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Research

Validated high performance liquid chromatography method for estimation of ofloxacin and satranidazole in bulk and tablet dosage form

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Abstract

A rapid and precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Satranidazole and Ofloxacin, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Hypersil C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol: Water (80:20v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 310 nm. The retention time of the Satranidazole and Ofloxacin was 3.0, 4.0 ±0.02min respectively. The method produce linear responses in the concentration range of 20-100µg/ml of Satranidazole and 15-75µg/ml of Ofloxacin. 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: Satranidazole, Ofloxacin, RP-HPLC, validation.

INTRODUCTION

Analytical chemistry

Analytical chemistry is a scientific discipline used to study the chemical composition, structure and behaviour of matter. The purposes of chemical analysis are together and interpret chemical information that will be of value to society in a wide range of contexts. Quality control in manufacturing industries, the monitoring of clinical and environmental samples, the assaying of geological specimens, and the support of fundamental and applied research are the principal applications. Analytical chemistry involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative and structural information on the nature of matter.[1]

- **Qualitative analysis** is the identification of elements, species and/or compounds present in sample.

- **Quantitative analysis** is the determination of the absolute or relative amounts of elements, species or compounds present in sample.

Structural analysis is the determination of the spatial arrangement of atoms in an element or molecule or the identification of characteristic groups of atoms (functional groups). An element, species or compound that is the subject of analysis is known as analyte. The remainder of the material or sample of which the analyte(s) form(s) a part is known as the matrix.[2]

The gathering and interpretation of qualitative, quantitative and structural information is essential to many aspects of human endeavour, both terrestrial and extra-terrestrials. The maintenance of an improvement in the quality of life throughout the world and the management of resources heavily on the information provided by chemical analysis. Manufacturing industries use analytical data to monitor the quality of raw materials, intermediates and finished products. Progress and research in many areas is dependent on establishing the chemical composition of man-made or natural materials, and the monitoring of toxic substances in the environment is of ever increasing importance. Studies of biological and other complex systems are supported by the collection of large amounts of analytical data. Analytical data are required in a wide range of disciplines and situations that include not just chemistry and most other sciences, from biology to zoology, butte arts, such as painting and sculpture, and archaeology. Space exploration and clinical diagnosis are two quite desperate areas in which analytical data is vital. Important areas of application include the following.[3]

Quality control

(QC) in many manufacturing industries, the chemical composition of raw materials, intermediates and finished products needs to be monitored to ensure satisfactory quality and consistency. Virtually all consumer products from automobiles to clothing, pharmaceuticals and foodstuffs, electrical goods, sports equipment and horticultural products rely, in part, on chemical analysis. The food, pharmaceutical and water industries in particular have stringent requirements backed by legislation for major components and permitted levels of impurities or contaminants. The electronic industry needs analyses at ultra-trace levels (parts per billion) in relation to the manufacture of semi-conductor materials. Automated, computer-controlled procedures for process-stream analysis are employed in some industries.

Monitoring and control of pollutants

The presence of toxic heavy metals (e.g., lead, cadmium and mercury), organic chemicals (e.g., polychlorinated biphenyls and detergents) and vehicle exhaust gases (oxides of carbon, nitrogen and sulphur, and hydrocarbons) in the environment are health hazards that need to be monitored by sensitive and accurate methods of analysis, and remedial action taken. Major sources of pollution are gaseous, solid and liquid wastes that are discharged or dumped from industrial sites, and vehicle exhaust gases.[4]

Clinical and biological studies

The levels of important nutrients, including trace metals (e.g., sodium, potassium, calcium and zinc), naturally produced chemicals, such as cholesterol, sugars and urea, and administered drugs in the body fluids of patients undergoing hospital treatment require monitoring. Speed of analysis is often a crucial factor and automated procedures have been designed for such analyses.[5]

Geological assays

The commercial value of ores and minerals are determined by the levels of particular metals, which must be accurately established. Highly accurate and reliable analytical procedures must be used for this purpose, and referee laboratories are sometimes employed where disputes arise.[6]

