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ORIGINAL ARTICLE

Spectrophotometric determination of adrenaline in pharmaceutical preparations

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KEYWORDS

Adrenaline;
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Abstract A new and effective spectrophotometric method was developed for the determination of adrenaline in pharmaceutical preparations for control purposes. The condensation of Mannish reaction between 1 mol of adrenaline with new ligand, 5-benzimino-1,3,4-thiodiazole-2-thione (I) in the presence of 1 mol of *p*-formaldehyde as the condensing agent with 15–25 ml of dioxin as solvent took place. The precipitate yield was yellowish-brown colored. The new ligand was prepared by the Schiff base reaction of 5-amino-3-H, 1,3,4-thiodiazole-2-thione and 1 mol of benzaldehyde in 15 ml of ethanol as solvent and one drop of glacial acetic acid. The accuracy and precision of this method were determined by analyzed laboratory samples of adrenaline, the results show absolute error ranging from −0.22 to +0.86 and relative errors ranging from ±1.4% to 8.6%. The calibration graph was linear in the range of 2–20 mg l^{-1} for adrenaline with an S.D equal to 2.17%, and RSD of 2.76% ($n = 5$; Conc. = 80 mg l^{-1}). This method was successfully applied for the adrenaline determination in commercial pharmaceutical injections for the quality control.

1. Introduction

Adrenaline is a hormone and a neurotransmitter (Berecek and Brody, 1982). Japanese chemist Jokichi Takamine and his assistant Keizo Uenaka independently discovered adrenaline in 1900 (Yamashima, 2003). Adrenaline is an active principle of the medulla of the suprarenal gland and is a drug used in the treatment of cardiac arrest, heart block, asthma, nasal congestion, hypotension, etc. It increases heart rate, constricts blood vessels, dilates air passages and participates in the fight-or-flight response of the sympathetic nervous system. Chemically, epinephrine is a catecholamine, a monoamine produced only by the adrenal glands from the amino acids phenylalanine and tyrosine. HPLC is regulated by US pharmacopeia (United State Pharmacopeia XXV, 2002) and China pharmacopeia (China Pharmacopeia, 1995) as the official method to assay the drug in injections. Epinephrine may be quantitated in blood, plasma or serum as a diagnostic aid, to monitor therapeutic administration or to identify the causative agent in a potential poisoning victim. Endogenous plasma

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epinephrine concentrations in resting adults are normally less than 10 ng L^{-1}, but may increase by 10-fold during exercise and by 50-fold or more during times of stress. Pheochromocytoma patients often have plasma epinephrine levels of 1000–10,000 ng L^{-1}. Parenteral administration of epinephrine to acute-care cardiac patients can produce plasma concentrations of 10,000–100,000 ng L^{-1} (Baselt, 2008). Epinephrine also leads to broad alterations throughout all organ systems (Sircar, 2007). Epinephrine acts by binding to a variety of adrenergic receptors. Adrenaline is a nonselective agonist of all adrenergic receptors, including α_1, α_2, β_1, β_2, and β_3 receptors (Howard, 2008). Adrenaline in acetate buffer reacts with a solid-phase reactor containing lead (IV) dioxide immobilized in a polyester resin and the yield was continuously monitored at 486 nm using a flow injection spectrophotometric procedure (Teixeira et al., 2002). A single channel FIA assembly is proposed for the spectrophotometric determination of adrenaline in aqueous sample solution, the method has been applied to the determination of adrenaline in a pharmaceutical formulation (Kojo and Martinez Calatayud, 1990). Berzas Nevado et al. proposed a new rapid and successfully applied method for the determination of adrenaline and dopamine in pharmaceuticals, on the basis of the hydrolysis of these compounds in alkaline medium. The method was optimized by using a spectrophotometer operating at $\lambda = 390$ nm as detector (Berzas Nevado et al., 1996). The best conditions for the optimization were established by square wave voltammetry. The best performance was obtained with 50%:20%:15%:15% (m/m/m/m) as the graphite powder:laccase:Nujol:ILs composition in 0.1 mol L^{-1} acetate buffer solution pH 4.0. The analytical curve was linear in the concentration range of 2.49×10^{-6}–2.27×10^{-4} mol L^{-1} with a detection limit of 5.34×10^{-7} mol L^{-1}. The recovery of adrenaline in injectable samples ranged from 96.3% to 101.6%. The results obtained for adrenaline using the proposed biosensor and the United States Pharmacopeia procedure were in agreement at the 95% confidence level (Franzoi et al., 2010). The design of an un segmented-flow injection manifolds for the simultaneous determination of adrenaline and nor adrenaline two structurally related compounds with overlapping spectra. An FIA manifold is proposed for the simultaneous determination in which the sample solution is directly injected into a carrier-reagent stream of aqueous NaOH. The selected wavelengths (first derivative) were 394 and 342 nm, for noradrenaline and adrenaline, respectively, with an integration time of 0.4 s. The calibration graphs are linear over the range of 2.0–30 mg L^{-1} for both drugs (Rivas et al., 1996).

