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RESEARCH ARTICLES

EFFECT OF SOLVENT STRENGTH ON THE CHROMATOGRAPHIC BEHAVIOR OF DRUGS IN REVERSED-PHASE HPLC

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ABSTRACT

The water content of the injected sample significantly influenced the chromatographic behavior of acidic, neutral and basic drugs on reversed-phase columns. The number of theoretical plates of each compound distinctly depends on the water concentration. Resolution of previously unresolved peaks could be achieved without change in retention. Application of this method to different medicament formulations are given.

INTRODUCTION

Reversed-phase high-performance liquid chromatography is an important mode for chromatographic separation of compounds by using the hydrophobic interaction mechanism.[1] A relatively unexplored and poorly understood phenomenon in this mode of chromatography is the peak distortions caused by the solvent used for injecting the test samples into the chromatogrphic column, which can significantly influence the elution of the compounds of interest.[2] A number of cases of such peak distortion or peak splitting caused by solvents has been documented: for example, acetaminophen, caffeine, acetylsalicylic acid, salicylic acid and phenacetin;[3] dihydroxybenzene;[4] bendroflumethiazide;[5] nadolol and captopril,[6] benzamides,[7] carotenoids[8] and aflatoxin M_1.[9] The phenomenon was mostly attributed to the solvent being "stronger" than the mobile phase, i.e., being able to elute the compounds of interest more faster than the mobile phase, and so decreasing the average capacity factor of the test samples during their elution.[10] However, a previous work has demonstrated analogous effect that gives better resolution and sensitivity of derivatized polyamines but without the change in the retention.[11] This is of interest as a mean of improving the separation capability and sensitivity in liquid chromatography. The aim of this work was to explore this effect for various underivatized drugs.

EXPERIMENTAL SECTION

Apparatus

The equipment consisted of a high-performance liquid chromatographic system (Model SP 8810 Isocratic pump, Spectra Physics, CA, U.S.A.; Spectra 100 UV-Vis deector, Spectra Physics, adjusted to 280 nm). The chromatograms were evaluated by means of a SP 4270 integrator (Spectra Physics). The stationary phase materials consisted of a prepacked Bio-Sil ODS-5s column, 5 μm, 250 mm×4 mm I.D. (Bio-Rad, Richmond, CA, U.S.A.) and a μ-Bondapak C_{18} column, irregular 10 μm, 300×3.9 mm I.D. (Waters, MA, U.S.A.). Both packing materials are porous silica with covalently bond n-octadecyl groups and endcapped. The optimized mobile phase was a mixture of methanol and water (60:40, v/v). The flow rate was adjusted to 1.0 ml/min. Injections were made using a 10 μl sample loop (Rheodyne valve 7125, Altech, U.S.A.).

Reagents

Phenol, p-nitrophenol, p-cresol, 4-chlorophenol, 2,4-dimethylphenol and 2,4,6-trimethylphenol were purchased from Sigma (St.Louis, MO, U.S.A.). Methanol (analytical grade) and hexane-1-sulfonic acid sodium salt were obtained from Merck (Darmstadt, Germany). Acetonitrile (HPLC grade) was supplied by May & Baker (Dagenham, England). Standards of acetaminophen, niacinamide, propylparaben, reserpine, ascorbic acid and diazepam were donated by the Government Pharmaceutical Organization of Thailand. Water was deionized and distilled.

Effect of solvent composition

A 100-ml methanolic stock solution of phenols contained 16 mg phenol, 5 mg p-nitrophenol, 10.6 mg p-cresol, 16 mg 4-chloro-phenol, 14 mg 2,4-dimethylphenol and 26 mg 2,4,6-trimethylphenol was prepared. Subsequently, 100 μl of the methanolic solution were mixed with various amounts of water (50 to 750 μl) and diluted to 1000 μl with methanol; and were employed as the sample solution. Different acidic, basic and neutral drugs were dissolved in either pure methanol or mobile phase and injected onto the reversed phase column. The number of theoretical plate (N) was calculated by the equation.

$$N = 16\ (t_R/W)^2$$

where t_R is the retention time and W is the peak width.

Applications

Different medicament formulations were analysed and the chromatographic conditions are depicted in Table 1.

TABLE 1. Chromatographic conditions for the various medicament formulations. The reversed-phase colum used is μ-Bondapak C_{18} (3.9 mm I.D.×30 cm)

Formulations	Mobille phase	Flow rate (ml/min)	Internal standard	Detection (nm)
Diazepam Tablets	methanol/water (65:35)	1.2	acetaminophen	254
Prednisolone cream	acetonitrile/ water (45:55)	1.0	propyl-paraben	239
Reserpine Tablets	methanol/water/ acteic acid (55:44:1)	1.2	propyl-paraben	267
Sulfadoxine and Pyrimethamine Tablets	dil. acetic acid (1 in 100)/ acetonitrile (80:20)	2.5	acetaminophen	254

The standard and assay preparations were prepared as follows:

Diazepam Tablets

Prepared standard and assay preparations as described in the literature[12] except propylparaben were used as an internal standard and the preparations were diluted to final volume with the mobile phase in place of methanol.

Prednisolone Cream

Internal standard solution- Transfer about 25 mg of propylparaben to a 100-ml volumetric flask, add methanol to volume, and mix.

Standard preparation- Transfer about 25 mg of standard prednisolone, accurately weighed, to a 100-ml volumeteric flask, add methanol to volume, and mix. Pipet 10 ml of this solution to a suitable stoppered vial, add 10.0 ml of Internal standard solution, and mix to obtain a solution having a known concentration of about 0.125 mg of prednisolone per ml.

