

## CHAPTER IV

### RESULTS AND DISCUSSION

#### 1. Concentration effect of acetonitrile in mobile phase on $\log k'$ within pH range 2.5 - 6.5

The chromatographic behaviors of each organic acid by percent of acetonitrile in mobile phase are studied. The results are shown in Figure 4.1- 4.5. The graphs show the trend of retention of chromatographic behavior of each organic acid in various mobile phases which are different solvent strength (comparison between 5% and 90% solvent strength). The relation of  $\log k'$  and percent of acetonitrile in mobile phase in pH range 2.5 - 4.5 are linearity while in pH range 5.0 - 6.5 have less linearity because at higher percent of acetonitrile in mobile phase (40 - 50% acetonitrile in mobile phase) are not much different. The correlation of  $\log k'$  and percent of acetonitrile in mobile phase of phenol, benzoic acid, acetylsalicylic acid and salicylic acid at various pH may be considered as in equation (4.1) and (4.2) while L-ascorbic acid shows linear character as others tested organic acids but its slope near zero.

$$\text{From linear equation} \quad y = A + B x \quad (4.1)$$

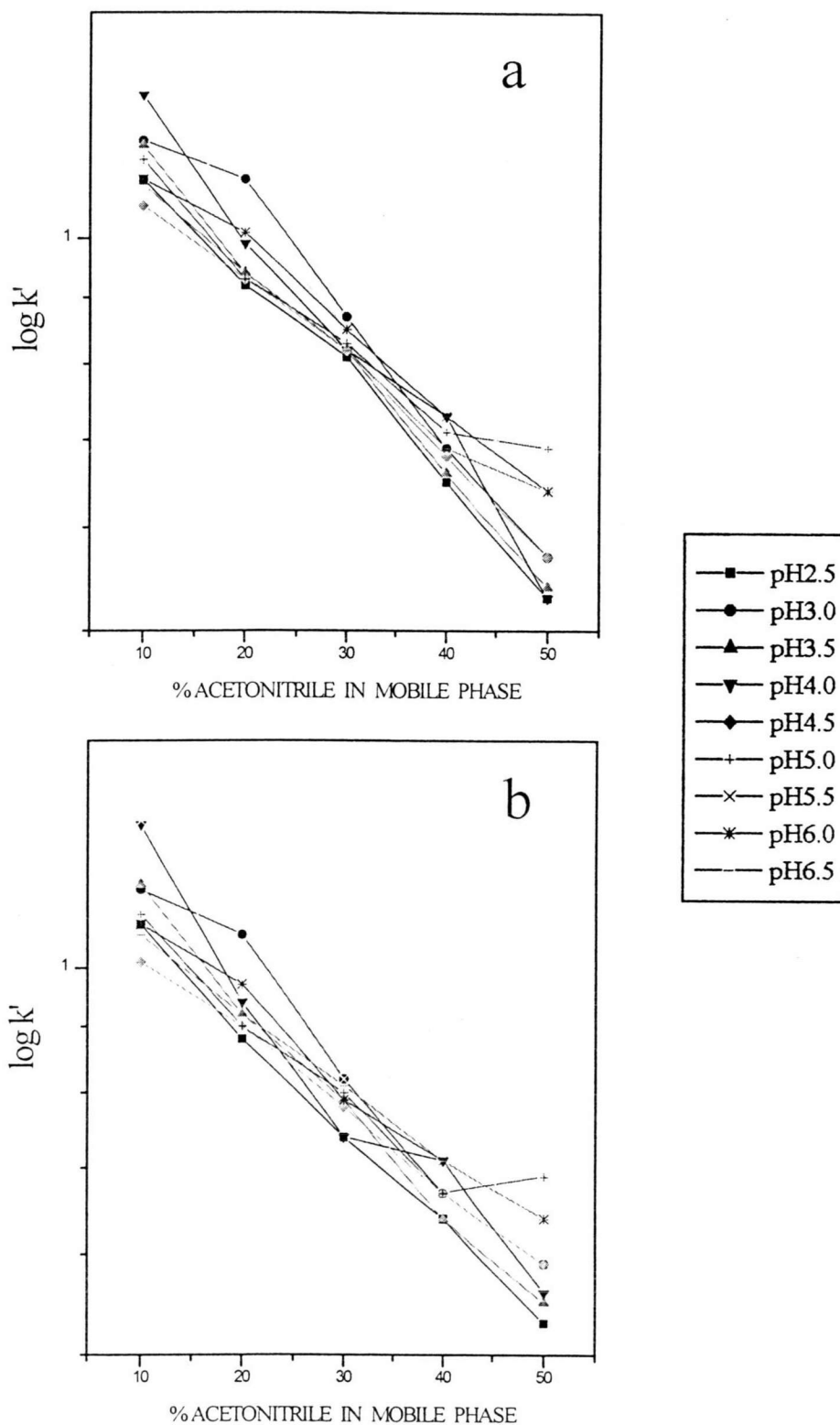
$$B = \text{slope}$$

$$A = \text{constant}$$

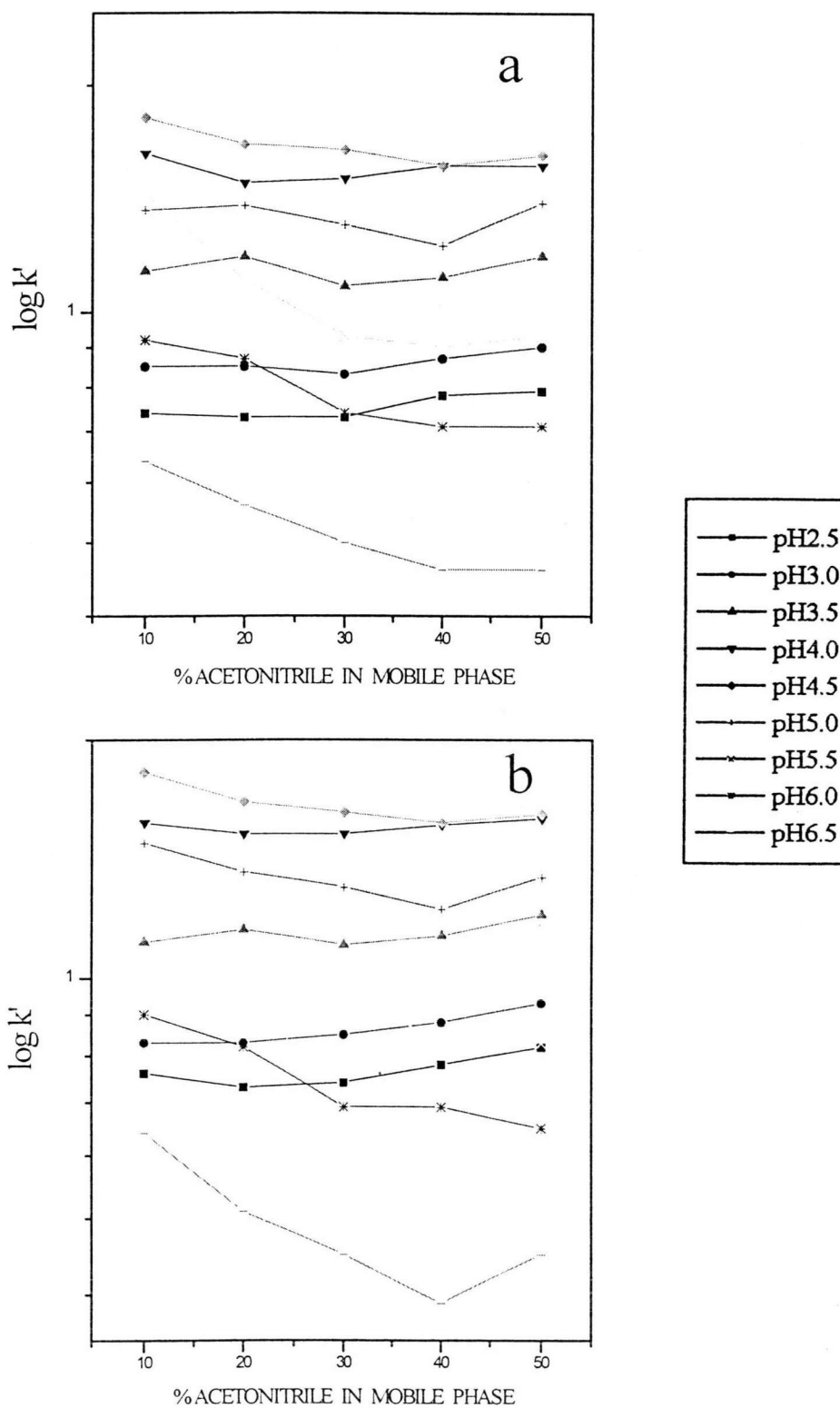
so,

$$\log k' = A + B * \text{AMP} \quad (4.2)$$

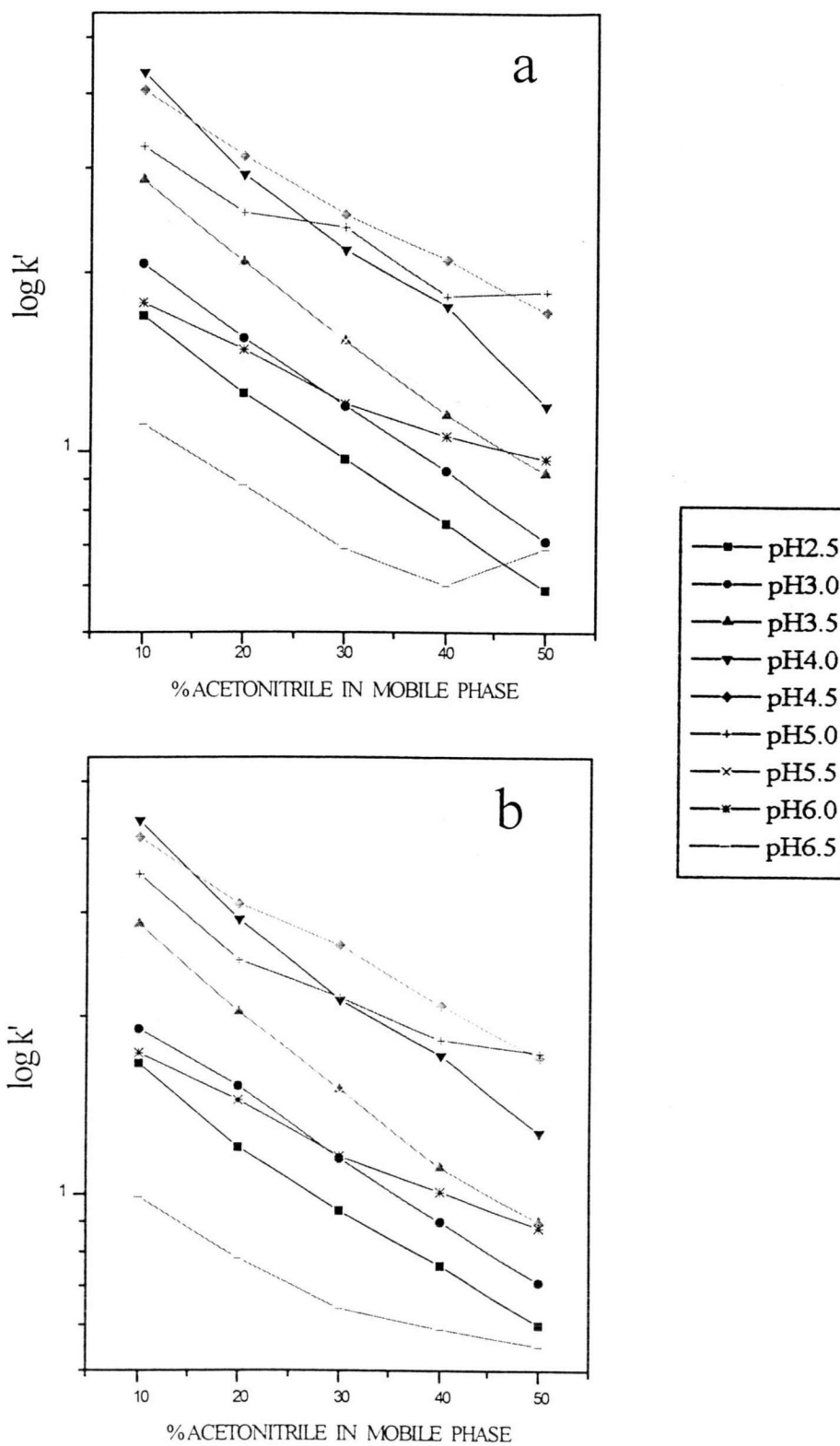
$$\text{AMP} = \% \text{ Acetonitrile in Mobile Phase}$$



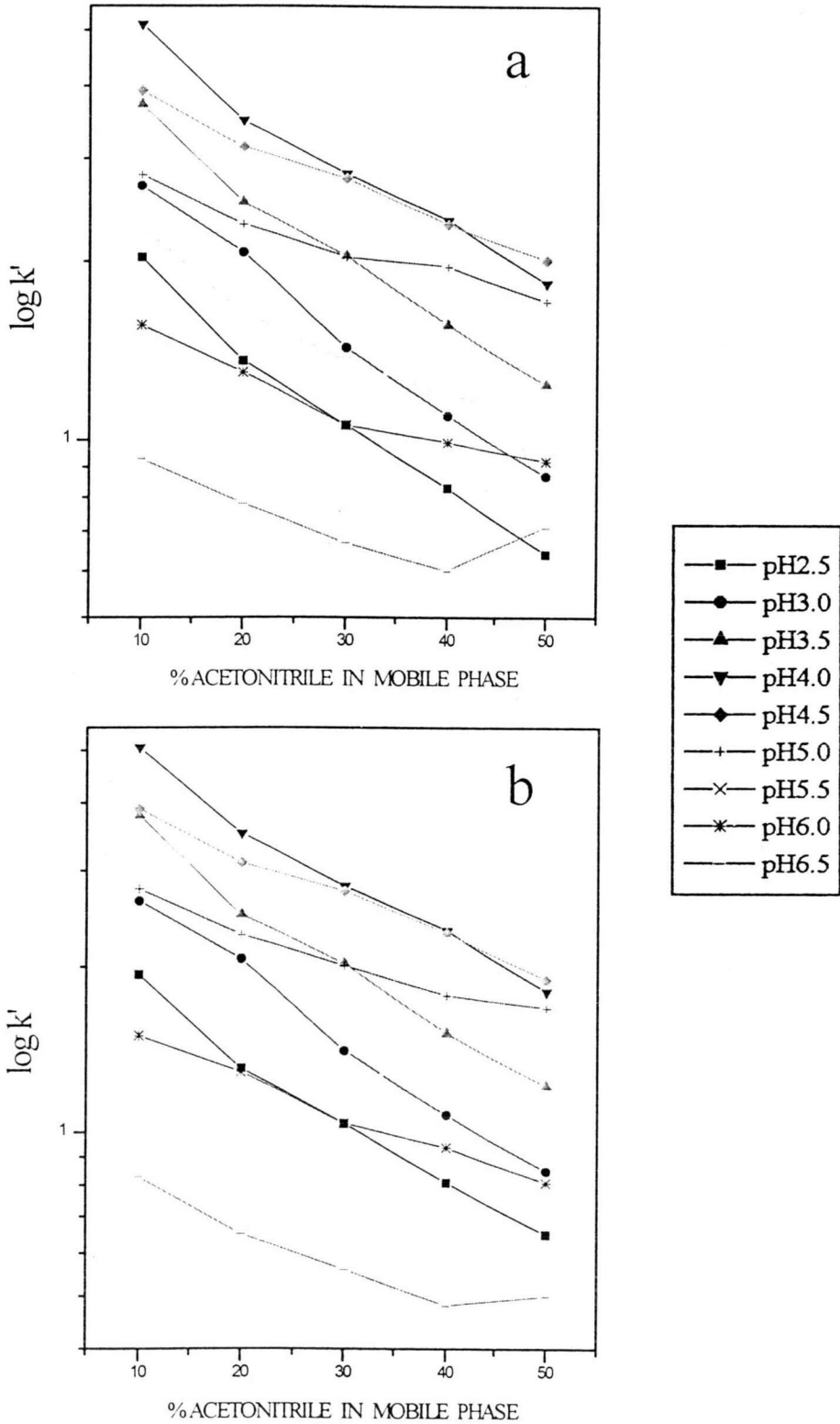
**Figure 4.1** The correlation between  $\log k'$  and percent of acetonitrile in mobile phase of phenol at pH 2.5 to 6.5 ; phenylpropanolamine column, 5  $\mu\text{m}$ , 150  $\times$  4.6 mm I.D ; flow rate 1 ml / min ; UV 254 nm ; solvent strength (a) 5% (v/v) and (b) 90% (v/v).



