Research Article

Spectrophotometric Quantitative Determination of Bromhexine Hydrochloride in Bulk and Pharmaceutical Dosage Form using p-nitrobenzaldehyde Reagent.

K. Siddappa*, Prashant C. Hanamshetty
Department of Studies and Research in Chemistry, Gulbarga University, Gulbarga, Karnataka, India.
***Corresponding author's E-mail:** siddappa_65@rediffmail.com

Accepted on: 20-06-2016; **Finalized on:** 31-07-2016.

ABSTRACT

An efficient, sensitive spectrophotometric method has been developed and validated for the determination of Bromhexine hydrochloride (BRH), in bulk drug and its pharmaceutical formulations. The method is based on the formation of Schiff base with p-nitrobenzaldehyde (PNBZ) the reaction of drug with reagent gives a bright yellow colour. The so formed coloured species absorbance was measured at its absorption maximum (λ_{max}) 410 nm. The Beer's law has been obeyed in the concentration range 5-25 µg/ml. The optical parameters were calculated as 2.01561×10^4 (L/mol/cm), 0.01866 (µg/cm^2), molar absorptivity and Sandell sensitivity respectively. The LOD and LOQ of the proposed method were calculated 0.1851 (µg/ml), 0.6165 (µg/ml) respectively. All the variables were studied to optimize the reaction conditions. No interference was observed in the presence of common pharmaceutical excipients. The validity of the method was tested by analyzing BRH in its pharmaceutical formulations and critically tested for its accuracy by statistical tests. Good recoveries were obtained by the developed method; the obtained results were critically analyzed and successfully employed for the determination of BRH in its pharmaceutical dosage forms.

Keywords: Bromhexine, PNBZ, Spectrophotometry, Mucolytic agent, Schiff base, Validation.

INTRODUCTION

Bromhexine HCl (BRH), chemically named 2-amino-3,5-dibromo-N-cyclohexyl-N-methyl benzenemethanamine hydrochloride Fig. 1, is a mucolytic agent used in the treatment of respiratory disorders associated with viscid or excessive mucus. This agent's mechanism is to increase the production of serous mucus in the respiratory tract and it makes the phlegm thinner and less viscous. BRH is a mucous modifying drug that helps to improve the flow properties of bronchial mucous and eases expectoration.[1,2]

The drug is official in IP and BP.[3,4] Because of its physiological importance, the drug has been quantified by exploiting its chemical[5,6] and physical properties. The different analytical methods used to quantify the drug as a single active pharmaceutical ingredient include flow injection analysis with ionselective electrodes,[7] inductively coupled plasma mass spectrometry,[8] electrokinetic chromatography,[9] electrochemical oxidation at the glassy carbon electrode,[10] liquidchromatography,[11] liquid gas chromatography,[12] GC with mass detection,[13] and voltammetry.[14] The drug has also been quantified in its combined formulations using HPLC,[15-18] direct and derivative UV spectrophotometry.[19-23] These methods involve scarcely available costly equipment and tedious experimentation.

Simple, accurate and precise methods using spectrophotometry have also been developed based on the production of chromophore by the interaction of the drug with an analytical reagent, as chromogen.[24-26] Availability of vast number of analytical reagents to serve as chromogens always leaves a lot of scope for pharmaceutical analysis. A thorough survey of the literature showed that the quantification methods of our interest are not reported yet and hence in the present communication we report a quantification method that has been developed and validated for quantification of Bromhexine HCl both in pure and pharmaceutical dosage forms. In the present research work an effort has been made to develop and validate a simple spectrophotometric determination of BRH in bulk and pharmaceutical formulations. Although there are several highly sophisticated instrumental methods were reported but are suffered by time of analysis, cost per analysis, sophistication and most importantly the skilled analyst to handle the instruments. The present method offers a simple, sensitive, cost effective method for the determination of BRH in any common QC laboratory.

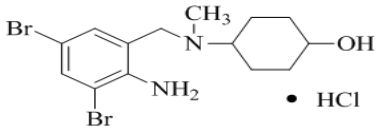


Figure 1: Chemical Structure of Bromhexine Hydrochloride.

MATERIALS AND METHODS

Apparatus

Spectral and absorbance measurements were carried out by using double beam UV-Spectrophotometer ELICO-SL-244.

