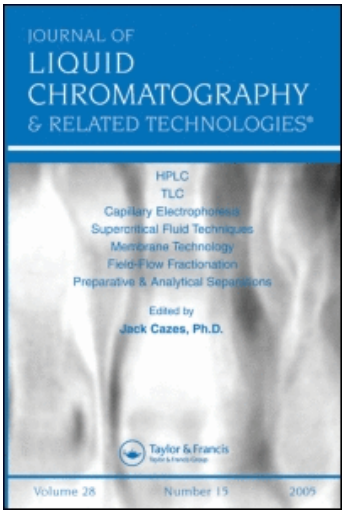

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DETERMINATION OF GLYPHOSATE AND SOME RELATED COMPOUNDS BY ION-EXCHANGE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Anion exchange liquid chromatographic methods are described for the determination of N-phosphonomethyl-glycine (PMG), which is known also as glyphosate, its monomethyl-ester (PMG-Met), N,N-bis(phosphonomethyl)-glycine (bis-PMG), N-methyl, N-phosphonomethyl-glycine (PMG-NMet) and glycine (Gly). Five different anion exchange columns were tested for the separation of the above mentioned compounds. Effects of the eluent pH, ionic strength and organic solvent composition were studied. It was found that only some of the tested columns were applicable for the separation of the PMG and PMG-NMet critical pair, which may be due to the different structure, the ratio of the hydrophobic and hydrophilic surface area of the ion exchanger. Analytical performance of the methods was investigated. Some examples are also shown for the application of these described methods.

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INTRODUCTION

N-phosphomonethyl glycine is widely used in herbicide formulations (e.g. Roundup or Rodeo from Monsanto or Glialka from Alkaloida Ltd) as a weedkiller. Different analytical methods were applied for the determination of PMG in a wide range of matrices. In herbicide formulations or as technical samples, glyphosate can be analyzed by ion exchange chromatography due to its ionic and polar character (1,2) and ultraviolet and refractive index detectors were used. However, analysis of glyphosate in biological (3) or environmental samples (4) requires pre- or post-column derivatization and fluorescence detection for the more sensitive and specific determination. For this purpose fluorenylmethyl chloroformate (FMOC), orto-phtaldialdehyde (OPA) or phenylisothiocyanate (PITC) has been successfully applied. Metabolism of PMG in water and soil was followed by thin-layer chromatography and metabolites, mainly aminomethyl-phosphonic acid (AMPA) were analyzed by gas chromatography-mass spectroscopy after derivatization (5,6). The sample preparation was carried out by column chromatography on ion exchanger columns. For the analysis of technical glyphosate and bis-PMG, complexometric determinations were also developed (7,8). Solid phase analysis of PMG and its salts has been carried out by Fourier-Infrared spectroscopy (9,10). NMR spectroscopy has also been applied to the analysis of PMG (1).

In our present work anion exchange liquid chromatographic method was developed for the analysis of technical PMG and formulations and for its impurities. We also evaluated the data obtained using different anion exchanger stationary phases and eluents, which will be discussed later.

EXPERIMENTAL

Chemicals and liquid chromatographic instrumentation

All chemicals were of analytical or chromatographic grade. Acetonitrile was obtained from Romil Chemicals (Shepsed, United Kingdom). Water was purified using a Milli-Q instrument (Millipore, Milford, USA). Potassium dihydrogen ortophosphate and ortophosphoric acid were obtained from Reanal (Budapest, Hungary). PMG reference standard and technical samples, Bis-PMG, PMG-Met, PMG-NMet and Gly were obtained from Alkaloida Ltd., (Tiszavasvári, Hungary). The liquid chromatographic system consisted of the following instruments: a Waters 600E pump with 3000A system controller, a Rheodyne 7010 injection valve (equipped with 20 µl sample loop), and a Biotronik BT 3030 variable wavelength ultraviolet detector operated at 195 nm

