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Development And Validation of Rp-Uplc Method For Simultaneous Estimation Of Iloperidone And Idebenone In Sublingual Bilayer Tablets

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ABSTRACT

Aim: Establishing and validating the stability indicating Reverse-Phase Ultra Performance Liquid Chromatography (RP-UPLC) technique for simultaneous measurement of Iloperidone and Idebenone in sublingual bilayer tablets is the main goal of this research work.

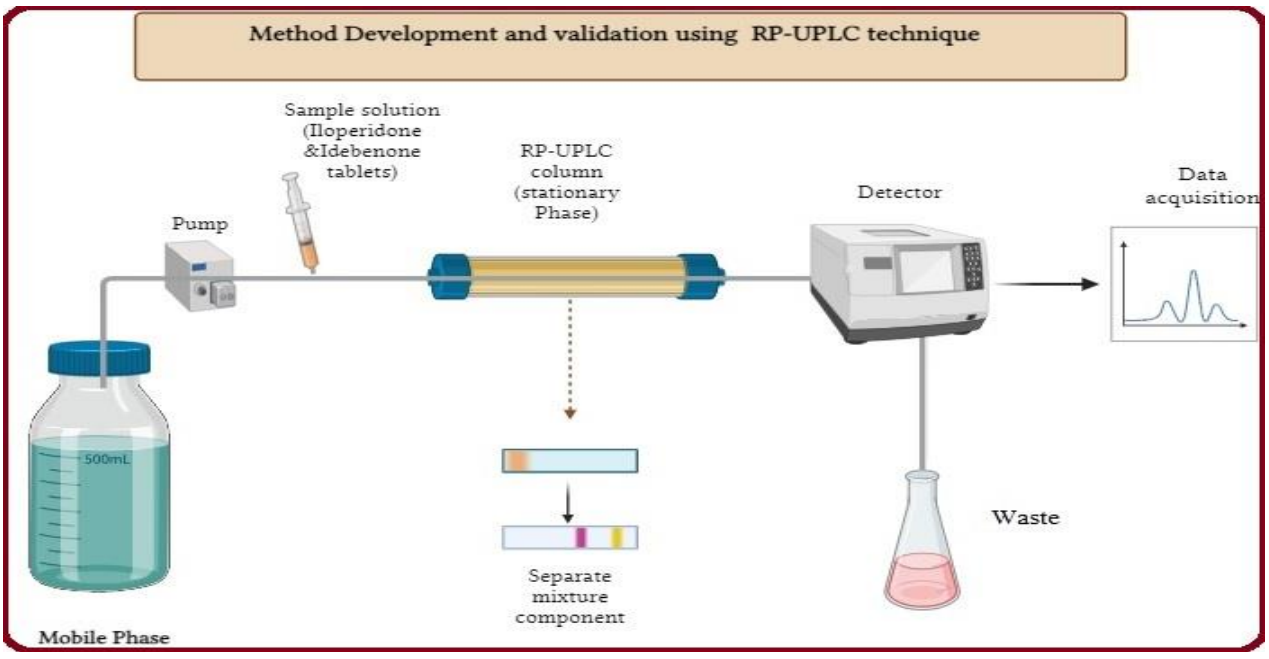
Methods: Acetonitrile as the mobile phase, 0.1% v/v orthophosphoric acid in water, a flow rate of 0.4 ml/min, and a detection wavelength of 240 nm were all necessary for the successful separation of Iloperidone and Idebenone utilizing an HSS column C18 (100 X 2.1 mm). According to ICH requirements, the stability of the active ingredients in mixed powder and sublingual bilayer tablets was evaluated under extreme circumstances such as heat degradation, peroxide oxidation, and acid-base hydrolysis.

Results and Discussion: Iloperidone and Idebenone are pharmaceutically significant combinations recommended in the treatment of numerous psychiatric and neurological conditions. Articulating these compounds into sublingual bilayer tablets enhances their bioavailability and efficacy. Therefore, an accurate and precise analytical method is imperative for their simultaneous estimation to ensure product quality and therapeutic effectiveness. Iloperidone and Idebenone had different retention times, with Iloperidone at 0.49 min and Idebenone at 0.93 min. The proposed method shows linear responses for Iloperidone (5–30 µg/mL) and Idebenone (2.5–15 µg/mL) within concentration ranges. The calculated limits for detecting and measuring Iloperidone were 0.22µg/mL & 0.47µg/mL, respectively, while for Idebenone they were 0.12µg/mL & 0.36µg/mL. All the validation parameters for the method confirmed according to the ICH Q2 acceptance limits. Significant resolution between Iloperidone, Idebenone, and their degradation products has been noted in the methodology's stability aspect.

Conclusion: The proposed RP-UPLC method demonstrated high sensitivity, precision, and stability indication, making it a potentially viable technique for the simultaneous estimation of Iloperidone and Idebenone in sublingual bilayer tablets.

Keywords: RP-UPLC, Idebenone, Iloperidone, sub-lingual bilayer tablets, method development and validation.

PICTORIAL ABSTRACT



INTRODUCTION

Historically, chromatographic methods have been recognized as essential tools in analytical chemistry since they provide a powerful method for separating complex mixtures and identifying individual components. Despite their widespread use, these techniques present a range of challenges and limitations that must be carefully considered to ensure accurate and reliable results. Chromatography skilled professional must track a complex landscape of potential obstacles to achieve their research objectives.[1,2] From issues related to selectivity and sensitivity to problems with sample preparation and instrument optimization, this landscape presents a range of challenges that must be carefully considered.

