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CONCURRENT ESTIMATION OF GEMIFLOXACIN, ATORVASTATIN, GLIMEPIRIDE AND MONTELUKAST SODIUM ACTIVE PHARMACEUTICAL INGREDIENTS BY RP-HPLC TECHNIQUE

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Abstract:

The objective of the experimental study was to developed a validated RP-HPLC analytical method for the simultaneous estimation of gemifloxacin in the presence of atorvastatinGlimepiride and montelukast sodium. The chromatographic analysis involved C18 (5 µm, 25×0.46 cm) column, mobile phase consisted of methanol : water (80:20 v/v with flow rate of 1.0 mL·min−1 and pH 3.0 was maintained. Retention time of gemifloxacin , atorvastatin,glimipiride and montelukast was found to be 2.09, 3.5 min , 4.4 min and 7.7 min respectively at 244 nm. The correlation coefficient of gemifloxacin , atorvastatin,glimipiride and montelukast was found to be 0.997, 0.998, 0.998 and 0.998 respectively. The results obtained through the employed method were validated according to the International Conference on Harmonization guidelines requirements. All the validated parameters were of great quality which indicated that the used method could successfully be applied in pharmaceutical preparations. The development of a simple, precise, less time consuming and accurate method is likely to be helpful for routine analysis

Key expressions: *Gemifloxacin, Atorvastatin calcium,Glimepiride, Montelukast sodium, HPLC, analytical method validation.*

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INTRODUCTION:

Drug-drug interactions are identified as a leading cause of hospitalization and death. However, it is unlikely that all clinically possible interactions can be predicted. Conditions under which drug-drug interactions occur, their clinical relevance, and mechanisms causing them, therefore represent a highly active field in pharmacological research. This paper clearly demonstrated, the method validation of different therapeutic classes of the drug and all the parameters related to the analytical method development . Atorvastatin have proven to be safe in numerous clinical trials as in its combined form, and used against high cholesterol levels (1-3). Gemifloxacin, a well known fluoroquinolone, has shown potent antibacterial activity against clinical isolates for *Streptococcus pneumoniae*.literature review revealed that there is no interaction found between the gemifloxacin and atorvastatin . It is approved by FDA for bronchitis and other respiratory tract infections(4, 5) Gemifloxacin has a good access into respiratory secretions with sufficient amount at the site of the infection(6,7).The other drug Glimepiride is an oral antidiabetic drug to control the blood sugar levels belonging to the class of sulfonylureas (8) .The drug is effectively used in type 2 diabetes together with diet and exercise(9). Insulin regimen is also used with Glimepiride for the treatment . literature review revealed that anti diabetic drug can be given to the patients with statins and fluoroqinolones with the strict monitoring and care but can be given a patient which having all these diseases .(10).On the other hand, seasonal allergies and asthma are mostly treated with Montelukast sodium and it can be generally prescribed with the statins , sulfonylureas and fluoroqinolones because there is no interaction found between these drug groups. (11, 12), but interms of analytical method development , Several HPLC methods has been reported in literature survey for determination of atorvastatin, gemifloxacin,Glimepiride and montelukast sodium individually and related substances , structures of these APIs are given in figure 1 (5),(13) but in combination with these four APIs there were no HPLC method was reported (13-17).For the therapeutic point of view and interactive effect of these drugs whenever used in combination, the identification of gemifloxacin, atorvastatin,Glimepiride and montelukast is essential and there were no previously reported method for combined determination of all these drugs and their were no data reported for their interaction. This research study demonstrated the RP-HPLC technique for the detection of gemifloxacin, atorvastatin calcium,Glimepiride and montelukast sodium (18) which is likely to benefit simultaneous determination of these for routine analysis in labs.

SCOPE OF THE STUDY:

The leading cause of death is drug-drug interaction and for the determination of interaction there is no data available for this combination. The review of the scientific literature revealed that there is no analytical method available for this combination. The efforts were to develop simple, rapid, accurate, reproducible and economical HPLC method for all the four APIs(Gemifloxacin, Atorvastatin calcium,Glimepiride and Montelukast sodium) in combined dosage forms their structures are expressed in figure 1.