Assay preparation- Transfer an accurately weighed portion of Prednisolone Cream, equivalent to about 2.5 mg of prednisolone, to a 50-ml centrifuge tube. Add 10.0 ml of the Internal standard solution and 10.0 ml of methanol. Insert the stopper into the tube, and place in a water bath held at 60°C until the specimen melts. Remove from the bath, and shake vigorously until the specimen resolidifies. Repeat the heating and shaking two more times. Place the tube in an ice bath for 20 minutes, then centrifuge to separate the phases. Decant the clear supernatant solution into a suitable stoppered flask, and allow to warm to room temperature.

Reserpine Tablets

Internal standard solution- Transfer about 10 mg of propylparaben to a 100-ml volumetric flask. Dissolve in methanol, dilute with methanol to volume, and mix well.

Standard preparation- Dissolve an accurately weighed quantity of standard reserpine in the mobile phase, and dilute quantitatively, and stepwise if necessary, with the mobile phase to obtain a solution having a known concentration of about 0.25 mg per ml. Pipet 2 ml of this solution and 2 ml of Internal standard solution into a 25-ml volumetric flask, dilute with the mobile phase to volume, and mix to obtain about 0.02 mg of reserpine per ml.

Assay preparation- Weigh and finely powder not less than 20 Reserpine Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1.25 mg of reserpine, to a 50-ml volumetric flask and add about 15 ml of the mobile phase. Dissove by sonication for 10 minutes, dilute with mobile phase to volume, and mix. Filter the solution, discarding the first 10 ml of the filtrate. Pipet 20 ml of the filtrate and 2 ml of Internal standard solution into a 25-ml volumetric flask, dilute with mobile phase to volume, and mix. Filter the solution through a 0.45 μm membrane filter and use the filtrate solution.

Sulfadoxine and Pyrimethamine Tablets

Proceed as direct for method in the literature[13] except acetaminophen was used as an internal standard.

RESULTS AND DISCUSSIONS

Fig. 1 shows chromatograms of standard mixtures of phenols. Under the optimized chromatographic conditions high resolution of the unresolved phenols (Fig. 1a) was achieved when an aqueous methanolic solution (40:60, v/v) was used as the solvent (Fig. 1b)

It is clearly demonstrated that the optimized dissolving solvent can increase both resolution capability and peak height of the phenols. Fig.2 shows the influence of varying water-to-methanol ratios on the chromatographic behaviors of the standard mixtures. The number of theoretical plates (N) of all compounds increases depending on the concentration of water in the injected sample and reaches a constant level at approx. 30% water. The solvent composition affects all compounds in the same way; increments in N of 8.4, 5, 2.2, 2.2, 1.8 and 1.3 times were obtained with p-cresol, phenol, 2.4-dimethylphenol, 2,4,6-trimethylphenol, 4-chlorophenol and p-nitrophenol, respectively. In all cases, peak fronting occurred when the compounds were dissolved in methanol. However, neither the retention time nor the capacity factor (k') of the investigated phenols were negatively affected. The effect of the solvent composition on peak area and peak height of the test compounds is demonstrated in Fig.3. Fig.3a shows increments in peak area of approximately 1.6 times for the phenols; increments in peak height of 1.6 times for phenol and 2,4,6-trimethylphenol, 1.8 times for p-nitrophenol and 2 times for p-cresol, 4-chlorophenol and 2,4-dimethylphenol in Fig.3b. In order to explore this peak compression phenomena for neutral and basic drugs, the two representative compounds, i.e. propylparaben and reserpine, were chromatographed on a reversed-phase column and the chromatograms are presented in Fig.4. Again, the effect is the same as that observed for the derivatized polyamines.[11] The opposite effect takes place for ascorbic acid in the separation of ascorbic acid and niacinamide

