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TO INVESTIGATE THE DEGRADATION PROPERTIES OF THE COMBINATION OF LEVOSULPIRIDE AND RABEPRAZOLE SODIUM USING LC–MS

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ABSTRACT

Levosulpiride, an atypical antipsychotic, is primarily used to treat psychiatric conditions, while Rabeprazole, a proton pump inhibitor, is indicated for managing various gastrointestinal disorders. When combined, Levosulpiride and Rabeprazole Sodium are commonly prescribed for the treatment of gastro-oesophageal reflux disease, indigestion, as well as duodenal and gastric ulcers. Studying the degradation properties of combined active pharmaceutical ingredients is a critical aspect of pharmaceutical research, essential for ensuring the safety, efficacy, and stability of drug formulations throughout their shelf life. Based on this, the main aim of the present research was to examine the degradation behaviour of Levosulpiride and Rabeprazole Sodium, in combination, using liquid chromatography combined with mass spectrometry. Chromatographic separation was achieved using a Hypersil BDS C18 column with a specified mobile phase flow rate of 1.0 mL/min. A gradient mode was employed for the separation of Rabeprazole Sodium and Levosulpiride using a mobile phase consisting of 2 mM ammonium formate (Mobile Phase A) and acetonitrile (Mobile Phase B), with detection carried out at 210 nm. Levosulpiride and Rabeprazole Sodium were exposed to various stress conditions, and their degradation products were subsequently analyzed using LC-MS. Few degradation products were successfully identified, corresponding to degradation under acidic, basic, and oxidative conditions, respectively. The characteristic degradation behaviour under each specific stress condition was quantified and expressed as a percentage. In conclusion, novel impurities in the selected combination provide crucial stability insights, supporting formulation, quality control, and future research.

Keywords: Degradation Properties, Forced Degradation Studies, Levosulpiride, Liquid Chromatography–Mass Spectrometry, Rabeprazole Sodium.

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INTRODUCTION

Levosulpiride (LSP) functions as an atypical antipsychotic and gastroprokinetic agent by antagonising dopamine D2 receptors.[1] Rabeprazole sodium (RAB) is a proton pump inhibitor commonly used for the treatment of gastric ulcers, peptic ulcers, gastroesophageal reflux disease, duodenal ulcers, and Zollinger-Ellison syndrome.[2] The combination of LSP and RAB is commonly prescribed for the treatment of gastroesophageal reflux disease, management of psychiatric disorders, and control of acid secretion linked to stress-related conditions.[3] The safety and therapeutic efficacy of pharmaceutical products are heavily dependent on the chemical stability of their active pharmaceutical ingredients. To assess this stability, regulatory authorities such as the FDA and ICH mandate the execution of forced degradation studies in accordance with established guidelines.[4] Forced or stress degradation studies involve evaluating the stability of a drug substance under intensified or accelerated conditions. These studies are essential for identifying potential degradation products and assessing the intrinsic stability of pharmaceutical molecules. Typically, forced degradation is performed by exposing the active pharmaceutical ingredient or drug product to various stress conditions, including acidic, basic, oxidative, photolytic, and thermal conditions. Several analytical methodologies have been reported for the estimation of LSP and RAB in both individual and combined dosage forms with other drugs.[5-9] A LC–MS/MS method for the quantification of LSP in human plasma and its application in bioequivalence studies has been documented.[10] Additionally, a high-throughput UPLC–MS/MS method has been developed for the simultaneous quantification of Esomeprazole, RAB, and LSP in human plasma.[11] Although the

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degradation products of LSP and RAB have been individually characterized in previous studies, the collective behaviour of stress-induced degradation and the resulting degradation products in the combined formulation has not yet been reported.[12,13,14] This gap highlights a critical research opportunity to elucidate the stability profile of RAB and LSP in their combined dosage form. In the present study, a rapid and highly sensitive LC-MS method was used along with a conventional HPLC method for investigating the degradation behaviour of both RAB and LSP in their combined dosage forms.

