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Development and Validation of a Stability-Indicating HPLC and RP-HPLC Method for the Simultaneous Quantification of Levosulpiride and Rabeprazole.

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ABSTRACT

In this work, stability-indicating HPLC and RP-HPLC techniques for the simultaneous measurement of levosulpiride and rabeprazole in pharmaceutical formulations are developed and validated. Clear separation with symmetrical peaks, low tailing factors, and high theoretical plates was the goal of both optimisation techniques. Levosulpiride (10–100 µg/mL) and Rabeprazole (5–50 µg/mL) were found to be linear in HPLC and 60–140% standard concentrations in RP-HPLC, respectively, with correlation coefficients >0.99. Accuracy and precision were confirmed by mean recoveries ranging from 98 to 102% and a %RSD of less than 2%. Stress degradation was minor (<5%) under acidic, basic, oxidative, UV, thermal, and humidity environments. HPLC offered faster analysis, whereas RP-HPLC offered improved peak resolution. Both techniques were successfully applied to commercial formulations without excipient interference and met ICH Q2(R1) requirements.

Keywords: Levosulpiride; Rabeprazole; Stability-Indicating; HPLC; RP-HPLC; Method Validation; Stress Degradation; Simultaneous Estimation; ICH Q2(R1).

INTRODUCTION

5-(aminosulfonyl)-N-[(1-ethyl-2-pyrrolidinyl) methyl]-2 methoxybenzamide is the chemical formula for levosulpiride . A refined levo-isomer of sulpiride is called levosulpiride. It is not included in any pharmacopoeia as official. The levo-form of sulpiride exhibits reduced acute toxicity, antiemetic and anti-dyspeptic actions, and more central anti-dopaminergic activity than the racemic and dextro-forms. 4-[[4-(3methoxypropoxy)-3-methyl-2-pyridinyl]-methyl] sulfinyl] is the chemical formula for rabeprazole.(1) Sodium salt of -1H-benzimidazole . Patients with functional dyspepsia, depression, and psychosis are frequently administered levosulpiride, a D2-dopamine receptor antagonist. By inhibiting dopamine autoreceptors, which prevents pre-synaptic dopamine synthesis and release, levosulpiride, at low doses, enhances dopaminergic neurotransmission .(7) The levo-form of sulpiride exhibits decreased acute toxicity, antiemetic and anti-dyspeptic actions, and stronger central anti-dopaminergic activity in comparison to the racemic and dextro-forms.[1]

Rabeprazole is an antiulcer medication that belongs to the proton pump inhibitor class and is used to treat duodenal ulcers and GERD . It is a prodrug because it changes into the active sulphenamide form in the parietal cells' acidic environment. The drug Rabeprazole prevents the K+AT, H+ The coating of the stomach cells and dose-dependent suppression of the basal and stimulated production of gastric acid.(1)

Few techniques have been documented for the simultaneous determination of RABE sodium and LEVO in a combination dose form, despite the fact that numerous methods, including HPLC, UV, LC-MS, HPTLC, and capillary zone electrophoretic method, have been published in the literature for the individual estimate of RABE and LEVO. The current study offers two novel techniques for RABE and LEVO simultaneous determination utilising reverse phase HPLC and HPLC.(1)

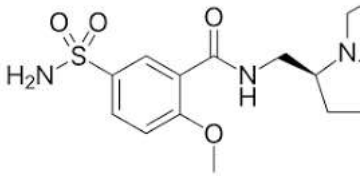

Fig.no.1 Levosulpiride

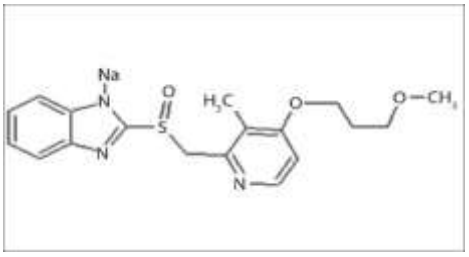

Fig.no.2 Rabeprazole

EXPERIMENTAL METHODS

Reagents and chemicals.

