Method Development of Mirabegron and Solifenacin by Novel HPLC Methods

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ABSTRACT

AIM- The aim of the present investigation is Method Development of Mirabegron and Solifenacin by Novel HPLC Methods. **MATERIAL & METHODS-** The pure sample of the Mirabegron and Solifenacin were obtained from IPCA Lab, Ratlam, M.P., India. Solubility of all three drugs was observed by dissolving them in different solvents according to IP. Melting point of drugs was determined by using Digital melting point apparatus. A drift of the drug and KBr (AR grade) was prepared and scanned in the range of 400-4000cm-1. The LC system consists of pump (Shimadzu LC 10AT VP) with universal loop injector (Rheodyne 7725 i) of injection capacity 20 µL. Detector consists of photodiode array detector (PDA) SPD-10 AVP UV-Visible detector, for separation column used was Phenomenex Luna C_{18} (5 µm x 25 cm x 4.6 mm i.d.). The procured standard samples of Mirabegron and Solifenacin were tested for purity and it complied the test between 98 % to 102 %.Pure samples of Mirabegron and Solifenacin were obtained from IPCA Laboratories ltd, Mumbai, Maharashtra, India. Methanol was used as a common solvent for these drugs. 50 mg each of SOL and MIB were accurately weighted and dissolved in 50 ml of solvent to get solution of 1000µg/ml. Form the standard stock solutions of 1000 µg/ml different dilutions were prepared for each drug having concentration as shown in Table 5.6 and 5.7 with solvent. Then 20µL of these solutions were injected into the LC system with the help of Hamilton syringe. **RESULTS-** From the study it was found that best result was obtained in a quality separation in terms of peak symmetry, resolution, reasole run time and other

parameters by use of 55:30:15 (v/v) ratio mixture of methanol: acetonitrile: water as mobile phase. The flow rate was determined by testing the effect of different flow rate on the peak area and resolution, flow rate of 0.6 ml/min found optimum. From the chromatograms it was observed that SOL and MIB were eluted at 4.918 and 3.256 respectively. **CONCLUSION-** The simplicity, rapidity, accurate and reproducibility of the proposed methods completely fulfill the objective of the research work of estimation of the drug in blood plasma.

KEYWORDS

Method Development, Mirabegron and Solifenacin, Novel HPLC Methods, Simplicity, Rapidity, Accurate, Reproducibility.

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INTRODUCTION

Chemical analysis can be defined as the resolution of a chemical compound into its proximate parts; the determining its elements or the foreign substances it may contain [1].

The drug products are required by law to confirm a minimum standard of quality. With the rapid development of pharmaceuticals and higher challenges of quality, the volume of analytical work is increasing day by day. This forces the development of analytical methods that are rapid, accurate, precise and reproducible.

Analytical chemistry plays vital role in development of science, which involves separation, identification and determination of the relative amounts of components in a sample of matter [2-4].

Pharmaceutical analysis is a branch of pharmacy and have a very significant role in quality control of pharmaceuticals, through the rigid check on raw materials used in manufacturing of formulation and on finished product .It plays an important role in building up the quality products through in process control.

Today in pharmaceutical industry, preparations containing two or more ingredients are more popular than those with single ingredient. As a consequence various problems are being faced by the pharmaceutical analyst who has to develop methods to analyze this mixture [5,6].

The advents of new instrumental technique are opening avenues for the analyst to circume the difficulties in mixture analysis. Some of these techniques commonly employed are Spectrophotometry, Gas Chromatography, High Performance Liquid Chromatography [7, 8]. The availability of very sophisticated instruments and their continual improvement has made instrumental method most widely used. Though use of chemical method of analysis has not become absolute, their use is on decline. The instrumental methods are more accurate, precise, sensitive, selective and less time consuming than classical methods [9].

As a result, simple, rapid and economical methods for the simultaneous analysis of multi-component formulation, which don't require extraction or separation of the analyte from themselves or from the excipient, becomes necessary for the pharmaceutical industry. In the present study, the combination of Solifenacin (SOL), and Mirabegron (MIB) have been selected which are recent combination.

