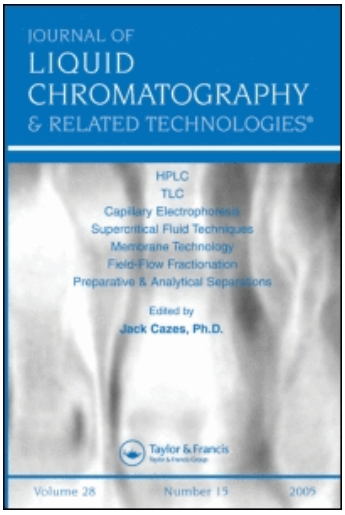

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DIRECT URINE INJECTION WITH MICELLAR LIQUID CHROMATOGRAPHY-AMPEROMETRIC DETECTION FOR DOPAMINE MONITORING

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ABSTRACT

Micellar liquid chromatography with amperometric detection was evaluated for the determination of dopamine in urine. The samples were injected directly without time-consuming protein precipitation. The separations were carried out in an analytical column packed with C18. The mobile phase was 0.01M SDS with 3% n-propanol added at pH 4.15 . The limit of detection for dopamine is 4 pg. Eight urine samples were analyzed. The results were in a reasonable range.

INTRODUCTION

A significant application of micellar chromatography is the ability to inject serum or urine directly onto a reverse-phase column with no tedious protein precipitation or pressure buildup problems, this is probably the result of the protein-surfactant complex being excluded from the pores of the stationary phase support, preventing partitioning and retention. Therefore, in micellar chromatography analytes be adsorbed onto stationary phase while micellar aggregates solubilize the serum or urine proteins in the mobile phase and cause them to elute with the void volume. Cline Love et al [1-3] have shown the utility of this extrmely important advantage of micellar mobile phases in therapeutic drug monitoring. However, all of their works use UV or fluorescence detector and sensitivity is limited. This is also a limitation for micellar chromatography in monitoring some drugs at very low therapeutic concentration ranges. In this work, we coupled micellar chromatography with an amperometric detector in real analytical application and the detection limit of dopamine is 4 pg. To date , the direct serum/urine injection method of micellar liquid chromatography are only used in monitoring therapeutic drug and added substance [4] in body fluid. We have circumvented these problems by developing a micellar liquid chromatography-amperometric detection method to directly inject urine for monitoring endogenous substance ---- dopamine which is one of the typical biogenic amines.

EXPERIMENT

Chemicals

The chemicals used were obtained from a variety of suppliers. The standard solution of dopamine (DA) was prepared according to the following procedure. Dissolve 0.1000 g of DA in 0.05 M hydrochloric acid in 100 ml brown volumetric flasks. Store the standard solutions at 4°C in the dark. Dilute them with 0.05M HCl to appropriate concentrations before use.

Mobile phase

The chromatographic mobile phase was a 0.01 M SDS aqueous solution, pH 4.15, with a buffer composition of 0.02M citrate, 0.03M HCl, 3% n-propanol and 1mM EDTA. The EDTA is added to the mobile phase to reduce the noise caused by the presence of trace metal impurities. The mobile phase was filtered and degassed through a G6 (<2.5μm) sintered glass funnel.

Apparatus

The Chromatographic separations were carried on with Model LC-4A liquid chromatograph equipped with a L-ECD-6A electrochemical detector in which the working electrode is glass carbon and the reference electrode is Ag/AgCl (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 x 4.6 mm I.D. column was packed with Micropak ODS (5 μm) (Varian Co., USA). In order to minimize dissolution of the analytical column packing, a precolumn (50 x 4.6 mm I.D.) packed with silica gel (15 - 25 μm) was placed between the pump and the injection valve to saturate the mobile phase with silica. The chromatographic experiments were carried out at 40°C.

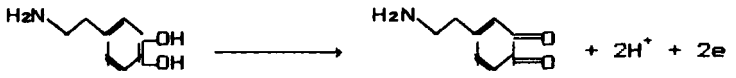
Collection and pretreatment of urine

DA are easily oxidized and sensitive to light, especially in an alkaline medium. A complete 24-hour urine sample was collected in a 2000 ml brown bottle. A 15 ml of 6M HCl as stabilizer was added. A 25 ml of each urine sample was kept for use after the total volume was measured.
Urine samples were filtered through a G6 (<2.5μm) sintered glass funnel to remove deposits. The filtrate was adjusted to about pH 1.5 with 6M HCl and stored at 4°C.

