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SINTERED THIN-LAYER CHROMATOGRAPHY WITH FLAME IONIZATION DETECTOR SCANNING*

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SUMMARY

Various organic compounds have been separated by thin-layer cromatography on silica gel sintered glass ceramic sticks with flame ionization detector scanning. Thermolabile or polar compounds not suitable for gas chromatography were successfully detected in amounts of 10^{-6}–10^{-7} g per spot. In this method, the determination of compounds, recovery and reactivation of the sintered sticks are possible in one scanning process.

INTRODUCTION

In a previous paper[1], we reported the preparation and standardization of silica gel and alumina sintered glass ceramic sticks for thin-layer chromatography (TLC) with flame ionization detector (FID) scanning. The application of silica gel sintered sticks to the quantitative determination of lipids in serum[2,3] and of ginseng and related crude drugs in plant components[4] was performed successfully.

In this paper, we describe the qualitative TLC on silica gel sintered sticks of various classes of organic compounds such as sulphonic acids and sulphonamides, alkaloids, amino acids, water-soluble vitamins, pesticides, polychlorinated biphenyls (PCBs), cardiac glycosides, genins, estrogens, progestins, androgens and corticosteroids.

EXPERIMENTAL

Materials and apparatus

Glass ceramic powder. A broken, uncrystallized glass ceramic was ground by ball-milling, screened through a 200-mesh sieve and fractionated by sedimentation in

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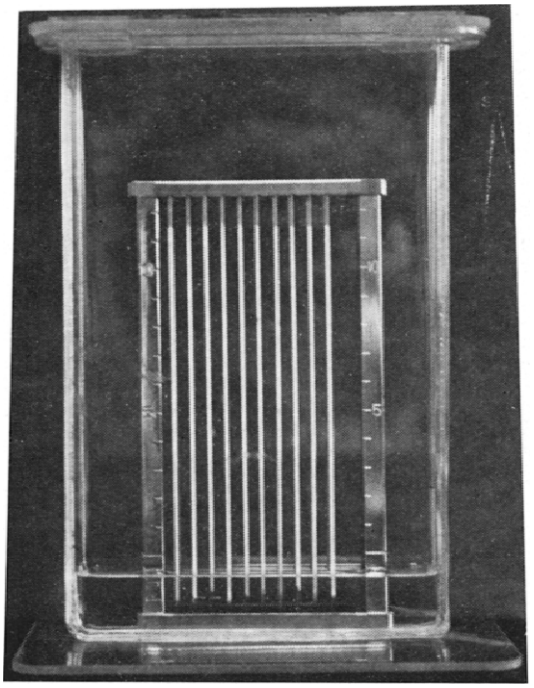

Fig. 1. Thin-layer stick chromatography (TLSC) on silica gel sintered sticks.

water. The glass ceramic powder thus obtained had the same particle size as that of silica gel and alumina for TLC.

*Preparation of sintered glass ceramic sticks**. A mixture of one part of silica gel for TLC and one to three parts of the glass ceramic powder prepared as above was suspended in a solvent such as acetone, ethanol or dioxane. A quartz glass stick (0.9 mm diameter × 153 mm length) was dipped into this suspension for few minutes, and then pulled out slowly. The stick coated with silica gel layer was air-dried. The layer was heated in an electric furnace at 800–1000° for several minutes so as to yield a silica gel–fused glass ceramic layer (layer thickness 50–100 μm) without decreasing the chromatographic activity of the silica gel. Similar sintered sticks were also prepared from alumina and other inorganic porous adsorbents.

FID scanner. A TLC autodetector (Thinchrograph TFG-10, Iatron Laboratories) was used.

Chromatography of organic compounds on silica gel sintered sticks

Developing chamber. For the separation of organic compounds on silica gel sintered sticks, a compact cubic chamber (165 mm height × 85 mm × 13 mm, Fig. 1) lined with silica gel sintered plate[5] to saturate the chamber with the solvent vapour was devised and used throughout this work. This chamber was made of a borosilicate glass in order to prevent contamination by metal oxides in the glass components such

* Sintered glass ceramic sticks are commercially available under the name of Thinchrod (Iatron Laboratories, 1-11-4, Higashi-Kanda, Chyiodaku, Tokyo 101, Japan).

