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SIMPLE SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF DRUGS AND PHARMACEUTICALS USING NBS-METHYL ORANGE DYE COUPLE

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Keywords:

Argatrobane monohydrate, Bortezomib, Chloroquine phosphate, Granisetron HCl, Ibandronate sodium, Estimation, NBS, Methyl orange

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ABSTRACT: Simple, sensitive, cost-effective and selective methods are designed and developed for the oxidative indirect spectrophotometric determination of drugs and pharmaceuticals, *viz*., Argatrobane Monohydrate (ARG), Bortezomib (BOR), Chloroquine Phospate (CHP), Granisetron HCl (GRA), Ibandronate Sodium (IBA) based on their reactivity towards n-bromo succinamide (NBS in excess). The method is based on the oxidation of drugs by n-bromo succinamide (excess) and estimating the amount of un-reacted NBS by Methyl Orange dye at λ_{max} 508 nm. The calibration curves obeyed Beer's law over the concentration range of 10-70 µg mL^{-1} (ARG), 8-56 µg mL^{-1} (BOR), 6-42 µg mL^{-1} (CHP), 4-28 µg mL^{-1} (GRA) & 5-35 µg mL^{-1} (IBA). This method has been applied for the determination of drugs in their pure form as well as in tablet formulations. 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. The method has been validated in terms of guidelines of the International Council of Harmonization.

INTRODUCTION: Argatrobane Monohydrate: Argatrobane monohydrate, ARG **Fig. 1** is chemically [((2R, 4R)-4-methyl-1,2,3,4-tetrahydro-8- quinolinesulfonyl)- L- arginyl- 2- piperidine-carboxylic acid mono hydrate]. It is a direct thrombin inhibitor under clinical development as adjunctive therapy to thrombolytic agents in acute myocardial infarction (AMI). Recent clinical trials have shown argatroban to be especially effective when administered in conjunction with a thrombolytic agent within 6 h of the onset of AMI symptoms.

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Biochemical studies have shown that argatroban is a potent and selective thrombin inhibitor; Argatroban is a direct thrombin inhibitor that reversibly binds to the thrombin active site. Argatroban does not require the co-factor antithrombin III for antithrombotic activity. Argatroban exerts its anticoagulant effects by inhibiting thrombin catalyzed or -induced reactions.

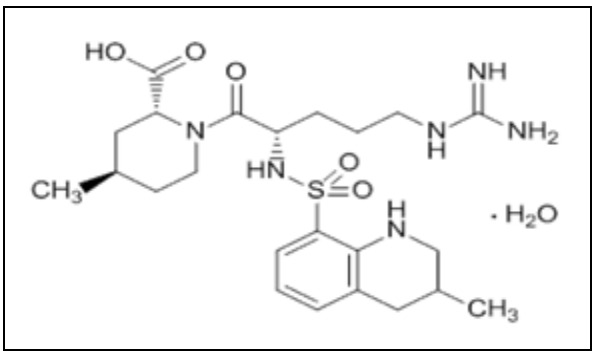

FIG. 1: CHEMICAL STRUCTURE OF ARGATROBAN MONOHYDRATE

Argatroban is capable of inhibiting the action of both free and clot-associated thrombin [1]. Several methods are known for the quantitative determination of ARG [2-8].

Bortezomib: Bortezomib is a novel cytotoxic chemical entity that potently and specifically inhibits the proteolytic activity of the proteasome and thus the degradation of poly-ubiquitinated proteins destined for catalysis by the proteasome. Bortezomib is a modified dipeptidyl boronic acid derived from leucine and phenylalanine. The chemical name for bortezomib, the monomeric boronic acid, is [(1R)-3- methyl-1-[[(2S)-1-oxo-3-phenyl- 2- [(pyrazinyl carbonyl) amino] propyl] amino]butyl]boronic acid, **Fig. 2** [9].

