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Separation of acetylated polypropylene glycol di- and triamines by gradient reversed-phase high-performance liquid chromatography and evaporative light scattering detection

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Abstract

Polypropylene glycol di- and triamines, the so-called Jeffamines, were reacted with a mixture of equal volumes of acetic anhydride and pyridine to give the corresponding acetamides prior to chromatographic separation by reversed-phase high-performance liquid chromatography using a linear binary solvent gradient and evaporative light scattering detection (ELSD). The procedure was applied to decrease silanophilic interactions of the solutes with the column matrix in order either to improve the peak resolution R_s or to decrease peak tailing. Removal of excess of derivatizing agent prior to sample injection is not required owing to the volatility of pyridine, which does not yield any ELSD response. Therefore, the signals of oligomers with lower retention that are hidden by the broad and strongly tailing pyridine peak when measured by UV detection, are now clearly detectable. Separation was performed on C_{18}, C_8, C_6, C_4 and C_1 stationary phases with either acetonitrile or methanol as organic modifiers. With acetonitrile either complete elution or excellent separation of the low-molecular-mass samples ($M_r \approx 400$) is achieved on the C_{18}, C_8, C_6 and C_4 matrices. However, R_s decreases with increasing M_r but the recoveries of high-M_r samples ($M_r \approx 2000$–5000) increase markedly with decreasing hydrophobicity of the stationary phase in the order $C_{18} < C_8 < C_6 < C_4$. Complete elution of the whole family of investigated polyether amines was accomplished with either acetonitrile on a C_1 column or methanol on all sorbents used in the study. The optimum peak resolution was obtained on a C_4 column with either acetonitrile or methanol. Complete elution of all samples in particular on matrices with either high or intermediate hydrophobicity with methanol compared with acetonitrile is presumably attributable to a better solvation of the polypropylene glycol backbone by hydrogen bonding between the ether oxygens and the hydroxyl group of the protic solvent.

1. Introduction

Polypropylene glycol di- and triamines (PPG amines) are synthesized starting with propylene oxide and 1,2-propylene glycol, yielding the linear PPG amines (Fig. 1a), and/or with glycerol–trimethylolpropane, yielding the branched PPG amines (Figs. 1b and c). They play an increasing role in the synthesis of polyamides, polyurethanes, polyureas and isocyanate prepolymers and as epoxy curing agents and flexibilizers [1]. Although they are commercially available under the trade name Jeffamine [1], precise information concerning their behaviour in liquid chromatography is still lacking. This may presumably be attributable to either the poor UV absorption as the native compounds in the usual wavelength range or to their strongly

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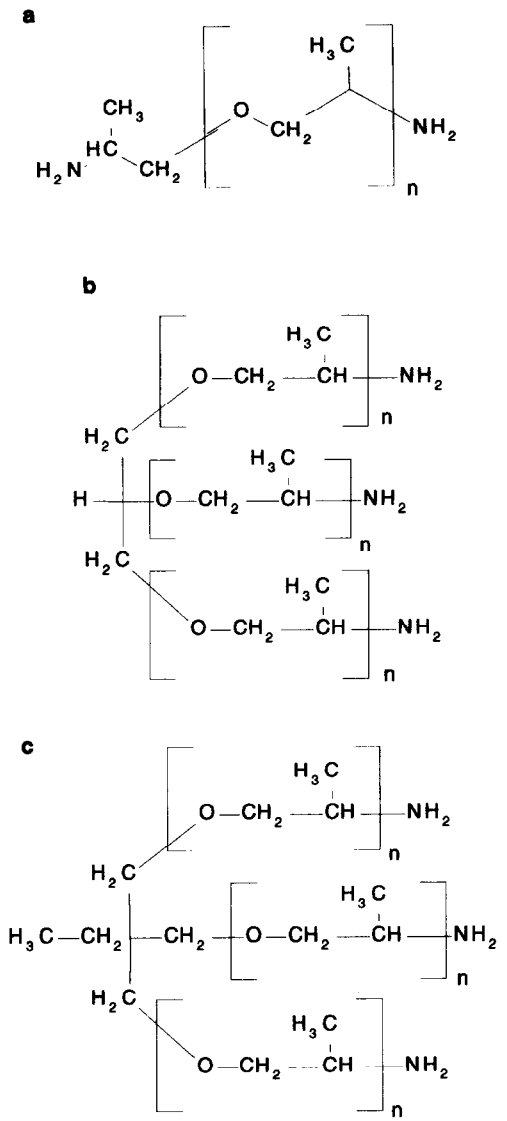

