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Chemometrics - Assisted UV Spectrophotometric Method for Determination of Ciprofloxacin and Ornidazole in Pharmaceutical Formulation

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Abstract: *This presented work is based on application of two multivariate calibration methods for simultaneous UV-Vis spectrophotometric determination of active substances in combined pharmaceutical formulation composed of Ciprofloxacin (CPX) and Ornidazole (ONZ). The methods used were Principal Component Regression (PCR) and Partial Least Square (PLS). The Spectra of both CPX and ONZ were recorded at concentrations within their linear ranges 2.0-12.0 µg/ml. 27 set of mixtures were used for calibration and 9 set of mixtures were used for validation in the wavelength range of 267 to 330 nm with the wavelengths intervals λ= 0.5 nm in methanol. International Conference on HarmonizationQ2 (R1) (ICH) guidelines were followed to validate the methods. Recovery study results indicate no interference of the excipients, thus methods were successfully applied for determination of drugs in pharmaceutical formulation. The methods can be used as alternative analytical tool in the quality control of these drugs.*

Keywords:*Ciprofloxacin, Ornidazole, PLS, PCR, Validation*

1. INTRODUCTION

Ciprofloxacin (CPX) chemically 1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid, [Fig.1 (a)] is a fluoroquinolone antibiotic used to treat a number of bacterial infections [1] Ornidazole(ONZ) chemically is 1-Chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol[Fig.1(b)]. Itis aantiamoebic agent that interacts with helical DNA strand which leads in a protein synthesis inhibition and cell death[2]. Several methods are reported for quantitative determination of CPX and ONZ in single and in combination such as UV[3-6] and RP-HPLC[7-10].

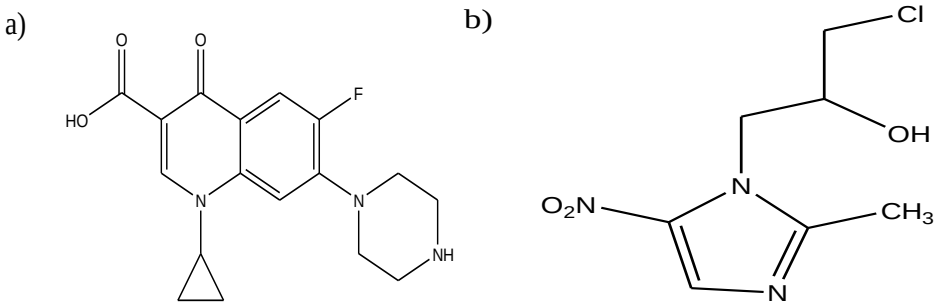

Figure1.*Structure of a) Ciprofloxacin (CPX) and b) Ornidazole (ONZ)*

Chemometrics was introduced in 1972 by SvanteWold [11].Chemometric is the science of extracting information from chemical system. For determination of mixtures including drugs combination; chemometric approaches like multiple linear regression (MLR), principle component regression (PCR) and partial least squares (PLS) utilizing spectrophotometric data can be used [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 CPX and ONZ in tablet dosage form.

2. MATERIALS AND METHODS

2.1. 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 400 nm/min and data pitch 0.5 nm. Unscrambler X (10.3) (64-bit) trial version and Microsoft Excel 2007 were used for model generation and application of chemometric.

2.2. Material and Reagents

Reference standard of CPX and ONZ were obtained from Cipla Ltd, Mumbai as gift samples and methanol used was of AR grade (LOBA Chemie, India). ZOXAN-OZ tablets manufactured by FDC Limited (Goa) containing Ciprofloxacin IP 500 mg and Ornidazole IP 500 mg were procured from local pharmacy shop.

2.3. One Component Calibration

One component calibration was studied in the concentration range of 2.0-12.0 µg/ml for both CPX and ONZ. Absorbance values wererecorded at $\lambda_{max}$of each drug (278 nm for CPX and 311 nm for ONZ) 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 CPX and ONZ and their mixture.

