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A NEW METHOD DEVELOPMENT AND VALIDATION OF METFORMIN HCL AND SITAGLIPTIN BY RP-HPLC AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple rapid and precise Rp-hplc method for metaformin HCL in a pharmaceutical solid dosage form has been developed and validated. Chromatography was performed on a 250 × 4.6mm i.d 4g particle C18 Column with di potassium hydrogen phosphate and Acetonitrile 55:45% $^{V}/_{V}$) PH-4.5 with orthophosphoric acid buffer as mobile phase at a flow rate of 1ml/min. UV detection was performed at 260nm.Run time 10 min; metaformin HCL retension time is 4.28. The method was validated for accuracy, precision, linearity, LOD, LOQ, Robustness, Ruggedness, specificity, and sensitivity in accordance with ICH guide lines. Validation revealed the method is specific, rapid, accurate, precise, reliable and reproducible. Calibration plot was linear over the concentration ranges 20mg/ ml for metaformin HCL. Limit of detection is1.05 µg and limit of quantification is 5.6 µg for metaformin HCL. The high recovery and low coefficients of variation confirm the suitability of the method for metaformin HCL in tablets. The validated method was successfully used for quantitative analysis of metaformin HCL tablets. Find out the impurities of given metaformin HCL by using the validated method with help of HPLC.

KEYWORDS: HPLC analysis, Method development, RP-HPLC, Validation, Impurities, metaformin HCL, di potassium hydrogen phosphate, orthophosphoric acid.

INTRODUCTION

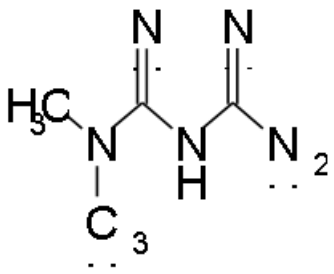
The working condition for the RP-HPLC method was established for Metformin HCL and Sitagliptin then was applied on pharmaceutical dosage forms. A simple reverse phase liquid chromatographic method has been developed and subsequently validated.

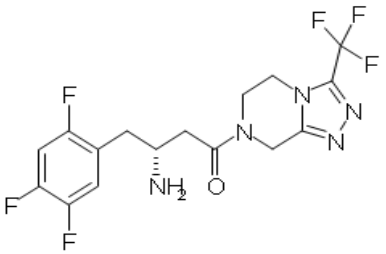
The separation method was carried out by using a mobile phase consisting of 0.02M dipotassium hydrogen phosphate and acetonitrile in the ratio 55:45. the detection was carried out by using UV – Visible SPD 20 A at 240nm. The column was phenominex Gemini C18 (250×4.6mm×5µ). The flow rate was selected as 1ml/min.

The retention time of Metformin HCL and Sitagliptin was found to be 4.285 and 7.485 respectively. The asymmetry factor or tailing 1.008 and 1.011 respectively, which indicates symmetrical nature of the peak. The number of theoretical plates of Metformin HCL and Sitagliptin was found to be 8840 and 12044 respectively, which indicates the efficiency performance of the column.

From the linearity studies, specified concentration levels were determined. It was observed that Metformin HCL and Sitagliptin was linear in the range of 80% to 120% for the target concentration. The linearity range of 10-50mg/ml for Metformin HCL and Sitagliptin were found to obey linearity with a correlation coefficient of 0.999 and 0.999 respectively.

The validation of proposed method was verified by recovery studies. The percentage recovery range was found to be satisfied which represent in results. The robustness studies were performed by changing the pH and wavelength. The ruggedness study was also performed.





METFORMIN HCL **SITAGLIPTIN**

Figure 1: Molecular structure of METFORMIN HCL and SITAGLIPTIN.