MATERIAL AND METHOD

Procurement of Drug Samples and Chemicals:

The pure sample of the Mirabegron and Solifenacin were obtained from IPCA Lab, Ratlam, M.P., India. The tablet dosage form (Label claim: 50 mg SOL and 50 mg MIB) was procured from the local market. Methanol (AR and HPLC grade), Acetonitrile (HPLC grade) and was purchased from Merck Limited, Mumbai, India, and Water (HPLC grade) was prepared in the college.

Identification and Characterization of drugs

Solubility

Solubility of all three drugs was observed by dissolving them in different solvents according to IP [10].

Table 1: Solubility of drug in different solvents

Solvent	**Mirabegron**	**Solifenacin**
Water	Insoluble	Freely soluble
Methanol/ Ethanol	Soluble	Soluble
Acetonitrile	Slightly soluble	Sparingly soluble

Ether	Soluble	Sparingly soluble
Dimethyl sulphoxide	Freely soluble	Practically insoluble

Identification of drugs by Melting point determination

Melting point of drugs was determined by using Digital melting point apparatus [11].

Table 2: Melting Point of Drugs

Drug Name	Melting Point	Standard Value
Mirabegron	139°C	138-140°C
Solifenacin	146-147°C	147°C

Identification of Drugs by IR Spectra

A drift of the drug and KBr (AR grade) was prepared and scanned in the range of 400-4000cm-1 [12,13].

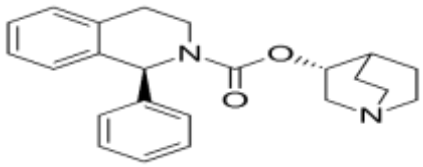

Figure 1: Chemical Structure of Solifenacin

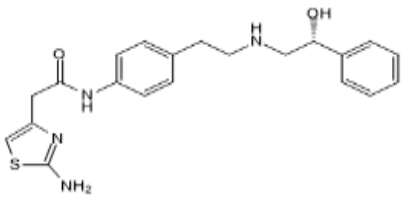

Figure 2: Chemical Structure of Mirabegron

RP-HPLC Method development for simultaneous estimation of Mirabegron (MIB) and Solifenacin (SOL).

Instrumentation

Shimadzu LC IOAT HPLC system

The LC system consists of pump (Shimadzu LC 10AT VP) with universal loop injector (Rheodyne 7725 i) of injection capacity 20 µL. Detector consists of photodiode array detector (PDA) SPD-10 AVP UV-Visible detector, for separation column used was Phenomenex Luna C_{18} (5 µm x 25 cm x 4.6 mm i.d.). The

equipment was controlled by a PC work station equipped with software CLASS-VP software (Shimadzu. Tokyo, Japan).

The volume capacity of the reservoir was greater than 500ml. The mobile phase velocity was within 1-2 ml/min [14].

Binary pump

It is mainly useful during gradient run and characterized by automatically varying solvent composition- A pump delivers a steady flow or more solvents to sample injection system. The binary pump works by pumping a filtered and degassed solvent into a proportioning value. In series binary pump there was two valves. Solvents are measured by percentage, specified by chemist, and mixed inside the pump head where a piston meters the flow of the mixture to an outlet tube. The pump outlet tubing then connects the solvent stream to a sampler [15].

Column

The column is usually a stainless-steel tube packed with octa-decylsilane or octa-acylsilane coated silica gel of average diameter 3, 5 or 10 μn [16].

Photodiode array detector

The detector used SPD-10 AVP UV-Visible detector was an advanced type of UV detector with the ability to monitor across the full UV range simultaneously using an array of photodiodes which detects light dispersed by a fixed monochromatic over a range of wavelength offering a resolution of Inm. It was useful for complex mixtures containing compounds with widely different absorbance ranges and mixtures where peaks overlap chrornatographically but can be separated in terms of UV absorbance. The detector gives full UV spectrum of peak in the chromatogram which adds in the identification of unknowns.

