# INFLUENCE OF CHEMICAL MODIFICATION OF POLYMERIC RESIN ON RETENTION OF POLAR COMPOUNDS IN SOLID-PHASE EXTRACTION

## ABSTRACT

Three polymeric sorbents, two of which had been chemically modified with different hydrophilic functional moieties and the third, which was the corresponding unmodified polystyrene-divinylbenzene (PS-DVB) resin, were compared for solid-phase extraction (SPE) of several polar pesticides and phenolic compounds from water samples. The SPE system was on-line coupled to a liquid chromatograph with UV detector.

Chemical modification of the PS-DVB resin with either 2-carboxy-3/4-nitrobenzoyl or 2,4-dicarboxybenzoyl, improved the efficiency of the SPE process by increasing polar interactions with the analytes. The sorbent with the nitro group gave higher recoveries, mainly for the most polar analytes. This sorbent enabled 100 ml river water to be preconcentrated to determine the target analytes in this matrix.

*Keywords:* Column liquid chromatography; Solid-phase extraction; Chemically-modified polymeric resins; Polar pollutants; Water analysis.

**INTRODUCTION**

Pollutants in water need to be determined to solve environmental and health problems. In recent years, solid-phase extraction (SPE) has become a very important technique for sample preparation in the environmental field. However, many types of organic compound, mainly polar ones, are not completely extracted from predominantly aqueous solutions with typical SPE materials (modified silicas, carbon blacks and styrene-divinylbenzene copolymers (PS-DVB)). Moreover, silica-based materials have considerable pH lability. These drawbacks can be overcome by using chemically derivatized polymeric resins which have enhanced surface hydrophilicity and improved extraction efficiencies, due to the possibility of polar interactions between the polar analytes and the functional groups of these resins.

Some of these derivatized polymers were used by Sun et al. in analytical columns, in reversed-phase liquid chromatography, and they were much better wetted by water and had higher retention times for most polar test compounds [1]. Best results were given by the acetyl resin although the hydroxymethyl resin also retained polar analytes more strongly than the underivatized resin. These results suggested that resins can be used as SPE sorbents. Several authors have used these acetyl [2-7] and hydroxymethyl [2,4] resins in SPE. Recoveries for the most polar analytes were higher than recoveries obtained with underivatized resin and other SPE sorbents, such as $C_{18}$-silica [2-4], PLRP-S (PS-DVB resin) and Envi-Chrom P (highly crosslinked PS-DVB) [7]. In previous papers, we described introduction of other hydrophilic groups, such as benzoyl [8] and *o*-carboxybenzoyl [9-12], into PS-DVB resins. These sorbents were compared with PLRP-S, Envi-Chrom P, LiChrolut EN (highly crosslinked PS-DVB) [8] and Envi-Carb (graphitized carbon black (GCB)) [10] in the extraction of polar phenolic compounds and pesticides from water samples, and they gave

higher breakthrough volumes in all cases, except for LiChrolut EN which gave slightly higher recoveries.

Bond Elut PPL is a commercially available, functionalized PS-DVB which has been developed to extract highly polar species, such as phenol, from large volumes of water. It has been used and compared with GCBs in the SPE of pesticides and phenolic compounds from natural waters, and breakthrough volumes were higher (for phenol: 2 ml with Envi-Carb, 14 ml with Bond Elut PPL and 14.2 ml for polystyrene-divinylbenzene resin modified with *o*-carboxybenzoyl moiety) [10]. It has also been compared with HYSphere-1 (highly crosslinked PS-DVB, but in this case the breakthrough volume was higher: 22 ml) [13].

Slightly sulfonated resins have also been used as SPE sorbents [4,14,15]. If a polymeric resin is sulfonated, it changes into a cation-exchanger. Moreover, hydrogen bonding between some analytes and the sulfonic acid group on the resin is also possible.

Other authors have synthesised a new functionalized crosslinked polymer with a carboxylic acid group using ring-opening-metathesis polymerization [16,17]. This sorbent is a crosslinked poly(norbornene-5,6-dicarboxylic acid), so hydrophilic analytes can be retained by the carbonyl groups, which increase the hydrophilic character of the resin. It has been used in the SPE of a wide range of organic compounds which are retained by both hydrophobic adsorption and ion-exchange.

