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SPECTROPHOTOMETRIC DETERMINATION OF DRUGS & PHARMACEUTICALS USING N-BROMOSUCCINIMIDE AND RHODAMINE-B COUPLE

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Keywords:

NBS, Quantification, Rodamine-B, Spectrophotometry, Drugs, Validation

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ABSTRACT: Simple, sensitive, and accurate spectrophotometry method one each for estimation of five drugs *viz*., chloroquine phosphate (CHP), granisetron hydrochloride (GRA), rizatriptan benzoate (RIZ), zoledronic acid (ZOL) and zolmitriptan (ZOT), have been developed. The method depends upon oxidation of each drug by excess N-Bromo succinimide (NS), and subsequent determination of UN reacted NBS by Rhodamine-B dye at λ_{max} 557 nm. Beers law is obeyed between 2-14 µg mL^{-1} (for CHP); 52-14 µg mL^{-1} (for GRA); 1-7 µg mL^{-1} (for RIZ); 1-7 µg mL^{-1} (for ZOL) & 2-14 µg mL^{-1} (for ZOT). This method has been applied for the determination of drugs in their pure form as well as in tablet formulations. All the methods have been validated as per ICH guidelines.

INTRODUCTION:

Chloroquine Phosphate: Chloroquine phosphate (CHP) is an antimalarial drug whose structure is shown in **Fig. 1A** and chemically knows as 7-chloro - 4 - 4 - diethylamino - 1 - methylbutyl amino-quinoline diphosphate. CHP is also used for the treatment of amebiasis, polymyositis, sarcoidosis rheumatoid arthritis, discoid lupus erythematosus, and photosensitive diseases [1]. The literature survey reveals that several analytical methods are reported for the quantification of CHP. Among these methods, British Pharmacopoeia (BP) [2], United States Pharmacopeia (USP) [3], UV-spectro-photometry [4], HPLC [5], HPTLC [6], gas chromatography [7], polarimetry [8] and electro-phoresis [9] are a few mention worth.

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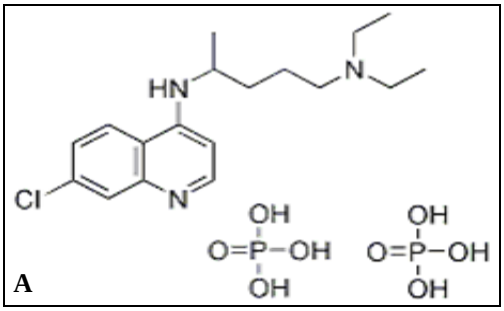
Granisetron Hydrochloride: Granisetron hydro-chloride (GRA) is an antiemetic drug. The structure of the GRA is shown in **Fig. 1B** and it is chemically known as endo-N- (9-methyl-9- aza-bicyclo 3.3.1 non-3-yl) -1-methyl-1H-indazole-3-carboxamide hydrochloride [10, 11]. It represents the class of selective 5-HT3 antagonists which is commonly employed as anti-emetic in combination with anti-ulcer and anti-cancer agents [12] an effective and potent antiemetic drug that is used in the treatment of vomiting and nausea resulting from cancer chemotherapy and radiotherapy in adults and children. The literature survey reveals that the drug has been estimated by tandem LC-MS [13], LC-MS [14, 15], HPLC [16, 17] and UV-visible spectrophotometry [18].

Rizatriptan Benzoate: Rizatriptan benzoate (RIZ) is used to treat migraines. **Fig. 1C** shows structure of the RIZ [19], chemically it is known as N, N-dimethyl-5- (1 H-1, 2, 4-triazol-1- ylmethyl) -1 H-indole-3-ethanamine mono-benzoat [20, 21]. RIZ also acts on sensory trigeminal nerves, reducing transmission along pain pathways.

