DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF ACEBROPHYLLINE AND DOXOFYLLINE IN COMBINED SOLID DOSAGE FORM

[1] *Samadhan Magar*, [2]*Dr. Kailas Biyani*
[1]*Research Scholar*, [2]*Supervisior*
[1,2] *School of Pharmacy, SunRise University, Alwar, Rajasthan, India*

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Abstract:

Acebrophylline with Doxofylline is used in the treatment of asthma and chronic obstructive pulmonary disorder. Market survey reveals that Spirodin-AB® combination manufactured by Koye Pharmaceuticals Pvt. Ltd. is recently introduced in market containing Acebrophylline (100mg) and Doxofylline (400mg) as solid dosage form. ACEBRO and DOXO were separated on silica gel 60F254TLC plate using Toluene : Methanol : Glacial acetic acid (6:2:2,v/v/v) as mobile phase. Chambersaturation time was 20 min. The optimum wavelength for detection and quantification used was 250nm. There tention factors for ACEBRO and DOXO were found to be 0.29± 0.05 and 0.64 ± 0.02 respectively. Straight-line calibration graphs were obtained in the concentration range 100-600 ng/band for ACEBRO and 400-2400 ng /band for DOXOwith high correlation coefficient. The method was applied to marketed tablet formulation and the % amount of drug estimated was in good relationship with label claim. The spectra of ACEBRO and DOXO standard and tablet formulation indicate there is no interference of excipients present in tablet formulation. The method was validated as per ICH guidelines for Linearity, accuracy, precision and robustness. The accuracy of methodwas studied by recovery studies at 80%, 100% and 120 %. The proposed method whenused for estimation of ACEBRO and DOXO from its pharmaceutical formulation afterover spotting with 80%, 100% and 120 % of additional drug showed good drug recoveryin the range of 100.36 % to 100.87 % for ACEBRO and 99.97 % to 100.66 % forDOXO (% RSD less than 2) indicates accuracy of method.

Keywords: Validation, Stability, Acebrophylline, Doxofylline, HP-TL

INTRODUCTION:

The purpose of stability testing is to provide evidence on how the quality of Drug substance or Drug Product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light, and to institute a e-testperiodforthedrugsubstanceorashelflifeforthedrugproductandrecommended storage conditions. Stress testing studies are conducted to challenge the specificity of stability-indicating methods as part of validation protocol.

The forced degradation studies are carried out for the following reasons:

Development and validation of stability-indicating methodology;

Determination of degradation pathways of drug substances and drug products;

Discernmentofdegradationproductsinformulationsthatarerelatedtodrugsubstancesversusthosethatarerelatedtonon-drugsubstances (e.g.,excipients);

Structure elucidation of degradation products;

Determination of the inherent stability of a drug substance in solution and solid state and

To reveal the thermolytic, hydrolytic, oxidative, and photolytic degradation mechanism of the drug substance and drug product.

In a quest to make drugs available forever increasing diseases, disorders and ailments, new drugs, drug combinations and formulations are being introduced on regular interval. It is the responsibility and duty of analytical chemist to develop and validate analytical methods for these drugs, drug combinations and formulations.

Aim of the current work is to develop and validate quantitative analytical methods for active pharmaceutical

ingredients(API)that are competent to meet up the requirements to be entitled as 'stability indicating method'. The developed method must be proficient for resolving potential interferents specifically degradation products which are formed during stability evaluation period. The extent of degradation of API understress condition swill be studied.

Extensive literature survey with respect to 'Stability-indicating analytical methods' revealed that stability indicating methods for selected drugs or drug combinations as bulk and/or pharmaceutical formulations are no reported.

Materials and Methods

Materials:

Ambroxol hydrochloride obtained from Amilife sciences Pvt. Ltd. Baroda, Gujrat, India.Loratadine was obtained from vasudha Pharmachem Ltd.Hyderabad, Telangana, India and all chemicals and reagents were purchased from S. D. Finechem, Mumbai and are of amalytical grade.

