Journal of Education and Science (ISSN 1812-125X), Vol: 31, No: 03, 2022 (17-26)

Spectrofluorimetric Determination of Adrenaline and Dopamine

Mohamed Y. Dhamra[1*], Theia'a N. Al-Sabha[2], Mohammed salim Al-Enizzi[3]

[1*,2]Chemistry Department, College of Education for Pure Science, Mosul University, Mosul, Iraq
[3]Chemistry Department, College of Education for girls, Mosul University, Mosul, Iraq

E-Mail:[1*]mohameddhamra@uomosul.edu.iq, [2]dr_theiaa@yahoo.co.uk, [3]Mohammmed.salim@uomosul.edu.iq

(Received May 12, 2022; Accepted June 18, 2022; Available online September 01, 2022)

DOI: 10.33899/edusj.2022.133845.1238,

Abstract

A sensitive fluorometric method, with few steps and suitable for the daily routine, was made for examining adrenaline hydrochloride and dopamine hydrochloride. The reliance in this paper was on the nucleophilic substitution interaction of the mentioned drugs with 1,2-naphthoquinone sulfonate (NQS) in an aqueous pH 6 to give a fluorescent product with a maximum emission wave at λ_{em} 471 nm after being excited at a maximum excitation wave at λ_{ex} 300 nm. The plots have complied within the range of 0.01- 4.0, 0.01-2 µg/ml, and The detection limits (0.0062, 0.0027) and quantitation limits were (0.0207, 0.0091) µg/ml, for adrenaline and dopamine respectively. The accuracy (% recovery) was between (99.21% - 100.72%) and the relative standard deviation (RSD%) is better than 0.95%. It was also found that the formed product was in a ratio of 1:2 reagent to the drug. The estimation of adrenaline and dopamine has been successfully tested on the injection, and it is in good agreement with its approved value and with that of the British Pharmacopoeia method.

Keywords: adrenaline; dopamine; substitution reaction; Spectrofluorimetry.

التقدير الفلورومتري للادرينالين والدوبامين

محمد يحيى ضمرة[1*], ضياء نجم الصبحة[2], محمد سالم شيت العنزي[3]

[2,1]قسم الكيمياء، كلية التربية للعلوم الصرفة، جامعة الموصل، موصل، العراق
[3] قسم الكيمياء، كلية التربية للبنات، جامعة الموصل، موصل، العراق

الملخص

تم إجراء طريقة قياس فلورومترية حساسة ودقيقة، مع خطوات عمل قليلة ومناسبة للروتين اليومي، لقياس الأدرينالين هيدروكلوريد و الدوبامين هيدروكلوريك. تعتمد الطريقة المطورة على تفاعل التعويض النيوكليوفيلي للأدوية المذكورة مع 2,1-نافثوكينون سلفونات (NQS) في محلول مائي ذي دالة الحامضية مقدارها 6 لإعطاء ناتج فلورة بموجة انبعاث قصوى عند λ_{em} 471 نانومتر بعد الإثارة بأقصى موجة إثارة عند λ_{ex} 300 نانومتر. كانت حدود تقدير المركبات المدروسة في نطاق 0.01 - 4.0، 0.01-2 ميكروغرام / مل، في حين بلغت حدود الكشف بين (0.0062 , 0.0027) والحدود الكمية (0.0207, 0.0091) ميكروغرام / مل على التوالي. كانت قيم الدقة (معدل الاسترداد٪) بين (99.21٪ - 100.72٪) والانحراف المعياري النسبي (RSD) أفضل من 0.95٪. وجد أيضًا أن ناتج الفلورة المتكون كان بنسبة 1: 2 كاشف إلى عقار. امكن تطبيق هذه الطريقة بنجاح لفحص الأدرينالين هيدروكلوريد والدوبامين هيدروكلوريد في المستحضرات الصيدلانية بشكل حقن، تعد هذه النتائج متوافقة بشكل جيد مع قيمتها المعتمدة وقيمة طريقة دستور الأدوية البريطانية.

الكلمات المفتاحية : ادرينالين; دوبامين; تعويض نيوكليوفيلي; قياس الفلورة.

Introduction

Adrenaline hydrochloride, $C_9H_{14}ClNO_3$, (R)-4-(1-hydroxy-2-(methylamino)ethyl)benzene-1,2-diol hydrochloride (figure 1). is classified as a hormone and is secreted by the adrenal gland (adrenaline gland). It was used as a medicine to treat some conditions, such as hypersensitivity caused by taking doses of medicines, especially penicillin, as a pupil dilatant, and to reduce intraocular pressure.[1-2] .

