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Determination of enantiomeric composition of ofloxacin in tablets by chemometric techniques applied to overlapped chromatograms

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ABSTRACT

This study demonstrates how chiral liquid chromatography combined with multivariate chemometric techniques, provides a powerful analytical methodology. Although enantiodiscrmination can be possible, the optimization of the analytical procedure to achieve this goal for a given molecule often requires expensive and time - consuming testing of different columns and chromatographic conditions and finally , the most usual scenario is to hardly get baseline resolution but with relatively low enantioseparation factors. Strongly overlapped chromatographic profiles of ofloxacin enantiomers in a sample could be successfully processed and enantiomeric purity could be accurately determined without requiring baseline enantioresolution between peaks by using multivariate chemometric techniques. The samples of ofloxacin were partially enantioseparated with a (R, R) - Whelk- O-2 chiral column under reversed phase condition. Mobile phase was 50 mM potassium dihydrogen phosphate (pH = 2.6) and methanol [60:40, v/v] using 20 µg/ml paracetamol as internal standard. The flow rate was 1.8 ml / min. Signals detected with a diode array detector within a wavelength range from 240 to 260 nm at 5 nm intervals. The developed methods were applied to the quantitative analysis of the investigated enantiomers in tablets. HPLC-chemometric techniques using DAD provide reliable results with high sensitivity, accuracy and robustness as well as high peak purity assessment via DAD empowered by PCR and PLS. Chiral HPLC-chemometric combination techniques using stronger mobile phases can elute earlier all interesting peaks and overcome the usual lower enantioresolution factors, therefore, it reduce analysis cost.

Key words: multivariate techniques; Chiral high performance liquid chromatography; overlappedchromatograms; ofloxacin enantiomers.

INTRODUCTION

Ofloxacin is (±)-9-Fluoro-2, 3-dihydro-3-methyl-10-(4-methylpiperazin-1-yl)-7oxo-7-Hpyrido [1,2,3-de]-1,4-benzoxazine-6-carboxylic acid (Fig. 1). Its chemical formula is $C_{18}H_{20}FN_3O_4$ and molecular weight is 361.4 g [1]. Ofloxacin is a second generation fluoroquinolone antimicrobial agent with a 6-fluoro substituent and a 7-piperazinyl substituent on the quinolone ring structure [2].

Levofloxacin is the (-)-S form of ofloxacin and it is an active antimicrobial agent. It is substantially more active than ofloxacin [3-5]. Ofloxacin melts at 270 to 273^{0}C [5]. The solubility of ofloxacin varies depending upon pH [6, 7].

The official method of assay of ofloxacin as reported in British Pharmacopoeia (B.P.) [8] is the non–aqueous titration method. Several spectrophotometric methods were reported for assay of ofloxacin in pharmaceutical formulations [9-12]. Ofloxacin was also determined by spectrofluorimetry [9, 13] and flow-injection chemiluminescence [14, 15]. Atomic absorption spectroscopy was also used for determination of ofloxacin [9, 16].

Different HPLC methods were reported for the assay of the fluoroquinolones in dosage forms, serum, urine and other biological fluids [17, 18]. Few methods were reported for separation and determination of ofloxacin

enantiomers such as ionic liquid – assisted ligand-exchange [19, 20], HPLC separation by a chiral stationary phase [21] and nano-liquid chromatography [22].

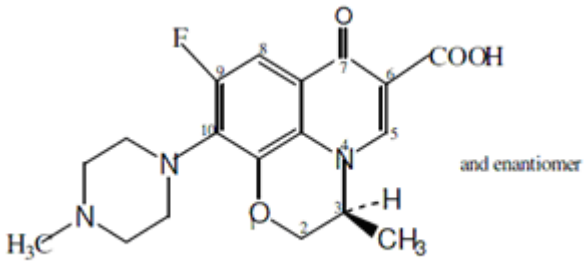

Fig. 1: Chemical structure of ofloxacin.

Strongly overlapped chromatographic profiles in a sample could be successfully processed and enantiomeric purity could be accurately determined without requiring a good baseline enantioresolution between peaks by using multivariate chemometric techniques [23].

DAD response will eliminate the errors of single regression equations based on single wavelength and provide multichromatographic data in single run. This procedure reduces the time of analysis and consumption of reagents [24].

Combination between HPLC and multivariate analysis provides a powerful technique for purity assessment of chromatographic peaks. DADdetector can analyze peak purity by comparing spectra within a peak. A pure peak has matching spectra throughout the peak (at all wavelengths) [25] in addition to purity assessment provided by the multivariate methods through principle component analysis (vector analysis) [26].