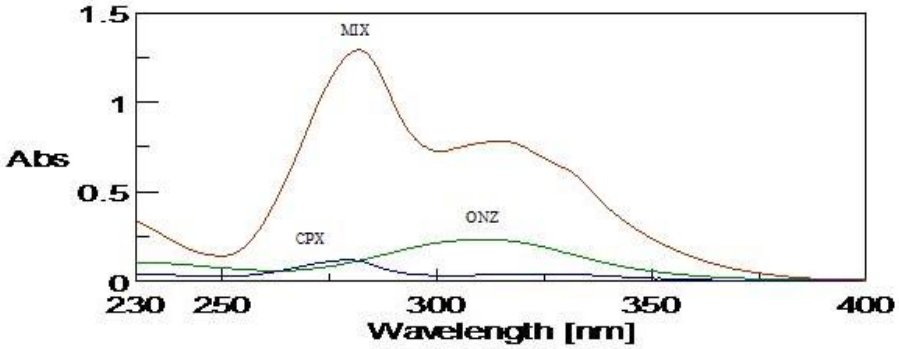

Figure2.*Overlay spectra of CPX, ONZ and mixture.*

2.4. Preparation of Standard Stock Solution

Stock solution of CPX and ONZ were prepared by dissolving accurately weighed 10 mg of standard drugs in 10 ml of methanol, separately. The concentration of CPX and ONZ were 1000 µg/ml from which further 5 ml was pipetted and diluted to 50 ml to achieve final concentration of 100 µg/ml of CPX and ONZ respectively.

2.5. 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 for both CPX and ONZ.

2.6. Construction of Calibration and Validation Set

By combining standard stock solution of CPX and ONZ,36 mixtures were prepared in their linear concentration range of 2.0-12.0 µg/ml (Table 1). Out of 36 mixtures,randomly selected 27 mixtures wereusedfor model development (calibration set) andremaining 9 mixtures were used for model validation (validation set). The absorbance spectra were recorded in range of 267- 330 nm with 0.5 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 Figure 3 and calculated using formula [13],

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

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

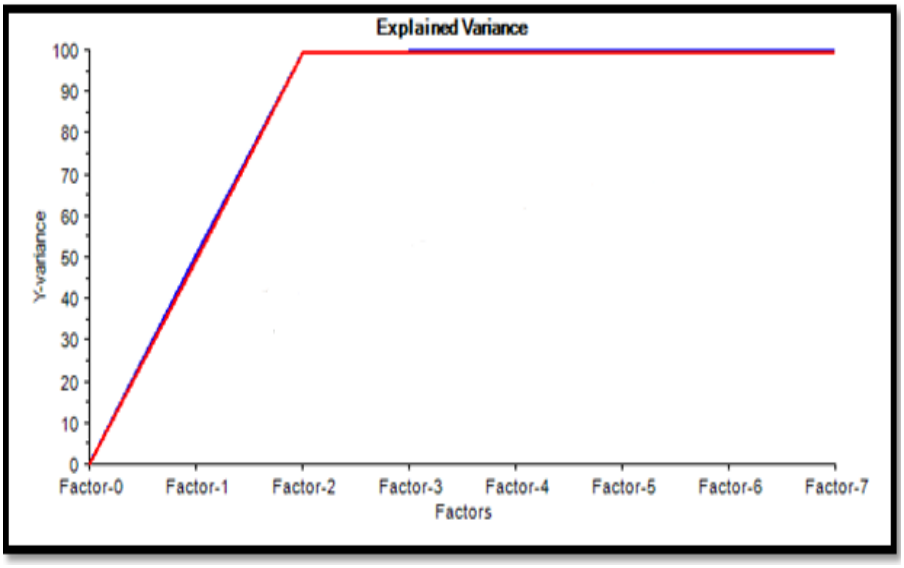

Figure3.*Explained variance describing number of optimum PCs (Principle Components).*

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 2). The validation of allmethods was performed as per ICH Q2 (R1) [14].

