

Study on fluorescence property of dopamine and determination of dopamine by fluorimetry

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Abstract

The property of dopamine in acid–alkaline medium was studied by spectrofluorimetry and spectrophotometry. Effects of organic solvents on dopamine fluorescence spectra were examined and the reason was discussed. Among the organic solvents tested, methanol was selected as sensitizing reagent. A fluorescence quenching reaction took place in basic medium. Mechanism of fluorescence quenching was discussed. A simple, rapid and highly sensitive fluorimetric method for the determination of dopamine in injection and urine is developed. The measurement of relative fluorescence intensity was carried out at 315 nm with excitation at 279 nm. Effects of pH, foreign ions on the determination of dopamine have been examined. A linear relationship was obtained between the relative fluorescence intensity and concentration of dopamine in the range of 0.10–3.50 $\mu\text{g ml}^{-1}$. The linear regression equation of the calibration graph is $C = 0.00447F - 0.00748$, with a correlation coefficient of linear regression of 0.9997 and relative standard deviation of 2.37%. The detection limit of this method is 0.082 $\mu\text{g ml}^{-1}$, and recovery is from 104.2 to 106.6%. This method can be used for determination of dopamine in injection and urine sample. The results obtained by this method agreed with those by the official method (high performance liquid chromatography (HPLC)). © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Dopamine; Spectrofluorimetry; HPLC; Injection; Urine

1. Introduction

Dopamine, a neurotransmitter, is one of the naturally occurring catecholamines, and its hydrochloride salt is being used in the treatment of acute congestive failure and renal failure [1]. Many analytical chemists try to find compendious

methods for the determination of dopamine in authentic and dosage forms. da Vieira and Fati-bello-Filho reported a spectrophotometric method for determining the dopamine using a crude extract of sweet potato root as enzymatic source [2]. Based on the oxidation of *N*-bromosuccinimide [3] and the charge transfer reaction between dopamine and tetrachlorobenzquinone [4], the spectrophotometric methods for determining dopamine in pharmaceutical formulations were developed. The *N*-hydroxyduccinimidyl 3-indolylacetate [5] and 1,2-bis(3-chlorophenyl)ethylene-

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diamine [6] as pre-column derivative reagents had been employed for determining catecholamines by HPLC. A sample mixture containing dopamine, catechol, adrenaline, noradrenaline was separated and determined by electrochemical method [7]. Micelles effect on electrochemistry of dopamine was studied, and dopamine was determined in the presence of 100 times excess of ascorbic acid [8]. Trihydroxyindole method [9] and improved trihydroxyindole method using iodine as oxidation reagent and sodium sulfite as stabilization reagent [10] are widely used for determining dopamine. Wang et al. published a fluorimetric method for determining dopamine in different parts of brain-stem and spinal cord [11]. Trihydroxyindole method has a good selectivity, but the stability and the detection limit are not satisfactory. Based on reaction of catecholamines with 1,2-diphenylethylenediamine, catecholamines were determined by fluorimetry, but reaction time was longer [12]. Terbium ion fluorescence probe for the determination of dopamine was reported [13]; it was a highly sensitive method.

In this paper, effects of organic solvent on the relative fluorescence intensity and the fluorescence spectrum of dopamine were examined. Among the organic solvents tested, methanol was strongest in increase of fluorescence intensity of dopamine. Therefore, methanol was selected as sensitizing reagent. The dopamine took place a fluorescence quenching reaction in alkaline medium; mechanism of fluorescence quenching was discussed. A simple, rapid and sensitive method for the determination of dopamine was developed. The principal advantages of the method are that it is simple, rapid, possesses a low detection limit. The method can be used for determining dopamine in injection solution and urine. The results obtained by this method agreed with those of the official method [14].

2. Experiment

2.1. Apparatus

Spectrofluorimetric measurements were made on LS-5B (Perkin–Elmer, USA) spectrofluorimeter

equipped with a xenon discharge lamp, 1 cm quartz cells.

Absorption spectra were obtained with UV–vis recording spectrophotometer with matched 1-cm quartz cells (Model UV-265, Shimadzu, Japan).

A pH meter (Model pH-3C, Shanghai Leici instruments Factory, China) was used for pH adjustment.

Silica gel plates of 0.20–0.25 mm thick, 100 × 200 mm area (Qingdao oceanic departed factory, China) were used to separate dopamine from urine sample.

2.2. Reagents and solutions

Dopamine hydrochloride (chemical correspondent product) was used standards (Chinese Drug and Biological Product Detection Institute). Methanol was a research grade and other reagents were of analytical-reagent grade. Doubly distilled water was used in all experiments.