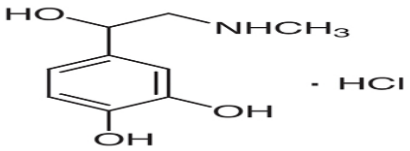


Figure 1. Chemical structure of Adrenaline hydrochloride, Molecular Weight:219.66 g/mol

Dopamine hydrochloride, $C_8H_{12}ClNO_2$, 4-(2-Aminoethyl) benzene-1,2-diol hydrochloride. (figure2). One of the catecholamine neurotransmitters in the brain. It is classified as a cardiac stimulant, and it is a hormone secreted from the adrenal gland and contributes to the fight-and-flight response processes, and it is called the happiness hormone. The right balance of dopamine in the human body is extremely important and vital, as it plays a role in controlling motor skills and emotional responses, making it essential for physical and mental health. The effect of dopamine lies in the vital areas of the brain, as it affects mood, sleep, study, focus, and learning, so its deficiency may lead to certain diseases, including; Parkinson's and depression [3- 4].

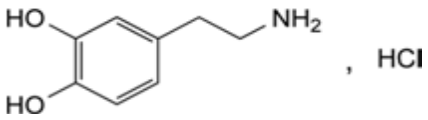


Figure 2. Chemical structure of Dopamine hydrochloride, Molecular Weight:189.64 g/mol

Numerous analytical methods are used to determine adrenaline and dopamine, such as chromatographic methods[5-8], voltammetry[9-14], capillary electrophoresis[15], flow injection[16-18], ion-selective electrode[19-23], spectrophotometric[24-30] and spectrofluorimetric[31-34] methods, It is known that the fluorescence measurement methods are sensitive, so in this paper, an easy method was described to measure the fluorescence of adrenaline and dopamine and it does not require a lot of materials, that is, it is economically feasible in addition to being in an aqueous medium, while most of the methods used expensive organic solvents and harmful to the environment.

Experimental

Apparatus

The fluorometric measurements were carried out using an RF-5301 PC-Spectrofluorophotometer xenon lamp with transparent quartz cells on all sides with a thickness of 1 cm. Heating processes were also carried out using a nüve NB 20 water bath, the acidity of the solutions was measured using a Philips PW 94 device connected to a CE 10-12 pH electrode, and the weighing processes were carried out using a sensitive balance of the type D0001.A&D Company Limited

Chemical Reagents

The reagents used are all of the analytic reagent grades supplied by BDH, Fluka, and Molekula companies. Standard adrenaline.HCl, dopamine.HCl solution was prepared at a concentration of 100 µg/ml by dissolving 0.0100 g in 2.0 ml of ethanol and then diluting it to the mark with distilled

water separately in two volumetric flasks of 100 ml capacity. Then different volumes were taken from them as needed. 1,2-Naphthoquinone-4-Sulfonic Sodium (NQS) 0.5% (w/v), prepared daily by dissolving 0.5 g of it in 100 ml of distilled water. Prepare 0.1 M sodium bicarbonate by dissolving 5.3 g in distilled water and then adjusting the volume to 500 ml in a volumetric flask. Hydrochloric acid was prepared by diluting 1.75 milliliters of acid at a concentration of 11.44 molar in 200 milliliters of distilled water and then taken from it as needed. All surfactants were prepared by dissolving 0.1 g of each one in 100 ml volumetric flasks.

General procedure

Aliquots containing 2 ml of 0.1 M HCl followed by optimal values (0.001-0.4, 0.001-0.2 ml) of adrenaline and dopamine hydrochloride were transferred then 3 and 2 ml of 0.5% NQS, respectively, were added to two sets of 10ml volumetric flasks. The mixture was then diluted to the mark with separately distilled water and kept at 50 °C for 15 min until fluorescence appeared. At 471 nm the fluorescence of the resulting solution was checked after excitation at 334 nm versus the blank solution.

Pharmaceutical preparation

Adrenaline.HCl

A solution of 100 µg/ml is prepared after diluting the content of five epinephrine injections (each injection contains 1.0 mg/1.0 ml of epinephrine (Misr Company)) with distilled water then the volume iscompleted to 50 ml. Different volumes were taken from it to get concentrations covering the area of the standard plot of adrenaline in its pure form.