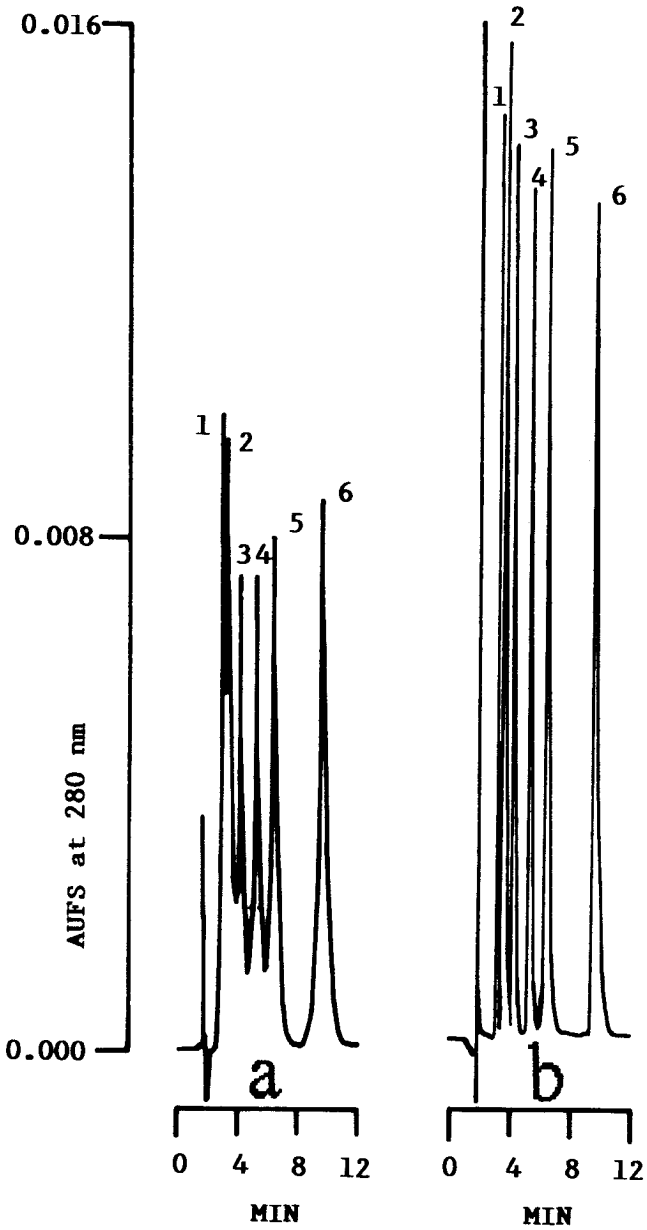


Fig.1 Chromatograms of standard mixtures of phenols on a reversed-phase column dissolved in either methanol (a) or water/methanol (b, 40:60 v/v). Peak identification : 1..phenol, 2...p-nitrophenol, 3...p-cresol, 4..4-chlorophenol, 5...2,4-dimethylphenol, and 6...2,4,6-trimethylphenol. Chromatographic conditions; column : Bio-Sil ODS-5s, mobile phase : methanol/water (60:40), flow rate 1.0 ml/min, detection at 280 nm.

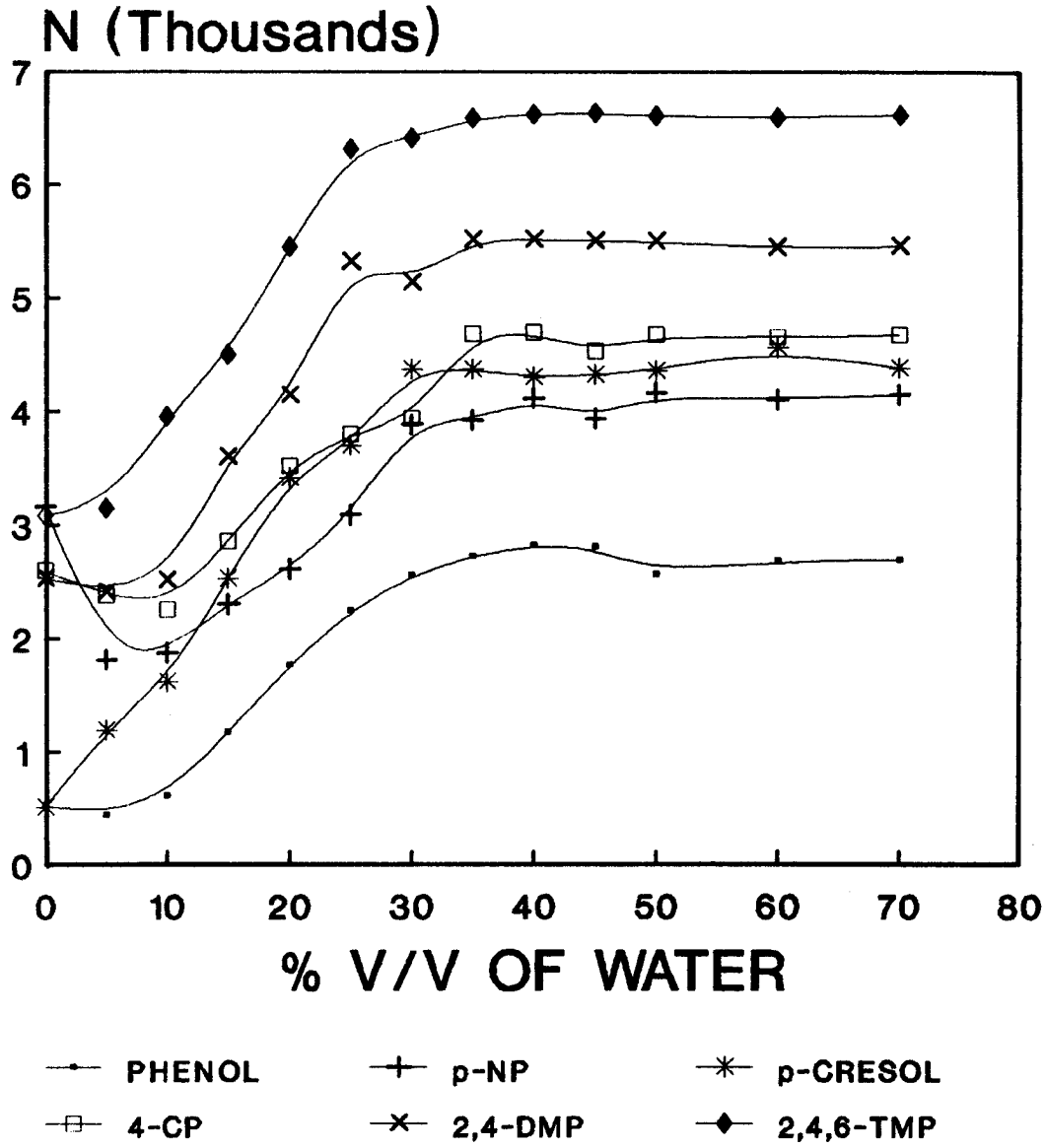


Fig.2 Effect of varying concentrations of water on the chromatographic behavior of phenols (Dependence of N on the water to methanol ratio). Chromatographic conditions as given in Fin. 1

