

Determination of Free Catecholamines in Urine by Direct Injection onto a Shielded Hydrophobic Phase Column

D. J. Wang¹ / Y. Qu¹ / P. Hu² / P. L. Zhu^{1*}

¹Department of Chemistry, Lanzhou University, Lanzhou, P. R. China

²Department of Chemistry, Northwestern Normal University, Lanzhou, P. R. China

Key Words

Column liquid chromatography
Shielded hydrophobic phase
Catecholamine in urine
Direct sample injection

Summary

A direct sample injection approach for the determination of free catecholamines in urine has been developed. The chromatographic separation is carried out on a 150 × 4.6 mm HisepTM shielded hydrophobic phase column, where proteins in biological fluids are not retained. The mobile phase consists of 0.03 M sodium citrate, 0.004–0.007 M sodium dodecylsulfate (SDS), 3 % n-propanol and 1 mM EDTA with pH 4.2. Amperometric detector is used, the working electrode of which is set at 0.35 V (vs. Ag-AgCl). Norepinephrine, epinephrine and dopamine have linear response ranges of 8 ppb–10 ppm, 5 ppb–10 ppm and 2 ppb–10 ppm, respectively. The analytical results obtained from eight urine samples are consistent with those in the references.

Introduction

A significant application of HPLC is to analyse endogenous substances, therapeutic drugs, and their metabolites in biological fluids, such as urine, serum and plasma. Since proteins existing in them can result in rapid deterioration of the chromatographic column, samples must undergo time-consuming pretreatment before injection to remove the proteins. This has led to more attention paid to direct sample injection techniques. Since the middle of the 1980s two approaches have been developed to avoid pretreating samples of biological fluids: micellar chromatography [1] and special stationary phases [2, 3].

Surfactants have the ability to solvate proteins in aqueous solutions. Under the circumstance of micellar chromatography analytes can be absorbed onto the stationary phase while proteins still remain in the mobile phase containing surfactants above the critical micelle concentration [1].

Some packing materials were prepared specially for direct injection analysis of biological fluids, including internal-surface reversed-phase (ISRP) [2] and shielded hydrophobic phase (SHP) [3]. Silica gel with 80 Å pore diameter is used as the support of ISRP. The diol-Gly-Phe-Phe internal bonded phase can retain analytes in terms of hydrophobic interaction, but proteins excluded from the internal region of the support are not absorbed by the diol external surface. The SHP packing consists of a polymeric bonded phase containing hydrophobic regions enclaved by a hydrophilic network and is similar to ISRP in the characters to exclude proteins and interact with small molecules.

The analysis of biogenic amines in biological fluids provides important informations for clinical diagnosis. The intension of this work is to develop a method for the determination of free catecholamines in urine by direct injection onto the SHP column. Several types of HPLC were used for this purpose [4]. In this lab micellar chromatography was examined to analyse catecholamines in urine. However, the retention of both proteins and analytes decrease simultaneously, due to the existence of the micelle; therefore, only the determination of dopamine in urine is possible which has stronger retention. On the SHP stationary phase it would be possible to determine simultaneously catecholamines in urine under selected chromatographic conditions, ignoring their influence on the retention of proteins.

Experimental

The chromatographic experiments were performed on a Model LC-4A liquid chromatograph equipped with a L-ECD-6A electrochemical detector (Shimadzu, Kyoto, Japan). The injection valve had a 10-μl loop. A Type R-112M recorder (Shimadzu, Kyoto, Japan) was connected to the 1 mV output of the detector. A 150 × 4.6 mm HisepTM SHP column (Supelco, Bellefonte, PA, USA) was used as the analytical column. A precolumn (50 × 4.6 mm) packed with silica gel (15–25 μm) was placed between the pump and the injection valve to protect the analytical column. The chromatographic experiments were carried out at 25 °C.

The chemicals used were obtained from a variety of supplies. The standard solutions of norepinephrine (NE), epinephrine

(E) and dopamine (DA) were prepared according to the following procedure. Dissolve 0.1000 g of the individual substances in 0.05 M hydrochloric acid in a 100-ml brown volumetric flask. Store the standard solutions at 4 °C, away from light. Dilute them with 0.05 M HCl to appropriate concentrations before use.