Dopamine.HCl

One injection (containing 200 mg/5 ml dopamine hydrochloride, Hospira, INC, LAKE FOREST, USA,) to make volume up to 200 ml was diluted with distilled water to get a solution at a concentration of 1000 µg/ml, from which solution was then prepared at a concentration of 100 µg/ml, different volumes of dopamine were taken within the standard curve range and treated according to the general procedure. The concentration of the medicinal compound in the syringe is found using the standard curve for the medicinal compound in its pure form.

Results and Discussion

Preliminary test

The coupling of adrenaline and dopamine with the NQS reagent for the formation of fluorescent products were examined in the presence of each constant amount of hydrochloric acid and sodium hydroxide measured at 471 nm after excitation at 300 nm. It was also found that there is an increase in the intensity of fluorescence when heating the mixture at 50 ° C and for a few minutes.

Optimization of conditions

Effect of acid and base

To obtain a high fluorescence intensity, the effect of adding several acids and bases (0.1M) such as sodium hydroxide, sodium carbonate, sodium bicarbonate, hydrochloric acid, and acetic acid was examined, later it was found that hydrochloric acid gave the highest intensity of fluorescence as shown in Table (1). The final pH of the final solution was measured and, it was 6.

Table 1. Effect of acid and base.

Type of acids and bases	Fluorescence	
	Adrenaline	Dopamine
NaOH	0	0
Na_2CO_3	0	0
$NaHCO_3$	15	35
CH_3COOH	18	40
HCl	20	55

Effect of buffer solution

To study the effect of buffer solutions such as citrate, phthalates, and phosphate with pH 6, these buffer solutions were examined. A decrease in fluorescence intensity was observed so hydrochloric acid was used instead. The addition of increasing amounts of hydrochloric acid at a concentration of 0.1 M was also studied, and 2 ml was preferable (Fig. 3), so it was used in subsequent studies

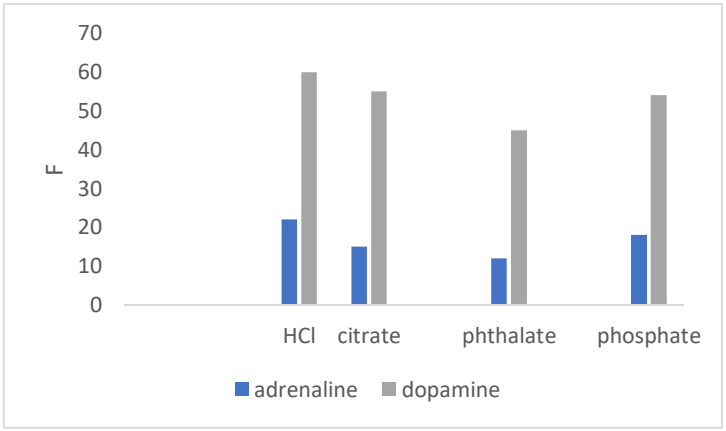


Figure 3. Effect of buffer solutions.

Effect of NQS amount

In this study, the results of adding different concentrations of NQS to the solution containing a fixed amount of studied drugs were examined, and it is clear that the fluorescence intensity increases with the increase in the concentration of NQS, and this increase reaches a maximum when using 3.2 ml of 0.5% of adrenaline and dopamine reagent respectively (Fig. 4). Therefore, these quantities were used in later studies.

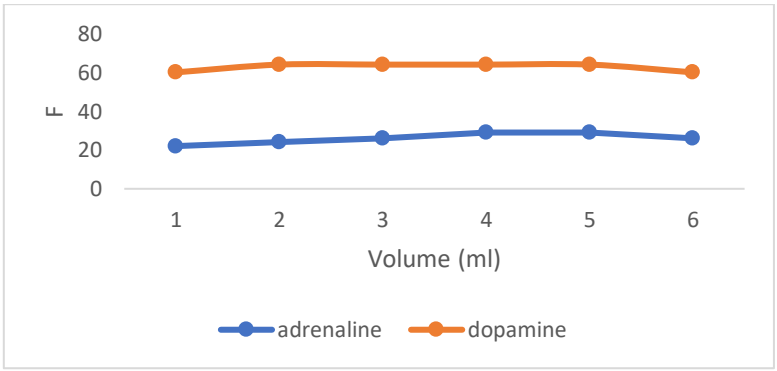


Figure 4. Effect of volume of NQS.