The objective of this research was aimed at developing a Spectrophotometric analytical method for the determination of adrenaline in pharmaceutical preparations for the quality control purpose.

2. Experimental

2.1. Materials and measurements

All reagents and solvents obtained from commercial sources were of high purity purchased from Fluka and BDH and no further purification was needed. Adrenaline hormone supplied by the BDH and used as received, adrenaline (Epinephrine) injection, 1 mg/1 mL, commercially available supplied by the MISR Co., Egypt. IR spectra were recorded using KBr and CsI discs in the range 4000–400 cm^{-1} on FT-IR, Tests cane Shimadzu model Spectrophotometer. UV–Vis spectra were recorded using Shimadzu UV–Vis spectrophotometer with quartz cells size 1 cm was used to measure the absorbance at λ_{max} of each adrenaline complex analyte.

3. Preparation of complexes

3.1. Synthesis of the ligand (Schiff base procedure)

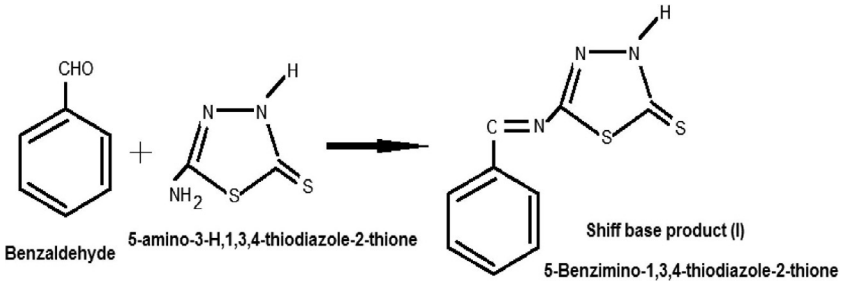
1 mol of 5-amino-3-H, 1,3,4-thiodiazole-2-thione in 15 ml ethanol as solvent and 1 mol of benzaldehyde in 15 ml of ethanol as solvent and one drop of glacial acetic acid was stirred and heated to reflux for 2 h (Ortega-luoni et al., 2007), The crude product was a yellowish-brown colored liquid, 5-benzimino-1,3,4-thiodiazole-2-thione (I), Scheme 1.

3.2. Synthesis of adrenaline complexes (Mannish procedure)

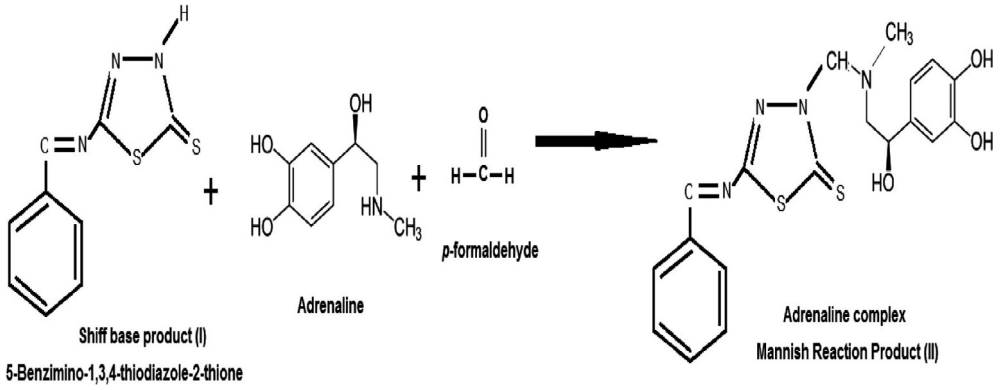
1 mol of adrenaline hormone and 1 mol of *p*-formaldehyde as condensing agent with 1 mol Schiff base yield, 5-benzimino-1,3,4-thiodiazole-2-thione (I) with 15–25 ml of dioxin as solvent, then heated to reflux for 2 h, Scheme 2.

