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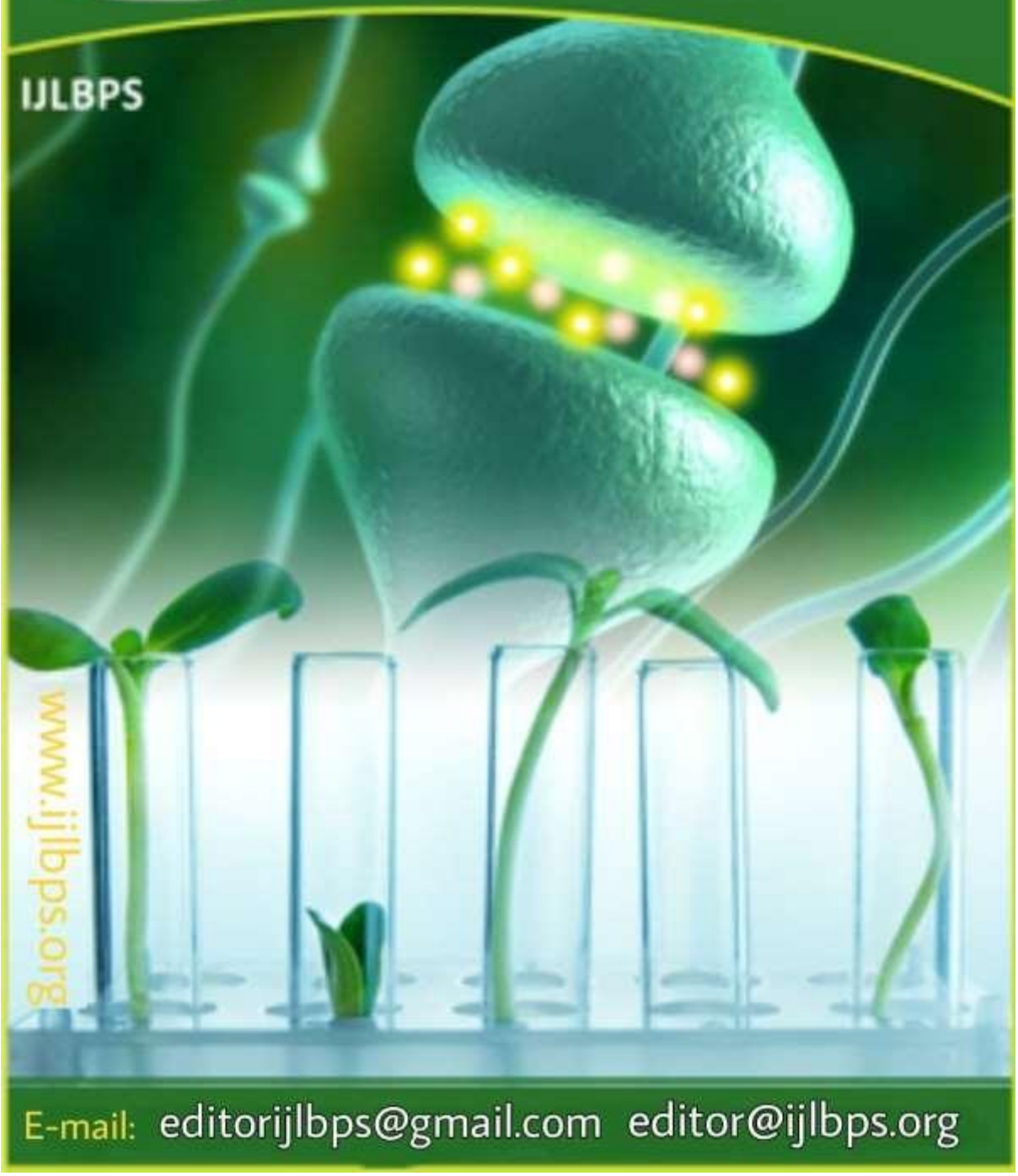

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Improving Levosulpiride and Rabeprazole's Bulk and Dosage Forms with the Use of High-Performance Liquid Chromatography and Its Validation