Protocol

The procured standard samples of Mirabegron and Solifenacin were tested for purity and it complied the test between 98 % to 102 %.Pure samples of Mirabegron and Solifenacin were obtained from IPCA Laboratories ltd, Mumbai, Maharashtra, India. All the chemical and reagents used were of HPLC grade and purchased from Merck, Mumbai, India.

Preparation of standard stock solutions

The equivalent of 10 mg each of Mirabegron and Solifenacin were accurately weighed in 100 ml volumetric flasks separately and dissolve in 25 ml of methanol to

prepare standard stock solutions. After the immediate dissolution, the volume was made up to the mark with solvent. These standard stock solutions were observed to contain 100 μg/ml of Mirabegron and Solifenacin [17].

Selection of sampling wavelengths

The equivalent of 10 mg each of Mirabegron and Solifenacin were accurately weighed in 100 ml volumetric flasks separately After the immediate dissolution, the volume was made up to the mark with solvent. These standard stock solutions were observed to contain 100μg/ml of Mirabegron and Solifenacin. From the above stock solution, working standard solutions having concentration 5 μg/ ml was prepared by appropriate dilution. Working standard solutions of 5 μg//ml of each of the drug were scanned in the range 400- 200 nm in the spectrum mode at the low scan speed to obtain the overlain spectra of these drugs.

Selection of mobile phase and optimization of method

Different column chemistry, solvent type, solvent strength (vol. fraction of organic solvent(s) in the mobile phase and pH of the buffer solution), detection wavelength and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimized so that the components were not interfered with the solvent and excipients [18].

Optimized chromatographic condition

The optimized chromatographic conditions are reported in Table.

Table 3: Optimized chromatographic conditions

Variable	Condition
Column	
Dimension.	250mm x 4.60mm
Particle Size	5μ
Bonded Phase	Octadecylsilane (C_{18})
Mobile Phase	
Methanol:	55
Acetonitrile:	30
Water	15
Flow rate	1 ml/min
Run time	10 min
Temperature	Ambient
Sample Size	20μl

Detection wavelength	239 nm
Retention time MIB SOL	 3.25± 0.2 min 4.91± 0.4 min

Assay of SOL and MIB in combination

Preparation of standard stock solutions

Methanol was used as a common solvent for these drugs. 50 mg each of SOL and MIB were accurately weighted and dissolved in 50 ml of solvent to get solution of 1000μg/ml.

Preparation of standard solutions for linearity study

Form the standard stock solutions of 1000 μg/ml different dilutions were prepared for each drug having concentration as shown in Table 5.6 and 5.7 with solvent. Then 20μL of these solutions were injected into the LC system with the help of Hamilton syringe. Then the chromatograms were recorded at 239 nm., from the chromatogram it was cleared that SOL relented at time 4.918 min and MIB at 3.256 min from which their area was noted and calibration curve was plotted between the peak area against their respective concentrations.

Analysis of mixed standard

From the standard stock solutions of 1000 μg/ml of the drugs different mixed standard solutions of known concentration were prepared and their 20uL solutions were injected into the LC system with the help of Hamilton syringe and their chromatograms were recorded after that the concentration of individual drugs were calculated by extrapolating the value of area from their calibration curves respectively.

Analysis of tablet

As the result of mixed standard analysis found satisfactory, the method was applied for the quantitative study of all the three drugs in commercially available tablet. For the preparation of the stock solution of tablet dosage form, 20 tablets were taken and their average weight was determined, they were crushed to fine powder. Then powder equivalent to 10 mg of SOL (respective quantity of MIB) was taken in 50ml volumetric flask and dissolved in 30ml of methanol with vigorous shaking for 5-10 minutes.

