**Determination of Norfloxacin and Tinidazole In Pharmaceutical Formulation by using Chemometric-Assisted UV-Spectrophotometric Method**

Ankit Bhalchandra More\(^2\)*, Rajendra B. Patil\(^1\), Sheetal Patil\(^2\)

1. JSPM’s Rajarshi Shahu College of Pharmacy and Research, Tathawade, Pune- 411033, Maharashtra, India
2. Alard College of Pharmacy, Marunje, Hinjewadi, Pune-411057, Maharashtra, India

**ABSTRACT**

This presented work is based on application of two multivariate calibration methods for simultaneous UV-Visible spectrophotometric determination of active substances in combined pharmaceutical formulation contained of Tinidazole (TINI) and Norfloxacin (NFX). The methods used were Partial Least Square (PLS) and Principal Component Regression (PCR). The spectra of both NFX and TINI were recorded at concentrations within their linear range 2.0-12.0 μg/mL for NFX and 5.0-30.0 μg/mL for TINI. The 29 set of mixtures were used for calibration and 07 set of mixtures were used for validation in the wavelength range of 260 to 320 nm with the wavelength interval λ= 0.2 nm in methanol. The methods were validated as per International Conference on Harmonization Q2 (R1) (ICH) guidelines. These methods were successfully applied for determination of drugs in pharmaceutical formulation (tablet) with no interference of the excipients as indicated by the recovery study results. The proposed methods are simple, rapid and can be easily used as an alternative analysis tool in the quality control as well as in process control of drugs and formulation.

**Keywords:** Norfloxacin, Tinidazole, PLS, PCR, Validation.

*Corresponding Author Email: ankit12more@gmail.com
Received 20 May 2018, Accepted 20 June 2018*
INTRODUCTION

Norfloxacin (NFX) is chemically 1-ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-1H-quinoline-3-carboxylic acid [Fig.1(a)]. NFX is a bactericidal agent used to treat variety of bacterial infections. It works by stopping the growth of bacteria. The main indication includes urinary tract infections [1]. Tinidazole (TINI) is chemically 1-(2-ethylsulfonylethyl)-2-methyl-5-nitro-imidazole [Fig.1(b)]. TINI is an anti-parasitic drug used against protozoan infections. It is widely known throughout Europe and the developing world as a treatment for a variety of amoebic and parasitic infections [2].

Several methods are reported for quantitative determination of NFX and TINI in single and in combination such as UV [3-5] and RP-HPLC [6-11].

Chemometric is the science of extracting information from chemical systems. Multivariate calibration methods e.g., multiple linear regression (MLR), principle component regression (PCR) and partial least squares (PLS), utilizing spectrophotometric data are the important chemometric approach for determination of mixtures including drugs combination [12]. As there are no reports on chemometric analysis of these drugs, this work was undertaken which presents simple, accurate and reproducible multivariate spectrophotometric methods for simultaneous determination of NFX and TINI in tablet dosage form.

![Figure. 1: Structure of a) Norfloxacin (NFX) and b) Tinidazole (TINI)](image)

MATERIALS AND METHOD

Instrumentation

Double beam UV- Vis spectrophotometer (Jasco V-550) with matched pair of 1cm quartz cells were used to record spectra of all solutions. The spectra were recorded at spectral band width of 2.0 nm, scanning speed 100 nm/min and data pitch 0.2 nm. Unscrambler X (10.3) (64-bit) trial version and Microsoft Excel 2013 were used for model generation and application of chemometric.
Material and Reagents
Reference standard of NFX and TINI were obtained from Cipla Ltd. and Aarti Drugs Ltd., Mumbai as gift samples and methanol (AR grade purchased from LOBA Chemie, India). NORFLOX-TZ tablets manufactured by Cipla Ltd. containing Norfloxacin IP 400 mg and Tinidazole IP 600 mg were procured from local pharmacy shop.

