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Coupling reaction and complex formation for the spectrophotometric determination of physiologically active catecholamines in bulk, pharmaceutical preparations and urine samples of schizophrenic patients

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A simple and sensitive spectrophotometric method for the determination of three catecholamines namely dopamine hydrochloride (DO.HCl), dobutamine hydrochloride (DOB.HCl) and vanillymandelic acid (VMA), in both pure form or in their commercially available pharmaceutical formulations or urine samples of schizopherinic patients is described. The method is based on the reaction of diazotized 4-aminoantipyrine (4-AAP) with catecholamines in a basic medium (pH = 10–11) to yield pink-coloured products having absorption maxima at 500, 505 and 480 nm for DO.HCl, VMA and DOB.HCl, respectively. Before carrying out Beer's Law, different experimental conditions, such as time, temperature, sequence of addition, and pH are optimized. The coloured species obeyed Beer's Law in the range of 47.4–417.2, 59.45–445.9 and 67.57–405.4 mg/L for DO.HCl, VMA and DOB.HCl, respectively. The molar absorptivity values as obtained from Beer's Law data were found to be 2.979×10^4, 4.39×10^4 and 1.036×10^4 L mol^{-1} cm^{-1}, while Sandell's sensitivity values were observed to be 3×10^{-3}, 4.4×10^{-3} and 1.3×10^{-3} μg cm^{2-} for DO.HCl, VMA and DOB.HCl, respectively. Common excipients did not interfere with the proposed method. The results of the proposed method compare favourably with those of official methods. The proposed method offers simplicity, reliability, rapidity, and accuracy compared to the existing methods.

Keywords: spectrophotometry; catecholamines; vanillymandelic acid; pharmaceutical preparations; urine samples; schizophrenic patients

Introduction

Catecholamines that have their own medicinal importance are aromatic vicdiols, in which either three or four positions are unsubstituted and these positions are sterically blocked. Catecholamines are widely used in the treatment of bronchial asthma, hypertension, Parkinson's disease, and cardiac surgery. Dopamine, a naturally occurring catecholamine, is a neurotransmitter responsible for controlling movement, the emotional response, and the ability to experience pleasure and pain. Hydrochloride salt of dopamine is used in the treatment of acute congestive failure and renal failure.[1] In Parkinson's disease, dopamine-transmitting neurons die in the area of the brain due to its deficiency. To treat this disease, levodopa (LDP) is used, which is easily converted to dopamine in the brain and readily crosses the blood-brain barrier.[2] Methyldopa is used in the treatment of hypertension and is readily converted to 1-methyl dopamine and methyl norepinephrine. Adrenaline is a potent vasoconstrictor and cardiac stimulant.[3] Due to the biological importance of catecholamines, efforts have been made towards the development of simple and reliable analytical techniques. Several methods, such as spectrofluorimetry,[4,5] gas chromatography,[6,7] high performance liquid chromatography (HPLC),[8,9] chemiluminescence,[10] voltammetry,[11] and flow-injection analysis[12] have been reported in the literature for the assay of catecholamines in various biological samples and pharmaceutical formulations, apart from spectrophotometric methods. Some of the reported spectrophotometric methods require non-aqueous media[13] and many require long heating times for colour development.[14–26] Some spectrophotometric methods have a very narrow range of the Beer's Law limit.[26–33] Some of them are based on the application of different oxidizing agents to catecholamines to form o-benzoquinone,[15] oxidation followed by coupling with compounds having electron-donating groups,[16] or nitration with sodium nitrite in the presence of tungstate or molybdate.[17] The diazotization method has been reported for the determination of dopamine, which involves the formation of the azo red compound with sulphamic acid in the presence of an alkali.[18] Another diazotization method was based on the interaction of diazotized sulphanilamide with catecholamines in the presence of molybdate ions.[19]

This paper reports a method based on the reaction of diazotized 4-AAP with catecholamines (DO.HCl and DOB.HCl) and VMA (Figure 1) in a basic medium and subsequent chelate formation with Cu(II) ion. This method offers the advantage of simplicity, needs no extraction, heating or cooling, in addition to having a higher sensitivity compared to the reported methods (Table 1). Moreover, the proposed method is totally free from the twin disadvantages of the interference and instability of the coloured

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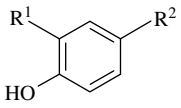
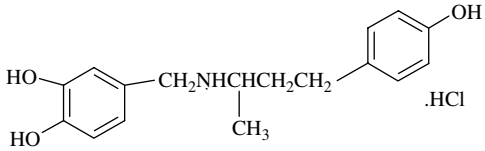


For DO.HCl, $R^1 = OH$, $R^2 = CH_2CH_2NH_2.HCl$
For VMA, $R^1 = OCH_3$, $R^2 = CH(OH)(COOH)$



DOB.HCl

Figure 1. Structures of DO.HCl, VMA and DOB.HCl.

