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Pharmaceutical Analysis using N-Bromo Succinamide-Amarnth Dye couple : A Spectrophotometric Study.

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Abstract: Simple, specific, accurate and precise UV–visible spectrophotometric methods have been developed for the estimation of five drugs *viz.*,Amlodepine besylate, Esmolol hydrochloride, Olmesartan medoxomil, Phenyl ephrine hydrochloride and Tramadol hydrochloride. These methods involve the addition of a known excess of NBS to the drugs in acid medium followed by estimation of residual NBS by reacting with a fixed amount of Amaranth and measuring the absorbance of the dye at 520nm. The proposed methods were found to be successful for the estimation of these drugs in bulk and their formulations. The results of analysis have been validated statistically for linearity, accuracy, precision, LOD and LOQ.
Keywords: Amlodepine besylate, Esmolol HCl, Olmesartan medoxomil, Phenyl ephrine HCl and Tramadol HCl, NBS-Amaranth, UV-visible spectrophotometry and validation.

Introduction:

Amlodepine besylate (AML) is a calcium channel blocker. Chemically it is 3-ethyl-5-methyl (4RS)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-methyl-1-dihydropyridine-3,5-dicorboxylate benzene sulfonate (Fig.1a). AML is a calcium antagonist that inhibits the transmembrane influx of calcium ions into vascular smooth muscles, which inturn affects their contractile process and results in reduced blood pressure . It is used in the treatment of hypertension and angina. European pharmacopeia describes assay of Amlodidine besylate by reversed phase high performance liquid chromatography in bulk and pharmaceutical formulations[1]. AML has been studied and determined by relatively other methods such as spectrophotometric[2,3], voltammetry[4], HPLC[5,6], HPTLC[7] and capillary zone electrophoresis[8].

Esmolol hydrochloride (ESM), Methyl 3-{4-[2-hydroxy-3-(isopropylamino) propoxy]phenyl} propionate hydro chloride (Fig.1b), is an ultra-short acting adrenergic receptor antagonist used for the rapid control of heart rate, it is important to develop and validate analytical methods for its determination in pharmaceutical dosage form. Review of literature has revealed that few methods have been reported for the estimation of Esmolol hydrochloride. Most HPLC methods reported are useful in estimating ESM in human plasma[9-11] and biological fluids[12].Extractive spectrophotometric methods[13] and HPLC[14] methods for determination of ESM in pharmaceutical injections have been reported.

Olmesartan medoxomil (OLM) is chemically (5-methyl-2-oxo-2H-1,3-dioxol-4-yl) methyl 4-(2-hydroxy propan-2-yl)-2-propyl-1-({4-[2-(2H-1,2,3.4-tetrazol-5-yl)phenyl]phenyl}methyl)-1H-imidazole-5-Carboxylate (Fig.1c). OLM blocks the vasoconstrictor effects of angiotensin 2 by selectively blocking the binding of angiotensin 2 to the AT_1 receptor in vascular smooth muscle. Its action is, therefore, independent of the pathways for angiotensin 2 synthesis. Liquid chromatography[15,16], UV spectrophotometry[17] and Capillary electrophoresis[18,19] are reported in the literature. Combination methods are reported for determination of OLM with Ramipril[20] and HPTLC[21] method for Hydrochloro thiazide and OLM.

Phenyl ephrine hydrochloride (PHE),[(R)-1-(3-hydroxy phenyl) 2-(methylamino) ethanol hydrochloride] (Fig.1d), is a white crystalline powder and belongs to the group sympathomimetics. It acts in stimulating the alpha receptors in certain areas of the body. It is used locally, as decongestant, for non-specific and allergic conjunctivitis, sinusitis and nasopharyngitis. PHE nasal drops are used for treating symptoms such as runny nose, sneezing, itching of the nose and throat. Various methods have been reported for analysis of PHE *viz.*, spectrophotometry[22,23], High performance liquid chromatography[24], micellar liquid chromatography[25] and Capillary zone electrophoresis[26].

