N- BROMOCAPROLACTAM-METHYLENE BLUE A NOVEL REAGENT FOR SPECTROPHOTOMETRIC DETERMINATION OF ANTIASTHAMATIC DRUG SALBUTAMOL AND ANTIULCER DRUG RANITIDINE IN PHARMACEUTICAL SAMPLES

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Abstract

An accurate, precise, and sensitive spectrophotometric method for the determination of H_2-receptor antagonists, ranitidine hydrochloride and the antiasthamatic drug salbutamol has been developed. The method is based on the reaction of these drugs with NBCL and subsequent measurement of the excess N-Bromocaprolactam (NBCL) by its reaction with methylene blue to give a violet-coloured product (λmax 664 nm). Decrease in the absorbance of the coloured product, due to the presence of the drug, was correlated with its concentration in the sample solution. Different variables affecting the reaction were carefully studied and optimized. Under optimal conditions, linear relationships with good correlation coefficients 0.9988–0.9998 were found between absorbance values and the corresponding concentrations of the drugs in a concentration range of 8–30, 6–22 µg mL^{-1}, the limits of detection were 1.22 and 1.01 mg mL^{-1} for ranitidine and salbutamol respectively. The proposed method was successfully applied to the analysis of the above-mentioned drugs in pharmaceutical dosage form. The % recoveries ranged from 98.5 ± 0.9 to 102.4 ± 0.8% without interference from the common excipients. The results obtained by the proposed method were comparable with those obtained by the official methods.

Keywords: H_2-receptor antagonists, N-Bromocaprolactam, spectrophotometry, pharmaceutical analysis, methylene blue

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Introduction

Histamine H_2-receptor antagonist drug ranitidine competitively inhibits the action of histamine on the H_2-receptors of parietal cells and thereby reduce gastric acid secretion under daytime and nocturnal basal conditions. Ranitidine is used for short-term treatment of active duodenal ulcers (1). Because of the therapeutic importance of H_2-RAs, several methods have been reported for their determination in bulk, pharmaceutical dosage forms and/or biological fluids. These methods include titrimetry (2), electrochemical methods (3), TLC (4), HPLC (5, 6), capillary electrophoresis (7), immunoassay (8), fluorimetry (9, 10), and spectrophotometry (11–17). However, spectrophotometric methods suffer from disadvantages such as having low sensitivity, take long reaction time for colour development >30 min, and require prior extraction of the coloured product.

Salbutamol sulfate is a sympathomimic bronchodilator useful in the treatment of bronchial asthma and related conditions. Most of the methods reported for the determination of salbutamol are spectrophotometric [18-19]. Its determination in pharmaceutical products and plasma by HPLC has also been reported [20-21]. The official method [22] involves two different methods for the analysis of pure drug and its formulations. The pure drug is analyzed by nonaqueous titration with perchloric acid using Oracet Blue B as indicator, while for the tablets analysis is carried out by UV spectrophotometric measurement after the solution has been passed through an anion exchange resin column. The present method of micro-determination of salbutamol by titration with N-Bromocaprolactam is simple, quick and is applicable to both the pure drug and its formulations.

N-Bromocaprolactam (NBCL) is formed by brominating caprolactam with liquid bromine which is the monomer of Nylon-6. NBCL contains unstably bound bromine (Br+) used for bromination and dehydrogenation in organic chemistry [23, 24]. The NBCL solution is useful for the resolution of mixture of thiols, xanthates, and sulphites [25-26] and for the determination of antimalarial drugs, sulfides and disulfides [27, 28]. It has already been reported that methylene blue is easily susceptible to oxidation with N-bromosuccinimide and gives a violet chromogenic product of λmax 664 nm [29]. The use of NBCL/methylene blue combination has not been investigated in the spectrophotometric determination of ranitidine and salbutamol. The present study describes, for the first time, the use of a NBCL/methylene blue combination in the development of a new simple spectrophotometric method for determination of ranitidine and salbutamol. The analytical procedure involved oxidation with excess NBCL and subsequent measurement of the remaining unreacted NBCL by its reaction with methylene blue to give a violet coloured product that was measured at 664 nm. The decrease in the absorbance at 664 nm, caused by the presence of the drug, was directly proportional to the amount of the drug in the sample solution.

EXPERIMENTAL

Apparatus

UVD-081604 (Spectronic Unicam, Mercers Row, Cambridge UK) ultraviolet-visible spectrophotometer with matched 1-cm quartz cells was used for all measurements.

