

CHROM. 21 177

Note

Cluster analysis in the comparison of two-dimensional chromatograms

DANIELA HEIMLER*

Dipartimento di Scienza del Suolo e Nutrizione della Pianta, Università degli Studi di Firenze, Piazzale delle Cascine 28, Florence (Italy)

and

VIERI BODDI

Istituto di Patologia Generale, Università degli Studi di Firenze, Viale G.B. Morgagni 50, Florence (Italy)

(Received December 5th, 1988)

Two-dimensional chromatography, notwithstanding its undoubted advantages from a theoretical point of view, is the least studied separation technique owing to the difficulty in interpreting the experimental results. The technique has two main drawbacks: the possibility of analysing only one sample on one sheet without the simultaneous spotting of test compounds¹ and the difficult interpretation of a two-dimensional chromatogram on the basis of R_F values obtained by one-dimensional developments. Even the quantitative analysis of two-dimensional chromatograms is difficult, as the spots are not arranged in vertical strips but occupy the whole layer. Nevertheless two-dimensional chromatography can separate very complex mixtures which are difficult to resolve by means of other techniques².

This paper describes a method which allows the use of chromatographic data in order to calculate similarity criteria without having quantitative data. The problem of sample classification on the basis of chromatographic results is generally carried out by means of pattern recognition techniques using the quantitative data from gas chromatography or high-performance liquid chromatography³.

EXPERIMENTAL

Flavonoids aglycones of elm and iris leaves were examined. The crushed leaves (1 g) were treated with 25 ml of boiling hydrochloric acid for 30 min. The flavonoids were extracted with 10 ml of ethyl acetate, which was evaporated to dryness under vacuum. The residue was dissolved in 2 ml of methanol and 2 μ l of this solution were spotted on Sil C₁₈-50 plates (Macherey, Nagel & Co.) and eluted in the first direction with *n*-hexane ethyl acetate acetic acid (72:27:1) and in the second direction with 1 *M* acetic acid in 50% methanol. The spots were sprayed with a 1% methanolic solution of ethanolamine diphenylborate and a 5% ethanolic solution of polyethylene glycol. The spots were observed under UV light (360 nm). Under these conditions the flavonoids give fluorescent spots of different colours. The spots were characterized by their positions on the layer by means of two coordinates, obtained by dividing the

distance of the spot from the origin lines by the distance of the two solvents from the same lines, and by their colour under UV light.

For elm leaves we considered only those compounds which migrate in both eluents, because compounds which remain at the starting point with the first eluent and migrate with the second eluent are difficult to identify owing to their incomplete separation. In this way every spot in all of the chromatograms were assigned to a definite group and the results in Table I were obtained. It should be noted that only in a few instances could the spots be identified⁴. The aim of this work, however, was to compare several two-dimensional chromatograms, regarded as "fingerprints" of different plants, in order to ascertain whether the thin-layer chromatographic (TLC) profile of phenolic compounds could be of help in the determination of differences among populations, provenances and species (that is, intra- and inter-specific differences).

The elm leaves were obtained from the germoplasm collection of the Centre for Forest Pathology of the National Research Council of Florence. The data for the two-dimensional chromatograms are reported in Table II.

The iris leaves belong to spontaneous species and were sampled in the Giardino dell'Iris in Florence. In this instance we also considered the compounds lying on the y-axis (that is, those compounds which migrate with the first eluent but remain at the origin with the second), as they are better characterized than in the case of elm leaves. The data are reported in Tables III and IV.

TABLE I
COORDINATES AND COLOURS UNDER UV LIGHT OF ALL THE SPOTS OBSERVED IN THE TWO-DIMENSIONAL CHROMATOGRAMS OF ELM LEAVES

The values of the coordinates are the means of 10-32 determinations. Letters A-U represent the different spots in the chromatograms.

	<i>Coordinates</i> <i>× 100</i>	<i>Colour</i>	<i>Name</i>
A	18-18	Orange	Quercetin
B	12-51	Red	—
C	13-37	Red	Myricetin
D	25-39	Red	—
E	13-50	Orange	—
F	29-74	Light blue	—
G	31-53	Light blue	—
H	39-70	Light blue	—
I	24-84	Light blue	—
L	29-61	Light blue	—
M	25-67	Yellow	Caffeic acid
N	25-75	Yellow	Caffeic acid
O	44-45	Yellow	—
P	14-48	Yellow	—
Q	19-55	Yellow	—
R	30-10	Green yellow	Kaempferol
S	23-35	Light blue	—
T	63-81	Light blue	—
U	42-82	Blue	—

TABLE II
DISTRIBUTION OF SPOTS IN THE ELM LEAVES

A-U as in Table I.

