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APPLICATION OF PHOTODIODE ARRAY ULTRAVIOLET DETECTOR TO UNRESOLVED PEAK ANALYSIS

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SUMMARY

Poorly resolved chromatographic peaks have been mathematically resolved to give all component peaks of a multicomponent sample, using a newly developed 32-channel multiwavelength spectrophotometric UV detector and its data processor. In order to isolate poorly resolved peaks quantitatively, three-dimensional chromatographic data were stored on a floppy disk and computed by a data processor. A mathematical method of simultaneous multicomponent analysis for UV spectra was applied to all the points along the time axis. The results obtained have been compared with those by conventional perpendicular-dropping and tangential-skimming methods for known amounts of components. Recoveries obtained by the new method were 100.7 and 101.1%, while those by the conventional methods wer 89.9 and 110.1%, and 126.8 and 26.4%, respectively, by perpendicular dropping and tangential skimming. The detection of a hidden component in a chromatogram and determination of its UV spectrum was also investigated.

INTRODUCTION

Accurate results in qualitative and quantitative high-performance liquid chromatographic (HPLC) analysis are fundamentally dependent on the degree of separation of the component peaks obtained. However, there are many cases in which one cannot separate the sample components completely, even when the chromatographic conditions are contrived skilfully. For instance, multicomponent samples, such as serum, urine and other physiological liquids, are extremely difficult to separate completely in a single chromatographic analysis. Even though these samples contain important target components which must be quantitatively analyzed with good accuracy and precision, they are often very small in quantity and allow only a few chromatographic experiments. In that case, a conventional HPLC detector, a fixed- or variable-wavelength UV photometric detector or a refractive index detector yields only a single chromatogram from an injection, which may include many un-

resolved peaks. To obtain good accuracy from such a single chromatogram is not generally possible.

In order to obtain more accurate results with those detectors, a number of methods for isolating incompletely separated peaks have been proposed, based on digital integrators. Most of these methods are based on two well known techniques: (1) perpendicular dropping, which simply divides at the valley between unresolved peaks, and (2) tangential skimming, which skims off small peaks from a larger peak. These two methods are quantitatively applicable under only limited conditions: when peak heights and widths are very similar for both peaks for perpendicular dropping, and when the second peak is small enough for tangential skimming. These conditions are quite unlikely to be realized in actual chromatographic experiments. Thus, a more sophisticated and precise peak-isolation technique is required for quantitative analysis of unresolved peaks from multicomponent samples.

In 1979, Klatt[1] reported an isolation technique for unresolved peaks, based on a rapid scanning multiwavelength detection system, interfaced with a microcomputer. At that time the system was very costly and required a high degree of skill. In the last 5 years the cost of microcomputers and photodiode arrays has been drastically reduced and they are now readily available. Multiwavelength UV detectors with 128, 256 or more element photodiode arrays are now commercially available and have already attracted many chromatographers. They offer great advantages for qualitative analysis, producing three-dimensional chromatograms[2–5]. However, it should be noted that the potential of microprocessor-controlled multiwavelength UV detectors is not limited to simple qualitative analysis for purity checks, but it can also be quantitative, because all the data, namely absorbance, wavelength and time, are generated in the three-dimensional chromatographic domain.

We built a new multiwavelength UV detector with a 32-element photodiode array and a data processor and used them for quantitative isolation of poorly resolved chromatographic peaks. Further, we developed computer software for the determination of an unknown and hidden component, by using the mathematical technique of simultaneous multicomponent analysis for UV spectra.

METHOD

Quantitative isolation of unresolved peaks

A chromatogram, obtained with a spectrophotometric detector is defined as a function of wavelength, λ, and retention time, t, as $A_s(\lambda,t)$ in a three-dimensional chromatographic domain. For a multicomponent sample, the chromatogram is expressed as a summation over each individual component, i, as follows

$$A_s(\lambda,t) = \sum A_i(\lambda,t) + e \tag{1}$$

where e represents the error due to noise. The chromatogram of each individual component, i, is expressed as

$$A_i = a_i \mathrm{f}_i(\lambda,t) \tag{2}$$

where $\mathrm{f}_i(\lambda,t)$ represents the chromatogram of a unit amount of the component i,

obtained by injecting a standard sample of the component i, and a_i is a coefficient of quantity for the component i.