Materials and reagent solutions

Ranitidine hydrochloride (Glaxo-Wellcome, UK) and salbutamol of FDC were obtained and used as received. Stock standard solution (1×10^{-2} M) were prepared. Working standard solutions were obtained by further dilution of the stock solution with water. Caprolactam (Merck, India Ltd), bromine ampoule (Merck India Ltd), methylene blue was 1000µg. All solvents, acids, and other chemicals used throughout the study were of analytical grade. Double distilled water was used throughout the study.

Synthesis of N-Bromocaprolactam (NBCL)

NBCL was synthesized as a relatively pure sample by suitably modifying the procedures of Hino and Taub [30]. In a round bottom flask fitted with a stirrer, 27.12 gm of caprolactam was taken and dissolved in a minimum amount (8-9 mL) of doubly distilled water. The reaction flask was placed in an ice bath to maintain the temperature 5° C. To the content of the flask, 5 mL of liquid bromine taken in a burette was delivered drop by drop and stirring was continued for a period 40-45 min. The completion of bromine addition is indicated by the smell of bromine and also by the yellow colour of the solution. Thereafter pale-yellow solution sodium chloride (8 g) was added in small fractions until it dissolves. The heavy amorphous oily layer appeared in the bottom of the flask when stirred for about 15-20 mins results into 7 g solid NBCL M.P 66-68° C. The reagent was standardized iodometrically.

General recommended procedure

Aliquots of ranitidine and salbutamol solution 0.5 - 3.5 mL and 0.2-4.9 mL (3.21×10^{-4} M and 3.98×10^{-4} M) were transferred into a series of 10 mL measuring flasks and the total volume was adjusted to 5 mL with double distilled water. To each flask, 1 mL of 1M HCl was added followed by adding an excess amount of NBCL solution (3.54×10^{-4} M). The contents were allowed to react for 10 min with occasional shaking. Finally, 1 mL of 50 µg methylene blue solution was added to each flask, diluted to the mark with double distilled water and the absorbance value of the solution was measured at 664 nm against a reagent blank after 10 min.

In each spectrophotometric method, the concentration of the unknown was calculated from the calibration graph or alternatively by computing the regression equation derived from Beer`s law data.

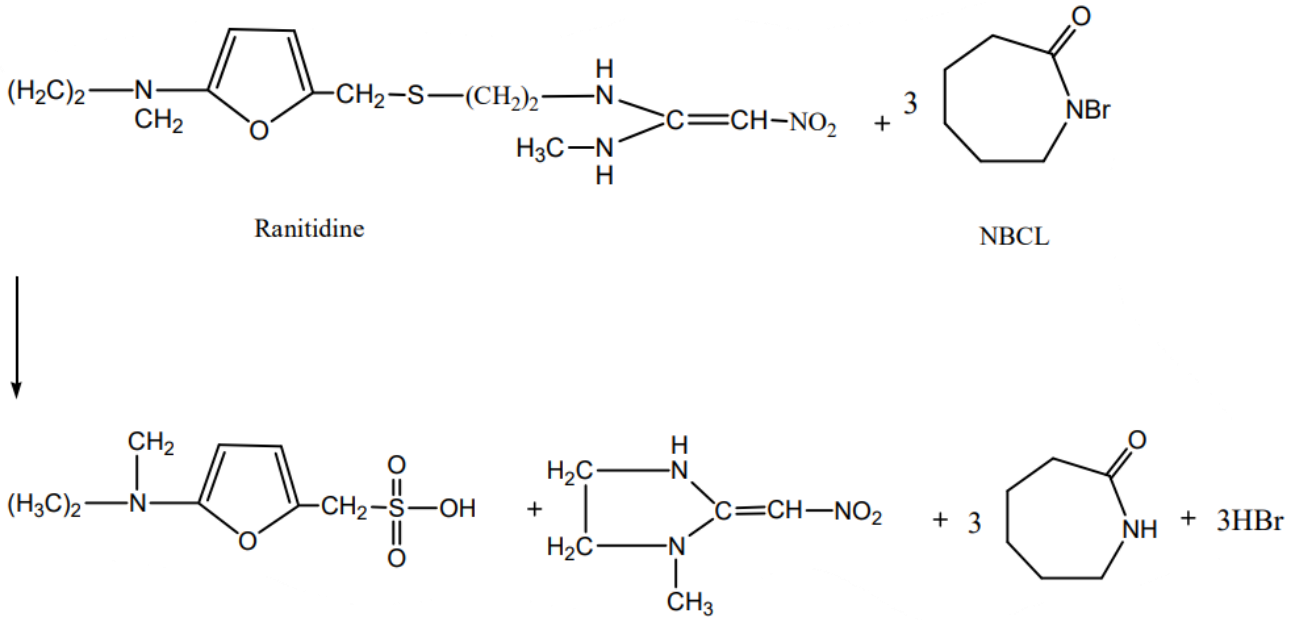
The limits of detection (LOD) and quantification (LOQ) were calculated according to the current ICH guidelines using the following formulae.

