to determine lipid malabsorption and the effect of diet on fecal lipids in patients.

2. Experimental

2.1. Reagents and chemicals

Cholic, chenodeoxycholic, deoxycholic, lithocholic and nor-deoxycholic acids and cholesterol, coprostanol, campesterol and sitosterol were purchased from Steraloids (Wilton, NH, USA). Ursodeoxycholic and ursocholic acids were gifts from Novartis (East Hanover, NJ, USA). Nor-cholic acid was prepared according to literature [9]. Fatty acids were purchased from Aldrich (Milwaukee, WI, USA). Anhydrous methanolic hydrochloric acid, *n*-butanol and acetyl chloride were purchased from Aldrich. Sil-Prep (hexamethyldisilazane–trimethylchlorosilane–pyridine, 3:1:9) used for preparation of TMS ether derivatives of the bile acid esters was purchased from Alltech Associates (Deerfield, IL, USA).

2.2. Preparation of n-butyl esters

(i) With *n*-butanol–concentrated hydrochloric acid: *n*-Butanol (200 μl) was added to 5–20 μg of the bile acid or fatty acid followed by addition of 20 μl of concentrated hydrochloric acid. The reaction mixture was heated at 60 °C for 4 h followed by evaporation of solvents at 60 °C under N_2.

(ii) With *n*-butanol–acetyl chloride: A mixture of *n*-butanol (200 μl) and acetyl chloride (20 μl) was added to 5–20 μg of the bile acid or fatty acid and the reaction mixture was heated at 60 °C for 4 h followed by evaporation of the solvents at 60 °C under N_2.

2.3. Trimethylsilylation

The sterols or the bile acid esters (5–20 μl) were treated with 100 μl of Sil-Prep for 30 min at 55 °C. Solvents were evaporated at 55 °C under N_2, the reaction product was dissolved in 100 μl hexane and an aliquot was used for GLC [10].

All compounds were >98% pure as judged by gas chromatography of the TMS ether derivatives and exhibited mass spectral fragmentation patterns compatible with their structures.

2.4. Gas chromatography (GLC)

A Hewlett-Packard Model 6890 gas chromatograph equipped with a flame ionization detector and an injector with a split/splitless device for capillary columns was used for all separations. The chromatographic column consisted of a chemically bonded fused-silica CP-Sil-5 CB (stationary phase, 100% dimethylsiloxane) capillary column (25 m×0.22 mm I.D.) (Chrompack, Raritan, NJ, USA) and helium was used as the carrier gas. The GC operating conditions were as follows: injector and detector temperatures were 260 and 290 °C, respectively. After injection, oven temperature was kept at 150 °C for 1 min, and then programmed at a rate of 7 °C/min to a final temperature of 272 °C. The retention times of various fatty acids, sterols and bile acids relative to that of nor-cholic acid obtained under these GLC conditions are given in Table 1. Nor-cholic acid was used as the internal recovery stan-

Table 1
GLC retention times of trimethylsilyl ether-*n*-butyl esters of bile acids, trimethylsilyl ethers of sterols and *n*-butyl esters of fatty acids on a CP-Sil-5-CB capillary column[a]

TMS ether of	RRT[b]	TMS ether of	RRT[b]
Nor-CA-*n*-butyl ester[c]	1.000	Campesterol	0.923
Nor-DCA-*n*-butyl ester	0.980	Campestanol	0.931
LCA-*n*-butyl ester	1.029	24-Ethylcoprostanol	0.910
Iso-DCA-*n*-butyl ester	1.052	Stigmasterol	0.940
DCA-*n*-butyl ester	1.061	Sitosterol	0.972
CDCA-*n*-butyl ester	1.077	Sitostanol	0.983
CA-*n*-butyl ester	1.082	Palmitic acid-*n*-butyl ester	0.537
UDCA-*n*-butyl ester	1.115	Heptadecanoic acid-*n*-butyl ester	0.564
UCA-*n*-butyl ester	1.132	Stearic acid-*n*-butyl ester	0.595
Coprostanol	0.842	Oleic acid-*n*-butyl ester	0.591
Cholesterol	0.885	Linoleic acid-*n*-butyl ester	0.588
Cholestanol	0.892	Eicosanoic acid-*n*-butyl ester	0.689
24-Methylcoprostanol	0.893	10-OH-stearic acid-*n*-butyl ester	0.648

