https://doi.org/ 10.33472/AFJBS.6.10.2024.5379-5387

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Simultaneous RP-HPLC Method Development and Validation for Levosulpiride and Rabeprazole

Dharmesh Sisodiya [1], Budagala Gayathri [2], Krishna Chandra Panda [3], P. Balan [4] Ramenani Hari Babu [5], Sridevi Ranjitha Karanam [6], Minaketan Sahoo [7], Patta Salomi [8]*

1. Associate Professor, Institute of Pharmacy,Vikram University,Ujjain M.P. Pin Code 456010
2. Assistant professor, Priyadarshini institute of pharmaceutical education and research 5th mile Guntur, 522017
3. Associate Professor, Roland Institute of Pharmaceutical Sciences, Khodasingi, Berhampur, Pin-760010
4. Professor & Head, Department of Pharmaceutical Chemistry, The Erode College of Pharmacy, veppampalayam Vallipurathanpalayam PO, Erode -638112, Tamilnadu
5. Professor, MB school of pharmaceutical sciences, Mohan Babu University, Tirupati, 517102
6. Associate Professor, Vignan Institute of Pharmaceutical Technology, Duvvada, Visakhapatnam -530049
7. Assistant Professor, Institute of Pharmacy and Technology Salipur, Cuttack
8. Professor and Head, P. Ramireddy Memorial College of Pharmacy, Kadapa

Corresponding Author: Dr. Patta Salomi
Designation and Affiliation: Professor and Head, P. Ramireddy Memorial College of Pharmacy, Kadapa
Email id: rayofhope01@gmail.com

Article History
Volume 6,Issue 10, 2024
Received:25 Apr 2024
Accepted : 30 May 2024
doi: 10.33472/AFJBS.6.10.2024.5379-5387

ABSTRACT

The aim of the project is to develop a fast, simple, precise, and cost-efficient RP-HPLC method for measuring the amounts of Levosulpiride and Rabeprazole simultaneously in both pharmaceutical products and bulk samples. This technique has effectively achieved the separation of Levosulpiride and Rabeprazolebulk. The separation was performed using a Phenomenex C18 150 x 4.6 mm, 5m analytical column at a wavelength of 280 nm. The mobile phase consisted of a mixture of Potassium dihydrogen orthophosphate, acetonitrile, and water in a ratio of 30:55:15. The pH of the buffer was adjusted to 6.0. The separation was carried out in isocratic elution mode with a flow rate of 1.2 ml/min. Levosulpiride had a retention time of 3.852 minutes, whereas Rabeprazoleshowed a retention duration of 5.987 minutes. Quantitative analysis of Levosulpiride and Rabeprazole was achieved using PDA detection at 280 nm using a linear calibration curve. The concentration ranges of 10-50 µg/ml (with a correlation coefficient of 0.9997) and 10-50 µg/ml (with a correlation coefficient of 0.9999) were used for reliable quantification for Levosulpiride and Rabeprazole respectively. The limit of detection (LOD) for Levosulpiride was 0.2345 µg/ml, whereas the LOD for Rabeprazole was 0.2456 µg/ml. The proposed method is highly suitable for use in quality-control laboratories for the bulk and pharmaceutical quantitative analysis of pharmaceuticals, whether used individually or in combination. This technique is characterized by its simplicity and efficiency, while yet ensuring a high level of accuracy and precision.

Keywords: Levosulpiride, Rabeprazole and RP-HPLC.

Introduction:

Levosulpiride (LEVO) belongs to the class of substituted benzamide antipsychotics. LEVO is a non-typical antipsychotic drug that inhibits the presynaptic D2 receptors in the brain. Similar to its parent chemical, LEVO has antagonistic effects on D3 and D2 receptors. LEVO is prescribed for the treatment of psychosis, anxiety disorder, premature ejaculation (not advised in some countries), vertigo, and irritable bowel syndrome. It dissolves readily in methanol, ethanol, and DMSO.