<i>Elm leaves</i>	A	B	C	D	E	F	G	H	I	L	M	N	O	P	Q	R	S	T	U
<i>U. pumilia:</i>																			
(1) S1	+	+	+			+	+	+	+	+	+								+
(2) S12	+		+				+		+		+								+
(3) S15	+						+	+	+		+								+
(4) PU1	+	+	+		+		+	+	+		+	+	+						+
(5) 73P	+	+	+			+	+	+	+		+	+							+
(6) 182P	+	+	+				+	+	+		+	+	+	+	+	+			+
<i>U. parvifolia:</i>																			
(7) PA1.1, PA1.2	+					+	+		+		+	+							+
(8) PA2	+		+				+		+		+	+		+					+
(9) 157P	+	+	+	+			+		+		+	+	+						+
(10) NA33	+		+				+				+	+	+	+					+
<i>U. japonica:</i>																			
(11) 3P	+	+	+		+		+	+	+		+	+							+
(12) 2P	+	+	+	+	+		+	+	+		+	+							+
(13) 127P	+	+	+		+	+	+	+	+		+	+	+						+
(14) 23P	+	+	+	+	+	+	+		+		+	+	+						+
(15) 57P	+	+	+	+			+		+	+	+	+	+	+	+				+
<i>U. carpinifolia:</i>																			
(16) C3	+	+	+				+	+			+	+	+						+
(17) C6	+	+	+			+	+	+			+	+	+						+
(18) 6-11	+	+	+			+	+	+			+	+							+
<i>U. xhollandica:</i>																			
(19) 274, P38, 275	+		+				+		+	+	+	+	+	+	+		+	+	+
(20) 405	+		+	+			+		+	+	+	+	+	+	+	+	+	+	+
<i>U. chemmoui:</i>																			
(21) 176P.2	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
(22) 176P.5	+	+	+		+		+	+	+		+	+	+	+	+	+	+	+	+
<i>U. villosa:</i>																			
(23) VII,P554	+						+		+		+	+		+		+			+
(24) <i>U. laevis</i>	+		+				+		+		+	+		+		+	+	+	+
(25) <i>U. glabra</i>	+		+				+		+		+	+		+	+	+			+
(26) <i>U. elliptica</i>	+	+	+	+			+	+	+		+	+		+		+			+
(27) <i>U. laciniata</i>	+		+				+		+		+	+		+	+	+			+
(28) <i>U. wilsoniana</i>	+	+	+				+	+	+	+	+	+				+		+	+

RESULTS AND DISCUSSION

The question of the comparison of qualitative data regarded as indicating the presence or absence of a compound can be solved by means of numerical indices expressed by equations which may change slightly from one case to another. The information in each line in Tables II and IV can be codified as either 0 or 1 (absence or presence of a spot). The result of the comparison of two sequences is characterized by four values: N_{11} (number of positive agreements), N_{00} (number of negative agreements), N_{10} and N_{01} (number of disagreements, that is, the presence of a spot in

TABLE III

COORDINATES AND COLOURS UNDER UV LIGHT OF ALL THE SPOTS OBSERVED IN THE TWO-DIMENSIONAL CHROMATOGRAMS OF IRIS LEAVES

	<i>Coordinates</i> <i>x 100</i>	<i>Colour</i>
A	9-27	Green
B	12-18	Green
C	17-10	Green
D	18-64	Light blue
E	18-77	Orange
F	24-0	Red
G	25-60	Light blue
H	30-10	Light blue
I	30-83	Blue
L	51-0	Red
M	56-0	Red
N	76-0	Red
O	12-15	Green
P	86-60	Light blue
Q	18-69	Light blue
R	37-49	Light blue
S	65-28	Light blue
T	51-73	Light blue

TABLE IV

DISTRIBUTION OF SPOTS IN THE IRIS LEAVES

A-T as in Table III.

