ISSN (Online) 2249-6084 (Print) 2250-1029

International Journal of Pharmaceutical and Phytopharmacological Research (eIJPPR)
[Impact Factor – 0.852]

Journal Homepage: www.eijppr.com

Research Article

Development and Validation of Spectroscopic Method for Simultaneous Estimation of Acebrophylline and Acetylcysteine in Capsule Dosage Form

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Article info

Article History:
Received 22 May 2014
Accepted 25 June 2014

Keywords:
Acetylcysteine, Acebrofylline, Simultaneous equation method, Validation, ICH.

Abstract

A novel combination of Acebrophylline and Acetylcysteine is used in the treatment of chronic obstructive pulmonary disease (COPD) and bronchial asthma. A simple, economic, sensitive, accurate and reproducible spectroscopic method has been developed and validated for the simultaneous estimation of Acebrophylline and Acetylcysteine in capsule dosage form by Simultaneous equation method. Acebrophylline and acetylcysteine were found to have absorbance maxima at 273 and 220 nm respectively in distilled water. Acebrophylline was found to be linear in the concentration range of 1 to 6 µg/ ml at 273 nm and Acetylcysteine was found to be linear in the concentration range of 6 to 36µg/ml at 220 nm. The LOD and LOQ were found to be 6.22 µg/ml and 18.84µg/ml (at 273nm) for Acebrophylline and 36.64µg/ml and 111.04µg/ml at (220nm) for Acetylcysteine respectively. The recovery of Acebrophylline and Acetylcysteine were found to be 101.86% and 99.54% respectively showing accuracy of the method. The assay of marketed tablet formulation (Pulmoclear Capsules) was found to be 101.81% and 99.82% for Acebrophylline and Acetylcysteine respectively. The method was validated statistically as per ICH guidelines. The method showed good reproducibility and recovery with % RSD less than 2. So, the proposed method was found to be simple, specific, precise, accurate and linear. Hence it can be applied for routine analysis of Acebrophylline and Acetylcysteine in pharmaceutical formulations.

1. INTRODUCTION

Chemically Acetylcysteine[1] is the N-acetyl derivative of the amino acid L-cysteine and a precursor in the formation of antioxidant glutathione in the body. The thiol (sulfahydryl) group confers antioxidants effects and is able to reduce free radicals. Acetylcysteine[1,2] IUPAC name is a (2R)-2-acetamido-3-sulfanylpropanic acid [Figure 1], represents mucolytic drug which decreases the viscosity of secretions by splitting of disulphide bonds in mucoproteins and it also promotes the detoxification of an intermediate paracetamol metabolite which is used in the management of paracetamol overdose.

Acebrofylline[3] IUPAC name is 4-[(2-amino-3,5-dibromophenyl) methylamino] cyclohexan-1-ol; 2-(1,3-dimethyl-2,6-dioxopurin-7-yl)acetic acid. Acebrofylline is the salt obtained by reaction of equimolar amounts of theophylline-7-acetic acid, a xanthine derivative with specific bronchodilator activity and ambroxol, a mucolytic and expectorant with molecular formula $C_{22}H_{28}Br_2N_6O_5$ and molecular weight 616.302 g/mol as shown in Figure 2. It is a novel drug with bronchodilating, anti-inflammatory and mucuregulating effect due to inhibition of phospholipase A, and phosphatidylcholine. Literature survey[4-14] reveals that some methods have been reported for the estimation of single and very few methods for the combinations, but still there is no UV-Visible simultaneous equation method developed for the determination of Acebrofylline and Acetylcysteine in capsule formulations. So the present method developed is relatively simple, rapid and highly sensitive and validated as per ICH guidelines[15] in the analysis of multicomponent of interest and it can be used for routine quality control analysis in laboratories.