Selection of detection wavelength

Stock solutions (10 µg/ml) of drugs were prepared in methanol and their isobestic point is observed at 250nm on UV-spectrophotometer shown in Fig. 1 Dry heat degradation studies

Dry heat study was performed by keeping drug sample separately in oven(1000C) for a period of 1 hour. Samples were withdrawn, dissolved in methanol and diluted appropriately to get concentration of 400 ng band-1 for ACEBRO and 1600 ngband-1 for DOXO. After Dry heat degradation, ACEBRO was stable without any degradation product and DOXO showed 11.61 % of degradation without any degradation product. Densitograms are shown in Fig.15 and Fig. 16

*Averageofsix determinations

Preparation of Standard stock solution

Standard stock solution of ACEBRO was prepared by dissolving 10 mg of drug in10 ml methanol to achieve concentration of 1 mg/ ml which was diluted further with same solvent to obtain final concentration100 ng/µl.

Standard stock solution of DOXO was prepared by dissolving 40 mg of drug in 10ml methanol to get concentration 4000 ng/µl. The resulting solution was diluted to get final concentration 400 ng/µl.

Selection of mobile phase and chromatographic conditions

Todevelopappropriatemethodfordeterminationofacebrophyllineanddoxofylline, different solvents like methanol, toluene, n-hexane, ethyl acetate, carbontetrachlorideandchloroforminvariouscombinations weretriedforseparationandresolution of drugs from their related substances and other excipients of formulation.Finally the combination Toluene: Methanol: Glacial acetic acid (6:2:2v/v/v) offered agood resolution. This mobile phase system was observed to give compact spots for bothacebrophylline and doxophylline and the Rf values were 0.29±0.05 and 0.64±0.02 forACEBROand DOXO respectivelyasshown in Fig. 2

Analysis of Tablet formulation

The proposed method was effectively used to estimate the amount of ACEBRO and DOXO from their combined tablet formulation (SpirodinAB®).Two microliter volume of prepared sample stock solution (100 ng band-1 and 400 ng band-1 for ACEBROand DOXO) was spotted on TLC plate followed by development and scanning. The content of drug was calculated from the peak areas recorded. Six determinations were carried out for analysis. ACEBRO and DOXO produced distinct peak at Rf 0.29±0.05,0.66±0.02resp. The results are shown in Table 1

Methodvalidation1

Linearity

Calibration was done by automatic sample applicator Linomat 5 on TLC plate togive concentration 100, 200, 300, 400, 500, 600 ng/band of ACEBRO and 400,800,1200, 1600, 2000, 2400 ng/band of DOXO. The plates were developed in mobile phase. The graph for calibration was plotted as peak area versus concentration.

