SHORT PAPERS

Separation of the *ortho*, *meta* and *para* Isomers of Aminophenol by High-performance Liquid Chromatography

Chris J. Dowle and Andrew P. Malyan
ICI Wilton Materials Research Centre, P.O. Box 90, Wilton, Middlesbrough, Cleveland TS6 8JE, UK
Alasdair M. Matheson
Department of Pure and Applied Chemistry, University of Strathclyde, 295 Cathedral Street, Glasgow G1 1XL, UK

Reversed-phase high-performance liquid chromatography on a polystyrene - divinylbenzene column was applied to the qualitative and quantitative analysis of aminophenol positional isomers. The method is simple, rapid and more reproducible than the normal-phase system used previously.

Keywords: *Reversed-phase high-performance liquid chromatography; normal-phase high-performance liquid chromatography; aminophenol analysis*

The analysis of aminophenol isomers present in hair-dye using normal-phase ion-pair high-performance liquid chromatography (HPLC)[1] has overcome some of the difficulties associated with earlier techniques.[2,3] However, this requires a complex, six-component mobile phase. Reversed-phase systems have also been developed using less common, expensive stationary phases.[4,5] A normal-phase method was developed in our laboratories to monitor the purity of *meta*-aminophenol as a starting product in a polymerisation process. However, this was not very successful for studying aminophenol isomers and is discussed further here.

This paper describes an alternative, simple, routine method for the qualitative and quantitative analysis of aminophenol positional isomers, using a polystyrene - divinylbenzene column.

Experimental

Reagents

HPLC-grade solvents were obtained from Fisons.

All standard reagents and chemicals were obtained in the highest available purity from Aldrich, BDH and Eastman Kodak.

Apparatus

The HPLC system consisted of an Altex Model 110A pump, a Rheodyne 7125 injection valve with a 10-mm^3 loop, a Spectra-Physics SP8400XR UV - visible detector (λ = 295 nm) and a Knauer oven unit. A Polymer Laboratories PLRP-S HPLC column (250 × 4.7 mm i.d.) and Hichrom S5NH column (250 × 4.7 mm i.d.) were used for the reversed- and normal-phase methods, respectively.

Procedure

Normal-phase method

This was performed isocratically at a flow-rate of 2 cm^3 min^{-1} at ambient temperature. Stock solution A consisted of 1.0 g of tetraethylenepentamine dissolved in 100 cm^3 of methanol - dichloromethane (10 + 90). The mobile phase was prepared by adding 20 cm^3 of stock solution A, 180 cm^3 of dichloromethane and 100 cm^3 of methanol to a 1000-cm^3 calibrated flask and making up to the mark with hexane.

The column was conditioned before use with approximately 50 cm^3 of 10% v/v stock solution - dichloromethane. All samples were dissolved in dichloromethane - methanol (80 + 20).

Reversed-phase method

This was performed isocratically at a flow-rate of 1 cm^3 min^{-1}

Table 1. Resolution of adjacent aminophenol peaks at different concentrations of organic modifier

Concentration of modifier in mobile phase, % v/v	R_s (*para* - *meta*)	R_s (*meta* - *ortho*)
5	1.36	2.72
10	1.37	3.52
11	1.06	2.91
15	0.94	2.46

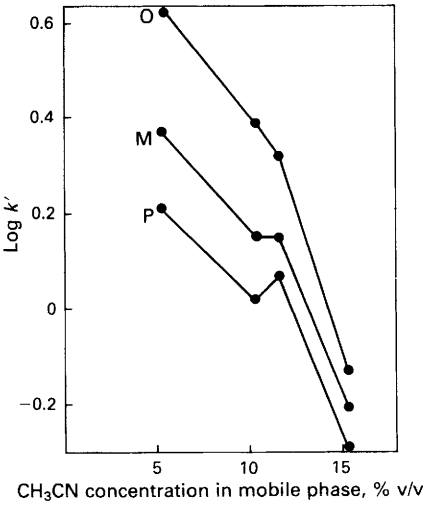

Fig. 1. Graph of logarithm of phase capacity ratio (k') *versus* modifier (CH_3CN) concentration at 80 °C, illustrating subsequent variation in solute retention. O, *ortho*-Aminophenol; M, *meta*-aminophenol; and P, *para*-aminophenol

over a range of temperatures with a water - acetonitrile eluent. Samples were dissolved in the appropriate eluent for the experiment being carried out.

A series of calibration standards covering the range 0–600 p.p.m. were prepared for both methods. Reproducibility was examined by repeated injections of 0.2% m/v *meta*-aminophenol in the eluent used in the optimised methods.

Results and Discussion

Two parameters were altered in the reversed-phase system: the modifier concentration and oven temperature.

Table 2. Resolution of adjacent aminophenol peaks at selected oven temperatures

Temperature/°C	R_s (*para* - *meta*)	R_s (*meta* - *ortho*)
24	0.98	3.01
40	1.23	3.01
60	1.06	2.71
80	1.37	3.52

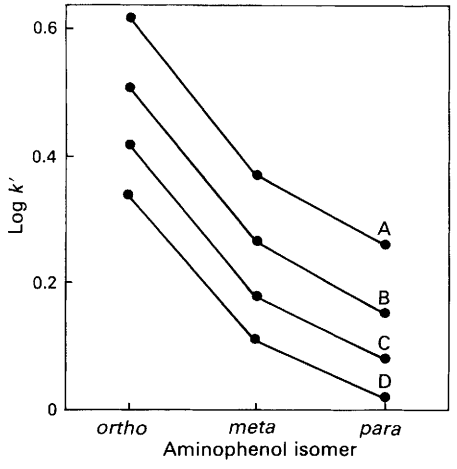

Fig. 2. Graph of logarithm of phase capacity ratio (k') against the different aminophenol isomers highlighting the effect of temperature on the system. Mobile phase: water - acetonitrile (90 + 10). Temperature: A, 24; B, 40; C, 60; and D, 80 °C