EXPERIMENTAL

Material and Methods

RAB and LSP were obtained from Dhamtech Pharma (Mumbai, India), while all other reagents were of analytical or HPLC grade and were procured from well-known commercial vendors. Method development and validation were performed on an HPLC system (Waters Alliance e2695) with PDA detection, and degradation product analysis was carried out using LC–MS systems (Thermo Fisher, Shimadzu, and Bruker) equipped with an ESI source in positive ion mode. Chromatographic separation was achieved on a Hypersil BDS C18 column. The mobile phase was optimised for LC–MS analysis using an LCQ Fleet system (Thermo Fisher Scientific, USA), and separation was achieved on a Hypersil BDS C18 column (250 × 4.6 mm, 5 µm). A gradient program consisting of Mobile Phase A (2 mM ammonium formate) and Mobile Phase B (acetonitrile) was employed, starting at 90:10 (A: B) and shifted to 30:70 before being returned to the initial conditions within a 20-minute run. Detection was carried out at 210 nm.

General Procedure

A mixed standard solution of RAB (40 µg/mL) and LSP (75 µg/mL) was prepared from accurately weighed quantities of the pure drugs. Further dilutions were prepared to obtain concentration ranges of 20–60 µg/mL for RAB and 37.5–112.5 µg/mL for LSP, respectively. For sample preparation, 20 capsules were taken, and the powdered sample equivalent to 40 mg of RAB (with the corresponding proportion of 75 mg of LSP) was dissolved in diluent, sonicated, and appropriately diluted to achieve final concentrations of RAB and LSP within the selected range. The resulting solution was then filtered through a 0.45 µm PTFE syringe filter prior to analysis. Acid and base degradation studies of RAB and LSP were carried out by treating 1 mL of the standard stock solution with 1 mL of 1N HCl or 1N NaOH, respectively, allowing the reaction to proceed for 6 hours, followed by neutralisation and dilution to obtain final concentrations of 40 µg/mL (RAB) and 75 µg/mL (LSP). Oxidative degradation was performed by treating 1 mL of the stock solution with 1 mL of 1% H_2O_2 for 10 minutes, followed by dilution to obtain the same final concentrations. For thermal degradation, 115 mg of the standard RAB–LSP mixture (equivalent to 40 mg of RAB and 75 mg of LSP) was exposed to 75 °C for 24 hours, and suitable amounts were dissolved and diluted to achieve the required concentrations. Similarly, photolytic degradation was carried out by exposing 115 mg of the RAB–LSP mixture to a photostability chamber for 24 hours, followed by solution preparation and dilution to final concentrations of 40 µg/mL (RAB) and 75 µg/mL (LSP). After all these treatments, conventional chromatography followed by the optimised LC-MS analytical methodology was applied for detailed investigation of the stability behaviour of both RAB and LSP in the presence of each other. Analytical method validation was performed according to ICH Q2 (R2) guidelines. Since the study focused on forced degradation and stability pathways of the RAB–LSP combination, these parameters were specifically evaluated to ensure method reliability.

RESULTS AND DISCUSSION

The individual APIs exhibited distinct degradation behaviours under various stress conditions. When subjected to forced degradation in combination, the interaction between RAB and LSP influenced their stability profiles. In some cases, the presence of one compound enhanced the formation of degradation products, whereas in others, it appeared to suppress impurity generation or reduce the intensity of degradation peaks. Based on this premise, the primary objective of the present study was to optimise a robust mobile phase for the simultaneous estimation of RAB and LSP using HPLC, perform forced degradation studies on their mixture, and further investigate the possible degradation products along with the percentage of degradation of both drugs in the presence of each other using LC–MS. Mobile phase

optimisation was successfully achieved through a gradient elution strategy, as detailed in the methodology, which enabled effective resolution and accurate quantification of both analytes. Under the optimised chromatographic conditions, distinct retention times of 3.306 minutes for RAB and 8.284 minutes for LSP were consistently obtained, confirming the suitability of the method for simultaneous estimation. The chromatogram of the RAB–LSP mixture was represented in Fig.-1, as shown below.

