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Development and Validation of RP-HPLC Method for Estimation of Anti-Diabetic Drug in Bulk and Tablet Dosage Form

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Abstract:

Imeglimin is an investigational first-in-class novel oral agent for the treatment of type 2 diabetes. Imeglimin's mechanism of action involves two distinct effects: (a) an increase in glucose-stimulated insulin secretion (GSIS) and preservation of cell mass; and (b) an improvement in insulin action, with the potential to reduce hepatic glucose output and enhance insulin signaling in both the liver and skeletal muscle. Imeglimin may address mitochondrial dysfunction, a frequent underlying factor in the pathophysiology of T2D, at the cellular and molecular level. Reduced reactive oxygen species formation (decreasing oxidative stress) and prevention of mitochondrial permeability transition pore opening (implicated in preventing cell death) have been observed as a result of the observed rebalancing of respiratory chain activity (partial inhibition of Complex I and correction of deficient Complex III activity).

Key words: Keywords: Type 2 diabetes, Imeglimin, Mechanism, Mitochondria.

Introduction:-

Imeglimin is an oral anti-diabetic drug sold under the trade name Twymeeg. In Japan, it was given the go-ahead in June 2021.

It is an inhibitor of oxidative phosphorylation that also works to improve muscle glucose absorption and restore regular insulin secretion. It is the first anti-diabetic drug of this type to receive approval. The prevalence of type 2 diabetes , which currently affects more than 380 million people worldwide, is expected to increase to more than 590 million people by the year 2035. Numerous organisations, both public and private, have committed a lot of time, energy, and resources to treatment, prevention, and education in order to solve this global issue. Despite best efforts, the prevalence of T2DM keeps increasing, especially when you take into account the growing elderly population, rising obesity rates, and growing numbers of high-risk ethnic groups. Additionally, in order to establish and sustain long-term glycemic control, diabetes' physiologic and progressive nature necessitates a combination of pharmaceutical therapy and lifestyle changes.

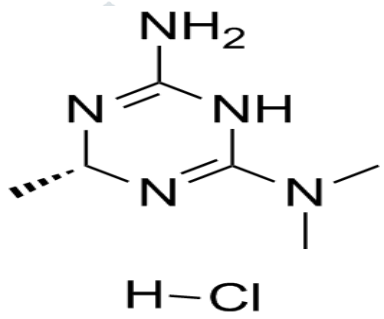
Objectives:

The main objectives of the study are:

To develop new, simple, sensitive, accurate, and economical analytical method for the determination of assay of of Anti-Diabetic drug in tablet dosage from by RP-HPLC. To Validate the proposed method in accordance with USP and ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form.

DRUG PROFILE:

Structure:-



General profile of imeglimin hydrochloride-

Category- Anti-diabetes Agent
Chemical Name- (R)-6-imino-N,N,4-trimethyl-1,4,5,6-tetrahydro-1,3,5-triazin-2-amine hydrochloride
Molecular Formula- $C_6H_{14}ClN_5$
Molecular Weight- 191.66 g/mol
pKa- 10.21

Mechanism of action: Imeglimin's mechanism of action involves two distinct effects: (a) an increase in glucose-stimulated insulin secretion (GSIS) and preservation of -cell mass; and (b) an improvement in insulin action, with the potential to reduce hepatic glucose output and enhance insulin signalling in both the liver and skeletal muscle.

Pharmacological action:
It is an oxidative phosphorylation blocker that acts to inhibit hepatic gluconeogenesis, increase muscle glucose uptake, and restore normal insulin secretion. It is the first approved drug of this class of anti-diabetic medication.

PLAN OF WORK:

Estimation of of Anti-Diabetic Drug in bulk and tablet dosage from will be done by following methods.

Selection of Drugs and Formulation

By literature and market survey

Online Journals, chemical and analytical abstracts were studied to find out drugs for which there were no reported RP-HPLC methods. Market survey was carried to check the availability of these drugs and their dosage forms.

Selection of analytical techniques

Estimation by UV-Visible spectroscopy.
Identification by IR Spectroscopy
HPLC method

Method development by RP-HPLC.

Selection of preliminary HPLC conditions.
Selection of preliminary HPLC conditions.
Selection of mobile phase.
Selection of column.
Selection of wavelength.
Selection of Flow rate.
Selection of Injection Volume.
Selection of column Temperature.
Selection of sample Temperature.
Analysis of laboratory mixture.