TABLE I

DEVELOPING SOLVENT SYSTEMS

Compound	*Solvent system*	
	No.	*Components**
Sulphonic acids and sulphonamides	I	*n*-Butanol–ethanol–0.1 *N* acetic acid (3:1:1)
Alkaloids	II	Chloroform–diethylamine (30:1)
Amino acids	III	*n*-Propanol–water (4:1)
Water-soluble vitamins	IV	Acetone–water (9:1)
Pesticides and PCBs	V	*n*-Hexane
Cardiac glycosides	VI	Chloroform–methanol (9:1)
Cardiac genins	VII	Ethyl acetate–acetone (3:1)
Estrogens	VIII	Benzene–methanol (9:1)
Progestins	IX	Chloroform–acetone (4:1)
Androgens	IX	Chloroform–acetone (4:1)
Corticosteroids	X	Chloroform–methanol (19:1)
	XI	Chloroform–methanol (9:1)

* Parts by volume.

as Na_2O, K_2O, CaO and MgO, which might cause a high background noise and interfere in the flame ionization detection of organic compounds. A developing frame for the sintered sticks was also devised, which permitted the simultaneous separation of ten sintered sticks (Fig. 1).

Developing solvent systems. Organic solvents and water were purified by distillation prior to use in order to remove non-volatile impurities that might hinder the flame ionization detection. The TLC separation of various classes of organic compounds were performed using 11 solvent systems (Table I).

Preliminary experiments indicated that separations on the sintered sticks were similar to those on the sintered plates[5,6]. We therefore selected solvent systems for use with the sintered sticks based on the results obtained with sintered plates.

Detection. After chromatography on the sintered sticks, the chromatograms were air-dried or heated in an electric oven for several minutes if necessary, and then attached to the FID scanner and passed through the FID flame for detection. Table II

TABLE II

STANDARD CONDITIONS FOR DETECTION

The amount of each compound detected was 0.1–1 μg per spot.

Parameter	*Value*
Quartz stick diameter	0.9 mm
Thickness of the sintered layer	50 μm
Distance between nozzle and stick	1 mm
Hydrogen pressure to FID	1.5 kg/cm²
Air flow-rate to FID	2.0 l/min
Electrometer setting	1×10^{-8} A scale
Scanning speed	210 mm/min
Chart speed	60 mm/min
Detector range	50 mV
Recorder range	100 mV

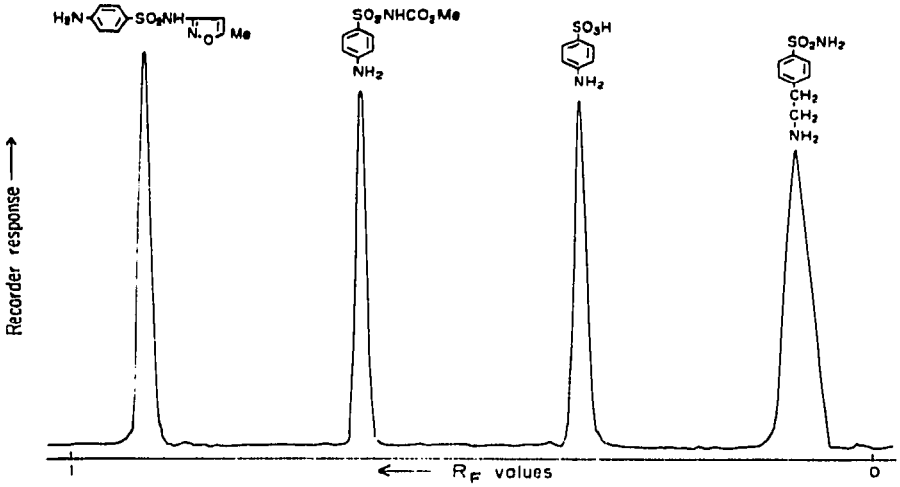

Fig. 2. TLSC separation of sulphanilic acid and sulphonamides on silica gel sintered sticks with an FID scanner. Developing solvent system I.

shows typical conditions for the analysis. It takes approximately 9 min to obtain results with ten sticks.

