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Quantification of Ambroxol Hydrochloride in Bulk and Oral Dosage Form using Spectrofluorimetry Method

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Abstract

Ambroxol Hydrochloride is a widely used mucolytic agent in the pharmaceutical industry. Accurate quantification of Ambroxol Hydrochloride is crucial for quality control and assurance in both bulk drug substance and oral dosage forms. Spectrofluorimetry is a highly sensitive and selective analytical technique that offers significant advantages for this purpose. In this study, a validated spectrofluorimetry method was developed for the quantification of Ambroxol Hydrochloride in bulk and oral dosage form. The spectrofluorimetry method involved excitation and emission wavelengths at specific ranges suitable for Ambroxol Hydrochloride detection. Calibration curves were constructed to establish the relationship between the drug concentration and fluorescence intensity. The method exhibited a linear range of concentrations with excellent correlation coefficients. The limit of detection and limit of quantification were determined to evaluate the method's sensitivity. The developed method was successfully applied to quantify Ambroxol Hydrochloride in both bulk drug substance and commercial oral dosage forms. The results obtained were consistent, accurate. This method provides a rapid, cost-effective, and precise means of quantifying Ambroxol Hydrochloride in pharmaceutical samples, making it a valuable tool for quality control and formulation development. The high sensitivity and selectivity of spectrofluorimetry further enhance its utility for routine analysis of Ambroxol Hydrochloride in pharmaceutical laboratories.

Key words: Preparation Of Standard Stock Solution, Preparation Of Sample Solution.

Introduction

Ambroxol Hydrochloride is chemically known as Trans-4-[(2-amino-3, 5-dibromobenzyl) amino] cyclohexanol hydrochloride [1]. It is an official drug in all pharmacopoeias. Ambroxolhcl acts as an expectoration improver and mucolytic agent. It decreases mucus viscosity by altering its structure. It depolymerizes mucopolysaccharides directly as well as liberating lysosomal enzymes and by breaking network of fibres in tenacious sputum. [2]

It was observed that various analytical methods such as, UV Visible spectrophotometry[3], LC-MS[4], HPTLC[5], UPLC[6], Gas chromatography[7] and electrochemical methods[8] have been reported. New drugs and drug combinations introduced into market is increasing every year. It is necessary to develop newer analytical methods for such drugs.

The spectrofluorimetric methods for determination are ordinarily utilized, as the spectrofluorimetic techniques has a higher responsiveness and selectivity than spectrophotometric techniques. Moreover, spectro fluorimeter instrumentation is viewed as more basic, accessible and less costly in determination with chromatographic and electrochemical instruments. Fluorescence is the emission of radiation when there is transition from singlet excited state to singlet ground state of compound. The fluorescence intensity is measured at variable wavelengths of excitation and emission, and fluorescence spectra are obtained fluorescence in spectro fluorimeter [9].

So, the aim of this study was to develop a simple, selective and sensitive spectrofluorimetric methods for determination of ambroxol hydrochloride in bulk and oral dosage forms. Fluorescence is the emission of radiation when there is transition from singlet excited state to singlet ground state of compound [10] .The fluorescence intensity is measured at variable wavelengths of excitation and emission, and fluorescence spectra are obtained fluorescence in spectro fluorimeters

Materials and methods

Ambroxol hydrochloride bulk drug was purchased in phoenix pharmaceutical pvt.ltd, Pondicherry. Ambroxol Hcl 30mg tablets were purchased in medical shop, villupuram, Tamil nadu.
Glacial acetic acid, sodium nitrite, catechol, resorcinol, β-naphthol, resorcinol, and sodium hydroxide of AR grade were used in the study.

PREPARATION OF STANDARD STOCK SOLUTION

A standard stock solution was prepared by dissolving 100mg of Ambroxol Hydrochloride in 100mL standard flask and the volume was made up with water to produce 1000µg/mL.

PREPARATION OF SAMPLE SOLUTION

The average weight of 20 Tablets of Ambroxol Hydrochloride was weighed and finely powdered. The powder equivalent to 10mg of Ambroxol Hydrochloride was taken in a 100mL volumetric flask and shaken with water to dissolve the active ingredient and made up the volume to produce 100µg/mL. The solution was then filtered, first few ml of the filtrate was discarded and the filtrate was used for further analysis [12].

INSTRUMENTATION

The fluorimetric measurements were made on JOBIN YVON FLUROLOG-3-11 Spectrofluorimeter reader instrument with DATA MAX/ GRAMS/ 31 software.