Then, the observed chromatogram of a multicomponent sample at the wavelength λ_j is expressed as $A_s(\lambda_j,t)$, and the observed spectrum at the time t_k is expressed as $A_s(\lambda,t_k)$. Thus, the observed spectrum of a multicomponent sample at the time t_k can be expressed by using eqns. 1 and 2 as follows

$$\begin{bmatrix} A_s(\lambda_1,t_k) \\ A_s(\lambda_2,t_k) \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ A_s(\lambda_{32},t_k) \end{bmatrix} = \begin{bmatrix} f_1(\lambda_1,t_k) & f_2(\lambda_1,t_k) & \cdots & f_n(\lambda_1,t_k) \\ f_1(\lambda_2,t_k) & f_2(\lambda_2,t_k) & \cdots & f_n(\lambda_2,t_k) \\ \vdots & \vdots & & \vdots \\ \vdots & \vdots & & \vdots \\ \vdots & \vdots & & \vdots \\ \vdots & \vdots & & \vdots \\ \vdots & \vdots & & \vdots \\ f_1(\lambda_{32},t_k) & f_2(\lambda_{32},t_k) & \cdots & f_n(\lambda_{32},t_k) \end{bmatrix} \begin{bmatrix} a_1 \\ a_2 \\ \\ a_n \end{bmatrix} + e \quad (3)$$

where $A_s(\lambda_1,t_k)$, $A_s(\lambda_2,t_k) \cdots$, $A_s(\lambda_{32},t_k)$ represent observed absorbances for all the wavelengths and e is the error due to noise. Isolated absorbances for the components $i \cdots, n$, *i.e.*, A_1, A_2, $\cdots$, A_n, can be determined by algebraically solving eqn. 3 by applying the multicomponent analysis technique to the UV spectrum obtained at the time t_k. Eqn. 3 consists of 32 independent equations for the time t_k and represents the cross-section along the wavelength and absorbance axes intersecting the time axis at t_k, as shown in Fig. 1. Therefore, in principle, the compositional ratio of as many as 32 components might be determined if their individual UV spectra could be obtained.

To correct for the error inherent in data collection, it is preferable to employ the least-squares method for determining A_1, $\cdots$, A_n. Once isolated absorbances for

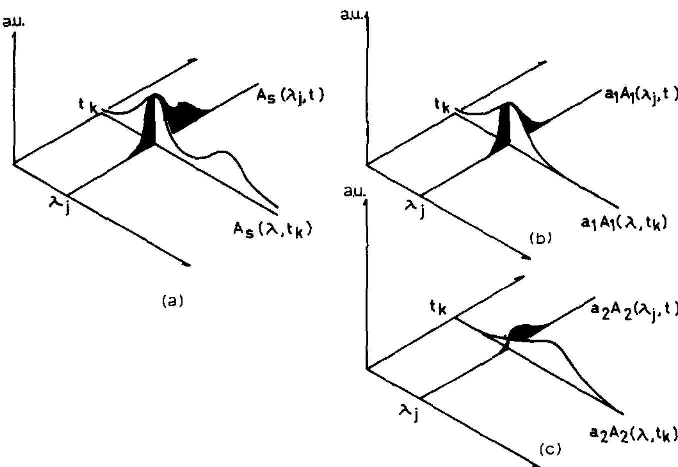


Fig. 1. Schematic representation of a three-dimensional chromatogram/spectrum of a multicomponent sample (a) and its components (b, c).

respective components at the time t_k have been obtained, isolated chromatograms at any wavelength for the components can be numerically derived from eqn. 2 by inserting the wavelength of interest, λ_j. Then, peak integration can be performed independently as with a single peak chromatogram.

Determination of UV spectrum and chromatogram of an unknown component

When chromatographic and spectral data contain unknown or hidden components they will appear as residual error from the equations due to a lack of correlation of the obtained data with calculated data. In performing the least-squares method, the poorer the agreement of the data, the greater is the deviation. However, this treatment does reflect hidden unknown components. A chromatogram of an unknown component, defined as $f_{UNK}(\lambda_j,t)$, can be included in eqn. 1 as

$$A_s(\lambda,t) = \sum A_i(\lambda,t) + f_{UNK}(\lambda_j,t) + e \quad (4)$$

and treated as one of the components. However, obtaining a spectrum of the unknown component means adding 32 unknowns besides the number of coefficients of the known components in eqn. 3. Because solving the set of equations algebraically is difficult, it is preferable to approximate the chromatogram of the unknown component by some simpler method. We found that the summation of the absolute values of the deviations as a function of time is suitable for an approximation. Thus the absolute values of the deviations of the observed spectrum from the spectrum calculated by the least-squares method were summed, and evaluated with eqn. 3 at the wavelength of interest, λ_j.

Once the chromatogram of the unknown component is approximated, coefficients of quantity for all the known components can be derived in the manner described in the previous section, by applying multicomponent analysis to the cross-section along the absorbance and time axes, intersecting the wavelength axis at λ_j.