MATERIALS AND METHODS

Instrumentation

S. No.	Name of instrument	Model	Make
1	Semi micro balance	CPA225D	Sartorius
2	pH meter	Metler Toledo	Thermo Orion
3	HPLC	LC-20 AT	Shimadzu
4	C 18 Column	Phenomenex	Gemini
5	Sonicator	USB	Spectro lab
6	UV	1700 series	Shimadzu

The separation and quantification of SITAGALIPTIN and METAFORMIN HCL was carried in ACETONITRILE spherisorb ODS1 C18 column (250 × 4.6mm, 5µ) equipped in isocratic LC-100 S-HPLC™ (Cyberlab- USA) LC 20AT pump for solvent delivery and variable wavelength programmable LC – 20AT UV- visible detector for detection. The samples were injected using Rheodyne manual inject port and data was analyzed by using WS-100 Workstation software (Cyberlab- USA). USB SPECTRO LAB electronic balance and ultrasonic batch sonicator (1.5 L) in the study.

Chemicals and Reagents

METAFORMIN HCL and SITAGILIPTIN active pharmaceutical ingredients (APIs) were obtained from Klokter life sciences, Himachal Pradesh. The marketed formulation duzallo (METAFORMIN HCL – 200mg and SITAGLIPTIN- 300mg) was purchased in local pharmacy. HPLC grade acetonitrile, methanol, glacial acetic acid, di potassium hydrogen phosphate and water were purchased from Merck chemicals, Mumbai. The membrane filter papers (0.2µ nylon) were purchased from millipore (India).

S. No.	Chemicals/Reagents	Make/grade
1	**Glacial acetic acid**	**Merck(HPLC Grade)**
2	**Dipotassium hydrogen phosphate**	**Merck(GR Grade)**
3	**Methanol**	**Merck(GR Grade)**
4	**water**	**Merck(GR Grade)**

Preparation of standard solutions

Weigh accurately about 50mg of Metformin, 50mg Sitagliptin working standard to a 100ml volumetric flask. Dissolve it completely and sonicate it. Make up to 100ml mobile phase. Take 3ml from the above flask and make up to 50ml with mobile phase.

Preparation of formulation solution

Weigh accurately 20 tablets equivalent to 92.4mg to a 100ml volumetric flask. mobile phase to dissolve it completely and sonicate for 10min with intermediate shaking Make up to 100ml with mobile phase and filter through 0.45µ GHP filter. Further dilute 3ml with 50ml mobile phase.

Method development

The method development for the identification and simultaneous quantification of metaformin HCL and sitagliptin in pharmaceutical formulations, different method development trails were performed. In the method development, composition of mobile phase, pH of mobile phase, configuration of stationary phase, UV detector wavelength and mobile phase flow rate was studied. In each trail condition, the system suitability parameters like peak shape, peak response, number of theoretical plates, tail factor and resolution were checked and the conditions that produce best results were considered as optimized and further validated.

Method validation

The developed method for the simultaneous quantification of sitagliptin and metaformin HCL was validated for the determination of range of analysis, sensitively, accuracy, precise, rugged and robust nature. The sensitivity of the developed method was determined by confirming detection and quantification limits.

Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to be present in the sample matrix. The other component may include excipients, impurities, degradation product etc.

Peak purity test may be useful to show that the analyte chromatographic peak is not contributed by more than one component (e.g. Diode array, mass, spectroscopy).

Force degradation studies

Forced degradation study was carried for the standard drugs sitagliptin and meatformin HCL in the develop method to evaluate the effectiveness of the developed method for the separation and identification of known and unknown impurities in the drug. 50mg of standard drug was mixed with 50mL of 0.1N NaOH in base hydrolysis study and 50mL of 3% hydrogen peroxide solution for oxidative degradation study. These conditions were carried

separately for both the drugs and the solutions were incubated 24 H and then neutralized separately. The equal volume of selected concentration of both the drugs were mixed and then neutralized. The neutralized solutions were analyzed in the developed method condition. In photolytic and thermal degradation conditions, standard drug was kept under UV light at 260nm and oven at 60 ^{0}C for 24 hours respectively. Then the standard drug was diluted to 300µg/mL and was analyzed in the developed method condition. The % degradation, number of degradation products formed in the degradation study and the % effectiveness of the method for the separation of degradation products was evaluated.

Formulation analysis

The formulation solution prepared from the formulation tablets of sitagliptin and metaformin HCL was analyzed in the developed method. The % assay of sitagliptin and metaformin HCL in the developed method was calculated in the developed method.