[a] The *n*-butyl ester of fatty acid, *n*-butyl ester-TMS ether of the bile acid or the TMS ether of the sterol (0.1 μg in 1 μl hexane) was injected onto a fused-silica CP-Sil-5 CB capillary column. The injector and detector temperatures were kept at 260 and 290 °C, respectively. After injection, oven temperature was kept at 150 °C for 1 min, and then programmed at a rate of 7 °C/min to a final temperature of 272 °C.
[b] Retention times are expressed relative to that of the *n*-butyl ester-trimethylsilyl ether of nor-cholic acid (retention time, 27.56 min).
[c] Nor-CA, nor-cholic acid; Nor-DCA, nor-deoxycholic acid; LCA, lithocholic acid; Iso-DCA, iso-deoxycholic acid; DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; CA, cholic acid; UDCA, ursodeoxycholic acid; UCA, ursocholic acid.

dard. Correction factors of the various compounds were calculated by injection of known amount of a fatty acid, sterol or bile acid together with a known amount of the internal standard and measurement of the area under the curves as shown by the integrator. Detector response was calculated by injection of various amounts (0.02–0.2 μg) of the respective fatty acid, sterol or bile acid and plotting area under the curve against the amount injected.

2.5. Gas chromatography–mass spectrometry (GC–MS)

Mass spectra of the various sterols, bile acids and fatty acids, whenever needed, were carried out on a Hewlett-Packard Model 5972A mass-selective detector coupled to a Model 6890 gas chromatograph using a 25 m CP-Sil-5 CB capillary column.

2.6. Quantitation of fecal fatty acids, sterols and bile acids

To 10–15 mg freeze–dried stool (exactly weighed) was added internal standard (nor-cholic acid, 20 μg) in 200 μl of *n*-butanol followed by 20 μl concentrated hydrochloric acid and the contents were subjected to *n*-butyl ester formation as described above. After complete evaporation of solvents at 60 °C under N_2, the esterified product was directly subjected to trimethylsilylation with 200 μl of Sil-Prep for 30 min at 55 °C. After evaporation of solvents at 55 °C under N_2, the dark colored residue containing the TMS ether derivatives was taken up in 200 μl of hexane, centrifuged to separate the stool debris, and 1–2 μl of the clear, light brown supernatant was injected into the GLC column in the 20:1 split mode.

In separate experiments, the above butyl ester derivatization procedure was repeated but 20 μl acetyl chloride was substituted for concentrated hydrochloric acid. The esterified product was subjected to trimethylsilylation and an aliquot in hexane was subjected to GLC exactly as described above for the *n*-butanol–hydrochloric acid derivatization procedure.

We have been routinely using this direct derivatization method for fecal bile acid analysis for the last 5 years. Since no prepurification of the fecal products is made in the method, the injection insert is cleaned after every 20–30 injections to remove the

nonvolatile fecal material. Further, as a precaution, approximately one foot of the column was discarded from the injection site after approximately 600 injections to remove any nonvolatile fecal material. We have already used the same chromatographic column for over 1000 injections to carry out fecal bile acid analyses, without apparent loss of resolution. The retention times of the various compounds are likely to change slightly as the column is shortened, but this can be corrected with appropriate temperature programming.

2.7. Quantitation of fecal fatty acids, sterols and bile acids by solvent extraction method

Freeze–dried stool (10–15 mg) was transferred to a small paper thimble, together with 20 μg of nor-cholic acid and 20 μg of 5α-cholestane, and was subjected to continuous extraction with 1% ammoniacal ethanol for 16 h in a Soxhlet extractor [11]. Ethanol was evaporated to dryness and the residue was taken up in 5 ml of 0.5 *M* sodium hydroxide. The neutral sterols were then extracted with *n*-hexane (4×5 ml). The *n*-hexane was evaporated to dryness and the residue was subjected to trimethylsilylation. An aliquot was used for GLC to quantitate the neutral sterols using 5α-cholestane as internal standard. The aqueous solution after removal of neutral sterols was acidified with 5 *M* hydrochloric acid to pH 1 and fatty acids and bile acids were extracted with ethyl acetate (4×5 ml). The ethyl acetate extract was washed with water to neutrality, evaporated to dryness and subjected to *n*-butyl ester derivatization followed by trimethylsilylation as described above. An aliquot was then used for GLC and fatty acids and bile acids were quantitated against nor-cholic acid as internal standard.