Fundamental and applied research

The chemical composition and structure of materials used in or developed during research programs in numerous disciplines can be of significance. Where new drugs or materials with potential commercial value are synthesized, a complete chemical characterization maybe required involving considerable analytical work. Combinatorial chemistry is an approach used in pharmaceutical research that generates very large numbers of new compounds requiring confirmation of identity and structure.[7,8]

Analytical techniques

There are numerous chemical or physico-chemical processes that can be used to provide analytical information. The processes are related to a wide range of atomic and molecular properties and phenomena that enable elements and compounds to be detected and/or quantitatively measured under controlled conditions. The underlying processes define the various *analytical techniques*. The more important of these are listed in Table.No.1 together with their suitability for qualitative, quantitative or structural analysis and the levels of analyte(s) in a sample that can be measured. *Atomic, molecular spectrometry* and *chromatography*, which together

comprise the largest and most widely used groups of techniques, can be further subdivided according to their physico-chemical basis. *Spectrometric techniques* may involve either the *emission or absorption* of *electromagnetic radiation* over a very wide range of energies, and can provide qualitative, quantitative and structural information for analytes from major components of a sample down to ultra-trace levels.[9,10]

MATERIALS AND METHODS

Satranidazole & Ofloxacin from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK), Acetonitrile for HPLC from Merck.

HPLC METHOD DEVELOPMENT
TRAILS
TRAILS
Preparation of standard solution

Accurately weigh and transfer 10 mg of Satranidazole and Ofloxacin 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 0.6ml of the Satranidazole and 0.45ml of the Ofloxacin from above 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 Water: Acetonitrile with varying proportions. Finally, the mobile phase was optimized to Methanol: Water in proportion 80:20 v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column, Symmetry and X-Bridge. Hypersil 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 PDA Detector 996 model.
Temperature	:	35ºC
Column	:	Hypersil C18 (4.6×150mm, 5µ)
Mobile phase	:	Methanol: Water (80:20v/v)
Flow rate	:	1ml/min
Wavelength	:	310nm
Injection volume	:	10 µl
Run time	:	8min

VALIDATION
PREPARATION OF MOBILE PHASE
Preparation of mobile phase

Accurately measured 800ml (80%) of Methanol and 200ml of Water (20%) a were mixed and degassed in digital ultrasonicater 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	:	Methanol: Water (80:20v/v)
Column	:	Hypersil C18 (4.6×150mm, 5µ)
Flow rate	:	1 ml/min
Wavelength	:	310 nm
Column temp	:	35ºC
Injection Volume	:	10 µl
Run time	:	8 minutes