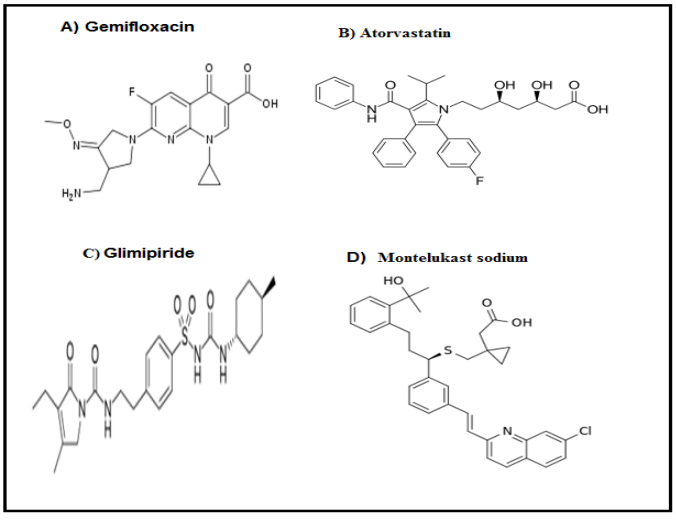

Figure 1. Structure of A= Gemifloxacin , B=Atorvastatin , C=Glimepiride and D= Montelukast Sodium

EXPERIMENTAL:

HPLC System used in this research study were of Shimadzu with a LC-10 AT VP pump with model # SPD-10 AV and rheodyne manual injector fitted by way of a 20 µL loop. The column arrangement utilized a C-18 column (250×4.6 mm) and double beam spectrophotometer (UV visible 1601 Shimadzu). Sonicator (model number WHC-67, Galvano scientific) used to degass water and solutions after filtering through 0.45 µm, p H meter(were galvano scientific ALP-104) used to maintain the p H of mobile phase . UV-VIS spectrophotometer were used for the determination of the suitable wavelength for the estimation of the drugs.

Selection of Wavelength:

In this research study UV spectra of individual drugs were overlapped at the wavelength range of 200 to 400 nm. It was observed that all the drugs showed highest response at 244 nm.

Material and Reagents:

Pharmaceutical grade atorvastatin was gifted by Bosch Lab Ltd, Karachi, Pakistan. Gemifloxacin andGlimepiride, were also received as a gift from Medisure Pvt Ltd, and montelukast sodium was gifted by Tabroz pharma. The supplier of Methanol, acetonitrile and Orthophosphoric acid 85% was E. Merck, Germany.

HPLC Conditions:

The chromatographic analysis involved C18 (5 µm, 25×0.46 cm) column. The mobile phase consisted of methanol : water (80:20 v/v with flow rate of 1.0 mL·min−1 and pH 3.0 was maintained and sample were analyzed at 244 nm.

Internal Standard stock preparation:

First the internal Standard(99.9% pure) stock solution was prepared by dissolving 10 mg each of gemifloxacin, atorvastatin,Glimepiride and montelukast sodium in the methanol , complete solubility of the drug into the solvent was done and than the volume made up to 100 mL with the same solvent. Preparation of serial dilution from the standard solution was done over the range of 0.62-100 µgmL^{-1} to obtain the required calibration curve.

Sample preparation of APIs:

Sample stock solution was prepared by dissolving 10 mg each of gemifloxacin, atorvastatin, Glimepiride and montelukast sodium in the methanol and the volume made up to 100 mL with the same solvent. Preparation of serial dilution from the sample solution was done over the range of 0.62-100 µgmL^{-1} to obtain the required calibration curve. The solution were filtered through 0.45 micrometer filter paper and after the filtration solution injected into system.

RESULTS:

In this study the different compositions of mobile phase and different buffers were tried to obtained the proper validated method for the identification of the gemifloxacin with atorvastatin, Glimepiride and montelukast in API by using C-18 column having the specifications 5 µm, 25×0.46 cm with UV detector with the HPLC technique, accurate results and better peak resolutions were obtained the mentioned column at 25 degree centigrade. For the selection of absorption wavelength of gemifloxacin with atorvastatin, Glimepiride and montelukast the UV spectra of analytes were performed, and the best results were recorded at 244 nm for all the active ingredients. chromatograms of the gemifloxacin with atorvastatin, Glimepiride and montelukast individual and in combined form are expressed in figure 3-4.

Method Validation:

This method was validated accordingly to the ICH guidelines (International conference on the harmonization). All the steps related to the validation were assessed for completion of this procedure. The chromatographic analysis involved C18 (5 µm, 25×0.46 cm) column. The mobile phase consisted of methanol : water (80:20 v/v with flow rate of 1.0 mL·min−1 and pH 3.0 was maintained.