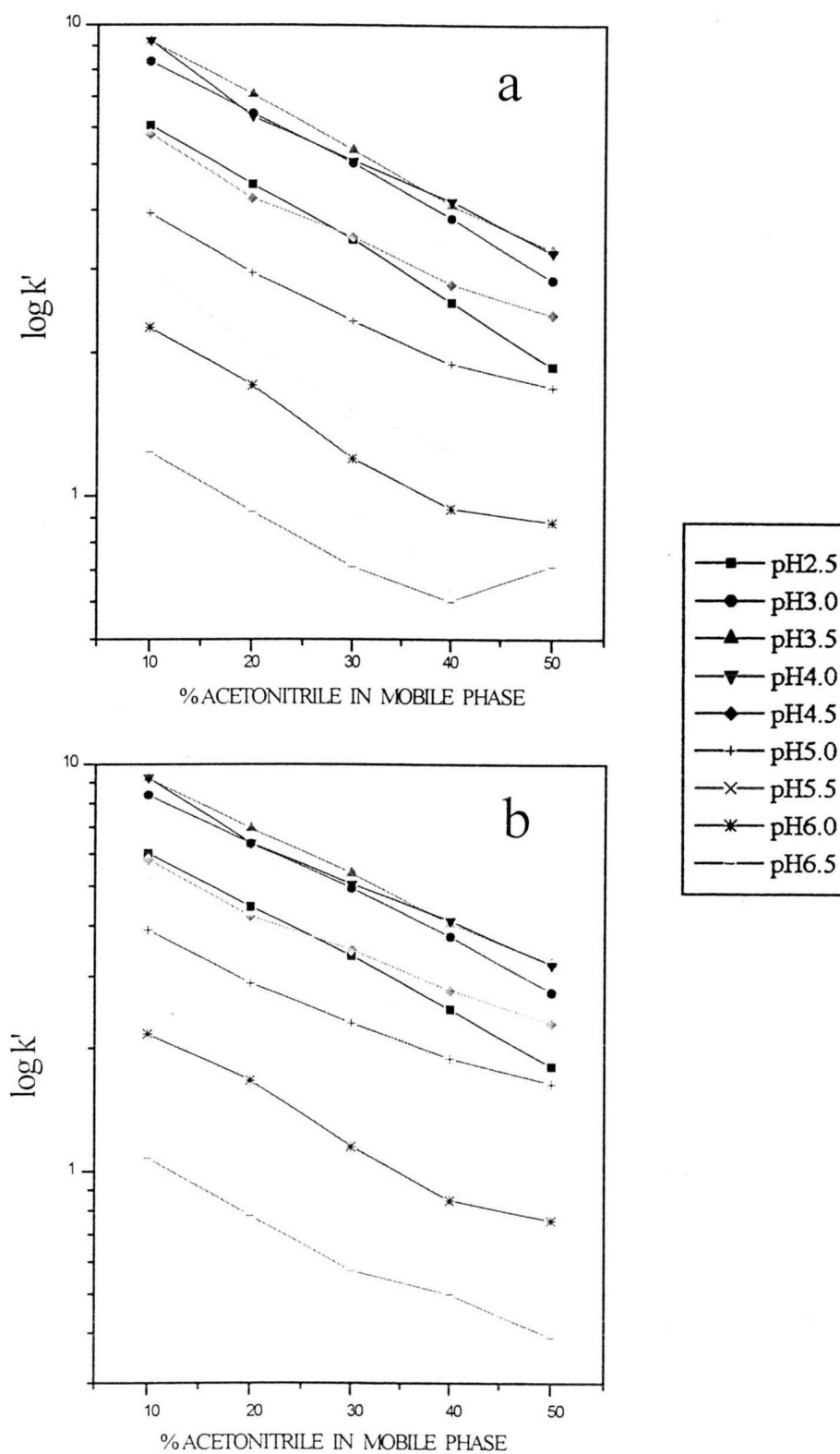
**Figure 4.2** The correlation between  $\log k'$  and percent of acetonitrile in mobile phase of L-ascobic acid at pH 2.5 to 6.5 ; phenylpropanolamine column, 5  $\mu\text{m}$ , 150 x 4.6 mm I.D ; flow rate 1 ml / min ; UV 254 nm ; solvent strength (a) 5% (v/v) and (b) 90% (v/v).



**Figure 4.3** The correlation between  $\log k'$  and percent of acetonitrile in mobile phase of benzoic acid at pH 2.5 to 6.5 ; phenylpropanolamine column, 5  $\mu\text{m}$ , 150 x 4.6 mm I.D ; flow rate 1 ml / min ; UV 254 nm ; solvent strength (a) 5% (v/v) and (b) 90% (v/v).



**Figure 4.4** The correlation between  $\log k'$  and percent of acetonitrile in mobile phase of acetylsalicylic acid at pH 2.5 to 6.5 ; phenylpropanolamine column, 5  $\mu\text{m}$ , 150 x 4.6 mm I.D ; flow rate 1 ml / min ; UV 254 nm ; solvent strength (a) 5% (v/v) and (b) 90% (v/v).



**Figure 4.5** The correlation between  $\log k'$  and percent of acetonitrile in mobile phase of salicylic acid at pH 2.5 to 6.5 ; phenylpropanolamine column, 5  $\mu\text{m}$ , 150 x 4.6 mm I.D ; flow rate 1 ml / min ; UV 254 nm ; solvent strength (a) 5% (v/v) and (b) 90% (v/v).

In reversed-phased chromatography, when percent of organic solvent in mobile phase was increased or ionic strength was decreased the hydrophobic interaction between analytes and organic portions in mobile phase was also increased so it was the cause of decreasing retention of analytes or reducing  $\log k'$  [7]. But in ion-exchange show contrast results when the same conditions were used, the ionic attraction between ionic portion of stationary phase and analytes was increased or increasing  $\log k'$  [23,30]. These results of mobile phase strength effects could be interpreted in terms of hydrophobicity changes. The retention of phenol, L-ascorbic acid, benzoic acid, acetylsalicylic acid, and salicylic acid in various percent acetonitrile in mobile phase are also investigated. The trend of retention of phenol, benzoic acid, acetylsalicylic acid, and salicylic acid show same results, retention of those organic acids are decreased when percent of acetonitrile in mobile phase increased and pH are rarely affected to its retention within pH range. This could be explained by partition distribution between analytes and acetonitrile in mobile phase which increasing partition to organic portions when organic part in mobile phase is increased. The graphs of phenol, benzoic acid, acetylsalicylic acid and salicylic acid show the trend of linearity at various pH curves as Figure 4.1-4.5. In pH range 2.5-4.5 the curves show more linearity than pH range 5.0-6.5 due to at lower pH, unionized form of analytes which is more active with aromatic part of mobile phase than weak secondary ammonium group of stationary phase after protonating of proton in solution while at higher pH, ionized form of analytes are increased and ionic attraction between ionized form of analytes and weak secondary ammonium group of stationary phase are increased. Although, the pH are increased in order to give higher ionisation of analytes, predominate mechanism is still hydrophobic interaction due to aromatic part or unionized form of analytes could be interacted with acetonitrile in mobile phase than the ammonium group of phenylpropanolamine when increasing acetonitrile in mobile phase. So, the predominate interaction of

phenol, benzoic acid, acetylsalicylic acid and salicylic acid are hydrophobic interaction.

For L-ascorbic acid, when acetonitrile in mobile phase is increased cause a little change in its retention (or  $\log k'$ ) but its plots at low pH range (pH 2.5-4.5) show different line curves when compare with other organic acids as above, acetonitrile in mobile phase is increased show increasing of retention which could be interpreted that ionic interaction predonant while at higher pH show hydrophobic interaction predominate as above organic acids. It could be explained by competition between ionized form of L-ascorbic acid and dissociate forms of orthophosphoric acid in buffer solution with in pH range (phosphoric acid could ionize to be a monovalent, divalent or trivalent anion ( $pK_{a1}=2.20$ ,  $pK_{a2}=7.10$  and  $pK_{a3}=12.00$ ). At lower pH range, polar groups of L-ascorbic acid which interact with ammonium group of stationary phase as well as  $H_2PO_4^-$  in buffer solution, coelute by ionic mechanism and hydrophobic altogather could be occured but ionic interaction show more dominate so predominated mechanism is ionic interaction while at higher pH,  $H_2PO_4^{2-}$  and  $HPO_4^{2-}$  from othophosphoric acid in buffer solution more active than ionized form of L-ascorbic acid when acetonitrile in mobile phase is increased cause reducing retention and chromatographic mechanism of L-ascorbic acid at higher pH is hydrophobic interaction predominate as the same as above organic acids.

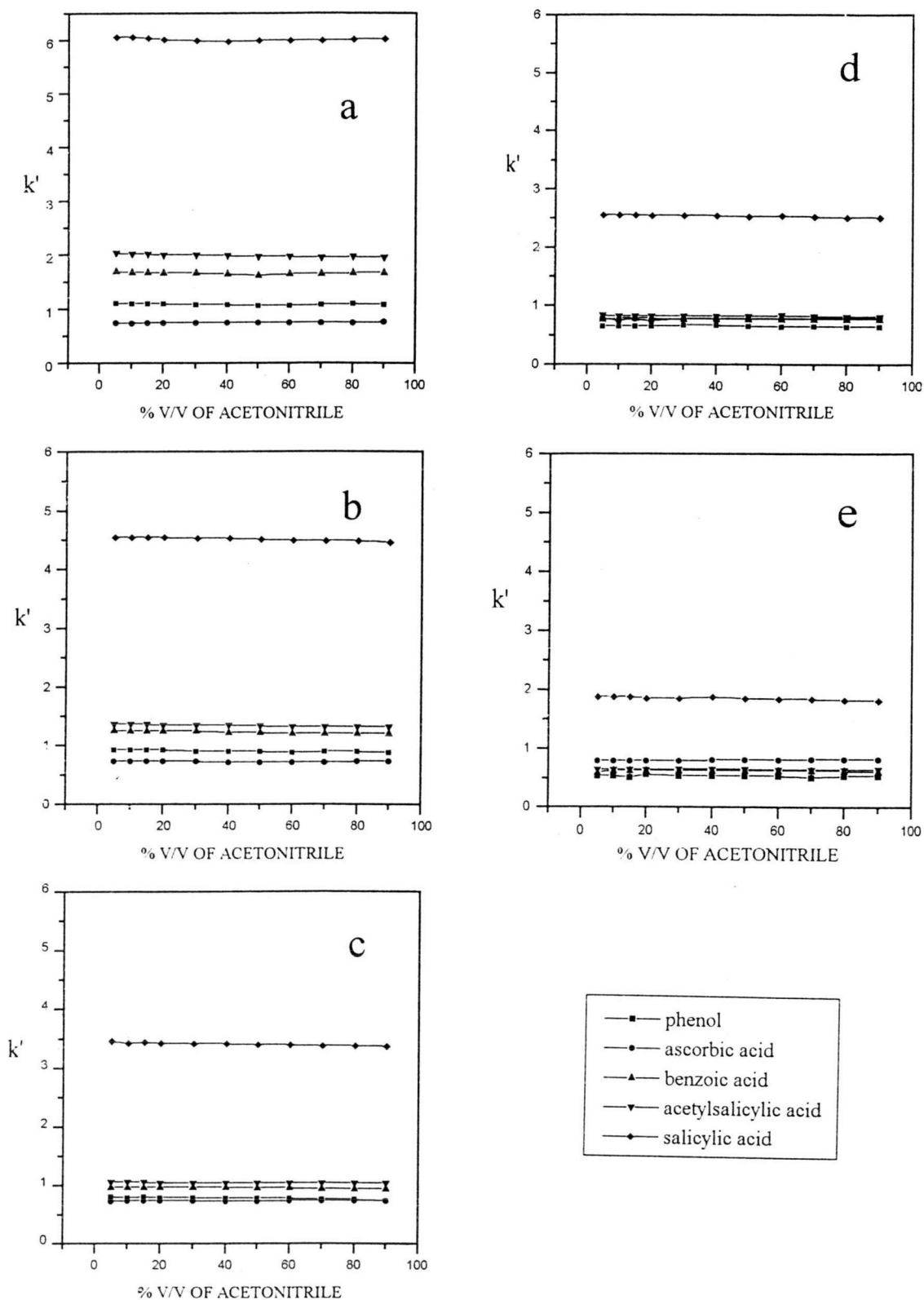
Percent of acetonitrile in solvent from 5 - 90% (v/v) by varying percent of acetonitrile in mobile phase and pH are studied to observe  $k'$ . The results are compared between 5% solvent strength (a) and 90% solvent strength (b) as Figure 4.1-4.5 which are not different retention by changing % solvent strength (v/v), the slope of curves (B) and the reliability of each fitting curve represented as the correlation coefficient (R). It is statistically accepted of phenol, benzoic acid, acetylsalicylic acid and salicylic acid at pH 2.5 to 6.5 when solvent strength are between 5-90% as shown in Table J 1- J 4 of Appendix J.



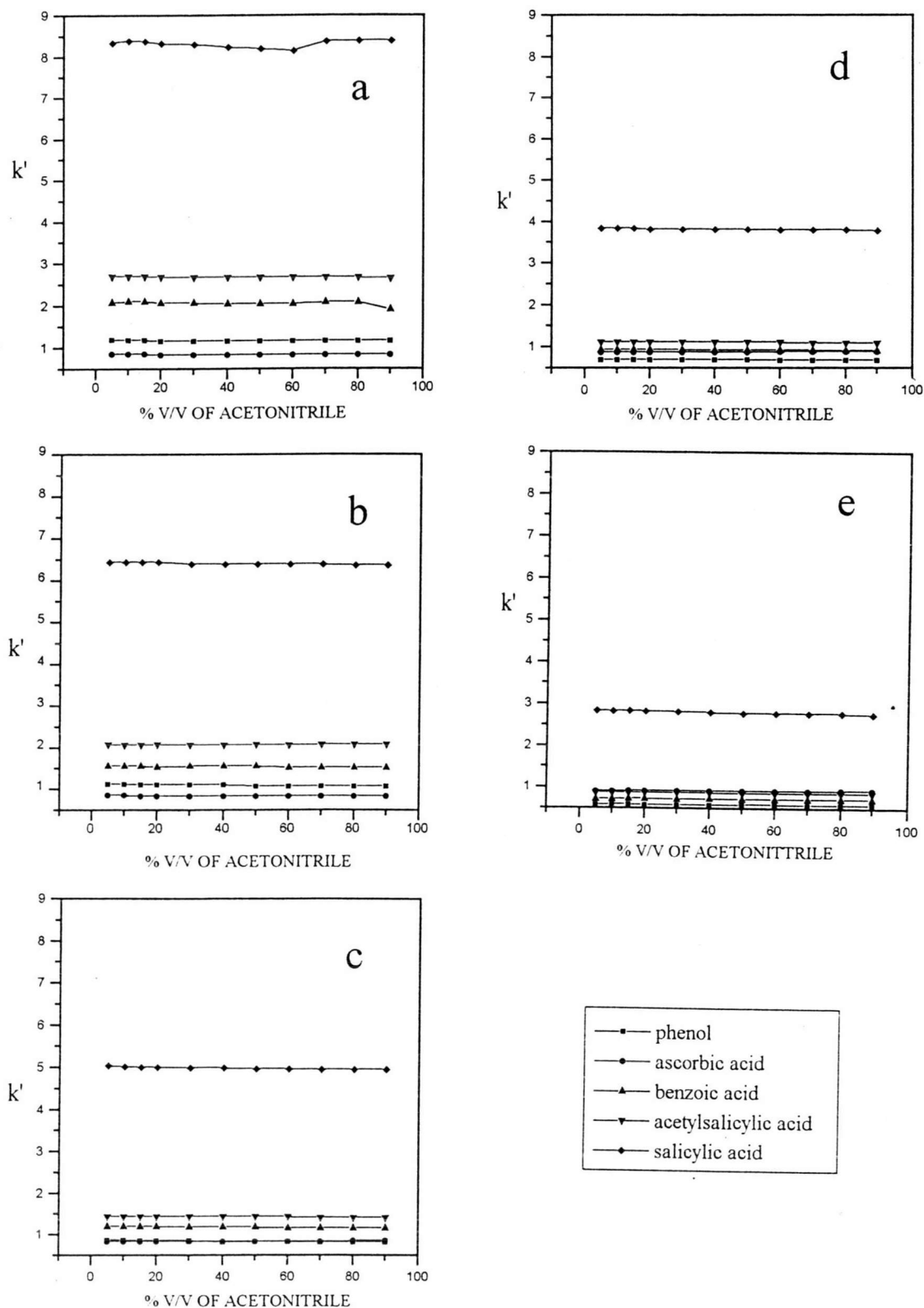
## 2. Effect of % acetonitrile (v/v) in dissolving solvent on $k'$ in pH range 2.5 - 6.5