One component calibration
To find linear concentration of each drug, one component calibration was performed. Linear dynamic ranges were studied in the concentration range of 2.0-12.0 μg/mL and 5.0-30.0 μg/mL for both NFX and TINI respectively. Absorbance values were recorded at $\lambda_{\text{max}}$ of each drug (273 nm for NFX and 311 nm for TINI) against methanol as blank. Linear dynamic range for each compound was determined by least-square linear regression of concentration and the corresponding absorbance. Fig. 2 represents overlain spectra of NFX and TINI and their mixture.

![Figure 2: Overlay Spectra of NFX, TINI, and Mixture](image)

Preparation of standard stock solution
Stock solution of NFX and TINI were prepared by dissolving accurately weighed 10 mg of standard drugs in 10 mL of methanol, separately. The concentration of NFX and TINI were 1000 μg/mL from which further 2.5 mL was pipetted and diluted to 25 mL to achieve final concentration of 100 μg/mL of NFX and TINI, respectively.

Preparation of working stock solution
Working standard solutions were prepared from standard stock solution of 100 μg/mL by appropriate dilution with methanol to obtain final concentration of 2, 4, 6, 8, 10, and 12 μg/mL and
5, 10, 15, 20, 25, and 30 μg/mL for NFX and TINI, respectively.

**Construction of calibration and validation set**

A total set of 36 mixtures were prepared by combining working standard of NFX and TINI in their linear concentration range of 2.0-12.0 μg/mL and 5.0-30.0 μg/mL (Table I). From these 29 mixtures were used for calibration set and 07 mixtures were used for validation set by random selection. The absorbance spectra were recorded in range of 260-320 nm with 0.2 nm interval. The spectra were saved as ASCII (.txt) format which were further extracted in MS-Excel as required by Unscrambler software for model generation. The PCR and PLS models were developed utilizing absorption data using Unscrambler software. Selection of proper number of latent variables for development of model was necessary to obtain good prediction. Leave-one-out (LOO) cross validation method was used to obtain necessary number of latent variables (LVs), as shown in Fig. 3 and calculated using formula [13],

\[
\text{RMSECV} = \sqrt{\frac{\sum (C_{\text{act}} - C_{\text{pre}})^2}{I_c}}
\]

Where,

- RMSECV = Root mean square error of cross validation
- Cact = actual concentration of calibration set
- Cpre = predicted concentration of validation set
- Ic = Total number of samples in calibration set

![Figure 3: Explained Variance Describing Number of Optimum PCS (Principle Components)](image-url)
After the PCR and PLS models have been constructed, it was found that the optimum number of LVs were two factors for both PCR and PLS. For validation of generated models, concentration in validation set was predicted by using proposed PCR and PLS models (Table II). The validation of all methods was performed as per ICHQ2 (R1)\textsuperscript{[14]}

**Table I: Composition of Calibration and Validation Sets.**

| MIX. NO. | NFX (μg/mL) | TINI (μg/mL) | MIX. NO. | NFX (μg/mL) | TINI (μg/mL) |
|----------|-------------|-------------|----------|-------------|-------------|
| 1        | 2           | 10          | 19       | 8           | 20          |
| 2        | 2           | 15          | 20       | 8           | 25          |
| 3        | 2           | 25          | 21       | 8           | 30          |
| 4        | 2           | 30          | 22       | 10          | 15          |
| 5        | 4           | 5           | 23       | 10          | 20          |
| 6        | 4           | 10          | 24       | 10          | 25          |
| 7        | 4           | 15          | 25       | 10          | 30          |
| 8        | 4           | 20          | 26       | 12          | 5           |
| 9        | 4           | 25          | 27       | 12          | 10          |
| 10       | 4           | 30          | 28       | 12          | 15          |
| 11       | 6           | 5           | 29       | 12          | 25          |
| 12       | 6           | 10          | 30       | 2           | 5           |
| 13       | 6           | 15          | 31       | 2           | 20          |
| 14       | 6           | 20          | 32       | 6           | 30          |
| 15       | 6           | 25          | 33       | 10          | 5           |
| 16       | 8           | 5           | 34       | 10          | 10          |
| 17       | 8           | 10          | 35       | 12          | 20          |
| 18       | 8           | 15          | 36       | 12          | 30          |