Fig. 1. Structural formulae of (a) the linear PPG amines (Jeffamine D 230, D 400 and D 2000) started with propylene-1,2-glycol and propylene oxide, (b) the branched PPG amines started with glycerol and propylene oxide (Jeffamine T 403) and (c) the branched PPG amines started with trimethylol propane and propylene oxide (Jeffamine T 3000 and T 5000).

basic properties, making them less amenable to a satisfactory chromatographic separation owing to interactions with residual silanols [2–8]. To compensate for the latter effect we converted the PPG amines into the corresponding acetyl derivatives by reacting them with an excess of acetic anhydride and pyridine as the catalyst.

We have investigated the separation of both linear (Jeffamine D 230, D 400 and D 2000) and branched (Jeffamine T 403, T 3000 and T 5000) samples over a wide molecular mass range by gradient RP-HPLC with different organic modifiers on several stationary phases differing widely in hydrophobicity and measurement of the column effluent by means of evaporative light-scattering detection (ELSD).

2. Experimental

2.1. Separation media, reagents and solvents

The PPG amine samples Jeffamine D 230[a], D 400 and D 2000 (linear) and Jeffamine T 403, T 3000 and T 5000 (branched) were purchased from Texaco Chemical (Bellaire, TX, USA). As the stationary phases Nucleosil $5C_{18}$, $5C_8$ and $5C_4$ (each column 125 × 4.6 mm I.D., 5 μm particle size, 100 Å pore diameter) from Macherey–Nagel (Oensingen, Switzerland) and Ultremex $5C_6$ and $5C_1$ (each column 150 × 4.6 mm I.D., 5 μm particle size, 80 Å pore diameter) from Phenomenex (Torrance, CA, USA) were used. Pyridine, acetic anhydride (both pro analysi), trifluoroacetic acid (purum), acetonitrile and methanol (both HPLC grade) were obtained from Fluka (Buchs, Switzerland). Water for use in HPLC was purified with a Milli-Q reagent water system from Millipore–Waters (Milford, MA, USA).

2.2. Derivatization reaction

An amount of *ca.* 25 mg of the PPG amine was dissolved in 100 μl of pyridine–acetic anhydride (1:1, v/v) and heated at 60°C for 30 min. Excess of reagent was reacted to give the methyl ester by addition of 1.5 ml of methanol and heating at 60°C for 10 min. The solution

[a] The numbers represent the average molecular mass, M_r, of the samples as specified by the manufacturer.

Table 1
Gradient programme for the elution of PPG amides with acetonitrile and methanol as organic modifiers

Time (min)	Organic solvent (%)	Water (%)
0	0	100
40	100	0
50	100	0
51	0	100
65	0	100

prepared in this manner was used for HPLC without further purification.

2.3. Analytical equipment

The HPLC apparatus consisted of an SP 8800 ternary HPLC pump, an SP 8880 autosampler equipped with a 10-μl sample loop and a PC 1000 data acquisition unit, all obtained from Spectra-Physics (San Jose, CA, USA). For ELSD a Sedex 45 apparatus from SEDERE (Vitry-sur-Seine, France) equipped with a 20-W iodine lamp was applied.

2.4. Chromatographic separation

The gradient system depicted in Table 1 was used for the separation with either acetonitrile or methanol as organic modifier. Separation was performed on the columns indicated above at ambient temperature (*ca.* 22°C) and a flow-rate of 1.5 ml/min. Aliquots of 10 μl of the solutions prepared as described were injected. For detection by means of ELSD the nebulization chamber was heated to 40°C and the nitrogen flow-rate was adjusted to 4.5 l/min, corresponding to an inlet pressure of 200 kPa.

3. Results and discussion

Detection of the polypropylene glycol di- and triamides (PPG amides) can be performed either by UV absorption at 210–220 nm owing to the amide chromophore or by ELSD. In the former instance complete removal of excess of pyridine is required (*i.e.*, an additional sample prepurification step) because the broad and strongly tailing peak of the catalyst overlaps with those of early-eluting low-M_r PPG amide oligomers. This drawback can, however, be successfully overcome by using ELSD owing to the high volatility of pyridine, which does not invoke any detector response. The ELSD method has a wide application range for non-volatile components, which easily form solid particles after loss of the surrounding solvent shell by nebulization and subsequent heating of the resulting droplets in the drift tube [9–20]. Further, a stable baseline is obtained, which is either independent of the type of organic modifier or the gradient shape [10–12, 19]. Optimization of the detection conditions for signal monitoring of polyethers was described recently [21] and was used in this study.