### 2.1) Effect of % acetonitrile (v/v) in solvent on $k'$ within a range of 2.5 - 6.5 of each weak organic acid.

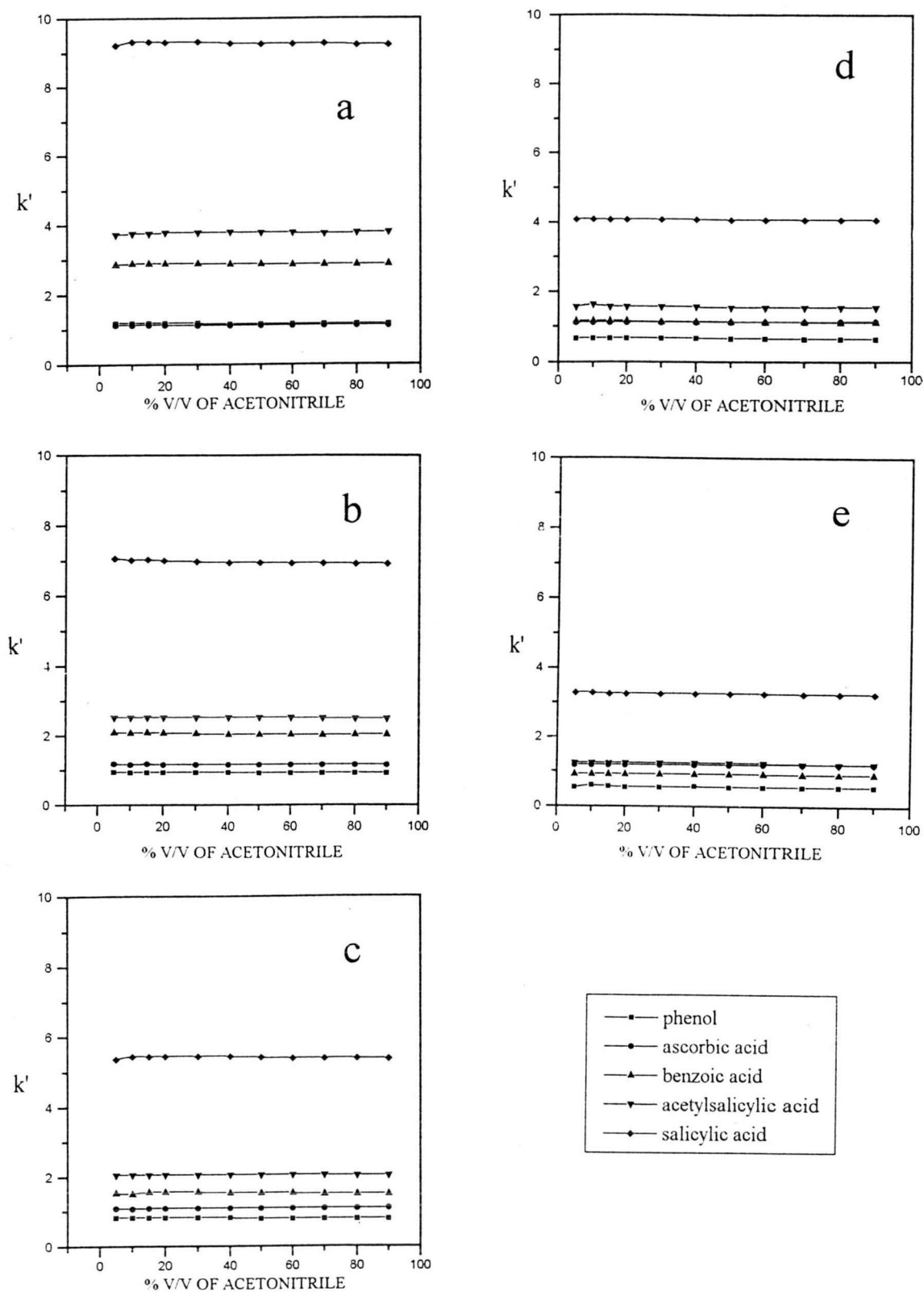
The effect of varying % of acetonitrile in solvent on the capacity factor ( $k'$ ) of each weak organic acid *i.e.* phenol, L-ascorbic acid, benzoic acid, acetylsalicylic acid and salicylic acid are investigated. The results of the studied on chromatographic behavior in terms of capacity factors ( $k'$ ) on the phenylpropanolamine column by varying % solvent strength at pH 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5 are shown in Figure 4.6 - 4.14 respectively. The plots show the relationship of the  $k'$  of each weak organic acid when varying acetonitrile-to-buffer ratios before injection. The results as shown in Figure 4.6 - 4.14, show that retention time ( $t_R$ ) or in term of capacity factor ( $k'$ ) of each weak organic acid in pH range 2.5 - 4.5 is not affected by % acetonitrile (v/v) in solvent (not more than  $\pm 0.17$  min.). But at higher pH range (pH 5.0 - 6.5) and mobile phase is high percent of acetonitrile as solvent (about 40 - 50% of acetonitrile which are represented in Figure d and e of each pH) have a little decrease affected in L-ascorbic acid, benzoic acid, acetylsalicylic acid and salicylic acid while phenol is rarely affected.



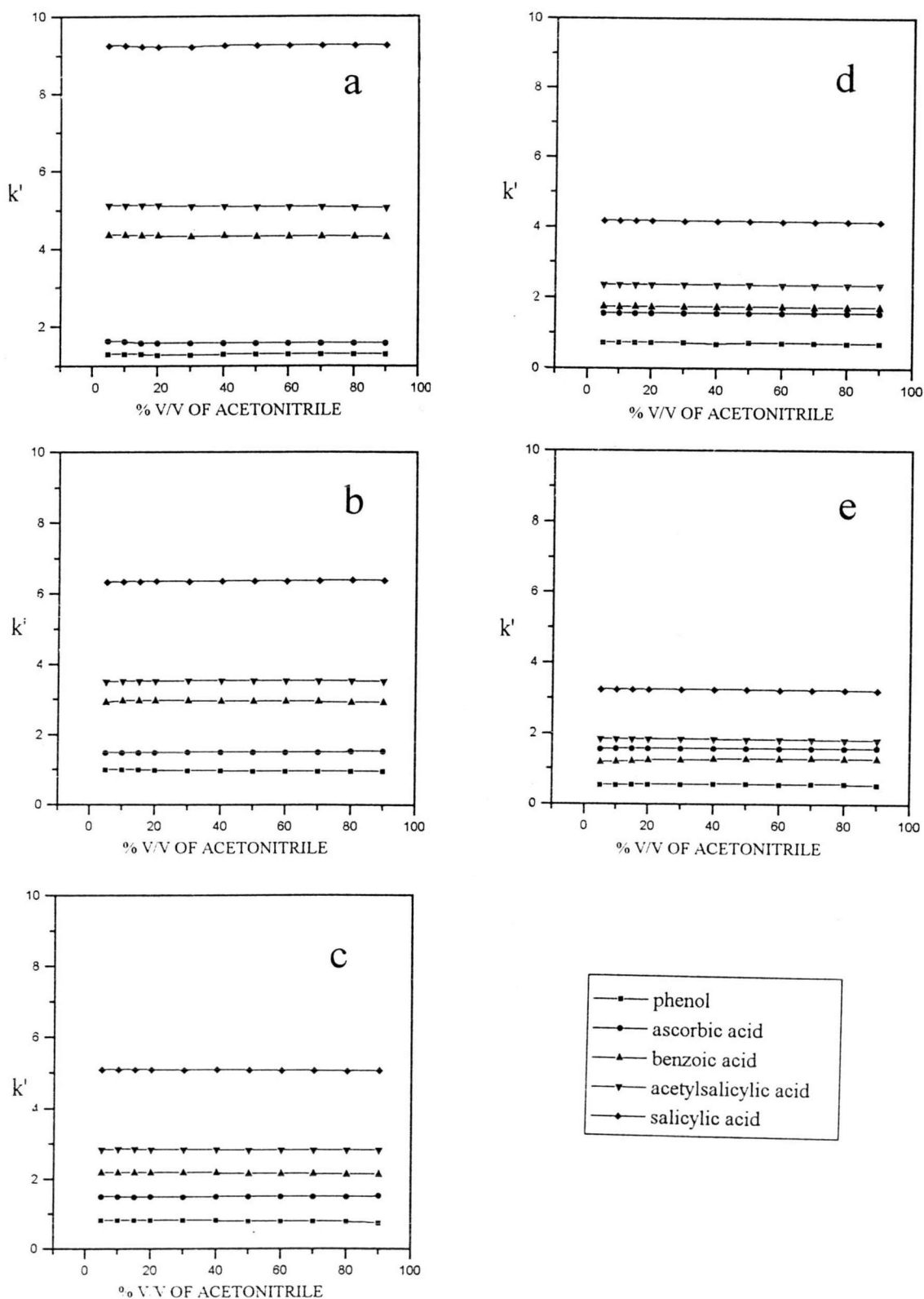
**Figure 4.6** Effect of solvent strength at pH 2.5 on capacity factors ( $k'$ ) of phenol, L-ascorbic acid, benzoic acid, acetylsalicylic acid and salicylic acid. Stationary phase : phenylpropanolamine column, 5  $\mu$ m, 150 x 4.6 mm I.D. ; mobile phase: (a) 10:90, (b) 20:80, (c) 30:70, (d) 40:60 and (e) 50:50 (v/v) of acetonitrile / 30 mM phosphate buffer, pH 2.5 ; flow rate 1 ml / min. ; UV 254 nm.



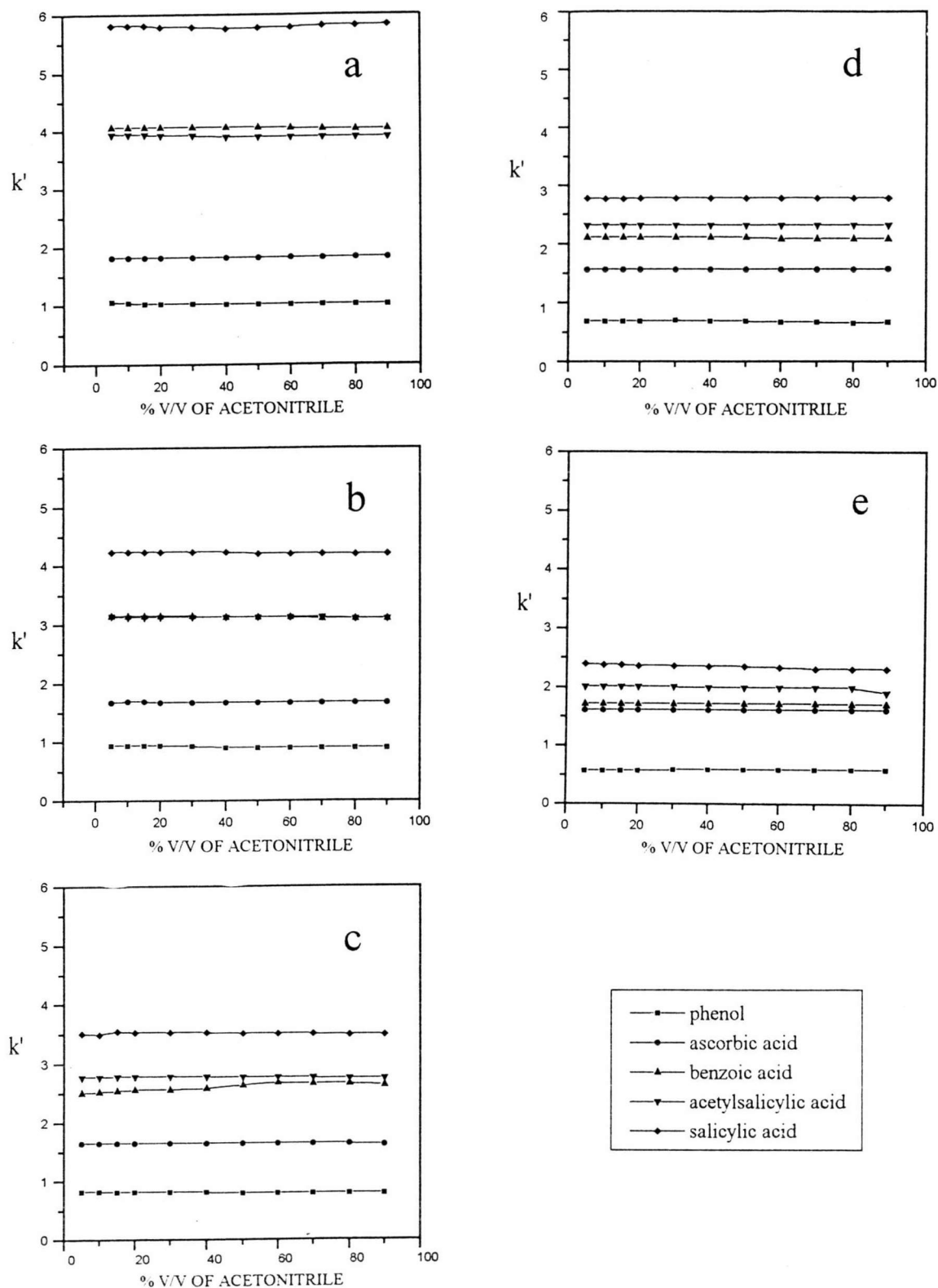
**Figure 4.7** Effect of solvent strength at pH 3.0 on capacity factors ( $k'$ ) of phenol, L-ascorbic acid, benzoic acid, acetylsalicylic acid and salicylic acid. phenylpropanolamine column, 5  $\mu$ m, 150 x 4.6 mm I.D.; mobile phase: (a) 10:90, (b) 20:80, (c) 30:70, (d) 40:60 and (e) 50:50 (v/v) of acetonitrile / 30 mM phosphate buffer, pH 3.0; flow rate 1 ml / min.; UV 254 nm.



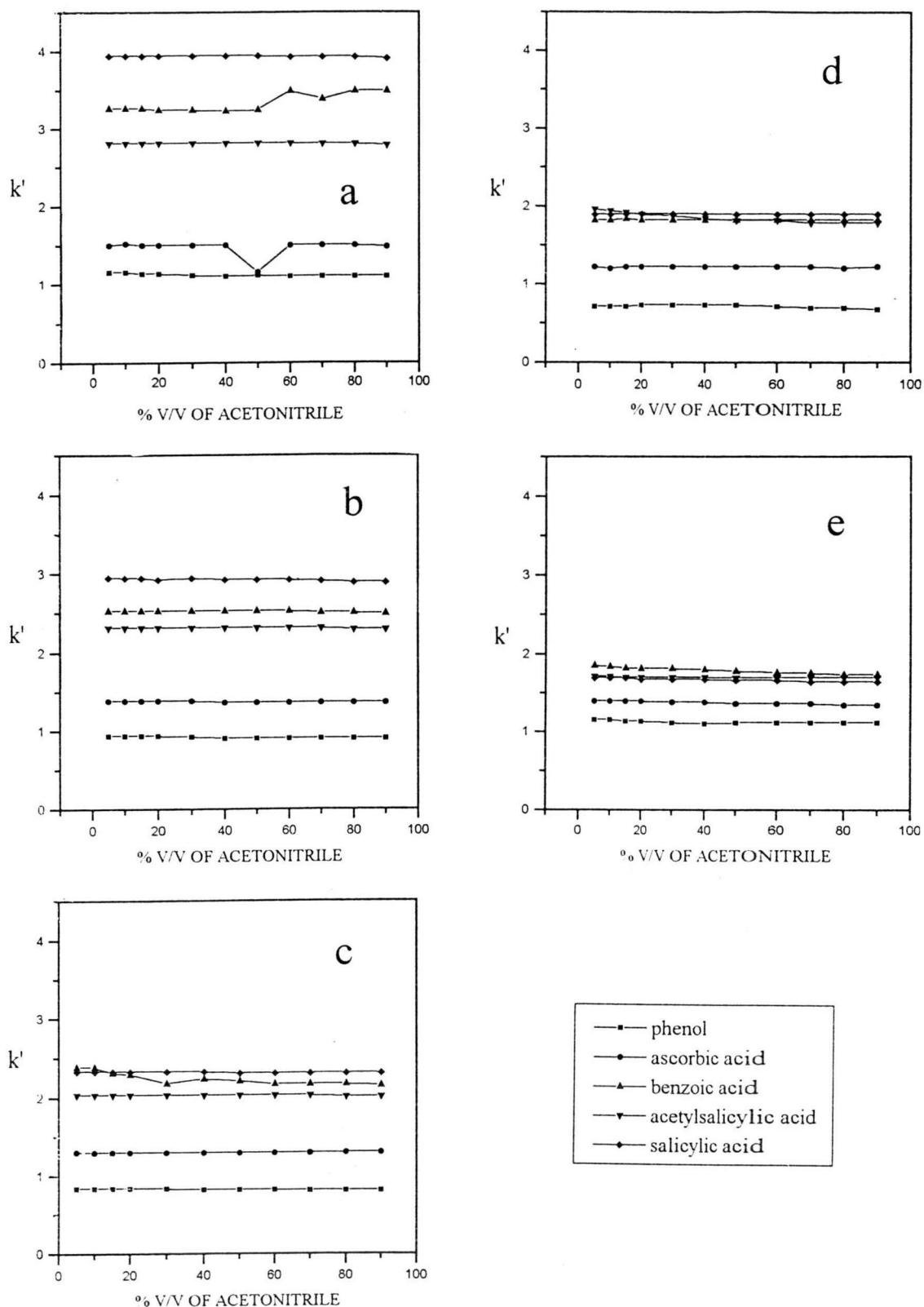
**Figure 4.8** Effect of solvent strength at pH 3.5 on capacity factors ( $k'$ ) of phenol, L-ascorbic acid, benzoic acid, acetylsalicylic acid and salicylic acid. phenylpropanolamine column, 5  $\mu\text{m}$ , 150 x 4.6 mm I.D. ; mobile phase: (a) 10:90, (b) 20:80, (c) 30:70, (d) 40:60 and (e) 50:50 (v/v) of acetonitrile / 30 mM phosphate buffer, pH 3.5 ; flow rate 1 ml / min. ; UV 254 nm.