Materials and Reagents

All the chemicals used were of analytical grade. All the solutions were prepared freshly and deionised water is

used throughout the experiment. Bromhexine hydrochloride bulk drug obtained from Sun Pharmaceutical (Mumbai, India) certified to contain 99.5% of active ingredient, which has been used as a reference substance, as received without further purifications. p-nitrobenzaldehyde procured from Sd-Fine chemicals 99.5% purity and HCl procured from Sd-Fine chemicals 35% purity. Methanol AR grade procured from Sd-Fine chemicals.

Bromhexine hydrochloride tablets were purchased from pharmaceutical store, different makes such as Ascoril® (8 mg BRH) and TusQ® (8 mg BRH) from Glenmark (India) laboratory Ltd. and Blue Cross Laboratory Ltd. respectively.

Preparation of Standard Stock Solution

Weighed accurately near (0.001g) 10 mg of the reference standard in a 10 ml volumetric flask; added 5 ml deionised water swirled to mix and brought to the mark with deionised water. The apparent concentration has been reached to 1000 µg/ml. further stepwise dilutions were made to obtain the working standard stock solution 100 µg/ml.

Preparation of Standard Stock Solution

Accurately weighed 2 g of p-nitrobenzaldehyde in a 100 ml volumetric flask, added 50 ml of methanol swirled to mix then the solution made up to the mark with methanol.

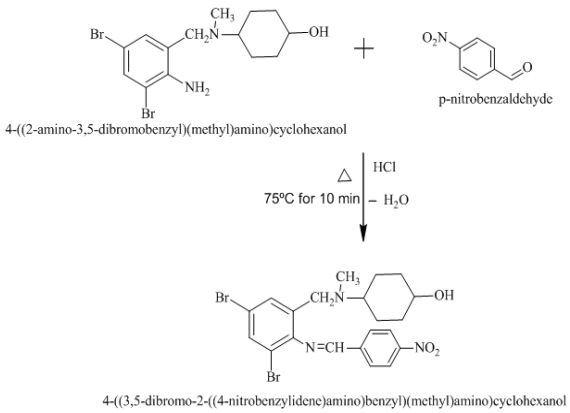
Preparation of Sample Solution

1. Ascoril® tablets (15 tablets) labelled claim to contain 8 mg of BRH, average weight of each tablet was 420 mg were triturated and made a fine powder, mixed it well for homogeneity. A portion (5250 mg) of the fine powder was transferred to contain 100 mg of the BRH in to a beaker and dissolved with 20 ml of deionised water and mixed well. This solution was filtered through a Whatmann filter paper No.41, in to a 100 ml volumetric flask. The filtrate was made up to the mark with deionised water.
2. TusQ® tablets (15 tablets) labelled claim to contain 8 mg of BRH, average weight of each tablet was 550 mg were triturated and made a fine powder, mixed it well for homogeneity. A portion (6875 mg) of the fine powder was transferred to contain 100 mg of the BRH in to a beaker and dissolved with 20 ml of deionised water and mixed well. This solution was filtered through a Whatmann filter paper No.41, in to a 100 ml volumetric flask. The filtrate was made up to the mark with deionised water.

General Procedure for the Determination of Bromhexine Hydrochloride

Variable aliquots of working standard solution containing 5-25 µg/ml of BRH were transferred in to series 10 ml volumetric flasks. To each flask 1 ml of concentrated HCl was added, mixed the solution mechanically followed by added 2 ml of 2% PNBZ solution, heated the contents on water bath at 70 ºC for 5 min then there was the formation of yellow coloured Schiff base. The yellow coloured species absorbance was measured at 410 nm using reagent as a blank. The formation of Schiff base was shown in Scheme 1. The calibration graph was prepared by plotting absorbance versus concentration of drug and the concentration of unknown was read from the calibration graph or computed from the regression equation derived from the Beer's law data.

The same procedure was followed for the determination of BRH in the tablet formulations and the content of the tablets were calculated by using regression equation.



Scheme 1

RESULT AND DISCUSSION

Determination of Absorption Maxima (λ_{max})

To determine the λ_{max} of the colored species, 1 ml of 100 µg/ml of the BRH was added to a 10 ml volumetric flask and 1 ml of concentrated HCl, mixed the contents mechanically then added 2 ml of 2% PNBZ solution, heated the contents on water bath at 70 °C for 5 min then there was the formation of yellow coloured Schiff base. The flasks are allowed to cool to room temperature and the solutions made up to the mark with water. The coloured species was measured against reagent blank in the range of 350 nm to 900 nm. The λ_{max} of the complex was found to be 410 nm. Absorption spectrum of the proposed method was shown in Fig.2. Under the experimental conditions each reagent blank showed a negligible absorbance at the corresponding λ_{max}.