<i>Iris leaves</i>	A	B	C	D	E	F	G	H	I	L	M	N	O	P	Q	R	S	T
(1) <i>I. pallida</i> ^a				+	+	+	+		+	+		+						
(2) <i>I. pallida</i> ^b				+	+	+	+		+	+		+					+	
(3) <i>I. pallida</i>				+	+	+			+	+		+					+	
(4) <i>I. cengialti</i>	+	+	+	+		+			+	+		+		+	+			
(5) <i>I. florentina</i>	+	+	+	+	+	+			+	+	+	+					+	
(6) <i>I. germanica</i>	+		+	+	+	+			+	+		+						
(7) <i>I. lutescens</i> (<i>Quercianella</i>)		+	+			+					+	+	+					+
(8) <i>I. lutescens</i> (<i>Monte Marcello</i>)	+	+	+			+					+	+	+					+
(9) <i>I. squalens</i>	+	+	+	+	+	+	+		+	+	+	+					+	
(10) <i>I. kockii</i>			+	+		+			+	+	+	+	+				+	
(11) <i>I. sambucina</i>	+	+	+	+	+	+			+	+		+					+	
(12) <i>I. aphylla</i>	+	+	+			+			+	+			+	+	+			
(13) <i>I. uinguicularis</i>	+	+	+	+	+	+	+	+	+	+	+	+	+					

^a Fertile form.^b Sterile form.

one sequence and its absence in the other). The most general similarity index of two sequences (simple matching coefficient) is⁵

$$S_{SM} = (N_{11} + N_{00}) / (N_{11} + N_{00} + N_{10} + N_{01})$$

The Jaccard-Sneath coefficient does not consider the negative agreements (N_{00}):

$$S_{JS} = N_{11} / (N_{11} + N_{10} + N_{01})$$

From the point of view of our data (TLC data), we deemed the Jaccard-Sneath coefficient to be more useful, as TLC can give information on the presence of one

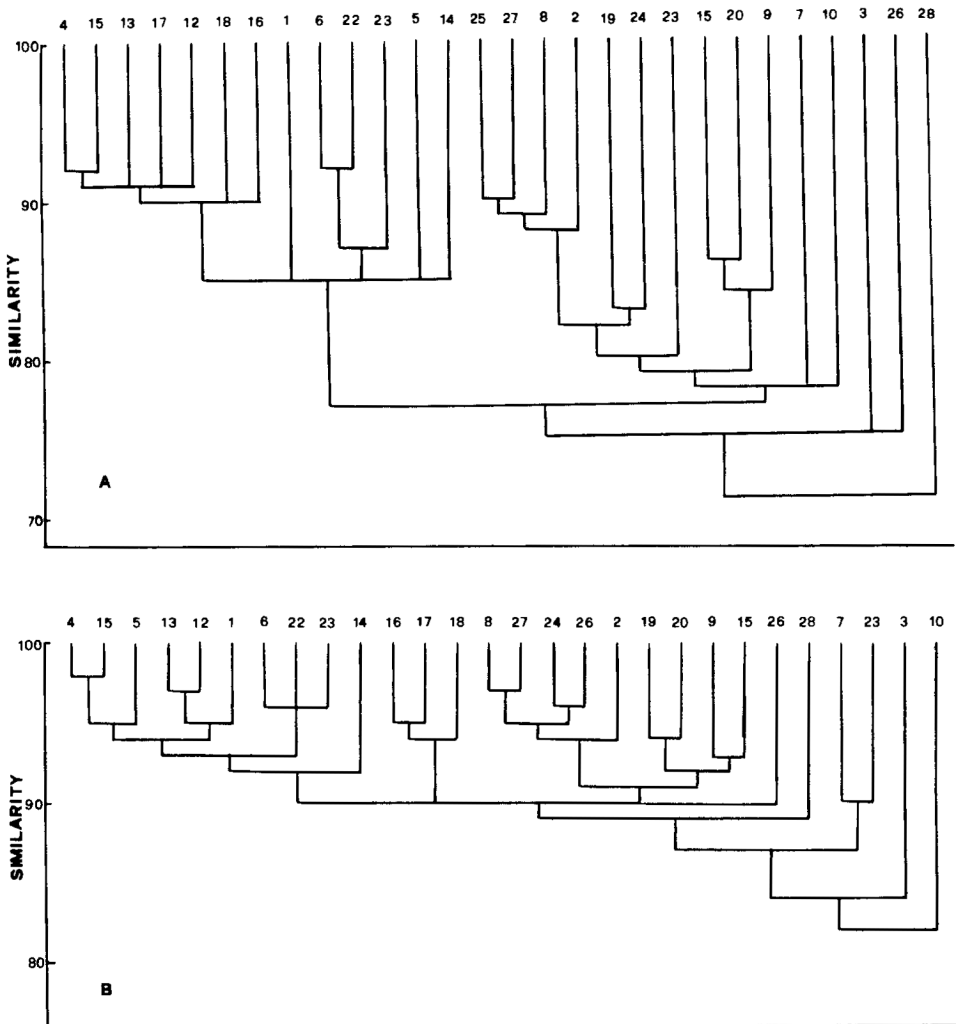


Fig. 1. Dendrograms obtained by the cluster analysis of: (A) S_{JS} coefficient and (B) S_w coefficient. The data refer to elm leaves. Numbers as in Table II.

