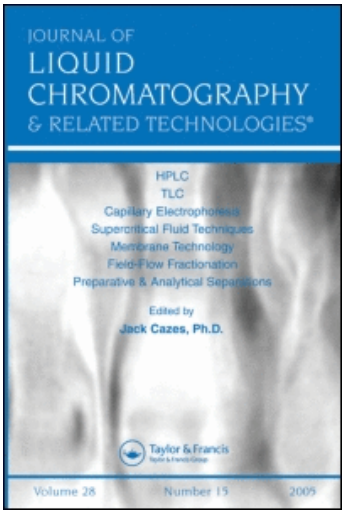

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CHROMATOGRAPHIC SEPARATION OF SYNTHETIC ISOFLAVONES ON STANNIC MOLYBDATE PAPERS

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A B S T R A C T

Chromatographic separation of 24 synthetic isoflavones on stannic molybdate papers has been described. Various analytically and biochemically important separations, such as separation of isomers and that of having same molecular weights have been achieved. Out of different solvent systems used aqueous methanol was found to be the best eluent.

I N T R O D U C T I O N

It is a well established fact now that papers impregnated with ion exchangers combine the advantages of ion exchange, adsorption and partition. Such papers are therefore particularly useful in difficult separations and are frequently being used. The chromatography of isoflavones has been reviewed

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by Harborne(1). Seshadri et.al.(2,3) have studied the paper chromatographic behaviour of isoflavones and these methods were used to investigate the isoflavone glycosides present in sophora japonica fruits(4). Afterwards, separation of soybeam isoflavones from their 5-hydroxy derivatives by thin layer chromatography has been reported by Chuanwang(5). In our laboratories chromatographic studies of both inorganic and organic substances on stannic molybdate papers (6,7,8) have revealed that the separations achieved are rapid, reproducible, selective and clean. However no attempt has been made to utilize these papers for the systematic separation of synthetic isoflavones. The present chapter deals with the effect of various inorganic and organic eluents on the chromatographic movement of synthetic isoflavones on these papers.

E X P E R I M E N T A L

Apparatus : Chromatography was performed on Whatman No.3 paper strips of 15x3.5 cm using 20x5 cm ordinary glass jars.

Materials: Stannic chloride pentahydrate (Poland) and sodium molybdate, Riedel (Germany) were used. All other reagents and chemicals were of B.D.H. AnalaR grade.

Preparation of Ion-Exchange Papers: Paper strips of required size were first impregnated in aqueous stannic chloride solution (0.25M) for 3 to 5 seconds. The excess of the reagent was removed by placing the strips over a filter sheet for 15 minutes. The dried strips were then dipped into sodium molybdate solution (0.25M in water) for 5 seconds and the

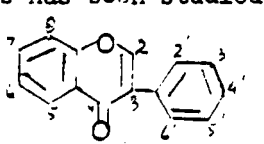
strips were redried as before. In order to remove excess reagent the paper strips were washed in distilled water, dried finally as usual and used as such.

Preparation of isoflavone solutions and Detector: Isoflavone solutions (1%) were prepared in 10% ethanol. An aqueous solution of 6% Ferric chloride in demineralized water was used for the detection of spots on the paper strips. The spots were generally visible on heating the strips at 60 to 80°C.

Procedure : Isoflavone solutions were applied to the strips two or three times with the help of fine glass capillaries and dried subsequently. The papers were first conditioned for 10 to 15 minutes in the jars. They were then dipped in the solvent and the solvent ascent was 11 cm in each case.

R E S U L T S

The chromatographic behaviour of the following 24 synthetic isoflavones has been studied:

1. Isoflavone i.e. 
2. 5,7,4'-Trimethoxy-6-methyl -
3. 2-Carboxy-5,7-dimethoxy-8-methyl-
4. 6-Carbmethoxy-5-hydroxy-6-methyl-
5. 6-Carboxy-5-hydroxy-8-methyl-
6. 2-Carbmethoxy-5,7,2-triethoxy-8-methyl -
7. 2-Carboxy-2',5-dimethoxy-8-methyl -
8. 5,7-Dihydroxy-8-methyl -

9. 2-Carbethoxy-5,7-dihydroxy-4'-methoxy-8-methyl -
10. 2-Methyl-5-hydroxy-5¦7-dimethoxy-8-methyl-
11. 6-Carboxy-7-hydroxy-8-methyl -
12. 2-Carboxy-5,7-dihydroxy-6-methyl-
13. 2-Carboxy-5,7-dihydroxy-8-methyl -
14. 2-Methyl-5-acetoxy-7,8-dimethoxy-8-methyl-
15. 2,6-Dimethyl-5-hydroxy-7-methoxy-
16. 2-Carbethoxy-5,7-dihydroxy-8-methyl-
17. 2-Carboxy-5,7-dihydroxy-4'-methoxy-8-methyl-
18. 5,7,2'-Triethoxy-8-methyl-
19. 6-Carboxy-8-hydroxy-
20. 2-Carbmethoxy-
21. 5-Carboxy-7-hydroxy -
22. 2-Carboxy-8-hydroxy -
23. 2-Carbethoxy-5,7-dihydroxy-4'-methoxy-6-methyl-,and
24. 5-hydroxy-7-methoxy-8-methyl -