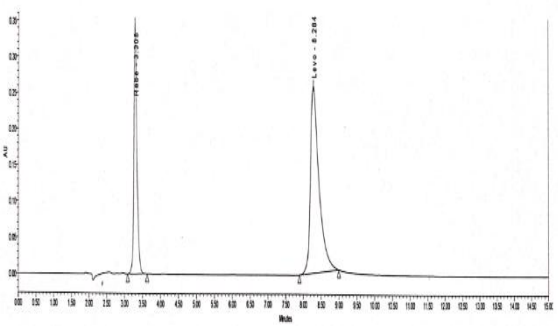

Fig.-1: Chromatogram of RAB-LSP Mixture

The forced degradation studies were performed under different stress conditions, including acidic, basic, oxidative, thermal, and photolytic environments, to assess the stability of the combined formulation. The degradation products were successfully identified and characterised using LC-MS, confirming the presence of new degradation impurities that have not been previously reported in the literature. The optimised conditions demonstrated high specificity, sensitivity, and reliability for both drug substances.

Analytical Method Validation

The developed chromatographic method was validated for key analytical parameters as mentioned in the ICH guidelines of AMV. As the primary objective of the study was to assess the forced degradation behaviour and stability profile of RAB and LSP, these validation parameters were critically evaluated to ensure the robustness and reliability of the analytical method employed. The developed method demonstrated excellent linearity for both analytes, with a concentration range of 20–60 µg/mL for RAB and 37.5–112.5 µg/mL for LSP, yielding correlation coefficients of 0.9986 and 0.9983, respectively. Method precision was confirmed by repeatability values of 1.14% RSD for RAB and 0.93% RSD for LSP. Interday precision was 0.81% RSD for RAB and 0.93% RSD for LSP, while intraday precision was 1.21% RSD and 0.86% RSD, respectively, all well within acceptable limits. Robustness studies under varied analytical conditions confirmed method reliability, with RSD values ranging from 0.98% to 1.51% for RAB and 0.48% to 1.38% for LSP. Accuracy results further supported the method's validity, with mean recoveries of 99.97 ± 0.91% for RAB and 100.3 ± 0.81% for LSP. The LOD and LOQ were found to be 1.19 µg/mL and 3.60 µg/mL for RAB, and 0.86 µg/mL and 2.62 µg/mL for LSP, respectively. System suitability parameters were also satisfactory, with retention times of 3.39 ± 0.002 minutes for RAB and 8.3 ± 0.015 minutes for LSP, theoretical plate counts of 9167 and 6955, and tailing factors of 1.17 and 1.64, respectively, for RAB and LSP. The forced degradation studies highlighted the chemical stability of the drug combination and offered critical insights into its potential degradation pathways under various stress conditions. These findings contribute to a deeper understanding of the degradation behaviour of LSP and RAB, providing valuable information for ongoing formulation development and current quality control practices in pharmaceutical applications.

Degradation Behaviour of RAB and LSP on LC/MS System

The combination of RAB and LSP was subjected to various stress conditions-including acidic, basic, oxidative, photolytic, and dry thermal stress conditions-for predetermined time intervals to assess their degradation behaviour. LC-MS analysis revealed that RAB is susceptible to acidic, basic, and oxidative stress, as evidenced by observable changes in its molecular mass. Conversely, RAB exhibits greater stability under photolytic and thermal stress, with no significant degradation detected. In contrast, LSP consistently maintains its molecular ion peak at 342.26 Da across all stress conditions. As a result, no