wavelength. Data acquisition was carried out using a Waters Maxima 820 data station running on NEC APC IV. Eluent (eluent A) for the routine determination of Gly, PMG-Met and PMG contained 5(v/v)% acetonitrile in 3 mM potassium dihydrogenphosphate solution, pH 3.15. Eluent (eluent B) for the determination of bis-PMG contained 5 (v/v)% acetonitrile in 50 mM potassium dihydrogenphosphate solution pH 2.40. In both case, pH of the eluent was adjusted to the desired value using 1 M phosphoric acid solution using a Jenway 3020 pH meter (Jenway Ltd., Felsted, Dunway, Essex, United Kingdom). For these routine determination a Dionex Omnipac PAX-500, 250x 4mm ID column was used. Other columns used for comparison are listed in Table 1. together with their main characteristics.

Sample preparation

Approximately 15-20 mg of the glyphosate sample was weighed with analytical precision and was dissolved in 10 ml of the mobile phase and was homogenized. Reference solutions were prepared by weighing of 2-3 mg of the reference materials with analytical precision and were dissolved in the mobile phase and it was homogenized. These samples were stable at least for 96 hours from the preparation. Both reference and sample solutions were prepared with the freshly prepared mobile phase. During the preparation of the eluent, the transmittance of the solvent was regularly checked and pH adjustments were made with a precision of 0.1 pH unit.

Evaluation of the method

Linearity

Linearity was tested by injecting solutions containing the target compounds in different, known concentrations. Two or three replicate injections were performed. We found that peak response-solute concentration functions were linear for all compounds between the concentrations of about 100 μg/ml and 1500 μg/ml.

Reproducibility

The reproducibility of injection was tested by injecting the same reference solution containing the analytes at 100 μg/ml. RSD of the injection reproducibility was found to be 0.90 % (n=5). Reproducibility of retention times was also calculated and RSDs of the retention times were better than 5 % except bis-PMG, where the reproducibility of the retention times was ca 9%. Retention times of the different compounds are given in Table 2.

Table 1. Stationary phases and columns used in the experiments

Stationary phase	Column dimensions	Functionality	Manufacturer
Dionex PAX-500	250x4 mm ID	agglomerated type anion exchanger, polymer	Dionex, Sunnyvale, California, USA
Chrompack Ionosphere 5A	100x3 mm ID glass cartridge	anion exchanger on silica	Chrompack, Raritan USA
Waters IC Pak A HC	150x4 mm ID	quaternary amine anion exchanger on methacrylate polymer	Waters, Milford, USA
Merck Polysphere AA NA	120x4.6 mm ID		Merck, Darmstadt, Germany
Hamilton PRP X-100	150x4.6 mm ID	quaternary amine anion exchanger on polystyrene-divinylbenzene polymer	Hamilton, Bonaduz, Switzerland

Table 2. Retention times of the solutes

Solute	Eluent	Retention time, min
glycine	A	1.82
glyphosate-monomethylester	A	2.59
glyphosate	A	5.72
gliphosate	B	2.05
bis-phosphonomethyl glycine	B	5.38

Detection limit, limit of quantitation

Detection limits were determined by injecting reference solutions at different concentrations. Detection limit for Gly was found to be 5 μg/ml, for PMG and PMG-Met 10 μg/ml, and for bis-PMG 25 μg/ml.

Recovery

Recoveries were tested in the concentration range of 0.11-2.81 percent of the main component PMG by the addition of the impurities to the reference PMG solution. The solutions were analyzed and recoveries were calculated from the found amount of the compound in question and the added amount of the same compound. Values of recovery varied between 91 % and 122 %, the average was 106 % (n=10).

Analysis of technical PMG

Using the methods described above, glyphosate batches obtained from the Alkaloida Ltd, were analyzed. Chromatogram of the reference solution containing PMG, PMG-Met and Gly is shown on Fig. 1. Comparing the chromatogram of th reference solution to the chromatogram of technical PMG, we concluded that unknown impurities are present in the technical PMG in low concentration (Fig.2.). Fig. 3. shows the typical impurity profile of the technical glyphosate. In order to identify the unknown compounds in the samples, PMG-NMet and aminonomethyl phosphonic acid were included in the tests. Unfortunately, using the described system and conditions, aminomethyl phosphonic acid was not detectable even at greater concentration. PMG- NMet gave similar retention as PMG, so the separation and determination was not possible. This fact will be discussed in more detail later. Solution of On Fig 4 separation of PMG and bis-PMG using eluent B is demonstrated.