To address these challenges, researchers have recently developed a novel technique termed RP-UPLC that promises to enrich analytical capabilities. RP-UPLC is a great analytical tool that has emerged as a critical technique in the field of chromatography. Its importance and significance derived from its ability to provide high-resolution separations of complex samples in a rapid and efficient manner. RP-UPLC achieved this through the use of small particle size stationary phases, high-pressure pumps, and advanced column technology, which allowed for the separation of compounds with similar physicochemical properties.[3] This technique has become a popular choice for a broad range of applications, covering pharmaceuticals, biotechnology, environmental monitoring and food analysis owing to its ability to provide accurate and reliable results with minimal sample preparation. Moreover, RP-UPLC has the potential to offer improved sensitivity, selectivity, and throughput over other chromatographic techniques, making it an indispensable tool in modern analytical chemistry.[4]

Additionally, RP-UPLC has outperformed traditional HPLC methods. A crucial aspect is the use of columns filled with sub - 2 micron particles and equipment that can handle high pressures.[5] This configuration allows for quicker separations and greater resolution in comparison with traditional methods. In particular, RP-UPLC differentiates molecules by their hydrophobic interactions with a non-polar stationary phase, resulting in improved resolution and selectivity compared to regular UPLC through a polar stationary phase.[6, 7] In addition, RP-UPLC includes a non-polar stationary phase that enables better separation of molecules with higher molecular weights, a capability that cannot be reached with UPLC. Moreover, RP-UPLC has the ability to process a more diverse array of sample types while also segregating polar and non-polar compounds at the same time, which boosts its flexibility compared to UPLC.[8]

RP-UPLC is utilized throughout the various stages of drug development, such as analysing impurities and degradants in drug formulations, and monitoring the bioavailability of drugs, quality assurance, and regulatory compliance.[9] In environmental analysis, RP-UPLC is exploited to analyse soil and water samples to assess the presence of pollutants and contaminants. In clinical research, RP-UPLC is applied for analysing biomarkers and metabolites in biological fluids like blood, saliva and urine.[10]

Iloperidone is approved by the USFDA in 2009 indicated for the treatment of schizophrenia and mania or mixed episodes in bipolar I disorder (Fig 1). Pharmacogenomics research has shown single nucleotide polymorphisms that increase the efficacy in the acute treatment of schizophrenia. Iloperidone has excellent affinity for the 5HT2A receptor than D2 receptor antagonistic effects.[9] Iloperidone presents itself as a finely crystalline free flowing powder with slight solubility in water, 0.1 N Hydrochloric acid, and excellent solubility in organic solvents (chloroform, ethanol, methanol, acetonitrile..etc.). It has an empirical formula of $C_{24}H_{27}FN_2O_4$ and a molecular weight of 426.5 g/mol.[11]

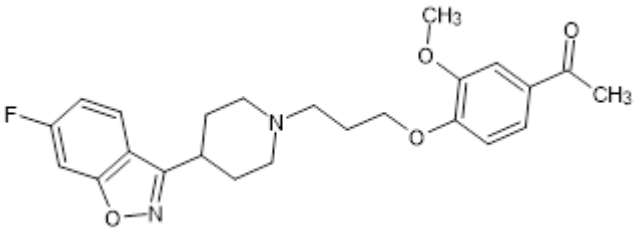


Figure 1: Molecular Structure of Iloperidone

Iloperidone is sold as tablets with the proprietary names Fanapt, Fanapta, and Zomaril, each of which has a different potency. The half-life of this medication is 12 - 14 hrs, and it reaches its peak blood levels within 2 to 4

hours after administration. However, due to its substantial first-pass metabolism and water insoluble nature, it has a poor bioavailability of about 36% in humans.

Idebenone, a widely recognized compound, was developed in the early 1980s by Takeda Pharmaceuticals for the treatment of cognitive decline or dementia. It is a benzoquinone, and its chemical name is 2-(10-hydroxydecyl)-5,6-dimethoxy-3-methyl-1,4-benzoquinone (Fig 2). Idebenone is a powerful antioxidant and it is an analog of ubiquinone, which is a well-known and commonly used antioxidant drug. It is also commercially promoted as a synthetic analogue of coenzyme Q10 (CoQ10). Idebenone exhibits considerable oral bioavailability, however, its extensive hepatic first-pass metabolism results in a negligible systemic exposure of 1%. The Empirical Formula of Idebenone is $C_{19}H_{30}O_5$ and its molecular mass is 338.444 g/mol.[12, 13]

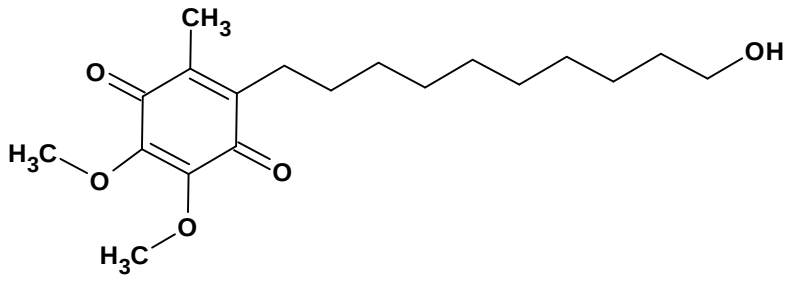


Figure 2: Molecular Structure of Idebenone

Idebenone is accessible in tablet form under the trademark names Norwayz, Ideben, and Idebest in diverse strengths.

