

CHAPTER IV



RESULTS AND DISCUSSIONS

The effect of solvent strength on the chromatographic behavior in terms of the number of theoretical plates, peak shape and retention time of each phenolic compound i.e., paracetamol, phenol, methylparaben, ethylparaben and propylparaben are studied. The results of the study in the solvent strength are presented in Appendix B Tables B1 to B20. The graphs showing the relationship between column efficiency in terms of number of theoretical plates (N) for each phenolic compound and varying concentrations of water are shown in Figures 4.1 to 4.8. The graphs showing the relationship between value of N_{max}/N_{min} for each phenolic compound and various temperatures of column are shown in Figures 4.9 and 4.10. The chromatograms of standard mixtures of phenolic compounds, on a reversed phase column, dissolved in either methanol or water/methanol (40:60, v/v) are shown in Figures 4.11 to 4.20.

All Chromatograms obtained from the experiments are presented in Appendix A.

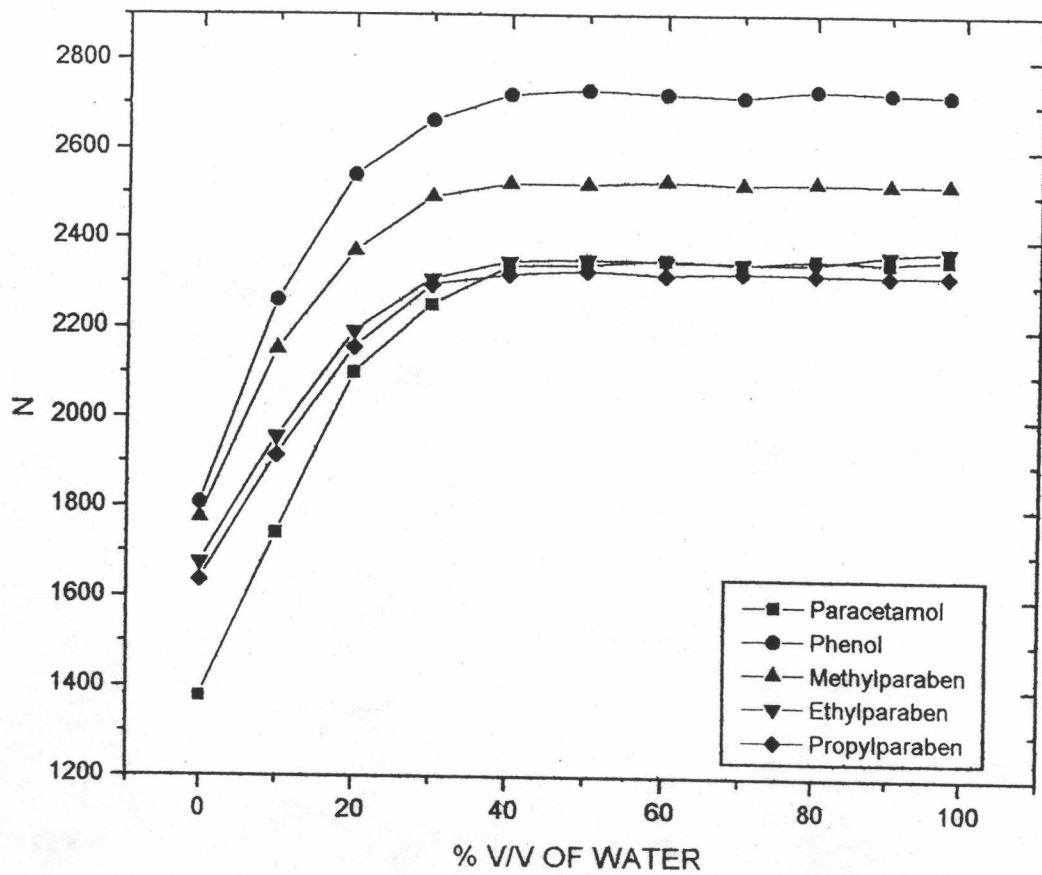


Figure 4.1 Effect of varying concentrations of water on the chromatographic behavior for phenols (Dependence of N on the water to methanol ratio). Chromatographic conditions as given in Table 3.1 at 20 °C.

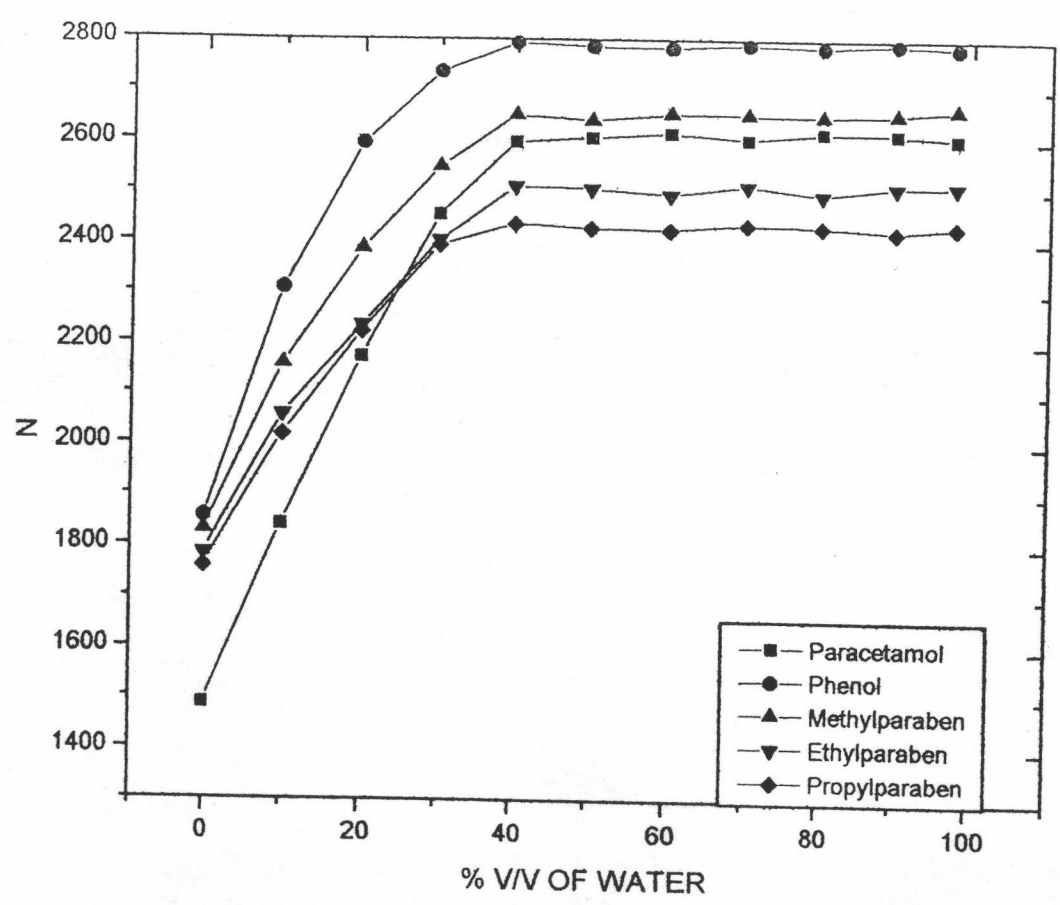


Figure 4.2 Effect of varying concentrations of water on the chromatographic behavior for phenols (Dependence of N on the water to methanol ratio). Chromatographic conditions as given in Table 3.1 at 25 °C.

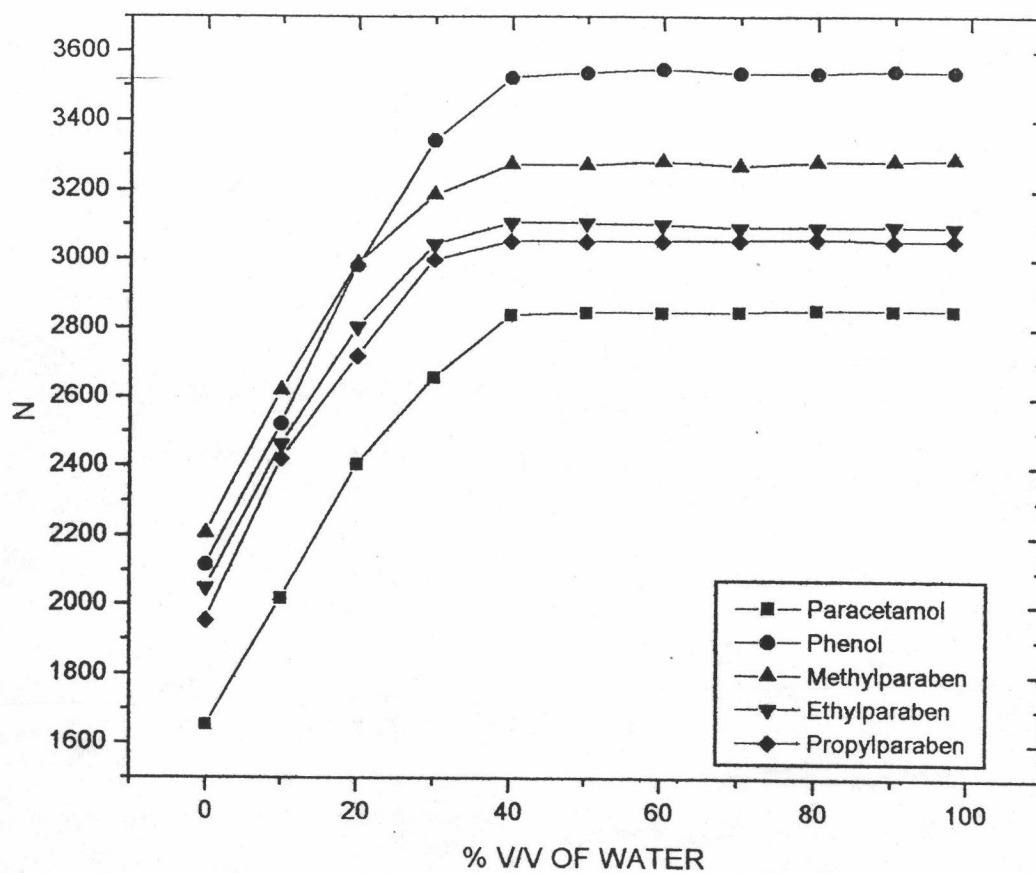


Figure 4.3 Effect of varying concentrations of water on the chromatographic behavior for phenols (Dependence of N on the water to methanol ratio). Chromatographic conditions as given in Table 3.1 at 30 °C.

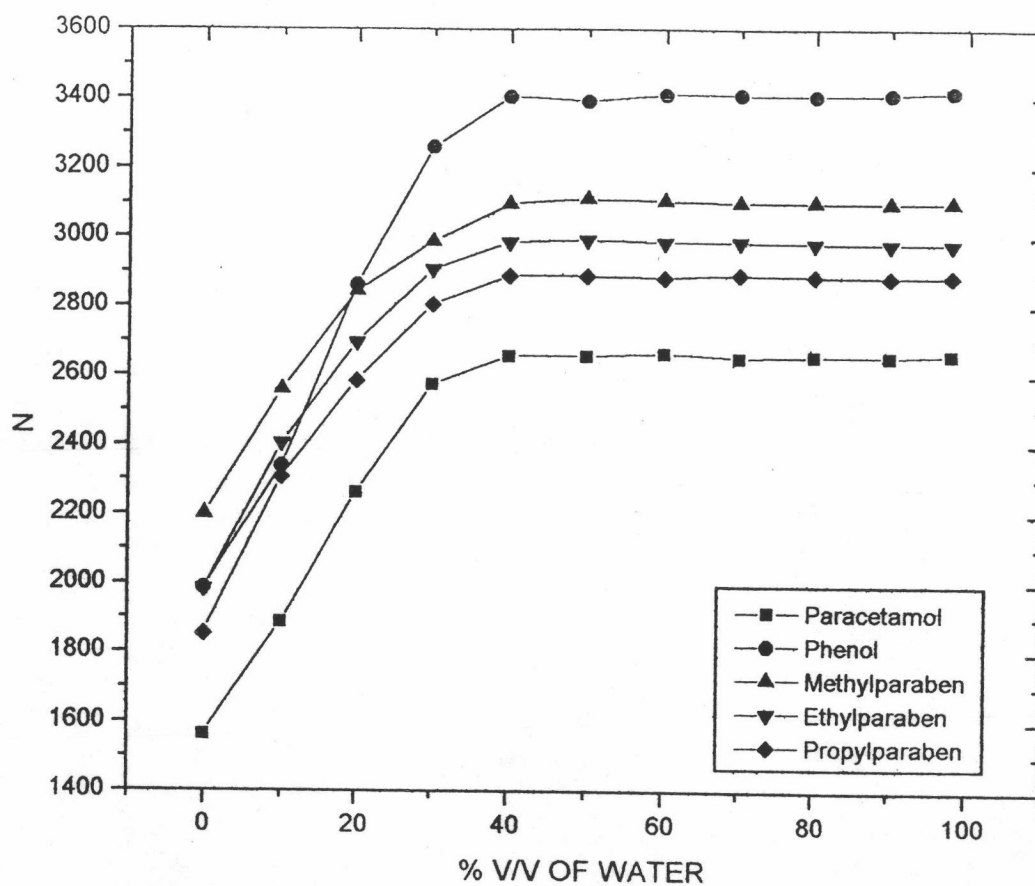


Figure 4.4 Effect of varying concentrations of water on the chromatographic behavior for phenols (Dependence of N on the water to methanol ratio). Chromatographic conditions as given in Table 3.1 at 35 °C.

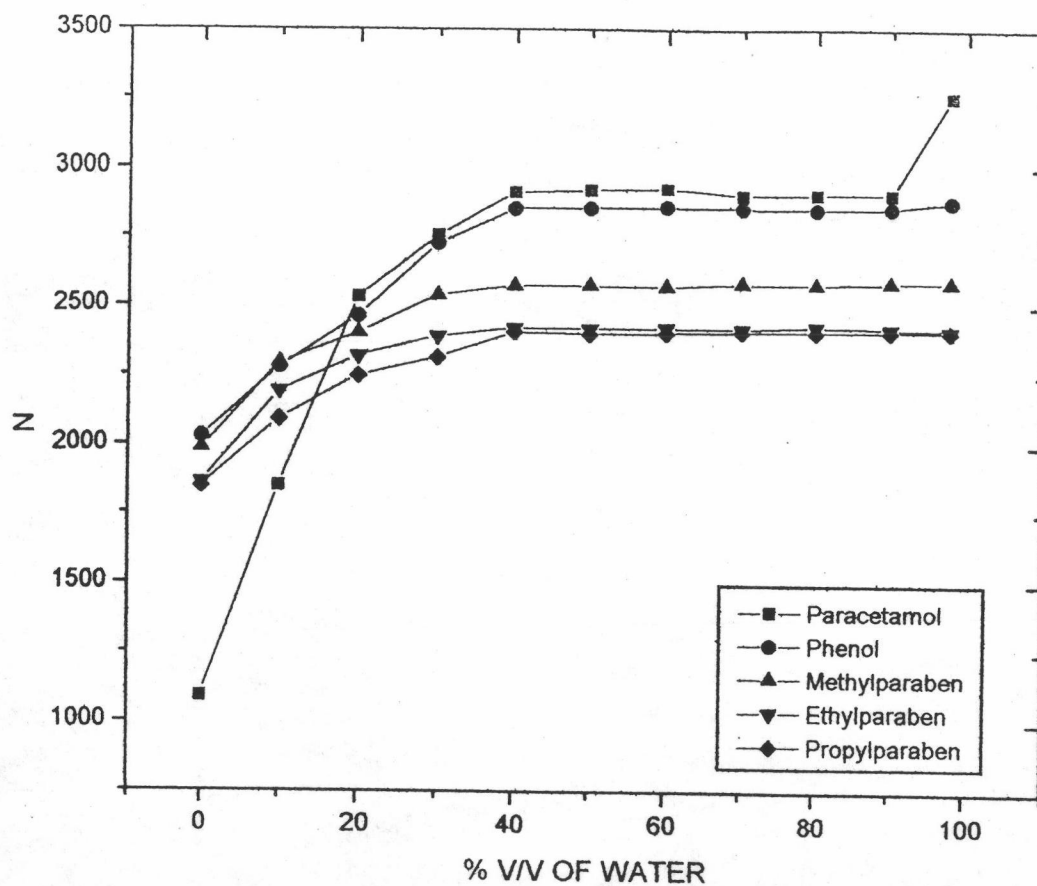


Figure 4.5 Effect of varying concentrations of water on the chromatographic behavior for phenols (Dependence of N on the water to methanol ratio). Chromatographic conditions as given in Table 3.2 at 20 °C.

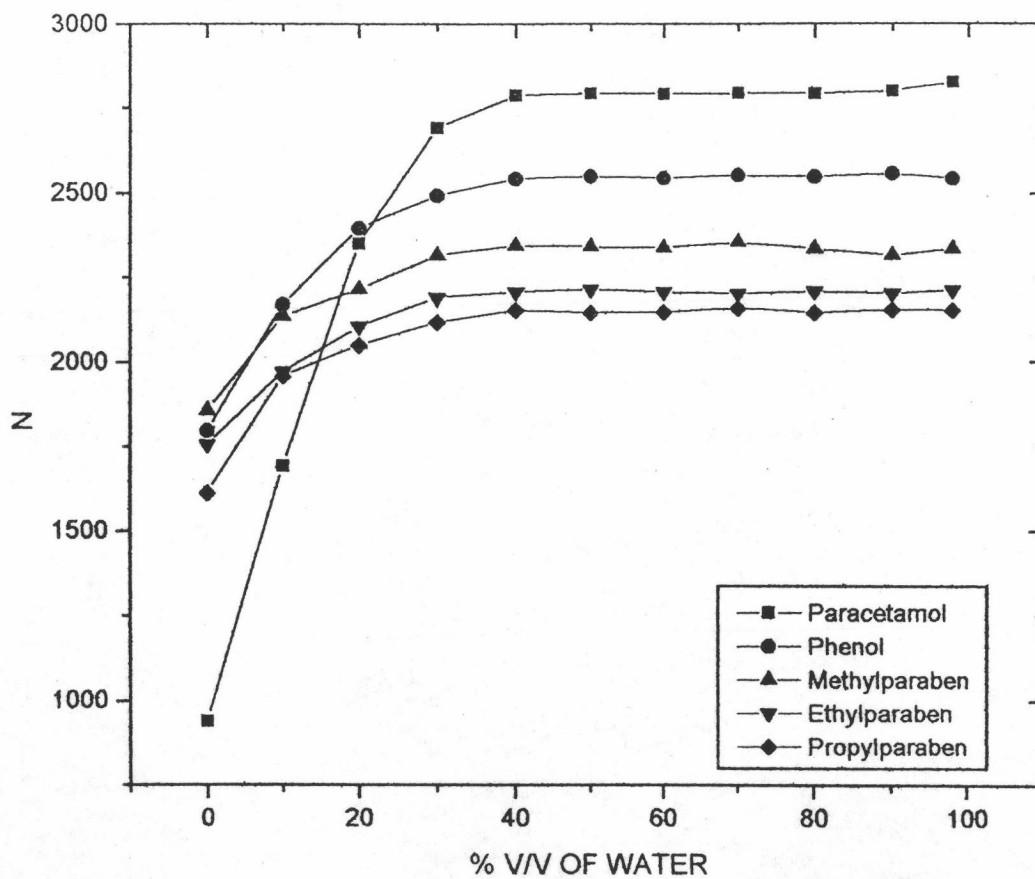


Figure 4.6 Effect of varying concentrations of water on the chromatographic behavior for phenols (Dependence of N on the water to methanol ratio). Chromatographic conditions as given in Table 3.2 at 25 °C.