RESULTS & DISCUSSION

IR Spectra of Solifenacin

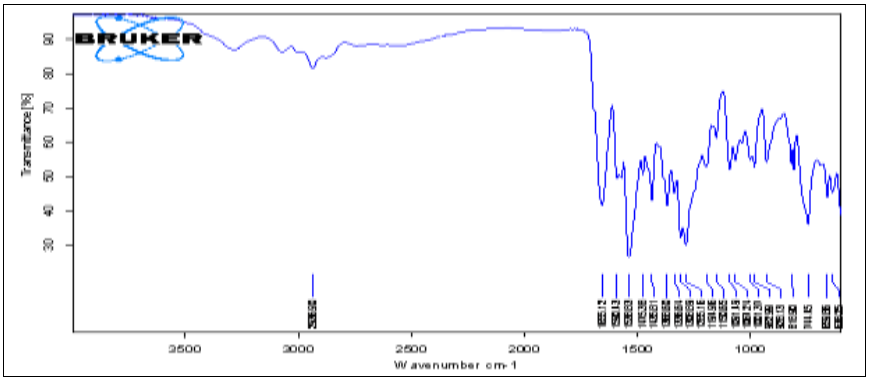

Figure 4: FT-IR Spectra of Solifenacin

Table 4: IR interpretation of Solifenacin

Group	**Observed Frequency (cm $^{-1}$)**	**Standard Range (cm^{-1})**
C=O str	1,655.12 cm^{-1}	1650-1780
O-H str	2,925.90 cm^{-1}	2700- 3800

IR Spectra of Mirabegron

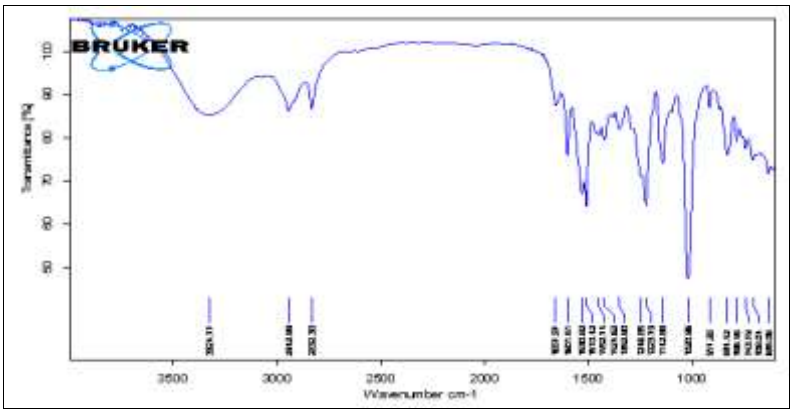

Figure 5: FT-IR Spectra of Mirabegron

Table 5: IR interpretation of Mirabegron

Group	**Observed Frequency (cm $^{-1}$)**	**Standard Range (cm^{-1})**
O–H s at c ring	3324.77	3300-3010
C=O s at b ring	1667.97	1744-1650
NH2 sc	1601.51	1661-1550