Method Precision for Metformin HCL

Sample. No	% Assay
Sample Preparation – 1	100.14
Sample Preparation – 2	100.1.8

RESULTS AND DISCUSSION

The present work is aimed to develop a simple, precise and accurate stability indicating HPLC method for the separation and quantification of sitagliptin and metaformin HCL in pharmaceutical formulations. The optimized separation was achieved using isocratic elution at a flow rate of 1.0 mL/min using mobile phase of methanol, acetonitrile and water in the ratio of 55:45% (v/v) at pH 4.5 and 7.3. Waters spherisorb ODS1 C18 column (250 mm x 4.6 mm, 5µ) was used as stationary phase and UV detection was monitored at 260 nm.

In the optimized conditions, sitagliptin and metaformin HCL were well resolved and retained at a retention time of 3.7 min and 6.1 min respectively and clear base line was observed with in run time of 10 min. The method obeys system suitability conditions (Table 1) for both sitagliptin and metaformin HCL. Figure 2 shows the optimized chromatogram of sitagliptin and metaforine HCL in the developed method.

Robustness study for Metformin HCL and Sitagliptin

Robustness Criteria	RT Metformin of	RT of Sitagliptin
Change in flow +0.2	3.707	6.100
Change in flow -0.2	4.790	7.560
Change in wavelength by -P^H	4.27	7.44
Change in wavelength by + P^H	4.28	7.48

Table 1: System suitability results.

System suitability parameters	Metformin HCL	Sitagliptin
Tailing factor	1.056	1.000
No. of theoretical plates	9226	11340
Resolution	-	13.861

CHROMATOGRAM-08

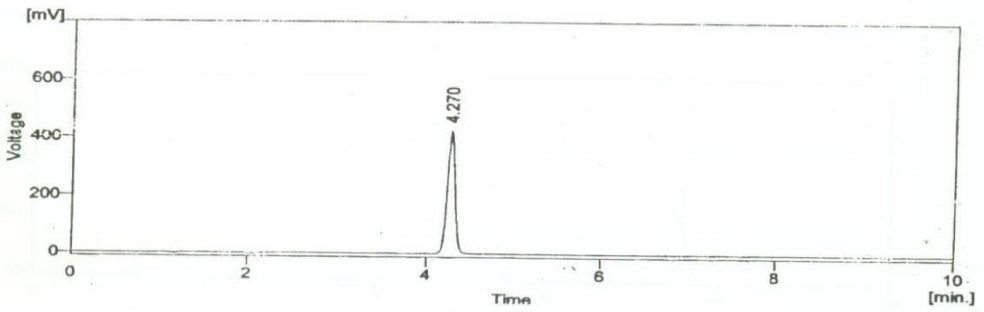
QUALITY CONTROL DEPARTMENT

SAMPLE NAME : METFFORMIN HCL

SYSTEM : HPLC

DETECTOR : UV-VIS

TYPE OF ANALYSIS : PERCENT ON AREA



Result Table (MET+SIT)

S.NO	Reten Time (Min)	Area (mV)	Area (%)
1	4.270	2231.765	100.0
	Total	2231.765	100.0

