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**Der Pharmacia Lettre, 2010, 2(2): 22-26**
***(http://scholarsresearchlibrary.com/archive.html)***

Der Pharmacia Lettre
Scholars Research Library
**ISSN 0975-5071**
**USA CODEN: DPLEB4**

# Method development and validation of simultaneous determination of Ofloxacin and Satranidazole in pharmaceutical dosage form by RP-HPLC

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## Abstract

*A simple, selective, rapid, and precise reverse phase HPLC method has been developed for the simultaneous estimation of Ofloxacin and Satranidazole in pharmaceutical dosage form. A Phenomenex Luna C18 (4.6*250 mm, 5µ) column was used for the separation. The mobile phase was Phosphate buffer: Acetonitrile (70: 30 % v/v) and pH 6.0 at a flow rate of 1.2ml/min with detection at 300nm.The retention time of Ofloxacin and Satranidazole was 3.720and 6.130min, respectively. The developed method was validated in term of accuracy, precision, specificity, system suitability, linearity, and robustness, limit of detection and limit of quantification. The proposed method is suitable for simultaneous determination of Ofloxacin and Satranidazole in pharmaceutical dosage form.*

**Keywords**: Ofloxacin, Satranidazole, Simultaneous estimation and RP-HPLC.

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## INTRODUCTION

Ofloxacin is a fluoroquinolone antibiotic considered to be a second-generation fluoroquinolone. Ofloxacin is a racemic mixture, which consists of 50% levofloxacin (the biologically active component) and 50% of its “mirror image” or enantiomer dextrofloxacin. Chemical name is (+/-)-9-fluoro-2, 3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1, 2, 3-de]-1, 4-benzoxazine-6-carboxylic acid. Satranidazole, a novel nitroimidazole possessing a C-N linkage at C2 of the imidazole.Chemical name is 1-(1-methyl-5-nitro-imidazol-2-yl)-3-methylsulfonyl-imidazolidin-2-one. Many methods have been described in the literature for the determination of Ofloxacin and Satranidazole individually and in combination with other drug[7-9]. Our present plan is to develop new, simple, precise, & accurate method for its analysis in formulation after a

detailed study a new RP-HPLC method was decided to be developed and validated. The method was validated according to the ICH ($Q_2A$ 1995) guidelines.[2-6]

## MATERIALS AND METHODS

Acetonitrile HPLC grade was procured from E.Merck (India) Ltd, Mumbai. Potassium Dihydrogen ortho phosphate AR grade were procured from Qualigens fine chemicals, Mumbai. Water HPLC grade was obtained from a Milli-QRO water purification system. A reference Standard of Ofloxacin and Satranidazole were procured from Alkem laboratories, Baddi, India.

*Preparation of standard solutions*

Accurately weighed quantity of 200.8mg Ofloxacin and 300.1mg Satranidazole was transferred to a 100ml volumetric flask, dissolved in 15ml of mobile phase, sonicated for 15 min and the volume was made up with mobile phase. From the standard stock preparation 10ml of standard stock solution was taken in 100ml volumetric flask and further diluted with mobile phase.

*Preparation of sample solutions*

For estimating the tablet dosage form, 20 tablets from a batch were randomly selected and powdered. Amount equivalent to 637.1 mg each of Ofloxacin and Satranidazole from powdered formulation were accurately weighed and taken in a 100 ml volumetric flask.15ml of mobile phase was added. The mixture was subjected to sonication for 10 min with intermediate shaking for complete extraction of drugs. Cool to room temperature and the solution was made up to the mark with mobile phase. The sample was centrifuged in tight enclosure for 10 min at 3000 RPM. Then 10ml of clear solution was transferred into a 100 ml volumetric flask and diluted with mobile phase. And 20µl of this solution was injected for HPLC analysis.

*Assay method*

With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solution was injected and the chromatogram was recorded. The retention time Ofloxacin and Satranidazole was 3.731and 6.165.min, respectively. This procedure was repeated for the sample solution obtained from the formulation. The response factor (peak area ratio of standard peak area and internal standard peak area) of the standard solution and sample solution were calculated.

*Recovery studies*

To study the accuracy, reproducibility and precision of the above methods, were carried out by addition of standard drug solution to pre-analyzed sample at different levels. Result of recovery studies were found to be satisfactory and are reported in [Table- 1]

**Table 1: Recovery Studies for Ofloxacin and Satranidazole**

| S. No: | Sample | Spike level | Amount Present | Amount Recovered | % Recovered |
|---|---|---|---|---|---|
| 1 | **Ofloxacin** | 80 % | 160.64 | 198.04 | 99.02 |
| 2 | | 100 % | 200.80 | 198.30 | 99.15 |
| 3 | | 120 % | 240.96 | 199.52 | 99.76 |
| 4. | **Satranidazole** | 80 % | 240.08 | 299.82 | 99.94 |
| 5 | | 100 % | 300.10 | 305.97 | 101.99 |
| 6 | | 120 % | 360.12 | 298.32 | 99.44 |