3. Results and discussion

Our recent gas chromatographic method, that involves simultaneous formation of the *n*-butyl ester-TMS ether derivatives of bile acids and TMS ethers of the sterols directly in the stool samples, completely eliminates multiple extraction steps. In this method, bile acids are well resolved from each other and from the TMS ethers of major fecal sterols (cholesterol, campesterol and sitosterol and their bacterial 5β-H derivatives) and the *n*-butyl esters of fatty acids present in the stool. Unlike the previously described methods, our method is applicable directly to the stool sample, without prior isolation of the fecal lipids or any pre-purification of the stool sample. The method also represents an improvement over the method of Child et al. that employs the less sensitive *n*-butyl ester-acetate derivatives of fecal lipids following elaborate extraction from the stool [3].

Mineral acids have been shown to cause partial dehydration of bile acids [7,8]. Since, our method involves heating stool with *n*-butanol–hydrochloric acid [5], it was important to determine optimum derivatization conditions with minimum losses of sterols, fatty acids or bile acids. We attempted various derivatization conditions and found that treatment of stool with concentrated hydrochloric acid–*n*-butanol (1:10, v/v) at 60 °C for 4 h resulted in complete *n*-butyl ester formation of fatty acids and bile acids employed in the study, with approximately 3–5% destruction of 3β-hydroxy-Δ^5-sterols or 7α-hydroxylated bile acids, chenodeoxycholic acid, cholic acid and nor-cholic acid. More rigorous derivatization conditions [increased concentration of hydrochloric acid (15% or above), increased derivatization time (6 h at 60 °C or overnight at 55 °C) or higher temperature (70 °C or above)] resulted in increased destruction of sterols and bile acids (10–30%), while shorter reaction time (less than 3 h), lower concentrations of hydrochloric acid (5%) or reaction temperature below 55 °C resulted in incomplete derivatization, in particular, of nor-cholic acid used as internal recovery standard, or lower overall recoveries from fecal samples. Stanols, fatty acids and the bile acids, lithocholic acid, deoxycholic acid, nor-deoxycholic acid, ursodeoxycholic acid and ursocholic acid, that lack 7α-hydroxy group, were stable under our optimal derivatization conditions. We have also demonstrated previously that fatty acid esters of cholesterol are transesterified under these derivatization conditions to produce fatty acid butyl esters and the liberated cholesterol can then be derivatized as the TMS ether derivative [5,6] so that both the fatty acid and cholesterol moieties can be quantitated.

We had employed aqueous hydrochloric acid in

our original method for fecal sterol and bile acid analysis with the contention that fecal bile acids will be more completely extracted on heating with *n*-butanol–aqueous hydrochloric acid [5]. In order to determine if it would be more beneficial to use anhydrous hydrochloric acid, we also generated hydrochloric acid in situ by addition of required amounts of acetyl chloride to *n*-butanol to obtain appropriate concentration of hydrochloric acid and used this mixture for derivatization of stool samples as well as standard fatty acids and bile acids. It was found that although standard fatty acids and bile acids were completely esterified in less than 15 min, extraction of bile acids from the stool samples with *n*-butanol–acetyl chloride was not as complete after heating for 4 h at 60 °C as with *n*-butanol–aqueous hydrochloric acid (Table 2). The dehydration of the sterols and bile acids was of similar order whether we employed aqueous or non-aqueous conditions for derivatization.