Attempts were made to analyze the bis-PMG content of PMG together with the other two impurities using gradient elution by changing the eluent pH or ionic strength. Unfortunately, the high baseline drift did not allow the sensitive detection of the analytes. Using more selective detection system, e.g. post column derivatization and fluorescence detection, gradient elution of the compounds either by pH or ionic strength gradient seems to be possible.

DISCUSSION

Comparison of different stationary phases for the separation of PMG and its derivatives

The analytical methods described in the previous part employed a Dionex PAX500 column, which is special, multifunctional stationary phase with anion exchanger groups and hydrophobic surface, which enables the separation of the analyte both in ion exchange and reversed phase mode. The packing material in this column is a so-called agglomerated type anion exchanger (11). To evaluate the effect of ionic strength and pH of the eluent, the separation was tested at different pH and buffer concentration. Fig. 5 shows the dependence of the logarithm of the capacity factors of the solutes on the reciprocal of the buffer concentration at constant pH of 3.15. This correlation was found to be linear except the highest and lowest buffer concentrations. Logarithm of the capacity factors showed linear dependence using the least square method on the logarithm of the buffer concentration. Slopes of these functions are equal to the ratio of the charge on the analyte and the counterion of the ion exchanger. These data together with the correlation coefficients are given in Table 3. However, using the initial chromatographic system,

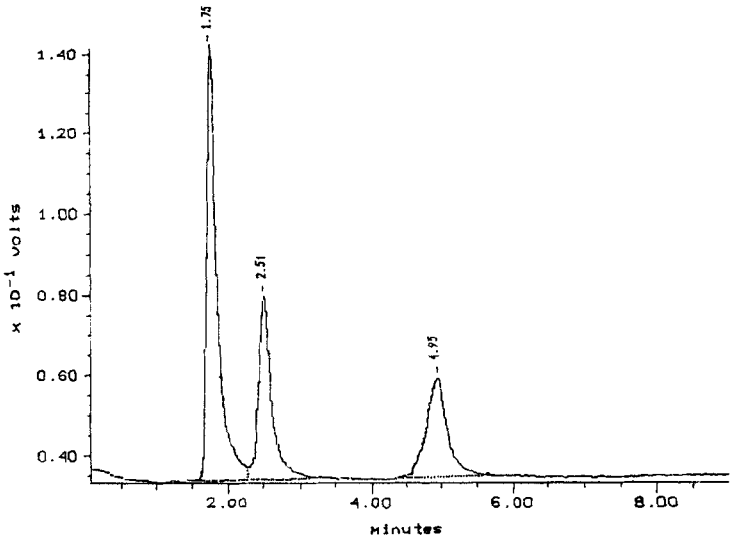


Fig. 1. Chromatogram of the reference solution
Gly (1.75 min), PMG-Met (2.51 min), PMG (4.95 min)