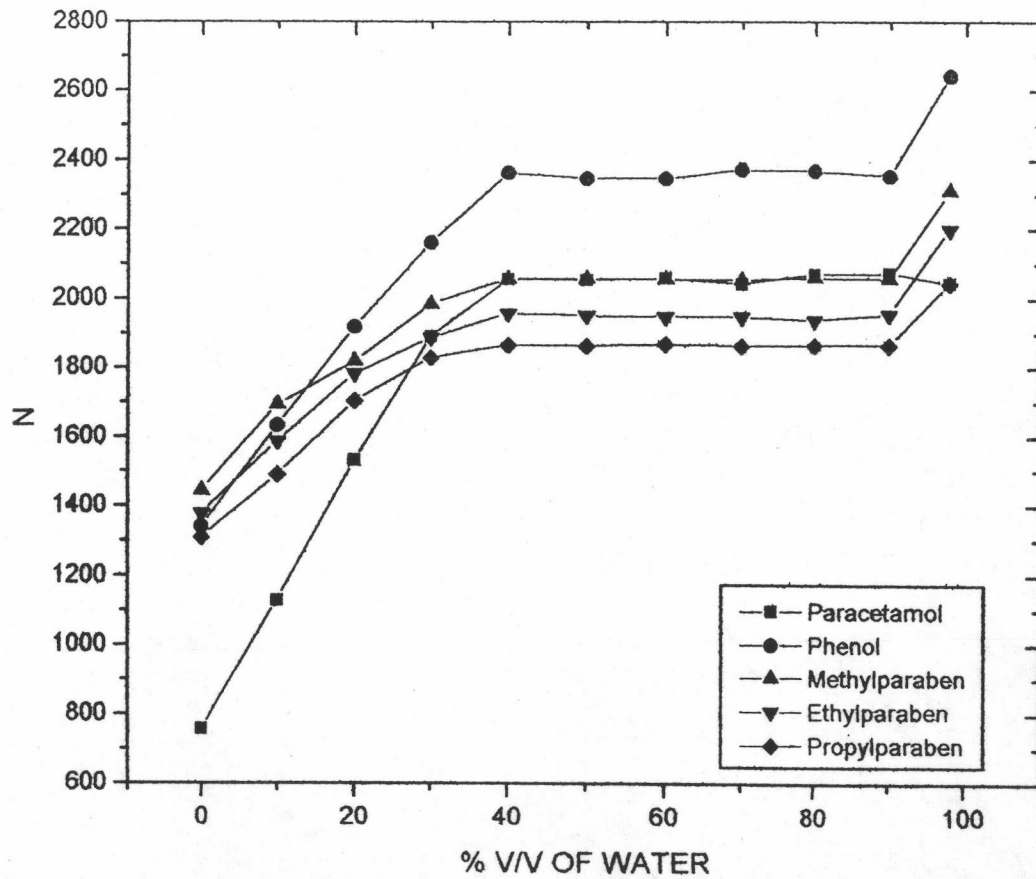


Figure 4.7 Effect of varying concentrations of water on the chromatographic behavior for phenols (Dependence of N on the water to methanol ratio). Chromatographic conditions as given in Table 3.2 at 30 °C.

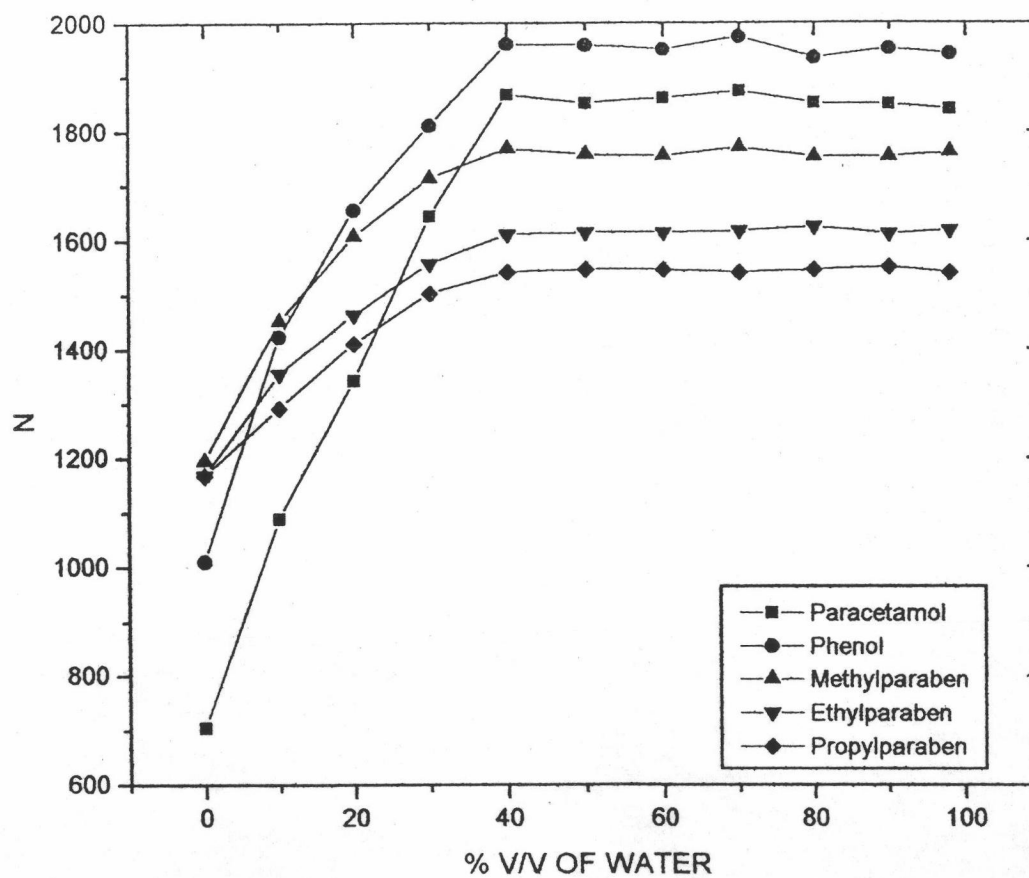


Figure 4.8 Effect of varying concentrations of water on the chromatographic behavior for phenols (Dependence of N on the water to methanol ratio). Chromatographic conditions as given in Table 3.2 at 35 °C.

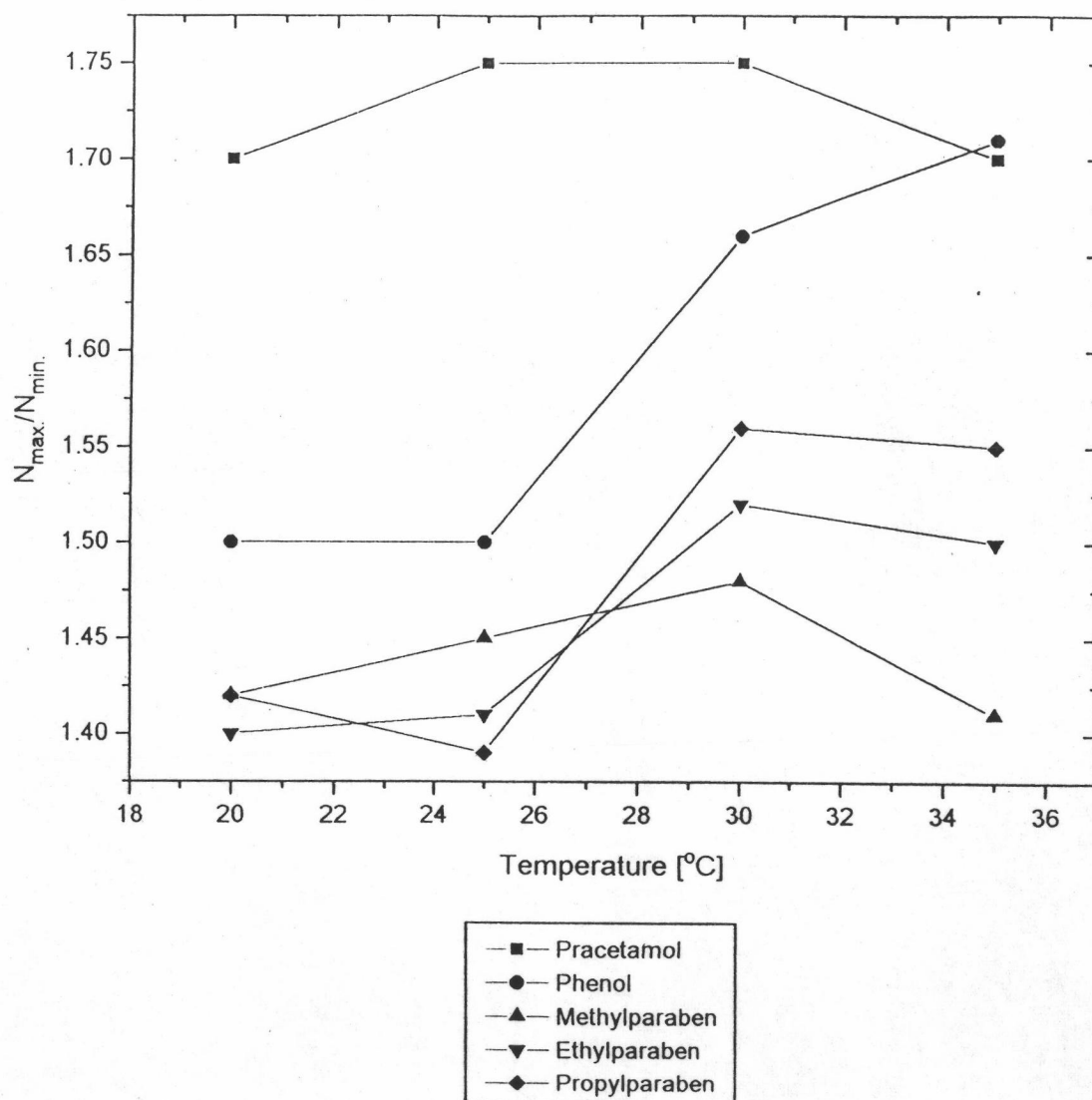


Figure 4.9 Effect of varying temperatures on the N_{max}/N_{min} ratio for phenols.

Chromatographic conditions as given in Table 3.1 .

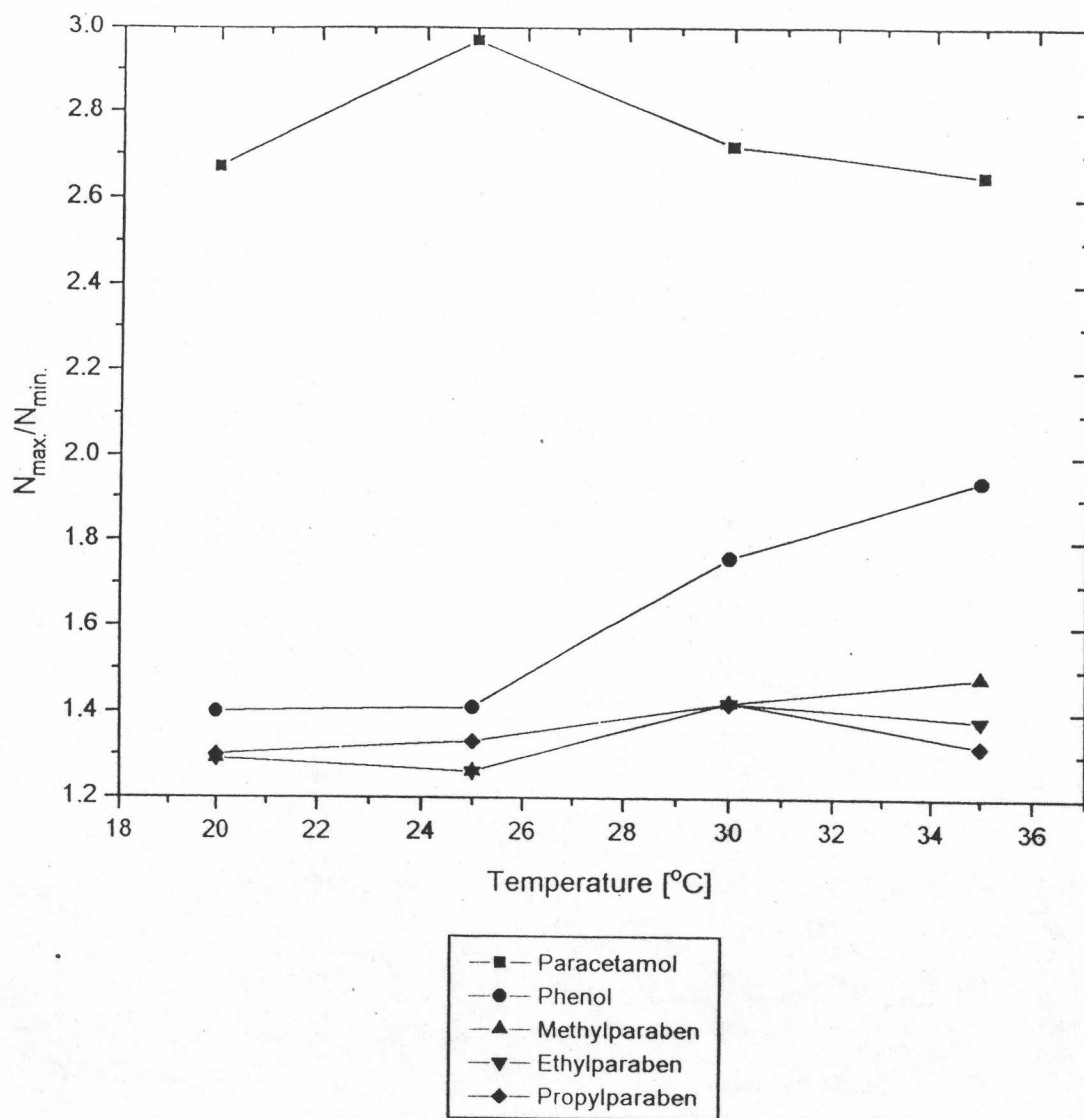


Figure 4.10 Effect of varying temperatures on the N_{max}/N_{min} ratio for phenols.

Chromatographic conditions as given in Table 3.2.

Figures 4.1 to 4.4 show the effect of varying water-to-methanol ratios on the chromatographic behavior in terms of number of theoretical plates (N) in RP-8 column of the standard mixtures at temperatures 20°, 25°, 30° and 35°C., respectively, and Figures 4.5 to 4.8 show the effect of varying water-to-methanol ratios on the chromatographic behavior in terms of number of theoretical plates (N) in RP-18 column of the standard mixtures at temperatures 20°, 25°, 30° and 35°C., respectively. The results as in Figure show that, at the same chromatographic behavior, the number of theoretical plates (N) of each phenolic compound increases depending on the concentration of water in the injected sample and reaches a constant level at approximate 40% water as the mobile phase. It can be seen in agreement with results of S. Wongyai (22) and B. Porsch (23) that the highest efficiency is reached when the injection is made in a solvent weaker than the mobile phase. This is a natural consequence of the well known on column enrichment principle, i.e., concentrating the solute injected under conditions of higher retention.

Figures 4.9 and 4.10 show the effect of various temperatures on the N_{max}/N_{min} ratio of each phenolic compound in RP-8 and RP-18, respectively. The results as in Figure show that at the higher temperature, the trend of N_{max}/N_{min} ratio of each studied compound is increased. It can be seen that, the effect of solvent strength on the studied compound will be increased with higher temperature.

As the experiments, it showed that the N_{max}/N_{min} ratio of paracetamol has a significant higher than the others. So the solvent strength affected on compound which retained in a short time.

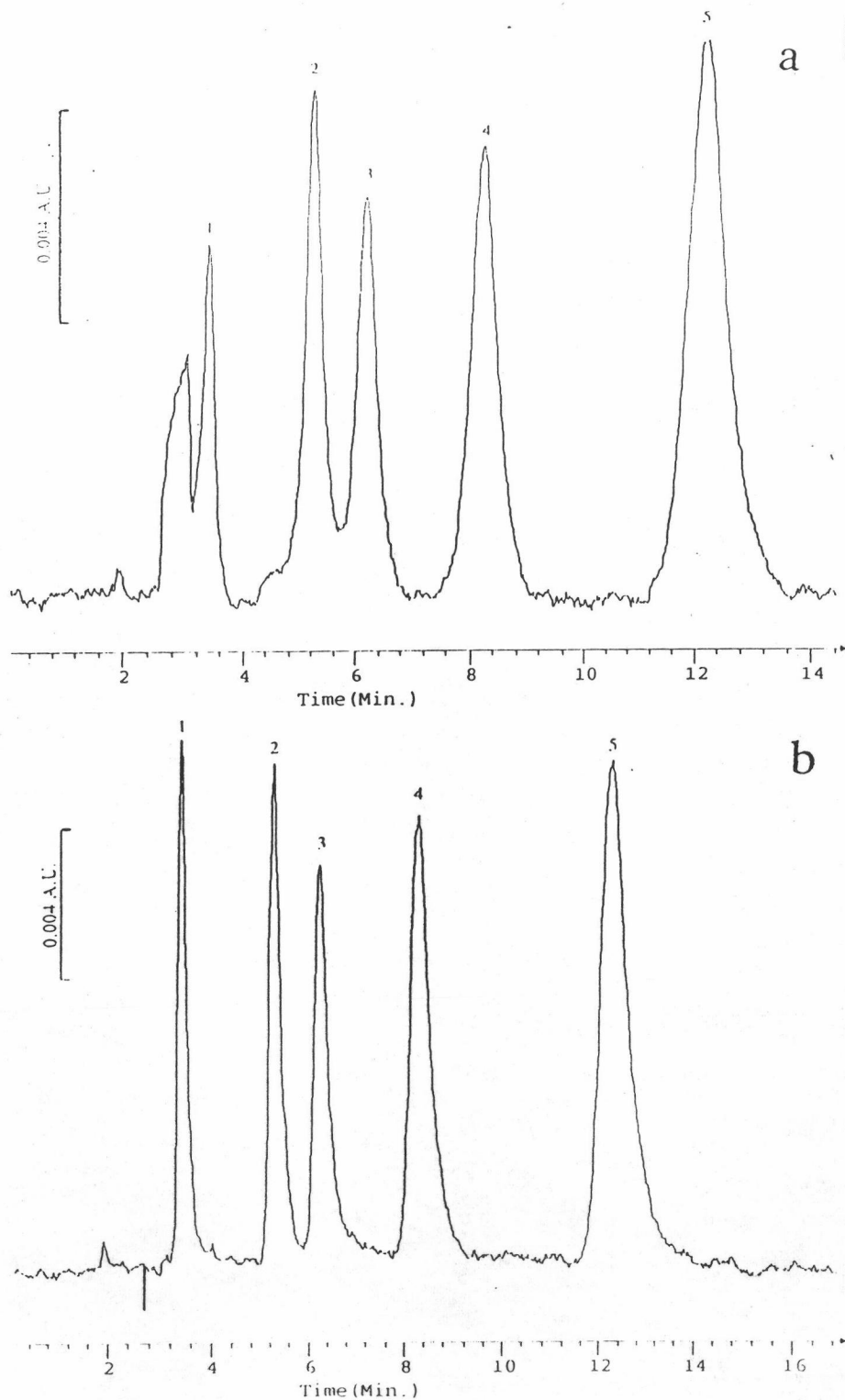


Figure 4.11 Chromatograms of standard mixtures of phenols dissolved in either methanol (a) or water/methanol (b, 40 : 60 v/v) on a reversed phase column. Peak identification : 1...paracetamol , 2...phenol , 3...methylparaben , 4...ethylparaben and 5...propylparaben. Chromatographic conditions as given in Table 3.1 at 20 °C.

