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Research

Development and validation of rp-hplc method for simultaneous estimation of imeglimin and metformin used for The treatment of diabetes

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Abstract

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Imeglimin And Metformin, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Hypersil C18 (4.6×250mm) 5µ column using a mixture of Water and Acetonitrile (50:50) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 244nm. The retention time of the Imeglimin and Metformin was 2.0, 4.0±0.02min respectively. The method produce linear responses in the concentration range of 20-100µg/ml of Imeglimin and 40-200µg/ml of Metformin. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Imeglimin, Metformin, RP-HPLC, Validation, Diabetes

INTRODUCTION

High Performance Liquid Chromatography (HPLC) was derived from the classical column chromatography and, is one of the most important tools of analytical chemistry today.[1]In the modern pharmaceutical industry, high-performance liquid chromatography (HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development, and production.[2] HPLC is the method of choice for checking peak purity of new chemical entities, monitoring reaction changes is in synthetic procedures or scale up, evaluating new formulations and carrying out quality control / assurance of the final drug products.[3]

The Goal of HPLC method is to try & separate, quantify the main drug, any reaction impurities, all available synthetic intermediates and any degradants.[4] High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantify the compounds that are present in any sample that can be dissolved in a liquid. HPLC is the most accurate analytical methods widely used for the quantitative as well as qualitative analysis of drug product and used for determining drug product stability. [5] HPLC principle is the solution of sample is injected into a column of porous material (stationary phase) and liquid phase (mobile phase) is pumped at higher pressure through the column. The principle of

separation followed is the adsorption of solute on stationary phase based on its affinity towards stationary phase. (Figure-1) The technique of HPLC has following features.[6] High resolution, Small diameter, Stainless steel, Glass column, Rapid analysis, Relatively higher mobile phase pressure, Controlled flow rate of mobile phase.

HPLC Method Development

Methods are developed for new products when no official methods are available. Alternate methods for existing (Non-Pharmacopoeial) products are to reduce the cost and time for better precision and ruggedness. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available. The goal of the HPLC-method is to try & separate, quantify the main active drug, any reaction impurities, all available synthetic inter-mediates and any degradants.[7]

Understanding the physicochemical properties of drug molecules

Physicochemical properties of a drug molecule play an important role in method development. For Method development one has to study the physical properties like solubility, polarity, pKa and pH of the drug molecule. Polarity is a physical property of a compound. It helps an analyst, to decide the solvent and composition of the mobile phase. [6] The solubility of molecules can be explained on the basis of the polarity of molecules. Polar, e.g. water, and nonpolar, e.g. benzene, solvents do not mix. In general, like dissolves like i.e., materials with similar polarity are soluble in each other. Selection of diluents is based on the solubility of analyte. The acidity or basicity of a substance is defined most typically by the pH value. Selecting a proper pH for ionizable analytes often leads to symmetrical and sharp peaks in HPLC.[7]

Selection of chromatographic conditions

During initial method development, a set of initial conditions (detector, column, mobile phase) is selected to obtain the first "scouting" chromatograms of the sample. In most cases, these are based on reversed-phase separations on a C18 column with UV detection. A decision on developing either an isocratic or a gradient method should be made at this point.

Selection of Column

Normal phase chromatography utilizes a polar stationary phase and a non-polar mobile phase. Generally, more polar compounds elute later than non-polar compounds. Commonly used reverse phase columns and their uses are listed below. Propyl (C3), Butyl (C4), and Pentyl (C5) phases are useful for ion-pairing chromatography (C4) and peptides with hydrophobic residues, and other large molecules. C3–C5 columns generally retain non-polar solutes more poorly when compared to C8 or C18 phases. Examples include Zorbax SB-C3, YMC-Pack C4, and Luna C5. These columns are generally less stable to hydrolysis than columns with longer alkyl chains. Octyl (C8, MOS) phases have wide applicability. This phase is less retentive than the C18 phases, but is still quite useful for pharmaceuticals, nucleosides, and steroids.[10]Selection of the stationary phase/column is the first and the most important step in method development. The development of a rugged and reproducible method is impossible without the availability of a stable, high performance column. To avoid problems from irreproducible sample retention during method development, it is important that columns be stable and reproducible. The separation selectivity for certain components vary between the columns of different manufacturer as well as between column production batches from the same manufacturer. Column dimensions, silica substrate properties and bonded stationary phase characteristics are the main ones.