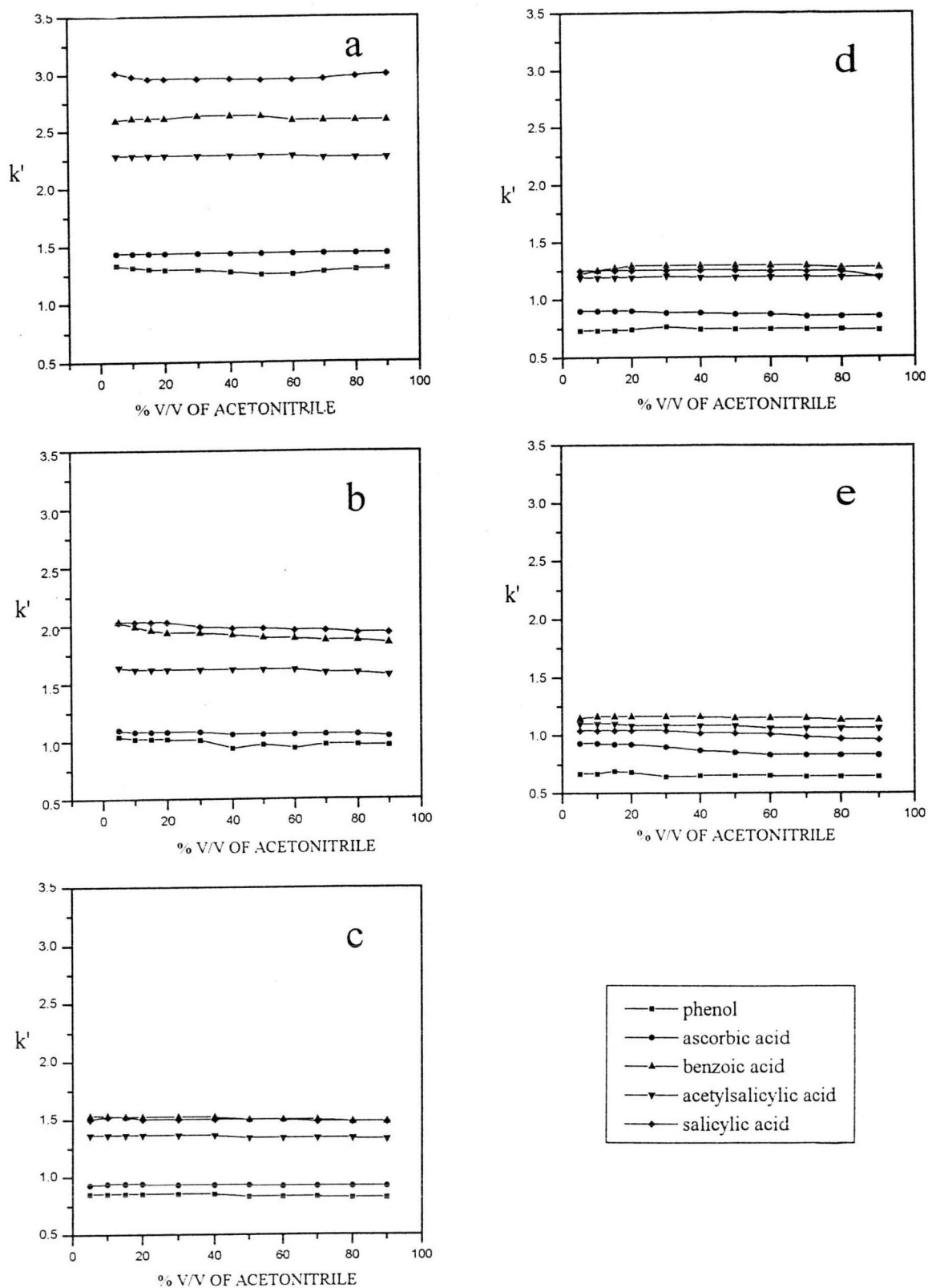
**Figure 4.9** Effect of solvent strength at pH 4.0 on capacity factors ( $k'$ ) of phenol, L-ascorbic acid, benzoic acid, acetylsalicylic acid and salicylic acid. phenylpropanolamine column, 5  $\mu$ m, 150 x 4.6 mm I.D. ; mobile phase: (a) 10:90, (b) 20:80, (c) 30:70, (d) 40:60 and (e) 50:50 (v/v) of acetonitrile / 30 mM phosphate buffer, pH 4.0 ; flow rate 1 ml / min. ; UV 254 nm.



**Figure 4.10** Effect of solvent strength at pH 4.5 on capacity factors ( $k'$ ) of phenol, L-ascorbic acid, benzoic acid, acetylsalicylic acid and salicylic acid. phenylpropanolamine column, 5  $\mu\text{m}$ , 150 x 4.6 mm I.D. ; mobile phase: (a) 10:90, (b) 20:80, (c) 30:70, (d) 40:60 and (e) 50:50 (v/v) of acetonitrile / 30 mM phosphate buffer, pH 4.5 ; flow rate 1 ml / min. ; UV 254 nm.

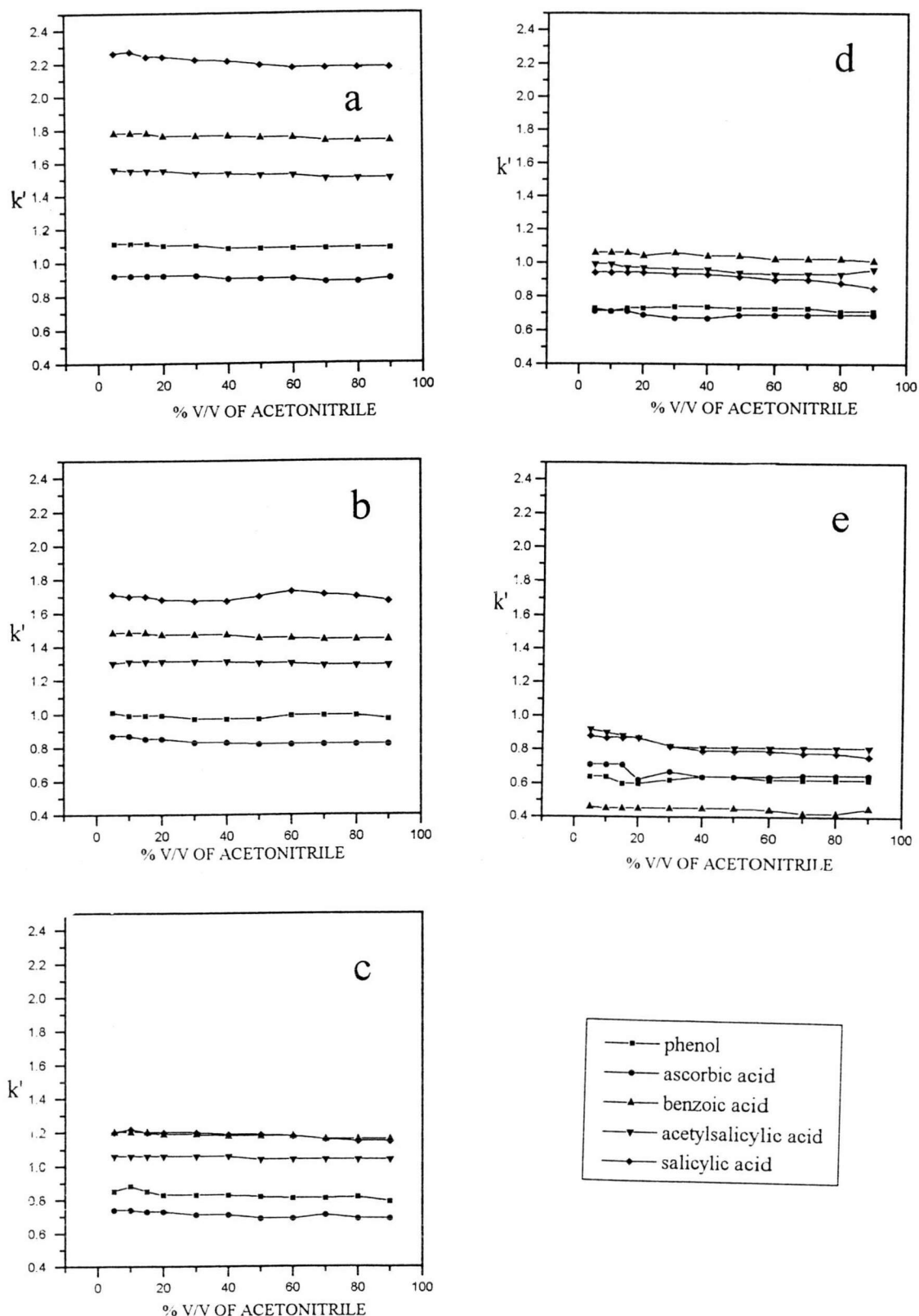


**Figure 4.11** Effect of solvent strength at pH 5.0 on capacity factors ( $k'$ ) of phenol, L-ascorbic acid, benzoic acid, acetylsalicylic acid and salicylic acid. phenylpropanolamine column, 5  $\mu$ m, 150 x 4.6 mm I.D. ; mobile phase: (a) 10:90, (b) 20:80, (c) 30:70, (d) 40:60 and (e) 50:50 (v/v) of acetonitrile / 30 mM phosphate buffer, pH 5.0 ; flow rate 1 ml / min. ; UV 254 nm.

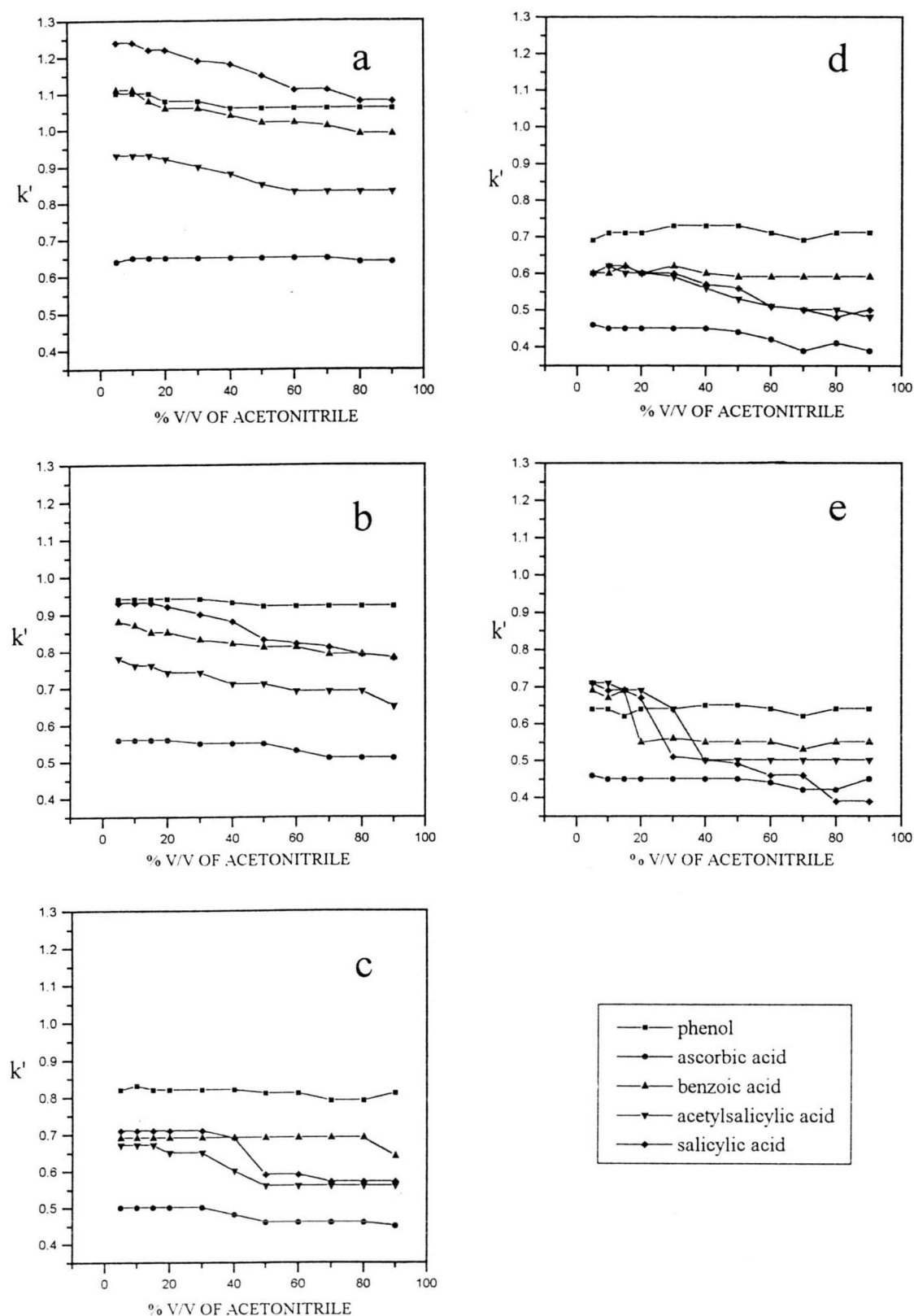


**Figure 4.12** Effect of solvent strength at pH 5.5 on capacity factors ( $k'$ ) of phenol, L-ascorbic acid, benzoic acid, acetylsalicylic acid and salicylic acid. phenylpropanolamine column, 5  $\mu$ m, 150 x 4.6 mm I.D. ; mobile phase: (a) 10:90, (b) 20:80, (c) 30:70, (d) 40:60 and (e) 50:50 (v/v) of acetonitrile / 30 mM phosphate buffer, pH 5.5 ; flow rate 1 ml / min. ; UV 254 nm.





**Figure 4.13** Effect of solvent strength at pH 6.0 on capacity factors ( $k'$ ) of phenol, L-ascorbic acid, benzoic acid, acetylsalicylic acid and salicylic acid. phenylpropanolamine column, 5  $\mu\text{m}$ , 150 x 4.6 mm I.D. ; mobile phase: (a) 10:90, (b) 20:80, (c) 30:70, (d) 40:60 and (e) 50:50 (v/v) of acetonitrile / 30 mM phosphate buffer, pH 6.0 ; flow rate 1 ml / min. ; UV 254 nm.



**Figure 4.14** Effect of solvent strength at pH 6.5 on capacity factors ( $k'$ ) of phenol, L-ascorbic acid, benzoic acid, acetylsalicylic acid and salicylic acid. phenylpropanolamine column, 5  $\mu$ m, 150 x 4.6 mm I.D. ; mobile phase: (a) 10:90, (b) 20:80, (c) 30:70, (d) 40:60 and (e) 50:50 (v/v) of acetonitrile / 30 mM phosphate buffer, pH 6.5 ; flow rate 1 ml / min. ; UV 254 nm.

2.2) Effect of % acetonitrile (v/v) in solvent on theoretical plates (N) and peak shape within a range of pH 2.5 - 6.5.

The effect of solvent strength on the chromatographic behavior of each organic acids *i.e.* phenol, L-ascorbic acid, benzoic acid, acetylsalicylic acid and salicylic acid were studied. The results of study on the solvent strength are presented in Figure 4.15 - 4.19. The graphs show the relationship between column efficiency in terms of number of theoretical plates (N) of each organic acid with varying concentrations of 30 mM phosphate buffer.

The results as Figure 4.15 (a) - (d) show that the number of theoretical plates (N) of phenol decrease depending on the concentrations of acetonitrile as dissolving solvent. The theoretical plate (N) rather stable until the solvent strength is stronger than mobile phase which are predominate hydrophobic interaction. Figure 4.15 (e) at pH 6.5 shows that increasing of N when solvent strength was weaker than mobile phase, ionic interaction are predominated because N is increased with increasing solvent strength but when solvent strength stronger than mobile phase, hydrophobic interaction are predominated because N is decreased with increasing solvent strength.

Figure 4.16 (a) - (b) L-ascorbic acid shows rather decreasing of N when solvent strength weaker than mobile phase at low pH (pH 2.5 - 4.5) while higher pH, ionic character is more dominant and ionic mechanism predominated. When % acetonitrile (v/v) in mobile phase is 30:70 (v/v) acetonitrile / 30 mM phosphate buffer as Figure 4.16 (c), L-ascorbic acid show ionic interaction at pH 6 while at pH 6.5 show ionic mechanism when solvent strength weaker than mobile phase and hydrophobic mechanism when pH stronger than mobile phase. Figure 4.16 (d) - (e) show ionic character are more dominant at low pH range (pH 2.5 - 5.5) while at higher pH show dual mechanism.

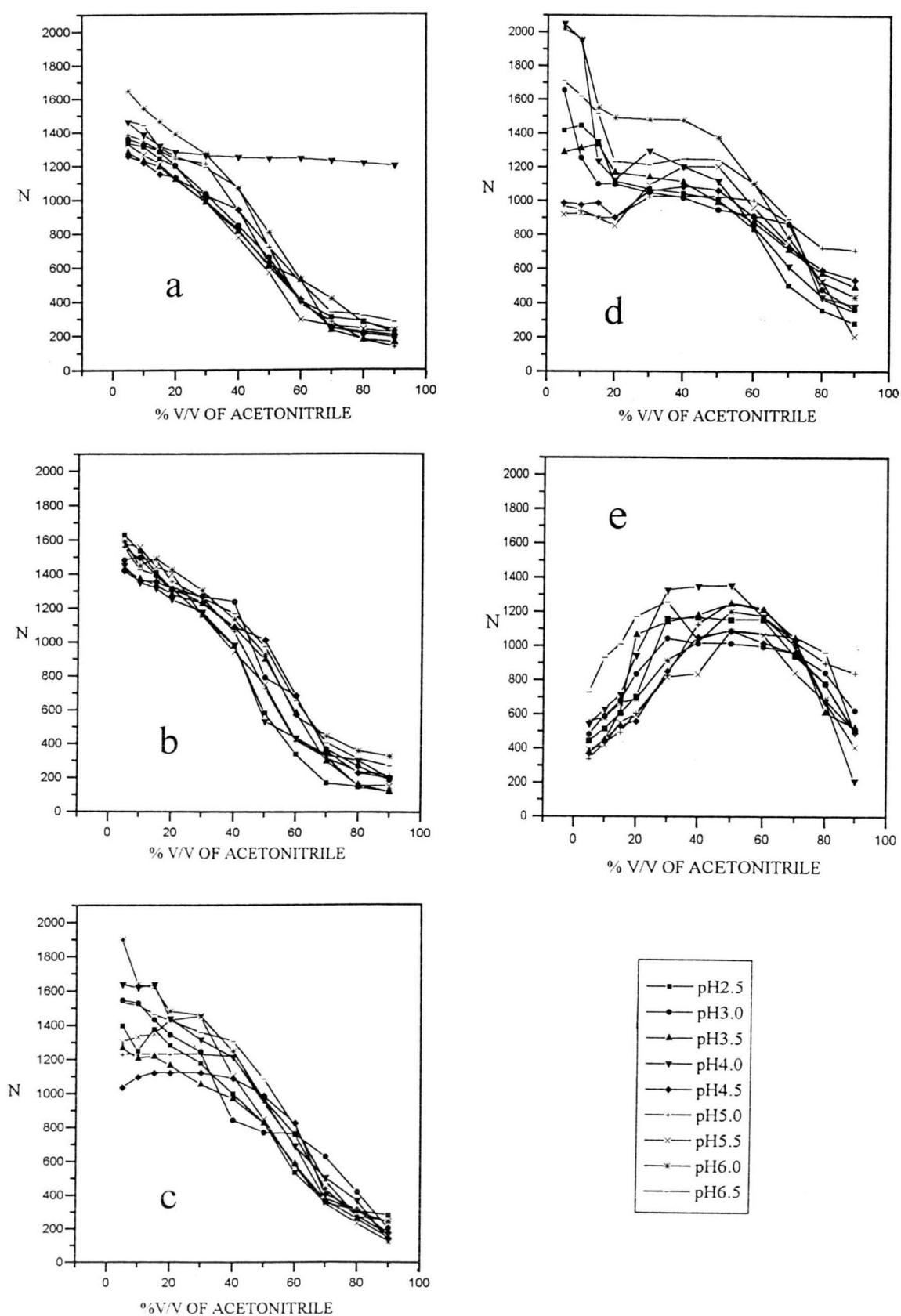
The results as Figure 4.17 (a) - (b) show the number of theoretical plates (N) of benzoic acid decrease depending on the concentrations of

acetonitrile in the injected sample which is increased from 5% to 90% (v/v) and hydrophobic interaction are predominated. Figure 4.17 (c) - (d) show that at low pH range, hydrophobic interaction are predominated because  $N$  is decreased with increasing solvent strength while at pH 6 shows ionic interaction predominated and at pH 6.5 show dual mechanism. Figure 4.17 (e) at pH 2.5, 3.0 and 6.5 show increasing of  $N$  when solvent strength is weaker than mobile phase, but when solvent strength stronger than mobile phase, hydrophobic interaction are predominated because  $N$  are decreased with increasing solvent strength as same as in pH range 3.5 - 5.5.