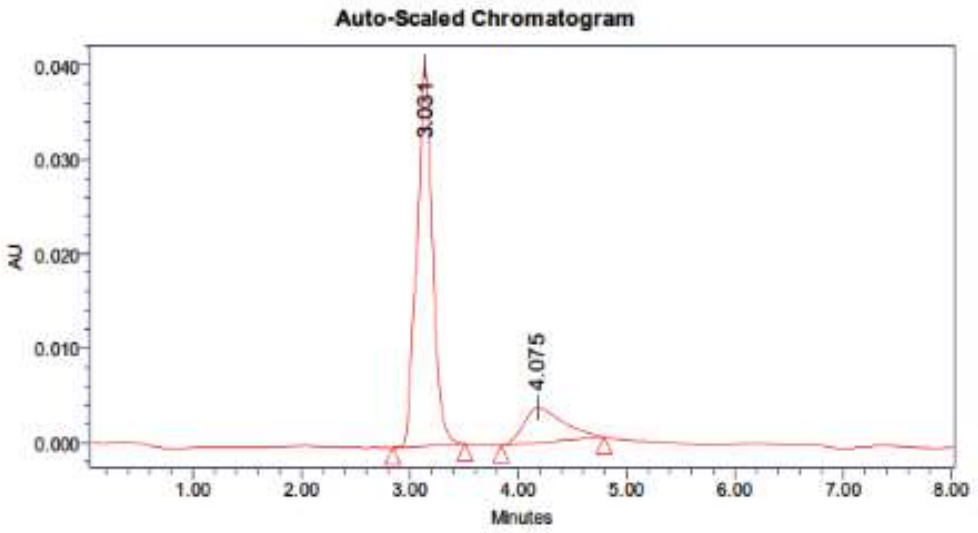

Fig 1: Optimized Chromatogram

Table 1: Peak results for optimized

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Satranidazole	3.031	822484	80339		1.2	6633
2	Ofloxacin	4.075	166402	7149		1.0	5833

Observation: From the above chromatogram it was observed that the Satranidazole and Ofloxacin peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

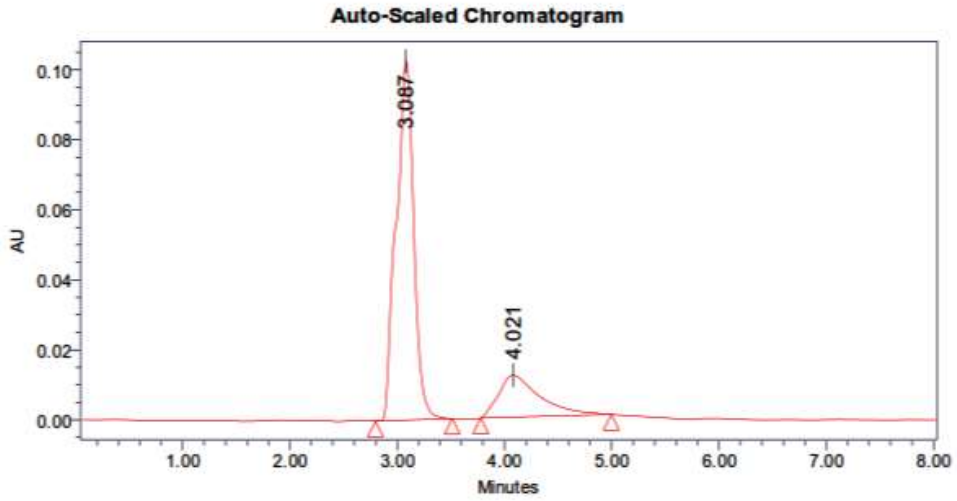

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Satranidazole	3.087	872579	87475		1.3	2939
2	Ofloxacin	4.021	164029	7143	3.5	1.0	5552

Assay (Standard)

Table 3: Peak results for assay standard

S.No	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Satranidazole	3.064	915566	82861		1.2	6574
2	Ofloxacin	4.045	173391	17456	5.9	1.1	7265
3	Satranidazole	3.094	947095	86365		1.2	6284
4	Ofloxacin	4.014	189816	17986	5.9	1.2	7264
5	Satranidazole	3.025	926537	110000		1.1	6292
6	Ofloxacin	4.047	177686	18280	5.9	1.2	7595

Assay (Sample)

Table 4: Peak results for Assay sample

S.No	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Satranidazole	3.046	930441	103285		1.1	6488
2	Ofloxacin	4.029	171452	18350	5.9	1.2	6927
3	Satranidazole	3.046	949593	102166		1.2	7218
4	Ofloxacin	4.029	176778	18561	5.9	1.1	6455
5	Satranidazole	3.016	968149	105311		1.1	6622
6	Ofloxacin	4.036	188857	18791	5.9	1.2	6844

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

The % purity of Satranidazole and Ofloxacin in pharmaceutical dosage form was found to be 98.7 %.