RESULTS

Separation and detection of various classes of organic compounds on silica gel sintered sticks

Sulphonic acid and sulphonamides. Fig. 2 shows the separation of sulphanilic acid and three sulphonamides with solvent system I. In spite of the presence of

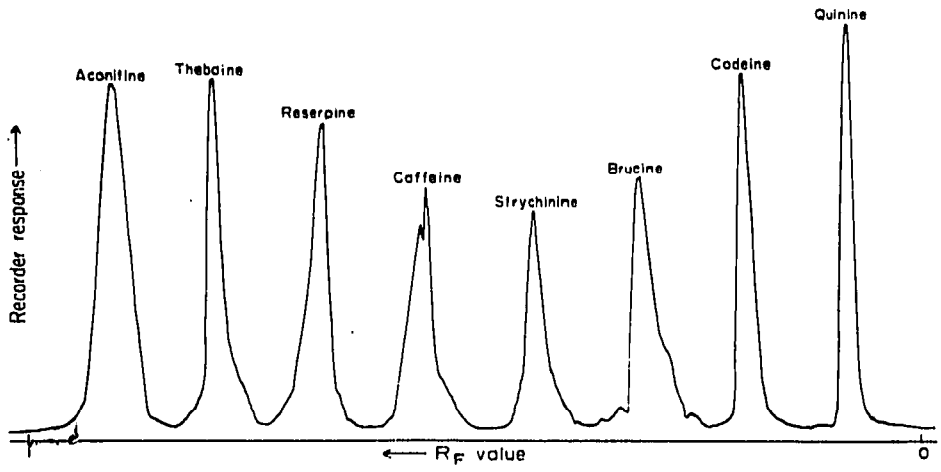

Fig. 3. TLSC separation of alkaloids on silica gel sintered sticks with an FID scanner. Developing solvent system II.

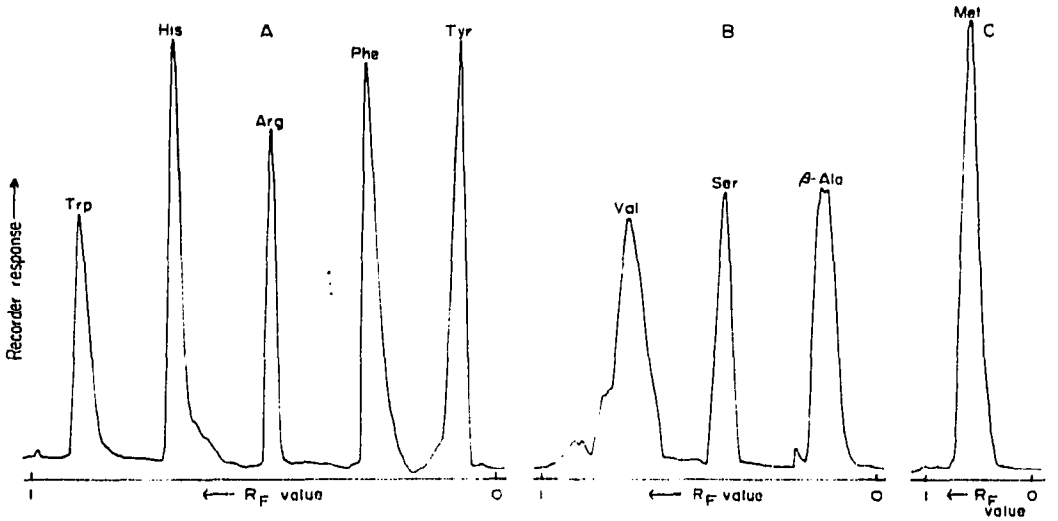

Fig. 4. TLSC separation of amino acids on silica gel sintered sticks with an FID scanner. Developing solvent system III.

nitrogen and sulphur atoms in the molecules, the recorder responses of these compounds were found to be very sensitive.

Alkaloids. Fig. 3 shows the separation of aconitine, thebaine, reserpine, caffeine, strychinine, brucine, codeine and quinine on silica gel sintered sticks with solvent system II and on alumina sintered sticks with the solvent system benzene–chloroform–diethylamine (36:8:1). It is recommended that after chromatography, the sintered sticks should be re-chromatographed with lipophilic solvents such as *n*-pentane in order to remove the excess of diethylamine.