Temperature and growing time

Fluorescence measurement of the solutions was tracked to determine the reaction time at room temperature and in a thermo-controlled water bath at various temperatures up to 70 °C. The fluorescence of the solutions was measured at 5-min intervals against the similarly treated blank reagent. The fluorescence was observed to peak after 15 min at 50 °C and remained constant for 80 min, while a decrease in fluorescence intensity was observed with increasing time and temperature (Fig. 5). Hence, 15 minutes at 50°C was the optimum temperature for this work.

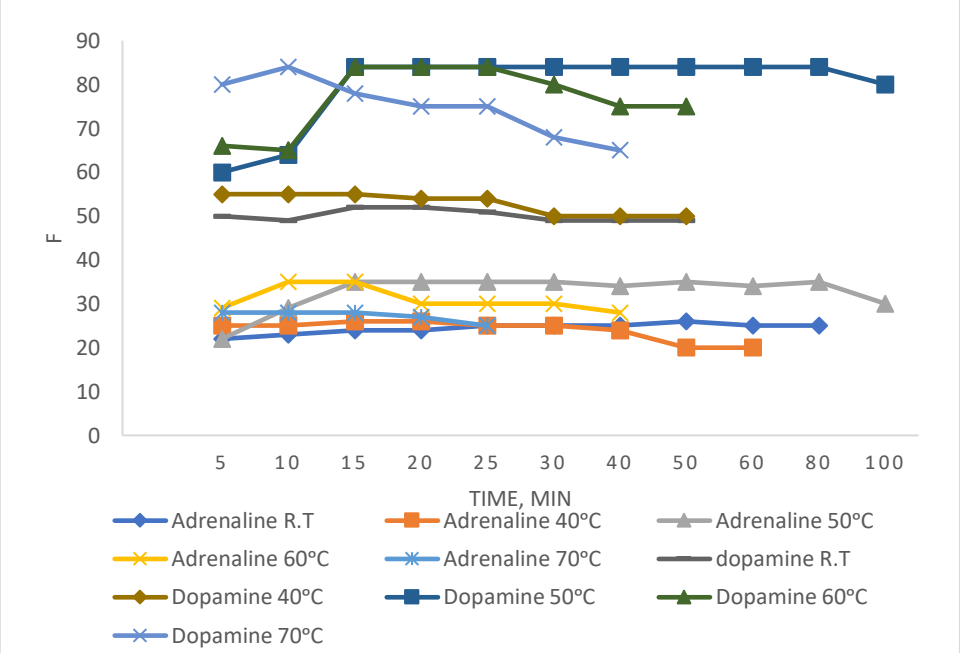


Figure 5. Effect of temperature and developing time.

Addition of surfactants

To raise the intensity of fluorescence, several surfactants such as cetyltrimethylammonium bromide (CTAB), cetylpyridinium chloride (CPC), Sodium Dodecyl Sulfate (SDS), Tween 80 (TW-80), and TritonX-100 (TX-100) have been added at a concentration of 0.1%, surfactants showed a negative effect Therefore, it was excluded in subsequent studies.

sequence of addition

To search for high sensitivity, the addition sequence of reagents under optimal conditions was tested. Table 2 shows that sequence III is optimal, so it was adopted in the next study.

Table 2. order of addition of components reaction.

Order of addition	Order no.	Intensity of fluorescence Adrenaline	Intensity of fluorescence Dopamine
Drug + NQS + acid	I	34	84
Drug + acid + NQS	II	34	83
Acid + drug + NQS	III	38	91
Acid + NQS + drug	IV	33	84
NQS +acid + drug	V	34	84
NQS + drug + acid	VI	34	83

Final spectra

The final spectrum for each adrenaline hydrochloride and dopamine hydrochloride with the reagent NQS was taken, which are nucleophilic substitutions. (Fig. 6), shows the fluorescence spectrum of the two drugs mentioned above.