In the search for new chemically modified polymeric resins that increase the retention of the most polar analytes, we have introduced two new hydrophilic functional moieties, 2-carboxy-3/4-nitrobenzoyl and 2,4-dicarboxybenzoyl, on a PS-DVB resin. To our knowledge these modifications have not been used for SPE. The aim of this work is to evaluate the effect of these modifications on the

retention of polar water pollutants in SPE. For this reason, these sorbents have been compared with the unmodified resin and with another chemically modified polymer, previously synthesised in our laboratory, to determine the effect of the different functional groups introduced.

## EXPERIMENTAL

### Equipment

The liquid chromatographic system consisted of two Shimadzu (Tokyo, Japan) LC-10AD pumps, a Shimadzu DGU-4A degasser, and a Shimadzu SPD-10A UV spectrophotometric detector. The analytical column was a 25 x 0.46 cm I.D. Kromasil 100 $C_{18}$, particle size: 5 μm, supplied by Teknokroma (Barcelona, Spain) the temperature of which was controlled by a Shimadzu CTO-10A oven. For direct injection, a Rheodyne valve (Cotati, CA, USA) with a 20 μl loop was used. A Hewlett-Packard (Waldbronn, Germany) dual-channel interface module HP-35900C connected the analytical system to the HP-3365 Series II Chemstation recording chromatographic data.

A Must column-switching device (Spark Holland, Emmen, The Netherlands) was used in the on-line SPE. A Waters (Milford, MA, USA) M45 pump was used to deliver the sample and to condition and clean the sorbents. Trace enrichment was by using 10 x 3 mm I.D. steel precolumns, laboratory-packed with the different sorbents studied.

### Reagents and Standards

The compounds studied (phenolic compounds and pesticides) can be divided into two groups. Group A is made up of oxamyl and methomyl (carbamate pesticides), atrazine and desisopropylatrazine (DIA) (triazine and metabolite),

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phenol (Ph), dimethoate (organophosphorus pesticide) and 2,4-dichlorophenoxy acetic acid (2,4-D) (aryloxyalkanoic acid pesticide). Group B is made up exclusively of highly-polar phenolic compounds, such as hydroquinone, resorcinol, catechol, orcinol and guaiacol. Phenolic compounds were from Aldrich-Chemie (Steinheim, Germany) and pesticides from Riedel-de Haën (Seelze, Germany). They were all 95-99% purity. Stock solutions of each compound (2 g $l^{-1}$) were prepared in methanol. Standard solutions were prepared weekly by diluting stock solutions with pure demineralized water from a Milli-Q filtration system (Millipore, Bedford, MA, USA) (Milli-Q water). They were used daily to spike water samples. All solutions were stored under refrigeration at 4°C.

Acetonitrile for chromatography (Lichrosolv) (Merck, Darmstadt, Germany) and Milli-Q quality water, adjusted to pH 3 with sulphuric acid (Probus, Badalona, Spain), were used to prepare the mobile phase. Hydrochloric acid (Probus) was added to adjust the pH of the sample to 2.5 before SPE in order to prevent analytes ionising. Real water samples were filtered through a 0.45 µm nylon membrane (Whatman, Maidstone, UK) before SPE to eliminate particulate matter. 1 ml 10% (*w/v*) $Na_2SO_3$ solution (Panreac, Barcelona, Spain) was added to 100 ml Ebro river water samples to reduce the frontal hump that appears in the chromatograms because of the presence of humic substances [9,11].

Reagents used in the synthetic procedures were nitrobenzene and acetic anhydride from Probus, anhydrous aluminium chloride and 3-nitrophathalic acid from Fluka (Buchs, Switzerland) and trimellytic acid (1,2,4-benzenetricarboxylic anhydride) from Aldrich-Chemie.

**Synthetic Procedures and Polymer Characterisation**

Both chemically-modified resins were prepared from porous crosslinked polystyrene-divinylbenzene (PS-DVB) beads. Amberchrom GC-161m (Tosohaas, Montgomeryville, PA, USA) is a spherical resin with an average particle and pore size of 50-100 μm and 110-175 Å, respectively, and a specific surface of 900 $m^2$ $g^{-1}$. Both reactions were carried out by a Friedel-Crafts reaction at room temperature and under mechanical stirring for 15 h. Magnetic stirring could not be used because it degraded the beads. Both reactions were quenched by adding acetone with 1% HCl. The filtered polymers were washed three times with methanol and dried under vacuum at 60ºC. Chemical structures of these sorbents are shown in Fig. 1.