Validation of proposed method.

System suitability test
Linearity and Range
Accuracy
Precision
a.Intermediate precision(Ruggedness)
b.Method precision
Robustness
Specificity

Probable outcomes:

A simple and accurate analytical technique can be developed for the determination of of Anti-Diabetic Drug in bulk and tablet dosage from
Method developed can be conveniently used for quality control and routine determination of drug in pharmaceutical industry.

Drug used in experiment:

Name of drug and drug product	Supplier and manufacturer by
Imeglimin hydrochloride	Lupin Ltd.
Imeglimin hydrochloride tablet	Lupin Ltd.

Reagent

List of Reagent

Sr.No	Chemical	Make
1	Water	Rankem
2	Methanol	Merck life science
3	Sodium dihydrogen phosphate	Merck life science
4	Phosphoric acid 88%	Merck life science
5	0.45 µ Nylon membrane disc filter	Mdi
6	0.45µ PVDF Syringe Filter	Mdi

INSTRUMENTS:-

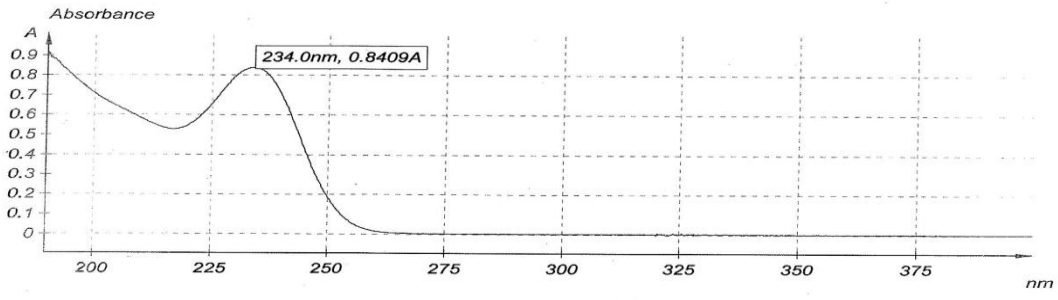
Sr No.	Instrument Name	Make	Model
1	Analytical balance	Mettler Toledo	XS205D0
2	Spectrophotometer	Shimadzu	UV 1900i
3	HPLC	Agilent	1260 Infinity
4	Ph meter	Lab India	PICO+

RESULTS AND DISCUSSION:

A simple, precise and economic RP-HPLC method was developed and validated for estimation of Imeglimin hydrochloride in bulk and tablet. The method was validated as per ICH guidelines by using various validation parameters such as System suitability, Linearity, accuracy, precision and robustness.

STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND OPTIMIZATION:

Selection of Wavelength



Spectra showing λ max of Imeglimin hydrochloride

Determination of λ max of Imeglimin hydrochloride

Sr. No.	Wavelength (nm)	Absorbance
1.	234	0.8409 A

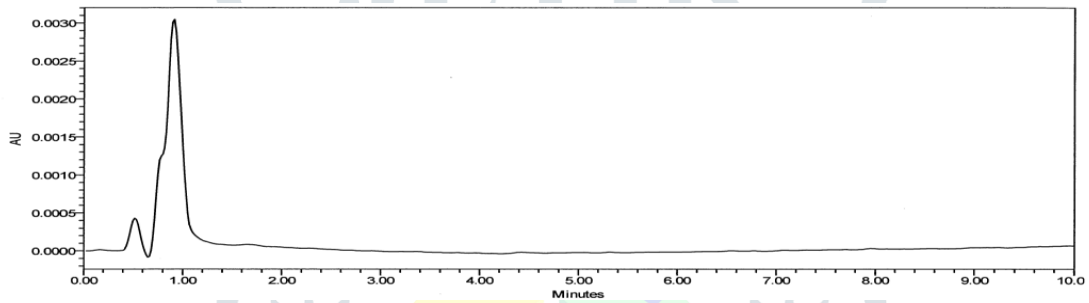
Reverse Phase High Performance Liquid Chromatography Method Development

Different trials taken were as follows

TRIAL: 1

Chromatographic Conditions:

Column	Purospher Star RP 18 Endcapped (250 mm X 4.6 mm), 5µm
Mobile phase	Water:Methanol (80:20)
Flow Rate	0.8 mL/min
Injection Volume	10 µL
Wavelength	234 nm
Column Temp.	25°C
Auto sampler Temp.	25°C
Run time	10.0 min.
Needle wash	Water:methanol (20:80 v/v)
Seal wash	Water:methanol (80:20 v/v)



Typical chromatogram for Trial- 1

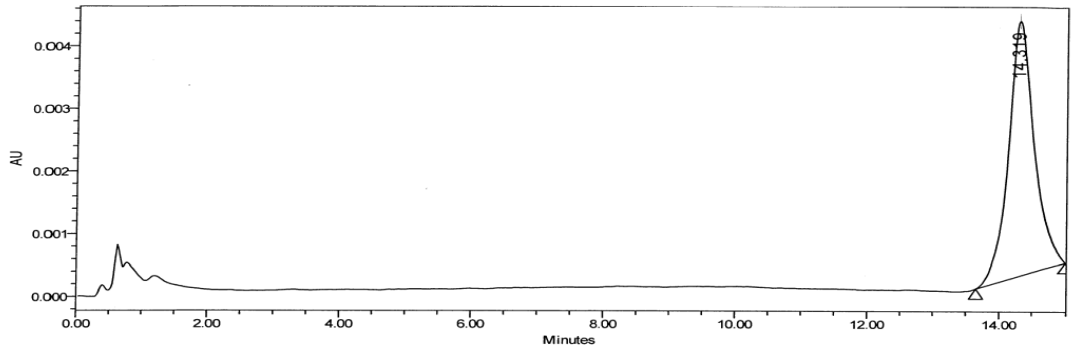
Conclusion:

Imeglimin hydrochloride peak not observed, hence method needs to be optimized.

TRIAL: 2

Chromatographic Condition:

Column	Purospher Star RP 18 Endcapped (250 mm X 4.6 mm), 5µm
Mobile phase	Water:methanol (80:20 v/v)
Flow Rate	0.8 mL/min
Injection Volume	10 µL
Wavelength	234 nm
Column Temp.	25°C
Auto sampler Temp.	25°C
Run time	15.0min.
Needle wash	Water:methanol (20:80 v/v)
Seal wash	Water:methanol (80:20 v/v)



Typical chromatogram for Trial- 2

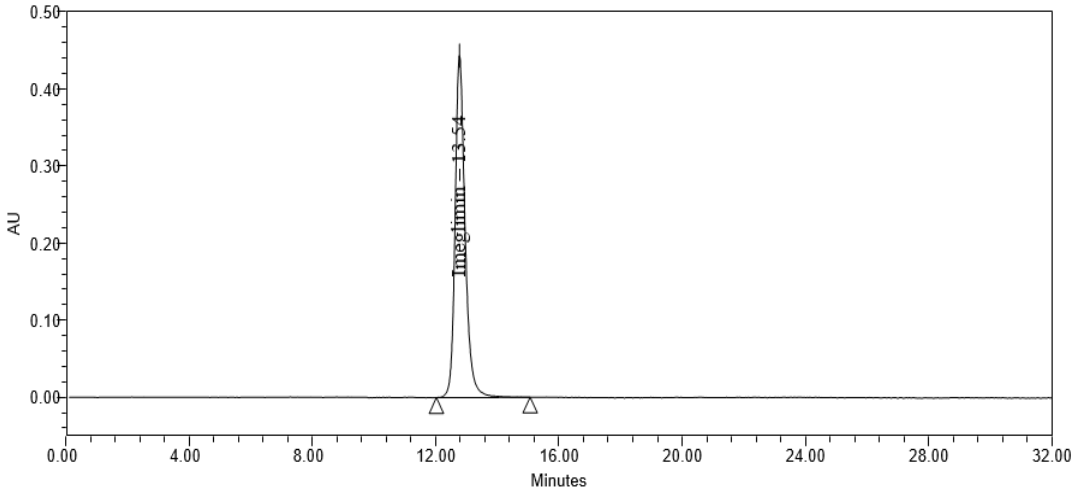
Conclusion:- Imeglimin hydrochloride peak observed at 14.319 min, but method needs to be optimized. Mobile phase needed to be **changed** further to decrease the retention time.

