Thin-layer systems giving maximum separation of α- and β-tocopherols

No thin-layer adsorption technique has been described which will resolve α- and β-tocopherol as effectively as zinc carbonate impregnated paper[1]. SEHER[2] has reported useful separations of tocopherols on thin layers of alumina with benzene as developing solvent and on silica gel with chloroform as solvent. DILLEY AND CRANE[3] separated α- and β-tocopherol on Silica Gel G using benzene and BOLLIGER[4] reported better separation on Silica Gel G with benzene–methanol (98:2) than with cyclohexane–diethyl ether (80:20). Secondary magnesium phosphate, which gives a useful separation of α- and β-tocopherols by column chromatography[5], proved slightly inferior to silica gel as a thin layer[4].

SCHMANDKE[6], however, reported an excellent separation of α- from γ-tocopherol (which should migrate close to β-tocopherol) on a mixed layer of alumina–zinc carbonate (3:1) with chloroform as developing solvent. A mixed layer of silica gel–zinc carbonate (1:1) with benzene–chloroform (1:1) as developing solvent gave poor resolution.

A search for thin-layer chromatographic systems giving maximum separations of α- and β-tocopherol has been conducted in this laboratory and the results are shown in Table I.

Aluminium oxide–zinc carbonate layers were prepared by mixing 30 g Aluminium Oxide G (Merck) and 10 g zinc carbonate (Basic, British Drug Houses Ltd.) with 60 ml water (or 60 ml 0.001 % dichlorofluorescein in water) in a blender for 15–30 sec and allowing the bubbles to break before spreading. Kieselgur–zinc carbonate layers were prepared by blending 16 g Kieselgur G (Merck) and 8 g zinc carbonate (2:1) or 12 g Kieselgur G and 12 g zinc carbonate (1:1) with 50 ml water (or 0.001 % dichlorofluorescein) for 15–30 sec and spreading as before. Silica Gel H (Merck) and zinc carbonate were mixed in the ratio of 10:1, slurried and layers prepared. Zinc sulphate was incorporated into Silica Gel G and H layers by slurrying the absorbent with 5 % zinc sulphate solution in place of water. In one instance zinc carbonate was incorporated into a Kieselgur G layer by slurrying 10 g Kieselgur G with 10 ml water + 10 ml zinc ammonium carbonate solution[1] which was also used to impregnate papers. Florisil layers were prepared by slurrying 15 g Florisil (Floridin Co,, W. Virginia thin-layer grade as supplied by Fisher Scientific Co., New Jersey) with 45 ml water (or 0.001 % dichlorofluorescein).

All layers were 250 μ thick and were activated at 120° for 30 min immediately before use. Spots were detected by viewing under U.V. light or by staining with iodine vapour.

Best separations were achieved using mixed layers of Aluminium Oxide G–zinc carbonate (3:1) and Kieselgur G–zinc carbonate (2:1) using the solvents chloroform and benzene–cyclohexane (30:70), respectively (Table I). The efficiency of these systems in resolving α- and β-tocopherol came close to that of zinc carbonate treated paper. Occasionally when using the alumina–zinc carbonate layer with chloroform as developing solvent, the resolution was greater than that ever obtained with freshly prepared zinc carbonate papers. Incorporation of zinc carbonate or zinc sulphate into layers of Silica Gel G and H did not improve the resolution over that obtained with the respective layers alone. Increasing the ratio of zinc carbonate to either aluminium oxide or Kieselgur to 1:1 (w/w) did not increase the resolution but did allow greater

J. Chromatog., 29 (1967) 293–295

TABLE I

APPROXIMATE $R_F \times 100$ VALUES OF α- AND β-TOCOPHEROLS ON VARIOUS ADSORBENTS

Absorbent	*Solvent*	$R_F \times 100$		*Difference*	*Reference*
		α-Tocopherol	*β-Tocopherol*		
Silica Gel G	Chloroform	58	35	23[b]	2
	Benzene	50	32	18[b]	3, 7
	Benzene–methanol (98:2)	65	61	14[b]	4
	Cyclohexane–ether (80:20)	32	30	2[b]	4
Silica Gel G–zinc carbonate (1:1)	Chloroform–Benzene (50:50)	83	80 (as γ)	3[b]	5
Aluminium oxide	Benzene	56	34	22[b]	2
Aluminium Oxide G–zinc carbonate (3:1)	Chloroform	72	42 (as γ)	30[b]	5
	Chloroform	92	54	38	
	Benzene	48	18	30	
Sec. magnesium phosphate	Petroleum ether–ether (85:15)	86	76	10[b]	4
Florisil	Benzene	56	41	15	
Silica Gel G–zinc sulphate[a]	Benzene	50	32	18	
Silica Gel H	Benzene	46	30	16	
Silica Gel–zinc sulphate[a]	Benzene	46	31	15	
Silica Gel–zinc carbonate (10:1)	Benzene	44	29	15	
Kieselgur G	Cyclohexane	54	37	17	
Kieselgur–zinc ammonium carbonate[a]	Cyclohexane	55	34	21	
Kieselgur–zinc carbonate (1:1)	Cyclohexane–benzene (70:30)	47	17	30	
	Cyclohexane–benzene (50:50)	60	27	33	
	Benzene	81	49	32	
Kieselgur–zinc carbonate (2:1)	Cyclohexane–benzene (70:30)	70	33	37	
Zinc carbonate impregnated paper	Cyclohexane	73	31	42	

[a] See text.

[b] From published data.

loading of the Kieselgur G–zinc carbonate layer. Florisil and Kieselgur G layers produced elongated spots and therefore poor resolution. Both the Aluminium Oxide G–zinc carbonate and Kieselgur G–zinc carbonate layers have been used in this laboratory to assess the purity of the α-tocopherol separated from a leaf extract by TLC. The α-tocopherol isolated after a single run on Silica Gel G with benzene as the developing solvent[3,7] was rechromatographed on one of the above layers. The homogeneity of the preparation was judged by the number and size of the spots observed in this second run. Possible interference with the assay could also be checked by comparing the values obtained before and after the run on the mixed absorbent.

The Aluminium Oxide G–zinc carbonate (with dichlorofluorescein) layer has been used in two dimensions in a manner analogous to that usually employed with paper chromatography in the estimation of tocopherols in animal and plant tissues[8,9]. Acetone–petroleum ether extracts of 0.3 g of white clover and Xanthium leaf tissue[7] were applied as a 4 cm band near one corner of a 20 cm^2 layer and development in one direction carried out with benzene. After allowing the layer to dry for 2–3 min the plate was dipped into solution of 2.5 % of liquid paraffin (B.P. grade) in petroleum ether so that the level of the paraffin solution almost reached the material separated in the first run. Following evaporation of the petroleum ether, the plate was developed in the second direction using methanol as developing solvent. When viewed under U.V. light the most prominent spot, R_F 0.50 in the first direction and 0.30 (measured from limit of paraffin impregnation) in the reverse phase direction, was α-tocopherol. This was readily recoverable for analysis.

α-Tocopherol was purified by preparative TLC from DL-α-tocopherol (C-Grade, Calbiochem, Los Angeles) and β-tocopherol was a generous gift from Dr. J. GREEN, Vitamins Ltd., Tadworth, England.

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Received February 20th, 1967