Table 1. Most suitable condidtions and different analytical parameters for the determination of DO.HCl, VMA and DOB.HCl

Parameters	DO.HCl	VMA	DOB.HCl
pH	11.0	10.5	10.2
Time (min)	30	33	15
Temperature (°C)	20 ± 2	27 ± 2	20 ± 2
Sequence of addition			
$CuSO_4$ + NH_4OH + 4-AAP + active ingredient	3 and 4	3 and 4	1
$CuSO_4$ + 4-AAP + NH_4OH + Active ingredient			
4-AAP + Active ingredient + NH_4OH + $CuSO_4$			
4-AAP + NH_4OH + Active ingredient + $CuSO_4$			
Active ingredient + NH_4OH + 4-AAP + $CuSO_4$			
λ_{max} (nm)	505	500	480
Detection range (mg/L)	47.41–417.2	59.45–445.9	67.57–405.4
Correlation coefficient	0.999	0.999	0.999
Molar absorbitivity (L mol^{-1} cm^{-1})	2.979×10^4	4.39×10^4	1.036×10^4
Percent recovery (%)	98.5–102.5	97.14–102.9	100.0–104.0
Standard deviation	0.06–0.3	0.07–0.25	0.26–0. 32
Relative standard deviation (%)	0.1–0.12	0.13–0.15	0.1–0.12
Sandell Sensitivity, µg cm^{-2}	3×10^{-3}	4.4×10^{-3}	1.3×10^{-3}

species, typical for most of the reported spectrophotometric methods.

Experimental

Apparatus

The spectrophotometric measurements were carried out using the manual Spectronic 601 (Melton Roy Company, USA), and Perkin-Elmar automated specrophotometer UV-Vis model Lambda 20 ranged from 200 to 900 nm with 1-cm matched quartz cells. HPLC measurements were undertaken using a Perkin-Elmer instrument with binary LC pumb (250), C18 analytical column (250 × 4.60 mm) and uv-vis spectrophotometric detector (LC 290). Measurements of pH were done using 716 DMS Titrino Metrohm connected with 728 Metrohm stirrer. This titrino had a combined electrode, which was more convenient to be used, where the glass electrode and a reference half-cell were arranged in the same equipment. For selecting accurate micro litre volumes, the calibrated transferpepitte (BRAND) was used in the range 100 to 1000 µL that transferred with disposable tips.

Reagents

The chemicals used in this investigation were of the highest purity available. They included dopamine hydrochloride (DO.HCl), vanillylmandelic acid (VMA) and dobutamine hydrochloride (DOB.HCl) supplied by Sigma (St Louis, MO, USA), and 4-aminoantipyrine (4-AAP) was supplied from Fluka Chemie AG, USA. Copper sulphate (Sigma, St Louis, MO, USA), ammonia solution (33%), sulphuric and glacial acetic acids, resorcinol, glucose from EL-Naser Pharmaceutical Chemical Co., Egypt phosphoric acid (90%) (Mallinckrodt Co., USA), sodium hydroxide (United Co. For Chem. and Med. Preparations, Industrial Area El-Salam City, Cairo, Egypt.), sodium dihydrogen phosphate (EL-Gomhoria Pharmaceutical Co., Egypt), boric acid, phenol (Riedel-de Haen, Germany), pyrogallol (Wardley Products Co. INC, USA), pyrocatechol, catechol, anhydrous formic acid, acetic anhydride and perchloric acid (Aldrich Chemical Co., USA). Deionized water was used throughout the study.

Source of pharmaceutical preparations

Dopamine hydrochloride present in pharmaceutical preparations as dopamine Fresenius and Dopamine Pierre Fabre concentrate ampoules for infusion (200 mg/5 mL), where they are supplied by Fresenius Kabi, (Bad Homburg, Germany) and Pierre Fabre Medicament Production (Boulogne, France) respectively. Dobutamine injection ampoules labeled (250 mg/20 mL) were supplied by Abbott Labs (Chicago, 1L, USA).

Preparation of standard solutions

0.01 M stock solutions of DO.HCl, DOB.HCl and VMA were prepared by dissolving 0.0474 g, 0.0844 g and 0.0494 g, respectively, in 25 mL bi-distilled water except DOB.HCl in methanol. 5×10^{-3} to 1×10^{-3} M solutions were prepared by accurate dilution from the stock solution. The stock and dilute solutions were stored in dark bottles under cold conditions (4 °C) in a fridge. 0.01 M solutions of copper sulphate and 4-AAP were prepared by dissolving 0.0624 g and 0.0508 g, respectively, in 25 mL deionized water. During the preparation of copper sulphate solution, about 50 µL of sulphuric acid was added to prevent the hydrolysis of the solution. Copper sulphate stock solution was standardized by recommended procedure.[34] All the required dilutions from reagents were prepared from stock one. 0.35 M ammonia solution was prepared from ingredient 33%. Series of universal buffer solutions covering the pH range from 2.0 to 12.0 were prepared

as recommended by Britton and Robinson.[35] A mixture of 0.04 M phosphoric, boric and acetic acids was titrated with 0.2 N NaOH to adjust the desired pH into the required value in 100 mL of the acid mixture using pH-meter. 0.07% (v/v) of acetone and 0.045 g, 0.044 g, 0.055 g, 0.024 g, 0.032 g, 0.028 g and 0.028 g of glucose, ascorbic acid, catechol, phenol, pyrogallol, resorcinol, and hydroquinone, respectively, were prepared by dissolving the accurate calculated weight in 25 mL deionized water to obtain a solution of 0.01 M.

1.01×10^{-3} M DO.HCl solution was prepared by pipetting 0.120 mL from ampoule and diluted up to 25 mL using deionized water. 2×10^{-3} M stock solution of DOB.HCl was prepared by pipetting 0.35 mL from ampoule and diluted up to 25 mL using deionized water. Diluted solutions were prepared by accurate dilution from the stock solution and incubated in cold, dark bottles, away from oxygen air to prevent oxidation or decomposition.