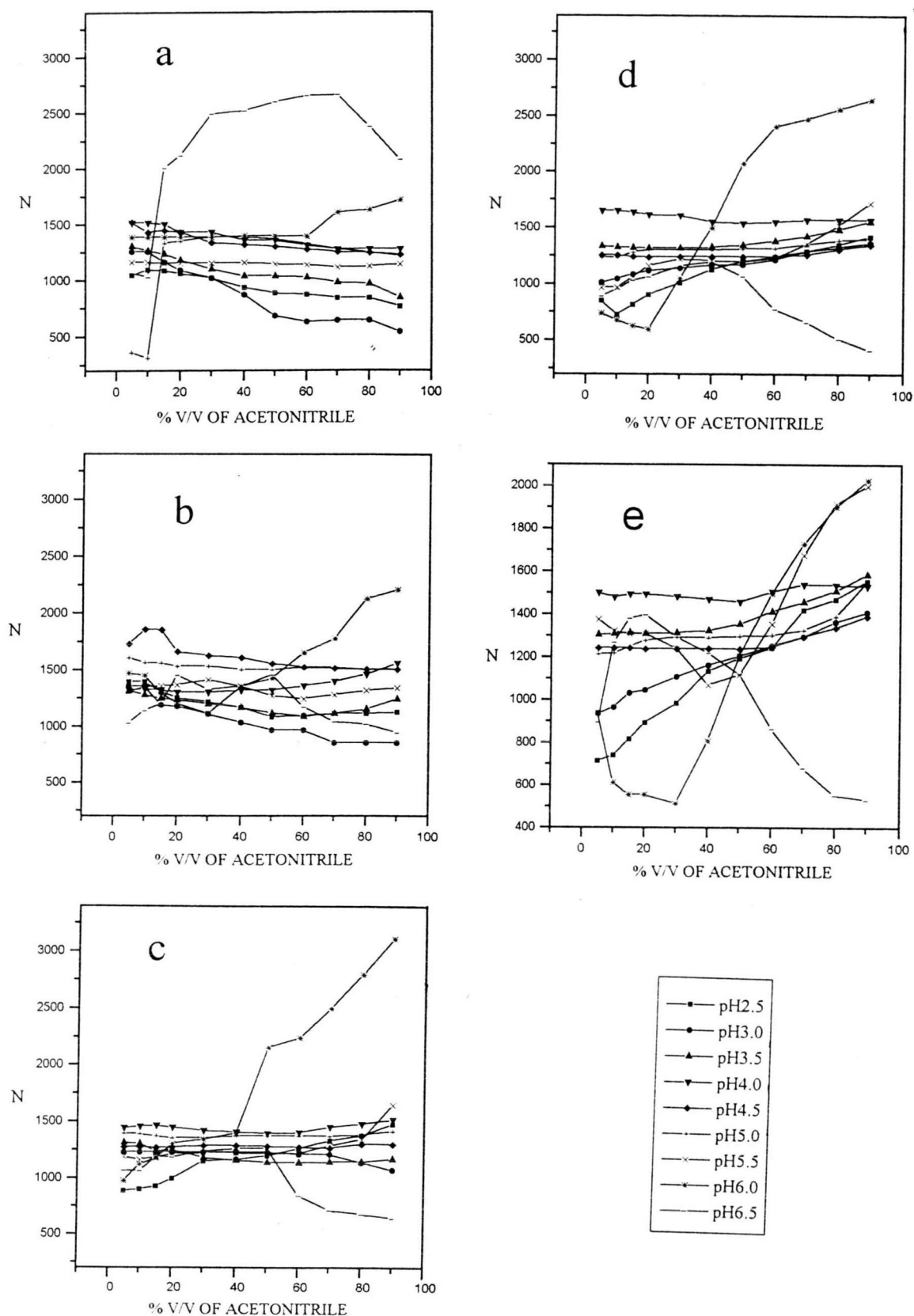
The results as Figure 4.18 (a) - (b) show that  $N$  of acetylsalicylic acid rapidly decrease at low pH range (pH 2.5 to 3.5) and few decrease at higher pH due to strength of hydrophobic mechanism at pH 2.5 to 3.5 are stronger than the others. Figure 4.18 (c) - (e) at pH 6.5,  $N$  of benzoic acid increase when solvent strength weaker than mobile phase which ionic interaction is predominated but when solvent strength stronger than mobile phase, hydrophobic interaction are predominated which as same as in low pH range.

At pH range 2.5 - 6.0 of Figure 4.19 (a) - (e) the number of theoretical plates of salicylic acid decrease when solvent strength is increased which hydrophobic mechanism are predominated while at pH 6.5 ionic interaction are predominated when solvent strength weaker than mobile phase but hydrophobic interaction are predominated when solvent strength stronger than mobile phase.

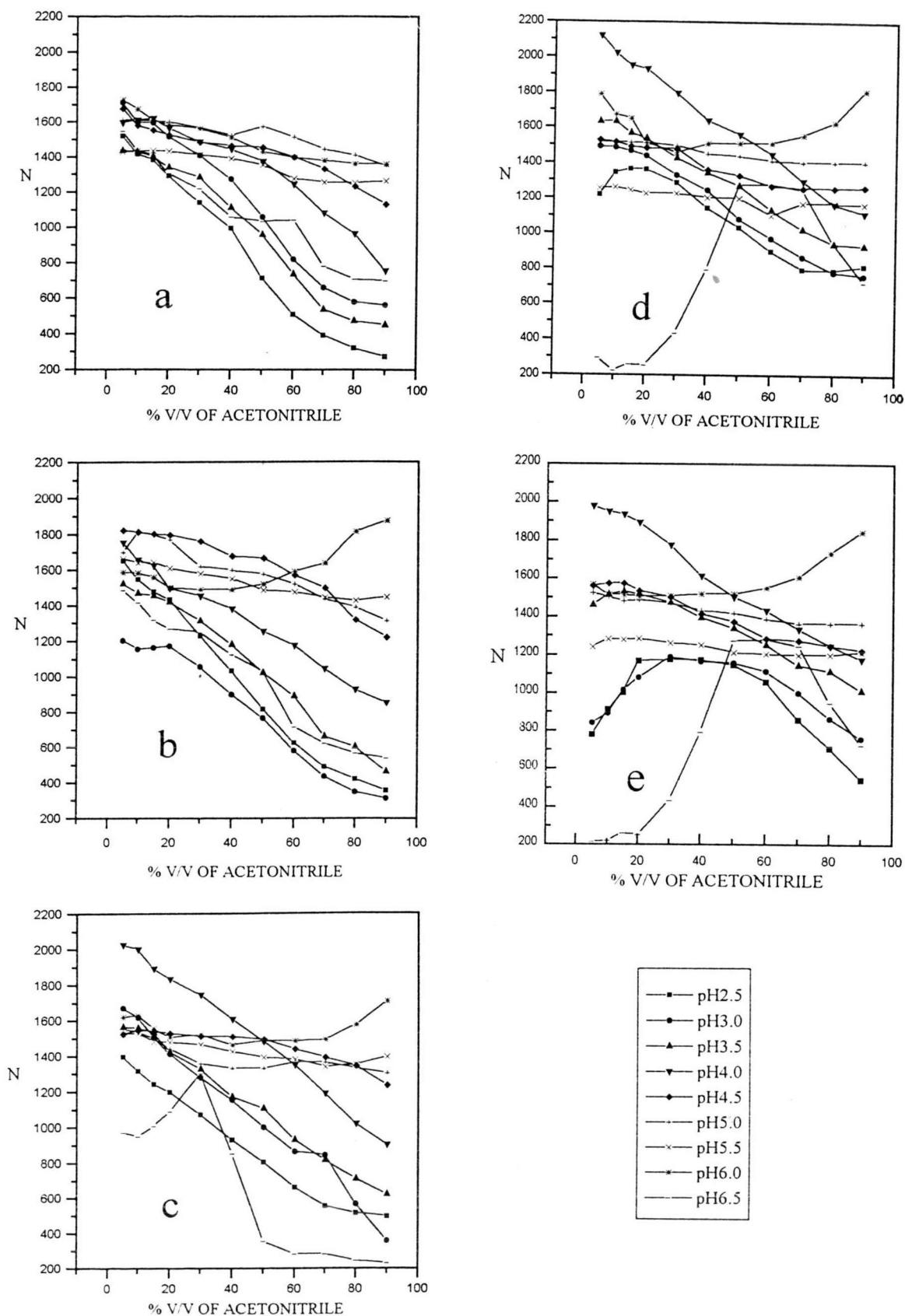
The chromatograms of standard mixtures of organic acids *i.e.* phenol, benzoic acid, acetylsalicylic acid and salicylic acid on the phenylpropanolamine column dissolved in acetonitrile/buffer (5:95, v/v), acetonitrile/30 mM phosphate buffer (the same as mobile phase *i.e.* 10:90, 20:80, 30:70, 40:60, 50:50 (v/v) respectively) and acetonitrile/buffer (70:30, v/v) at various mobile phase are shown in Appendix A - I .



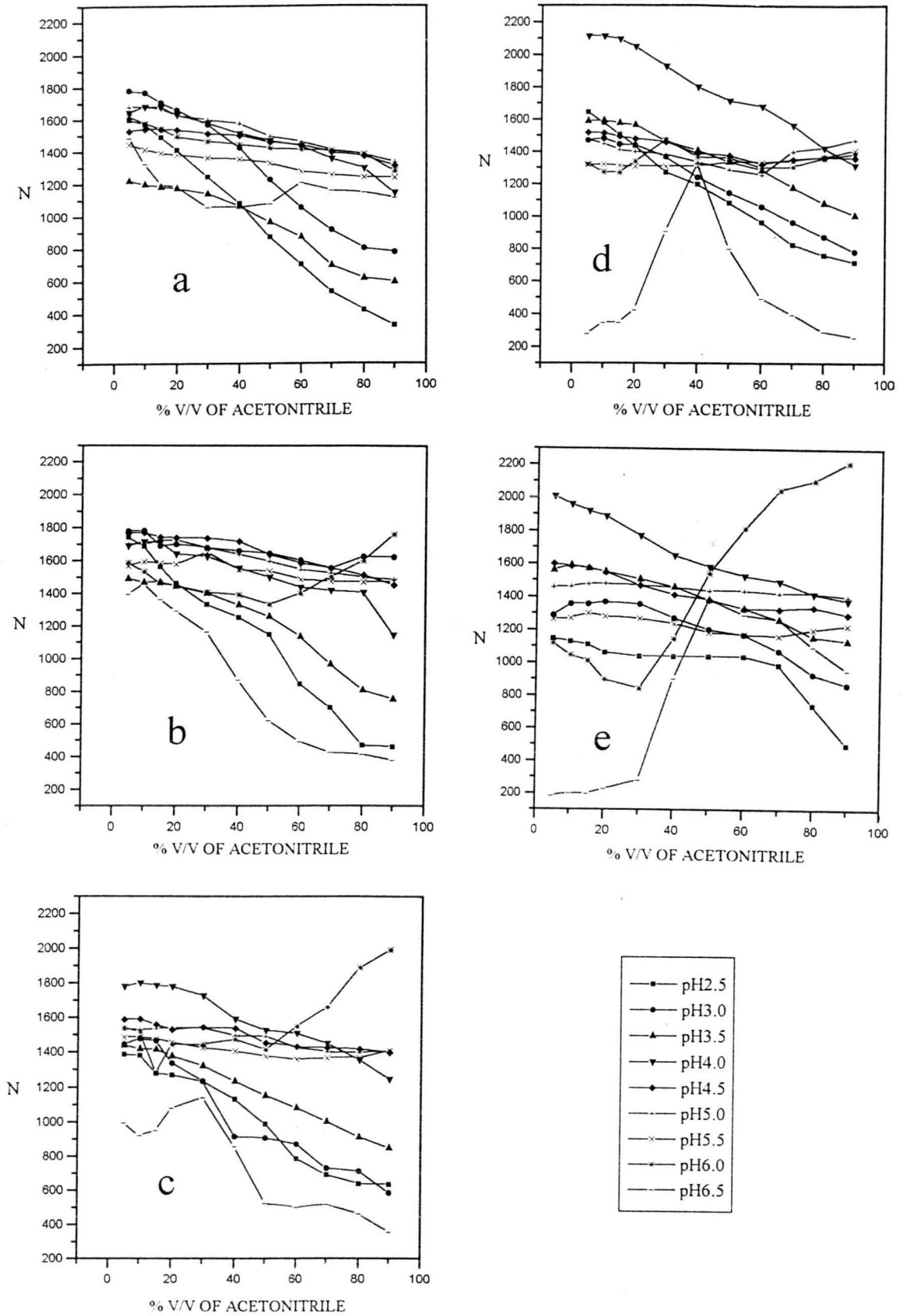
**Figure 4.15** Effect of solvent strength on the theoretical plates (N) of phenol in the range of pH 2.5-6.5 ; phenylpropanolamine column, 5  $\mu$ m, 150 x 4.6 mm I.D. ; mobile phase : (a) 10:90, (b) 20:80, (c) 30:70, (d) 40:60 and (e) 50:50 (v/v) of acetonitrile / 30 mM phosphate buffer ; flow rate 1 ml / min. ; UV 254 nm.



**Figure 4.16** Effect of solvent strength on the theoretical plates (N) of L-ascorbic acid in the range of pH 2.5-6.5 ; phenylpropanolamine column , 5  $\mu\text{m}$  , 150 x 4.6 mm I.D.; mobile phase : (a) 10:90, (b) 20:80, (c) 30:70, (d) 40:60 and (e) 50:50 (v/v) of acetonitrile / 30 mM phosphate buffer ; flow rate 1 ml / min. ; UV 254 nm.

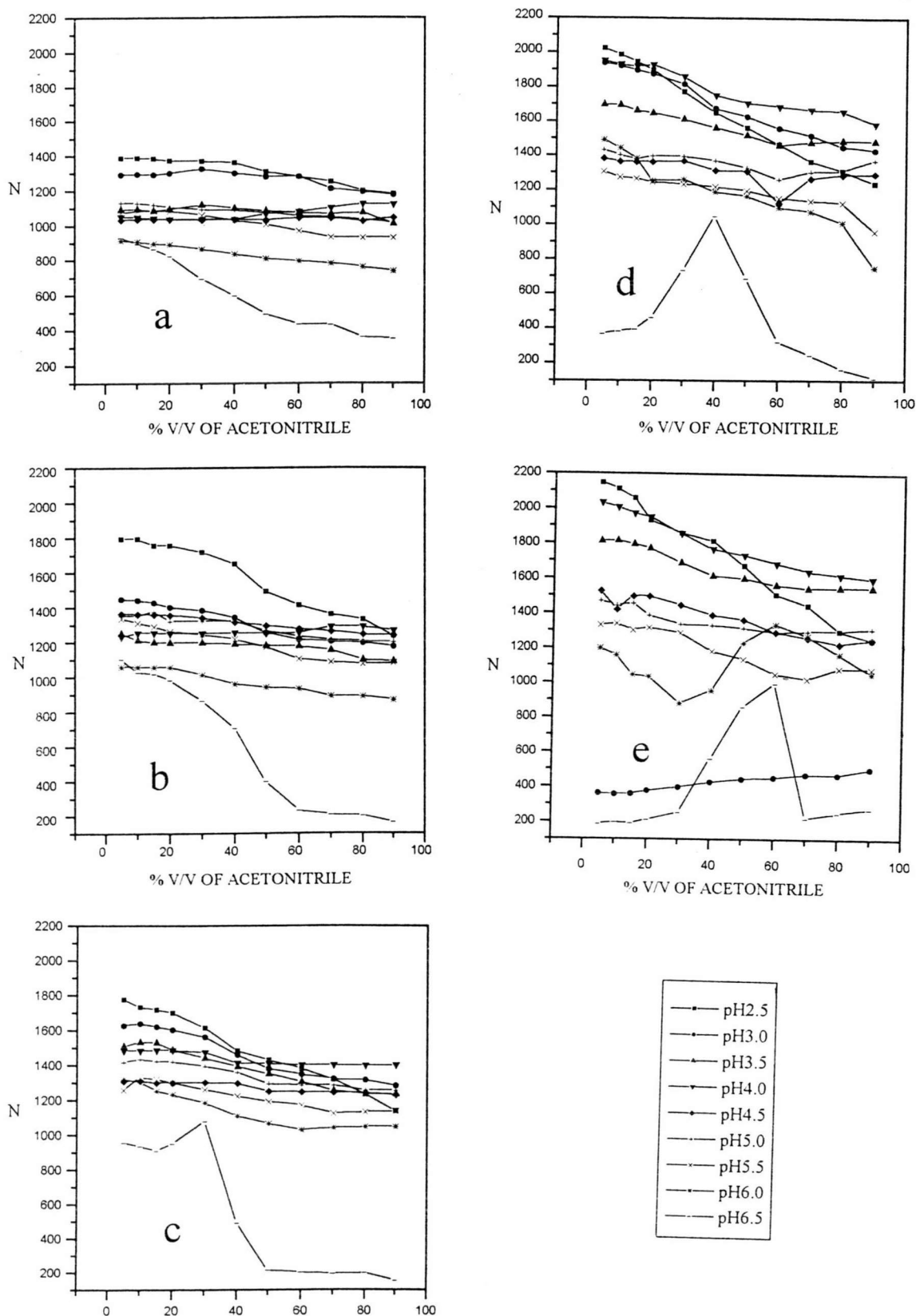


**Figure 4.17** Effect of solvent strength on the theoretical plates (N) of benzoic acid in the range of pH 2.5-6.5 ; phenylpropanolamine column, 5  $\mu\text{m}$ , 150 x 4.6 mm I.D. ; mobile phase : (a) 10:90, (b) 20:80, (c) 30:70, (d) 40:60 and (e) 50:50 (v/v) of acetonitrile / 30 mM phosphate buffer ; flow rate 1 ml / min. ; UV 254 nm.