Classical least squares (CLS), sometimes known as K-matrix calibration, can be applied to simple systems where the concentration values of all the components present in the training samples are provided. Principal component regression (PCR) is sometimes described as performing a least squares regression of the projections of the data onto the basis vectors of a factor space using inverse least squares. Partial least squares (PLS) method is a multivariate calibration based on factor analysis [26- 30]. The basic concept of PLS regression was originally developed by Wold [31]. A detailed description of the mathematical principles of the PLS algorithm was reported by Martens and Naes [32]. PLS method involves simultaneously the independent and the dependent variables in the data compression and decomposition operations [26, 28, 33].

Chemometric calibration techniques have been utilized for the resolution of overlapping spectra for the determination of active compounds in samples containing two or more compounds [34-38]. HPLC-chemometric combination techniques have been applied to simultaneous determination of some drugs in a multicomponent dosage form [39- 41].

MATERIALS AND METHODS

2.1. Apparatus and software

A Dionex UltiMate 3000 RS system was used, (Thermo Scientific™, Dionex™, Sunnyvale, CA, USA), equipped with Quaternary RS pump, RS auto-sampler injector, Thermostated RS Column Compartment and RS Diode array detector (DAD). The instrument was connected to a Dell compatible PC, bundled with Chromeleon® 7.1 Chromatography Data System software. The HPLC column was (R, R)-Whelk-O 2 (10 µm, 4.6 mm × 250 mm), (Regis Technologies, Inc. Morton Grove, IL, USA). Hanna HI 8314 pH Meter was used to adjust the pH of the buffer used in the mobile phase. Data acquisition was performed on Chromeleon® 7.1 Chromatography Data System software at selected five wavelengths (240nm, 245nm, 250nm, 255nm and 260 nm). CLS, PCR, and PLS analyses were carried out using the Chemometrics Toolbox 3.02 software [42] for use with MATLAB 6.

2.2. Chromatographic conditions

The samples of ofloxacin were partially enantioseparated with a (R, R) -Whelk- O-2 chiral column under reversed – phase condition. Mobile phase consists of 50 mM potassium dihydrogen phosphate (pH = 2.6) and methanol [60:40, v/v] using 20 µg / ml paracetamol as internal standard. The flow rate was 1.8 ml / min at 25 ^{0}C. Signals detected with a diode – array detector within a wavelength range from 240 to 260 nm at 5 nm intervals.

2.3. Materials

Pure drugs

Racemic (±) ofloxacin (99.90%), levofloxacin (99.60%) and paracetamol (99.80%) were kindly supplied by Sigma Company for Pharmaceutical Industries, Egypt.

Pharmaceutical preparations

Ofloxin ® 200mg film coated tablets product of Kahira Pharm.&Chem. Ind. Co., Cairo-Egypt.

Levofloxacin ® 750 mg film coated tablets product of EPCI- The Egyptian Co. for Pharmaceutical & Chemical Industries, Industrial Zone, Bayad El-Arab, Beni Suief, Egypt.

2.4. Reagents

Methanol was HPLC grade. Potassium dihydrogen phosphate and phosphoric acid were of analytical grade. Phosphate buffer was 50 mM potassium dihydrogen phosphate adjusted to pH 2.6 using phosphoric acid then filtered through a membrane filter 0.22µm and degassed using sonication.

2.5. Standard solutions and calibrations

Ofloxacin, levofloxacin and paracetamol (internal standard) were weighed (50 mg each) and transferred to three separate 50ml volumetric flasks and dissolved in 20 ml of methanol and make up the volume to the mark with the mobile phase. Aliquots from the stock solutions of each drug were appropriately diluted with the mobile phase to obtain working standard solutions of 100µg/ml for each drug. A training set of ten synthetic mixture solutions in different combinations containing 0-36 µg/ml (+)-R- ofloxacin(I) and 13-50 µg/ml (-)S- ofloxacin (II) was used to develop the chemometric calibrations. A validation set containing ten synthetic binary mixtures in the range of 5-34µg/ml and 16-50 µg/ml for (I) and (II), respectively, was prepared from the above stock solutions. Triplicate 20 µl injections were made for each solution and chromatographed under the specified conditions using 20 µg/ml paracetamol as internal standard (IS).

2.6. Sample preparation

Ten Ofloxin® film coated tablets were accurately weighed and finely powdered in a mortar. An amount equivalent to one tablet content [200 mg of (I) and 200mg of (II)] was dissolved in 30 ml of methanol. After 30 min of mechanical shaking, the solution was filtered in a 100 ml volumetric flask using Whatman® filter paper. The residue was washed thrice, each with 10 ml of the solvent. Then the volume was completed to 100 ml using mobile phase. Suitable dilutions were made using the mobile phase. The same procedures were performed for sample preparation of Levofloxacin® film coated tablets. An amount of the tablet equivalent to one tablet content [750mg of (II)] was taken and the solution was prepared by the same procedures as in Ofloxin®.