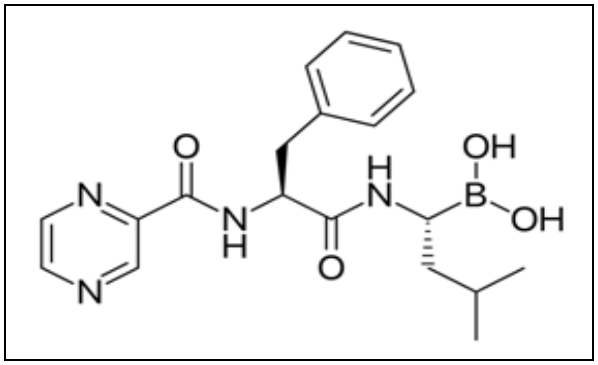

FIG. 2: CHEMICAL STRUCTURE OF BORTEZOMIB

Because of its physiological significance, it has been quantified by several methods which are enumerated in the recent reference [10-16].

Chloroquine Phosphate: Chloroquine phosphate (CQP), chemically known as 7-chloro-4[{4-(diethylamino)-1-methylbutyl} amino] quinoline, is a 4-aminoquinolein antimalarial drug **Fig. 3**. It is the prototype synthetic antimalarial drug most widely used to treat all types of malaria infections.

The drug is also prescribed to decrease the symptoms of rheumatoid arthritis and to treat systemic and discoid lupus erythematosus in adults [17]. Determination of chloroquine concentrations in biological samples is important for several reasons, such as assessment of patient compliance, evaluation of pharmacokinetic data and prevention of toxic blood concentrations after prolonged use, especially in the case of treatment of rheumatoid arthritis. Reliable analysis methods are also required for quality control of chloroquine preparations.

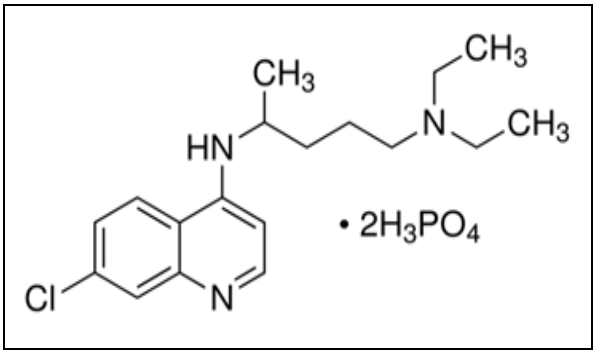

FIG. 3: CHEMICAL STRUCTURE OF CHLOROQUINE PHOSPHATE

Other analytical methods reported for the assay of CLQ in pharmaceuticals include visible spectrophotometry [18-21], spectrofluorimetry [22], high-performance liquid chromatography [23-25].

Granisetron Hydrochloride: Granisetron hydrochloride (as shown in **Fig. 4**) is an effective and potent antiemetic drug which is used in the treatment of vomiting and nausea resulting from cancer chemotherapy and radiotherapy in adults and children. Granisetron hydrochloride is also effective in the management of postoperative nausea and vomiting due to the anesthetics [26], Chemically, it is endo-N(9-methyl-9- azabicyclo [3.3.1] non- 3- yl)- 1- methyl- 1H- indazole- 3-carboxamide hydrochloride. GTH selectively blocks type 3 serotonin (5-HT3) receptors, is an antinauseant, antiemetic and specific selective serotonin 5-HT3 receptor antagonist **Fig. 4**. The molecular formula is $C_{18}H_{24}N_4O$•HCl and its molecular weight is 348.9.

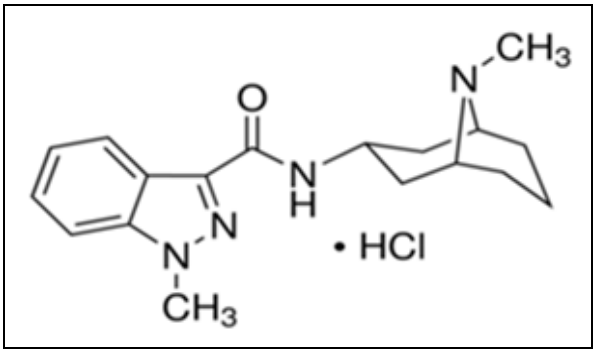

FIG. 4: CHEMICAL STRUCTURE OF GRANISETRON HYDROCHLORIDE

A few analytical methods *viz*; RP-HPLC [27, 28], HPTLC [29], and spectrophotometry [30, 31] have been developed for the determination of phenylephrine hydrochloride. The aim of this study is to develop simple, selective, accurate, precise and sensitive

stability-indicating UV method for the determination of Granisetron hydrochloride in bulk and in pharmaceutical dosage forms (tablets) suitable for routine quality control analysis.