Rabeprazole sodium (RABE) is a medication that belongs to the class of drugs known as proton pump inhibitors. It is often used as a medication for treating ulcers. It belongs to the class of proton pump inhibitors. This medication is used for the management of gastric ulcer, peptic ulcer, ulcerative gastroesophageal reflux disease, duodenal ulcer, Zollinger-Ellison syndrome, and the eradication of Helicobacter pylori when combined with amoxicillin. The compound is chemically known as 2-([4-(3-methoxypropoxy)-3-methylpyridin2-yl] methylsulfinyl)-1H-benzo[d]imidazole. It has high solubility in water and mayalso dissolve in methanol, ethanol, and ethyl acetate [1-3].

The literature study indicates that UV [4, 5] and HPLC [6, 7] techniques have been used to analyze Levosulpiride alone or in combination with other drugs, whereas different UV [8, 9], HPTLC [10, 11], and HPLC [12, 13] methods have been used to analyze Rabeprazole sodium alone or in combination with other drugs. The objective of this work was to provide reliable and specific reverse phase HPLC procedures for the simultaneous analysis of Levosulpiride and Rabeprazole sodium.

Materials and Methods:

The drug material used in this study consists of Levosulpiride and Rabeprazole. The chemicals used for the analysis are Potassium dihydrogen orthophosphate (HPLC grade), Orthophosphoric acid (HPLC grade), Water (HPLC grade), and acetonitrile (HPLC grade). The HPLC system used for the analysis is the Shimadzu SPD-20A from Tokyo, Japan.

Instrumentation:

The HPLC investigations were carried out using a Shimadzu SPD-20A HPLC system manufactured by Shimadzu Corporation in Tokyo, Japan. The system included a separation module and a photodiode array detector. The experiments were conducted in isocratic mode with the assistance of an Autosampler. The data collection and processing were performed using laboratory solution software. The separation was conducted with a Kromosil C18 150 x 4.6 mm, 5m analytical column. The supplementary apparatus used consisted of a pH meter (Eutech), precision balance (Shimadzu), and ultrasonicator (Unichrome, UCA701).

Preparation of mobile phase:

The mobile phase was created by combining Potassium dihydrogen orthophosphate, acetonitrile, and water in a proportion of 25:60:15. The pH of the buffer was then modified to 6.0, and the resulting solution was filtered using a 0.45µ membrane.

Preparation of standard stock solution:

Using a digital microbalance, 10.0 mg of Levosulpiride and 10.0 mg of Rabeprazole were measured and placed into a 10 millilitre volumetric flask. After introducing seven millilitres of diluent, the mixture was subjected to sonication in order to facilitate dissolution. Subsequently, the solution was diluted to its full volume using the diluent, and ultimately, it was further diluted to get the desired final volume by adding more diluent.

Chromatographic conditions:

High Performance Liquid Chromatography equipped with PDA detector.

For Levosulpiride and Rabeprazole (isocratic)

Column : Phenomenex C18 150 x 4.6 mm, 5m analytical column
Wavelength : 280 nm
Injection Volume : 20µl
Column Temperature : Ambient
Flow rate : 1.2 ml/min

The LEVO peak was detected during a retention time of 3.852 minutes. It exhibited an area of 749276 units and a tailing factor of 1.42. Figure 1 and Table 1 demonstrate that the RABE peak appeared at a retention time of 5.987 minutes, with a peak area of 1099116, a tailing factor of 1.22, and a resolution of 2.25. This experiment was considered ideal since it yielded favorable results and had a shorter time of retention. LEVO has a retention time of about 3.852 minutes, whereas RABE has a retention duration of 5.987 minutes.

Table 1: System suitability parameters

S.No.	Name of the Peak	Retention Time (Mins)	Peak Area	Tailing Factor	Resolution	Plate Count
01	Levosulpiride	3.852	749276	1.42	---	4242
02	Rabeprazole	5.987	1099116	1.22	2.25	5262

