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ORIGINAL PAPER

Spectrofluorimetric Estimation of Norepinephrine Using Ethylenediamine Condensation Method

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Abstract A simple and sensitive method for the determination of norepinephrine is described. Norepinephrine (NE) was oxidized by mercury (II) nitrate and the oxidation product was condensed with ethylenediamine (EDA) to form a strong fluorescent compound. The addition of acetone enhances the light intensity. The measurement was carried out at 507 nm with excitation at 420 nm. A linear relationship was obtained between the fluorescence intensity and norepinephrine concentration in the range of 0.01 μM–0.014 mM; the correlation coefficient and the detection limit are 0.99813 and 2.5 nM, respectively. The interference from dopamine (DA) can be eliminated by first derivative synchronous fluorimetric method using peak to zero technique. The recovery efficiency was performed using known amounts of norepinephrine in urine sample and the results indicate a 95–98.62% recovery. The proposed method was also applied to the determination of norepinephrine in injections solution. The reaction mechanism was also described.

Keywords Fluorescence · Norepinephrine · Ethylenediamine · Intramolecular michael addition reaction

Introduction

Norepinephrine (Noradrenaline) (Fig. 1) represents a endogenous catecholamine which acts hormone and or neurotransmitter. It has an aromatic portion (catechol) and amine group on a chain of two-carbon atom meta or para position to the phenolic hydroxyl groups. This biogenic amine is known to occur in vivo. In human body, norepinephrine (NE) is formed by the following sequence of reactions: tyrosine → 3, 4-dihydroxyphenylalanine (DOPA) → dopamine → norepinephrine → epinephrine. It has been strongly implicated in some physiological conditions like anxiety, stress, sleep, and memory. The concentrations of norepinephrine and its metabolites in plasma and/or urine are used as an index for several diseases such as hypertension, pheochromocytoma and neuroblastoma. Septic shock, systemic inflammation or pharmacological vasodilatation (caused by phosphodiasterase inhibitors, sedative drugs, epidural or spinal block) are usually associated with systemic hypotension [1]. Potent systemic vasopressor agents, such as norepinephrine can then be used to restore an acceptable mean arterial blood pressure [2–4]. Therefore, quantification of norepinephrine has appeared of great importance. Many methods have been developed for its determination in different matrices and at different levels with high sensitivity and precision.

Jeong et al. [5] developed a modified glassy carbon electrode by electropolymerization of tetrakis-(2-aminopheny) porphyrin for the determination of norepinephrine in the presence of ascorbic acid. Norepinephrine, serotonin, and 5-hydroxyindole-3-acetic acid in microdialysis samples from rat brain were simultaneously determined by microbore column liquid chromatography with fluorescence detection using derivatization with benzylamine [6].

A method [7] coupling capillary electrophoresis to time-of-flight mass spectrometric (TOFMS) detection for the simultaneous analysis of catecholamines (dopamine, norepinephrine and epinephrine) and their o-methoxylated metabolites (3-methoxytyramine, normetanephrine and metanephrine) was demonstrated. High-performance liquid

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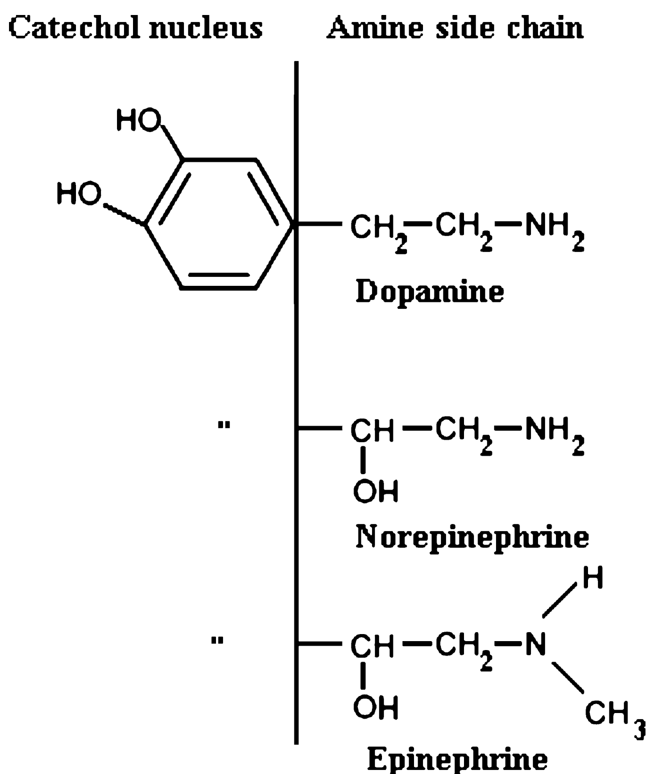


Fig. 1 Structure of catecholamines

chromatographic method [8] using terbium sensitized fluorescence as a post-column detection for the simultaneous determination of catecholamines norepinephrine (NE), epinephrine (E) and dopamine (DA) was reported. Most currently used methods in clinical chemistry use HPLC with electrochemical detection. However the methods are not completely specific and lack in insufficient sensitivity [7].