The administration of Iloperidone (ILP) and Idebenone (IDB) as a combination may show complimentary for the effective management of psychosis, considering that Idebenone has shown exceptional capability to cross the blood-brain barrier and induce protection from neuronal damage by inhibiting lipid peroxidation.[14] However, attaining therapeutic levels in the bloodstream proves challenging since both Iloperidone and Idebenone have low bioavailability due to their huge first-pass metabolism. Since sublingual administration has been demonstrated to improve the bioavailability of several medications by avoiding first-pass metabolism and boosting absorption through the sublingual mucosa, one strategy to solve this problem is to manufacture both as sublingual bilayer tablets.[15, 16]

This chromatographic technique has a hydrophobic stationary phase and an aqueous mobile phase. This made it ideal for analysing drugs that are poorly soluble in water. The sample is injected into the column, and the components of the mixture interact with the stationary phase in a manner that depends on their polarity. The separation occurs based on the differential retention of the individual components of the sample in the stationary phase, which results in a series of peaks with distinct retention times.[17]

Previous reports on analytical methods for estimating ILP or IDB individually have not yielded a validated method for their simultaneous determination in sublingual bilayer tablet formulations. This study bridges that gap by introducing a new RP-UPLC method that is precise, rapid, simple, specific, accurate, and validated for simultaneous determination. The presented information also emphasizes the potential benefits of using the combination of Iloperidone and Idebenone in the treatment of psychosis, and the need to develop a validated RP-UPLC method that is precise, rapid, simple, specific, accurate, and specific for the concurrent estimation of ILP and IDB in sublingual bilayer tablet formulations. Furthermore, with the continuous advancements in technology, RP-UPLC is expected to become even more widely used in the future, and the development of a validated RP-UPLC method for the simultaneous determination of ILP and IDB in sublingual layered tablet formulations will be a significant step forward in this regard. As a result, the objective of this study was to develop a new stability indicating RP-UPLC method that is precise, rapid, simple, specific, accurate, and validated for the simultaneous estimation of ILP & IDB in Physical mixture and sublingual bilayer tablet formulations.[18-20]

MATERIALS AND METHODS

Idebenone gift sample received from Xi'an Bosheng Biological Technology Co Ltd, China and Iloperidone gift sample received from Lupin Ltd., Visakhapatnam, India. ILP and IDB sublingual tablets In-house prepared for method development purpose. All chemicals and reagents handled were of UPLC grades are procured from Fine chemicals, India. Nylon filter paper of 0.45 mm (Millipore) was purchased from Pall life science, Mumbai, India. Throughout the analysis, double-distilled water filtered through a 0.45 mm membrane filter was used.

Chromatographic conditions:

A UPLC system with a Photodiode Array detector was used for method development. A mobile phase using Orthophosphoric acid in water (0.1%) and acetonitrile with a ratio of 70:30 v/v was used in conjunction with an HSS C18 column to separate Iloperidone & Idebenone with exceptional resolution. A wavelength of 240 nm was used to identify the Iloperidone and Idebenone that were eluted from the column after the mobile phase was put into it at a rate of 0.4 mL per minute. When preparing the standard and sample solutions, acetonitrile and water were mixed in a 50:50 ratio to act as a diluent. Table 1 provides an explanation of the chromatographic conditions that yielded the best results.

Stock solution preparation:

To make the stock solution, Iloperidone (20 mg) and Idebenone (10 mg) API powders were added to a volumetric flask. 100 mL was measured to produce 200 µg/mL of Iloperidone and 100 µg/mL of Idebenone, respectively.

Standard solution preparation:

One milliliter of the stock solution stated above should be transferred into a 10-milliliter volumetric flask. Add diluent to make up the volume to get a solution of 20 microgram per mL and 10 microgram per mL of ILP and IDB, which was called a 100% level solution. The accuracy and system applicability of the established technique, which was created utilizing the provided standard solution, were confirmed

Preparation of sample solution:

The prepared 05 Sublingual tablets equal to 20 mg of Iloperidone and 10 mg of Idebenone were powdered to obtain a mixture. Diluent was used to make up the remaining volume such that the solutions for Iloperidone and Idebenone were 200 microgram per mL and 100 microgram per mL, respectively. 1 millilitre of the resultant solution was put into a 10-milliliter volumetric flask, and the remaining volume was adjusted using a diluent to get 10 microgram per mL for Idebenone and 20 microgram per mL for iloperidone. The debris of un-dissolved particles was filtered out using 0.25 µm nylon filters. The produced sample solution was utilized to ascertain the percentage assay of the manufactured combination dosage forms and to confirm the specificity of the devised procedure.

Method validation:

The validation of the current technique was verified in accordance with the International Council of Harmonisation guidelines (ICH Q2) .[15]

System suitability test:

The standard solution, which included 20 micrograms per milliliter of ILP and 10 micrograms per milliliter of IDB, was injected into six duplicates into the RP UPLC. The proportion of relative standard deviation (%RSD), USP plates (N), and USP tailing (T) were among the system suitability criteria evaluated.

Linearity:

This linearity demonstrates a direct proportionality between the technique's peak regions and the input concentrations. Concentrations ranging from 2.5microgram per mL to 15 microgram per mL of Idebenone and 5 microgram per mL to 30 microgram per mL of Iloperidone, linear graphs between concentrations and peak areas were produced for both compounds using the current approach. The values of the regression coefficient (r2) were evaluated.

Precision:

The measurement is typically expressed in terms of system and method precision. Six replicates of standard solution used for evaluation of system precision using standard solutions containing 20 microgram per mL and 10 microgram per mL of Iloperidone and Idebenone, similar to the assessment of system suitability. Method precision was tested by assessing the %relative standard deviation value from six replicate injections of the sample solution.