Levosulpiride and Rabeprazole sodium were obtained as gift samples from Dr. Reddy's Laboratories for the HPLC process, which used acetonitrile, methanol, and Milli-Q water as solvents.

Buffers were prepared using potassium dihydrogen orthophosphate and sodium hydroxide.[1]

In the RP-HPLC method, Levosulpiride (gift from Unichem Laboratories) and Rabeprazole (Sigma-Aldrich) were used, with methanol and Milli-Q water as solvents. Orthophosphoric acid and glacial acetic acid were employed for pH adjustment, and RABONIK® tablets were analyzed as the marketed formulation.[2]

Instrumentation.

An LC-10ATvp pump, SIL-10ADvp autosampler, CTO-10Avp oven, and SPD-10Avp UV-Vis detector were all part of the Shimadzu Class VP system used for HPLC. An autosampler and Empower2 software were used for data processing in a Shimadzu LC-2010 system with a UV/VIS or PDA detector and a Hypersil BDS C18 (250 × 4.6 mm, 5 µm) column for RP-HPLC.

Chromatographic conditions.

Both methods employed C18 columns but with variations in mobile phase and detection. The HPLC method used an Agilent Polaris C18 (150 × 4.6 mm, 5 µm) with a mobile phase of methanol–0.1% orthophosphoric acid (60:40 v/v) at a flow rate of 1.0 mL/min, detecting at 232 nm. Another HPLC approach reported a buffer–acetonitrile system (72:28 v/v), pH adjusted with sodium hydroxide, filtered and sonicated, with a higher flow rate of 1.5 mL/min and detection at 282 nm. In contrast, the RP-HPLC method employed a longer C18 column (250 × 4.6 mm, 5 µm) with methanol–water (70:30 v/v, pH 4.0 with orthophosphoric acid), pre-filtered and sonicated, at a flow rate of 1.2–1.5 mL/min, with detection at 254–282 nm. The injection volume was consistently 20 µL.

Preparation of standard solution ,calibration standard and quality control of standard

In HPLC method, stock solutions of Levosulpiride and Rabeprazole (1 mg/mL each) were prepared in methanol and diluted with 50:50 methanol–Milli-Q water. Calibration curves were constructed over 5–50 µg/mL for Rabeprazole and 10–100 µg/mL for Levosulpiride with eight non-zero standards. Specificity was evaluated using single-drug, blank, and mixed solutions. Quality control samples were prepared from separate stocks at low, medium, and high concentrations (LQC, MQC, HQC) to validate accuracy and precision.In the RP-HPLC method, a combined standard stock was prepared by dissolving 25 mg of Rabeprazole sodium and 75 mg of Levosulpiride in methanol, sonicated, and diluted to volume. Further dilutions were made with mobile phase after filtration. For sample analysis, powdered tablets equivalent to one dose were dissolved in methanol, sonicated, filtered, and diluted with mobile phase before injection.

Method Validation

System Suitability

Six replicate injections of 100% levosulpiride and rabeprazole sodium were used to assess the system appropriateness for RP-HPLC, and the percentage RSD of the peak regions was noted. Six replicates of a 50 µg/mL mixture of both medications were examined for HPLC, and system compatibility was determined if the retention time %CV was less than 1%.

Linearity (Calibration curve)

Six replicate injections of 100% levosulpiride and rabeprazole sodium were used to assess the system appropriateness for RP-HPLC, and the percentage RSD of the peak regions was noted. Six replicates of a 50 µg/mL mixture of both medications were examined for HPLC, and system compatibility was determined if the retention time %CV was less than 1%

Specificity

Six replicate injections of 100% levosulpiride and rabeprazole sodium were used to assess the system appropriateness for RP-HPLC, and the percentage RSD of the peak regions was noted. Six replicates of a 50 µg/mL mixture of both medications were examined for HPLC, and system compatibility was determined if the retention time %CV was less than 1%

Precision

Repeatability (intra-day) and intermediate precision (inter-day) were used to establish the assay's precision for HPLC. The application of the analytical process in the lab over a shorter time frame that was assessed by assaying the QC samples on the same day is referred to as repeatability. Comparing the assays conducted on three distinct days allowed for the evaluation of intermediate precision. Precision for RP-HPLC was evaluated based on application and measurement repeatability. Six repetitions of the same sample concentration were used to test the repeatability of sample application.