3.3. Calibration graph

A standard calibration graph for adrenaline complexes (Fig. 1) in the concentration range of 2–20 mg L^{-1} was prepared and used to determine the adrenaline concentration. Using the Method of Least Squares (Miller and Miller, 2000) the regression equation ($Y = Xb \pm a$), where b is the slope = 0.0056, a is the intercept = +0.00479, Y is the absorbance and X is the concentration, was utilized for the calculation of unknown concentrations in medicinal adrenaline injection samples. The validity of the regression equation was tested by analyzing laboratory injections. Beers law is valid within the concentration



Scheme 1 The Schiff base yield (I).



Scheme 2 The adrenaline complex, Mannish yield (II).

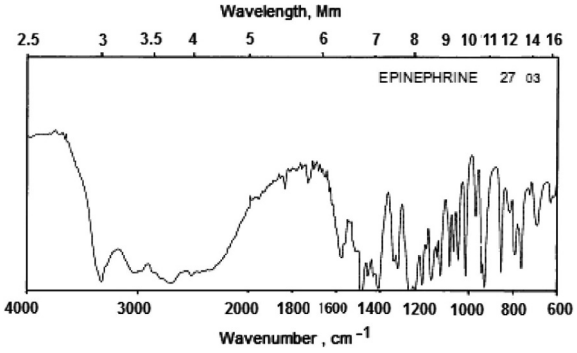


Figure 1 FT-IR spectrum of standard adrenaline.

Table 1 Analysis of laboratory standard adrenaline samples.

Sample No.	Abs.	Conc. (mg L^{-1})	Av. Conc.	Absolute error	Relative error %
1	0.059	9.68	9.68	+0.32	+3.2
2	0.062	10.22		−0.22	−2.2
3	0.058	9.50		+0.50	+5.0
4	0.056	9.14		+0.86	+8.6
5	0.060	9.86		+0.14	+1.4

Table 2 Analysis of adrenaline (epinephrine) injection, 1 mg/1 mL commercially available supplied by the MISR Co., Egypt.

Sample No.	Abs.	Conc. (mg L^{-1})	Av. Conc. (mg L^{-1})	S.D	R.S.D%
1	0.451	79.68	78.50	2.17	2.76
2	0.442	78.07			
3	0.438	77.36			
4	0.455	80.39			
5	0.436	77.00			

ranges of adrenaline. The results are summarized in Table 1 for laboratory injections. Samples of medicinal adrenaline injections samples were analyzed, the results are given in Table 2.

3.4. Stoichiometric ion complex formation

The adrenaline complexes with the ligand, 5-benzimino-1,3,4-thiodiazole-2-thione was studied in solution using methanol as solvent, in order to determine M:L ratio in the prepared complexes following continuous variation (Job's) method (Skooge et al., 2004). A series of solutions were prepared having varied concentration, of adrenaline and 5-benzimino-1,3,4-thiodiazole-2-thione ligand at total constant concentration of 10^{-3} M. The M:L ratio was found from the plot of absorbance at λ_{max} 312 nm of the formation complexes versus the mole fraction.

3.5. Results and discussion

Adrenaline is a hormone and neurotransmitter (Berecek and Brody, 1982). It increases heart rate, contracts blood vessels, dilates air passages and participates in the fight-or-flight response of the sympathetic nervous system (United State Pharmacopeia XXV, 2002).