Owing to the large differences in M_r and the concomitant large differences in the retention of the samples, gradient RP-HPLC proved to be superior to the isocratic technique and was therefore applied. With acetonitrile as organic modifier, only the low-M_r oligomers Jeffamine D 230, D 400 and T 403 eluted quantitatively from the C_{18}, C_8, C_6 (results not shown) and C_4 stationary phases, but a marked improvement in the elution efficiency of the high-M_r samples Jeffamine D 2000, T 3000 and T 5000 was achieved in the range $C_{18} < C_8 < C_6 < C_4$. Whereas the peak resolution R_s {$R_s = 2[(t_2 - t_1)/(w_1 + w_2)]$, where t_1 and t_2 are the retention times of two adjacent peaks and w_1 and w_2 their base widths} of the low-M_r samples was excellent on these four matrices, the R_s of the high-M_r oligomers was markedly lower but increased substantially in the order $C_{18} < C_8 < C_6 \approx C_4$. Optimum results were obtained on the C_4 column (Fig. 2a–f) and, in contrast to the C_6 matrix (results not shown), nearly complete elution of Jeffamine D 2000 and T 3000 was achieved (Fig. 2d and e). Nevertheless, the recovery of Jeffamine T 5000 still remained low (Fig. 2e). The C_1 matrix permits quantitative elution of the whole family of high-M_r PPG amides (results not shown) but a marked leveling effect of analyte retention does not make the method suitable for investigations of sample mixtures, which more or less differ in M_r. Further, the peak resolution of