UV spectra of Mirabegron and Solifenacin

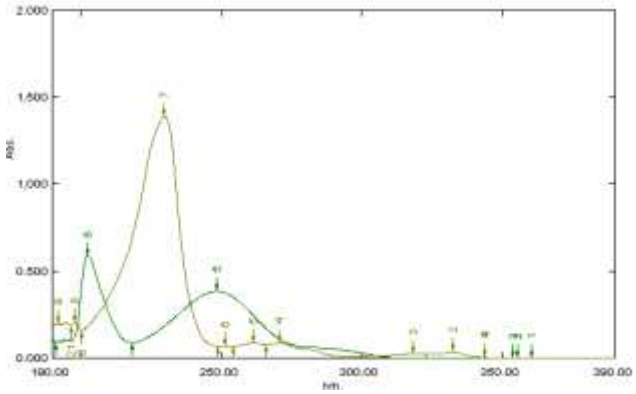

Figure 6: Overlain spectra of Mirabegron and Solifenacin

Selection of Mobile Phase

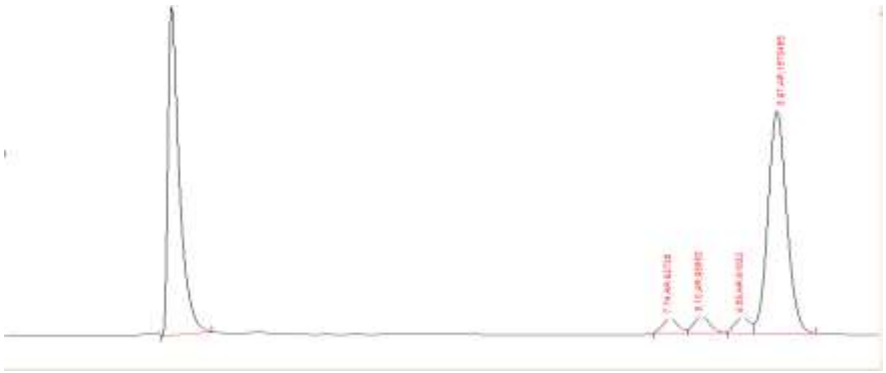

Figure 7: MIB-SOL in methanol: acetonitrile: water (75:22:05)

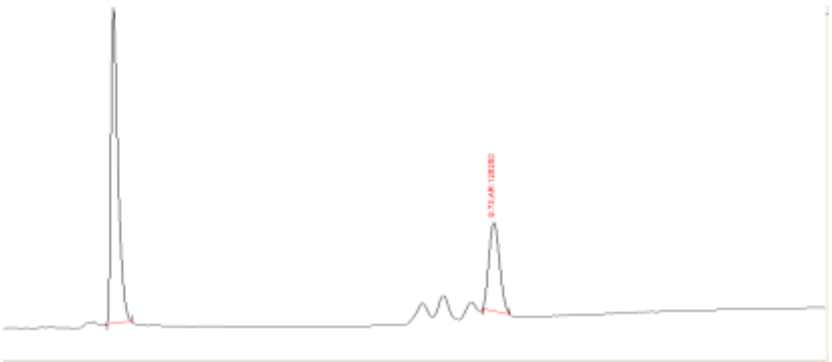

Figure 8: MIB-SOL in methanol: acetonitrile: water (60:30:10)

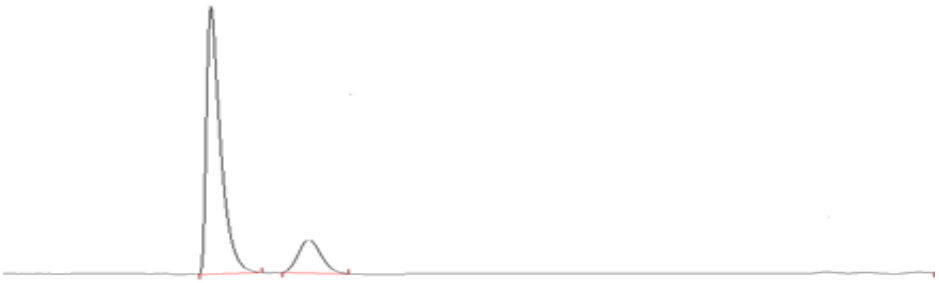

Figure 9: MIB-SOL in methanol: acetonitrile: water (55:30:15)

