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A Sensitive Spectrophotometric Determination of Ritodrine, Pentazocine, Isoxsuprine Hydrochlorides and Amoxicillin in Pure and Pharmaceutical Samples

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Abstract: A simple, accurate and highly sensitive spectrophotometric method for the determination of ritodrine hydrochloride (RTH), pentazocine hydrochloride (PZH), isoxsuprine hydrochloride (ISH) and amoxicillin (AMX) is described. The method is based on the oxidation of the studied drugs by a known excess of chloramine – T (CAT) in hydrochloric acid medium and subsequent determination of the unreacted oxidant by reacting it with iodide in the same acid medium liberates iodine, which subsequently react with starch to form a stable starch-iodine complex. The reacted oxidant corresponds to the drug content. The coloured complex exhibits a maximum absorption at 590 nm. The apparent molar absorptivity values and Sandell's sensitivity values are in the range 6.96×10^4 - 1.43×10^5 L mol⁻¹ cm⁻¹ and 2.45-4.30 ng cm⁻², respectively. The method was successfully applied to the studied drugs in their dosage forms. The results are reproducible within ± 1 % and compare favorably with those of official methods of British Pharmacopoeia and the United States Pharmacopoeia.

Keywords: Spectrophotometry, Iodine - starch reagent, Drug analysis.

Introduction

Ritodrine hydrochloride (RTH), chemically 1-(4-hydroxy phenyl)-2-[2-(4- hydroxy phenyl) ethyl amino] propanol, is a β_2 - adrenergic agonist used to arrest preterm delivery in pregnant woman^{1,2}. Pentazocine hydrochloride (PZH), (2R*,6R*,11R*)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-(3-methyl-2-butenyl)-2,6-methano-3-benzazocin-8-ol, is an analgesic with antagonist action. Amoxicillin (AMX), 6-(*p*-hydroxy- α -aminophenyl acetamido) penicillanic acid, is used as an antibacterial drug. Isoxsuprine hydrochloride (ISH), *p*-hydroxy-*N*-(1-methyl-2-phenoxyethyl)

norephedrine hydrochloride, is an active peripheral and cerebral vasodilator and it has a direct relaxant effect on the smooth muscular tissue of the blood vessels and uterus. In view of the increased pharmaceutical applications of RTH, PZH, AMX and ISH, their assay and quality control are very important. Spectrophotometric methods have been reported in the literature for the determination of RTH³⁻⁵, PZH⁶⁻⁷, AMX^{5,8,9} and ISH^{3,10,11}. For the determination of studied drugs, other methods include HPLC,¹²⁻¹⁴ titrimetric¹⁵ and fluorimetric^{16,17} are also reported in the literature. Some of these methods required expensive reagents, less sensitive, poor selective, time consuming and tedious experimental procedures. To overcome these limitations in the existing method, there is still a need for a sensitive and cost-effective method for the determination of these drugs that can be adopted for the routine analysis of pharmaceutical samples. In the present investigation, a highly sensitive indirect spectrophotometric determination of cited drugs with chloramine-T-iodine and starch is described, and the proposed method has been employed to the determination of studied drugs in pure and in dosage forms. This new procedure is accurate, highly sensitive, rapid, simple and completely different from the existing methods.

Experimental

Apparatus

All absorbance measurements were made with an Elico - model SL-171 digital spectrophotometer with 1 cm matched cells.

Reagents

All chemicals used were of analytical reagent grade. Chloramine-T ($\cong 0.01 \text{ mol L}^{-1}$) was prepared by dissolving 0.28 g of CAT in 100 mL of distilled water and standardized iodometrically. This solution was then diluted subsequently to get $100 \mu\text{g mL}^{-1}$ solution. Starch 1%: It was prepared by dissolving 1.0 g of starch in 100 mL of hot distilled water. Solutions of potassium iodide (0.5 %) and hydrochloric acid (2.0 mol L^{-1}) were used.

Standard solution

Aqueous solutions of pentazocine hydrochloride (PZH) (Sigma Laboratories Pvt. Ltd., Mumbai), amoxicillin (AMX) (Cadila Health Care Ltd. India), ritodrine hydrochloride (RTH) and isoxsuprine hydrochloride (ISH) (Duphar-Interfran Ltd., India) were prepared by dissolving the requisite amount of the samples in distilled water, working solutions prepared as required by dilution.