*Modification with a 2-Carboxy-3/4-Nitrobenzoyl Moiety*

The 2-carboxy-3/4-nitrobenzoyl derivative (we will use -3/4- in all paper to indicate a mixture of isomers with the nitro group in positions 3 and 4), A-$NO_2$, was prepared by aromatic electrophilic substitution with 3/4-nitrophthalic anhydride using distilled nitrobenzene as the solvent and anhydrous aluminium chloride as the catalyst. The 3/4-nitrophthalic anhydride was previously prepared in our laboratory by heating, under magnetic stirring, a mixture of 21 g 3-nitrophthalic acid and 40 g redistilled acetic anhydride until a clear solution was obtained. The hot mixture was allowed to cool before being filtered. The crystalline mass was washed four times with diethyl ether and dried under vacuum at 60ºC. 2.5 g Amberchrom was swollen in 70 ml nitrobenzene at room temperature under mechanical stirring for 8 h. Afterwards, 4.5 g $AlCl_3$ was added slowly and, when it had dissolved, 6.8 g 3/4-nitrophthalic anhydride and 75 ml of nitrobenzene were added.

The presence of this moiety was proved by a broad H-bonded $\nu$OH at c.a. 3500 $cm^{-1}$, $\nu$C=O of the ketonic carbonyl at 1670 $cm^{-1}$, COOH absorption at c.a. 1700

cm$^{-1}$ and a small band of $\nu NO_2$ at c.a. 1550 cm$^{-1}$ on the IR spectrum. The extent of the modification, 7%, was established by elemental analysis.
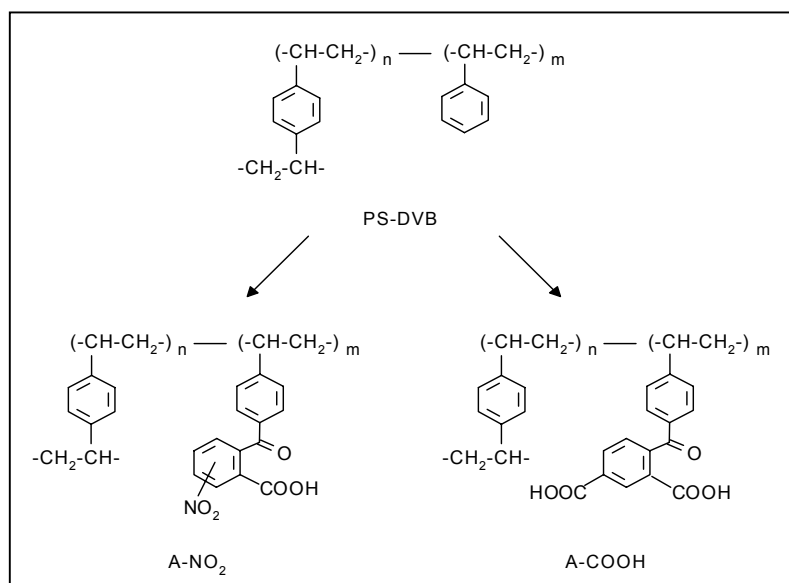


**Fig. 1.** Structures of functionalized polymeric sorbents.

*Modification with a 2,4-Dicarboxybenzoyl Moiety*

The 2,4-dicarboxybenzoyl derivative, A-COOH was prepared like the previous one. That is, 2 g Amberchrom was swollen in 70 ml nitrobenzene at room temperature under mechanical stirring for 8 h. Subsequently, 11.2 g AlCl$_3$ was added slowly. When it had dissolved, 7.86 g trimellytic acid, ground and dissolved in nitrobenzene by heating and 75 ml nitrobenzene were added.

This polymer was also characterised by IR spectroscopy. The salient features of the infrared spectra show a broad H-bonded $\nu OH$ at c.a. 3500 cm$^{-1}$ and another broad band assignable to the COOH absorption at c.a. 1730 cm$^{-1}$. Elemental analysis showed that the extent of modification was 6%.