Selectivity and Specificity:

The method for gemifloxacin, atorvastatin, Glimepiride and montelukast sodium was checked for selectivity and specifity.The overall results were found to be satisfactory according to the ICH guidelines results are expressed in table 2 system suitability parameters and HPLC chromatogram are expressed in figure 3 and 4.

Linearity and Range:

For calibration curves concentration of 0.62 - 100 µg·mL^{-1} were constructed and excellent linearity was obtained in all cases. The correlation coefficient, however ranged from <0.997 to <0.998 as expressed in table I and calibration curves are expressed in figure 2.

Accuracy and Recovery:

The method was found to be accurate over the range of 99.7% - 101.3%, at low, medium and high levels for all investigated analytes. The data given in table shows that there is no significant difference amongst the amount of all the drugs and in their formulations results are expressed in table II

Precision:
For intra and inter batch precision the samples were assayed and results were in accordance to the acceptable limits of ICH guidelines which shows that the method was accurate and precise. There were no deviations observed in the procedure and method was therefore termed capable and highly précised results are expressed in table III

Robustness:

The analytical method was performed by making few changes like mobile phase(±2%) , p H (±0.05) and flow rate (± 2ml) to checked the method accuracy and reproducibility results are revealed in table IV .

Limit of Detection and Quantification:
The detection and quantification limit of an individual analytical procedure shows the accuracy of this developed method. Results are expressed in table I.

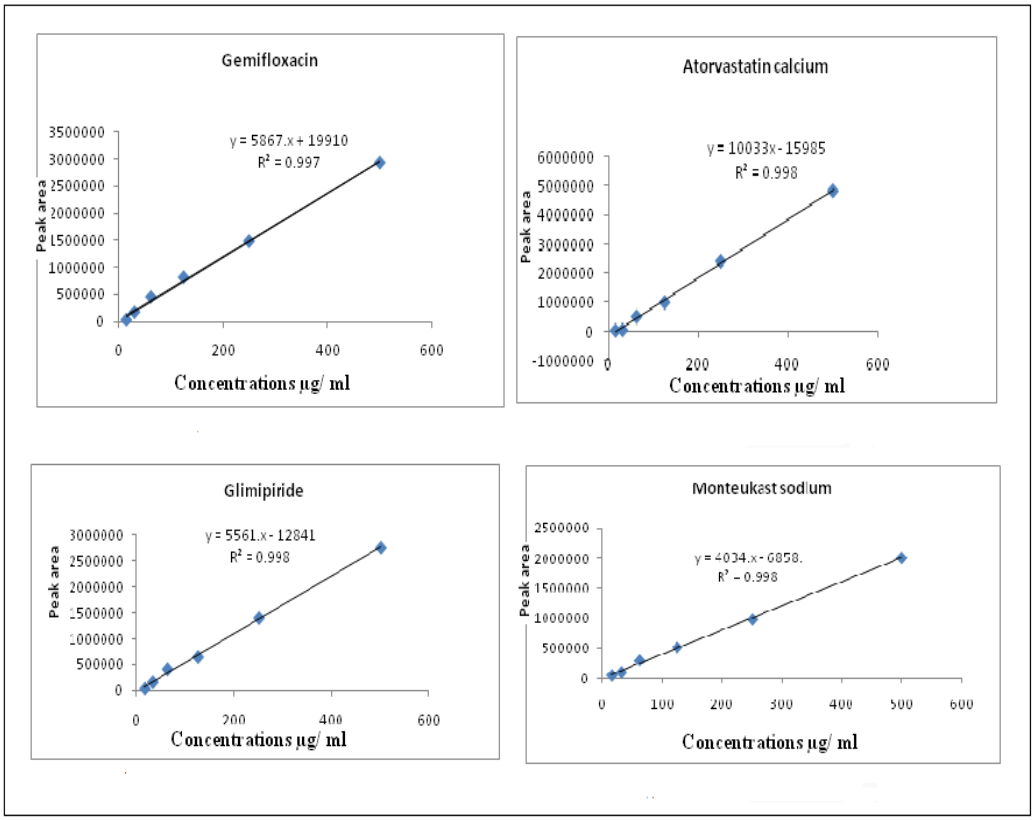

Figure 2. Standard calibration curves of Gemifloxacin , Atorvastatin ,Glimepiride and Montelukast Sodium