Collection and Pretreatment of the Urine Sample

Catecholamines are easy to be oxidized and sensitive to air and light, especially in an alkaline medium. A complete 24-hour urine sample was collected in a 2000-ml brown bottle. 15 ml of 6N HCl was added as the stabilizer. 25 ml urine sample was left for use. After the total volume was measured, a 25-ml aliquot of the urine was filtrated through a G5 (2–5 μm) sintered glass funnel to remove any deposit present. The filtrate was adjusted to about pH 3 with 6N HCl and stored at 4 °C.

Results and Discussion

Mobile Phase

Catecholamines are retained by SHP in terms of hydrophobic interaction. The composition of the mobile phase containing ion-pair reagent, buffer and organic solvent was adjusted in order that the retention of the catecholamines should be within a reasonable range and a high column efficiency should be obtained.

Effect of pH

The mobile phase consisting of 0.05 M sodium citrate, 0.01 M sodium dodecylsulfate and 3 % n-propanol was adjusted with HCl to the different pH values. The variation of the capacity factors (k') of the catecholamines with the pH of the mobile phase is shown in Figure 1. With the catecholamines being weak bases, their protonation and the formation of ion pairs are weakened with increasing pH value. As a result, there is a decline in k' values as the pH of the mobile phase increases.

At a higher acidity the competition between H^+ and protonated catecholamines for the anion of SDS to form an ion pair also leads to a small decrease in the value of the capacity factor.

Effect of SDS Concentration

The existence of SDS in the mobile phase results in an increase of the capacity factors of catecholamines, due to the formation of ion pairs. In micellar chromatography a surfactant concentration above the critical micelle concentration must be kept in the mobile phase for proteins not to be retained by the stationary phase. The high micelle concentration in the mobile phase can also reduce the retention of catecholamines, resulting in the difficulty to resolve solute peaks which have low k' values from those of proteins. When ISRP or SHP is used, with proteins excluded outside the pores of the packing, the surfactant concentration can be independently adjusted to optimize the retention and resolution of solutes. Figure 2 shows how the retention

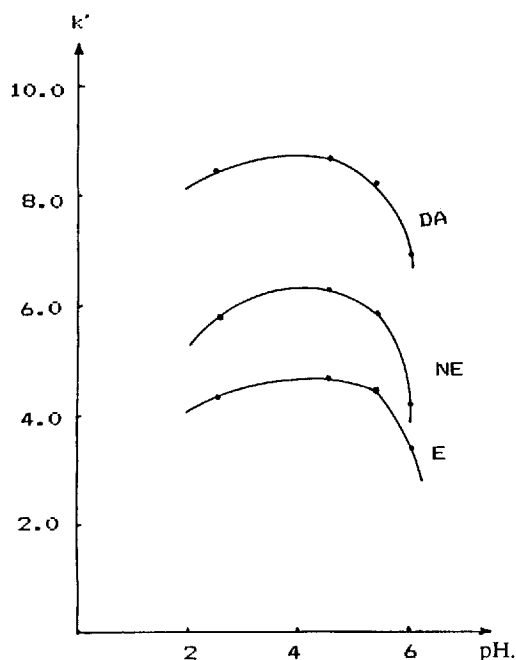


Figure 1

Capacity factors (k') of catecholamines plotted against the pH. Mobile phase: 0.01 M SDS, 0.05 M sodium citrate and 3 % n-propanol. NE = norepinephrine; E = epinephrine; DA = dopamine.

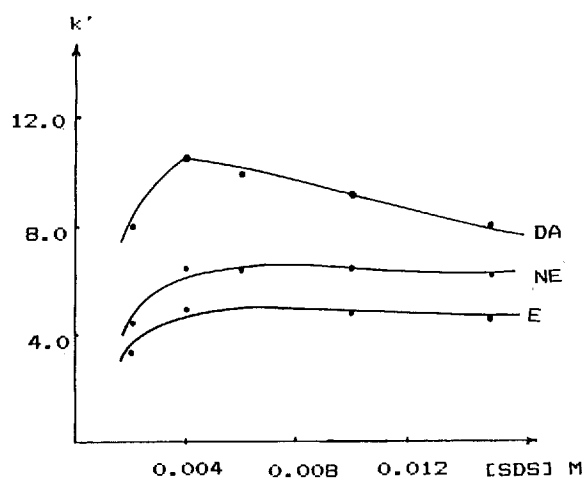


Figure 2

Capacity factor (k') of catecholamines plotted against the SDS concentration.