compound but not on its absence. The similarity coefficients were calculated from all the pairs of sequences in Table II and were used for the cluster analysis. We used one of the simplest classification methods, that is, the single linkage cluster analysis⁵. The results of this kind of analysis are visualized by means of a dendrogram (see Fig. 1A). The data for 30 different two-dimensional chromatograms can be easily correlated.

A general consideration should, however, be made before discussing in detail the results in Fig. 1. The Jaccard-Sneath coefficient (S_{JS}) ascribes the same weight to each positive agreement and to each disagreement. It seemed interesting to ascribe a different weight to each spot depending on its frequency in the whole data matrix. In this way we consider the presence in one sequence of a compound which is present in a large number of sequences to be more important than the presence of one compound which rarely appears in the whole data matrix. For this reason we attributed a weight to each spot equal to the number of times that the spot appears in the whole sequences matrix. The resulting coefficient is

$$S_w = W_{11}/(W_{11} + W_{10} + W_{01})$$

where W_{11} is the sum of the weights of the spots present in both sequences and W_{01} and W_{10} are the sums of the weights of the spots present in one of the two sequences considered.

The value of a similarity coefficient S_w , such as that of Jaccard-Sneath, changes from 0 to 1. The S_w coefficient allows the introduction into each coefficient of information concerning the whole data matrix, in contrast to all the other similarity coefficients which consider only two sequences.

In order to test the validity of the S_w coefficient, Fig. 2 shows the correlation

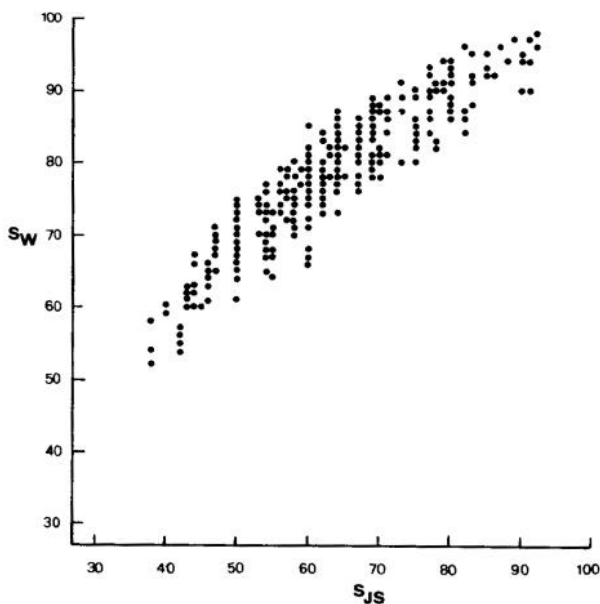


Fig. 2. Correlation between S_{JS} and S_w coefficients of the elm data set.

between the values of the S_{JS} and S_w coefficients for the same data matrix (Table II). It should be noted that the S_w coefficients generally have a higher value and exhibit a better differentiation. In fact, in many instances, one value of the S_{JS} coefficient corresponds to different values of the S_w coefficient; this occurrence could be of help in giving a better differentiation overall in those instances in which the chromatographic data are very similar. Fig. 1B shows the dendrogram obtained by the cluster analysis of the S_w coefficients. Comparison of the two dendrograms in Fig. 1A and B indicates that in Fig. 1B the similarity among the sequences due to the higher mean values of the S_w coefficients is increased with respect to Fig. 1A. However, from a general point of view, such an occurrence does not affect the dendrogram, as Fig. 1 must be considered as a whole.

