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Indirect spectrophotometric determination of some catecholamine drugs

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ABSTRACT

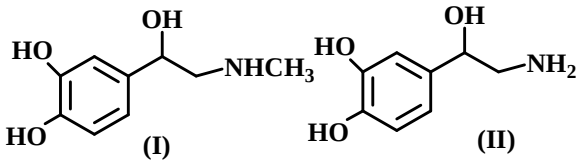
A simple, fast and sensitive indirect spectrophotometric method is proposed for the determination of epinephrine and norepinephrine. The method is based on the oxidation of the drugs by a known excess of chromium(VI) in the presence of sulfuric acid. The residue of chromium(VI) is then reacted with 1,5-diphenylcarbazid to form a red-violet color which shows an absorption maximum at 542 nm. Calibration graphs are linear in the range of 0.2-3.20μg ml^{-1} for epinephrine and 0.3-3.2μg ml^{-1} for norepinephrine with detection limits of 0.1μg ml^{-1} and 0.15μg ml^{-1} for epinephrine and norepinephrine, respectively. The relative standard deviations at the level of 1μg ml^{-1} and 2μg ml^{-1}(n=10) were 1.8% and 2.1% for epinephrine and 1.2% and 2.8% for norepinephrine, respectively. The method was successfully applied to the determination of these catecholamines in pharmaceutical preparations.

KEYWORDS

Catecholamine drugs;
Dichromate oxidation;
1,5-Diphenylcarbazide;
Epinephrine determination;
Norepinephrine determination.

INTRODUCTION

Catecholamine drugs are aromatic vic-diols in which both the 3- or 4-position is unsubstituted and these positions are not sterically blocked. Epinephrine(EP) or adrenaline**(I)** and norepinephrine(NE) or noradrenaline **(II)** are catecholamine drugs that are widely used in the treatment of allergic emergencies, status asthmatics, bronchial asthma, ventricular bradycardia, cardiac arrest, glaucoma, and styptic[1].



The amount of catecholamines in biological fluids is very small and its determination requires a method with high sensitivity and low detectability such as high performance liquid chromatography with fluorimetric[2] or electrochemical detection[3,4]. However pharmaceutical formulations containing relatively larger amount of drugs can be analyzed with batch spectrophotometric method. Several spectrophotometric procedures have been developed, most of which are based on the oxidation of catecholamines prior to the absorbance measurement. The oxidants such as periodate[5-7], bismuthate[8], metavanadate[9], bromate[10], phenanthroline-iron(III) complex[11] and N-bromosuccinimide[12] had been used. In this work, an improved, rapid and sensitive method for indirect determination of epinephrine and norepinephrine is described. Chromium is used as a mark element. The Cr(VI) is reduced by the drug to Cr(III) in

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acidic media. The excessive Cr(VI) then react with 1,5-diphenylcarbazide(DPC) to form a red complex which absorbed at 542nm. The absorbance of this solution is inversely related to the epinephrine or norepinephrine concentration.

EXPERIMENTAL

Apparatus

A JASCO(model 7800) equipped with 1-cm matched quartz cells was used for recording the absorption spectra. A Jenway(model 6300) single beam spectrophotometer with 1cm glass cell has been used for measuring the absorbance at 542nm.

Reagents

All reagents were of analytical-reagent grade and were used without further purification. Distilled water was used for preparing all solutions.

The stock solutions of epinephrine and norepinephrine (100mg l^{-1}) were prepared by dissolving the appropriate amount of the bitartarate salts of ephinephrine and norepinephrine(Aldrich Chemical Company) in 0.1% sodium metabisulphite solution and stored in amber bottles. The solutions were kept in a refrigerator and used within 2 weeks of preparation. Working solutions were prepared daily by appropriate dilution of stock solutions with the same solvent.

1,5-diphenylcarbazide(0.1%) was prepared by dissolving 0.05g of it in 25ml acetone and diluting to 50 ml with distilled water.

A solution of Cr(VI)(1000μg ml^{-1}) was prepared by dissolving 0.2850g of potassium dichromate in water and diluting to 100ml in a volumetric flask. Suitable dilutions were made to obtain a concentration of 10μg ml^{-1} Cr(VI).

Sulfuric acid 1M was prepared by diluting the appropriate volume of concentrated acid with water.

Procedures

In each of the series of 10ml volumetric flask standard solution containing 2-32μg of epinephrine or 3-32μg of norepinephrine and 1ml of acetone was placed. Then, 1ml of potassium dichromate solution(10μg ml^{-1}) and 1ml sulfuric acid(1M) was added to each flask. After 12min, when the reaction was complete, 1ml of 1,5-diphenylcarbazide solution(0.1%) was added and the volumes were adjusted with distilled water. The solutions were transferred into 1cm glass cells and all absorbance measurements were made against water at 542nm.

The content of 3 ampoules(1mg/ml^{-1}) of injection solution of commercial pharmaceutical of either epinephrine(Darou-Pakhsh, Iran) or norepinephrine (Sanofi Winthrop Medication system, England) was mixed. 1ml of this solution was diluted to 100ml with sufficient water. Then, 1ml of resultant solution was used and analyzed with general procedure.

RESULTS AND DISCUSSION

1,5-Diphenylcarbazide is a well-known spectrophotometric reagent for Cr(VI). It forms a red complex with Cr(VI) with a maximum absorption at 542nm, but does not react with Cr(III). This concept has been used for chromium speciation in spectrophotometric technique[13]. In this study, the same concept coupled to redox reaction between dichromate and epinephrine or norepinephrine was used for indirect determination of these catecholamine drugs. Epinephrine and/or norepinephrine when added in increasing amount to the solution of Cr(VI) prior to the addition of 1,5-diphenylcarbazide, consumes chromium(VI) in a redox reaction. This causes a proportional decrease in the absorbance of the red complex(Figure 1).