Mobile phase: SDS, 0.05 M sodium citrate and 3 % n-Propanol; pH 4.25.

NE = norepinephrine; E = epinephrine; DA = dopamine.

of catecholamines varies with the SDS concentration. A concentration range of 0.004–0.01 M SDS is appropriate for the separation of catecholamines.

Effect of Ionic Strength

The variation of k' with the sodium citrate concentration at a given pH value is shown in Figure 3. The solute retention decreases with increasing sodium citrate concentration.

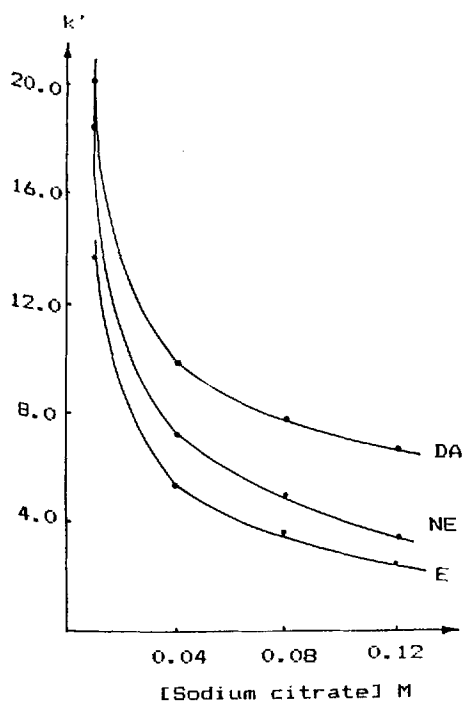


Figure 3
Capacity factor (k') of catecholamines plotted against the sodium citrate concentration.
Mobile phase: 0.008 M SDS, sodium citrate and 3 % n-propanol; pH 4.0.
NE = norepinephrine; E = epinephrine; DA = dopamine.

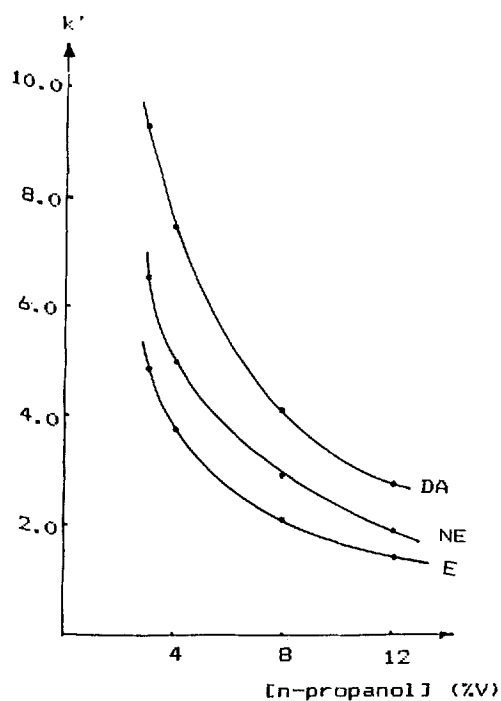


Figure 4
Capacity factor (k') of catecholamines plotted against the n-propanol concentration.
Mobile phase: 0.008 M SDS, 0.05 M sodium citrate and n-propanol; pH 5.4.
NE = norepinephrine; E = epinephrine; DA = dopamine.

The observed result is consistent with the way how the existence of sodium citrate in the mobile phase influences the activity coefficient of the solutes. Various buffer systems, such as sodium citrate, potassium citrate, ammonium acetate and sodium dihydrogen phosphate, were examined. Among them sodium citrate can give the best resolution for the separation of catecholamines.

