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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF AMBROXOL HYDROCHLORIDE AND LORATADINE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT:

Ambroxol Hydrochloride in combination with Loratadine is used in the treatment of cough. Stability indicating HPTLC method has been developed for simultaneous estimation of AMB and LORA. AMB and LORA were separated on silica gel 60 F_{254} TLC plate using *Chloroform: Methanol (9:1* v/v) as mobile phase. Chamber saturation time was 15 min. The optimum wavelength for detection and quantitation used was 216 nm. Both drugs were separated well from each other with R_f values 0.36±0.003for AMB and 0.68±0.002 for LORA. Linearity for AMB and LORA was observed in the range of 600-3600ng/ band and 50-300ng/band respectively. The correlation of coefficient value nearer to 1 also indicates linearity of the method. The method was applied to marketed tablet formulation and the % amount of drug estimated was in good relationship with label claim. The method was validated as per ICH guidelines fo rLinearity, accuracy, precision, and robustness. The accuracy of method was studied by recovery studies at 80%, 100 % and 120 %.The proposed method when used fo restimation of AMB and LORA from its pharmaceutical formulation after over spotting with 80 %, 100 % and 120 % of additional drug showed good drug recovery in the rangeof 99.93 % to 100.01 % for AMB and 99.61 % to 100.86 % for LORA (% RSD lessthan 2) indicates accuracy of method. The precision of the method was expressed as %RSD and observed within limit indicate method is precise. The low value of LOD and LOQ indicates sensitivity of the method. The method robustness was studied by alteration in chromatographic conditions and results were concluded in terms of % RSDand found within accepatable limit for each parameter which express method is robust.

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 re-test period for the drug substance or a shelf life for the drug product and recommended storage conditions. Stress testing studies are conducted to challenge the specificity of stability-indicating methods as part of validation protocol.

To develop and validation of stability-indicating methodology, to determination of degradation pathways of drug substances and drug products, to discernment of degradation product in formulations that are related to drug substances versus those that are related to non-drug substances (e.g.,excipients), Structure elucidation of degradation products, to determine of the inherent stability of a drug substance in solution and solid state and to reveal the therm olytic, hydrolytic, oxidative, and photolytic degradation mechanism of the drug substance

and drug product.

In a quest to make drugs available for ever 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 competen to meet up the requirements to be entitled as 'stability indicating method'. The developed method mus tbe proficient for resolving potential interferents specifically degradation products which are formed during stability evaluation period. The extent of degradation of API understress conditions will be studied.

Extensive literature survey with respect to 'Stability-indicating analytical methods'revealed that stability indicating methods for selected drugs or drug combinations as bulkand/or pharmaceutical formulations are not 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.

Methods:

Solvent

As AMB and LORA both are soluble in methanol, methanol was selected as a solvent.

Instrumentation

HPTLC	Make/Specification
System	A Camag TL Csystem (Muttenz,Swizerland)
Sample Applicator	CamagLinomat5sampleapplicator
Densitometry Scanner	Camag TLC Scanner
Data Processor	Camagwin CATS software (Version 1.4.2
)Development Chamber	Camag twin troughs (20 x10 cm; 10 cm
x10 cm)Syringe	Hamilton syringe(100µl)
Slit Dimension	Set at 6mm
Weighing Balance	ANAMED AA-2200
UV-Chamber	Camag UV-Cabinet

Selection of Detection Wavelength

Stock solutions (10µg/ml) of drugs were prepared in methanol and their isobestic point is observed at216 nm on UV-spectrophotometer shown in **Fig.1**

Preparation of Standard stock solution

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Standard stock solution of LORA was prepared by dissolving 5 mg of drug in 10ml of methanol to get concentration of 500 µg/ml from which 1 ml was further diluted with methanol to get the final concentration 50ng/µl.

Standard stock solution of AMB was prepared by dissolving 60 mg of drug in 10ml of methanol to get concentration of 6000 µg/ml from which 1 ml was further diluted with methanol to get the final concentration 600ng/µl.

Selection of mobile phase and chromatographic conditions

Chromatographic separation studies were carried out on the working standard solution of LORA(10µg/ml)andAMB(10µg/ml).Initially,differentsolventslikeToluene,Methanol,EthylAcetateandBenzeneindifferentproportionsweretried.Finally the combination **Chloroform: Methanol (9:1 v/v)** offered good resolution. This mobile phase system was observed to give compact spots for both AMA and LORA and the R_f values were **0.36±0.003 and 0.68±0.002** for AMB and LORA respectively as showed in **Fig.2.** The chamber saturation time for mobile phase was 15 min and activation of plate was done at 110^0C for five min to obtain distinct spots.