The new coefficients of quantity derived from the multicomponent analysis of the chromatogram are used for the evaluation of the previous approximation. By using the new ones again for multicomponent analysis of the UV spectrum at the time, t_k, the accuracy of the primary approximation can be evaluated. The least-squares method can be applied to evaluate the deviations of the obtained chromatogram/spectrum from the synthesized one, which was derived from eqn. 3 with the new coefficients of quantity and the approximated chromatogram/spectrum. As the approximation improves with repeated computations, more of the deviations should match the zero surface in the three-dimensional chromatographic/spectral domain.

EXPERIMENTAL

JASCO Model MULTI-320 photodiode array multiwavelength detector

The optical system of the detector (JASCO, Tokyo, Japan) employs dual-beam optics and is designed so that both the sample and the reference cells are irradiated by exactly the same point of the light source by using a beam-splitter mirror. The same point of the diode array is alternately irradiated by the sample and the reference beams as a result of a chopper and a beam-mixing mirror. This arrangement minimizes noise and drift due to the optical elements. The optical diagram is shown in

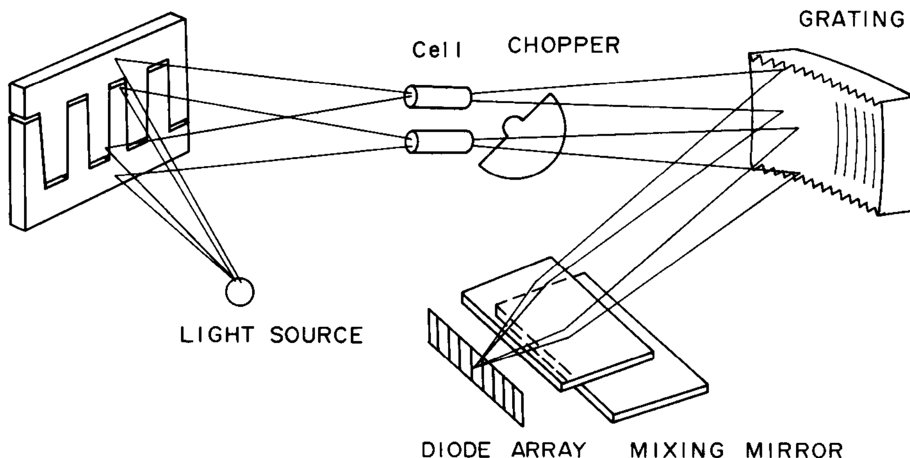


Fig. 2. Optical diagram of the MULTI-320 detector.

Fig. 2. Although, a 128, 256 or more element photodiode array is now commercially available, it cannot be effectively used unless the spectral resolution in terms of spatial width is less than the element width. Thus, it should be emphasized that a spectral band width of a diode array UV detector is generally determined, not by the number of elements, but rather by the optics employed. Therefore, we have adopted a 32-element discrete parallel photodiode array, which covers a spectral band width of 5 nm which is ideally suited to the inner diameter of the flow cell, which functions as an entrance slit, and to the focal length of the polychromator. The signal-processing system incorporates a photodiode array, a photo chopper and a multichannel lock-in amplifier to minimize noise and drift, generated in the signal-processing system, as shown in Fig. 3. The configuration of the JASCO Model MULTI-320/DS-L800

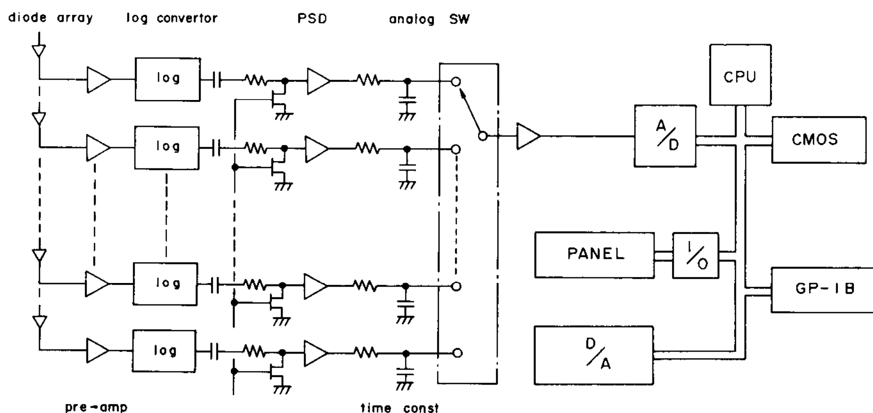


Fig. 3. Block diagram of the signal-processing system of the MULTI-320 detector. Pre-amp = Pre-amplifier; PSD = phase-sensitive detector; SW = wavelength switching; time const = time constant; D/A = digital-to-analog converter; A/D = analog-to-digital converter; I/O = input/output; CMOS = random access memories; GP-IB = interface for data processor.