TRIAL: 3

Chromatographic Condition:

Column	Hypersil ODS (150 mm X 4.6 mm), 5µm
Mobile phase	buffer pH 3.0 and methanol (80:20 v/v)
Flow Rate	0.8 mL/min
Injection Volume	20 µL
Wavelength	234 nm
Column Temp.	25°C
Auto sampler Temp.	25°C
Run time	10.0 min.

Needle wash	Water: methanol (20:80 v/v)
Seal wash	Water: methanol (80:20 v/v)



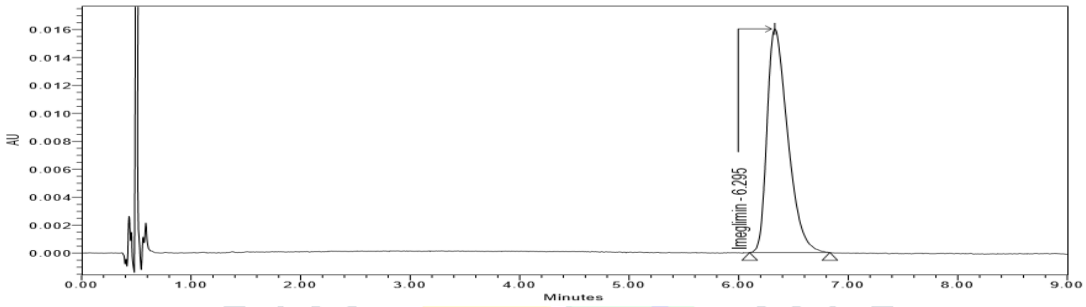
Typical chromatogram for Trial- 3

Conclusion:- Imeglimin hydrochloride peak observed at 13.54 min, but method needs to be optimized. Mobile phase needed to be **changed** further to decrease the retention time.

TRIAL: 4

Chromatographic Condition:

Column	Hypersil ODS (150 mm X 4.6 mm), 5µm
Mobile phase	buffer pH 3.0 and methanol (75:25 v/v)
Flow Rate	1.0 mL/min
Injection Volume	20 µL
Wavelength	234 nm
Column Temp.	30°C
Auto sampler Temp.	25°C
Run time	5.0 min.
Needle wash	Water: methanol (20:80 v/v)
Seal wash	Water: methanol (80:20 v/v)



Typical chromatogram for Trial- 4

Conclusion:- Keeping rest of the chromatographic conditions constant, Imeglimin hydrochloride peak was eluted at RT 6.295 min. peak shows peak purity. Theoretical plate, USP plate count, symmetry was found to be satisfactory. So conditions of trial 4 were selected as optimized chromatographic conditions

METHOD VALIDATION

The following parameters were considered for the analytical method validation of title ingredients.

System Suitability.
Linearity.
Accuracy.
Precision.
Method Precision.
Intermediate Precision.
Robustness.
Specificity

SYSTEM SUITABILITY: System suitability test is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done.

System suitability test

Tailing factor	**1.0**
Theoretical plates	**6357**
Sr. No.	**Area**
1	695247
2	700214
3	699858
4	698685
5	700054
Mean	699181
% RSD	0.3

Conclusion:

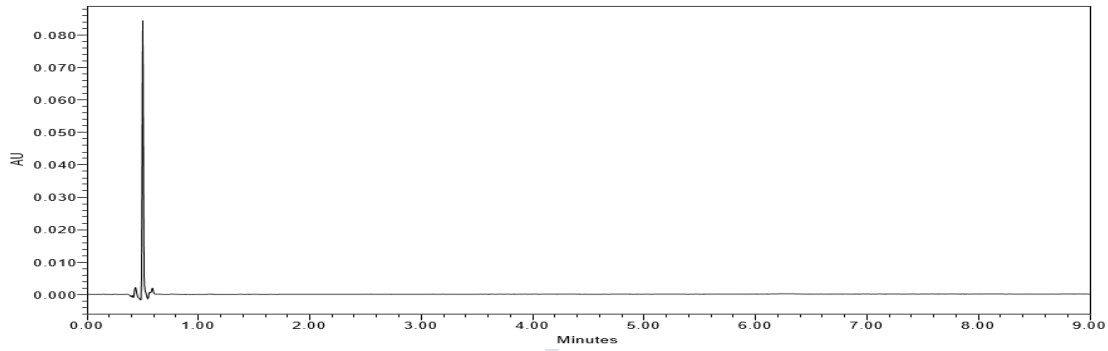
The data demonstrates that the system suitability is within the acceptance criteria, thus the system is suitable.

