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Simultaneous UV-spectrophotometric estimation of bromhexine hydrochloride and salbutamol sulphate by area under curve method in combined dosage form

Rajan V. Rele

Central Research Laboratory, D. G. Ruparel College, Matunga, Mumbai
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ABSTRACT

The objective of the study was to develop a simple, accurate, precise and rapid UV spectrophotometric, area under curve (AUC), method for simultaneous estimation of bromhexine hydrochloride and salbutamol sulphate from combined dosage form. The validation was carried out by using ICH guidelines for the determination of bromhexine hydrochloride and salbutamol sulphate by using 0.1N hydrochloric acid as the solvent in pharmaceutical dosage form. The proposed area under curve method involves the measurement of area at selected analytical wavelength ranges and performing the analysis using "Cramer's rule and Matrix method" .The two analytical wavelengths ranges were used i.e. 240-250 nm and 220-230 nm for estimation of bromhexine hydrochloride and salbutamol sulphate respectively. The linearity of the proposed method was found in the concentration range of 2- 14 µg /ml (r^2 = 0.9998) for bromhexine hydrochloride and salbutamol sulphate (r^2=0.9999) respectively. The percentage mean recovery was found to be 100.083% for bromhexine hydrochloride and 100.111 % for salbutamol sulphate respectively. The method was statistically validated for its linearity, accuracy and precision as per ICH guidelines. Both intra and inter day variation showed less percentage (%) RSD values indicating high grade of precision of this method.

Keywords: UV spectrophotometric estimation, area under curve method, bromhexine hydrochloride, salbutamol sulphate.
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INTRODUCTION

In this communication the present work proposes UV spectrophotometric method (area under curve method) for assay of bromhexine hydrochloride and salbutamol sulphate from combined pharmaceutical dosage form.

Bromhexine Hydrochloride is chemically named 2-amino-3,5- dibromo-N-cyclohexyl-N-methyl benzenemethan amine hydrochloride, is a mucolytic agent used in the treatment of respiratory disorders associated with viscid or excessive mucus . The drug is official in IP [1] and BP [2].

Salbutamol sulphate is, chemically known as bis [(1RS)-2-[(1, 1-Di-methyl-ethyl) amino]-1-[4-hydroxy- 3-(hydroxyl methyl) phenyl] ethanol] sulphate. It is beta-adrenoceptor agonist used for the relief of broncho-spasm in conditions such as asthma and chronic obstructive pulmonary disease. The drug is official in Indian pharmacopoeia [1]. Literature survey reveals HPLC [3], spectrophotometric [4,5] method for simultaneous determination of bromhexine hydrochloride and salbutamol sulphate in combined dosage form. Combination of bromhexine

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hydrochloride and salbutamol sulphate is used for the treatment of asthma and bronchitis. This simple method can also be used for the routine analysis of this combination formulation. In the proposed work development, optimization and validation of the method are presented.

MATERIALS AND METHODS

Instrument and reagents
Spectral scan was made on a Shimadzu UV-spectrophotometer, model 1800 (Shimadzu, Japan) with spectral band width of 0.5 nm with automatic wavelength corrections by using a pair of 10 mm quartz cells. All spectral measurements were done by using UV-Probe 2.42 software. Reference standards of bromhexine hydrochloride and Salbutamol sulphate were obtained from reputed firm with certificate analysis.

Preparation of standard drug solution
A 10 mg standard bromhexine hydrochloride was weighed accurately and transferred to a 10 ml volumetric flask and sonicated with 5 ml of ethanol for 15 minutes. The volume was made up to the mark with ethanol to give a stock solution of concentration 1000 μg /ml. From this solution, 1 ml of solution was pipetted out and transferred into 10 ml volumetric flask. The volume was made up to mark with ethanol to give a working standard solution of concentration 100 μg/ml.

A 10 mg standard Salbutamol sulphate was weighed accurately and transferred to a 10 ml volumetric flask and sonicated with 5 ml of ethanol for 15 minutes. The volume was made up to the mark with ethanol to give a stock solution of concentration 1000 μg /ml. From this solution, 1 ml of solution was pipetted out and transferred into 10 ml volumetric flask. The volume was made up to mark with ethanol to give a working standard solution of concentration 100 μg/ml.