Kuhlenbeck et al. [9] reported a first stable-isotope based gas chromatography–tandem mass spectrometry–negative ion chemical ionization (GC–MS–MS–NICI) method for the analysis of norepinephrine in small volumes (10–100 ml) of rat and dog plasma.

Ion chromatography (IC) with direct conductivity detection (CD) [10] based on the ionization of catecholamines in acidic medium without chemical suppression was developed for simultaneous determination of three catecholamines (norepinephrine, epinephrine and dopamine). Liquid chromatography with chemiluminescence detection was reported by Ragab et al. [11] for the determination of norepinephrine, epinephrine and dopamine in human blood plasma. 1, 2-bis(3-chlorophenyl)ethylenediamine was used as pre-column derivatizing reagent in this method. Another 1, 2-diarylethylenediamine was used as pre-column derivatizing agent for chemiluminescence detection in HPLC method [12] for the determination of norepinephrine, epinephrine and dopamine.

Spectrophotometric method [13] for the determination of norepinephrine and epinephrine based on the development of a red colour with sodium bismuthate in aqueous medium at pH 3.0 was described and peroxidase-based (enzymatic) spectrophotometric method [14] was also well suited for pharmaceutical formulations.

Spectrofluorimetry is simple and highly sensitive method of analysis used for the assay of a large number of drugs and metals [15–18] and permits the selective and sensitive determination of low concentrations of analytes.

Several fluorimetric methods have been published for catecholamines and they can be classified into three separate groups. These are based either on the native fluorescence e.g. Synchronous and derivative spectrofluorimetry [19], an ethylenediamine condensation and the trihydroxy indole. Catecholamines were determined using trihydroxy indole reaction. The method was essentially based on the classic aluminum oxide trihydroxyindole (THI) procedure [20–22], certain modifications have been introduced. Norepinephrine and epinephrine were isolated from urine by absorption on aluminum oxide and subsequently eluted with diluted acid. The amines were then converted by oxidation to their trihydroxyindole derivatives, which give a high and specific fluorescence. The oxidation of epinephrine and norepinephrine was carried out at different pH values. Ethylenediamine condensation involves oxidizing the amines to adrenochrome or noradrenochrome with oxidizing agents followed by condensation with ethylene diamine. Using this concept epinephrine with o-phenylene diamine [23] and dopamine with ethylenediamine [24] reactions have been reported. In addition to ethylenediamine condensation two fluorimetric methods [25, 26] were also reported for the determination of 3, 4-dihydroxyphenylalanine and epinephrine. The results obtained with ethylenediamine method in different laboratories show a considerable variation, but the discrepancies among workers using trihydroxyindole method are just as great: while some find practically no epinephrine and a value for norepinephrine of about 0.3 $\mu g\ l^{-1}$ [27, 28].

Catecholamines can be oxidized chemically to their o-benzoquinones that are quite reactive and can be attacked by side chain amine group to form cyclic o-quinone via intramolecular Michael addition reactions [29].

Norepinephrine can be easily oxidized but the oxidation mechanism is complex. According to the result of our experiment, the probable oxidation mechanism for norepinephrine is as follows: First, norepinephrine (A) is oxidized to its corresponding open chain quinone (B) (norepinephrinequinone) by Hg $(NO_3)_2$ which then undergoes 1, four Michael addition of amine group with quinone ring to give noradrenochrome (C). The rate of formation of noradrenochrome is very slow at acidic pH values. The alkaline solution induces tautomerisation and trihydroxyindole (D) which is condensed

with ethylenediamine to form a fluorescent compound (E). Experiment indicates that the method is sensitive. It is used in determining norepinephrine in both injection and simultaneous determination of the synthetic mixture of norepinephrine and dopamine by first derivative synchronous fluorimetric method using peak to zero technique with satisfactory results. The reaction pathway is shown in the Scheme 1.