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ABSTRACT

The analytical technique is the bedrock of pharmaceutical analysis. A sensitive, easy-to-understand, and accurate method for concurrently assessing levosulpiride (LEVO) and rabeprazole (RABE) was the objective of the present investigation. The mobile phase, which consisted of acetonitrile and buffer at a ratio of 70:30, was more than enough. Our analysis found that LEVO could be recovered from 80%, 100%, and 120% sample solutions with percentages of 99.98%, 100.06%, and 100.1%, respectively. Results showed that RABE values of 98.99%, 99.46%, and 100.08% were obtained from the 80%, 100%, and 120% sample solutions, respectively. Both drugs' percentage recoveries were within the expected range. This indicates that the proposed technique was more precise than the existing ones. The method ensures accuracy by analyzing the sample enough to provide statistically significant results. Next, the precision is shown by the percentage of the relative standard deviation. A relative standard deviation (RSD) of no more than 2% is considered acceptable for this method's accuracy. Results showed that RABE had an intermediate accuracy percent RSD of 0.242 and LEVO had a value of 0.344, according to the present study. The % RSD value indicates a high degree of accuracy within the provided range.

INTRODUCTION

During last few decades, analytical chemistry has witnessed extensive development in terms of sophistication, quantitation, and instrumentation. Consequently, newer analytical techniques such as hyphenated techniques Fourier transform infrared spectroscopy (FT-IR), Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography-Mass Spectrometry (LCMS), high performance liquid chromatography (HPLC), High-performance Thin Layer Chromatography (HPTLC) etc. and their areas of application have increased considerably because of the stringent requirements for testing and monitoring of the drugs for approval; the demand on quality, validation data and performance of analytical methods have gained importance. Nowadays, most of the people are suffering from various types of disease conditions. This can be happened due to the changed lifestyle. This means improper intake of food, lack of exercise can lead to the disease conditions. Due to this many pharmaceutical industries introduce the new drug molecules or drug combinations in every year, to treat such types of diseases. In this one of the worldwide diseases is gastric acidity, which can further lead to Gastroesophageal Reflux Disease (GERD), or Gastric/peptic Ulcers. Hence by taking market survey, considering peoples need we have tried to promote the search-related recent drugs and

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combinations. Many industries work on the drugs used to treat gastric acidity, gastroesophagealreflux disease (GERD), or gastric/peptic ulcers. Instead of single-drug physicians prefer a combination of drugs. Hence by referring the articles on this combination at laboratory level we tried to develop a method for this combination of antiulcer drugs by following ICH guidelines. HPLC is the fastest growing analytical technique for the analysis of drugs individually and in combination too. Its simplicity, high specificity and wide range of sensitivity make it ideal for the analysis of many drugs. Therefore, it was thought worldwide to develop such methods of analysis, which can estimate both the drugs in combination without prior separation. Hence present work was attempted to develop accurate, simple and sensitivemethod for simultaneous estimation of rabeprazole and levosulpiride.[1-6]

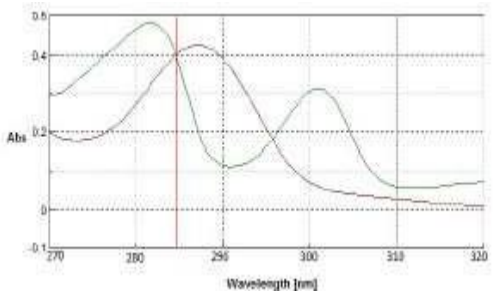

Fig. 1: Determination of Maximum Wavelength

Table 1: List of materials for the work

S. No.	Material	Company
Drugs		
1	Rabeprazole	Swapnroop Drugs and Pharmaceuticals, Aurangabad
2	Levosulpiride	Swapnroop Drugs and Pharmaceuticals, Aurangabad
3	Rabeprazole & Levosulpiride Tablet (Cyra-LS)	Local pharmacy
Reagents		
4	Acetonitrile (HPLC grade)	Merck
5	Acetonitrile (UV grade)	Loba Chemicals
6	Methanol (UV grade)	Loba Chemical
7	Purified Water	Milli Q
8	Potassium dihydrogen phosphate (A.R. grade)	Fisher Scientific
9	O -phosphoric acid (A.R. grade)	Fisher Scientific