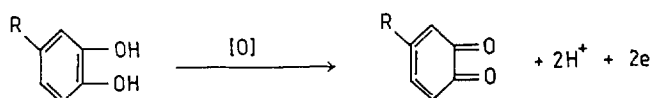
Effect of Organic Solvent

The organic solvent in the mobile phase plays a role in both increasing the eluent strength and improving solute mass transfer. As shown in Figure 4 the retention of catecholamines decreases with increasing the concentration of n-propanol.

Balancing all of the factors, an eluent was chosen which consisted of 0.03 M sodium citrate, 0.005–0.007 M SDS, 3 % n-propanol and 1 mM EDTA with pH 4.2. If an electrochemical detector is used, a small amount of EDTA will exist in the mobile phase to restrain the noise caused by the presence of trace metal impurities.

Conditions of Detection

In view of its sensitivity and selectivity, it is usual to use an electrochemical detector for the chromatographic separation of catecholamines the electrode reaction of which is the following:



The response produced by the oxidation of the catecholamine on the electrode increases with an increase of the potential of the working electrode. However, at a potential above 0.4 V (vs. Ag-AgCl) some of the components in the urine might be oxidized at the working electrode and interfere with the catecholamine determination. Thus the potential of the working electrode is set at 0.35 V (vs. Ag-AgCl). Under this circumstance the interfering peaks will disappear and simultaneous determination of catecholamines becomes possible. The chromatograms shown in Figure 5 were obtained at different potentials of the working electrode. It can be seen that at a higher potential the peaks of norepinephrine and epinephrine are seriously interfered.

Calibration Curve and Detection Limit

Under the specified chromatographic conditions the peak heights were plotted against the concentrations of NE, E and DA, to give the calibration curves shown in Figure 6. The linear ranges of NE, E and DA are 8 ppb–10 ppm, 5 ppb–10 ppm and 2 ppb–10 ppm, respectively, the detection limits being 0.08 ng, 0.05 ng and 0.02 ng (Table I).

On the basis of the calibration curves, catecholamines were added into the urine samples to calculate recoveries of NE, E and DA, giving the values of 105.2, 106.8 and 108.2 % with relative standard deviation values of 3.6, 3.0 and 2.4 %, respectively. Figure 7 shows the chromatograms obtained before and after adding the standard solutions.