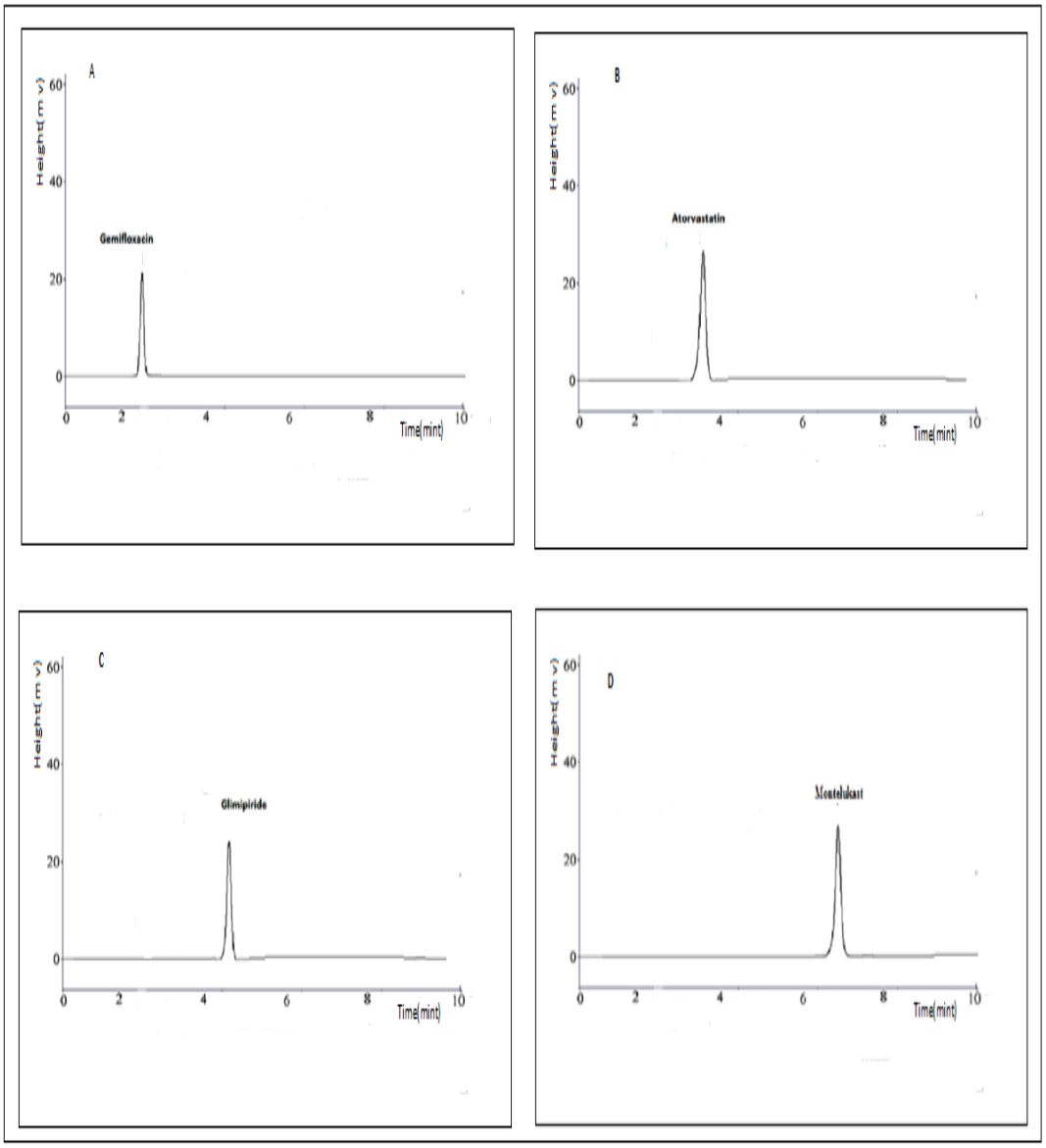

Figure 3: Individual HPLC chromatogram of A= Gemifloxacin, B= Atorvastatin Calcium , C =Glimepiride, D= Montelukast Sodium