RESULT AND DISCUSSION

Condition of detection

Because of its sensitivity and selectivity, an electrochemical detector is commonly used for the chromatographic separation of DA. The electrode reaction is the following :



However, there is a paucity of information about electroanalysis in micellar solutions. One fundamental process that must be considered in any discussion of electrochemistry in micellar solutions is adsorption of surfactant on the electrode surface. The adsorbed surfactant can change the double-layer structure, the rate of electron transfer (by both acceleration and inhibition) and the apparent $E_{1/2}$ value of an electroactive specie. It is clear that electrochemical conditions developed for a hydroorganic separation are probably not directly transferable to micellar mobile phases. New DA hydrodynamic voltammograms are necessary to determine the optimum operating potential. Figure 1 shows the cyclic voltammograms of the DA in 0.05 M HCl and SDS micellar solution, respectively. According to Fig.1, in micellar mobile phases, DA are oxidized at lower operating potentials, which should be a benefit for selectivity. Also the oxidizing current is lower. Because there is generally a higher local viscosity in micellar solutions, the rate of molecular diffusion toward the electrode is lower. According to the Fig. 1 and calculation, an operating potential of +0.5 V (vs. Ag/AgCl) was chosen.

Separation Condition

At first, an adequate separation condition was chosen by orthogonal design tests. The concentration of surfactant, pH

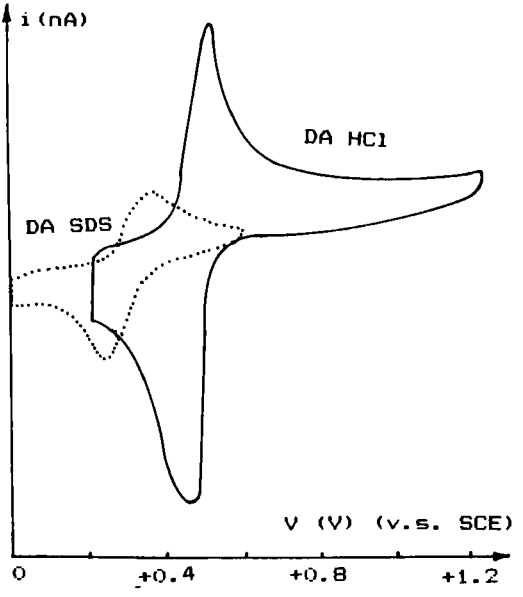


Fig.1 The cyclic voltammograms of the dopamine.
the cyclic voltammograms of dotted lines and solid lines were obtained in 0.1M SDS and 0.05M HCl, respectively. (reference electrode: SCE).

Table I The factors and the levels of the orthogonal design

Factor	level			
	1	2	3	4
[SDS] (M)	0.005	0.01	0.10	0.20
pH	2.0	3.7	4.6	6.2
n-propanol (v/v)	1%	3%	7%	10%

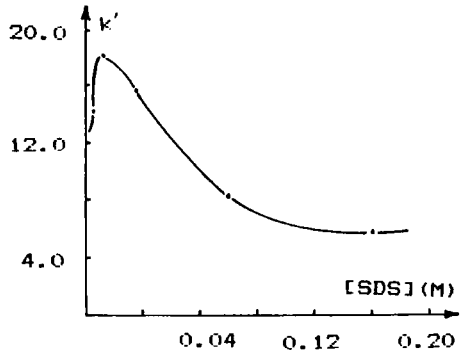


Fig.2 Capacity factor of DA (k') plotted aganist SDS concentration.

value and the concentration of organic solvent (n-propanol) are the three factors of orthogonal design and four levels of each factor were chosen, as shown in Table I. Tests for sixteen groups were conducted according to a $L_{16}(4^8)$ orthogonal table. The optimum result is 0.1M SDS aqueous solution and 3% n-propanol with pH 4.15. To increase the mass transfer rate, The column temperature was set at 40°C.