2.2.1. Stock standard solution of dopamine hydrochloride ($100 \mu\text{g ml}^{-1}$)

An accurately weighed 0.1000 g standard sample of dopamine hydrochloride was dissolved in water, transferred into 100 ml volumetric flask and diluted to the mark with water and mixed well. The solution was stored at 4 °C. The stock standard solution was diluted to $10.0 \mu\text{g ml}^{-1}$ before being used.

2.2.2. Walpole buffer solution (pH 3.6)

A total of 0.2 mol l^{-1} HOAc and 0.2 mol l^{-1} NaOAc were mixed well according to volume ratio of 18.5:1.5.

2.2.3. Developing reagent

A total of butyl alcohol and glacial acetic acid and water were mixed well according to volume ratio of 4:1:5. The solution was stood for half an hour; the upper layer solution was used as developing reagent.

2.3. Procedure

2.3.1. Procedure I

A 1.0 ml portion of $10 \mu\text{g ml}^{-1}$ dopamine

hydrochloride standard solution was transferred into a 10.0 ml volumetric flask, 2.0 ml of pH 3.6 HOAc/NaOAc buffer solution and 4.0 ml of methanol were added, diluted to the mark with water and mixed well. The relative fluorescence intensity of the solution was measured at 320 nm with excitation at 279 nm against a reagent blank prepared with the same reagent concentrations, but no dopamine hydrochloride. All fluorescence measurements were made using 5 nm excitation and emission windows. The fluorescence spectra were recorded at scan rate of 240 nm min⁻¹.

2.3.2. Procedure II

A 0.50 ml portion of 10.0 µg ml⁻¹ of dopamine standard solution was transferred into a 10.0 ml volumetric flask, 2.0 ml pH 3.6 buffer solution and different volume of methanol were added, diluted to the mark with water and mixed well. The emission spectra of these solutions were recorded. Ethanol, 2-propanol, dimethylsulfoxide (DMSO), dimethylformamide (DMF) were tested separately according to same procedure.

2.3.3. Procedure III

Fifteen microliter of dopamine, adrenaline and noradrenaline standard solutions were subjected to a silica gel plate by microdropper, respectively. The plate was developed in developing reagent for 3 h at room temperature, and dried in a desiccator, and then merged into the vaporation of iodine. The spots of dopamine, adrenaline and noradrenaline were colored, respectively. The R_f values were measured.

2.3.4. Procedure IV

Urine specimens from volunteers were collected in 10 ml of 6 mol l⁻¹ hydrochloric acid over a 24-h period. Ten milliliter of such urine sample were taken out and evaporated to dry in vacuum at room temperature; the residue is dissolved in 2 ml methanol. This solution was used as sample solution. Fifteen microliter of sample solution was subjected to a silica gel plate by microdropper. The plate was developed in developing reagent for 3 h at room temperature, and dried in a desiccator. The spot of dopamine on the plate was scratched off (to ensure accuracy of sampling, the

place of spot of sample should be confirmed based on the standard dopamine), transferred into 10 ml centrifuge tube, 0.6 ml buffer solution (HOAc/NaOAc), 1.2 ml methanol, 1 ml sodium sulfate (0.05 mol l⁻¹), 0.2 ml water were added separately. Stirred for 10 min, stood for half an hour, and centrifuged for 2 min at 1500 rpm, and the supernatant was analyzed according to the procedure I.

3. Result and discussion

3.1. Excitation and emission spectra

As can be seen (Fig. 1), the maximum excitation and emission wavelength of dopamine are at 279 and 315 nm, respectively (lines a and b). However, maximum emission wavelength is at 320 nm in the presence of methanol. Maximum emission wavelength shifted to the long wave (from 315 to 320 nm). What is more, the relative fluorescence intensity of dopamine was increased obviously in the presence of methanol as compared with in the absence of methanol because methanol changed the polarity of the medium for determination of dopamine. Therefore, methanol was selected as sensitizing reagent for determination of dopamine. Wavelengths of 279 and 320 nm were selected as excitation and emission wavelengths, respectively. In addition, reagent blanks have no effect on determination of dopamine whatever the presence of methanol or not (lines e and f).