Analysis of Tablet formulation

Twenty tablets were weighed accurately and finely powdered. A quantity of powder equivalent to 5 mg of LORA and 60 mg of AMB was weighed and transferred to a 10 ml volumetric flask containing approximately 5 ml methanol. The mixture wassonicated for 5 min and then the solution was made to the mark with same solvent. Thesolution was filtered using Whatman paper no. 41. One millilitre of the above solutionwas further diluted with methanol to obtain the concentration 50 ng/band for LORA and600 ng/band for AMB. Two microliter volume of this solution was applied on TLC platetofurnishconcentration100ng/band forLORAand1200ng/bandfor AMB.Afterchromatographic development the peak areas of the bands were measured. The same analytical procedure was repeated for six times. AMB and LORA produced distinct peak at R_f 0.35 ±0.003, 0.67 ± 0.002 respectively when scanning was done at 216 nm. The results are given in **Table. 1**. The result also shows that there isno interference between the drug and the excipients presents in tablet formulation.

Validation of Analytical Method[1]Linearity

Calibration was done by automatic sample applicator Linomat 5 on TLC plate togive concentration 600, 1200, 1800, 2400, 3000, 3600 ng/band of AMB and 50, 100, 150,200, 250, 300 ng/band of LORA. The plates were developed in mobile phase. The graphfor calibration was plotted as peak areas versus concentrations. The results are shown in **Table. 2** and Calibration curve in **Fig. 3. and Fig.4.**

Precision

The precision study was performed by intra-day and inter-day variation study. Inthe intraday study, three replicates of three different concentrations of AMB (1800, 2400,3000 ng/band) and of LORA (150,200,250ng/band) were analyzed in a day and percentage RSD was calculated. The % RSD was found to be in the range of0.27-0.66for AMB and 0.84-1.24 for LORA. For the inter day variation study, three

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replicates of three different concentrations were analyzed on three consecutive days and percentage RSD was calculated. The % RSD was found to be in the range of 0.45-0.65 for AMB and0.44-1.02forLORA. The results are shown in **Table.3**

Accuracy

The consistency and accuracy of technique was ensured by recovery study. This study was carried out by spotting a mixture of standard drug solution to pre-analyzed sample solution at three different levels 80, 100 and 120 %.Basic concentration of sample chosen was 1200 ng/band for AMB and 100 ng/band for LORA. The experiment wa carried out in triplicate. Results are depicted in **Table.4.**

Limit of Detection(LOD)

LODiscalculatedfromtheformula:-

$$LOD = \frac{3.3X\sigma}{S}$$

Where,

σ = standard deviation of response for the lowest concentration in the range

S=slope of the calibration curve.

The results of LOD and LOQ are given in **Table. 5.**

Specificity

Specificity of the developed method was ascertained by analyzing standard AMBand LORA and AMB and LORA extracted from tablets. The band for AMB and Lora inthe sample wasconfirmed by comparing the R_f and spectra of the band with thoseobtainedfrom thestandard.The Spectraisshownin **Fig. 5** and **Fig. 6.**

Robustness

As per the ICH, method robustness expresses its capacity to remain unalteredthrough small,deliberatevariationsinparameters ofmethod. The parameters alteredwere change in mobile phase composition (± 1%) and chamber saturation time (□ 2 min).The effects on result are observed by applying 3600 ng/band for AMB and 300 ng/bandforLORA. Results areshown in **Table. 6.**

Forced degradation studies [2-8]

Forced degradation studies were carried under condition of acid, base, neutral hydrolysis, oxidation, photolytic and dry heat in order to evaluate the ability of the proposed method to separate both the drugs from their degradation products.Dryheatand photo degradation study was carried out in solid state. The results are shown in **Table .7**

Acid degradation

From the standard solution of AMB (6000 µg/ml) 1 ml solution was mixed with1ml of 0.1N HCl and 8 ml of methanol. The solution was refluxed at 60°C for 4 hrs. 3 µl volume was applied on TLCplatetogetconcentration1800 ng/band.