Accuracy:

Precision of the designated approach was confirmed using the conventional addition methodology. This procedure involved adding different concentrations of standard solution (50, 100, and 150%) to a predetermined

amount of sample solution (20 microgram per mL and 10 microgram per mL of Iloperidone & Idebenone, respectively). Average percentage recovery of the amount of standard solution recovered from the spiked solutions was determined. Analysis was done in triplicate for each spiked solution.

Specificity:
The unique feature of the analytical method lies in its ability to distinguish the substance of interest from other elements such as degradation products, impurities, and placebo substances without any disruption. To ensure the specificity of the current method, a series of solutions including blank, placebo, standard, test, and degraded forms of Iloperidone and Idebenone were carefully prepared and analyzed. Observations were made to identify any overlap in the retention times of the analytes (Iloperidone and Idebenone), degradation products, and placebo substances.

Sensitivity:
By applying the standard deviation approach to measure the limit of detection (LOD) and limit of quantification (LOQ), the sensitivity of the current procedure was assessed.

$$LOD = 3\sigma/S$$

$$LOQ = 10\sigma/S$$

where S is the linearity curve's average slope value (n = 3), and σ is the intercept's standard deviation (n = 3).

Robustness:
The robustness approach was assessed through minor adjustments in the optimal method parameters, including variations in the mobile phase ratio (± 1 mL), temperature (± 5°C), and flow rate (± 0.1 mL/min). To assess the robustness of the approach provided, the % relative standard deviation values for the peak areas obtained under the modified method circumstances were calculated.

Degradation studies:
When compared to accelerated stability testing, the ILP and IDB were subjected to far more severe stress conditions during the forced degradation approach. The evaluation of the ILP and IDB's intrinsic stability, it is crucial for creating a dosage form that remains stable over time, is supported by these studies. Forced deterioration investigations adhered to accepted standards such ICH Q1A, Q1B, and Q2B.[21]

Acid and base hydrolysis:
Equal parts of the stock solution (200 microgram per mL of Iloperidone and 100 microgram per mL of Idebenone) and Hydrochloric acid (2N) were combined evenly, refluxed for 30 minutes at 60° C, and then cooled to room temperature before being neutralized with sodium hydroxide (2N). Iloperidone and Idebenone concentrations of 20µg/mL and 10µg/mL, respectively, were obtained by further diluting the resulting solution. The solution mentioned above was thought to be an acid degradation solution. Similarly, in the acid hydrolysis process, 2N hydrochloric acid was substituted with 2N sodium hydroxide to create an alkali or base degradation solution. The produced solutions were added to the UPLC system after a 24-hour period.

Oxidative degradation:
Stock solution containing 200 microgram per mL of Iloperidone and 100 microgram per mL of Idebenone, as well as 10% Hydrogen Peroxide, were mixed evenly, refluxed for 30 minutes at 60°C, and then allowed to cool to room temperature. Iloperidone and Idebenone concentrations of 20 microgram per mL and 10 microgram per mL, respectively, were obtained by further diluting the resulting solution. The produced solutions were added to the UPLC system after a 24-hour period.

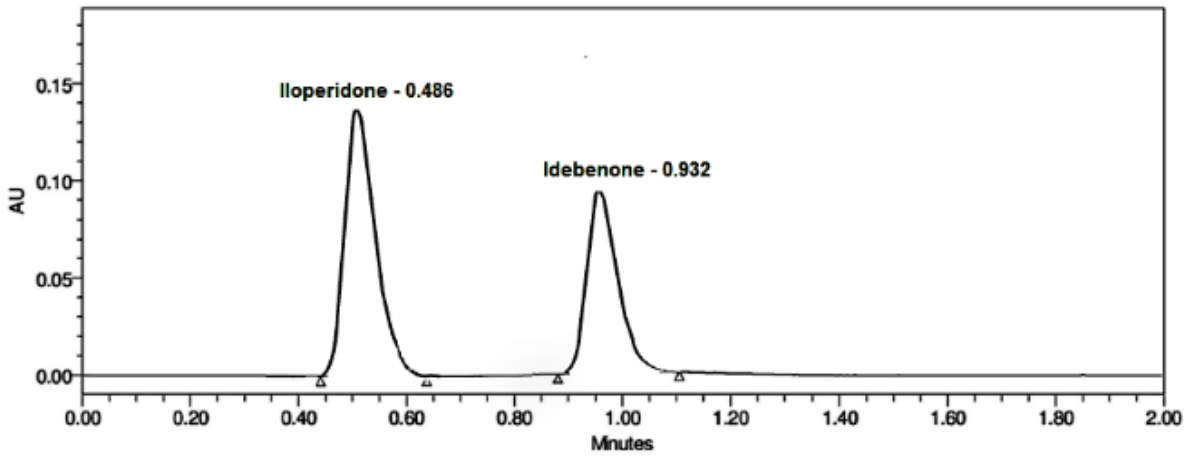
Thermal degradation:
The stock solution, which included 200 microgram per mL of Iloperidone and 100 microgram per mL of Idebenone, were maintained in the heating chamber for 24 hours at 80°C/75% RH. To create a solution with 20 microgram per mL of Iloperidone and 10 microgram per mL of Idebenone, the resulting solution had been further diluted.

Photo degradation:
A Ultraviolet chamber set at 254 nm with dark control was used to hold the standard stock solution, which included 200 microgram per mL of Iloperidone and 100 microgram per mL of Idebenone, for 24 hours. The resultant solution had been further diluted to produce a solution with 20 micrograms per milliliter of Iloperidone and 10 micrograms per millilitre of Idebenone.