Table 2: Instruments used for the work

S. No.	Instrument	Manufacturer
1	Ultra-violet spectroscopy	JASCO UV- V-530
2	HPLC	Dionex-Summit
3	PH meter	Global DBH-500
4	Sonicator, Mumbai	PCI, Mumbai

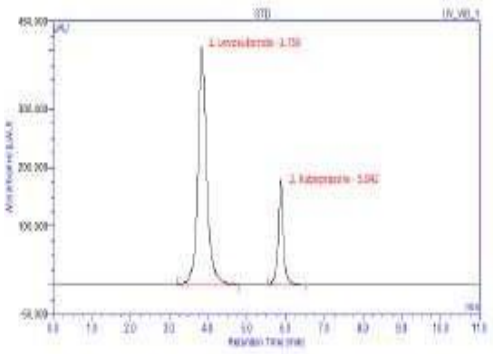

Fig. 2: Chromatograph of Standard Solution of LEVO and RABE

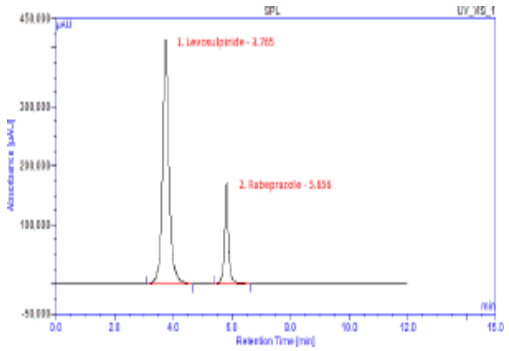

Fig. 3: Chromatograph of Sample Solution of LEVO and RABE

Material and Methods

HPLC Apparatus

The liquid chromatographic system consists of following components: HPLC system of make Dionex and model summit with vwd detector and chromeleon 6.8 SR11 as data processing software. Analysis performed on 250 mm × 4.6 mm, hypersil BDS C18 column with particle size 5 µ.

Selection of Common Solvents

Phosphate buffer 6.5: Acetonitrile (70:30) was selected as a common solvent for developing spectral characteristics of the drug. The selection

was made after assessing the solubility of both drugs in a different solvent.

Procedure for Determining the Sampling Wavelength [7-9]

Levosulpiride and rabeprazole (10 µg/mL) each were scanned separately in a wavelength range of 200-400 nm against phosphate Buffer pH 6.5: acetonitrile (70:30) as blank to determine the wavelength of maximum absorption of drug. The wavelength was selected for levosulpiride and rabeprazole from overlain spectra of both drugs at 284 nm. The Spectrum shown in Fig 1.

Selection of Mobile Phase

From this solution mixed solution of both drugs was prepared. For that less than 20 µL of levosulpiride and Rabeprazole was injected in to HPLC system. Solution was analyzed by using different mobile phases.

Preparation of Mobile phase- Buffer: Acetonitrile (70:30)[10,11]

This mobile phase was prepared by dissolving 6.8 gm of potassium dihydrogen phosphate in 1000 mL of water (pH was adjusted to 6.5 with NaOH): Acetonitrile. Then this solution was sonicated and filter through 0.45 µ nylon filter paper & then mixed solution of both drugs was injected in to Hplc system. The column is further changed to obtain good results. (250×4.6 mm, 5µ). It was found that there is proper separation of peak was observed and get sharp peak with less tailing effect.This mobile phase shown retention time 3.756 and 5.842 from that result this mobile phase was selected as Final optimized Phase. Chromatogram shown in Fig. 2**.**

Table 3: Finalized chromatographic condition

Chromatographic mode	*Chromatographic condition*
Standard solution	320 µg/mL ofLevosulpiride, 200 µg/ mL of Levosulpiride
Stationary Phase	C-18 (4.6×250 mm) Hypersil BDS
Mobile Phase	Buffer:Acetonitrile (70:30)
Detection of wavelength	284 nm
Flow rate	1.0 mL/min
Sample concentration	20 µL