General procedure

1×10^{-3} and 2×10^{-3} mmole of copper sulphate and 4-AAP solutions, respectively, and 0.35 M ammonia solution were kept constant and mixed with 1×10^{-4} to 2.2×10^{-3} mmole or 1×10^{-4} to 1.2×10^{-3} mmole DO.HCl or VMA, respectively. 3×10^{-3} mmole from copper sulphate and 4-AAP and 0.35 M ammonia solutions were mixed with 2×10^{-4} to 1.2×10^{-3} mmole DOB.HCl. The total volume was completed up to 10 mL in measuring flask using deionized water. The pH of the reaction mixture was adjusted using ammonia solution as buffering medium to pH = 10.5, 10.2 and 10.5 in case of DO.HCl, VMA and DOB.HCl, respectively. The reaction mixture was incubated at 20, 33 and 20 °C for 30, 33 and 15 min for DO.HCl, VMA and DOB.HCl, respectively. The mixtures were shaken well and the absorbance was measured at $\lambda_{max} = 505$, 500 and 480 nm for DO.HCl, VMA and DOB.HCl, respectively.

Assay of DO.HCl and DOB.HCl in pharmaceutical formulations

0.4 and 0.8 mL of DO.HCl ampoule solutions (1.01×10^{-3} M) were mixed with 1×10^{-3} mmole of copper sulphate, 2×10^{-3} mmole of 4-AAP and 0.35 M ammonia solution was added to adjust the pH at 10.5. 0.2 and 0.5 mL of DOB.HCl ampoule solutions were mixed with 3×10^{-3} mmole of each of copper sulphate and 4-AAP. The pH was adjusted to 10.3 using ammonia solution, and the final volume of mixture was completed up to 10 mL in a measuring flask using deionized water. The procedure was completed as mentioned earlier.

Standard addition method was also applied for quantitative microdetermination of all active ingredients, which are present in pharmaceutical preparations (DO.HCl and DOB.HCl in ampoules). 0.4 or 0.1 mL of DO.HCl or DOB.HCl ampoules was added to serial volumes from standard solution of DO.HCl (1×10^{-4}–1×10^{-3} mmole) or DOB.HCl (5×10^{-4}–1.1×10^{-3} mmole). All the mixtures were mixed with 0.02 M of each of copper sulphate and 4-AAP solutions and the pH was adjusted to 10.5 for DO.HCl and DOB.HCl using 0.35 M ammonia solution. The procedure was completed as mentioned earlier.

Collection of urine samples

Urine samples were collected from 10 healthy males aged between 27 and 38, with a mean weight of 67–99 kg and mean height of 175–187 cm. Urine samples were also collected from 15 untreated schizophrenic male patients. Then other urine samples were collected from the same individuals after 15 and 30 days of treatment to monitor dopamine concentration. In addition, urine samples were collected three different times from normal people eating about 300–350 g of banana per day. The collections were applied before eating, after 1h of eating and the last one after 2h of eating. In those individuals, we had the ability to monitor vanillymandelic acid secretion. From each individual we needed to collect only one sample of about 50 mL. All samples were acidified with 0.6 N HCl, then centrifuged for 5 min at 2000 rpm in order to remove cells and other particulate matters. The supernatants were transferred into 10 mL clean and dry vessels. The preservatives were used to reduce bacterial action to minimize chemical decomposition and to decrease atmospheric oxidation of catecholamines. The most satisfactory form of preservation of urine specimens is to refrigerate immediately in clean and sterilized vessels after voiding and acidification with 6 N HCl.

Assay of DO.HCl in urine samples of healthy individuals and schizophrenic patients

Before pipetting, urine samples were allowed reach room temperature. To be sure of complete homogeneity, a rapid vortex was done for 30 s. Certain volumes from urine samples were mixed with 0.2 M 4-AAP and 0.4 M copper tetramine at pH 10.5 adjusted with ammonia solution. The total volume was completed up to 10 mL measuring flask using deionized water. The method was completed under optimum conditions as described earlier. From the calibration curve, the concentration of dopamine in different urine samples was calculated.

Assay of VMA in urine samples of normal individuals and normal people eating bananas

Urine samples removed from the fridge and allowed to reach room temperature. To be sure of complete homogeneity, a rapid vortex was done for 30 s. Accurate volumes from urine samples were mixed with 0.2 M from 4-AAP and 0.4 from copper sulfate. pH was adjusted at 10.5 using 0.035 M ammonia solution. These contents were mixed well then diluted up to 10 mL using deionized water in a calibrated measuring flask. The procedure was completed as mentioned earlier. VMA concentration in each sample was determined from the calibration curve.

Results and Discussion

The method involves the reaction of catecholamines (DO.HCl and DOB.HCl) and VMA with Cu(II) and 4-AAP in a basic medium to produce red-coloured chromophores. The factors affecting the colour development (reproducibility, sensitivity, and adherence to Beer's Law) were investigated.

Spectral characteristics

A red-coloured chromophore was formed when catecholamines (DO.HCl and DOB.HCl) and VMA were treated with Cu(II) and 4-AAP solutions in a basic medium. These coloured species exhibited absorption maxima at 505, 500 and 480 nm for DO.HCl, DOB.HCl and VMA, respectively, as shown in Figures 2–4. The corresponding reagent blanks show a negligible absorbance at these wavelengths.