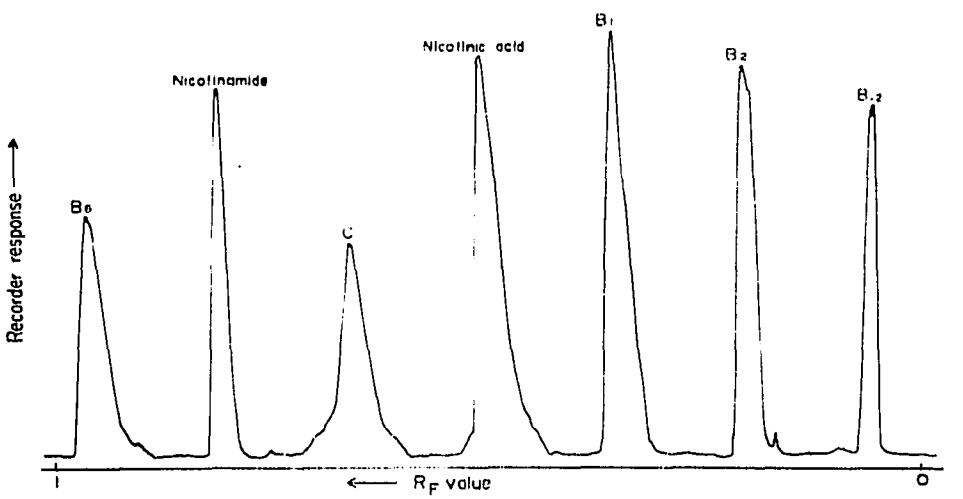

Fig. 5. TLSC separation of water-soluble vitamins on silica gel sintered sticks with an FID scanner. Developing solvent system IV.

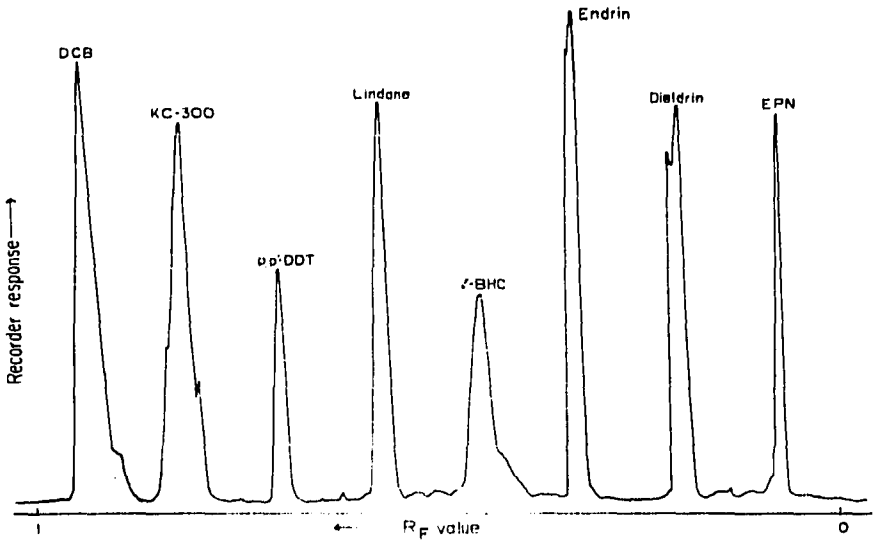

Fig. 6. TLSC separation of PCBs and pesticides on silica gel sintered sticks with an FID scanner. Developing solvent V.

Amino acids. Fig. 4 shows the separation of histidine, arginine (basic), tryptophane, phenylalanine, tyrosine, valine, serine, β-alanine and methionine (neutral) with solvent system III. Sensitive recorder responses were obtained, as in the case of sulphonamides.

Water-soluble vitamins. Fig. 5 shows the separation of pyridoxine (vitamin B_6), nicotinamide, ascorbic acid (vitamin C), nicotinic acid, thiamine (vitamin B_1), ribo-