Experimental

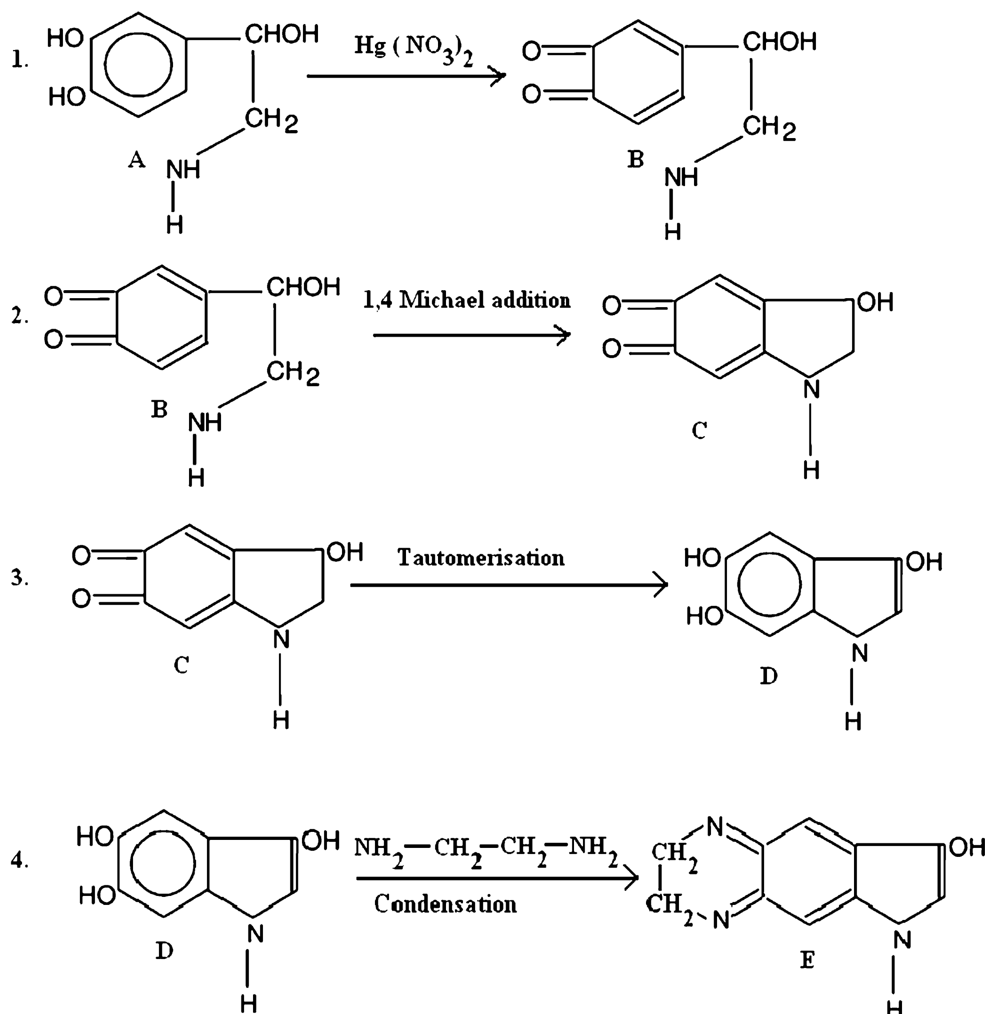
Apparatus

Sepectrofluorimetric measurements were made on SPEX (Edison, NJ, USA) FL 111 spectrofluorometer equipped with a 450-W xenon lamp, quartz cells having a 1 cm optical path, 0.50 mm slits on monochromators. Fluorescence was collected and detected by a Hamamatsu R928 photomultiplier tube. All spectral data were obtained by SPEX DM 3000F spectroscopy computer. A pH meter (Model Orion 520A USA) was used for monitoring pH adjustment. Origin version 6.0 professional software was used for data processing.

Reagents

Norepinephrine bitartrate was used as standard (Sigma, USA). All reagents were of analytical-reagent grade. Stock solution of 0.10 M norepinephrine was prepared in DI water and preserved at 4°C. The standard solutions were prepared from stock solution just before use. Mercury (II) nitrate (Junsei, Japan) solution (0.30%, m/v) was prepared by adding 0.30 g of Hg $(NO_3)_2$ to 1 ml of 0.01 M HCl, diluting to 100 ml with deionized water and mixing well. 1 mol l^{-1} ethylenediamine was prepared from 99% (v/v) ethylenediamine (Junsei chemical Co ltd) and 15% (v/v) acetone was prepared from 95% (v/v) assay. Boric buffer solution of pH 9.0 was made as follows: 50 ml of 0.1 M boric acid and 0.1 M KCl was transferred into a 20.80 ml 0.1 M NaOH solution and diluted to 100 ml with DI water.