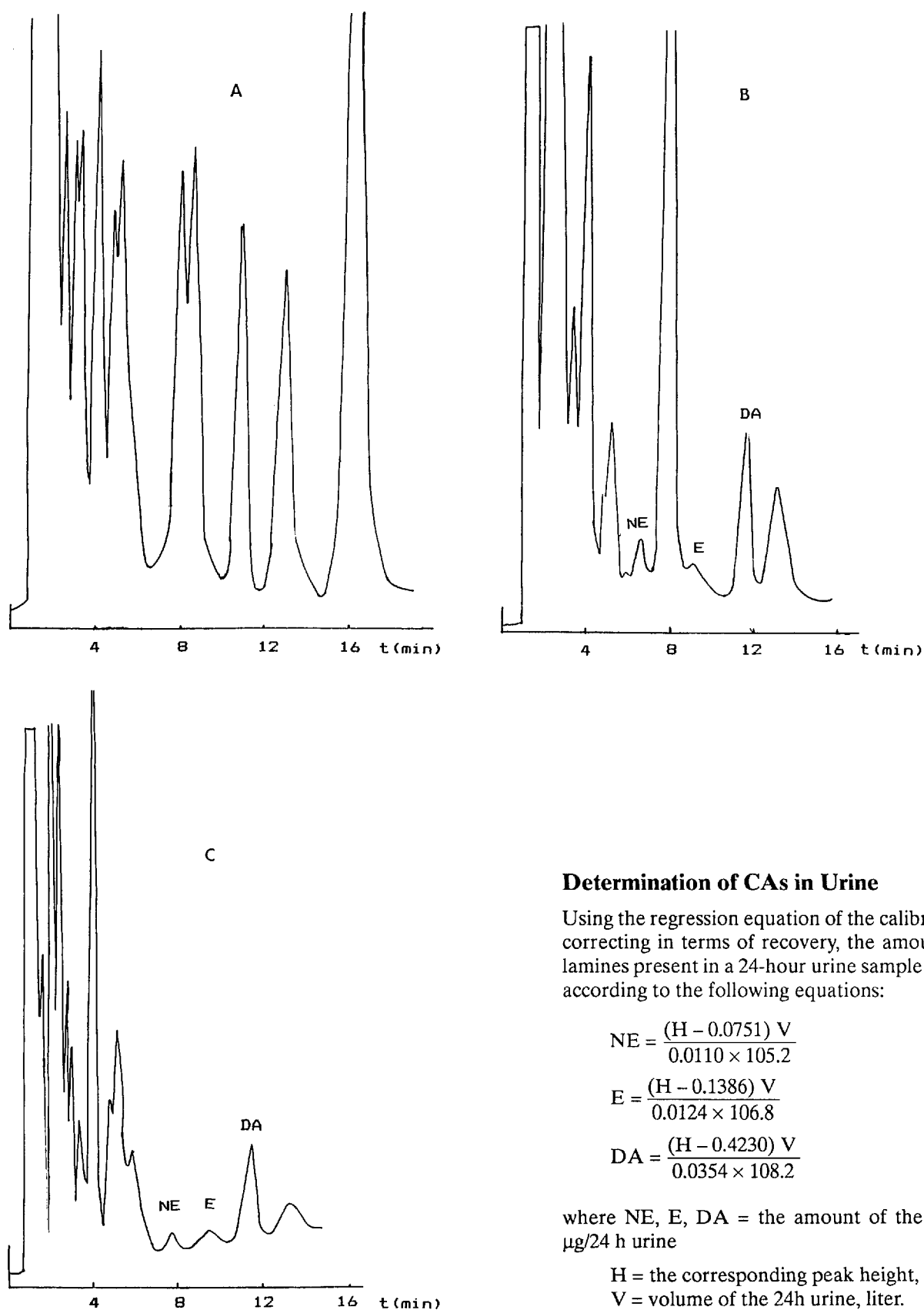


Figure 5
 Chromatograms of catecholamines obtained at different potentials of the working electrode.
 Mobile phase: 0.007 M SDS, 0.03 M sodium citrate, 3 % n-propanol and 1 mM EDTA; pH 4.2. Amperometric detector: (a) +0.55 V, (b) +0.40 V, (c) +0.35 V (vs. Ag-AgCl).
 NE = norepinephrine; E = epinephrine; DA = dopamine.

Determination of CAs in Urine

Using the regression equation of the calibration curve and correcting in terms of recovery, the amounts of catecholamines present in a 24-hour urine sample were calculated according to the following equations:

$$NE = \frac{(H - 0.0751) V}{0.0110 \times 105.2}$$

$$E = \frac{(H - 0.1386) V}{0.0124 \times 106.8}$$

$$DA = \frac{(H - 0.4230) V}{0.0354 \times 108.2}$$

where NE, E, DA = the amount of the catecholamine, $\mu\text{g}/24 \text{ h}$ urine

H = the corresponding peak height, cm

V = volume of the 24h urine, liter.

The constants in the equations (e.g. 0.0751, 0.0110 and 105.2 in the NE equation) are the intercept and slope of the calibration curve and the percent recovery, respectively.

The results for the determinations of eight urine samples are listed in Table II. These are consistent with those given in the literature [5-8].

Table I Linear response range and detection limit.

Abbreviation	Catecholamine	Linear range	Correlation coefficient	Regression equation	Detection limit*
NE	Norepinephrine	8 ppb-10 ppm	0.9997	$y = 0.0751 + 0.0110 x$	80 pg
E	Epinephrine	5 ppb-10 ppm	0.9998	$y = 0.1386 + 0.0124 x$	50 pg
DA	Dopamine	2 ppb-10 ppm	0.9950	$y = 0.4230 + 0.0354 x$	20 pg

* Signal-to-noise ratio: 2

Table II Analytical results of the analysis of catecholamines in urine.