Preparation of sample solution
Twenty tablets were weighed accurately and average weight of each tablet was determined. A powder equivalent to 8 mg of standard bromhexine hydrochloride and 2 mg of salbutamol sulphate were weighted accurately and transferred in 10 ml volumetric flask. To this flask 5 ml of ethanol was added and sonicated for 10 minutes. Such solution is diluted with ethanol to mark to give concentration as 800 μg /ml of bromhexine hydrochloride and 200 μg /ml. of salbutamol sulphate respectively. The working standard solution was prepared by diluting 1 ml of 800 μg /ml of bromhexine hydrochloride and 200 μg /ml of salbutamol sulphate solution to 10 ml with diluent to get concentration 80 μg /ml of bromhexine hydrochloride and 20 μg /ml. of salbutamol sulphate respectively. Such solution was used for further analysis.

EXPERIMENTAL
Method: Area under curve method
Area under curve method involves the calculation of integrated values of absorbance with respect to the wavelength between two selected wavelengths such as λ_1 and λ_2. The area under curve between λ_1 and λ_2 was calculated by UV probe 2.42 software.

(a) For bromhexine hydrochloride
For the selection of analytical wavelength range, 100 μg/ml solution of bromhexine hydrochloride was scanned in the spectrum mode from 400 nm to 200 nm by using 0.1N hydrochloric acid as blank. On examination of the spectra, 240-250 nm wavelength range was selected as working wavelength range for bromhexine hydrochloride.

(b) For Salbutamol sulphate
For the selection of analytical wavelength range, 100 μg/ml solution of Salbutamol sulphate was scanned in the spectrum mode from 400 nm to 200 nm by using 0.1N hydrochloric acid as blank. On examination of the spectra, 220-230 nm wavelength range was selected as working wavelength range for Salbutamol sulphate.

Preparation of calibration curves
Series of solutions containing 1- 14 µg/ ml of bromhexine hydrochloride and 1-16 µg/ ml Salbutamol sulphate µg/ ml were used to determine linearity of the proposed method respectively. Area under curve of above solutions of bromhexine hydrochloride and Salbutamol sulphate were measured at their respective selected analytical

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wavelength ranges. [Fig. 1(a), 1(b)]. This area under curve (AUC) was then divide by concentration in g/lit to get X_{amox} for bromhexine hydrochloride and X_{carbo} for Salbutamol sulphate.

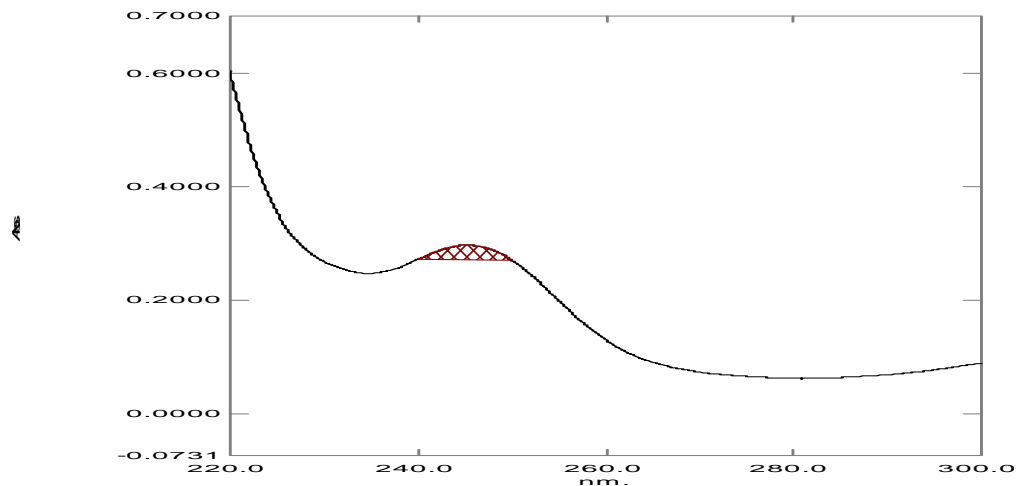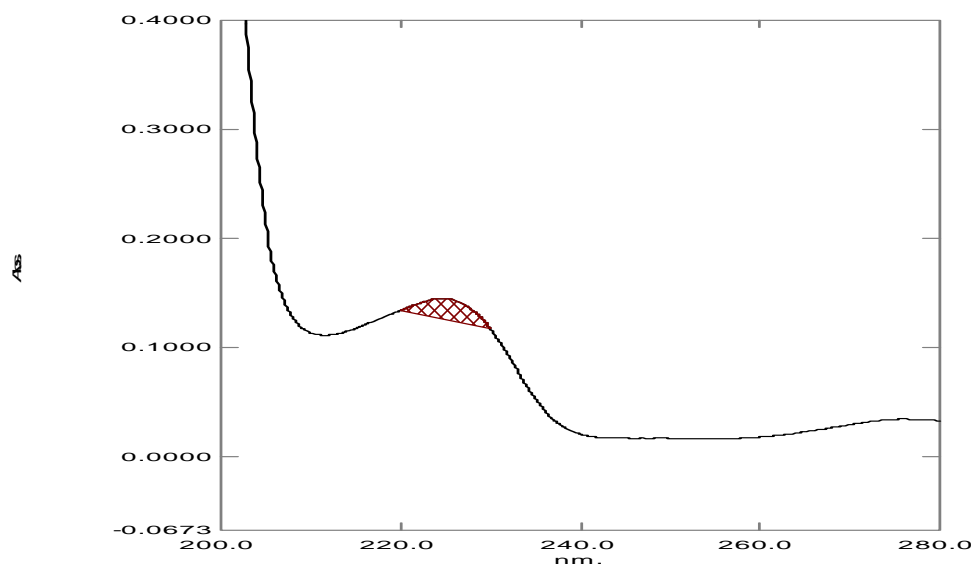**Fig. 1(a): Spectrum showing area under curve of bromhexine hydrochloride in the concentration of 10 µg/ ml at 240-250 nm**