3.2. Influence of pH

Effect of pH on the relative fluorescence intensity of dopamine in the absence of methanol has been tested, comparative tests at various pH values showed that the relative fluorescence intensity of dopamine changes with pH. The result is shown in Fig. 2. Variation of the pH from 2 to 12 was investigated. It can be seen that the relative fluorescence intensities of dopamine do not have notable change in acidic environment, and then reduces gradually above pH 7. Because the relative fluorescence intensity of dopamine is maximal at pH 3.6, and an excellent linear

relationship exists between fluorescence intensity and concentration of dopamine at pH 3.6, the pH 3.6 HOAc/NaOAc buffer solution was selected to control pH of the system. Obviously, the buffer solution had no effect on the determination of dopamine.

3.3. Mechanism of dopamine fluorescence quenching in alkaline medium

Fig. 2 showed that the relative fluorescence intensities of dopamine had no notable change in acidic medium, but reduced obviously in alkaline. It is possible that chemical reaction takes place in basic medium. The acid–base behavior of dopamine was investigated by spectrophotometry, and the absorption spectra of dopamine at different pH were shown in Fig. 3.

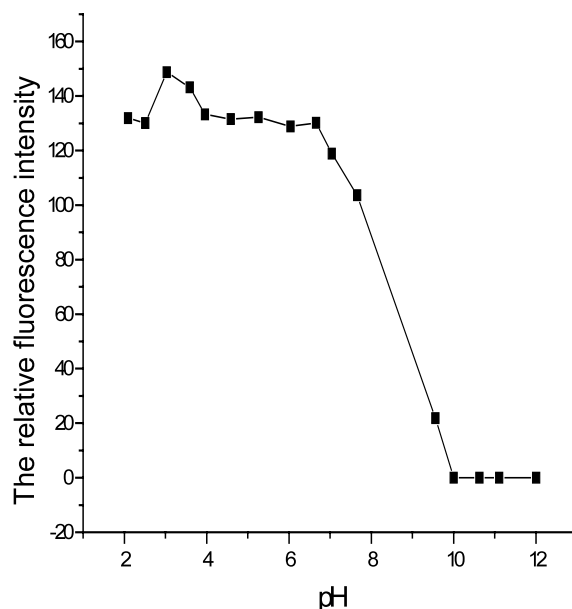


Fig. 2. Effect of pH on the fluorescence intensity of dopamine. The concentration of dopamine was $1.0 \mu\text{g ml}^{-1}$, $\lambda_{\text{ex}} = 279$ and $\lambda_{\text{em}} = 320$ nm.

As can be seen (Fig. 3), λ_{max} of dopamine was located at 280 nm (lines from 1 to 3) when pH was below 8.45. When pH values of the solution was above 8.45, the λ_{max} of dopamine changed to 296 nm (lines from 4 to 8). It is emphasized that the absorbance of dopamine is different at the different pH conditions. The absorbance increases with a rise in pH, achieving a maximum at pH 11.87. However, the fact that the dopamine had no fluorescence when pH value above 10 (see Fig. 2) indicated that a reaction of fluorescence quenching occurred in alkaline medium. The quenching reaction may be a static quenching, i.e. dopamine was oxidized to form a compound that was not fluorescence substance. Therefore, the following reaction was suggested to explain the reason of the fluorescence quenching in alkaline solution.

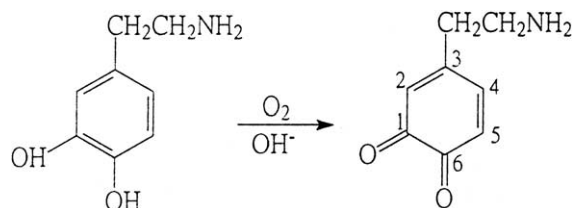


Fig. 1. Excitation and emission spectra of dopamine. Excitation spectrum (a) ($\lambda_{\text{ex}} = 279$ nm) and emission spectrum (b) ($\lambda_{\text{em}} = 315$ nm) of dopamine in the absence of methanol. Excitation spectrum (c) ($\lambda_{\text{ex}} = 279$ nm) and emission spectrum (d) ($\lambda_{\text{em}} = 320$ nm) of dopamine in the presence of methanol. Excitation spectrum (e) and emission spectrum (f) of reagent blank. Concentration of dopamine: $0.5 \mu\text{g ml}^{-1}$. pH 3.6 HOAc/NaOAc buffer solution.

