Spectrophotometric Estimation of Ambroxol Hydrochloride in Tablets

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Accepted 15 October 2002
Revised 12 October 2002
Received 10 January 2002

Two spectrophotometric methods in visible region have been developed for estimation of ambroxol hydrochloride in bulk drug and in tablets. In method A, the $-NH_2$ group of ambroxol reacts with sodium nitrite and forms a diazo compound which couples with N- (1-naphthyl)- ethylene diamine dihydrochloride to form a pinkish red chromogen which exhibits maximum absorption at 500 nm against a reagent blank. Method B is based on the reduction of ferric ions in its salt to ferrous ions by the drug, producing a yellowish orange chromogen with absorption maximum at 400 nm against the reagent blank. Beer's law was obeyed in the range of 10 to 50 μg/ml for method A and 40 to 240 μg/ml for method B. The results of analysis have been validated statistically and by recovery studies. The recovery ranged between 100.6 and 100.3% for method A and between 99.6 and 100.2% for method B. This paper describes new, simple and sensitive colourimetric methods for the estimation of ambroxol hydrochloride in bulk drug and pharmaceutical dosage forms.

Ambroxol hydrochloride, chemically 4-[[(2-Amino-3,5-dibromophenyl)-methyl]-amino]-cyclohexanol or N-[(trans-p-hydroxycyclohexyl), (2-amino-3,5-dibromobenzyl)]-amine[1], is an active metabolite of bromhexine, a mucolytic agent used in the treatment of respiratory disorders associated with viscid or excessive mucus[1,2]. It is official in Martindale-The Extra Pharmacopoeia[1]. Literature survey reveals that HPLC methods are reported for the estimation of ambroxol hydrochloride in pharmaceutical formulations[3,4].

The present work describes two simple and sensitive spectrophotometric methods for the quantitative estimation of ambroxol hydrochloride. A GBC UV/vis 911A spectrophotometer with 1 cm-matched cuvettes was used for spectrophotometric estimation. Hydrochloric acid solution (5 N in distilled water), sodium nitrite solution (0.1% in distilled water), ammonium sulphamate solution (0.1% in distilled water), N- (1-naphthyl)- ethylene diamine dihydrochloride (NEDD) solution (0.1% in distilled water), ferric nitrate solution (20 % in distilled water) and concentrated nitric acid were used. Standard solutions of ambroxol hydrochloride (100 μg/ml) for method A (solution A) and (1000 μg/ml) for method B (solution B) were prepared in methanol.

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Twenty tablets (each containing 30 mg ambroxol hydrochloride) were weighed and average weight was determined. The tablets were powdered in glass mortar and amount equivalent to 100-mg ambroxol hydrochloride was transferred to the 100 ml volumetric flask and then shaken with 50 ml methanol for 15 min. The mixture was filtered through Whatman filter paper no. 40 and the residue was washed thoroughly with methanol. The filtrate was made up to 100 ml with methanol (1 mg/ml), used for method B. Further dilutions were made with methanol to have 100 mcg/ml concentration, which was used for method A.

For the method A aliquots of standard solution (solution A) ranging between 1 and 5 ml were taken in a series of 10 ml volumetric flasks, to which 1 ml of hydrochloric acid (5 N) was added, followed by 1 ml of sodium nitrite solution (0.1 %). The mixture was allowed to stand for 5 min. Then 1ml of ammonium sulphamate solution (0.1 %) was delivered into it and after 5 min, 1.5 ml of 0.1 % NEDD solution was added. The mixture was then heated on a boiling water bath for 10 min, cooled to room temperature and volume was made up to the mark with distilled water. The absorbance values were measured at 500 nm against the reagent blank and plotted against concentration to obtain the standard calibration curve and found to be linear over the con-

centration range of 10 to 50 μg/ml. Similarly, the absorbance of the sample solution was measured and the amount of ambroxol hydrochloride was determined from the standard calibration curve.

For the method B, aliquots of ambroxol hydrochloride standard solution (solution B) ranging between 0.4 to 2.4 ml were transferred in a series of 10 ml volumetric flasks. To each flask, 4 ml 20% ferric nitrate solution, followed by 0.4 ml of concentrated nitric acid, were added. After 5 min, the volume was brought to the mark with distilled water and the absorbance of yellowish orange coloured species was measured at 400 nm against the reagent blank. The calibration curve was plotted and found to be linear over the concentration range of 40 to 240 μg/ml. Similarly, the absorbance of the sample solution was measured and the amount of ambroxol hydrochloride was determined from the standard calibration curve.

To test the accuracy and reproducibility of the proposed methods, recovery experiments were performed by adding known amount of drug to the preanalyzed formulation and reanalyzing the mixture by the proposed methods. The results are shown in Table 1.