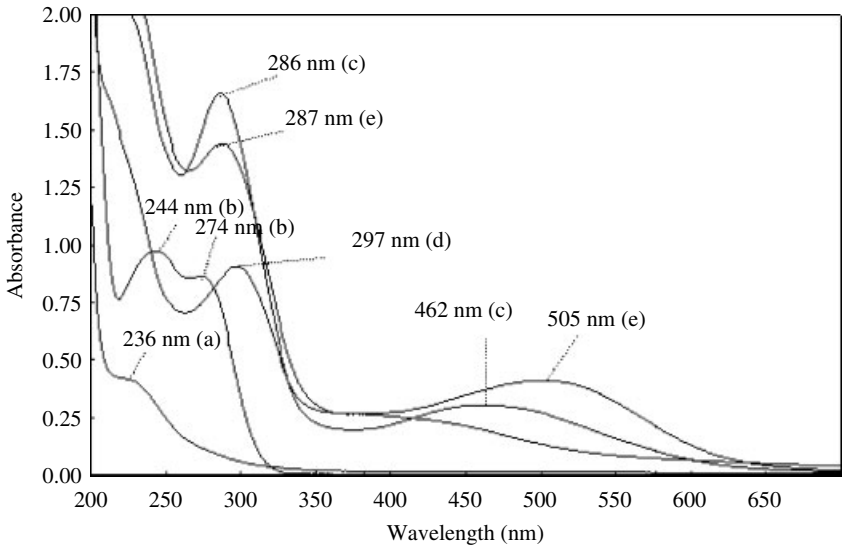


Figure 2. Absorption spectra of (a) Cu(II) + ammonia solution, (b) 4-AAP + ammonia solution, (c) 4-AAP + DO.HCl + ammonia solution, (d) Cu(II) + DO.HCl + ammonia solution, (e) 4-AAP + DO.HCl + Cu(II) + ammonia solution.

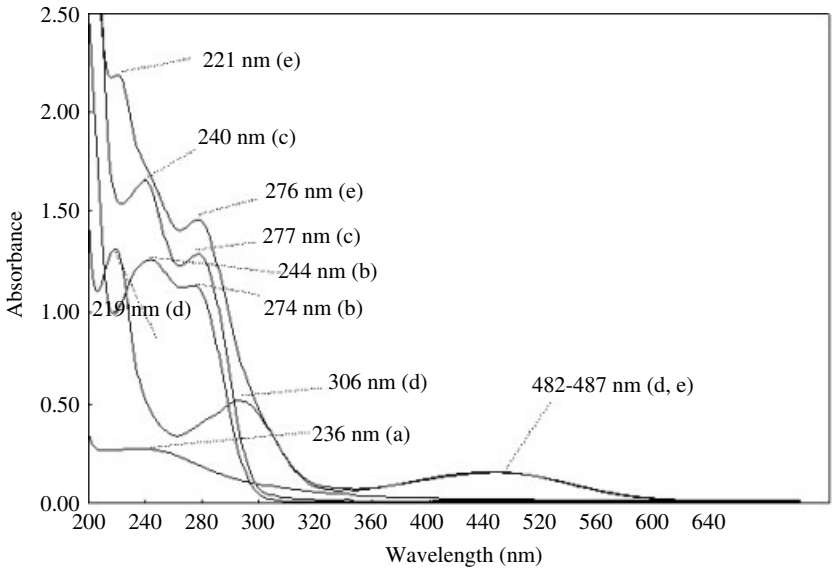


Figure 3. Absorption spectra of (a) Cu(II) + ammonia solution, (b) 4-AAP + ammonia solution, (c) 4-AAP + DOB.HCl + ammonia solution, (d) Cu(II) + DOB.HCl + ammonia solution, (e) 4-AAP + DOB.HCl + Cu(II) + ammonia solution.

Optimum reaction conditions

The effect of pH on the reaction of different active ingredients was studied using ammonia solution as buffering media in the pH range from 9.5 to 12. It was found that the most suitable pH values for microdetermination of DO.HCl, VMA or DOB.HCl in pure, urine or pharmaceutical forms using copper tetramine and 4-AAP reagents are 11.0 ($\varepsilon = 2.979 \times 10^4$ L mol^{-1} cm^{-1}), 10.5 ($\varepsilon = 4.39 \times 10^4$ L mol^{-1} cm^{-1}) or 10.3 ($\varepsilon = 1.036 \times 10^4$ L mol^{-1} cm^{-1}), respectively, as given in Table 1. The effect of time on the reaction between DO.HCl, VMA or DOB.HCl with copper tetramine complex and 4-AAP reagents was studied at $\lambda_{max} = 505$, 500 or 480 nm, respectively, at different time intervals in order to choose the proper time needed for complete complex formation and hence microdetermination of these drugs in pure and different available commercial sources. From these results, it is obvious that the most suitable times for spectrophotoetric microdetermination of DO.HCl, VMA and DOB.HCl are 30, 33 and 15 min, respectively. Due to the pronounced effect of temperature on the stability of the new coloured products, the selection of optimum conditions for microdetermination is necessary. The effect of temperature on these reactions was studied in the temperature range from 10 to 60 °C. It is found that 20 ± 2, 27 ± 2 and 20 ± 2 °C are the most suitable temperatures selected for determination of DO.HCl, VMA and DOB.HCl, respectively, with copper tetramine and 4-AAP reagents in alkaline medium.

Determination of the stoichiometric ratio

On applying molar ratio method, it is found that the two extended lines are intersected at ratio 1 : 2, 1 : 1.5 and 1 : 1 as [Cu(II)]:[DO.HCl], [Cu(II)]:[VMA] and [Cu(II)]: [DOB.HCl], respectively, which are the most suitable ratios for complete complex formation.

