ANALYSIS OF FECAL STEROLS BY GAS CHROMATOGRAPHY*

R. S. ROSENFELD, M. C. LEBEAU, S. SHULMAN AND J. SELTZER
Sloan-Kettering Institute for Cancer Research, New York, N.Y. (U.S.A.)

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The quantitation of fecal sterols is of major importance in sterol balance studies designed to determine the effects of changes in diet or to evaluate cholesterol regulating agents. Gravimetric methods or procedures which involve colorimetric determinations are difficult to carry out accurately with stool extracts[1], while chromatographic analysis of the fecal non-saponifiable material[2], although sufficiently precise, is lengthy and cumbersome. The feasibility of gas chromatography for the analysis of sterol mixtures has recently been reported[3]. Cholesterol and coprostanol comprise more than 90% of the sterols in feces and it is the purpose of this communication to present a method for analysis of these substances by gas chromatography.

EXPERIMENTAL

Gas chromatography

The analyses were carried out with glass columns, 1.8 m × 5 mm i.d., packed with 100–140 mesh Gas Chrom P** coated with SE-30*** (3% by weight), maintained at 235° and run with argon pressure 30 p.s.i.; an ionization detection system was used. Relative retention times compared with cholestane (8.8 min) were 1.66 for coprostanol and 1.78 for cholesterol.

Calibration curves

Cholesterol purified by regeneration from the dibromide and coprostanol purified by procedures earlier described[2], were employed as reference standards. Appropriate volumes of ethanol solutions of each sterol (1 and 2 μg per μl) were injected on the column so that from 1 to 20 μg of the reference compounds were introduced. The area under each peak was calculated from the half band width[4]. The amount of cholesterol and coprostanol in terms of the area under the corresponding peak are shown in Fig. 1. Mixtures of pure cholesterol and coprostanol were chromatographed in the same manner; typical curves are illustrated in Fig. 2. Each component was calculated

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** Applied Science Laboratories, State College, Pa.
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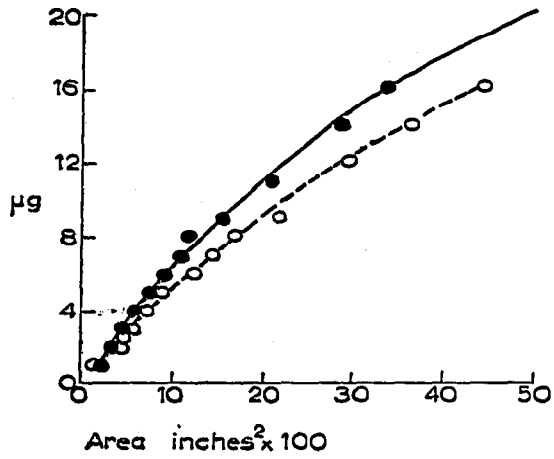

Fig. 1. Sterol standards for gas chromatography. —●—●— coprostanol; --○--○-- cholesterol.

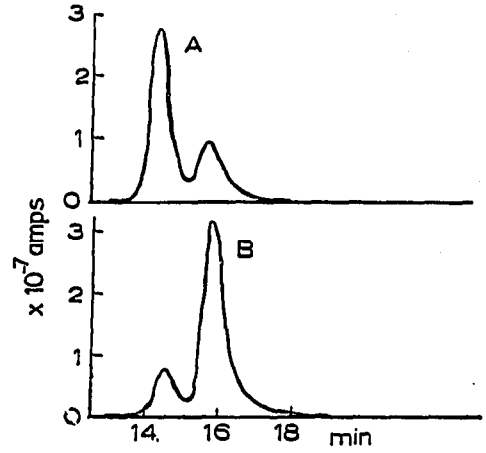

Fig. 2. Gas chromatographic analysis of sterol mixtures. (A) Coprostanol 14 μg; cholesterol 6 μg. (B) Coprostanol 6 μg; cholesterol 14 μg.

from the calibration curves in Fig. 1 and the results are compared with the weights of the known mixtures (Table I).

