[Reprinted from the Journal of Pharmaceutical Sciences,
Vol. 58, No. 12, December, 1969, ©1969 by the American Pharmaceutical Association]

Polyamide-Kieselguhr Thin-Layer Chromatography of Antioxidants

HUNG-CHEH CHIANG and REN-GUU TSENG

Abstract ☐ Eight fat antioxidants are identified on the polyamide-kieselguhr G (2:1) mixed layer, which is firmly bonded and easy to handle. The sharp separation is achieved by use of both aqueous and nonaqueous solvent systems.

Keyphrases ☐ Antioxidants—identification ☐ TLC—separation, identification ☐ UV light—TLC spot visualization ☐ Iodine vapor—TLC spot visualization

Thin-layer chromatography of fat antioxidants has been the subject of numerous investigations. The separation by TLC on alumina (1), silica gel (2, 3), silica gel-kieselguhr mix layer (4), acetylcellulose (5), polyamide (6), and polyamide-starch mix layer (7), has been reported, but the application of a mixed polyamide-kieselguhr layer in the chromatographic separation has not. In this note, the separation of eight fat antioxidants by this mixed layer procedure is described.

EXPERIMENTAL

Materials—The solvents and chemicals are reagent grade (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Thin-Layer Plates—Twenty-two grams of polyamide[1] chip was dissolved in a mixture (80:20) of 90% formic acid and distilled water. After obtaining a homogeneous solution with gentle warming and stirring, 10 g. of kieselguhr G (E. Merck) was added. Two hundred milliliters of the above solution was added to a dish (14.5 × 19.5 × 2.5 cm.) and a glass plate (12 × 16 × 0.1 cm.) dipped into it. Both sides of the glass were covered evenly. The glass was placed over the dish for 2 min. to let the excess solution drain. It was then air-dried for 3 hr. and heated at 100° for 30 min.

Chromatographic Procedure—A 0.5% alcoholic solution of antioxidants was applied to the start line 1.5 cm. from the bottom of the layer and developed by ascending techniques. The chamber was equilibrated with the respective solvent for 30 min. before use.

Visualization—The layers were sprayed with 0.07% rhodamine B alcoholic solution and deep violet spots were observed under the UV light. The spots were detected by exposing the plate to iodine vapor.

RESULTS AND DISCUSSION

R_f values obtained with the five solvent systems are given in Table I. It is very interesting to note that the R_f values of the gallic acid ester in the two solvent groups (*N* and *A*) are reversed.

In a nonaqueous solvent system (Group *N*), the R_f values of the gallic acid ester increase with an increase in length of the carbon chain. In this case, the separation is mainly based on the adsorption or partition between the kieselguhr and the ester. In aqueous solvent systems (Group *A*), the R_f values decrease with an increase in length of the carbon chain. The separation mechanism may depend on the reversible formation of hydrogen bonds between the carbonyl-oxygen atom of polyamide and the hydrogen atom of phenolic group in the ester.

The layer is firmly bonded, does not crack, and can be stored easily. Both sides of the glass are independent of each other and chromatography can be performed simultaneously on each side.

Table I—Chromatographic Data

	Group				
	N			*A*	
	Solvent[a]				
	I	II	III	IV	V
	Time, hr.[b]				
Substance	9	2	2.5	2.5	3
n-Propyl gallate	0.26	0.22	0.27	0.63	0.63
Isoamyl gallate	0.36	0.31	0.33	0.61	0.52
Lauryl gallate	0.56	0.54	0.48	0.44	0.45
Cetyl gallate	0.68	0.65	0.53	0.21	0.19
Stearyl gallate	0.72	0.67	0.56	0.08	0.08
Butylated Hydroxyanisole	0.69	0.87	0.74	0.70	0.54
Butylated Hydroxytoluene	0.80	0.95	0.63	0.10	0.14
Ethyl protocatechuate	0.42	0.38	0.37	0.35	0.64

[a] I, isoamyl alcohol; II, isoamyl acetate-acetone (5:1); III, isoamyl acetate-acetone-ethanol (20:1:1); IV, acetone-water (5:3); V, dioxane-water-ethanol (10:7:5). [b] Required to ascend 10 cm. from origin.

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ACKNOWLEDGMENTS AND ADDRESSES

Received June 9, 1969 from the *Department of Pharmacy, Taipei Medical College, Taipei, Taiwan (Republic of China).*

Accepted for publication August 6, 1969.

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[1] Nylon 6, type 1022B of UBE Industrial Ltd., Osaka, Japan. U. S. supplier: American Enka Corp.