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# Analytical Method Development and Validation for The Simultaneous Estimation of Azithromycin and Levofloxacin by RP-HPLC

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## Abstract

A new method was established for simultaneous estimation of Azithromycin and Levofloxacin by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Azithromycin and Levofloxacin by using ZODIAC –SIL RP C18 column 4.6×100 mm 3.0µm, flow rate was 1.0 ml/min, mobile phase ratio was (75:25 v/v) acetonitrile:phosphate buffer ($KH_2PO_4$ and $K_2HPO_4$) pH 9 ( pH was adjusted with orthophosphoric acid), detection wave length was 292 nm. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Azithromycin and Levofloxacin was found in concentration range of 6.25µg-37.5µg and 0.5µg-3.0µg and correlation coefficient ($r^2$) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.27 and 0.40, % RSD for intermediate precision was 0.27 and 0.94 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.17 and 6.60, and LOQ value was 0.032 and 0.1125 respectively.

**Keywords**
Azithromycin, Levofloxacin, RP-HPLC, Validation, Methanol.

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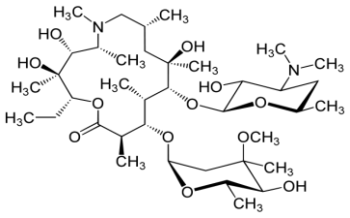
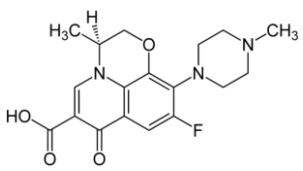
**INTRODUCTION:**

Azithromycin is an antibiotic medication used for the treatment of a number of bacterial infections. This includes middle ear infections, strep throat, pneumonia, traveler's diarrhea, and certain other intestinal infections. Along with other medications, it may also be used for malaria.



**Azithromycin**

Levofloxacin, sold under the brand name Levaquin among others, is an antibiotic medication. It is used to treat a number of bacterial infections including acute bacterial sinusitis, pneumonia, H. pylori urinary tract infections, chronic prostatitis, and some types of gastroenteritis.



**Levofloxacin**

**MATERIALS AND METHOD:**

**Instrumentation**

System Alliance Waters 2690 separation module, Pump Analytical HPLC isocratic pump, Detector Photo diode array detector, Software Empower 2 software, Column Agilent (250×4.6mm, 5µ) C-18 RP-column, Sonicator Analytical Technologies Limited-Ultrasonic cleaner. U.V double beam spectrophotometer LABINDIA, UV 3000+pH meter, Weighing machine

**Chemicals**

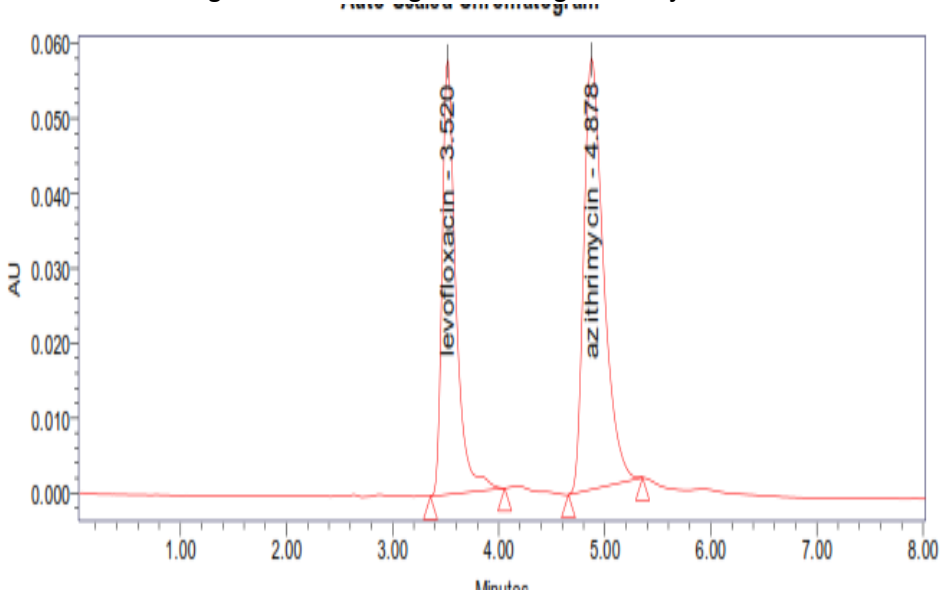
Azithromycin and Levofloxacin Potassium dihydrogen orthophosphate, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid.