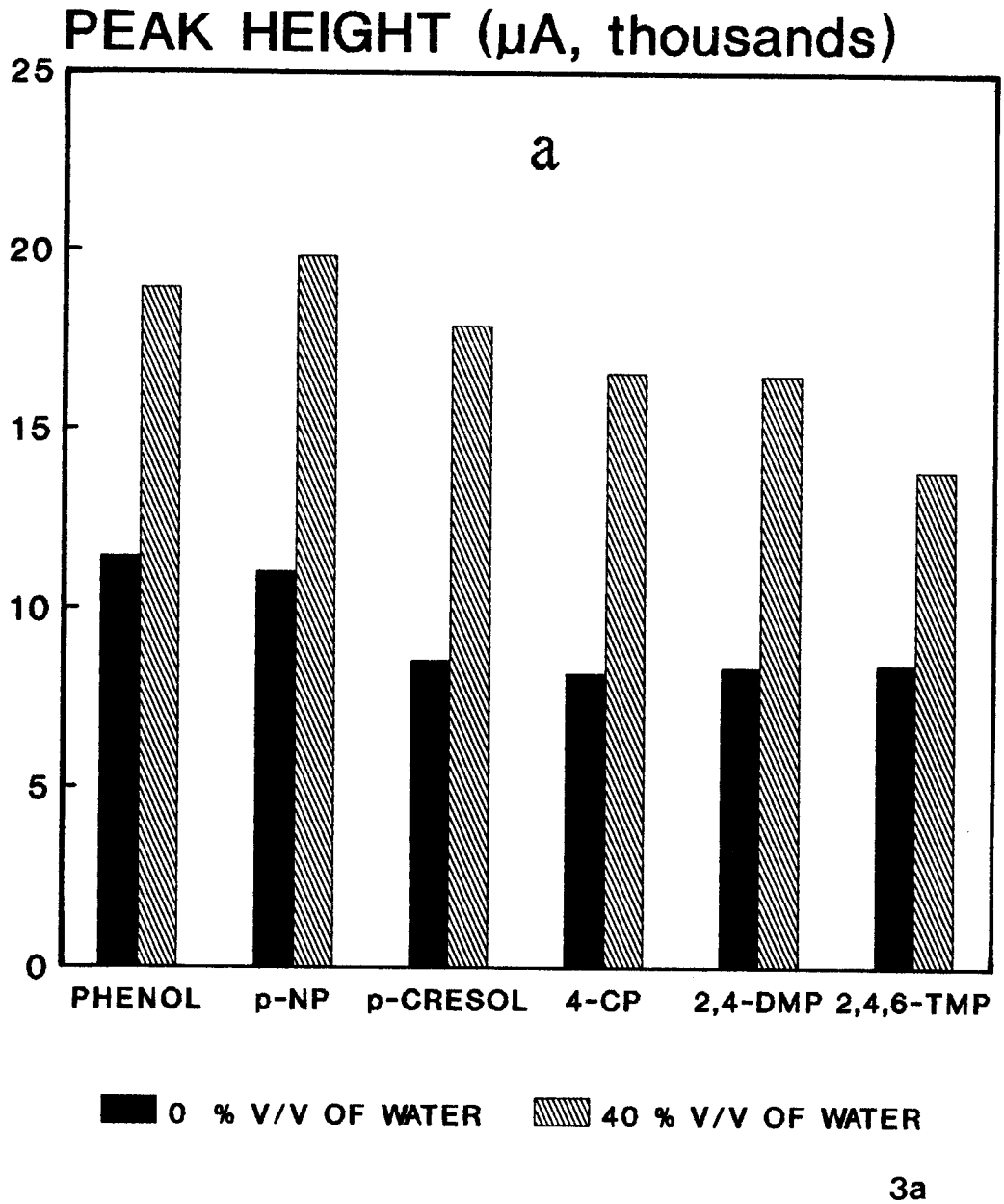
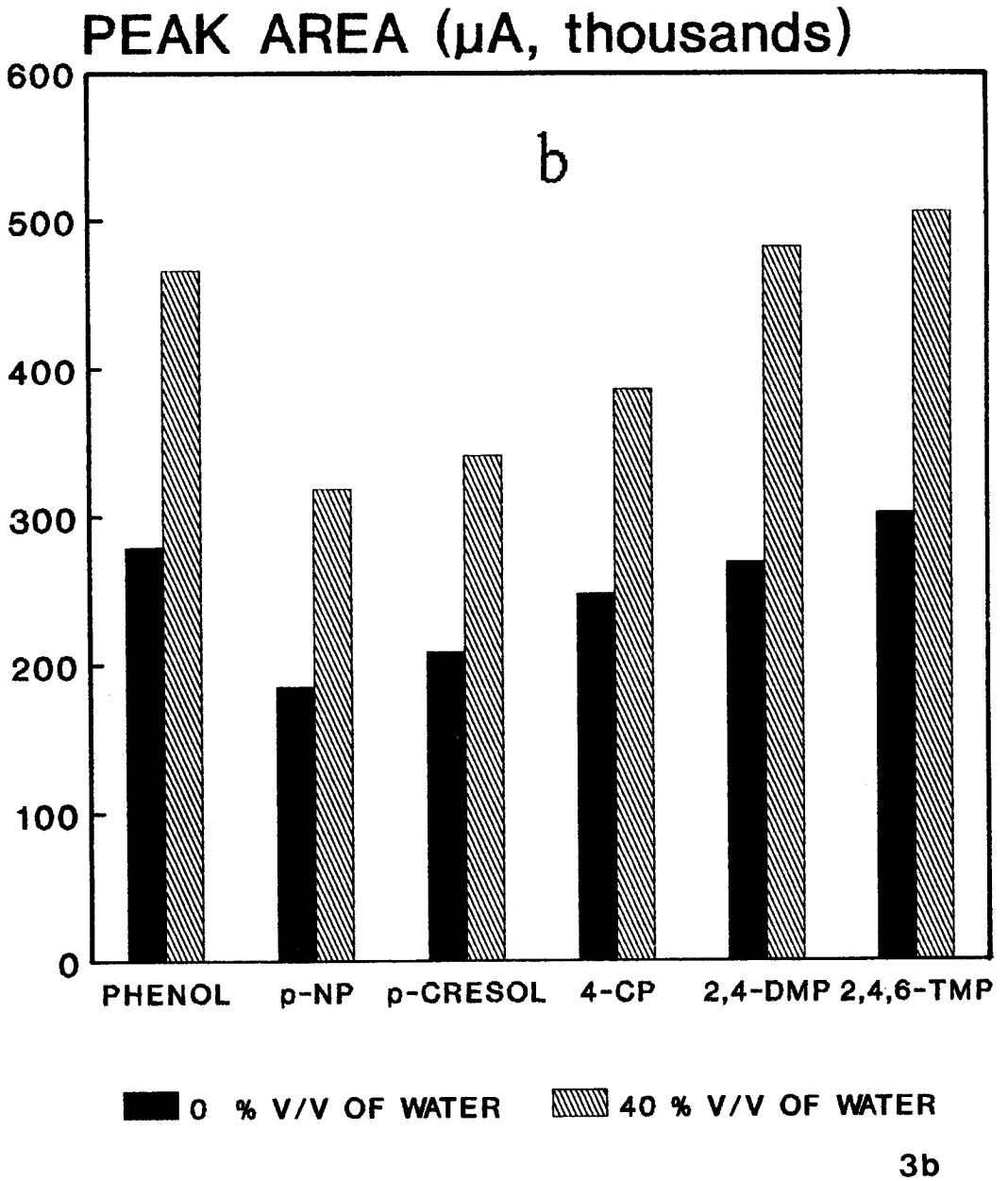


