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MULTI-CHANNEL DETECTORS FOR MICRO HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY: EXAMINATION OF FLOW CELL STRUCTURES

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SUMMARY

The use of a UV–visible multi-channel spectrophotometer with a photodiode array in micro high-performance liquid chromatography was investigated. Parallel and cross flow cells for a multi-channel detector were prepared and their characteristics were examined. The geometry of the parallel flow cell provided a relatively long optical path length, leading to a higher sensitivity of detection. The cross flow cell permitted the use of narrow-bore columns with minimal extra-column band broadening. The system was applicable to the separation of antioxidants in gasoline.

INTRODUCTION

The use of multi-channel detectors with a photodiode array has improved the qualitative ability of high-performance liquid chromatography (HPLC), which is poor when a conventional spectrophotometer or a refractometer is used as a detector. They are capable of obtaining spectral information from a single chromatographic run, which offers various advantages over conventional single-wavelength spectrophotometers. We have developed a multi-channel photodiode array UV–visible detector for micro HPLC in cooperation with Union Giken (Osaka, Japan)[1] and applied the system to the separation of components of a drug[2].

The concentration of solutes in the column effluent is higher in micro HPLC, owing to the low dispersion, which offers the possibility of highly sensitive detection. Provided that the same column efficiency is achieved independent of the column bore, the concentration of the solutes is inversely proportional to the cross-sectional area of the column. The cross-sectional area of conventional columns of 4.6 mm I.D. is 200–500 times that of micro columns of 0.2–0.35 mm I.D., which have generally been employed in our work. Therefore, this results in an increase in mass sensitivity in micro HPLC in spite of short optical path length detection. It should be noted that the mass sensitivity can increase with increasing ratio of the optical path length to

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the square of the column diameter. However, the dimensions of the flow cell, such as the optical path length and the cell volume, should be designed in relation to the column dimensions. An increase in mass sensitivity is especially favourable for the analysis of samples available in restricted amounts. The sample volume to be loaded on the column should be reduced accordingly to the column dimensions in micro HPLC, which leads to a decrease in concentration sensitivity. This drawback can be overcome by means of on-column sample enrichment or pre-column concentration techniques.

Miniaturization of flow cells is highly desirable when they are coupled with high-resolution capillary techniques, such as isotachophoresis, zone electrophoresis or open-tubular liquid chromatography. Two kinds of micro flow cells, parallel-flow and cross-flow cells, were prepared for a multi-channel photodiode array detector and their characteristics were examined in this work. The system was applied to the analysis of the real samples.

EXPERIMENTAL

The apparatus consisted of a Micro Feeder (Azumadenkikogyo, Tokyo, Japan) equipped with an MS GAN-050 gas-tight syringe (Terumo, Tokyo, Japan) or a MicroMetric metering pump (LDC/Milton Roy, Riviera Beach, FL, U.S.A.), an ML-422 micro valve injector (19 nl) [Japan Spectroscopic (JASCO), Tokyo, Japan], a micro packed fused-silica column and a micro gate photodiode array detector (MGPD) system (Union Giken).

The MGPD system was the same as in previous work[1], consisting of a multi-channel photodiode array detector, a monitorscope, a plotter and a micro computer. The number of active photodiode array elements was 840 and two elements controlled one channel. The spectral range and the bandwidth of the diode array detector were 200–800 and 1.4 nm, respectively. A deuterium lamp was employed as the light source and covered the range between 200 and 600 nm. Although accumulation times could be changed from 1 to 250, eight times was adopted for this work. The sampling time was changed from 25 to 75 msec in order to keep the light intensity reaching the detector nearly constant. When the three-dimensional information (time, wavelength and absorbance) were stored during the chromatographic run, each spectrum was stored every 2 sec. The dark current noise was around $8 \cdot 10^{-4}$ absorbance units (a.u.). The measurements were carried out with a resolution of 1.6 nm.

The separation column was prepared according to previous work[3]. Silica ODS SC-01 (5 μm) (JASCO) and fused-silica tubing of 0.34 mm I.D. $\times$ 0.42 mm O.D. (Hewlett-Packard, Avondale, PA, U.S.A.) were selected as the materials for the separation columns.