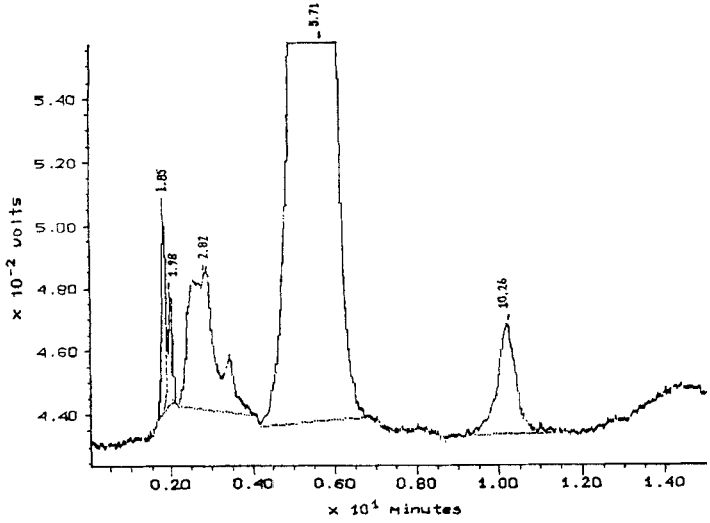


Fig. 2. Chromatogram of the technical PMG sample

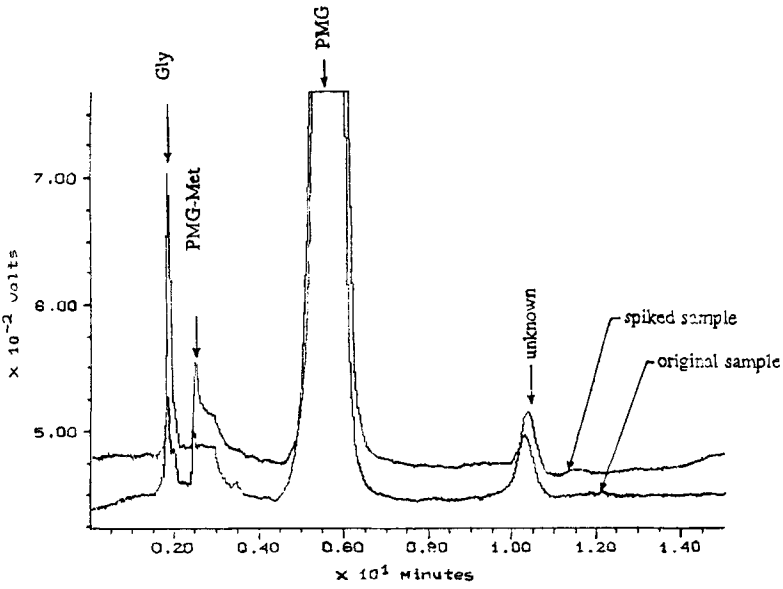


Fig. 3. Typical impurity profile of the technical PMG. Solutes are marked on the figure. Lower trace: original sample; upper trace: sample after spiking with Gly and PMG-Met

separation of PMG and PMG-NMet was not achieved. Thus, capacity factors were measured as a function of pH (Fig. 6b). We found that by using the Dionex column, separation of PMG and PMG-NMet is possible only at higher pH.

The retention of the solutes was not influenced significantly by varying the acetonitrile concentration in the eluent between 1 and 20 (v/v)%. However, the peak response decreased gradually as the acetonitrile content of the eluent was increased according to the poor solubility of the analytes in acetonitrile-water mixtures with higher acetonitrile content.

For the other columns, the dependence of the logarithm of the capacity factor on pH was also studied (Fig. 6a,c,d,e). The five column can be roughly divided into three groups: (1) Dionex PAX-500 and Chrompack Ionosphere A; (2) Waters IC-Pak A and Hamilton PRP X-100; (3) Merck Polysphere AA NA. Using the columns in the second group, separation of PMG and PMG-NMet is not possible in the pH and ionic strength range studied. These