Samples Assay testing:
In order to assay the In-house-made sublingual tablets, prepared sample solutions were injected along with individual standards. By calculating the peak areas of Iloperidone and Idebenone present in the chromatograms, the percentage purity of these compounds was ascertained.

RESULTS

Method optimization:
To obtain chromatographic peaks with acceptable tailing, resolution, and adherence to USP plate standards, a various solvent systems, solvent ratios, and flow rates were investigated. To separate Iloperidone and Idebenone with unusually high resolution, an High Strength Silica C18 column and a mobile phase consisting of 0.1% v/v Ortho-phosphoric acid (70% v/v) and Acetonitrile (30% v/v) were finally chosen. This methodology was validated by implementing the previously optimized parameters. A satisfactory resolution was achieved with Iloperidone and Idebenone eluting at RT of 0.49 minutes and 0.93 minutes, respectively, under these chromatographic conditions (Fig. 3).



Peak Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
Iloperidone	0.49	396584	2654	1.71	
Idebenone	0.93	287546	3254	1.53	6.5

Figure 3: Optimized method chromatogram of Iloperidone and Idebenone

Method validation:
The criteria to determine approach acceptability, including USP plates, USP tailing values, and % relative standard deviation (RSD), met the ICH (Q2) acceptance range as shown in (Table 1, Table 2). The r^2 values for Iloperidone and Idebenone at the specified concentration ranges were found to be 0.999 and 0.999, respectively (Fig.4a & Fig.4b). Therefore, the method demonstrates a strong linear correlation within the defined concentration range. Based on the average % recovery of Iloperidone and Idebenone in spike solutions ranging from 98.5 to 100.8% for both compounds (Table 3), it is evident that the methodology is accurate. The % RSD was found to be lower than 2 in (Table 4) during the evaluation of system and method accuracies. The results that were determined show the method's precision without a doubt. The method's strength was greatly confirmed by the considerably produced %RSD values within the ICH acceptance criteria (Table 5), even when minor and deliberate changes in the control variables did not affect the efficacy of the method. Other method suitability parameters, including plate count and tailing, as well as the relative standard deviation, provided strong evidence of the method's robustness.

Table 1: Optimized chromatographic conditions of current method

Mobile Phase:	0.1% OPA in water: Acetonitrile (70:30 v/v)
Column:	HSS C18 (100 × 2.1 mm, 5 µm)
Flow Rate:	0.4 mL/min
Temperature:	Ambient
Volume:	2µL
Wavelength:	240 nm
Diluent:	Water: Acetonitrile (50:50)
Retention time:	Iloperidone – 0.49 min
Idebenone – 0.93 min	

Table 2: System suitability data of Iloperidone and Idebenone in standard solution

Drug Name (n=6)	RT (Min)	Area *Mean ± SD	%RSD	Tailing (T)	Plate Count (N)	Resolution
Iloperidone	0.49	396584±4125.3	1.3	1.71	2680	-
Idebenone	0.93	287546±1815.4	0.6	0.15	3321	5.9

*****mean of six replicates of standard solution*

Table 3: Percentage recovery results of Iloperidone and Idebenone

Name of the drug	% Level of standard	*Amount spiked (µg/ml)	*Amount recovered (µg/ml)	%Recovery *(Mean ± SD)
Iloperidone	50	10	9.9	99.0 ± 0.98
	100	20	19.7	98.5 ± 0.50
	150	30	29.83	99.43 ± 0.49
Idebenone	50	5	5.04	100.8 ± 0.74
	100	10	9.87	98.7 ± 0.16
	150	15	14.99	99.93 ± 0.63

**Mean of three replicates*

Table 4: Precision of Iloperidone and Idebenone

Name of the drug	Precision type	*Mean ± SD (n=6)	%RSD	Acceptance limit
Iloperidone	System precision	389584 ± 2154.1	0.6	
	Method Precision	99.1 ± 0.74	0.75	
Idebenone	System precision	251784 ± 1654.2	0.26	
	Method Precision	98.8 ± 0.32	0.32	

**Mean of six replicates*

Table 5: Results of robustness of Iloperidone and Idebenone

Parameter	Variation	Iloperidone				Idebenone			
		Peak Area *(Mean± SD)	%RSD	Plate count	Tailing	Peak Area *(Mean± SD)	%RSD	Plate count	Tailing
Mobile Phase	71:29	626632±4266.0	0.7	2787	1.73	260172±1968.9	0.8	3435	1.57
(± 1 mL)	69:31	401774±1345.3	0.3	2612	1.74	242845±760.9	0.3	3302	1.66
Temperature	25°C	315006±2069.0	0.1	2691	1.71	195916±534.7	0.3	3283	1.63
(± 5°C)	35°C	343675±2069.0	0.6	2659	1.75	211199±1182.7	0.6	3039	1.59
Flow rate (±	0.2	323962±1868.4	0.6	2710	1.68	204923±843.0	0.4	3372	1.65
0.1mL/min)	0.4	285488±661.6	0.2	2602	1.75	181521±359.1	0.2	3231	1.53