Ibandronate Sodium: Ibandronate sodium (IBA) is one of the nitrogen carrying Bisphosphonate. According to IUPAC nomenclature it is 3-(N-methyl-N-pentyl) amino-1-hydroxypropane-1, 1-diphosphonic acid, sodium salt, monohydrate **Fig. 5**. It prevents osteoclast-conciliate boneresorption [32]. It is precious for the cure of hypercalcemia of malignancy [33], Paget's disease, postmenopausal osteoporosi and corticosteroid-induced osteoporosis metastatic bone disease [34].

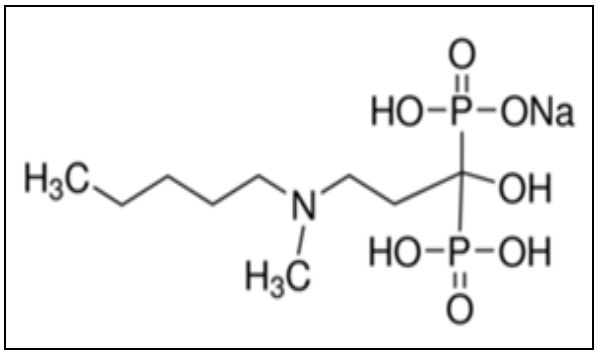

FIG. 5: CHEMICAL STRUCTURE OF IBANDRONATE SODIUM

Several methods have been reported for quantitative determination of IBD in UV-Vis spectrophotometry [35], Ion chromatography and ion pair chromatography [36-37], RP-HPLC method [38] for bulk drug.

MATERIALS:

Instrument: All absorbance measurements were recorded on Shimadzu 140 double beam spectrophotometer as well as on Thermo Nicolet 100 & Elico double beam SL210 UV- Visible spectrophotometers using matched pair of Quartz cells of 10mm path length.

Materials and Reagents: All the reagents used were of analytical reagent grade and distilled water was used throughout the investigation. 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 µg mL^{-1} NBS for use in the spectrophotometric method.

A stock solution of Methyl Orange (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 hydrochloric acid (S.D. Fine Chem., Mumbai, India; sp. gr. 1.18) was diluted appropriately with water to get 1 M acid.

The pharmaceutical grade drugs were supplied by Arabindo pharmaceuticals and hetero drugs Pvt. Ltd Hyderabad. A stock standard solution of drugs was prepared by dissolving accurately weighed 10 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 2-70 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 was followed by 1 mL of 1M HCl and contents were shaken well. After 30 minutes, 1 mL of methyl orange dye added to the content. Then contents were shaken well and diluted up to the mark. The absorbance of each solution was measured at 508 nm against the corresponding reagent blank.

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. 6**.

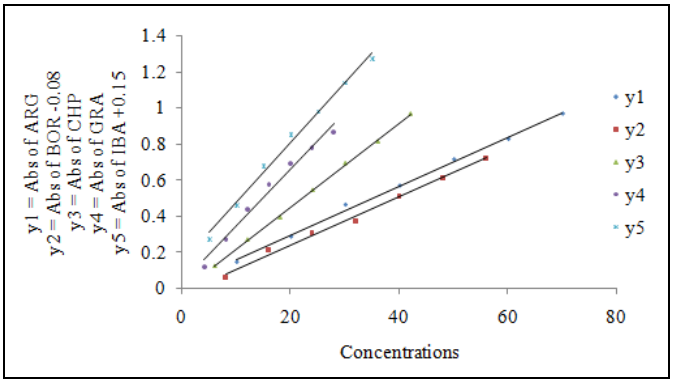

FIG. 6: CALIBRATION CURVES OF THE DRUGS

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 chosen and recovery are 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 1**.