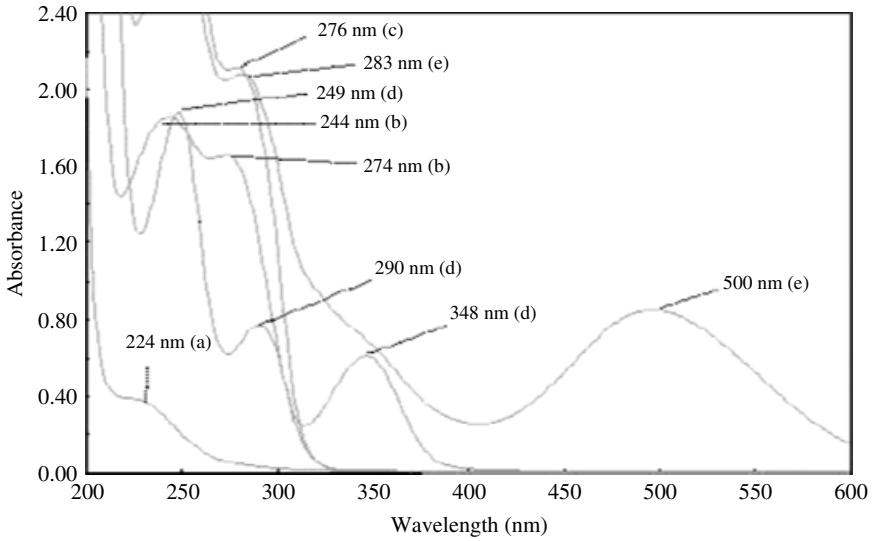


Figure 4. Absorption spectra of (a) Cu(II) + ammonia solution, (b) 4-AAP + ammonia solution, (c) 4-AAP + VMA + ammonia solution, (d) Cu(II) + VMA + ammonia solution, (e) 4-AAP + VMA + Cu(II) + ammonia solution.

It is found that molar ratio 1: 1 [4-AAP]:[active ingredients] is selected as suitable ratio for quantitative determination of DO.HCl, VMA and DOB.HCl in pure and pharmaceutical preparations.

This can be explained in terms of formation of coupled dye product of 4-AAP and DO.HCl or VMA in 1 : 1 ratios then complex formation with Cu(II) tetramine complex in the ratio 1 : 2 : 2 or 1 : 1.5 : 1.5 [Cu(II)]Λ[DO.HCl]:[4-AAP]) or [Cu(II)]Λ[VMA]:[4-AAP]), respectively. While in case of DOB.HCl, 4-AAP acts as a 2nd ligand and the DOB.HCl is 1st ligand forming mixed ligand complex with 1 : 1 : 1 ratio as [Cu(II)]Λ[DOB.HCl]:[4-AAP]).

Sequence of addition

The effect of sequences of addition on the quantitativeness of the reaction of the drugs being studied with copper tetramine complex and 4-AAP reagents is given in Table 1. The most suitable sequences of addition in case of DO.HCl or VMA are No. 3 and and No. 4 which involve mixing of 4-AAP with one of them then adding copper tetramine, where 4-AAP forms a coupling product with DO.HCl or VMA and they act as a one unit called dye ligand.[36,37] The addition of copper tetramine complex resulted in the formation of metal-chelate which absorbs maximally at λ = 505 and 500 nm for DO.HCl and VMA, respectively. In the case of DOB.HCl, the most proper sequence of addition is No. 1 involving formation of copper tetramine complex then the addition of DOB.HCl and 4-AAP. This may be explained by the fact that copper tetramine forms mixed ligand complex with DOB.HCl as a primary ligand, and 4-AAP as secondary ligand. The probable reaction mechanism is shown in Scheme 1.

Stability

The addition of 4-AAP to catecholamines in alkaline medium yielded red-coloured products within 2 min. The red-coloured products are found to be stable in the temperature range of 20–30 °C for 24–32 h.

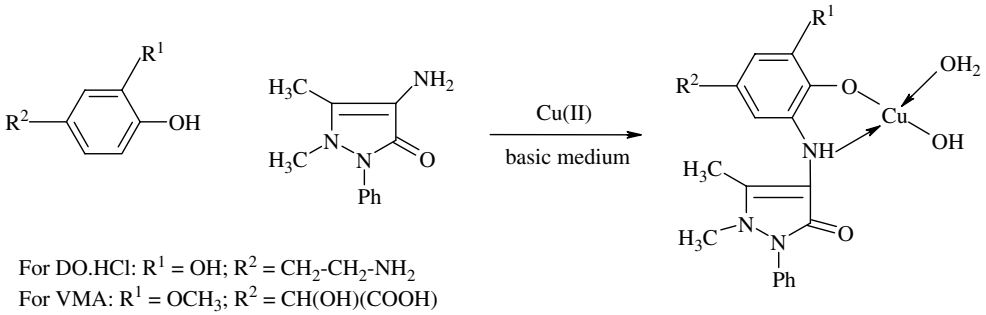
Quantification

The Beer's law limits, molar absorptivity, and Sandell's sensitivity values were evaluated and are given in Table 1. Regression analyses of the Beer's Law plots at their respective maxima absorption values revealed a good correlation between the concentration and absorbance values. The relation between absorbance and concentration (mg/L) are linear in the concentration range 47.41–417.2, 59.45–445.9 and 67.57–405.4 mg/L for DO.HCl, VMA and DOB.HCl, respectively. The recovery percent is found to be in the range 98.50–102.5, 97.14–102.9 and 100.0–104% for DO.HCl, VMA and DOB.HCl, respectively.

