Spectrophotometric Determination of Ambroxol

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Two simple and sensitive spectrophotometric methods developed for the determination of ambroxol using the reagents 3-methyl -2-benzothiazolinone hydrazone (MBTH) and ferric chloride ($FeCl_3$) and potassium ferricyanide [$K_3Fe(CN)_6$] for bulk samples and pharmaceutical preparations.

AMBROXOL hydrochloride (ABH) is chemically known as cyclohexanol, trans-4-(2- amino -3, 5-dibromo benzyl amino) monohydrochloride[1] and since it is a metabolite of bromhexine, has similar mode of action and used[2]. It has a significant importance as a potent expectorant and mainly used as a mucolytic agent in the treatment of respiratory disorders associated with viscid mucus.

The drug is not official any pharmacopoeia and few analytical methods[3-7] appeared in the literature for the determination of ambroxol in biological fluids and pharmaceutical formulations. The authors have developed two spectrophotometric methods for the determination of ABH. They are based on the oxidative coupling [MBTH - Ce(IV)] and oxidation followed by complex formation [Fe (III) - $K_3Fe(CN)_6$].

Since the pharmaceutical preparations containing ambroxol are not available in the market, model preparations (syrups) were prepared and 1 mg/ml solution of standard ABH and its dosage forms (syrup) were prepared in distilled water. The stock solutions were further diluted with distilled water to get working standard solutions, 500 µg/ml for method A and 100 µg/ml for method B.

Aqueous solutions of MBTH (0.2 %), ferric chloride (0.5 %), potassium ferricyanide (0.2 %) and 1N hydrochloric acid were prepared in distilled water in the usual manner. While ceric ammonium sulphate (1 %) was prepared by dissolving in 0.72 M sulphuric acid. To avoid photochemical reaction, ferric chloride solution was prepared freshly and stored in an amber coloured bottle. Optical measurements were made on Systronics spectrophotometer model-106.

For method A, aliquots of working standard solution of ambroxol hydrochloride (500 µg/ml) solution ranging from 0.4 to 1.4 ml were transferred into a series of 10 ml graduated test tubes. A 1 ml portion of MBTH (0.2 %) solution was added to each test tube and shaken gently for 2 min. Then 1 ml of Ce (IV) solution (0.1 %) was added to each test tube and after 10 min, appropriate volume of distilled water was added to each test tube to bring the total volume to 10 ml, and the absorbances were measured at 545 nm against a reagent blank. The amount of ambroxol present in the sample solution was computed from the calibration curve.

For method B, to series of 10 ml graduated test tubels, aliquot samples of working standard solution of ambroxol hydrochloride (100 µg/ml) solution ranging from 0.1 to 1 ml, 1 ml of $FeCl_3$ (0.5 %), 2 ml of $K_3Fe(CN)_6$ (0.2 %) were added. After 10 min. 1 ml of 1 N HCl was added to each test tube and appropriate volume of distilled water was added to each test tube to bring the total volume to 10 ml. The absorbance of the bluish green coloured species was measured at 740 nm against a reagent blak and the amount of ambroxol present in the sample was computed from the calibration curve.

The Beer's law limits, Molar extinction coefficient, Sandell's sensitivity, correlation coefficient, slope and intercept of regression analysis using least square method were incorporated in Table - 1. The percent relative standard deviation and percent range of error (0.05 confidence

Table-1 : Optical characteristics of proposed methods

Parameters	Method A	Method B
Beer's law limits (μg/ml)	10-70	1-10
Molar extinction coefficient (1 $mole^{-1}$ cm^{-1})	$2.57x10^3$	$2.49x10^4$
Sandell's sensitivity (μg/cm^2/0.001 absorbance unit)	0.161	0.015
Regression equation (Y*)	—	—
Slope (b)	$0.625x10^{-2}$	$6.66x10^{-2}$
Intercept (a)	$6.0x10^{-4}$	$-3.7x10^3$
Correlation coefficient (r)	0.9998	0.9999
Percent relative standard deviation**	0.772	1.043
Percent range of error (0.05 level)	0.645	0.729

* Y= a+bc; Where c is concentration in μg/ml and Y is absorbance unit.
** Eight replicate samples.