In the GLC conditions reported in our earlier publication, the peaks due to the major fecal fatty acids overlapped on each other [5]. We have now adopted different temperature programming so that the fatty acids are eluted over a longer time period and several C_{16}–C_{20} fatty acids were resolved from each other and from sterols and bile acids employed in the chromatography (Table 1; Fig. 1). The retention times of all compounds were highly reproducible under identical chromatographic conditions and for amounts of bile acids, sterols or fatty acids ranging from 0.02 to 0.2 μg injected onto the column, the detector response, as shown by the integrator, was linear. Nor-cholic acid was employed as the internal standard and the peak-area correction factor of 1–1.1 was needed for quantitation of bile acids and sterols and approximately 0.6 for quantitation of fatty acids.

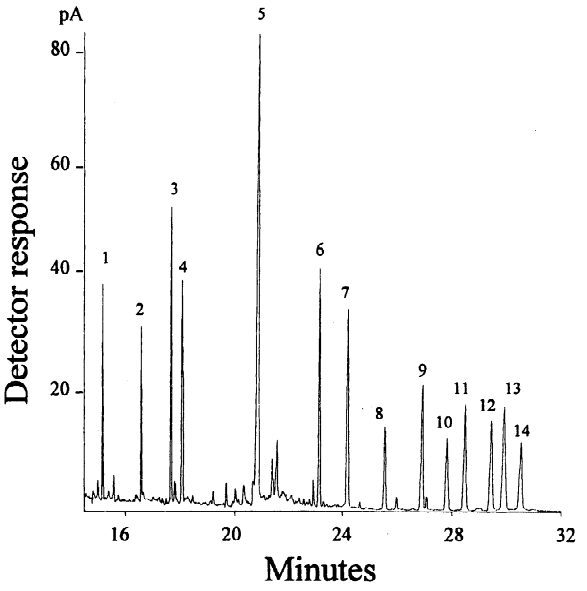

Fig. 1. Gas–liquid chromatogram of standard fatty acids, sterols and bile acids. A 5-20-μg amount of each compound was subjected to *n*-butyl ester-trimethylsilyl ether derivatization as described in the Experimental section. After adding 100 μl hexane, 1 μl was injected into the GC column. Chromatographic conditions were as described in the Experimental section. Peak identification, derivatives of: (1) palmitic acid; (2) stearic acid; (3) 12-hydroxystearic acid; (4) eicosanoic acid; (5) 5α-cholestane; (6) coprostanol; (7) cholesterol; (8) campesterol; (9) sitosterol, 10, nor-cholic acid; (11) lithocholic acid; (12) deoxycholic acid; (13) cholic acid; (14) ursodeoxycholic acid.

Fig. 2 shows a typical gas chromatogram of the

Table 2
Bile acid analysis in human stool after derivatization with *n*-butanol–HCl and *n*-butanol–acetyl chloride[a]

Compound	μg/mg dry stool	
	n-Butanol–HCl[b]	*n*-Butanol–acetyl chloride[b]
Lithocholic acid	1.8±0.1	1.2±0.3
Iso-deoxycholic acid	0.5±0.05	0.4±0.1
Deoxycholic acid	3.5±0.2	2.9±0.4
Others[c]	0.5±0.05	0.4±0.1

[a] Gas–liquid chromatographic conditions are given in Table 1.

[b] Stool (10–15 mg), to which 20 μg of nor-cholic acid was added as internal recovery standard, was directly subjected to *n*-butyl ester formation followed by TMS ether derivatization (for conditions for derivatization, see Experimental). After addition of hexane (200 μl), the contents were vortexed and 2 μl of the clear supernatant was used for GLC.

[c] Include: chenodeoxycholic acid, cholic acid, ursodeoxycholic acid, ursocholic acid, 12-ketolithocholic acid and 12-ketodeoxycholic acid.