CHROMATOGRAM-09

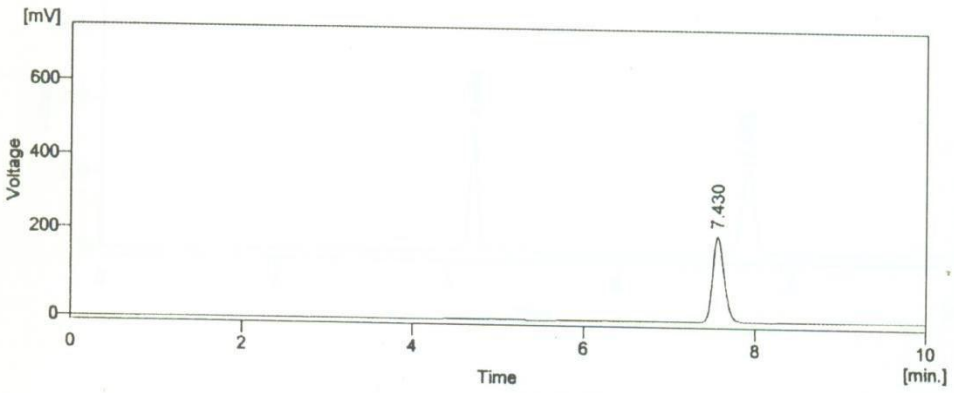
QUALITY CONTROL DEPARTMENT

SAMPLE NAME : SITAGLIPTIN

SYSTEM : HPLC

DETECTOR : UV-VIS

TYPE OF ANALYSIS : PERCENT ON AREA



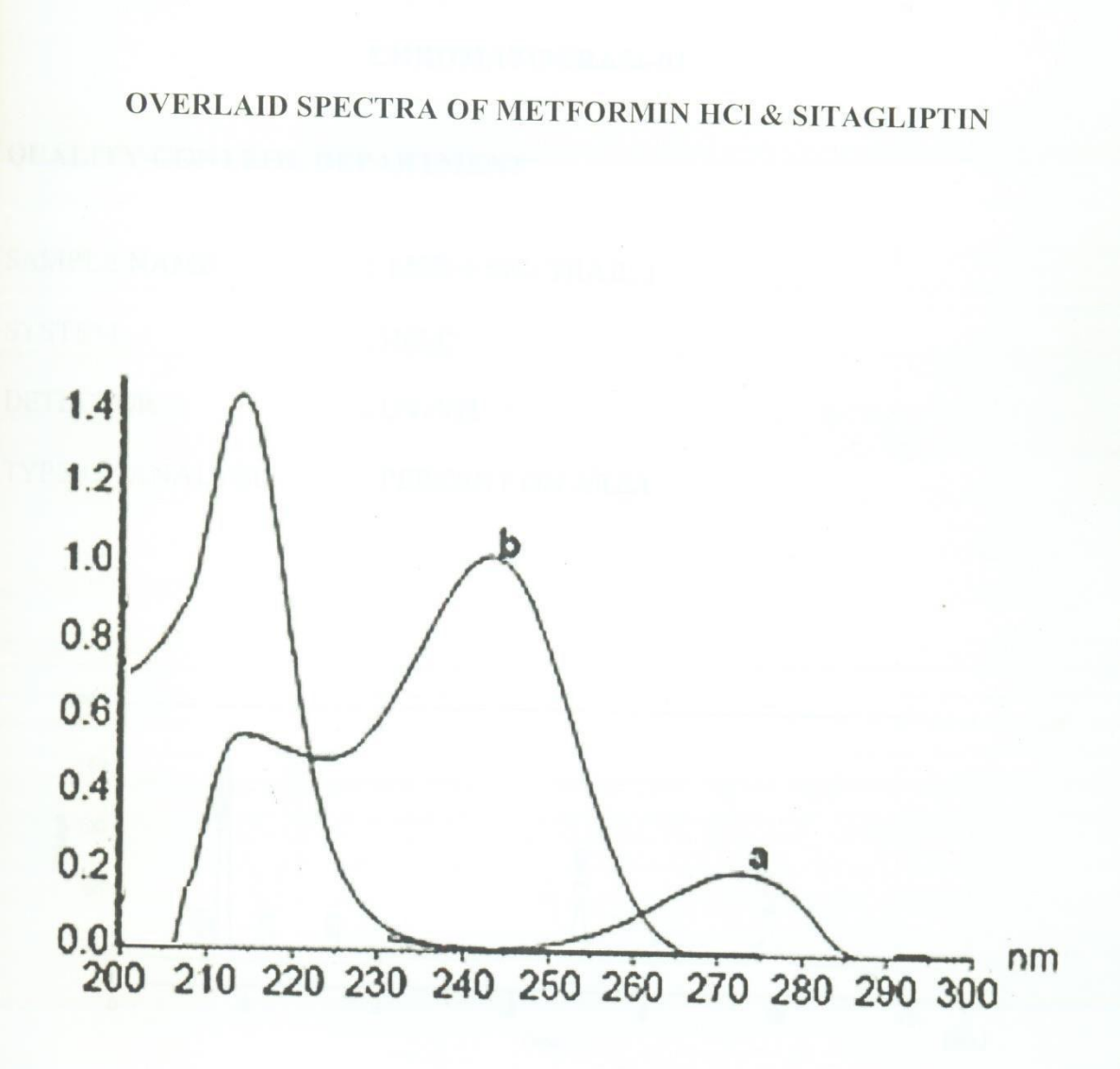
Result Table (MET+SIT)

S.NO	Reten Time (Min)	Area (mV)	Area (%)
1	7.430	2102.11	100.0
	Total	2102.11	100.0

Figure 2: Standard chromatogram in the optimized conditions.