Scheme 1 Reaction pathway of norepinephrine



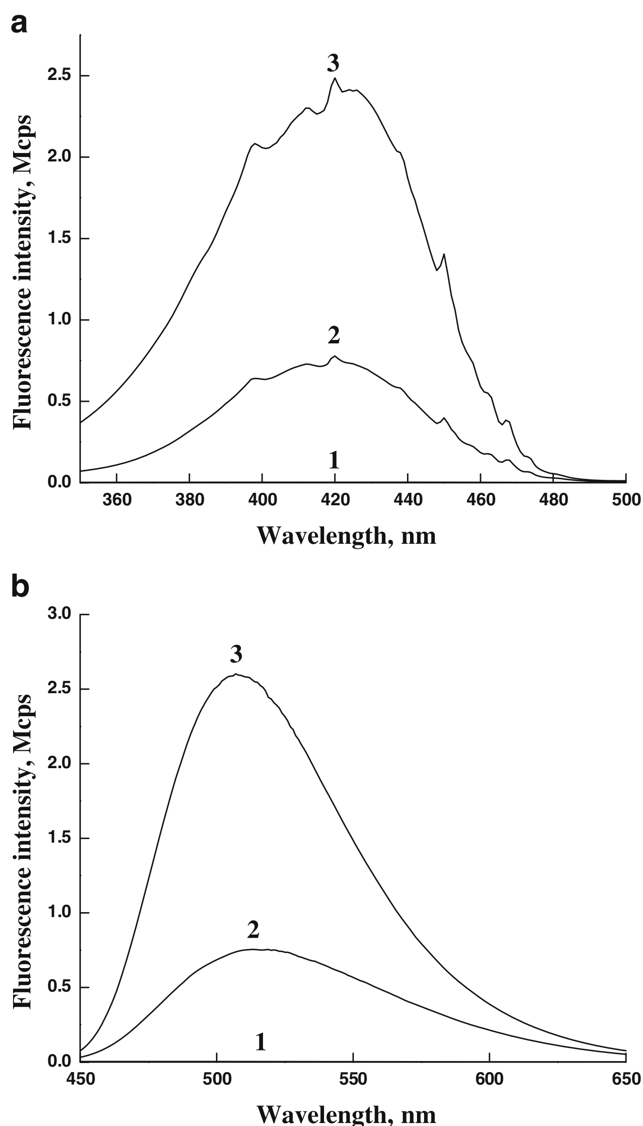


Fig. 2 Excitation and emission spectra of norepinephrine derivative. **a** Excitation spectra of blank (1), norepinephrine derivative in the absence of acetone (2), and in the presence of acetone (3) using an emission wavelength of 507 nm. **b** Emission spectra of blank (1), norepinephrine derivative in the absence of acetone (2), and in the presence of acetone (3) using an excitation wavelength of 420 nm. Conditions: Norepinephrine, 1 mM; ethylenediamine, 1 M; Hg $(NO_3)_2$, 0.30% (m/v); acetone, 15% (v/v); buffer, pH=9.0

Basic procedure

Apparent fluorescence excitation and emission spectra were measured at room temperature and optimum excitation and emission wavelengths were found from these spectra. 0.50 ml portion of 1 mM norepinephrine standard solution was transferred into a 10 ml standard flask. 0.50 ml of 0.30% (m/v) Hg $(NO_3)_2$, 1 ml 15% acetone and 0.50 ml of 1 M ethylenediamine solutions were added successively. The mixture was shaken roughly and 2 ml of 0.40 M HCl was added to the mixture to neutralize excess ethylenediamine and 2 ml of boric buffer pH 9 was added. The mixture was heated for 10–15 min at 30°C and then cooled in tape water. The solution was diluted to mark with DI water, mixed well and put into the quartz cell for measuring fluorescence spectra and intensities. The fluorescence intensity of the solution was measured at 507 nm with excitation at 420 nm against a reagent blank prepared with the reagent concentrations without norepinephrine. For each standard solution, three successive measurements were carried out.

Results and discussion

Spectral characteristics

The excitation and emission spectra of mercury nitrate-acetone-ethylenediamine-buffer (blank) (1), norepinephrine-mercury nitrate-ethylenediamine-buffer (2), norepinephrine-mercury nitrate-acetone-ethylenediamine-buffer (3) systems are shown in Fig. 2a–b. From this figure, it can be seen that the fluorescence signal for the systems of (1)–(3) is located at 507 nm with excitation peak at 420 nm. The behavior can be explained taking account of the basic principle of fluorescence. In common with the report by Moldovan et al. [30] we can propose that after excitation the molecule will be promoted from the lowest vibrational levels of the ground state (S_0) to various vibrational levels of excited singlet states (S_1, S_2), followed by rapid, non-radiative processes to the lowest vibrational level of S_1.