As we all know that *o,p*-dihydroxybenzene can easily be oxidized in alkaline solution [15]. Benzoquinone can be regarded as the oxidation product of *o,p*-dihydroxybenzene [16]. Obviously, 280 nm is $\lambda_{\max}$ of dopamine in acidic solution. In an alkaline solution, dopamine is oxidized by oxygen in air to form an oxidation product. The 296 nm is $\lambda_{\max}$ of oxidation product. The $\lambda_{\max}$ of oxidation product is calculated as follows according to Woodward rule [17] (numbers in bracket indicate number of carbon atom of product molecule): base value, 215 nm; one conjugated double bond (6), add 30 nm; one homoannular diene, add 39 nm; two double bond outside of the ring (1, 6) add 10 nm; one alkyl (3), add 12 nm; solvent is water, minus 8 nm; total is 298 nm. The measured value (296 nm) agreed with calculated value (298 nm) for $\lambda_{\max}$ of oxidation product. It should be noted that $\lambda_{\max}$ of oxidation product is longer than that of dopamine, this is due to increase of conjugation of the oxidation product according to the molecule structure of oxidation product. Obviously, the oxidation product is not fluorescence substance due to its benzoquinonyl structure.

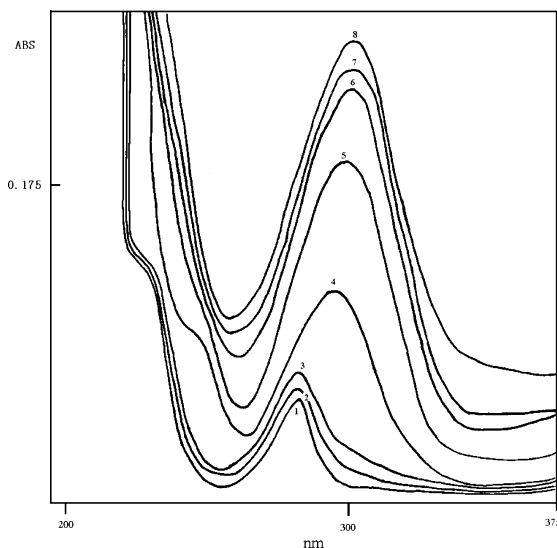


Fig. 3. Absorption spectra of $5.0 \mu\text{g ml}^{-1}$ dopamine hydrochloride at different pH. pH: 5.28 (line 1); 3.21 (line 2); 8.45 (line 3); 9.57 (line 4); 9.90 (line 5); 10.65 (line 6); 11.42 (line 7); 11.87 (line 8).

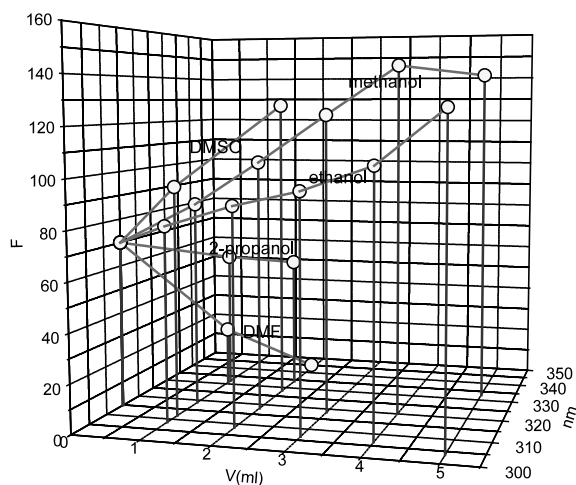


Fig. 4. Graphical 3D perspective of effect of organic solvent on fluorescence spectra of dopamine.

3.4. Solvent effect

3.4.1. Effect of solvent on the emission wavelength of dopamine

According procedure II, effects of different solvent on the emission wavelength of dopamine were tested. The results are shown in Fig. 4. As can be seen (Fig. 4), the maximum emission wavelengths of dopamine shift to long wavelength in present of methanol. The relative fluorescence intensity increases with a rise in methanol, achieving a maximum at 320 nm when 4 ml of methanol added. Effects of DMSO and ethanol on the emission wavelengths of dopamine are similar to methanol, but maximum emission wavelengths of dopamine shift to short wavelength in present of ethanol. It was noted that DMF quenched fluorescence of dopamine. Obviously, sensitization of methanol is strongest in the organic solvents we tested. Therefore, methanol was selected as sensitizing reagent for analytical application.