3.6. IR spectra

The standard adrenaline shows an overlapped band in the region of 3400–3200 cm^{-1} which could be assigned to V(NH) and V(OH) vibrations (Tajen et al., 2004; Socrates, 1980). Another absorptions at 2962 cm^{-1} and (1500–1600 cm^{-1}) referred to aliphatic V(C–H) and aromatic V(C═C) vibrations modes (Tajen et al., 2004; Socrates, 1980; Silversten and Webster, 1998) (Fig. 1). Upon condensation of 5-benzimino-1,3,4-thiodiazole-2-thione (Schiff base (I) with adrenaline in the presence of condensing agent (*p*-formaldehyde), the appearance of a V(C═N) band at 1669 cm^{-1}, V(C═S) at 1226 cm^{-1} and the broadening of V(O–H) mode in the range of 3518–3200 cm^{-1} confirm the formation of the Mannish base (II). As well as the isomethene group in thiodiazole ring at 1535 cm^{-1} as a strong band and C–N band at 1375 cm^{-1} together with the above absorptions suggests good proof for the formation of N–CH_2–N mode, Fig. 2.

3.7. Electronic absorption spectra (UV–Vis)

Complex of adrenaline standard with Schiff base (I) of 5-benzimino-1,3,4-thiodiazole-2-thione caused either bathochromic or hypsochromic shifts. The standard solution of adrenaline in acidified methanol (0.1 N) shows three peaks related to charge transfer, and $\pi \rightarrow \pi^*$ of benzenoid group at 205 and 282 nm, respectively, Fig. 3 (Dibbern et al., 2002; Kannappan et al., 2006; Beraldom and Tosi, 1986). Upon condensation of

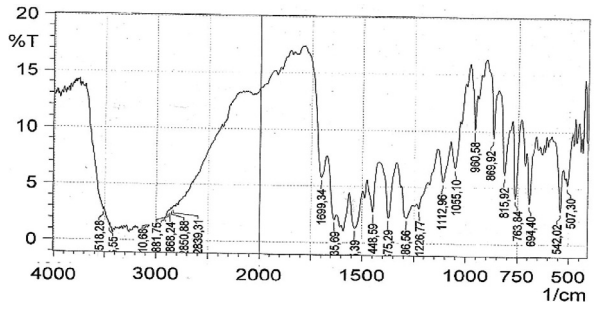


Figure 2 FT-IR spectrum of adrenaline complex.

adrenaline with Schiff base (I), 5-benzimino-1,3,4-thiodiazole-2-thione, the adrenaline complex exhibits two distinct peaks at 240 and 310 nm which are attributed to $\pi \rightarrow \pi^*$ and overlapping of $n \rightarrow \pi^*$ with charge transfer due to the presence of chromophore groups like C═N, C═S conjugates with heterocyclic groups such as those observed in 1,3,4-thiodiazole, Fig. 4 (Kannappan et al., 2006; Silversten and Webster, 1998).

3.8. Analysis

A standard calibration graph for adrenaline complex solutions Fig. 5 in the concentration range of 2–20 ppm was prepared and used to determine the concentration of adrenaline. In order to examine the accuracy and precision of the analysis method, Tables 1 and 2 summarize the analysis of laboratory sample and samples of medicinal adrenaline injections, the results show an absolute error ranging from −0.22 to +0.86 and relative errors ranging from ±1.4% to 8.6%. The

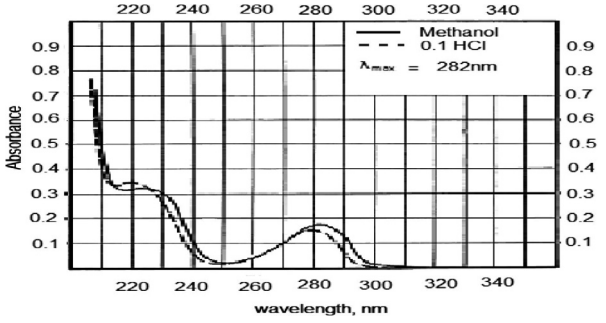


Figure 3 UV–Vis spectrum of standard adrenaline.