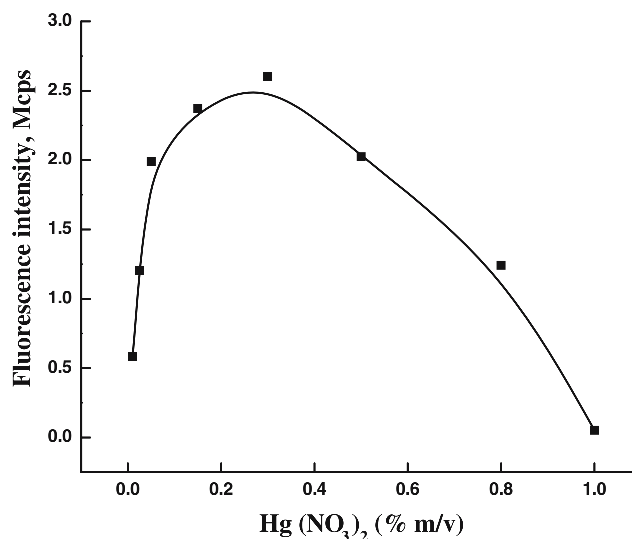


Fig. 3 Variation of fluorescence intensity as a function Hg $(NO_3)_2$. Norepinephrine treated with increasing concentration of Hg $(NO_3)_2$ was derivatized with 1 M ethylenediamine at pH 9 under 30°C for 10–15 min in the presence of 15% (v/v) acetone. Data were obtained using an emission wavelength of 507 nm at an excitation wavelength of 420 nm

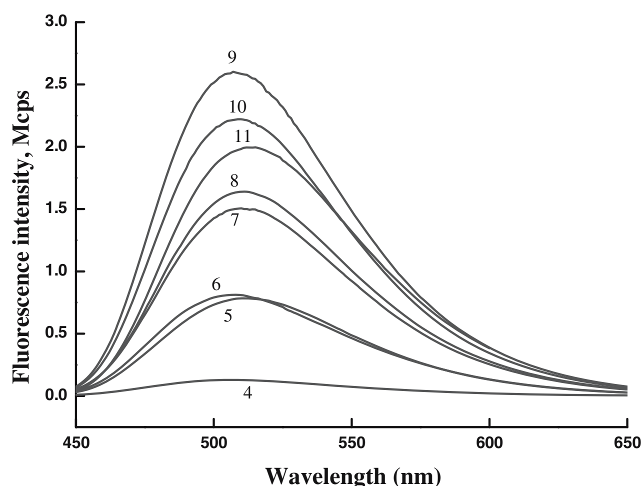


Fig. 4 Emission spectra of the norepinephrine derivative with increasing pH of the solution. Norepinephrine treated with 0.30% (m/v) Hg $(NO_3)_2$ was derivatized with 1 M ethylenediamine at increasing pH of the solution under 30°C for 10–15 min in the presence of 15% (v/v) acetone. Spectra were recorded at a fixed excitation wavelength of 420 nm

Afterwards, with very few exceptions, emission always results from the return of molecule from lowest vibrational level of the electronically excited singlet state (S_1) to any of the vibrational levels of the ground state (S_0). This means that only one emission band may be observed from the final product. Fluorescence of norepinephrine-mercury nitrate-ethylenediamine-buffer solution-system is very weak but when 15% acetone is added to the system the intensity is enhanced by more than 3.5-fold. Using an excitation wavelength of 420 nm, the reagent blank is low and stable, therefore the peaks of $\lambda_{(em)}$=507 nm and $\lambda_{(ex)}$=420 nm were chosen for the further study which did not interfere with the determination.

Effect of oxidant on fluorescence intensity

The procedure was based on the norepinephrine reacted with oxidant to form an indole derivative and the indole derivative condensed with ethylenediamine to a form strong fluorescent compound. The oxidant had a vital role in the formation of fluorescence. In our work, mercury (II) nitrate, sodium nitrate, potassium hexacyanoferrate (IV), hydrogen peroxide, cerium (IV) sulfate were used. The blank value was high and unstable when hydrogen peroxide or potassium hexacyanoferrate (IV) or sodium nitrate was used as oxidant while high fluorescence with excitation and emission wavelengths at 298 and 358 nm, respectively, was observed when cerium (IV) sulfate was used and it interfered with the determination. Because Ce (IV) was reduced to Ce (III) which emitted fluorescence. Only mercury (II) nitrate enhances the intensity comparing to the other oxidants mentioned. The effects of different concentrations of mercury (II) nitrate [0.01–1% (m/v)] on the fluorescence intensity of final product have been investigated under the optimized concentrations of norepinephrine 1 mM; ethylenediamine, 1 M; acetone, 15% (v/v); buffer, pH=9.0. The results are shown in the Fig. 3. As can be seen from the figure, when the concentration of mercury (II) nitrate was in the range of 0.01–0.3%, remarkable enhancing effect was observed. This means that in this concentration range of mercury (II) nitrate, oxidation between the oxidant and norepinephrine was initiated and most stable norepinephrinequinone was formed at 0.3% mercury (II) nitrate showing maximum fluorescence intensity. Cyclization was enhanced simultaneously via intramolecular Michael addition reaction due to the nucleophilicity of amine group. But beyond this range an evident inhibition was observed. The probable reason is that Hg^{2+} is an extremely quencher and at higher concentration collisional quenching of the excited-state fluorophore proceed predominately via multitude of interactions like heavy atom effect [31]. Therefore, 0.3% mercury (II) nitrate was selected for further experiments.