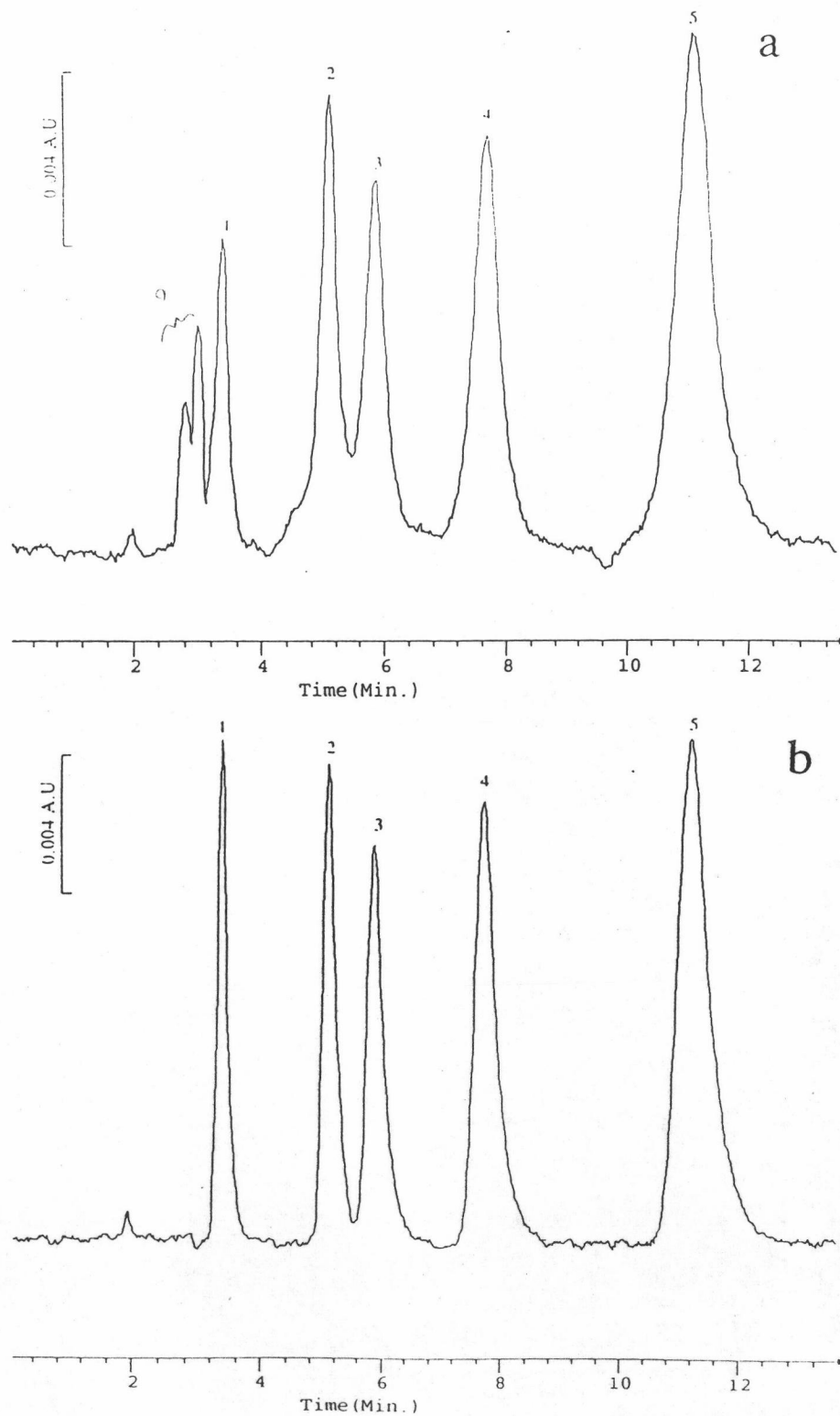


Figure 4.12 Chromatograms of standard mixtures of phenols dissolved in either methanol (a) or water/methanol (b, 40 : 60 v/v) on a reversed phase column. Peak identification : 1...paracetamol , 2...phenol , 3...methylparaben , 4...ethylparaben and 5...propylparaben. Chromatographic conditions as given in Table 3.1 at 25 °C.

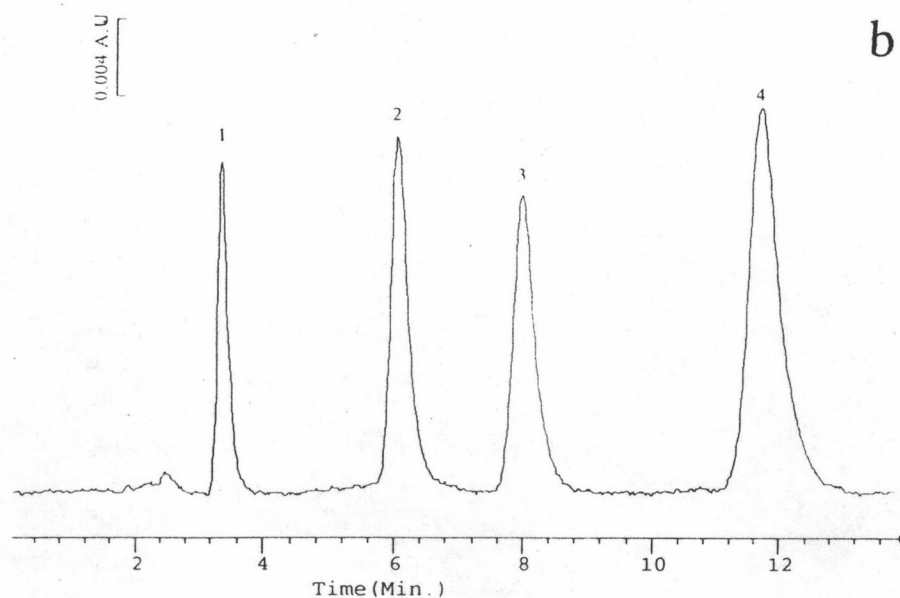
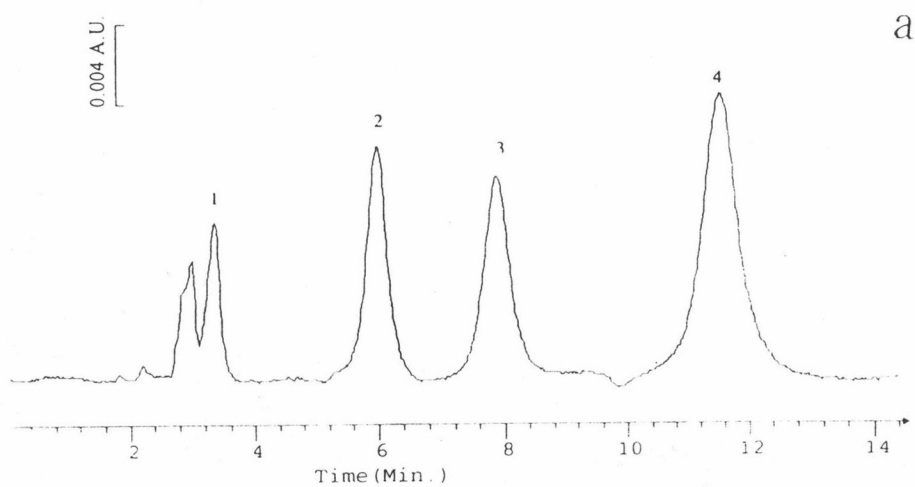


Figure 4.13 Chromatograms of standard mixtures of phenols dissolved in either methanol (a) or water/methanol (b, 40 : 60 v/v) on a reversed phase column. Peak identification : 1...paracetamol , 2...phenol , 3...methylparaben , 4...ethylparaben. Chromatographic conditions as given in Table 3.1 at 20 °C.

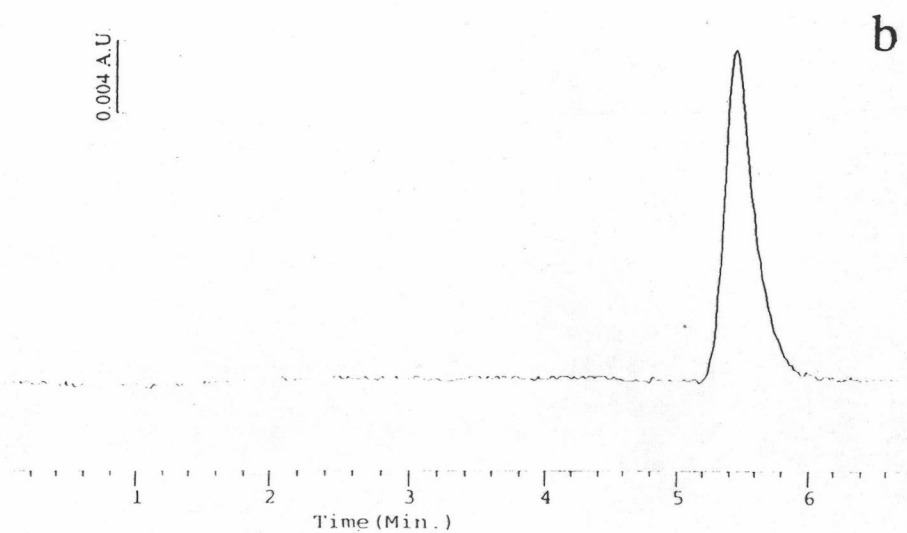
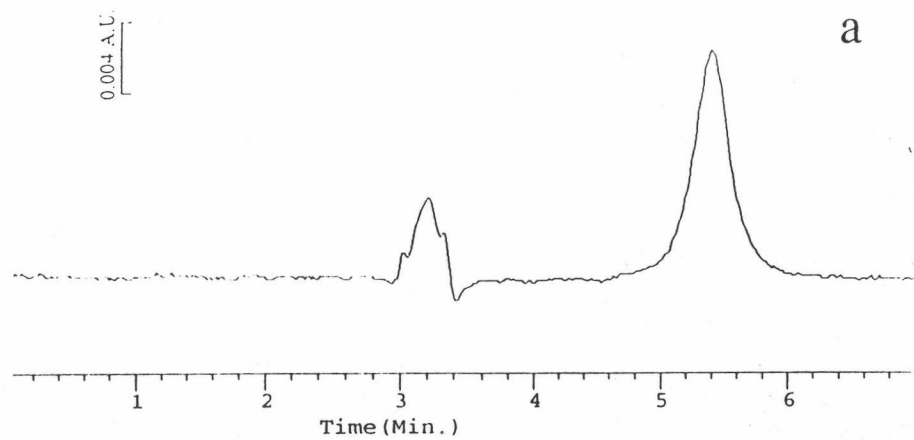


Figure 4.14 Chromatograms of phenol dissolved in either methanol (a) or water/methanol (b, 40:60 v/v) on a reversed phase column. Chromatographic conditions as given in Table 3.1 at 30 °C.

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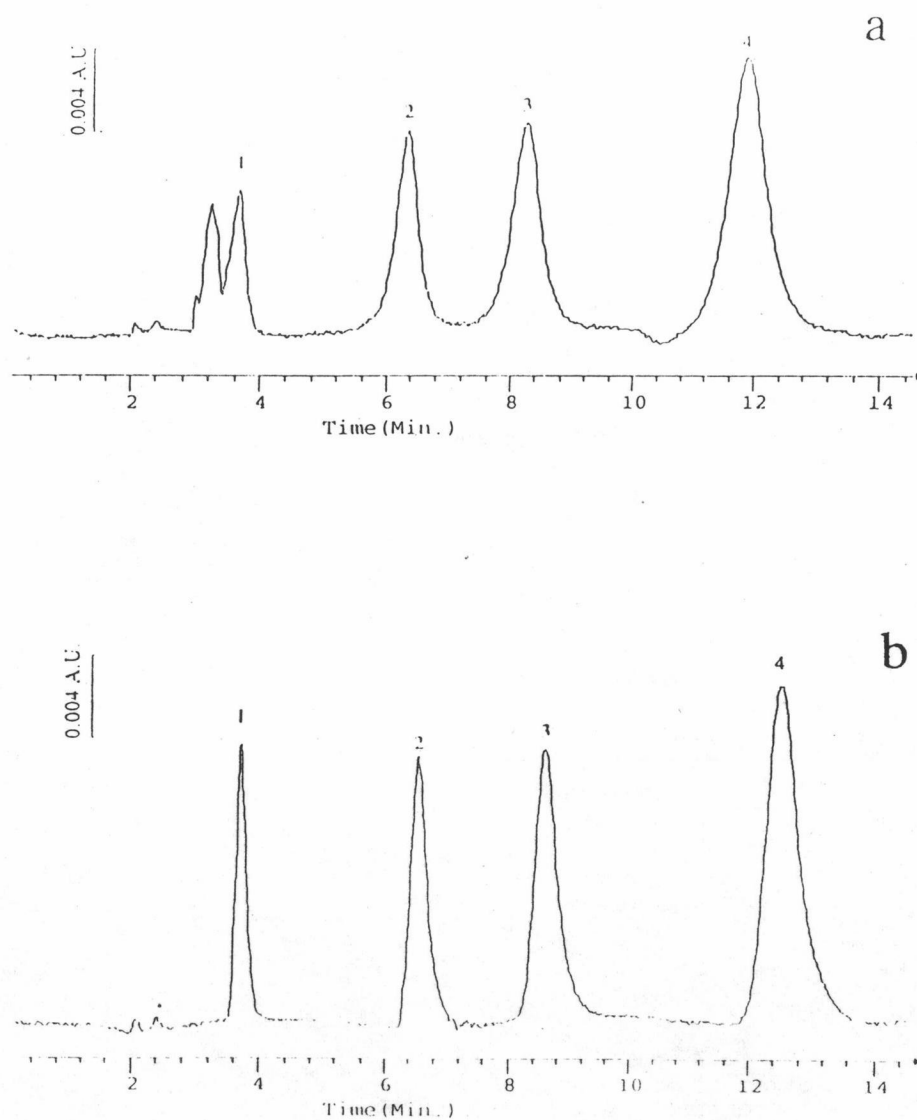


Figure 4.15 Chromatograms of standard mixtures of phenols dissolved in either methanol (a) or water/methanol (b, 40 : 60 v/v) on a reversed phase column. Peak identification : 1...paracetamol , 2...phenol , 3...methylparaben , 4...ethylparaben. Chromatographic conditions as given in Table 3.1 at 20°C.

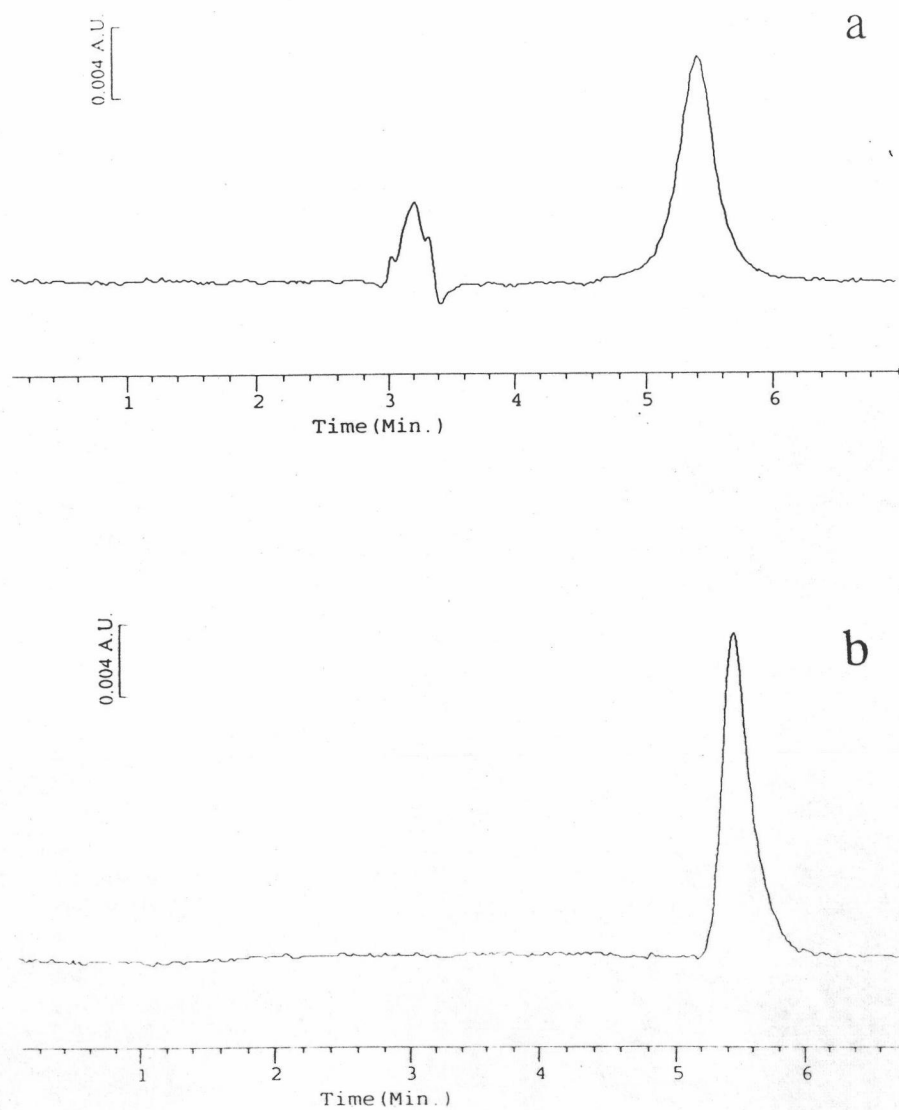


Figure 4.16 Chromatograms of phenol dissolved in either methanol (a) or water/methanol (b, 40:60 v/v) on a reversed phase column. Chromatographic conditions as given in Table 3.1 at 35 °C.

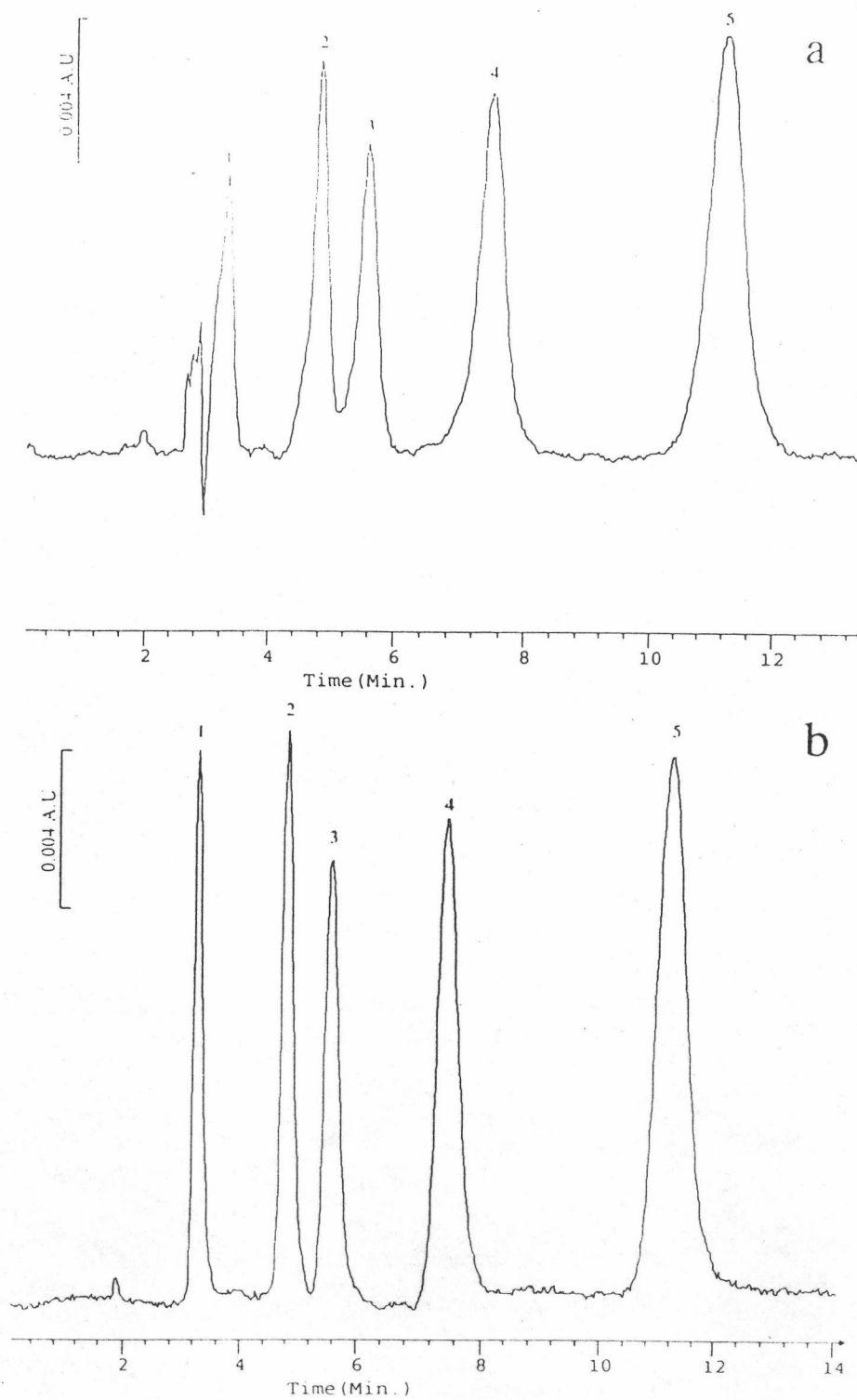


Figure 4.17 Chromatograms of standard mixtures of phenols dissolved in either methanol (a) or water/methanol (b, 40 : 60 v/v) on a reversed phase column. Peak identification : 1...paracetamol , 2...phenol , 3...methylparaben , 4...ethylparaben and 5...propylparaben. Chromatographic conditions as given in Table 3.2 at 20 °C.