Accuracy

Using triplicate analysis of the QC samples, the accuracy of the assay method for HPLC was assessed for both intra-day and inter-day fluctuations. Regarding RP-HPLC, % Recovery tests were conducted in triplicate at three distinct concentrations of standard solution (i.e., levosulpiride and beta-adrenazole sodium API added to the placebo): 80%, 100%, and 120%.

Robustness

Robustness was evaluated for HPLC by adjusting the mobile phase composition (±5%) and flow rate (1.0 ± 0.1 mL/min). Reduced flow rate resulted in somewhat higher tailing factors (1.27–1.33) and longer retention durations (Rabeprazole: 4.63 ± 0.10 min; Levosulpiride: 2.48 ± 0.10 min). Levosulpiride: 2.06 ± 0.02 min; Rabeprazole: 3.79 ± 0.01 min; higher flow rate shortened retention. Retention durations with 75:25 methanol:20 mM orthophosphoric acid (pH 3.0) were 2.48 ± 0.03 and 4.63 ± 0.02 minutes (n = 6). The robustness of the suggested approach for RP-HPLC was assessed by analysing aliquots from homogenous lots using varying physical factors, such as flow rate, column temperature, and buffer composition, which may vary yet still produce responses that fell within the predetermined ranges.

Stability.

Levosulpiride and Rabeprazole sodium stability in analytical solution for rphplc was confirmed by analysing the sample solution both initially and at various intervals of roughly 2, 4, 8, and 12 hours while being stored at 250c. The results were expressed as the percentage RSD of peak regions. For HPLC, the drug's stability is assessed by storing the MQC samples for a maximum of 12 hours at room temperature, after which the peak area is compared to that of a freshly prepared sample that was prepared similarly. Additionally, auto-sampler stability for a whole day was investigated and determined.

Stress degradation studies

For HPLC stress degradation, 1 mL MQC samples (in duplicate) were exposed for 24 h to 0.1 N HCl (acidic), 0.1 N NaOH (alkaline), 5% H_2O_2 (oxidative), or UV light (photolytic). Treated samples were analyzed and compared with fresh controls in triplicate.For RP-HPLC, By analysing the sample solution both initially and at various intervals of roughly 2, 4, 8, and 12 hours while storing at 250c, the stability of levosulpiride and rabeprazole sodium in analytical solution was confirmed. The results were expressed as the percentage RSD of peak regions.

Results and Discussion

The stability-indicating HPLC and RP-HPLC methods for simultaneous estimation of Levosulpiride and Rabeprazole were optimized and validated according to ICH guidelines. Both methods exhibited clear separation with symmetrical peaks, minimal tailing, and good resolution.

System Suitability:

For RP-HPLC, Levosulpiride and Rabeprazole showed retention times of 2.23 min and 7.27 min, respectively, with tailing factors of 1.34 and 1.07 and theoretical plates of 4282 and 10362. For HPLC, retention times were 2.1 ± 0.10 min (Levosulpiride) and 4.1 ± 0.10 min (Rabeprazole) with theoretical plates ~5153 and ~10883 and tailing factors ~1.25.

Linearity:

RP-HPLC displayed linearity over 60–140% of the standard concentration with correlation coefficients of 0.9943 (Levosulpiride) and 0.99998 (Rabeprazole). HPLC showed linearity between 10–100 µg/mL (Levosulpiride) and 5–50 µg/mL (Rabeprazole) with $r^2 \geq 0.99$.