System suitability

Table 5: Results of system suitability for Satranidazole

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Satranidazole	3.045	697722	115333	9747	1.2
2	Satranidazole	3.014	691653	110394	7684	1.4
3	Satranidazole	3.045	697662	127464	8646	1.3
4	Satranidazole	3.044	697154	117493	5763	1.2
5	Satranidazole	3.064	697558	115079	4537	1.4
Mean			696349.8			
Std. Dev			2634.92			
% RSD			0.37839			

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

Table 6: Results of system suitability for Satranidazole

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Ofloxacin	4.021	124736	10404	6181	1.4	5.9
2	Ofloxacin	4.074	124947	10428	7464	1.4	5.9
3	Ofloxacin	4.052	124665	10466	6276	1.3	5.9
4	Ofloxacin	4.062	124088	10492	6184	1.2	5.9
5	Ofloxacin	4.085	124653	10491	6774	1.2	5.9
Mean			124617.8				

Std. Dev	318.7863
% RSD	0.255811

- *%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.*

LINEARITY
Satranidazole

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	20	308034
66.6	40	616134
100	60	898253
133.3	80	1166477
166.6	100	1490568

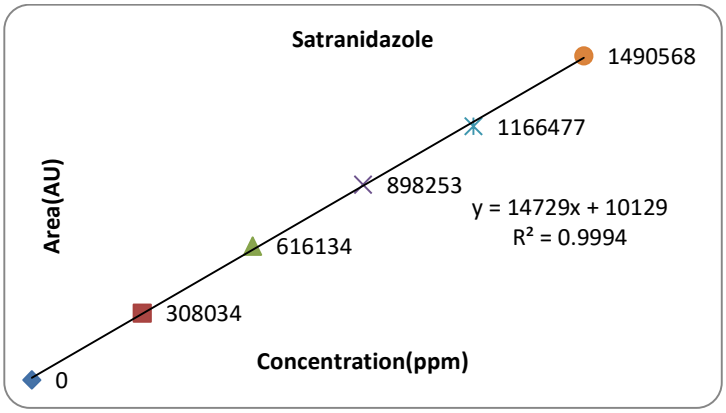

Fig 3: calibration graph for Satranidazole

Ofloxacin

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	15	93033
66	30	181522
100	45	261170
133	60	357887
166	75	446895

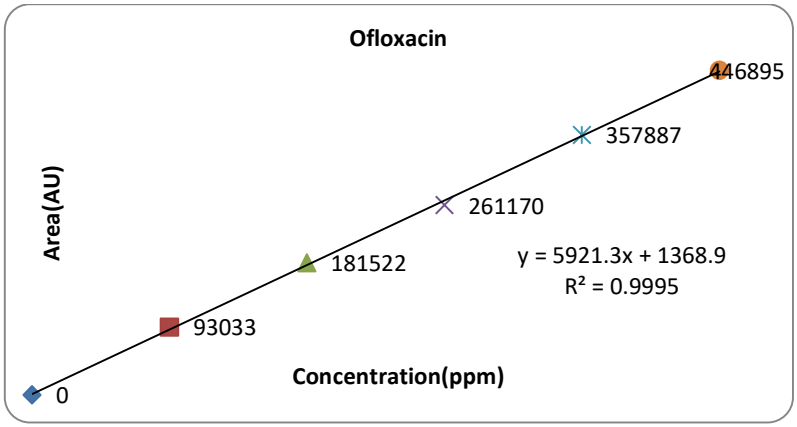

Fig 4: calibration graph for Ofloxacin

REPEATABILITY

Table 7: Results of repeatability for Satranidazole

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Satranidazole	3.021	918705	108721	8363	1.2
2	Satranidazole	3.073	910361	108276	7465	1.2
3	Satranidazole	3.021	912476	108351	9282	1.2
4	Satranidazole	3.041	910894	106872	8664	1.2
5	Satranidazole	3.095	917558	102421	8726	1.2
Mean			913998.8			
Std. Dev			3873.258			
% RSD			0.423771			

- *%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 8: Results of method precession for Ofloxacin