In the chromatogram obtained with Standard,

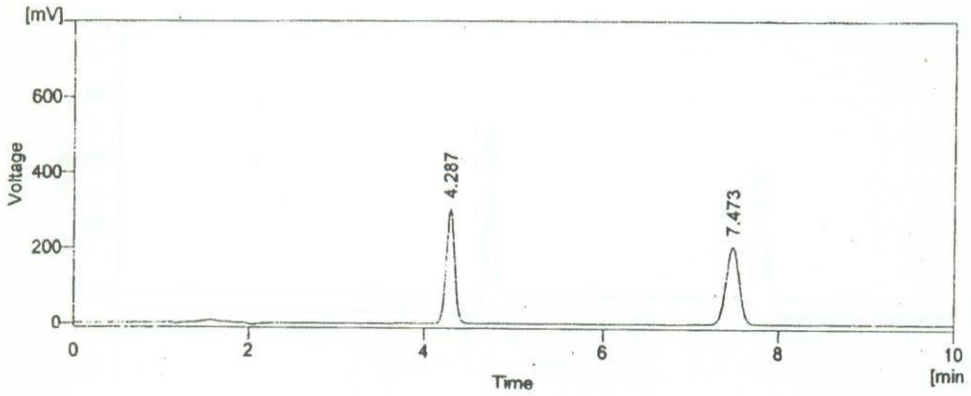
- The % RSD of area of Metformin HCL and Sitagliptin in replicate injections of standard solution should not be more than 2.0.
- The tailing factor of Metformin HCL and Sitagliptinpeak should not be more than 2.0
- The theoretical plates of Metformin HCL and Sitagliptin peak should be
- more than 2000.



CHROMATOGRAM-17

QUALITY CONTROL DEPARTMENT

SAMPLE NAME : LINEARITY 1
SYSTEM : HPLC
DETECTOR : UV-VIS
TYPE OF ANALYSIS : PERCENT ON AREA



Result Table (MET+SIT)

S.NO	Reten Time (Min)	Area (mV)	Area (%)
1	4.287	1773.545	52.0
2	7.473	1640.741	48.0
	Total	3414.286	100.0

Figure 4: Linear calibration curve for metaformin HCL.

The repeatability and reproducibility were studied by intraday, interday precision and ruggedness study. The standard solution at a concentration of 20 µg/mL of sitagliptin and 20 µg/mL of metaformin HCL was analysis six times in the same day for intraday precision, six times in three successive days for interday precision and six times for change in analyst for ruggedness study. The % RSD in each study was calculated for both the drugs and was found to be 0.79, 0.562 in intraday precision, 0.44, 1.091 in interday precision and 0.593, 0.849 in

ruggedness study for metaformin HCL and sitagliptin respectively. This confirms that the method developed was found to be precise and rugged for the simultaneous analysis of metaformin HCL and sitagliptin.

The standard concentration of metaformin HCL and sitagliptin were analyzed by change in analytical conditions i.e mobile phase composition (±5 %), mobile phase pH (±0.1) and detector wavelength (±5 nm). The % change was calculated in each changed condition for both the drugs and was found to be within the acceptable limit of less than 2 confirms that the method was found to be robust. The spiked recovery at 80%, 100% and 120% spiked levels at a target concentration of 20 µg/mL of metaformin HCL and 20 µg/mL of sitagliptin were studied. The % Recovery and the % RSD of recovery in each spike level for 30 µg/mL was calculated (table 3 and 4) and was found to be within the acceptable limits for both metaformin HCL and sitagliptin confirms that the method was found to be accurate.

Recovery values of Sitagliptin

Table 3: Linearity results for sitagliptin.