**Trial-6 (Optimized)**

**Chromatographic conditions**

Column: Zodiac sil RP C18 4.6×100mm 3.0µm
Mobile phase ratio: ACN: pH 9buffer (75: 25 % v/v0
Detection wavelength: 292nm
Flow rate: 0.6ml/min
Injection volume: 20µl
Column temperature: Ambient
Auto sampler temperature: Ambient
Run time: 4.5 min
Retention time: 3.520 and 4.878 mins.

**Fig.No.1. Chromatogram showing trial-6 injection**




| S.No | Peak Name | $R_t$ | Area | Height | USP plate count |
|---|---|---|---|---|---|
| 1 | Levofloxacin | 3.520 | 10966728 | 1412054 | 3445 |
| 2 | Azithromycin | 4.878 | 1397231 | 177886 | 5441 |

**Sample solution preparation**

161.2 mg of Levofloxacin and Azithromycin tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 7 ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent (Stock solution). Further pipette 1.5 ml of the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent. The chromatograms are shown in Fig. and results are tabulated in Table.

**METHOD VALIDATION:**

- System Suitability
- Linearity
- Specificity
- Precision
- Intermediate Precision
- Accuracy
- Limit of Detection and Limit of Quantification
- Robustness

**RESULTS AND DISCUSSION:**

**Linearity**

**Fig.2 Showing calibration graph for Levofloxacin**

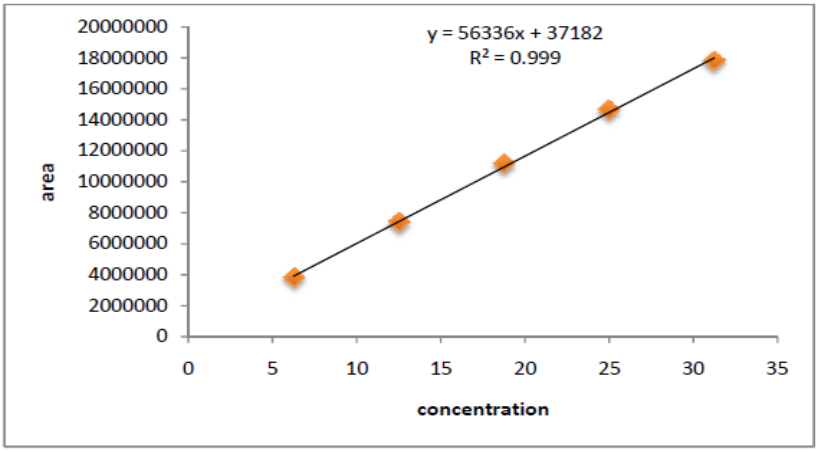
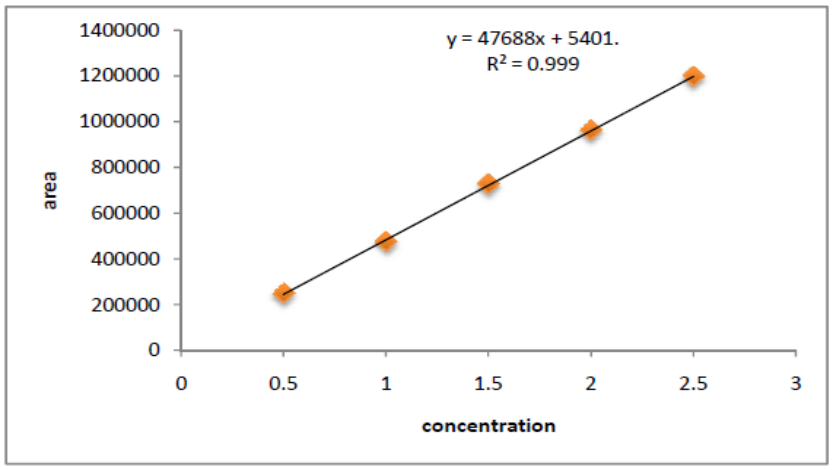



**Fig.3 Showing calibration graph for Azithromycin**




**Accuracy**

**Table.No.1. Showing accuracy results for Levofloxacin**

| %Concentration (at specification level) | Average area | Amount added (mg) | Amount found (mg) | % Recovery | Mean recovery |
|---|---|---|---|---|---|
| 50% | 7371253 | 5 | 4.9 | 99.91% | |
| 100% | 14634226.7 | 10 | 9.98 | 99.18% | 99.56% |
| 150% | 2243270.7 | 15 | 14.89 | 99.60% | |

**Table.No.2. Showing accuracy results for Azithromycin**

| %Concentration (at specification level) | Average area | Amount added (mg) | Amount found (mg) | % Recovery | Mean recovery |
|---|---|---|---|---|---|
| 50% | 484733 | 5.0 | 4.9 | 99.53% | |
| 100% | 967998 | 10.0 | 9.59 | 99.38% | 99.47% |
| 150% | 145437 | 15.0 | 14.85 | 99.52% | |