Standard procedure

Accurately measured volumes of drug solutions equivalent to 0.2-1.2, 0.2-1.6, 0.0 -1.5 and 0.5-2.5 $\mu\text{g mL}^{-1}$ of final solution of RTH, PZH, AMX and ISH, respectively were transferred into a series of 10 mL standard flasks. Then a volume of 0.7 mL of $100 \mu\text{g mL}^{-1}$ CAT was added to each flask followed by acidification by 1.0 mL of 2.0 mol L^{-1} hydrochloric acid. After 10 min, 1.5 mL of 0.5 % KI was added to each flask. After 2.0 min 1.0 mL of 1 % starch was added and the contents were diluted to the mark with distilled water and mixed well. The absorbance of the coloured complex was measured at 590 nm against distilled water after 5.0 min. Blank was prepared similarly omitting the drug and its absorbance was measured against distilled water. The decrease in absorbance corresponding to consumed CAT and in turn, to drug concentration, obtained by subtracting the absorbance of a test solution from that of the blank solution. The calibration graph was drawn by plotting the difference in absorbance (absorbance values of test and blank solutions) of the complex against the amount of the drug. The amount of drug was determined from the concurrent calibration graph.

Procedure for pharmaceutical formulations

In a 100 mL standard flask, an accurately weighed amount (from the mixed and powdered contents of 20 tablets or mixed contents of 10 capsules), equivalent to 50 mg of the respective drug, was dissolved in 5.0 mL of methanol and completed to volume with distilled water and filtered. Appropriate aliquots of the drug solution were taken and the standard procedure was followed for analyzing the drug content.

To analyze the injection solution and syrup, the requisite amount was transferred to a 100 mL standard flask and dissolved in 5.0 mL of methanol and completed to volume with distilled water. The drug content in the diluted solution was determined as described above, and the results of the analysis are given in Table 1.

Table 1. Results of assay of RTH, PZH, AMX and ISH in dosage forms

Drug and Formulation	Amount taken $\mu\text{g mL}^{-1}$	Proposed Method ^a		Reference Method ^{18,19}		t-value ^b	F-value ^c
		Amount found $\mu\text{g mL}^{-1}$	% Rec \pm SD	% C V	% Rec \pm SD		
RTH	0.4	0.390	99.85 \pm 0.22	0.56	100.06 \pm 0.34	1.48	2.38
Yutopar tab	0.8	0.805	100.68 \pm 0.37	0.20	100.30 \pm 0.16	2.26	5.35
10 mg / tab	1.2	1.206	100.54 \pm 0.44	0.36	100.14 \pm 0.19	2.40	5.48
Yutopar inj	0.4	0.401	100.28 \pm 0.32	0.79	99.70 \pm 0.55	2.32	2.95
50 mg/10 mL	0.8	0.798	99.79 \pm 0.34	0.42	100.09 \pm 0.58	1.47	2.91
	1.2	1.203	100.25 \pm 0.13	0.10	100.17 \pm 0.26	1.88	4.0
PZH	0.4	0.399	99.94 \pm 0.32	0.80	100.06 \pm 0.39	0.66	1.48
Penzyl inj	1.0	1.002	100.20 \pm 0.17	0.16	100.15 \pm 0.12	1.21	2.0
30 mg/ mL	1.6	1.603	100.22 \pm 0.20	0.12	100.06 \pm 0.12	1.97	2.78
Fortwin inj	0.4	0.399	99.85 \pm 0.35	0.87	100.15 \pm 0.59	1.16	2.84
30 mg/ mL	1.0	1.004	100.43 \pm 0.64	0.63	100.19 \pm 0.32	1.68	4.00
	1.6	1.608	100.54 \pm 0.66	0.41	100.43 \pm 0.31	0.90	4.50
AMX	0.5	0.500	100.18 \pm 0.33	0.66	99.81 \pm 0.55	2.46	2.77
Amokid tab	1.0	1.001	100.13 \pm 0.22	0.21	100.01 \pm 0.43	1.21	3.82
250 mg/tab	1.5	1.502	100.15 \pm 0.21	0.13	99.99 \pm 0.48	1.69	5.20
Hipen inj	0.5	0.498	99.69 \pm 0.41	0.82	100.03 \pm 0.59	1.73	2.07
250 mg/mL	1.0	1.001	100.18 \pm 0.33	0.32	99.81 \pm 0.61	2.46	3.41
	1.5	1.502	100.16 \pm 0.37	0.24	99.88 \pm 0.78	1.69	4.44
Amoxipen	0.5	0.499	99.91 \pm 0.34	0.68	99.60 \pm 0.54	2.38	2.52
Syrup	1.0	0.999	99.93 \pm 0.24	0.24	99.80 \pm 0.42	1.25	3.06
125 mg/5mL	1.5	1.505	100.35 \pm 0.17	0.11	100.17 \pm 0.35	2.26	4.23
ISH	1.0	0.995	99.56 \pm 0.44	0.44	100.01 \pm 0.59	1.73	1.64
Duvadilan tab	1.5	1.500	100.06 \pm 0.67	0.44	99.80 \pm 0.49	0.86	1.86
10 mg/ tab	2.0	2.001	100.09 \pm 0.65	0.32	99.50 \pm 0.43	1.96	2.28
Tidilan inj	1.0	1.003	100.30 \pm 0.45	0.45	100.31 \pm 0.52	0.86	1.34
5 mg/mL	1.5	1.506	100.43 \pm 0.64	0.31	100.19 \pm 0.47	0.85	1.85
	2.0	2.00	100.00 \pm 0.90	0.45	99.73 \pm 0.56	0.71	2.58