Concentration	Avg Area	Amount Recovery	% Recovery
80	1679.874	3.96	99.78
100	2086.258	4.96	99.24
120	2565.329	6.08	101.18
		Mean	100.06
		SD	0.79
		%RSD	0.79

Recovery values of Metformin

Table 4: Linearity results for metaformin HCL.

Concentration	Avg Area	Amount Recovery	% Recovery
80	1777.467	39.89	99.93
100	2210.760	49.50	99.05
120	2694.174	59.99	100.03
		Mean	99.66
		SD	0.44
		%RSD	0.44

In the stress degradation study, the % degradation of metaformin HCL was found to be 8.61 (acidic), 10.67 (basic), 4.72 (peroxide), 5.38 (thermal) and 7.28 (UV light) where as the % degradation of sitagliptin was found to be 7.76 (acidic), 8.78 (basic), 3.34 (peroxide), 6.46

(thermal) and 5.28 (UV light). Less % degradation was observed for both the drugs in peroxide conditions where as the % degradation was found to be high in acidic and base conditions. In the stress degradation studies, both the standard drugs were retained in the same retention time compared with un stressed conditions and the additional degradation products formed were effectively separated and retained in the developed method. Hence the method can be used for the identification of known or unknown impurities formed during the stress study. Hence the method was considered as stability indicating method.

The analytical method validation was carried as per ICH guidelines and given below are the tables are the summary of the result.

S.No	Parameters	Limit	Observations	Passes/ fails
1	Specificity	No Interferences at retention time of the analyte peak.	No Interference at retention time of the analyte peak	Passes
2	System Precision	RSD NMT 2.0%	Metforminn:0.3397% Sitagliptin:0.385	Passes
3	Method Precision	RSD NMD 2.0%	Metforminn:0.46% Sitagliptin:0.25%	Passes
4	Linearity ‘of detector response	Correlation coefficient NLT 0.999	Metforminn:0.999 Sitagliptin:0.999	Passes
5	Accuracy	% Recovery range 98-102%	Metforminn:99.59100.71% Sitagliptin:99.11101.18%	Passes
6	Ruggedness	% Recovery range 98-102%	Within limits	Passes
7	Robustness	RSD NMT 2.0%	Within limits	Passes
8	Limit detection (LOD) of	Based on SD of the Response and slope	Metforminn :1.052µg/ml Sitagliptin:7.10µg/ml	Passes
9	Limit quantitation (LOQ)	Based on SD of the Response and slope	Metforminn :5.7µg/ml Sitagliptin 3.4µg/ml	passes

CONCLUSION

RP-HPLC method was developed. It was validated for the estimation of Metformin HCL and Sitagliptin in tablet dosage form using HPLC Shimadzu Prominence with UV-Visible SPD 20A Detector and Phenominex C18 (250x4.6mm, 5µ) column, injection of 20 µl is injected and eluted with the mobile phase of dipotassium hydrogen phosphate buffer, and acetonitrile in the ratio 55:45, which was pumped at a flow rate of 1ml at 260 nm. The peak of Metformin HCL and Sitagliptin are found well separated at 4.285 and 7.485 respectively. The developed method was validated for various parameters as per ICH guidelines like Accuracy, Precision, Linearity, Specificity, Ruggedness, Robustness, LOQ and LOD.

The analytical method validation of Metformin HCL and Sitagliptin by RP HPLC method was found to be satisfactory and could be used for the routine pharmaceutical analysis of Metformin HCL and Sitagliptin.