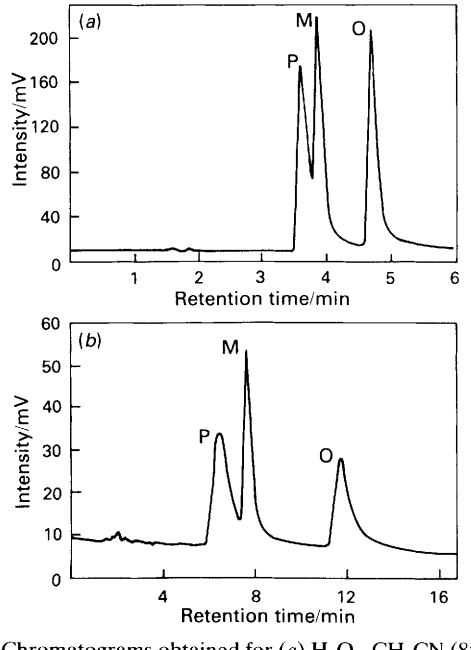

Fig. 3. Chromatograms obtained for (*a*) H_2O - CH_3CN (85 + 15) at 80 °C; and (*b*) H_2O - CH_3CN (90 + 10) at 24 °C. O, *ortho*-Aminophenol; M, *meta*-aminophenol; and P, *para*-aminophenol

The modifier concentration was adjusted from 95 + 5 to 85 + 15 v/v water - acetonitrile. The variation of the phase capacity ratio (k') with respect to the modifier content is shown in Fig. 1. This indicates that the polystyrene - divinylbenzene column interacts with the solute more strongly when the amount of modifier is decreased. The optimum resolution between pairs of adjacent isomers appeared to be obtained with water - acetonitrile (90 + 10). This was confirmed by calculation of the R_s values[6] (the relative resolution between pairs of solutes at 10% peak height) as given in Table 1.

Varying the oven temperature had a considerable impact on the chromatography as can be seen from Fig. 2. The results follow a predictable trend, namely, that at higher oven temperatures, the rate of mass transfer is more efficient, thus giving rise to peaks that elute more rapidly with greater sensitivity. Accordingly, from the values of R_s calculated from these results (Table 2), 80 °C was chosen as the optimum oven temperature. The R_s values of adjacent aminophenol peaks for the normal-phase method were: *ortho* - *para* 3.32 and *para* - *meta* 1.99.

Representative chromatograms for the two methods are shown in Figs. 3 and 4. The normal-phase method offers improved resolution, but has several serious drawbacks in comparison with the reversed-phase system, namely, severe drifting of peak position, a laborious conditioning step and long equilibration times. The relative standard deviations of the normal- and reversed-phase methods were 11.6 and 1.4%, respectively, for nine successive measurements of the retention time of the *meta* isomer. This highlights the superiority of

Table 3. Values of the correlation coefficient obtained from calibrations of each isomer for the normal- and reversed-phase methods

Analyte	Correlation coefficient: Normal-phase method	Correlation coefficient: Reversed-phase method
ortho-Aminophenol . .	0.9995	0.9996
meta-Aminophenol . .	0.9987	0.9980
para-Aminophenol . .	0.9997	0.9993

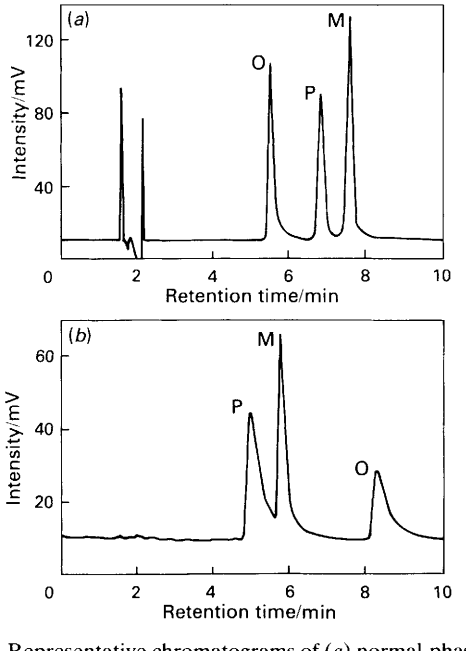

Fig. 4. Representative chromatograms of (*a*) normal-phase; and (*b*) optimised reversed-phase methods. O, *ortho*-Aminophenol; M, *meta*-aminophenol; and P, *para*-aminophenol

the polystyrene - divinylbenzene system in terms of reproducibility. Additionally, the system can be set up and running within 10 min as opposed to 45–60 min for the normal-phase method. A further disadvantage of the normal-phase system is that a residue forms in the mobile phase if it is left overnight.

Calibrations were very good for the *ortho*-, *meta*- and *para*-aminophenols for both the reversed- polystyrene - divinylbenzene and normal-phase methods, with similar values for the linear correlation coefficients, as can be seen from Table 3. Linear calibrations of peak area *versus* concentration were achieved for all isomers over the range 0–600 p.p.m.

Conclusions

A simple and rapid reversed-phase method suitable for the qualitative and quantitative analysis of *ortho*-, *meta*- and *para*-aminophenol has been developed. The system uses a polystyrene - divinylbenzene column to overcome the drawbacks associated with the previously preferred method. Although separation of the first pair of peaks is slightly poorer than in the previous normal-phase method, the reversed-phase system proved to be simpler, faster and more reproducible than the former method.

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