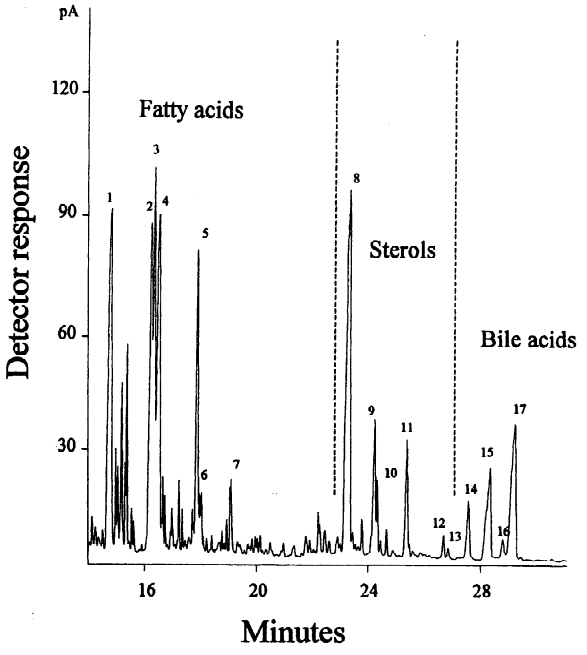

Fig. 2. Gas–liquid chromatogram of fatty acids, sterols and bile acids present in stool from a healthy control. A 10-mg amount of freeze–dried stool containing 20 μg nor-cholic acid was subjected to *n*-butyl ester-trimethylsilyl ether derivatization as described in the Experimental section. After adding 200 μl hexane, the contents were vortexed, centrifuged and 1 μl of the clear supernatant was injected into the GC column. Chromatographic conditions were as described in the Experimental section. Peak identification, derivatives of: (1) palmitic acid; (2) linoleic+ linolenic acids; (3) oleic acid; (4) stearic acid; (5) 10-hydroxystearic acid; (6) eicosanoic acid; (7) docosanoic acid; (8) coprostanol; (9) cholesterol; (10) 24-methylcoprostanol; (11) 24-ethylcoprostanol; (12) sitosterol, (13) sitostanol; (14) nor-cholic acid; (15) lithocholic acid; (16) iso-deoxycholic acid; (17) deoxycholic acid.

fecal lipids in a healthy control subject. The fatty acids were the major lipids found in the stool with the C_{18} saturated and unsaturated fatty acids predominating. Due to the presence of large proportions of the unsaturated C_{18} fatty acids, oleic acid, linoleic acid and linolenic acid, the peaks due to these compounds overlapped on each other. However, if necessary, these compounds can be resolved from each other with appropriate temperature programming, and will still be eluted from the GLC column earlier than any fecal sterols or bile acids. The major fecal sterols were shown to be cholesterol and its bacterial product, coprostanol, but significant amounts of plant sterols, campesterol, stigmasterol and sitosterol and their bacterial products were also present. All sterols were eluted before the bile acid internal standard, nor-cholic acid, following which the fecal bile acids were eluted. As expected, the 7α-dehydroxylated bile acids, lithocholic acid and deoxycholic acid were the predominant bile acids in the stool sample, although several other bile acids were also present. Repeat analysis of this stool sample was carried out in order to determine reproducibility of the analysis, and the coefficient of variability of the individual sterols or bile acids was found to be less than 10% from the mean value while it was of the order of 5% for the fatty acids (Table 3). Also, the method was comparable to the extraction method, where the sterols, fatty acids and bile acids were first exhaustively extracted from the stool with ammoniacal ethanol, the neutral sterols were separated from the fatty acids and bile acids and each fraction was subjected to derivatization/gas chromatography (Table 3).

Table 4 shows the application of our method for determination of mean values of fecal fatty acids, sterols and bile acids in stool sample from the subject shown in Table 3 and five additional healthy volunteers. A lot of variability was observed in fecal components in individual persons. However, coprostanol was the major fecal sterol component, while the C_{18} fatty acids were the predominant fatty acids in all samples. 10-Hydroxystearic acid (structure confirmed by mass spectrum) was present in all samples in amounts ranging from 1 to 7% of the total fatty acids [12–14]. No attempt was made to quantitate C_{12-15} fatty acids due to overlap by peaks due to unknown compounds. However, small amounts of the C_{12}- and C_{14}-fatty acids were detected by GC–MS. Similarly, C_{22}- and C_{24}-fatty acids were also detected in small amounts in most samples. Even though the primary bile acids, chenodeoxycholic acid and cholic acid and the 7β-hydroxy bile acids, ursodeoxycholic acid and ursocholic acid were detected in most samples, the 7-dehydroxylated bile acids, lithocholic acid and deoxycholic acids were the predominant fecal bile acids. Variable amounts of oxo-bile acids, particularly, 12-ketolithocholic acid, were often present.