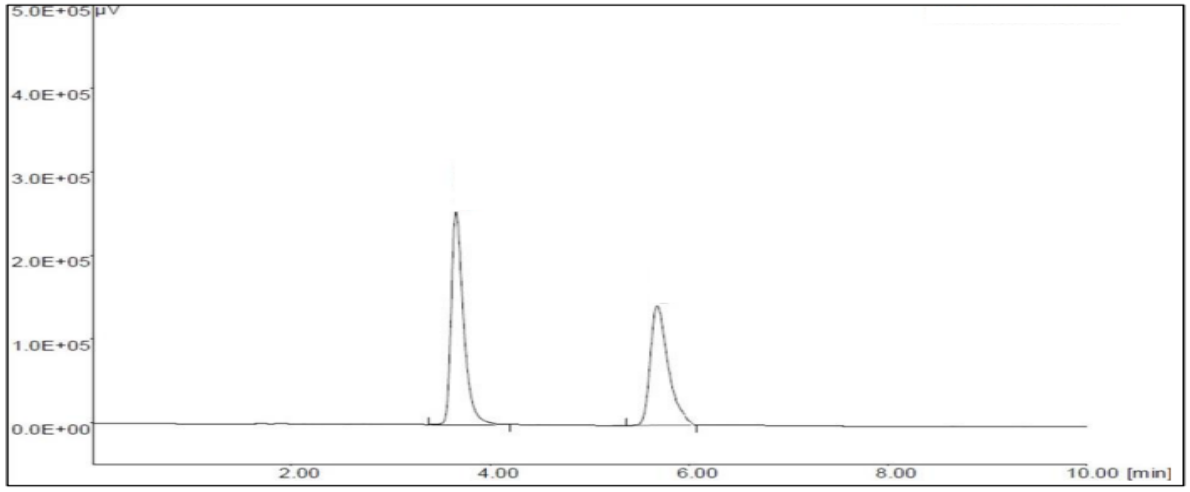

Fig.No. 01 : Typical Chromatogram of Levosulpiride and Rabeprazole

Preparation of sample solution:

A quantity of 10 milligrams of the sample was measured and placed into a volumetric flask with a capacity of 10 milliliters. Subsequently, 7 milliliters of diluent were introduced. Subsequently, the mixture was subjected to sonication in order to dissolve the substance, and

then further diluted with diluent till reaching the desired volume. The solution was further reduced to a volume of 10 ml by adding the diluent and then passed through a 0.45µ Nylon syringe filter.

Procedure:

Five injections, each containing 20 µl, were administered using active LEVO and RABE standard solutions. Chromatograms were acquired and the peak responses were assessed. The system's appropriateness was assessed by analyzing its parameters. The determination of the amounts of LEVO and RABE in the sample was accomplished by analyzing the peak responses.

Method Validation:

The current investigation assessed many criteria to confirm the accuracy of the HPLC technique in measuring the quantities of LEVO and RABE, following the prescribed process. This demonstrates that the approach is appropriate for its intended purpose. The validation criteria were implemented in accordance with the standards established by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH).

Linearity and Range:

The concentrations of LEVO and RABE that exhibited a direct correlation with peak area were within the range of 10 to 50 µg/ml for both substances. The results are shown in Figures 2 and 3, Tables 2 and 3, and the linearity of the calibration curve is verified by the strong correlation coefficient of the regression equation.

Table 2: Linearity data of LEVO

S.No.	Concentration (µg/ml)	Peak Area
1	0	0
2	10	374325
3	20	749276
4	30	1147143
5	40	1509715
6	50	1875135
Slope		**37621**
Intercept		**2501.1**
Regression		**0.9997**