Fused-silica tubing with various dimensions was obtained from Hewlett-Packard, SGE (Melbourne, Australia) or SpecTran (Sturbridge, MA, U.S.A.), and employed as a micro flow cell and/or connecting tubing.

Gasoline samples were prepared according to previous work[4]. Gasoline was diluted 300-fold with *n*-hexane and 300 μl of the resulting solution were introduced into the concentration column. The concentration column was composed of PTFE tubing, 10 $\times$ 0.2 mm I.D., packed with Develosil 60-10 (Nomura Chemical, Seto-

shi, Japan). After collecting the samples, the concentration column was purged of *n*-hexane in a stream of nitrogen and connected to the head of the separation column before the chromatographic analysis.

Reagents were supplied by Wako (Osaka, Japan) and Tokyo Chemical Industry (Tokyo, Japan), unless indicated otherwise.

RESULTS AND DISCUSSION

Most UV–visible detectors used in conventional HPLC have a parallel flow cell, *i.e.*, the light passes parallel to the direction of the flow in the cell, and this geometry provides a relatively long optical path length. On the other hand, a cross flow cell has mostly been employed in micro HPLC, as additional band broadening

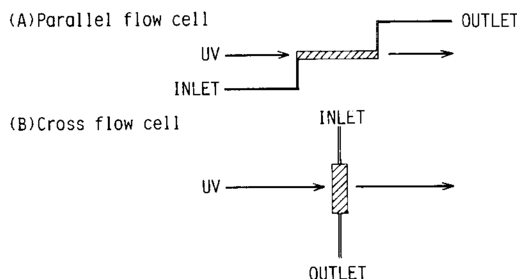

Fig. 1. Structures of the flow cells for UV detection.

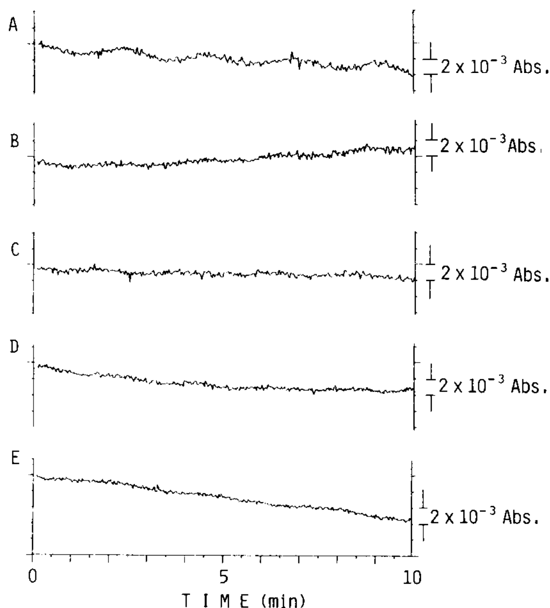

Fig. 2. Noise level of the photodiode array detector. Mobile phase: acetonitrile–water (7:3). Pump: MicroMetric metering pump. Flow-rate: 5 μl/min. Flow cells: (A) cross, 0.26 mm I.D. × 1.9 mm, 40 μm slit width; (B) cross, 0.26 mm I.D. × 1.6 mm, 100 μm slit width; (C) cross, 0.26 mm I.D. × 0.45 mm, 220 μm slit width; (D) cross, 0.34 mm I.D. × 0.96 mm, 100 μm slit width; (E) parallel, 1 mm × 0.25 mm I.D. Sampling time: (A) 75 msec × 8; (B) 25 msec × 8; (C) 40 msec × 8; (D) 45 msec × 8; (E) 35 msec × 8.

in the flow channel between the column and the cell can easily be minimized. The structures of both types of flow cells are illustrated in Fig. 1.

The parallel flow cell had the dimensions 1 × 0.25 mm I.D. The eluent from the column was passed into the fused-silica connecting tube (15 cm × 55 μm I.D.) and the narrow channel (0.2 × 0.2 × 8 mm) and then into the optical path. On the other hand, a narrow-bore connecting tube (15 cm × 55 μm I.D.) could be connected directly to the flow cell when using the cross flow cell. Further, cross flow cells with various dimensions could easily be prepared in relation to the column dimensions.