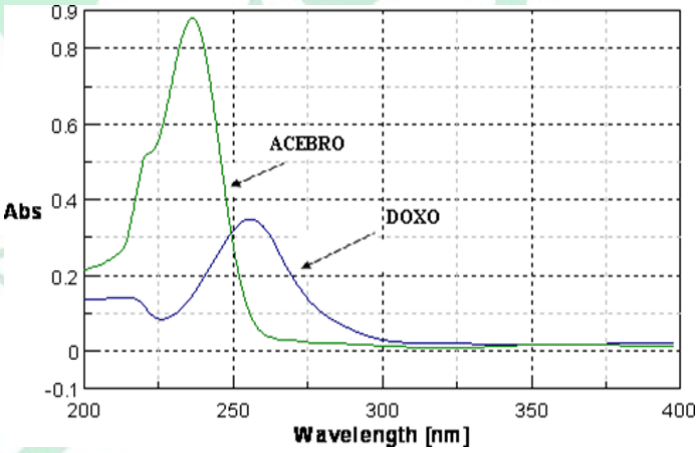

Fig. 1:Overlain UV spectrum of Acebrophylline and Doxofylline

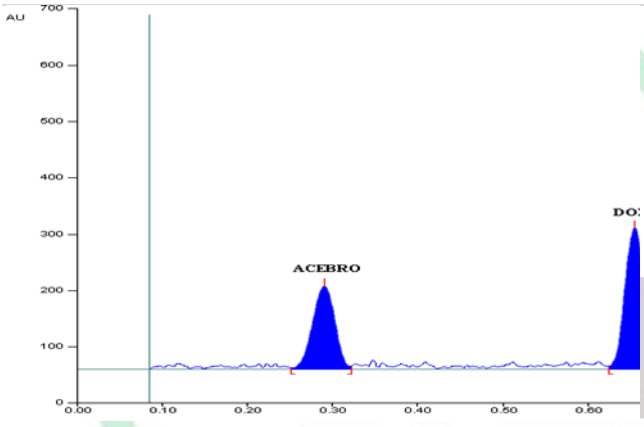

Fig. 2: Densitogram for Acebrophylline (400 ng band-1, Rf = 0.29±0.05) and Doxofylline (1600ngband-1, Rf=0.64±0.02)

DISCUSSION:

Acebrophylline with Doxofylline is used in the treatment of asthma and chronic obstructive pulmonary disorder. Market survey reveals that Spirodin-AB®combination manufactured by Koye Pharmaceuticals Pvt. Ltd. is recently introduced in market containing Acebrophylline (100mg) and Doxofylline (400mg) as solid dosageform. ACEBRO and DOXO were separated on silica ge l 60F254TLCplateusing Toluene:Methanol:Glacia lacetic acid (6:2:2,v/v/v) as mobil ephase.Chamber saturation time was 20 min. The optimum wavelength for detection and quantification used was 250nm.There tention factors forACEBRO and DOXOwere found tobe 0.29 ± 0.05 and 0.64 ± 0.02 respectively. Straight-line calibration graphs were obtained in the concentration range 100-600 ng/band for ACEBRO and 400-2400 ng /band for DOXO with high correlation coefficient. The method was applied to marketed tablet formulation and the % amount of drug estimated was in good relationship with label claim. The spectra of ACEBRO and DOXO standard and tablet formulation indicate there is no interference of excipient spresent in tablet formulation.Themethodwasvalidatedasper

ICH guidelines for Linearity, accuracy, precision and robustness. The accuracy of methodwas studied by recovery studies at 80%, 100% and 120 %. The proposed method when used for estimation of ACEBRO and DOXO from its pharmaceutical formulation after over spotting with 80%, 100% and 120 % of additional drug showed good drug recovery in the range of 100.36 % to 100.87 % for ACEBRO and 99.97 % to 100.66 % forDOXO (% RSD less than 2) indicates accuracy of method. The precision of the methodwas expressed as % RSD and observed within limitindicate method is precise. The lowvalue of LOD and LOQ indicates sensitivity of the method. The method robustness was studied by changing inc hromatographic conditions and results were concluded interms of % RSD. Found less than 2 for each parameter which express method is robust. Method summary given in Table 8

ACEBRO and DOXO were exposed to various stress degradation conditions. Peaks procured from the samples degraded by acid, alkali, neutral, hydrogen peroxide,dry heat and photo treatment showed well separated spots of the pure drugs and few degradation spots at various Rf values. ACEBRO showed degradation product peak under acid (0.42) and alkali (0.46) conditions but did not show any observable peak in neutral, oxidation, dry heat and photo condition. DOXO showed degradants peaks for acid (0.51),alkali (0.55), neutral (0.48), oxidation (0.78) and photo (0.75) condition but did not showany observable peak in dry heat stress condition. The degradation peaks developed under various stress condition for both ACEBROand DOXO were well separated from the peak of the intact drugs. The peaks of the ACEBRO and DOXO were not remarkably shifted in the presence of the degradation peaks, which specify the stability-indicating character of the developed method.

Conclusion:

ACEBRO and DOXO were well separated from the peak of the intact drugs. The peaks of the ACEBRO and DOXO were not remarkably shifted in the presence of the degradation peaks, which specify the stability-indicating character of the developed method.

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