RESULTS AND DISCUSSION

3.1. Method Development and Optimization

Many trials were done to get enantiomeric separation of ofloxacin isomers with good chromatographic resolution using a (R, R) –Whelk- O-2 chiral column under normal phase and reversed phase conditions using several mobile phase systems with different chromatographic conditions varying the type of the solvent, organic modifier and buffer at different pH, percentage and flow rate. Baseline resolution was hardly achieved but with low enantioseparation factors (not more than 1.18) and the run time was too late (more than 40 min).

The mobile phase was chosen after several trials with methanol and buffer solutions in various proportions and at different pH to achieve partial enantioseparation within a shorter run time. Ofloxacin enanaiomers were partially separated with a (R, R) - Whelk- O-2 chiral column under reversed –phase condition. A mobile phase consists of 50 mM potassium dihydrogen phosphate (pH = 2.6) and methanol [60:40, v/v] at flow rate was 1.8 ml / min with injection volume 20 µl was found to be suitable for partial enantioseparation of ofloxacin isomers in their synthetic mixtures (Fig.2) and in their tablet (Fig.3) with in a run time less than 14 min using a (R, R)- Whelk- O-2 chiral column under reversed –phase condition at 25^0C using 20 µg/ml paracetamol as internal standard. Under these chromatographic conditions, chromatograms were obtained for (+) -R- ofloxacin (I) and (-) - S- ofloxacin (II) with retention times 13.501 ± 0.065 and 12.062± 0.043 min for (I) and (II), respectively and 2.463 ± 0.110 for IS. Chromatograms corresponding to the training and validation sets (Table 1) using 20 µg/ml paracetamol as IS by DAD at five wavelengths set, 240 nm, 245 nm, 250 nm, 255 nm and 260 nm were obtained.

It is difficult to determine component purity from a chromatogram because the peak shape itself does not reveal that it actually corresponds to two or even more components. However, DAD detector can analyze peak purity by comparing spectra within a peak. A pure peak has matching spectra throughout the peak (at all wavelengths).

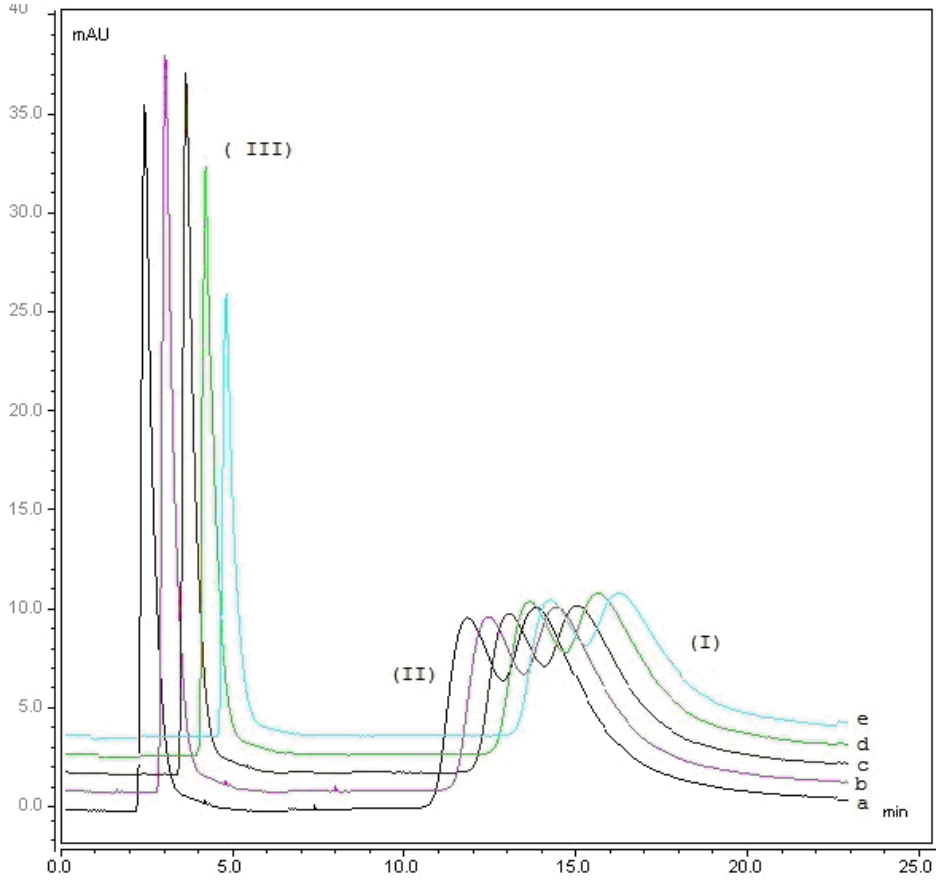

Fig.2: HPLC chromatograms of 20 µl injection of a mixture in training set containing 36 µg/ml (+) -R- ofloxacin (I) and 36 µg/ml (-) - S- ofloxacin (II) using 20 µg/ml paracetamol as IS (III) at five different wavelengths [a) 240 nm, b) 245nm , c) 250nm, d)255nm and e)260nm]