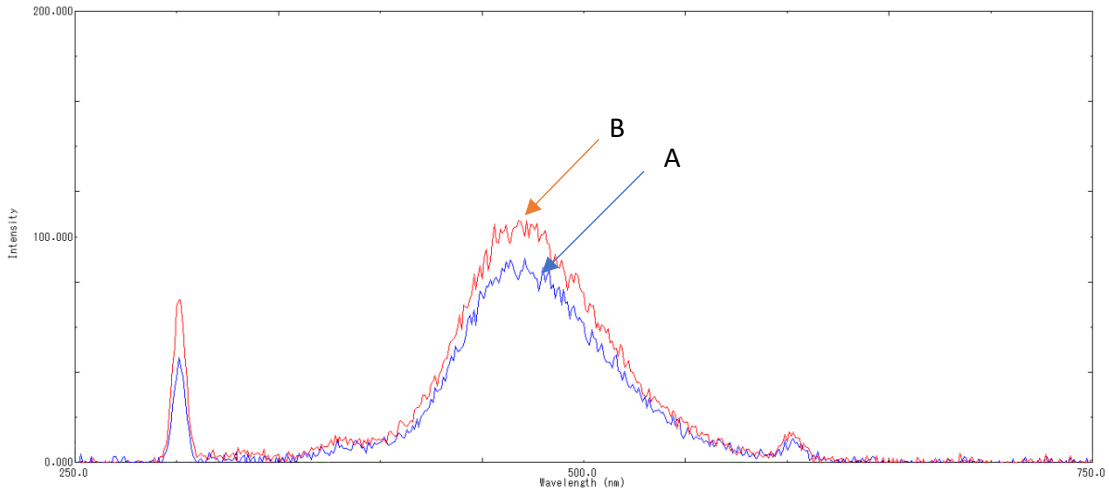


Figure 6. Fluorescence spectrum for each of 2.5 µg/ml adrenalin hydrochloride, and 1.25 µg/ml dopamine hydrochloride.

Calibration plot and results

Calibration graphs were drawn using Optimum experimental conditions by plotting the fluorescence intensity (F) as a function of Adrenaline and dopamine hydrochloride ppm concentrations, Excellent linearity graphs are shown in the range 0.01-4.0 and 0.01-2 µg/ml for the above drugs, respectively (Fig. 7). The specification of the curve is shown in Table 3.

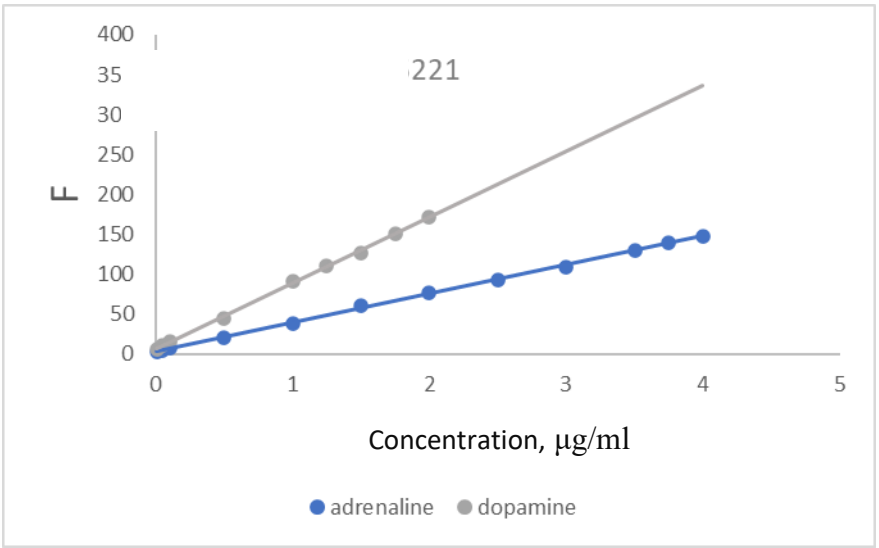


Figure 7. calibration curves of adrenaline.HCl and dopamine.HCl

Table 3. Analytical parameters for adrenaline and dopamine

Parameters	Adrenaline	Dopamine hydrochloride
Liner range, µg/ml	0.01-4.0	0.01-2.0
Slope	36.335	82.635
Intercept	2.5752	5.5221
LOD ($\mu g.ml^{-1}$)	0.0062	0.0027
LOQ ($\mu g.ml^{-1}$)	0.0207	0.0091
Average recovery (%)	100.72	99.21
RSD%*	0.9421	0.7412
R^2	0.9993	0.9993

* Average of five determinations.

Application of methods

To prove the efficiency of the proposed method and its success in estimating both adrenaline HCl and Dopamine HCl in their pharmaceutical preparations by injection, a comparison was made between the present analytical method and the standard method contained in British pharmacopeia using t and F tests at a confidence level of 95% with Six degrees of freedom by applying the statistical laws the results of the experimental t-test and the F-test were less than the calculated value (t = 2.45, F = 6.39). This results in the proposed method being free of significant differences in comparison with the standard method. The results of the two methods are shown in the table (Table 4).

Table4.Application of methods.