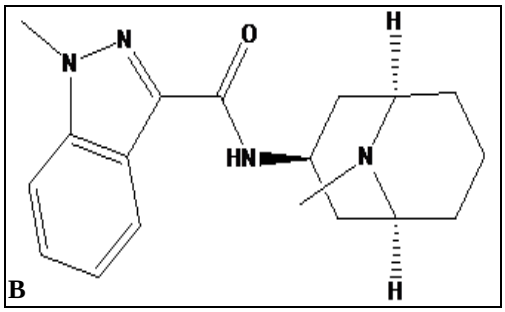
RIZ is orally administered. The literature survey reveals that several analytical methods are reported for the determination of RIZ. Among these methods, liquid chromatography [22, 23, 24], LC-MS/MS [25, 26], HPLC [27, 28] are a few mention worth.

Zoledronic Acid: Zoledronic acid (ZOL) is an inhibitor of osteoclastic bone resorption drugs. **Fig. 1D** shows the structure of ZOL and is chemically known as (1-hydroxy-2-imidazole-1-yl-phospho-noethyl) phosphonic acid. ZOL is a bisphosphonic acid [29] ZOL is clinically used for the handling of malignant and benign bone diseases, *e.g.*, Osteoporosis. ZOL is given by injection. The literature survey reveals that the drug has been estimated by HPLC [30], including solid-state 13C and 31P CPMAS and solution state, IR spectroscopy, powder X-ray diffraction, NMR spectroscopy, thermal analysis and scanning electron microscopy.

Zolmitriptan: Zolmitriptan (ZOT) is an anti-migraine drug. **Fig. 1E** shows the structure of ZOT and is chemically known as (4S)-4-3-2-(dimethylamino) ethyl-1H-indol-5-yl methyl-2-oxazolidinone [31]. It acts by stimulating serotonin receptors in the brain. Serotonin is a natural substance in the brain that, among other things, causes blood vessels in the brain to narrow. It is used to treat severe migraine headaches [32].



Chloroquine phosphate



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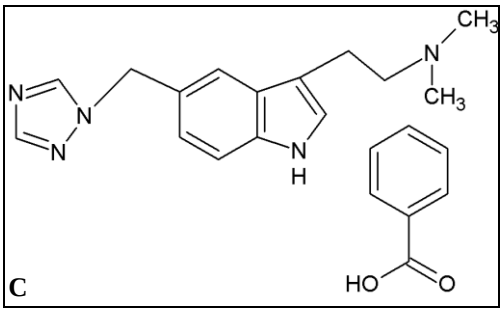
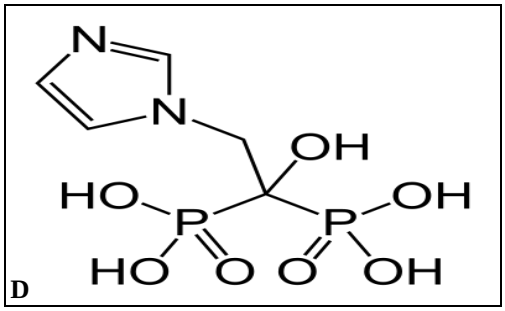
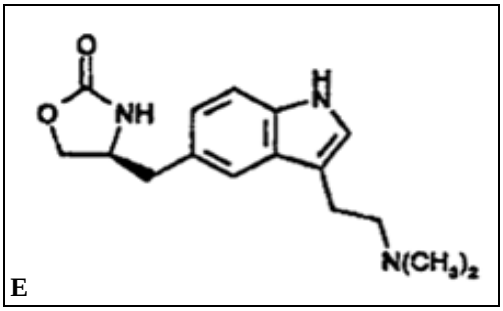


Rizatriptan benzoate



Zoledronic acid



Zolmitript

FIG. 1: STRUCTURE OF DRUGS

The literature survey reveals that several analytical methods are reported for its quantification. Among these methods, HPLC with mass spectrometry detection [33], with coulometric detection [34], electrospray ionization mass spectrometry [35], tandem mass spectrometry [36], fluorescence detection [37, 38] in pharmaceutical preparations and biological fluids and spectrophotometric methods [39, 40], mass spectrometry detection [41] and ultra-performance liquid chromatography (UPLC) [42] are a few mention worth.