In summary, we have shown that fecal fatty acids, sterols and bile acids can be quantitated in a single

Table 3
Quantitation of fecal fatty acids, sterols and bile acids by direct derivatization method—comparison with solvent extraction method[a]

Compound	μg/mg dry stool	
	Current method[b]	Solvent extraction[c]
LCA[d]	1.8±0.1	1.7±0.2
Iso-DCA	0.5±0.05	0.5±0.1
DCA	3.5±0.2	3.3±0.4
Coprostanol	5.9±0.3	5.4±0.5
Cholesterol	1.2±0.05	1.2±0.06
24-Methylcoprostanol	0.7±0.05	0.8±0.2
Campesterol	0.2±0.01	0.2±0.01
24-Ethylcoprostanol	1.3±0.1	1.5±0.1
Sitosterol	0.4±0.02	0.4±0.04
Palmitic acid	20.9±0.4	20.3±0.4
Stearic acid	12.2±0.5	12.6±0.6
Oleic+linoleic+linolenic acids	30.2±1.6	31.1±1.9

[a] Mean±SD of analysis performed on three separate samples weighed from a homogenate of freeze–dried human stool used in Fig. 2. Gas chromatographic conditions are described in Table 1.

[b] Stool (10–15 mg), to which 20 μg of each of nor-cholic acid and 5α-cholestane were added as internal recovery standard, was directly subjected to *n*-butyl ester formation followed by TMS ether derivatization. After addition of hexane (200 μl), the contents were centrifuged and 2 μl of the clear supernatant was used for GC. Sterols were quantitated against 5α-cholestane and fatty acids and bile acids were quantitated against nor-cholic acid as internal recovery standard.

[c] Stool (10–15 mg), to which 20 μg of each of nor-cholic acid and 5α-cholestane were added, was extracted for 18 h with ammoniacal ethanol in a Soxhlet extractor. Sterols were extracted with *n*-hexane and bile acids and fatty acids were then extracted with ethyl acetate. Sterol fraction was subjected to trimethylsilylation and the bile acid+fatty acid fraction was subjected to *n*-butyl ester formation followed by TMS ether derivatization. After addition of hexane (200 μl) to each fraction, 2 μl was used for GC as described above. Sterols were quantitated against 5α-cholestane and fatty acids and bile acids were quantitated against nor-cholic acid as internal recovery standard.

[d] LCA, Lithocholic acid; Iso-DCA, iso-deoxycholic acid; DCA, deoxycholic acid.

gas chromatographic run as the *n*-butyl ester/trimethylsilyl ether derivatives. The method avoids solvent extractions and the derivatization of the lipids is carried out in the same vial so that losses are minimized. We have also shown that under optimal conditions, the method is reproducible and is at least as accurate as the conventional rigorous solvent extraction method (Table 3). Due to the simplicity of the method, it is highly suitable for routine screening of fecal samples, e.g., to compare the fecal lipid profiles and secondary/primary bile acid ratios in healthy individuals vs. patients with colon polyps or

Table 4
Sterols, fatty acids and bile acids in freeze–dried stool from healthy volunteers[a]

Sterol	mg/day	Fatty acids	mg/day	Bile acids	mg/day
Coprostanol	566.8±213.8	Palmitic	637.0±114.4	LCA	127.4±25.2
Cholesterol	62.7±26.6	Stearic	381.0±97.9	Iso-DCA	7.6±8.7
24-Methyl-coprostanol	48.3±17.6	Oleic+linoleic+linolenic	843.5±78.0	DCA	177.2±45.7
Campesterol	8.0±5.9	10-Hydroxystearic	57.2±11.0	CDCA	6.4±6.5
24-Ethyl-coprostanol	150.0±38.7	C_{20}-fatty acids	82.0±20.4	CA	7.7±5.7
Stigmasterol	8.0±3.8	Other fatty acids[b]	52.0±35.5	UDCA	1.4±3.3
Sitosterol	18.5±10.7			UCA	7.3±3.8
Sitostanol	14.3±8.1			Others[c]	15.5±10.0