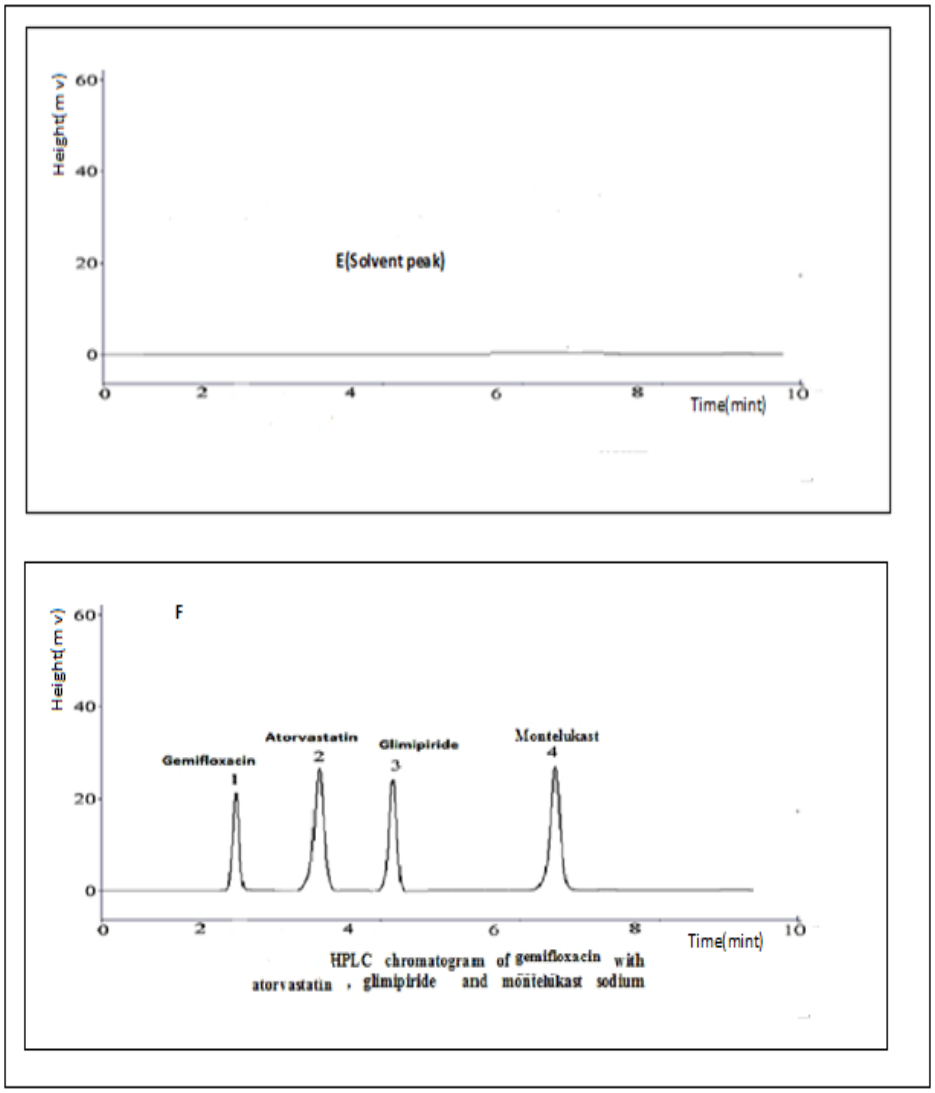

Figure 4: HPLC injection peak of solvent E= Solvent injection peak , F= Combined HPLC chromatograms of Gemifloxacin, Atorvastatin Calcium ,Glimepiride and Montelukast sodium.

Table I. Limit of quantification , limit of detection , regression equation and co relation coefficient (R^2) of gemifloxacin , atorvastatin, Glimepiride and montelukast sodium

Parameters	Drugs			
	Gemifloxacin	Atorvastatin	Glimepiride	Montelukast Sodium
STANDARD DEVIATION	1	1	1	1
LOD(ng*ml)	0.00056241	0.000329	0.00059	0.0008
LOQ(ng*ml)	0.00170427	0.000997	0.0018	0.0025
SLOPE	5867.632	10033.21	5561.13	4034.8
REGRESSION EQUATIONS	y = 5867.x + 19910	y = 10033x - 15985	y = 5561.x – 12841	y = 4034.x - 6858
R^2 (Correlation co-efficient)	0.997	0.998	0.998	0.998

Table II Accuracy measurements and system suitability parameters of all API (active pharmaceutical ingredients)