The low degree of modification may be due to restricted accessibility to reactive sites or to the fact that the anhydrides are not as reactive as other reagents [12].

## Chromatographic Conditions and On-Line SPE Procedure

Both groups of analytes were separated using the same elution gradient. Solvent A was Milli-Q water acidified at pH 3 with sulphuric acid and solvent B was acetonitrile (ACN). The gradient profile was 15-25% ACN from 0-9.5 min, 50% ACN at 14 min, 70% ACN at 18 min, 100% ACN at 20 min and then isocratic elution for 2 min. Afterwards, the mobile phase was returned to the initial conditions in 2 min. The post-run time was 10 min. The flow-rate of the mobile phase was 1 ml min$^{-1}$ and the column temperature 65ºC.

Group A was detected at 240 nm, except phenol and dimethoate which were detected at 210 nm and 2,4-D at 220 nm. All analytes in group B were monitored at 280 nm.

Stainless-steel precolumns (10 x 3mm I.D.), laboratory-packed with the unmodified or modified polymeric sorbents, were used to perform on-line trace enrichment. The sorbents were conditioned with 2 ml ACN and 2 ml Milli-Q water acidified (pH 2.5) at a flow-rate of 2.5 ml min$^{-1}$. Once activated, the sample was percolated at 4 ml min$^{-1}$. In the next step, analytes were desorbed using only the organic solvent (ACN) of the mobile phase (backflush mode; 1 min), to reduce band broadening, and transferred on-line to the analytical column. Both components of the mobile phase were mixed before they reached the analytical column [18].

## RESULTS AND DISCUSSION

### On-line Trace Enrichment

Before on-line SPE, the wavelength program and gradient elution were optimised to separate the analytes studied. Optimum conditions are already described under Chromatographic Conditions. The linearity of response by direct injection was good: 0.25-20 mg l$^{-1}$ for all compounds, and regression coefficients were higher than 0.9995.

Recoveries and breakthrough volumes in the SPE process were determined for analytes in group A by percolating different sample volumes (4, 20, 50, 100 and 200 ml) of standard solutions spiked with different concentrations of analytes through the different precolumns, so that the amount of each analyte in the samples was constant (0.1 μg injected). Recoveries were calculated by measuring the peak area for each compound and dividing it by the area obtained by direct injection. Table 1 shows the recoveries obtained with the two new sorbents and the unmodified resin.

By comparing the three SPE sorbents, it can be seen that introducing hydrophilic groups into the PS-DVB resin increased recoveries of analytes, mainly those of the most polar, because polar interactions (hydrogen bonds and dipolar interactions) were possible between these new resins and analytes. This is the case especially at high sample volumes. For example, when 200 ml was preconcentrated, all the compounds had recoveries of more than 70% with the functionalized polymers, except for phenol which gave 36% with A-COOH and 60% with A-NO$_2$. However, with Amberchrom, the underivatized resin, recoveries were between 53% for oxamyl and 20% for phenol (except for dimethoate, 2,4-D and atrazine which had recoveries of more than 77%,

although these recoveries were slightly lower than with the modified resins) (see Table 1).

As results with the modified resins were better, we tested these for the SPE of highly polar compounds, group B, for which low recoveries with some commercial sorbents (PLRP-S, Amberchrom, Envi-Chrom P and LiChrolut EN) were obtained previously [7]. Recoveries of these analytes were also better with the synthesised resins, as seen in Table 2, but only 4 ml could be extracted which involves high limits of detection with the detector used. So, this group was not considered in the application to real samples.