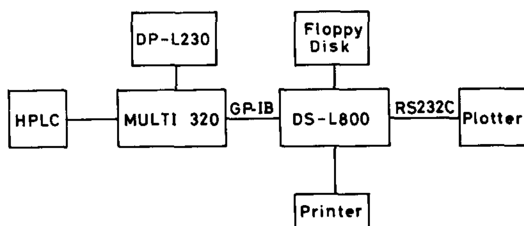


Fig. 4. Configuration of the JASCO MULTI-320/DS-L800 multiwavelength detection system, including peripherals and interconnections. The printer and the dual floppy disk drive unit are integrated in the DS-L800 data processor.

multiwavelength detection system, including peripherals, is as shown in Fig. 4. A printer and a dual floppy disk drive unit were integrated in the JASCO DS-L800 data processor.

HPLC system

The JASCO HPLC system used consisted of a Model TRI ROTAR-V HPLC pump, a Model VL-614 injection valve and reversed-phase columns Finepak SIL C_{18} and Finepak SIL C_8. A conventional digital integrator, JASCO Model DP-L230, was used to compare the accuracy of the results.

Chemicals

Methanol and acetonitrile were LC grade from Wako Pure Chemical Industries (Osaka, Japan). Water was purified from tap-water, by using a Milli-Q/R and Milli-Q water purification system from Millipore Corporation (Bedford, MA, U.S.A.). Phosphoric acid and sodium 1-pentanesulphonate, used for buffer solutions were reagent grade, obtained from Tokyo Chemical Industry Co (Tokyo, Japan). All the eluents were filtered and degassed before use. The test compounds, naphthalene, anthracene and pyrene, were obtained from Wako; acetaminophen and benforitamine were obtained from Sankyo Co. (Tokyo, Japan).

Procedures

The linear dynamic range and the minimum detection limits were examined by common HPLC techniques. To evaluate the applicability of the method, separate chromatograms of known amounts of compounds were mathematically superimposed by computer so that they had different levels of resolution, and the multicomponent analysis method was performed for the superimposed chromatogram to isolate the components quantitatively. This experiment was performed for two different combinations of components in order to examine the contribution of the coefficient of correlation between spectra of those components. Then, poorly resolved actual chromatographic peaks were quantitatively isolated by using the method. The results were compared with those from a conventional integrator, obtained simultaneously during the experiment.

The determination of chromatogram/spectrum of an unknown and hidden component was also investigated by using summations of the absolute values of deviations as an approximation. Fused chromatographic peaks, containing three

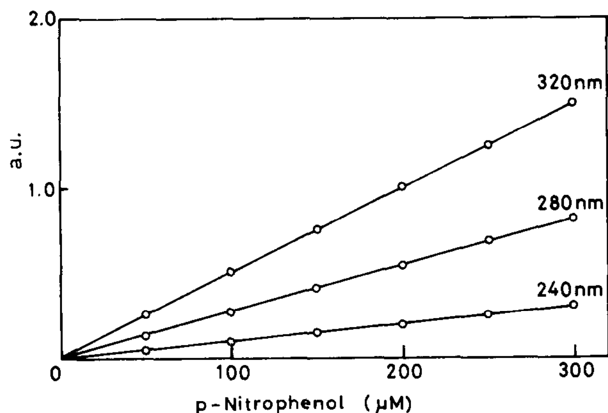


Fig. 5. Linear dynamic range of the MULTI-320 detector. The detector response *vs*. the amount of *p*-nitrophenol is linear up to 1.8 a.u. at 320 nm.

components, were examined, treating one of the known components as the unknown component to evaluate the approximation.

RESULTS AND DISCUSSION

Detector performance

The linear dynamic ranges and the detector response *vs*. sample amount were examined for *p*-nitrophenol at three wavelengths. The detector response was linear up to 1.8 absorbance units (Fig. 5). The noise levels of the detector output at all

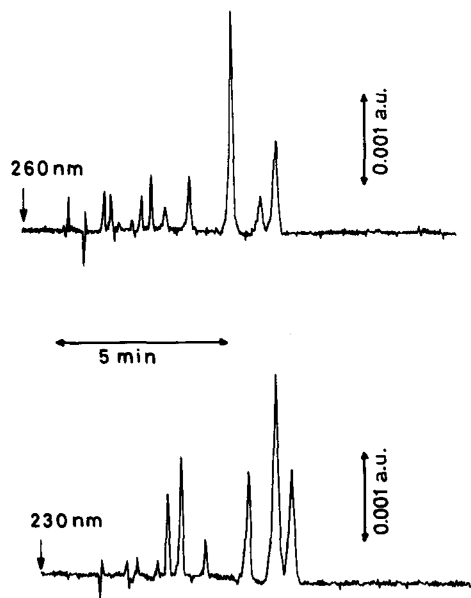


Fig. 6. Typical chromatograms in the high-sensitivity range, obtained by separating aromatic hydrocarbons on a Finepak SIL C_{18} column (250 × 4.6 mm I.D.). Time constant: 0.4 sec.

wavelengths were also examined. The noise level was less than $1 \cdot 10^{-4}$ a.u. according to the standard method of the ASTM[6] for all the wavelengths, as shown in Table I and in Fig. 6. The extremely low noise and drift are considered to be due to employment of well-matched dual-beam optics and a single array of discrete photodiodes.