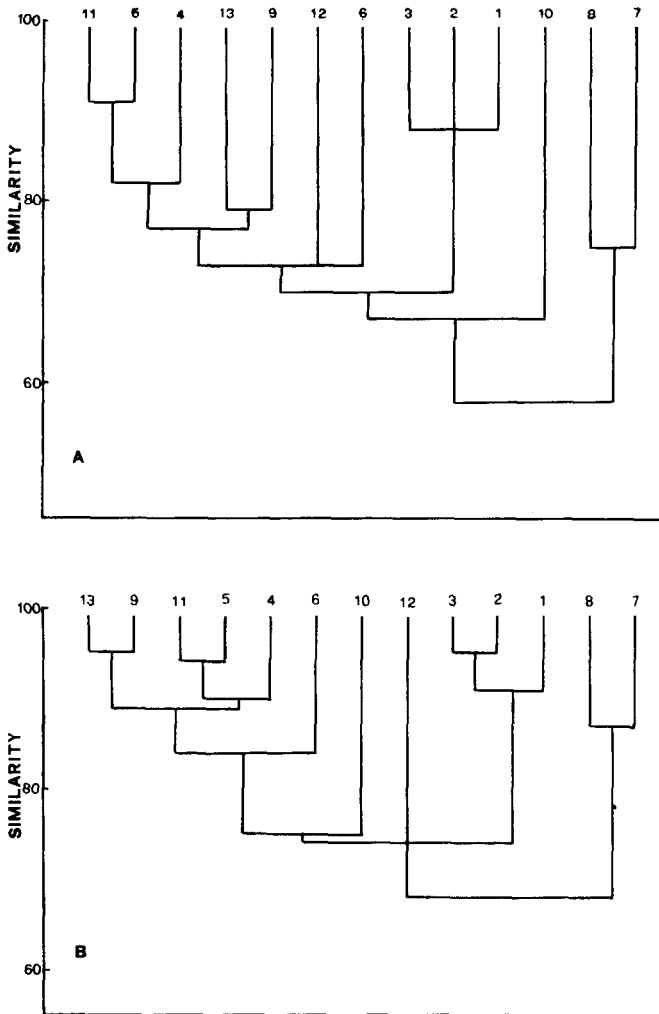


Fig 3. Dendrograms obtained by the cluster analysis of (A) S_{JS} coefficient and (B) S_w coefficient. The data refer to iris leaves. Numbers as in Table IV.

Let us now see what kind of information can be obtained from these dendrograms. *U. pumila* and *U. parvifolia* exhibit a high intra-specific variation owing to their provenance from a wide geographic area; in both instances, however, all *U. parvifolia* populations are in the cluster on the right. *U. japonica* and *U. carpinifolia* are very similar species from a botanical point of view⁶; in the dendrogram in Fig. 1A they appear in the same cluster. In Fig. 1B, however, the three populations of *U. carpinifolia* are gathered in one cluster, offering more detailed information in this instance where the differences between the samples are very small. The species *U. xhollandica* is very similar in all its populations (it should be noted that three of the four populations studied are identical) and in Fig. 1B (but not in Fig. 1A) the four populations are in one cluster.

As a further demonstration of the higher resolving power of the S_w coefficient for very similar sequences, it is interesting to consider the data for two populations of *U. japonica* (2P and 3P) which come from a restricted area of southern Japan; in Fig. 1B the two populations are linked in a more evident way than in Fig. 1A. From a botanical point of view other considerations could be made on the way in which the different species are linked, but this is beyond the aims of this paper.

As can be seen from the data in Table II, in all elm samples five spots were constantly found; in order to go deeper into the question of the interpretation of chromatographic data by means of cluster analysis, we considered the matrix in Table II without the five common columns. Apart from an expected translation towards lower values of similarity, there are no substantial differences with respect to Fig. 1A and B.

Fig. 3 shows the data relating to the iris leaves; Fig. 3A refers to the cluster analysis of the S_{JS} coefficients and Fig. 3B to that of the S_w coefficient. The only notable difference between the two dendrograms is found where the similarity between the sequences is higher, resulting in a better differentiation of clusters in Fig. 3B.

ACKNOWLEDGEMENT

This work was performed with financial support from the National Research Council (CNR).

REFERENCES

- 1 F. Geiss, *Fundamentals of Thin Layer Chromatography*, Hüthig, Heidelberg, 1987.
- 2 G. Guiochon, M. F. Gonnord, A. Siouffi and M. Zakaria, *J. Chromatogr.*, 250 (1982) 1-20.
- 3 S. D. Brown, T. Q. Arker, R. J. Larivee, S. L. Monfre and H. R. Wilk, *Anal. Chem.*, 60 (1988) 252R-273R.
- 4 D. Heimler and V. Vidrich, *Agrochimica*, in press.
- 5 P. H. A. Sneath and R. R. Sokal, *Numerical Taxonomy*, Freeman, San Francisco, CA, 1973.
- 6 G. Gambi, R. Gellini and L. Brogi, *Inf. Fitopatol.*, 30 (1980) 27-47.