Fig. 1(b): Spectrum showing area under curve of Salbutamol sulphate in the concentration of 40 µg/ ml at 220-230 nm

After measuring the area under curve of bromhexine hydrochloride at 240-250 nm and 220-230 nm for Salbutamol sulphate by using UV-Probe software 2.42, the calibration curves were plotted of area under curve against concentrations [Fig. 2 (a), 2(b)].

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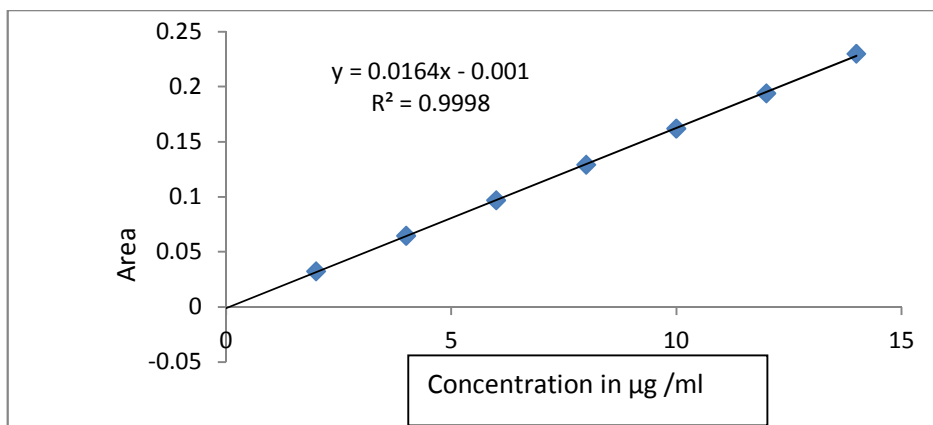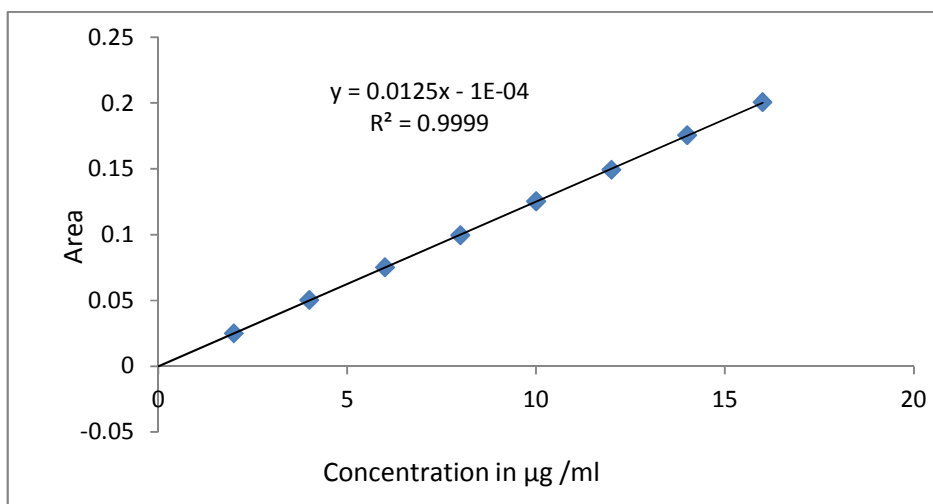**Fig.2 (a): Calibration curve of bromhexine hydrochloride in the concentration range of 2-14 µg/ml**