**Precision**

**Table.3 Showing% RSD results for Levofloxacin**

| | Peak Name | RT | Area(µV*sec) | Height(µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Levofloxacin | 3.517 | 520512 | 55970 | 3743.5 | 1.5 |
| 2 | Levofloxacin | 3.514 | 521717 | 56909 | 3777.3 | 1.5 |
| 3 | Levofloxacin | 3.513 | 521846 | 57014 | 3776.0 | 1.5 |
| 4 | Levofloxacin | 3.514 | 522710 | 56972 | 3822.2 | 1.5 |
| 5 | Levofloxacin | 3.514 | 528234 | 56798 | 3777.8 | 1.5 |
| Mean | | | 522013.9 | | | |
| Std.Dev | | | 1057.0 | | | |
| %RSD | | | 0.2 | | | |

**Table .4 Showing %RSD results for Azithromycin**

| | Peak Name | RT | Area(µV*sec) | Height(µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Azithromycin | 4.864 | 787229 | 56504 | 3081.3 | 1.6 |
| 2 | Azithromycin | 4.864 | 789690 | 56992 | 3144.1 | 1.6 |
| 3 | Azithromycin | 4.863 | 790187 | 56962 | 3118.1 | 1.6 |
| 4 | Azithromycin | 4.862 | 791763 | 56934 | 3147.3 | 1.6 |
| 5 | Azithromycin | 4.862 | 794118 | 57105 | 3101.8 | 1.6 |
| Mean | | | 790597.2 | | 3118.5 | 1.6 |
| Std.Dev | | | 2553.9 | | | |
| %RSD | | | 0.3 | | | |

**Intermediate precision/Ruggedness**

| | Peak Name | RT | Area(µV*sec) | Height(µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Levofloxacin | 4.863 | 790742 | 56569 | 3075.9 | 1.6 |
| 2 | Levofloxacin | 4.860 | 794791 | 56512 | 3043.2 | 1.7 |
| 3 | Levofloxacin | 4.862 | 796445 | 56415 | 3049.7 | 1.6 |
| Mean | | | 793992.9 | | | |
| Std.Dev | | | 2934.1 | | | |
| %RSD | | | 0.4 | | | |

| | Peak Name | RT | Area(µV*sec) | Height(µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Azithromycin | 3.513 | 521817 | 56358 | 3075.9 | 1.6 |
| 2 | Azithromycin | 3.515 | 522684 | 56384 | 3043.2 | 1.7 |
| 3 | Azithromycin | 3.516 | 522921 | 56456 | 3049.7 | 1.6 |
| Mean | | | 522473.9 | | | |
| Std.Dev | | | 581.3 | | | |
| %RSD | | | 0.1 | | | |

**Detection limit**

**Table.5 Showing results for Limit of Detection**

| Drug name | Standard deviation(σ) | Slope(s) | LOD(µg) |
|---|---|---|---|
| Levofloxacin | 371827.90 | 563365963 | 2.17 |
| Azithromycin | 5401.60 | 479884400 | 0.0372 |

**Quantitation limit**

**Table.6 Showing results for Limit of Quantitation**

| Drug name | Standard deviation(σ) | Slope(s) | LOQ(µg) |
|---|---|---|---|
| Levofloxacin | 371827.90 | 563365963 | 6.60 |
| Azithromycin | 5401.60 | 479884400 | 0.112 |

**Robustness**

**Table.7 Showing system suitability results for Levofloxacin**

| S. No | Flow rate (ml/min) | System suitability results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 0.8 | 3696 | 1.8 |
| **2** | **1.0** | **3646** | **1.4** |
| 3 | 1.2 | 3657 | 1.8 |

**Table.No.8. Showing system suitability results for Azithromycin**

| S. No | Flow rate (ml/min) | System suitability results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 0.4 | 3108 | 1.8 |
| **2** | **0.5** | **3348** | **1.4** |
| 3 | 0.6 | 3057 | 1.9 |

**Table.9 Showing system suitability results for Levofloxacin**

| S. No | Change in organic composition in the mobile phase | System suitability results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 5 % less | 3706 | 1.75 |
| 2 | ***Actual** | **3646** | **1.4** |
| 3 | 5 % more | 3627 | 1.8 |

**Table.No.10. Showing system suitability results for Azithromycin**

| S. No | Change in organic composition in the mobile phase | System suitability results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 5 % less | 3309 | 1.86 |
| 2 | ***Actual** | **3348** | **1.4** |
| 3 | 5 % more | 3220 | 1.9 |

**SUMMARY AND CONCLUSION:**

This method is simple, specific, and easy to perform and requires short time to perform to analyse the samples. The developed method was validated in terms of Linearity, precision, robustness, LOD, LOQ and accuracy.

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