Fig. 2 shows the baselines observed at 254 nm with several flow cells. The noise level of the baseline for the parallel flow cell was lower than that of the cross flow cells. In the latter instance, the noise level was slightly dependent on both the slit

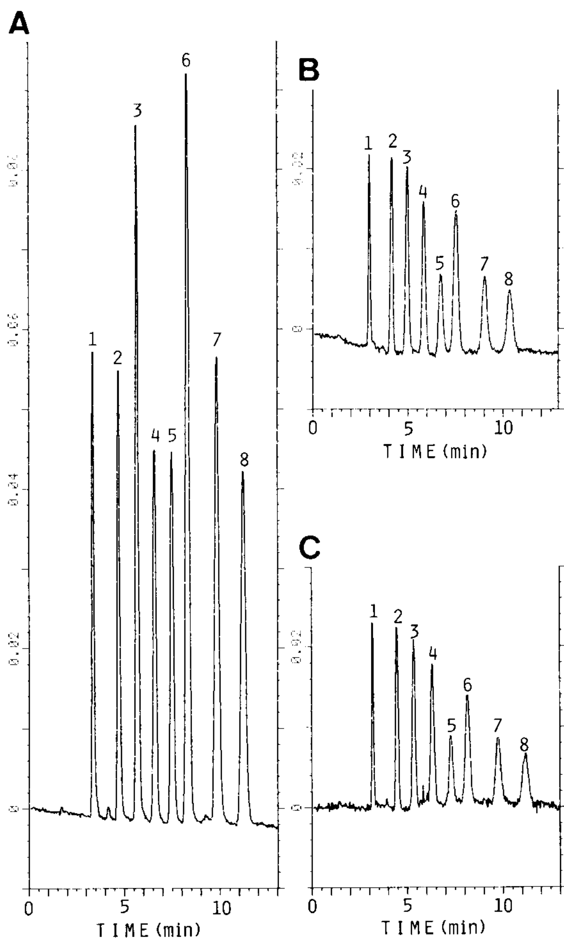

Fig. 3. Chromatograms of PAHs with different cell structures. Column: silica ODS SC-01, 150 × 0.34 mm I.D. Mobile phase: acetonitrile–water (7:3). Pump: MicroMetric metering pump. Flow-rate: 5 μl/min. Wavelength: 254 nm. Flow cells: (A) parallel, 1 mm × 0.25 mm I.D.; (B) cross, 0.34 mm I.D. × 0.96 mm, 100 μm slit width; (C) cross, 0.26 mm I.D. × 1.6 mm, 100 μm slit width. Samples: 1 = benzene (280 ng); 2 = naphthalene (21 ng); 3 = biphenyl (5.5 ng); 4 = fluoranthene (5.3 ng); 5 = phenanthrene (1.3 ng); 6 = anthracene (1.2 ng); 7 = fluoranthene (5.9 ng); 8 = pyrene (5.8 ng).

height and width, the optical path length and the sampling time. The noise level of the baseline can be estimated to be less than $5 \cdot 10^{-3}$ a.u./cm for the parallel flow cell and $1 \cdot 10^{-2}$–$4 \cdot 10^{-2}$ a.u./cm for the cross flow cells. These values are greater than those of a single-wavelength spectrophotometer for micro HPLC. In addition, the drift of the baseline was hardly dependent on the cell structure and wavy drift was observed when the slit window was narrow.

The chromatograms of polynuclear aromatic hydrocarbons (PAHs) in Fig. 3 were obtained with three kinds of flow cells. High sensitivity of the parallel flow cell can be seen. It should be noted that spectra of *ca.* 1 ng of phenanthrene or anthracene can be measured even with the small flow cell. The relative peak heights of solutes eluted earlier are small for the parallel flow cell compared with those for the cross