Generally, methanol, 2-propanol, and water are donors of hydrogen bond. But donation of methanol or 2-propanol is weaker in comparison with water. With addition of methanol or 2-propanol, the charge transfer action was strengthened between the isolated double electrons on the oxygen atom and the benzene ring of excited state. This action leads to increase of conjugation

to dopamine molecule. Therefore, $\lambda_{\max \text{ em}}$ of dopamine shifts to red. DMSO and DMF are hydrogen bond acceptors. It is helpful to strengthen conjugation between the isolated double electrons of the oxygen atom and the benzene ring when DMSO or DMF are added. Because DMSO and DMF are solvents of hydrogen bond acceptors, DMSO or DMF also reduced the donation of water and can increase intramolecular conjugation of dopamine. Therefore, DMSO or DMF can lead $\lambda_{\max \text{ em}}$ of dopamine to the red too [18]. Ethanol does not obey this rule although it is also donor of hydrogen bond (see Fig. 4); the reason needs to research further.

3.4.2. Effect of solvent on the fluorescence intensity of dopamine

Dielectric constant n was used to reflect polarity of solvent (n values of water, methanol, ethanol, 2-propanol, DMSO, DMF are 80.103, 33.1, 23.8, 18.3, 48.9, 36.71, respectively [19]). According to the following formula, the n value of the binary system can be calculated [19].

$$K_{\text{mixture}} = \frac{K_1 Q_1 + K_2 Q_2}{100}.$$

K_{mixture} value is dielectric constant of the mixture; K_1 and K_2 are dielectric constants of two pure compositions, respectively; Q_1 and Q_2 are the volume percentage of the two pure compositions, respectively. A relationship was obtained between the relative fluorescence intensity of dopamine and dielectric constant. Results were shown in Fig. 5.

Generally, as can be seen (Fig. 5), the relative fluorescence intensity of dopamine increases with the decrease of the polarity of solution except for DMF and 2-propanol. DMF quenched fluorescence of dopamine and 2-propanol has no obvious change. Fig. 5 show that relative fluorescence intensity of dopamine is different at different organic solvent although polarities of solutions are same. Methanol was selected as sensitizing reagent for determining dopamine in this paper. DMSO was not selected because it is expensive.

3.5. Stability of fluorescence of dopamine

According to the procedure I, the relative fluorescence intensity of the mixture solution of $0.5 \mu\text{g ml}^{-1}$ of dopamine was measured after standing for different time. The results shown that relative fluorescence intensity of dopamine was stable at least in 24 h.

3.6. Effect of foreign ions

A systematic study was carried out on the effects of foreign ions on the determination of $0.5 \mu\text{g ml}^{-1}$ of dopamine. A 500 mg l^{-1} level of each potentially interfering species was tested first. If interference occurred, the ratio was reduced progressively until interference ceased. The tolerance level was defined as an error not exceeding $\pm 5\%$ in the determination of the analyte. Results are summarized in the Table 1.

3.7. Calibration graph of dopamine

Under the selected conditions, a linear relationship was obtained between the fluorescence intensity and the concentration of dopamine in the

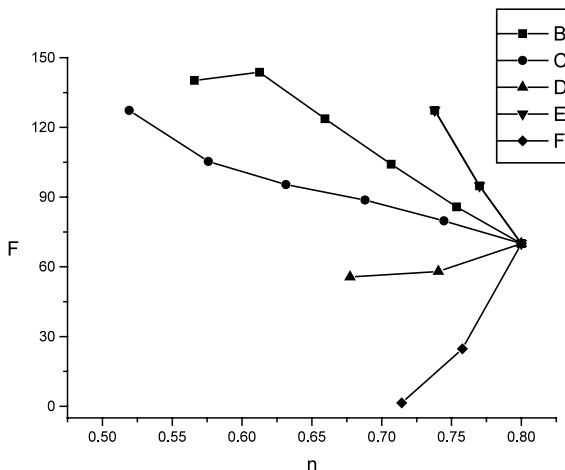


Fig. 5. The relationship between the relative fluorescence intensity of dopamine and n (dielectric constant). Concentration of dopamine: $0.5 \mu\text{g ml}^{-1}$; pH = 3.6 HOAc/NaOAc buffer solution. Line B: methanol and water; line C: ethanol and water; line D: 2-propanol and water; line E: DMSO and water; line F: DMF and water.

Table 1
Effect of foreign ions on the determination of $0.5 \mu\text{g ml}^{-1}$ of dopamine

Foreign ions or species	Tolerance level ($\mu\text{g ml}^{-1}$)	Foreign ions or species	Tolerance level ($\mu\text{g ml}^{-1}$)
Na^+ , Cl^- , K^+	500	Cu^{2+} , Hg^{2+}	2.5
SO_4^{2-} , HPO_4^{2-}	500	PO_4^{3-}	400
Ca^{2+} , Mg^{2+} ,	10	Zn^{2+} , Al^{3+}	25
Pb^{2+}	20		

range of $0.10\text{--}3.50 \mu\text{g ml}^{-1}$. Linear regression equation of calibration graph is $C = 0.00447F - 0.00748$, with a correlation coefficient of linear regression of 0.9997.