METHOD 1: DIRECT FLUORIMETRIC METHOD [11]

EMISSION SPECTRUM

The standard stock solution was suitably diluted in distilled water to yield a concentration of 0.6µg/mL. This solution was scanned in the spectrofluorimeter between 300-800nm using distilled water as blank. It was found that AMB exhibited an intense maximum absorption at about 381nm. [**Figure 1]**

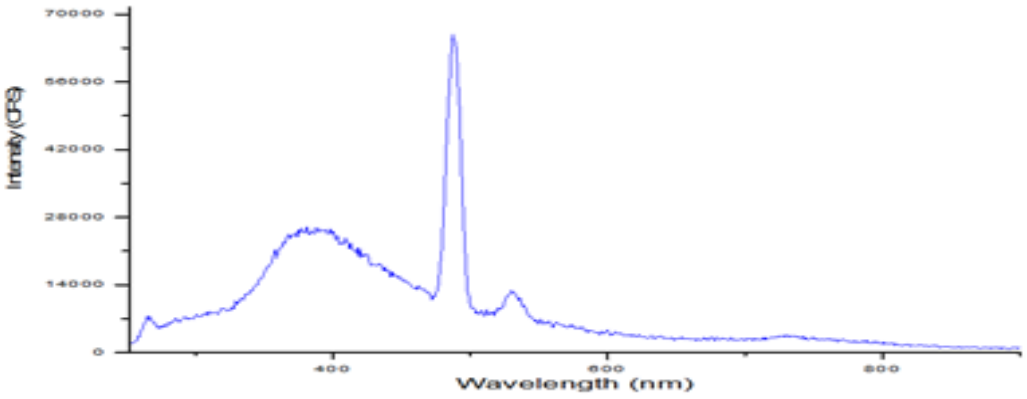


Figure 1: Fluorescence spectra of AMB at 381nm

FLUORESCENSE CONCENTRATION TO CONFIRM THE LINEARITY RANGE

Aliquots of standard solution of AMB were suitably diluted to give varying concentrations ranging of 0.2–1.0µg/mL. The solution were scanned in the range of 300-800nm and the relative fluorescence was measured an emission wavelength of 381nm with excitation wavelength of 242nm. A calibration graph was obtained by plotting fluorescence intensity versus concentration. (**Table1**)

Table 1: Fluorescence intensity of AMB at 381nm

S.NO	CONCENTRAION (µg/mL)	FLUORESCENSE INTENSITY*
1	0.2	112670
2	0.4	227340
3	0.6	339010

4	0.8	435280
5	1.0	568350

*Each value is the mean of three determination

CALIBARATION GRAPH

A graph of fluorescence intensity against concentration was plotted. From the graph the fluorescence concentration for the analytic was found to be between 0.2-1.0µg/mL (**Figure 2)**