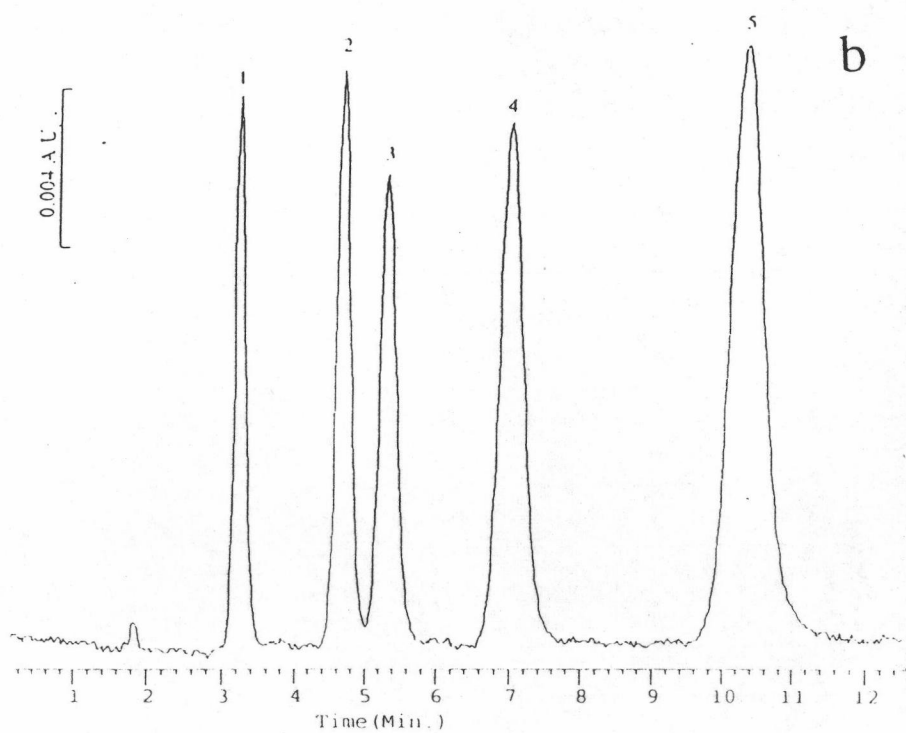
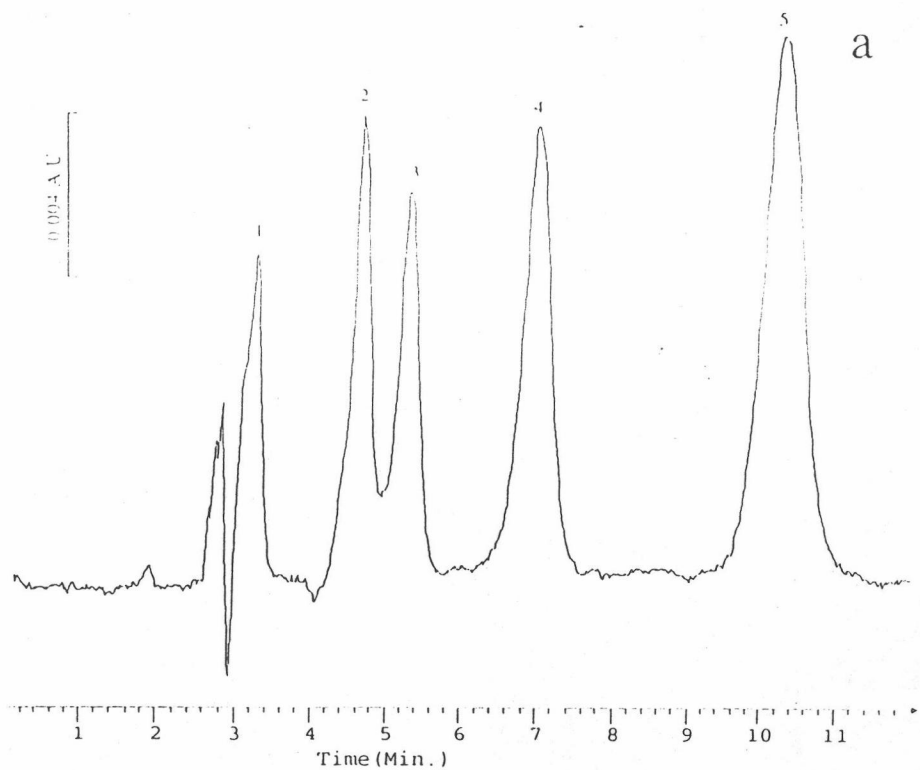
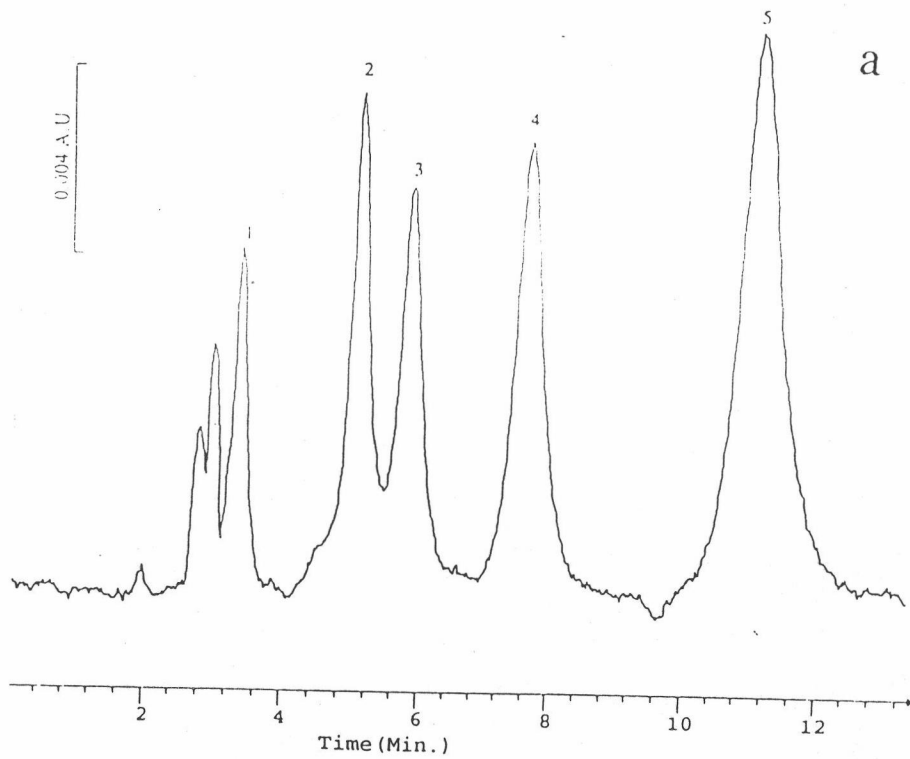


Figure 4.18 Chromatograms of standard mixtures of phenols dissolved in either methanol (a) or water/methanol (b, 40 : 60 v/v) on a reversed phase column. Peak identification : 1...paracetamol , 2...phenol , 3...methylparaben , 4...ethylparaben and 5...propylparaben. Chromatographic conditions as given in Table 3.2 at 25 °C.



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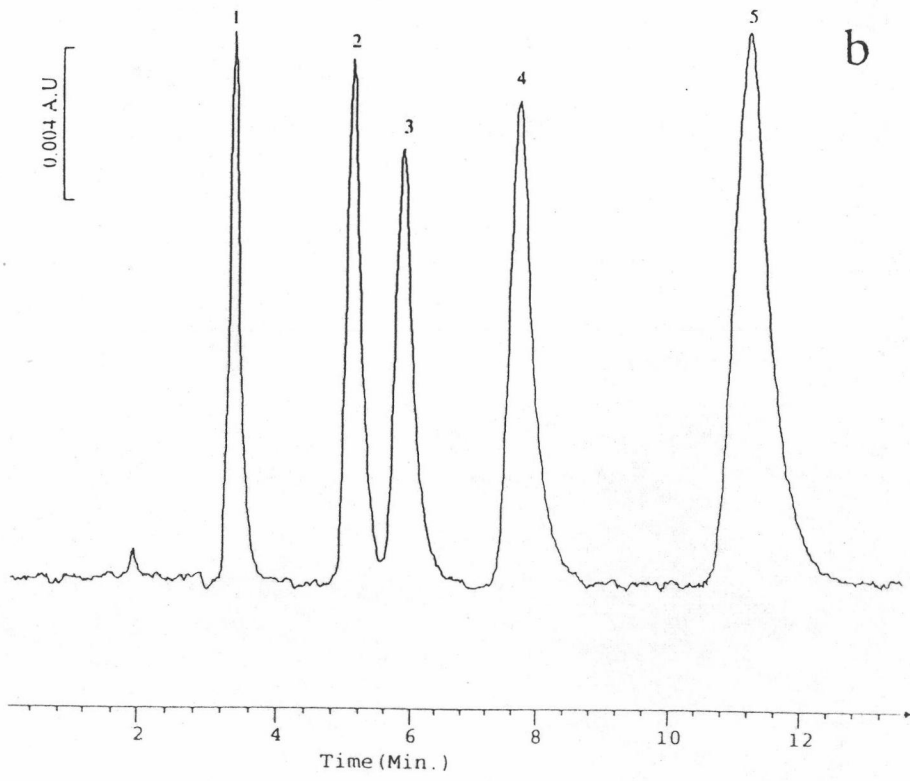


Figure 4.19 Chromatograms of standard mixtures of phenols dissolved in either methanol (a) or water/methanol (b, 40 : 60 v/v) on a reversed phase column. Peak identification : 1...paracetamol , 2...phenol , 3...methylparaben , 4...ethylparaben and 5...propylparaben. Chromatographic conditions as given in Table 3.2 at 30 °C.

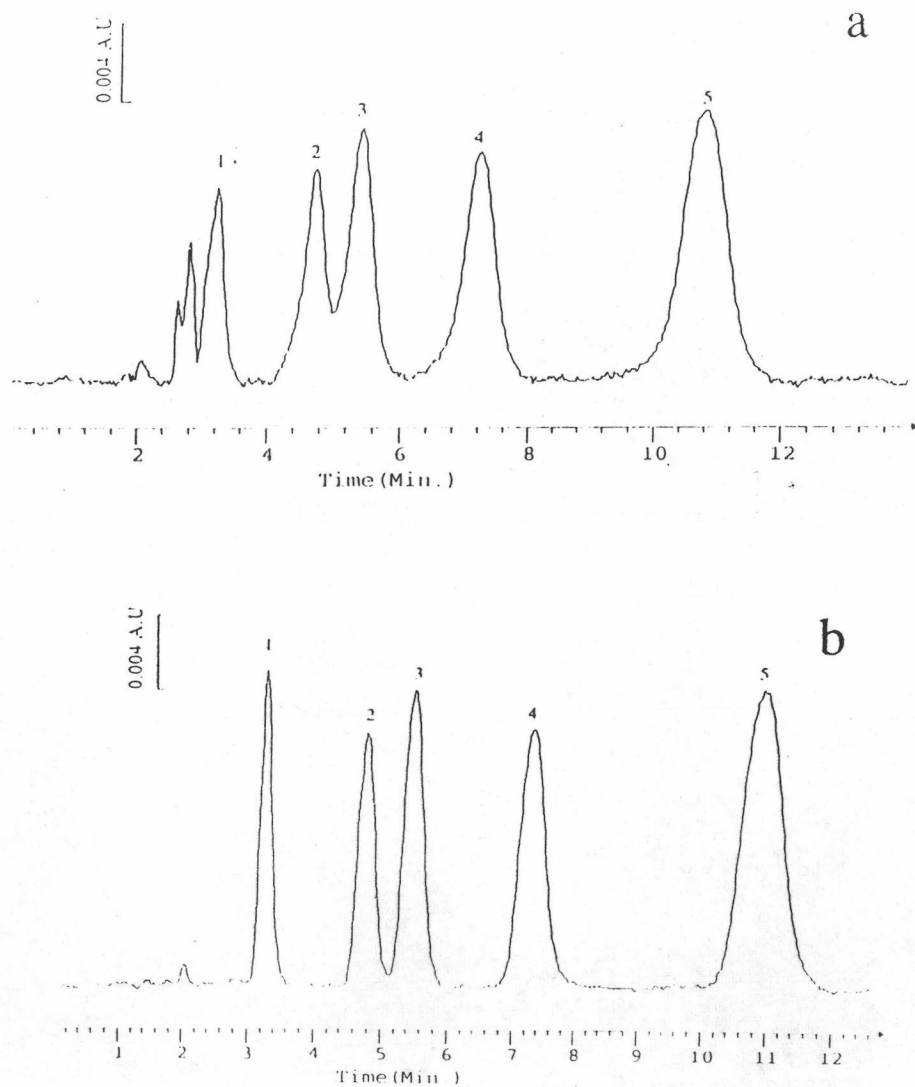


Figure 4.20 Chromatograms of standard mixtures of phenols dissolved in either methanol (a) or water/methanol (b, 40 : 60 v/v) on a reversed phase column. Peak identification : 1...paracetamol , 2...phenol , 3...methylparaben , 4...ethylparaben and 5...propylparaben. Chromatographic conditions as given in Table 3.2 at 35 °C.

Figures 4.11 to 4.16 show chromatograms of standard mixtures of phenols on RP-8 column at various temperatures and Figures 4.17 to 4.20 show chromatograms of standard mixtures of phenols on RP-18 column at various temperatures. Under the optimal chromatographic conditions high resolution of the unresolved phenols (Figures (a) in Figures 4.11 to 4.20) were achieved when an aqueous methanolic solution (40:60, v/v) was used as the solvent (Figures (b) in Figures 4.11 to 4.20). It is clearly demonstrated that the optimal amount of dissolved can increase both resolution capability and peak height for the phenols.

The results as in the Figure show that solvents which are stronger than those of the mobile phase cause deterioration of peak shapes. The deterioration of peak shape are caused by mixing or the distortion of the sample zone at the head of the column. The reason is that when a sample plug is injected into an HPLC column an elute band skewness should develop immediately because of the drop in concentration of the elute in the plug as it moves into the column. The concentration should be highest at the very beginning at the column and dropped proceedingly down the column. Therefore, the degree of skewness should depend on the water-to-methanol ratio in the sample.

As above, the effect of solvent strength on the peak shape of sample affects only the head of column. Because the mobile phase will reduce the solvent strength in the sample solution when it move through the column.

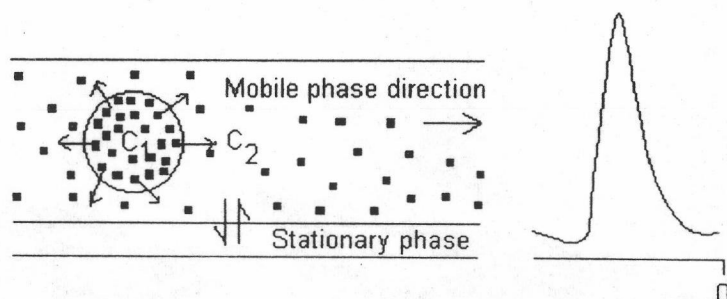
Form the experiment , three models are proposed to explain these phenomena as follows :

Model I : the percentage of methanol in injection solvent is greater than the percentage of methanol in mobile phase ,

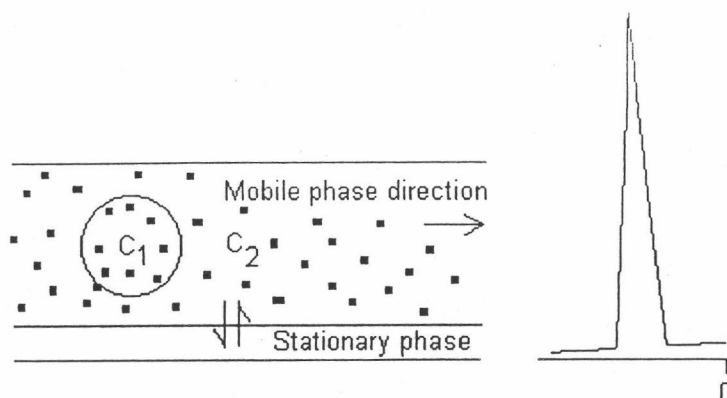
Model II : the percentage of methanol in injection solvent is equal to the percentage of methanol in mobile phase ,

Model III : the percentage of methanol in injection solvent is lower than the percentage of methanol in mobile phase .

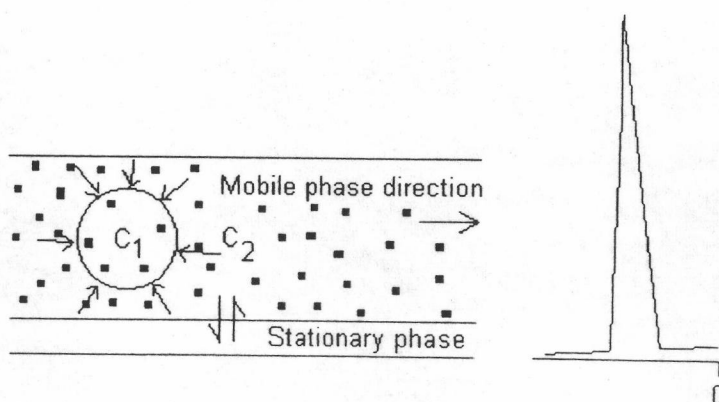
Model I



When the percentage of methanol in the sample zone is greater than percentage of methanol in the mobile phase. The shape of peak will be distorted. This phenomena can be explained that it is dued to a natural consequence of concentration gradient between methanol in the sample zone and methanol in the mobile phase. Methanol in the sample will diffuse to the mobile phase , so the shape of the sample zone will be distorted. By this case , it also affects the analytes in the sample zone that makes the shape of the peak to be broadening and/or fronting.

Model II

The peak shape from this model is almost symmetrical because the concentration gradient between methanol in the sample zone and the methanol in the mobile phase does not give any effect to this system. However, peak still have small fronting which is probably due to the mobility of the mobile phase.

Model III

From the experiment, it can be seen that peaks shapes obtained from the model II and III are not so different. This is because the methanol concentration in the mobile phase is greater than that in the sample zone. Thus methanol will diffuse into the sample zone, as the consequence the analytes in the sample zone cannot diffuse to the mobile phase. This phenomena is called "compression effect".

