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DEVELOPMENT AND VALIDATION OF NEW RP HPLC METHOD FOR CONCURRENT DETERMINATION OF ACEBROPHYLLINE AND ERDOSTEINE IN SYNTHETIC MIXTURE

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ABSTRACT
A novel, simple, accurate and precise method for concurrent determination of Acebrophylline (ACB) and Erdosteine (ERB) in a blend by using RP-HPLC was developed. The analysis of the analyte was done by employing Inertsil ODS-3V C18 (5µ, 250 × 4.6 mm) as the stationary phase. The quantification of the ACB and ERB was done by using eluent as acetonitrile and a 50 mM potassium dihydrogen phosphate buffer with a 1.0 ml/min flow rate. The detection was set up at 250 nm. In compliance with ICH criteria (ICH Q2 (R1)), the analytical approach was successfully validated. By using the gradient method of acetonitrile and buffer, the peak of ACB and ERD was found to be at 5.47, 7.96 and 3.41 min, respectively. ACB and ERD showed linear response from 50-150 µg/mL and from 150 -450 µg/mL, with correlation coefficient (R^2) as 0.9997 and 0.9995, respectively. The percentage recovery for ACB and ERD was also determined to be 99.1–102.2% and 99.6–101.7%, respectively, falling well within the range of 98–102.2%. The current approach was found to be precise, accurate, robust and could be beneficially utilised for the concurrent analysis of ACB and ERD in dosage form.
Keywords: Acebrophylline, Erdosteine, RP HPLC, Validation, Simultaneous Estimation.
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INTRODUCTION

The disorder known as COPD, or chronic obstructive pulmonary disease, is brought on by injury to the airways or other lung structures that block airflow and make breathing difficult.[1] COPD ranked third among causes of death in 2019, accounting for more than three million deaths globally.[2] Worldwide, 0.544 billion persons suffered from chronic respiratory disorders in 2017, with COPD making up 55.1 per cent of cases in men and 54.8 per cent in women. By 2015, 0.174 billion individuals were living with COPD, and the disease was responsible for approximately 3.2 million deaths globally.[3] Acebrophylline (ACB) (Fig.-1A) and Erdosteine (ERD) (Fig.-1B) both are mucolytic agents. ACB contains theophyllinate-7, acetic acid and ambroxol base. Theophylline is a bronchodilator, and Ambroxol is a mucolytic agent.[4] ERD also has a mucolytic effect.[5] So the combination of ACB and ERD can help in reducing the frequency and severity of COPD exacerbation. The fixed-dose combination of ACB and ERD was recently approved by CDSCO for phase-3 trials on 20 Dec 2023.[6] As per the literature survey carried out, various UV and HPLC methods were documented for the estimation of ACB[4,7-12] and ERD[5,13-18] separately. But to date, no single RP HPLC method was documented for the concurrent quantitation of ACB and ERD in formulation. Therefore, it was thought of interest to develop an accurate, novel, reproducible, simple and specific RP-HPLC method for concurrent determination of ACB and ERD in a synthetic mixture following the guidelines outlined in ICH Q2 (R1).[19]

EXPERIMENTAL

Material and Methods

ACB (99.53 % purity) and ERD (99.86 % purity) were obtained as a gift sample from Aarpik Pharma Pvt. Ltd., Ahmedabad, Gujarat and Reine Lifescience, Ankleshwar, Bharuch, Gujarat, respectively. Milli-Q

Water, Methanol (MeOH), Acetonitrile (ACN) and Potassium Dihydrogen phosphate (KH_2PO_4; HPLC Grade) were bought from Rankem Chemicals. The excipients required for the blend were bought from S. D. Fine Chemicals Ltd, Mumbai.