REFERENCES

1. Alfonso genera in Remington's Pharmaceutical series, 18th -Edn., Mack publishing company, 1990; 648.
2. Amini et al., Determination of Ofloxacin in Plasma by HPLC with UV detection, *Journal of Applied Sciences*, 2005; 5(9): 1655-1657.
3. Beckett, A.H., and Stenlake, J.B., Practical Pharmaceutical Chemistry, CBS Publishers and Distributors, 1997; 157.
4. Berezkin, V.G and Horwood, E., Chromatographic Adsorption Analysis, 1990.
5. Bhusari KP et al., Spectrophotometric methods were developed for the estimation of ofloxacin and ornidazole simultaneously in tablet dosage form, Asian Journal of Research Chemistry, 2009; 60-62.
6. C Bala Sekaran and A Prameela Rani. Development and Validation of Spectrophotometric method for the determination of DPP-4 inhibitor, sitagliptin, in its pharmaceutical dosage, *Int J Pharm Pharm Sci*, 2010; 2(4): 138142.
7. CDER Reviewer Guidance: Validation of Chromatographic Methods, 1994.
8. Chung' Chow Chan., et al., Analytical Method Validation and Instrument Performance Verification, 35-45.
9. Dan Diaconu et al., Studies regarding the stability of pharmaceutical formulation released through association of beta lactamic antibiotic. Stability of an oral suspension powder containing cefadroxil and cefixime,
10. Roumanian Society of Biological Sciences, 2006; 2(3): 27732780.
11. David C. Lee, Michael Webb, Pharmaceutical Analysis, 1: 32-44.
12. David G. Watson, Pharmaceutical Analysis, A textbook for Pharmacy students and Pharmaceutical chemists, 2nd edition, 286.
13. Dharuman J et al., RPHPLC method development and validation for the simultaneous estimation of ofloxacin and tinidazole in tablets, *International Journal of Pharmatech Research*, 2009; 1: 121-124.
14. Douglas A. Skoog., Donald, M. West, and James Holler, F., Fundamentals of Analytical Chemistry. 7lh Edn., 1-3: 628-641.

15. Dragica Zendelovska et al., Developed in high-performance liquid chromatographic method for the determination of Ofloxacin and Lomefloxacin in human plasma, Journal of the Serbian Chemical Society, 2005; 70: 1451 – 1460.
16. Elsevier ICH Topic Q2A, "Validation of Analytical Procedures": Methodology, 6th Nov, www.ich.org, 1996.
17. G. Mubeen, Khalikha Noor and MN Vimala. Spectrophotometric Method for Estimation of Metformin Hydrochloride, *International Journal of ChemTech Research*, 2010; 2(2): 1186-1187.
18. Galen, W. E., Instrumental Methods of Chemical Analysis., 5th Edn., Me Graw-Hill International Editions, 1.
19. Garry 'D.Christian, Analytical Chemistry, 4th edition, University of Wellington, A.W.Sons, London, 1-4: 469-475.
20. Goodman and Gilman's The Pharmacological basis of Therapeutics 11th edition by Kaball, 2006.
21. Gurdeep R Chatwal, Sham, K. and Anand, Instrumental methods of Chemical Analysis, 5th Edn., Himalaya publishing House, New Delhi, 561-567.
22. Hawk, G.GL and Kingston, H.M., Laboratory Robotics and Trace Analysis in quantitative Trace Analysis of Biological Materials, 1988.
23. ICH Stability Testing: Photo stability Testing of New Drug Substances and Products, ICH, Geneva, Switzerland, Nov., www.ich.org, 1996.
24. Indian Pharmacopoeia Vol II A-185. Introduction to chemical analysis by Robert D.Braun, 3,8: 145. 24.
25. Jeffery, G.H. and Basselt, J., VogeFs Text Book of Quantitative pharmaceutical Chemistry, 21-23.
26. Jitendra D et al., Simultaneous spectrophotometric estimation of ofloxacin and ketorolac tromethamine in ophthalmic dosage form, *International Journal of Chem Tech Reserarch*, 2009; 1(2): 189-194.
27. K.S. Lakshmi, T. Rajesh and Shrinivas Sharma. Simultaneous determination of metformin and pioglitazone by reversed phase hplc in pharmaceutical dosage forms, *International journal of pharmacy and pharmaceutical sciences*, 2009; 1(2): 162-166.
28. Kalta RR et al., Simultaneous RPHPLC Determination of Nitazoxanide and Ofloxacin in Combined Tablet Dosage Form, *Indian Journal of Pharmaceutical Sciences*, July – August 2008; 491-493.

