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Note

Paper chromatography of phenolic compounds using buffered acetonitrile

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A rapid high-resolution paper chromatographic procedure was reported by Hendrick and co-workers[1,2] for the separation of purine and pyrimidine bases using aqueous acetonitrile buffer systems. Gabriel[3] studied the application of such systems to nucleic acids and found them to be very useful and versatile. Heimer and co-workers[4,5] applied acetonitrile–aqueous ammonium acetate as a solvent system for amino acids and carbohydrates and found it to be efficient in the rapid paper chromatography of these compounds, particularly for teaching purposes. Special features[3] of the system are: (1) rapid development time (10 cm travel by the solvent takes less than 1 h on Whatman 3MM paper); (2) small spot area; (3) simplicity of preparation compared with conventional systems; (4) short drying times; and (5) papers do not have to be pre-equilibrated. The useful features attributed to buffered acetonitrile systems for paper chromatography prompted us to confirm these findings and to evaluate their applicability in the rapid analysis of certain other classes of compounds. We present in this paper the results of the analysis of amino acids, carbohydrates, anthocyanins, flavonoid glycosides and their aglycones and other phenolic compounds.

EXPERIMENTAL

Chromatography was carried out at room temperature on Whatman 1MM and 3MM paper strips by the ascending technique without prior equilibration. As chromatographic chambers, we used readily available rectangular and cylindrical specimen jars with glass covers. The compounds were spotted at one end of the paper and the other end was fixed to the cover plate by means of adhesive tape and then suspended in the chamber. This apparatus is simple and can be conveniently assembled by students. When the solvent front had moved about 20 cm, the papers were removed from the jars and dried. The spots were rendered visible by spraying with ninhydrin for amino acids[6] and diazotised benzidine[7] for phenols and flavonoid glycosides. The flavonoid glycosides were also detected by their colour with ammonia vapour and their fluorescence under UV irradiation. Anthocyanins and anthocyanidins were located by their own colour, carbohydrates by spraying with aniline hydrogen phthalate[8] and cyclitols and sugar alcohols with alkaline silver nitrate solution[9,10].

Solvent system

Acetonitrile was distilled over phosphorus pentoxide before use. A 0.1 *M* aqueous solution of ammonium acetate was used as the buffer. The following proportions of acetonitrile to 0.1 *M* ammonium acetate solution were used: 7:3, pH 7 (solvent A); 7:3, pH 4 (solvent B); 7:3, pH 9 (solvent C); 6:4, pH 7 (solvent D); 6:4, pH 4 (solvent E). The pH of the developing solvent was determined by using BDH (Poole, Great Britain) pH papers and adjusted by the addition of acetic acid or ammonia solution. The pH of these solvents did not change over a period of 24 h.

RESULTS AND DISCUSSION

Chromatographic paper

Both Whatman 1MM and 3MM paper gave similar results. The developing time for a 20-cm movement of solvents A, B and C was about 2 h for Whatman 1MM and 1¾ h for 3MM paper. The development time was a little longer with solvents that contained a greater proportion of water. The chromatographic data given in the tables are based on triplicate determinations and are the values obtained on Whatman 1MM paper.

Amino acids and carbohydrates

Our results agree with those previously published[4,5]. Good resolutions were obtained by using solvent system A for both amino acids and carbohydrates. Table I lists the R_G values of several sugars and their derivatives.

TABLE I

R_G VALUES OF MONOSACCHARIDES, DISACCHARIDES, SUGAR ALCOHOLS AND CYCLITOLS USING ACETONITRILE–0.1 *M* AMMONIUM ACETATE (7:3), pH 7, AS SOLVENT SYSTEM

Sugars	R_G *value**	*Sugars*	R_G *value**
Monosaccharides		*Sugar alcohols*	
Glucose	1.00	Arabinitol	1.00
Galactose	0.87	Galactitol	0.96
Fructose	1.00	Glycerol	1.28
Arabinose	1.07	Mannitol	1.06
Xylose	1.18	Sorbitol	0.96
Mannose	1.11		
Rhamnose	1.27	*Cyclitols*	
		Bornisitol	1.22
Disaccharides		Inositol	0.65
Lactose	0.51	Quebrachitol	1.00
Maltose	0.73		
Sucrose	0.82		
Cellobiose	0.60		

* $R_G = \frac{\text{distance travelled by substance}}{\text{distance travelled by glucose}}$

Anthocyanins and anthocyanidins

Anthocyanins and anthocyanidins would be expected to behave in a similar manner to amino acids because of their ionic nature, and this was found to be so.