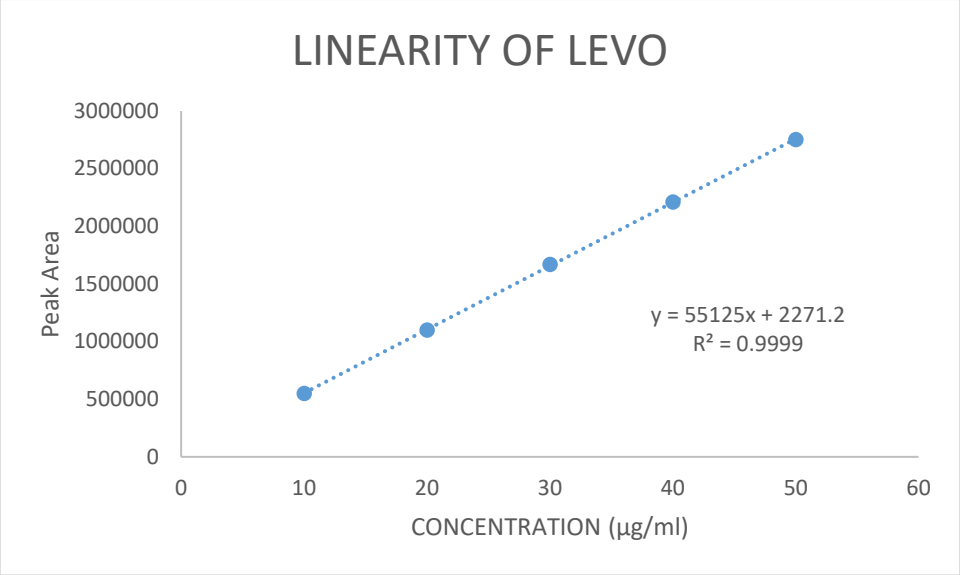

Fig.No. 02 : Linearity of Levosulpiride

Table 3: Linearity data of RABE

S.No.	Concentration (µg/ml)	Peak Area
1	0	0
2	10	550265
3	20	1099116
4	30	1669802
5	40	2209612
6	50	2751245
Slope		**55125**
Intercept		**2271.2**
Regression		**0.9999**

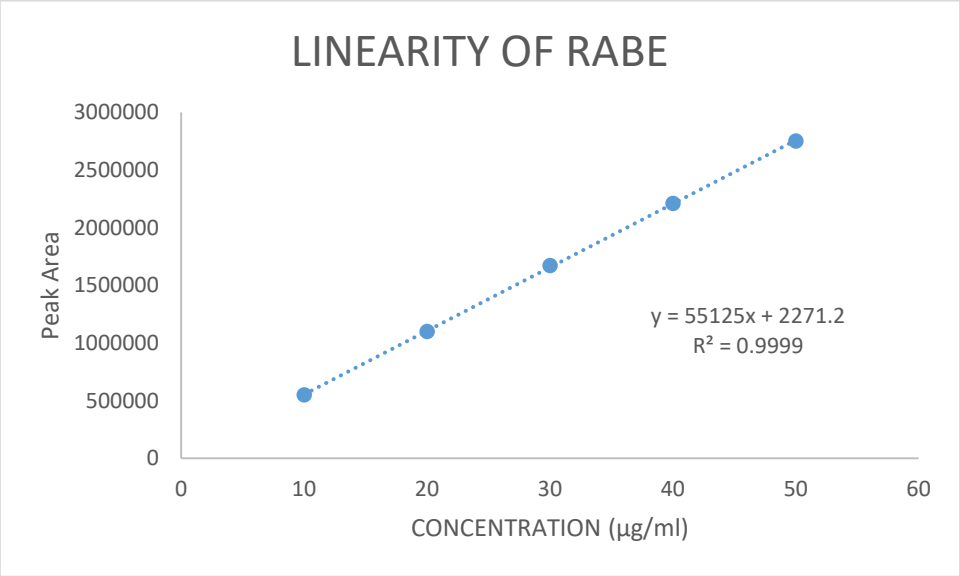

Fig.No. 03 : Linearity of Rabeprazole

Accuracy and Precision:

The accuracy of recovery was assessed by adding additional Standard drug at three different concentration levels to a previously tested test solution. Based on our observations, we found that the recommended approach is very accurate for simultaneously estimating both LEVO and RABE. The relative standard deviation (RSD) was less than 2%. Additionally, we achieved a recovery rate of 99.97% for LEVO and 100.24% for RABE. The Method's reliability is shown by its great repeatability and low RSD readings. The tables labeled as 4 and 5.

Table 4: Precision data of LEVO and RABE

	LEVO				**RABE**			
Injection Number	**Retention Time**	**Peak Area**	**Plate Count**	**Peak Symmetry**	**Retention Time**	**Peak Area**	**Plate Count**	**Peak Symmetry**
1	3.852	1403270	8767	1.42	5.987	1375623	8921	1.19
2	3.842	1404432	9865	1.42	5.876	1375720	9654	1.21
3	3.831	1405678	9645	1.39	5.869	1376234	9876	1.2
4	3.82	1405234	8975	1.52	5.854	1377756	9454	1.34
5	3.811	1415823	9642	1.43	5.964	1378214	8782	1.41
6	3.801	1414765	9743	1.44	5.958	1386723	8898	1.34
Average	3.826	1403270			5.918	1378378		
Standard Deviation	0.019	5565.42			0.058	4226.22		
% RSD	0.5018	0.40			0.98	0.31		

