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Original Article

ABSORPTION CORRECTION METHOD FOR THE SIMULTANEOUS ESTIMATION OF N-ACETYL-L-CYSTEINE AND AMBROXOL HYDROCHLORIDE IN BULK AND IN COMBINED TABLET DOSAGE FORM

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ABSTRACT

Objective: The objective of this research was to estimate the concentrations of N-Acetyl-L-cysteine (NAC) and Ambroxol Hydrochloride (AMB) simultaneously, in bulk and combined tablet formulation using a new, simple, precise and accurate absorption correction method using UV Spectrophotometer.

Methods: The concentrations of both the drugs (NAC and AMB) were determined using absorption correction method as; at 244 nm only AMB gave substantial absorbance and at 220 nm both NAC and AMB gave absorbance. Distilled water was used as a common solvent for both the drugs and the method was developed. Further statistical evaluations were carried out and the method was validated.

Results: In the range of 3-18 µg/ml for AMB at 244 nm and 20-120 µg/ml for NAC at 220 nm, the Beer's law was obeyed. Percentage recovery for AMB was in the range 100.50-101.10% and for NAC it was 99.85-100.20%. The % RSD values reported were less than 2. The developed method was validated and was found to be linear, accurate, precise and also rugged.

Conclusion: The results obtained clearly demonstrated that the proposed method of analysis was simple, sensitive, accurate, precise, rapid and also economical and could be applied successfully for the simultaneous estimation of NAC and AMB in bulk and combined tablet formulation.

Keywords: N-Acetyl-L-cysteine, Ambroxol Hydrochloride, Absorption correction method, Mucolytic, UV spectrophotometer

INTRODUCTION

N-Acetyl-L-cysteine (NAC), ((2R)-2-(acetylamino)-3-sulfanyl propanoic acid), (fig.1) is used in bronchitis, pulmonary diseases and other respiratory diseases as a mucolytic agent [1].

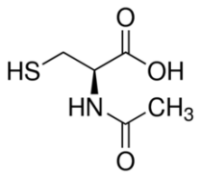

Fig. 1: Chemical structure of N-Acetyl-L-cysteine

It reduces the viscosity of pulmonary secretions by depolymerizing the mucopolysaccharides. Along with significant mucolytic effect, it also has anti-oxidant and anti-inflammatory effects and also used as an antidote in Paracetamol poisoning [2]. As per the recent animal and human studies of NAC, it has shown it to be a powerful antioxidant and a potential therapeutic agent in the treatment of HIV infections, heavy metal toxicity, cancer, heart disease, and other diseases characterized by free radical and oxidant damage [3]. A number of spectrophotometric [4], colorimetric [5], potentiometric [6], chemiluminescence [7], HPLC with electrochemical [8-11], fluorimetric [12], mass [13, 14] and ultraviolet [15-17] detectors and GC [18] have been widely applied as the main methods of detection in biological samples and pharmaceuticals.

Ambroxol hydrochloride (AMB) is chemical, trans-4-((2-amino-3, 5-dibromobenzyl) amino) cyclohexanol hydrochloride (fig. 2). AMB helps in reducing the bronchial hyper-reactivity and acts as a cough suppressant and also mucolytic [19, 20].

AMB is a metabolite of bromhexine. It acts an expectorant and mucolytic agent which can be used in the treatment of acute and chronic disorders related to the production of excess or thick mucus [21, 22]. For the determination of AMB, various methods like HPLC [23-26], GC [27, 28], LC-MS [29] and Capillary electrophoresis [30] are available.

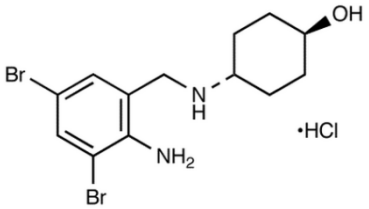

Fig. 2: Chemical structure of Ambroxol Hydrochloride

Both the drugs are official in Indian Pharmacopoeia (IP) and the United States Pharmacopoeia (USP). An extensive literature survey was performed which revealed that there are no methods reported for the assay of NAC and AMB in combined tablet dosage form by UV spectrophotometry. Methods are reported for estimation of NAC and AMB alone or in combination with other drugs using UV Spectrophotometer [31-33]. The study was thus performed with an aim to develop a simple, sensitive, precise, rapid, accurate and economic method for determination of NAC and AMB in combined tablet dosage form. The tablet formulation containing AMB and NAC manufactured by Tablets (India) Ltd. named AMBRONAC was used. The content of the tablets was 200 mg of NAC (USP) and 30 mg of AMB (IP) in each tablet.