The following system were used as the developer reagents:

I. Sodium nitrate: (a) 0.01M (b) 0.05M (c) 0.1M (d) 0.5M (e) 1.0M

II. Nitric Acid: (a) pH=0, (b) pH=1 (c) pH=2) (d) pH=3 (e) pH=4 (f) pH=5

III. Methanol : (a) 5% (b) 10% (c) 25% (d) 50% (e) 75%(f) 100%

IV. Ethanol : 50%

V. Propanol : 50%

On the basis of the differential migration of the isoflavones on stannic molybdate papers binary,ternary and

quarternary separations have been achieved using different solvent systems (Table I).

TABLE I

SEPARATIONS ACHIEVED ON STANNIC MOLYBDATE PAPERS

S.No.	Mixture	R_f Value	Eluent
	BINARY		
1.	6-Carboxy-5-hydroxy-8-methyl-	0.00	Sodium nitrate
	2-Carboxy-2',5-dimethoxy-8-methyl-	0.42	(1.0M)
2.	6-Carboxy-5-hydroxy-8-methyl-	0.00	Sodium nitrate
	2-Carbethoxy-5,7-dihydroxy-8-methyl-	0.45	(1.0M)
3.	2-methyl-5-hydroxy-5,7'-dimethoxy-	0.00	Methanol, (5%)
	2-Carboxy-5,7-dihydroxy-6-methyl -	0.83	
4.	2-methyl-5-hydroxy-5,7'-dimethoxy-	0.00	Methanol (5%)
	2-Carboxy-5,7-dihydroxy-8-methyl -	0.84	
5.	2-Carboxy-5,7-dihydroxy-8-methyl-	0.20	Methanol (10%)
	2-Carboxy-5,7-dihydroxy-6-methyl-	0.43	
6.	2-Carboxy-8-hydroxy -	0.00	Methanol (50%)
	5-Carboxy-7-hydroxy -	0.75	
7.	2-Carboxy-8-hydroxy -	0.00	Methanol (50%)
	2-Carboxy-5,7-dihydroxy-8-methyl-	0.76	
8.	6-Carboxy-7-hydroxy -	0.40	Methanol (50%)
	2-Carboxy-5,7-dihydroxy-8-methyl-	0.67	
9.	5-hydroxy-6-carbmethoxy-6-methyl-	0.00	Methanol (100%)
	2,6-dimethyl-5-hydroxy-7-methoxy-	0.78	
10.	2-Carboxy-5,7-dimethoxy-8-methyl-	0.00	Methanol (100%)
	2-Carbethoxy-5,7-dihydroxy-8-methyl-	0.80	
11.	2-Carboxy-2',5-dimethoxy-8-methyl-	0.00	Methanol (100%)
	2-Carbethoxy-5,7-dihydroxy-8-methyl-	0.78	

(continued)

TABLE I (continued)

S.No.	Mixture	R_f Value	Eluent
	TERNARY		
12.	2-Carbethoxy-5,7-dihydroxy-8-methyl-	0.02	Sodium nitrate(1.0M
	2-Carboxy-5,7-dihydroxy-8-methyl-	0.22	
	2-Carboxy-8-hydroxy-	0.64	
13.	2-Carbethoxy-5,7-dihydroxy-8-methyl-	0.02	Sodium nitrate(1.0M
	2-Carboxy-5,7-dihydroxy-4'-methoxy-8-methyl-	0.41	
	6-Carboxy-8-hydroxy -	0.72	
	QUARTERNARY		
14.	2-Carbethoxy-5,7-dihydroxy-8-methyl-	0.02	Sodium nitrate(1.0M
	2-Carboxy-5,7-dihydroxy-8-methyl-	0.22	
	2-Carboxy-5,7-dihydroxy-4'-methoxy-8-methyl-	0.41	
	6-Carboxy-8-hydroxy-	0.72	
15.	6-Carboxy-5-hydroxy-8-methyl-	0.00	Sodium nitrate(1.0M
	2-Carboxy-8-hydroxy-	0.20	
	2-Carboxy-5,7-dihydroxy-6-methyl-	0.50	
	2-methyl-5-hydroxy-5-7-dimethoxy-	0.70	

DISCUSSION

The superiority of stannic molybdate papers over plain papers can easily be judged from the resolving capabilities and the compactness of the spots. These special features make these papers promising for the separation of isoflavones.

The best results were achieved by eluting with water-methanol solutions, whereas in inorganic solvents or water

alone it is not possible to obtain a migration for most of the isoflavones from the starting point. The inorganic solvents also proved unsatisfactory as they produce elongated spots. Methanol-water system gave the best results in a short elution duration. Moreover, with this eluent compact spots are obtained.

