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Research Article

CHEMOMETRICS-ASSISTED UV SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF CEFIXIME 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 Cefixime (CEF) and Ornidazole (ONZ). The methods used were Principal Component Regression (PCR) and Partial Least Square (PLS). The Spectra of CEF and ONZ were recorded at concentrations within their linear ranges 2.0-12.0 µg/ml and 5.0-30.0 µg/ml, respectively. 27 set of mixtures were used for calibration and 9 set of mixtures were used for validation in the wavelength range of 260 to 330 nm with the wavelengths intervals λ= 0.5 nm in methanol. The methods were validated as per International Conference on HarmonizationQ2 (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 controlas well as in process control of drugs and formulation.

Keywords: Cefixime, Ornidazole, PLS, PCR, Validation.

INTRODUCTION

Cefixime (CEF) Chemically, it is (6R, 7R)-7-{[2-(2-amino-1,3-thiazol-4-yl)-2(carboxymethoxy-imino) acetyl]amino}-3-ethenyl-8-oxo-5-thia-1azabicyclo-[4.2.0] oct-2-ene-2) carboxylic acid [Fig. 1(a)]. Clinically used in the treatment of susceptible infections including gonorrhea, otitis media, pharyngitis, lower respiratory-tract infections such as bronchitis, and urinary-tract infections [1]. Ornidazole (ONZ) is 1-Chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol [Fig. 1(b)] is a antiamoebic agent that interacts with helical DNA structure and strand leading to a protein synthesis inhibition and cell death and used for amoebic dysentery[2].Several methods are reported for quantitative determination of CEF and ONZ in single and in combination such as UV [3-6] and RP-HPLC [7-9].

Chemometrics was introduced in 1972 by Svante Wold [10]. Chemometric is the science of extracting information from chemical system. Multivariate calibration method (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[11].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 CEF and ONZ in tablet dosage form.

MATERIALS AND METHODS

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)

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(64-bit) trial version and Microsoft Excel 2007 were used for model generation and application of chemometric.

Material and Reagents

Reference standard of CEF and ONZ were obtained from Cipla Ltd, Mumbai as gift samples and methanol used was of AR grade (LOBA Chemie, India). tablets manufactured by Relax Pharmaceutical Pvt. Ltd (49-A-B, GondpurIdustrial Area, Paonta Sahib, Dist. Sirmour India) containing Cefixime IP 200 mg and Ornidazole IP 500 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 for CEF and 5.0-30.0 µg/ml for ONZ. Absorbance values were recorded at $\lambda_{max}$of each drug (289 nm for CEF 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 CEF and ONZ and their mixture.

Preparation of standard stock solution

Stock solution of CEF and ONZ were prepared by dissolving accurately weighed 10 mg of standard drugs in 10 ml of methanol, separately. The concentration of CEF 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 CEF and ONZ, 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 for CEF and 5, 10, 15, 20, 25 and 30 µg/ml for ONZ, respectively.

Construction of calibration and validation set

A total set of 36 mixtures were prepared by combining working standard stock solution of CEF and ONZ in their linear concentration range of 2.0-12.0 µg/ml and 5.0-30.0 µg/ml, respectively (Table I). From these randomly 27 mixtures were used for development of model (calibration set) and 9 mixtures were used for validation of model (validation set). The absorbance spectra were recorded in range of 260- 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 Fig. 3 and calculated using formula [12],

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

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.

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 ICH Q2 (R1) [13].

Assay of marketed preparation

20 tablets of MAHACEFIA-OZ were accurately weighed and finely powdered. Tablet powder equivalent to 10 mg of CEFIXIME (25 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 then filtered with help of whatman filter paper no. 41. 1 ml of filtrate solution 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 and 10 µg/ml respectively of CEF and ONZ. The procedure was repeated 6 times for tablet formulation. The assay results are presented in Table. III.

Accuracy study

The accuracy study was carried out at three levels 50 %, 100 % and 150 % of assay concentration. Calculated amount of CEF and ONZ from standard solutions were spiked into sample solution and scanned in range of 260-330 nm. Concentrations were predicted by using developed PCR and PLS models. Accuracy data is presented in Table IV and Table V.

Precision (Intraday and Interday)

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

LOD and LOQ

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

RESULT

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 VIII.