MATERIALS AND METHODS

Instrumentation

The present work was carried out on Shimadzu - 1800 double beam UV - Visible spectrophotometer which was equipped with a pair of

10 mm matched quartz cells. The glass wares used were of 'A' grade. They were all thoroughly cleaned and rinsed with double distilled water and dried in hot air oven prior to the use. The software used for UV determination was UV PROBE.

Reagents and chemicals

Pure active pharmaceutical ingredients were obtained as a gift sample from Kores (India) Pharmaceutical Division Pvt. Ltd., Roha, Raigad (AMB) and Pallav Chemicals, Mumbai (NAC). Tablet formulation of a combination of the drugs AMB (30 mg) and NAC (200 mg) named AMBRONAC, manufactured and marketed by tablets (India) Ltd. was obtained. All other chemicals required of analytical grade, for the experiment were procured from SD Fine Chemicals, Mumbai.

Experimental conditions

Distilled water was the solvent used to dissolve both the drugs. Thus, all the stock solutions and further all dilutions were prepared using distilled water. The analysis was completely carried out in controlled conditions of temperature, humidity and pressure.

Preparation of standard stock solution

100 mg of NAC and 15 mg of AMB were weighed accurately and transferred into a 100 ml volumetric flask. The stock solution was prepared by dissolving both the drugs in distilled water by sonication technique and then made up to the mark with distilled water. The final concentrations obtained, were 1000 µg/ml and 150 µg/ml for NAC and AMB respectively.

Spectral studies

The stock solution of NAC was diluted to make 100 µg/ml solutions and AMB to 15 µg/ml. Distilled water was used as a blank, and the solutions were scanned in a UV Spectrophotometer in the range of 200 nm to 400 nm in 1 cm cell. The spectrum so obtained was recorded.

It was observed from the spectrum, that at 244 nm only AMB has substantial absorbance, whereas, at 220 nm, both AMB and NAC showed substantial absorbance. Thus, estimation of AMB was carried at 244 nm without any interference, as NAC has zero absorbance at 244 nm. At 220 nm the absorbance of AMB was deducted from the total absorbance of the mixture which gave the corrected absorbance for NAC.

The concentrations of the solutions were calculated using the Beer-Lamberts Law:

$$A = a \times b \times c$$

Where,

'A' is the absorbance of the solution, 'a' is the absorptivity, 'b' is the path length and 'c' is the concentration.

Thus, concentration at different wavelengths was found using the equation:

$$Cx = Ax \div ax$$

$$Cy = (Axy - Ax) \div ay$$

Where,

Cx is the concentration of the drug 'x' at a wavelength, *Cy* is the concentration of the drug 'y' at a wavelength, *Ax* is the absorbance of drug 'x', *Axy* is the absorbance of mixture of drugs 'x and y', *ax* is the absorptivity of drug 'x' at a wavelength, *ay* is the absorptivity of drug 'y' at a wavelength.

From the standard stock solutions of AMB and NAC, the aliquots were transferred into 10 ml calibrated volumetric flasks and diluted using distilled water, to make final concentrations of the range 3 to 18 µg/ml for AMB and 20 to 120 µg/ml for NAC and the calibration curves were obtained at their respective wavelengths, 244 nm and 220 nm. Also, the calibration curve for AMB was plotted at 220 nm for the concentration range 3 to 18 µg/ml.

Analysis of synthetic mixture of AMB and NAC

From the stock solutions of AMB and NAC, different mixtures of the two drugs were prepared into 10 ml calibrated volumetric flask and diluted using distilled water. All the mixtures prepared, were then measured for absorbance at wavelengths 244 nm and 220 nm and their concentrations were determined using absorbance correction method.

Analysis of tablet formulation

The average weight of 20 tablets was determined accurately. The tablets were powdered into fine particles by triturating them using a mortar and pestle. Powder equivalent to 100 mg of NAC was weighed accurately and transferred into a 100 ml calibrated volumetric flask. A minimum quantity of distilled water was added to the volumetric flask to dissolve the powder using sonication for about 20 min, after which the volume was made up to the mark using the same solvent. The solution was then filtered using a 0.45 µ Whatmann filter paper using a vacuum pump. The filtrate in the form of a clear solution was then subjected to further dilutions to obtain 100 µg/ml solution of NAC and 15 µg/ml solution of AMB theoretically. At all the selected wavelengths the absorbance of the sample solution was accurately measured. The concentrations of AMB and NAC in the tablet formulation were thus calculated. Six solutions using the same procedures were analyzed.