notable variations in the mass of LSP are observed under the applied stress conditions. Thus, it can be inferred that the behaviour of the RAB–LSP combination differed significantly when compared to their individual responses under various stress conditions. This variation may be attributed to possible drug-drug interactions within the mixture, wherein the presence of one analyte influences the stability of the other. Such interactions can either accelerate degradation by promoting additional pathways or, conversely, exert a protective effect by reducing impurity formation. These findings highlight the importance of evaluating drug substances not only individually but also in combination, particularly when they are intended for fixed-dose formulations, as the stability profile of the mixture may not simply reflect the sum of the individual components. The mass spectra of the standard samples revealed molecular ion peaks at m/z 342.26 Da for LSP and 360.19 Da for RAB, aligning with their respective theoretical molecular weights. Figure-2 represents the mass spectra of RAB and LSP.

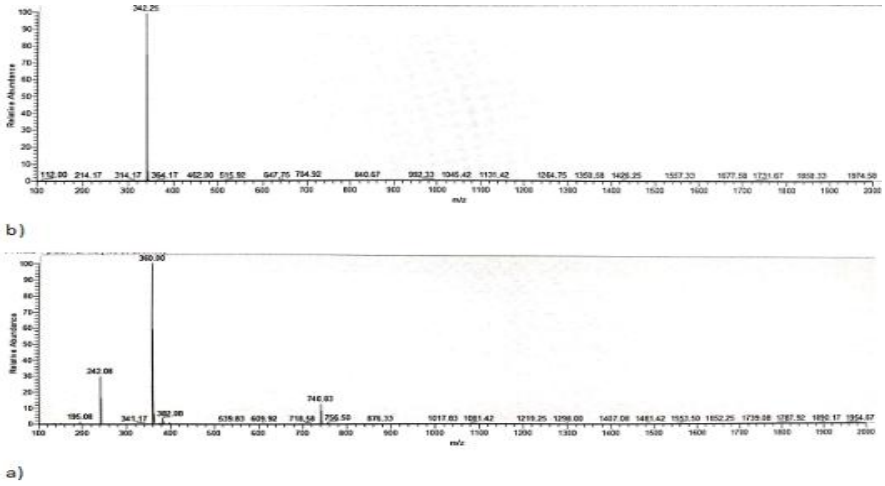

Fig.-2: Mass Spectra of Pure RAB (360.19) and LSP (342.26 Da)

The RAB–LSP mixture was subjected to various stress conditions to assess its stability profile. Under acid (1N HCl, 6h) and base (1N NaOH, 6 h) degradation, HPLC revealed the formation of two degradation products in each case (DP1–DP2 and DP3–DP4, respectively), while LC-MS confirmed the intact molecular mass of LSP (342.24 Da) with no detectable degradation. In contrast, RAB exhibited significant degradation under both conditions, with a reduction in molecular mass from 360.19 Da to 312.22 Da. Oxidative degradation (1% H_2O_2, 10 min) led to the formation of a single degradation product (DP5) in HPLC, though it was not observed in LC-MS, likely due to gradient elution. LSP again remained stable, showing the same molecular mass (342.22 Da), whereas RAB showed oxidative transformation with an increased mass of 376.23 Da, suggesting the incorporation of an additional oxygen atom. Thermal and photolytic stress studies showed no significant changes, as no extra peaks were detected in the HPLC chromatograms, confirming the stability of the RAB–LSP mixture under these conditions. The chromatograms of the RAB–LSP mixture obtained under the respective stress conditions are presented in Fig.-3. The corresponding MS spectra following hydrolytic degradation are shown in Fig.-4, while those obtained after oxidative degradation are depicted in Fig.-5.