Fig.3 Effect of concentration of water in sample solvent on peak area (a) and peak height (b) of the phenols, dissolved in either methanol (0% V/V of water) or methanol/water (60:40, 40 %V/V of water). Chromatographic conditions as given in Fig.1.


PEAK AREA (μA, thousands)
600
500
400
300
200
100
0
b
PHENOL
p-NP
p-CRESOL
4-CP
2,4-DMP
2,4,6-TMP
0 % V/V OF WATER
40 % V/V OF WATER

3b

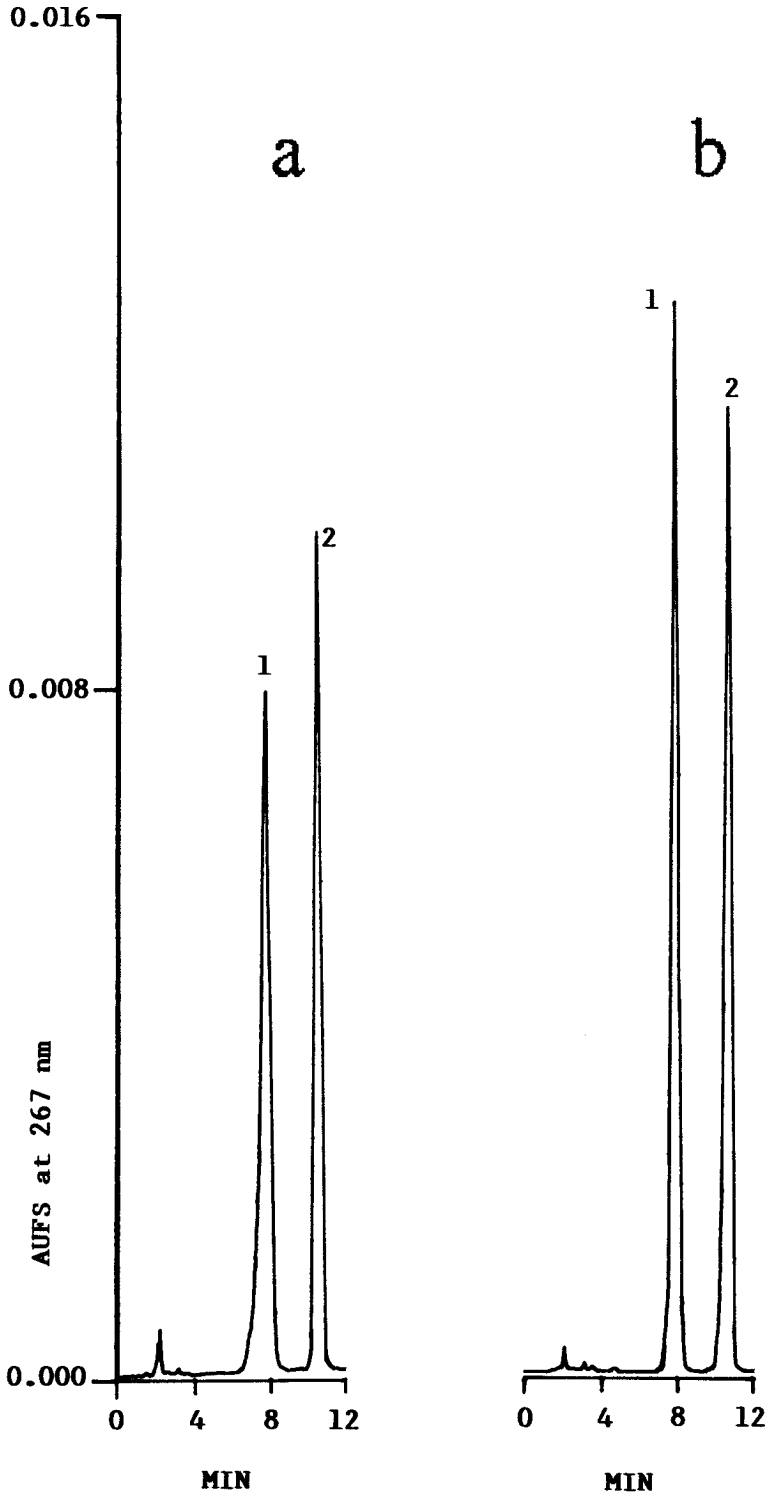


Fig.4 Chromatograms of standard mixtures of propyl paraben (1) and reserpine (2) dissolved in either methanol (a) or water/methanol (b, 45:55 v/v). Chromatographic conditions; column : μ-Bondapak C_{18}, mobile phase : methanol/water/acetic acid (55:44:1), flow rate 1.0 ml/min, detection at 267 nm.

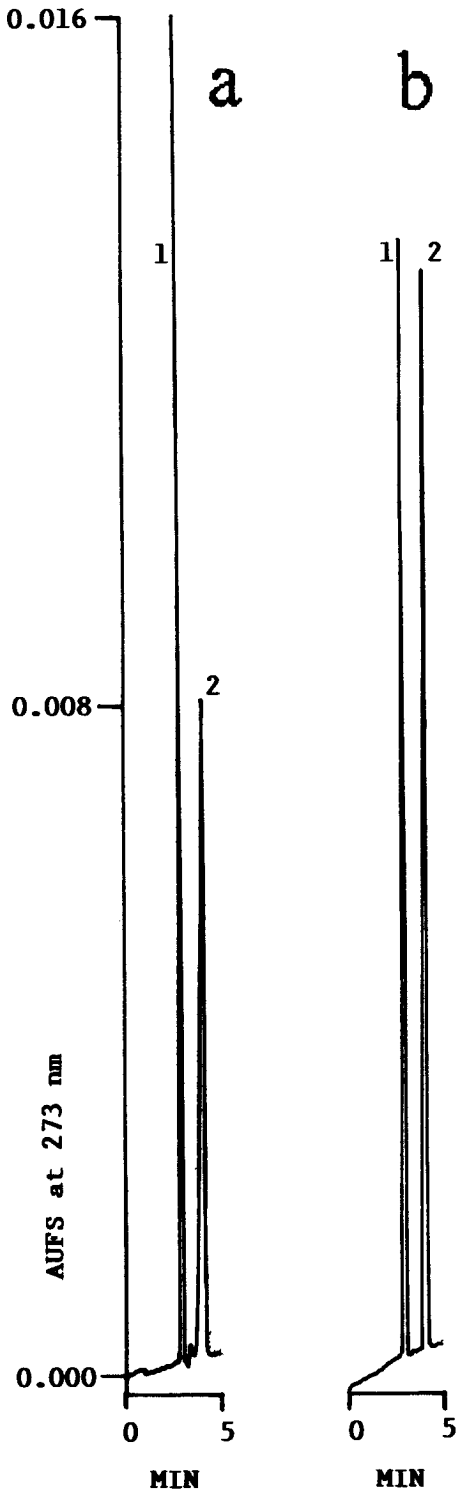


Fig.5 Chromatograms of standard mixtures of ascorbic acid (1) and niacinamide (2) on a reversed phase column dissolved in either methanol (a) or mobile phase. Chromatographic conditions; column : μ-Bondapak C_{18}, mobile phase : methanol/10 mM hexanesulfonate in 1% acetic acid (35:65, pH 2.50), flow rate 1 ml/min detection at 273 nm.