Method validation

Following were the parameters which were performed in order to validate the method-Linearity, Accuracy, Precision, LOD, LOQ, and Ruggedness [34].

Linearity

Six different concentrations were checked for linearity by diluting the stock solutions. The final concentrations for AMB were in the range of 3-18 µg/ml and for NAC in the range 20-120 µg/ml and absorbance were measured at wavelengths 244 nm and 220 nm respectively. Concentrations of AMB were also measured at 220 nm. Calibration curves (n=6) were plotted between the concentration of solution and absorbance of drugs.

Sensitivity

Sensitivity was checked by determination of limit of detection (LOD) and limit of quantification (LOQ) using the following equations for six replicates;

$$LOD = 3.3\sigma \div s \text{ And } LOQ = 10\sigma \div s$$

Where,

'σ' is the standard deviation of y-intercept of calibration curve and 's' is the slope of regression equation.

Precision

Repeatability and intermediate precision were performed to check the precision of the method. Six replicates of the same concentration were used for determining the repeatability, and intermediate precision was determined by interday and intraday analysis where three replicates of same concentration were analyzed on the same day and also on successive three days. The quantity of each drug present in the tablet formulation was calculated and percentage relative standard deviation was also obtained.

Accuracy

The recovery studies were carried out to check the interference of excipients and other interferences at three different concentrations for which standard addition method was used. The percentage recovery was calculated from the total amount of the drug found and the procedure was repeated three times for all the concentrations. Percentage relative standard deviation was also found out.

Ruggedness

It is the degree of reproducibility of results obtained after analysis of samples at a different place, different time, by different analyst, etc. Variations between the conditions and its effect on the results are

determined by ruggedness. The determination of AMB and NAC was thus carried out using different analyst and using a different instrument. Percentage relative standard deviation was then calculated.

RESULTS AND DISCUSSION

A rapid, sensitive, precise, accurate and economic analytical method has been proposed in the article, for the simultaneous estimation of AMB and NAC in pure form and tablet formulation. The method is based on absorbance correction where at a certain wavelength only one drug possesses absorbance whereas at other wavelength both the drugs show substantial absorbance. Simultaneous estimation was carried out for AMB and NAC using distilled water at wavelengths 220 nm and 244 nm (fig. 3). At 244 nm only AMB showed absorbance and thus its concentration was determined directly from the absorbance, whereas at 220 nm both AMB and NAC showed absorbance. Thus correction of absorbance helps estimate the concentration of NAC, the other drug in the combination by subtracting the known absorbance of AMB at 220 nm. Solution stability was performed at different intervals using the same solution, and it was found that the solution is stable up to 6 h.

In the range of 3-18 µg/ml for AMB at 244 nm and 220 nm and 20-120 µg/ml for NAC at 220 nm, the Beer's law was obeyed. The absorbances of the drugs were found to be linear with concentration as the values for correlation coefficient were above 0.999 at all the wavelengths. LOD and LOQ values of 0.3840 µg/ml and 1.16 µg/ml were observed for AMB and 1.525 µg/ml and 4.62 µg/ml were observed for NAC (table 1). The values indicated that the method is sensitive and thus can even be used for determination of drugs even with lower concentrations.

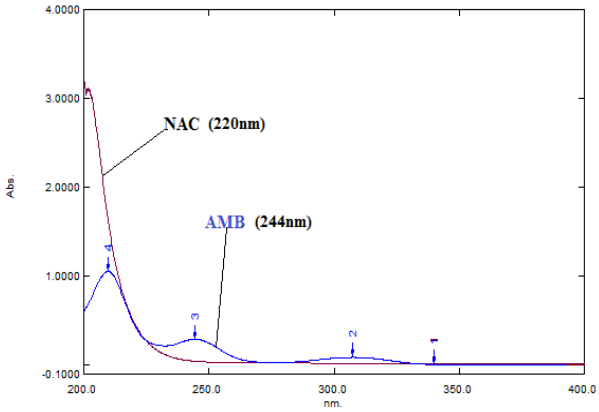

Fig. 3: Overlain spectrum of NAC and AMB (100 µg/ml and 15 µg/ml)

Table 1: Data for spectral and linearity characteristics

Parameters	AMB*	NAC*
λ_{max}	244 nm	220 nm
Linearity Range (µg/ml)	3-18	20-120
Correlation coefficient (r^2)	0.9991	0.9993
Slope (m)	0.027	0.004
Intercept (c)	0.003	0.009
Regression equation ($y = mx + c$)	y= 0.027x+0.003	y= 0.004x+0.009
LOD (µg/ml)	0.3840	1.525
LOQ (µg/ml)	1.16	4.622

*Mean of six observations

Synthetic mixtures of the drugs at various concentrations were analyzed to study the mutual interference in the absorbance if any, using the proposed method. The % recovery of AMB was 100.33% to 101.66% and for NAC it was 98.89% to 101.11% (table 2) which indicated that there is no mutual interference between these drugs.