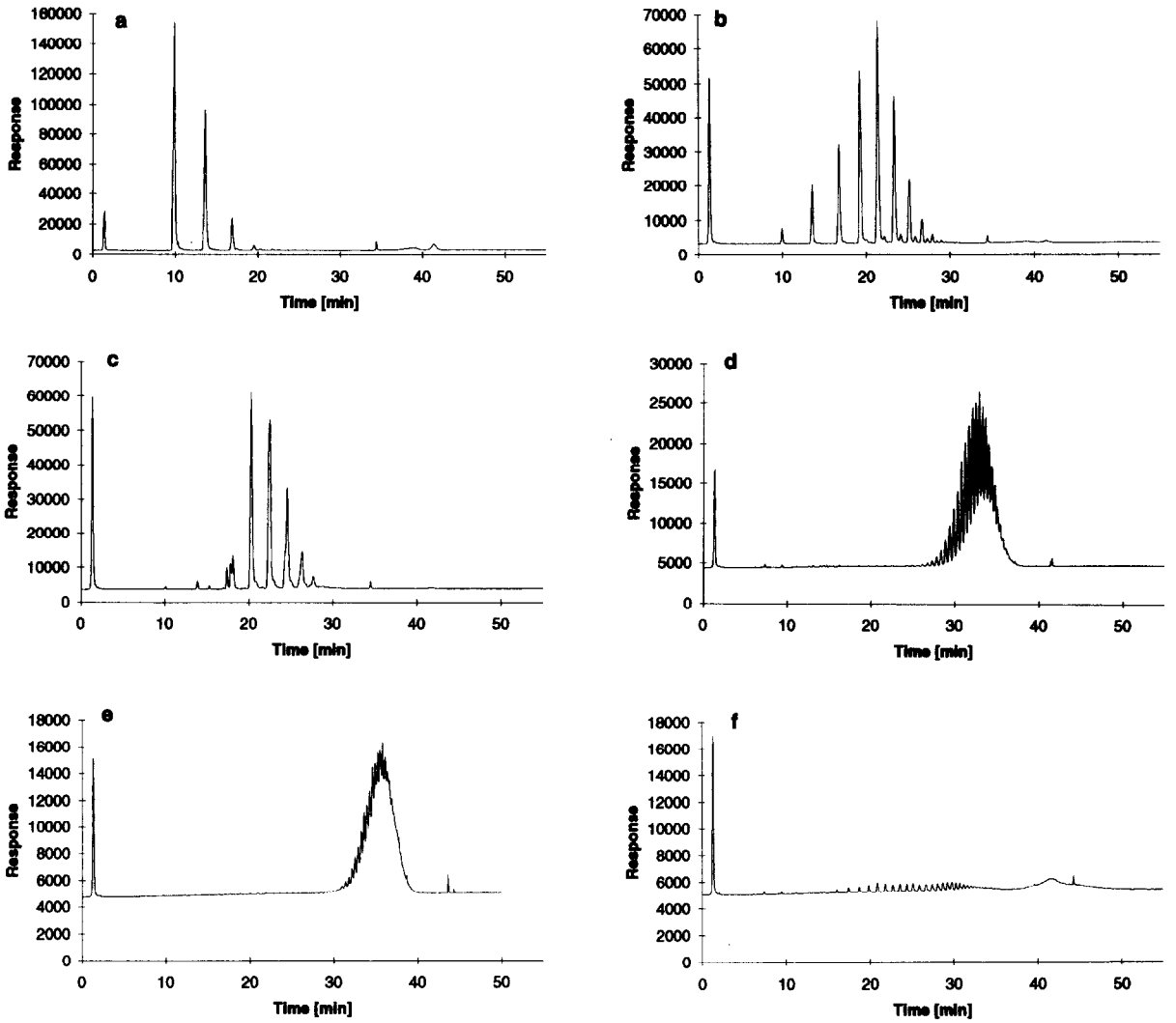

Fig. 2. Chromatograms of (a) Jeffamine D 230, (b) Jeffamine D 400, (c) Jeffamine T 403, (d) Jeffamine D 2000, (e) Jeffamine T 3000 and (f) Jeffamine T 5000 on a C_4 column with acetonitrile as organic modifier.

low-M_r oligomers is unsatisfactory and thus contrasts markedly with the separation profile of the other stationary phases. When methanol was used as the organic modifier, quantitative elution of all the investigated PPG amide samples was achieved on the C_{18}, C_8, C_6, C_1 (results not shown) and C_4 matrices (Fig. 3a–f). As already observed with acetonitrile, the C_4 stationary phase also exhibited either the best R_s of oligomers or selectivity with respect to an individual assignment of samples within mixtures.

Addition of 0.05% of trifluoroacetic acid (TFA) to the mobile phase and thus conversion of the native samples into the corresponding trifluoroacetates provides another means for measurement by ELSD without prior derivatization owing to the high volatility of excessive TFA. However, the peak broadening is markedly higher and, as a consequence, the R_s much lower compared with the corresponding amides (results not shown). Further, a "levelling effect" of retention occurs for the higher M_r samples