TABLE 1: 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
ARG	10	9.98	0.02	99.92		99.89
	20	20.01	0.05	100.05	0.1094	± 0.109
	30	29.96	0.13	99.86		
	40	39.94	0.15	99.95		
BOR	8	7.98	0.25	99.75		99.93
	16	16.02	0.12	100.125	0.1732	±0.173
	24	23.96	0.16	99.83		
	32	32.01	0.03	100.03		
CHP	6	5.96	0.66	99.33		99.70
	12	11.98	0.16	99.83	0.285	±0.284
	18	18	0.00	100.00		
	24	23.92	0.33	99.66		
GRA	4	4.02	0.50	100.50		100.01
	8	7.98	0.25	99.75	0.380	±0.382
	12	11.96	0.33	99.66		
	16	16.02	0.12	100.12		
IBA	5	4.98	0.40	99.60		99.95
	10	10.01	0.10	100.10	0.284	±0.283
	15	14.98	0.13	99.86		
	20	20.05	0.25	100.25		

Procedure for Tablets:

Argatrobane Monohydrate:

Argatroban monohydrate injection is a sterile non-pyrogenic, clear, colorless, to a pale yellow isotonic solution. It is available as a single-use polyolefin bag containing 250 mg of argatroban in 250 ml sodium chloride solution (1 mg/ml). Convenient aliquots were taken from this for the determination of argatroban.

Bortezomib: For the analysis of pharmaceutical formulations ten tablets (Velcade, 3.5mg) were weighted, powered and equivalent to 10 mg of Bortezomib was transferred into 100 mL volumetric flask. 60.0 mL of distilled water was added and ultrasonicated for 20 min, then made up to the mark with distilled water. The resulting solution was mixed and filtered through Whatman filter paper no. 42. From the filtrate, the solution was diluted appropriately with distilled water in order to obtain the working concentration of drug used for the analysis.

Chloroquine Phosphate: Ten tablets of (Kamquine 150mg) were weighed accurately and powdered. The powder equivalent to 50 mg of CHP 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 mints and the solution was filtered using Whatman no. 41 filter paper and further diluted with water to obtain a working standard solution.

Granisetron Hydrochloride: About ten to fifteen tablets (Kytril, 2mg) were powdered and equivalent to about 10 mg of Granisetron hydrochloride had been taken into a 100 ml of volumetric flask and dissolved in 40 ml of distilled water by using 0.5M HCl. This solution was t filtered through Whatman filter paper no. 42. The residue was dissolved in 100 mL of distilled water. It was used as a stock sample solution. The aliquot portions of this stock solution were further diluted with distilled water to get the final concentration required for the determination of the drug.

Ibandronate Sodium: Two tablets (Bondria, 150 mg) were weighed accurately and grounded. A quantity equivalent to 20mg of Ibandronate Sodium was weighed and transferred into a 100ml

calibrated flask and the volume was finally diluted to the mark with water, mixed well and filtered using a Whatman no. 42 filter paper. It was used as a stock sample solution and was further diluted with water to get a working solution.

RESULTS AND DISCUSSION: Each method developed for quantification of drugs has been validated in terms of precision, accuracy, the limit of detection, the limit of quantification, linearity, selectivity, and ruggedness. Beer's law limits, slope, intercept, correlation coefficient, Sandell's sensitivity and regression equations for each drug are tabulated in **Table 2**. 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 is less than 2 for each drug demonstrates the accuracy and precision of the methods.

