SEPARATION OF GALLIC ACID AND ITS ESTERS ON THIN LAYERS OF POLYAMIDE POWDER

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In recent years gallic acid and its esters have found increasing application as stabilizers of oils and fats, especially those of animal origin. These substances are preferred in cases when a prolonged storage period of the prepared fat before its consumption is anticipated. In most countries only certain specified antioxidants in very low concentrations (0.01–0.05%) are permitted. Consequently reliable methods for detection and determination of these substances appeared necessary.

In the methods generally used, the isolation of antioxidants from oil or fat is effected by extraction with dilute alcohol or another suitable organic solvent, and the extracts thus obtained separated by chromatography. A series of paper chromatographic methods have been described, *e.g.* ZIJP[1], GANDER[2], DEHORITY[3], and others. Acetylated paper, or a paper impregnated with a fixed, less polar phase, usually paraffin or olive oil is employed. One disadvantage of these paper chromatographic methods lies in the prolonged development time of the chromatogram, usually about 3–9 hours, according to the complexity of the sample to be separated. Antioxidants can be detected much more quickly using thin-layer chromatography. The method was first used by SEHER[4,5], who succeeded in separating some antioxidant mixtures on a thin layer of silica gel. The same author separated mixtures of even 10 antioxidants using two-dimensional chromatography.

In our laboratory a method using chromatography on thin layers of polyamide powder has recently been elaborated[6]. Polyamide had already been used as a chromatographic adsorbent, especially for the isolation of phenolic substances. The advantages of polyamide chromatography lie not only in the high adsorption capacity, but also in the reversibility of the sorption process which allows the use of this adsorbent for analytical purposes. We used thin-layer chromatography on polyamide powder some time ago for separation of some flavonoids and antioxidants[7,8], and in the present paper the chromatographic separation of gallic acid and its esters is described.

EXPERIMENTAL

Solutions of gallic acid and its methyl, ethyl, *n*-propyl, lauryl, and *n*-octyl esters were prepared in a concentration of about 1% in pure methanol.

As the solvent system the following solvents or their mixtures were used: methanol, anhydrous ethanol, *n*-butanol, acetone, petroleum ether (b.p. 60–70°), ether, benzene, chloroform and carbon tetrachloride.

TABLE 1

R_F VALUES OF GALLIC ACID AND ITS ESTERS

Chromatographic system	R_F*					
	GA	MG	EG	PG	OG	LG
Methanol	0.32	0.60	0.70	0.62	0.71	0.62
Ethanol	0.31	0.53	0.67	0.55	0.80	0.80
Chloroform	0.0	0.0	0.0	0.0	0.09	0.12
Carbon tetrachloride	0.0	0.0	0.0	0.0	0.0	0.08
Ether	0.04	0.07	0.13	0.28	0.45	0.67
Butanol–acetic acid–water (4:1:5)	0.28	0.60	0.70	0.68	0.89	0.89
Carbon tetrachloride–methanol (7:3)	0.06	0.29	0.39	0.46	0.63	0.80
Carbon tetrachloride–ethanol (7:3)	0.05	0.19	0.26	0.44	0.62	0.80
Carbon tetrachloride–methanol (3:2)	0.10	0.39	0.52	0.52	0.70	0.77
Carbon tetrachloride–ethanol (3:2)	0.08	0.33	0.44	0.47	0.73	0.85

* GA = gallic acid; MG = methyl gallate; EG = ethyl gallate; PG = propyl gallate; OG = octyl gallate; LG = lauryl gallate.