However, when this condition was used in the analysis urine , the blank urine produced a background response that completely overlaped the peak of the dopamine . Therefor, it was necessary to increase the retention time of the DA. According to the orthogonal test, Fig. 2 shows a plot of capacity factor (k') vs. SDS mobile phase concentration for DA. From this, we know, when the SDS concentration is below the critical micelle concentration (CMC), CMC of SDS is 0.008M, the ODS column is modified by adsorption of SDS monomers with the negative head

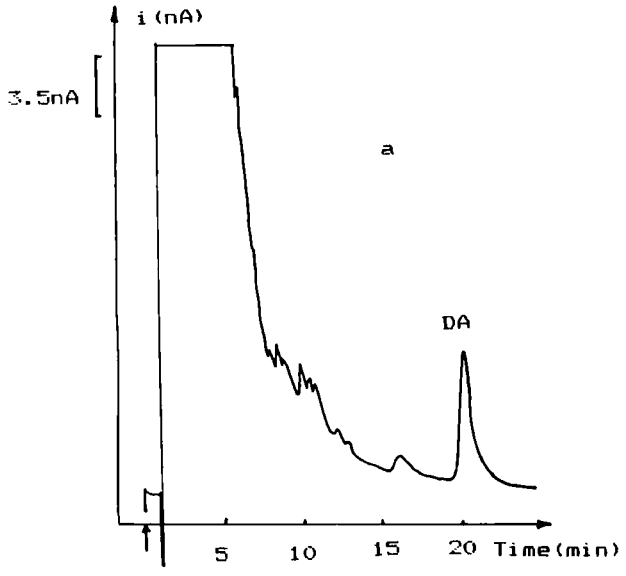


Fig.3(a)

Fig.3 Chromatograms of urine samples

(a) urine + 200ppb DA, pH 1.5, (b) urine sample, pH 1.5, (c) urine sample, pH 3.5.

Mobile phase: 3% n-propanol, pH 4.15, 1mM EDTA, 0.01M SDS. Flow rate: 0.7 ml/min.

Amperometric detector: +0.5 V (v.s. Ag/AgCl).

groups in contact with the mobile phase, such that the surface is charged and electrostatic force is predominant. Increasing the SDS concentration results in longer retention time for the solutes oppositely charged to the SDS, such as dopamine. k' increase with SDS concentration. The operative mechanism is similar to that of ion-pair chromatography. At the CMC, the

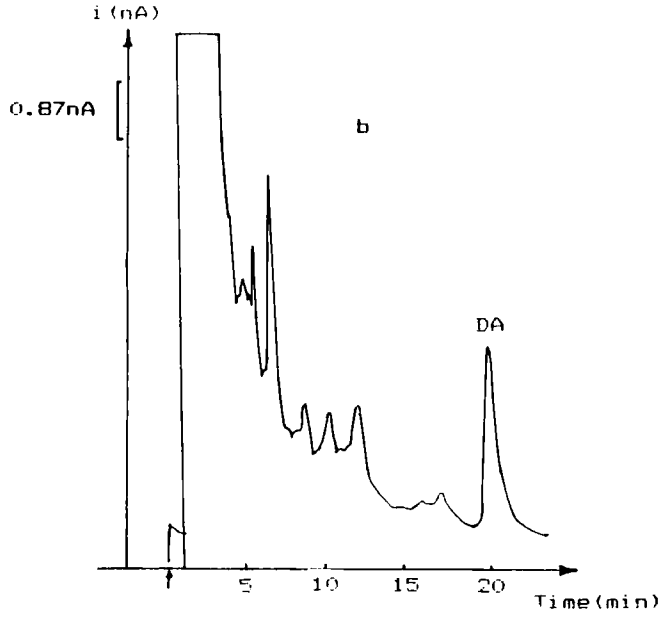


Fig.3(b)