Applicability to chromatographic peaks at various resolutions and compositional ratios

To evaluate the applicability of the method to components which have very similar spectra, chromatograms of acetaminophen and benfotiamine, obtained by separate injections, were superimposed on each other by the computer so that the resolution, R_s, was varied. Computer simulation was carried out to evaluate the accuracy and the detection limit in the multicomponent analysis. Acetaminophen and benfotiamine had a coefficient of correlation of $R = 0.96$ for their spectra. Chromatograms of 500 ng and 50 ng, respectively, of benfotiamine were superimposed on that of 500 ng of acetaminophen to obtain the following resolutions: $R_s = 0.2$, completely fused; $R_s = 0.6$, shoulder distinguishable; $R_s = 1.25$, well separated. The superimposed chromatograms with different resolution factors are shown in Fig. 7, the UV spectrum of each component in Fig. 8 and quantitative results are shown in Table II. The results show that the recoveries obtained by the multicomponent analysis are independent of the level of resolution, even though the coefficient of correlation was $R = 0.96$. However, the compositional ratio slightly affected the recoveries; this effect may be due to the lower signal-to-noise ratio of the spectrum of benfotiamine.

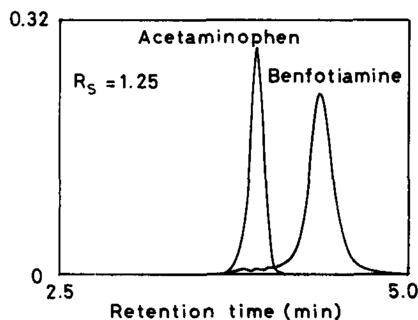
To evaluate the detection limit of the component in smaller quantity in a multicomponent sample, chromatograms of the component, obtained by separately injecting various amounts of pyrene, were superimposed by the computer on the chro-

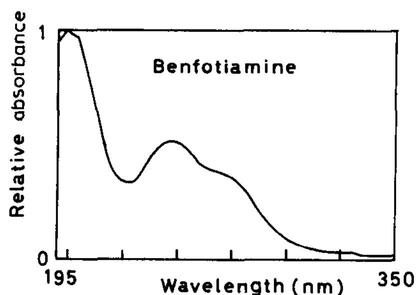
TABLE I

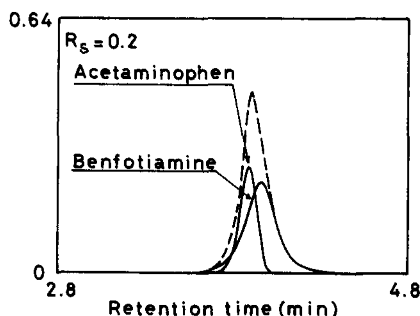
NOISE LEVEL OF EACH WAVELENGTH OUTPUT SIGNAL

Noise level was measured according to ref. 6.

Element number	*Wavelength (nm)*	*Noise (a.u. × 10^{-5})*	*Element number*	*Wavelength (nm)*	*Noise (a.u. × 10^{-5})*
1	195	6.6	17	275	3.2
2	200	6.6	18	280	4.8
3	205	8.3	19	285	5.0
4	210	6.6	20	290	3.2
5	215	5.0	21	295	6.4
6	220	6.6	22	300	4.8
7	225	6.6	23	305	3.2
8	230	5.0	24	310	6.4
9	235	5.0	25	315	6.4
10	240	5.0	26	320	6.4
11	245	6.6	27	325	6.4
12	250	3.3	28	330	6.4
13	255	8.3	29	335	6.4
14	260	5.0	30	340	4.8
15	265	5.0	31	345	6.4
16	270	5.0	32	350	4.8







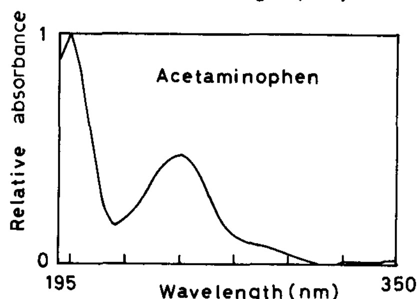


Fig. 7. Superimposed chromatograms with different resolution factors. Separate chromatograms of 500 ng of benfotiamine and acetaminophen were superimposed by the computer so that the resolution could assume arbitrary values. Component chromatograms are shown by solid lines and the superimposed one by a broken line.