Effect of pH on fluorescence intensity

Figure 4 shows the effect of pH on the fluorescence intensity of product. Analyses have been carried out in a wide range of pH 4–11 under the optimized concentrations of norepinephrine 1 mM; ethylenediamine, 1 M; acetone, 15% (v/v). The fluorescence intensity of product itself in basic media was stronger and more stable than that in acid

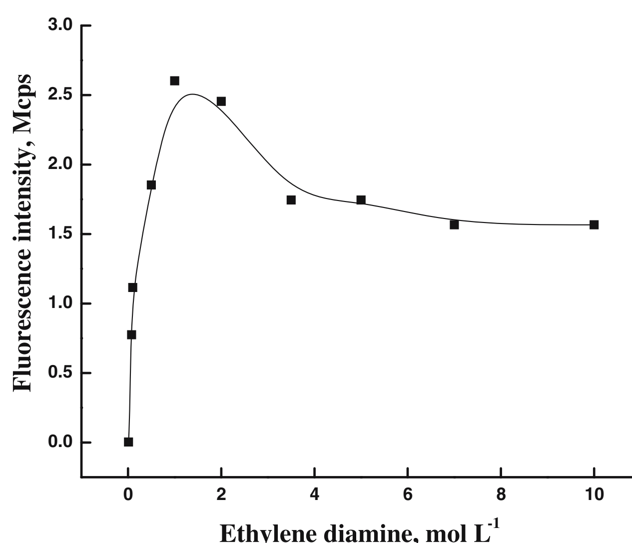


Fig. 5 Variation of fluorescence intensity as a function ethylenediamine. 1 mM norepinephrine treated with 0.30% (m/v) Hg $(NO_3)_2$, was derivatized with increasing concentration of ethylenediamine at pH 9 under 30°C for 10–15 min in the presence of 15% (v/v) acetone. Data were obtained using an emission wavelength of 507 nm at an excitation wavelength of 420 nm

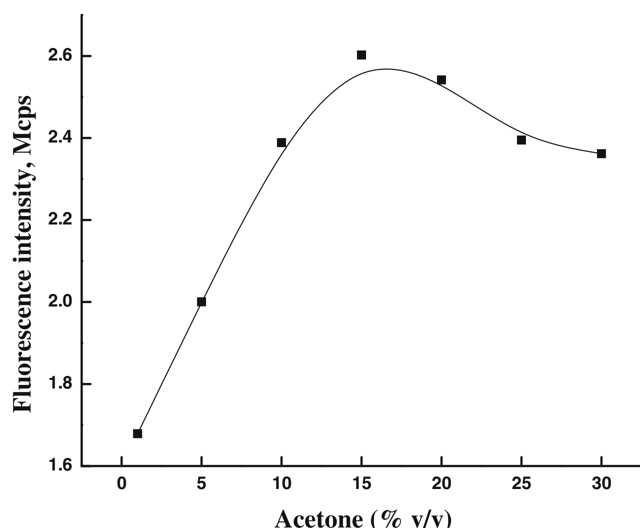


Fig. 6 Variation of fluorescence intensity as a function acetone. 1 mM norepinephrine treated with 0.30% (m/v) Hg $(NO_3)_2$, was derivatized with 1 M ethylenediamine at pH 9 under 30°C for 10–15 min in the presence various concentrations of acetone. Data were obtained using an emission wavelength of 507 nm at an excitation wavelength of 420 nm

media, and it was relatively maximum when the final solution pH was 9. The emission wavelength is 3 nm blue shift with the pH value increasing from 4 to 6 but intensity continued to rise up to pH 9 and thereafter decreased to a very small extent within the investigated pH of 11.0. It is likely that different spectra were obtained from protonated and unprotonated form of the fluorescent compound. It can be proposed that amine group seems to be protected by protonation at low pH values and the intramolecular Michael reaction does not take place but when the media turns to alkaline environment the reaction is initiated and the rate of the reaction increases by increasing pH up to 9. From our experiment it was also observed that the more alkaline solution (> pH 9) weakens the emission intensity. The reason for this behavior is the fact that the partial precipitation of Hg (II) to HgO under strong basic condition might decrease the actual concentration of Hg (II) in the system, which might cause the decrease of the fluorescence intensity of the final product. Therefore pH of 9 was selected with the using of 0.1 M boric acid-0.1 M KCl-0.1 M NaOH buffer solution for further study.