Procedure applied	Dosage form	Drug amount present (ppm)	Recovery[a] (%)	Drug content found (mg)	Average recovery (mg)	Certified value (mg)
Proposed NQS method (adrenalin.HCl)	Injection	1.0 2.0 3.0	102.04 99.500 103.50	1.020 0.995 1.035	1.016	1 mg/1ml
Proposed NQS method (dopamine.HCl)	Injection	0.5 1.0 1.5	100.01 100.10 102.5	200.02 200.20 205.00	201.74	200mg/5ml
British Pharmacopoeia (adrenalin.HCl)	Pure	1.0 2.0 3.0	100.25 99.450 100.10	10.02 9.945 10.01	9.99	10mg
British Pharmacopoeia (dopamine.HCl)	Pure	0.5 1.0 1.5	100.15 100.01 99.035	200.30 200.02 198.07	199.46	200mg

a average of three determinations.

The nature of complex and the mechanism of reactions

To predict the final products, a stoichiometric study of the interaction of adrenaline and dopamine with the NQS reagent was used by the mole ratio method using $1x10^{-2}$ M solutions for each drug and NQS reagent. As shown in (Fig. 8 A and B), the results that 1:2 reagents to 2 of each drugs were formed using both of the above-mentioned methods. This indicates that a nucleophilic substitution product was formed in the weak acidic medium, as shown in the proposed mechanism [35] (Fig.9).

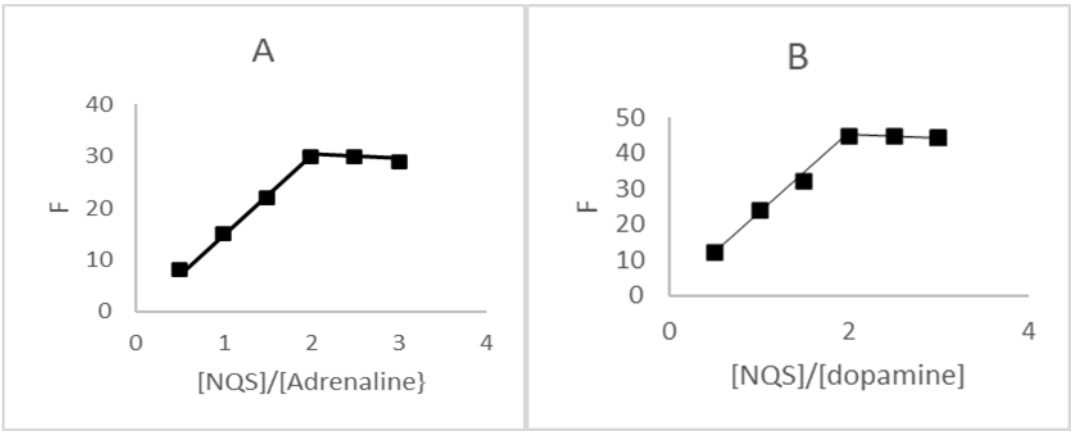
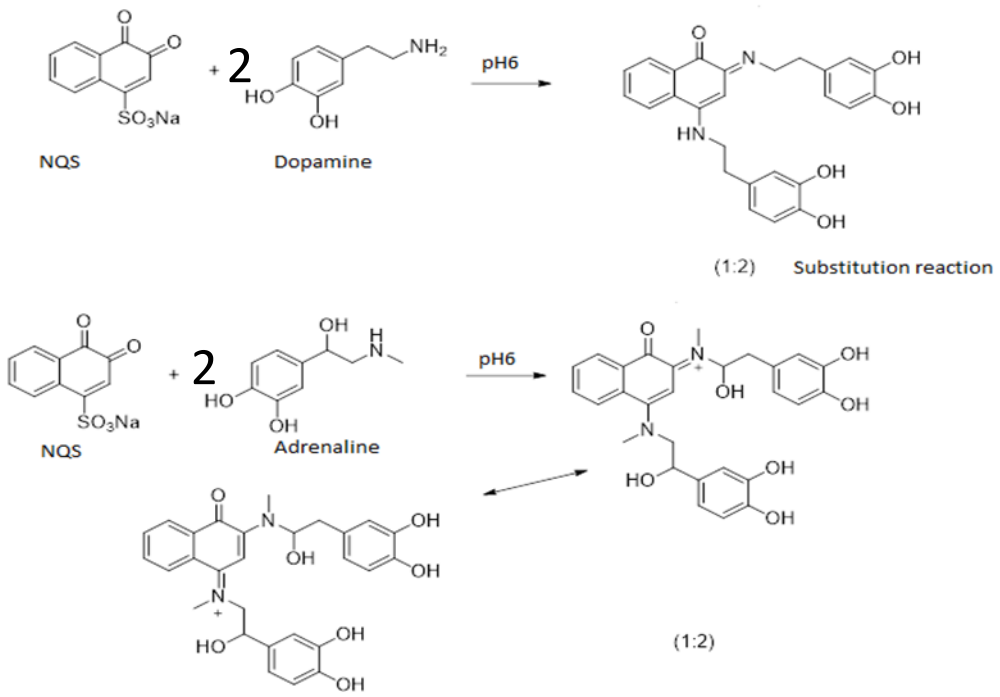