The obtained multiwavelength detections produce different peak area information about qualitative and quantitative properties of the analyzed compounds. Simultaneous data collection at multiwavelengths provides the application of multivariate calibration techniques, to these HPLC data for quantitative studies. The application of multivariate methods, including CLS, PCR and PLS, to the chromatographic data is a new approach for the simultaneous quantitative analysis of ofloxacin enantiomers sets were obtained by using the peak-area ratio of each compound to IS versus its concentration. Afterwards, these peak area ratios as HPLC data sets were used to construct the multivariate calibrations as HPLC-CLS, HPLC-PCR and HPLC-PLS.

Table 1 Mixtures of (+) -R- ofloxacin (I) and (-) - S- ofloxacin (II) used in the training and validation sets

Mixture no.	Training set		Validation set	
	(I) µg/ml	(II) µg/ml	(I) µg/ml	(II) µg/ml
1	0	50	5	50
2	8	13	12	16
3	13	16	16	21
4	15	15	20	22.5
5	17	22	24	24
6	20	25	26	31
7	22	27	27	27
8	28	33	24	29
9	36	36	30	30
10	36	41	34	34

In this study, we explored the role of multivariate techniques as a mean of extracting information about enantiomeric composition of ofloxacin samples from chromatographic matrix data obtained after partial resolution of peaks in a typical chiral chromatographic separation followed by diode-array detection. Using multivariate chemometric techniques, strongly overlapped chromatographic profiles of ofoxacin enantiomers in a sample could be successfully processed and enantiomeric purity could be accurately determined without requiring a good baseline enantioresolution between peaks [23].

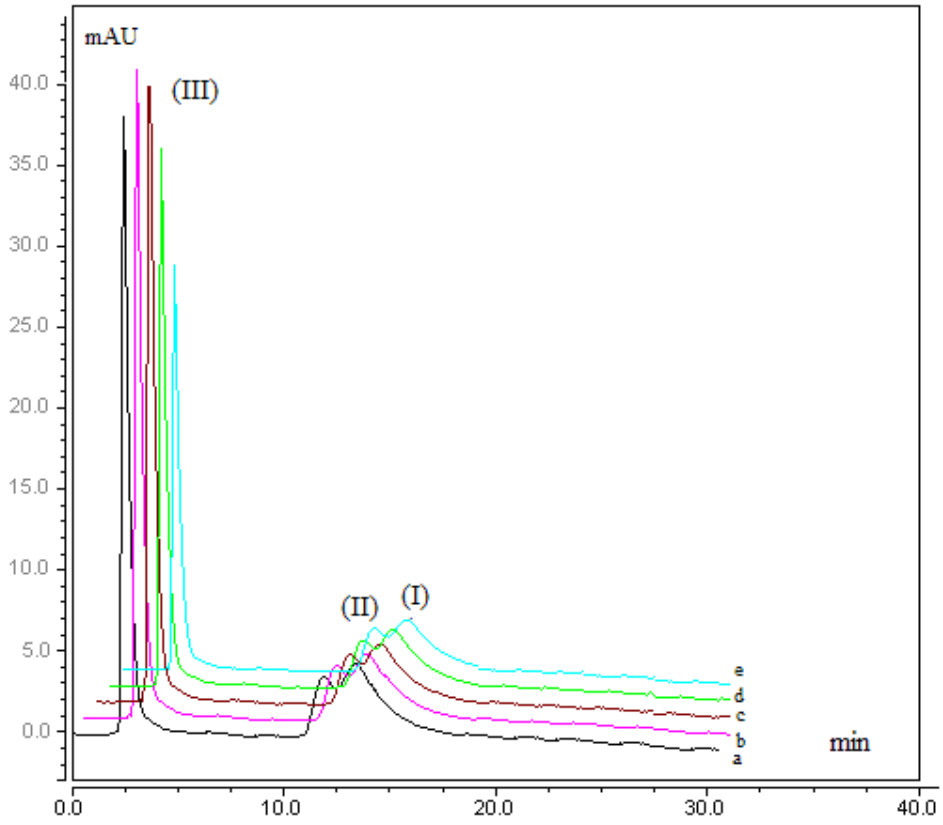

Fig.3: HPLC chromatograms of 20 µl injection of assay solution of Ofloxin® film coated tablet solution in presence of 20 µg/ml paracetamol (III) at five different wavelengths [a) 240 nm, b) 245nm , c) 250nm, d)255nm and e)260nm]