Sample no.	Total value of 24-h urine (ml)	Catecholamine amount ($\mu\text{g}/24\text{-h}$)		
		Norepinephrine	Epinephrine	Dopamine
1	750	111.7	6.31	248.1
2	2480	69.6	11.6	247.7
3	1185	156.1	44.0	235.8
4	1100	125.4	21.7	343.4
5	1050	107.4	15.1	143.2
6	1200	94.5	7.6	355.3
7	1320	147.7	38.1	270.5
8	620	92.1	19.1	220.1

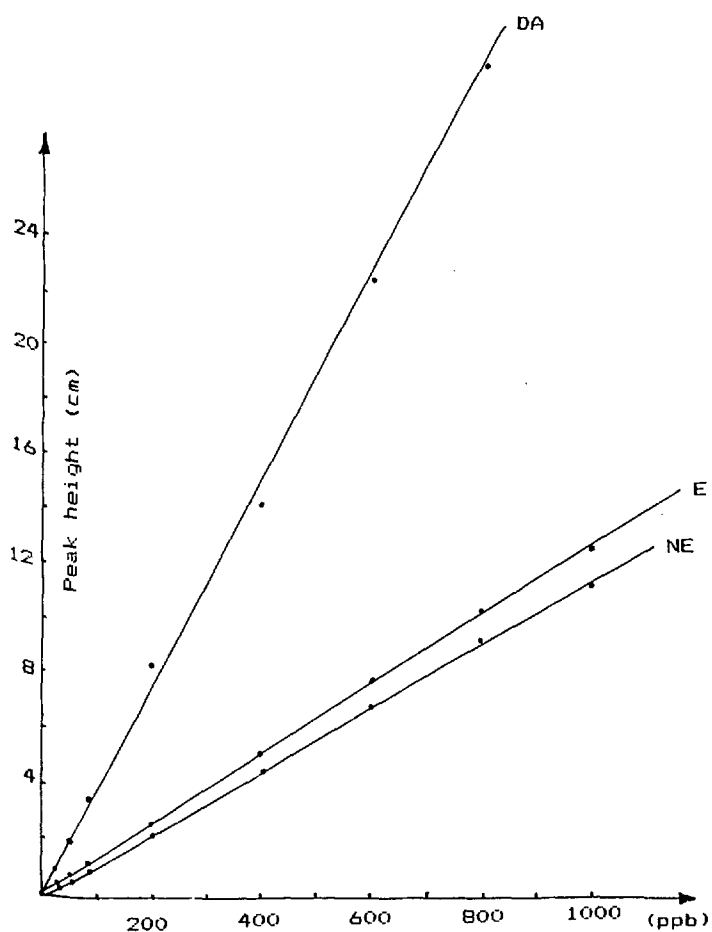


Figure 6

Calibration curve of peak height against catecholamine concentration.

Mobile phase: 0.005 M SDS, 0.03 M sodium citrate, 3 % n-propanol and 1 mM EDTA; pH 4.2. Amperometric detector, 0.35 V (vs. Ag-AgCl), range 2.

NE = norepinephrine;

E = epinephrine;

DA = dopamine.