Chromatogram of Drugs individually and combination

Figure 10: Typical chromatogram of MIB

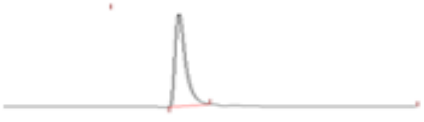

Figure 11: Typical chromatogram of SOL

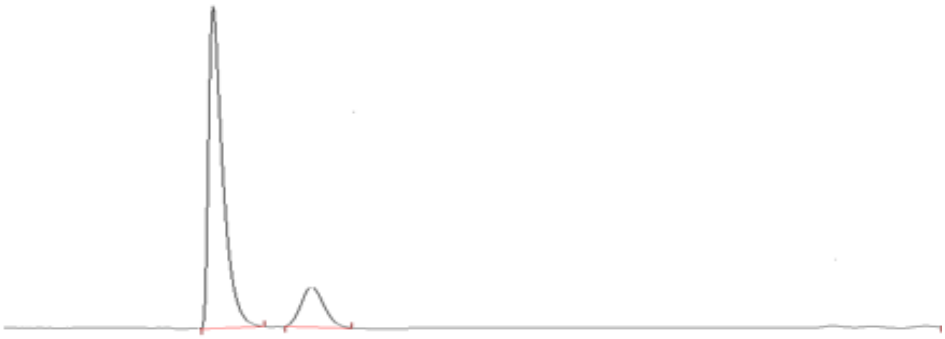

Figure 12: Typical Chromatogram of MIB and SOLin Mixed Standards

Other criteria like lime required for analysis. Appropriate k range for eluted peaks, assay sensitivity, solvent noise and use of the same solvent system for extraction of drug from formulation matrices during drug analysis were also considered [19].

A series of aqueous mobile phases containing methanol, acetonitrile and water were also tested. The best results were obtained when above three mentioned solvents were used. Further the method was optimized by changing the concentration of mobile phase and the results are reported. From the study it was found that best result was obtained in a quality separation in terms of peak symmetry, resolution, reasole run time and other parameters by use of 55:30:15 (v/v) ratio mixture of methanol: acetonitrile: water as mobile phase. The flow rate was determined by testing the effect of different flow rate on the peak area and resolution, flow rate of 0.6 ml/min found optimum [20].

Linearity of SOL & MIB

From the calibration curve it was cleared that SOL and MIB has linearity range between 5-70 µg/ml respectively.

Table 6: Linearity of SOL

Conc.	**Peak Area**						**Mean ± SD**
	Replica 1	**Replica 2**	**Replica 3**	**Replica 4**	**Replica 5**	**Replica 6**	
5	1006076	1017095	999913	1010687	1024862	1031318	1014992 ± 11779.49

10	2064509	2055823	2058860	2049876	2078374	2066234	2062279 ± 9867.805
15	3030965	3031193	3036464	3028496	3026468	3048034	3033603 ± 7825.547
20	4011512	4004965	4012549	4038380	4009315	4043755	4020079 ± 16552.16
25	5102162	5164099	5187894	5156518	5171163	5161573	5157235 ± 29097.92

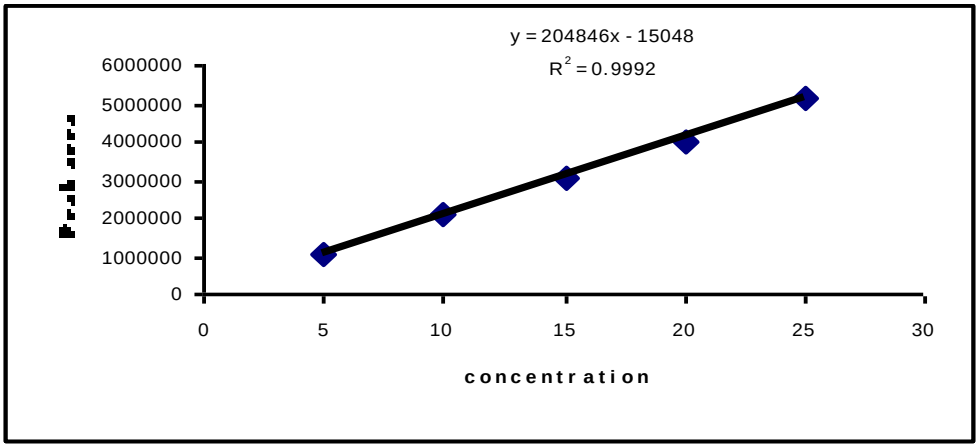

Figure 13: Calibration curve of SOL

Table 7: Linearity of MIB

Conc.	Peak Area						Mean ± SD
	Replica 1	Replica 2	Replica 3	Replica 4	Replica 5	Replica 6	
5	1278319	1251698	1261677	1256021	1286251	1277015	1268497 ± 3918.35
10	2430400	2456558	2423352	2472280	2406928	2445956	2439246±23712.02
15	3532244	3531129	3591347	3572418	3519401	3548518	3549176±27633.15
20	4696147	4681402	4693653	4684429	4660450	4666927	4680501±14285.02
25	5953012	5938715	5973641	5992403	5988563	5981579	5971319±21216.58