DAD provides multichromotographic data in one run thus reduces the number of injections, saves time and reagents. Multivariate – HPLC combination techniques permit the removal of errors and residuals of calibration of classic HPLC based on a single wavelength. Thus, the sensitivity, accuracy and precision of the HPLC-chemometric calibrations are higher than that provided by the classic- HPLC method. HPLC-chemometric methods provide high purity assessment via DAD empowered by PCR and PLS. Implementation of the multivariate calibration algorithms is applied as shown in the following section.

3.2. Processing of HPLC Data

A training set consisting of the mixture solution in the concentration range of 0-36 µg/ml (+) -R- ofloxacin (I) and 13-50 (-) - S- ofloxacin (II) with 20µg/ml IS was prepared. The ratio of peak area for the training set was obtained at a five wavelength set (240 nm, 245nm, 250nm, 255nm and 260nm) for the partially separated peaks. The chemometric calibration techniques, CLS, PCR, and PLS, were applied to the prepared training set and its measured HPLC data set. The concentration of each enantiomer in samples was determined by the HPLC-chemometric calibrations.

3.2.1. HPLC-CLS Approach

The coefficient matrix (*K*) was calculated using the linear equation system based on the relationship between the data of the ratio of peak areas and training set. By replacing the coefficient matrix (*K*) into the linear equation system, HPLC-CLS calibration was obtained. The prediction of an unknown concentration of the enantiomers of ofloxacin in samples was carried out by the HPLC-CLS calibration. The calibration and data treatment were done by CLS algorithm by means of the Chemometrics Toolbox 3.02 software [42] for use with MATLAB 6.

3.2.2. HPLC-PCR Approach

The HPLC-PCR calibration was constructed using the PCR algorithm. In this case, the square matrix of the ratio of peak areas data was obtained by decomposition of peak area values. Linear correlation between the training set and decomposed peak area values was used to obtain the HPLC-PCR calibration. The obtained HPLC-PCR calibration was applied to the determination of both enanatiomers in the synthetic mixtures and tablets. Chemometrics Toolbox 3.02 software [42] for use with MATLAB 6 was used for both the calculation of calibration and data treatment.

3.2.3. HPLC-PLS Approach

In this calibration model, both peak area data and concentration set were decomposed. HPLC-PLS calibration was obtained using the relationship between the decomposed peak area data and concentration set. The amount of ofloxacin enantiomers in samples was determined using the HPLC-PLS calibration. The mathematical treatments have been performed by means of the Chemometrics Toolbox 3.02 software [42] for use with MATLAB 6. In order to validate the developed calibrations, an independent set of validation synthetic mixtures containing (+)-R-ofloxacin (I) and (-) - S- ofloxacin (II) compositions given in Table 1, was prepared and analyzed. The mean percentage recoveries, standard deviations S.D. and relative standard deviations (R.S.D.) are indicated in Table 2. The results contributed to the high accuracy and precision of the developed HPLC-chemometric methods.

Table 2 Assay results of (+)-R- ofloxacin (I) and (-)-S- ofloxacin (II) combinations in synthetic mixtures (validation mixtures) by the proposed HPLC-chemometrics methods

Validation mixture no.	Found %					
	HPLC-CLS		HPLC-PCR		HPLC-PLS	
	(I)	(II)	(I)	(II)	(I)	(II)
1	101.00	99.92	101.00	99.90	101.00	99.90
2	100.67	99.56	100.58	99.56	100.58	99.56
3	100.69	99.57	100.69	99.52	100.69	99.52
4	100.10	99.91	100.10	99.91	100.10	99.91
5	99.25	100.38	99.21	100.33	99.21	100.33
6	99.42	100.19	99.39	100.19	99.39	100.19
7	98.93	101.00	98.89	101.00	98.89	101.00
8	100.63	99.72	100.63	99.72	100.63	99.72
9	100.97	99.43	100.93	99.43	100.93	99.43
10	99.97	100.03	99.94	100.03	99.94	100.03
Mean	100.163	99.971	100.136	99.959	100.136	99.959
±S.D.	0.749	0.468	0.755	0.468	0.755	0.468
R.S.D.%	0.748	0.468	0.754	0.468	0.754	0.468

S.D. standard deviation.
R.S.D. relative standard deviation.