Table 1. Composition of calibration and validation sets.

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

*Mix no. 1-27 calibration set

*Mix no. 28-36 validation set

Table 2. Predicted results for validation set by PCR and PLS method.

METHOD		PLS				PCR			
CEF	***ONZ***	***CEF***		***ONZ***		***CEF***		***ONZ***	
Actual (µg/ml)		Predicted	% R*	Predicted	% R*	Predicted	% R*	Predicted	% R*
2	5	2.029	101.4	4.876	97.53	2.029	101.4	4.876	97.53
2	30	2.069	103.4	29.969	99.89	2.069	103.4	29.969	99.89
4	5	4.125	103.1	5.134	102.6	4.125	103.1	5.134	102.6
6	10	5.988	99.80	9.809	98.09	5.988	99.80	9.809	98.09
6	30	6.778	112.9	29.743	99.14	6.778	112.9	29.743	99.14
8	25	7.738	96.73	25.040	100.1	7.738	96.73	25.040	100.1
10	15	9.890	98.90	15.168	101.1	9.890	98.90	15.168	101.1
10	10	9.987	99.87	10.109	101.0	9.987	99.87	10.109	101.0
12	5	11.883	99.00	5.059	101.1	11.883	99.00	5.059	101.1

Table 3. Assay result for ONZ and CEF in tablet (MAHACEFIA-OZ) by proposed methods

METHOD		PLS				PCR			
CEF	***ONZ***	***CEF***		***ONZ***		***CEF***		***ONZ***	
Actual (µg/ml)		Predicted (µg/ml)	% R	Predicted (µg/ml)	% R	Predicted (µg/ml)	% R	Predicted (µg/ml)	% R
4	10	3.998	99.95	10.138	101.38	3.999	99.97	10.137	101.37
4	10	4.046	101.1	10.155	101.55	4.043	101.0	10.156	101.56
4	10	3.965	99.12	10.088	100.88	3.963	99.07	10.086	100.86
4	10	4.036	100.9	10.447	104.47	4.033	100.8	10.449	104.49
4	10	4.013	100.3	10.048	100.48	4.015	100.3	10.048	100.48

4	10	4.108	102.7	10.389	103.89	4.107	102.6	10.378	103.78
MEAN		4.027	100.6	10.210	102.10	4.026	100.6	10.209	102.09
SD		0.0487	1.218	0.1658	1.658	0.0483	1.209	0.1644	1.644

Table 4. Accuracy data of CEF 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	6.113 6.004 6.048	6.113 6.002 6.046	101.88 100.06 100.80	101.88 100.03 100.76	0.905	0.923
100 %	4	4	8	8.065 8.188 8.267	8.063 8.189 8.267	100.81 102.35 103.33	100.78 102.36 103.33	1.245	1.259
150%	4	6	10	10.082 10.351 10.288	10.081 10.353 10.287	100.82 103.51 102.88	100.81 103.53 102.87	1.373	1.385

Table 5. 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	14.784 14.867 14.889	14.782 14.868 14.889	98.56 99.11 99.26	98.54 99.12 99.26	0.372	0.381
100 %	4	4	8	19.677 19.862 19.998	19.661 19.849 19.985	98.38 99.31 99.99	98.30 99.24 99.92	0.811	0.820
150 %	4	6	10	24.898 24.343 24.982	24.899 24.348 24.999	99.59 97.37 99.92	99.59 97.39 99.99	1.403	1.416

Table 6. 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	*CEF*	*ONZ*	*CEF*	*ONZ*	*CEF*	*ONZ*	*CEF*	*ONZ*	*CEF*	*ONZ*	*CEF*	*ONZ*	*CEF*
10	4	10.03	4.104	10.02	4.108	100.3	102.6	100.2	102.7				
10	4	10.13	4.013	10.13	4.017	101.3	100.3	101.3	100.4	0.846	1.144	0.858	1.140
10	4	10.20	4.043	10.20	4.048	102.0	101.0	102.0	101.2				
15	6	14.91	5.991	14.91	5.998	99.40	99.85	99.42	99.96				
15	6	15.01	5.892	15.01	5.892	100.0	98.20	100.0	98.20	0.620	1.419	0.620	1.427
15	6	15.09	6.061	15.09	6.061	100.6	101.0	100.6	101.0				
20	8	20.26	7.985	20.26	7.984	100.3	99.81	101.3	99.80				
20	8	20.02	8.189	20.02	8.192	100.1	101.3	101.1	102.4	1.099	1.482	1.123	1.496
20	8	20.46	8.198	20.48	8.197	102.3	102.4	101.4	102.4				