Proposed Degradation Pathways and Summary of Percentage Degradation of RAB and LSP in the RAB–LSP Mixture

Forced degradation studies on the RAB–LSP mixture demonstrated distinct stability patterns for the two drugs under various stress conditions. Under acidic stress (1N HCl, 6 h), RAB showed extensive degradation (98.96%), with LC–MS confirming the conversion of its molecular ion from m/z 360.19 Da to 312.22 Da, whereas LSP exhibited 42.67% degradation without any shift in its molecular ion (m/z 342 Da). In alkaline conditions (1N NaOH, 6 h), RAB degraded by 19.52% with the same m/z shift (360.19 Da → 312.22 Da), while LSP underwent 47.16% degradation, again retaining its molecular ion at m/z 342 Da.Under oxidative stress (1% H_2O_2, 10 min), RAB degraded by 5.61%, showing an increase in mass

from 360.19 Da to 376.23 Da, suggestive of oxygen incorporation, while LSP degraded by 7.13% with no change in its m/z value. Thermal stress (75 °C, 24h) caused only slight degradation of RAB (3.11%) and LSP (2.22%), with no detectable mass fragments. Similarly, photolytic exposure (UV light, 24 h) resulted in minimal degradation, 2.31% for RAB and 0.94% for LSP, with no detectable LC–MS changes.

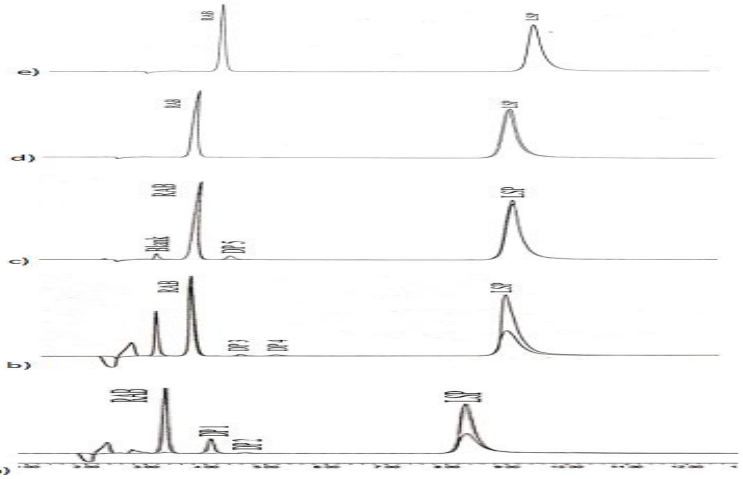

Fig.-3: Overlay Chromatograms of RAB and LSP before and after Forced Degradation: (a) Acid Degradation with 1N HCl for 6 h, (b) Base Degradation with 1N NaOH for 6 h, (c) Oxidative Degradation with 1% H_2O_2 for 10 min, (d) Thermal Degradation at 75 °C for 24 h, and (e) Photolytic Degradation for 24 h.

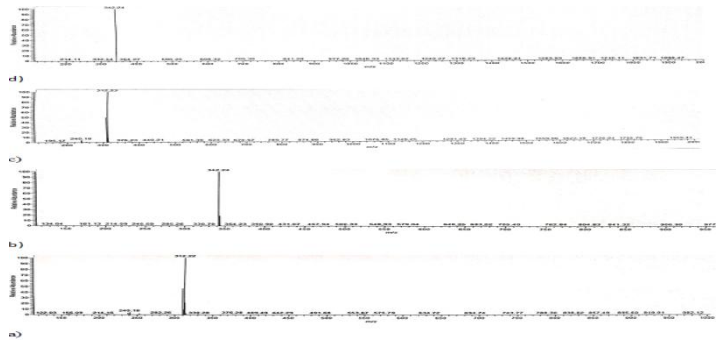

Fig.-4: Mass Spectra of RAB and LSP in the RAB–LSP Mixture After Hydrolytic Forced Degradation: (a) RAB in 1N HCl for 6 h, (b) LSP in 1N HCl for 6 h, (c) RAB in 1N NaOH for 6 h, and (d) LSP in 1N NaOH for 6 h