$$LOD = \frac{3.3\ SD_B}{a} \qquad LOQ = \frac{10SD_B}{a}$$

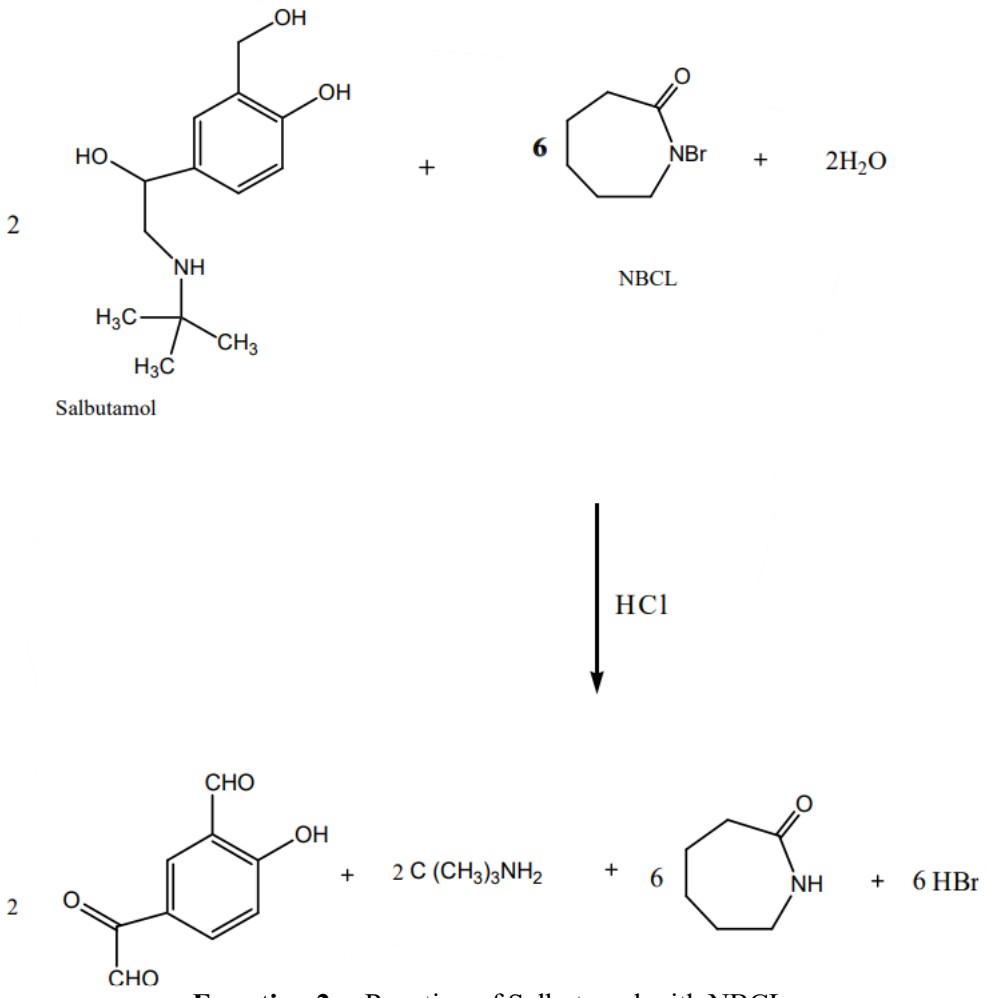
where SD_B is the standard deviation of seven reagent blank determinations and a is the slope of the calibration curve.

RESULTS AND DISCUSSION

In the spectrophotometric method for the determination of ranitidine and salbutamol, a fixed amount of NBCL was added to the drug solution. Thereafter, the completion of the reaction, NBCL was determined by reacting with a fixed amount of methylene blue dye. The reaction between rantidine and salbutamol with methylene blue is as follows.