EXPERIMENTAL:

Instruments: All absorbance measurements were recorded on thermo nicolet 1000 single beam as well as on Shimadzu 140 and Elico SL 210 double beam UV-Visible spectrophotometers using matched pair of quartz cells of 10 mm path length.

Materials and Reagents: All the reagents used were of analytical reagent grade, and dounle distilled water was used throughout the invest-tigation. NBS solution (0.01%) was prepared by dissolving N-bromosuccinimide (Himedia Laboratories Pvt. Ltd, Mumbai) in water with the aid of heat and standardized. The solution was kept in an amber-colored bottle and was diluted with distilled water appropriately to get 70 µ gmL^{-1}.

A stock solution of rhodamine-B (5×10^{-4} M) was prepared by dissolving the dye (S. d. Fine Chem. Ltd., Mumbai) in water and filtered using glass wool. The dye solution was diluted to 50 µg mL^{-1}.

Hydrochloric Acid (1 M): Concentrated hydro-chloric acid (S. D. Fine Chem., Mumbai, India; sp. gr. 1.18) was diluted appropriately with water to get 1 M HCl acid. The pharmaceutical grade drugs were procured from NATCO pharmaceuticals and MSN Drugs Pvt. Ltd. Hyderabad.

A stock standard solution of each drug was prepared by dissolving accurately weighed 30 mg of pure drug in water and diluting to 100 mL in a calibrated flask with water. The solution was diluted stepwise to get working concentrations.

Assay Procedure: Aliquots containing 2-25 µg mL^{-1} of the drug were transferred into a series of 10 mL standard flasks using a micro burette. To this, 1 mL of NBS, followed by 1 mL of 1M HCl was added, and contents were shaken well.

After 30 min, 1 mL of rhodamine-B dye was added to each flask. Then contents were shaken well and made up to the mark.

The absorbance of each solution was recorded between 480 and 630 nm against the corresponding reagent blank **Fig. 2**.

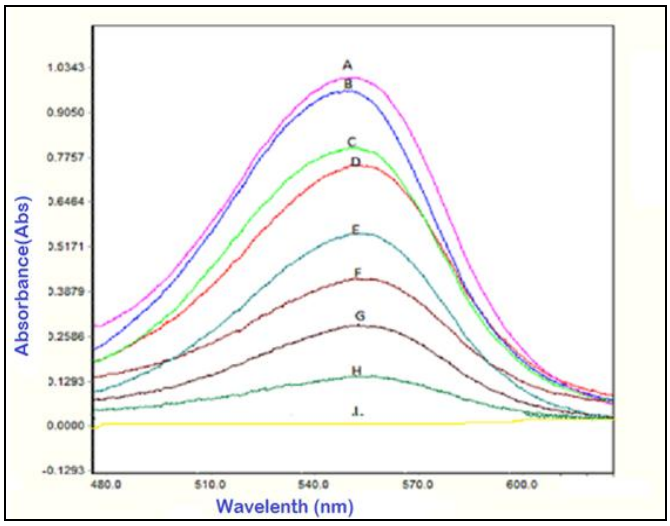


FIG. 2: ABSORPTION SPECTRUM OF RHODAMINE-B DYE (B-H) ABSORPTION SPECTRUM OF RHODAMINE-B WITH NBS AT DIFFERENT CONCENTRATIONS OF CHLOROQUINE PHOSPHATE (I) ABSORPTION SPECTRUM OF RHODAMINE-B WITH NBS AT EQUI-VALENCE POINT