Tramadol hydrochloride (TRA) is chemically [2-(dimethylaminomethyl)-1-(3-methoxyphenyl) cyclo hexanol] (Fig.1e). TRA is a centrally acting analgesic, used for treating moderate to severe pain. It possesses agonist actions at the μ-opioid receptor and effects reuptake at the noradrenergic and serotonergic systems. TRA is used to treat moderate to moderately severe pain and most types of neuralgia, including trigeminal neuralgia. Literature survey reveals that, several spectrophotometric methods[27-29], TLC-densitometry[30],HPLC[31], Extractive spectrophotometry[32] and spectrofluorimetric method[33] were reported in the literature for the determination of Tramadol in pharmaceutical formulations.

A comparision of various techniques used for estimation of above drugs in terms of sensitivity and reproducibility are presented in Table-1.

Thorough survey of literature revealed that simple spectrophotometric methods are not yet reported for the above drugs. In this communication we present simple, accurate, precise methods for the quantification of above drugs.

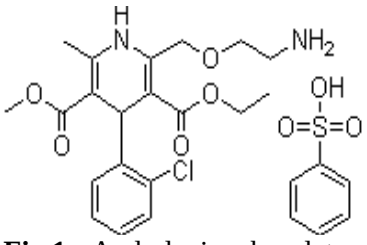


Fig.1a: Amlodepine besylate

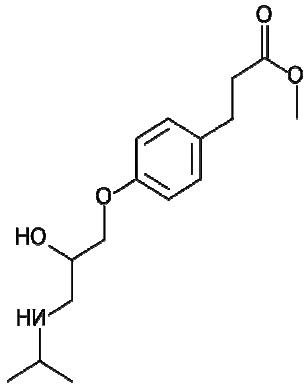


Fig.1b: Esmolol hydrochloride

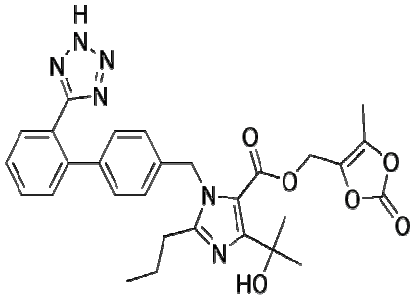
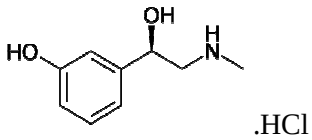
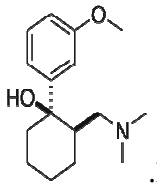


Fig.1c: olmesartan medoxomil



.HCl

Fig.1d: Phenyl ephrine hydrochloride



.HCl

Fig.1e: Tramadol hydrochloride

Table-1:Comparision of various techniques

Drug	Method	Sensitivity	Recovery
AMl	1)Spectrophotometry 2HPLC 3)HPLC-UV 4)Voltammetry 5)HPTLC	50-250 µg mL^{-1} 50-200 µg mL^{-1} 0.5-50 ng mL^{-1} 1.5-3.38 µg mL^{-1} 200-1000 µg mL^{-1}	99.07% 99.09% 97.6-98.53% 100.24%
ESM	1)RPHPLC 2)RPHPLC 3)Exractive spectrophotometry a)BTB b)BPB c)BCG	1-50 µg mL^{-1} 0.035-12 µg mL^{-1} 2.5-25 µg mL^{-1} 2.5-25 µg mL^{-1} 2.5-25 µg mL^{-1}	99.8% 94.8-95.5% 100.31% 99.58% 100.32%
OLM	1)UV-spectrophotometry 2)RP-HPLC 3)Capillary zone electrophoresis 4)HPLC	5-50 µg mL^{-1} 32-160 µg mL^{-1} 2.0-50 µg mL^{-1} 80-320 µg mL^{-1}	99.75-100.43% 99.42% 99.09%
PHE	1)RPHPLC 2)UV spectrophotometry 3)Flow-injection spectrophotometry	15-32 µg mL^{-1} 0-35 µg mL^{-1} 2.0-50 µg mL^{-1}	100.3% 99% 99.89%
TRA	1)Spectrophotometry 2)TLC-Densitometry	50-250 µg mL^{-1} 2.5-32.5 µg mL^{-1}	100.74% 101.8%

Methods and Materials:

The pharmaceutical grade drugs were supplied by Arabindo pharmaceuticals and Hetero drugs Pvt.Ltd, Hyderabad. Amaranth, HCl were purchased from S.d fine chem. Pvt. Ltd, Mumbai,India. N-bromo succinamide (NBS) is purchased from SRL chemicals, Mumbai, India. Whatman filter paper no.42 was used for filtration purpose. All the reagents used were of analytical-reagent grade and distilled water was used throughout the investigation. Tablets were purchased from the local market.