In method A, the amino group of ambroxol hydrochloride reacts with sodium nitrite and forms a diazo compound, which couples with NEDD to form a pinkish red chromogen. In method B, ambroxol hydrochloride is estimated on the basis of reduction of ferric ions in its salt to ferrous ions by the drug, producing a yellowish orange chromogen. The colour intensity of chromogen in method A was intensified by 1 ml each of hydrochloric acid (5 N), sodium nitrite solution (0.1 %), ammonium sulphamate solution (0.1%) and 1.5 ml of NEDD (0.1 %) for the concentration range mentioned above. For method B, 4 ml of ferric nitrate solution (20 %) and 0.4 ml of concentrated nitric acid were sufficient for the achievement of the maximum colour intensity for the concentration range mentioned above. Stability of the coloured complex was determined by measuring absorbance value of chromogen at time intervals of 15 min and was found to be stable for 2 h for method A and 1 h for method B.

The calibration curve yielded correlation coefficient (r) of 0.9997 for method A and 0.9999 for method B over the beer's range of 10 to 50 μg/ml and 40 to 240 μg/ml respectively. The regression equation for method A was found to be y=0.0144x+0.0219 and y=0.0037x+0.0023 for method B. The molar absorptivity (lit/mole.cm) for method A was found to be 5.991×10^3 and 1.551×10^3 for method B. Sandell's sensitivity ($\mu g/cm^2/0.001$) was found to be 0.0692 and 0.2672 for method A and method B, respectively. The values of molar absorptivity and sandell's sensitivity indicate high sensitivity of the methods. The low values of % relative standard deviation and 95 % confidence limits of 0.1937 and 0.0009 for method A and 0.2509 and 0.0014 for method B, respectively, indicate that the proposed methods are highly precise.

The results of analysis of marketed formulations are shown in Table 1. The reproducibility and accuracy of the methods was found to be good, which is evidenced by low standard deviation. The percent recovery values obtained indicate non-interference from excipients used in the formulations. Hence, the proposed methods reported here are new, simple, rapid, sensitive, accurate and precise and can be successfully applied in the estimation of ambroxol hydrochloride in pharmaceutical dosage forms.

TABLE 1: RESULTS OF ANALYSIS OF MARKETED TABLETS.

Formulations	**Label claim (mg/tab)**	**Method**	**% of Label claim* ± Standard deviation**	**% C.O.V**	**Std. Error**	**% Recovery***
Tablet 1	30	A	99.2±1.03	1.0419	0.517	100.1
		B	99.4±0.97	0.9787	0.486	99.6
Tablet 2	30	A	99.5±1.09	1.0937	0.544	100.1
		B	99.5±0.66	0.667	0.328	100.2
Tablet 3	30	A	100±1.42	1.4126	0.706	100.3
		B	99.2±0.25	0.2461	0.123	99.9

*Mean of five determinations. Tablet 1: AMBRODIL (Aristo Pharma) 30 mg, Tablet 2: MUCOLYTE (American Remedies) 30 mg and Tablet 3: AMBRIL (Sigma Laboratories Ltd.) 30mg.

ACKNOWLEDGEMENTS

Thanks are extended to The Principal, Govt. College of Pharmacy, Karad for their invaluable assistance and encouragement.

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Antimicrobial activity of *Dioscorea bulbifera* bulbils

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Accepted 24 October 2002
Revised 9 September 2002
Received 14 January 2002

The successive extracts of *Dioscorea bulbifera* (bulbils) has been investigated for *in vitro* antimicrobial activity against *Klebsiella pneumoniae, Escherichia coli, Bacillus aureus, Proteus vulgaris, Staphylococcus aureus, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and *Rhizopus nigricans.* The petroleum ether and chloroform extracts showed significant activity against *A. fumigatus* and *R. nigricans.* The petroleum ether and distilled water extract showed good activity against *K. pneumoniae.* The chloroform extract showed feeble activity against *S. aureus.*

Dioscorea bulbifera L. (Dioscoreaceae) is a climber widely distributed in India, Ceylon, Malay peninsula, Australia, E. Africa and Brazil. *D. bulbifera* is one of the major Indian medicinal plants used in the three indigenous systems of medicine[1]. Traces of diosgenin (4%) are present in *D. bulbifera*[2,3,4]. *D. bulbifera* has diuretic and antiinflammatory activity[5]. Sterols and diterpenoids have been reported from this plant[6]. This communication reports the antimicrobial activity of bulbils of *D. bulbifera.*

The plant was collected from Gulbarga University Campus, Gulbarga in January 2001 and authenticated at the Botany Department, Gulbarga University with the help of Flora of Gulbarga District[7] where a voucher specimen is deposited (Voucher No. HGUG-785). The bulbils were cut, shade dried and coarsely powdered. The powdered plant material was subjected for successive extraction with petroleum ether, chloroform, ethanol (95%) and distilled water using Soxhlet extractor. The extracts were concentrated to dryness *in vacuo.* Four milligrams of each extract is dissolved in 1 ml of distilled dimethylformamide. The antimicrobial activity was assayed by agar well diffusion method[8]. The *in vitro* screening was carried out using *Klebsiella pneumoniae, Escherichia coli, Bacillus aureus, Proteus vulgaris, Staphylococcus aureus, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and *Rhizopus nigricans.*

Streptomycin sulphate (4 mg/ml of distilled water) and nystatin (4 mg/ml of distilled water) was used as a standard for bacteria and fungi respectively. The petroleum ether extract showed significant activity against *A. fumigatus* (16.5

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