* Mix No. 1-28 calibration set
* Mix No. 29-36 validation set

**Table II: Predicted Results for Validation Set by PCR and PLS Method.**

| Method | NFX | TINI | PLS | NFX | TINI | PCR | NFX | TINI |
|--------|-----|------|-----|-----|------|-----|-----|------|
| Actual(μg/mL) | Predicted | % R* | Predicted | % R* | Predicted | % R* | Predicted | % R* |
| 2      | 5   | 2.1936 | 109.68 | 6.0944 | 121.888 | 2.1936 | 109.68 | 6.0944 | 121.888 |
| 2      | 20  | 2.4391 | 121.955 | 20.3889 | 121.955 | 2.4391 | 121.955 | 20.3889 | 121.955 |
| 6      | 30  | 6.7805 | 113.0083 | 29.9226 | 99.742 | 6.7805 | 113.0083 | 29.9226 | 99.742 |
| 10     | 5   | 10.3159 | 103.159 | 7.0969 | 141.938 | 10.3159 | 103.159 | 7.0969 | 141.938 |
| 10     | 10  | 10.6433 | 106.433 | 9.1402 | 91.402 | 10.6433 | 106.433 | 9.1402 | 91.402 |
| 12     | 20  | 12.4715 | 103.9292 | 21.0889 | 105.4445 | 12.4715 | 103.9292 | 21.0889 | 105.4445 |
| 12     | 30  | 12.4351 | 103.6258 | 31.0103 | 103.3677 | 12.4351 | 103.6258 | 31.0103 | 103.3677 |

* % R: percent recovery

**Assay of marketed preparation**

20 tablets of NORFLOX-TZ were accurately weighed and finely powdered. Tablet powder equivalent to 10 mg of TINI (6.66 mg of NFX) was taken and transferred to 10 mL volumetric
flask and was diluted to 10 mL with methanol. The solution was sonicated for 10 minutes. This solution was then filtered with help of Whatman filter paper no. 41. The 1 mL of filtrate solution was diluted to 10 mL with methanol. Further 0.7 mL and 1 mL of this solution was diluted to 10 mL with methanol to get final concentration of 7 μg/mL and 10 μg/mL of NFX and TINI, respectively. The procedure was repeated 6 times for tablet formulation. The results of assay are presented in Table III.

### Table III: Assay Result for NFX and TINI in Tablet (NORFLOX-TZ) by Proposed Methods

| Method | Actual (μg/mL) | NFX | TINI | Predicted (μg/mL) | Percentage Recovery | Predicted (μg/mL) | Percentage Recovery | Predicted (μg/mL) | Percentage Recovery | Predicted (μg/mL) | Percentage Recovery |
|--------|----------------|-----|------|-------------------|---------------------|-------------------|---------------------|-------------------|---------------------|-------------------|---------------------|
| NFX    | 6.66           | 10  | 100  | 6.669             | 99.96               | 6.645             | 99.775              | 9.998             | 99.98              |
| TINI   | 10             |     |      | 6.621             | 99.414              | 6.561             | 98.514              | 10.3345           | 103.345            |
| NFX    | 6.66           | 10  | 99.264 | 6.611             | 10.2686             | 6.683             | 100.345             | 10.2689           | 102.689            |
| TINI   | 6.66           | 10  | 104.441 | 6.589             | 104.441             | 6.528             | 98.018              | 10.4512           | 104.512            |
| NFX    | 6.66           | 10  | 99.114 | 6.601             | 102.217             | 6.652             | 99.880              | 10.2245           | 102.245            |
| TINI   | 6.66           | 10  | 104.091 | 6.7001            | 104.901             | 6.6385            | 99.677              | 10.4945           | 104.945            |
| MEAN   | 6.63185        | 0.65029 | 0.176531 | 1.765308         | 0.059798            | 0.897873          | 0.178672            |
| SD     | 0.043309       | 0.65029 | 0.176531 | 1.765308         | 0.059798            | 0.897873          | 0.178672            |