Table1.*Composition of calibration and validation sets.*Mix no. 1-27 calibration set; *Mix no. 28-36 validation set.*

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

Table2. *Predicted results for validation set by PCR and PLS method.*

METHOD		PLS				PCR			
CPX	*ONZ*	*CPX*		*ONZ*		*CPX*		*ONZ*	
Actual (µg/ml)		Predicted	% R*	Predicted	% R*	Predicted	% R*	Predicted	% R*
2	8	2.071	103.5	8.186	102.3	2.071	103.5	8.186	102.3
4	6	3.829	95.74	6.171	102.8	3.829	95.72	6.171	102.8
4	12	3.95	98.17	12.31	102.6	3.951	98.79	12.39	102.61
6	6	5.353	89.22	5.841	97.35	5.352	89.2	5.842	97.37
8	6	7.538	94.23	6.069	101.1	7.537	94.22	6.07	101.1
10	4	9.932	99.32	4.054	101.3	9.932	99.22	4.054	101.3
10	10	9.194	91.94	9.542	95.42	9.193	91.93	9.542	95.42
12	4	11.822	98.51	3.974	99.29	11.822	98.52	3.971	99.28
12	8	12.026	100.2	7.602	95.03	12.027	100.2	7.6	95.01

2.7. Assay of Marketed Preparation

20 tablets of ZOXAN-OZ were accurately weighed and finely powdered. Tablet powder equivalent to 10 mg of CPX (10 mg of ONZ) 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 filtered and 1 ml of filtrate was diluted to 10 ml with methanol. Further 0.4 ml of this solution was diluted to 10 ml with methanol to get final concentration of 4 µg/ml of both CPX and ONZ. The procedure was repeated 6 times for tablet formulation. The results of assay are presented in Table3.

Table3.*Assay result for CPX and ONZ in tablet (ZOXAN-OZ) by proposed methods.*

METHOD		PLS				PCR			
CPX	*ONZ*	*CPX*		*ONZ*		*CPX*		*ONZ*	
Actual(µg/ml)		Predicted (µg/ml)	% R	Predicted (µg/ml)	% R	Predicted (µg/ml)	% R	Predicted (µg/ml)	% R
4	4	4.1372	103.4	4.1493	103.7	4.1325	103.3	4.1496	103.7
4	4	4.0184	100.4	4.0411	101.0	4.0137	100.3	4.0416	101.0
4	4	4.1234	103.0	4.0011	100.0	4.1248	103.1	4.0013	100.0
4	4	3.9872	99.68	4.1542	103.8	3.9885	99.71	4.1543	103.8
4	4	4.1284	103.2	3.9912	99.78	4.1267	103.1	3.9945	99.86
4	4	4.1032	102.5	4.0541	101.3	4.1024	102.5	4.0542	101.3
MEAN		4.0829	102.0	4.0651	101.6	4.0812	102.0	4.0659	101.6
SD		0.0638	1.596	0.0711	1.777	0.0636	1.559	0.0704	1.732

2.8. Accuracy study

The accuracy study was carried out at three levels 50 %, 100 % and 150 % of assay concentration. Calculated amount of CPX and ONZfrom standard solutionswere spiked into sample solution and scanned in range of 267-330 nm. Concentrations were predicted by using developed PCR and PLS models. Accuracy data is presented in Table 4 and Table 5.

Table4. *Accuracy data of CPX 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 %	4	2	6	6.187 6.024 6.155	6.187 6.023 6.165	103.13 100.41 102.59	103.13 100.39 102.75	1.409	1.456
100 %	4	4	8	7.942 8.054 8.012	7.942 8.053 8.013	99.27 100.67 100.15	99.27 100.67 100.16	0.708	0.706
150 %	4	6	10	9.974 9.994 10.243	9.984 9.972 10.23	99.74 99.94 102.43	99.84 99.72 102.33	1.488	1.463

Table5.*Accuracy data of ONZ 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 %	4	2	6	6.223 6.225 6.102	6.222 6.224 6.101	103.71 103.75 101.71	103.76 103.73 101.68	1.139	1.148
100 %	4	4	8	7.973 8.053 8.169	7.971 8.054 8.175	99.63 100.63 102.11	99.63 100.67 102.18	1.235	1.222
150 %	4	6	10	10.223 10.255 10.437	10.221 10.256 10.438	102.23 102.55 104.37	102.21 102.56 104.38	1.120	1.130

2.9. Precision

Precision was carried at three concentration levels (4,6,8µg/ml of both CPX and ONZ) in three replicates at each level. The results of which are presented in Table6 and Table7.