Graph with equation $y = 0.0164x - 0.001$, $R^2 = 0.9998$. Y-axis: Area. X-axis: Concentration in µg /ml.

Fig.2 (b): Calibration curve of Salbutamol sulphate in the concentration range of 2-16 µg/ml

Graph with equation $y = 0.0125x - 1E-04$, $R^2 = 0.9999$. Y-axis: Area. X-axis: Concentration in µg /ml.

Results of the analysis are given in table 1

**Table 1: Values of results of optical and regression of drugs**

Parameter	Bromhexine hydrochloride	Salbutamol sulphate
Detection Wavelength range (nm)	240-250	220-230
Beer Law Limits (µg/ml)	1-14	1-16
Correlation coefficient(r^2)	0.9998	0.9999
Regression equation (y=b+ac)		
Slope (a)	0.0164	0.0125
Intercept (b)	-0.001	-0.0001

Estimation from tablets

Twenty tablets were weighed accurately and average weight of each tablet was determined. A powder equivalent to 8 mg of standard bromhexine hydrochloride and 2 mg of salbutamol sulphate were weighted accurately and transferred in 10 ml volumetric flask. To this flask 5 ml of ethanol was added and sonicated for 10 minutes. Such solution is diluted with ethanol to mark to give concentration as 800 µg /ml of bromhexine hydrochloride and 200 µg /ml. of salbutamol sulphate respectively. The working standard solution was prepared by diluting 1 ml of 800 µg /ml of bromhexine hydrochloride and 200 µg /ml of salbutamol sulphate solution to 10 ml with diluent to get concentration 80 µg /ml of bromhexine hydrochloride and 20 µg /ml. of salbutamol sulphate respectively.   Such solution was used for further analysis.

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The spectrum of sample solution containing bromhexine hydrochloride and Salbutamol sulphate was recorded and areas under curves were recorded in wavelength ranges of 240-250 and 220-230 nm. The areas under curves were analyzed by applying "Crammer's rule and "Matrix method". It is defines as "The total area under curve of mixture at particular wavelength range is equal to sum of area under curve of individual component at same wavelength range." (Fig. 3).