Figure 8. A and **B** The mole ratio of adrenaline.HCl, dopamine.HCl (1×10^{-2}M) and NQS (1×10^{-2}M) respectively under the optimum conditions.



Scheme 1: Probable products formation mechanisms.

Conclusion

The proposed method are uncomplicated and does not require additional steps or extraction processes, in addition to being in an aqueous medium. Also, its recovery values are good. The method has been applied successfully in pharmaceutical preparations, and the results were consistent with the pharmacological content of the studied compounds.

References

1. Johnston, S.L., J. Unsworth, and M.M. Gompels, Adrenaline given outside the context of life threatening allergic reactions. Bmj, 2003. 326(7389): p. 589-590.
2. Sheikh, A., et al., Adrenaline (epinephrine) for the treatment of anaphylaxis with and without shock. Cochrane Database of Systematic Reviews, 2008.(4)
3. Goldberg, L.I., Dopamine—clinical uses of an endogenous catecholamine. New England Journal of Medicine, 1974. 291(14): p. 707-710.

4. Marshall, V. and D. Grosset, Role of dopamine transporter imaging in routine clinical practice. Movement disorders: official journal of the Movement Disorder Society, 2003. 18(12): p. 1415-1423.
5. Bergh, M.S.-S., et al., Determination of adrenaline, noradrenaline and corticosterone in rodent blood by ion pair reversed phase UHPLC–MS/MS. Journal of Chromatography B, 2018. 1072: p. 161-172.
6. Kongkiatpaiboon, S., et al., Development and validation of stability indicating HPLC method for determination of adrenaline tartrate. Journal of King Saud University-Science, 2019. 31(1): p. 48-51.
7. Siddiqui, M.R., et al., High throughput ultra performance liquid chromatography-mass spectrometric method for determination of adrenaline and characterization of its stress degradation products: a mechanistic approach. Journal of the Chilean Chemical Society, 2018. 63(1): p. 3803-3810.
8. Zhao, H.-X., et al., A rapid method for the determination of dopamine in porcine muscle by pre-column derivatization and HPLC with fluorescence detection. Journal of pharmaceutical analysis :(3)1 .2011 ,p. 208-212.
9. Cincotto, F.H., et al., Simultaneous determination of epinephrine and dopamine by electrochemical reduction on the hybrid material SiO 2/graphene oxide decorated with Ag nanoparticles. Analyst, 2014. 139(18): p. 4634-4640.
10. Zhang, Y., W. Ren, and S. Zhang, Simultaneous determination of epinephrine, dopamine, ascorbic acid and uric acid by polydopamine-nanogold composites modified electrode. Int. J. Electrochem. Sci, 2013. 8(5): p. 6839.
11. Alpat, Ş., K. Özdemir, and S. Kılınç Alpat, Voltammetric determination of epinephrine in pharmaceutical sample with a tyrosinase nanobiosensor. Journal of Sensors, 2016. 2016.
12. Xu, G., et al., Sensitive, selective, disposable electrochemical dopamine sensor based on PEDOT-modified laser scribed graphene. Biosensors and Bioelectronics, 2018. 107: p. 184-191.
13. Yang, H., et al., Sensitive determination of dopamine and paracetamol based on carbon nanotubes-supported Pd nanoparticles. Journal of Nanoscience and Nanotechnology, 2018. 18(1 :(p. 500-509.
14. Pourghobadi, Z. and D. Neamatollahi, Voltammetric determination of dopamine using modified glassy carbon electrode by electrografting of catechol. Journal of the Serbian Chemical Society, 2017. 82(9): p. 1053-1061.
15. Weng, Y., et al ,.Separation and determination of dopamine and epinephrine in serum by capillary electrophoresis with inkjet introduction system. Chromatography, 2013. 34(1): p. 33-40.
16. UKEDA, H., FIA Bibliography (37). AnaL Lett, 2002. 35: p. 282.
17. Michałowski, J .and P. Hałaburda, Flow-injection chemiluminescence determination of epinephrine in pharmaceutical preparations using raw apple juice as enzyme source. Talanta, 2001. 55(6): p. 1165-1171.
18. Zheng-Hua, S. and Z. Ni, Flow-injection Chemiluminescence Determination of Reserpine in Medicine and Biological Fluids with Controlled-Reagent-Release Technology. Chinese Journal of Chemistry, 2003. 21(2): p. 175-180.
19. Zaidi, S.A., Utilization of an environmentally-friendly monomer for an efficient and sustainable adrenaline imprinted electrochemical sensor using graphene. Electrochimica Acta, 2018. 274: p. 370-377.
20. Shankar, S.S., R.M. Shereema, and R. Rakhi, Electrochemical determination of adrenaline using MXene/graphite composite paste electrodes. ACS applied materials & interfaces, 2018. 10(50): p. 43343-43351.