Determining how many factors to be used in the calibration is a key step in factor based techniques (PCR and PLS). Only those factors that contain analytical information must be kept. The discarded factors should contain only noise [26, 43]. The Chemometrics Toolbox 3.02 Software offers several indicators which could be used for determining the optimum number of factors (rank). The cross validation procedure leaving out one sample at a time was used for this purpose [26, 44] and the predicted residual error sum-of-squares, (PRESS) was calculated.

$$PRESS= \sum_{i=1}^{n} (C_i^{Predicted} - C_i^{True})^2$$

Where $C_i^{Predicted}$ denotes the predicted concentration, C_i^{True} represents the true concentration and n is the total number of validation samples. A better way for selecting the optimum number of factors involved the generation of a calibration for every possible rank. Each calibration was used to predict the concentrations for a set of independently measured, independent validation samples. Then the PRESS was calculated [26]. Another way to determine the optimum number of factors was the two-way F-test on reduced eigenvalues (REV) according to the method of Malinowski [26].

To develop the HPLC-PCR model for partially enantioseparated chromatograms, the following indicator functions have been used to select the optimum number of factors: PCRPRESS, PCRREV, PCRFIT and PCRFITV illustrated in Fig.4. A rank of two factors was found to be the optimum system rank according to all the studied indicators.

To develop the HPLC-PLS model for partially enantioseparated chromatograms, a rank of two factors was also found to be the optimum system rank according to PLSPRS (PRESS for PLS), PLSREV and PLSCRS indicators.

According to the studied indicators, the HPLC-PCR and HPLC-PLS models were constructed using two factors succeeded to span nearly all the data leaving only negligible residuals. The predictive ability of a model could be defined using several validation diagnostics. These include the standard error of prediction (SEP), the mean squared error of prediction (MSEP), the root mean standard error of prediction (RMSEP) and the variance of prediction (s^2) [26,33]. The MSEP and RMSEP characterize both the accuracy and the precision of prediction [33].

$$SEP= [\sum_{i=1}^{n} (C_i^{Predicted} - C_i^{True})^2 / n-1]^{½}$$
$$MSEP= \sum_{i=1}^{n} (C_i^{Predicted} - C_i^{True})^2 / n$$
$$RMSEP= [\sum_{i=1}^{n} (C_i^{Predicted} - C_i^{True})^2 / n]^{½}$$
$$s^2 = \sum_{i=1}^{n} (C_i^{Predicted} - C_i^{True} - bias)^2 / n-1$$

where $C_i^{Predicted}$ is the predicted concentration, C_i^{True} is the true concentration and n is the total number of validation samples. The numerical values of SEP, MSEP, RMSEP and s^2 are indicated in Table 3. The small values of the calculated validation diagnostics indicate the negligible error of prediction and the high predictive ability of the proposed methods.