29. Liu GL et al., Determiniation of cefixime in human plasma and urine using high performance liquid chromatography column switching technique. *Yao Xue Xue Bao*, 1993; 28(3): 216-218.
30. Lloyd R. Snyder., Joseph J. Kirkland., Jopseph 1. Glajch., Practical HPLC Method Development, 2nd edition, 3-4, 234-242, 351-352, 25-27, 42, 653-656.
31. Maria-Cristina Ranetti, Mihaela Ionescu and Lavinia. Validation of a hplc method for the simultaneous analysis of metforminand gliclazide in human plasma, Farmacia, 2009; 57(6): 728-736.
32. Martindale The Complete Drug Reference. Mendham, J., Denney, R.C., Barnes, J.D. and Thomas. M.J.K., Vogel's, Text book of Quantitative Chemical Analysis., 5th Edn., Addison Wesley Longman Ltd, 2003.
33. Mohsen Amini et al., Simple high performance liquid chromatographic method for determination of ciprofloxacin in human plasma. *Iranian Journal of Pharmaceutical Research*, 2004; 2: 99-101.
34. Nanda RK et al., Simultaneous Spectrophotometric Estimation of cefixime and Ornidazole in Tablet Dosage, *International Journal of Pharmtech Research*, 1(3): 488-491.
35. Natarajan Sunder*, Raman Bhanu, *et al.*, Developed method and validation of a stability indicating HPLC method for simultaneous estimation of ofloxacin and ornidazole, *The Indian Pharmacist*, 2005; 4: 79-84.
36. Priyabrata Pattanayak et al., Simultaneous spectrophotometric estimation of Nitazoxanide and Ofloxacin in combined tablet dosage form, *Reserarch Journal of Pharmacy and Technology*, 2009: 291-293.
37. *Ranjit Singh et al.*, Method development and validation for simultaneous estimation Ofloxacin, and Ornidazole in tablet formulations, *Eurasian Journal Analytical Chemistry, 2009; 4(2): 161-167.*
38. Rathinavel G et al., A validated RP – HPLC method for Simultaneous Estimation of Cefixine and Cloxacilline in Tablets, *E. Journal of Chemistry*, July 2008; 5(3): 648-651.
39. S. Havele and S. Dhaneshwar. Validation of A Hplc Method For The Determination Of Metformin Hydrochloride, Gliclazide And Piogliglitazone Hydrochloride in Multicomponent Formulation, *Webmedcentral Pharmaceutical Sciences*, 2010; 1(10): 1-16.
40. Salama F.M et al., 13-Spectrophotometric determination of cefixime in presence of its hydrolysis products.

41. Satinder Ahuja, Chromatography and Separation Science, Volume 4 of the Separation Science and Technology Series, 153-156.
42. Sethi, P.D. Quantitative Analysis of Pharmaceutical Applications, 1997; 23-65.
43. Sharma, B.K., Instrumental methods of chemical Analysis, 18th Edn., Krishna prakashan media (p) Ltd, Meerut, 1999; 10-30.
44. Siva Kumar R et al., Simultaneous RPHPLC Estimation of Nitazoxanide and Ofloxacin in Tablet Dosage Form, *Asian Journal of Research Chemistry*, 2009; 43-45.
45. Solangi R et al., Quantitative analysis of eight cephalosporin antibiotics in pharmaceutical products and urine by capillary zone electrophorosis, *ACTA Chromatographica*, 19: 2007.
46. Stockwell, P.B and Corns, W.T Automatic Chemical Analysis, Taylor and Francis, London, 1996.
47. Studies regarding the stability of pharmaceutical formulations released through association of beta lactamic antibiotic. Stability of an oral suspension powder containing cefadroxil and cefixime.
48. T.Higuchi, E.Brochmann, H.Hanssen Pharmaceutical analysis 1st edition, 1-9.
49. Tadashi ohkubo et al., High Performance Liquid Chromatographic Determination of Ofloxacin in Serum, *Analytical Science*, Oct. 1991; 7.
50. Wankhede SB* et al, Developed in simultaneous reverse phase HPLC Estimation of Ofloxacin and Satranidazole in tablet dosage form , Asian Journal of research Chemistry, 2008; 9 – 11.
51. www.wikepedia.com Zeng S et al., High-performance liquid chromatography separation and quantitation of ofloxacin enantiomers in rat microsomes, *Journal of Chromatography, B, Biomedical Science and Application*, 1999; 728: 151-155.