21. Charithra, M. and J. Manjunatha, Electrochemical sensing of adrenaline using surface modified carbon nanotube paste electrode. Materials Chemistry and Physics, 2021. 262: p. 124293.
22. Yuan, D ,.et al., N-Doped carbon nanorods as ultrasensitive electrochemical sensors for the determination of dopamine. RSC advances, 2012. 2(21): p. 8157-8163.
23. Beitollahi, H., M. Safaei, and S. Tajik, Different electrochemical sensors for determination of dopamine as neurotransmitter in mixed and clinical samples: A review. Analytical and Bioanalytical Chemistry Research, 2019. 6(1): p. 81-96.
24. Ekhtesasi, A. and M.R. Shishehbore, Sensitive determination of epinephrine using kinetic spectrophotometric method .Orient J Chem, 2016. 32: p. 467-472.
25. Shishehbore, M.R., A. Asgharpoor, and N. Nasirizadeh, A novel kinetic spectrophotometric method for the determination of dopamine in biological and pharmaceutical samples. Journal of Chemistry, 2013. 2013.
26. Al Abachi, M.Q. and H. Hadi, A new kinetic and thermodynamic study of spectrophotometric method for determination of adrenaline in its pharmaceutical formulations. Pharmaceutical Chemistry Journal, 2014. 48(8): p. 558-563.
27. Zhu, S., et al., Simple and fast determination of catecholamines in pharmaceutical samples using Ag+–3, 3′, 5, 5′-tetramethylbenzidine as a colorimetric probe. Analytical Methods, 2015. 7(16): p. 6785-6790.
28. Guo, L., Y. Zhang, and Q. Li, Spectrophotometric determination of dopamine hydrochloride in pharmaceutical, banana, urine and serum samples by potassium ferricyanide-Fe (III). Analytical sciences, 2009. 25(12): p. 1451-1455.
29. El-Zohry, A.M. and E. Hashem, Environmental method to determine dopamine and ascorbic acid simultaneously via derivative spectrophotometry. Journal of Spectroscopy, 2013. 2013.
30. Al-Ameri, S.A., Spectrophotometric determination of adrenaline in pharmaceutical preparations. Arabian Journal of Chemistry, 2016. 9: p. S1000-S1004.
31. Underberg, W. and G .Wiese, Spectrofluorimetric determination of adrenaline and related compounds in pharmaceutical preparations. Pharmaceutisch Weekblad, 1980. 2(1): p. 1438-1443.
32. Zhu, X., X. Ge, and C. Jiang, Spectrofluorimetric determination of dopamine using chlorosulfonylthenoyltrifluoroacetone–europium probe. Journal of fluorescence, 2007. 17(6): p. 655-661.
33. Nohta, H., A. Mitsui, and Y. Ohkura, Spectrofluorimetric determination of catecholamines with 1, 2-diphenylethylenediamine. Analytica chimica acta, 1984. 1 :65p. 171-176.
34. Honda, S., et al., Spectrofluorimetric determination of catecholamines with 2-cyanoacetamide. Analytica Chimica Acta, 1983. 149: p. 297-303.
35. Ribeiro, R.C.B., et al., *1, 2-Naphthoquinone-4-sulfonic acid salts in organic synthesis.* Beilstein Journal of Organic Chemistry, 2022. 18(1): p. 53-69.