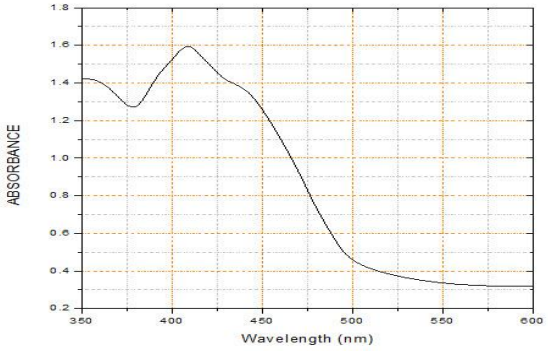


Figure 2: Absorption Spectrum of the Coloured Species

Investigation of Assay Parameters

Optimum reagent concentrations required for the formation of sensitive and quantitative coloured products were determined by varying one reagent concentration and fixing the concentrations of other reagents and its effect on absorbance was measured at 410 nm.

Effect of Heating Time

To study the effect of heating time for the development of maximum colour, the contents of the mixture were heated for up to 20 min. at 70±1°C. The intensity of the colour developed was measured at room temperature after the dilution to 10 ml with deionised water. It is apparent from the investigation that the maximum intensity of colour was obtained after 10 min of heating and remained constant. Therefore the optimum heating time was fixed to 10 min.

Effect of Reagent Concentration

The effect of concentration of PNBZ solution and HCl were studied on the related absorbance values. Different concentrations of PNBZ solutions from 0.5% to 5% were studied. Volumes of 0.5–3.0 ml of PNBZ (2%) and 0.5–3.0 ml of concentrated HCl were examined. The investigations showed that 2.0 ml of PNBZ and 1.0 ml of concentrated HCl gave maximum absorbance. There is no change in intensity of the colour any further with the increasing amounts of PNBZ and concentrated HCl. So the 2.0 ml of PNBZ and 1.0 ml of concentrated HCl were chosen throughout the experiment.

Interference Studies

To study the potential interference from the commonly used excipients and other additives such as glucose, lactose, starch, sodium starch glycolate, cellulose, magnesium stearate and ascorbic acid recovery studies were carried out. Under the experimental conditions employed, to a known amount of drug. The recovery studies suggest that there was no significant interference from the excipients on the assay of the drug.

Validation of the Method

Detection and Quantification Limits

According to the Analytical Methods Committee the detection limit (LOD) is the concentration of drug corresponding to a signal equal to the blank mean (Y_B) plus three times the standard deviation of the blank (S_B). Quantification limit (LOQ) is the concentration of drug corresponds to the blank mean plus ten times the standard deviation of the blank. The LOD and LOQ values for BRH were found to be 0.1851 µg/ml and 0.6165 µg/ml respectively.[27-30]

Quantification

The optical characteristics such as Beer's law limits, Sandell sensitivity and molar absorptivity were calculated for the proposed method and the results are summarized in Table 1. Regression analysis of the Beer's law plot at their λ_{max} revealed a good correlation as shown in Fig 3. For the regression analysis we have selected different concentration sets but the best fit curve was obtained in the concentration range 5 to 25 µg/ml of standard BRH. For the verification of Beer's law we have taken a series of 10 ml volumetric flasks and added the working standard solution (100 µg/ml) serially from 0.5 ml to 2.5 ml followed by added all the reagents as mentioned in the assay procedure. Graph of absorbance versus concentration plotted and are described by the regression equation Y= bx+a (where 'Y' is the absorbance, 'b' is the slope, 'x' is the concentration of the drug in µg/ml and 'a' is the intercept) obtained by least squares method. The results were summarized in Table 1.