^a Average of five determinations, ^b Tabulated value 2.78, ^c Tabulated value 6.39

Results and Discussion

Preliminary experiment was performed to fix the linear range (Beer's law curve) for chloramine-T (CAT) in the optimum experimental conditions, with the use of iodine-starch reagent. Under experimental conditions, the concentration range of CAT was found to be 0 -7.0 $\mu\text{g mL}^{-1}$. In the present work, known but excessive CAT was utilized to oxidize the studied drugs in 2.0 mol L^{-1} hydrochloric acid medium and the unreacted CAT was determined by reacting it with iodine - starch reagent in the same acidic system. The coloured complex shows a maximum absorption at 590 nm. This formed the basis for the determination of studied drugs in microgram quantities. The studied drugs, when added in increasing amounts to fixed amount of chloramine-T, drugs consume CAT and there is a concomitant decrease in the absorbance of the coloured complex on increasing the concentration of drugs (Figure 1).

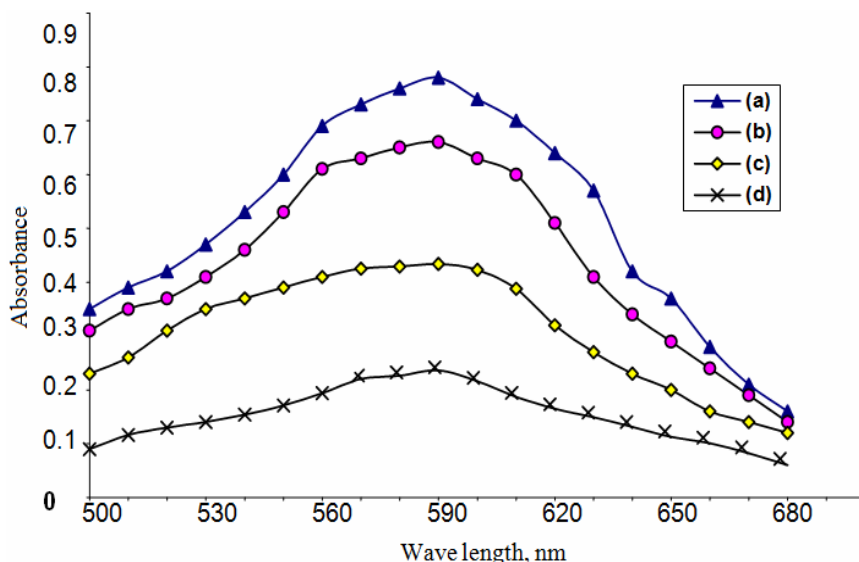
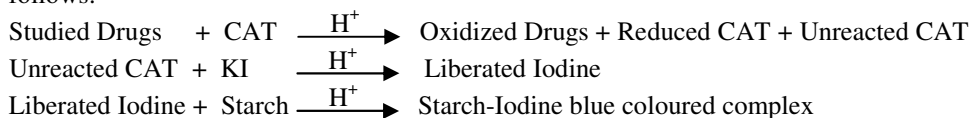


Figure 1. Absorption spectra of the CAT - iodine -starch complex with ISH (a) Blank (without ISH) (b) 0.5 $\mu\text{g mL}^{-1}$ (c) 1.5 $\mu\text{g mL}^{-1}$ (d) 2.5 $\mu\text{g mL}^{-1}$ measured against water.

The general reaction scheme of the method for studied drugs with CAT is represented as follows:



Effects of acid and reagents

Hydrochloric acid was the medium of choice for oxidation of the drugs by CAT as well as the latter's determination with iodine-starch reagent. A 1.0 mL of 2.0 mol L^{-1} concentration of HCl was found optimum for the oxidation of the drugs within 10 min, and hence the same concentration was employed for the determination of CAT with iodine-starch reagent. The volume of 1.5 mL of 0.5% KI and 1.0 mL of 1.0% starch solution in a total volume of 10 mL of reaction mixture were found suitable for the analysis.

Reaction time and stability of colour

The time taken for complete oxidation of the drugs is not critical. Any delay up to 45 min in the determination of unreacted CAT had no effect on the absorbance. The colour of the formed iodine – starch complex was stable for a period of more than 90 min for the studied drugs.