Drugs	Concentration µgmL^{-1}	%Relative Standard Deviation	% Recovery	Retention.Time	Flow Rate	Theoritical Plates
Gemifloxacin	80%	0.01	99.9	2.303	1	4839.05
	100%	0.63	100.36			
	120%	0.63	99.9			
Atorvastatin	80%	0.01	101.3	3.5	1	5297.03
	100%	0.32	99.9			
	120%	0.63	100.36			
Glimepiride	80%	0.22	99.8	4.4	1	4286.58
	100%	0.96	100.3			
	120%	0.53	99.7			
Montelukast sodium	80%	0.33	99.7	7.7	1	5367.35
	100%	0.88	101.2			
	120%	0.63	100.2			

Table III Interday and Intraday precission of Gemifloxacin , Atorvastatin, Glimepiride and Montelukast sodium

Drugs	Concentration. Injected	Inter-day		Intra-day	
	µg·mL^{-1}	%RSD	%Recovery	%RSD	%Recovery
GEMIFLOXACIN	0.625	0.23	100.32	0.26	99.9
	1.25	0.36	99.94	0.64	99.9
	2.5	0.26	100.23	0.098	100.23
	6.25	0.29	99.99	0.12	100.02
	12.5	0.26	100.23	0.56	100.02
	25	0.69	100	0.032	100.03
	50	0.23	100.01	0.069	100.9
	100	0.21	100.7	0.076	100.6
ATORVASTATIN	0.625	0.21	99.8	0.08	99.3
	1.25	0.30	99.4	0.09	99.2
	2.5	0.12	99.93	0.08	99.9
	5	12	100.2	0.09	100
	10	0.23	100.06	0.07	100.1
	25	0.52	100.17	0.062	100.2
	50	0.26	100.02	0.059	100
	100	0.23	100.1	0.089	100.23
GLIMEPIRIDE	0.625	0.65	101.12	0.48	101.1
	1.25	0.36	100.03	0.098	100
	2.5	0.56	100.01	0.089	100.23
	6.25	0.36	99.91	0.5	99.9
	12.5	0.36	100.32	0.045	100
	25	0.59	100	0.23	100
	50	0.16	100.9	0.050	100.7
	100	0.20	100.7	0.07	100.0
MONTELUKAST SODIUM	0.625	0.89	99.87	0.09	99.9
	1.25	0.32	99.86	0.01	99.9
	2.5	0.02	99.97	0.089	100
	6.25	0.0012	100.23	0.089	100.2
	12.5	0.0004	99.97	0.58	100
	25	0.023	100	0.99	100.3
	50	0.19	99.8	0.06	99.8
	100	0.12	100.2	0.07	100.6

Table IV. Robustness testing of Gemifloxacin, Atorvastatin,Glimepiride and Montelukast sodium

Drugs	Flow Rate	p H	Mobile Phase Composition
	%RSD	%RSD	% RSD
Gemifloxacin	0.562	0.752	0.587
Atorvastatin	0.892	0.28	0.538
Glimepiride	0.764	0.254	0.697
Montelukast Sodium	0.234	0.647	0.348

DISCUSSION:

In recent years HPLC methods has received significant attention indicated by its usage in routine analysis in pharmaceutical industry, quality control and other areas like R & D. The purpose of this study was to develop a unique and least time-consuming method for the identification of gemifloxacin with atorvastatin,Glimepiride and montelukast in API by using C-18 column with UV detector. The instrumentation technique of HPLC with the proper column wash proved that the method has been validated for the reproducibility , accuracy, precision and repeatability and the prepared solutions of all the actives pharmaceuticals has been found to b stable for the proper experimentation. This strategy can be utilized for a dose type of all the four actives and for the treatment of elevated cholesterol, occasional hypersensitivities , respiratory contamination with insulin routine treatment simultaneously this technique was assessed by HPLC procedure in a combined form since, all the four ailment conditions are exceptionally regular in the Asian nations particularly in Pakistan along these lines, through this strategy pharmaceutical organizations can built up another new combined dosage type of all the four APIs. Previously there were no such a technique were created by any researcher utilizing these chromatographic conditions and APIs.

CONCLUSION:

Gemifloxacin has been proved to be safe with atorvastatin ,Glimepiride and with montelukast too, but there were no reported data available for their simultaneous identification through HPLC, therefore, Simultaneous identification and quantification of four actives- gemifloxacin, atorvastatinGlimepiride and montelukast sodium were performed according to the ICH guidelines, RP-HPLC method proved to be simple, linear, précised, accurate and reproducible. This method therefore can be used in pharmaceutical industries for various applications in wide disciplines in combined dosage form.

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