**Accuracy study**

The accuracy study was carried out at three levels 50 %, 100 %, and 150 % of assay concentration. Calculated amount of NFX and TINI from standard solutions were spiked into sample solution and scanned in range of 260-320 nm. Concentrations were predicted by using developed PCR and PLS models. Accuracy data is presented in Table IV and Table V.

### Table IV: Accuracy Data of NFX by PCR and PLS Models.

| Level % | Sample Conc. (μg/mL) | Amount added (μg/mL) | Total Conc. (μg/mL) | Predicted Conc. (μg/mL) | % Recovery | % RSD | PCR | PLS | PCR | PLS | PCR | PLS | PCR | PLS |
|---------|----------------------|----------------------|---------------------|-------------------------|------------|-------|-----|-----|-----|-----|-----|-----|-----|-----|
| 50 %    | 6.66                 | 2                    | 8.66                | 8.4544                  | 97.626     | 1.2513 | 1.472|
|         | 8.5023               | 8.7011               | 98.179              | 100.47532               | 569        |
|         | 8.3254               | 8.5261               | 99.985              | 98.454                  |
| 100 %   | 6.66                 | 4                    | 10.66               | 10.5633                 | 99.09      | 0.9449 | 0.499|
|         | 10.4589              | 10.4588              | 98.11               | 98.113                  | 31         |
|         | 10.3597              | 10.3596              | 97.18               | 97.182                  |
| 150 %   | 6.66                 | 6                    | 12.66               | 12.548                  | 99.115     | 1.1728 | 1.168|
|         | 12.5646              | 12.5644              | 99.246              | 99.24                   | 771        |
|         | 12.562               | 12.5621              | 99.226              | 99.23                   |

www.ajptr.com
Table V: Accuracy data of TINI by PCR and PLS models

| LEVEL % | Sample Conc. μg/mL | Amount added μg/mL | Total Conc. μg/mL | Predicted Conc. μg/mL | % Recovery | % RSD |
|---------|--------------------|--------------------|-------------------|-----------------------|------------|-------|
|         |                    |                    |                   | PCR                   | PLS        | PCR   | PLS | PCR   | PLS | PCR   | PLS | PCR | PLS |
| 50 %    | 10                 | 5                  | 15                | 15.346                | 15.356     | 102.307 | 102.373 | 1.62116 | 1.573311 |
|         |                    |                    |                   | 15.456                | 15.458     | 103.040 | 103.053 |          |          |
|         |                    |                    |                   | 15.8269               | 15.8213    | 105.513 | 105.475 |          |          |
| 100 %   | 10                 | 10                 | 20                | 20.8456               | 20.8458    | 104.228 | 104.229 | 1.266973 | 1.2615   |
|         |                    |                    |                   | 20.5745               | 20.570     | 102.87  | 102.85  |          |          |
|         |                    |                    |                   | 20.15                 | 20.198     | 100.75  | 100.99  |          |          |
| 150 %   | 10                 | 15                 | 25                | 25.215                | 25.2105    | 100.860 | 100.842 | 0.708   | 0.699    |
|         |                    |                    |                   | 25.4896               | 25.4863    | 101.958 | 101.945 |          |          |
|         |                    |                    |                   | 25.153                | 25.156     | 100.612 | 100.624 |          |          |

### Precision

Precision was carried at three concentration levels (4, 6, 8 μg/mL for NFX and 10, 15, 20 μg/mL for TINI) in three replicates at each level. The results of which are presented in Table VI.