Fig.3: Spectrum showing area under curve of mixture at 220-230 nm and 240-250 nm
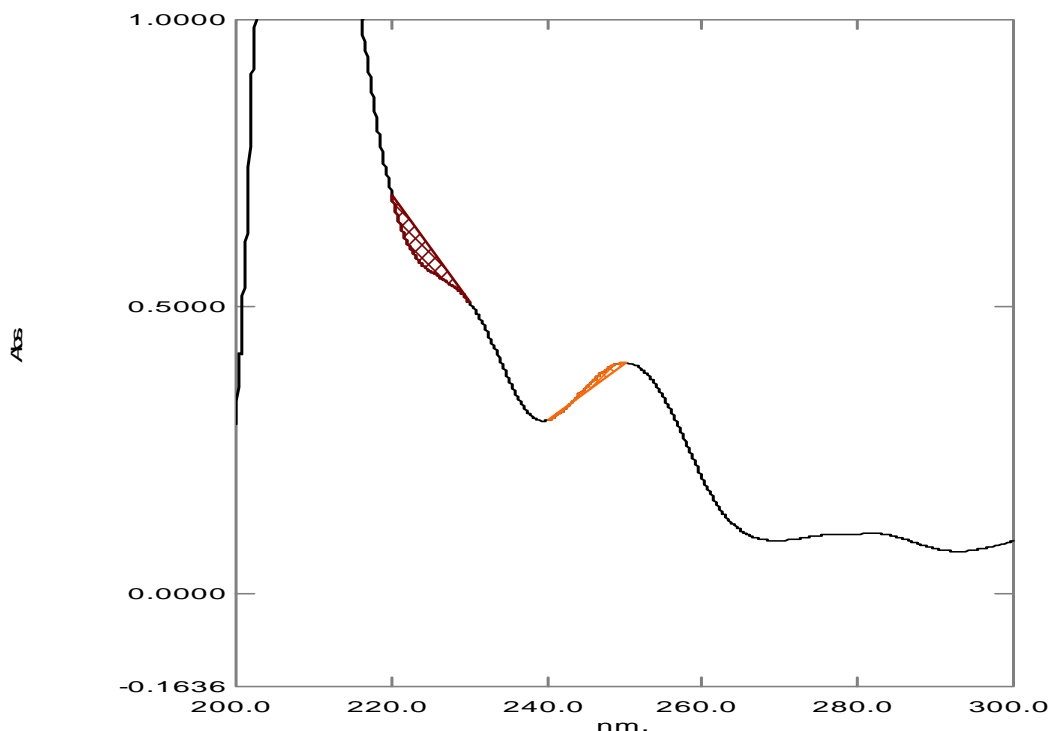
$X = \dfrac{\text{AUC of component between selected wavelength ranges}}{\text{Concentration of that component in mg/lit}}$

$$C_{Brom} = \frac{(X^{Sal2}.AUC^{M220\text{-}230}) - (X^{Sal1}.AUC^{M240\text{-}250})}{(X^{Sal1}.X^{Brom2}) - (X^{Sal2}.X^{Brom1})}$$

$$C_{Sal} = \frac{(X^{Brom1}.AUC^{M240\text{-}250}) - (X^{Brom2}.AUC^{M240\text{-}250})}{(X^{Sal1}.X^{Brom2}) - (X^{Sal2}.X^{Brom1})}$$

Where,

C_{Brom} = Concentration of Bromhexine hydrochloride
C_{Sal} = Concentration of Salbutamol sulphate
X^{Brom1} = Area under curve of Bromhexine hydrochloride at wavelength 220-230 nm
X^{Brom2} = Area under curve of Bromhexine hydrochloride at wavelength 240-250 nm
X^{Sal1} = Area under curve of Salbutamol sulphate at wavelength 220-230 nm
X^{Sal2} = Area under curve of Salbutamol sulphate at wavelength 240-250 nm
AUC^{M} = Area under curve of mixture

Method Validation
These methods were validated according to ICH guidelines.

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Accuracy

To ascertain the accuracy of proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%). Percent recovery for bromhexine hydrochloride was found in the range of 99.98% to 100.05% and Salbutamol sulphate was found in the range of 99.99% to 100.33%. (Table 2).

Table 2: Statistical evaluation of the data subjected to accuracy

Level of % recovery	Amount present in µg/ml		Amount added in µg/ml		Amount found in µg/ml		% Recovery		Mean % recovery	
	Sal	Brom	Sal	Brom	Sal	Brom	Sal	Brom	Sal	Brom
80%	2.0	8.0	1.6	6.4	3.601	14.404	100.01	100.03		
	2.0	8.0	1.6	6.4	3.596	14.423	99.89	100.08	99.98	100.03
	2.0	8.0	1.6	6.4	3.601	14.397	100.04	99.98		
100%	2.0	8.0	2.0	8.0	4.007	16.012	100.18	100.08		
	2.0	8.0	2.0	8.0	4.021	16.054	100.54	100.34	100.05	99.99
	2.0	8.0	2.0	8.0	3.978	15.912	99.45	99.56		
120%	2.0	8.0	2.4	9.6	4.403	17.726	100.07	100.72		
	2.0	8.0	2.4	9.6	4.419	17.663	100.45	100.36	100.07	100.33
	2.0	8.0	2.4	9.6	4.386	17.585	99.69	99.92		
Confidence Interval							100.04±0.22	100.12±0.21		

Brom = Bromhexine hydrochloride,   Sal = Salbutamol sulphate

Linearity

The linearity of measurement was evaluated by analyzing different concentration of the standard solutions of bromhexine hydrochloride and salbutamol sulphate. For both the drugs concentration range was found to be 1- 14 µg/ml for bromhexine hydrochloride and 1-16 µg/ml for Salbutamol sulphate respectively.

