Development and validation of an high-performance liquid chromatographic, and a ultraviolet spectrophotometric method for determination of Ambroxol hydrochloride in pharmaceutical preparations

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Abstract

A high-performance liquid chromatographic (HPLC) and ultraviolet (UV) methods were developed and validated for the quantitative determination of Ambroxol hydrochloride (AMH) in pharmaceutical dosage form. HPLC was carried out by reversed phase (RP) technique on an RP-18 column with a mobile phase composed of acetonitrile and water (pH 3.5 adjusted with orthophosphoric acid [60:40, v/v]). UV method was performed with the λ_{max} at 250 nm. Both the methods showed good linearity, reproducibility, and precision. No spectral or chromatographic interferences from the tablet excipients were found in UV and HPLC. The method was successfully applied to commercial tablets. Validation parameters such as linearity, precision, accuracy, and specificity were determined. The HPLC Limit of detection (LOD) and Limit of quantification (LOQ) for Ambroxol were found to be 1 and 5 ng/ml, respectively. The UV LOD and LOQ for Ambroxol were found to be 1 and 4 µg/ml, respectively. The results were statistically compared using one-way analysis of variance. The proposed economical method could be applicable for routine analysis of AMH and monitoring of the quality of marketed drugs.

Key words: Ambroxol hydrochloride**,** high-performance liquid chromatographic, tablets, ultraviolet

INTRODUCTION

Ambroxol hydrochloride (AMH) is chemically 1 ({[2-amino-3,5 dibromophenyl]-methyl} amino) cyclohexanol monohydrochloride,[1] which is a semi-synthetic derivative of vasicine from the Indian shrub "*Adhatoda vasica*." It is a mucolytic agent. AMH is an N-desmethyl metabolite of bromohexine. Only limited analytical methods were reported in the literature for Ambroxol.[2-10] The aim of this work was to develop and validate a simple, fast, and reliable isocratic reversed phase high-performance liquid chromatographic (HPLC) and ultraviolet (UV) methods for the determination of AMH in pharmaceutical dosage forms. The important features and novelty of the proposed method included sample treatment with sonication of small amount of powder sample at ambient temperature, centrifugation, dilution; short elution time with internal standard eluted prior to AMH; and good precision (relative standard deviation Relative standard deviation [RSD] <5%) and high recovery (>95%). Confirmation of the applicability of the developed method validated according to the International Conference on Harmonisation (ICH) to determine the AMH in pharmaceutical preparations. The objective of this study was to develop and validate an assay for the estimation of Ambroxol using HPLC.

MATERIALS AND METHODS

Chemicals

HPLC grade acetonitrile (ACN) and triethylamine (AR grade) was purchased from Merck, Kualalmpur

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Malaysia. Water HPLC grade was obtained from a Milli-QRO water purification system. AMH standard and dexibuprofen (internal standard) were provided by Noven life sciences private limited, Hyderabad, India.

Instrumentation and Analytical Conditions

HPLC separation was performed on a Shimadzu liquid chromatographic system equipped with a LC-20 AD solvent delivery system (pump), SPD-20A photo-diode array detector, and SIL-20 ACHT injector with 50 μl loop volume. LC solution version 1.25 was applied for data collecting and processing (Shimadzu, Japan). The HPLC was carried out at a flow rate of 1.0 ml/min using a mobile phase constituted of ACN–water (pH 3.5 adjusted with orthophosphoric acid [60:40, v/v]) and detection was made at 250 nm. The mobile phase was prepared daily, filtered through a 0.45-μm membrane filter (Millipore®) and sonicated before use. A Princeton SPHER C_{18} column (250 × 4.6 mm internal diameter i.d., 5 μ) was used for the separation. UV method was performed on a UV–visible spectrophotometer, Beckman Coulter-DU800 with the λ_{max} at 250 nm and using 1.0-cm quartz cell.

Preparation of Standard Solutions
HPLC method

For the calibration curve, accurately weighed 100 mg of AMH was transferred to a 100-ml volumetric flask and dissolved in a mixture of water and methanol in the ratio 1:1 v/v. From this solution, other solutions with concentrations of 2, 4, 6, 8, 10, and 12 ng/ml were obtained by diluting adequate amounts in triplicate.

UV method

For the calibration curve, accurately weighed 100 mg of AMH was transferred to a 100-ml volumetric flask and dissolved in a mixture of water and methanol in the ratio 1:1 v/v. From this solution, other solutions with concentrations of 5, 10, 15, 20, 25, and 30 μg/ml were obtained by diluting adequate amounts in triplicate.