Fig. 8. UV spectra of benfotiamine and acetaminophen obtained by separately injecting standard samples.

matogram of a fixed amount (500 ng) of acetaminophen. Then the multicomponent analysis was applied to the superimposed chromatogram. This experiment was performed with resolution factors of R_s = 0.0 (completely fused) and R_s = 1.25 (well resolved). The coefficient of correlation between the spectra of acetaminophen and pyrene is R = 0.49 (compare Figs. 8 and 10). The superimposed chromatogram and component chromatograms with a resolution of R_s = 0.0 are shown in Fig. 9. Quantitative results are shown in Table III. It is seen that the recoveries obtained are totally independent of the level of resolution. Note that recoveries were maintained, even when peaks were completely fused. They were within 100–108% for 100–150 ng of pyrene, but were higher when the amounts of pyrene were less than 50 ng. The reason for poorer recoveries was considered to be the lower signal-to-noise ratio for the lower amounts of pyrene. Even though the noise level of the detector was less than $1 \cdot 10^{-4}$ a.u., 50 ng of pyrene produced only 0.024 a.u. at the wavelength of maximum absorption. Accordingly, it is suggested that 10% of a component, totally fused into the chromatographic peak of another component, could be quantitatively isolated with 10–20% accuracy and a coefficient of correlation of R = 0.49–0.96.

It should be noted that, in order to obtain better quantitative results, it is very important to obtain as high an accuracy as possible in characteristic parts of spectra

TABLE II

EFFECT OF RESOLUTION ON RECOVERY FOR SIMILAR COMPOUNDS AS DETERMINED BY MULTICOMPONENT ANALYSIS

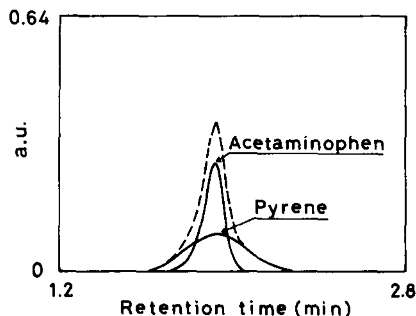
Chromatograms of 500 ng and 50 ng of benfotiamine, respectively, were superimposed by the computer on that of 500 ng of acetaminophen to give resolutions R_s of 0.2, 0.6 and 1.25. The coefficient of correlation between the UV spectra of those compounds is R = 0.96.

R_s	*Area (a.u. · sec)*	
	Acetaminophen	*Benfotiamine*
1:1 mixture		
	2.060 (500 ng)	3.231 (500 ng)
1.25	1.902 (92.3%)	3.400 (105.2%)
0.60	1.869 (90.7%)	3.424 (106.0%)
0.20	1.880 (91.3%)	3.417 (105.8%)
1:0.1 mixture		
	2.060 (500 ng)	0.323 (50 ng)
1.25	2.006 (97.4%)	0.374 (115.8%)
0.60	2.012 (97.7%)	0.379 (117.3%)
0.20	2.023 (98.2%)	0.372 (105.9%)

that distinguish a compound from another. Therefore, a greater number of absorbance data at different wavelengths does not result in higher accuracy.

Comparison of quantitative accuracy

The quantitative accuracy for poorly separated peaks in the new multicomponent analysis was compared with that obtained by a conventional integrator. Mixtures of naphthalene and antrhacene (1:1) and 1:3, w/w) were separated with a resolution of R_s = 0.7 on a Finepak SIL C_8 column with acetonitrile–water (90:10) as eluent (Fig. 11). The chromatograms were analyzed by the newly developed data processor by using multicomponent analysis, and by a conventional integrator. Each



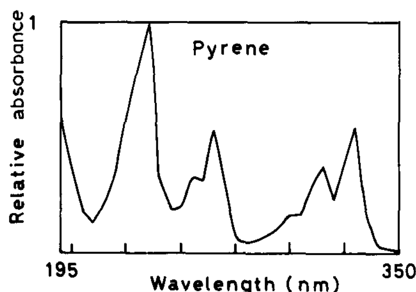


Fig. 9. Superimposed chromatograms and component chromatograms with resolution of R_s = 0.0. Chromatograms obtained by separately injecting pyrene were superimposed by the computer on the chromatogram of 500 ng of acetaminophen so that the resolution was R_s = 0.0. Component chromatograms are shown by solid lines and the superimposed one by a broken line.

Fig. 10. UV spectrum of pyrene obtained by injecting a standard sample.