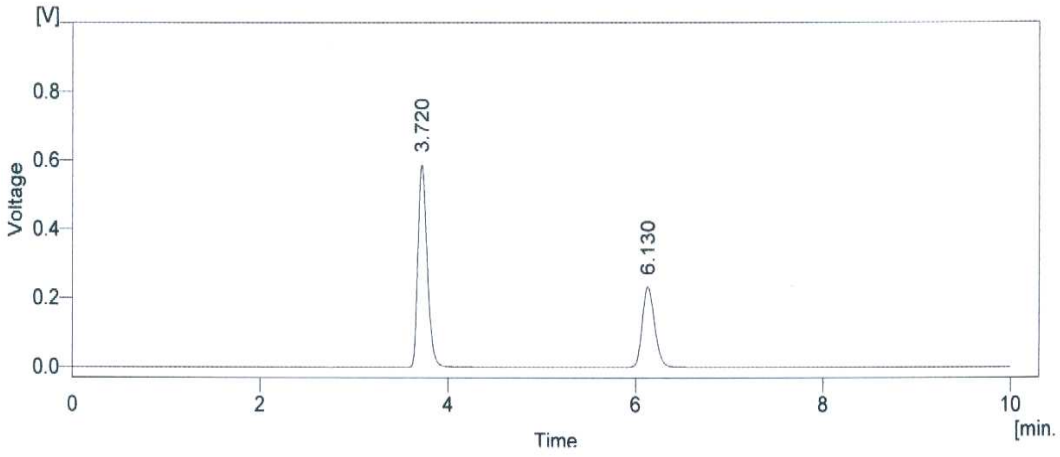


**Figure-1 Chromatogram for formulation**

## RESULTS AND DISCUSSION

Several mobile phase compositions were tried to resolve the peaks of Ofloxacin and Satranidazole. The optimum mobile phase containing Buffer: Acetonitrile (70:30V/V) was selected because it was found ideal to resolve the analyte peaks of both the drugs. Quantification was achieved with UV detections at 300 nm based on peak area and absorbance. As per USP requirements system suitability studies were carried out and freshly prepared standard solutions are Ofloxacin and Satranidazole. Various parameters obtained with 20 µl of injection volume are summarized in the table given below [1].

**Table 2: Validation of Ofloxacin and Satranidazole**

| S.No | Para-meters | Observations |
|---|---|---|
| 1 | Specificity | No Interference at retention time of the analyte peak |
| 2 | Precision<br>Method precision | Ofloxacin : 0.421 %<br>Satranidazole: 0.452 % |
| 3 | Linearity of detector response | Ofloxacin:0.9999<br>Satranidazole:0.9999 |
| 4 | Accuracy | Ofloxacin: 99.15 %<br>Satranidazole :101.99% |
| 5 | Limit of detection (LOD) | Ofloxacin: 1.0µg/ml<br>Satranidazole : 1.5µg/ml |
| 6 | Limit of quantiotion (LOQ) | Ofloxacin: 3.0 µg /ml<br>Satranidazole: 4.5 µg /ml |

*Solution stability*

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 5 h at room temperature. The results show that for both solutions, the retention time and peak area of Ofloxacin and Satranidazole remained almost unchanged (% R.S.D less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 5 h, which was sufficient to complete the whole analytical process.

**Table 3: Stability Studies**

| Stability | Retention time of Ofloxacin (mV) | Retention time of Satranidazole (mV) |
|---|---|---|
| Room temperature | 3941.58 | 2070.42 |
| After 8 hour | 3939.25 | 2069.95 |

## CONCLUSION

The proposed RP-HPLC method for the simultaneous estimation of Ofloxacin and Satranidazole in pharmaceutical dosage form is accurate, precise, linear, rugged, robust, simple and rapid. Hence the present RP-HPLC method is suitable for the quality control of the raw materials, formulation and dissolution studies.

**Acknowledgements**

The authors are thankful to the secretary and correspondent Smt. N. Sendamaraai**,** J. K. K. Rangammal charitable trust and Dr. P. Perumal, Principal, J. K. K. Nataraja College of Pharmacy, Komarapalayam, for the support rendered.

## REFERENCES

[1] Validation of Analytical Procedures: Methodology, ICH Harmonized tripartite Guideline, **1996**, 1-8.
[2] Snyder K.L, Krikland J.J and Glajch J.L: Practical HPLC Method Development, 2nd Edn, Wiley – Interscience Publication, USA, **1983**, 24.
[3] U.S.P Asian Edition, United Pharmacopoeial Convention Inc, Rockville, **2005**, 1694-95
[4] FDA Review Guidance, Analytical Procedures and Method Validation (II), **2000**,
[5] J. S. K. Nagarajan etal., *Der Pharmacia Lettre*., **2009,** 1 (1):162-168.
[6] Skoog, D.A., Holler, F.J., Timothy, A., Nieman, in; Principals of instrumental Analysis, 5th edition, M.Sherman, Bortel, F. Messina (EDS), Saunders College Publication, London, **1998**,32.
[7] Wankhede SB et al., *Asian J. Research Chem*., **2008**, 1, (1), 9-11.
[8] Shashikant R Pattanet al, *Research J. Pharm. and Tech*., **2009**,2 (3),531-536.
[9] Khoo Lay See et al., *Biomedical Chromatography*., **2009**,23(12), 1283 – 1290.