Precision

The method precision was established by carrying out the analysis of tablets powder blend containing 8 mg of bromhexine hydrochloride and 2 mg of Salbutamol sulphate. The assay was carried out for the drugs by using proposed analytical method in six replicates. The values of relative standard deviation were 0.1754 for bromhexine hydrochloride and 0.2317 for salbutamol sulphate respectively indicating the sample repeatability of the method. The results obtained are tabulated in table 3.

Table 3: Statistical evaluation of the data subjected to method of precision

Sr. No.	Sample No.	% Assay	
		Salbutamol sulphate	Bromhexine hydrochloride
1	1	100.08	100.18
2	2	100.14	99.78
3	3	100.47	100.12
4	4	99.87	100.27
5	5	100.12	100.09
6	6	99.82	100.23
Mean % assay		100.083	100.111
%R.S.D.		0.2317	0.1754

Intra-day precision was estimated by assaying powder blend of capsules containing 8 mg of bromhexine hydrochloride and 2 mg of salbutamol sulphate. The assay was carried out for the drugs by using proposed analytical method in six replicates. The results were average for statistical evaluation.

Inter-day precision was estimated by assaying powder blend containing 8 mg of bromhexine hydrochloride and 2 mg of Salbutamol sulphate for three consecutive days (i.e. 1st, 3rd and 5th days). The statistical validation data for intra and inter day precision is summarized in table 4.

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<p align="center">**Table 4: Summary of validation parameter for intra-day and inter-day**</p>

Sr. No.	Parameters	Bromhexine hydrochloride	Salbutamol sulphate
1	Intra-day precision (N=3)amount found ± % R.S.D.	100.08% 0.1917	100.11% 0.1163
2	Inter-day precision (N=3)amount found ± % R.S.D.	98.45% 0.1714	98.63% 0.1855

Both intra- day and inter-day precision variation found to be less in % RSD values. It indicates high degree of precision of the method.

<p align="center">**RESULTS AND DISCUSSION**</p>

The developed area under curve spectrophotometric method for simultaneous determination of bromhexine hydrochloride and Salbutamol sulphate in pharmaceutical formulation was found to be simple and convenient for the routine analysis of two drugs. The proposed method is accurate, precise and reproducible. It is confirmed from validation data as given in tables 1 to 4. The % RSD was found to be less than 1, which indicates validity of method. Linearity was observed by linear regression equation method for bromhexine hydrochloride and Salbutamol sulphate in different concentration range. The correlation coefficient of these drugs was found to be close to 1.00, indicating good linearity figure 2 (a) and 2 (b).

The assay results obtained by proposed method is shown in table 2 are in good agreement. Hence proposed method can be used for routine analysis of these two drugs in combined dosage form. Method is simple, accurate, precise, reliable, rapid, sensitive, reproducible and economical. It is validate as per ICH guidelines.

<p align="center">**CONCLUSION**</p>

The proposed method is simple, precise, accurate and rapid for the determination of bromhexine hydrochloride and Salbutamol sulphate in combined dosage form. This method can be adopted as an alternative to the existing methods. It can be easily and conveniently adopted for routine quality control analysis.

<p align="center">**REFERENCES**</p>

[1]Indian Pharmacopoeia, Controller of Publication, Delhi, **2010** volume I, II, III.2224.
[2] British Pharmacopoeia, Her Majesty's Stationary Office, London, **2010**,Volume I, II, and III.
[3] P. N. S Pai, G. K. Rao, M. S. Murthy, A. Agarwal, S. Puranik, *Indian J. Pharm. Sci*., **2009**, 71 (1), 53-55
[4] K. Susmitha, M. Thirumalachary, and G. Venkateshwarlu, *ISRN Analytical Chemistry,* **2013**,1-7.
[5] G.Vijaya Raja, G. Venu gopal, V. Mounika, S.Satyavathi, Ch.Lavanya, *International Journal of Pharma Sciences and Research*, **2010**, 1 (2), 90-94