TABLE III

EFFECT OF AMOUNT OF COMPONENT ON RECOVERY, AS DETERMINED BY MULTICOMPONENT ANALYSIS

Chromatograms of various amounts of pyrene were superimposed by the computer on that of 500 ng or acetaminophen. Resolution factors were R_s = 0.0 and 1.25. The coefficient of correlation between the UV spectra of those compounds is R = 0.49.

Area (a.u. · *sec)*			
Pyrene added		*Acetaminophen*	*Pyrene (recovery in %)*
Area	*Amount (in ng)*		
R_s = *0.0*			
0.182	(20)	2.050	0.230 (126.4)
0.404	(50)	2.054	0.457 (113.1)
0.690	(100)	2.048	0.738 (107.0)
1.108	(150)	2.047	1.168 (105.4)
1.521	(200)	2.046	1.586 (104.3)
1.898	(250)	2.046	1.972 (103.9)
R_s = *1.25*			
0.182	(20)	2.055	0.231 (126.9)
0.404	(50)	2.054	0.457 (113.1)
0.690	(100)	2.049	0.741 (107.4)
1.108	(150)	2.047	1.169 (105.5)
1.521	(200)	2.047	1.590 (104.5)
1.898	(250)	2.047	1.980 (107.9)

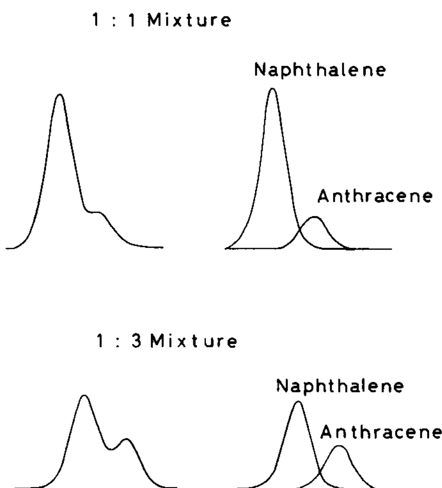


Fig. 11. Chromatograms of 1:1 and 1:3 mixtures of naphthalene and anthracene with a resolution of R_s = 0.7. The chromatograms of 1:1 were obtained by separating these mixtures on a Finepak SIL C_8 column with an eluent of acetonitrile–water (90:10) monitored at 215 nm. They were quantitatively analyzed by the multiwavelength detection system and a conventional integrator. Chromatograms on the left are the observed ones and those on the right are quantitatively isolated chromatograms of the respective components from the multicomponent analysis.

TABLE IV

COMPARISON OF QUANTITATIVE ACCURACY

Quantitative accuracy of poorly separated peaks in the new multicomponent analysis was compared with that obtained by a conventional integrator.

Method	*Mixture*	*Recovery (%)*	
		Naphthalene	*Anthracene*
Multicomponent analysis	1:1	100.7	101.1
	1:3	92.8	101.4
Conventional integrator			
Perpendicular dropping	1:1	89.8	110.1
	1:3	73.5	92.5
Tangent skimming	1:1	126.8	26.4

chromatogram was mathematically isolated by using experimentally determined coefficients of quantity by the multicomponent analysis of absorbance data at 215 nm as a function of time, and then areas of isolated chromatographic peaks were calculated. In Fig. 11 the chromatograms on the left are the observed ones and on the right side are the quantitatively isolated chromatograms of components from the multicomponent analysis. The integrator was set to perform both the perpendicular-dropping and the tangential-skimming methods. As shown in Table IV, the recoveries by the new method are 100.7 and 101.1% for the 1:1 mixture of naphthalene and anthracene, and 92.8 and 101.4% for the 1:3 mixture, even for poorly separated peaks of R_s = 0.7. On the other hand, these were not in accord with recoveries obtained by the conventional method: 89.9 and 110.1% for the 1:1 mixture and 73.5 and 92.5% for the 1:3 mixture by perpendicular dropping, and 126.8 and 26.4% for the 1:1 mixture by tangential skimming.

In this experiment, quantitative analysis was carried out by using mathemat-

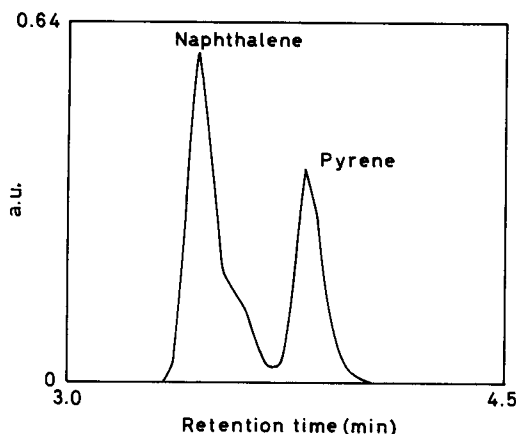


Fig. 12. Chromatogram of naphthalene, pyrene and an unknown component, which is completely fused into those peaks.