Analysis of Tablet formulation [15,16]

Crush and make fine powder of 20 tablets. Weigh and transfer tablet powder containing rabe 20 mg and LEVO 75 mg in 100 mL volumetric flask. Add about 70 mL of mobile phase, sonicate for 20 minutes. Allow to attend room temperature. Dilute up to mark with mobile phase. Filter with Whatman filter paper 41. Dispose first 10 mL of filtrate. Further dilute 5 mL to 50 mL with mobile phase. From the peak area obtained concentration (label claim) of the drug was calculated using their respective slope and intercept values from calibration data.

RESULT AND DISCUSSION

Method Validation

Accuracy (% recovery)

It is the measure of closeness between the actual value and the analytical value that is calculated by applying the test procedure for a number of times. Recovery was done at three different levels *viz.,* 80%, 100% and 120%, within the beer's limit for both the drugs. The previously analyzed sample of concentration 10 µg/mL was spiked with known concentrations of the pure samples and then reanalyzed using the proposed methods. Percentage recovery was calculated using the equations for both the methods. Percentage recovery for Levosulpiride and Rabeprazole were shown in Table. 4 and Table. 5. Chromatograms of Levo and Rabe for 80%, 100%, 120% are shown in Fig. 4, 6.

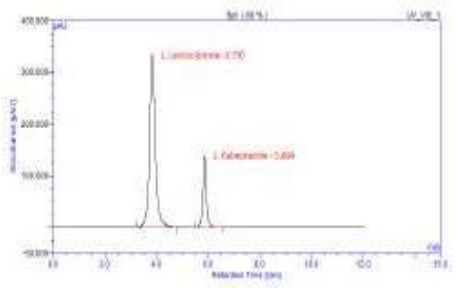

Fig. 4: Chromatograph of Levo and Rabe at 80%

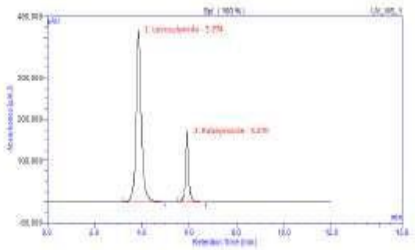

Fig. 5: Chromatograph of Levo and Rabe at 100%

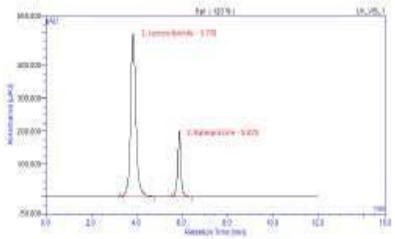

Fig. 6: Chromatograph of Levo and Rabeprazole at 120%

Table 4: Result of Recovery Study of Levo

Parameter	Area of LEVO	Mean Area	% Recovered	Std. Dev	% R.S.D.
	Inj1-293945				
80%	Inj2-294864	293879	99.68	0.34	0.38
	Inj3-294228				
	Inj1-333351				
100%	Inj2-333285	332951.33	100.06	0.28	0.32
	Inj3-333218				
	Inj1-376392				
120%	Inj2-377172	376700	100.1	0.38	0.42
	Inj3-376536				

*Average of six determination, % R.S.D. relative standard deviation, S.D. standard deviation

Table 5: Result of Recovery Study of Rabeprazole

Parameter	Area of RABE	Mean Area	% Recovered	Std. Dev	% R.S.D.
	Inj1-173245				
80%	Inj2-173587	173481.33	98.99	0.23	0.26
	Inj3-173612				
	Inj1-182356				
100%	Inj2-182738	182914	99.46	0.34	0.38
	Inj3-183648				
	Inj1-191518				
120%	Inj2-192274	192128.66	100.08	0.40	0.44
	Inj3-192594				

*Average of six determination, % R.S.D. relative standard deviation, S.D. standard deviation