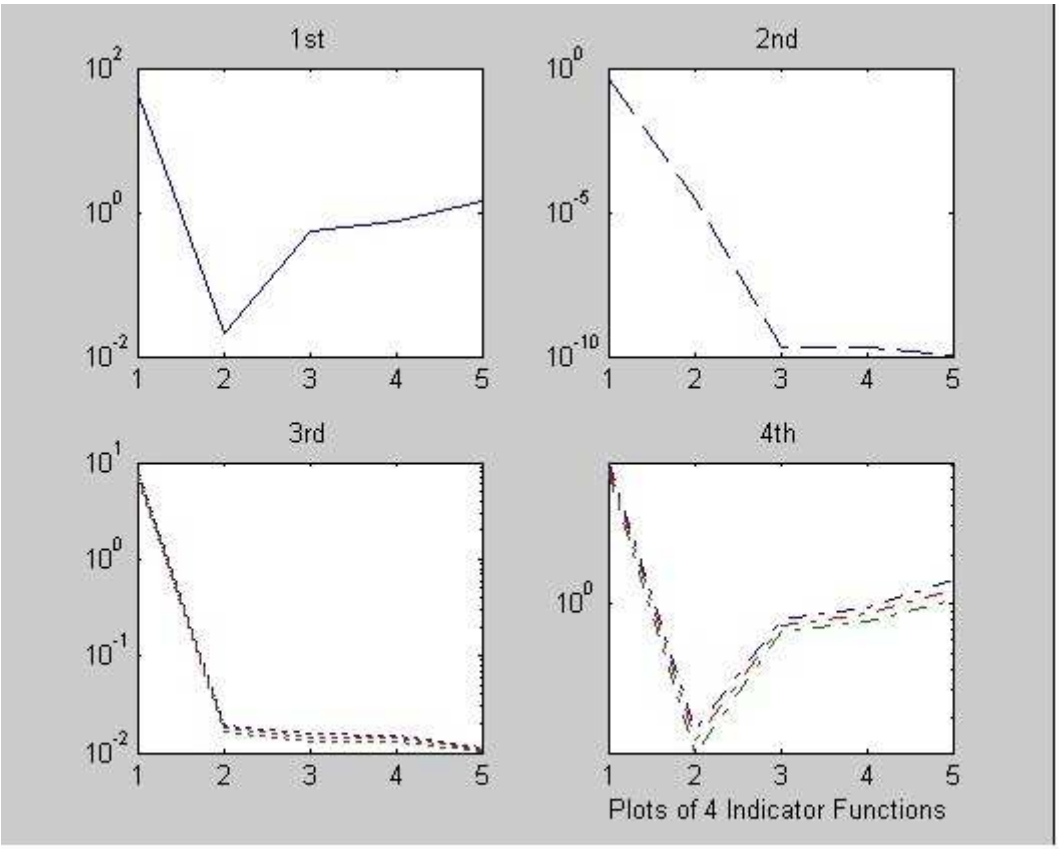

Fig.4: Plots of 4 indicator functions (1st: PRESS, 2nd: REV, 3 rd: FIT and 4th: FITV) versus number of factors for the PCR model of ofloxacin enantiomers

Another way to validate the models and to examine the results is the predicted versus true concentration plot. In this plot, points are expected to fall on a straight line with a slope of one and a zero intercept [44]. The correlation coefficient (r) is calculated for each calibration to indicate the quality of fit of all data to a straight line. The regression analysis for these linear relationships was carried out and the results are shown in Table 3. The absence of bias was proved by determining the confidence limits for the intercept, a, and the slope, b, at the 95% significance level [45]. The upper and lower confidence limits are shown in Table3.

Table 3 Statistical parameters of the validation synthetic mixtures of (+)-R- ofloxacin (I) and (-) - S- ofloxacin (II) using the proposed HPLC - chemometrics methods

Method	SEP	MSEP	RMSEP	s^2	a	Lower * 95%	Upper* 95%	b	Lower** 95%	Upper** 95%	r
(I)											
CLS	0.172	0.0267	0.1634	0.0340	0.0784	-0.2913	0.4481	0.9967	0.9809	1.0126	0.99981
PCR	0.174	0.0273	0.1652	0.0356	0.0795	-0.2936	0.45256	0.9964	0.9804	1.0124	0.99981
PLS	0.174	0.0273	0.1652	0.0356	0.0795	-0.2936	0.45256	0.9964	0.9804	1.0124	0.99981
(II)											
CLS	0.122	0.0135	0.1162	0.0211	-0.0006	-0.3214	0.32017	0.9999	0.9891	1.0107	0.99991
PCR	0.123	0.0136	0.1167	0.0217	-0.0005	-0.3217	0.32160	0.9998	0.9890	1.0106	0.99991
PLS	0.123	0.0136	0.1167	0.0217	-0.0005	-0.3217	0.32160	0.9998	0.9890	1.0106	0.99991

SEP, standard error of prediction; MSEP, mean squared error of prediction; RMSEP, root mean standard error of prediction; s^2, variance of prediction; a, intercept; b, slope; r, correlation coefficient.

** Lower and upper confidence limits for the intercept at the 95% confidence level, ** Lower and upper confidence limits for the slope at the 95% confidence level.*

For each enantiomer, using the three developed multivariate models, the 95% confidence interval of the intercept included the ideal value of zero and that of the slope included the ideal value of one. This gave indication of good fitness and absence of bias which confirmed the trueness of the developed methods. Furthermore, no sample(s) appeared to be unusually far from the line than the rest of the data.

3.3. Analysis of pharmaceutical dosage forms

The developed HPLC-CLS, HPLC-PCR and PLS-HPLC methods were applied to the simultaneous determination of ofloxacin enantiomers in commercial Ofloxin ® film coated tablets. Satisfactory results were obtained for each compound in good agreement with labeled claim (Table4).

The developed multivariate – HPLC methods were also applied to determination (-) - S- ofloxacin in commercial Levofloxacin ® film coated tablets under the same chromatographic conditions (Fig.5). Satisfactory results were obtained in good agreement with labeled claim (Table 5).