Preparation of Sample Solutions
HPLC method

Twenty tablets, 30 mg of AMH were weighed and finely powdered; a quantity of powder equivalent to 30 mg of AMH was weighed and transferred to a sintered glass crucible. To this, 5.0 ml of 1 mg/ml solution of AMH was added and the drug was extracted with suitable mixture solvent methanol and water (1:1 v/v). The extract was made up to 100 ml with mobile phase and further dilutions were made to get a concentration of 10 ng/ml of AMH, 50 μg/ml of dexibuprofen as internal standard and this solution was used for the estimation. 1 shows an HPLC chromatogram of AMH in pharmaceutical tablets [Figure 1].

UV method

Accurately weighed amount of powder equivalent to 30 mg
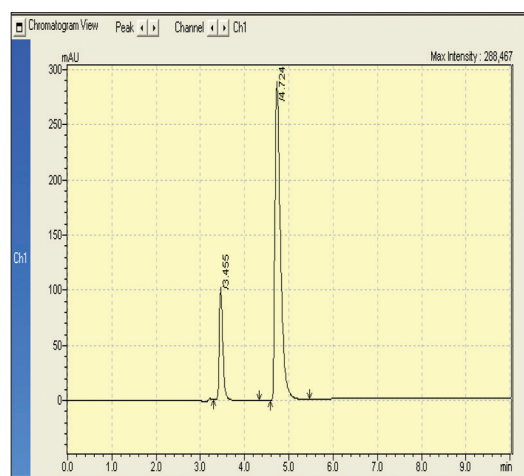

Figure 1: Typical sample chromatogram of Ambroxol and internal standard

of AMH was transferred to a 100-ml volumetric flask and dissolved in the mobile phase to obtain concentration of 10 ng/ml. An aliquot of this solution was diluted in mobile phase to obtain a solution with final concentration.

Method validation

The objective of method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines.[11] The method was validated for linearity, precision (repeatability and intermediate precision), accuracy specificity, short-term stability, and system suitability. Standard plots were constructed with six concentrations in the range of 2.0-12 ng/ml prepared in triplicates to test linearity. The ratio of peak area signal of AMH to that of internal standard (IS) was plotted against the corresponding concentration to obtain the calibration graph. The linearity was evaluated by linear regression analysis that was calculated by the least square regression method. The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from six replicate injections of freshly prepared AMH solution in the same equipment at a concentration 10 ng/ml of the intended test concentration value on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on 2 consecutive days to determine intermediate precision. Peak area ratios of AMH to that of IS were determined and precision was reported as %RSD. Method accuracy was tested (% recovery and %RSD of individual measurements) by analyzing samples of AMH at three different levels in pure solutions using three preparations for each level. The results were expressed as the percentage of AMH recovered in the samples. Specificity was assessed by comparing the chromato grams obtained from sample of pharmaceutical preparation and standard solution with those obtained from excipients which take part in the commercial tablets and verifying the absence of interferences. Sample solution short-term stability was

tested at ambient temperature (20 ± 1°C) for 3 days. In order to confirm the stability of both standard solutions at 100% level and tablets sample solutions, both solutions protected from light were re-injected after 24 and 48 h at ambient temperature and compared with freshly prepared solutions. A system suitability test was performed by six replicate injections of the standard solution at a concentration of 10 ng/ml verifying IS/AMH resolution > 2; %RSD of peak area ratios of AMH to that of IS ± 2%; and %RSD of each peak retention time ± 2%.

RESULTS AND DISCUSSION

Validation of Method
Linearity
Six point's calibration graphs were constructed covering a concentration range of 10-60 ng/ml. Three independent determinations were performed at each concentration. Linear relationships between the ratio of the peak area signal of AMH to that of IS versus the corresponding drug concentration were observed, as shown by the results presented in Table 1. The standard deviations of the slope and intercept were low. The determination coefficient (r^2) exceeded 0.99 [Figure 2]. The UV calibration is presented in Figure 3.

Precision
The repeatability study ($n = 6$) carried out showed a RSD of 0.74% for the peak area ratios of AMH to that of IS obtained,

thus showing that the equipment used for the study worked correctly for the developed analytical method and being highly repetitive. For the intermediate precision, a study carried out by the same analyst working on 2 consecutive days ($n = 3$) indicated a RSD of 0.69%. Both values were far below 5%, the limit percentage set for the precision and indicated a good method precision.

Accuracy
The data for accuracy were expressed in terms of percentage recoveries of AMH in the real samples. These results are summarized in Table 2. The mean recovery data of AMH in real sample were within the range of 99.89-100.38%, mean %RSD was 0.63%, satisfying the acceptance criteria for the study.

Specificity
The HPLC recorded for the mixture of the drug excipients revealed no peak within a retention time range of 6 min. The results showed that the developed method was specific as none of the excipients interfered with the analytes of interest.