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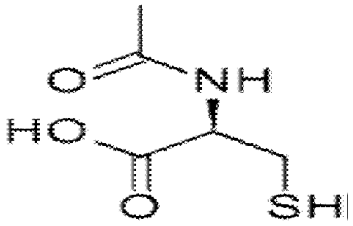

Figure 1: Chemical structure of Acetylcysteine

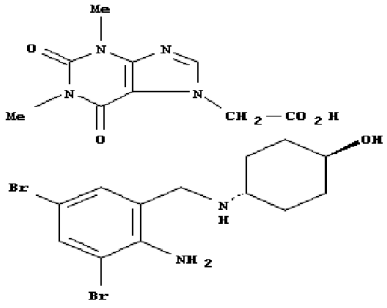

Figure 2: Chemical structure of Acebrofylline

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Acebrofylline (99.67%) and Acetylcysteine (99.69%) are samples were obtained from SL Drugs and Pharmaceuticals, Hyderbad, India and all other chemicals were of analytical grade. The commercial Pulmoclear (brand name) capsule formulation contain Acebrofylline 100mg and Acetylcysteine 600mg Manufactured by Fourrts(India) Laboratories Pvt. Ltd were obtained from local retail pharmacy.

2.2 Instrumentation
A PG Instruments double beam UV–visible spectrophotometer, Model: T-60, with a UV win Software 5.1.1 version. It has 1cm quartz cell used for the spectral and absorbance measurements.

2.3 Determination of λmax
By appropriate Aliquots of drugs with solvent containing 4µg/ml of Acebrofylline and 24 µg/ml of Acetylcysteine were scanned separately in the UV range of 400-200 nm to determine the λ max of Acebrophylline (273nm) and Acetylcysteine (220nm).

2.4 Preparation of standard stock solution
100mg of Acebrophylline and 600mg of Acetylcysteine Standards were weighed and transferred into 100 ml volumetric flasks separately and dissolved in distilled water and made up to the volume with same. These solutions were observed to contain 1000 and 6000 µg/ml concentration respectively. From the above stock solution 0.5ml was pipetted out into a 10 ml volumetric flask and made upto 10ml with diluent. From the above stock solution 0.8ml was pipetted out into a 10 ml volumetric flask and made upto 10ml with diluent to get concentration 4µg/mlAcebrophylline and 24µg/ml Acetylcysteine.

2.5 Preparation of sample solution and formulation Analysis
Twenty tablets of formulation (Acebrophylline 100 mg and 600 mg of Acetylcysteine) were weighed accurately and powdered. The tablet powder equivalent to Acebrophylline 100 mg and 600 mg of Acetylcysteine was weighed and transferred into 100 ml volumetric flask and a minimum quantity of distilled water was added to dissolve the substance by using ultra sonication for 15 min and made up to the volume with the same diluent (5000 µg/ml). The content was filtered through Whattman filter paper No. 41. From the cleared solution, further dilutions were made by diluting 2 ml to 100ml volumetric flask, further diluted 0.4 ml to 10 ml to obtain 4 µg/ml of Acebrophylline and 24µg/ml of Acetylcysteine theoretically. The absorbance measurements were made for the formulation at 273 nm, 220 nm. From the absorptivity values of Acebrophylline and Acetylcysteine at 273 nm, 220 nm, the amount of Acebrophylline and Acetylcysteine were determined by using Simultaneous equation method.

2.6 Simultaneous Equation Method
From the standard preparation, various dilutions were made at concentration range from 1-6µg/ml and 6-36µg/ml.
The simultaneous equations formed were,

At λ1 A1 = ax1bCx + ay1bCy ------ (1)
At λ2 A2 = ax2bCx + ay2bCy ------- (2)

Where A1 and A2 are the absorbance of sample solution at 273 and 220 nm respectively. Cx and CY are the concentration of Acebrofylline and Acetylcysteine respectively (µg /ml) in sample solution. The absorbance's (A1and A2) of the sample solution were recorded at 273 and 220nm respectively and concentration of both the drugs were calculated using above mentioned equation.