Effect of excipients

In pharmaceutical analysis, it is important to test the accuracy of the method, so recovery experiments were performed using a synthetic mixture of each drug (RTH, PZH, ISH and AMX) with several excipients such as talc, stearic acid, gum acacia, dextrose, sodium alginate etc. by the proposed method and recoveries obtained were in the range 99.8-101.2 %. The results suggested that the usual tablet diluents and excipients were found not to interfere with the analysis by the proposed method.

Analytical data

The Beer's law limit, molar absorptivity, Sandell's sensitivity, correlation coefficient, detection and quantitation limits obtained by least square treatment of the results are given in Table 2.

Table 2. Optical characteristics and precision data

Parameter	RTH	PZH	ISH	AMX
Beer's law limit, $\mu\text{g mL}^{-1}$	0.2 - 1.2	0.2 - 1.6	0.5 - 2.5	0.0 - 1.5
Molar absorptivity, $\text{L mol}^{-1}\text{cm}^{-2}$	1.32×10^5	8.08×10^4	6.96×10^4	1.43×10^5
Sandell's sensitivity, $\mu\text{g cm}^{-2}$	0.0024	0.0035	0.0043	0.0029
Correlation coefficient [r]	0.999	0.999	0.999	0.999
Regression equation [y*]				
Slope [b]	0.3665	0.2493	0.2126	0.3381
Intercept [a]	0.0220	0.0232	0.0216	0.0031
Detection limit [DL], $\mu\text{g mL}^{-1}$	0.0427	0.0504	0.1126	0.0354
Quantitation limit [QL], $\mu\text{g mL}^{-1}$	0.1296	0.1529	0.3414	0.1183

*Y= a+bx. where x is the concentration in $\mu\text{g mL}^{-1}$.

Applications

The proposed method was applied to the quantitative determination of studied drugs in pharmaceutical formulations and the results (Table 1) compare favorably with the official methods of the United States Pharmacopoeia¹⁸ and British Pharmacopoeia.¹⁹ A statistical analysis of the results by f- and t-tests at 95 % confidence level showed no significant difference in the accuracy between the proposed method and official methods (Table 1). To ascertain the ruggedness of the method, four replicate determinations at two different concentration levels of the drugs were carried out. The within-day RSD values were less than 1 %. The values of between-day RSD for different concentrations of drugs, obtained from four determinations carried out over a period of 4 days, are given in Table 3, and indicate that the proposed method has reasonable ruggedness.

Table 3. Between-day precision of the determination of cited drugs by the proposed method

Drug	Amount taken, μg	Amount found ^a , μg	RSD, %
RTH	4.0	3.96	0.39
	10.0	9.97	0.22
PZH	6.0	5.95	0.50
	12.0	11.99	0.35
AMX	5.0	4.90	1.40
	12.5	12.49	0.27
ISH	10.0	9.90	0.35
	20.0	20.01	0.25

^a. Average value of four determinations carried out over a period of 4 days.

Conclusions

The method developed is simple, selective and offer the advantages of high sensitivity and a wide range of determination without the need for heating or extraction. The colour developed is stable for a sufficient interval of time and the method unaffected by slight variations in the experimental conditions such as acidity and other reagents. The proposed method was compared with other reported spectrophotometric methods and found to be superior (Table 4). The proposed method can serve as an alternative method for the determination of the studied drugs in pure and in dosage forms.

Table 4. Comparison with other reported methods

Reagent	Beer's law limit $\mu\text{g mL}^{-1}$	ϵ $\text{L mol}^{-1} \text{cm}^{-1}$	Remarks	Reference
N-bromosuccinimide	[AMX] 1 - 20	1.90×10^4	Less sensitive	[8]
Benzocaine	[AMX] 2 - 16	2.26×10^4	Less sensitive and poor selective	[9]
4-aminoantipyrine	[RTH] 2 - 22	0.98×10^4	Less sensitive	[3]
	[ISH] 1-18	1.20×10^4		
Sodium nitroprusside and hydroxyl- ammonium chloride	[PZH] 1-10	1.28×10^4	Less sensitive	[6]
3-Methyl-1- benzothiazolin-2-one hydrazone[MBTH]	[AMX] 0.5 -16	2.35×10^4	Costly reagent and less sensitive	[5]
	[RTH] 0.6 -12	1.27×10^4		
Chloramine-T-iodine- starch	[RTH] 0.2 -1.2	1.32×10^4	Highly sensitive and cost- effective	Present method
	[PZH] 0.2 -1.6	8.08×10^4		
	[ISH] 0.5 - 2.5	6.96×10^4		
	[AMX] 0.0- 1.5	1.43×10^5		

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