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# CHROMATOGRAPHY OF FOOD PRESERVATIVES ON POLYAMIDE LAYERS AND COLUMNS

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SUMMARY

The separation of eight kinds of food preservative by one- and two-dimensional chromatography on polyamide layers was investigated. A comparison was made between the detection limits on polyamide and silica gel layers, and the superiority of the polyamide thin layers was confirmed.

The separation of the food preservatives on a polyamide column with two types of elution system was established. The recovery of these compounds was satisfactory.

## INTRODUCTION

After establishing the efficacy of polyamide in chromatography[1-7], it has been applied extensively to column and thin-layer chromatography where it was found that the separation was very much influenced by the hydrogen bond formed between the HO-group in phenolic compounds and the -CONH-group in polyamide[1,2,8].

This paper deals with the separation of food preservatives (aromatic acids with and without a phenolic HO-group and its alkyl esters, and some other conventional preservatives) by column and thin-layer chromatography using polyamide as the chromatographic medium.

## EXPERIMENTAL

### *Materials*

Polyamide powder from two sources, Polyamide Woelm TLC and Wakō Polyamide B-10* were used for thin-layer chromatography while for the column chromatography only Polyamide Woelm was used.

* "Wakō Polyamide B-10" contains 10% anhydrous calcium sulfate as a binder (Wakō Pure Chemical Industries, Ltd., Nihonbashi-honcho, Chuō-ku, Tokyo, Japan).

TABLE I

WAVE LENGTHS AND MOLAR ABSORBANCY INDICES OF FOOD PRESERVATIVES AT THEIR UV ABSORPTION MAXIMA

| *Sample* | *λmax (mμ) and ε × 10⁻³* | | | | | |
|---|---|---|---|---|---|---|
| | *In water* | | *In 1% (w/w) $NH_3$* | | *In $H_2O$–AcOH–MeOH (5.5:0.5:4, v/v)* | |
| Salicylic acid | 232<br>298 | 6.66<br>3.37 | | | 305[a] | 3.60[a] |
| *p*-Hydroxybenzoic acid | 251 | 11.8 | 280 | 17.5 | 256 | 15.0 |
| Ethyl *p*-hydroxybenzoate | 256 | 15.0 | 297 | 23.4 | 256 | 15.9 |
| *n*-Propyl *p*-hydroxybenzoate | 256 | 15.0 | 297 | 24.1 | 256 | 15.9 |
| *n*-Butyl *p*-hydroxybenzoate | 256 | 15.5 | 297 | 24.2 | 256 | 15.9 |
| Benzoic acid | 227.5 | 9.18 | | | —[b] | —[b] |
| Sorbic acid | 260 | 23.6 | | | 262 | 29.3 |
| Dehydroacetic acid | 227.5<br>306 | 14.5<br>12.1 | | | 308 | 14.1 |

[a] λ max and its molar absorbancy of salicylic acid were measured in $H_2O$–AcOH–MeOH (3:1:6).

[b] λ max and its molar absorbancy of benzoic acid could not be measured in this medium.

The food preservatives used in this experiment are of a specially refined grade. The chemical and optical characteristics of the preservatives are as listed in Table I.

*Preparation of the polyamide chromatoplates*

(a) One gram of Polyamide Woelm TLC was mixed with 12 ml of ethanol to give a homogeneous suspension, which was spread evenly with a suitable applicator on a glass plate (20 × 20 cm) to a thickness of 0.25 mm. The plate was dried in air at room temperature and stored in a desiccator.

(b) Fourteen grams of Wakō Polyamide B-10 were made into a homogeneous suspension by mixing it with 56 ml of distilled water. The chromatoplate was prepared in the same way as (a).

*Solvent systems for thin-layer chromatography*

The solvent systems (A–F) used in the experiments are listed in Table II.

*Application of samples and development of chromatoplates*

For one-dimensional chromatography, 0.5 $\mu$l of an 0.2 % (w/v) ethanolic solution of each preservative was applied except for dehydroacetic acid and benzoic acid where 1.5 $\mu$l of a sample of the same concentration was used. Sample application was made on a line 3 cm from the lower edge, and the sample was developed by the ascending method.