Specificity: (Identification, Interference & Peak Purity)

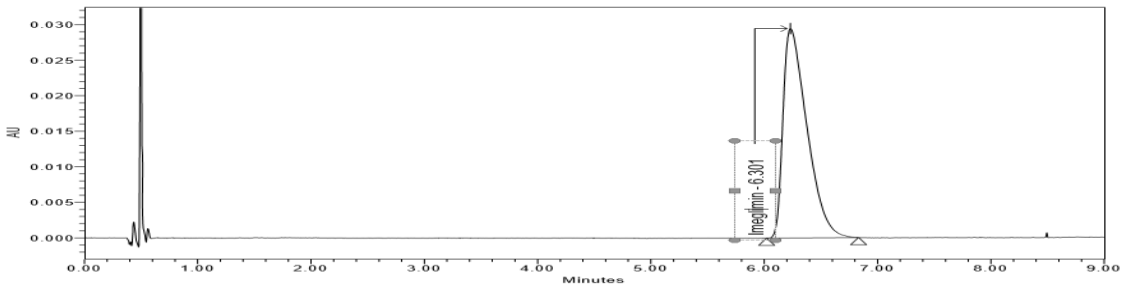
Inject Blank (Diluent), standard solution, impurity Solution, placebo solution and sample solution .The data obtained is summarized in Table

Specificity (Identification and Interference)

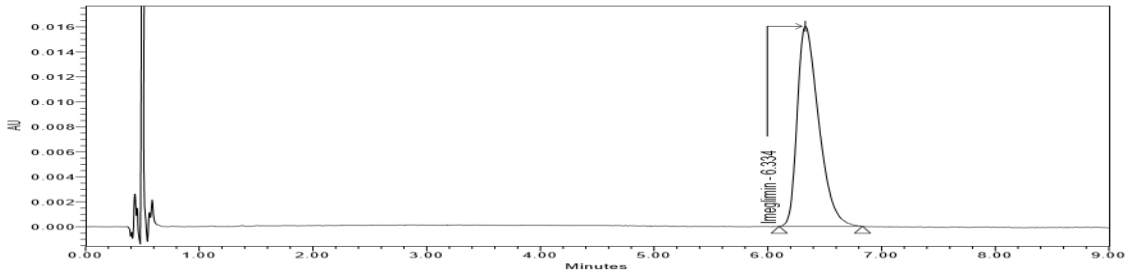
Sr. no.	**Component**	***RT (min)**	**Tailing factor**	**Purity angle**	**Purity threshold**
1	Blank	-	-	-	-
2	Placebo solution	-	-	-	-
3	Standard solution	6.301	1.0	2.36	5.24
4	Sample solution	6.334	1.0	2.01	4.98



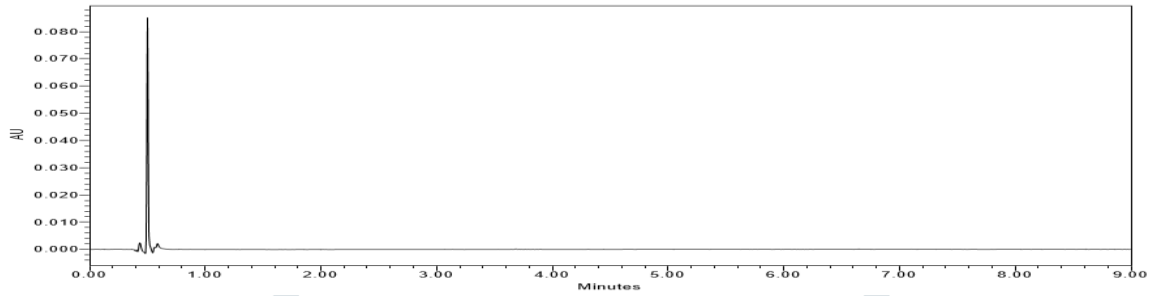
Chromatogram of Blank



Chromatogram of Standard



Chromatogram of Sample



Chromatogram of Placebo

Conclusion:

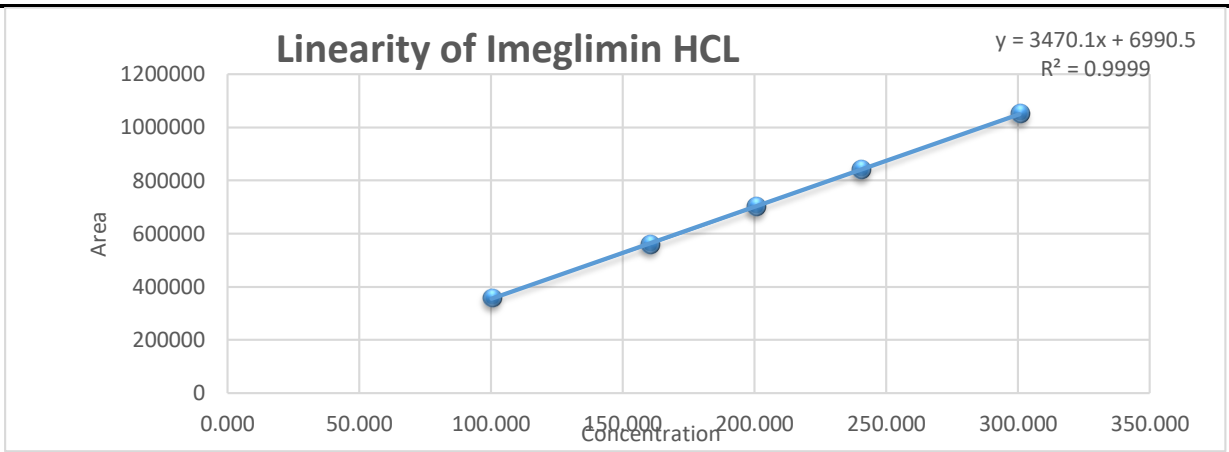
Retention time of sample solution found comparable with retention time of Imeglimin hydrochloride standard solution.
The data demonstrates that there is no interference in Blank Placebo tablet should not show any peak at the Retention time of Imeglimin hydrochloride peak.
Standard and sample peak is pure at working concentration level.
Purity angle is less than purity threshold in both chromatograms obtained from Standard and Sample solution.
Standard peak is pure at working concentration level.

LINEARITY:

Linearity was evaluated in the range of 50% to 150% of the working concentration level. As the working concentration level of Imeglimin hydrochloride.

Linearity

Level (%)	Concentration (ppm)	Response		
		1	2	Mean
50	100.160	356841	358014	357428
80	160.256	559758	561004	560381
100	200.320	700145	701236	700691
120	240.384	839998	840628	840313
150	300.480	1050365	1053256	1051811
Co-relation coefficient (r^2)				**0.9999**
SLOPE				**3470.1**
Y-INTERCEPT				**6990.5**



Linearity plot

Conclusion:

The data demonstrates that the system suitability is within the acceptance criteria, thus the system is suitable.
The data shows Response linear.
The data shows that the response found is linear; Correlation coefficient (R) is more than 0.999.
The data shows that correlation of determination (R^2) is more than 0.999.

Accuracy (Recovery):

Accuracy was evaluated three levels 50%, 100% and 150% of the working concentration level for Imeglimin hydrochloride. As the working concentration level of Imeglimin hydrochloride, Each level prepared in triplicates.

% Recovery

Level (%)	Theoretical concentration (mcg/mL)	Area	% Recovery	Mean recovery%
50	100	351633	100.6	100.6
	100	350305	100.2	
	100	349152	99.9	
100	200	701064	100.2	100.3
	200	700154	100.1	
	200	699822	100.1	
150	300	1054139	100.5	100.5
	300	1046941	99.8	
	300	1043930	99.5	
Mean recovery				**100.5**

Conclusion:
The data demonstrates that the system suitability complies with the acceptance criteria.
The data shows that the % mean and individual recovery at each level is within the acceptance criteria.
The data shows that the Mean recovery for 50% to 150% is in the range of 98.0%-102.0%and individual recovery for 50% to 150% is in the range of 97.0% - 103.0%.

Precision:

Method Precision:

Single injection of blank (Diluent), Standard solution (six replicates) and sample solution (six preparations) was injected on the system.

Method precision

Sample No	Area	% Assay
1	699181	99.3
2	702547	99.8
3	708547	100.6
4	706354	100.3
5	705248	100.2
6	699852	99.43
Mean		**99.9**
% RSD		**0.5**

Conclusion:

The data shows that system suitability is fulfilled.
The data shows that % RSD for % Assay is within the acceptance criteria and hence the method is precise.