Solvent system B gave an excellent resolution of anthocyanins and anthocyanidins (Table II). A pH of 4.5 was used because flavylium chlorides are stable only in acidic solution. The following are the most important observations: (1) a development time of 15 min (10 cm movement of solvent) is sufficient to effect excellent resolution; (2) a distinction is possible between the glycosides and the corresponding aglycones, the aglycones having a greater mobility than their glycosides, and among the most important aglycones, namely pelargonidin, cyanidin, delphinidin and petunidin; (3) the R_F values of the glycosides decrease in the order pelargonin > cyanin > petunin > violanin, and the order is the same for the aglycones.

TABLE II

R_F VALUES OF ANTHOCYANINS AND THEIR AGLYCONES USING ACETONITRILE–0.1 *M* AMMONIUM ACETATE (7:3), pH 4.5, AS SOLVENT SYSTEM

Compounds	R_F *value*	*Colour*
*Anthocyanins**		
Pelargonin	0.63	Orange
Cyanin	0.48	Pink
Petunin	0.43	Pink
Violanin	0.33	Purple
*Anthocyanidins***		
Pelagonidin	0.91	Orange
Cyanidin	0.64	Pink
Petunidin	0.49	Pink
Delphinidin	0.45	Purple

* Cyanin from roses, violanin from *Solanum melongena*, petunin from *Petunia* and pelargonin from *Punica granatum*.

** Anthocyanidins were prepared by the acid hydrolysis of the glycosides.

Flavonoids

The utility of this solvent system for flavonoid compounds was examined for some typical flavonoid glycosides (Table III). Solvent system A again gave an acceptable resolution. The non-planar flavonoids, *e.g.*, dihydroquercetin, seemed to

TABLE III

R_F VALUES OF FLAVONOIDS USING ACETONITRILE–0.1 *M* AMMONIUM ACETATE (7:3), pH 7, AS SOLVENT SYSTEM

Glycoside	R_F *value*	*Colour under UV irradiation*	*Colour on exposure to ammonia vapour*
Kaempferol	0.91	Yellow	Yellow
Quercetin	0.77	Yellow	Yellow
Quercetrin	0.45	Purple	Yellow
Rutin	0.44	Purple	Yellow
Quercimeritrin	0.82	Yellow	Yellow
Naringenin	0.99	—*	—
Naringin	0.86	Pink	Pink
Dihydroquercetin	0.98	—	—
Catechin	0.82	—	—
Epicatechin	0.82	—	—

* — = Colourless.

have higher R_F values than the planar compounds. Solvent systems D and E were not satisfactory as considerable streaking occurred.

Plant phenolic acids

Some simple and commonly occurring plant phenolic acids were examined (Table IV). Solvents A and D gave similar results. Solvents B and E caused most of the compounds to move closer to the solvent front, with very close R_F values. Some of the compounds were well resolved and the solvent system appears to be satisfactory for the rapid analysis of these compounds.

TABLE IV

R_F VALUES OF PHENOLIC ACIDS USING ACETONITRILE–0.1 *M* AMMONIUM ACETATE (7:3), pH 7, AS SOLVENT SYSTEM

Compound	R_F *value*	*Compound*	R_F *value*
Gallic acid	0.06	Sinapic acid	0.19
Protocatechuic acid	0.11	Ferulic acid*	0.29
p-Hydroxybenzoic acid*	0.32	Caffeic acid*	0.19
2,4-Dihydroxybenzoic acid	0.25	*p*-Coumaric acid	0.21
2,6-Dihydroxybenzoic acid	0.55	Chlorogenic acid	0.13

* Elongated spot.

CONCLUSION

The above results with amino acids and carbohydrates confirm, in general, the observations of Heimer and co-workers[4,5], and paper chromatography with the buffered acetonitrile solvent system has also been shown to be suitable for the analysis of anthocyanins, flavonoids and phenolic acids. Good resolutions together with high migration rates are advantageous features of the method. The paper chromatographic analysis of amino acids and sugars, which are not normally included in undergraduate practical courses owing to the long development time required with conventional systems, can now easily be included in such courses.

In general, acetonitrile–0.1 *M* ammonium acetate (7:3), at pH 7 was found to be the best solvent system, except for anthocyanins, which require an acidic pH. While an increase in the acetonitrile content did not alter the resolving ability of the solvent, an increase in the proportion of the buffer gave an inferior system. For normal work, the less expensive Whatman 1MM paper is recommended.

In the present study, we noted that good and consistent results were obtained by using freshly prepared solvent mixtures. Solvents that remain in the chamber should be used within 3–4 h as their efficiency subsequently decreases, owing partly to loss of acetonitrile by evaporation.

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