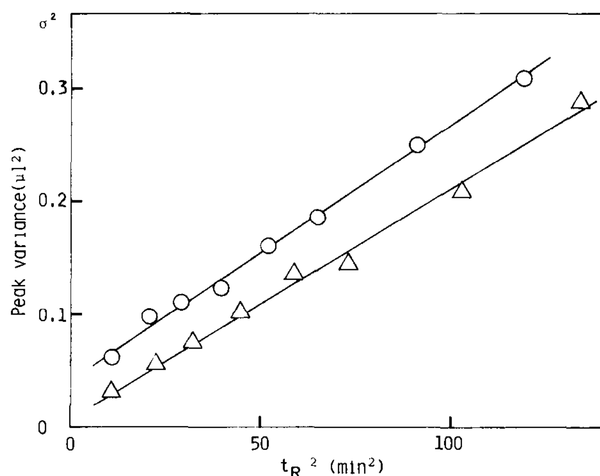

Fig. 4. Observed peak variance *versus* the square of the retention time. Operating conditions as in Fig. 3 except the flow cells. Flow cells: ○, parallel, 1 mm × 0.25 mm I.D.; △, cross, 0.26 mm I.D. × 1.9 mm, 40 μm slit width.

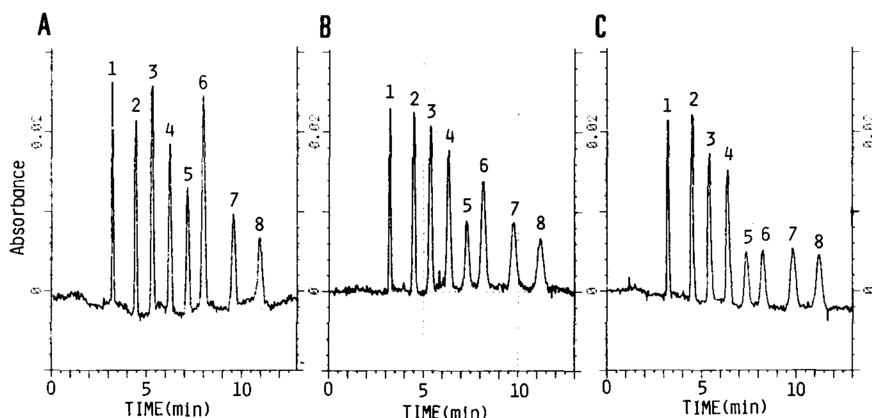

Fig. 5. Chromatograms of PAHs with different slit dimensions. Operating conditions as in Fig. 3 except the flow cell. Flow cells: (A) 40 μm slit width, 0.26 mm I.D. × 1.9 mm; (B) 100 μm slit width, 0.26 mm I.D. × 1.6 mm; (C) 220 μm slit width, 0.26 mm I.D. × 0.45 mm.

flow cells. This result is primarily due to the large effect of extra-column band broadening in the former cell. The noise level of the cross flow cell of 0.34 mm I.D. was lower than that of the 0.26 mm I.D. cell.

Fig. 4 shows peak variance (σ^2) *versus* the square of the retention time (t_R). The observed curve for the parallel flow cell is above that for the cross flow cell, which indicates that the extra-column effect is larger in the former. This distortion of the parallel flow cell is caused by broadening in the flow channel (0.2 × 0.2 × 8 mm) close to the optical path. The extra-column variances measured without the separation column were 0.049 μl^2 for the parallel flow cell and 0.011 μl^2 for the cross flow cell. As the extra-column band broadening is serious for weakly retained solutes, the plate height increased with decreasing retention time when the parallel flow cell was used. The structure of the flow channel should be improved so that it does not cause additional band broadening.

The noise level of the baselines decreased with increasing slit height and width for the cross flow cells. The slit height determines the cell volume, so that its dimensions are restricted by the column dimensions. On the other hand, the slit width can affect the linearity of detection, the resolution of spectra and the mean optical path length; they have a tendency to decrease with increasing slit width. Chromatograms of PAHs obtained with different slit dimensions are shown in Fig. 5.

The system permitted the recording of three-dimensional spectrochromatograms and other new methods. Fig. 6 illustrates a three-dimensional spectrochromatogram of PAHs obtained with the cross flow cell. The noise level, even at low