2.7 Linearity
Linearity of the concentrations was determined in the range of 1-6µg/ml at 273nm and 6-36µg/ml at 220nm for acebrofylline and acetylcysteine respectively. And Slope, intercept and correlation coefficient (R^2) was calculated from the calibration curve.

2.8 Precision
The repeatability of the method was confirmed by the formulation analysis, repeated for six times with the same concentration. The amount of each drug present in the tablet formulation was calculated. The percentage RSD was calculated. The intermediate precision of the method was confirmed by intra-day and inter-day analysis i.e. the analysis of formulation was repeated three times in the same day and on three successive days, respectively. The amount of drugs was determined and % RSD was calculated.

2.9 Accuracy
Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of standard drug to formulation samples. The recovery was performed at three different concentrations levels (i.e. 50%, 100% and 150%). This procedure was repeated for three times for each concentration. The results of recovery studies were calculated for %RSD.

2.10 Specificity
Specificity is the ability of the method to measure the analyte in the presence of other relevant components. The evaluation of specificity of the method was determined against placebo.

2.11 Selectivity
It is confirmed by determining the Limit of Detection (LOD) and Limit of Quantitation (LOQ).

2.12 Limit of Detection (LOD) and Limit of Quantitation (LOQ)
The limit of detection (LOD) and the limit of quantitation (LOQ) of all selected combination of drugs were derived by calculating the signal to-noise ratio using the following equations as per the ICH guidelines. LOD=3.3xσ/S, LOQ=10xσ/S,where σ-standard deviation of the response and S- slope of calibration curve.

3. RESULTS AND DISCUSSION
Overlay spectra of Acebrofylline and Acetylcysteine (Figure 3) shows that the estimation of both the drug can be possible using simultaneous equation method at the wavelength of 273nm and 220nm. Using appropriate dilutions of standard stock solution, the two solutions were scanned separately. A critical evaluation of proposed method was performed by statistical analysis of data where slope, intercept, correlation coefficient were studied. Beer's law is obeyed in the concentration range 1-6 µg/ml and 6-36µg/ml and correlation coefficient of 0.9997 and 0.9995 for Acebrofylline and Acetylcysteine respectively. The results and Optical parameters for the developed and validated method of Acetylcysteine (ACST) and Acebrofylline (ACBF) was given below Table 4, The Linearity data for the absorbance of Acebrofylline and Acetylcysteine drugs are given in Table 2 and Table 3 as well as respective calibration curve shown in figure 4 and figure 5 respectively. The recovery was performed at three different concentrations levels (i.e. 50%, 100% and 150%). The results of recovery studies were calculated for %RSD and shown in Table 3.

3.1 Specificity
The method was found to be specific in presence of Ambroxol which is the impurity of the drug.

3.2 Sensitivity
The limit of detection value for Acebrophylline and acetylcysteine was calculated and reported in Table 4. It was found to be selective method for this combination of drug.

3.3 Linearity and Range
The developed method was found to be Linear in it range and the slope, intercept and correlation coefficient was reported in Table 4.

3.4 Precision
It is expressed as the percentage coefficient of variation (% CV)/ %RSD which is calculated as per the following expression:

% CV= (standard deviation /mean)*100

It was found to be less than 2%. The results for interday and intraday precision were reported in Table 4.

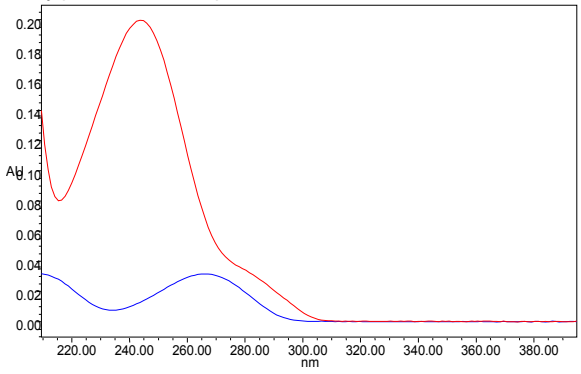

Fig. 3: Overlay spectra of Acebrophylline and Acetylcysteine

Table 1: UV Spectrophotometric assay Results for Acebrophylline and Acetylcysteine