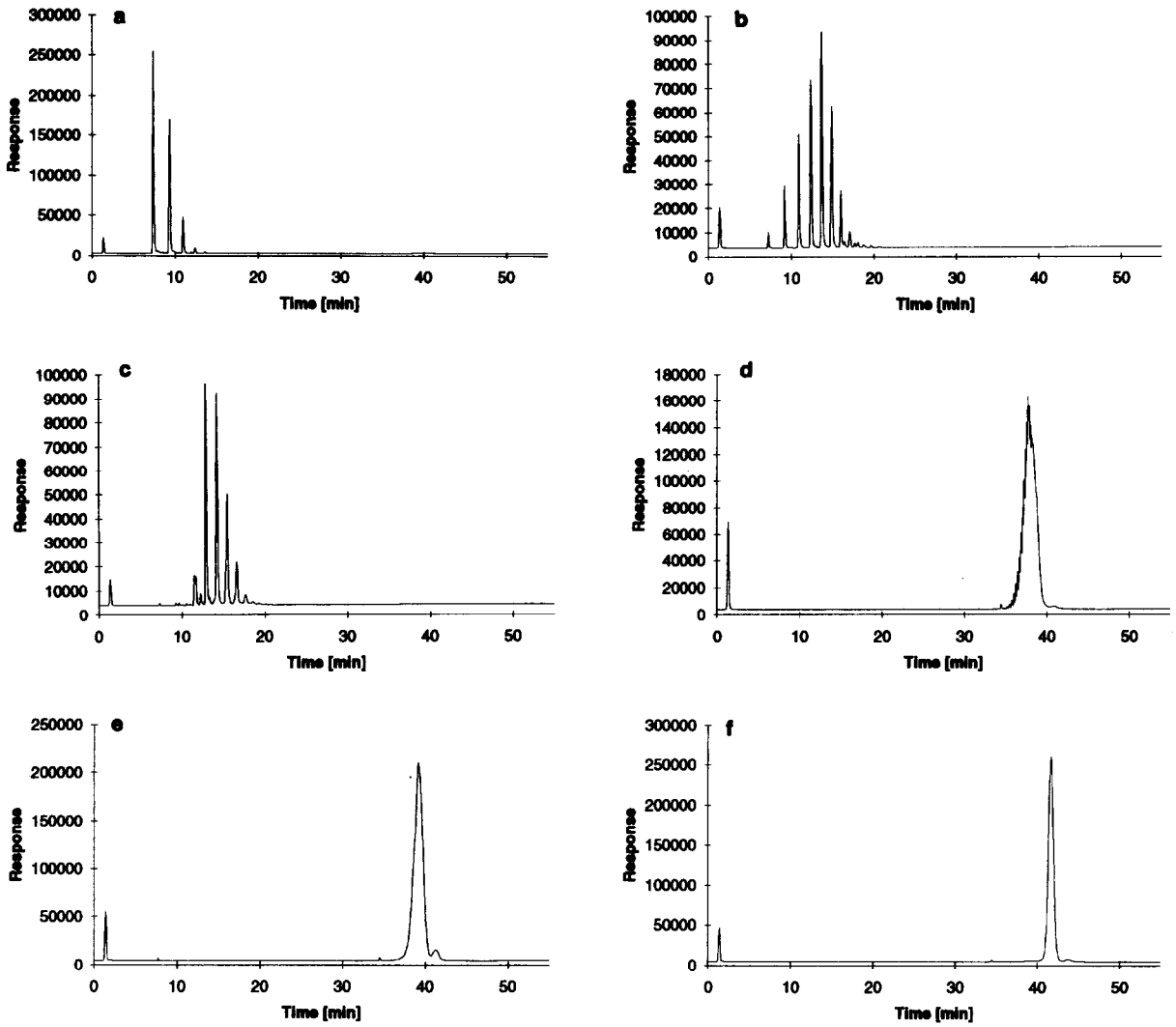

Fig. 3. Chromatograms of (a) Jeffamine D 230, (b) Jeffamine D 400, (c) Jeffamine T 403, (d) Jeffamine D 2000, (e) Jeffamine T 3000 and (f) Jeffamine T 5000 on a C_4 column with methanol as organic modifier.

(*e.g.*, Jeffamine D 2000, T 3000 and T 5000), thus making an assignment to individual PPG amine species nearly impossible. Additionally it is well known that TFA accelerates the dissociation of octadecylsilyl residues from the column matrix by increasing the concentration of free silanol groups, which in turn are responsible for a relatively rapid deterioration of chromatographic performance [22] due to marked silanophilic interactions [2–8]. In contrast, the underivatized PPG amines cannot be eluted on either reversed-phase materials (C_{18}, C_8, C_6, C_4, C_{phenyl}, C_1) or on so-called bonded-phase sorbents such as cyanopropyl (CN), aminopropyl (NH_2) and 2,3-propanediol (diol) stationary phases under "neutral" conditions, *i.e.*, no mobile phase additives such as acids, buffers and ion-pairing agents were used to suppress or at least to modulate the silanophilic solute–matrix interactions. This conclusion can be drawn from the observation that no responses were obtained from either ELSD or liquid chromatographic–

mass spectrometric (LC–MS) investigations. In the latter instance no nitrogen-containing ions of homologues differing from each other by 58 u and thus attributable to the propoxy monomeric structural unit were observed, although ionization of the polar solutes should not give rise to any problems (results not shown). However, trace amounts of polyproypylene glycol oligomers lacking nitrogen were found, which obviously have not quantitatively reacted to the corresponding PPG diamines, and their extremely low concentrations prevented detection by means of ELSD.