The different analytical data, namely coefficient of variation (CV), molar absorptivity (ε), Sandell sensitivity (S), standard deviation (SD) are listed in Table 1. The values of standard deviation (SD = 0.06–0.3, 0.07–0.25 and 0.26–0.32) and coefficient of variation (CV = 0.1–0.12, 0.13–0.15 and 0.1–0.12%) for DO.HCl, VMA and DOB.HCl, respectively, were calculated for three to five replicates, with correlation coefficient 0.999. The low valves of SD and CV indicate the accuracy and precision of the suggested procedure. They also indicate the sensitivity of copper tetramine and 4-AAP for microdetermination of DO.HCl, VMA and DOB.HCl.

Interference

In order to assess the possible analytical applications of the proposed method, the effects of some foreign ions, which often accompany these drugs in pharmaceutical products, were studied by adding different amounts of foreign ions to catecholamines. The colour was developed following the procedure described earlier. For this reason, the effect of some interfering materials, such as glucose, acetone, urea, ascorbic acid, catechol, pyrogallol, resorcinol and hydroquinone were studied. From Table 2, it is obvious that the presence of glucose, acetone or urea as tenfold does not affect the determination of DO.HCl (percent recovery = 99.3–100.7%) or slightly affect the determination of VMA (percent recoveries = 97.7–99.8%). While the presence of ascorbic acid as tenfold affects the determination of DO.HCl and VMA, but approximately has no effect when its concentration is present



For DO.HCl: R^1 = OH; R^2 = CH_2-CH_2-NH_2
For VMA: R^1 = OCH_3; R^2 = CH(OH)(COOH)

Scheme 1. Probable reaction mechanism for the formation of red-coloured product.

Table 2. Effect of different tolerant on the determination of DO.HCl, VMA and DOB.HCl

Tolerant	Fold	% Recovery			Tolerant	Fold	% Recovery		
		DO.HCl	VMA	DOB.HCl			DO.HCl	VMA	DOB.HCl
Glucose	10	100.7	97.70	99.10	**Pyrogallol**	10	138.7		
	5		97.50			1	113.7		
	1		97.90	101.7		0.5	107.4		
Acetone	10	99.30	96.30		**Resorcinol**	10	374.9		
	5		97.90			1	93.40		
	1		99.80			0.5	95.94		
Ascorbic acid	10		0.0		**Hydroquinone**	10	239.8		
	1	104.1				1	161.6		
	0.5	102.6	97.40			0.5	136.5		
Urea	10	97.85	99.70		**Maltose**	10			97.40
	1	98.90	99.76			1			103.0
	0.5	99.60	99.53						
Catechol	10	370.8	249.3		**Starch**	10			
	1					5			96.10
	0.5	186.7	187.0			1			94.40
Phenol	10	808.8							
	0.5	189.3							

as 1 : 1 or less. By decreasing the concentration of ascorbic acid to half of VMA concentration, the percent recovery increase to 97.40%. High deviation in percent recovery is obtained with the other organic compounds (catechol, phenol, pyrogallol, resorcinol, and hydroquinone). The presence of catechol, as tenfold or halffold, has a pronounced effect on the percent recovery of VMA (174–184%) which recommended that it is impossible to determine VMA in the presence of this material. This may be explained according to the fact that catechol can possibly compete with the dye (formed as coupling product of VMA with 4-AAP) during the complex formation with copper tetramine complex.

Since DOB.HCl present only in injection form, glucose, maltose and starch were tested as interfering materials during determination as shown in Table 2. The presence of glucose and maltose slightly affected microdetrmination of DOB.HCl. Starch forms viscous and turbid solution when present in high concentration, so it affects the quantitative determination of DOB.HCl. By decreasing the concentration to fivefold, the percent recovery of determination increases to 96.10%. Thus, the proposed method is free from interferences by various substances and ions

Application on Pharmaceutical Preparations

Determination of DO.HCl and DOB.HCl in pharmaceutical preparations (ampoules)

Before carrying out microdetermination, the absorption spectra of the drugs using suggested procedure were carried out. It was found that there is no shift in the λ_{max} whatever presence of other constituents in the dosage forms. Two different ampoule concentrations of DO.HCl (76.61 and 153.23 mg/L) were determined applying official HPLC technique[38] and proposed procedure under selected optimum conditions. The data listed in Table 3 show the precision and reproducibility of the results with a percent error 1.4–2.8% and SD = 0.11–0.19. The low values of SD indicate the successful application of our proposed methods for the microdetermination of DO.HCl in pharmaceutical preparations. The results were compared with those obtained by applying the HPLC as official method.[38] The results obtained are compared statistically using F- and *t*- tests with those obtained by official method at the 95% confidence level and their values are found to be 3.36–4.41 and 1.05–1.5. It is clear from Table 3 that the calculated F- and *t*-test values did not exceed the theoretical tabulated values indicating that there is no significant difference between accuracy of the proposed and the official methods.