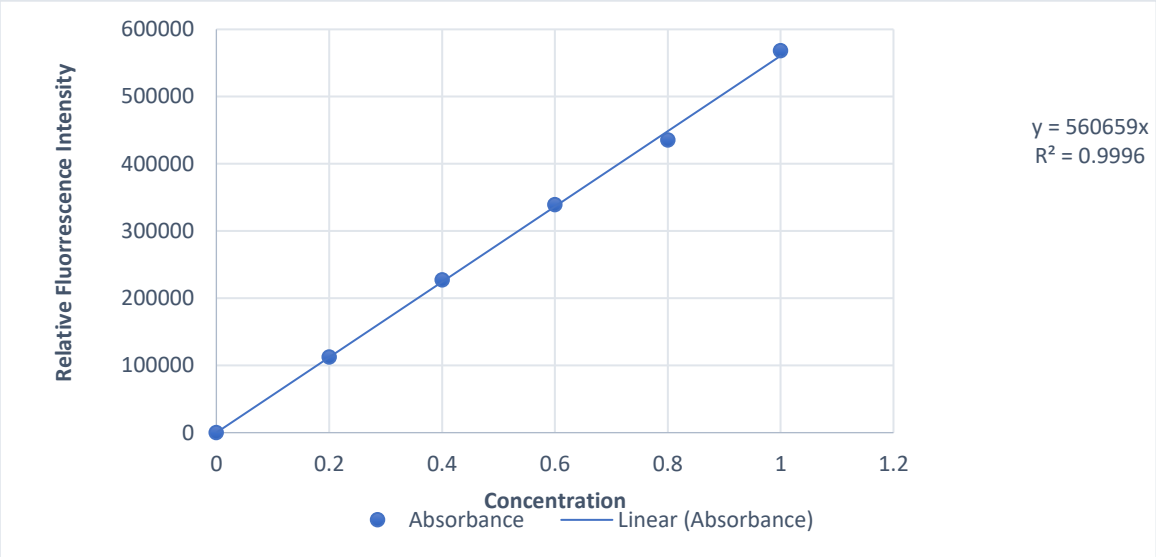


Figure 2: Calibration graph of AMB by spectrofluorimetry

Analysis Of Sample

The sample solution was further diluted with distilled water to the required concentration. The solution was scanned in the range of 300-800nm and the relative fluorescence was measured at λ_{em}= 521 nm with λ_{ex}= 385 nmThe amount of drug present in each tablet was calculated and the assay results are presented in the **Table 3.**

Table 3: Results of analysis of formulation and statistical parameters for AMB by Spectrofluorimetric methods

S.No	Methods	Label Claim	Amount found by proposed method (mg)*	% Label Claim	SD	SE	% RSD
1	Direct Fluorimetric method	30mg	29.90	99.97	0.023094	0.013334	0.005775
2	Oxidative spectrofluorimetric method using potassium permanganate		29.69	99.92	0.560119	0.323394	0.140136

*Each value is the mean of three reading

Recovery Studies

To study the accuracy, precision and reproducibility of the proposed method, the recovery studies were carried out on spiked sample by adding predetermined amount of standard drugs to the respective sample. About 50 and

100% of standard drugs were added to the sample solution and the absorbance was measured against method blank. The percentage recovered was calculated and presented in **Table4.**

Table 4: Recovery studies for Amb by spectrofluorimetric methods

S.No	*Methods*	*Label Claim*	*Amount of drug added(mg)**	*Amount of drug recovered (mg)**	*% Recovery*
1	*Direct fluorimetric method*	*30mg*	*0.2* *0.4*	*0.198* *0.396*	*99.00* *99.00*
2	*Oxidative spectrofluorimetric method using potassium permanganate*		*0.2* *0.4*	*0.198* *0.398*	*99.00* *99.50*

*Each value is the mean of three reading

METHOD 2: OXIDATIVE SPECTROFLUORIMETRIC METHOD USING POTASSIUM PERMANGANATE

EMISSION SPECTRUM

The standard stock solution was suitably diluted in distilled water to yield a concentration of 0.6µg/mL; 1.0 ml of potassium permanganate solution was added and the solution was scanned the range of 300-800nm. It was found that AMB exhibited an intense maximum fluorescence intensity at about 420nm. (**Figure. 3)**