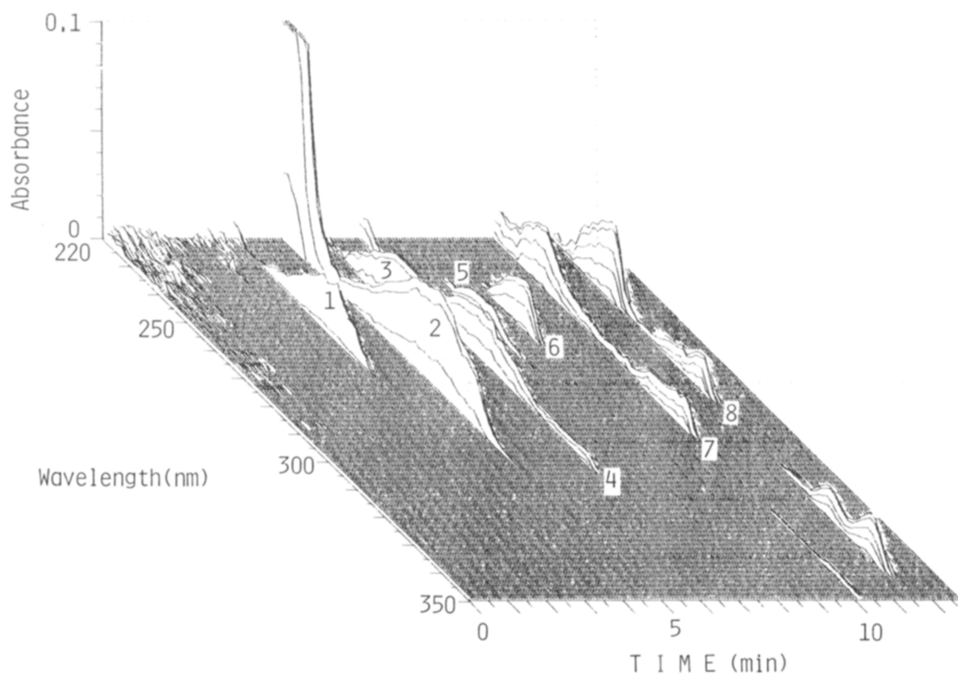

Fig. 6. Three-dimensional spectrochromatogram of PAHs with the cross flow cell. Operating conditions as in Fig. 3 except the flow cell. Flow cell: 0.26 mm I.D. × 1.9 mm, 40 μm slit width.

wavelength, is satisfactorily small. Three-dimensional plots with a better signal-to-noise ratio were provided by the parallel flow cell.

A multi-channel photodiode array detector is suitable for analysing real samples and the system was applied to the separation of antioxidants in gasoline. *p*-Phenylenediamines and phenols are usually employed as antioxidants in gasoline. Fig. 7A demonstrates the separation of an artificial mixture of standard compounds, *viz.*, N,N'-di-*sec.*-butyl-*p*-phenylenediamine (BPA), N-phenyl-N'-*sec.*-butyl-*p*-phenylenediamine (PBPA), N,N'-disalicylidene-1,2-propanediamine (MDA) and 2,6-di-*tert.*-butyl-*p*-cresol (DBPC). BPA, PBPA and DBPC were obtained from Sumitomo Chemical (Tokyo, Japan). MDA functions as a metal deactivator. The amount of PBPA corresponds to that in 1 μl of gasoline. The purity of the peak could be checked from spectral information.