Note

Separation and determination of organoarsenic compounds with a microbore column and ultraviolet detection

MASAHIRO MARUO, NAOKI HIRAYAMA, HIROO WADA^a and TOORU KUWAMOTO*

Department of Chemistry, Faculty of Science, Kyoto University, Kyoto 606 (Japan)

(First received July 12th, 1988; revised manuscript received December 21st, 1988)

Ion chromatography has been widely investigated in the analysis of inorganic anions and organic anions^{1–6}. Also of interest in this context is the use of a capillary column and an UV detector^{7–10} in order to enhance the efficiency of separation and enable the use of small amounts of samples. We have investigated the separation and determination of small amounts of organoarsenic compounds by use of ion chromatography^{15–17} with various eluents and a conductivity detector, but did not obtain satisfactory results.

In this study the separation and determination of organoarsenic acids, such as *o*-aminophenylarsonic acid (*o*-APA), *p*-aminophenylarsonic acid (*p*-APA) and *o*-nitrophenylarsonic acid (*o*-NPA), which have an aromatic group, were undertaken. It was found that the retention times of samples changed remarkably by changing the pH of the eluent. The effect of the eluent pH on the elution of arsenic compounds was investigated in detail by using phosphate as an eluent. Samples were completely separated at the pH values close to the pK_a values of the samples, and a concentration of at least 0.06 ppm using a sample injection of 1 μ l was detectable and determined.

EXPERIMENTAL

Apparatus

The chromatographic system consisted of a Tosoh double plunger pump CCPD, a Rheodyne injection valve 7520 (sample injection volume 1 μ l), a Shimadzu UV detector SPD-6AV (cell volume 0.6 μ l) and a stainless-steel microbore column (40–200 mm \times 0.5 mm I.D.) packed with Tosoh TSKgel IC-Anion-PW (particle size 10 μ m, exchange capacity 30 μ equiv./ml bed).

Eluents

100 mM Phosphoric acid, 100 mM potassium hydroxide and sodium hydroxide stock eluent solutions were prepared by dissolving special grade reagents in deionized distilled water (D.D.W.), diluting to the appropriate concentration and deaerating at a water-jet pump and by supersonic vibration (Bronson).

^a Present address: Shinwa Kako Co., Ltd., Kyoto 613, Japan.

Standard solutions

Standard 1000 ppm (as As) sample solutions were respectively prepared by dissolving of p-APA ($pK_{a1} = 2$, $pK_{a2} = 4.02$, $pK_{a3} = 8.92^{18}$), o-APA ($pK_{a1} = 2$, $pK_{a2} = 3.77$, $pK_{a3} = 8.66^{18}$) and o-NPA ($pK_{a1} = 3.37$, $pK_{a2} = 8.62^{18}$) in D.D.W. All other solutions were prepared from analytical reagent grade or reagent grade salts. Working standard solutions were obtained by diluting the stock solutions in the phosphate solution at the same pH as that of the eluent.

RESULTS AND DISCUSSION

Chromatography of organoarsenic species using potassium hydroxide as the eluent

The charges on the arsenic species in solution are controlled by the pK_a values of each species. Thus, the elution behaviours of these arsenic compounds have been investigated in acidic and alkaline solutions. Potassium hydroxide was selected as the alkaline eluent.

Concentration of the eluent. In potassium hydroxide solution (pH 11.0–11.6), it is estimated from the pK_a values that the charge on the arsenic acids is -2 . The eluent concentration was varied in order to investigate the separation of samples. On decreasing the eluent concentration from 4 to 1 mM, the retention times of o-APA and p-APA were increased and the resolution between them was changed from 0.28 to 0.58 on a 120-mm column at an eluent flow-rate of 20 $\mu\text{l}/\text{min}$. p-APA and o-NPA were completely overlapped.

Flow-rate of the eluent. With 2 mM potassium hydroxide as the eluent, the resolution between p-APA and o-APA varied from 0.58 to 0.37 by changing the flow-rate from 10 to 80 $\mu\text{l}/\text{min}$, on a 120-mm column as shown in Fig. 1. As a result, it was concluded that a low flow-rate gives a better separation.