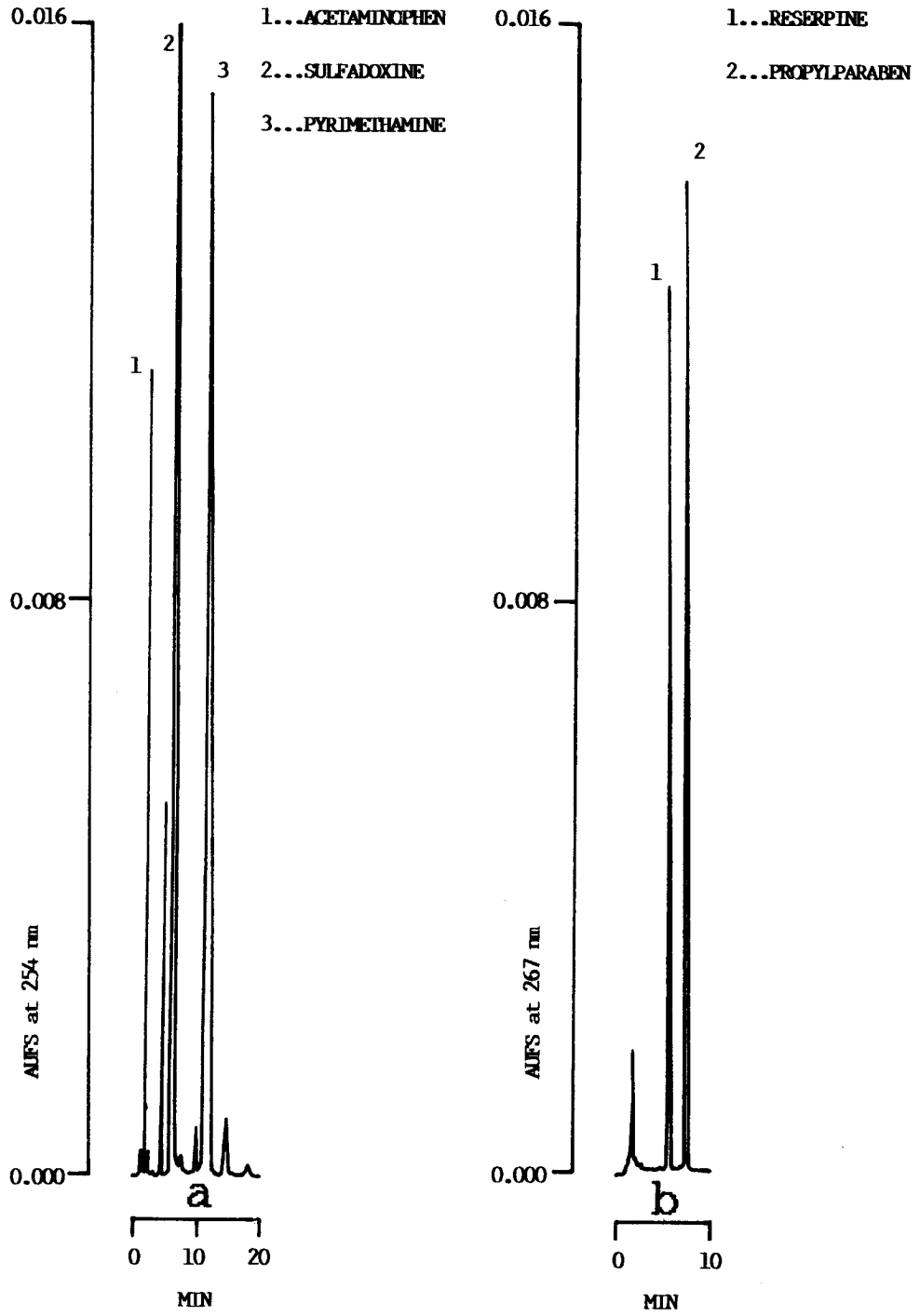
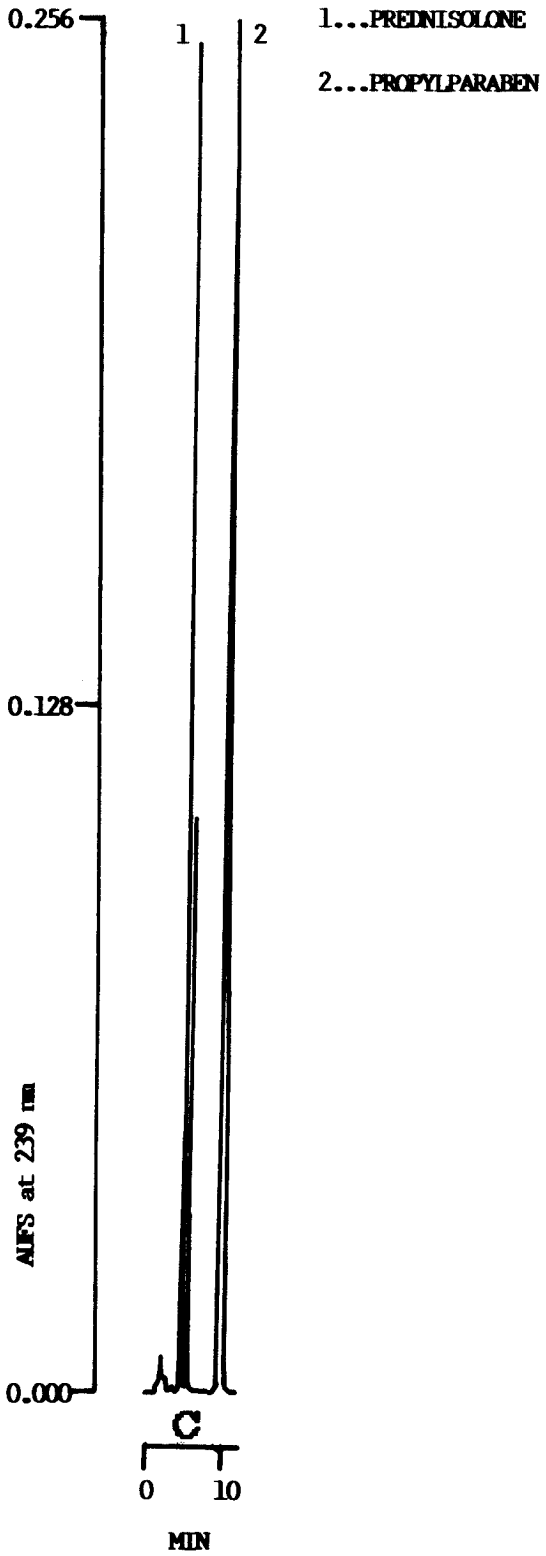