**Figure 4.18** Effect of solvent strength on the theoretical plates (N) of acetylsalicylic acid in the range of pH 2.5-6.5 ; phenylpropanolamine column, 5  $\mu$ m, 150 x 4.6 mm I.D.; mobile phase (a) 10:90, (b) 20:80, (c) 30:70, (d) 40:60 and (e) 50:50 (v/v) of acetonitrile / 30 mM phosphate buffer (v/v) ; flow rate 1 ml / min. ;UV 254 nm.





**Figure 4.19** Effect of solvent strength on the theoretical plates (N) of salicylic acid in the range of pH 2.5-6.5 ; phenylpropanolamine column , 5  $\mu\text{m}$  , 150 x 4.6 mm I.D.; mobile phase (a) 10:90, (b) 20:80, (c) 30:70, (d) 40:60 and (e) 50:50 (v/v) of acetonitrile / 30 mM phosphate buffer ; flow rate 1 ml / min. ; UV 254 nm.

### 3. Effect of pH on $k'$

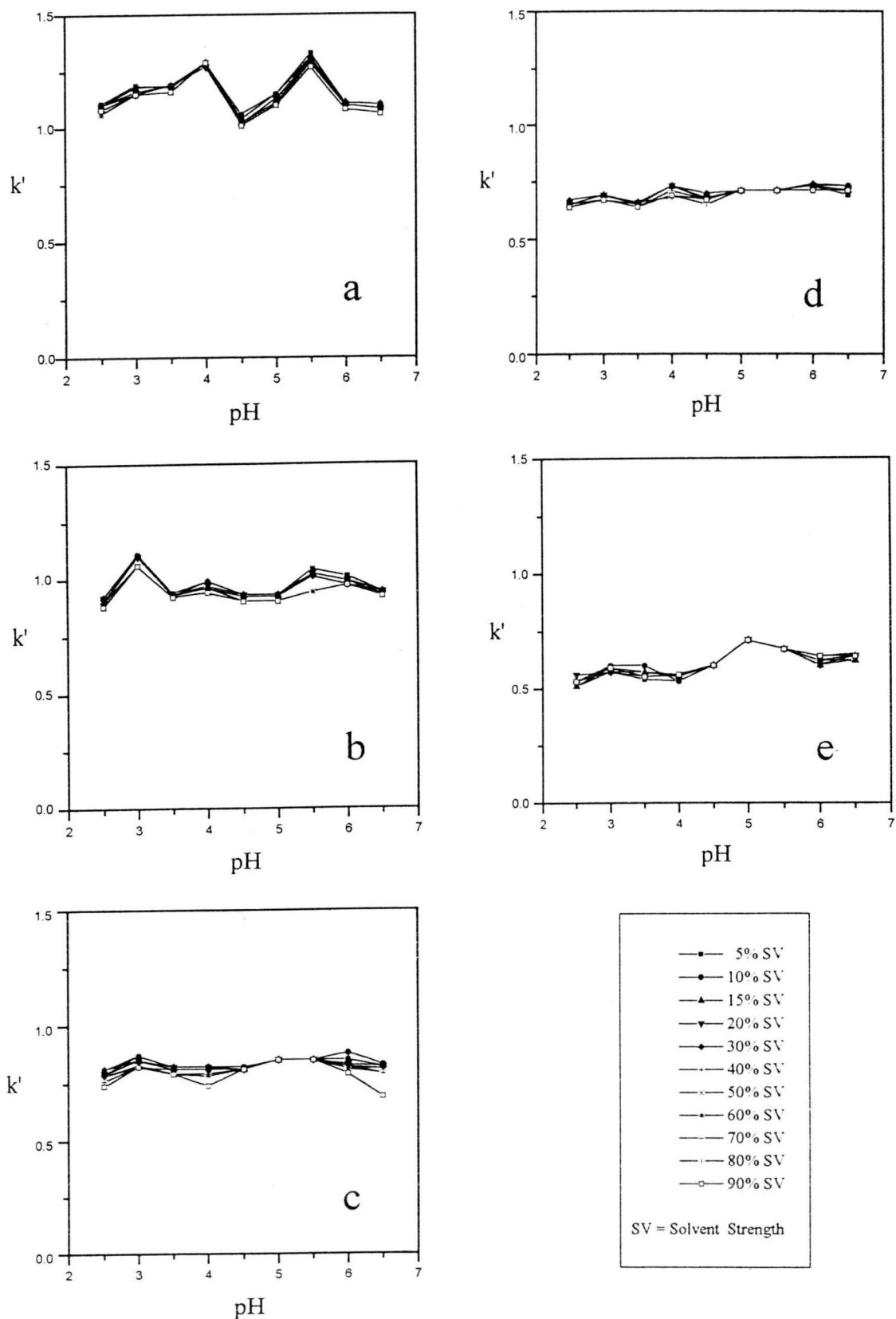
The aqueous component of the mobile phase used in this part consisted of a 30 mM phosphate buffer adjusted to pH 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5 and mixed to 10:90, 20:80, 30:70, 40:60, 50:50 (v/v) ratio with acetonitrile, respectively. The degassed eluents by replacing of helium gas were allowed to equilibrate on the column.

Variation of the eluent pH alters the degree of ionisation of organic acids, resulting in different proportions of neutral and ionized forms. As only the unionized form of the compounds will partition into the hydrophobic portion of the stationary phase, and the ionized form of the bases will interact with the ionic portion or ammonium group and the residual silanol group. Figure 4.20 (a) - (e) to 4.24 (a) - (e) show effect of eluent pH on capacity factors ( $k'$ ) of each organic acid by changing of pH on phenylpropanolamine column at 10:90, 20:80, 30:70, 40:60 and 50:50 (v/v) of acetonitrile / 30 mM phosphate buffer. The results were shown that pH greatly affects on retention of all studied weak acids. The  $k'$  of phenol ( $pK_a = 10.00$ ), L-ascorbic acid ( $pK_a = 4.19$ ), benzoic acid ( $pK_a = 4.21$ ), acetylsalicylic acid ( $pK_a = 3.48$ ) and salicylic acid ( $pK_a = 3.00$ ) reach their maximum at pH near its  $pK_a$  of each weak organic acid and decreased at a pH lower or higher than its  $pK_a$ . The effect can be explained both by the weakening of ionic interaction due to the decreased dissociation of the weak organic acids and an increase in ionic interaction between the competing phosphate buffer and the amine group of phenylpropanolamine which is stationary phase. Phosphoric acid could ionize to be a monovalent, divalent or trivalent anion ( $pK_{a1} = 2.20$ ,  $pK_{a2} = 7.10$  and  $pK_{a3} = 12.00$ ) depends on the pH and only pH range 2.5 to 6.5 were observed. When pH of mobile phase higher than the  $pK_a$  values of L-ascorbic acid, benzoic acid, acetylsalicylic acid and

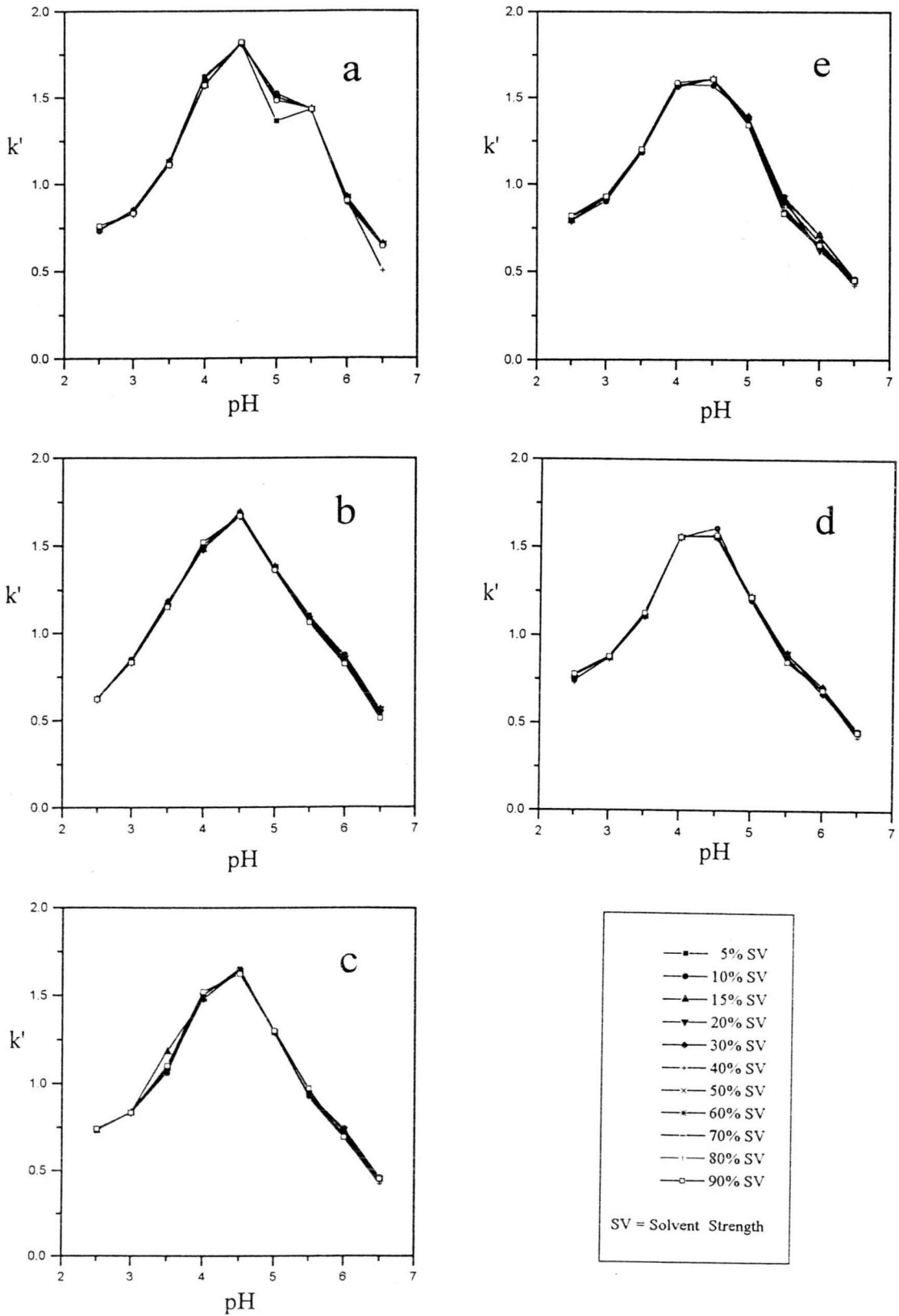
salicylic acid were shown again less retain. The explanation is that the buffer has increased  $\text{H}_2\text{PO}_4^{2-}$  form with this pH range, resulting in a loss of capacity for weak acids due to the higher affinity of the column for the mobile phase buffer anions. At pH higher than the pKa, hydrophobic interaction predominates. On the other hand, the retention of phenol, which is a very weak organic acid was unaffected over the mobile phase pH range investigated. It could be concluded that the analytes had roughly half the retention that would have been expected for the single dominated phase within a pH range 2.5 - 6.5.

The plots of capacity factors ( $k'$ ) by changing of pH of organic acids in different ratio of acetonitrile in mobile phase are shown in Figure 4.25 - 4.29 by using solvent strength as same as mobile phase.

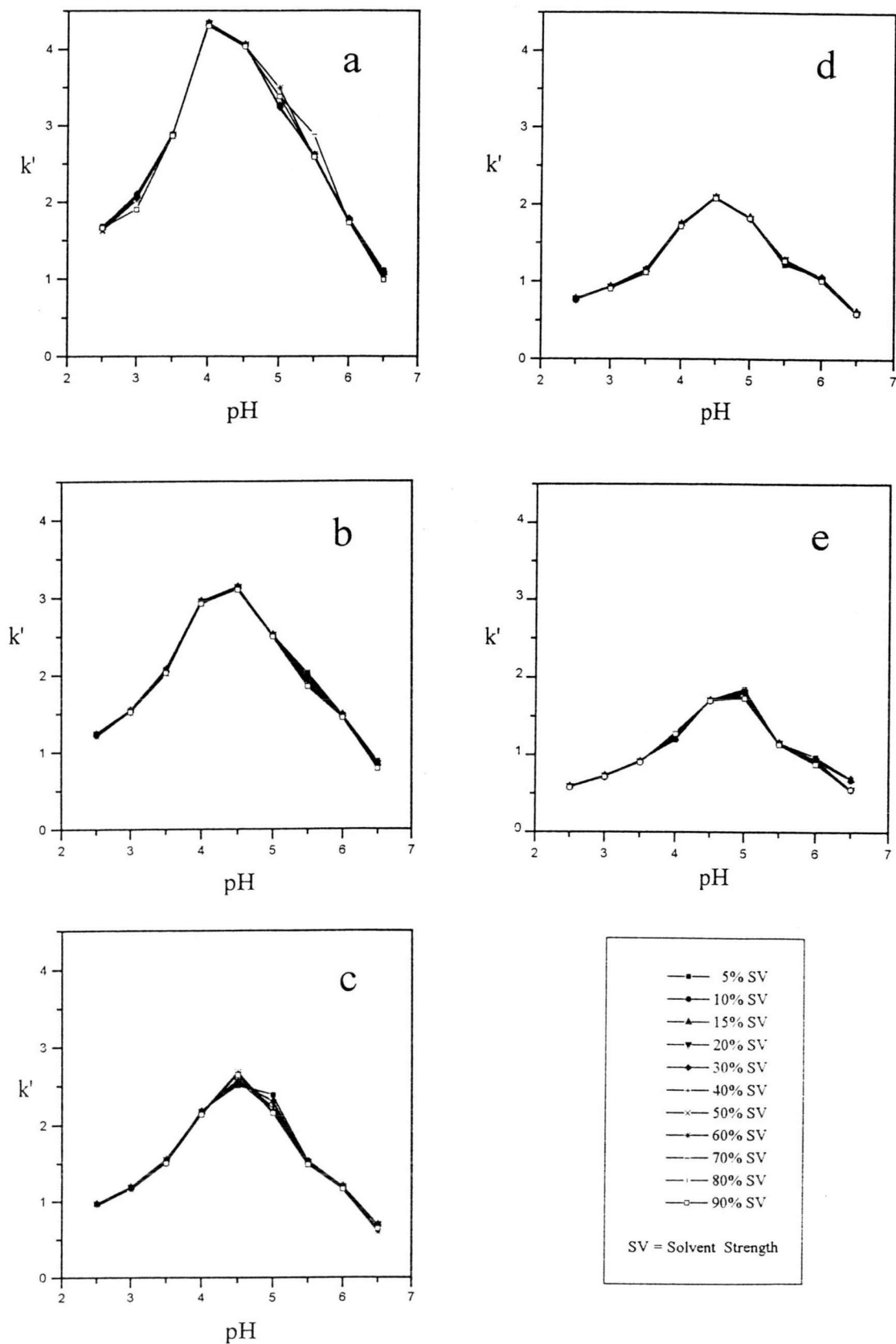
Effects of eluent pH on capacity factors ( $k'$ ) by changing of pH are compared between 5% and 90% solvent strength and data were demonstrated as Table J 1- J 4. From Table J 5 - J 9 of Appendix J the results show that changing of pH has no effect to the retention of phenol ( $\text{pK}_a = 10.00$ ), but L-ascorbic acid ( $\text{pK}_a = 4.19$ ), benzoic acid ( $\text{pK}_a = 4.21$ ), acetylsalicylic acid ( $\text{pK}_a = 3.48$ ) and especially salicylic acid ( $\text{pK}_a = 10.00$ ) are greatly effect from pH and these analytes are the most retain when pH is near its pKa.