Table6.*Precision results obtained using developed PCR and PLS models (Intraday precision).*

Amount Taken µg/ml		Predicted Conc. µg/ml				% Recovery				% RSD			
		PCR		PLS		PCR		PLS		PCR		PLS	
ONZ	*CPX*	*ONZ*	*CPX*	*ONZ*	*CPX*	*ONZ*	*CPX*	*ONZ*	*CPX*	*ONZ*	*CPX*	*ONZ*	*CPX*
4	4	4.100	3.956	4.100	3.956	102.5	98.90	102.5	98.9				
4	4	4.088	3.964	4.087	3.964	102.2	99.10	102.1	99.10	1.321	0.119	1.318	0.120
4	4	4.001	3.964	4.002	3.964	100.0	99.10	100.0	99.10				
6	6	6.021	6.006	6.021	6.006	100.3	100.1	100.3	100.1				
6	6	6.159	6.118	6.159	6.187	102.6	103.1	102.6	103.1	1.371	1.502	1.370	1.496
6	6	6.173	6.118	6.173	6.118	102.8	101.9	102.8	101.9				
8	8	8.124	8.141	8.124	8.141	101.5	101.7	101.5	101.7				
8	8	7.957	8.002	7.958	8.002	99.46	100.0	99.47	100.0	1.121	1.205	1.122	1.208
8	8	7.984	7.955	7.985	7.955	99.80	99.44	99.80	99.44				

Table7.*Precision results obtained using developed PCR and PLS models (Interday Precision).*

Amount Taken µg/ml		Predicted Conc. µg/ml				% Recovery				% RSD			
		PCR		PLS		PCR		PLS		PCR		PLS	
ONZ	*CPX*	*ONZ*	*CPX*	*ONZ*	*CPX*	*ONZ*	*CPX*	*ONZ*	*CPX*	*ONZ*	*CPX*	*ONZ*	*CPX*
4	4	3.98	3.99	3.98	3.99	99.53	99.87	99.51	99.8				
4	4	4.02	4.11	4.02	4.11	100.7	102.7	100.7	102.7	1.649	1.643	1.674	1.628
4	4	4.16	3.99	4.16	3.99	104.1	99.92	104.1	99.95				
6	6	6.13	5.90	6.13	5.90	102.2	98.44	102.2	98.46				
6	6	6.25	5.98	6.25	5.98	104.3	99.81	104.3	99.80	1.468	1.691	1.473	1.685
6	6	6.08	6.10	6.08	6.10	101.3	101.8	101.3	101.8				
8	8	7.90	7.95	7.90	7.94	98.85	99.41	98.86	99.2				
8	8	8.05	8.11	8.05	8.10	100.6	101.4	100.6	101.4	1.772	1.349	1.777	1.352
8	8	8.25	8.15	8.25	8.15	103.2	101.9	103.2	101.8				

2.10.LOD and LOQ

LOD (Limit of detection) and LOQ (Limit of quantitation) were calculated using the formula 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation (y-intercept) and S is the slope of the calibration plot.

3. RESULTS

Out of 36 mixtures, 27 set of mixtures were used for calibration and 9 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= 0.5 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 8.

Table8.*Summary of results.*

Parameters	CIPROFLOXACIN(CPX)		ORNIDAZOLE (ONZ)	
	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)	267-330	267-330	267-330	267-330
Data interval (Δλ)	0.5	0.5	0.5	0.5
Factors / PC's	2	2	2	2
% Recovery	102.0	102.0	101.6	101.6
LOD	0.53	0.53	0.49	0.49
LOQ	1.52	1.52	1.49	1.49
Correlation Coefficient (r^2)	0.9893	0.9894	0.9949	0.9949
Intercept	0.0723	0.0722	0.0350	0.0351
Slope	0.9893	0.9894	0.9949	0.9949
RMSECV	0.3470	0.3395	0.26024	0.26032
RMSEP	0.3469	0.3465	0.26023	0.26032

4. CONCLUSION

A study of the use of UV spectrophotometric in combination with PLS and PCR for the simultaneous determination of Ciprofloxacin (CPX) and Ornidazole (ONZ) in a binary mixture has been accomplished. The results were obtained which confirmed that the proposed method is suitable for simple, accurate and precise analysis of ONZ and CPX in pharmaceutical dosage form without separation of ONZ and CPX before analysis. The above method can also be applied for analysis of drugs in quality control lab as well as for in process quality control.

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