Stability
The stability of AMH in standard and sample solutions containing IS was determined by storing the solutions at ambient temperature (20 ± 1°C) protected from light. The solutions were checked in triplicate after 3 successive days of storage and the data were compared with freshly prepared samples. In each case, it could be noticed that solutions were stable for 72 h, as during this time the results did not

Table 1: Results of regression analysis of data for the quantitative determination of Ambroxol hydrochloride by the proposed methods

Statistical parameters	HPLC	UV
Concentration range	2-12 (ng/ml)	5-30 (μg/ml)
Regression equation	$y=0.001x+3\times10-5$	$y=53,204x+7, 142$
Correlation coefficient (r)	0.999 0.996	

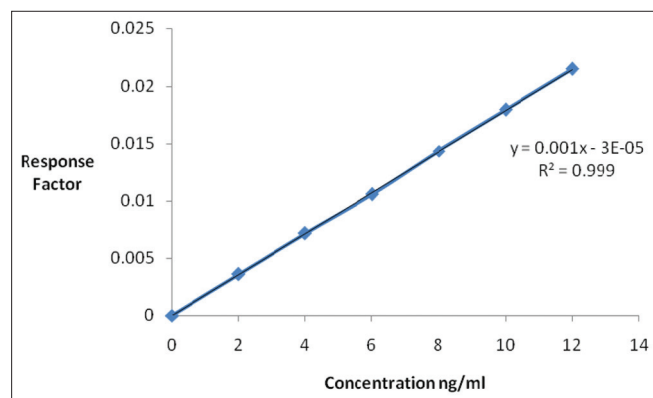HPLC: High-performance liquid chromatographic, UV: Ultraviolet

Figure 2: Calibration curve of Ambroxol by High-performance liquid chromatographic

Table 2: Accuracy study for Ambroxol hydrochloride ($n=5$)

Nominal concentration ng/ml (HPLC)	Nominal concentration μg/ml (UV)	Mean recovery (%)		RSD (%)	
		HPLC	UV	HPLC	UV
2.00	5	1.92	1.85	0.31	0.13
8.00	15	7.85	7.55	0.68	0.55
12.00	30	11.99	11.78	0.91	0.97

HPLC: High-performance liquid chromatographic, UV: Ultraviolet, RSD: Relative standard deviation
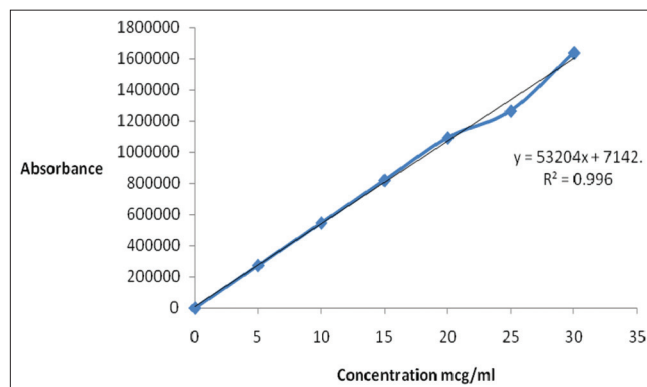

Figure 3: Calibration curve of Ambroxol by Ultraviolet

Table 3: Results obtained for the determination of Ambroxol hydrochloride in marketed formulations

Drug	HPLC Amount mg/tab		UV Amount mg/tab		HPLC %	UV Recovery
	Labeled	Found	Labeled	Found		
Ambroxol	30.0	29.10	30.0	28.89	98.30	97.89

HPLC: High-performance liquid chromatographic, UV: Ultraviolet

decrease below 97%. This denotes that AMH is stable in standard and sample solutions for at least 3 days at ambient temperature, protected from light and is compatible with IS.

System suitability

The resolution factor between IS and AMH, in the developed method, was above 2. The %RSD of peak area ratios of AMH to that of IS and retention times for both drug and IS were within 2% indicating the suitability of the system. These results indicate the applicability of this method to routine with no problems, its suitability being proved. The statistical evaluation of the proposed method revealed its good linearity, reproducibility, and its validation for different parameters and led us to the conclusion that it could be used for the rapid and reliable determination of AMH in pharmaceutical forms.

Assay of tablets

The validated method was applied for the assay of commercial tablets containing 30 mg of AMH; each sample was analyzed in triplicate after extracting the drug as mentioned in assay sample preparation of the experimental section and injections were carried out in triplicate. None of the tablets ingredients interfered with the analyte peak. The results presented in Table 3 are in good agreement with the labeled content.

CONCLUSION

A validated isocratic HPLC and UV methods have been developed for the determination of AMH in dosage forms. The proposed methods are simple, rapid, accurate, precise, and specific. Its chromatographic run time of 6.0 min allows the analysis of a large number of samples in a short period of time. Therefore, it is suitable for the routine analysis of AMH in pharmaceutical dosage forms. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments such as Liquid chromatography tandam mass spectrometry LC-MS/MS or Gass chromatography GC-MS/MS that are complicated, costly, and time-consuming rather than a simple HPLC–UV method. Considering the possible worldwide development of counterfeit Mukonil, the proposed method could be useful for the national quality control laboratories in developing countries.

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