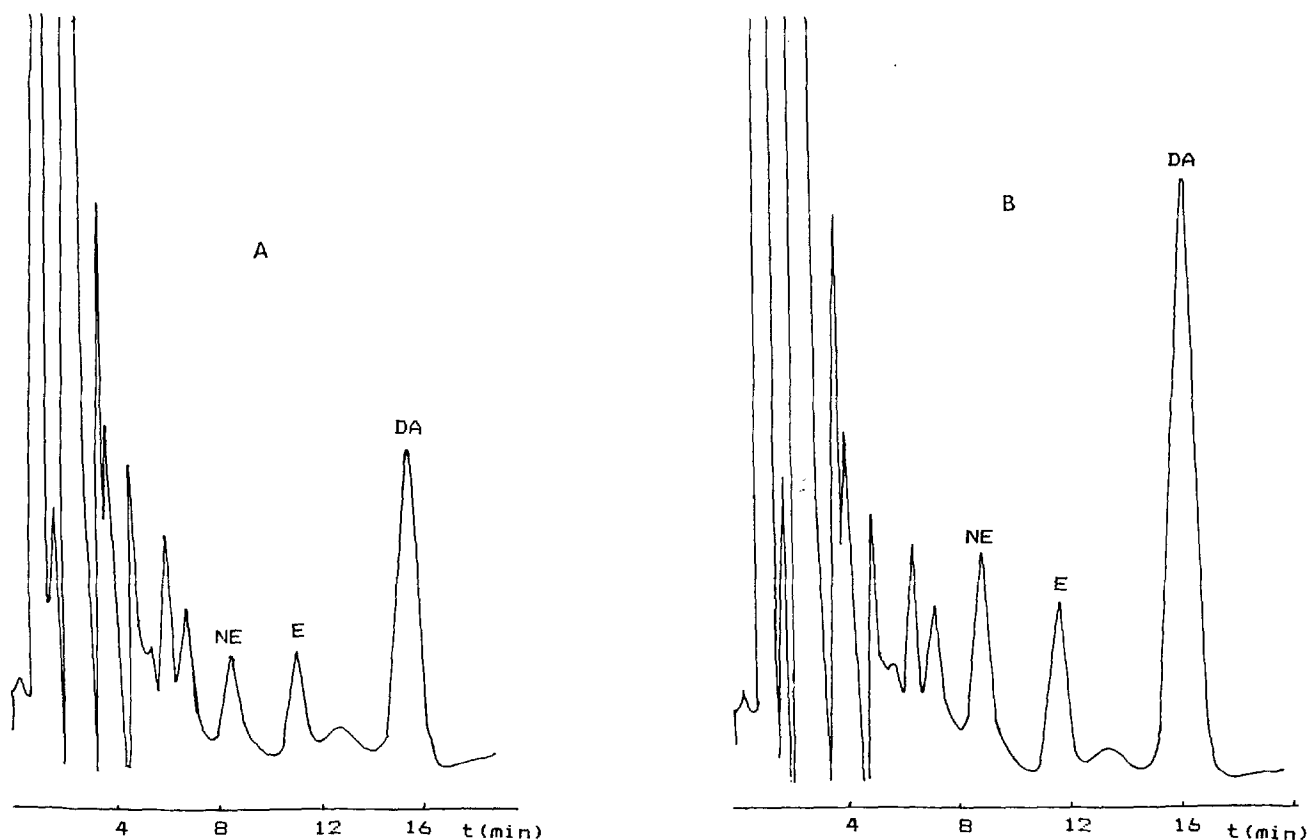


Figure 7 Chromatograms of catecholamines. (a) urine sample, (b) after adding 100 ppb of norepinephrine, epinephrine and dopamine. For chromatographic conditions see Figure 6.

Acknowledgement

We sincerely thank Dr. B. Feibush, Supelco, Inc. (Bellefonte, PA, USA) for presenting us with the HisepTM SHP column used in this work.

This work was supported by the National Science Foundation of China.

References

- [1] L. J. Cline Love, S. Zibas, J. Noroski, M. Arunyanart, *J. Pharm. Biomed. Appl.*, **3**, 511 (1985).
- [2] I. H. Hagestam, T. C. Pinkerton, *Anal. Chem.*, **57**, 1757 (1985).
- [3] D. J. Gisch, B. T. Hunter, B. Feibush, *J. Chromatogr.*, **433**, 264 (1988).
- [4] S. Allenmark, *J. Liq. Chromatogr.*, **5** (suppl. 1), 1 (1982).
- [5] R. T. Peaston, *J. Chromatogr.*, **424**, 263 (1988).
- [6] E. D. Schleicher, F. K. Kees, O.H. Wieland, *Clin. Chim. Acta.*, **129**, 295 (1983).
- [7] H. Nohta, A. Mitsui, Y. Ohkura, *J. Chromatogr.*, **380**, 229 (1986).
- [8] C. Julien, C. Rodriguez, G. Cuisinaud, N. Bernard, J. Sassard, *J. Chromatogr.*, **344**, 51 (1985).

Received: May 10, 1990
 Revised manuscript
 received: Sept. 15, 1990
 Accepted: Oct. 12, 1990
 A