Table 7. Precision results obtained using developed PCR and PLS models (Interday Precision)

Amount Taken µg/ml		Predicted Conc.µg/ml				% Recovery				% RSD			
ONZ	*CEF*	PCR		PLS		PCR		PLS		PCR		PLS	
		ONZ	*CEF*	*ONZ*	*CEF*	*ONZ*	*CEF*	*ONZ*	*CEF*	*ONZ*	*CEF*	*ONZ*	*CEF*
10	4	10.26	4.128	10.26	4.129	102.6	103.2	102.6	103.2				
10	4	10.35	4.132	10.35	4.131	103.5	103.3	103.5	103.2	0.535	1.666	0.530	1.679
10	4	10.26	4.012	10.26	4.011	102.6	100.3	102.6	100.2				
15	6	14.98	6.021	14.98	6.022	99.88	100.3	99.88	100.3				
15	6	14.95	5.857	14.95	5.857	99.69	97.61	99.70	97.61	0.648	1.486	0.657	1.495
15	6	15.13	5.997	15.13	5.998	100.9	99.95	100.9	99.96				
20	8	20.78	8.085	20.78	8.085	103.9	101.0	103.9	101.0				
20	8	20.45	7.953	20.45	7.956	102.2	99.41	102.2	99.45	1.327	1.723	1.333	1.724
20	8	20.24	7.811	20.24	7.811	101.2	97.63	101.2	97.63				

Table 8. Summary of results

Parameters	Cefixime (CEF)		Ornidazole (ORZ)	
	PCR	PLS	PCR	PLS
Range (µg/ml)	2.0-12.0	2.0-12.0	5.0-30.0	5.0-30.0
Wavelength (nm)	260-330	260-330	260-330	267-330
Data interval (Δλ)	0.5	0.5	0.5	0.5
Factors / PC's	2	2	2	2
% Recovery	100.6	100.6	102.09	102.10
LOD	0.628	0.628	0.592	0.592
LOQ	1.904	1.904	1.792	1.792
Correlation Coefficient (r^2)	99.73	99.73	99.92	99.92
Intercept	0.0190	0.0190	0.0139	0.0139
Slope	0.9973	0.9973	0.9992	0.9992
RMSECV	0.1765	0.1764	0.2158	0.2158
RMSEP	0.1765	0.1764	0.2158	0.2158

Fig 1. Structure of a) Cefixime (CEF) and b) Ornidazole (ONZ)

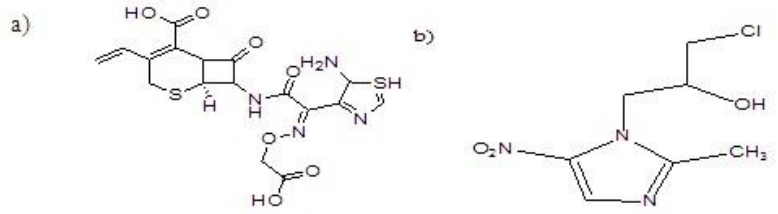


Fig 2. Overlay spectra of CEF, ONZ and mixture.

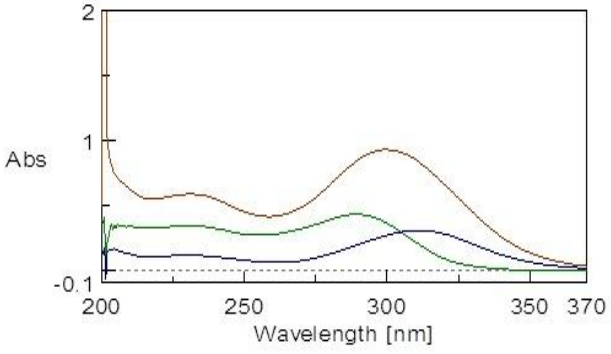
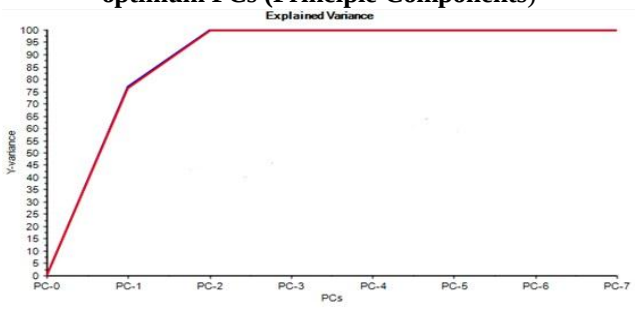