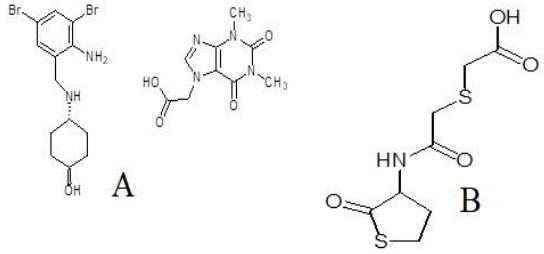

Fig.-1: Structure of (A) ACB and (B) ERD

Instruments and Experimental Condition

A Shimadzu HPLC (LC 2010 CHT) equipped with a detector (UV) was utilised for the analysis. Inertsil ODS-3V C18 (5µ, 250 × 4.6 mm) was the analytical column employed for the separation. LC solution software was utilised for data interpretation. 25°C was set as the column temperature. A mixture of 50 mM KH2PO4 Buffer and ACN was finalised as eluent in a gradient mode. A 1.0 mL/min was set as the flow rate (FR). The detection was accomplished at 250 nm. The injection volume was 10 µL.

Preparation of Solutions

50 mM Phosphate Buffer Preparation

Dissolve 6.8 g of potassium dihydrogen phosphate (KH_2PO_4) in 1 L of Milli-Q water and sonicate for 10 min. Finally, filter the Solution through a 0.45µ filter under vacuum.

Preparation of Eluent

50 mM Phosphate Buffer and ACN were used as eluent in a gradient program set-up.

Diluent Preparation

A Water and MeOH (50:50 %) were utilised.

Preparation of Solution (Stock) of ACB and ERD

Weighed 50 mg ACB and 150 mg ERD in a 50 ml volumetric flask (VF). Make the rest of the volume with MeOH. This solution (stock) corresponded to 1000 µg/ml of ACB and 3000 µg/ml of ERD.

Mixed Standard Solution Preparation

Aliquoted 1 ml from the stock solution of ACB and ERD in 10 ml VF and diluted using diluent to get the mixed solution containing ACB (100 µg/ml) and ERD (300 µg/ml).

Sample Preparation

The blend was formulated by combining 100 mg ACB and 300 mg ERD with routinely utilised excipients like polyvinyl alcohol, lactose, talc, magnesium stearate and sodium starch glycolate for one Tablet. Approximately 550 mg of the synthetic blend (equivalent to the weight of one tablet) was added to a 50 ml VF, and 25 ml of methanol was added. For 15 min, the solution was sonicated to dissolve the contents. Following that, methanol was added to the remaining volume while shaking occasionally. To filter the final solution, a Whatman 0.45µm filter was employed. A final concentration of ACB (100 µg/ml) and ERD (300 µg/ml) was attained by aliquoting 1 ml solution in a 10 ml VF and diluting with diluent.

Method Validation

The current approach was authenticated as recommendations provided in ICH Q2 (R1)[19], for parameters like linearity, system suitability, limit of detection (LOD), specificity, precision, limit of quantification (LOQ), robustness, and accuracy. To ensure the system's suitability, six replicate injections (10 µL) of ACB (100 µg/mL) and ERD (300 µg/mL) were analysand. The % RSD was determined after recording the theoretical plates (N), tailing factor (T_f), retention time (R_t), and resolution (R_s). Specificity was carried out by injecting blank, placebo, standard and sample to study the interference of excipients and the

analytes. Chromatograms were recorded, and R_t for blank, placebo, standard and sample were compared for identification of analytes. Selectivity was assessed using peak purity to confirm that the peak is purely attributed to the analyte. The calibration standards were prepared across 50-150 µg/mL for ACB and 150-450 µg/mL for ERD. ACB and ERD stock solutions (0.5, 0.75, 1, 1.25, and 1.5 mL) were added to a set of 10 mL flasks individually and diluted with diluent. The graph of response (peak area) vs. amount of analyte was used to find the R^2, slope, and Y-intercept. For repeatability, 6 determinations using the full (100 %) test concentration was carried out. For within-day precision, three concentrations of ACB (50, 100, 150 µg/mL) and ERB (150, 300, 450 µg/mL) were examined on the same day. While in between-day precision, three concentrations of ACB (50, 100, 150 µg/mL) and ERD (150, 300, 450 µg/mL) were examined on 3 consecutive days. Then, the % RSD was calculated. The approach's accuracy was assessed employing the placebo recovery method. A fixed amount (0.150 g) placebo was weighed, and the standard was spiked at 50 %, 100 %, and 150 %. Utilising dilutions, final concentrations of 50, 100, 150 µg/mL ACB and 150, 300, 450 µg/mL ERD were achieved. Mean slope and standard deviation of the Y-intercepts were employed to determine LOD and LOQ for ACB and ERD. The robustness was carried out three times (n=3) by changing various factors like temperature (± 5 °C), FR (± 0.1 mL/min), composition of organic ratio (± 2 %) and wavelength (± 2 nm). Stability of ACB and ERD solutions was tested by storing the sample and standard solutions separately in volumetric flasks with tightly sealed caps for a day at ambient temperature. The solution has been examined periodically for over 1 day.