TABLE 1: SPECTRAL AND ANALYTICAL PARAMETERS OF SPECTROPHOTOMETRIC STUDY

Parameter	CHP	GRA	RIZ	ZOL	ZOT
λ_{max}, nm	557	557	557	557	557
Beer's law limits, µg mL^{-1}	2-14	2-14	1-7	1-7	2-14
Molar absorptivity, L mol^{-1} cm^{-1}	2.94×104	1.95×10^4	4.4×10^4	2.78×10^4	1.66×10^4
Sandell sensitivity, µg cm^{-2}	0.0208	0.0196	0.0094	0.0101	0.02
Limit of detection, µg mL^{-1}	0.2052	0.4795	0.1400	0.1449	0.420
Limit of quantification, µg mL^{-1}	0.6221	1.4530	0.4245	0.4393	1.275
Regression equation, Y	=0.048x +0.069	=0.052x +0.033	=0.104x +0.027	=0.100x+-0.012	=0.052x +0.051
Intercept, (a)	0.069	0.033	0.027	0.012	0.051
Slope, (b)	0.048	0.052	0.104	0.100	0.052
Correlation coefficient, (r)	0.996	0.998	0.999	0.999	0.999
Standard deviation of intercept, (Sa)	0.0136	0.0069	0.0045	0.0043	0.0063
Standard deviation of slope, (Sb)	0.0077	0.0021	0.0022	0.0018	0.0009

The spectral and analytical parameters of spectro-photometric study **Table 1.** Calibration curves were constructed for all the drugs by plotting the absorbance versus the concentration of drugs. The absorbance data was collected for six replicate experiments, and absorbance to concentration ratio called the relative response was determined. The relative responses between 95% to 105% of average only are considered for construction of the calibration curves **Fig. 3**.

TABLE 2: DETERMINATION OF ACCURACY AND PRECISION OF THE METHODS ON PURE DRUG SAMPLES

Drug	Taken (µg/mL)	Found (µg/mL)	ER (%)	Recovery (%)	RSD (%)	Proposed method mean ± SD
CHP	2	1.97	1.5		0.6925	99.42 ± 0.688
	6	5.96	0.66	99.33		
	10	9.98	0.2	99.8		
	14	14.01	-0.07	100.07		
GRA	2	1.98	1	99	0.3185	99.55 ± 0.379
	6	5.98	0.33	99.66		
	10	9.97	0.3	99.7		
	14	13.98	0.14	99.85		
RIZ	1	0.99	1	99	0.4846	99.46 ± 0.482
	3	2.98	0.66	99.33		
	5	4.97	0.6	99.4		
	7	7.01	-0.14	100.14		
ZOL	1	0.99	1	99	0.5181	99.56 ± 0.515
	3	2.98	0.66	99.33		
	5	5.01	-0.2	100.2		
	7	6.98	0.28	99.71		
ZOT	2	1.99	0.5	99.5	0.1671	99.74 ± 0.166
	6	5.99	0.16	99.83		
	10	9.98	0.2	99.8		
	14	13.98	0.14	99.85		

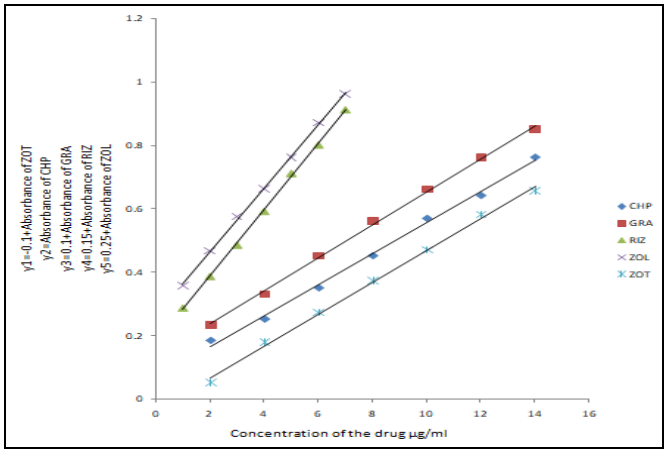


FIG. 3: CALIBRATION CURVES OF THE DRUGS

Procedure for Assay of Pure Drug: Sample solutions of each drug in the Beer's law limits were chosen, and recovery experiments were performed to check the accuracy and precision. The concentration was chosen, and recovery is tabulated in **Table 2**. For this purpose, the standard deviation method also adapted. Excellent recovery and % RSD being less than 2 speaks about the precision and accuracy of the method, **Table 2**.