Table 5: Accuracy data of LEVO and RABE

Sample Preparation No.	**LEVO Assay (%)**	**RABE Assay (%)**
1	99.41	101.44
2	99.91	100.54
3	98.91	98.27
4	100.13	99.86
5	100.83	100.32
6	100.34	100.85
Mean	99.92	100.21
SD	0.6825	1.0890
RSD (%)	0.6830	1.0867

Robustness:

The outcomes of the resilience assessment are shown in Table 6. Both components had similar tailing factors, elution orders, resolutions, relative standard deviations, and recoveries. The research indicated that the relative standard deviation (RSD) of the peak locations was much less than 2.0%.

Table 6: Robustness data of LEVO and RABE

Condition	Levosulpiride			Rabeprazole		
	% RSD	**Tailing Factor**	**% Recovery**	**% RSD**	**Tailing Factor**	**% Recovery**
1) Change in Flow rate						
Normal Condition (1.2 ml per minute)	0.17	1.41	99.21	0.22	1.22	99.20
Flow rate (1.0 ml per minute)	0.32	1.31	99.42	0.23	1.24	99.68
Flow rate (1.4 ml per minute)	0.52	1.20	100.21	0.35	1.21	98.90
2) Change in minor component in the mobile phase						
Normal Condition (Potassium dihydrogen orthophosphate, acetonitrile, and water in a ratio of 25:60:15)	0.44	1.46	100.21	0.55	1.20	99.82
(Potassium dihydrogen orthophosphate, acetonitrile, and water in a ratio of 30:55:15)	0.55	1.33	100.23	0.55	1.30	99.32
(Potassium dihydrogen orthophosphate, acetonitrile, and water in a ratio of 20:65:15)	0.77	1.32	100.32	0.85	1.20	101.22
3) Change in Wave Length						
Normal:Wave Length 280 nm	0.24	1.43	100.42	0.35	1.26	100.41
Wave Length 285 nm	0.44	1.33	98.72	0.65	1.36	100.41
Wave Length 275 nm	0.74	1.43	100.71	0.75	1.26	100.91
4) Change in pH						
Normal:pH 6.0	0.36	1.64	99.90	0.55	1.29	99.97
pH 5.5	0.46	1.84	98.90	0.75	1.29	98.57
pH 6.5	0.76	1.24	98.80	0.95	1.19	100.87

Ruggudness:

Levosulpiride and Rabeprazole had mean peak areas of 749156 and 1098956, respectively, with a relative standard deviation (RSD) of 0.35% and 0.28%, respectively.

SUMMARY

A novel and validated reverse-phase high-performance liquid chromatography (RP-HPLC) method has been developed to accurately determine the levels of LEVO and RABE in bulk

and pharmaceutical samples. Based on the results of the literature review, which showed a lack of methodologies for accurately calculating LEVO and RABE in large amounts, there is a pressing need for a direct, cost-effective, and accurate solution to solve this problem.

The concentrations of LEVO and RABE were quantified by injecting a solution containing Potassium dihydrogen orthophosphate, acetonitrile, and water (in a ratio of 30:55:15) with a pH of 6.0 onto a Phenomenex C18 column measuring 150 x 4.6 mm and having a particle size of 5m. The flow rate was adjusted to 1.2 milliliters per minute, while the injection volume was 20 microliters. The LEVO peak had a retention time of 3.852 minutes, whereas the RABE peak had a retention time of 5.987 minutes.

Following its improvement, the method was verified in accordance with ICH guidelines to assess its compatibility with the system, linearity, sensitivity parameters, precision, accuracy, and resilience. All validation parameters yielded results that were within acceptable ranges. The tests exhibited relative standard deviation (RSD) values below 2. The range of recoveries was between 98% and 102%.

CONCLUSION:

The suggested RP-HPLC technology provides a time-efficient and straightforward method that is both simple and speedy. It also ensures precision, accuracy, specificity, resilience, and cost-effectiveness. Thus, it is a preferred technique for the concurrent quantification of Levosulpiride and Rabeprazole. The developed approach was rigorously validated in compliance with ICH criteria in all aspects.

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