**Average of six replicates of standard solution*

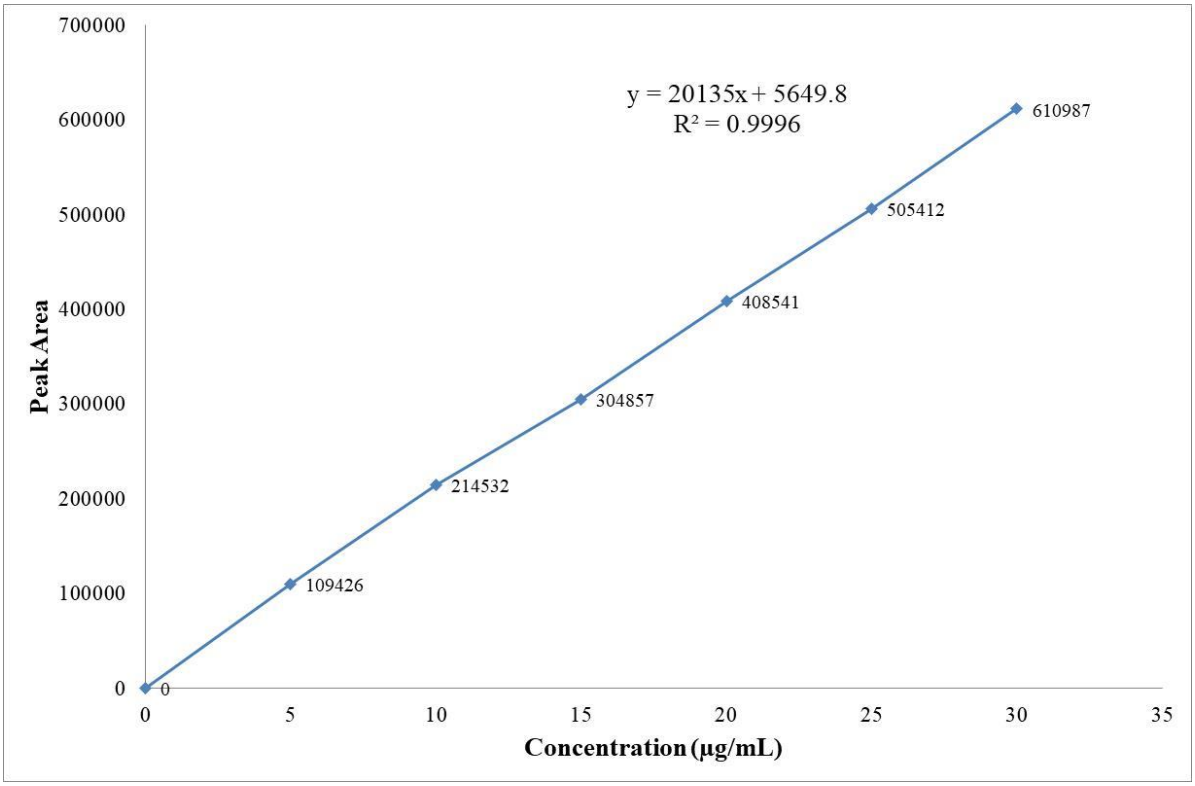


Figure 4a: Linearity results of Iloperidone

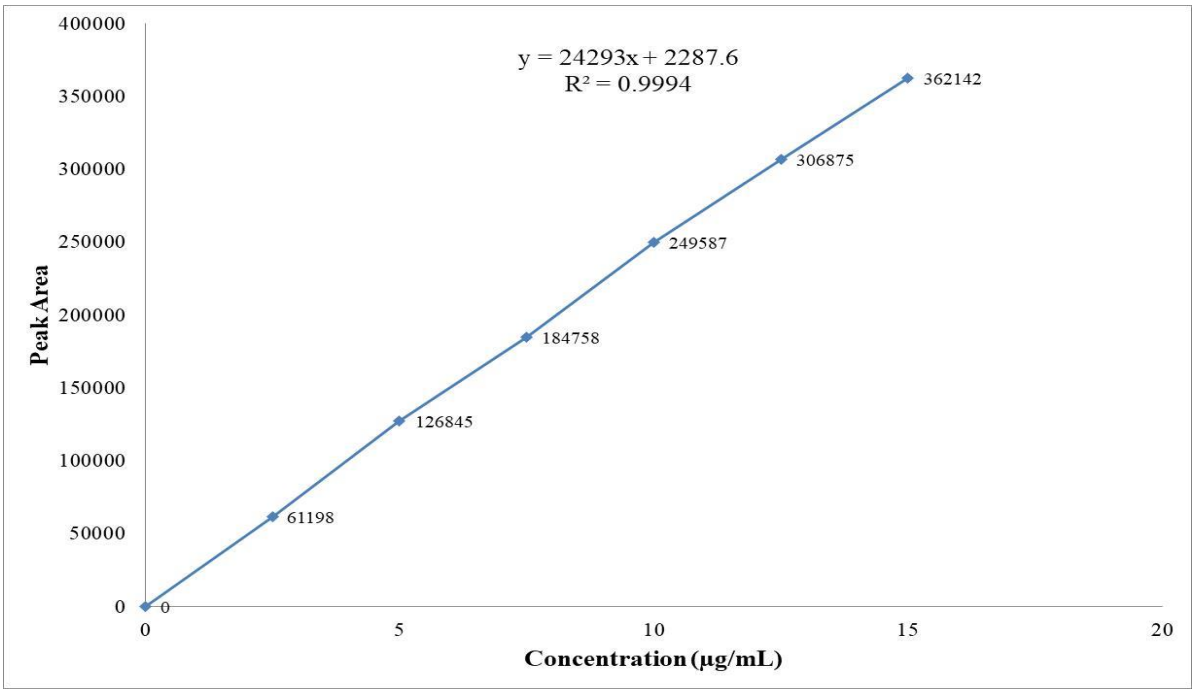


Figure 4b: Linearity results of Idebenone

Interference with the retention times of blank, degradants, and placebo did not occur in the presence of Iloperidone and Idebenone, demonstrating the method's specificity only toward these two compounds. The frontiers of detection and quantification for Iloperidone are found to be 0.22 microgram per mL & 0.47 microgram per mL and Idebenone are found to be 0.12 microgram per mL & 0.36 microgram per mL.