Table VI: Precision Results Obtained Using Developed PCR and PLS Models.

| Amount Taken μg/mL | Predicted Conc. μg/mL | % Recovery | % RSD |
|--------------------|-----------------------|------------|-------|
|                    | PCR TINI              | PLS TINI   | NFX   | PCR TINI | PLS TINI | NFX   | PCR TINI | PLS TINI | NFX   |
| 10 10 15 20        | 4 4 6 8              | 4 4 6 8    | 4 4 6 8 | 10.0002  | 10.0002  | 4.0001 | 99.998  | 99.998  | 99.998 |
| 10 10 15 20        | 9.916 9.9825 15.3462 | 3.9664 3.993 6.1385 | 4.0001 4.0001 4.0001 | 99.998 99.998 99.998 | 100.847 100.175 97.744 | 100.847 100.175 97.744 | 0.446396 0.446396 1.736026 |
| 15 15 20 20        | 6 6 8 8              | 5.9297 6.0459 7.8551 7.9486 | 5.9297 6.0459 7.8551 7.9486 | 101.185 101.186 101.845 101.845 | 101.185 101.186 101.845 101.845 | 101.185 101.186 101.845 101.845 | 1.736026 1.736026 1.694982 1.694982 |
| 20 20 20 20        | 8 8 8 8              | 7.8551 7.9486 8.1227 8.1227 | 7.8551 7.9486 8.1227 8.1227 | 101.845 101.845 101.845 101.845 | 101.845 101.845 101.845 101.845 | 101.845 101.845 101.845 101.845 | 1.736026 1.736026 1.694982 1.694982 |
RESULTS AND DISCUSSION

Out of 36 mixtures, 29 set of mixtures were used for calibration and 07 set of mixtures were used for validation. The models were tried to develop with varying Δ λ. The best results were obtained with the wavelengths intervals λ= 0.2 nm in methanol. The developed method found to be accurate as results are close to 100% and precise with % RSD less than 2. Summary of results is presented in Table VII.

Table VII: Summary of Results

| Parameters                  | Norfloxacin (NFX) | Tinidazole (TINI) |
|-----------------------------|-------------------|-------------------|
| PCR                         | 2.0-12.0          | 2.0-12.0          | 2.0-12.0 |
| PLS                         | 2.0-12.0          | 2.0-12.0          | 2.0-12.0 |
| Range (µg/mL)               | 2.0-12.0          | 2.0-12.0          | 2.0-12.0 |
| Wavelength (nm)             | 260-320           | 260-320           | 260-320 |
| Data interval (Δλ)          | 0.2               | 0.2               | 0.2     |
| Factors / PC’s              | 2                 | 2                 | 2       |
| % Recovery                  | 99.368            | 99.577            | 102.9527|
| Correlation Coefficient (r²)| 0.9903            | 0.9948            | 0.9921  |
| Intercept                   | 0.0802            | 0.0413            | 0.1244  |
| Slope                       | 0.9893            | 0.9943            | 0.9929  |

CONCLUSION

A study of the use of UV spectrophotometric in combination with PLS and PCR for the simultaneous determination of Tinidazole (TINI) and Norfloxacin (NFX) in a binary mixture has been accomplished. The PLS and PCR approaches used in this work are simple to perform, with adequate software support, and provides a clear example of the high resolving power of this technique. The results obtained confirmed the suitability of the proposed method for simple, accurate and precise analysis of TINI and NFX in pharmaceutical preparations. The proposed methods do not need separation of TINI and NFX before analysis. In addition, the proposed methods can be applied for analysis of drugs in quality control lab as well as for in process quality control. The results obtained using chemometric methods (PCR and PLS) were compared to those of the proposed UV-method and no significant difference was observed between the methods. The results obtained were found to be within the limits for all the validation parameter. Percentage recovery was found to be within percent limit, thus method was accurate.