Equation1: - Reaction of Ranitidine with NBCL



Equation 2: - Reaction of Salbutamol with NBCL

Preliminary experiments were conducted to determine the maximum concentrations of methylene blue spectrophotometrically by measuring the absorbance of their acidic solutions at their respective λmax, and the upper limits were found to be 5 and 4 μgmL^{-1} for methylene blue respectively. NBCL concentration of 7 μgmL^{-1} was found to bleach the red colour due to 5 μgmL^{-1}. NBCL was sufficient to destroy the blue colour of 4 μgmL^{-1} methylene blue. Hence, different amounts of ranitidine and salbutamol reacted with 7 μgmL^{-1} NBCL.

Hydrochloric acid was found to be a convenient medium for the two steps involved in both methods. For a quantitative reaction between ranitidine and salbutamol with NBCL, a contact time of 10 min was found sufficient in both methods. Constant absorbance readings were obtained when the reaction times were extended upto 20 min for ranitidine and 15 min for salbutamol and a standing time of 5-10 min was necessary for the bleaching of dye colour by the residual NBCL. The measured colour was stable for several hours even in the presence of the reaction product.

Quantitation Parameters

A linear correlation was found between absorbance at λmax 664 nm and ranitidine and salbutamol concentration are described by the regression equations.

A= 0.0014+ 0.4114x; R=0.9998

A= 0.0047 + 0.1187x; R = 0.9998

where A is the absorbance and x is the concentration in μgmL^{-1}, R is the correlation coefficient and n is the number of concentration levels. Beer`s law is obeyed for 0.25-1.75 and 0.5-5.0 μgmL^{-1} respectively. The calculated apparent molar absorptivity values were found to be 2.10 x 10^5 and
6.16 x 10^4 $Lmol^{-1}cm^{-1}$ for ranitidine and salbutamol, respectively.(Table 1 and 2)

TABLE 1: Microdetermination of Ranitidine with NBCL in presence of Methylene Blue at 664 nm

S. No	Weight Taken (in µg)	Weight Found (in µg)	Standard Deviation	Coefficient of Variation %		Regression Eq * Statistical data
1.	$2.30 \times 10^{+01}$	$2.99 \times 10^{+01}$	1.48×10^{-01}	4.94×10^{-01}		
2.	$6.89 \times 10^{+01}$	$7.35 \times 10^{+01}$	2.56×10^{-01}	3.37×10^{-01}		
3.	$1.15 \times 10^{+02}$	$1.29 \times 10^{+02}$	1.71×10^{-01}	1.36×10^{-01}		
4.	$1.61 \times 10^{+02}$	$1.79 \times 10^{+02}$	1.71×10^{-01}	9.60×10^{-02}		Slope b = 0.4114
5.	$2.07 \times 10^{+02}$	$2.09 \times 10^{+02}$	3.08×10^{-01}	1.44×10^{-01}		
6.	$2.53 \times 10^{+02}$	$2.60 \times 10^{+02}$	2.26×10^{-01}	8.72×10^{-02}		
7.	$2.99 \times 10^{+02}$	$3.19 \times 10^{+02}$	5.19×10^{-01}	1.66×10^{-01}		Intercept a = 0.0014
8.	$3.45 \times 10^{+02}$	$3.58 \times 10^{+02}$	1.48×10^{-01}	4.15×10^{-02}		
9.	$3.91 \times 10^{+02}$	$4.09 \times 10^{+02}$	2.56×10^{-01}	6.29×10^{-02}		
10.	$4.37 \times 10^{+02}$	$4.55 \times 10^{+02}$	8.53×10^{-02}	1.87×10^{-02}		Correlation Coefficient r = 0.998
11.	$4.83 \times 10^{+02}$	$4.92 \times 10^{+02}$	3.72×10^{-01}	7.48×10^{-02}		
12.	$5.29 \times 10^{+02}$	$5.49 \times 10^{+02}$	2.95×10^{-01}	5.42×10^{-02}		
13.	$5.75 \times 10^{+02}$	$5.91 \times 10^{+02}$	2.26×10^{-01}	3.82×10^{-02}		
14.	$6.20 \times 10^{+02}$	$6.34 \times 10^{+02}$	4.43×10^{-01}	7.06×10^{-02}		
15.	$6.66 \times 10^{+02}$	$6.85 \times 10^{+02}$	2.26×10^{-01}	3.30×10^{-02}		
16.	$7.12 \times 10^{+02}$	$7.17 \times 10^{+02}$	3.08×10^{-01}	4.28×10^{-02}		
17.	$7.58 \times 10^{+02}$	$7.79 \times 10^{+02}$	5.19×10^{-01}	6.73×10^{-02}		
18.	$8.04 \times 10^{+02}$	$8.09 \times 10^{+02}$	2.95×10^{-01}	3.63×10^{-02}		
19.	$8.50 \times 10^{+02}$	$8.57 \times 10^{+02}$	5.59×10^{-01}	6.50×10^{-02}		
20.	$8.96 \times 10^{+02}$	$9.01 \times 10^{+02}$	4.43×10^{-01}	4.88×10^{-02}		