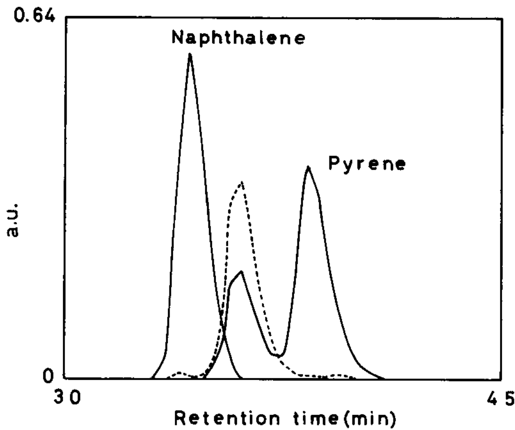


Fig. 13. Mathematically obtained chromatograms of naphthalene and pyrene, and the summation of the absolute values of deviations plotted *vs.* time as an approximate chromatogram for the unknown component (---).

ically obtained chromatograms at 215 nm. However, since absorbance data at all wavelengths between 195 and 350 nm are stored, in order to obtain higher sensitivity, the wavelength of maximum absorption can be used, and to obtain a wider dynamic range, a wavelength at which there is lower absorbance can be used.

Determination of UV spectrum and chromatogram of unknown component

This was investigated by treating one of the known components as an unknown component, fused with the known components. A mixture of naphthalene, pyrene and anthracene (treated as an unknown component) was separated on Finepak SIL C_8 with an eluent of acetonitrile–water (90:10), as shown in Fig. 12. Fig. 13 shows the chromatograms for naphthalene and pyrene, obtained by the multicomponent analysis by using their spectra, shown by solid lines. The summation of the absolute values of deviations of the observed spectrum from that calculated by the least-

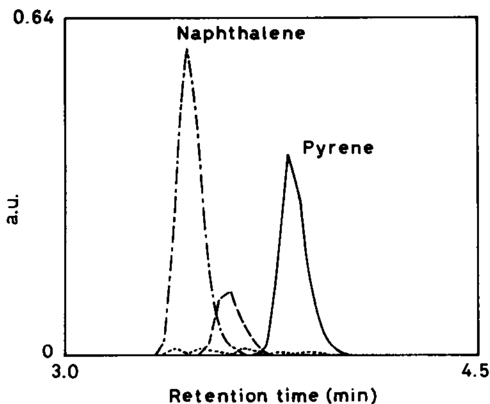


Fig. 14. Mathematically obtained chromatograms of all components including an unknown component; ——, pyrene; - · -, naphthalene; ----, unknown component. The dotted line shows the summation of the absolute values of deviations, and reflects the mismatch between obtained and calculated spectra.

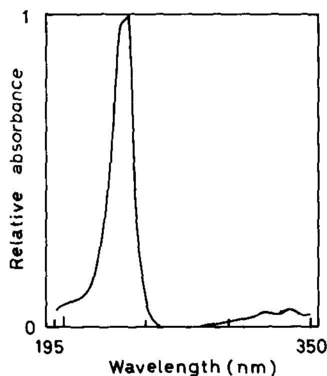


Fig. 15. Mathematically obtained UV spectrum of the unknown component. This spectrum is highly correlated (R_s = 0.96) with that of anthracene.

squares method is also plotted as a function of time. This curve was considered to reflect residual data which did not match either spectrum. Fig. 14 shows the respective chromatograms that were mathematically isolated by the multicomponent analysis by using this curve as an approximation to the chromatogram of the unknown component and standard chromatograms/spectra of napthalene and anthracene, as described under *Procedures*. Fig. 15 shows the mathematically obtained UV spectrum for the unknown component. In order to evaluate the results, the observed chromatogram/spectrum was again mathematically decomposed into each component chromatogram by using the above mathematically obtained chromatogram of the unknown component and the standard chromatograms/spectra of naphthalene and anthracene. Then, the summation of the absolute values of deviations was again plotted as a function of time, as shown in Fig. 14. This curve shows some low peaks. However, the height of any peak is less than 1/10 of the peak height of any component. Considering the fact that the curve was derived from summations of deviations along the whole wavelength axis, mismatch of the spectrum at each wavelength can be much less than that which appears in Fig. 14. Finally, when the mathematically obtained spectrum in Fig. 15 was compared with the standard spectrum of anthracene, it was found that the coefficient of correlation of those spectra was R = 0.96. Thus, it is clear that the approximation is close enough to determine the chromatogram and the UV spectrum of the unknown and hidden component.

We conclude that a photodiode array multiwavelength detector, together with multicomponent analysis, can be a very efficient tool for quantitative and qualitative determination of multicomponent samples in chromatographic analysis.

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