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Similarly from the standard stock solution of LORA (500 µg/ml) 1mlsolutionwas mixed with 1ml of 0.1N HCl and 8 ml of methanol. The solution was refluxed at60°C for 4 hrs. 3µl volume was applied on TLC plate to get concentration 150 ng/band. After acid treatment, 12.53 % of degradation was observed for AMB with additional products at R_f 0.48 and 0.52 and 11.78 % degradation was observed for LORA without appearance of degradation productshowninFig.7 and **Fig. 8.**

Alkaline degradation

From the standard solution of AMB (6000 µg/ml) 1 ml solution was mixed with1ml of 0.1N NaOH and 8 ml of methanol. The solution was refluxed at 60°C for 4 hrs. 3µlvolume was applied onTLC platetogetconcentration1800 ng/band.

Similarly from the standard stock solution of LORA (500µg/ml) 1mlsolutionwas mixed with 1ml of 0.1N NaOH and 8 ml of methanol. The solution was refluxed at60°C for 4 hrs. 3µl volume was applied on TLC plate to get concentration 150ng/band. After alkali treatment, 10.9% of AMB was found to undergo degradation with additional degradation product at R_f 0.45 and 7.56% degradation was observed for LORA without additional degradation product shown in **Fig.9** and **Fig. 10.**

Neutral Hydrolytic Degradation

From the standard solution of AMB (6000 µg/ml) 1 ml solution was mixed with1ml of water and 8 ml of methanol. The solution was refluxed at 60°C for 4 hrs. 3µlvolumewas applied on TLCplatetogetconcentration1800 ng/band.

Similarly from the standard stock solution of LORA (500µg/ml) 1ml solution wasmixed with 1ml of water and 8 ml of methanol. The solution was refluxed at 60°C for 4hrs. 3 µl volume was applied on TLC plate to get concentration 150ng/band. On neutral hydrolysis,18.82% degradation was observed for AMB with peak of degradationatR_f

0.19 and 0.24 and 15.70% of degradation was observed for LORA withpeak of degradation at R_f0.58 shown in **Fig.11** and **Fig. 12.**

Oxidative degradation

From the standard solution of AMB (6000 µg/ml) 1 ml solution was mixed with 1ml of 3% solution of H_2O_2 and 8 ml of methanol. The solution was refluxed at 60°C for 4hrs.3 µl volume was applied on TLC plate to get concentration 1800ng/band.

Similarly from the standard stock solution of LORA (500µg/ml) 1ml solution wasmixed with 1 ml of 3% solution of H_2O_2 and 8 ml of methanol. The solution was refluxedat60°Cfor4hrs.3µl volumewasappliedonTLCplatetogetconcentration150ng/band. In the oxidative condition, 9.07% degradation was observed for AMB with no peak of degradation and 19.22 % of degradation was observed for LORA with peak of degradation at R_f0.61 shown in **Fig.13** and **Fig. 14.**

Degradation under dry heat

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Dryheatstudieswereperformedbykeepingdrugsamplesseperatelyinoven(80^0C)foraperiodof2hour.Samples were with drawn after 2hr, dissolved in methanol and diluted appropriately to get concentration of 600µg/ml for AMB and 50µg/ml for LORA. 3µl volume was applied on TLC plate to get concentration 1800ng/ band for AMB and 150ng/ band for LORA.Under dry heat degradation condition,AMB showed 8.87% of degradation with additional product at R_f 0.40 and 18.39 % LORA was found to be degraded with peak of degradation at R_f values 0.78 shown in **Fig. 15** and **Fig. 16.**

Photo-degradation studies

Photolytic study was carried out by exposure of drug individually to UV light upto 200 watt hours/square meter for period of 4 hrs. Sample was weighed, dissolved anddiluted to get 1800 ng/band and 150 ng/band concentration for AMB and LORA resp.After exposing to light, 11.02% of AMB was found to be degraded with additional peakof degradation at R_f .0.48 and 9.57 % of LORA was found to be degraded with peak ofdegradation at R_f0.80 shownin **Fig.17** and **Fig. 18.**