Table 1: Optical and Regression characteristics, Precision and Accuracy of the Proposed Method

S. No.	Parameters	Value
1	λ_{max} (nm)	410
2	Beer's law limit (µg/ml)	5-25
3	Sandell sensitivity (µg/cm^2/0.001 abs. unit)	0.01866
4	Molar absorptivity (L mole^{-1}.cm^{-1})	2.01561x10^4
5	Stability of Color (hours)	5
6	Regression equation	y = 0.0535x + 0.00127
7	Correlation coefficient	0.999
8	% RSD	0.314
9	% Range of errors 0.05 % 0.01%	 0.911±0.0035 0.911±0.0027
10	Limit of detection (µg/ml)	0.1851
11	Limit of quantification (µg/ml)	0.6165

Table 2: Evaluation of the Accuracy and Precision of the Proposed Method by Intra-day Assay

Concentration of BRH (µg/ml)	Observed Concentration of BRH (µg/ml)			
	Intra-day			
	Mean*	Error (%)	RSD (%)	Recovery (%)
14	14.002	0.163	0.485	100.0
20	20.013	0.181	0.378	100.1
25	24.933	0.392	0.658	99.73

*For five determinations

Table 3: Determination of BRH in Pharmaceutical Formulation by Standard Addition Technique

Amount of Drug before Addition (µg)	Amount of Drug Added (µg)	Theoretical Amount (µg)	Mean Amount Recovered (µg) (n=5)	Mean % of Recovery (n=5)	RSD%
5	5	10	9.994	100	0.978
5	8	13	13.07	100.5	1.56
5	11	16	15.99	100	0.363

Table 4: Results of Analysis of Tablet Formulation containing BRH

Formulation	% Found ± SD				
	Labeled Amount (mg)	Reference Method[a]	Proposed Method*	%Recovery of Proposed Method	t-test**
Ascoril®	8	7.984	8.001±0.051	99.85±0.60	0.074
TusQ®	8		8.020±0.024	99.78±0.57	2.299

*Recovery amount was the average of five determinations

**The t-value from table is 2.776 at 95% level; 'a' is the contents of BRH in reference method.

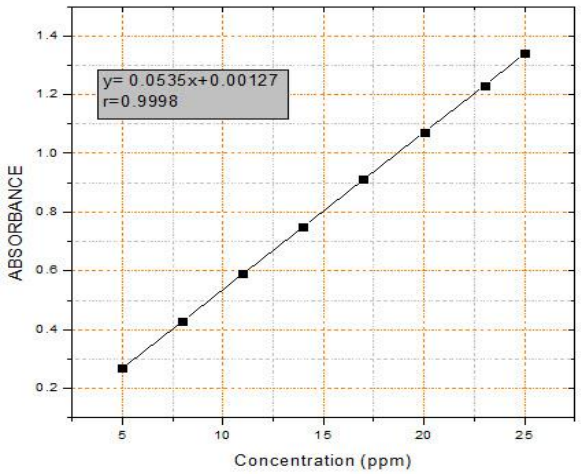


Figure 3: Beer's Law Calibration Curve

Accuracy Precision and Recovery Studies

The accuracy and precision of the proposed method was evaluated by performing five replicate determination of BRH in pure form at three different concentrations (14, 20 and 25 µg/ml) by short term (intra-day) precisions as shown in Table 2. The standard analytical errors, relative standard deviations (%RSD) and recoveries obtained in the intra-day analysis for the proposed method were found to be acceptable. Thus the proposed method is effective for the determination of BRH.

The accuracy of the proposed method was further checked by performing recovery experiments through standard addition technique.

For this purpose, a known amount of pure BRH was added to pre-analyzed dosage forms and then determined by the recommended procedure.

The results are as shown in Table 3. The values of mean recovery and relative standard deviation (%RSD) were in the range of 100-100.5 % and 0.363-1.56 % respectively. This indicates the reproducibility of the method. No interference was observed from the common excipients of tablet.

Applicability of the Method

The proposed method applied to the analysis of BRH in pharmaceutical dosage forms and the results were statistically compared with reference method by calculating the student's t -values. The evaluated t- values were less than the tabulated values at the 95% confidence level for five degrees of freedom, as revealed by the results complied in Table 4. This actually suggests that the proposed method is accurate and precise as the reference method.

CONCLUSION

The proposed method was quite simple and do not require any pre-treatment of the drug and tedious extraction procedure. The method has a wider range with good accuracy and precision. Hence, the data presented in the manuscript demonstrate that the proposed method was accurate, precise, linear, selective and offers advantages of reagent availability and stability, less time consumption and highly sensitive. Thus it can be extended for routine analysis of BRH in pharmaceutical industries and hospitals and research laboratories. Unlike the LC/MS procedure and HPLC procedures, the UV-visible spectrophotometer instrument is simple and not of highly expensive on the other hand in simplicity and user friendly the method could be considered superior in comparison with the previously reported methods. Moreover the method is free from interferences by common additives and excipients.