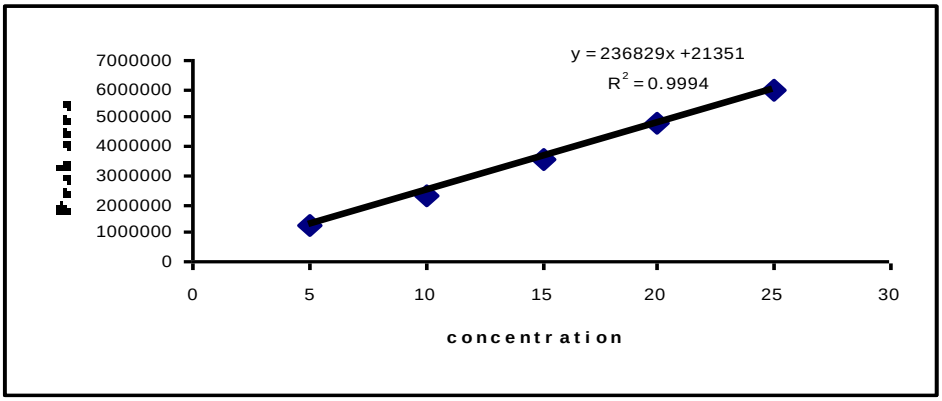

Figure 14: Calibration curve of MIB

Analysis of Tablet Formulation

The results are reported in Table 8.

Table 8: Analysis of mixed standard

S. No.	SOL			MIB		
	Amount Present	Amount Found (µg/ml)	(%)	Amount Present	Amount Found (µg/ml)	(%)
1	5	4.99	99.8	5	5.10	101.02
2	10	10.02	100.02	10	9.99	99.95
3	15	14.94	99.63	15	14.54	96.93
4	20	19.49	97.45	20	19.99	101.1
5	25	25.30	101.2	25	25.25	102.1
Mean	-	-	99.62	-	-	100.3
S.D.	-	-	1.36	-	-	1.81
%COV	-	-	1.37	-	-	1.80
S.E.	-	-	0.60	-	-	0.73

From the chromatograms it was observed that SOL and MIB were eluted at 4.918 and 3.256 respectively. The concentrations of these drugs were extrapolated from their respective calibration curves by using the area. The results and its statistical results are reported in Table [21].

Table 9: Result analysis and statistical validation of Tablet

S. No.	SOL			MIB		
	Amount Present	Amount Found		Amount Present	Amount Found	
		(µg/ml)	(%)		(µg/ml)	(%)
1	10	10.11	101.1	10	9.97	99.73
2	10	10.03	100.3	10	10.06	100.6
3	10	9.98	99.8	10	9.96	99.63
4	10	9.98	99.8	10	10.01	100.1
5	10	10.03	100.3	10	9.98	99.86
6	10	9.93	99.3	10	9.98	99.84
Mean	-	10.01	100.1	-	9.993	99.93
± S.D.	-	-	0.616	-	-	0.351
%CV	-	-	0.62	-	-	0.35
S.E.	-	-	0.252	-	-	0.143

The supernatant liquid was transferred to 100ml of volumetric flask through a whatman #41 filter paper. The residue was washed twice with solvent and the combined filtrate was made up to 100ml mark. After that 10 ml of the above solution was diluted up to 100 ml with solvent. Six replicates of sample solutions were prepared of required concentrations of the three drugs. Then 20uL of each replicate were injected into the system and their chromatograms were recorded [22].

CONCLUSION

In the present research work, a successful attempt was made for "Development & Validation of novel HPLC method for the estimation of selective drugs in bulk and pharmaceutical formulations" which was developed by experimentation based on thorough literature survey and ascertained by statistical parameters of sampling.

The simplicity, rapidity, accurate and reproducibility of the proposed methods completely fulfill the objective of the research work of estimation of the drug in blood plasma.

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