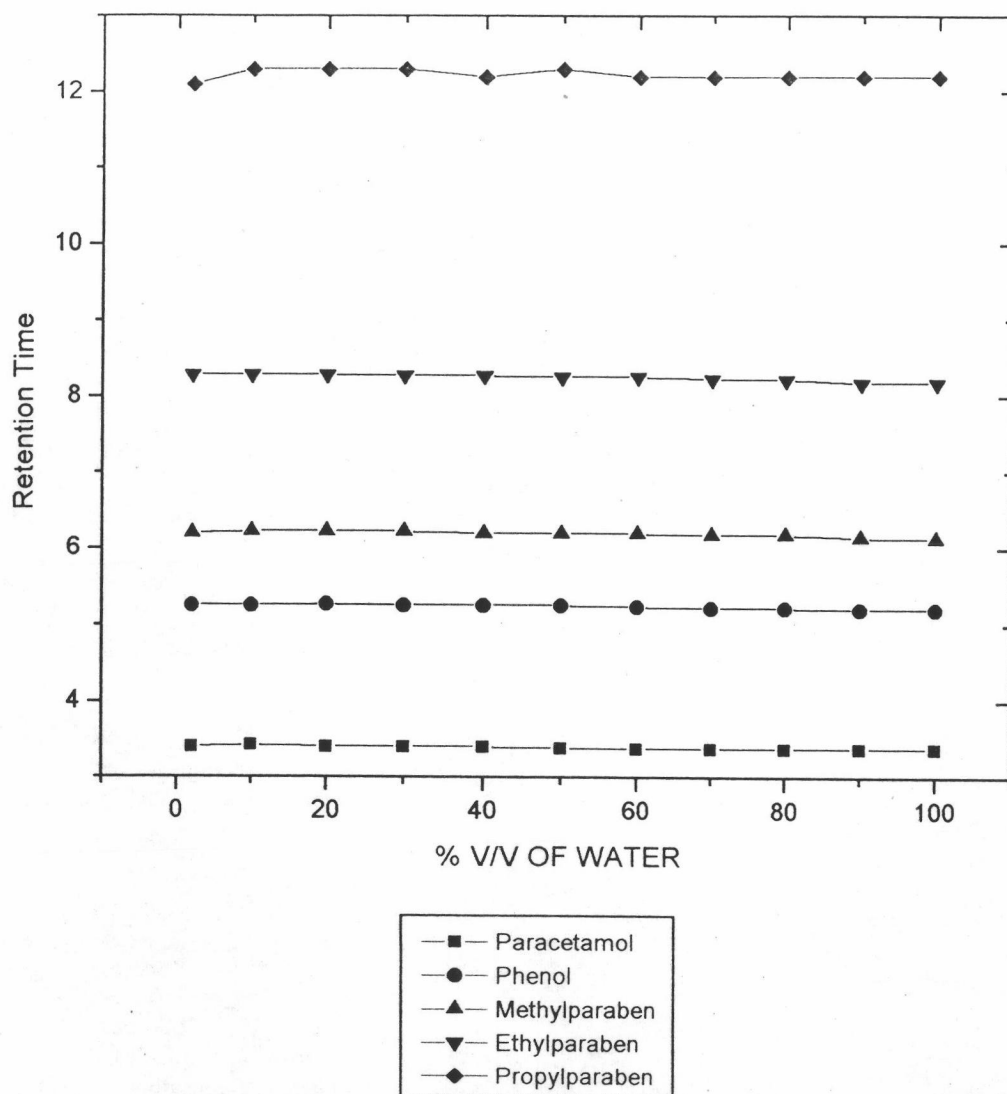


Figure 4.21 Effect of varying concentration of water on the retention time of phenols at 20 °C. Chromatographic conditions as given in Table 3.1

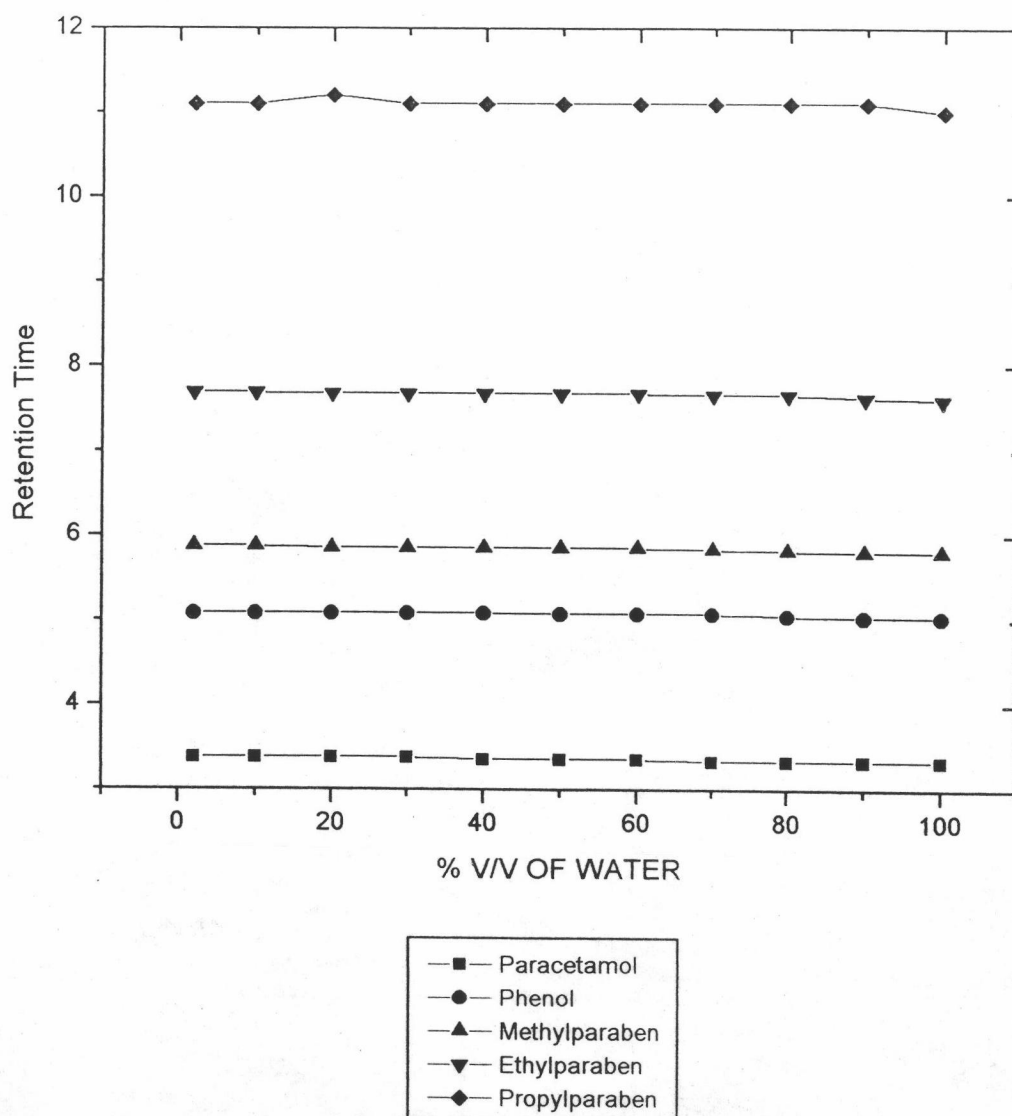


Figure 4.22 Effect of varying concentration of water on the retention time of phenols at 25 °C. Chromatographic conditions as given in Table 3.1

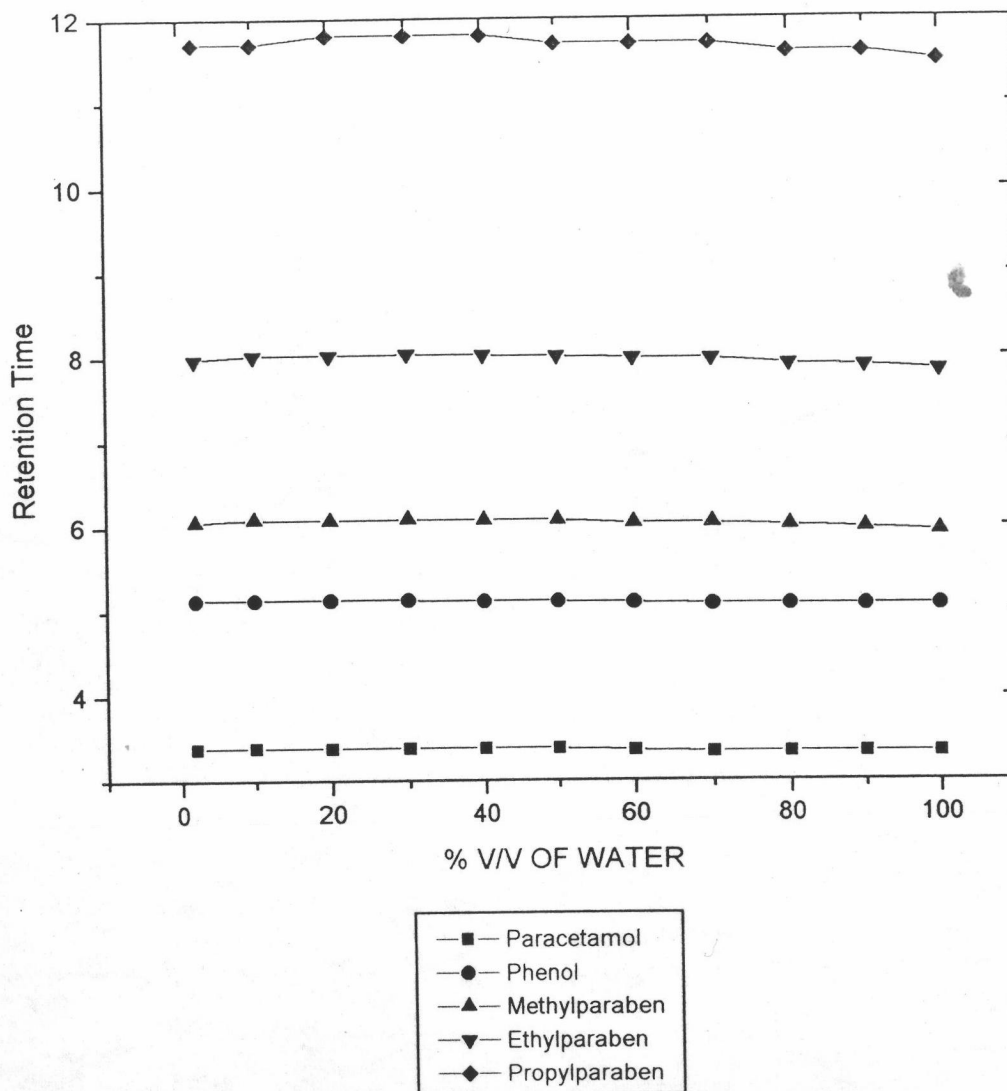


Figure 4.23 Effect of varying concentration of water on the retention time of phenols at 30 °C. Chromatographic conditions as given in Table 3.1

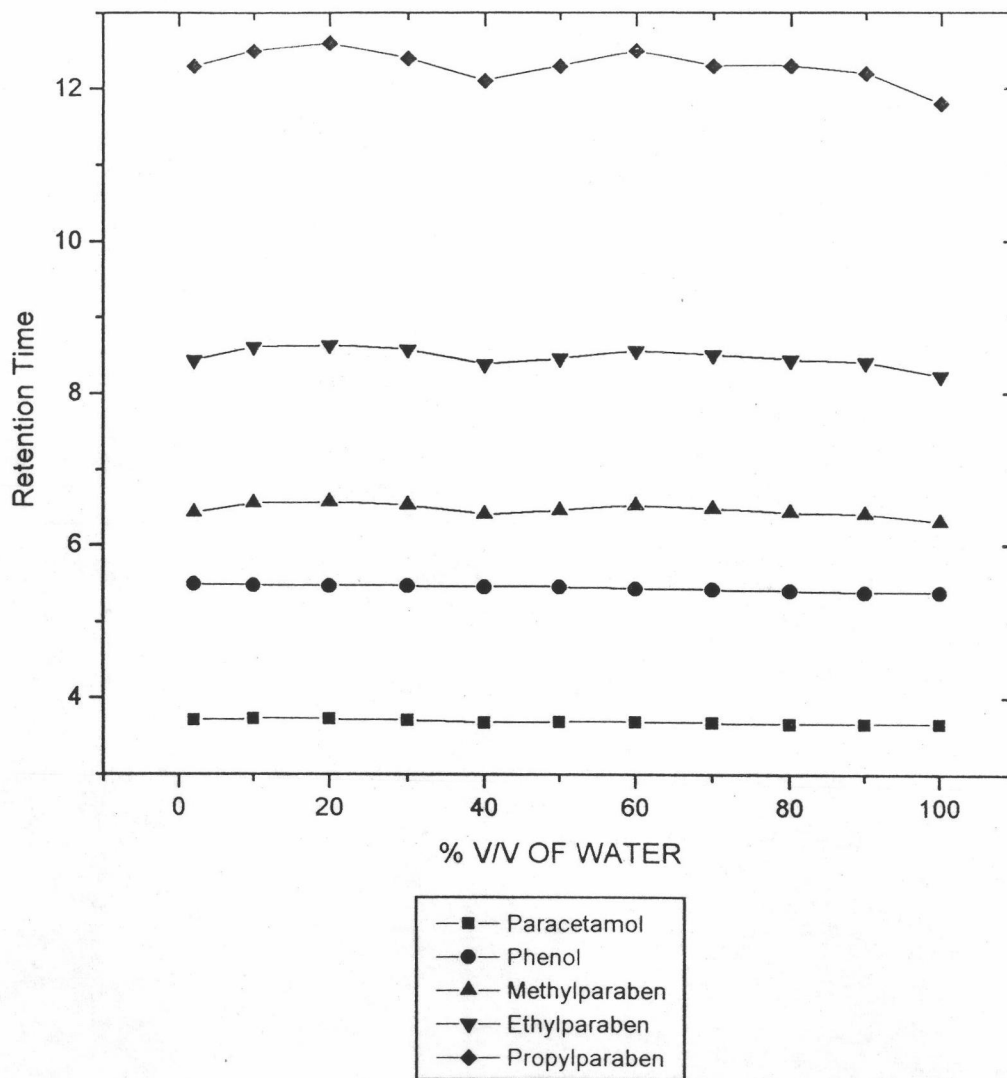


Figure 4.24 Effect of varying concentration of water on the retention time of phenols at 35 °C. Chromatographic conditions as given in Table 3.1

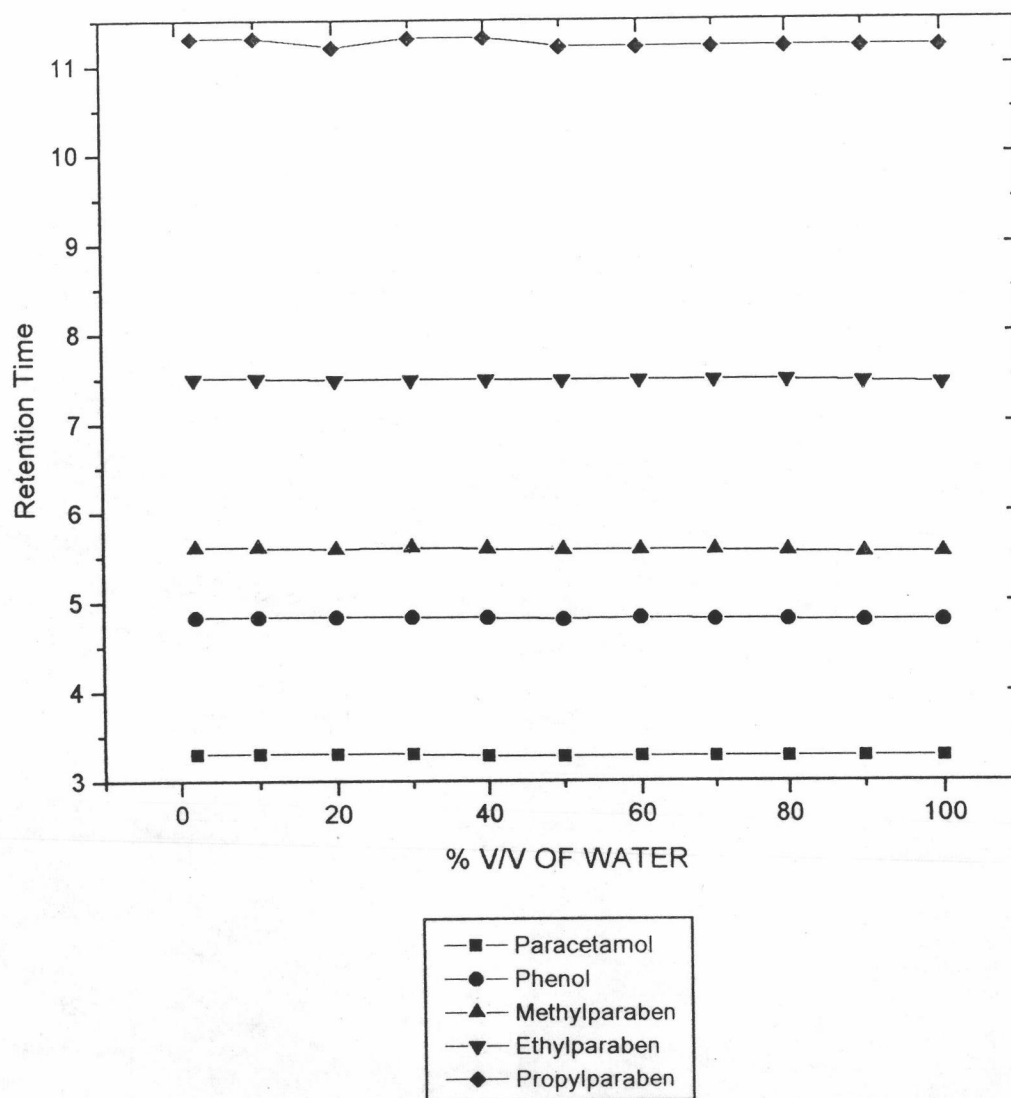


Figure 4.25 Effect of varying concentration of water on the retention time of phenols at 20 °C. Chromatographic conditions as given in Table 3.2