All absorbance measurements were recorded on Shimadzu 140 double beam spectrophotometer as well as on Thermo Nicolet 100 & Elico 159 UV-Visible single beam spectrophotometers using matched pair of Quartz cells of 10mm path length. A high precision Analytical balance was used for weighing the reagents.

Preparation of Standard stock solutions:

N-bromo succinamide (NBS): A stock solution of 0.01*M* was prepared by dissolving 0.1779 g of NBS in 100 ml distilled water. It is diluted appropriately to get 124µg mL^{-1}.

Amaranth [$0.8x10^{-3}M$]: A stock solution was prepared by dissolving 0.0484g of Amaranth in100 ml distilled water. From this stock solution,test solution containg 353µg mL^{-1} of Amaranth was prepared.

Drugs: A standard solutions of drugs were prepared by dissolving accurately weighed 30 mg of pure drug in water and diluted to the mark in 100 ml calibrated volumetric flasks.The stock solutions of AML, ESM, OLM, PHE and TRA were diluted with water to obtain 14µg

mL^{-1}, 7.5µg mL^{-1}, 36µg mL^{-1}, 16µg mL^{-1} and 9.0µg mL^{-1} respectively.

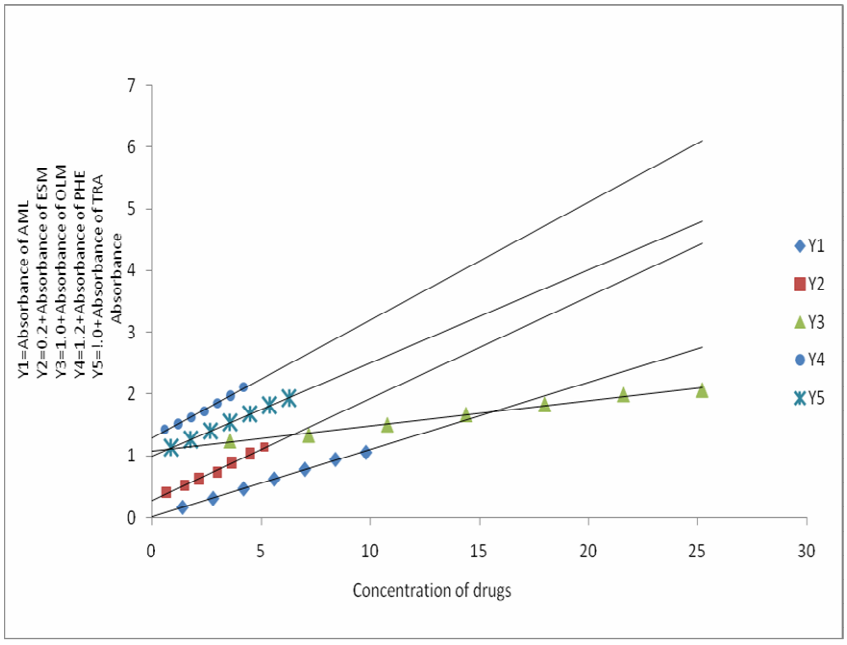
Hydrochloric acid (HCl): conc HCl is diluted appropriatelywith distilled water to get 1 *M* HCl solution.

Construction of calibration curve:

Aliquots of pure drug solution (1.0-7.0ml) were transferred into a series of 10ml calibrated flasks. To each flask 1ml of 1*M* HCl acid was added followed by 1.0ml of NBS solution. The flasks are stoppered and contents were mixed and the flasks are set aside for 15 min under occasional shaking. Finally, 1.0 ml of Amaranth solution was added to each flask and the volume was adjusted to the mark with water and mixed well. The absorbance of each solution was measured at 520 nm after 5 min.

A standard graph was prepared by plotting the absorbance versus the concentration of drugs.The concentration of the unknown were read from the calibration graph or computed from the regression equation derived using Beer's law data. Calibration curve for each drug was drawn in (Fig.2).