Acknowledgement: The authors are grateful to Sun pharmaceuticals India Ltd. Mumbai for providing gift sample of reference grade BRH and also the Chairman of the Department of Chemistry, Gulbarga University, Gulbarga, for providing all facilities, support and encouragement to carry out the study. Authors are thankful to USIC Department Gulbarga University, Gulbarga, for necessary instrumentation help. One of the authors extends his sincere acknowledgment to the UGC for providing financial assistance in the form of BSR Fellowship (No.F.7-226/2009 (BSR) Dated: 06-08-2014).

REFERENCES

1. L. Parvez M. Vaidya A. Sakhardande S. Subburaj, and T.G. Rajagopalan. Evaluation of antitussive agents in man, Pulmonary Pharmacology, 9, 1996, 299–308.
2. D. M. Cobbin, F. M. Elliott, and A. S. Rebuck. The mucolytic agent bromhexine (bisolvon) in chronic lung disease. A doubleblind crossover trial, Australian and New Zealand Journal of Medicine, 1, 1971, 137–140.
3. Indian Pharmacopoeia 1996 and Addendum 2000 (Government of India, Ministry of Health and Family welfare. Controller of publications. New Delhi, India).
4. British Pharmacopoeia, HMSO, London, UK, 2, 1996.
5. S. S. Zarapker, R. V. Rele, and V. J. Doshi. Simple spectrophotometric methods for estimation of bromhexine hydrochloride, Indian Drugs, 26, 1988, 38–41.
6. M. I. R. M. Santoro, M. M. Dos Santos, and J. F. Magalhaes. Spectrophotometric determination of bromhexine hydrochloride in pharmaceutical preparations, Journal of the Association of Official Analytical Chemists, 67, 1984, 532–534.
7. N. T. Abdel-Ghani, Y.M. Issa, and H.M. Ahmed. Potentiometric flow injection analysis of bromhexine hydrochloride and its pharmaceutical preparation using conventional and coated wire ion-selective electrodes, Scientia Pharmaceutica, 74, 2006, 121–135.
8. B. P. Jensen, B. Gammelgaard, S. H. Hansen, and J. V. Andersen. HPLC-ICP-MS compared with radiochemical detection for metabolite profiling of 3H-bromohexine in rat urine and faeces, Journal of Analytical Atomic Spectrometry, 20, 2005, 204–209.
9. H. Okamoto, T. Nakajima, Y. Ito, T. Aketo, K. Shimada, and S. Yamato. Simultaneous determination of ingredients in a cold medicine by cyclodextrin-modified microemulsion electrokinetic chromatography, Journal of Pharmaceutical and Biomedical Analysis, 37, 2005, 517–528.
10. M. Posp´ısilov´a, M. Polasek, and V. Jokl. Determination of ambroxol or bromhexine in pharmaceuticals by capillary isotachophoresis, Journal of Pharmaceutical and BiomedicalAnalysis, 24, 2001, 421–428.
11. E. V. Rao, G. R. Rao, S. Raghuveer, and P. Khadgapathi, Gas-liquid chromatographic and ion-pair high-performance liquid chromatographic determination of pseudoephedrine hydrochloride and bromhexine hydrochloride in pharmaceuticals, Analyst, 112, 1987, 871–874.
12. O.W. Lau and Y.M. Cheung. Simultaneous determination of some active ingredients in cough cold syrups by gas liquid chromatography, Analyst, 115, 1990, 1349–1353.
13. C. E. Uboh, J. A. Rudy, L. R. Soma. Characterization of bromhexine and ambroxol in equine urine: effect of furosemide on identification and confirmation, Journal of Pharmaceutical and Biomedical Analysis, 9, 1991, 33–39.
14. M. Turch´an, P. Jara-Ulloa, S. Bollo, L. J. Nunez-Vergara, J.A. Squella, and A. Alvarez-Lueje. Voltammetric behaviour of bromhexine and its determination in harmaceuticals, Talanta, 73, 2007, 913–919.
15. J.P. Rauha, H. Salomies, and M. Aalto. Simultaneous determination of bromhexine hydrochloride and methyl and propyl p-hydroxybenzoate and determination of dextromethorphan hydrobromide in cough-cold syrup by high-performance liquid chromatography, Journal of Pharmaceutical and Biomedical Analysis, 15, 1996, 287–293.
16. M. Vasudevan, S. Ravisankar, M. George, and J. Ravi. Simultaneous estimation of terbutaline, bromhexine and guaiphenesin in soft gelatin capsules by HPLC method, Indian Drugs, 37, 2000, 489–492.
17. H. N. Dave, R. C. Mashru, and A. K. Patel. Thin layer chromatography method for the determination of ternary mixture containing salbutamol sulphate, bromhexine hydrochloride and Etofylline, Journal of Pharmaceutical Sciences and Research, 2, 2010, 143–148.
18. A. El-Gindy, S. Emara, and H. Shaaban. Development and validation of chemometrics-assisted spectrophotometric and liquid chromatographic methods for the simultaneous