3.8. Comparison of fluorimetric and high performance liquid chromatography method for analysis of sample

The injection solutions of dopamine hydrochloride (different batch number) were diluted to different concentration with water. These solutions were used as sample solutions. According to the procedure I, the relative fluorescence intensity of the sample solutions were measured. The concentration of dopamine in sample was calculated according to linear regression equation of calibration graph. At the same time, dopamine in these sample solutions was measured by HPLC according to British Pharmacopeia [14]. Results are shown in Table 2. As can be seen (Table 2), the results by fluorimetry agreed with those of by HPLC. The results in Table 2 are mean value of replicating determination three times. In addition, the three groups of data of the two methods in Table 2 were tested with the Cochran method [20]. At a confidence level (P) equal to 95%, there was no significant difference between the two methods.

3.9. Recovery of dopamine

The recovery of dopamine added to the different concentration of sample solutions is shown in Table 3, the recovery is from 104.2 to 106.6%. The confidence intervals were estimated to results of determination in Table 3 based on $P = 95\%$ and $n = 3$. P and n are the confidence level and

times of replicating determination, respectively. It should be noted that the recovery of dopamine are all higher than 100%, it may be due to the matrix is not same between standard solution and sample solution.

3.10. Reproducibility and detection limit

A portion of the sample solution of dopamine was transferred into a 10 ml volumetric flask, the reagent was added and measured according to the procedure I. This sample solution was measured 10 times ($n = 10$), the mean value was $0.509 \mu\text{g ml}^{-1}$ with a R.S.D. of 2.37%. According to the procedure, a reagent blank was measured 10 times ($n = 10$), the detection limit was $0.082 \mu\text{g ml}^{-1}$, based on the blank plus three times the standard deviation of the blank [21].

3.11. Determination of R_f values of dopamine, adrenaline and noradrenaline

The dopamine, adrenaline and noradrenaline are similar in molecule structure. If existence of adrenaline and noradrenaline in sample, the interference will occur for determination of dopamine. Therefore, adrenaline and noradrenaline should be separated from dopamine. It was found that butyl alcohol–glacial acetic acid–water as devel-

Table 2
Results of determination of dopamine in injection

Sample no.	Fluorimetric method (mg ml^{-1})	HPLC method (mg ml^{-1})
1	0.10 ± 0.02	0.11 ± 0.02
2	0.44 ± 0.02	0.42 ± 0.03
3	0.85 ± 0.01	0.83 ± 0.02

Table 3
Results for the recovery of dopamine from samples

Sample no.	Sample content ($\mu\text{g ml}^{-1}$)	Dopamine ($\mu\text{g ml}^{-1}$)		Recovery (%) ($\mu\text{g ml}^{-1}$)
		Added	Found	
1	1.593 ± 0.003	0.500	2.114 ± 0.005	104.2 ± 0.4
2	1.062 ± 0.002	1.000	2.128 ± 0.004	106.6 ± 0.2
3	0.531 ± 0.002	1.500	2.100 ± 0.016	104.6 ± 0.9

oping reagent was optimum for separation of dopamine. According to procedure III, their R_f value were measured. The R_f values of dopamine, adrenaline and noradrenaline were 0.66, 0.43 and 0.94, respectively. The results shown that dopamine was separated entirely from adrenaline and noradrenaline.

3.12. Analysis of urine sample

According to procedure IV, the dopamine in urine sample was measured. The results were shown in Table 4. The results obtained by this method agreed with those by the HPLC. The result in Table 4 was mean value of replicating measurement of three times.

4. Conclusion

The results presented in this paper clearly demonstrate that dopamine can be determined by the fluorimetric method we proposed. The results obtained by the fluorimetric method agreed with those by the HPLC method. The principal advantage of the proposed method is rapid and simple. This method can be used for determination of the dopamine in the injection solution of dopamine hydrochloride and in urine sample.

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Table 4
Results of determination of dopamine in urine

Sample no.	Fluorimetric method (mg ml^{-1})	HPLC method (mg ml^{-1})
1	0.0901 ± 0.006	0.100 ± 0.004
2	0.0925 ± 0.007	0.103 ± 0.005
3	0.102 ± 0.011	0.098 ± 0.005

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