**Table 1**

Recoveries (% R) of group A analytes obtained by preconcentrating sample volumes with three sorbents

| Compounds | 20 ml | | | 50 ml | | | 100 ml | | | 200 ml | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | A-COOH | A-NO$_2$ | A | A-COOH | A-NO$_2$ | A | A-COOH | A-NO$_2$ | A | A-COOH | A-NO$_2$ |
| Oxamyl | 75 | 80 | 83 | 77 | 80 | 88 | 69 | 82 | 86 | 53 | 80 | 80 |
| Methomyl | 73 | 78 | 77 | 71 | 78 | 80 | 55 | 78 | 81 | 33 | 71 | 77 |
| DIA | 73 | 77 | 74 | 71 | 77 | 76 | 56 | 77 | 80 | 34 | 71 | 75 |
| Ph | 63 | 72 | 78 | 56 | 68 | 78 | 47 | 55 | 64 | 20 | 36 | 60 |
| Dimethoate | 79 | 79 | 78 | 83 | 80 | 86 | 76 | 81 | 85 | 86 | 81 | 78 |
| 2,4-D | 76 | 75 | 72 | 81 | 85 | 77 | 79 | 86 | 86 | 78 | 82 | 86 |
| Atrazine | 70 | 74 | 77 | 78 | 79 | 81 | 78 | 83 | 87 | 77 | 87 | 86 |

A : Amberchrom GC-161m
RSDs < 7% in all instances (n= 4).

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**Table 2**

Recoveries (% R) of group B analytes obtained by preconcentrating sample volumes with three adsorbents

| Compounds | 4 ml | | | 20 ml | | | 50 ml | | | 100 ml | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | A-COOH | A-NO$_2$ | A | A-COOH | A-NO$_2$ | A | A-COOH | A-NO$_2$ | A | A-COOH | A-NO$_2$ |
| Hydroquinone | 15 | 31 | 35 | — | 11 | 10 | — | — | — | — | — | — |
| Resorcinol | 36 | 63 | 68 | 5 | 22 | 23 | — | 9 | 7 | — | — | — |
| Catechol | 52 | 73 | 79 | 25 | 59 | 70 | 18 | 46 | 46 | 16 | 19 | 16 |
| Orcinol | 62 | 72 | 71 | 41 | 67 | 74 | 18 | 49 | 56 | 8 | 32 | 30 |
| Guaiacol | 73 | 80 | 78 | 80 | 86 | 83 | 81 | 81 | 83 | 77 | 83 | 84 |

A : Amberchrom GC 161m

RSDs < 12% in all instances (n= 4).

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From these results, we can conclude that A-NO$_2$ is more suitable than A-COOH for the SPE of polar pollutants from water samples, since recoveries were slightly higher. Phenol also gave a much better recovery with A-NO$_2$ (60% versus 36% with A-COOH) when a 200 ml sample was preconcentrated, as mentioned previously. This may be due to the fact that the hydrophilicity of the 2-carboxy-3/4-nitrobenzoyl moiety is greater than that of the 2,4-dicarboxybenzoyl moiety, since the extent of modification was similar in the two procedures. The difference between these moieties is the presence of a nitro or a carboxylic group (see Fig. 1). The nitro group has a higher polarity than the carboxylic group, hence analytes may interact better with the resin. Besides, if these new resins are compared with a *o*-carboxybenzoyl (the same moiety as in the new sorbents but without the nitro or carboxylic groups) modified sorbent (FC1), synthesized (11% modified) and used previously [12], we conclude that the presence of nitro or carboxylic groups led to more polar interactions with the analytes and, therefore, recovery values were better. These recoveries can be compared in

Table 3.

**Table 3**
Mean recoveries (%R) obtained with three chemically modified sorbents in preconcentration of 200 ml standard solution spiked at 0.5 µg l$^{-1}$.

| Compounds | 200 ml | | |
|---|---|---|---|
| | FC1 | A-COOH | A-NO$_2$ |
| Oxamyl | 62 | 80 | 80 |
| Methomyl | 59 | 71 | 77 |
| DIA | 64 | 71 | 75 |
| Ph | 31 | 36 | 60 |
| Atrazine | 70 | 87 | 86 |

RSDs <10% in all instances (*n*=4).