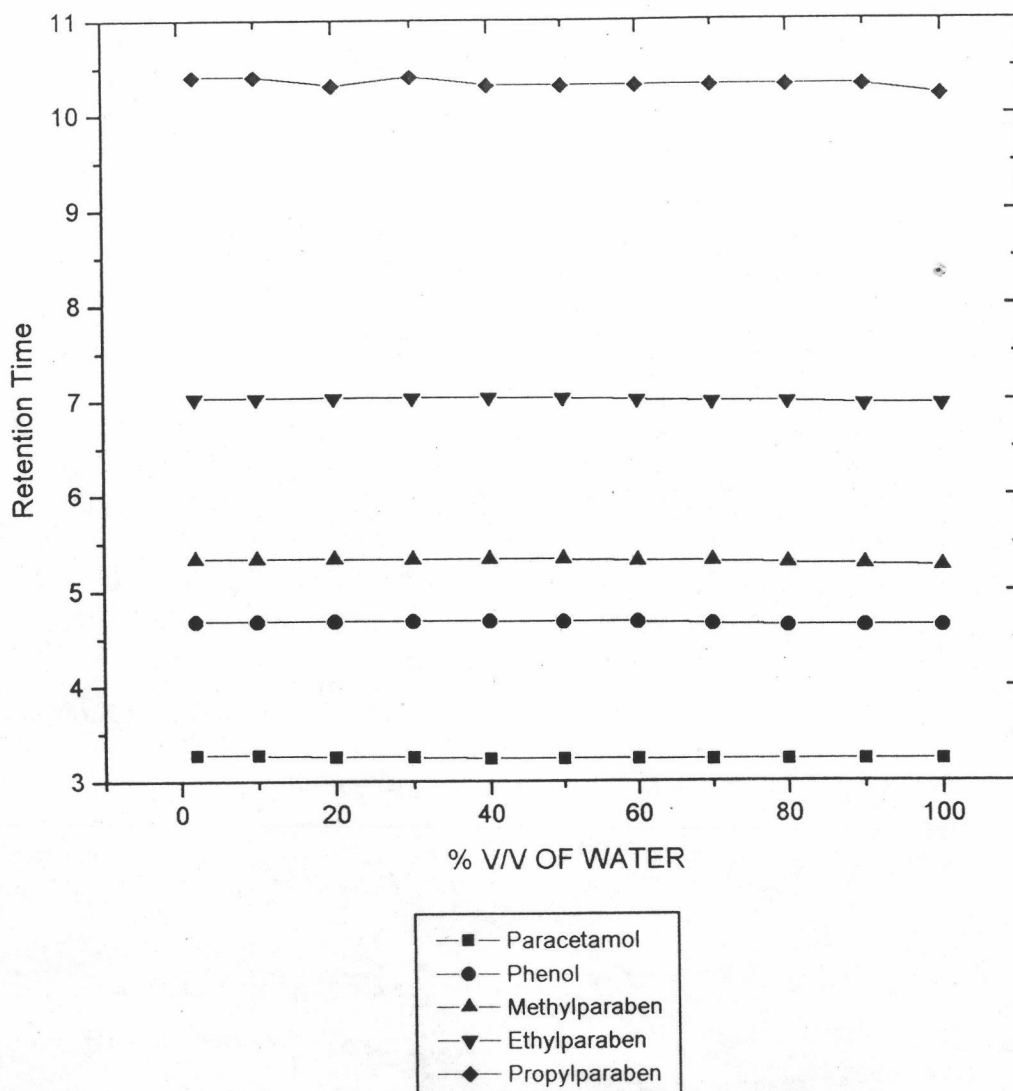


Figure 4.26 Effect of varying concentration of water on the retention time of phenols at 25 °C. Chromatographic conditions as given in Table 3.2

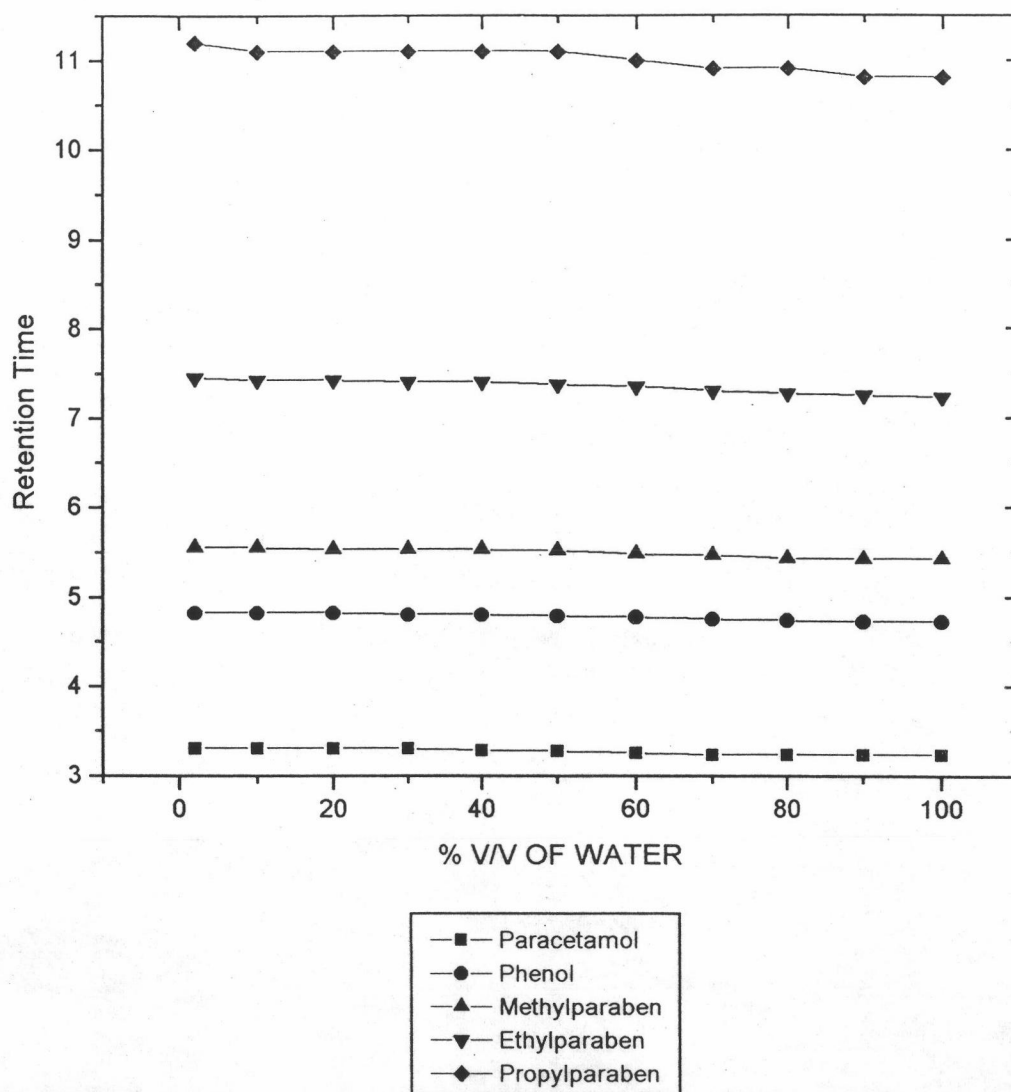


Figure 4.27 Effect of varying concentration of water on the retention time of phenols at 30 °C. Chromatographic conditions as given in Table 3.2

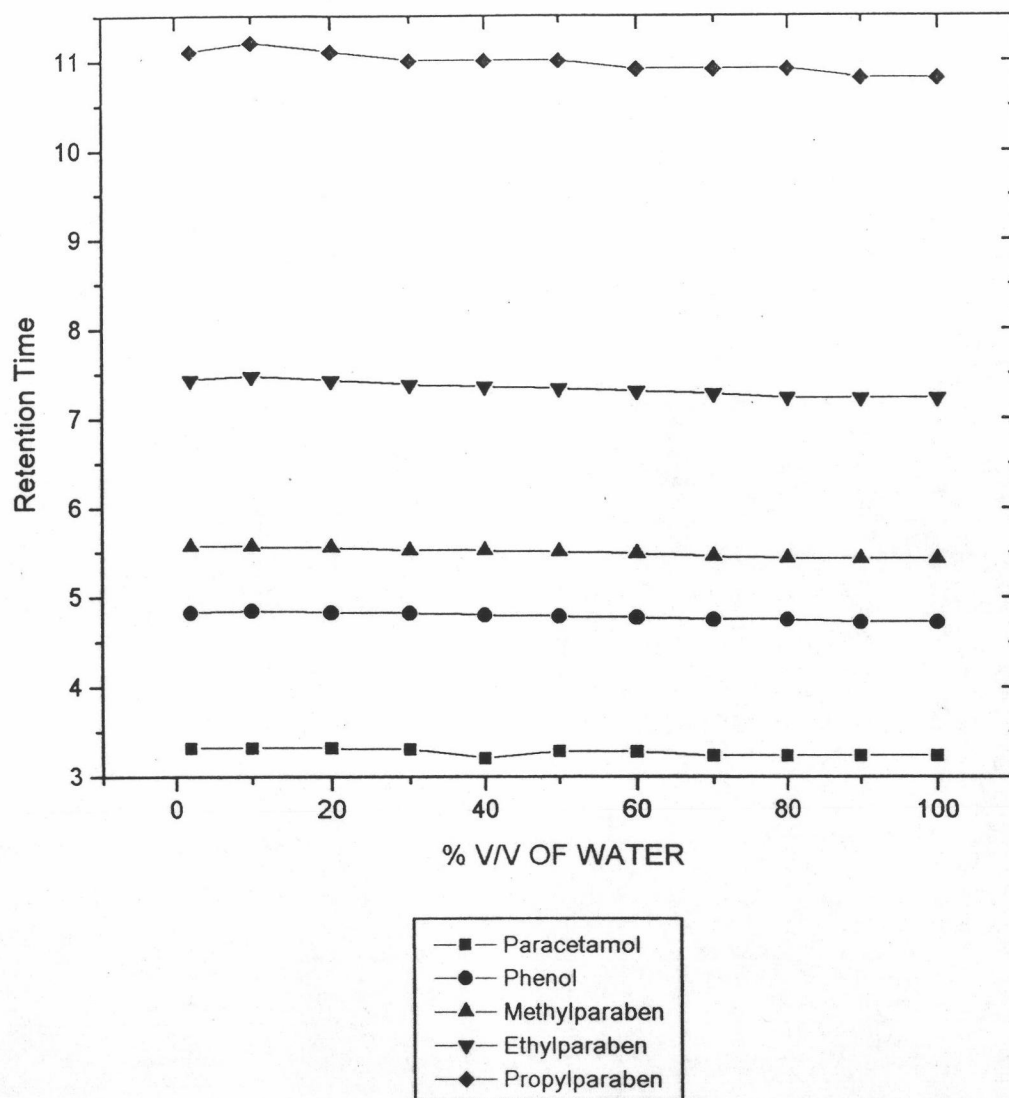


Figure 4.28 Effect of varying concentration of water on the retention time of phenols at 35 °C. Chromatographic conditions as given in Table 3.2

The effect of varying water-to-methanol ratios on the chromatographic behavior in terms of retention time (t_R) of each phenolic compound i.e., paracetamol, phenol, methylparaben, ethylparaben and propylparaben is studied. The results are presented in Appendix B Tables B9 to B12 on RP-8 column at temperatures 20°, 25°, 30° and 35 °C., respectively, and Tables B13 to B16 on RP-18 column at temperatures 20°, 25°, 30° and 35°C., respectively. The graphs showing the relationship of the retention time (t_R) of each phenolic compound with varying water-to-methanol ratios are shown in Figures 4.21 to 4.24 on RP-8 column at temperatures 20°, 25°, 30° and 35 °C., respectively, and Figures 4.25 to 4.28 on RP-18 column at temperatures 20°, 25°, 30° and 35°C., respectively. It can be seen that at the same temperature, retention time of each phenolic compound is not affected by the solvent strength in sample solution. The retention time of each studied compound is decreased at higher temperature because the viscosity of the mobile phase decreased.

Table 4.1 Statistical fitting parameters of appropriate model for paracetamol in RP-8 at various temperatures , where the following relationship in equation (4.1).

Temperature (K)	A_0	A_1	A_2	R^*
293	-15710.278	4144.233	-236.032	0.9950
298	-6197.757	1466.091	-46.001	0.9982
303	-6821.046	1643.173	-54.762	0.9975
308	-7267.622	1767.763	-65.717	0.9931

* correlation coefficient

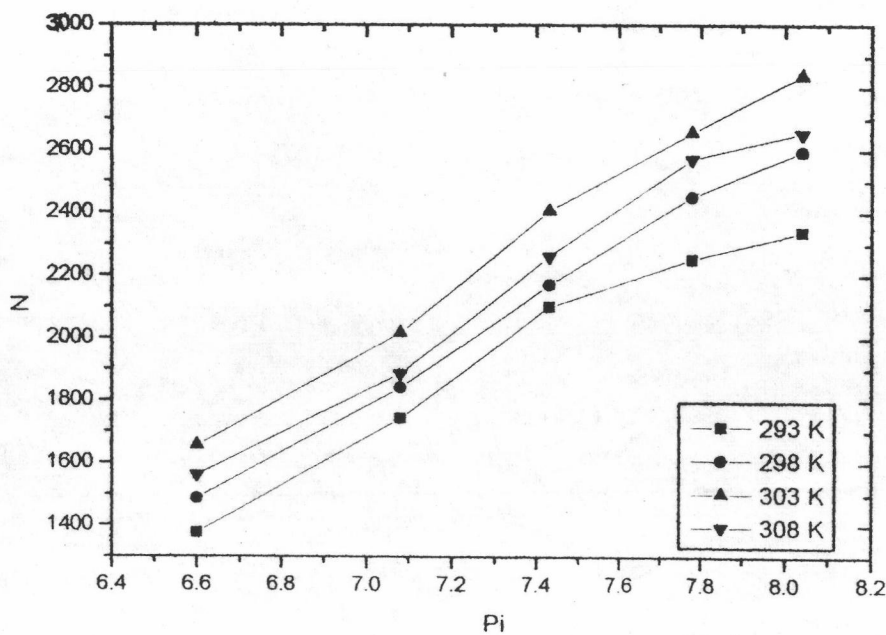


Figure 4.29 The relationship between number of theoretical plates (N) for paracetamol in RP-8 at various temperatures and polarity index (P_i) in the injection solvent.

Table 4.2 Statistical fitting parameters of appropriate model for phenol in RP-8 at various temperatures, where the following relationship in equation (4.1).

Temperature (K)	A_0	A_1	A_2	R^*
293	-22075.714	6065.409	-370.811	0.9992
298	-21202.535	5820.536	-352.679	0.9993
303	-5499.779	1262.197	-16.916	0.9966
308	-3917.691	758.537	19.797	0.9915

* correlation coefficient

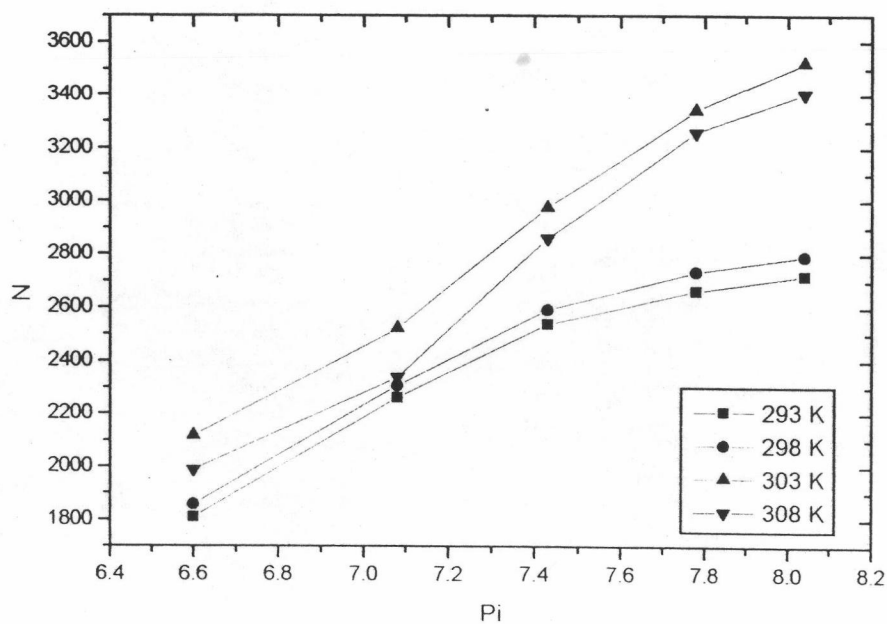


Figure 4.30 The relationship between number of theoretical plates (N) for phenol in RP-8 at various temperatures and polarity index (P_i) in the injection solvent.

Table 4.3 Statistical fitting parameters of appropriate model for methylparaben in RP-8 at various temperatures, where the following relationship in equation (4.1)

Temperature (K)	A_0	A_1	A_2	R^*
293	-18012.127	5025.645	-307.331	0.9996
298	-9597.369	2681.452	-144.034	0.9998
303	-15542.254	4274.993	-240.589	0.9971
308	-12493.283	3535.046	-198.509	0.9983

* correlation coefficient

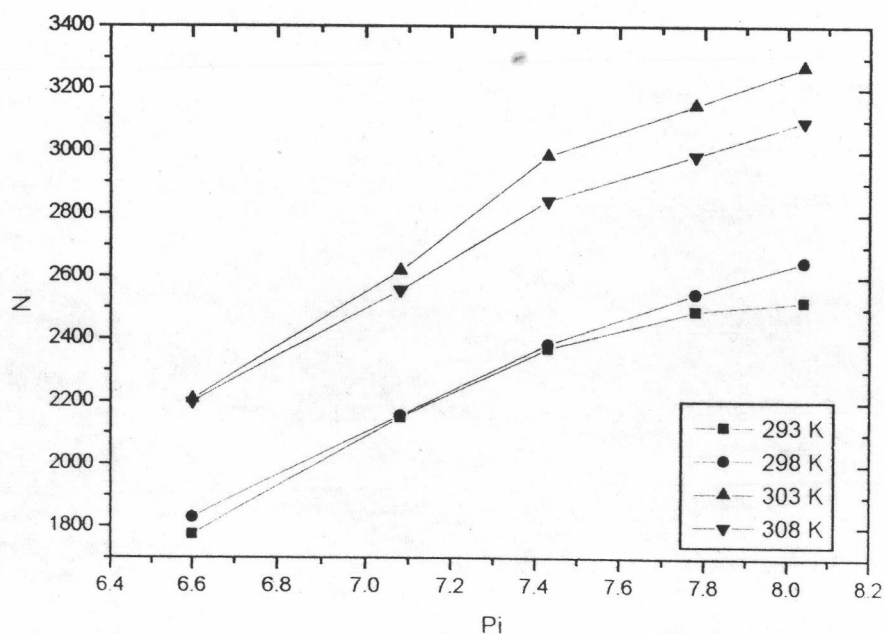


Figure 4.31 The relationship between number of theoretical plates (N) for methylparaben in RP-8 at various temperatures and polarity index (P_i) in the injection solvent.

Table 4.4 Statistical fitting parameters of appropriate model for ethylparaben in RP-8 at various temperatures , where the following relationship in equation (4.1)

Temperature (K)	A_0	A_1	A_2	R^*
293	-12299.678	2456.750	-203.156	0.9965
298	-5204.129	1514.117	-69.005	0.9999
303	-15512.161	4214.195	-235.759	0.9971
308	-15405.791	4212.984	-239.320	0.9991

* correlation coefficient

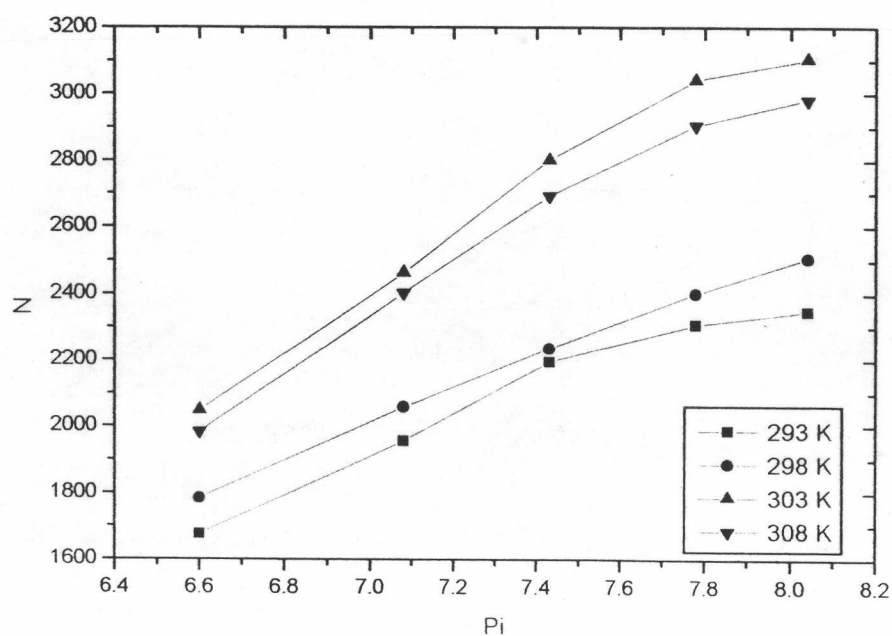


Figure 4.32 The relationship between number of theoretical plates (N) for ethylparaben in RP-8 at various temperatures and polarity index (P_i) in the injection solvent.