Table 4 Determination of (+) -R- ofloxacin (I) and (-) - S- ofloxacin (II) in Ofloxin® film coated tablets by the developed HPLC-chemometrics methods

Sample	Recovery%*					
	(+) -R- ofloxacin			(-) - S- ofloxacin		
	HPLC-CLS	HPLC-PCR	HPLC-PLS	HPLC-CLS	HPLC-PCR	HPLC-PLS
1	98.72	98.72	98.72	101.12	101.12	101.12
2	100.87	100.87	100.87	99.23	99.23	99.23
3	101.12	101.12	101.12	99.27	99.28	99.27
4	100.60	100.59	100.59	99.49	99.48	99.49
5	99.48	99.48	99.48	100.46	100.46	100.46
Mean	100.158	100.156	100.156	99.914	99.914	99.914
± S.D.	1.019	1.018	1.018	0.839	0.838	0.839
R.S.D.%	1.018	1.017	1.017	0.840	0.839	0.840

** Ofloxin ® film coated tablets labled to contain 200 mg (±) ofloxacin per tablet.*

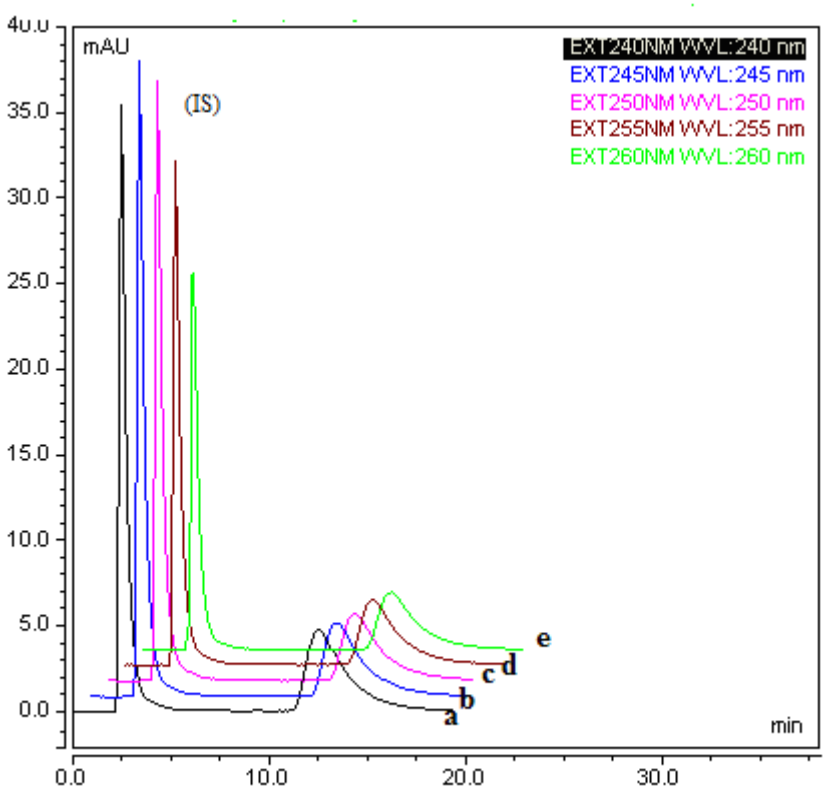

Fig. 5: HPLC chromatograms of 20 µL injection of assay solution of Levofloxacin® film coated tablet solution in presence of 20 µg/mL paracetamol (IS) [a) 240 nm, b) 245nm , c) 250nm, d)255nm and e)260nm]

Table 5 Determination of (-) - S-ofloxacin in Levofloxacin ® film coated tablets by the developed HPLC-multivariate methods

Sample	Recovery %		
	HPLC-CLS	HPLC-PCR	HPLC-PLS
1	101.34	101.33	101.33
2	101.63	101.63	101.63
3	99.26	99.26	99.26
Mean	100.743	100.74	100.74
±S.D.	1.293	1.291	1.291
%R.S.D.	1.283	1.281	1.281

** Levofloxin ® film coated tablets labled to contain 750 mg (-)-S-ofloxacin per tablet.*

CONCLUSION

This study explored the role of multivariate techniques, as a mean of extracting information about enantiomeric composition of ofloxacin samples from chromatographic matrix data obtained after partial resolution of peaks in a chiral chromatographic separation followed by diode-array detection. Using multivariate chemometric techniques, strongly overlapped chromatographic profiles of investigated enantiomers in a sample could be successfully processed and enantiomeric purity could be accurately determined without requiring baseline enantioresolution between peaks. The developed methods were applied to the quantitative analysis of the investigated enantiomers in their synthetic mixtures and in tablets. HPLC-chemometric techniques using DAD do not require tedious validation steps and eliminate the errors of single regression equations based on single wavelength and provide reliable results with high sensitivity, accuracy and robustness as well as high peak purity assessment via DAD empowered by PCR and PLS. DAD provides multichromatographic data in single run. This procedure reduces the time of analysis and consumption of reagents. HPLC-chemometric techniques applied to strongly overlapped enantiomeric chromatographic profiles reduce analysis time by using stronger mobile phases to elute earlier all interesting peaks and overcome the usual lower enantioresolution factors and, therefore, it reduce analysis cost.

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