Selection of Chromatographic mode: chromatographic modes based on the analyte's molecular weight and polarity. All case studies will focus on reversed-phase chromatography (RPC), the most common mode for small organic molecules. Ionizable compounds (acids and bases) are often separated by RPC with buffered mobile phases (to keep the analytes in a non-ionized state) or with ion-pairing reagents.[8]

Method optimization

Most of the optimization of HPLC method development has been focused on the optimization of HPLC conditions.[14] The mobile phase and stationary phase compositions need to be considered. Optimization of mobile phase parameters is always considered first as this is much easier and convenient than stationary phase optimization. To minimize the number of trial chromatograms involved, only the parameters that are likely to have a significant effect on selectivity in the optimization must be examined. Primary control variables in the optimization of liquid chromatography (LC) methods are the different components of the mobile phase determining acidity, solvent, gradient, flow rate, temperature, sample amounts, injection volume, and diluents solvent type. This is used to find the desired balance between resolution and analysis time after satisfactory selectivity has been achieved. The parameters involved include column dimensions, column-packing particle size and flow rate. These parameters may be changed without affecting capacity factor or selectivity.

Method Validation

Validation of an analytical method is the process by which it is established by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical application. Validation is required for any new or amended method to ensure that it is capable of giving reproducible and reliable results, when used by different operators employing the same equipment in the same or different laboratories. The type of validation program required depends entirely on the method and its proposed applications.[15] Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice. Use of equipment that is within specification, working correctly and adequately calibrated is fundamental to the method validation process. Analytical methods need to be validated or revalidated.[16]

- Before their introduction into routine use;
- Whenever the conditions change for which the method has been validated
- Whenever the method is changed

MATERIALS

Imeglimin-Sura labs, Metformin-Sura labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC- Merck, Triethylamine-Sura labs.

METHODOLOGY

Trails

Preparation of standard solution: Accurately weigh and transfer 10 mg of Imeglimin and Metformin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.2ml of the Imeglimin and o.4ml of the Metformin stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization: Initially the mobile phase tried was Methanol: Water and Methanol: TEA Buffer with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Water in proportion 65:35 v/v respectively.

Optimization of Column: The method was performed with various columns like Symmetry and Phenomenex. Gemini C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions:

Instrument used : Waters HPLC with auto sampler and PDADetector 996 model.
Temperature : 35ºC
Column : Hypersil C18 (4.6×250mm) 5µ
Mobile phase : Acetonitrile: Water (50:50v/v)
Flow rate : 1ml/min
Wavelength : 235 nm
Injection volume : 10 µl
Run time : 10 min

Method validation

Preparation of mobile phase

Preparation of mobile phase: Accurately measured 500 ml (50%) of Water, 500ml of Acetonitrile (50%) were mixed and degassed in digital ultra sonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase ratio : Acetonitrile: Water (50:50v/v)

Column : Hypersil C18 (4.6×250mm) 5µ
Column temperature : 40ºC
Wavelength : 235nm
Flow rate : 0.9ml/min
Injection volume : 10µl
Run time : 8minutes