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Ofloxacin	4.068	177255	8393	5542	1.2	5.9
2	Ofloxacin	4.021	177532	8664	6762	1.1	5.9
3	Ofloxacin	4.061	177993	6874	6823	1.2	5.9
4	Ofloxacin	4.064	177433	9633	8363	1.1	5.9
5	Ofloxacin	4.054	174028	6443	7644	1.2	5.9
Mean			176848.2				
Std. Dev			1599.934				
% RSD			0.904693				

- *%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 9: Results of Intermediate precision for Satranidazole

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Satranidazole	3.074	898654	108546	8754	1.2
2	Satranidazole	3.087	898219	108347	4546	1.2
3	Satranidazole	3.034	898032	108113	8896	1.2
4	Satranidazole	3.084	898132	108656	5678	1.2
5	Satranidazole	3.087	898014	108432	9845	1.2
6	Satranidazole	3.056	898044	108942	8658	1.2
Mean			898182.5			
Std. Dev			243.4829			
% RSD			0.027108			

- %RSD of five different sample solutions should not more than 2

Table 10: Results of Intermediate precision for Ofloxacin

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Ofloxacin	4.035	122487	19115	6076.6	1.2	6.0
2	Ofloxacin	4.025	122555	19066	8676	1.2	6.0
3	Ofloxacin	4.042	122076	19023	9567	1.2	6.0
4	Ofloxacin	4.065	122547	19545	5446	1.2	6.0
5	Ofloxacin	4.051	122067	18343	8668	1.2	6.0
6	Ofloxacin	4.012	122079	18477	9766	1.2	6.0
Mean			122301.8				
Std. Dev			250.7145				
% RSD			0.204997				

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

- *The %RSD obtained is within the limit, hence the method is rugged.*

Table 11: Results of Intermediate precision Day 2 for Satranidazole

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Satranidazole	3.094	898477	108361	7764	1.2
2	Satranidazole	3.047	898264	108554	9844	1.2
3	Satranidazole	3.061	898264	108653	4874	1.2
4	Satranidazole	3.072	898017	108611	9874	1.2
5	Satranidazole	3.069	898454	108747	9847	1.2
6	Satranidazole	3.095	898166	108938	8877	1.2
Mean			898273.7			
Std. Dev			174.0812			
% RSD			0.01938			

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

Table 12: Results of Intermediate precision for Ofloxacin

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Ofloxacin	4.034	269911	9562	5983	1.2	5.9
2	Ofloxacin	4.097	268464	9673	9465	1.2	5.9
3	Ofloxacin	4.034	268636	9616	8264	1.2	5.9
4	Ofloxacin	4.078	269773	9637	8644	1.2	5.9
5	Ofloxacin	4.074	264573	9278	9747	1.2	5.9
6	Ofloxacin	4.041	260484	9487	8764	1.2	5.9
Mean			266973.5				
Std. Dev			3723.644				
% RSD			1.394762				

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

ACCURACY

The accuracy results for Satranidazole

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	453153	30	30	100%	100%
100%	910568.3	60	60.2	101%	
150%	1351727	90	90.2	101%	

The accuracy results for Ofloxacin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	134989.7	22.5	22.5	100%	99.6%
100%	265939	45	44.8	99%	
150%	401572.3	67.5	67.5	100%	

- *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
Satranidazole

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	822484	3.031	8465	1.1
Less Flow rate of 0.9 mL/min	699451	3.215	6453	1.2
More Flow rate of 1.1 mL/min	718432	2.894	4736	1.2

Less organic phase	695412	3.185	3728	1.2
More organic phase	695471	2.954	7464	1.1

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

Ofloxacin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	166402	4.075	9766	1.1
Less Flow rate of 0.9 mL/min	126954	4.197	8263	1.2
More Flow rate of 1.1 mL/min	129542	3.825	8754	1.2
Less organic phase	128256	4.152	7664	1.2
More organic phase	131654	3.932	6474	1.1

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 Satranidazole and Ofloxacin 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. Satranidazole and Ofloxacin was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: Water (80:20v/v) 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 Satranidazole and Ofloxacin in bulk drug and in Pharmaceutical dosage forms.

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