Effect of ethylenediamine on fluorescence intensity

Ethylenediamine is used for condensation reaction to form fluorescent compound. So the study of ethylenediamine concentration on fluorescent intensity is important. Figure 5 presents the results expressed as a function of fluorescence intensity and ethylenediamine concentrations. When the concentration of ethylenediamine is low the fluorescence intensity is low owing to the incomplete reaction with trihydroxyindole. The fluorescent intensity increased with the increasing concentration of ethylenediamine up to 1 M. However, when the concentration of the ethylenediamine is over this value, intensity decreased. Therefore, 0.50 ml of 1 M ethylenediamine solution was recommended. Ethylenediamine is a bidentate ligand that can form two coordinate covalent bonds with a metal atom using the lone pair electrons on both nitrogens. It can exit in aqueous solution in three different forms [32]: $^{+}H_3N\text{-}CH_2\text{-}CH_2\text{-}NH_3^{+}$ (H_2En^{2+}) in acidic media, $^{+}H_3N\text{-}CH_2\text{-}CH_2\text{-}NH_2$ (HEn^{+}) exists at intermediate pH values and $H_2N\text{-}CH_2\text{-}CH_2\text{-}NH_2$ (En) predominates at alkaline media. When the concentration of ethylenediamine is high, it apparently predominated as $Hg(en)_2^{2+}$ with the use of excess of ethylenediamine between the alkaline range [33]. Therefore, consumption of Hg (II) facilitates the non-radiative deactivation which results in decreasing the fluorescence intensity of the fluorophore.

Effect of acetone on fluorescence intensity

In Fig. 6, the dependency of peak emission intensity on the presence of acetone in the aqueous solution can be seen. The fluorescence intensity of the final product reaches the maximum when the solution contains 15% (v/v) acetone and a significant decrease of the emission intensity with the increment of acetone concentration is observed. The increased behavior of the intensity may be due to the acetone acting as proton transmitter to accelerate the transformation of trihydroxyindole to

Table 1 Effect of the sequence of acetone added

Sequence of addition							
No.	1	2	3	4	5	6	Rfi[c]
1	NE[a]	$Hg(NO_3)_2$	Acetone	EDA[b]	HCl	Buffer	100
2	NE[a]	Acetone	$Hg(NO_3)_2$	EDA[b]	HCl	Buffer	100
3	NE[a]	$Hg(NO_3)_2$	EDA[b]	HCl	Acetone	Buffer	29
4	NE[a]	$Hg(NO_3)_2$	EDA[b]	HCl	Buffer		29

[a] Norepinephrine; [b] Ethylenediamine; [c] Relative fluorescence intensity

Table 2 Tolerance limit of some foreign substances on the determination of norepinephrine (0.1 μM)

SL	Species added	Maximum tolerable concentration ratio
1	Na^+, K^+	2,500
2	Pb^{2+}, Fe^{3+}, Mn^{2+}, Zn^{2+}	3,500
3	Cl^-, SO_4^{2-}, NO_3^-	1,200
4	Glucose, citric acid and resorcinol	950
5	Ascorbic acid and quinol	100
6	Sucrose and Fe^{2+}	100
7	Sodium metabisulfite	2,000
8	Dopamine	1
9	Epinephrine	1

o-quinone. Furthermore, when acetone is added the polarity of the solution is increased. The lone pair on nitrogen of the final product is solvated which lowers its energy relative to the π* orbital; as a consequence, the π π* becomes the lowest lying state and fluorescence is favored. So at 15% of acetone, the solution has greater fluorescence signal. But to be regretted, we could not explain the descending tendency of fluorescence intensity with the use of acetone more than 15% (v/v) in the system. On the basis of the maximum fluorescence intensity, the fluorimetric experiments were carried out using 15% (v/v) acetone. Experimental results in Table 1 also showed that acetone should be added prior to ethylenediamine in order to obtain more intense emission peak. However, intensities obtained following the sequences (1) and (2) were the same.

Effect of heating time on fluorescence intensity

The effects of oxidization temperature and time on the development of fluorescence were investigated. The reaction speed accelerates with the increase of reaction temperature. It can be seen that when the temperature is low, oxidization is slow and the degree of oxidization is low. At higher temperature, the fluorescence develops more rapidly and the degree of oxidization is higher. In our experiment, the heating time of 10–15 min at 30°C was recommended under the optimized conditions.

Interference study

In a real sample, the analyte of interest will be in the presence of other species. These other species, although they themselves have no apparent effect on the fluorescence intensity of the final product, may suppress or enhance the signal due to the analyte. Pharmaceutical norepinephrine bitartrate injections contain sodium chloride, sodium metabisulfite as an antioxidant, citric acid anhydrous and sodium citrate dehydrate as buffers. Interference effects of these additives and several other substances such as catecholamines (epinephrine, dopamine), glucose, amino acids, and familiar metal ions which are expected to be present in urine were thoroughly examined at 0.1 μM norepinephrine. The tolerable limit of foreign species was taken if it caused a relative error of <5%. The results are shown in the Table 2. It can be shown that most of the substances tested do not interfere or have little interference; but epinephrine and dopamine have significant interferences. The normal concentrations of epinephrine in urine are 0.06, 0.13 and 0.2 μM for normal personnel, patients with hypertension and patients with phaeochromocytoma, respectively. The interference of epinephrine can be eliminated by sample dilution. After ten times dilution, the concentration of epinephrine is in the range of 0.01 μm which is lower than the normal concentrations mentioned and norepinephrine concentration of 0.01 μM falls in the linear range for the determination.