* Summation of three determinations

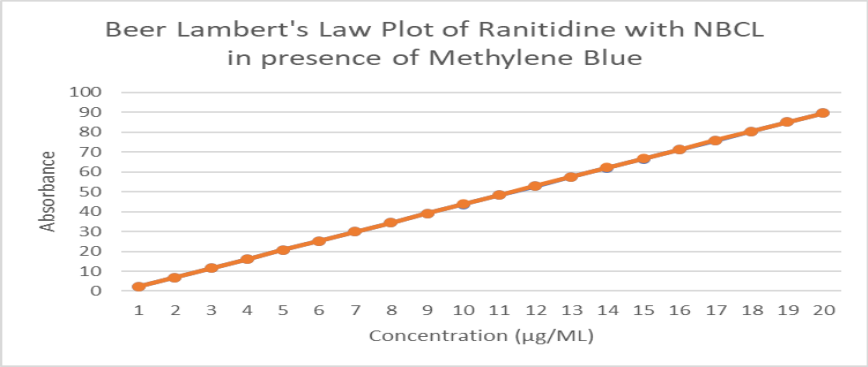
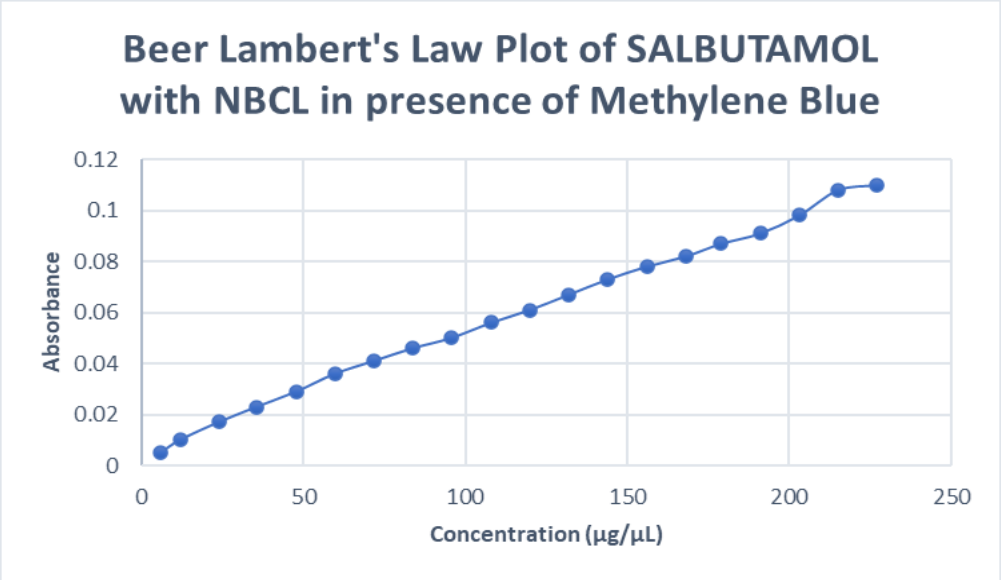


TABLE 2: Microdetermination of Salbutamol with NBCL in presence of Methylene Blue at 664nm