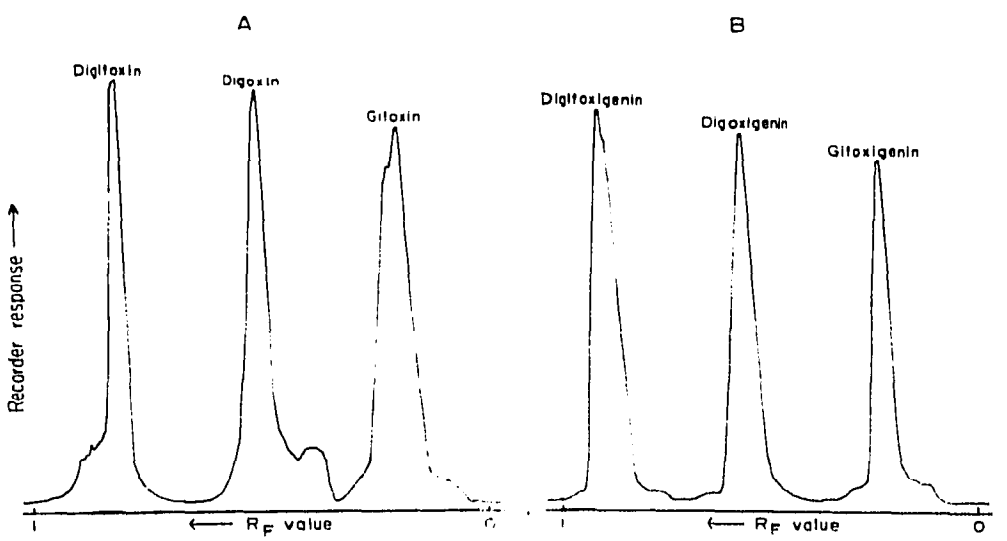

Fig. 7. TLSC separation of cardiac glycosides and genins on silica gel sintered sticks with an FID scanner. Developing solvent systems: A, VI; B, VII.

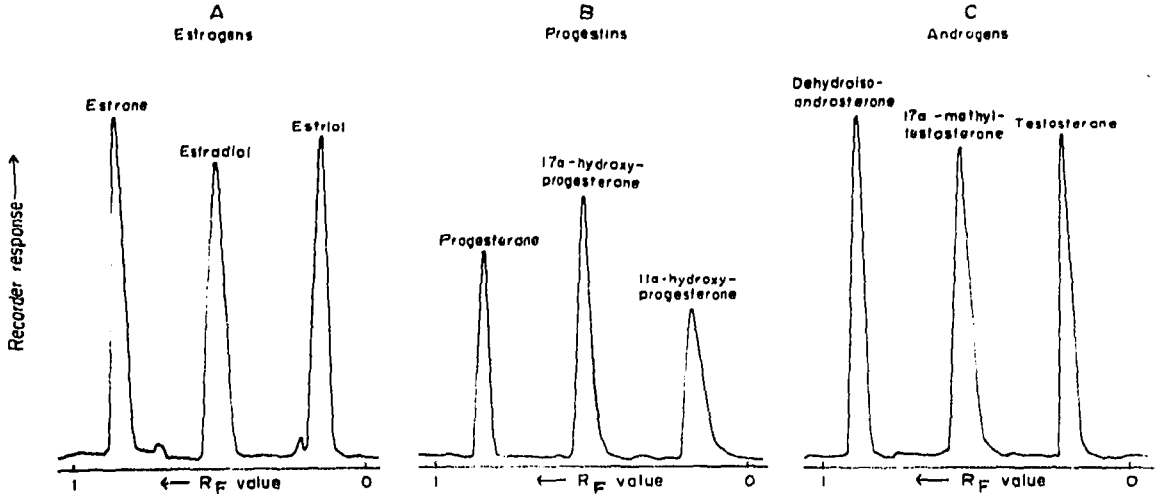

Fig. 8. TLSC separation of steroidal hormones on silica gel sintered sticks with an FID scanner. Developing solvent systems: A, VIII; B and C, IX.

flavin (vitamin B_2) and cyanocobalamin (vitamin B_{12}) with solvent system IV. It was possible to detect the chemically labile compounds such as thiamine and ascorbic acid under the thermal conditions described above.

Pesticides and polychlorinated biphenyls. Fig. 6 shows the separation of decachlorinated biphenyl (DCB), Kanechlor (KC)-300, *p,p'*-dichlorodiphenyltrichloroethane (DDT), lindane, γ-benzenehexachloride (BHC), endrin, dieldrin and phenylphosphonothioic acid O-ethyl O-*p*-nitrophenyl ester (EPN) with solvent system V. The halo-compounds were combustible in the FID flame.