These results show that A-NO$_2$ is the best of the sorbents studied for the SPE of polar pollutants from environmental water samples. For this reason, it was selected to carry out the SPE of these samples.
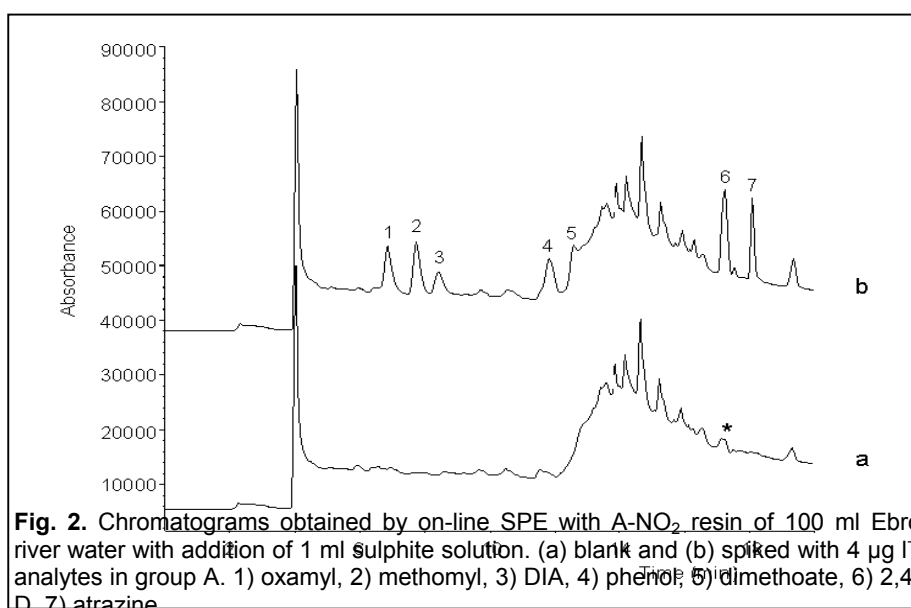
**Analysis of Real Water Samples**

Real water was preconcentrated in samples of 100 ml because when this volume of standard solution was preconcentrated with A-NO$_2$, recoveries for all compounds in group A, were higher than 80%, except for Ph which had a recovery of 64% (see Table 1).

When the sample volume had been established, the linearity, detection limits, repeatability and reproducibility (between days) of the method were determined for the analytes in group A with Ebro river water. The linearity of the method was between 0.4-50 µg l$^{-1}$ and 1-50 µg l$^{-1}$ for most of the analytes, except for DIA, the linearity of which was 2-50 µg l$^{-1}$, and for dimethoate, which had a linear response 4-50 µg l$^{-1}$. The correlation coefficients were good, with values ranging 0.9981-0.9996. Detection limits (LOD) were calculated by Winefordner and Long's method using the statistical program ULC (Univariate Linear Calibration) with $k$=6 [19,20]. They were between 0.2-1 µg l$^{-1}$ (1 µg l$^{-1}$ was for dimethoate which eluted on a big interfering peak of the matrix). Repeatability and reproducibility between days of the method, expressed as the RSD of 5 analyses of 100 ml of Ebro river water spiked at 4 µg l$^{-1}$ were lower than 10% for all compounds.

Figure 2 shows the chromatograms of 100 ml of Ebro river water (July 1998) and the same sample spiked with a standard solution of 4 µg l$^{-1}$ of the analytes in group A when they were preconcentrated with A-NO$_2$. It can be seen that a peak with the same retention time as 2,4-D appears in the blank chromatogram.

This signal may be tentatively assigned to this compound but it was near the LOD and it could not be quantified.



**Fig. 2.** Chromatograms obtained by on-line SPE with A-NO$_2$ resin of 100 ml Ebro river water with addition of 1 ml sulphite solution. (a) blank and (b) spiked with 4 µg l$^{-1}$ analytes in group A. 1) oxamyl, 2) methomyl, 3) DIA, 4) phenol, 5) dimethoate, 6) 2,4-D, 7) atrazine.

## CONCLUSIONS

This paper demonstrates that the introduction of the 2-carboxy-3/4-nitrobenzoyl or the 2,4-dicarboxybenzoyl moieties into a PS-DVB resin makes the SPE of

polar pollutants from environmental waters more efficient because the surface becomes more hydrophilic. The better of the two sorbents was the one containing a nitro group in the moiety introduced. Recoveries were also better when the moiety contained a carboxylic group than when it did not. Some polar pesticides and phenolic compounds were efficiently extracted from 100 ml water samples by on-line SPE with the functionalized resin with the 2-carboxy-3/4-nitrobenzoyl moiety, and recoveries were higher than 80%, except for phenol, 64%.

## ACKNOWLEDGEMENTS

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