RESULTS AND DISCUSSION

Method Development and Optimisation

To get a better resolution of ACB and ERD, different solvents like MeOH, ACN, water and phosphate buffer were tried. Different proportions of MeOH and water, as well as ACN and water, were tried, but the peak shape was not proper, and the N obtained was not within the limit. Then, instead of water, a phosphate buffer was tried with MeOH and ACN; still, the shape of the peak was not proper, and the peak was found in the dead volume. The experiments were conducted in the isocratic elution mode; however, the separation was insufficient, prompting the shift to the gradient elution mode due to the polarity influence of the Acebrophylline that includes ambroxol and theophyllinate-7-acetic acid. The trials were then done with buffer and MeOH in the gradient elution mode, but the peak shape, N, and peak R_s were not within the limits. ACN was used instead of methanol as an organic modifier. Acetonitrile and 50mM potassium dihydrogen phosphate buffer was found to be the best mobile phase mixture compared to the other mobile phase mixtures in terms of T_f, N, R_s, and peak shape (Fig.-2). Trials were done utilising 2 different stationary phases, i.e. Nucleosil ODS C18 (5µ, 150 × 4.6 mm) and Inertsil ODS-3V C18 (5µ, 250 × 4.6 mm), but effective separation was obtained by employing Inertsil ODS-3V C18 (250 × 4.6 mm, 5µ). Both analytes exhibited good response (absorbance) at 250 nm, so it was chosen for the analysis. FR of 1 ml/min was selected since it gives better resolution and a good peak shape. The separation was achieved at 25 °C. ACB consists of theophylline-7-acetate and Ambroxol; therefore, it gives two peaks when analysed by HPLC. ACB gets hydrolysed at pH 7 and breaks down into its constituent compounds, and hence gives 2 peaks.

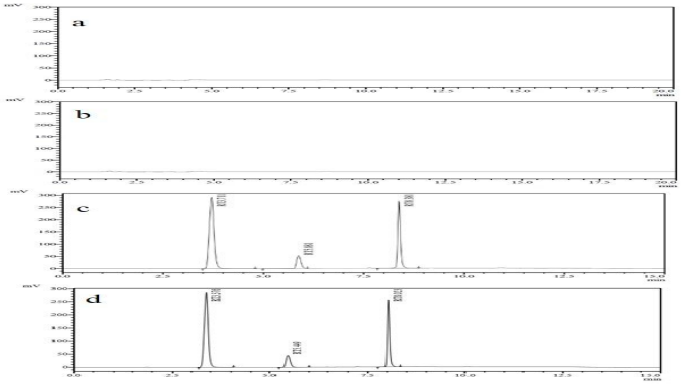

Fig.-2: Chromatogram of (a) Eluent; (b) Placebo; (c) Chromatogram (Standard) of ACB and ERD; (d) Chromatogram (Sample) of ACB and ERD