determination of two multi component mixtures containing bronchodilator drugs, Journal of Pharmaceutical and Biomedical Analysis, 43, 2007, 973–982.

19. V. Gupta, M. Verma, U. Misra, and R. K. Nema. Simultaneous spectrophotoetric estimation of bromhexine hydrochloride and pseudoephedrine hydrochloride in tablet dosages, Asian Journal of Chemistry, 21, 2009, 1633–1635.
20. A. K. Gupta and S. G. Kaskhedikar. Derivative spectrophotometric estimation of amoxycillin and bromhexine hydrochloride in tablets, Asian Journal of Chemistry, 15, 2003, 977–980.
21. S. K. Panda, A. K. Sharma, and L. K. Sahu. Simultaneous analysis of henylpropanolamine, chlorpheniramine and bromhexine in syrups by derivative spectrophotometry, Indian Journal of Pharmaceutical Sciences, 64, 2002, 540–544.
22. S. Gangwal and P. Trivedi. Simultaneous determination of terbutaline sulphate, bromhexine hydrochloride and guaiphenesin in three-component tablet formulation by UV spectrophotometry, Indian Journal of Pharmaceutical Sciences, 61, 1999,128–130.
23. X. Song, X.Wang, and D. Ze. Determination of aminophylline and bromhexine hydrochloride in Dingchuanzhike tablets by UV spectrophotometry, Zhongguo Yaoke Daxue Xuebao, 30, 1999, 100–102.
24. S. V. Murali Mohan Rao, I. Nageswara Rao, T. Rama Subba Reddy, and C. S. P. Sastry. Assay of bromhexine hydrochloride in pharmaceutical formulations by extraction spectrophotometry, Indian Journal of Chemical Technology, 12, 2005, 170–174.
25. S. V. MuraliMohan Rao, U. Viplava Prasad, I. Nageswara Rao, and M. Satya Babu. Sensitive spectrophotometric methods for the determination of bromhexine hydrochloride, Acta Ciencia Indica, Chemistry, 30, 2004, 105–108.
26. S. Kanna Babu, P. A. V. Ganapathi, N. V. S. N. Raju, and G. MadhuKumar. Spectrophotometric estimation of bromhexine hydrochloride in pharmaceutical preparations, Eastern Pharmacist, 42, 1999, 135–136.
27. International Conference on Harmonization (ICH) of Technical Requirement for the Registration of Pharmaceuticals for Human use, Validation of analytical procedures: definitions and Terminology Genera, 1996.
28. K. Siddappa, P. C. Hanamshetty. Spectrophotometric quantitative determination of Ambroxol hydrochloride in bulk and pharmaceutical dosage forms using PDAB reagent, International Journal of Pharmaceutical Science and Research, 5, 2014, 4188-94. doi: 10.13040/IJPSR.0975-8232.5(10).4188-94.
29. K. Siddappa, P. C. Hanamshetty. Application of charge transfer reactions for the quantitative spectrophotometric determination of cyclophosphamide in pure and pharmaceutical formulation, Current Pharmaceutical Analysis, 11, 2015, 131-138.
30. K. Siddappa, P. C. Hanamshetty. A simple spectrophotometric quantitative determination of Cilostazol in bulk and pharmaceutical dosage forms using DNPH reagent. Journal of Applied Pharmaceutical Science, 5, 2015, 117-121.

Source of Support: Nil, **Conflict of Interest:** None.