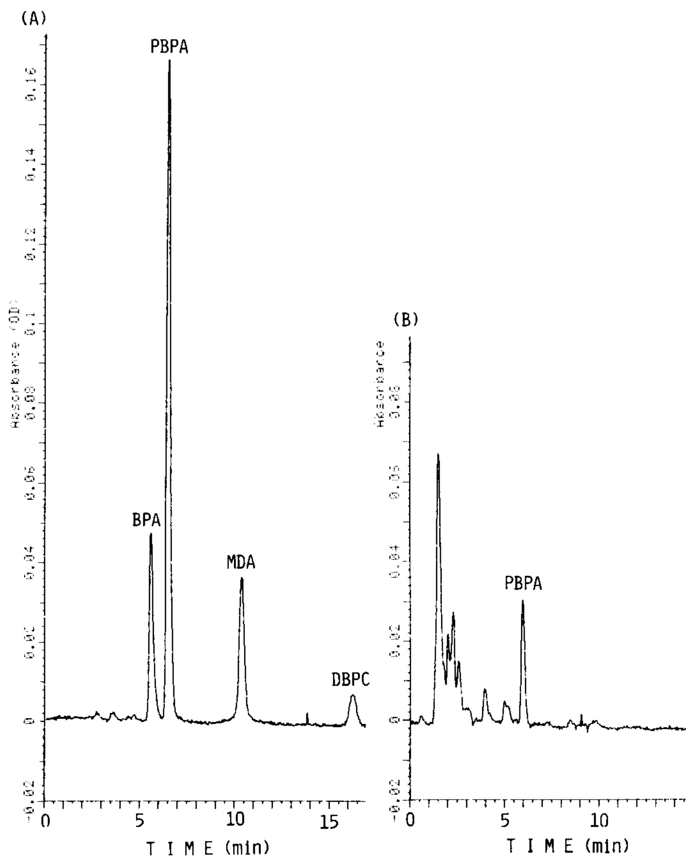

Fig. 7. Separation of antioxidants and a metal deactivator. Column: silica ODS SC-01, 150 × 0.34 mm I.D. Pre-column: Develosil 60-10, 10 × 0.2 mm I.D. Mobile phase: acetonitrile–water–*n*-hexylamine (65:35:1). Pump: Micro Feeder. Flow-rate: 5.6 μl/min. Samples: (A) standard mixture; (B) gasoline (1 μl). Wavelength: 290 nm.

Fig. 8 illustrates a contour plot of the gasoline sample. This plot is useful for selecting optimal conditions for the determination by single-wavelength detection.

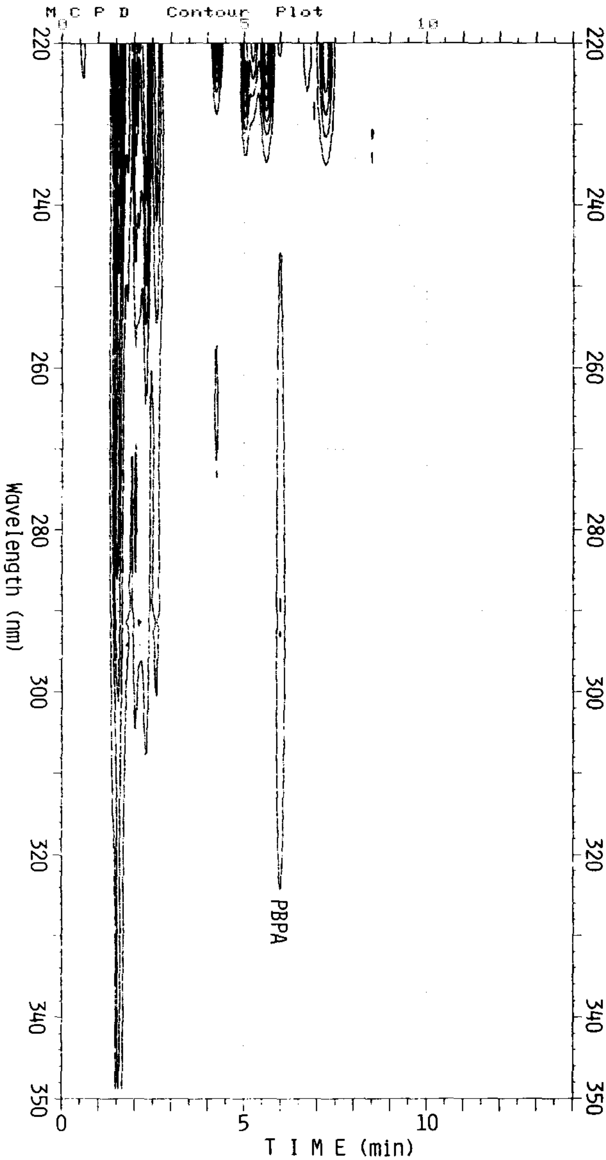

Fig. 8. Contour plot of additives in gasoline. Operating conditions as in Fig. 7B.

CONCLUSION

Micro HPLC could be coupled with a UV–visible photodiode array detector by using both parallel and cross flow cells. The former cell provided high sensitivity and the latter caused no deterioration of the column efficiency. The flow cell dimensions should be designed in relation to the column dimensions. With the cross flow cell, multi-channel photodiode array detector can be applied to other types of low-

dispersion separation techniques, *e.g.*, electrophoresis or capillary liquid chromatography. The cross flow cell will enable the above detection system to be applied to micro-scale supercritical-fluid chromatography with carbon dioxide as the mobile phase.

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