Fig.2: Calibration curve



Analysis of commercial Dosage forms:

A quantity of finely ground tablets powder equivalent to 10 mg of drug AML (Norvasc-10mg), OLM (Benicar-20mg), PHE (Dolgencorp-10mg)and TRA (Doltram-20mg,capsules) were accurately weighed and taken in 60 ml distilled water in 100ml volumetric flask and left for 10 min for complete dispersion and then filtered through Whatman No.42 filter paper. First 10 ml portion of the filtrate was rejected and a convenient aliquot of filtrate was further diluted for the analysis within the limits of Beer's law. Two ESM (Clol-50mg/10ml) injections were taken and combined, then diluted further to get required concentration.

Drug+known excess of NBS ⟶ Reaction product of the drug+Unreacted NBS

Unreacted NBS+ Fixed amount of Amaranth ⟶ Absorbance measured at 520nm.

Results and discussion:

N-bromo succinamide (NBS) has been used widely as a brominating and oxidizing agent for organic compounds. The proposed methods are indirect and are based on the oxidation and bromination reaction between drug and NBS and determination of residual NBS after allowing the reaction between drug and measured amount of NBS to be complete. The amount of NBS reacted corresponds to the drug content in all the methods.

The calibration curves for AML, ESM, OLM, PHE and TRA, over a concentration range of1.4-9.8 µg mL^{-1}, .75-5.25µg mL^{-1}, 3.6-25.2µg mL^{-1}, 0.8-4.82 µg mL^{-1}, 0.6-4.2µg mL^{-1} and 0.9-6.3µg mL^{-1} respectively, were plotted and molar absorptivity for drugs were calculated at the wavelength of 520nm. The regression characteristics were reported in Table-2.The result of assay is reported in Table-3.The accuracy of the proposed method was evaluated by percentage recovery studies of the drugs. The %RSD was less than 2%, showing high degree of precision of the proposed method. The results of the method lie within the prescribed limit, showing that method is free from interference from excipients.

Method development:

Many dyes are irreversibly destroyed to colorless species by oxidizing agents in acidic medium. The proposed spectroscopic methods are based on the reaction between the drug and measured excess of NBS and subsequent determination of the latter by reacting it with a fixed amount of amaranth and measuring the absorbance at 520 nm.These methods make use of the bleaching action of NBS on the dyes, the decolourization being caused by the oxidative destruction of the dyes. The drug when added in increasing concentrations to a fixed concentration of NBS consumes the later and there will be a concomitant decrease in the concentration of NBS. When a fixed concentration of amaranth dye is added to decreasing concentrations of NBS, a concomitant increase in the concentration of dye is obtained. Consequently, a proportional increase in the absorbance at the respective λmax is observed with increasing concentration of drug.

Preliminary experiments were performed to fix the upper concentrations of the dyes that could be determined spectrophotometrically in acidic medium, and these were found to be 35.3 µg/ml. A NBS concentration of 12.4 µg/ml was found to irreversibly destroy the red colour of 35.3 µg/ml amaranth in acid medium. Hydrochloric acid was found to be a convenient medium for these methods. However, since 1 *M* acid concentration was found optimum for the oxidation and bromination reaction in a reasonable time of 15min.The same concentration was maintained for the determination of unreacted NBS with dye, and even this reaction time is not critical. Any delay up to 30 min had no effect on the absorbance. A contact time of 5 min is necessary for the complete destruction of dye by the residual NBS.

Method validation:

The proposed methods were validated according to guidelines of international confrance on Harmonization (ICH). Under the described experimental conditions, standard experimental conditions, standard calibration curves for the studied drugs were constructed by plotting absorbance versus concentration. Conformity with Beer's law was evident in the concentration range cited in Table-2.The linear regression equations, molar

absorptivity, Sandell's sensitivity, limits of detection (LOD) and limits of quantification (LOQ) were listed in the same Table. Standard deviation, relative standard deviation, variance and standard error were calculated.

The accuracy of the method was established by analyzing the pure drug at three levels (within working limits) and the precision was ascertained by calculating the relative standard deviation of six replicate determinations on the same solution containing the drug at three levels in Table-3. The analytical results for accuracy and precision showed that the proposed methods have good repeatability and reproducibility.

The percentage recoveries of the pure drugs using the proposed methods compared with that given by reference methods are illustrated in Table-4. The validity of the proposed method in literature is evaluated by statastical analysis between the results obtained and that of reference methods. student's t-test and variance ratio F-test are chosen for the comparision of the results. Values are within the permissible range reported in literature.