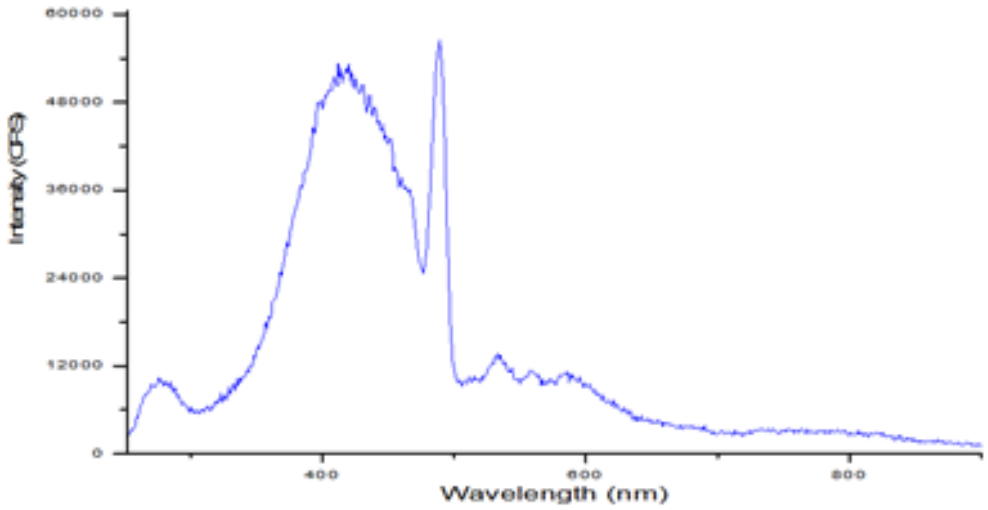


Figure. 3: Fluorescence spectra of AMB at 420nm

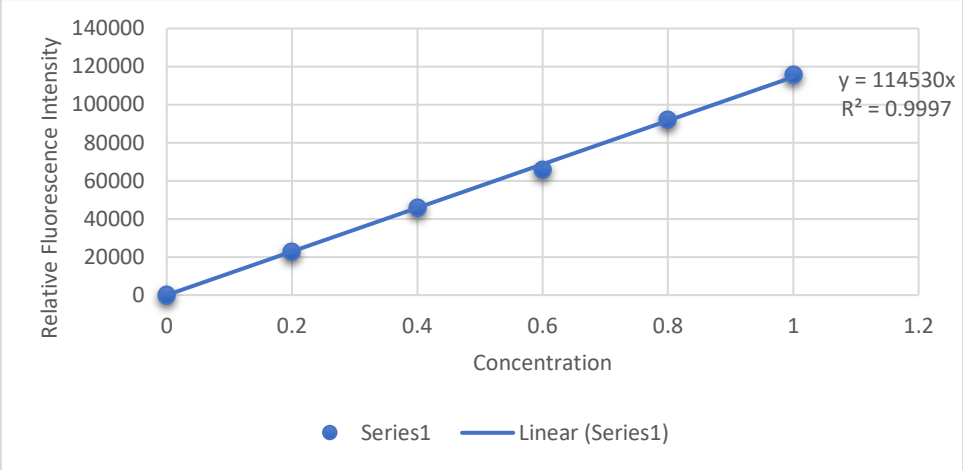
FLUORESCENCE CONCENTRATION TO CONFIRM THE LINEARITYRANGE

Aliquots of standard solution of AMB were suitably diluted to give varying concentrations ranging of 0.2 – 1.0µg/mL; 1.0 ml potassium permanganate solution was added and the solution were scanned the range of 300-800nm. The relative fluorescence intensity was measured at an emission wavelength of 420nm with excitation wavelength of 242nm.

Calibration Graph

A graph of fluorescence intensity against concentration was plotted. From the graph the fluorescence concentration for the analytic was found to be between 0.2-1.0µg/Ml. **(FIGURE 4)**

Fig 4: Calibration graph of AMB by spectrofluorimetry



ANALYSIS OF SAMPLE

The sample solution was further diluted with distilled water to the required concentration. 1.0 ml potassium permanganate solution was added and the solution was scanned the range of 300-800nm and the relative fluorescence was measured at an emission wavelength of 420nm with excitation wavelength of 242nm. The amount of drug present in each tablet was calculated and the assay results are presented in the **Table 3.**

RECOVERY STUDIES

To study the accuracy, precision and reproducibility of the proposed method, the recovery studies were carried out on spiked sample by adding predetermined amount of standard drugs to the respective sample. About 50 and 100% of standard drugs were added to the sample solution and the absorbance was measured against method blank. The percentage recovered was calculated and presented in **Table 4.**

Discussion

Spectrofluorimetry

The quantitative spectrofluorimetric methods for estimation of Ambroxol Hydrochloride step one also novel method. The drug showed linearity between 0.2 – 1.0 µg/mL for direct fluorimetric method and 0.2-1.0µg/mL for oxidative spectrofluorimetric method using potassium permanganate. The correlation coefficients were within the Limit, and RSD percentage was low. Thus the methods were precise, sensitive, highly specificity and accurate**. (Table 5).** Therefore all the methods developed and validated could be used for routine analysis and they are devoid of interference by some excipients.

Table 5: Optical characteristics of AMB by spectrofluorimetric methods

S.No	***Parameters***	***Spectrofluorimetric methods***	
		direct fluorimetric method	***Oxidative spectrofluorimetric method using potassium permanganate***
1	*Excitation and emission wave length (nm)*	*385& 521*	*405 & 469*
2	*Fluorescence concentration range (µg/mL)*	*0.2– 1.0*	*0.2 – 1.0*

3	*Regression equation (y=mx+c)*	560659x+0.00	114530x+0.00
4	*Slope (m)*	560659	114530
5	*Intercept (c)*	*0.00*	*0.00*
6	*Correlation coefficient*	*0.998*	*0.999*
7	*LOD (µg/mL)*	0.008727	-0.03632
8	*LOQ (µg/mL)*	0.026445	-0.11007

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