Upon assessing the peak areas from the freshly made standard solution with the Forced degradation solution, the amount of degradation for Iloperidone & Idebenone was measured as a percentage. The peak purity and percentage degradation of Iloperidone and Idebenone are displayed in (Table 6). The minimal degradation of both analytes in pH 7.0 conditions proved the stability of analytes in these conditions. Because of a notable

percentage of degradation in comparison to other stressful situations, both Iloperidone and Idebenone were vulnerable to an acidic setting. Both Iloperidone and Idebenone exhibited minimal degradation in the defined thermal and photo degradation conditions, indicating a high level of stability for both substances. Hence, it is argued that the novel method is stability-indicative for assessing API and dosage form stability. The chromatograms created from stressed samples can be found in (Figure 4a & 4b)The peak purity of Iloperidone, Idebenone, and degradants was indicated by the purity threshold value for each peak exceeding the peak's purity angle. The results of FD investigations amply illustrate the stability-indicating character of the approach.

Table 6: %Degradation of Iloperidone and Idebenone at different forced degradation conditions

Stress degradation	% Degradation	
	Iloperidone	Idebenone
Acidic degradation	5.11	5.48
Alkali degradation	4.55	4.07
Oxidative degradation	3.84	3.80
Thermal degradation	2.35	2.64
Photo degradation	1.62	1.83
Neutral degradation	0.01	0.09

Assay of the prepared sublingual tablets:

According to (Table 7), the purity of the in-house made sublingual bilayer tablets of Iloperidone and Idebenone was determined to be 99.2% ± 0.28% and 98.24% ± 0.34%, respectively.

Table 7: %Assay of prepared sublingual tablets

Drug	Chromatogram name	RT	Area	*% Assay ± SD	%RSD
Iloperidone	Standard	0.464	408647	99.2 ± 0.28	0.28
	Test	0.487	406214		
Idebenone	Standard	0.926	242900	98.24 ± 0.24	0.24
	Test	0.936	246981		

Average weight of tablet- 124.5mg, Label claim: Iloperidone – 20 mg & Idebenone – 10mg

**Mean of six measurements*

DISCUSSION

Both qualitative and quantitative drug confirmations can be achieved using the stability indicator LC method. Sub-lingual dosage form with a set dosage of Iloperidone & Idebenone, there isn't any RP-UPLC procedures available at the moment. In previous HPLC techniques, there was an issue with extended runtime and response time for Iloperidone (7.7 min). Idebenone 45 and 83 microgram per mL and Iloperidone 27 and 42 microgram per mL showed reduced sensitivity levels of LOD & LOQ in an alternative method. During forced degradation studies, the described approach did not exhibit any degradation peaks. To overcome earlier restrictions and shortcomings, research was done to develop an RP-UPLC technique with a shorter retention period, higher sensitivity, and a simpler solvent composition. In the proposed method, Iloperidone and Idebenone were observed at RT of 0.49 minutes and 0.93 minutes, respectively, indicating a shorter elution time with reduced run time. The mixture of Acetonitrile (30 % v/v) and 0.1% OPA (70 v/v) ratios, with high sensitivity and reduced run time, demonstrates the cost-effective aspect of the procedure. The present technique is capable to speed up the time it takes to analyze samples. The method offers a high level of precision when analyzing Iloperidone and Idebenone, as indicated by the statistical data on the validation parameters. The Iloperidone LOD 0.22 microgram per mL and LOQ 0.47 microgram per mL and Idebenone LOD 0.12 microgram per mL and LOQ 0.36 microgram per mL exhibited significantly improved results compared to the method discussed earlier. Assessing the quality of mixed powder and tablets is primarily determined by conducting stability studies. The potential of this method approach to assess the stability of Iloperidone and Idebenone is proven by measuring their degradation under various stress environments. Both the analytes remained extremely sensitive to acidic and alkaline conditions, but very stable in neutral conditions. The reported findings depict the nature of stability indicating.[22-25]

CONCLUSION

Iloperidone and Idebenone in physical combination form and their fixed dosage sublingual bilayer tablets were tested using an accurate, precise, and effective stability indicating RP-UPLC technique with enhanced sensitivity and fast analytical time. The method's robustness and dependability were demonstrated by the validation parameters, which were well within the acceptable range. Examining Iloperidone and Idebenone under a range of stressful circumstances validates the method's dependability. Excellent resolution was achieved by the method's successful separation of Iloperidone and Idebenone as well as any possible degradation products from each. To ensure the safety and effectiveness of Idebenone and Iloperidone in sublingual bilayer tablets, the suggested approach might be helpful for routine quality control analysis.

SUMMARY
The Reverse Phase Ultra-Performance Liquid Chromatography (RP-UPLC) technique holds an immense significance in the field of pharmaceutical drug estimation. This inventive chromatographic method is depicted by its high efficiency, rapid analysis, and unique sensitivity, making it tremendous in the pharmaceutical industry. Furthermore, its high throughput nature, superior resolution, and peak capacity, as well as precise separation, contribute to the overall quality assurance of pharmaceutical products. In this research study, a meticulous method was developed for the simultaneous estimation of Iloperidone and Idebenone in sublingual bilayer tablets using RP-UPLC analysis. The developed method was validated as a stability-indicating approach through complete analytical method validation, including forced degradation studies for commercial use.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

RP-UPLC: Reverse Phase – Ultra Performance Liquid chromatography; HSS column: High Strength Silica column; ICH: International Council for Harmonisation; PDA detector: Photo diode array detector; **µg:** Microgram.