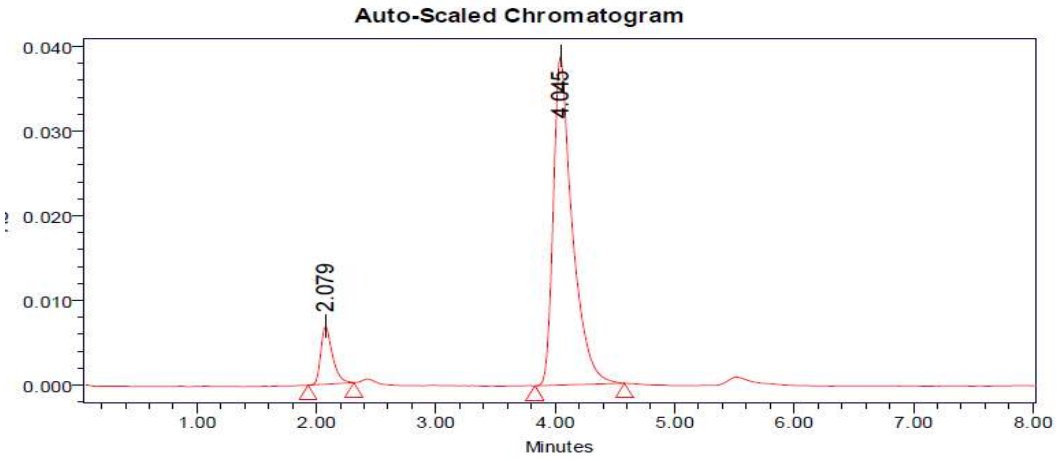

Fig 1: Optimized Chromatogram of Standard

Table 1: Various Parameters of Standard Chromatogram

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Imeglimin	2.079	46168	6841	1.33	4251
2	Metformin	4.045	429069	38885	1.59	5224

Optimized Chromatogram (Sample)

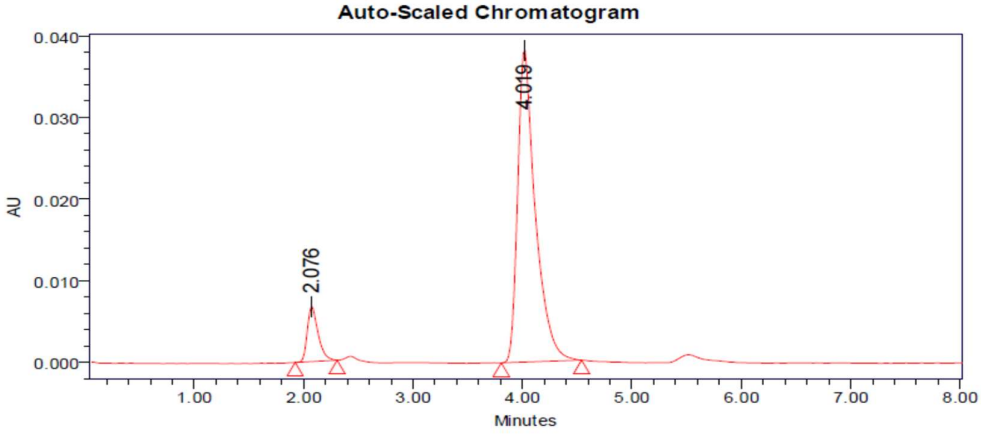

Fig 2: Optimized Chromatogram of Sample

Table 2: Various Parameters of Sample Chromatogram

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Imeglimin	2.076	46150	6766	1.36	5152
2	Metformin	4.019	427826	38246	1.58	6071

- *Theoretical plates must be not less than 2000. Tailing factor must be not less than 2.*
- *It was found from above data that all the system suitability parameters for developed method were within the limit.*

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantities Imeglimin and Metformin in drug product.

Assay (Standard)

Table 3: Peak results for assay standard of Imeglimin

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Imeglimin	2.078	49569	6811	6945	1.51
2	Imeglimin	2.080	49649	6999	6149	1.57
3	Imeglimin	2.078	49731	6972	6473	1.49
4	Imeglimin	2.079	49479	6971	6190	1.49
5	Imeglimin	2.082	49684	6841	6294	1.49
Mean			49607			
Std. Dev.			107.963			
% RSD			0.217637			

- *%RSD of five different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Table 4: Peak results for assay standard of Metformin

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Metformin	4.041	423328	44147	7672	1.35
2	Metformin	4.033	423805	44538	7786	1.13
3	Metformin	4.050	423229	44964	5772	1.34
4	Metformin	4.045	423876	44959	5191	1.35
5	Metformin	4.032	423575	38885	5137	1.35
Mean			423559.5			
Std. Dev.			328.2606			
% RSD			0.0775			

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Table 5: Peak results for Assay sample of Imeglimin

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Imeglimin	2.078	46684	6918	1.34	5217	1
2	Imeglimin	2.079	46168	6841	1.33	5251	2
3	Imeglimin	2.077	46088	6851	1.37	7127	3

Table 6: Peak results for Assay sample of Metformin

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Metformin	4.050	430575	39127	1.60	6197
2	Metformin	4.045	429069	38885	1.59	6224
3	Metformin	4.037	429543	38892	1.58	8203

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Imeglimin and Metformin in pharmaceutical dosage form was found to be 98.2%

Linearity

Table 7: Chromatographic Data for Linearity Study of Imeglimin

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	20	15065
66.6	40	31009
100	60	46166
133.3	80	60569
166.6	100	76862

