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Magnesol thin-layer chromatography of flavonoid compounds*

Recent investigations in this laboratory concerned with the distribution of anthocyanin and its precursors in corn (*Zea mays* L.) revealed the presence of quercetin and its 3-glycoside, isoquercitrin in seed and pollen. Furthermore, a glycosyltransferase enzyme was discovered in pollen which catalyzed the addition of glucose to quercetin. The characterization of the enzyme required a simple, efficient, quantitative method of separating quercetin from isoquercitrin.

Of the several chromatographic techniques that have been used in the separation and identification of flavonoid compounds, paper chromatography has been the most extensively used[1]. ICE AND WENDER[2] and WATKIN[3] have used column chromatography with Magnesol as the adsorbent for the large scale separation and purification of these compounds. Although their methods are reliable they require too much material and consume too much time to assay for particular flavonoids in small quantities. Numerous thin-layer systems have been developed in recent times for the chromatographic separation of flavonoid compounds[4–12]. Although these systems were effective in the qualitative separation of quercetin from isoquercitrin they were not readily adapted to the quantitative estimation of isoquercitrin. The successful use of Magnesol as an adsorbent in the column chromatographic separation of flavonoid compounds[2,3] strongly suggested its use as a thin-layer adsorbent if it could be applied to the thin-layer plates.

This communication describes a method for the preparation of Magnesol thin-layer plates and their use in the separation and quantitative estimation of flavonoid compounds.

Materials and methods

Magnesol** (magnesium acid silicate) used in these studies was obtained from Waverly Chemical Company, Guilford, Conn. The product obtained commercially was used satisfactorily without any further treatment. Five 20 × 20 cm thin-layer plates were prepared by vigorously mixing 36 g of Magnesol, 4 g of anhydrous calcium sulfate and 110 ml of distilled deionized water in a mortar with pestle for 1 min. This slurry was applied to the plates with a Desaga Brinkman applicator to a thickness of 0.5 mm. The plates were then air dried overnight after which they were ready for use. The plates were divided into strips 2 cm wide by 12 cm in length measured from the base of the plate with the origin at a distance 2 cm from the base of the plate.

Standard flavonoid compounds, with the exception of isoquercitrin, were obtained from commercial sources (*trans*-cinnamic acid, *p*-coumaric acid, caffeic acid from K. & K. Laboratories, Plainview, N.Y.; quercetin from Sigma Chemical Company, St. Louis, Mo.; quercitrin from Calbiochem, Los Angeles, Calif. and chlorogenic

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acid from General Biochemicals, Chagrin Falls, Ohio) and were used without further purification. Isoquercitrin was prepared by the hydrolysis of rutin and isolated by Magnesol column chromatography according to the method of Fox *et al.*[14]. A 10 μl quantity of each of these standards suspended in ethanol was applied to the thin-layer plate with a 0.01 ml Aloe Technipet (Aloe Scientific, St. Louis, Mo.).

Commercially available solvents were used without purification, toluene (T) and formic acid (FA) were reagent grade and ethyl formate (EF) was practical grade. The solvent system used was T–EF–FA (5:4:1). After addition of fresh solvent the development tanks were allowed to equilibrate overnight before use. Chromatograms were developed at room temperature (*ca.* 23°) without protection from normal room lighting. Development time was approximately 40 min. Following development the spots were located by scanning the chromatogram with a short wave UV light (254 nm). A more pronounced fluorescence or absorption could be obtained by spraying the chromatogram with 20 % sodium carbonate.

Quantitative estimations were made by scanning the developed air dried plates with UV light to locate the isoquercitrin. The Magnesol area containing the isoquercitrin was then scraped from the plate and suspended in 1 ml of distilled water and the suspension acidified with 0.1 ml of 3 *N* HCl. Three milliliters of ethyl acetate (Fisher certified) were added and the suspension mixed well after which it was centrifuged to separate the organic and aqueous layers. The organic layer was drawn off and its absorbance determined at 360 nm in a Beckman DB-G spectrophotometer. Isoquercitrin content in the ethyl acetate fraction was then determined by reading the absorbance from a previously prepared standard curve for a concentration series of pure isoquercitrin in ethyl acetate.

Photographic records of the chromatograms were made using a Polaroid MP-3 Industrial View Land Camera (Polaroid Corporation, Cambridge, Mass.) fitted with a 127 mm lens to which was attached a Wratten 2A filter. Black and white Polaroid type 47 film was used in these studies, with a shutter setting of f16 and time of exposure of 5 sec.

A Chromato-Vue fluorescence analysis cabinet (model C-5, Ultraviolet Products, Inc., San Gabriel, Calif.) equipped with two short wave UV lights was used in photographing the plates under incident UV radiation. The filter in the visor, for protection of the eyes, was removed during photography. The plate was positioned 1.5 in. below the light sources during photography. A similar arrangement for photography under UV radiation is described by Jones *et al.*[15].

Chromatograms were developed, air dried, sprayed with 20% sodium carbonate and again air dried before photographing them.