Table-2: Analytical and regression parameters of spectrophotometric methods

Parameters	**AML**	**ESM**	**OLM**	**PHE**	**TRA**
λmax nm	520	520	520	520	520
Beer's law limit(µg mL^{-1})	1.4-9.8	0.75-5.25	3.6-25.2	0.6-4.2	0.9-6.3
Sandell sensivity*(µg/cm^2)	0.0092	0.0060	0.025	0.00546	0.0066
Limit of detection(µg mL^{-1})	0.55	1.34	0.61	1.69	0.088
Limit of quantification(µg mL^{-1})	1.66	4.085	1.85	5.13	0.266
Regression equation**					
Intercept(a)	0.018	0.067	0.0074	0.094	0.004
Slope(b)	0.108	0.164	0.040	0.183	0.150
Correlation coefficient(r)	0.998	0.995	0.991	0.994	0.999
Standard deviation of intercept(S_a)	0.038	0.060	0.018	0.019	0.020
Variance(S_a^2)	$1.444x10^{-3}$	$3.6x10^{-3}$	$0.324x10^{-3}$	$0.361x10^{-3}$	$0.4x10^{-3}$
Standard deviation of slope(S_b)	0.00316	0.00856	0.00336	0.00151	0.00650

*Limit of determination as the weight in µg per mL of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 cm^2 and path length of 1 cm. Y** = a+bX, where Y is the absorbance and X concentration of drugs in µg per ml.

Table 3:Determination of accuracy and precision of the methods on pure drug samples:

Drug	Taken(µg mL^{-1})	Found(µg mL^{-1})	% error	%Recovery	%RSD	Proposed method mean±S.D
AML	1.5 4.0 5.5	1.49 4.02 5.48	0.66 0.5 0.36	99.33 100.5 99.63	0.608	99.82±0.607
ESM	1.5 3.0 4.5	1.49 3.02 4.49	0.66 0.66 0.22	99.33 100.6 99.77	0.644	99.9±0.644
OLM	3.0 5.0 6.5	3.01 4.9 6.6	0.33 2.0 1.53	100.3 98 101.5	1.778	99.93±1.779
PHE	1.0 4.0 7.0	1.02 3.98 7.01	2.0 0.5 0.14	102 99.5 101	1.248	100.8±1.25
TRA	2.0 4.0 6.0	2.01 4.04 5.9	0.5 1.0 1.6	100.5 101 98.3	1.439	99.33±1.43

Table-4 :Results of assay of tablets by the proposed methods and statistical evaluation and recovery experiments by standard addition method.

Tablet	Drug taken ($\mu g\ mL^{-1}$)	Drug found ($\mu g\ mL^{-1}$)	Error (%)	Recovery (%)	RSD (%)	Reference Method mean±SD	Proposed Method mean±SD	t-test	F-test
Norvasc (AML)	2.1 3.5 4.9	2.11 3.49 4.91	0.476 0.285 0.204	100.4% 99.71% 100.2%	0.349	98.53±0.67	100.1±0.35	0.96	0.272
Clol (ESM)	1.0 2.0 3.0	1.004 2.004 2.997	0.4 0.2 0.1	100.4% 100.2% 99.9%	0.249	100.1±0.17	100.4±025	0.72	2.16
Benicar (OLM)	5.4 9.0 12.6	5.42 9.01 12.59	0.37 0.111 0.079	100.3% 100.1% 99.92%	0.189	99.7±0.619	100.1±0.19	1.17	0.094
Dolgen corp (PHE)	1.0 1.65 2.3	1.01 1.66 2.28	1 0.606 0.86	101% 100.6% 99.13%	0.978	96.03±.097	100.2±0.98	0.02	1.0
Dolotram (TRA)	1.35 2.25 3.15	1.348 2.252 3.14	0.148 0.088 0.31	99.85% 100.08% 99.68%	0.2	99.95±0.82	96.5±0.2	2.99	0.05

*Average of six replicate determinations.

Conclusion:

The obtained results from the method for the determination of mentioned drugs indicates that method is simple,accurate and precise. The method is economical compared to other sophisticated analytical instruments. Hence can be used for routine analysis of commercially available formulations. The method is suitable for the determination of these drugs in tablet formulation without interference from commonly used excipients.The solvents used for the method are inexpensive and simple to prepare, and could be used in a quality control laboratory for routine drug analysis.

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