Drug name	Label claim (mg/tab)	Estimated Amount (mg/tab)	% of Lable claim S.D (n=6)
Acebrophylline	100	101.81	101.81
Acetylcysteine	600	598.968	99.828

Table 2: Linearity data for Acebrofylline

Sr. No	Concentration in (µg/ml)	Absorbance
1.	1	0.04
2.	2	0.082
3.	3	0.129
4.	4	0.167
5.	5	0.21
6.	6	0.256

Table 3: Linearity data for Acetylcysteine

Sr. No	Concentration in (µg/ml)	Absorbance
1.	6	0.167
2.	12	0.317
3.	18	0.456
4.	24	0.628
5.	30	0.755
6.	36	0.919

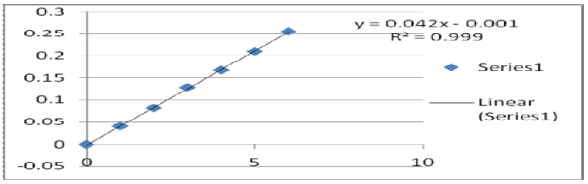

Fig.4: Calibration Curve for Acebrofylline

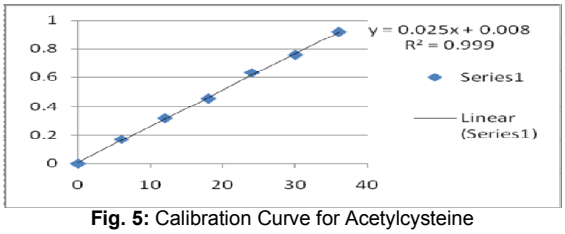

Fig. 5: Calibration Curve for Acetylcysteine

Table 4: Acetylcysteine and Acebrophylline data of validation parameter

Parameter	Acebrophylline	Acetylcysteine
Absorption maxima (λ_{max})	273	220
Beers law limit (µg/ml)	1-6 µg/ml	6-36 µg/ml
Correlation coefficient(r^2)	0.9997	0.9995
Slope	0.0429 (at 273nm)	0.0249(at 220nm)
	0.1300(at 220nm)	0.00706(at 273nm)
Intercept	-0.002866(at 273nm)	0.01573(at 220nm)
	-0.000133(at 220nm)	0.00786(at 273nm)
LOD(µg/ml)	0.166 (at 273nm)	1.275 (at 220nm)
	0.166(at 220nm)	1.275(at 273nm)
LOQ (µg/ml)	0.503(at 273nm)	3.866(at 220nm)
	0.5037(at 220nm)	3.866(at 273nm)
Recovery study	101.86±0.252	99.54±0.299
Interday precision	0.190	0.306
Intraday precision	0.315	0.293

4. CONCLUSION

The Spectroscopic method was developed and validated for simultaneous determination of Acebrofylline (ACBF) and Acetylcysteine(ACST) in capsule dosage forms. The method was found to be simple, specific, Precise and economical and can be applied for the routine quality control analysis for commercially available formulation.

5. ACKNOWLEDGEMENTS

The authors are thankful to ultra college of pharmacy, Madurai for providing necessary facilities for carrying out this work.