Table 6: Linearity data of Levosulpiride

S. no	Concentration µg/mL	Average Area of LEVO	S.D.	%R.S.D.
1	14	295076	0.14	0.145
2	20	310745	0.27	0.269
3	26	325498	0.34	0.345
4	32	341056	0.41	0.421
5	38	356786	0.44	0.445
6	44	373412	0.49	0.499

*Average of six determination, % R.S.D. relative standard deviation, S.D. standard deviation

Linearity

Linearity was demonstrated by analyzing six different concentrations of active compound. Peak areas were recorded for all peaks and calibration plot was constructed by plotting peak area vs concentration of Levosulpiride and Rabe which were found to be linear in range of 14-44 µg/mL, and 2-12 µg/mL, respectively. coefficient of correlation was 0.9999 and 0.9996 Calibration curve for levo was shown in Fig. 7. Calibration curve for Rab was shown in Fig. 8.

Intermediate Precision

To demonstrate agreement among result, a series of measurement were done with LEVO and RABE six replicate injection of the specific standard atvarious time of trials. The result of intermediate precision was shown in Table 16.

Robustness

The study of robustness was carried out to evaluate the influence of small but deliberate variations in the chromatogram conditions on the determinations of both drugs. A robustness study was carried out by changing the wavelength of both drugs and changing the flow rate of both drugs. The result of robustness after changing the flow rate were shownin Table 9 and the chromatogram are shown in Fig. 9 and 10. The result of robustness after changing the wavelength was shown in Table 10 and chromatogram was shown in Fig. 11 and 12.

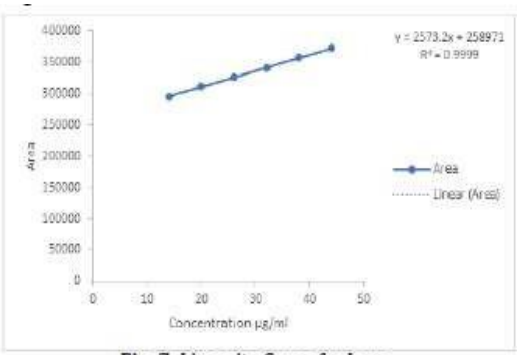

Fig. 7: Linearity Curve for Levo

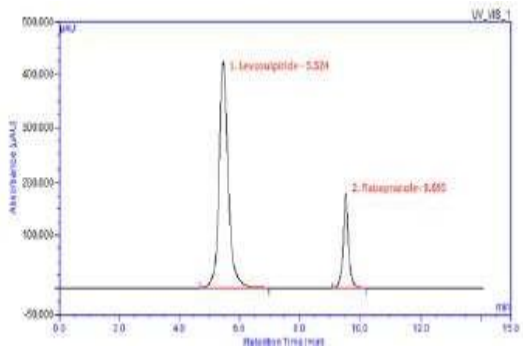

Fig. 9: Chromatograph of Levo and Rabe at Flow rate 0.8 mL/min

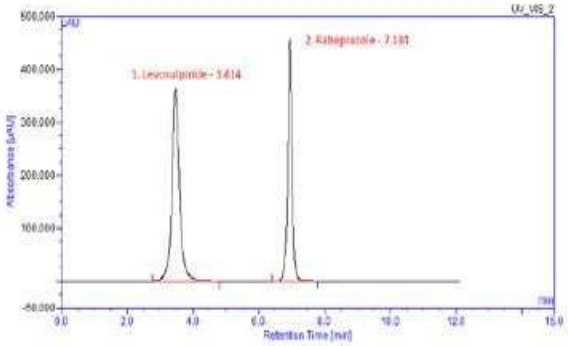

Fig. 11: Chromatograph of Levo (288 nm) and Rabe (282 nm) at Wavelength change + 2 nm