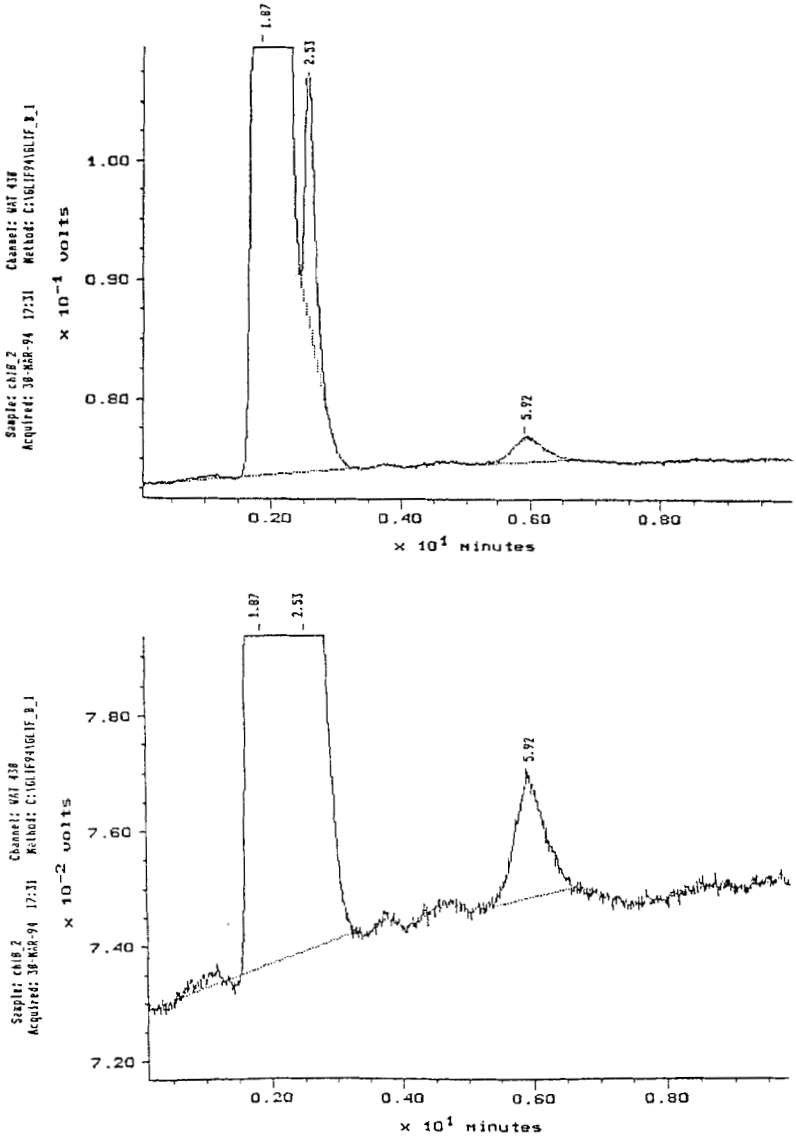


Fig. 4. Separation of PMG and bis-PMG in a technical PMG sample

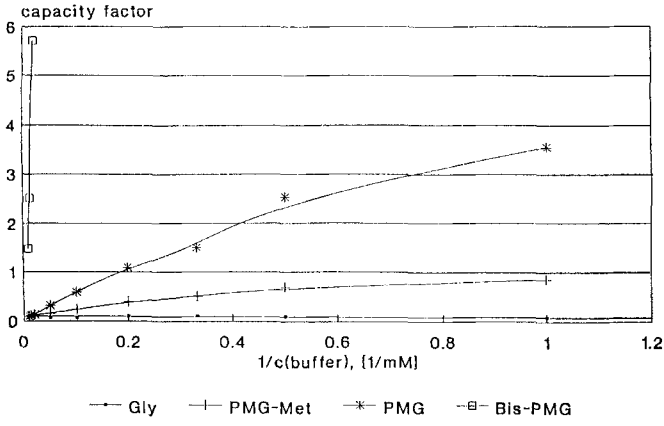


Fig. 5. Plot of the capacity factors for the different solutes versus the reciprocal of the potassium dihydrogenphosphate concentration in the eluent

Table 3. Slopes and regression coefficients for the linear regression of log k vs. logarithm of potassium-dihydrogenphosphate concentration (expressed in mM) in the eluent at pH 3.15