S.No	Weight Taken (in µg)	Weight Found (in µg)	Standard Deviation	Coefficient of Variation	Regression Eq * Statistical data
1.	5.98	5.03	3.38×10^{-02}	1.06×10^{-01}	
2.	$1.20 \times 10^{+01}$	$1.29 \times 10^{+01}$	1.69×10^{-02}	1.06×10^{-01}	
3.	$2.39 \times 10^{+01}$	$2.46 \times 10^{+01}$	4.25×10^{-02}	9.99×10^{-02}	
4.	$3.59 \times 10^{+01}$	$3.66 \times 10^{+01}$	5.44×10^{-02}	9.30×10^{-02}	
5.	$4.79 \times 10^{+01}$	$5.00 \times 10^{+01}$	2.58×10^{-02}	9.70×10^{-02}	Slope b = 0.00454
6.	$5.98 \times 10^{+01}$	$6.15 \times 10^{+01}$	7.81×10^{-02}	9.18×10^{-02}	
7.	$7.18 \times 10^{+01}$	$7.42 \times 10^{+01}$	9.32×10^{-02}	1.03×10^{-01}	
8.	$8.38 \times 10^{+01}$	$8.59 \times 10^{+01}$	4.88×10^{-02}	9.18×10^{-02}	Intercept a = 0.0157
9.	$9.57 \times 10^{+01}$	$9.81 \times 10^{+01}$	8.69×10^{-02}	9.61×10^{-02}	
10.	$1.08 \times 10^{+02}$	$1.06 \times 10^{+02}$	1.01×10^{-01}	1.06×10^{-01}	
11.	$1.20 \times 10^{+02}$	$1.24 \times 10^{+02}$	8.69×10^{-02}	9.61×10^{-02}	Correlation Coefficient r = 0.998
12.	$1.32 \times 10^{+02}$	$1.36 \times 10^{+02}$	8.34×10^{-02}	9.23×10^{-02}	
13.	$1.44 \times 10^{+02}$	$1.44 \times 10^{+02}$	9.32×10^{-02}	9.22×10^{-02}	
14.	$1.56 \times 10^{+02}$	$1.57 \times 10^{+02}$	4.47×10^{-02}	9.34×10^{-02}	
15.	$1.68 \times 10^{+02}$	$1.69 \times 10^{+02}$	4.25×10^{-02}	9.99×10^{-02}	
16.	$1.79 \times 10^{+02}$	$1.86 \times 10^{+02}$	1.46×10^{-01}	5.97×10^{-02}	
17.	$1.91 \times 10^{+02}$	$1.97 \times 10^{+02}$	1.31×10^{-01}	9.47×10^{-02}	
18.	$2.03 \times 10^{+02}$	$2.04 \times 10^{+02}$	5.73×10^{-02}	1.20×10^{-01}	
19.	$2.15 \times 10^{+02}$	$2.16 \times 10^{+02}$	6.84×10^{-02}	9.89×10^{-02}	
20.	$2.27 \times 10^{+02}$	$2.34 \times 10^{+02}$	1.22×10^{-01}	9.55×10^{-02}	



Application of the dosage forms

The proposed methods were applied for the analysis of ranitidine and salbutamol in tablets and the results were statistically compared with those obtained by the reported method [9], which consisted of measuring the absorbance of blue chromogen at 670 nm after treatment of tablet extract with Folin Ciocalteau reagent in alkaline medium. The calculated t-and F-values were lower than the tabulated values at 95% confidence level, revealing that the proposed method and the reference method have similar accuracy and precision in a few cases the t- calculated values are deviant and this can be ascribed to random errors.

From the recovery experiment, it was found that the percent recovery of the pure drug added to tablet/capsule powder ranged from 97.5 and 104.5, and the absorbance measurement was not affected by tablet excipients such as talc, starch, lactose, magnesium stearate, sodium alginate, calcium gluconate and calcium dihydrogenorthophosphate.

The proposed methods are simple, rapid, and reliable compared to most existing methods. In contrast to the direct titration method [16] reported earlier, the proposed method is more sensitive with a determinable range of 0.1-0.5 $mgmL^{-1}$ (1.73 x 10^{-4} - 8.68 x 10^{-4} $molL^{-1}$) and can be applied to a single tablet. The methods are accurate to -3.0 to 2.3 % when applied to the determination of ranitidine and salbutamol in formulations and the relative standard deviation varied from 0.9 - 1.4 %. Thus, the method in the present study was found to be highly sensitive compared to the existing spectrophotometric method, as shown by the molar absorptivity values. Furthermore the method is based on the measurement of dye colour, which is found to be exceptionally stable under the described experimental conditions, and the measurement is made at longer wavelengths where the interference from the excipients is far less compared to the shorter wavelengths used in many reported procedures. The proposed methods use ecofriendly and inexpensive chemicals and seldom employ organic solvents.

CONCLUSION

The results of the assay demonstrate that the proposed method can be used to determine the content uniformity of ranitidine and salbutamol tablets. Besides the simplicity of the procedures, the relative cheapness of the apparatus demonstrates their advantageous characteristics in addition to their high accuracy and precision. The spectrophotometric method described in the present work has the clear advantage of sensitivity comparable to that achieved by an expensive technique like HPLC. Applicability of spectrophotometric determination of ranitidine and salbutamol in urine and blood samples, clearly after appropriate sample pretreatment, will be the topic of our further research.

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