Table 3. Determination of DO and DOB in pharmaceutical preparations (ampoules) using proposed and standard addition methods

Drug	Name of preparation	[Drug], mg/L		Proposed method		Official method			
		Taken	Found[#]	% Recovery	SD	% Recovery	SD	t*-test	F*-test
Proposed method: DO	- Dopamine Fresenius	76.61	77.34	100.9	0.10	100.8	0.21	1.18	4.41
		153.2	153.6	100.2	0.11	99.17	0.25	1.05	5.16
	- Dopamine Pierre Fabre	76.61	78.05	101.8	0.17	102.2	0.20	1.1	1.38
		153.2	153.6	100.2	0.13	101.1	0.17	1.5	1.71
DOB	-Abbott Labs., N. Chicago. 1L 60064, USA.	100.0	104.5	104.4	0.12	103.6	0.25	1.22	4.34
		250.0	260.9	104.3	0.09	102.4	0.18	0.97	4.00
Standard addition method: DO	- Dopamine Fresenius	76.62	77.00	100.4	0.15	100.8	0.21	1.01	0.51
	- Dopamine Pierre Fabre	38.3	38.00	99.20	0.11	99.45	0.16	0.67	0.47
DOB	-Abbott Labs., N. Chicago. 1L 60064, USA.	50.0	51.0	102.0	0.25	99.60	0.29	1.05	0.74

Tabulated *t*-value at 95% confidence level is 2.78 for $n = 5$.
Tabulated *F*-value at 95% confidence level is 6.39 for $n = 5$.
[#] mean of individuals.

The data in Table 3 show that the estimated concentrations of DOB.HCl drug by the proposed method is 104.5–260.9 mg/L, close to that calculated by the applied official method[39] with percent error 2.1–4.1%. In order to check the confidence and correlation between the suggested spectrophotometric procedure and the official method[39] for microdetermination of DOB.HCl, it is better to calculate F-and *t*-test values for all the results. The F and *t*-test values at 95% confidence level were found to be 4.00–4.34 and 0.97–1.22, respectively, which do not exceed the theoretical values. The calculated standard deviation (SD = 0.09–0.12), indicates the reliability, accuracy and precision of the suggested procedure.

Quantitative determination of pharmaceutical preparations using standard addition method

The standard addition method was applied to support the validity of the application of our proposed procedure for determination of DO.HCl and DOB.HCl in their pharmaceutical preparations and to minimize the effect of any matrix interference's in quantitative determination of active ingredient in the commercial available drugs.

From Table 3, it is concluded that the x-intercept of the extrapolated line is the concentration of unknown DO.HCl (77.0 and 38.0 mg/L) and DOB.HCl (51.0 mg/L) in pharmaceutical ampoules. DO.HCl and DOB.HCl are determined with percent error 0.6–0.8 and 2%, and SD = 0.11–0.15 and 0.25, respectively. So we can conclude that the standard addition method reduces the interference effect of most additives that may present as fillers in pharmaceutical preparations. The high values of the percent recovery reflect the high efficiency of applying standard addition method for microdetermination of the drugs under investigation in their pharmaceutical preparations. The calculated values of *t* and *F* did not exceed the theoretical values at the 95% confidence level indicating that the results of the proposed method are not significantly different from those of the official and reported methods.

Spectrophotometric determination of dopamine in urine samples of schizophrenic patients

In the treatment of schizophrenia, more than in many other diseases, individual patients respond differently to medication. Despite recent advances in the treatment of schizophrenia there remains a number of unmet needs in therapy for schizophrenia management like low response, high relapse rates,[40] relapse,[40,41] non-response,[40–43] non-adherence[40] or challenging road ahead.

Nowadays, with our new option, an analytical test is ordered after a psychiatrist performs a clinical examination[44] on the patient and suspects that the patient has schizophrenic symptoms such as hallucination, delusion, deterioration, disturbed thinking, lack of insight and disturbed judgement and abstract. Actually, it can be used as a monitoring tool to follow up patient treatment and confirm the psychiatrist's findings. In addition, it can be applied before treatment, after 15 and 30 days of treatment. From the data in Table 4, it is concluded that the DO.HCl concentration before treatment ranged between 350.8 and 240.7 mg/L (high concentration) detected using proposed and official methods[38] with SD = 0.15–0.21, *t*-test = 0.62–0.87 and F-test = 4.5–5.0. Then, after two weeks of treatment, the concentration of DO.HCl in urine decreased and was found to be 107.6–188.7 mg/L. In the third urine samples collected after one month of treatment, it is observed that the concentrations of DO.HCl decreased to be 48.2–97.85 mg/L. In this way, a psychiatrist will have the facility to evaluate the schizophrenic patient as a whole and to follow up the patient's response to the recommended treatment. Moreover, it can be ordered as a routine analysis for early detection when a patient has a family history of schizophrenia.

In our proposed procedure, only one urine sample is collected from patient before treatment to monitor the dopamine concentration with the next collected after 15 days of medication. The result of the analysis is compared with the one before medication. If the level of dopamine is the same before treatment as after 15 days of treatment, this may reflect that the treatment is not effective. This helps the psychiatrist to regulate the treatment dose before relapse and is also helpful in assessing the suitability of each patient's drug dosage, assessing patients' compliance and avoiding over dosage. The urine analysis is repeated every 15 days to be sure that the medication is effective and that there is no disturbance in dopamine secretion.

