Silica gel TLC does not throw any light on the homogeneity of individual *o*- or *p*-alkylphenols having different chain lengths. However, reversed-phase TLC, with the solvent system acetic acid–water (80:20), separates the *o*- or *p*-alkyl phenol homologues due to the difference in the chain length of the alkyl groups. Fig. 3 shows that this technique effectively separates dodecyl, tetradecyl, hexadecyl and octadecyl phenols among the *ortho* or *para* class of compounds (items A, B, C and D; G, H, I and J) but does not distinguish isomeric alkyl phenols (items A and J; B and I; C and H; D and G) which can be separated only by silica gel TLC (Fig. 2). These separations also indicate that resolution of some of the lower and higher homologues is possible. This method is useful in determining their purity. The results of all the separations are summarised in Table I.

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Simple and rapid polyamide chromatography of antioxidants

The use of paper or thin-layer chromatography for the identification of antioxidants had been widely discussed[1–3]. A simple and rapid method for the analysis of antioxidants by polyamide layer chromatography is presented in this note. The results described are obtained with universally used spray reagents and two developing solvent systems.

Experimental

Chemicals. Eight kinds of antioxidant of F.D.A. standard were used. The solvents and chemicals are the first grade of Katayama Chemical Industries, Ltd., Osaka, Japan.

Thin-layer sheets. All the polyamide thin-layer sheets used were 15 × 15 cm and produced by Cheng Chin Trading Co. Ltd., Taipei, Taiwan.

Chromatography. The standard techniques of ascending thin-layer chromatography[4] was employed. The solvent systems were: petroleum ether (30–70°)–benzene–acetone (8:2:5) and acetone–ethyl alcohol–water (4:1:2).

The two spray reagents listed below were sprayed in the order indicated. After spraying the first reagent the chromatogram was dried and observed before spraying with the second reagent.

(1) 5 % (v/v) bromide soln. Dissolve 5 ml of bromine water in 100 ml of carbon tetrachloride.

(2) 0.25 % (w/v) fluorescein sodium soln. Dissolve 250 mg of fluorescein sodium in 5 ml of N,N-dimethylformamide and then add anhydrous ethanol to 100 ml.

TABLE I

CHROMATOGRAPHIC DATA OF PURE COMPOUNDS

System (I) petroleum ether (30–70°)–benzene–acetone (8:2:5); (II) acetone–ethyl alcohol–water (4:1:2).

No.	*Antioxidant*	R_F *values in system*		*Color* of spot at each stage of spraying*	
		I	*II*	*Bromine*	*Fluorescein sodium soln.*
1	Butyl hydroxytoluene	0.98	0.33	B	Y
2	Butyl hydroxyanisole	0.96	0.46	B	Y
3	Stearyl gallate	0.64	0.08	B	Y
4	Cetyl gallate	0.48	0.20	B	Y
5	Lauryl gallate	0.37	0.30	B	Y
6	Ethyl protecatechuate	0.31	0.57	B	Y
7	Amyl gallate	0.17	0.49	B	Y
8	*n*-Propyl gallate	0.09	0.53	B	Y

* B = Brown; Y = yellow.

Results and discussion

The R_F values of the antioxidants are given in Table I and show an excellent separation. The minimum amounts of the compounds detectable by the method are: *n*-propyl gallate and amyl gallate — 0.5 μg; ethyl protocatechuate, lauryl gallate, stearyl gallate and butyl hydroxyanisole — 1 μg; cetyl gallate — 2.5 μg; and butyl hydroxytoluene — 4 μg. In all cases visible spots are obtained after spraying with bromine and fluorescein sodium solutions. This method produces brown or yellow spots on a pink background.

The chromatographic results are highly reproducible. Another important feature of the polyamide layer chromatogram is its capability of direct filing.

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