Table-2 : Analysis of Pharmaceutical formulations of ABH by proposed methods

Formulation	Labeled amount (mg)	Reported[4] method (mg)	Proposed method (mg)		% Recovery	
			Method A	Method B	Method A	Method B
Syrup	25	24.89	24.90	24.92	99.82	98.91
	25	24.90	25.01	24.98	99.94	99.89
	50	49.78	49.88	49.86	98.90	99.24
	50	49.89	49.97	49.89	99.82	98.94

limits) calculated from the eight separate samples containing 3/4 of the amount of upper Beer's law limit in each method were also summarized in Table -1. The values obtained by the proposed and the reported methods for pharmaceutical preprations were compared in Table - 2. The results of recovery experiments of the proposed method were also summarized in Table-2.

Interference studies revealed that the common excipients usually present in the dosage forms such as methyl paraben, propyl paraben, glucose, sucrose, maltose, sodium benzoate, sodium phosphate, calcium gluconate, gelatin, phosphoric acid and alcohol do not interfere in the proposed methods.

The formation of coloured species by ambroxol with MBTH and ceric ammonium sulphate is probably due to the oxidised MBTH (by ceric ammonium sulphate) would be coupled with ambroxol gives pink colour product. While in the case of second method, ferric chloride is reduced to ferrous form by ambroxol and further, ferrous form would be coupled with potassium ferricyanide to form a bluish green coloured product of potassium ferro-ferrous complex.

The results indicate that both the proposed methods are simple, rapid and sensitive with reasonable precision and accuracy and can be used for the routine determination of ambroxol and its dosage forms.

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Determination of Glycyrrhizin in *Glycyrrhiza glabra* and its extract by HPTLC

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A simple reproducible HPTLC method for the determination of glycyrrhizin in *Glycyrrhiza glabra* and its extract was developed and is described. The sensitivity was found to be linear in the range of 0.2 to 1.0 μg. The proposed method being precise, sensitive and reproducible can be used for detection, monitoring and quantification of glycyrrhizin in *g. glabra* and its extract.

GLYCYRRHIZA glabra Linn, commonly known as Mulethi, is a highly reputed ayurvedic plant and is used in herbal preparations as a tonic, expectorant, demulcent, mild laxative and for allaying cough and catarrhal affections[1,2].

Not many methods for quantitative estimation of glycyrrhizin have been reported in the literature. Some of these methods are gravimetric and colorimetric[3,4] which are not very precise. A HPLC method[5,6] has also been reported for the estimation of glycyrrhetenic acids, aglycone of glycyrrhizin which involves critical steps such as hydrolysis. The method presented in this paper is quick, simple, accurate and provides a clear resolution and separation of peaks.

Dried and powdered roots (1 g) were extracted with water (35 ml x 3). The extracts were filtered, pooled and dried over a steam water bath to make the final volume to 100 ml. In case of *G. glabra* extract, around 400 mg of dried powder extract was accurately weighed and dissolved in 100 ml distilled water. Two and 5 ul of these test samples were applied on a aluminium TLC plate precoated with Silica gel 60 F 254 (E. Merck) alongwith 2, 5, 7 and 10 ul of standard glycyrrhizin (concentration 0.10 mg/ml) from about 1 cm edge of TLC plate using a band width of 6 mm and 5 mm distance between tracks using a sample applicator Linomat IV (M/s Camag, Switzerland).

The chromatogram was developed in n-Butanol:Acetic acid:Water 5:1:4, (upper layer) upto 80 mm. The plate was

Table-1 : Estimation of Glycyrrhizin in *G. glabra* and its extract

Name of Sample	% of Glycyrrhizin
1. Crude *G. glabra*	9.054
2. *G. glabra* extract DP	17.48

DP = dried powder
Each value is the average of three replicates

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