Table 4.5 Statistical fitting parameters of appropriate model for propylparaben in RP-8 at various temperatures , where the following relationship in equation (4.1)

Temperature (K)	A_0	A_1	A_2	R^*
293	-12177.513	3400.602	-198.399	0.9953
298	-8036.291	2298.902	-123.669	0.9973
303	-16980.543	4576.811	-258.989	0.9979
308	-16688.874	4516.611	-258.774	0.9996

* correlation coefficient

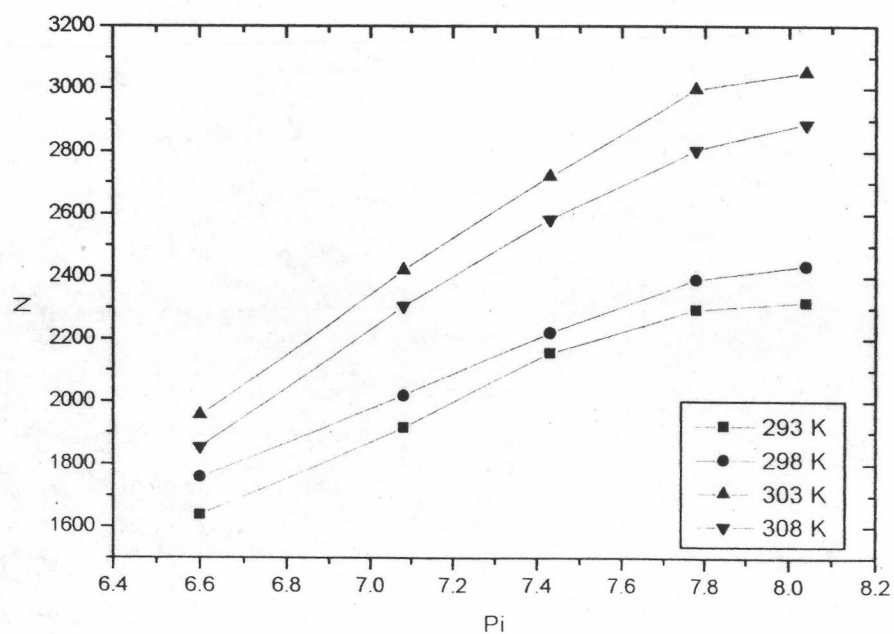


Figure 4.33 The relationship between number of theoretical plates (N) for propylparaben in RP-8 at various temperatures and polarity index (P_i) in the injection solvent.

Table 4.6 Statistical fitting parameters of appropriate model for paracetamol in RP-18 at various temperatures , where the following relationship in equation (4.1)

Temperature (K)	A_0	A_1	A_2	R^*
293	-38684.026	9903.601	-588.137	0.9953
298	-36337.837	9179.967	-535.898	0.9960
303	-4948.986	798.783	9.508	0.9969
308	-2396.865	197.239	41.389	0.9998

* correlation coefficient

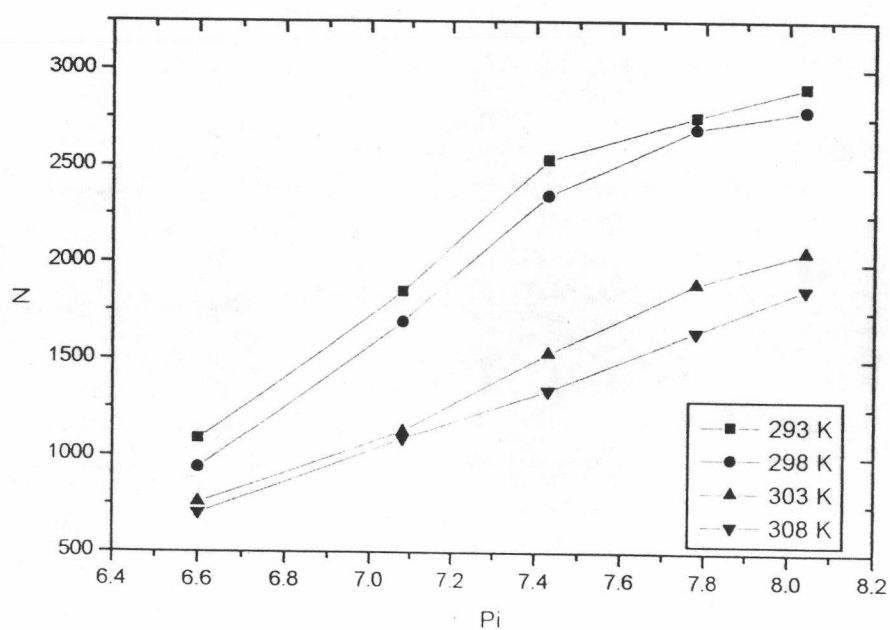


Figure 4.34 The relationship between number of theoretical plates (N) for paracetamol in RP-18 at various temperatures and polarity index (P_i) in the injection solvent.

Table 4.7 Statistical fitting parameters of appropriate model for phenol in RP-18 at various temperatures, where the following relationship in equation (4.1)

Temperature (K)	A_0	A_1	A_2	R^*
293	1255.398	-264.142	57.745	0.9984
298	-17816.154	4985.053	-305.122	0.9994
303	-360.595	-119.237	56.988	0.9995
308	-13333.076	3427.360	-189.913	0.9990

* correlation coefficient

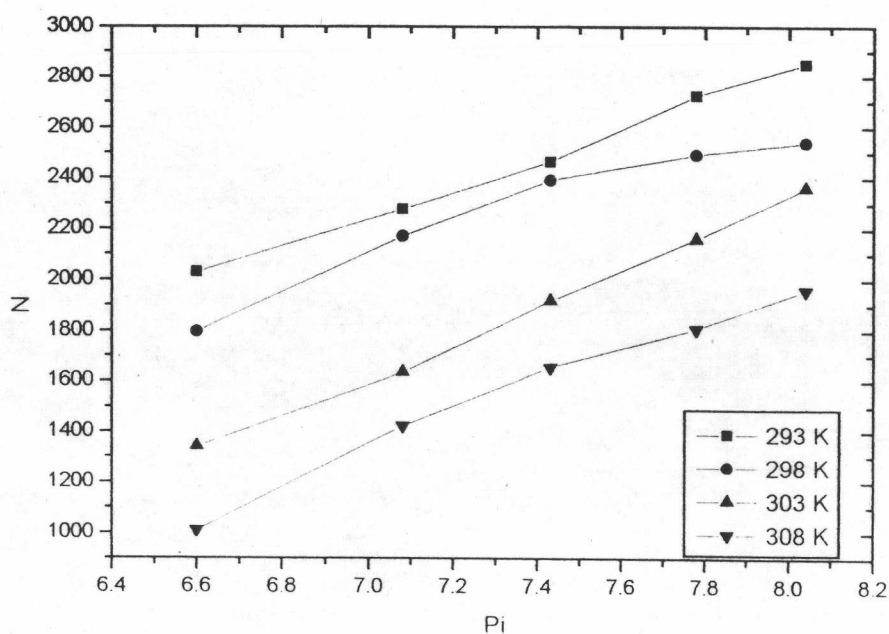


Figure 4.35 The relationship between number of theoretical plates (N) for phenol in RP-18 at various temperatures and polarity index (P_i) in the injection solvent.

Table 4.8 Statistical fitting parameters of appropriate model for methylparaben in RP-18 at various temperatures, where the following relationship in equation (4.1).

Temperature (K)	A_0	A_1	A_2	R^*
293	-11019.529	3257.090	-194.835	0.9978
298	-10719.655	3200.702	-196.101	0.9961
303	-5062.512	1444.921	-69.533	0.9987
308	-9672.866	2669.241	-154.989	0.9999

* correlation coefficient

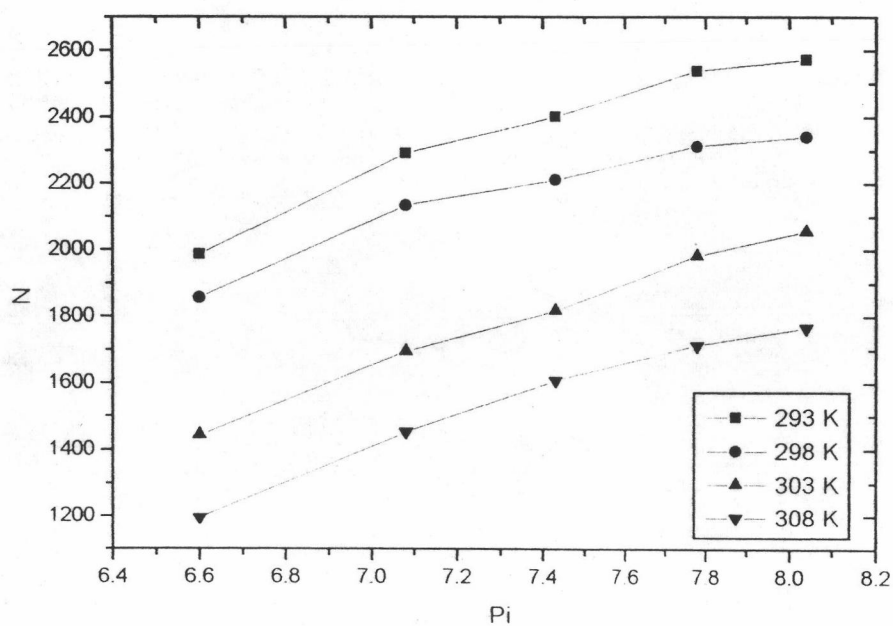


Figure 4.36 The relationship between number of theoretical plates (N) for methylparaben in RP-18 at various temperatures and polarity index (P_i) in the injection solvent.

Table 4.9 Statistical fitting parameters of appropriate model for ethylparaben in RP-18 at various temperatures , where the following relationship in equation (4.1).

Temperature (K)	A_0	A_1	A_2	R^*
293	-12764.908	3728.699	-228.928	0.9941
298	-9135.699	2741.781	-165.457	0.9992
303	-6408.881	1808.948	-95.486	0.9973
308	-5198.286	1504.272	-81.767	0.9999

* correlation coefficient

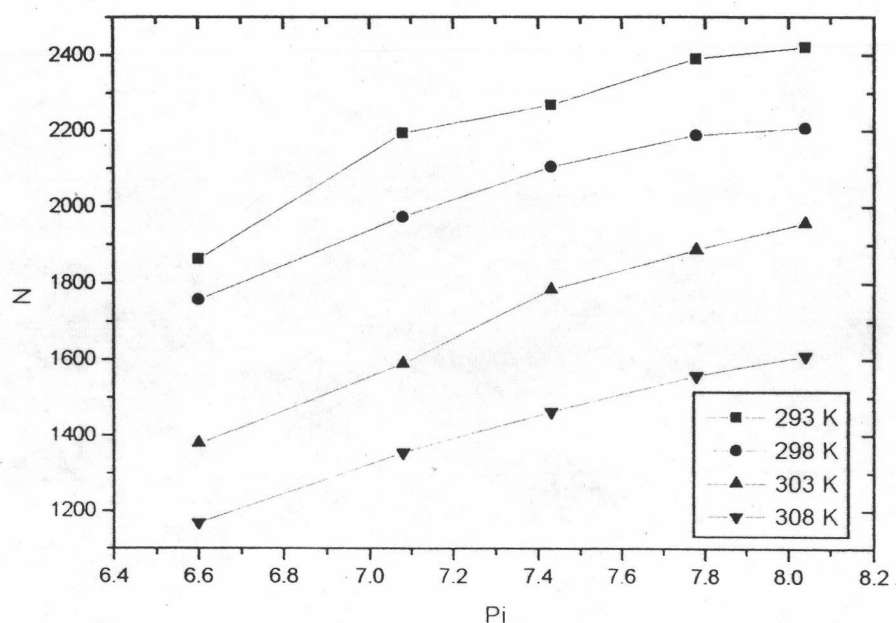


Figure 4.37 The relationship between number of theoretical plates (N) for ethylparaben in RP-18 at various temperatures and polarity index (P_i) in the injection solvent.

Table 4.10 Statistical fitting parameters of appropriate model for propylparaben in RP-18 at various temperatures, where the following relationship in equation (4.1).

Temperature (K)	A_0	A_1	A_2	R^*
293	-7971.079	2397.797	-137.875	0.9975
298	-16136.639	4605.328	-290.079	0.9954
303	-6002.025	1677.535	-86.649	0.9921
308	-2377.733	755.923	-33.254	0.9973

* correlation coefficient

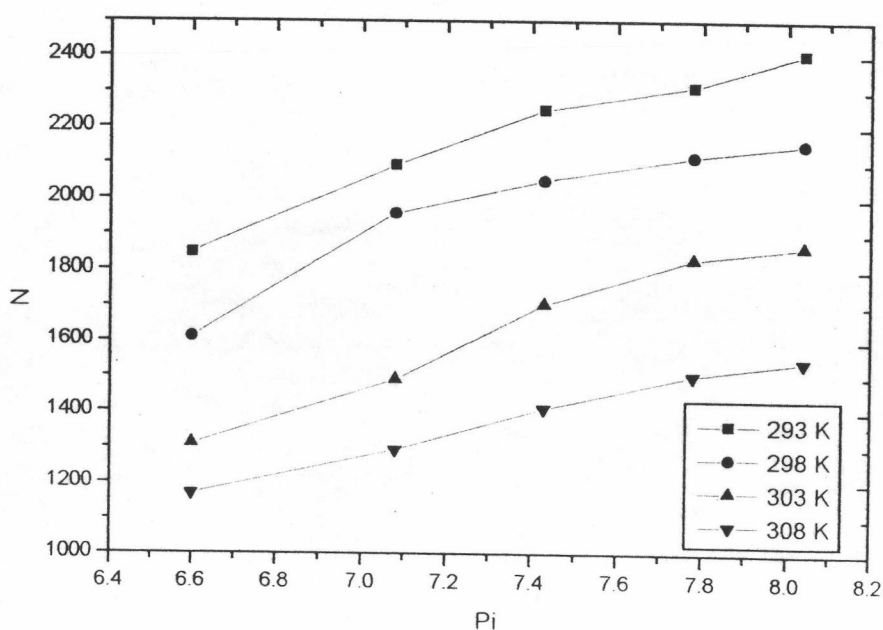


Figure 4.38 The relationship between number of theoretical plates (N) for propylparaben in RP-18 at various temperatures and polarity index (P_i) in the injection solvent.

The correlation between the number of theoretical plates (N) and polarity index (P_i) of the injection solvent for phenolic system are obtained from the fits by equation (4.1) :

$$N = A_0 + A_1P_i + A_2P_i^2 \quad (4.1)$$

where A₀ , A₁ and A₂ are the fitted parameters depending on the stationary phase , type of modifier , and column porosity. The fitted parameters of each phenolic compound in RP-8 column at various temperatures are shown in Tables 4.1 to 4.5 . The fitted parameters of each phenolic compound in RP-18 column at various temperatures are shown in Tables 4.6 to 4.10 .

Figures 4.29 to 4.33 show the number of theoretical plates (N) for paracetamol phenol , methylparaben , ethylparaben and propylparaben, respectively, on RP-8 at various temperatures are plotted against the polarity indexes (P_i) in the injected sample. Figures 4.34 to 4.38 show the number of theoretical plates (N) for paracetamol , phenol , methylparaben , ethylparaben and propylparaben , respectively , on RP-18 at various temperatures are plotted against the polarity indexes (P_i) in the injected sample.

From the regression equation (4.1) , the number of theoretical plates (N) coefficients were calculated for all investigated phenolic compounds at the point corresponding to the proportion (v/v) of 40:60 water : methanol as mobile phase. The correlation coefficients , R obtained in all phenolic compounds are satisfactory.

Table 4.11 Statistical fitting parameters of appropriate model for paracetamol in RP-8 at at temperature 25 °C. and 35 °C. , where the following relationship in equation (4.2).

Temperature (K)	B_0	B_1	B_2	R^*
298	717.959	1397.070	-104.196	0.9993
308	801.343	1645.065	-47.580	0.9961

* correlation coefficient

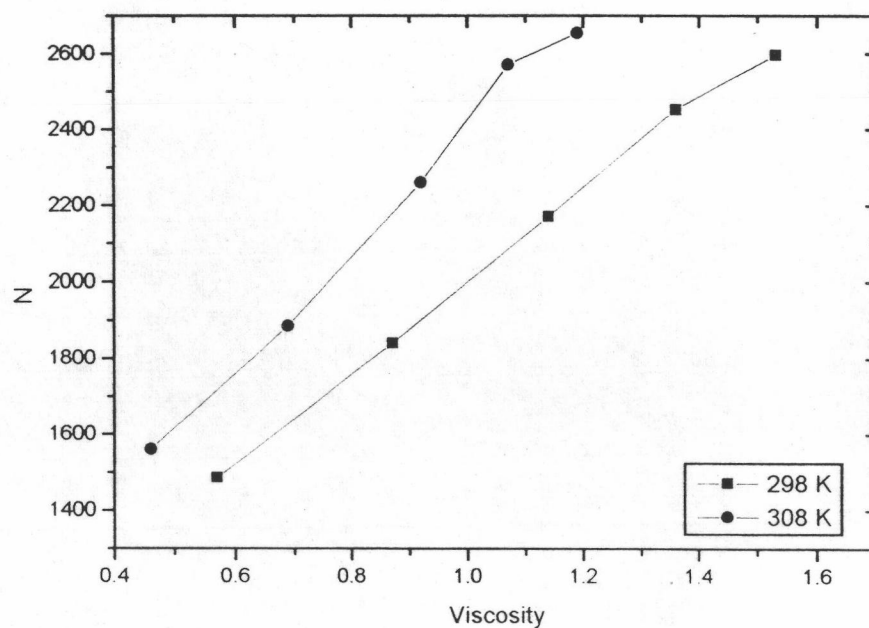


Figure 4.39 The relationship between number of theoretical plates (N) for paracetamol in RP-8 at temperature 25 °C and 35 °C and viscosity in the injection solvent.

Table 4.12 Statistical fitting parameters of appropriate model for phenol in RP-8 at temperature 25 °C. and 35 °C. , where the following relationship in equation (4.2).

Temperature (K)	B ₀	B ₁	B ₂	R [*]
298	594.886	2672.957	-808.214	0.9998
308	1226.717	1435.211	366.768	0.9959

* correlation coefficient

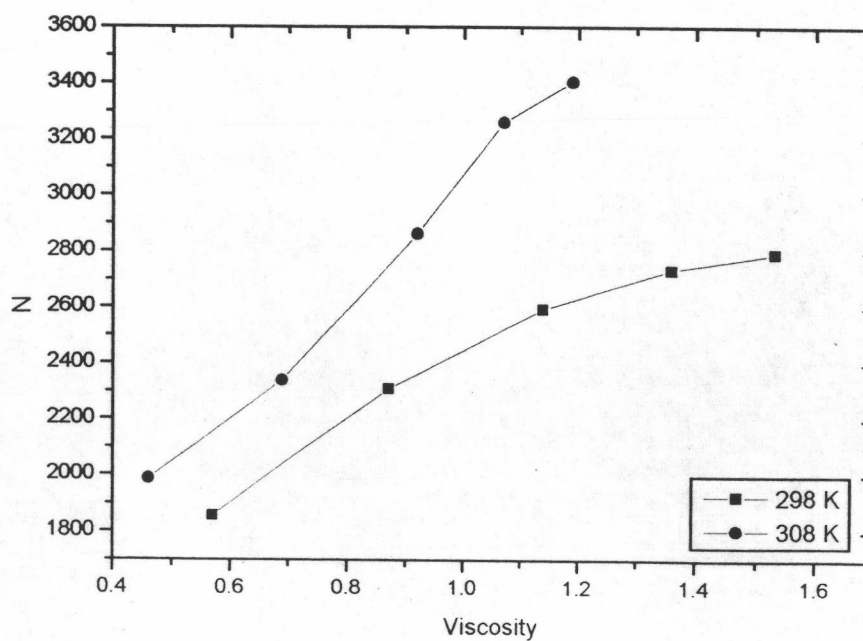


Figure 4.40 The relationship between number of theoretical plates (N) for phenol in RP-8 at temperature 25 °C and 35 °C and viscosity in the injection solvent.