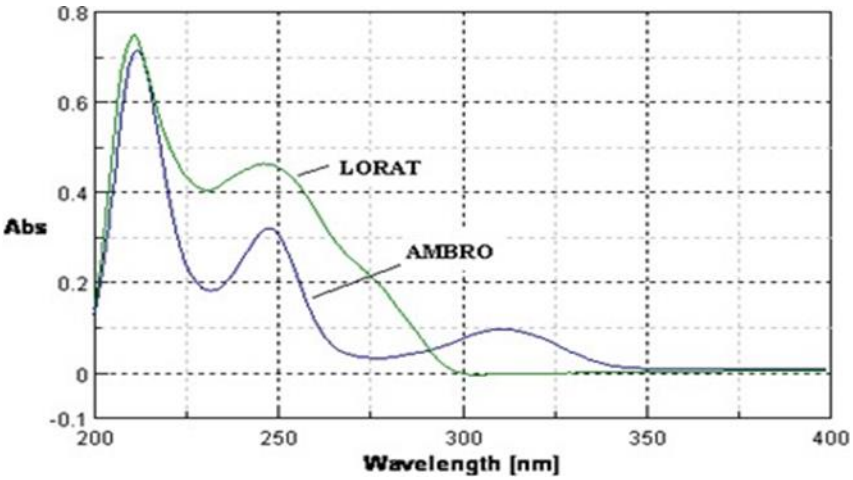
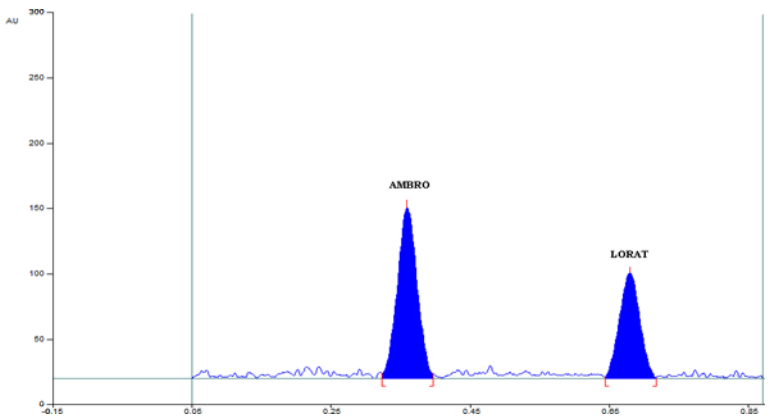


Fig. 1. Overlain UV Spectra of AMB and LORA



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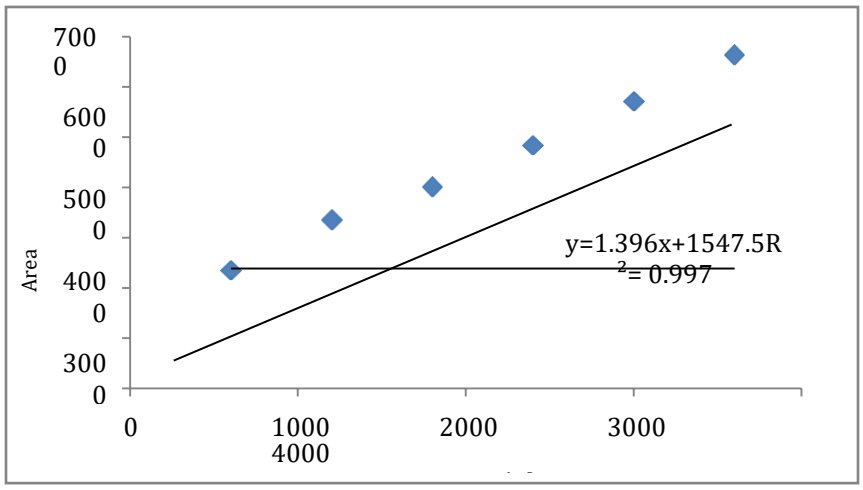