Compound	Slope	r	Concentration range
PMG-Met	-0,486	-0,987	1-100 mM
bis-PMG	-1,957	-0,999	10-100 mM
PMG	-0,8315	-0,997	1-100 mM

columns have quaternary alkylammonium groups bound to polymer surface (in case of IC-Pak A this polymer is polymethacrylate, in case of PRP X-100 the polymer skeleton is polystyrene-divinylbenzene copolymer). Columns in the first group are applicable for the separation of PMG and PMG-NMet. One of these columns, namely the Chrompack Ionosphere A is based on silica gel, where secondary interaction may occur between the silanol groups and the secondary and tertiary amino groups of PMG and PMG-NMet, respectively. In the case of the PAX-500 column, differentiation between PMG and PMG-NMet can not be explained easily. This column - as it was mentioned before - is of agglomerated type based on a highly crosslinked core and a functionalized latex layer

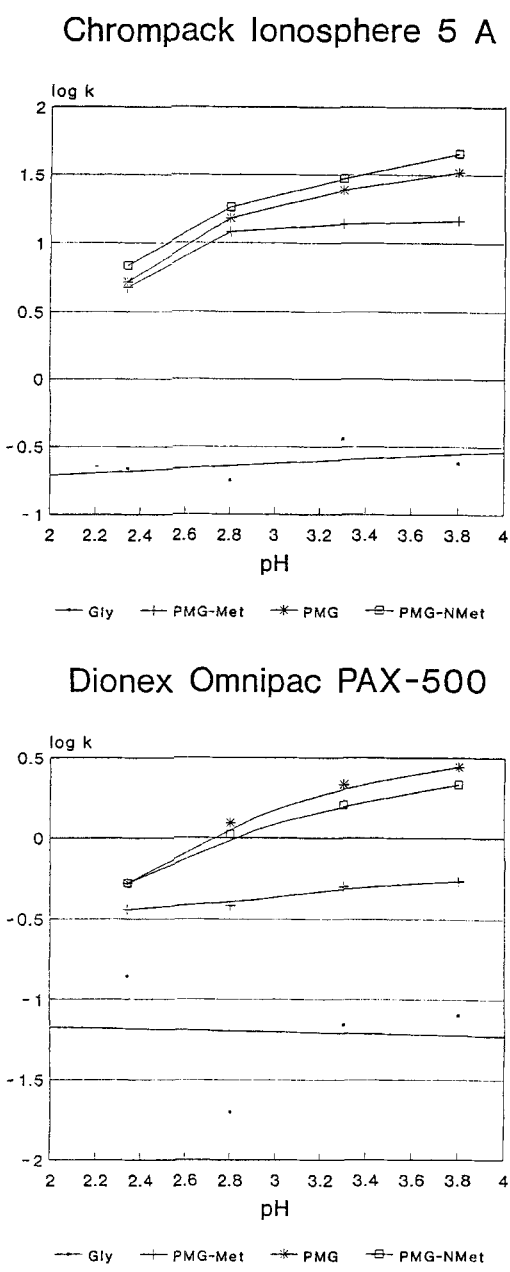
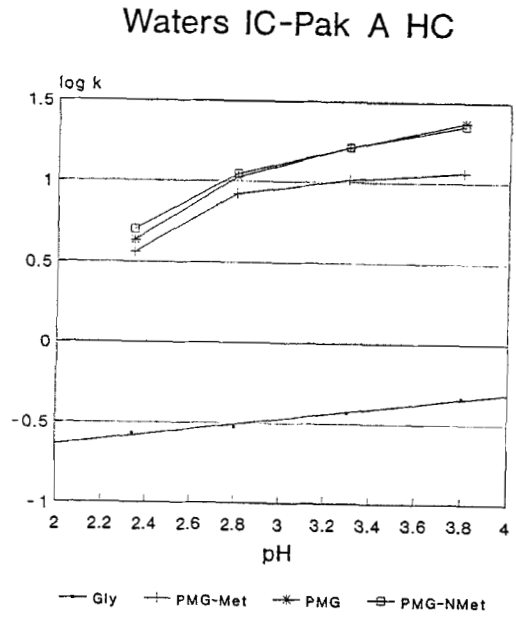


Fig. 6. Plot of the log k for PMG, Gly, PMG-Met and PMG-NMet versus the eluent pH on different anion exchanger columns