[a] $n=6$. Freeze–dried stool (10–15 mg exactly weighed), to which 20 μg of nor-cholic acid was added as internal recovery standard, was directly subjected to *n*-butyl ester formation followed by TMS ether derivatization. After addition of hexane (200 μl), the contents were centrifuged and 2 μl of the clear supernatant was used for GC. Chromatographic conditions as described in Table 1.

[b] Include: saturated C_{22}- and C_{24}-fatty acids.

[c] Include: 12-ketolithocholic acid and 12-ketodeoxycholic acid.

colon cancer (with increased concentrations of fecal secondary bile acids), and the effect of diet on fecal lipids in patients. Sterols, fatty acids and bile acids can be determined in both freeze–dried stool samples and fecal water. In the latter case, the additional step of evaporation of the fecal water needs to be carried out. As little as 10 mg of dried stool and 100 μl of aqueous phase stool (data not shown) give reproducible determination of the sterols, fatty acids and bile acids. It may, however, be noted that some information is lost by use of our method. Thus, the method cannot estimate fecal bile acid conjugates (which do not get deconjugated under our derivatization conditions, and therefore, cannot be analyzed by GLC), and fatty acid glycerides and fatty acid esters of sterols are quantitated together with the free fatty acids and sterols. Due to the highly complex lipid profile in the stool, contaminating peaks due to unknown fecal products may sometimes overlap peaks due to the compounds of interest. Thus, the identification of fecal fatty acids with less than 16 carbons may require mass spectral analysis, and these fatty acids will not be accurately quantitated by the current method. Also, fatty acids present in very small amounts may, at times, be overestimated.

4. Nomenclature

The following abbreviations and trivial names have been used:

GLC	Gas–liquid chromatography
GC–MS	Gas–liquid chromatography–mass spectrometry
TMS	Trimethylsilyl
Sil-Prep	Hexamethyldisilazane–trimethylchlorosilane–pyridine (3:1:9)
Lithocholic acid	3α-Hydroxy-5β-cholanoic acid
Deoxycholic acid	3α,12α-Dihydroxy-5β-cholanoic acid
Iso-deoxycholic acid	3β,12α-Dihydroxy-5β-cholanoic acid
Chenodeoxycholic acid	3α,7α-Dihydroxy-5β-cholanoic acid
Cholic acid	3α,7α,12α-Trihydroxy-5β-cholanoic acid
Ursodeoxycholic acid	3α,7β-Dihydroxy-5β-cholanoic acid
Ursocholic acid	3α,7β,12α-Trihydroxy-5β-cholanoic acid
Nor-deoxycholic acid	3α,12α-Dihydroxy-24-nor-5β-cholan-23-oic acid
nor-cholic acid	3α,7α,12α-Trihydroxy-24-nor-5β-cholan-23-oic acid
12-Oxo-lithocholic acid	12-Oxo,3α-hydroxy-5β-cholanoic acid
Coprostanol	5β-Cholestan-3β-ol
Cholesterol	5-Cholesten-3β-ol
24-Methylcoprostanol	24-Methyl-5β-cholestan-3β-ol
Campesterol	24-Methyl-5-cholesten-3β-ol
24-Ethylcoprostanol	24-Ethyl-5β-cholestan-3β-ol
Stigmasterol	24-Ethyl-5,22-cholestadien-3β-ol
Sitosterol	24-Ethyl-5-cholesten-3β-ol
Sitostanol	24-Ethyl-5α-cholestan-3β-ol
Palmitic acid	Hexadecanoic acid
Stearic acid	Octadecanoic acid
Oleic acid	Δ^{9}-Octadecanoic acid
Linoleic acid	$\Delta^{9,12}$-Octadecanoic acid
Linolenic acid	$\Delta^{9,12,15}$-Octadecanoic acid
10-Hydroxystearic acid	10-Hydroxy-octadecanoic acid.

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