In order to ensure the equilibration of the vapor in the chamber, the inside was lined with filter paper soaked in the solvent.

The development was continued until the solvent reached 12 cm from the point of sample application.

For two-dimensional chromatography, 1 $\mu$l of an 0.2 % (w/v) ethanolic solution of the samples was applied; as above increased amounts (2 $\mu$l) of dehydroacetic acid and benzoic acid were applied. Samples were spotted at a point 3 cm from both edges.

*Detection of the spots on the chromatoplates*

The spots can be seen under UV light as dark areas against a bright background, but for confirmation's sake *p*-hydroxybenzoic acid, its esters, and salicylic acid were detected as reddish violet spots by spraying with diazotized sulfanilic acid reagent; and dehydroacetic acid, sorbic acid and benzoic acid gave reddish spots against a yellow background with Methyl Red reagent.

The composition of the reagents used are as follows:

*Diazotized sulfanilic acid.* One gram of sulfanilic acid, dissolved in 8 ml of conc. HCl and 100 ml of distilled water, is added to the same volume of 0.69 % $NaNO_2$ solution.

*Methyl Red.* A mixture of 10 ml of 0.1 % ethanolic solution of Methyl Red and 20 ml of 0.1 *M* phosphate buffer (pH 7.0).

*Preparation of the polyamide column and application of samples*

Forty-five grams of Polyamide Woelm were washed with distilled water, methanol and chloroform, successively. After drying in air, the washed polyamide powder was suspended in the same solvent as that used for elution, and the powder was packed into a glass column (1.7 × 50 cm) to form a column bed of 45 cm height. The samples

TABLE II

SOLVENT SYSTEMS FOR CHROMATOGRAPHY OF FOOD PRESERVATIVES ON POLYAMIDE LAYERS

| *Symbol* | *Components* | *Ratio (v/v)* |
|---|---|---|
| A | MeOH–$H_2O$ | 6:4 |
| B | Acetone–$H_2O$ | 5:5 |
| C | MeOH–AcOH–$H_2O$ | 5:1:4 |
| D | MeOH–conc. $NH_3$–$H_2O$ | 2:1:7 |
| E | AcOH–$H_2O$ | 3:7 |
| F | *n*-Hexane–AcOH | 8:2 |

TABLE III

$R_F$ VALUES OF FOOD PRESERVATIVES ON POLYAMIDE LAYERS

(*a*) *$R_F$ values on polyamide (Woelm)*

| *Sample* | *Solvent system* | | | | | |
|---|---|---|---|---|---|---|
| | *A* | *B* | *C* | *D* | *E* | *F* |
| Salicylic acid | 0.05 | 0.05 | 0.18 | 0.56 | 0.14 | 0.25 |
| *p*-Hydroxybenzoic acid | 0.13 | 0.18 | 0.30 | 0.96 | 0.16 | 0.04 |
| Ethyl *p*-hydroxybenzoate | 0.32 | 0.41 | 0.38 | 0.64 | 0.16 | 0.11 |
| *n*-Propyl *p*-hydroxybenzoate | 0.27 | 0.37 | 0.32 | 0.55 | 0.10 | 0.15 |
| *n*-Butyl *p*-hydroxybenzoate | 0.22 | 0.32 | 0.29 | 0.44 | 0.07 | 0.19 |
| Benzoic acid | 0.14 | 0.21 | 0.46 | 0.85 | 0.23 | 0.56 |
| Sorbic acid | 0.20 | 0.29 | 0.56 | 0.92 | 0.32 | 0.64 |
| Dehydroacetic acid | 0.18 | 0.25 | 0.73 | 0.79 | 0.54 | 0.45 |