Table 2: Data of analysis of synthetic mixtures for linearity

Concentration of AMB (µg/ml)		% Recovery	Concentration of NAC (µg/ml)		% Recovery
Theoretical	Experimental*	(AMB)	Theoretical	Experimental*	(NAC)
3	3.03	101.23	20	20.22	101.11
6	6.10	101.66	40	40.13	100.32
9	9.13	101.44	60	59.78	99.63
12	12.08	100.66	80	79.63	99.53
15	15.19	101.26	100	98.89	98.89
18	18.06	100.33	120	119.23	99.35

*Mean of three replicates

The label claim present in the tablet formulation was found to be 101.18±0.1778 for AMB and 98.95±0.1300 for NAC and their % RSD were 0.18% and 0.13% respectively (table 3) which indicated that the method is precise as the values were in good agreement with the label claim.

The precision of the method was also carried out using intraday and interday analysis. The values of % RSD for AMB were 0.29% for intraday and 0.85% for inter-day and for NAC they were 0.35% for intraday and 0.82% for inter-day analysis which indicated that the method is precise. For the estimation of the ruggedness of the method, the analysis was performed on different instruments and by a different analyst. The values of % RSD were less than 2% (table 4) which indicated that the method is rugged and can be suitably used for estimation of NAC and AMB in combination and parameters like the difference in days, instruments or analyst won't affect the method.

% Recovery studies were performed in order to check the accuracy of the method, at three different concentrations at 80%, 100% and 120% of the standard drugs AMB and NAC. To the pre-analyzed sample, the three concentrations were added and analyzed. % Recovery for AMB was in the range 100.50-101.10% and for NAC it was 99.85-100.20%. The % RSD values obtained were 0.32% and 0.18% for AMB and NAC respectively (table 5). Lower % RSD values indicate that the drugs can be successfully recovered from the formulation and estimated. This implies that the method is accurate for estimation of NAC and AMB.

Table 3: Results of analysis of tablet formulation

Parameters	AMB	NAC
Label claim (mg)	30	200
% Assay*	101.18	98.95
SD	0.1778	0.1300
%RSD	0.18%	0.13%

*Mean of six determinations

Table 4: Results for intermediate precision and ruggedness of method

Parameters	% Label claim estimated (mean±% RSD)	
	AMB	NAC
Intraday precision (n=3)	100.86±0.29	100.07±0.35
Inter-day precision (n=3)	100.32±0.85	99.993±0.82
Instrument I (n=3)	100.67±0.33	99.98±0.48
Instrument II (n=3)	99.93±0.26	100.10±0.52
Analyst I (n=3)	100.42±0.30	100.03±0.32
Analyst II (n=3)	100.28±0.21	99.90±0.41

n= number of replicates

Table 5: Results of recovery studies

Drug	Amount present (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	Amount recovered (µg/ml)	% Recovery*	S. D	%RSD
AMB	7.67	5.91	13.61	5.940	100.50	0.3235	0.32%
	7.67	7.58	15.29	7.624	100.59		
	7.67	9.13	16.90	9.230	101.10		
NAC	49.86	39.92	89.85	39.99	100.20	0.1789	0.18%
	49.86	49.84	99.74	49.88	100.09		
	49.86	60.06	109.92	59.96	99.85		

*Mean of three observations

The results obtained from above set of observations prove that the method is useful in determining the concentration of the drugs from the synthetic mixture and tablet formulation. The developed method is based on the use of very economical solvent and hence can be performed with ease. Simultaneous estimation of the drugs can be achieved by various other techniques like simultaneous equation method or absorbance ratio method [35], but absorbance correction method was found to be most important in the case of drugs which show zero absorbance at maximum absorption of another drug. Estimation of NAC and AMB thus can be economically and simply done by absorption correction method.

CONCLUSION

The method was developed and validated which provided an inference that the method is sensitive, linear, simple, accurate, precise, rugged and also economical. Thus, the proposed method can be applied effectively for routine analysis of AMB and NAC bulk dosage forms as well as in combined tablet dosage form.

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CONFLICTS OF INTERESTS

All authors have none to declare

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