Method Validation

The proposed technique was validated by ICH Q2 (R1), which includes parameters such as accuracy, LOQ, robustness, precision, linearity and LOD. All the parameters (R_t, N, T_f, and R_s) fell within the acceptance range. The outcomes were presented in Table-1. The chromatograms of placebo, blank, sample and standard were shown in Fig.-2. It was confirmed that no more peaks were seen at both drugs' R_t, and this approach is specific for the concurrent estimation of ACB and ERD in a prepared blend. The peak purity test was also performed to assess the selectivity of the approach. The value of the single point threshold for ACB (Theophylline-7- acetate and Ambroxol) and ERD was found to be 0.99989, 0.99984 and 0.99981, respectively. To assess linearity, the approach shows a straight-line response for concentrations between 50 - 150 µg/mL, with R^2 = 0.9997 for ACB and the 150 – 450 µg/mL level range, with R^2 = 0.9995 for ERD. The % RSD of ACB and ERD for repeatability were 0.96 and 0.51, respectively. The outcomes for the intermediate precision study for ACB and ERD indicate that the % RSD results were within limits. The method was accurate because the % recovery fell within the expected range from 99.1 – 101.2 % for ACB and 99.6 % - 101.7 % for ERD, respectively. The results were presented in Table-2. The LOD is 9.42 and 29.12 µg/mL & LOQ is 29.15 and 88.32 µg/mL for ACB and ERD, respectively. The % RSD for the ACB and ERD peak areas was less than 2%, indicating that the approach is robust. The outcomes were mentioned in Table-2. An analysis of a synthetic blend was used to determine the applicability of the suggested approach. The average findings were 100.13 % ± 0.831 and 100.40 % ± 1.298 for ACB and ERD, respectively. The solution of ACB and ERD was found stable for one day, as the % assay difference was stated to be less than 2 %.

Table-1: System Suitability Test

S.No.	System Suitability Parameters	ACB-1		ACB-2		ERD	
		Mean ± SD (n=6)	% RSD	Mean ± SD (n=6)	% RSD	Mean ± SD (n=6)	% RSD
1	Theoretical Plates	11284.4 ± 212.82	1.88	69830.8 ± 586.612	0.84	4349.2 ± 61.98	1.43
2	Retention Time	5.47 ± 0.012	0.21	7.96 ± 0.011	0.14	3.41 ± 0.016	0.47
3	Tailing Factor	1.282 ± 0.004	0.31	1.398 ± 0.013	0.93	1.076 ± 0.009	0.83
4	Resolution	0.0	0.0	10.16 ± 0.089	0.88	15.56 ± 0.15	0.97

Table-2: Summary of Validation Parameters (*n=3, $^\#$ n=6)

S. No.	Parameter		ACB	ERD
1	Specificity		No interference	No interference
2	Range for linearity (µg/ml)*		50 - 150	150 - 450
3	Equation of Regression		y = 14789x - 11791	y = 7522.4x - 65 36.5
4	Coefficient of determination (R^2)		0.9997	0.9995
5	Precision (% RSD)	Repeatability$^\#$	0.96	0.51
		Within - day*	0.18-1.56	0.09-0.82
		Between day*	0.63-1.36	0.44-1.38
6	Accuracy (% recovery)*		99.1-101.2	99.6-101.7
7	Robustness (% RSD)	Change in wavelength (± 2 nm)*	(+) 0.01, (-) 0.03	(+) 0.20, (-) 0.25
		Change in FR (± 1 mL/min)*	(+) 0.36, (-) 0.89	(+) 0.07, (-) 0.21
		Change in Column Temperature (± 5 ºC)*	(+) 0.38, (-) 0.37	(+) 0.18, (-) 0.38

CONCLUSION

The study of quantitation of ACB and ERD in a synthetic blend has been achieved through the development and validation of a novel, precise, reliable, and simple RP-HPLC approach. All parameters, including selectivity, accuracy, specificity, precision, sensitivity, robustness and linearity, were performed and determined to be within acceptable limits. For all parameters, the % RSD values were less than 2 %, indicating that the outcomes obtained by this methodology are in good accord. As a result, this reverse-phase high-performance liquid chromatography approach may be used for regular QC assessment of ACB and ERD in formulation, which ensures the drug quality and safety.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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