Fig. 3. Calibration curve of Ambroxol Hydrochloride

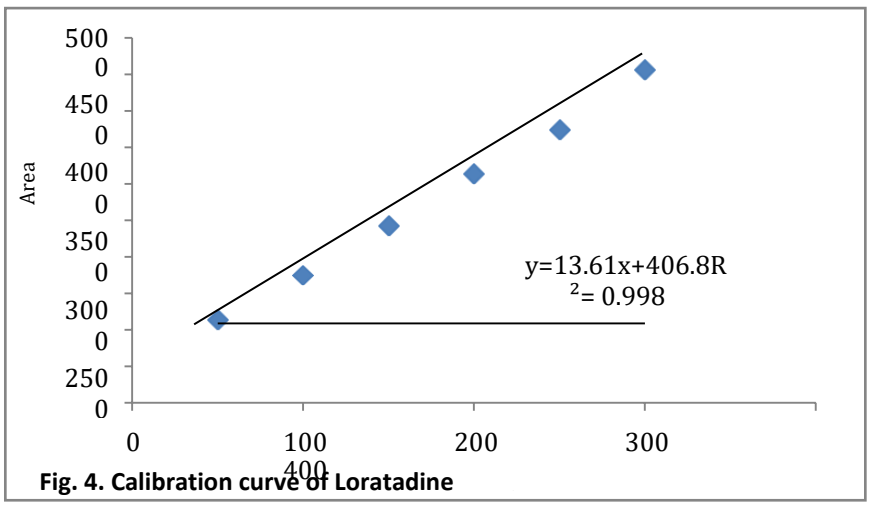

Fig. 4. Calibration curve of Loratadine

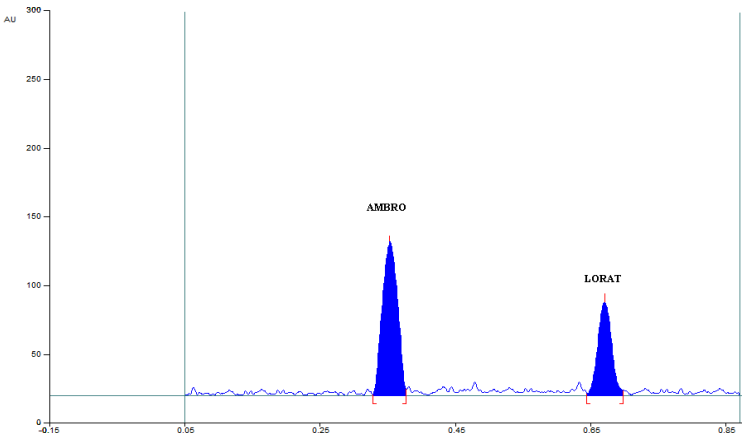

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Fig. 5. Densitogram of Sample (AMB of 1200 ng/band, R_f = 0.35 ±0.003 and LORA of 100ng/band, R_f=0.67 ±0.002)

DISCUSSION:

Ambroxol Hydrochloride in combination with Loratadine is used in estimation of AMB and LORA. AMB and LORA were separated on silica gel 60 F_{254} TLC plate using ***Chloroform: Methanol (9:1 v/v)*** as mobile phase. Chamber saturation time was 15 min. The optimum wavelength for detection and quantitation used was 216 nm. Both drugs were separated well from each other with R_f values **0.36±0.003**for AMB and **0.68±0.002** for LORA. Linearity for AMB and LORA was observed in therangeof600-3600ng/bandand50-300ng/bandrespectively.Thecorrelationofcoefficient value nearer to 1 also indicates linearity of the method. The method was applied to marketed tablet formulation and the % amount of drug estimated was in goodrelationshipwithlabelclaim.ThemethodwasvalidatedasperICHguidelinesforLinearity, accuracy, precision, and robustness. The accuracy of method was studied by recovery studies at 80%, 100 % and 120 %.The proposed method when used for estimation of AMB and LORA from its pharmaceutical formulation after over spotting with 80 %, 100 % and 120 % of additional drug showed good drug recovery in the range**of 99.93 % to 100.01 % for AMB and 99.61 % to 100.86 % for LORA** (% RSD lessthan 2) indicates accuracy of method. The precision of the method was expressed as %RSD and observed within limit indicate method is precise. The low value of LOD and LOQ indicates sensitivity of the method.The method robustnes s was studied by alteration in chromatographic conditions and results were concluded in terms of % RSDand found within accepatable limit for each parameter which express method is robust. Method.

Conclusion:

AMB and LORA were exposed to various stress degradation conditions. Peaks obtained from the samples degraded by acid, alkali, water, hydrogen peroxide, dry heat and Photo treatment showed well separated spots of pure drugs and few degradation products spots at various Rf values. AMB showed degradation product peak under acid (**0.48, 0.52**), alkali (**0.45**), neutral (**0.19, 0.24**), dry heat (**0.40**) and Photo (**0.48**) conditions but did not show any observable peak in oxidation condition. LORA showed degradants peaks for neutral (**0.58**), oxidation (**0.61**), dry heat (**0.78**) and Photo (**0.80**) condition but did not show any observable peak in acid and alkali stress condition. The degradation peaks developed under various stress conditions for both AMB and LORA were well separated from the peak of the intact drugs. The peaks of the AMB and LORA 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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