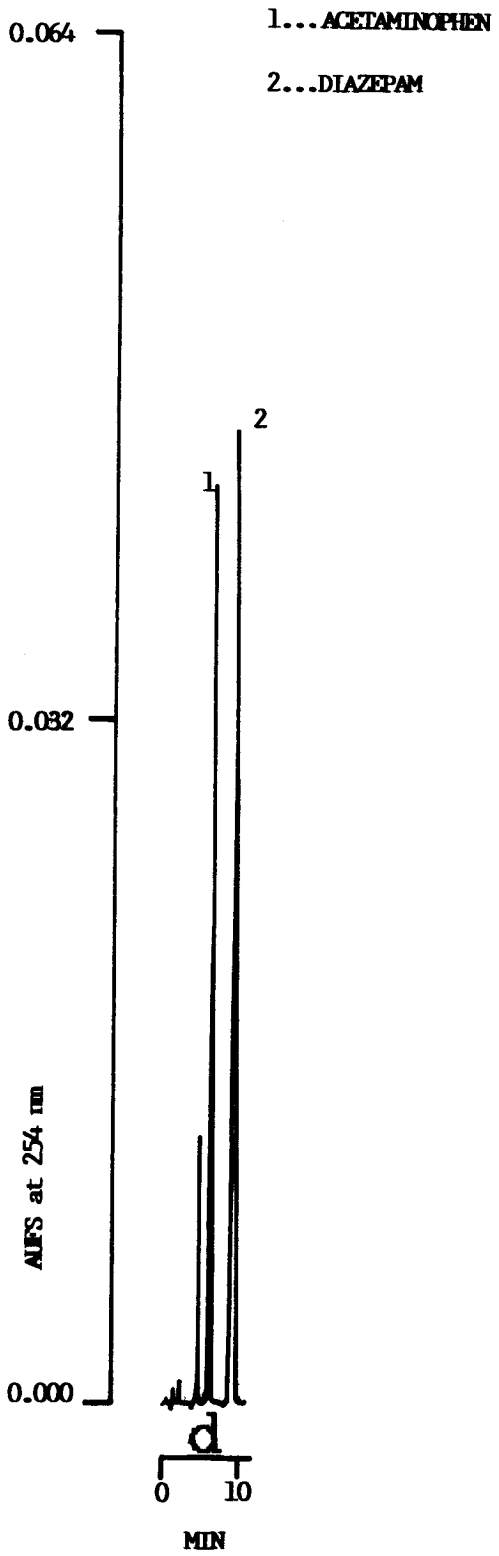