(*b*) *$R_F$ values on polyamide (Wakō B-10)*

| *Sample* | *Solvent system* | | | | | |
|---|---|---|---|---|---|---|
| | *A* | *B* | *C* | *D* | *E* | *F* |
| Salicylic acid | 0.12 | 0.16 | 0.25 | 0.75 | 0.29 | 0.40 |
| *p*-Hydroxybenzoic acid | 0.31 | 0.34 | 0.53 | 0.95 | 0.33 | 0.06 |
| Ethyl *p*-hydroxybenzoate | 0.53 | 0.63 | 0.61 | 0.76 | 0.34 | 0.15 |
| *n*-Propyl *p*-hydroxybenzoate | 0.49 | 0.58 | 0.56 | 0.68 | 0.25 | 0.21 |
| *n*-Butyl *p*-hydroxybenzoate | 0.44 | 0.54 | 0.52 | 0.58 | 0.18 | 0.28 |
| Benzoic acid | 0.34 | 0.33 | 0.66 | 0.91 | 0.43 | 0.62 |
| Sorbic acid | 0.43 | 0.44 | 0.72 | 0.93 | 0.55 | 0.68 |
| Dehydroacetic acid | 0.45 | 0.40 | 0.83 | 0.86 | 0.73 | 0.59 |

(each 10 $\mu$moles) were dissolved in 5 ml of the same solvent and applied on the top of the column. The elution was performed at a flow rate of 0.22–0.25 ml per min, and each 3.5 ml fraction was collected. The concentration of the food preservatives in the fractions was determined by measuring the absorbancy at its $\lambda$ max* in the medium used for elution.

* In the case of benzoic acid, the absorbancy was measured at 240 m$\mu$, as benzoic acid has no absorption maximum in $H_2O$–AcOH–MeOH (5.5:0.5:4).

## RESULTS AND DISCUSSION

### *Thin-layer chromatography*

The solvent systems suitable for the separation of the food preservatives on polyamide layers are summarized in Table II. They are classified into two types, solvents A–E and solvent F, according to the polarity of the constituent solvents. Tables III a and b show the $R_F$ values of the food preservatives in the above solvent systems.

It may generally be concluded from these tables that the Woelm Polyamide possesses stronger adsorption properties than the Wakō Polyamide B-10.

Hydrogen bonding effects can be observed from the $R_F$ values of salicylic acid and *p*-hydroxybenzoic acid which are known to form an intra-molecular hydrogen bond and inter-molecular hydrogen bond, respectively.

Comparison of the differences in the $R_F$ values of these compounds in any two types of solvent A–E or one of these and solvent F shows that a complete separation could be obtained by two-dimensional development in either of the following combinations:

Solvent B–Solvent F
Solvent B–Solvent E

Of all the possible combinations of the solvent systems listed in Table II, these two combinations gave the best distribution of spots on the chromatograms (Fig. 1).

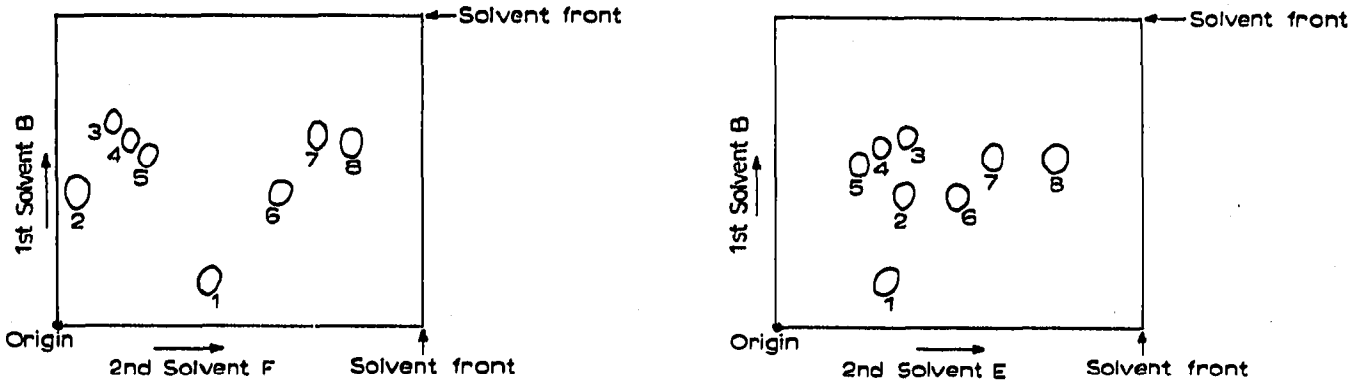


Fig. 1. Two-dimensional chromatograms of food preservatives on polyamide layers. 1 = salicylic acid; 2 = *p*-hydroxybenzoic acid; 3 = ethyl *p*-hydroxybenzoate; 4 = *n*-propyl *p*-hydroxybenzoate; 5 = *n*-butyl *p*-hydroxybenzoate; 6 = benzoic acid; 7 = sorbic acid; 8 = dehydroacetic acid.