TABLE 2: ANALYTICAL AND REGRESSION PARAMETERS OF SPECTROPHOTOMETRIC METHOD

Parameter	ARG	BOR	CHP	GRA	IBA
λ_{max}, nm	510	510	510	510	510
Beer's law limits, µg mL^{-1}	10-70	8-56	6-42	4-28	5-35
Molar absorptivity, L mol^{-1} cm^{-1}	7.70×10^3	7.036×10^3	1.05×10^4	1.031×10^4	7.68×10^3
Sandell sensitivity, µg cm^{-2}	0.083	0.076	0.043	0.032	0.04
Limit of detection, µg mL^{-1}	2.278	0.699	1.954	0.7445	1.0704
Limit of quantification, µg mL^{-1}	6.90	2.118	5.922	2.2561	3.243
Regression equation, Y	=0.012x +0.073	=0.013x +0.049	=0.023x - 0.016	=0.031x +0.031	=0.026x +0.068
Intercept, (a)	0.073	0.049	-0.016	0.031	0.068
Slope, (b)	0.012	0.013	0.023	0.031	0.026
Correlation coefficient,(r)	0.982	0.993	0.999	0.984	0.944
Standard deviation of intercept, (Sa)	0.0082	0.0027	0.0136	0.0069	0.0081
Standard deviation of slope, (Sb)	0.0007	0.02899	0.0077	0.0021	0.000707
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Factors Effecting Absorbance:

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 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 µgmL^{-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 methyl orange dye 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 were 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 addition of dye.

Effect of Oxidant Concentration: When a study on the effect of NBS on color development was performed, it was observed that in both cases the absorbance increased with increase in the volume of NBS. It reached maximum when 1 mL of 70 µg mL^{-1} NBS solution was added to a total volume of 10 mL for drugs solutions. The color intensity decreased above the upper limits. Therefore, 1 mL of 70 µg mL^{-1} NBS was used for all measurements.

Effect of Dye Concentration: In order to ascertain the linear relationship between the volume of added NBS and the decrease in absorbance of Methyl Orange dye, experiments were performed using 1 mL of 1M HCl with varying volumes of NBS. The decrease in absorbance was found to be linear up to the 1 mL of NBS with the optimum volume of 1.0 mL of methyl orange dye for fixed concentration drug solution. The color was found to be stable up to 24 h.

Application to Formulations: The proposed methods were applied to the determination of drugs in tablets. The results in **Table 3** 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**.

TABLE 3: RESULTS OF ASSAY OF TABLETS BY THE PROPOSED METHODS AND STATISTICAL EVALUATION AND RECOVERY EXPERIMENTS BY STANDARD ADDITION METHOD

Drug	Taken (µg/mL)	Found (µg/mL)	Er (%)	Recovery (%)	RSD (%)	Proposed method Mean ± SD	Reference Method Mean ±SD
ARG	26	25.98	0.076	99.92		99.95	99.42
	36	35.96	0.111	99.88	0.06	± 0.059	±.7718
	56	56	0.00	100			
	76	76.01	0.013	100.013			
BOR	4	3.98	0.50	99.5		99.86	99.82
	12	12	0.00	100	0.25	±0.2501	±0.3
	20	20.01	0.05	100.05			
	24	23.98	0.08	99.62			
CHP	6	5.98	0.33	99.66		99.83	99.96
	8	8.01	0.125	100.125	0.2182	±0.21	±0.732
	12	11.96	0.33	99.66			
	16	15.98	0.125	99.875			
GRA	14	13.98	0.142	99.86		99.83	100.94
	20	20.01	0.05	100.05	0.082	±0.0821	±1.042
	32	32	0.00	100			
	42	41.98	0.04	99.96			
IBA	4	3.96	1	99		99.73	100.01
	8	8.01	0.125	100.25	0.508	±0.50	±0.763
	10	10	0	100			
	12	11.98	0.16	99.84			

TABLE 4: STUDENT'S T-TEST AND F-TEST VALUES FOR PHARMACEUTICAL ANALYSIS

Name of the Drug (Tablet)	Student's t-test	Variant F-test
ARG (ARGANO)	0.413	1.622
BOR (VELCADE)	0.449	0.590
CHP (KAMQUINE)	0.304	0.702
GRA (KYTRIL)	0.398	0.628
IBA (IMODIUM)	0.478	0.570

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, rapid, 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 that achieved by a sophisticated and expensive technique like HPLC. Thus, they can be used as alternatives for rapid and routine determination of bulk sample and tablets.

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CONFLICT OF INTEREST: Nil

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