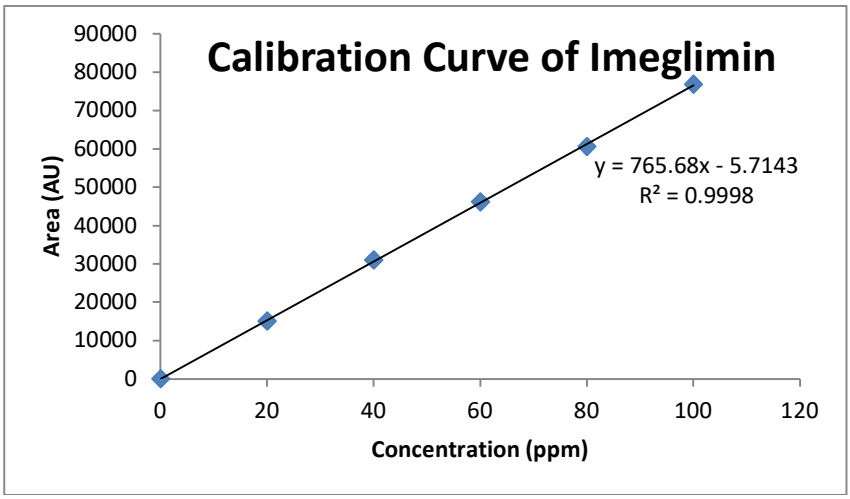

Fig 3: Chromatogram showing linearity level

Table 8: Chromatographic Data for Linearity Study of Metformin

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	40	131289
66.6	80	284775
100	120	427559
133.3	160	555861
166.6	200	712514

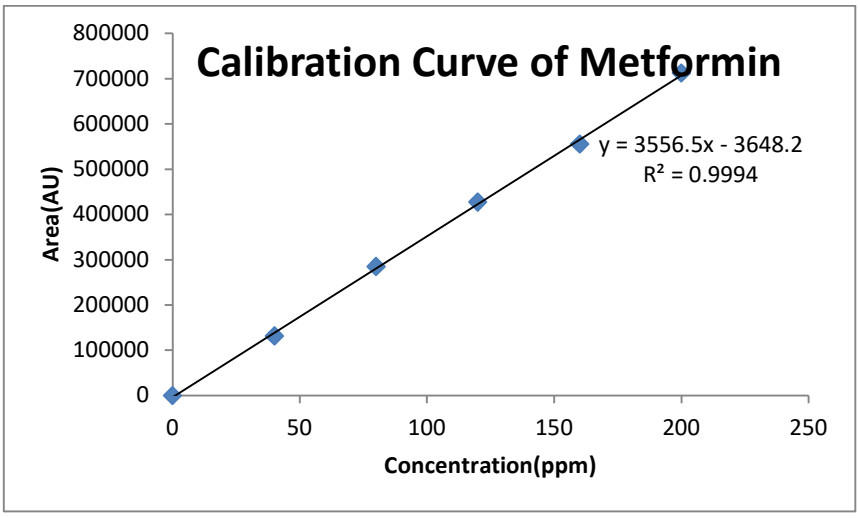

Fig 4: Chromatogram showing linearity level

Repeatability

Table 9: Results of repeatability for Imeglimin

S. No.	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Imeglimin	2.077	46054	6784	4208	1.32
2	Imeglimin	2.076	46803	6867	6088	1.34
3	Imeglimin	2.076	46150	6766	4152	1.36
4	Imeglimin	2.077	46056	6715	4184	1.32
5	Imeglimin	2.074	46247	6746	4065	1.33
Mean			46262			
Std.dev			312.7099			
%RSD			0.675954			

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Table 10: Results of repeatability for Metformin

S. No	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Metformin	4.031	427962	38634	5158	1.57
2	Metformin	4.024	429623	38673	5092	1.58
3	Metformin	4.019	427826	38246	5071	1.58
4	Metformin	4.016	427829	38310	5046	1.58
5	Metformin	4.014	429559	38181	5036	1.58
Mean			428559.8			
Std.dev			943.2246			
%RSD			0.220092			

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Intermediate precision
Day 1

Table 11: Results of Intermediate precision day1 for Imeglimin

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Imeglimin	2.075	46204	6673	5117	1.33
2	Imeglimin	2.074	46300	6735	5043	1.36
3	Imeglimin	2.075	46259	6652	5087	1.28
4	Imeglimin	2.075	46223	6667	5134	1.31
5	Imeglimin	2.075	46205	6674	5151	1.32
6	Imeglimin	2.074	46189	6703	5157	1.33
Mean			46230			
Std. Dev.			41.88556			

%RSD of Six different sample solutions should not more than 2.