ACKNOWLEDGMENT

Authors are thankful to the Principal, Alard College of Pharmacy for providing necessary facilities to carry out the experiment. Authors are also thankful to Cipla Pharmaceuticals Ltd and Aarti Drugs Ltd, Mumbai for providing a working standard of Tinidazole and Norfloxacin.
REFERENCE:

1. https://www.drugbank.ca/drugs/DB01059 (16.09. 2016).
2. https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/019384s066lbl.pdf (16.09. 2016).
3. Singh L, Nanda S. Method for determination of tinidazole using direct UV-visible spectrophotometry and differential spectrophotometry in pure and tablet dosage forms. East and Central African Journal of Pharmaceutical Sciences 2011;14(1):75-80.
4. Nayak Khushboo, Khare Navin K, Sayare Atul, Ghode Prashant, Lawrence Raymond M. New validated UV spectrophotometric methods for estimation of norfloxacin and tinidazole in bulk and tablet dosage forms. Der Pharmacia Lettre 2012;4(1):192-8.
5. Gummadi Sowjanya, Thota Devi, Varri Sri Valli, Vaddi Pratyusha, Jillella Venkata Lakshmi Narasimha Seshagiri Rao. Development and validation of UV-spectroscopic methods for simultaneous estimation of ciprofloxacin and tinidazole in tablet formulation. International Current Pharmaceutical Journal 2012;1(10):317-21.
6. Dharuman J, Vasudevan M, Somasekaran KN, Dhandapania B, Ghodea Prashant D, Thiagarajan M. Rp-Hplc method development and validation for the simultaneous estimation of ofloxacin and tinidazole in tablets. Int. J. PharmTech Res. 2009;1(2):121-24.
7. Srinivasa Rao A, Pavankumar KLNNSVK, Satyanarayana P, Sastry Subrahmanyana G. Method development and validation for simultaneous estimation of norfloxacin, tinidazole and loperamide in bulk and pharmaceutical formulations using Rp-Hplc method. World J Pharm Pharm Sci. 2015;4(11):1112-24.
8. Sharma Ravi, Kaur Amandeep, Malhotra Dipali. Rp-Hplc method development and validation of norfloxacin in bulk form. World J Pharm Res. 2014 Aug;3(5):1145-54.
9. Singh Raghabaendra Narayan, Sahoo Shisir, Mishra Umashakar, Garnaik Bamakanta, Sahoo Sudhir Kumar, Hati Deepak. Stability indicating Rp-Hplc method development and validation of norfloxacin. American Journal of Advanced Drug Delivery 2013;1(5):743-58.
10. Murugan S, Sunil kumar V, Vineela Ruth Madhuri P, Niranjan Babu M, Kathiravan MK. Method development and validation of Tinidazole and Ciprofloxacin HCl in bulk and tablet dosage form by Rp-Hplc. IJNTPS 2014 Oct;4(2):130-9.
11. Sebaiy Mahmoud M, El-Shanawany Abdullah A, El-Adl Sobhy M, Abdel-Aziz Lobna M, Hashem Hisham A. Rapid Rp-Hplc method for simultaneous estimation of norfloxacin and tinidazole in tablet dosage form. Asian J. Pharm. Ana 2011 Oct-Dec;1(4):79-84.
12. Dinc E, Baleanu D. Spectrophotometric quantitative determination of cilazapriland hydrochlorothiazide in tablets by chemometric methods. J Pharm Biomed Anal 2002;81(4):715-23.

13. Gandhi SV, Pahade AR, Tapale SR. Chemometrics - Assisted UV spectrophotometric methods for determination of metoprolol succinate and hydrochlorothiazide in oralloyhi tablets. Pharm Analysis & Quality Assurance 2016;4(1):177-181.

14. International Conference on Harmonization. ICH harmonized tripartite guideline validation of analytical procedures text and methodology Q2 (R1) ICH, Geneva; 2005: 1-17.