Table 4.13 Statistical fitting parameters of appropriate model for methylparaben in RP-8 at temperature 25 °C. and 35 °C. , where the following relationship in equation (4.2).

Temperature (K)	B_0	B_1	B_2	R^*
298	1059.788	1540.197	-328.493	0.9999
308	1283.406	2287.782	-645.681	0.9999

* correlation coefficient

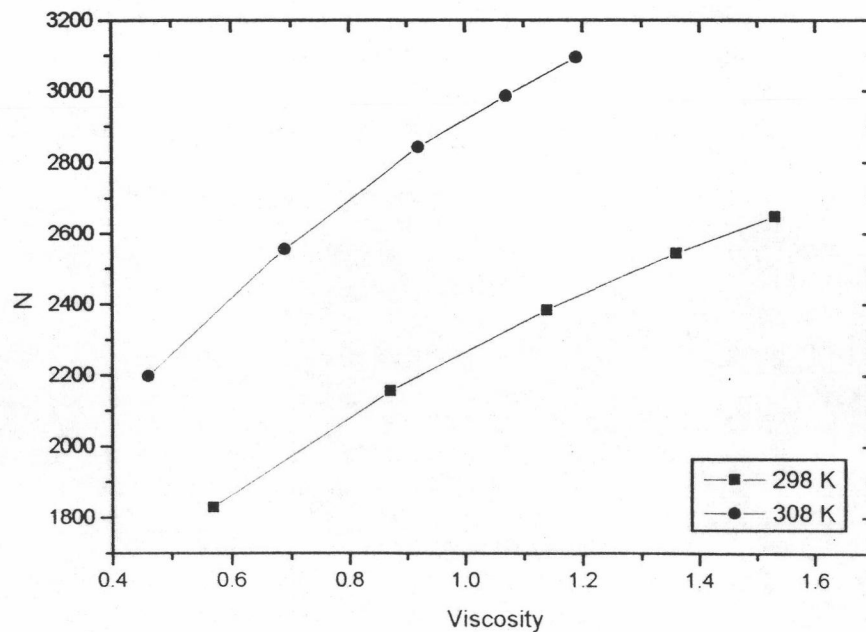


Figure 4.41 The relationship between number of theoretical plates (N) for methylparaben in RP-8 at temperature 25 °C and 35 °C and viscosity in the injection solvent.

Table 4.14 Statistical fitting parameters of appropriate model for ethylparaben in RP-8 at temperature 25 °C. and 35 °C. , where the following relationship in equation (4.2).

Temperature (K)	B_0	B_1	B_2	R^2
298	1226.113	1074.036	-155.441	0.9993
308	920.654	2677.052	-787.992	0.9991

* correlation coefficient

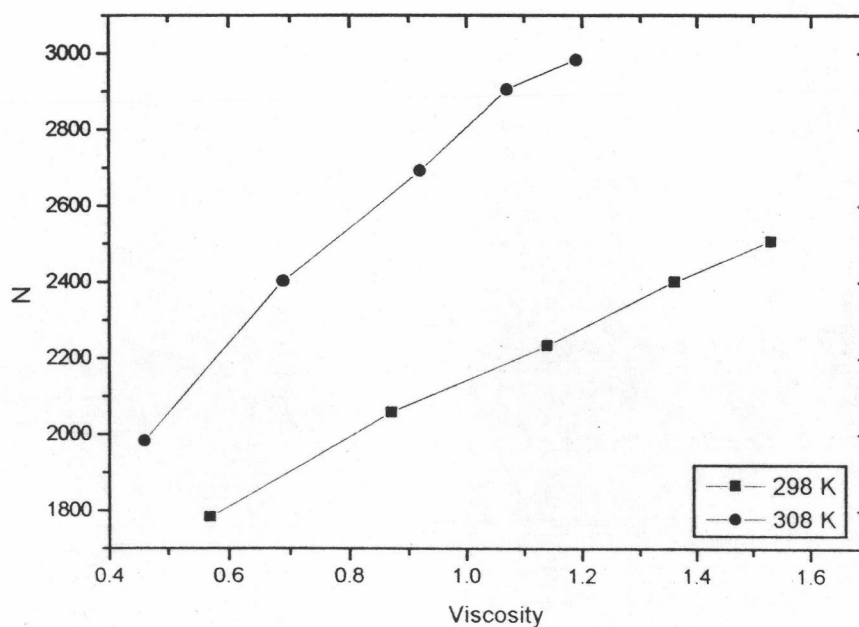


Figure 4.42 The relationship between number of theoretical plates (N) for ethylparaben in RP-8 at temperature 25 °C and 35 °C and viscosity in the injection solvent.

Table 4.15 Statistical fitting parameters of appropriate model for propylparaben in RP-8 at temperature 25 °C. and 35 °C. , where the following relationship in equation (4.2).

Temperature (K)	B_0	B_1	B_2	R^*
298	1092.729	1320.114	-283.124	0.9982
308	737.004	2837.921	-864.257	0.9986

* correlation coefficient

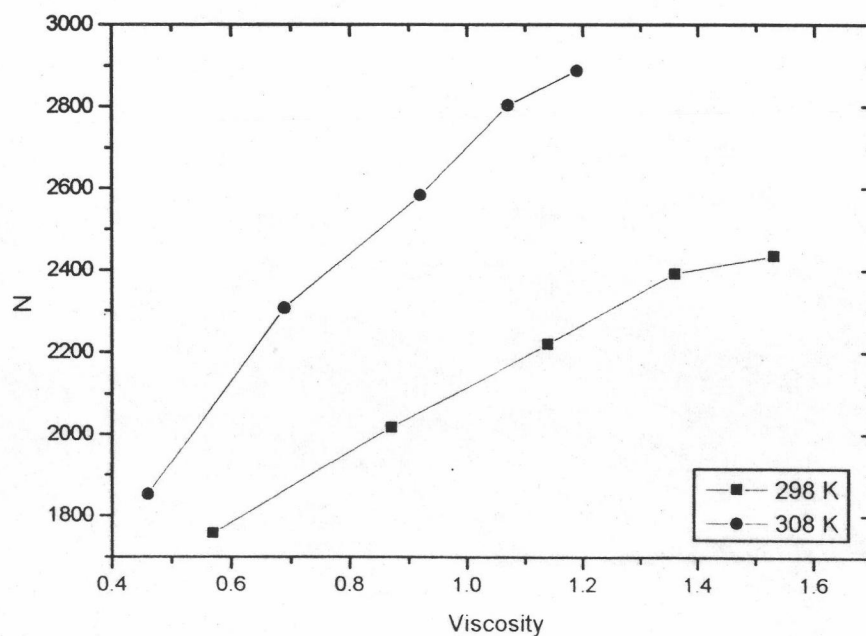


Figure 4.43 The relationship between number of theoretical plates (N) for propylparaben in RP-8 at temperature 25 °C and 35 °C and viscosity in the injection solvent.

Table 4.16 Statistical fitting parameters of appropriate model for paracetamol in RP-18 at temperature 25 °C. and 35 °C. , where the following relationship in equation (4.2).

Temperature (K)	B_0	B_1	B_2	R^*
298	-1290.689	4572.169	-1232.265	0.9983
308	207.666	949.369	364.802	0.9967

* correlation coefficient

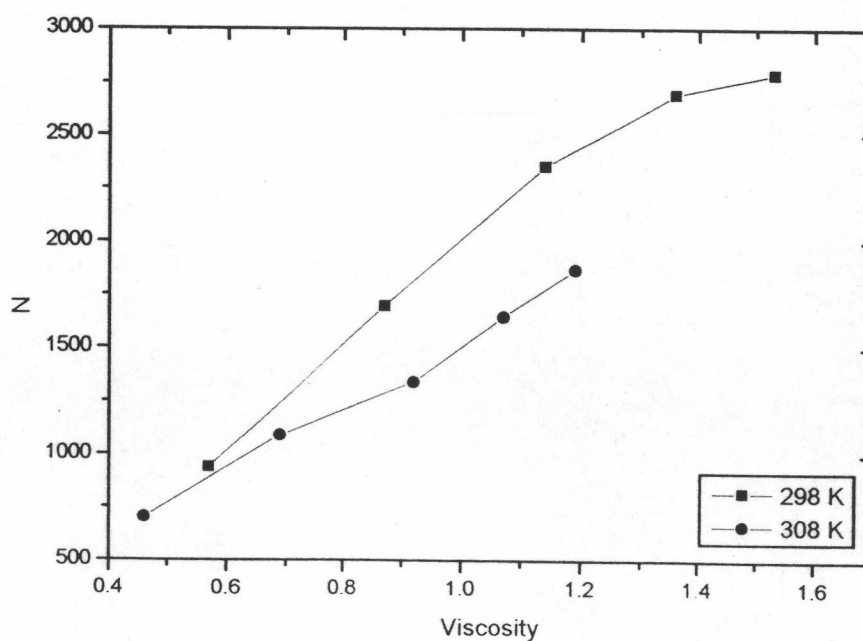


Figure 4.44 The relationship between number of theoretical plates (N) for paracetamol in RP-18 at temperature 25 °C and 35 °C and viscosity in the injection solvent.

Table 4.17 Statistical fitting parameters of appropriate model for phenol in RP-18 at temperature 25 °C. and 35 °C. , where the following relationship in equation (4.2).

Temperature (K)	B ₀	B ₁	B ₂	R [*]
298	750.739	2236.871	-699.095	0.9998
308	112.126	2266.707	-614.508	0.9970

* correlation coefficient

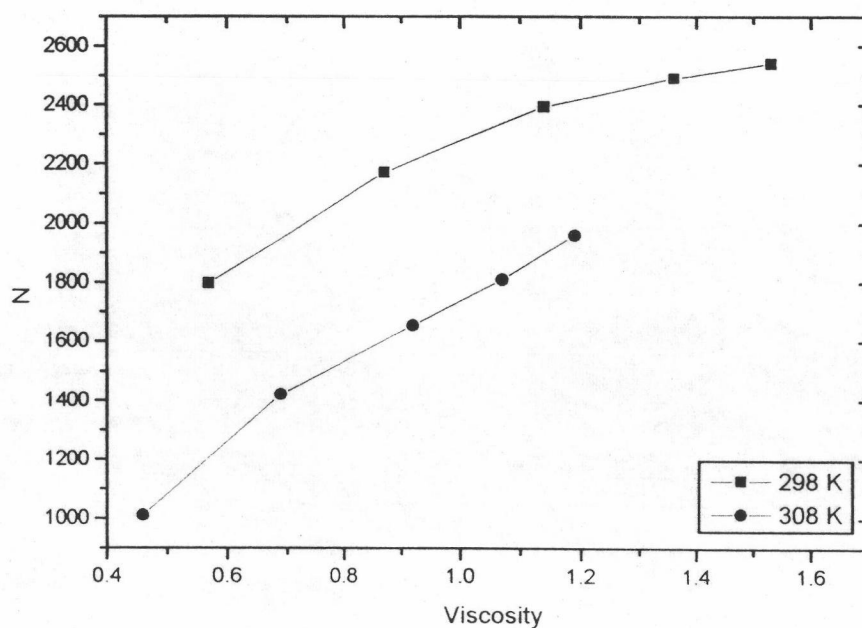


Figure 4.45 The relationship between number of theoretical plates (N) for phenol in RP-18 at temperature 25 °C and 35 °C and viscosity in the injection solvent.

Table 4.18 Statistical fitting parameters of appropriate model for methylparaben in RP-18 at temperature 25 °C. and 35 °C. , where the following relationship in equation (4.2).

Temperature (K)	B ₀	B ₁	B ₂	R [*]
298	1200.136	1424.490	-446.239	0.9923
308	552.518	1647.726	-528.252	0.9989

* correlation coefficient

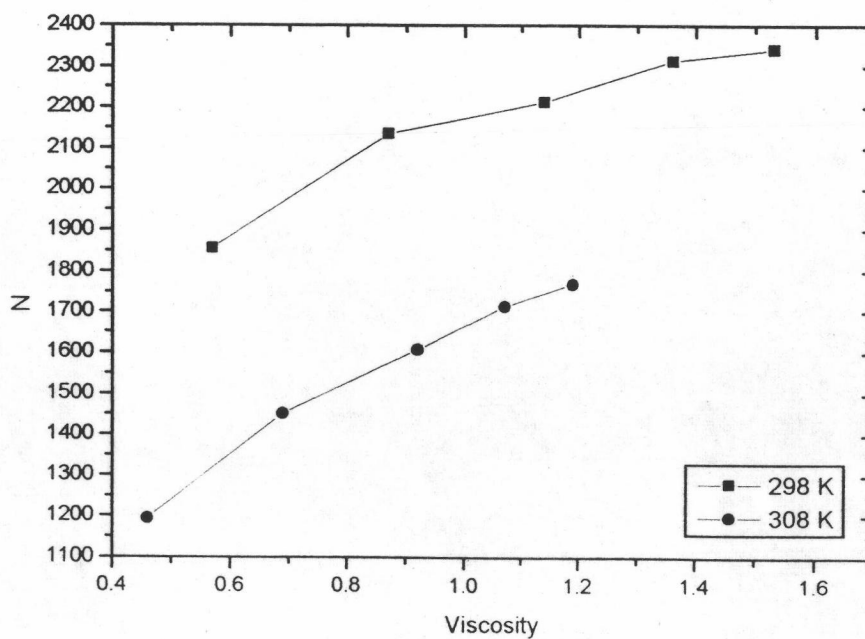


Figure 4.46 The relationship between number of theoretical plates (N) for methylparaben in RP-18 at temperature 25 °C and 35 °C and viscosity in the injection solvent.

Table 4.19 Statistical fitting parameters of appropriate model for ethylparaben in RP-18 at temperature 25 °C. and 35 °C. , where the following relationship in equation (4.2).

Temperature (K)	B ₀	B ₁	B ₂	R [*]
298	1154.729	1270.051	-379.011	0.9996
308	759.665	1015.854	-255.176	0.9980

* correlation coefficient

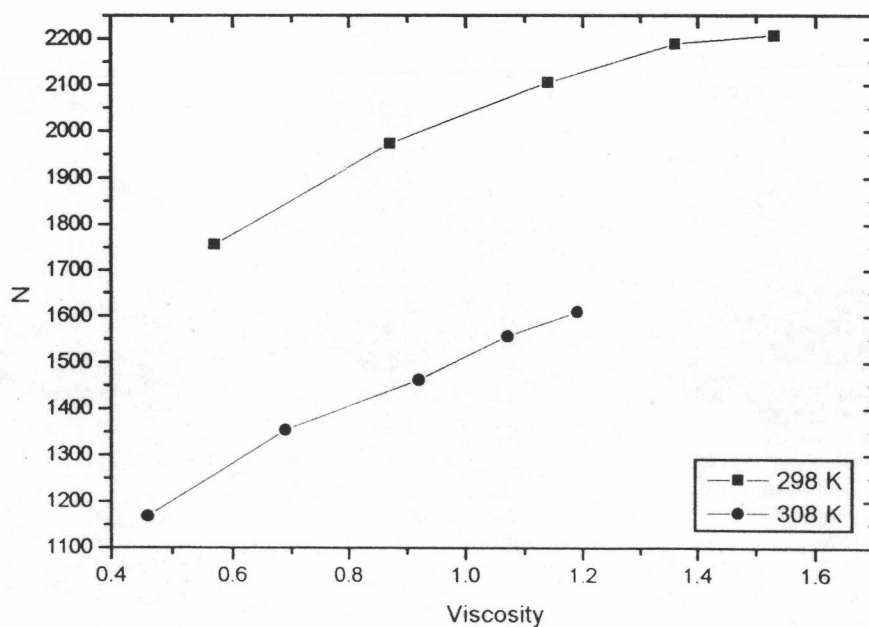


Figure 4.47 The relationship between number of theoretical plates (N) for ethylparaben in RP-18 at temperature 25 °C and 35 °C and viscosity in the injection solvent.

Table 4.20 Statistical fitting parameters of appropriate model for propylparaben in RP-18 at temperature 25 °C. and 35 °C. , where the following relationship in equation (4.2).

Temperature (K)	B ₀	B ₁	B ₂	R [*]
298	748.502	1919.146	-661.641	0.9917
308	891.070	628.533	-64.198	0.9989

* correlation coefficient

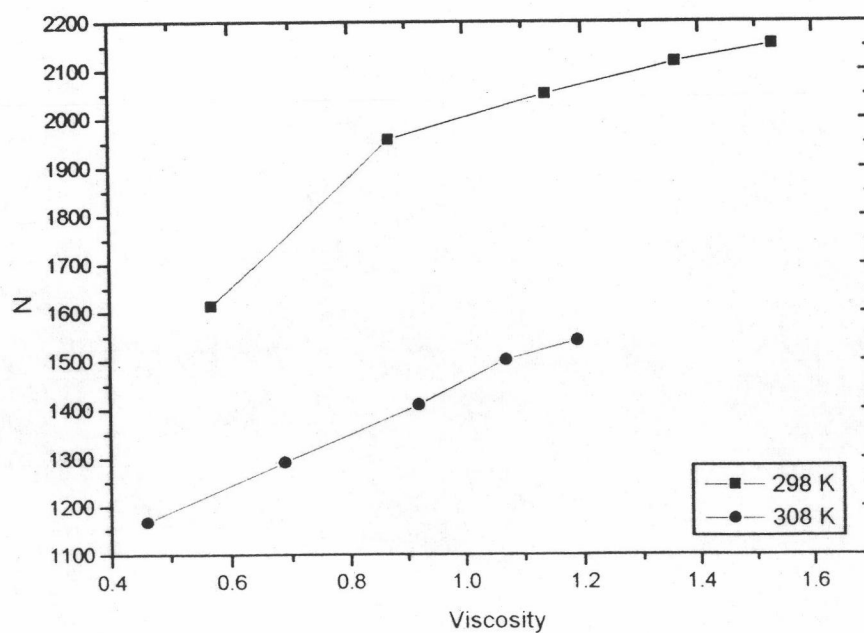


Figure 4.48 The relationship between number of theoretical plates (N) for propylparaben in RP-18 at temperature 25 °C and 35 °C and viscosity in the injection solvent.

The correlation between the number of theoretical plates (N) and viscosity (η) of the injection solvent for phenolic system are obtained from the fits by equation (4.2) :

$$N = B_0 + B_1\eta + B_2\eta^2 \quad (4.2)$$

where B_0 , B_1 and B_2 are the fitted parameters depending on the stationary phase , type of modifier , and column porosity. The fitted parameters of each phenolic compound in RP-8 column at temperatures 25°C and 35°C are shown in Tables 4.11 to 4.15 , respectively. The fitted parameters of each phenolic compound in RP-18 column at temperatures 25°C and 35°C are shown in Tables 4.16 to 4.20 , respectively.

Figures 4.39 to 4.43 show the number of theoretical plates (N) for paracetamol phenol , methylparaben , ethylparaben and propylparaben, respectively, on RP-8 at various temperatures are plotted against the viscosity values in the injected sample. Figures 4.44 to 4.48 show the number of theoretical plates (N) for paracetamol , phenol , methylparaben , ethylparaben and propylparaben , respectively , on RP-18 at various temperatures are plotted against viscosity values in the injected sample.

From the regression equation (4.2) , the number of theoretical plates (N) coefficients were calculated for all investigated phenolic compounds at the point corresponding to the proportion (v/v) of 40:60 water : methanol as mobile phase. The correlation coefficients , R obtained in all phenolic compounds are satisfactory.

From Tables B17 to B20 in Appendix B , the viscosity of injection solvent will affect the number of theoretical plates in the range of 0% to 40% (v/v) water but in the other range (40% - 98% v/v water) the number of theoretical plates are not dependent on the viscosity of injection solvent , namely , the number of theoretical plates will be constant although viscosity is changed.