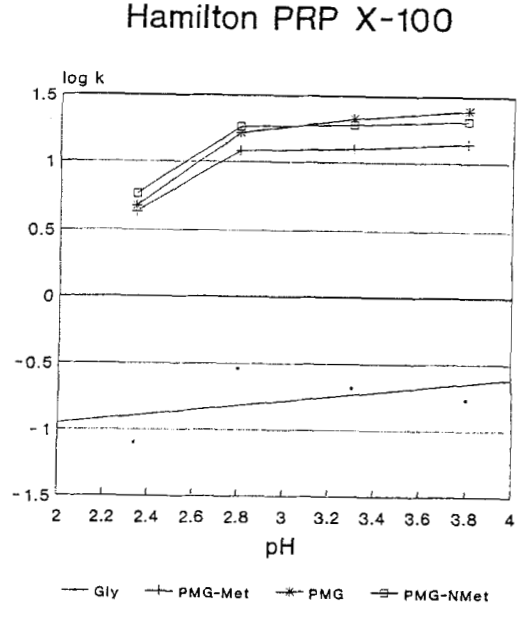


Fig. 6 (continued)

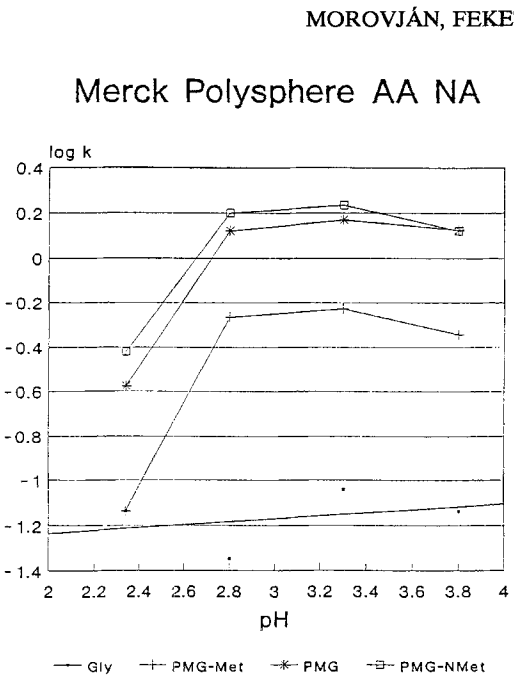


Fig. 6 (continued)

bound by electrostatic forces on the surface of the cores. The latex on the surface of the cores has quaternary ammonium groups but the alkyl groups attached to the quaternary ammonium contain hydroxy functions as well (11). Separation of PMG and PMG-NMet may be due to the residual sulphonated groups of the core which are not covered by the aminated latex, or, by a secondary interaction of the weakly acidic (pKa ca. 10) hydroxy functions on the latex-coated hydroxy groups by secondary equilibria. However, the experimental data show that separation of the two compounds depends on the pH in the pH range 2.2-3.0 but remains approximately constant between pH 3.0-3.6. In this pH range the ionization of strongly acidic sulphonic acid groups become complete and these dissociated groups can retain PMG and PMG-NMet by their different basicity. However, concentration of such uncovered groups must be low because the peak shape was not distorted due to secondary equilibria.

Polysphere AA NA exhibited unique retention of the tested solutes. The retention was relatively small compared to the other column but good efficiency and fair good separation of the analytes was provided. This column was originally offered for the analysis of amino

acids. Unfortunately, information are not available on the structure of functional group structure of this column except that this is an anion exchanger on polymer skeleton.
Summarizing, we can conclude that columns from the first group are the best choice for the detailed analysis of PMG including PMG-NMet. Silica based packing materials can be used in the pH range where this separation is made, and show very good mechanical stability over polymer based columns.

SUMMARY
Liquid chromatographic methods using anion exchanger columns were developed for the determination of the herbicide glyphosate and some related compounds. These methods were applied to the analysis of technical glyphosate batches. Anion exchanger columns obtained from different vendors were tested and compared. Further development of these methods has to be made mainly by improving the detection of the compounds and application of gradient-compatible detection methods.

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