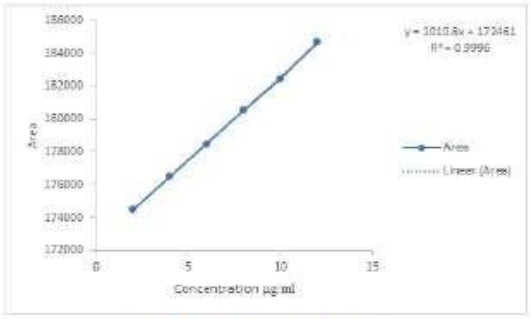

Fig. 8: Linearity Curve for Rabe

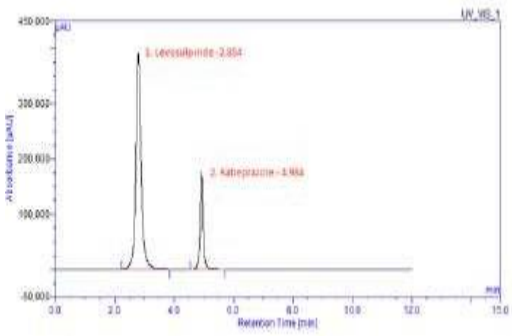

Fig. 10: Chromatograph of Levo and Rabe at Flow rate 1.2 mL/min

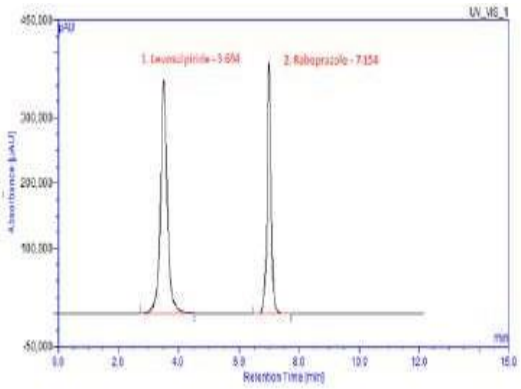

Fig. 12: Chromatograph of Levo (288 nm) andRabe (282 nm) at Wavelength change - 2 nm

Analysis of Tablet Formulation

For HPLC Method, Chromatographic conditions were optimized to obtain, an adequate separation of eluted compound. Initially, various mobile phase composition was tried for better separation of drugs. The mobile phase was consisted of Buffer: Acetonitrile, with ratio of (70:30) at flow rate was quite satisfactory. In our study, the percentage recovery of LEVO was found to be 99.98, 100.06, and 100.1% from 80, 100, 120% sample solution, respectively. For RABE was found to be 98.99, 99.46, and 100.08% from 80, 100, 120% sample solution, respectively. The obtained percentage recovery of both drugs was found to be within the range. This indicates the proposed method was more accurate than the existing methods. Precision is determined by using the method to assay a sample for a sufficient number of times to obtain statistically valid results. The precision is then expressed as the percentage relative standard deviation. Acceptance criteria for the precision of method is that % RSD should not be more than 2%.In the present study for intermediate precision % RSD for LEVO and RABE was found to be 0.344, 0.242, respectively. % RSD value indicate a good degree of precision within specified range. In the present study, it was observed that there was no significant change in peak area with change in flowrate and wavelength.

CONCLUSION

The proposed HPLC method allows for simple, reliable, precise and accurate measurement of Rabeprazole and Levosulpiride simultaneously in combined dosage form. Hence easily adopted for routine quality control analysis. The developed methods were found to be simple, rapid, precise, and accurate for the determination of drugs inrespective two component tablet dosage form of Rabe and Levo. The additives usually present in the pharmaceutical formulations of the assayed

samples did not interfere with determination of Rabe and Levo. The methods were evaluated with best condition such as linear relation, including coefficient of correlation, robustness, accuracy and precision. The % RSD for all parameter was found to be less than 2, which indicate validity of method and assay results obtained by this method are in fair of agreements. The interday and intraday precision was found to be within limits. The percentage recovery of both drugs for all methods was found to be within the range. These results show that the proposed UV spectroscopic and HPLC methods are simple, rapid, economic, precise and accurate; therefore, they are suitable for analyzing Rabe and Levo in the bulk and tablet dosage form without the interference of excipients. These methods can be applied successfully for the determination of Rabe and Levo in pharmaceutical tablet dosage form without interference and with good sensitivity.

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