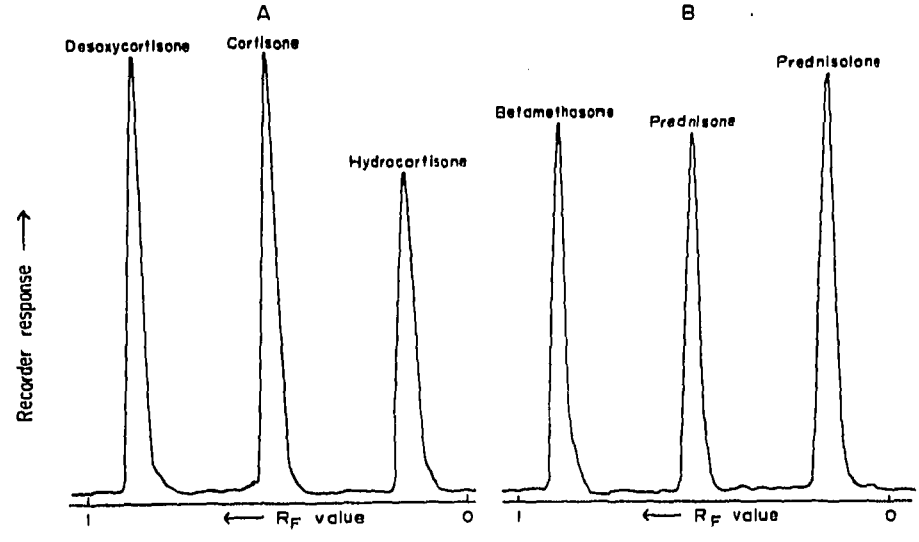

Fig. 9. TLSC separation of corticosteroids on silica gel sintered sticks with an FID scanner. Developing solvents: A, X; B, XI.

Cardiac glycosides and genins. Fig. 7 shows the separation of digitoxin, digoxin and gitoxin with solvent system VI and of digitoxigenin, digoxigenin and gitoxigenin with solvent system VII. Unlike GC, these cardiac components could be determined as the free forms without conversion into volatile silyl ethers.

Steroids. The steroids considered here are stable compounds and are suitable for FID measurement.

Fig. 8A shows the separation of estrone, estradiol and estriol with solvent system VIII, Fig. 8B shows the separation of progesterone, 17α-hydroxyprogesterone and 11α-hydroxyprogesterone with solvent system IX and Fig. 8C shows the separation of dehydroisoandrosterone, 17α-methyltestosterone and testosterone with solvent system IX.

Fig. 9A shows the separation of desoxycortisone, cortisone and hydrocortisone with solvent system X and Fig. 9B shows the separation of betamethasone, prednisone and prednisolone with solvent system XI.

DISCUSSION

For the determination of organic compounds by this FID scanning method, careful pre-treatment of the sintered sticks and the FID is indispensable. The quality and purity of the developing solvents must be checked. In particular, non-volatile organic materials, which greatly influence the recorder response, must be eliminated. After chromatographic separation, satisfactory removal of the developing solvent from the chromatogram is necessary. Room atmospheres contaminated with cigarette smoke or organic solvent vapours are harmful to the FID and must be avoided. In order to eliminate minor heterogeneous substances that are often adsorbed on the sintered sticks, the sintered sticks must be passed through the FID flame prior to TLC separation. In order to avoid such contamination, the sintered sticks must be stored in a desiccator.

In spite of these difficulties connected with the experimental conditions, the thin-layer stick chromatography (TLSC)–FID scanning method has various advantages. It permits rapid qualitative and quantitative TLC separations and FID detection of organic materials to be achieved. Unlike thin-layer densitometry, detection with the FID is easily performed without troublesome detection by colour reagents such as sulphuric acid.

This method will probably have wide applications in clinical, biological, natural-products, medicinal and synthetic chemistry.

REFERENCES

1 T. Okumura and T. Kadono, *Bunseki Kagaku (Jap. Anal.)*, 22 (1973) 980.
2 E. Nakano and T. Kawai, *8th World Congress of Anatomical and Clinical Pathology, Munich, September 1972*, 1972, p. 12.
3 M. Tokunaga, S. Andoh and N. Ueda, *Proc. Jap. Conf. Biochem. Lipids*, 15 (1973) 195.
4 T. Namba, M. Yoshizaki, T. Tomimori, K. Kobayashi, K. Mitsui and J. Hase, *Yakugaku Zasshi (J. Pharm. Soc. Jap.)*, 94 (1974) 252.
5 T. Okumura, T. Kadono and M. Nakatani, *J. Chromatogr.*, 74 (1972) 73.
6 T. Okumura and T. Kadono, *J. Chromatogr.*, 86 (1973) 57.