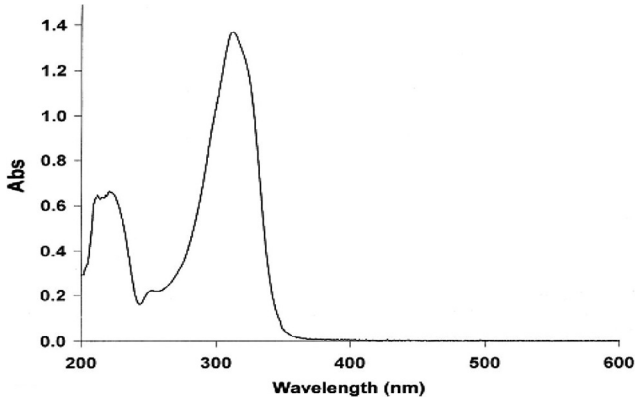


Figure 4 UV–Vis spectrum of adrenaline complex.

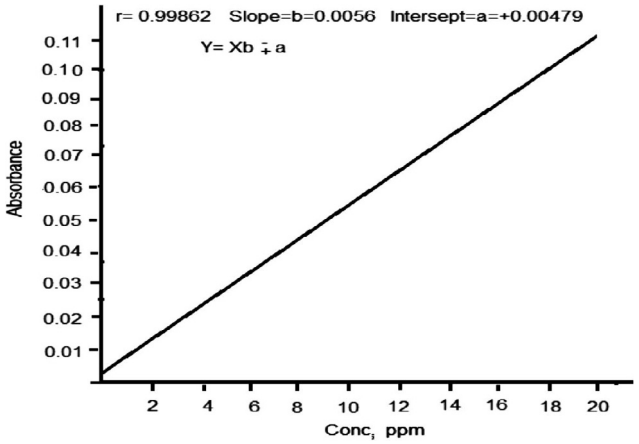


Figure 5 Standard calibration graph for adrenaline complex.

calibration graph was linear in the range of 2–20 mg L^{-1} for adrenaline with an S.D equal to 2.17%, and RSD of 2.76% ($n = 5$; $C = 80\ mg\ l^{-1}$). The proposed method was successfully applied to the determination of adrenaline in various injections used in commercial pharmaceutical formulations for the quality control purpose. The concentration measurement was done using UV–Vis spectrophotometer at λ_{max} 312 nm using 1 cm quartz cell (Fig. 4).

3.9. Structure of the product

This study concerns with the Spectrophotometric identification and determination of the adrenaline hormone as complex (II) in pharmaceutical injections using Mannish reaction after the preparation of Schiff base (I), 5-benzimino-1,3,4-thiodiazole-2-thione, Scheme 1, heated to reflux for 2 h of benzaldehyde and 5-amino-3-H, 1,3,4-thiodiazole-2-thione with 1 drop of glacial acetic acid. Upon condensation of Schiff base (I), 5-benzimino-1,3,4-thiodiazole-2-thione with 1 mol adrenaline in the presence of 1 mol of *p*-formaldehyde as condensing agent with 15–25 ml of dioxin as solvent. The precipitate yield was yellowish-brown in color, Scheme 2. The stoichiometry of the reaction between each adrenaline and ligand was investigated under the recommended conditions and applying Job's method. The obtained results in Fig. 6 showed that a 1:1 product was formed between adrenaline and ligand at λ_{max} 312 nm, and due to the phenolic nature of the adrenaline, it can be readily coupled with the ligand according to Scheme 2, Figs. 1 and 2 (Dibbern et al., 2002).

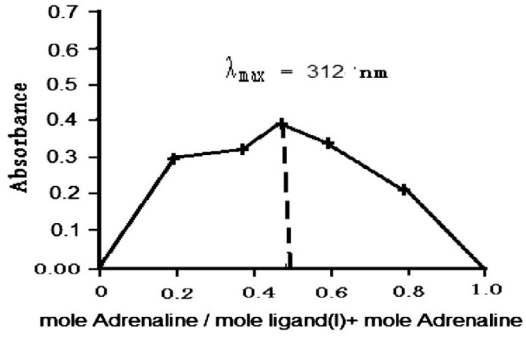


Figure 6 Continuous variation (Job's method) for adrenaline: ligand complex.

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