Procedure for Assay of Tablets:

Chloroquine Phosphate (CHP): Ten tablets of arquin were weighed correctly and powdered. The powder equivalent to 250 mg was transferred into a 100 mL volumetric flask, containing a mixture of distilled water (10.0 mL) and HCl (2.0 mL). The flask was shaken for 5 min, and the solution was filtered using Whatman no. 41 filter paper and further diluted with water to obtain a standard working solution.

Granisetron Hydrochloride (GRA): Ten tablets of granisol containing 1 mg each amounting about 10 mg of GRA was accurately weighed, dissolved in water and diluted to volume 100 mL calibrated flask. This solution was diluted stepwise to give a series of concentrations proper for the construction of the calibration graph.

Rizatriptan Benzoate (RIZ): Four tablets of Rizact containing 5 mg each amounting about 20 mg of RIZ was exactly weighed, dissolved in water and diluted to volume 100 mL calibrated flask. This solution was diluted stepwise to give a chain of concentrations suitable for the construction of the calibration curve.

Zoledronic Acid (ZOL): One Rokfos t injection (50 mg/100 mL/ injection)s of ZOL were placed in a boiling tube and worked out to get working standard solutions of 5 µgmL^{-1}. Quantification was performed using 2, 3, 5 & 6 µg mL^{-1} of Zoledronic acid.

Zolmitriptan (ZOT): Ten Zolmitriptan tablets, 2.5 mg/ tablet were weighed and powdered. Exactly weighed quantity of tablet powder equivalent to about 25 mg of zolmitriptan was transferred into 100 mL volumetric flask, shaken for ten minutes, the volume was then adjusted to mark with, the solution was filtered through Whatman filter paper no. 42, and the filtrate was then appropriately diluted to get a final concentration of 4 µg mL^{-1} of zolmitriptan.

RESULTS AND DISCUSSION: Each method developed for quantification of drugs has been validated in terms of precision, accuracy, limit of quantification, the limit of detection, linearity, selectivity, and ruggedness.

The Beer's law limits, correlation coefficient, slope, Intercept, sandell's sensitivity, and regression equations for each drug are tabulated in **Table 3**. To assess the precision, each experiment was repeated at least 6 times, and accuracy is estimated in terms of percent recovery and percent RSD. Excellent percent recovery and RSD being less than 2 for each drug demonstrates the accuracy and precision of the methods.

TABLE 3: SPECTRAL AND ANALYTICAL PARAMETERS OF SPECTROPHOTOMETRIC STUDY

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Limit of quantification, µg mL^{-1}	0.6221	1.4530	0.4245	0.4393	1.275
Regression equation, Y	=0.048x +0.069	= 0.052x +0.033	=0.104x+ 0.027	= 0.100x +-0.012	= 0.052x +0.051
Intercept, (a)	0.069	0.033	0.027	0.012	0.051
Slope, (b)	0.048	0.052	0.104	0.100	0.052
Correlation coefficient,(r)	0.996	0.998	0.999	0.999	0.999
Standard deviation of intercept, (Sa)	0.0136	0.0069	0.0045	0.0043	0.0063
Standard deviation of slope, (Sb)	0.0077	0.0021	0.0022	0.0018	0.0009

TABLE 4: RESULTS ASSAY OF TABLETS OF ACCURACY AND PRECISION OF THE METHODS ON PURE DRUG (TABLET) SAMPLES & STUDENT'S T-TEST AND F-TEST VALUES FOR PHARMACEUTICAL ANALYSIS