Fig.6 Chromatograms of different drug dosage forms (a) Sulfadoxine and Pyrimethamine Tablets, (b) Reserpine Tablets, (c) Prednisolone Cream and (d) Diazepam Tablets. Chromatographic conditions as given in Table 1


0.256
0.128
0.000
AUFS at 239 nm
1
2
1...PREDNISOLONE
2...PROPYLPARABEN
c
0
10
MIN


0.064
0.032
0.000
AUFS at 254 nm
1
2
1...ACETAMINOPHEN
2...DIAZEPAM
d
0
10
MIN

as shown in Fig.5. In Fig. 5a, methanol produces a positive effect for ascorbic acid peak when compared to Fig 5b. This phenomenon can be explained as ascorbic acid is a polar compound, and when elution was done on a reversed-phase column, the competing ionic interaction can also prevail like a normal phase mechanism, i.e. if the injecting solvent is weaker or less polar than the mobile phase, peak distortion occurred.[14]

The water-to-methanol ratio in the sample affects only resolution of the compounds. In all cases, the best resolution was achieved when the samples were dissolved in solvents of a strength similar to that of the mobile phase. Fig.1 clearly demonstrates the optimized solvent strength should not be more than 10% of that of the mobile phase. In case where the mobile phase can not be employed as the sample solvent, the sample should be dissolved in smallest volume of a suitable solvent which is compatible with mobile phase and diluted to final volume with chromatographic eluent. If the solvent strength is still much different from mobile phase, dilute solution of the sample should be prepared especially in case, where solubility is the main problem. Fig.6 shows chromatograms of different medicament formulations. All samples were diluted to final volume with the mobile phases except Prednisolone Cream which is extracted with methanol (polarity index = 6.6) in stead of the mobile phase (polarity index = 7.74). However, the chromatographic behaviour is not negatively affected.

CONCLUSION

The strength of dissolving solvents affects chromatographic peaks of acidic, neutral and basic drugs on reversed-phase columns. No change in retention time were observed and increment of theoretical plate is the advantage of this high resolution techniques for separation of different medicaments in formulations. The best result is to dissolve the samples in mobile phase prior to injection.

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บทคัดย่อ

อัตราส่วนของน้ำในตัวทำละลายสำหรับสารตัวอย่างมีผลต่อลักษณะของ peak ของตัวยาที่มีคุณสมบัติเป็นกรด กลาง หรือเบส เป็นอย่างมากเมื่อแยกสารเหล่านี้โดยใช้คอลัมน์ชนิด reversed-phase กล่าวคือจำนวน plate ทางทฤษฎีเพิ่มสูงขึ้นตามความเข้มข้นของน้ำและจะคงที่เมื่ออัตราส่วนของน้ำเท่ากันกับใน mobile phase สามารถนำเทคนิคนี้ไปแยกตัวยาต่าง ๆ ออกจากกันได้เป็นอย่างดีโดยที่ retention ไม่เปลี่ยนแปลง ดังตัวอย่างการประยุกต์ใช้ในการวิเคราะห์ยาหลายชนิดในตำหรับ