One of the characteristics of the polyamide thin layers is to give a bright background in transmitted UV light, and this phenomenon provides a highly sensitive method of detection of UV-absorbing substances on the chromatoplate[9,10]. As shown in Table I, all the food preservatives tested absorb the UV light, thus they are all detectable as dark spots under transmitted UV light. In Table IV, a comparison is made between the detection limit of the spots on polyamide layers and on silica gel layers. It can be seen from Table IV that the detection of these substances is more sensitive on polyamide layers than on silica gel layers. With regard to other methods of detection of the spots, the Methyl Red reagent and the diazotized sulfanilic acid reagent can be used on the polyamide layers as well as on silica gel layers (see EXPERIMENTAL).

TABLE IV

DETECTION LIMITS OF FOOD PRESERVATIVES BY UV ABSORPTION ON POLYAMIDE LAYERS AND SILICA GEL LAYERS

| *Sample* | *Polyamide in μg* | *Silica gel*[a] *in μg* |
|---|---|---|
| Salicylic acid | 0.1 | 0.5 |
| *p*-Hydroxybenzoic acid | 0.25 | 5–10 |
| Ethyl *p*-hydroxybenzoate | 0.25 | 5–10 |
| *n*-Propyl *p*-hydroxybenzoate | 0.25 | 5–10 |
| *n*-Butyl *p*-hydroxybenzoate | 0.25 | 5–10 |
| Benzoic acid | 3[b] | 50[b] |
| Sorbic acid | 0.25 | 5–10 |
| Dehydroacetic acid | 2[b] | 25–50[b] |

[a] Fluka Silica gel (Fluka AG, Chemische Fabrik, Switzerland) was used.

[b] The low sensitivities of both these compounds are due to the large discrepancy between their absorption maxima and the wave length (2537 Å) of the UV light (see Table I).

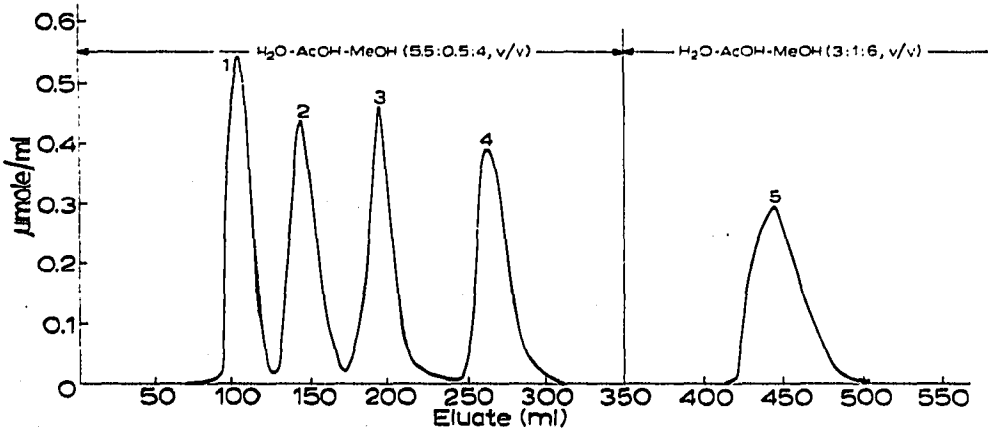


Fig. 2. Separation of food preservatives by polyamide column chromatography. Samples: 1 = dehydroacetic acid; 2 = sorbic acid; 3= benzoic acid; 4 = *p*-hydroxybenzoic acid; 5 =salicylic acid; each 10 μmoles applied. Column: Polyamide Woelm for column use; 1.7 × 45 cm.