Effect of variables

Parameters, which effect the redox reaction and absorbance of the complex, were studied. The absorbance was dependent on concentration of sulfuric acid in the sample, and a maximum absorption was observed at concentration of 0.075 to 0.125M(Figure 2). There-

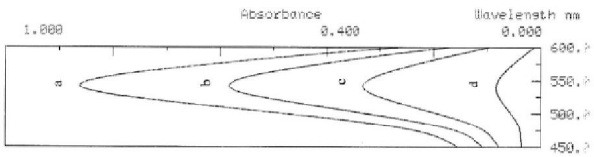

Figure 1 : Absorption spectra of chromium(VI)-1,5-diphenylcarbazide complex in the presence of different concentration of norepinephrine: chromium(VI), 1μg ml^{-1}; sulfuric acid, 0.1M; 1,5-diphenylcarbazide, 0.1mg ml^{-1}; norepinephrine: a, 0; b, 1; c, 2 and d, 3μg ml^{-1}

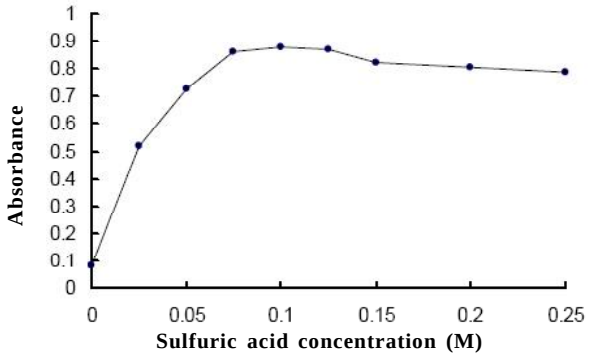

Figure 2 : Effect of sulfuric acid concentration on the absorbance: chromium(VI), 1μg ml^{-1}; 1,5-diphenylcarbazide, 0.2mg ml^{-1}

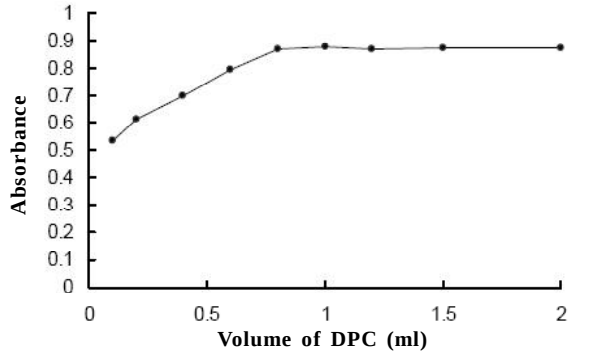

Figure 3 : Effect of 1,5-diphenylcarbazide concentration on the absorbance: chromium(VI), 1μg ml^{-1}; sulfuric acid, 0.1M

TABLE 1 : Determination of epinephrine and norepinephrine in pharmaceutical preparations

	Label claim	Proposed method		Accepted method	
	(μgml^{-1})	(μg ml^{-1})[a]	RSD%	(μg ml^{-1})[a]	RSD%
Epinephrine	1000	1000.2±4.5	1.4	999.5±5.6	0.6
Norepinephrine	1000	995.6±17.1	1.7	997.3±9.8	1.0

[a]Each number is the average of five independent analysis.

fore, a concentration of 0.1M of sulfuric acid was selected for subsequent work.

In order to optimize the concentration of 1,5-diphenylcarbazide, different volumes of 1,5-diphenylcarbazide solution(1%) were added to the mixture under study. It was found that 0.8ml of 1,5-diphenylcarbazide solution was sufficient for maximum color development. There was a decrease in absorbance at lower concentration of 1,5-diphenylcarbazide, whereas no change in absorbance was observed at higher concentration. An optimum volume of 1ml was then chosen.

The time required for complete oxidation of epinephrine and norepinephrine was studied and found that at least 12min is required for complete oxidation.

The absorbance was found to be independent of temperature in the range of 10-30^{0}C. Therefore the reaction was carried out at room temperature.

Furthermore it was considered that sodium metabisulphite, which is usually added to epinephrine and norepinephrine injection as antioxidant may interfere. Metabisulphite ion can reduce Cr(VI) to Cr(III) and cause a positive error. However it was found that addition of 1ml acetone overcome this possible interference.

Analytical performance

The calibration graph obtained under the optimum conditions for EP and NE were linear over the range of 0.2-3.20μg ml^{-1} and 0.3-3.2μg ml^{-1} with a correlation coefficient of -0.9994 and -0.9996, respectively. The limit of detection based on three times the standard deviation of the blank was 0.1μg ml^{-1} for EP and 0.15μg ml^{-1} for NE. The relative standard deviation(n=10) at 1μg ml^{-1} and 2μg ml^{-1} of epinephrine were 1.8% and 2.1%, respectively. The same figure of merit for norepinephrine at the level of 1μg ml^{-1} and 2μg ml^{-1} were calculated to be 1.2 and 2.8% RSD, respectively.

APPLICATION

The procedure was then successfully applied to the determination of EP and NE solution of commercial pharmaceutical formulations. The results of which are demonstrated in TABLE 1. As can be seen the results compared well with those obtained by accepted method[14].

The proposed method promises a simple, reliable, and economical method for the determination of EP and NE, which can be applied to the analysis of pharmaceutical preparations.

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