REFERENCES

1. Wikipedia, the free encyclopedia, en.wikipedia.org/wiki/Acetylcysteine
2. Pubchem compound USA., National centre for biotechnology information., 25 march 2005
3. Jignesh Maniya, Hasumati Raj, Hasmukh Vaghani, Manoj Mangukiya, Pradip Dudhat.,Development and Validation of Spectroscopic Method for Simultaneous Estimation of Acebrophylline and Montelukast Sodium in Combined Dosage Form., Indo American Journal of Pharmaceutical Research. 2012, 2(10), 1027-1036
4. Bhagavati Solanki, Harshal Trivedi, Ankita Khodiyar, Falguni Tandel, Lalit Lata Jha, Rajesh KS; Development And Validation of Derivative Spectroscopic Method For Estimation of Acebrophylline In Bulk And Its Dosage Form and In Presence of Impurity, Ambroxol HCl;; Pharmagene; 2013; 1(1); 1-11.
5. S. Ramanjaneyulu, G.Vijay Kumar, K.B.Chandrasekhar , S. Jyothirmai; Development and validation of RP- HPLC method for the estimation of Acebrophylline in capsules; Int.j.inv.pharm.sci; 2013; 1(5); 404-408.
6. Jignesh Maniya, Hasumati Raj, Hasmukh Vaghani, Manoj Mangukiya, Pradip Dudhat; Development and Validation of Spectroscopic Method for Simultaneous Estimation of Acebrophylline and Montelukast Sodium in Combined Dosage Form; Indo American Journal of Pharmaceutical Research; 2012; 2(10); 1027-1036.
7. Acelino Cardoso de Sá, Leonardo L. Paim, Urquisa de Oliveira Bicalho, Devaney Ribeiro do Carmo; Determination of N-Acetylcysteine by Cyclic Voltammetry Using Modified Carbon Paste Electrode with Copper Nitroprusside Adsorbed on the 3–Aminopropylsilica; Int. J. Electrochem. Sci; 2011; 6; 3754 – 3767.
8. Rim Haggag, Saied Belal, Rasha Shaalan; Derivatization With 4-Chloro-7-Nitro-2,1,3-Benzoxadiazole For The Spectrophotometric And Differential Pulse Polarographic Determination Of Acetylcysteine And Captopril; Sci Pharm. 2008; 76: 33–48.
9. Sravani Takkarusu, Sridhar Thota, Venisetty Raj Kumar, Venumadhav Neerati; RP-HPLC Analysis of Acebrophylline in API and Capsule Dosage Form; Research Journal of Pharmaceutical, Biological and Chemical Sciences; 2014; 5(1); 480-486.
10. Kotkar TA , Parihar YP, Mahajan MP, Sawant SD; Development and Validation of Spectroscopic Method for Simultaneous Estimation of Acebrophylline and Montelukast Sodium by Absorbance ratio method in bulk and Combined Dosage Form; 2014;2(3); 46-58
11. Y. Vander Heyden, D. Mangelings, J. Van Brempt, and H. Spapen**;** Development And Validation Of An Hplc Method With Post-Column Derivatisation For Assay Of N-Acetylcysteine In Plasma; Acta Chromatographica; 2014;12;149-164.
12. Aline Ferreira Ourique, Karine Coradini, Paula dos Santos Chaves, Solange Cristina Garcia, Adriana Raffin Pohlmann, Silvia Stanisçuaski Guterres and Ruy Carlos Ruver Beck; A LC-UV method to assay *N*-acetylcysteine without derivatization: analyses of pharmaceutical products; Analytical Methods ; 2013; 5**,** 3321-3327
13. Toussaint B, Pitti C, Streel B, Ceccato A, Hubert P, Crommen J; Quantitative analysis of N-acetylcysteine and its pharmacopeial impurities in a pharmaceutical formulation by liquid chromatography-UV detection-mass spectrometry; J Chromatogr A ; 2000; 896(1-2):191-199
14. Lu C, Liu G, Jia J, Gui Y, Liu Y, Zhang M, Liu Y, Li S, Yu C; Liquid chromatography tandem mass spectrometry method for determination of N-acetylcysteine in human plasma using an isotope-labeled internal standard; Biomed Chromatogr; 2011;25(4); 427-431
15. International Conference on Harmonisation (ICH), Validation of Analytical Procedures: Methodology, Q2B (CPMP/ICH/281/95), 1995