Dopamine was also oxidized by mercury (II) nitrate and the oxidation product was condensed with ethylenediamine to form a strong fluorescent compound under the experimental conditions. So, dopamine is a strong candidate for interference with the determination of norepinephine by the proposed method. In order to minimize the interference of dopamine with the determination of norepinephrine, the combined technique of first derivate synchronous fluorimetric method (peak to zero method) [15] is used and the results are shown in the Table 3. As can be seen, the error

Table 3 Determination of results of recovery of DA and NE in synthetic mixture

Mixture composition (μg l^{-1})			Found (μg l^{-1})		Recovery (%)		Error (%)	
Mixture no.	DA	NE	DA	NE	DA	NE	DA	NE
1	30	3	29.55	2.88	98.50	96	−1.5	−4
2	15	3	14.55	2.94	97	98	−3	−2
3	3	3	3.00	2.91	100	97	0	−3
4	3	6	2.95	5.76	98.33	96	−1.67	−4
5	6	30	5.88	29.58	98	98.60	−2.0	−1.4

Table 4 Summary of several methods for the determination of norepinephrine

Sl	Method	Linear range	LOD	References
1	Electrochemical	1 μM–0.05 mM		[5]
2	TOFMS		0.3 μM	[7]
3	HPLC -FL		0.01μM	[8]
4	IC-CD	0.049–0.243 μM	4.9 nM	[10]
5	CL-LC		42.6fM	[11]
6	Spectrophotometric	4.8–600 μM	2.46μM	[13]
7	Peroxidase based spectrophotometric	2.96 μM–0.0592 mM		[14]
8	Derivative synchronous spectroscopy	5.92 nM–0.177 μM		[19]
9	Ethylenediamine condensation- FL	0.01 μM–0.014 mM	2.5 nM	Proposed

Converted values in molarities; *LOD*, Limit of detection

made never exceeded 4% in case of each catecholamine. It proves that the proposed method has a potential for the determination of norepinephrine in real sample.

Analytical application

Calibration graph and detection limit

Under the optimum experimental conditions, a linear relationship was obtained between the fluorescence intensity and the concentration of norepinephrine in the range of 0.01 μM–0.014 mM with a correlation coefficient of 0.99813, and a detection limit of 2.5 nM. A comparison between the sensitivity of the method and other common methods is shown in the Table 4. The results summarized in the table indicate that the sensitivity of the method is higher than most other methods.

Recovery of norepinephrine in injection solution

Test of the recovery efficiency for known amounts of norepinephrine added to different concentrations of sample solution (Norepinephrine Bitartrate, 1 mg/ml, 4 ml ampoule) was performed and the results are shown in the Table 5. The recovery ranged from 96.67 to 102%.

Recovery of norepinephrine in urine sample

Standard addition method was applied for the recovery test considering the effects of foreign species on the fluorescence intensity of the system. Tests of the recovery efficiency for known amounts of norepinephrine in urine were made. Recoveries from urine spiked with NE (0.08, 0.14 and 0.8 μM) varied from 95 to 98.62% (Table 6).

Personal protection

$Hg(NO_3)_2$ is highly toxic. Inhalation, ingestion or skin absorption may be fatal. It is also neurological hazard and readily absorbed through the skin. Safety glass, rubber gloves, and a fume cupboard should be used while handling $Hg(NO_3)_2$.

Conclusion

The results presented in this paper clearly demonstrated that norepinephrine can be determined by fluorimetric method proposed. The linear range, detection limit and correlation coefficient are 0.01 μM–0.014 mM, 2.5 nM and 0.99813, respectively. The method is very easy, rapid and sensitive.

Table 5 Recovery of norepinephrine from injection solution of norepinephrine (Norepinephrine Bitartrate, 1 mg/ml, 4 ml ampoule)

Sample	Sample quantity (μg/ml)	Norepinephrine (μg/ml)		Recovery (%)
		Added	Observed[a]	
1	0.10±0.02	0.10	0.197±0.002	97.0±1.5
2	0.50±0.01	0.20	0.704±0.003	102±2.3
3	0.75±0.03	0.30	1.04±0.005	96.67±1.6

[a] Average of five measurements (±SD)

Table 6 Assay for recovery in urine sample

Sample	Added (×0.01 μM)	Found[a] (Mean±SD) (× 0.01 μM)	Recovery, %
Urine	0.8	0.76±0.064	95.00
	1.4	1.38±0.108	98.57
	8.0	7.89±0.169	98.62

[a]Average of five measurements (±S.D)

It has low detection limit and can be applied for the determination of norepinephrine in pharmaceutical formulation and biological samples.

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