Fig 3. Explained Variance describing number of optimum PCs (Principle Components)



CONCLUSION

A study of the use of UV spectrophotometric in combination with PLS and PCR for the simultaneous determination of Ornidazole (ONZ) and Cefixime (CEF) in a binary mixture has been accomplished. The results obtained confirmed the suitability of the proposed method for simple, accurate and precise analysis of ONZ and CEF in pharmaceutical preparations. The proposed methods do not need separation of ONZ and CEF before analysis. In addition, the proposed methods can be applied for analysis of drugs in quality control lab as well asfor in process quality control.

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CONFLICT OF INTEREST

No interest

REFERENCES

1. The drug bank Website.http://www.drugbank.ca/drug/DB00201.
2. http://www.genericpedia.com/generic/ornidazole/ accessed.
3. Nanda RK, Gaikwad J and Prakash A. Simultaneous Spectrophotometric Estimation of Cefixime and Ornidazole in Tablet Dosage Form. *International Journal of Pharm Tech Research,* 1(3), 2009, 488-491.
4. Chinmaya CB, Punasiya S. Validated spectrophotometric methods for estimation of Cefixime and Ornidazole in combined dosage form. *Pharmaceutical and Biological Evaluations,* 3(4), 2016, 444-449.
5. Nanda R, Gaikwad J and Prakash A. Estimation of cefixime and ornidazole in its pharmaceutical dosage form by spectrophotometric method. *Journal of Pharmacy Research,* 2(7), 2009, 1264-1266.
6. Dey S, *et al*. UV Spectrophotometric Determination of Cefixime in Bulk and its Dosage Form. *Journal of Pharmacy Research,* 5(12), 2012, 5419-5422.
7. Kher GJ, Vijay RR, Pandiya GG and Hitendra JS. Validated HP LC Method for Simultaneous analysis of Cefixime and Ornidazole in commercial tablets. *International Journal of Chem Tech Research,* 4(3), 2012, 1124-1136.
8. Ramesh R, Malothu R, Vasanth PM. Development and Validation of an Rapid RP-HPLC Method for Simultaneous Determination of Drug Cefixime and Ornidazole In Tablet Dosage Form. *International Journal of Biological & Pharmaceutical Research,* 3(6), 2012, 747-751.
9. Shirisha M, Dhani R, *et al.* Analytical Method Development and Validation by RP-HPLC for Simultaneous Estimation of Cefixime and Ornidazole in Combined Tablet Dosage Form. *International Journal of Research in Pharmacy and Life Sciences,* 3(7), 2015, 330-335.
10. Gandhi SV and Tapale SR. Chemometric-assisted UV-spectrophotometric method for simultaneous estimation of paracetamol, chlorzoxazone and ibuprofen in multicomponent formulation. *Ash Ese Journal of Pharmacy,* 1(1), 2016, 001-008.
11. Gandhi SV, Pahade AR, Sutar AS, Kuchekar BS, Tapale SR. Simultaneous estimation of beclomethasonedipropionate and salbutamol sulphatein capsules by chemometric assisted UV-spectrophotometric method. *International Journal of Pharmaceutical Chemistry and Analysis,* 3(2), 2016, 84-89.
12. Gandhi SV, Pahade AR, Tapale SR. Chemometrics - Assisted UV Spectrophotometric Methods for Determination of Metoprolol Succinate and Hydrochlorthiazide in Oral Tablets. *Inventi Impact: Pharm Analysis & Quality Assurance*, 3(4), 2016, 177-181.
13. International Conference on Harmonization. ICH harmonized tripartite guideline validation of analytical procedures text and methodology Q2 (R1) ICH, *Geneva,* 2005, 1-17.