Table 4. Determination of DO.HCl in urine samples of schizopherenic patients using proposed method

Code of case	Gender	Age	General feature		Active ingredient used in treatment	Dose used in treatment	[DO], mg/l		
			Hight (cm)	Weight (Kg)			A	B	C
1	Male	40	176.5	94	Chlorpromazine (100 mg/tablet)	One tablet 3 times per day	283.6	107.6	61.60
2	Male	33	170.3	79	Thioridazine (200 mg/tablet)	One tablet 3 times per day	287.4	138.5	49.50
3	Male	28	170.8	88	Haloperidol (50 mg/ampoule)	One ampoule I.M./2 weeks	300.5	181.5	48.20
4	Male	41	179.1	82	Thioridazine (200 mg/tablet)	One tablet 4 times per day	290.0	166.2	57.80
5	Male	40	188.0	97	Haloperidol (50 mg/ampoule)	One ampoule I.M./2 weeks	244.9	145.3	54.60
6	Male	36	181.6	90	Trifluoperazine (5 mg/tablet)	One tablet 4 times per day	301.8	157.7	67.90
7	Male	30	194.4	88	Chlorpromazine (100 mg/tablet)	One tablet 3 times per day	288.4	163.2	60.80
8	Male	45	185.8	84	Thioridazine (200 mg/tablet)	One tablet 3 times per day	310.3	174.8	58.10
9	Male	24	179.0	69	Trifluoperazine (5 mg/tablet)	One tablet 4 times per day	289.4	152.7	60.86
10	Male	31	185.0	71	Haloperidol (50 mg/ampoule)	One ampoule I.M./2 weeks	277.1	126.1	54.25
11	Male	37	160.5	65.5	Clozapine (100 mg/tablet)	One tablet 4 times per day	240.7	139.2	95.22
12	Male	32	167.2	70	Chlorpromazine (100 mg/tablet)	One tablet 3 times per day	305.2	167.7	85.07
13	Male	25	171.0	65	Thioridazine (200 mg/tablet)	One tablet 4 times per day	307.5	163.6	97.85
14	Male	38	172.1	75	Haloperidol (50 mg/ampoule)	One ampoule I.M./2 weeks	350.8	175.5	68.90
15	Male	43	187.9	90	Clozapine (100 mg/tablet)	One tablet 3 times per day	323.6	188.7	84.30

A = Concentration of DO in the urine sample of schizopherenic patients before medication.
B = Concentration of DO in the urine sample of schizopherenic patients after two weeks of medication.
C = Concentration of DO in the urine sample of schizopherenic patients after one month of medication.

Table 5. Quantitative determination of VMA in urine samples of normal people eating bananas

Code of Case	Gender	Age	*General feature*		Banana produced (g)	Concentration of VMA (mg/L)		
			Height (cm)	Weight (Kg)		Before eating	After 1 hour from **eating**	After 2 hours from eating
1	Male	50	175.7	84	310	Nil	57.6	80.1
2	Male	30	164.2	72	300	Nil	58.1	85.9
3	Male	27	153.5	60	320	**Nil**	57.4	88.3
4	Male	21	191.2	105	340	Nil	59.6	97.6
5	Male	16	177.8	94	300	Nil	58.9	99.8
6	Female	45	173.5	85	300	Nil	59.2	86.9
7	Female	25	189.9	110	350	Nil	58.7	87.5
8	Female	20	169.5	98	300	Nil	57.5	91.7
9	Female	18	188.3	101	315	Nil	58.4	89.9
10	Female	16	168.5	77	300	Nil	59.8	90.5

Modification in spectrophotometric determination of dopamine in urine samples

Early detection, diagnosis, follow up, and relapse prevention for schizophrenic patients can be carried out via modification in spectrophotometric determination of DO.HCl using copper tetramine and 4-AAP reagents. The modification is based on the formation of a coloured stripe sheet, which is picked from the calibration curve of DO.HCl with copper tetramine and 4-AAP under optimum conditions. The collected urine sample is mixed with copper tetramine and 4-AAP and allowed to stand under optimum conditions till complete complex formation; then the colored end-product is compared with colours which present in the coloured stripe sheet. In this way we have the ability to easily detect the concentration of DO.HCl in the urine sample without needing to recording absorbance at certain wavelengths using a spectrophotometer. DO.HCl concentration was also recorded in urine samples collected from normal people using our proposed and official method. It is obvious that the concentration of DO.HCl is less than 47.0 mg/L, so we can conclude that modification in our procedure facilitated quantitation of DO.HCl in urine samples without the need to form a calibration curve from time to time for standard DO.HCl material using a spectrophotometer. Moreover, early detection, diagnosis, follow up, and relapse prevention in schizophrenic patients will be quick and easy.

Quantitative determination of VMA in urine samples of healthy people

In our proposed procedure, we use a spectrophotometer as an easier instrument for quantitation of VMA in standard material and in the urine of healthy people eating a certain amount from banana (eating banana is one of the main factors that increases the concentration of VMA in the human body). Moreover, it is one of the precaution decided before carrying out any one of catecholamine analysis.

From the analytical data, which are presented in Table 5, it is obvious that VMA in the urine samples before eating banana cannot be detected (i.e., Nil). There is a slight increase after 1 h of eating, ranging from 57.4 to 59.8 mg/L. After 2 h of eating, the concentration of VMA rises up to 80.1–99.8 mg/L. The concentration of VMA in urine samples of normal people eating banana after 2 h was examined using HPLC as an official method.[38] By comparing these results, it is obvious that the concentration ranged between 80.1 and 99.8 mg/L with SD = 0.15–0.22, *t*-test = 1.06–1.4 and F-test = 5.1–5.4. So we can conclude that our proposed procedure suggested for quantitative determination of VMA can be recommended and applied as a routine analysis to detect and follow up pheochromocytoma.

Conclusion

The proposed method is found to be simple and sensitive compared to other reported methods. The statistical parameters and recovery data clearly indicate the reproducibility and accuracy of the proposed method. Furthermore, the analytical reagents are inexpensive, have excellent shelf life, and are available in many analytical laboratories. Hence, the proposed method could be used as a better alternative to the existing methods. Therefore, the methods are practical and valuable for routine application in quality control laboratories for analysis of DO.HCl, VMA and DOB.HCl.

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