Intermediate Precision:

Five independent sample preparations were prepared on different day and by different analyst and injected on the HPLC.

Intermediate Precision

Parameter	Method Precision(Analyst-I)	Intermediate Precision(Analyst-II)
HPLC Instrument No.	**LC-01**	**LC-04**
Date of analysis	**xxxx**	**xxxx**
HPLC column No.	**HC-35**	**HC-07**
Sample No.	**% Assay**	
1	99.3	99.3
2	99.8	99.5
3	100.6	99.4
4	100.3	100
5	100.2	99.6
6	99.43	100.1
Mean	**99.9**	**99.7**
Average	**99.8**	
% RSD of all determinations	**0.4**	

Conclusion:

The data shows that RSD of six determinations is within the acceptance criteria.
The data shows that Cumulative % RSD for % assay of twelve independent samples preparation of two analysts is within the acceptance criteria.

Robustness:

This parameter was studied by making small, deliberate changes in the chromatographic conditions and Assay parameters, observing the effect of these changes on the system suitability and results obtained by injecting the standard and sample solutions.

Robustness

Parameters	Values	%Assay	Absolute difference
Control	As per method	100.1	NA
Flow rate (± 0.1 mL/min)	0.9mL/min	99.3	-0.8
	1.1mL/min	100.5	0.4
Change in Wavelength(± 5 nm)	229nm	100.3	0.2
	239 nm	100.1	0
Column temperature (± 5°C)	25°C	99.9	-0.2
	35°C	100.5	0.4

Conclusion:

System suitability criteria were fulfilled.
The difference of % assay value in each modified condition is within acceptance criteria.

CONCLUSION:

RP-High Performance Liquid Chromatography (HPLC) Method:

HPLC has gained the valuable position in the field of analysis due to ease of performance, specificity, sensitivity and the analysis of sample of complex nature. This technique was employed in the present investigation for estimation of Dolutegravir tablet formulation. HPLC Water2469 with Inertsil ODS -3v C18(250 mm X 4.6 mm), 5µm column and UV/PDA detector with empower pro Software was used for the study. The standard and sample solution of Dolutegravir were prepared in diluent. Different pure solvents of varying polarity in different proportions were tried as mobile phase for development of the chromatogram.

The mobile phase that was found to be most suitable was Buffer and Methanol, the wavelength 261 nm were selected for the evaluation of the chromatogram of Dolutegravir respectively. The selection of the wavelength was based on the λ max obtained by scanning of standard laboratory mixture in water: methanol.

This system gave good resolution and optimum retention time with appropriate tailing factor (<2).

After establishing the chromatographic conditions, standard laboratory mixture was prepared and analysed by procedure described under Materials and methods. It gave accurate, reliable results and was extended for estimation of drugs in tablet formulation.

The results from table clearly indicate that the RP-HPLC technique can be successfully applied for the estimation of above-mentioned drugs in their formulation.

Validation:

Validation of these methods was performed as per the ICH guidelines for these following parameters.

1) **Accuracy** - Accuracy of the proposed method was ascertained from the recovery studies by standard addition method.
2) **Precision** - Replicate estimation of tablet analysed by the proposed method has yielded quite consistent result indicating repeatability of method. Study showed % R.S.D. < 2.
3) **Specificity** – Studies shows that there is no interference of peak from the component of matrix showing retention time
4) **Robustness** - Studies were carried out for the different parameters like Change in Temperature, Wavelength, pH and Flow Rate. Results of estimation by proposed method are very much similar under variety of conditions. This study signifies the Robustness of the method under varying condition of its performance.
5) **Solution Stability** – The % difference of standard and sample were found to be within range.

6) **Linearity and Range**- Dolutegravir were found to be linear in the range of 50%to 150 % of test concentration with R2 ≈ 0.999 at selected wavelength.

From the studies it can be concluded that RP-HPLC technique can be successfully used for the estimation of the Dolutegravir tablet Formulations.

The method shows good reproducibility; more over the RP-HPLC method is accurate, precise, specific, reproducible and sensitive. The analysis of single dose formulation of Dolutegravir bulk and tablet can also be successfully performed by the RP-HPLC method. No interference of additives, matrix etc. is encountered in these methods. Further studies on other pharmaceutical formulations would throw more light on these studies. Suitability of these methods on biological samples needy also studies.

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