TABLE I

ANALYSES OF MIXTURES OF REFERENCE COMPOUNDS BY GAS CHROMATOGRAPHY

Mixture	*Composition*			
	Added (μg)		*Found (μg)*	
	Coprostanol	*Cholesterol*	*Coprostanol*	*Cholesterol*
1	18	2	17.4	2.3
2	14	6	13.8	6.4
3	10	10	9.5	9.2
4	6	14	6.1	13.4
5	2	18	2.0	16.6

Extraction and measurement of fecal sterols

Stool specimens were homogenized, refluxed with potassium hydroxide and the non-saponifiable fraction was extracted as described earlier[2]. A small amount of the non-saponifiable fraction was analyzed by gas chromatography while the balance was

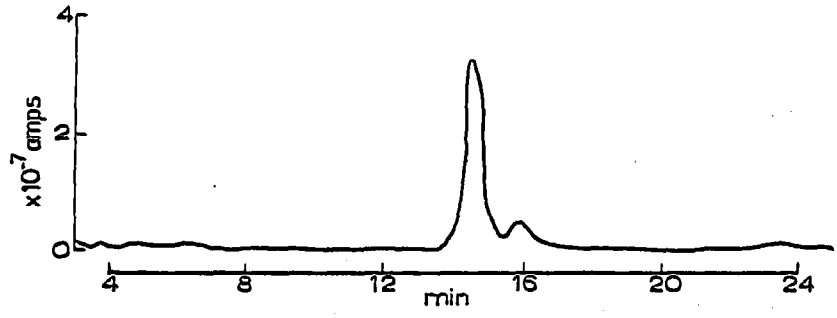

Fig. 3. Gas chromatographic analysis of fecal sterols.

chromatographed on alumina[2]. A typical gas chromatogram of the non-saponifiable fraction of stool is illustrated in Fig. 3, and a comparison of the stool analyses by each method appears in Table II.

TABLE II

CHROMATOGRAPHIC ANALYSES OF FECAL STEROLS

Subject	N-S* (mg)	Per cent of non-saponifiable			
		Gas		Column	
		Coprostanol	Cholesterol	Coprostanol	Cholesterol
Fl-1	900	56	< 5	56	2.1
MO-2	740	63	13	60	14
MO-3	2860	61	11	57	11
KA-1	266	< 3	30	3.4	27

* N-S = Non-saponifiable fraction.

DISCUSSION

The relation between quantity of sterol and area is not linear with the ionization detection system (Fig. 1) but is nearly so over the range examined. Reference compounds on different days, under identical conditions on the same column fall on the curves; however, when the detector cell, column, or conditions are changed, new calibration curves must be constructed. Since the columns operate from 6 weeks to 2 months with continuous use, recalibration is not a serious consideration. The analyses calculated for the mixtures of cholesterol and coprostanol from data illustrated in Fig. 2 and Table I are in good agreement with the actual amounts and demonstrate the feasibility of the method for the sterol mixtures.

Gas chromatography on the non-polar phase SE-30 under these experimental conditions does not separate cholestanol from cholesterol and the second peak at 15.8′ (Fig. 3) could contain both constituents; however, analysis of the cholesterol fraction of stool showed cholestanol to be present only in very small amounts[5]. VANDEN HEUVEL, HAAHTI AND HORNING[6] have recently reported the separation of these substances on a fluorinated alkyl silicone polymer.

Phytosterols are efficiently separated from the C_{27} sterols[7]; under the conditions of these experiments, sitosterol had a retention time of 23 min. Fig. 3 contains a small peak with a retention time similar to phytosterols and their products of biochemical reduction which probably represents these constituents. Mineral oil, a possible contaminant of the non-saponifiable fraction in some samples emerged from the SE-30 column before coprostanol. Aside from these, the non-saponifiable material contained little which interferes with the measurement of cholesterol and coprostanol under the usual experimental conditions and the analyses of sterols in this fraction by gas chromatography agreed well with the amounts actually isolated by column chromatography (Table II).

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SUMMARY

A method has been described which permits the quantitative analysis of cholesterol and coprostanol in feces by gas chromatography. The data have been compared with those of column chromatography and the technique appears useful in the measurement of these substances in sterol balance studies.

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