It is important to note that addition of buffers and other mobile phase additives will not be compatible with ELSD. This is due to the huge background invoked by the continuous generation of solid particles from the mobile phase in the heating zone of the detector after loss of the solvent shell, which completely suppresses signal monitoring of the analyte. For this reason, all solvents, even if they are supplied as "HPLC-grade" materials, have to be tested prior to use because in our experience not all of these solvents are suitable for ELSD and wide batch-to-batch and manufacturer-to-manufacturer variances exist. On the other hand, buffers will not be convenient additives for gradient elution (even if they show only negligible absorbance when UV detection is applied) owing to their generally decreasing solubility with increasing percentage of organic solvent. Therefore, derivatization of the primary amine groups of the PPG amines with an appropriate reagent offers an attractive alternative way to achieve both optimum separation efficiency and detection sensitivity. Although the 3,5-dinitrobenzoyl derivatives [21,23,24] allow signal monitoring at 254 nm, at least for low-M_r samples, partial overlapping of peaks from the (less volatile) excess of reagent and polyether have to be taken into account. For this reason, we applied the more suitable method of acetylation using equal volumes of acetic anhydride and pyridine, which yields mostly volatile components after post-derivatization methanolysis of the excess of reagent. Only the signal presumably attributable to pyridine acetate is observed, which however elutes near the column void volume and therefore does not interfere with chromatographic separation.

Whereas complete retention of underivatized PPG amines on chemically modified silica gel stationary phases can be ascribed to marked silanophilic interactions [2–8], the poor refractive index responses of the samples obtained by gel permeation chromatography (results not shown) are surprising because extensive signals should have been expected owing to the presumed negligible solute–matrix interactions between the polar PPG amines and the strongly hydrophobic divinylbenzene cross-linked polystyrene (DVB–PS) matrix used for gel permeation chromatography (GPC) A similar effect was observed by Mukoyama *et al.* [25] when they subjected polyamic acid (obtained by polycondensation of 4,4′-diaminodiphenyl ether and pyromellitic dianhydride) to GPC. Complete retention of the extremely polar solute takes place on the non-polar DVB–PS matrix in dimethylformamide as the solvent, whereas addition of phosphoric acid to the mobile phase effected its elution. At present we cannot give a reasonable answer, but "aggregation" via hydrogen bonding yielding poorly soluble "complexes" may be at least one hypothesis for an explanation. Indeed, the corresponding PPG amides exhibit a large increase in detector response, which may be explained by marked disruption of hydrogen bonding after amide formation. Perhaps a phenomenon such as this may partially also be operating in the RP-HPLC of underivatized samples and thus superimposes silanophilic interactions. The preferred retention of high-M_r PPG amides on C_{18}, C_8, C_6 and C_4 columns with acetonitrile and their quantitative release with methanol is in accordance with our recent observations reported elsewhere [21]. In these instances an increase in solubility of polyethers in methanol owing to the formation of hydrogen bonds between ether oxygens and the hydroxyl group of the protic compared with the aprotic solvent was postulated. This effect elicits a higher concentration of polyether in the methanolic solvent layer compared with its concentration in the adjacent region of hydrophobic

octadecylsilyl chains of the column matrix and thus markedly affects analyte retention. In contrast, a decrease in retention of the high-M_r PPG amides with increasing polarity of the stationary phase can be explained by smaller hydrophobic interactions of the relatively non-polar polypropylene glycol backbone with the column matrix, which decrease in the order $C_{18} > C_8 > C_6 > C_4 > C_1$ [21].

Although the samples with higher M_r show increasingly lower peak resolution, their retention depends markedly on the M_r ($R_s \sim 1/M_r$, but $t_R \sim M_r$). Thus PPG amides with a broad M_r range can be well separated in one run and a selective assignment within sample mixtures is achieved. Further, a dependence of peak resolution on the type of polyether is observed and, at least on reversed-phase matrices, R_s decreases markedly in the order polybutylene glycol > polypropylene glycol > polyethylene glycol for a given M_r [21]. PPG amides behave chromatographically similarly to polypropylene glycols, implying that retention is preponderably governed by the polyoxypropylene backbone.

4. Conclusions

Prior derivatization of PPG amines to the corresponding acetamides yields strong suppression of interactions with residual silanol groups of the column matrix, which normally invoke irreversible adsorption of the strongly basic solute. This procedure makes them amenable to separation by RP-HPLC with purely aqueous organic solvents. For this reason ELSD can be applied for signal monitoring and responses are measured with high sensitivity. The alternative method of using the native PPG amines and small amounts of TFA in the mobile phase proves to be inferior owing to marked peak broadening, lower R_s and a decreased column lifetime. PPG amides of different M_r are separated according to increasing M_r, which allows an individual assignment of samples within mixtures. Linear solvent strength gradient RP-HPLC with either acetonitrile or methanol as modifiers and a C_4 stationary phase provides an optimum chromatographic system.

5. Acknowledgements

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