REFERENCES

1. Vij I, Pathania A. An Overview-Advance in Chromatographic Techniques in Photochemistry. EDP Sciences. 2023;391:01038.
2. Lorenc W, Kruszka D, Kachlicki P, Kozłowska J, Barałkiewicz D. Arsenic species and their transformation pathways in marine plants. The usefulness of advanced hyphenated techniques HPLC/ICP-MS and UPLC/ESI-MS/MS in arsenic species analysis. Talanta. 2020;220:121384.
3. González-Burgos E, Fernández-Moriano C, Gómez-Serranillos MP. Current knowledge on Parmelia genus: Ecological interest, phytochemistry, biological activities, and therapeutic potential. Phytochemistry. 2019; 165:112051.
4. Parys W, Dołowy M, Pyka-Pająk A. Current strategies for studying the natural and synthetic bioactive compounds in food by chromatographic separation techniques. Processes. 2021; 9(7): 1100.
5. G TD. Novel Analytical Techniques used in Identification and Isolation of Impurities in Pharmaceuticals an Overview. J Pharm Sci Res. 2020; 12(1):37-42.
6. Gordillo R. Supercritical fluid chromatography hyphenated to mass spectrometry for metabolomics applications. J Sep Sci. 2021; 44(1):448-463.
7. Penmatsa VK, Kanakapura B, Nagaraju S. Development and validation of a stability-indicating RP-UPLC method for the determination of fluconazole in bulk drug and in pharmaceutical dosage forms. Pharmaceuticals. 2014;31: 32.
8. Ahmed S, Islam S, Ullah B, Biswas SK, Azad AS, Hossain S. A review article on pharmaceutical analysis of pharmaceutical industry according to pharmacopoeias. Orient J Chem. 2020; 36.
9. Coskun O. Separation techniques: Chromatography. North Clin Istanb. 2016; 3(2):156–160.
10. Landge SB, Jadhav SA, Vishwambar SP, Solanki PV, Bembalkar SR, Mathad VT. Development and validation of RP-UPLC method for the determination of Iloperidone, its related compounds and degradation products in bulk and dosage form. American Journal of Analytical Chemistry. 2014; 5(14):969.
11. Atila Karaca S, Uğur DY. Determination of Iloperidone in Pharmaceuticals by Validated High-Performance Liquid Chromatography. Analytical Letters. 2018; 51(1-2):24-32.
12. Gueven N, Ravishankar P, Eri R, Rybalka E. Idebenone: When an antioxidant is not an antioxidant. Redox Biol. 2021;38:101812.
13. Nohara Y, Suzuki J, Yamazaki Y, Kubo H. Determination of idebenone in plasma by HPLC with post-column fluorescence derivatization using 2-cyanoacetamide. Chem Pharm Bull. 2012;60(5):598 602.
14. Londhe V, Shirsat R. Formulation and characterization of fast-dissolving sublingual film of iloperidone using Box–Behnken design for enhancement of oral bioavailability. AAPS PharmSciTech. 2018; 19:1392-400.
15. Bhowmik D, Chiranjib B, Krishnakanth P, Chandira RM. Fast dissolving tablet: an overview. J Chem Pharm Res.2009;1(1):163–77.
16. Batinic B, Ristic I, Zugic M, Baldwin DS. Treatment of symptom clusters in schizophrenia, bipolar disorder and major depressive disorder with the dopamine D3/D2 preferring partial agonist cariprazine. Front Psychiatry. 2021;23(12):784370 4.
17. Citrome L, McEvoy JP, Todtenkopf MS, McDonnell D, Weiden PJ. A commentary on the efficacy of olanzapine for the treatment of schizophrenia: the past, present, and future. Neuropsychiatr Dis Treat. 2019; 15:2559–69.
18. Rafi S. A new validated method for the estimation of olanzapine and Samidorphan using high performance liquid chromatography and of its degradation. Biosci Biotechnol Res Commun. 2021;14(9):198–204.
19. Batinic B, Djokic V, Ivkovic M. Assessment of cognitive function, social disability and basic life skills in euthymic patients with bipolar disorder. Psychiatr Danub. 2021;33(3):320-327.
20. Green MF. Cognitive impairment and functional outcome in schizophrenia and bipolar disorder. J Clin Psychiatry. 2006; 67(10):e12.
21. ICH. Quality Guidelines ICH. https:// www. ich.org/ page/quality- guide lines. Accessed 18 Nov 2023.
22. Berrettini W. Evidence for shared susceptibility in bipolar disorder and schizophrenia. Am J Med Genet C Semin Med Genet. 2003; 123C (1):59–64.
23. Alam M, Jahan K. Informative review of layered technology of tablet-an emerging innovation in oral drug delivery system. EJPMR, 2023;10(12): 667-675.
24. P. BR, P JB. A Comprehensive Review on Technological Aspects in Bilayer Tablet Manufacturing. World Journal of Pharmaceutical Research. 2022; 11(3):1947–63.
25. S. R, T P. A Recent Review on Dual Release Bilayered Tablet. Critical Review in Pharmaceutical Sciences. 2016; 5(4):1–11.