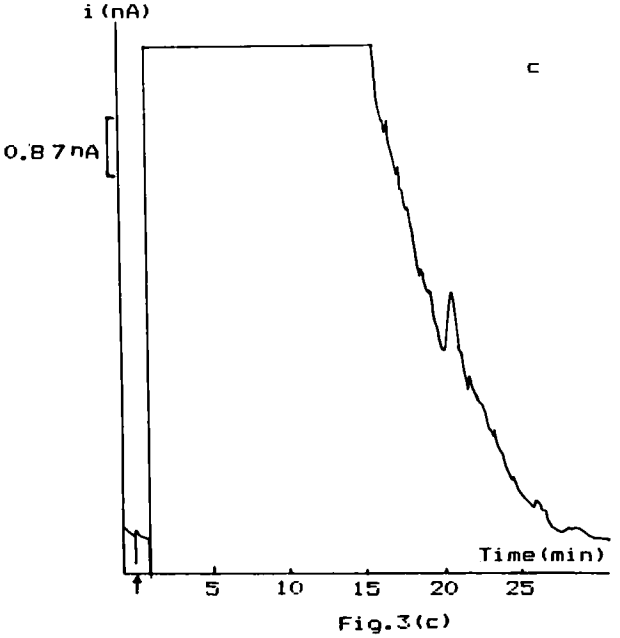


Fig.3(c)

concentration of adsorbed SDS monomers and attracted electrostatic force reach to the maximum value, so the solute have the highest k' value. Above CMC, the adsorbed SDS monomers on stationary phase keep a constant and the micelle concentration in mobile phase increase with the increasing the SDS concentration. The solute can partition from the stationary phase to micellar aggregates and elutes in shorter time. So k' decrease with SDS concentration. According to above conclusion, the concentration of SDS was changed into 0.01M. Other separation conditions remained unchanged. Under this condition . Dopamine in urine can thus be successfully separated and determined, as shown in Fig.5(a) and (b).

Calibration curve and detection limit

In the above-mentioned chromatographic condition the peak heights were plotted aganist the concentration of DA, to give a calibration curve in which correlation coefficient is 0.9998 and regression equation is:

$$Y = 0.0222\ X + 0.233$$

The linear range of DA is 1 ppb - 10 ppm, the detection limit is 4.0 pg.

Recovery and determination of DA in Urine

Different quantities of dopamine were added to urine samples to calculate recoveries according to the following

equation:

$$\text{recovery} = \frac{S_m - B}{S} \times 100\%$$

Where : S = the peak height of standard solution of DA,

B = the peak height of DA in blank urine,

S_m = the peak height of DA that was added to the urine

The results are shown in Table II.

In addition, before injection, the urine samples were adjusted to about pH 1.5 with 6M HCl so that the blank urine produced a rapid elution of unretained protein species at the solvent

Table II Recovery of DA

Concentration of standard DA in 10 ml urine	Recovery	Average recovery	CV%
50 ng/ml	94.8 91.8 99.7	95.4	4.2
100 ng/ml	92.3 92.7 96.7	93.9	2.6
200 ng/ml	92.7 97.6 97.6	96.0	2.9

Table III Analytical results of DA in Urine

No. of sample	Age	* Sex	Total volumn of 24-h urine(ml)	Amount of DA (μg/24-h)
1	27	m	1805	315
2	24	m	1130	285
3	26	m	1250	305
4	17	f	995	419
5	26	f	2385	377
6	28	m	2675	610
7	25	m	1955	533
8	25	f	2290	561

* m = male, f = female.

front ,then returned completely to the baseline within the mininum time . This is probably because some substances in urine irreversely reacted under acidic condition to produce other subtances which can eluted in shorter time . Fig.5(b) and (c) show the urine sample chromatograms at pH 1.5 and pH 3.5, respectively.

Using the regression equation of the calibration curve and correcting in terms of recovery, the amounts of DA in 24-hour urine samples were calculated according to the following equation:

$$DA = \frac{(H - 0.233)\ V}{0.222 \times 95.1}$$

where DA = the amount of the dopamine, μg/24hour

H = the corresponding peak height, cm

V = volume of 24-h urine, liter

Numbers are the intercept and slope of the calibration curve and recovery, respectively.

The results for the determinations of eight urines samples are listed in Table III and are consistent with those given in the literature [5-8].These results shown , micellar liquid chromatography with amperometric detection is a simple, rapid, reliable and sensitive method not only suitable for the analysis of free dopamine in urine, but also hopeful for the determination of other endogenous substance and low level therapeutic drug in body fluid .

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