Table 12: Results of Intermediate precision day1 for Metformin

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Metformin	4.013	428922	38004	7038	1.58
2	Metformin	4.011	428524	37935	7999	1.57
3	Metformin	4.010	427239	37850	7003	1.57
4	Metformin	4.008	427667	37780	7982	1.57
5	Metformin	4.006	427826	37824	7983	1.57
6	Metformin	4.006	427093	37970	7042	1.58
Mean			427878.5			
Std. Dev.			718.1952			
% RSD			0.16785			

- *%RSD of Six different sample solutions should not more than*

Day 2

Table 13: Results of Intermediate precision Day 2 for Imeglimin

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Imeglimin	2.076	46803	6867	5149	1.57
2	Imeglimin	2.076	46056	6715	5190	1.13
3	Imeglimin	2.077	46252	6652	6088	1.58
4	Imeglimin	2.075	46205	6674	5184	1.58
5	Imeglimin	2.075	46940	7249	5087	1.57
6	Imeglimin	2.072	46727	6983	5151	1.57
Mean			46497.17			
Std. Dev.			369.4739			
% RSD			0.794616			

%RSD of Six different sample solutions should not more than 2 Table:

Table 14: Results of Intermediate precision Day 2 for Metformin

S.No.			Area	Height (µV)		
1	Metformin	4.024	429623	38673	6789	1.49
2	Metformin	4.024	427829	38310	5772	1.34
3	Metformin	4.016	427263	37850	5092	1.32
4	Metformin	4.010	427826	37824	6046	1.28
5	Metformin	4.006	421284	40752	6003	1.32
6	Metformin	4.008	421832	40281	6983	1.33

Mean	425942.8
Std. Dev.	3492.681
% RSD	0.819988

- *%RSD of Six different sample solutions should not more than 2.*

Accuracy

Table 15: The accuracy results for Imeglimin

%Concentration	Area	Amount	Amount	% Recovery	Mean
50%	22938.33	30	29.9655	99.88	
100%	45426	60	59.33511	98.89	100.166
150%	70096.67	90	91.55572	101.7285	

- *The percentage recovery was found to be within the limit (98-102%).*

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Table 16: The accuracy results for Metformin

%Concentration	Area	Amount	Amount	% Recovery	Mean
50%	209357	60	59.8	99%	
100%	420697.7	120	119.8	99%	99%
150%	631550.7	180	179.8	99%	

- *The percentage recovery was found to be within the limit (98-102%).*

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table 17: Results for Robustness -Imeglimin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical	Tailing factor
Actual Flow rate of 0.9mL/min	46168	2.079	4251	1.33
Less Flow rate of 0.8mL/min	51177	2.29	5269	1.38
More Flow rate of 1.0mL/min	42190	1.890	5126	1.32
Less organic phase	42402	1.885	5126	1.19
More organic phase	42112	1.908	5854	1.36

Table 18: Results for Robustness-Metformin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical	Tailing factor
Actual Flow rate of 0.9mL/min	429069	4.045	5224	1.59
Less Flow rate of 0.8mL/min	472673	4.450	6328	1.58
More Flow rate of 1.0mL/min	392497	3.660	6217	1.54
Less organic phase	391379	4.251	6996	1.61
More organic phase	391703	3.239	6120	1.50

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Imeglimin And Metformin in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Imeglimin was found to be very slightly soluble in water (0.9 mg/mL). Imeglimin is soluble in methanol (ca. 60 mg/mL), sparingly soluble in ethanol (ca. 10 mg/mL), very slightly soluble in isopropanol (<1 mg/mL), and very slightly soluble in acetone. Metformin was found to be freely soluble in water; slightly soluble in alcohol; practically insoluble in acetone and in methylene chloride, freely-soluble in water, slightly soluble in ethanol, but almost insoluble in acetone, ether, or chloroform. Water and Acetonitrile (50:50) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Imeglimin and Metformin in bulk drug and in Pharmaceutical

dosage forms.

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