Drug	Taken µg/mL	Found µg/mL	Er (%)	Recovery (%)	RSD (%)	Proposed method mean ± SD	Reference method mean ± SD	T-Test	F-Test
CHP	4	3.97	0.75	99.25	0.3836	99.66 ± 0.382	99.9 ± 0.85	1.2301	0.2019
	7	7.01	-0.14	100.14					
	10	9.95	0.5	99.5					
	13	12.97	0.23	99.76					
GRA	4	3.98	0.5	99.5	0.2861	99.77 ± 0.285	100 ± 0.75	1.4196	0.1444
	7	6.97	0.42	99.57					
	10	10.01	-0.1	100.1					
	13	12.99	0.07	99.92					
RIZ	2	1.98	1	99	0.5629	99.66 ± 0.561	100 ± 0.33	0.8693	2.89
	3	3.01	-0.33	100.33					
	4	3.98	0.5	99.5					
	6	5.99	0.16	99.83					
ZOL	2	2.01	-0.5	100.5	0.458	99.76 ± 0.458	100 ± 0.53	2.2295	0.0074
	3	2.99	0.33	99.66					
	5	4.98	0.4	99.6					
	6	6.02	-0.33	100.33					
ZOT	4	3.97	0.75	99.25	0.377	99.76 ± 0.376	99.9 ± 0.52	0.0946	1.1155
	7	6.99	0.14	99.85					
	10	9.98	0.2	99.8					
	13	13.02	-0.15	100.15					

Effect of Acid Concentration: To study the effect of acid concentration, different types of acids were examined (H_2SO_4, HCl and H_3PO_4 and CH_3COOH) to achieve maximum yield of a redox reaction.

The results indicated that the hydrochloric acid was the preferable acid with NBS as oxidant. The reaction was performed in a series of 10 mL volumetric flask containing 8.0 µg mL^{-1} of the cited drugs, different volumes (0.5-2.5 mL) of 1M HCl, and 1 mL of NBS (0.01%) were added. After 5.0 min of heating time at 60 ± 2 °C in a water bath, the solution was cooled for about 3.0 min, 1 mL of Rhodamine-B were added, then complete to 10 mL total volume with water.

It was found that the maximum absorbance was obtained at 1 mL of 1M HCl. Above this volume, the absorbance decreased. Therefore, a volume of 1 mL of 1M HCl was used for all measurements.

Effect of Heating Time: In order to obtain the highest and most stable absorbance, the effect of heating time on the oxidation reaction of drugs was catalyzed by heating in a water bath at 60 ± 2 °C for the periods ranging for 5-10 min. The time required to complete the reaction and maximum absorbance were obtained after 5.0 min of heating. After the oxidation process, the solution must be cooled at least for 3.0 min before the addition of dye.

Application to Formulations: The proposed methods were applied to the determination of drugs in tablets. The results in **Table 4** showed that the methods are successful for the determination of drugs and that the recipients in the dosage forms do not interfere.

Statistical analysis of the results using Student's t-test for accuracy and F-test for precision revealed no significant difference between the proposed methods and the literature method at the 95% confidence level with respect to accuracy and precision **Table 4**.

Recovery experiment was performed *via* standard addition technique to ascertain the accuracy and validity of the proposed methods. To a fixed and known amount/concentration of drug in tablet powder, the pure drug was added at three levels (50, 100, and 150 % of the level present in the tablet), and the total was found by the proposed methods. Each experiment was repeated six times, and the percent recovery of pure drugs added was within the permissible limits showing the absence of interference by the inactive ingredients in the assay.

CONCLUSION: This is simple, fast, and cost-effective methods for the determination of drugs have been developed and validated. The proposed method is more sensitive, and the methods depend on the use of simple and cheap chemicals and techniques but provide sensitivity comparable to those achieved by a sophisticated and expensive technique like HPLC. Thus, they can be used as alternatives for rapid and routine determination of bulk samples and tablets.

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CONFLICTS OF INTEREST: Nil

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