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

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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.

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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.

a) b)

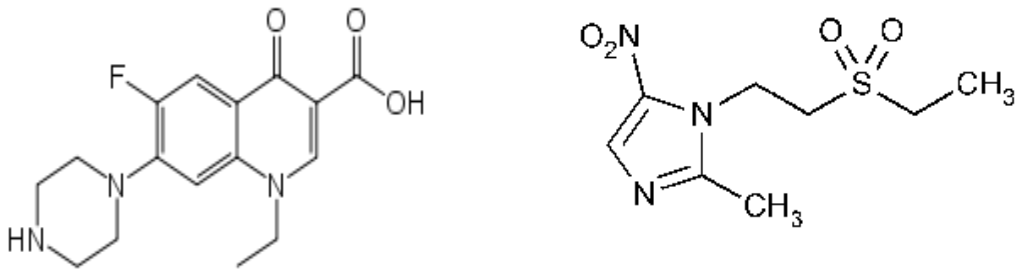

Figure. 1: Structure of a) Norfloxacin (NFX) and b) Tinidazole (TINI)

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 λ_{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.

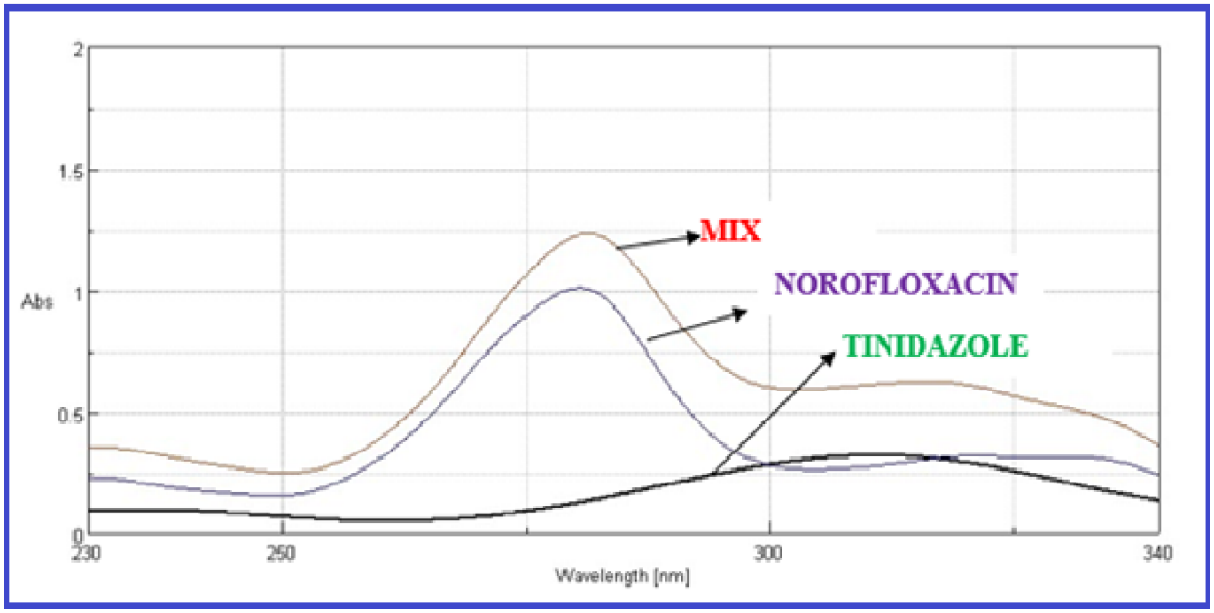

Figure 2: Overlay Spectra of NFX, TINI, and Mixture

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],

$$\text{RMSECV} = \sqrt{\sum \frac{(\text{Cact}-\text{Cpre})^2}{\text{Ic}}}$$

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

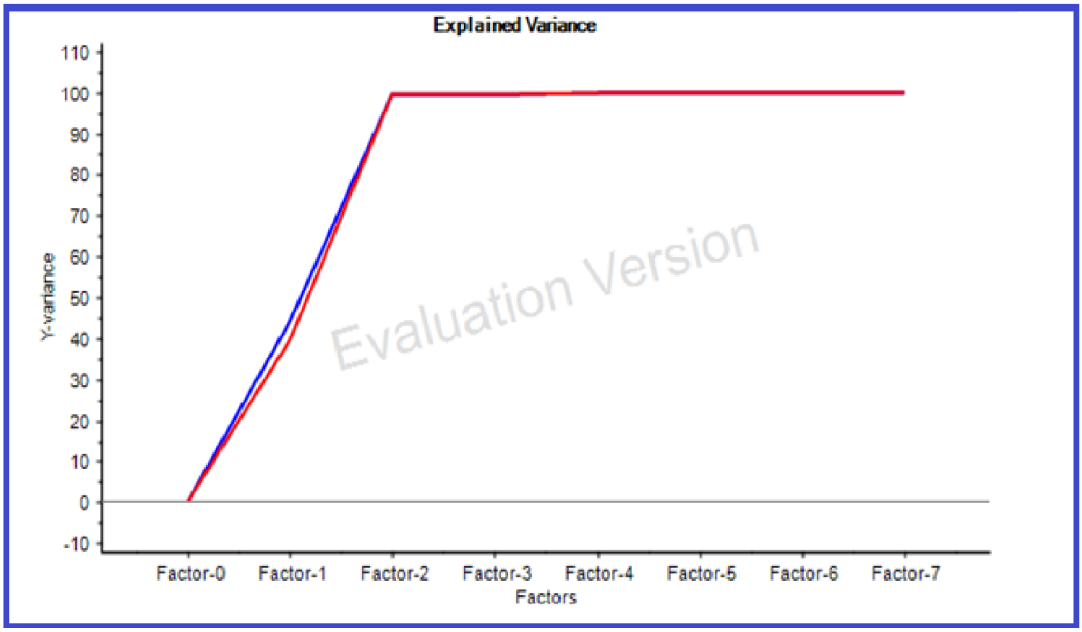

Figure 3: Explained Variance Describing Number of Optimum PCS (Principle Components)

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) [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		PLS				PCR			
NFX	*TINI*	*NFX*		*TINI*		*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	101.9445	2.4391	121.955	20.3889	101.9445
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		**PLS**				**PCR**			
NFX	**TINI**	**NFX**		**TINI**		**NFX**		**NFX**	
Actual (µg/mL)		**Predicted (µg/mL)**	**% R**	**Predicted (µg/mL)**	**% R**	**Predicted (µg/mL)**	**% R**	**Predicted (µg/mL)**	**% R**
6.66	10	6.669	100.135	9.996	99.96	6.645	99.775	9.998	99.98
6.66	10	6.621	99.414	10.3119	103.119	6.561	98.514	10.3345	103.345
6.66	10	6.611	99.264	10.2686	102.686	6.683	100.345	10.2689	102.689
6.66	10	6.589	98.934	10.4441	104.441	6.528	98.018	10.4512	104.512
6.66	10	6.601	99.114	10.2217	102.217	6.652	99.880	10.2245	102.245
6.66	10	6.7001	100.602	10.4901	104.901	6.6385	99.677	10.4945	104.945
MEAN		6.63185	99.577	10.28873	102.8873	6.617917	99.368	10.29527	102.9527
SD		0.043309	0.65029	0.176531	1.765308	0.059798	0.897873	0.178672	1.786723

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
50 %	6.660	2	8.66	8.4544	8.4564	97.626	97.649	1.2513	1.472