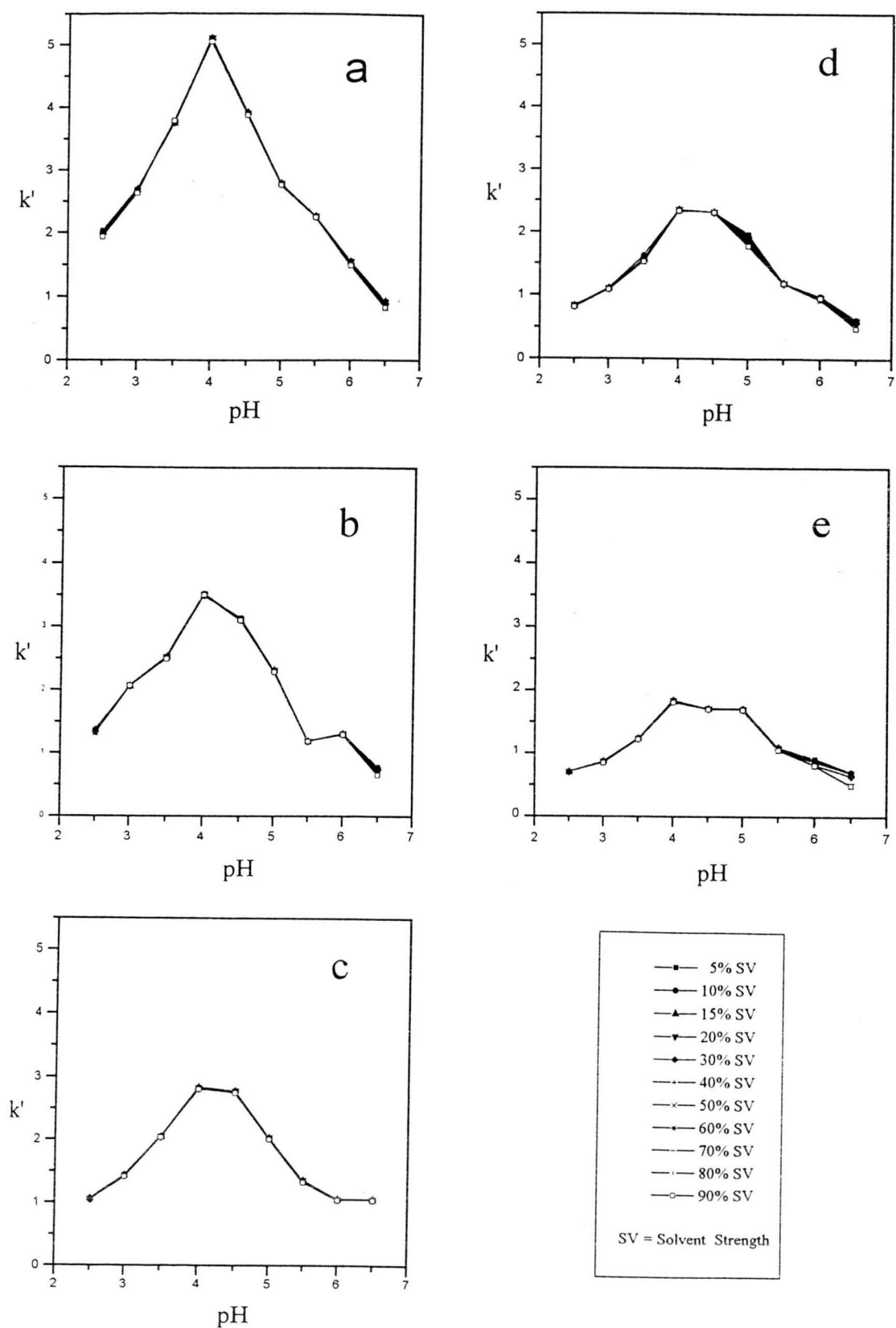
**Figure 4.20** Effect of eluent pH on capacity factors ( $k'$ ) of phenol by changing of pH ; phenylpropanolamine column, 5  $\mu$ m, 150 x 4.6 mm I.D. ; mobile phase : (a) 10:90, (b) 20:80, (c) 30:70, (d) 40:60 and (e) 50:50 (v/v) of acetonitrile in mobile phase / 30 mM phosphate buffer ; flow rate 1 ml/min. ; UV 254 nm.



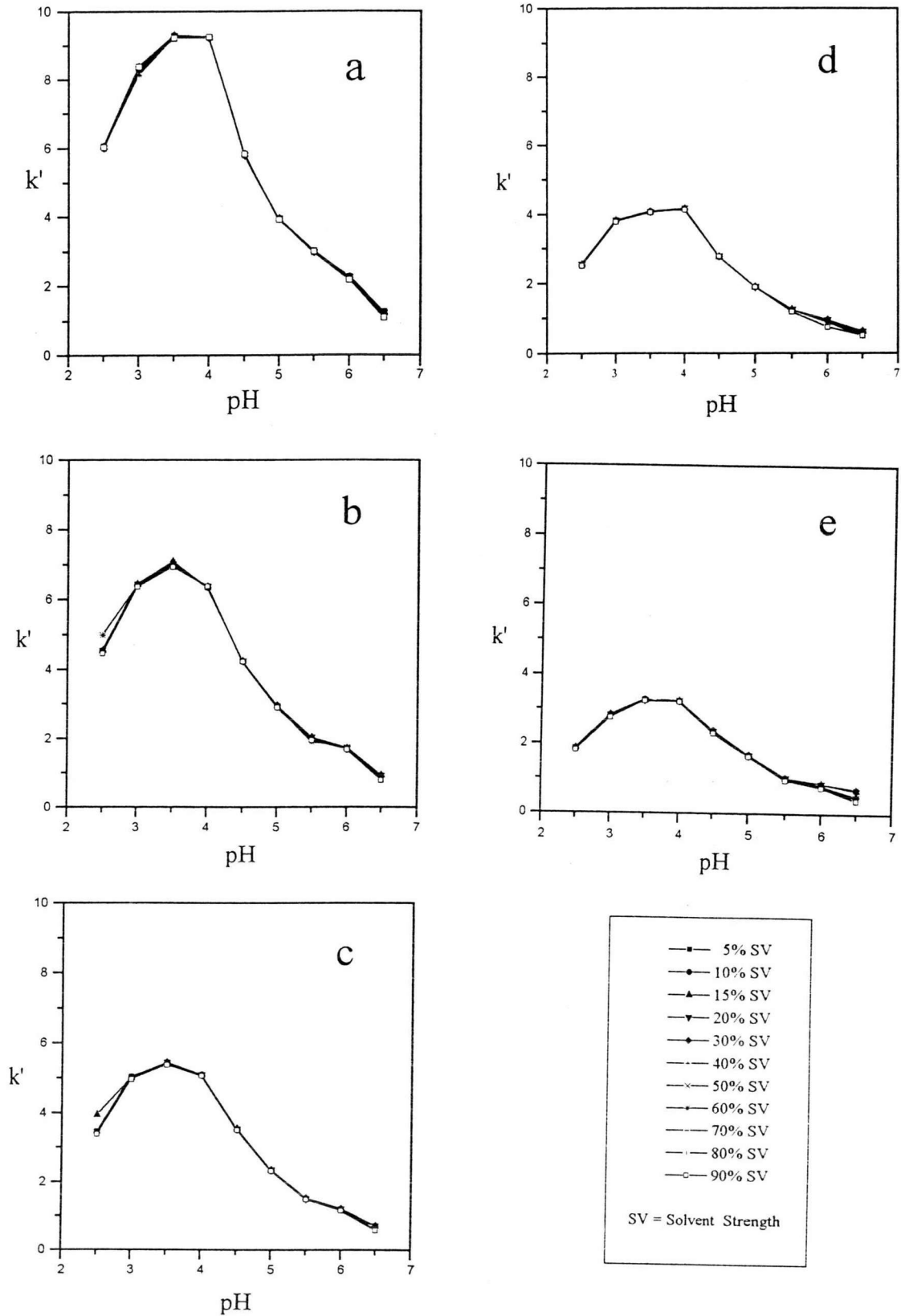
**Figure 4.21** Effect of eluent pH on capacity factors ( $k'$ ) of L-ascorbic acid by changing of pH. Chromatographic conditions as given in Figure 4.20.



**Figure 4.22** Effect of eluent pH on capacity factors ( $k'$ ) of benzoic acid by changing of pH. Chromatographic conditions as given in Figure 4.20.

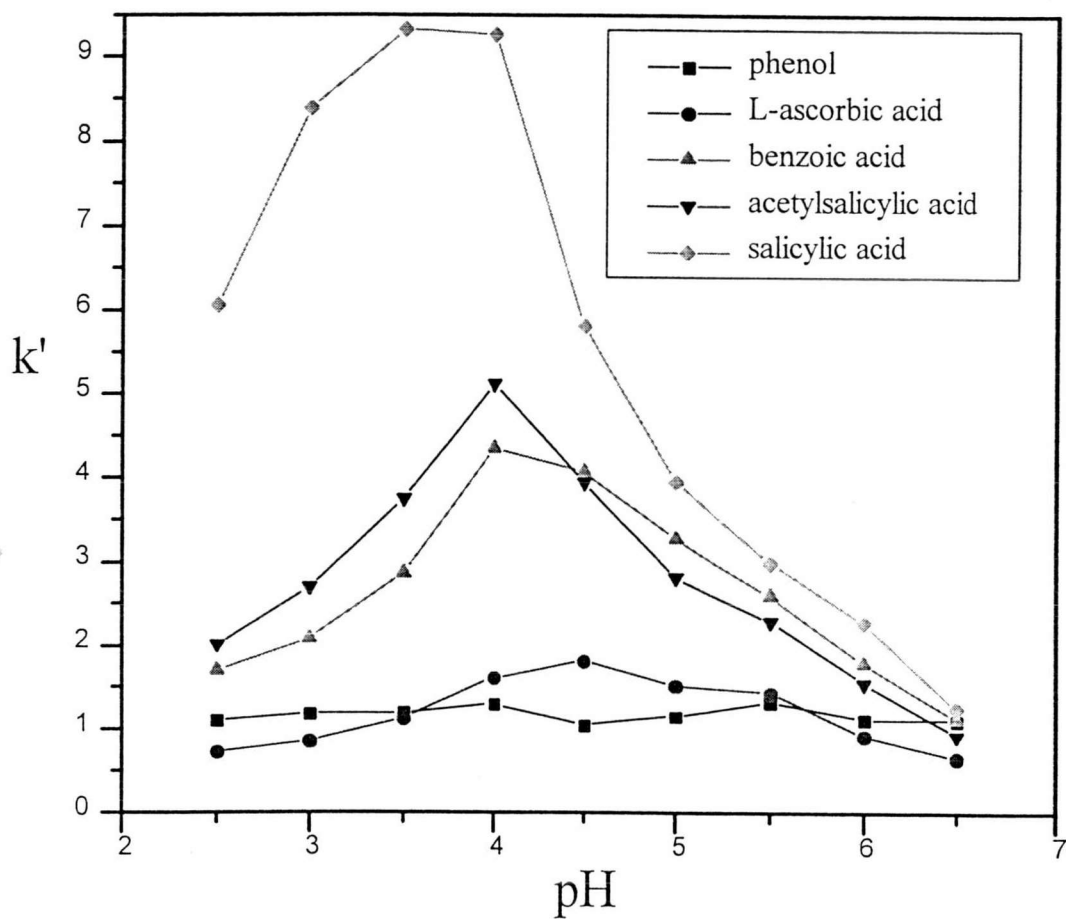


**Figure 4.23** Effect of eluent pH on capacity factors ( $k'$ ) of acetylsalicylic acid by changing of pH. Chromatographic conditions as given in Figure 4.20.

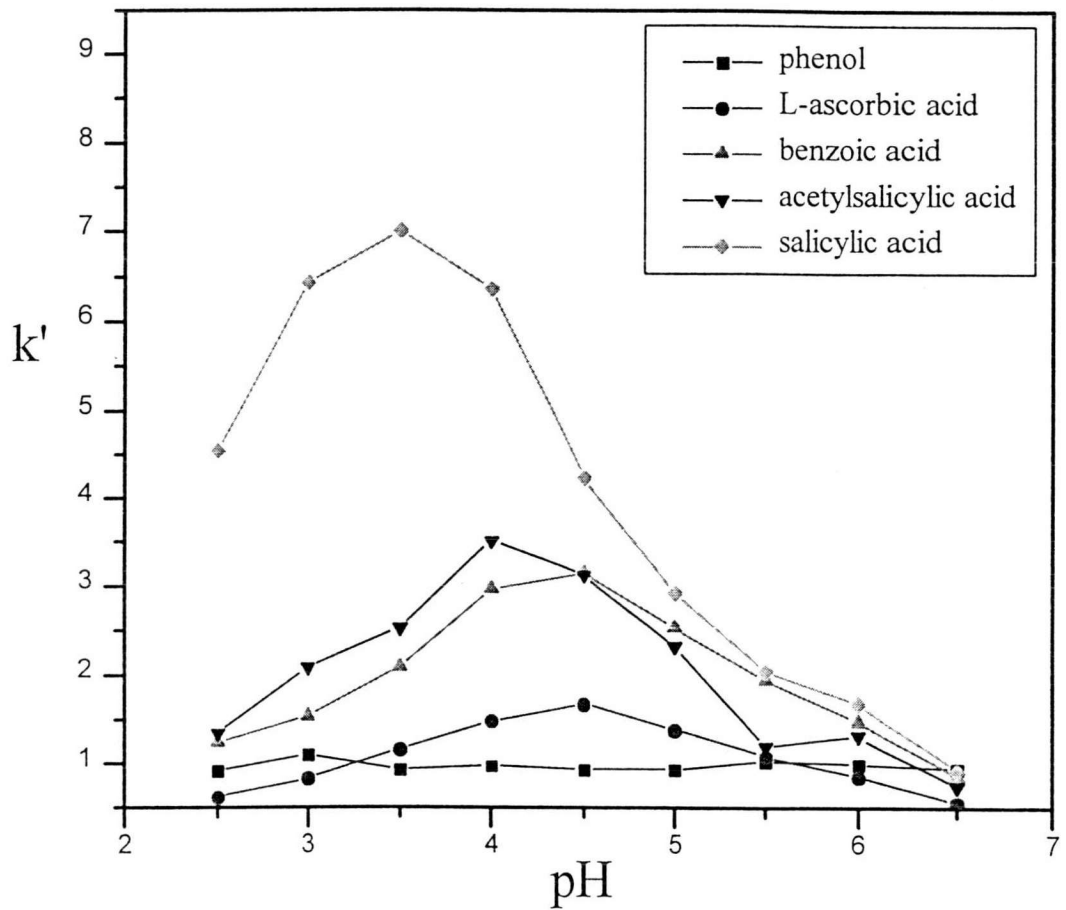


**Figure 4.24** Effect of eluent pH on capacity factors ( $k'$ ) of salicylic acid by changing of pH. Chromatographic conditions as given in Figure 4.35.