Column length. With 2 mM potassium hydroxide as the eluent and a flow-rate of 20 $\mu\text{l}/\text{min}$, the resolution between p-APA and o-APA was increased from 0.58 to 0.98 by increasing the column length from 120 to 200 mm. However, the use of the latter column was not practical because the sample was strongly retained and the pressure

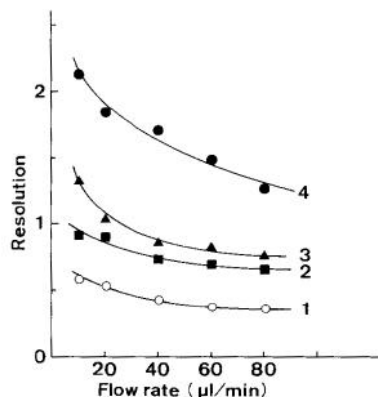


Fig. 1. Dependence of the resolution on the eluent flow-rate. 1, p-APA and o-APA in 2 mM KOH (pH 11.3); 2, o-NPA and o-APA; 3, o-NPA and p-APA and 4, p-APA and o-APA in 5 mM phosphate (pH 3.8). Column: 120 mm \times 0.5 mm I.D.

was too high for elution with conventional flow-rates. Although p-APA and o-APA were separated under these conditions, they overlapped under conventional conditions. On the other hand, o-NPA and p-APA completely overlapped.

Chromatography using phosphate as the eluent

Choice of the eluent. In order to investigate the elution behaviour of organo-arsenic species in acidic solutions, a phosphate eluent, adjusted to pH 3.8 by sodium hydroxide, was selected. Chloride and sulphate eluents were excluded because of difficulties in adjusting the pH, and some organic acids were excluded because of the poor separation of p-APA and o-NPA.

Concentration of the eluent. Fig. 2 shows the relationship between the retention times of samples and the concentration of the eluent at pH 3.8. The changes in the retention times of o-NPA with changes in the eluent concentration were very large compared with those of p-APA and o-APA: namely, o-NPA was eluted close to o-APA in a low concentration eluent, but close to p-APA in a high concentration eluent. From the results, it was found that 5 mM eluent is preferable for a good separation of p-APA, o-NPA and o-APA.

Effect of eluent pH on the retention times of samples. By using phosphate solution as the eluent, the retention times of samples were measured in the range pH 3.4–10.3 adjusted with sodium hydroxide. Fig. 3 shows the relationship between the eluent pH and the retention times of p-APA, o-APA and o-NPA. When the pH was above 9, the charge on all samples was -2 and the samples were strongly retained. It may be concluded that o-APA has a different character and behaviour from those of p-APA and o-NPA, because it forms a six-membered ring by intramolecular hydrogen bonding between the hydrogen of the amino group and the oxygen of the arsenic group.

When the eluent pH was near 8.5, the charge on each sample was changed from -2 to -1 , and the retention times of samples were decreased. When the pH was near to 7.5, the elution order of p-APA and o-NPA was reversed. The elution order was unchanged in the range pH 7.5–4.5.

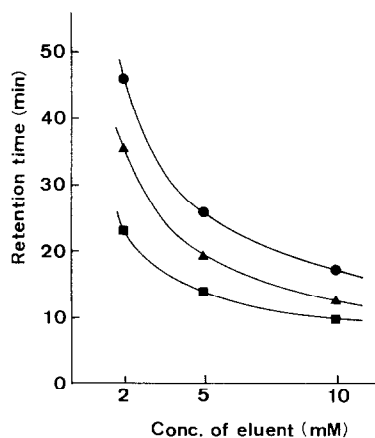


Fig. 2. Dependence of the retention time on the eluent concentration. (■) p-APA; (▲) o-NPA; (●) o-APA. Eluent: phosphate (pH 3.8); flow-rate, 20 μ l/min. Column: 120 mm \times 0.5 mm I.D.

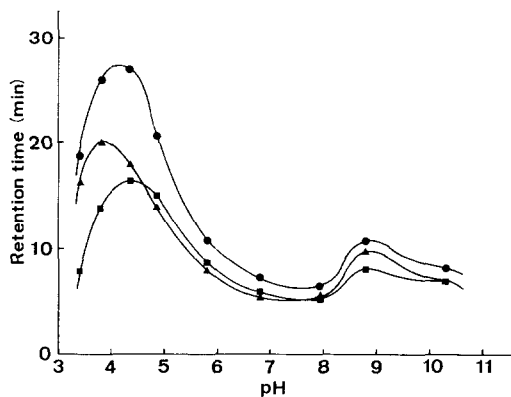


Fig. 3. Dependence of the retention time on the eluent pH. (■) p-APA; (▲) o-NPA; (●) o-APA. Eluent: 5 mM phosphate; flow-rate, 20 μ l/min. Column: 120 mm \times 0.5 mm I.D.