Accuracy and Precision:

Mean recoveries were 99.54% (Levosulpiride) and 98.94% (Rabeprazole) for RP-HPLC, and 99.85% (Levosulpiride) and 101.25% (Rabeprazole) for HPLC. %RSD values for both methods were <2%, confirming precision.

Robustness:

Both methods were robust against small changes in flow rate (±0.1 mL/min), column temperature, and mobile phase composition (±5%). Retention times varied slightly (e.g., Rabeprazole: 3.79–4.63 min; Levosulpiride: 2.06–2.48 min), but system suitability remained within acceptance limits.

Stress Degradation Studies

Under acid, base, oxidative, UV, thermal, and humidity stress, both drugs showed minimal degradation (<5%) in most conditions, except notable alkaline instability for both compounds in HPLC analysis. RP-HPLC confirmed degradation peaks were well-resolved from the main analyte peaks, ensuring specificity.

Solution Stability:

Samples remained stable up to 12–24 hours at room temperature with %RSD <2% in both methods.

Assay of Marketed Formulations:

Both methods successfully quantified Levosulpiride and Rabeprazole in tablets with assays near 100%, and no excipient interference was observed.

Conclusion

The developed HPLC and RP-HPLC methods are accurate, precise, robust, and stability-indicating for simultaneous estimation of Levosulpiride and Rabeprazole. HPLC offers a simpler and faster approach for routine analysis, while RP-HPLC provides superior resolution and robustness, particularly in the presence of degradation products. Both methods meet ICH Q2(R1) requirements and are suitable for quality control, stability studies, and regulatory compliance of combined formulations.

Reference

1. Atulita Mullapud et.al.,(2013) Development and Validation of a stability indicating method for the simultaneous determination of Levosulpiride and Rabeprazole by High Performance Liquid Chromatography,Indian Journal of Pharmaceutics and analysis.vol:1.
2. Nandakishore agarwal et.al.,(2012)Development and Validation of Stability indicating RP-HPLC method for simultaneous estimation of levosulpiride and rabeprazole sodium, International Journal of Pharma and Bio Sciences.
3. Atul Baravkar et.al.,UV Spectroscopic Method Development and Validation of Rabeprazole and Levosulpiride in its Bulk and Dosage Form
4. Shreenidhi Surve et.al.(2013),HPTLC and HPLC Method Development and Validation for Simultaneous Estimation of Rabeprazole Sodium and Levosulpiride in Bulk and Its Pharmaceutical Dosage Form, International Journal of Pharmacy and Pharmaceutical Sciences Vol 5, Suppl 3.
5. Pravin Devidas Pawar et.al., (2014) Simultaneous quantification of levosulpiride and rabeprazole in tablet dosage form by validated normal phase high performance thin layer chromatographic method,Journal of Chemical and Pharmaceutical Research, , 6(5):1193-1199
6. Agarwal, N., & Jagdigsh, B. (2012). Development and validation of stability indicating RP-HPLC method for simultaneous estimation of levosulpiride and rabeprazole sodium. *International Journal of Pharma and Bio Sciences*, *3*(4), 718-726.
7. Tonini M, Cipollina L, Poluzzi E, Crema F, Corazza G and De Ponti F: Clinical implications of enteric and central D2 receptor blockade by antidopaminergic gastrointestinal prokinetics. Aliment Pharmacology and Therapeutics 2004; 19: 379-90
8. Agarwal N, Jagdigsh B. Development and validation of stability indicating RP-HPLC method for simultaneous estimation of levosulpiride and rabeprazole sodium. International Journal of Pharma and Bio Sciences. 2012;3(4):718-26.
9. Mullapudi, A., Pingali, S. and Santosh, T., 2013. Development and Validation of a stability indicating method for the simultaneous determination of Levosulpiride and Rabeprazole by High Performance Liquid Chromatography. *Development*, *1*(1).
10. Agarwal, Nandkishor, and B. Jagdigsh. "Development and validation of stability indicating RP-HPLC method for simultaneous estimation of levosulpiride and rabeprazole sodium." *International Journal of Pharma and Bio Sciences* 3, no. 4 (2012): 718-726.