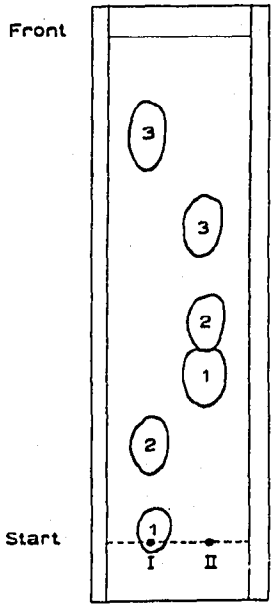

Fig. 1. Chromatogram of a mixture of gallic acid and its esters. Solvent system: carbon tetrachloride–ethanol (7:3). Development time: 45 min. Temperature: 20°. Polyamide powder graining: 0.15 mm. Layer thickness: 1 mm. (I) Spot No. 1 = gallic acid; 2 = methyl gallate; 3 = lauryl gallate. (II) Spot No. 1 = ethyl gallate; 2 = propyl gallate; 3 = octyl gallate.

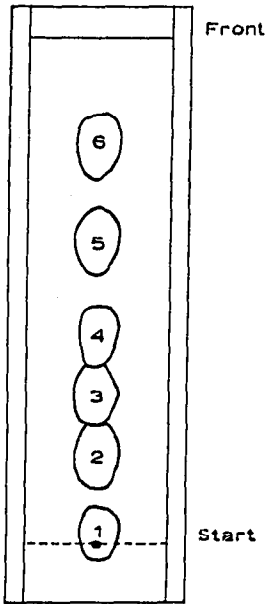

Fig. 2. Chromatogram of a mixture of gallic acid and its esters. Solvent system: carbon tetrachloride–ethanol (7:3). Development time: 50 min. Temperature 20°. Polyamide powder graining: 0.15 mm. Layer thickness: 1 mm. Spot No. 1 = gallic acid; 2 = methyl gallate; 3 = ethyl gallate; 4 = propyl gallate; 5 = lauryl gallate; 6 = octyl gallate.

The polyamide powder used was the commercial product manufactured by the firm Severočeské chemické závody Lovosice, n.p., factory "Rudník". Before use the product was sieved through standard sieves. The optimal grain size for the preparation of the plates is 0.1–0.2 mm.

The chromatographic plates were prepared according to DAVÍDEK AND PROCHÁZKA[6] by applying the polyamide powder to plates of sheet glass 60 × 350 mm, and spreading it roughly with a spatula. Perfect smoothness was achieved by carefully rolling the layer with a simple roller consisting of a glass rod fitted at both ends with a piece of rubber tubing. The breadth of the prepared plate was controlled by the distance between the two pieces of tubing, and the thickness of the chromatographic layer depended on the wall thickness of the tubing.

The sample was applied, taking care not to damage the layer (holding the micropipette at a distance of about 1–2 mm). After the sample had dried the plate was placed into the chamber at an angle of 20–30°, and was developed by the ascending technique until the solvent front had penetrated for some 30 cm (or less, according to the complexity of the mixture to be separated). The wet plate was sprayed in the usual manner with the reagent; this operation, however, required great care in order to avoid disturbing the thin layer by the stream of spray mist.

DISCUSSION

In preliminary experiments we found this method suitable for the separation of gallic acid and its esters. It was first necessary to establish the simplest solvent systems which would give separation of the greatest possible number of the gallates examined. The results of these experiments are shown in Table I and Figs. 1 and 2.

The most advantageous solvent systems for the separation of gallic acid and its esters were mixtures of methanol or ethanol with carbon tetrachloride, in various ratios. Best results were obtained with the mixtures ethanol–carbon tetrachloride, 3:7. Using this system, all the substances studied could be separated.

CONCLUSION

The newly elaborated method for the separation of gallic acid and its esters on a thin layer of polyamide powder shows several advantages, particularly in speed, in which it surpasses all previous methods. The preparation of the chromatographic plates is very rapid and simple, and the time required for development even of a very complex mixture (6 components) does not in any case exceed 60 minutes. The method is feasible in any routine laboratory without special and expensive equipment.

SUMMARY

Chromatographic separation of gallic acid and its esters on a thin layer of polyamide powder has been described. The method is rapid, convenient and feasible even in laboratories with minor equipment. The method was successfully tested in the separation of substances isolated from stabilized fats.

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