Approaching a pH of 3.8, the retention time of p-APA only was shortened, because the eluent pH was lower than the pK_{a2} of p-APA. Thus, the elution order of p-APA and o-NPA was reversed again. However, the retention times of o-APA and o-NPA were not shortened. Approaching pH 3.4, the retention times of all samples were shortened, and the difference in retention time between o-NPA and o-APA were small. From the results, it was found that the range pH 4.0–3.5 is suitable to determine p-APA, o-APA and o-NPA in the phosphate eluent.

It may be concluded that the separability of samples is increased when the eluent pH is near to the pK_a values of the samples.

Recommended procedure. p-APA, o-APA and o-NPA were completely separated by using the phosphate eluent adjusted to pH 3.8. A 200-mm column was selected to get a good separation, because the pressure was decreased under acidic conditions. The flow-rate was selected to be 20 μ l/min in order to get high resolution and appropriate retention times (Fig. 1). Fig. 4 shows an ion chromatogram of organoarsenic compounds in 5 mM phosphate eluent, pH 3.8, on a 200 mm \times 0.5 mm I.D. column, with a detection wavelength of 210 nm, cell volume 0.6 μ l and flow-rate 20 μ l/min.

Calibration graph and interference. Calibration graphs were obtained by plotting the peak heights (Abs.) against the concentrations of samples (ppm as As). The graphs

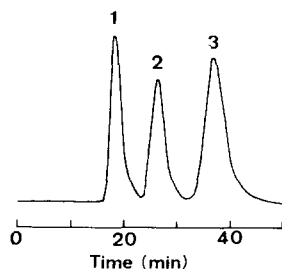


Fig. 4. Chromatogram of organoarsenic compounds. Sample: 1 = p-APA; 2 = o-NPA; 3 = o-APA. Eluent: 5 mM phosphate (pH 3.8); flow-rate, 20 μ l/min. Detection wavelength: 210 nm. Column: 200 mm \times 0.5 mm I.D.

were linear in the range 50–0.4 ppm. Detection limits obtained from the plots were 0.06 ppm for p-APA, 0.07 for o-APA and 0.08 ppm for o-NPA (three times the noise level). As(III) and As(V) did not interfere because the former was not dissociated in the pH range used and the latter did not absorb in the UV range used.

ACKNOWLEDGEMENT

This work was supported by a Grant-in-Aid for Scientific Research No. 63540450, from the Ministry of Education, Science and Culture, Japan.

REFERENCES

- 1 H. Small, T. S. Stevens and W. L. Bauman, *Anal. Chem.*, 47 (1975) 1801.
- 2 D. T. Gjerde, G. Schmuckler and J. S. Fritz, *J. Chromatogr.*, 187 (1980) 35.
- 3 P. R. Haddad and A. L. Heckenberg, *J. Chromatogr.*, 300 (1984) 357.
- 4 T. H. Jupille and D. T. Gjerde, *J. Chromatogr. Sci.*, 24 (1986) 427.
- 5 J. S. Fritz, *Anal. Chem.*, 59 (1987) 335A.
- 6 J. S. Fritz, *J. Chromatogr.*, 439 (1988) 3.
- 7 R. P. W. Scott and P. Kučera, *J. Chromatogr.*, 185 (1979) 27.
- 8 P. Kučera and G. Manius, *J. Chromatogr.*, 216 (1981) 9.
- 9 F. J. Yang, *J. Chromatogr.*, 236 (1982) 265.
- 10 T. Takeuchi and D. Ishii, *J. Chromatogr.*, 238 (1982) 409.
- 11 J. C. Gluckman, A. Hirose, V. L. Guffin and M. Novotony, *Chromatographia*, 17 (1983) 303.
- 12 M. Novotony, M. Alasandro and M. Konishi, *Anal. Chem.*, 55 (1983) 2375.
- 13 R. Gill, *J. Chromatogr.*, 354 (1986) 169.
- 14 H. B. Brooks, C. Thrall and J. Tehrani, *J. Chromatogr.*, 385 (1987) 55.
- 15 T. Okada and T. Kuwamoto, *Anal. Chem.*, 55 (1983) 1001.
- 16 T. Okada and T. Kuwamoto, *J. Chromatogr.*, 284 (1984) 149.
- 17 N. Hirayama and T. Kuwamoto, *J. Chromatogr.*, 447 (1988) 323.
- 18 A. J. Dean, *Lange's Handbook of Chemistry*, McGraw-Hill, New York, 13th ed., 1985.