Gas Chromatographic Microdetermination of Indole and Skatole in Gastrointestinal Contents of Domestic Animals[†]

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It is generally recognized that indole and skatole(3-methylindole) are not formed in the normal metabolism of tryptophan in animal body, but formed in the digestive tract by the action of putrefying bacteria on tryptophan or on tryptophan-containing proteins. Therefore, indole and skatole in gastrointestinal contents are the appropriate indicators of putrefaction in the digestive tract. But a suitable method for their determination has not been reported, although the colorimetric co-determination of them¹⁾ and some gas chromatographic determinations of indole have been reported.²⁻⁴⁾ Hermann and Post⁵⁾ reported that direct aqueous-injection gas chromatography is desirable to overcome the disadvantages of conventional concentration-extraction procedures and to facilitate a research program. For the determination of indole and skatole in gastrointestinal contents of domestic animals by this method, it is necessary to detect trace amounts of them in steam distillates of gastrointestinal contents.

This paper deals with the application of direct aqueous-injection gas chromatography with flame-ionization detection to the determination of 0.1~0.8 μ mole of indole and 0.2~1.0 μ mole of skatole in gastrointestinal contents.

A Shimadzu GC-3BF gas chromatograph was used in this study. Nitrogen was employed as the carrier gas. A 10 μ l capacity microsyringe was employed to inject 4 μ l of steam distillates of samples. Glass columns, 2.1 m $\times$ 3 mm, were packed with 7% silicone SE-30 on 60~80 mesh Chromosorb W. Other operating conditions were as follows: flow rate of nitrogen, 70 ml/min; flow rate of hydrogen, 50 ml/min; temperature of column oven, 170°C; temperature of injection port, 240°C; range and sensitivity, 8×10^4 .

The retention times and peak heights of indole and some derivatives were studied by injecting 4 μ l of each 10 μ M of aqueous solution in order to obtain reference

TABLE I. RETENTION TIMES AND PEAK HEIGHTS OF INDOLE AND SOME DERIVATIVES

Indoles	t_R (min)	Peak height ^{a)} (cm)
Indole	1.03	12.60
3-Methylindole	1.38	8.89
2-Methylindole	1.37	8.72
2,3-Dimethylindole	1.91	3.33
2,5-Dimethylindole	1.88	1.50
5-Chloroindole	2.25	4.37

^{a)} Peak heights were measured as shown in Fig. 2.

data to select an internal standard. The results are shown in Table I. Although the retention time of skatole is similar to that of 2-methylindole, the latter is not an obstacle for the purpose of our experiment, not only because its biological formation has not been shown, but also because it is unlikely to be formed considering the structure of tryptophan. 5-Chloroindole was selected as an internal standard, because it was confirmed in our experiments mentioned below that any substance, which showed the same retention time as that of 5-chloroindole, was not found in the steam distillates of gastrointestinal contents.

0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 μ mole of indole and skatole were steam-distilled from about 60 ml of aqueous solutions adjusted to pH 8.5~9.0 by the addition of 10% sodium hydroxide solution and the distillates were collected in 100 ml volumetric flasks. Five milliliters of 160 μ M of 5-chloroindole were added to each 100 ml of distillate, mixed thoroughly and 4 μ l of each solution was injected.

FIG. 1. Peak Height Ratio as a Function of the Amount of Indole or Skatole in the Distillation Flask.

●—●, indole; ×---×, skatole.

[†] On the Methods for the Study on Digestion and Absorption. Part IV. For Part III, see *Nippon Nôgeikagaku Kaishi*, 34, 563 (1960).

The peak height ratios of indole and skatole to 5-chloroindole showed linear responses in the range of 0.1~0.8 μ mole and 0.2~1.0 μ mole, respectively (Fig. 1) and regression equations were as follows:

$$x = 0.365y + 0.029 \text{ (indole)}$$

$$x = 0.443y + 0.031 \text{ (skatole)}$$

where, x is mole of indole or skatole in the distillation flask and y is the peak height ratio of indole or skatole to 5-chloroindole.

It is desirable that indole and skatole in a weighed sample are steam-distilled thoroughly in 100 ml of distillate. Therefore experiments were carried out as follows: after the addition of about 20 ml of water to the adequate amount of gastrointestinal contents and stirring, the suspension was adjusted to pH 8.5~9.0 by the addition of 10% sodium hydroxide solution and quantitatively transferred to 500 ml distillation flask with 40 ml of water and steam-distilled, till the first and second 100 ml of the distillates were separately collected in 100 ml volumetric flasks. By the injection of each 4 μ l of the first and second 100 ml, the appropriateness of 5-chloroindole to an internal standard and

the thorough distillation of indole and skatole in the first 100 ml were confirmed. Then 5 ml of 160 μ M of 5-chloroindole were added to the first 100 ml and mixed thoroughly. Four microliters of this test solution were injected and calculations were carried out according to the regression equations (Fig. 2). Recoveries of indole and skatole added to 1 g of rectal contents were estimated three times. The results of the recovery test and the determination of indole and skatole were shown in Tables II and III, respectively.

The results of recovery test were satisfactory, and in our laboratory this method has also been successfully employed for the determination of indole and skatole in gastrointestinal contents of growing pigs. This method may be useful for analyses of foods and polluted water samples. Generally speaking, the adequate amounts of gastrointestinal contents to be weighed for the determination are as follows: approximately 5 g of cecal contents, 4 g of colonic and rectal contents of growing pigs and 30 g of rumen liquor of goats. Indole and skatole were not or scarcely detected in 15 g of the contents of stomach, jejunum and ileum

FIG. 2. Gas Chromatogram of Steam Distillate of Rumen Liquor, Injected before (A) and after (B) the Addition of Internal Standard, and an Example of Peak Height Measurement.

For experimental details, see the text.

(1) indole, (2) skatole, (3) 5-chloroindole (internal standard), (4) peak height of 5-chloroindole.

TABLE II. RECOVERIES OF INDOLE AND SKATOLE ADDED TO RECTAL CONTENTS

Indole			Skatole		
Added ^{a)} (μ g)	Found (μ g)	Recovery (%)	Added ^{a)} (μ g)	Found (μ g)	Recovery (%)
46.86	47.25	100.8	52.47	54.22	103.3
46.86	45.26	96.6	52.47	49.82	94.9
46.86	47.55	101.5	52.47	52.04	99.2

^{a)} 10 ml of 40 μ M solution was added to 1 g of rectal contents of a growing pig.

of growing pigs and 7 g of feces of germ-free rats.

TABLE III. AMOUNTS OF INDOLE AND SKATOLE IN GASTROINTESTINAL CONTENTS OF A GROWING PIG AND A CONVENTIONAL AND A DEFAUNATED GOAT

Gastrointestinal contents	Indole ($\mu\text{g/g}$)	Skatole ($\mu\text{g/g}$)
Cecal contents (pig)	trace	8.71
Contents of upper colon (pig)	very small amount	29.80
Contents of middle colon (pig)	4.45	34.70
Contents of lower colon (pig)	6.25	30.30
Rectal contents (pig)	5.65	37.81
Rumen liquor (conventional goat)	1.05	1.73
Rumen liquor (defaunated goat)	1.37	3.80

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