Results and discussion

A Polaroid print of a typical Magnesol chromatogram is shown in Fig. 1. Dividing the thin layer into equal strips as shown in Fig. 1 eliminated the problem of uneven solvent migration during development of the chromatogram. Fig.1 illustrates the type of separation routinely obtained with cinnamic acid (No. 1), its 3-hydroxy derivative (No. 2) and its 3,4-dihydroxy derivative (No. 3). The remaining portion of the plate shows the chromatographic separation of quercetin (No. 4), its rhamnoside (No. 5) its glucoside (No. 6) and the quinic acid ester of caffeic acid (No. 7). In Fig. 1 the absorbent spot at R_F 0.72 in the quercitrin run is an impurity in the sample. With the

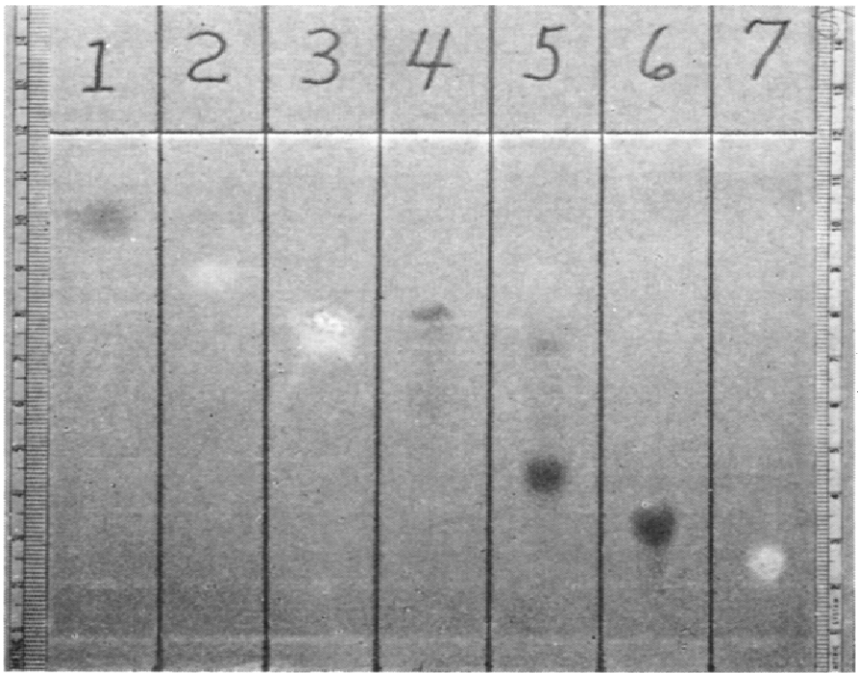

Fig. 1. Polaroid print of an ascending Magnesol chromatogram of flavonoid compounds. Solvent: toluene–ethyl formate–formic acid (5:4:1). Sample indentity: 1, *trans*-cinnamic acid; 2, *p*-coumaric acid; 3, caffeic acid; 4, quercetin; 5, quercitrin; 6, isoquercitrin; 7, chlorogenic acid. Film: black and white type 47. Exposure: f16, 5 sec, Polaroid MP-3 camera. Origin at 2 cm.

Magnesol thin-layer system these aromatic compounds are easily detected with or without indicator spray under UV light.

In these studies the Magnesol thin-layer system proved to be superior to other methods[4–12] employing silica gel, polyamide or cellulose for the separation of flavonoid compounds. In addition several other advantages of this procedure may be noted. Magnesol plates were easily prepared as the thin-layer material can be used without any pre-treatment and the plates required no oven drying. The plate surface was quite

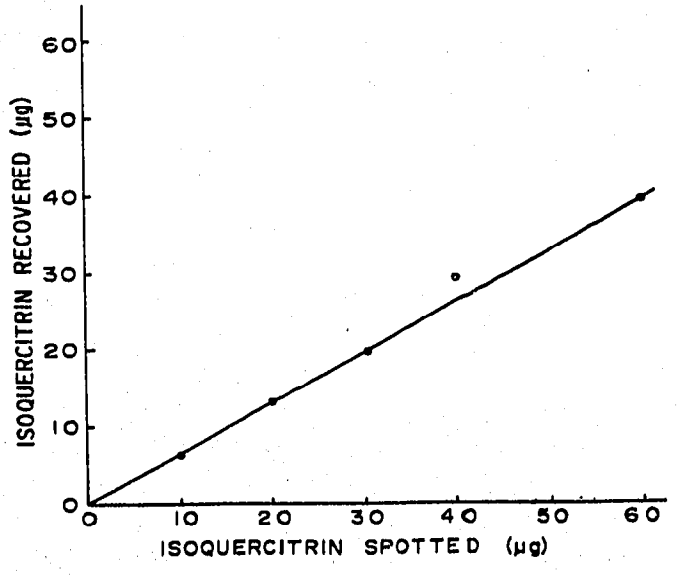

Fig. 2. Recovery of standard isoquercitrin from the Magnesol thin layer.

stable to handling and the samples were easily spotted without disturbing the surface of the layer.

Recoveries obtained from the elution of concentration series of standard isoquercitrin from the Magnesol thin-layer plates are shown in Fig. 2. The data incorporated in this plot were collected from the elution of ten separate concentration series over a period of time and the average recovery for the combined series was 66%. The ease with which quantitative as well as qualitative data can be obtained with the Magnesol thin-layer system should make it useful in the assay of flavonoid compounds in chemotaxonomic studies.

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