Fig.1 shows some peculiar trends of R_F values with increasing percentage of methanol in the eluent. The greatest change in R_F value occurs at alcohol contents between 25% and 50%. When alcohol content is less than 25% a series of straight lines is obtained. The trend of these curves can be explained by assuming that the addition of methanol to the eluent causes, other than an increase in the solubility in the mobile phase, a decrease in interactions between the isoflavones and the exchanger. Such interactions seem to be completely eliminated when methanol content in the eluent exceeds 50%.

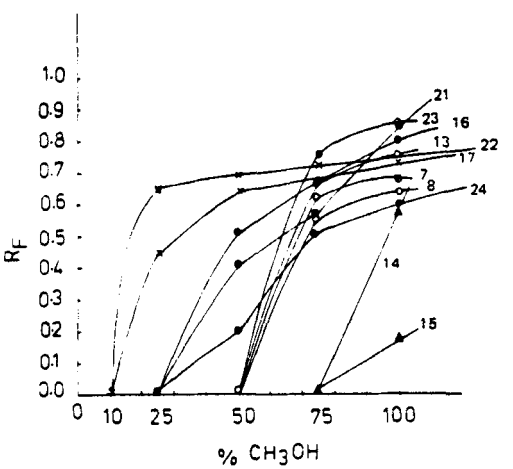


FIG.1 Plots of R_F vs % CH_3OH

Other important point which emerge from these studies is that on changing from methanol to propanol a general increase in the R_F values is observed (Fig.2). The behaviour of isoflavones at different pH values is particularly interesting. Most of the isoflavones remain at starting point when eluted with nitric acid, while those which more show an increase in R_F values as the pH of the eluent increases. This trend shows that affinity of isoflavones decreases as the pH of eluent increases (Fig.3).

Table 1 shows some analytically and biochemically important separation i.e. separation of somers and separation of isoflavones having same molecular weights. Following mixtures of isomers have separated:

1. 2-Carboxy-5,7-dihydroxy-8-methyl isoflavone from 2-Carboxy-5, 7-dihydroxy-6-methyl isoflavone in 10% methanol.

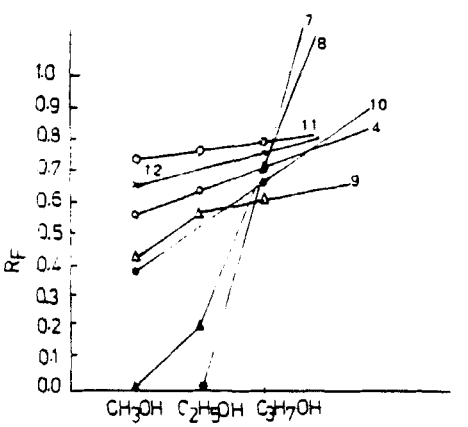


FIG.2 Effect of various alcohols on R_F values

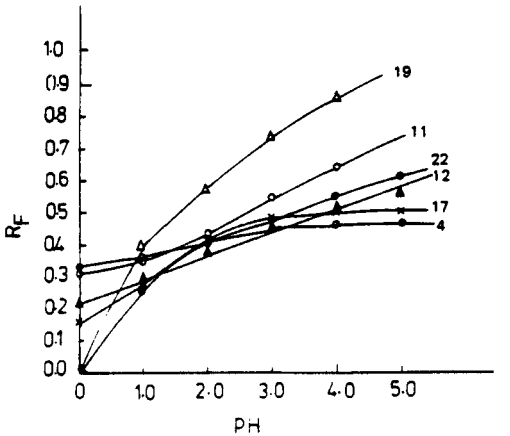


FIG.3 Effect of pH on R_F values

2. 2-Carboxy-8-hydroxy isoflavone from 5-carboxy-7-hydroxy isoflavone in 50% methanol.

Following mixtures of isoflavones having same molecular weight have been separated successfully:

1. 2-methyl-5-hydroxy-5',7-dimethoxy-8-methyl isoflavone from 2-carboxy-5,7-dihydroxy-6-methyl isoflavone in 5% methanol.
2. 2-methyl-5-hydroxy-5' 7-dimethoxy-8-methyl isoflavone from 2-carboxy-5,7-dihydroxy-8-methyl isoflavone in 5% methanol and
3. 2-Carboxy-2',5-dimethoxy-8-methyl isoflavone from 2-Carbethoxy-5,7-dihydroxy-8-methyl isoflavone in 100% methanol.

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REFERENCES

1. Harborne, J.B., Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, Chapman & Hall Ltd., London, 1975, p.79
2. Ahluwalia, V.K., Bhasin, M.M., and Seshadri, T.N., Current Sci., (India), 22, 363, 1953.
3. Krishnamurti, M., and Seshadri, T.N., J.Sci.Ind.Res., (India), 14B, 258, 1955.
4. Szabo, V., Bognar, R., and Puskas, M., Acta Chem.Acad. Sci (Hung), 15, 103, 1958.
5. Chuanwang, L., Anal. Biochem., 42(1), 296, 1971.
6. Qureshi, M., and Rawat, J.P., Separation Sci., 7(3), 297, 1972.
7. Rawat, J.P., and Singh, P., Ann.Chim., 64, 873, 1974.
8. Rawat, J.P., and Singh, P., Z.Anal.Chem., 279, 368,1976.