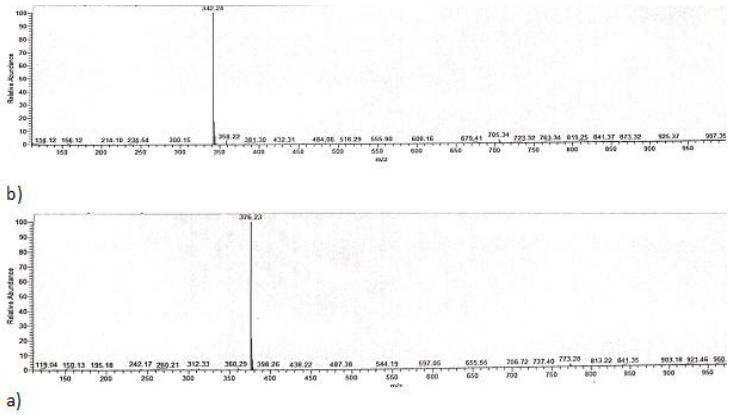

Fig.-5: Mass Spectra of RAB and LSP in the RAB–LSP Mixture After Oxidative Forced Degradation with 1% H_2 O_2 for 10 min: (a) RAB and (b) LSP

These findings highlight that RAB is highly acid-labile, undergoing almost complete degradation through elimination and rearrangement reactions, forming a degradation product (DP1) at m/z 312.22 Da. In

contrast, LSP was comparatively more sensitive under alkaline conditions. Under oxidative stress, RAB formed an oxidised product, with LC–MS confirming a mass shift from 360.19 Da to 376.23 Da, consistent with the incorporation of an additional oxygen atom. The proposed degradation pathways and structures of RAB under acidic and oxidative conditions in the RAB–LSP mixture are depicted in Fig.-6.

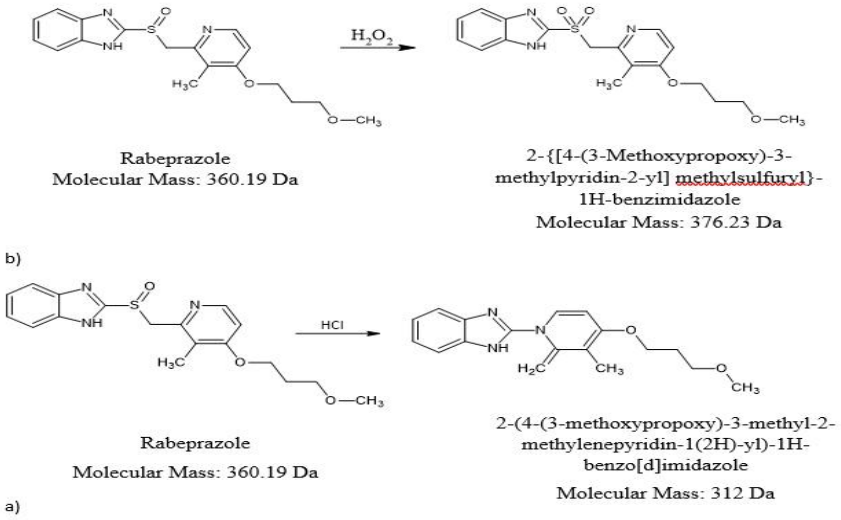

Fig.-6: The Proposed Structures of RAB Degradation Products Under Acidic and Oxidative Conditions in the RAB–LSP Mixture

CONCLUSION

This study systematically evaluated the degradation behaviour of Levosulpiride and Rabeprazole Sodium in combination using LC–MS. Forced degradation studies confirmed the formation of novel impurities under acidic, basic, and oxidative stress conditions, thereby elucidating critical aspects of their stability profile. RAB was found to be highly susceptible to acidic and oxidative degradation, whereas LSP demonstrated greater vulnerability under alkaline conditions. In contrast, both drugs exhibited negligible degradation under thermal and photolytic stress. These findings underscore the importance of comprehensive stability assessment in fixed-dose combinations to ensure formulation quality, therapeutic efficacy, and patient 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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