				8.5023	8.7011	98.179	100.475	32	569
				8.3254	8.5261	99.985	98.454		
100 %	6.66	4	10.66	10.5633	10.5621	99.09	99.082	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	12.546	99.115	99.10	1.1728	1.168
				12.5646	12.5644	99.246	99.24		771
				12.562	12.5621	99.226	99.23		

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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
50 %	10	5	15	15.346 15.456 15.8269	15.356 15.458 15.8213	102.307 103.040 105.513	102.373 103.053 105.475	1.621116	1.573311
100 %	10	10	20	20.8456 20.5745 20.15	20.8458 20.570 20.198	104.228 102.87 100.75	104.229 102.85 100.99	1.266973	1.2615
150 %	10	15	25	25.215 25.4896 25.153	25.2105 25.4863 25.156	100.860 101.958 100.612	100.842 101.945 100.624	0.708	0.699

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			
TINI	NFX	PCR TINI	NFX	PLS TINI	NFX	PCR TINI	NFX	PLS TINI	NFX	PCR TINI	NFX	PLS TINI	NFX
10 10 10	4 4 4	10.0002 9.916 9.9825	4.0001 3.9664 3.993	10.0002 9.916 9.9825	4.0001 3.9664 3.993	99.998 100.847 100.175	99.998 100.847 100.175	99.998 100.847 100.175	99.998 100.847 100.175	0.446396	0.446587	0.446396	0.446587
15 15 15	6 6 6	14.8243 15.1148 15.3462	5.9297 6.0459 6.1385	14.8243 15.1148 15.3462	5.9297 6.0459 6.1385	101.185 99.240 97.744	101.186 99.241 97.744	101.185 99.240 97.744	101.186 99.241 97.744	1.736026	1.736341	1.736026	1.736341
20 20 20	8 8 8	19.6377 19.8716 20.3068	7.8551 7.9486 8.1227	19.6377 19.8716 20.3068	7.8551 7.9486 8.1227	101.845 100.646 98.489	101.845 100.647 98.489	101.845 100.646 98.489	101.845 100.647 98.489	1.694982	1.69478	1.694982	1.69478

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	PLS	PCR	PLS
Range (µg/mL)	2.0-12.0	2.0-12.0	2.0-12.0	2.0-12.0
Wavelength (nm)	260-320	260-320	260-320	260-320
Data interval (Δλ)	0.2	0.2	0.2	0.2
Factors / PC's	2	2	2	2
% Recovery	99.368	99.577	102.9527	102.8873
Correlation Coefficient (r^2)	0.9903	0.9948	0.9921	0.9947
Intercept	0.0802	0.0413	0.1244	0.1218
Slope	0.9893	0.9943	0.9929	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.

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