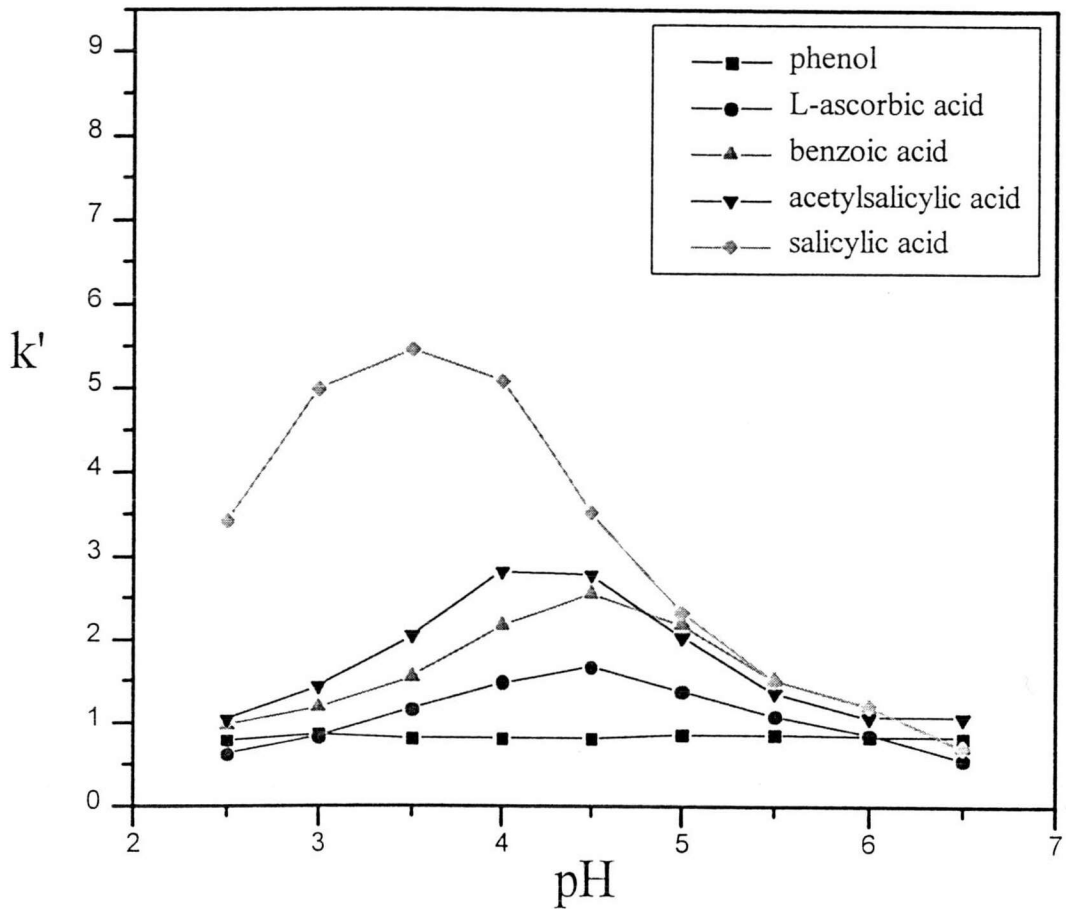




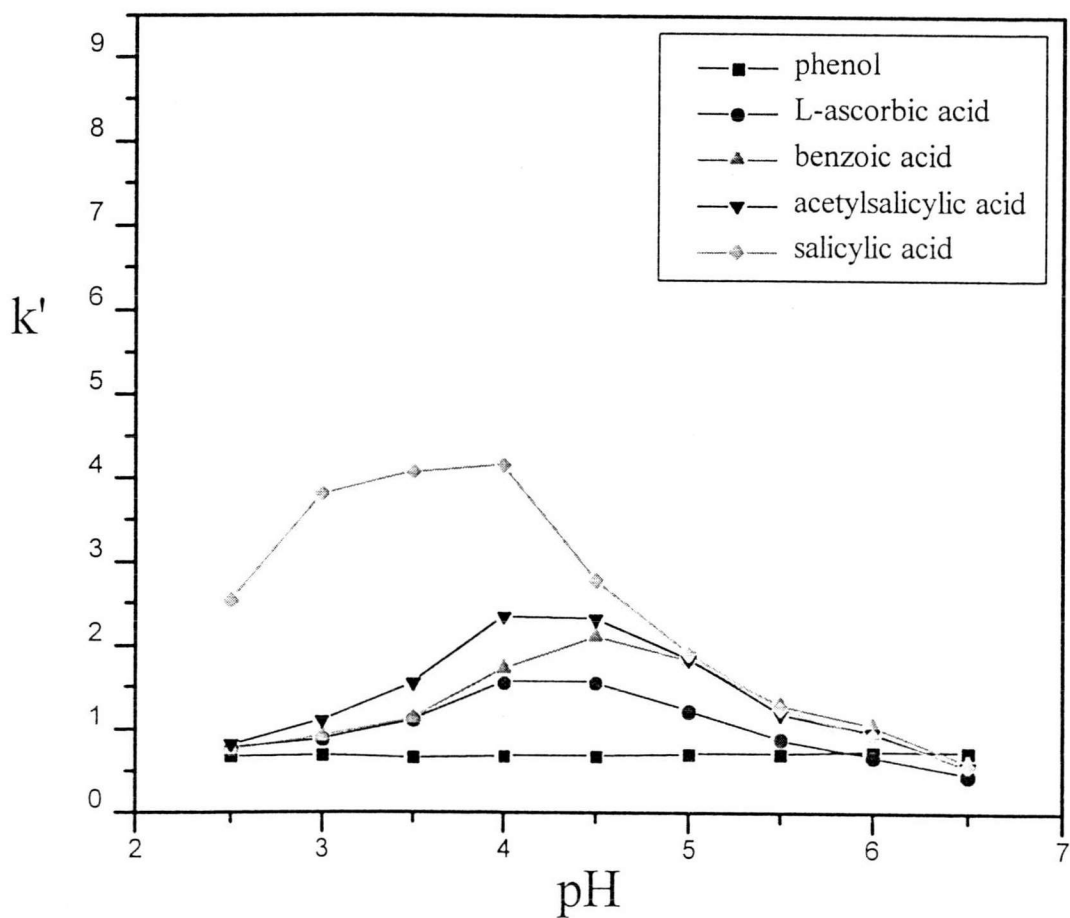
**Figure 4.25** Effect of eluent pH on capacity factors ( $k'$ ) by changing of pH. Stationary phase : phenylpropanolamine column, 5  $\mu\text{m}$ , 150 x 4.6 mm I.D. ; mobile phase : acetonitrile / 30 mM phosphate buffer (10:90, v/v) ; flow rate 1 ml/min. ; UV 254 nm.



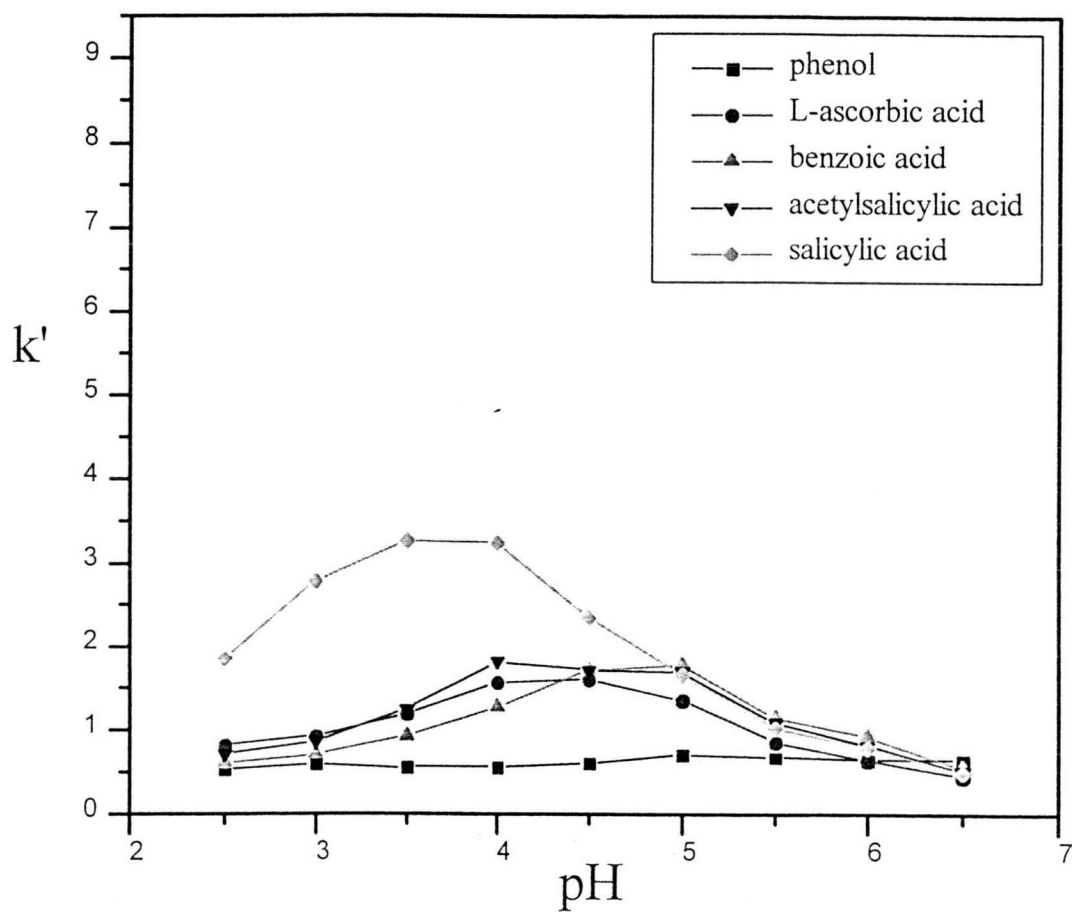
**Figure 4.26** Effect of eluent pH on capacity factors ( $k'$ ) by changing of pH. Stationary phase : phenylpropanolamine column, 5  $\mu\text{m}$ , 150 x 4.6 mm I.D. ; mobile phase : acetonitrile / 30 mM phosphate buffer (20:80, v/v) ; flow rate 1 ml/min. ; UV 254 nm.



**Figure 4.27** Effect of eluent pH on capacity factors ( $k'$ ) by changing of pH. Stationary phase : phenylpropanolamine column, 5  $\mu\text{m}$ , 150 x 4.6 mm I.D. ; mobile phase : acetonitrile / 30 mM phosphate buffer (30:70, v/v) ; flow rate 1 ml/min. ; UV 254 nm.



**Figure 4.28** Effect of eluent pH on capacity factors ( $k'$ ) by changing of pH. Stationary phase : phenylpropanolamine column, 5  $\mu\text{m}$ , 150 x 4.6 mm I.D. ; mobile phase : acetonitrile / 30 mM phosphate buffer (40:60, v/v) ; flow rate 1 ml/min. ; UV 254 nm.



**Figure 4.29** Effect of eluent pH on capacity factors ( $k'$ ) by changing of pH. Stationary phase : phenylpropanolamine column, 5  $\mu\text{m}$ , 150 x 4.6 mm I.D. ; mobile phase : acetonitrile / 30 mM phosphate buffer (50:50, v/v) ; flow rate 1 ml/min. ; UV 254 nm.

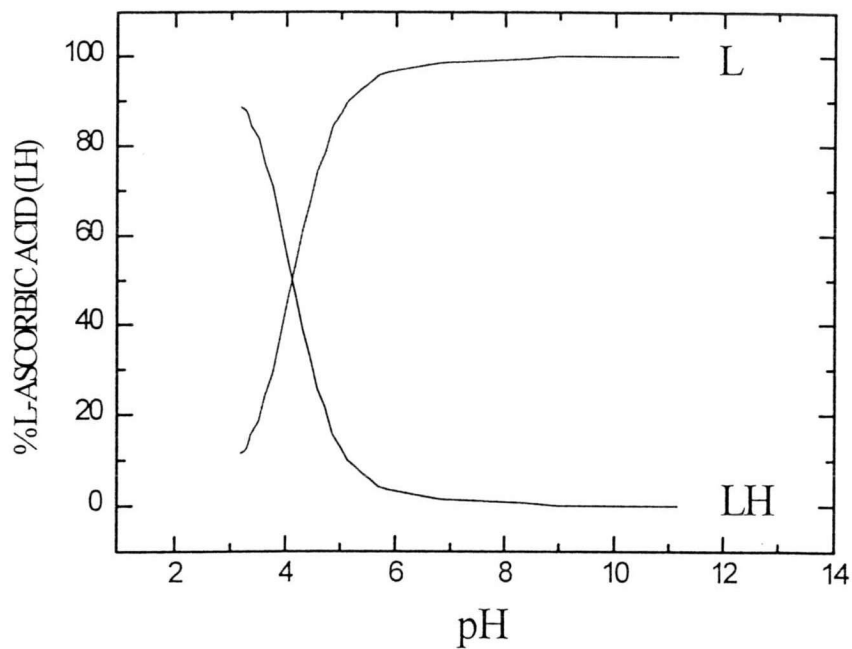
#### 4. Speciation study of L-ascorbic acid, benzoic acid, acetylsalicylic acid and salicylic acid by potentiometric method.

Speciation of L-ascorbic acid, benzoic acid, acetylsalicylic acid and salicylic acid were studied within a pH range of 2 to 11 by potentiometric titration. Superquad program [37] was used for evaluation of acidity constants of those acids. The species distribution of each system were constructed from acidity constant obtained corresponding experimental data. Data used in the computer refinement for studied compounds and their results are shown in Figure 4.30 to 4.33. Titration data are shown in Table 4.1. The data from computer refinement show that L-ascorbic acid, benzoic acid and acetylsalicylic acid have  $pK_a$  values near  $pK_a$  values in reference (39).

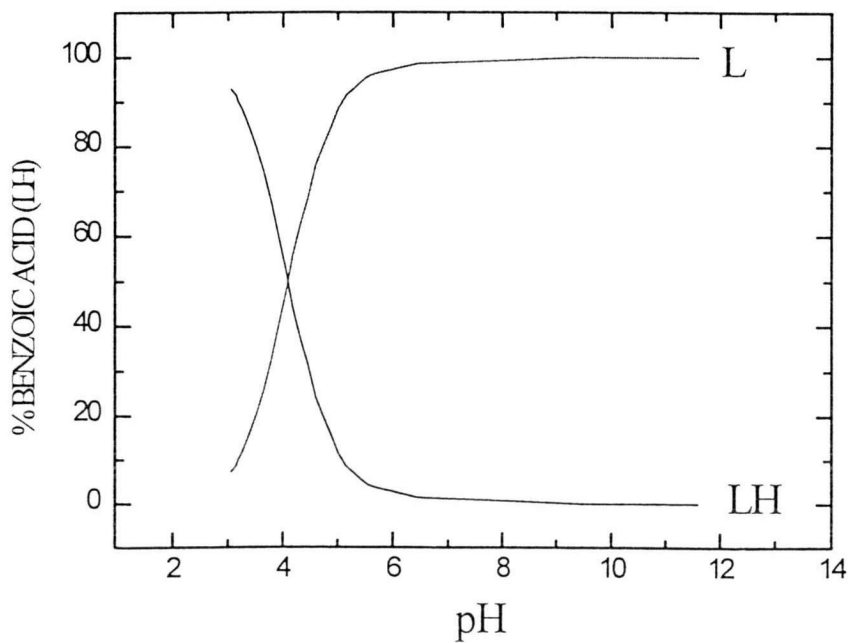
The chromatographic behavior of 10:90 (v/v) acetonitrile / 30 mM phosphate buffer may be considered by ionized and unionized species of tested compounds *i.e.* L-ascorbic acid, benzoic acid, acetylsalicylic acid and salicylic acid. The results show that at pH lower than  $pK_a$  produces more percent of unionized species than ionized species and major retention mechanism is hydrophobic interaction. whereas at pH higher than  $pK_a$ , percent of unionized species is less than ionized species which could be interacted with weak anion exchanger (secondary amino group) and increase ionic interaction.

**Table 4.1** Data used in the computer refinement for studied compounds and their results.

log K of acids	No.	initial concentration (mM)	pH range	data points	pKa from computer refinement	pKa from ref. 39
L-ascorbic acid	1	0.02	3.17-11.35	43	$4.36 \pm 0.07$	4.19
	2	0.02	3.17-11.35	44		
benzoic acid	1	0.02	3.07-11.59	39	$4.20 \pm 0.02$	4.21
	2	0.02	3.05-11.61	40		
acetylsalicylic acid	1	0.02	2.72-12.28	40	$3.76 \pm 0.07$	3.48
	2	0.02	2.72-11.61	40		
salicylic acid	1	0.02	2.31-11.51	43	$2.94 \pm 0.06$	3.00
	2	0.02	2.33-11.57	43		

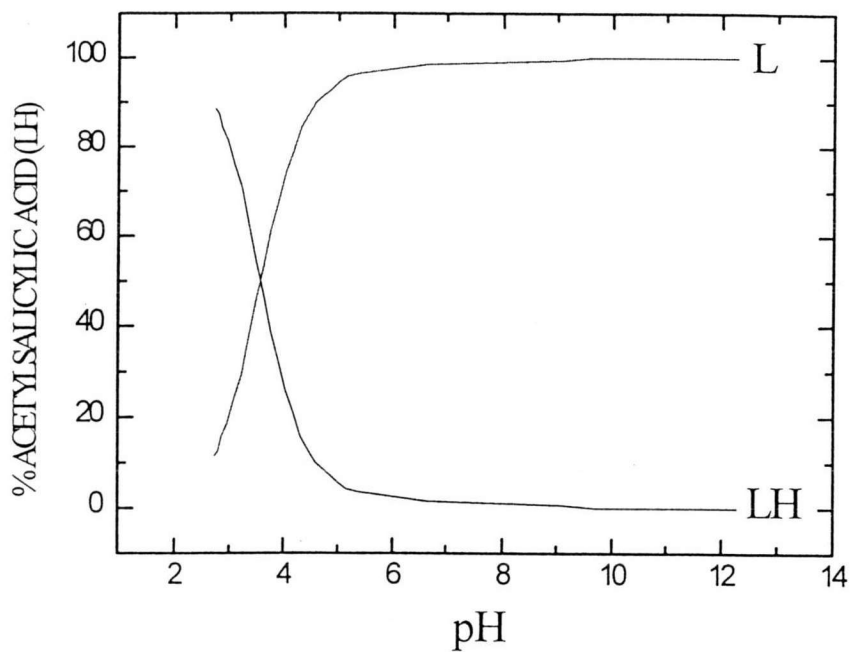


**Figure 4.30** Species distribution of L-ascorbic acid (LH) in 0.1M  $\text{KNO}_3$  of 10% acetonitrile solution at 25 °C

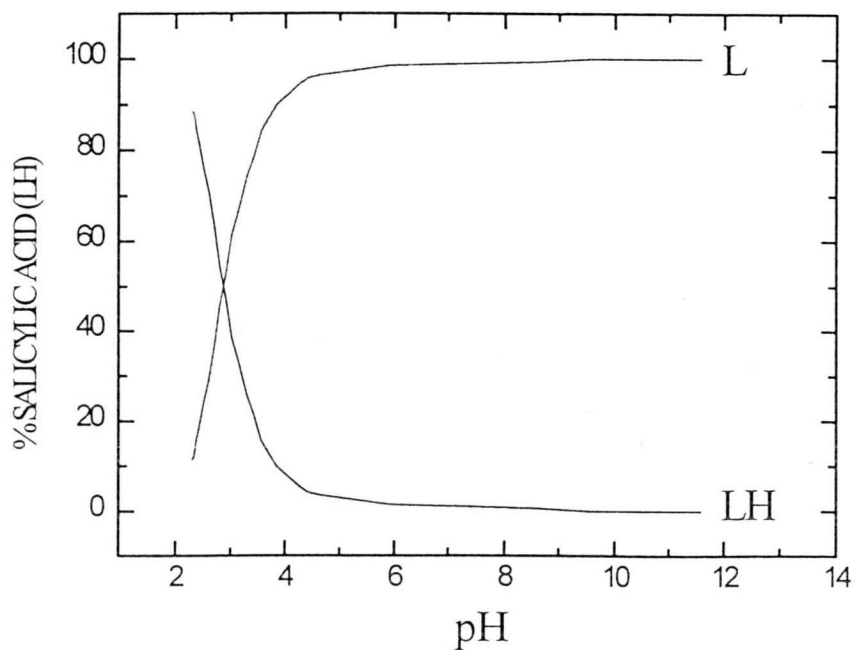


**Figure 4.31** Species distribution of benzoic acid (LH) in 0.1 M  $\text{KNO}_3$  of 10% acetonitrile solution at 25 °C





**Figure 4.32** Species distribution of acetylsalicylic acid (LH) in 0.1 M  $\text{KNO}_3$  of 10% acetonitrile solution at 25 °C



**Figure 4.33** Species distribution of salicylic acid (LH) in 0.1 M  $\text{KNO}_3$  of 10% acetonitrile solution at 25 °C