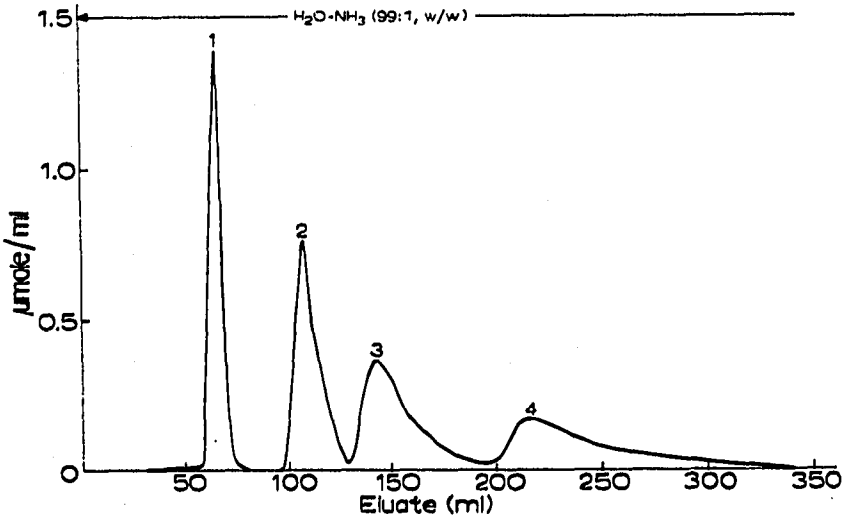


Fig. 3. Separation of food preservatives by polyamide column chromatography. Samples: 1 = *p*-hydroxybenzoic acid; 2 = ethyl *p*-hydroxybenzoate; 3 = *n*-propyl *p*-hydroxybenzoate; 4 = *n*-butyl *p*-hydroxybenzoate; each 10 μmoles applied. Column: Polyamide Woelm for column use; 1.7 × 45 cm.

*Column chromatography*

Suitable conditions for the separation of the food preservatives on column chromatography have been established as shown in Figs. 2 and 3. A mixture of salicylic acid, *p*-hydroxybenzoic acid, benzoic acid, sorbic acid and dehydroacetic acid was separated on a polyamide column by stepwise elution using two elution systems: (1) $H_2O$–AcOH–MeOH (5.5:0.5:4) and (2) $H_2O$–AcOH–MeOH (3:1:6) successively (Fig. 2). The recovery of the samples in this separation system was satisfactory, as is shown: dehydroacetic acid 95.5 %, sorbic acid 96.5 %, benzoic acid 101.2 %, *p*-hydroxybenzoic acid 100.1 %, salicylic acid 103.2 %. An elution procedure using water containing $NH_3$ (1 %, w/w) was selected for the separation of a mixture of *p*-hydroxybenzoic acid and its alkyl esters. Separation was attained as shown in Fig. 3 and recovery was also good: *p*-hydroxybenzoic acid 92.0 %, ethyl *p*-hydroxybenzoate 96.0 %, *n*-propyl *p*-hydroxybenzoate 98.3 %, *n*-butyl *p*-hydroxybenzoate 91.0 %.

Because neither of the elution media above used has any absorption in the UV, the concentration of each preservative in the fractions was directly determined by measuring the absorbance at the $\lambda$ max (see Table I).

## CONCLUSION

Eight kinds of food preservative, which include five phenolic hydroxyl compounds, one enolic hydroxyl compound and two carboxylic compounds, were separated on polyamide layers. From the correlation of the mobility of the samples and the solvents used, it was noticed that some hydrogen bonding forces have a definite effect on the interaction between samples having phenolic hydroxyl groups and the polyamide molecule, and that the polarity of the solvents exerts a considerable influence on the hydrogen bonding. The detection of the spots on the polyamide layers by the UV absorption method is 5–20 times more sensitive than for those on silica gel layers. This confirms that polyamide layer chromatography is suitable for the micro-analysis of food preservatives.

The conditions for the separation of these substances on a polyamide column were established. The simultaneous separation of all the samples was not possible, but by combining two types of elution system, *viz.*, a $H_2O$–AcOH–MeOH system and a $H_2O$–$NH_3$ system, good separations in two groups of samples were attained. The recovery of the samples separated on the polyamide column was also satisfactory.

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