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SIMULTANEOUS DETERMINATION OF ESOMEPRAZOLE MAGENISUM TRIHYDRATE AND NAPROXEN BY COMBINED HPLC-CHEMOMETRIC TECHNIQUES AND RP- HPLC METHOD

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ABSTRACT

New chemometric approaches were applied to high performance liquid chromatography (HPLC) for simultaneous determination of esomeprazole magenisum trihydrate and naproxen in their synthetic mixture. These chemometric approaches were applied to the multiwavelength HPLC peak area ratio obtained by plotting the chromatograms at the five wavelengths using diclofenac sodium as internal standard (IS). The multichromatograms were obtained by using the diode array detector (DAD). A mixture of 20 mM acetate buffer (pH=4.2) and acetonitrile (35:65, v/v) was used as a mobile phase on a 5µm (4.6 x 250mm) Inertsil® C18 Column at a flow rate of 1.5 mL/min to separate and determine the investigated drugs in their synthetic mixture. For comparison purposes, a new validated developed classic HPLC method at 299 nm was used to confirm the results obtained from HPLC-chemometric calibration techniques. There was no significant difference between the methods compared.

Key words: esomeprazole magenisum trihydrate, naproxen, HPLC-chemometric combined techniques

INTRODUCTION

Esomeprazole magnesium trihydrate is a proton pump inhibitor. Chemically, it is bis(5-methoxy-2-[(S)- [(4-methoxy-3,5- dimethyl-2 pyridinyl)methyl] sulfinyl] - lH-benzimidazole-1 -yl) magnesium trihydrate as shown in figure 1. Esomeprazole is the S-isomer of omeprazole [1]. Omeprazole is official in The Merk Index [2], Martindale [3], The Indian Pharmacopoeia [4], BP [5], USP [6]. Esomeprazole, the S-enantiomer of omeprazole, shows improved efficacy over the racemic mixture of omeprazole. In vivo investigations demonstrated that ESO is chirally stable after administration. ESO is 97% bound to plasma proteins [7,8]. Esomeprazole is a proton pump inhibitor, which reduces acid secretion through the inhibition of ATPase in gastric parietal cells, by inhibiting the functioning of this enzyme, so the drug prevents formation of gastric acid. The primary uses of esomeprazole are for gastroesophageal reflux disease, treatment of duodenal ulcers caused by H. pylori, prevention of gastric ulcers in those on chronic NSAID therapy, and treatment of gastrointestinal ulcers associated with Crohn's disease [9]. Naproxen is a member of arylacetic acid group of nonsteroidal anti-inflammatory drugs (NSAIDS). Chemically, it is (S)-6-methoxy-α-methyl-2-naphthaleneacetic acid as shown in figure 2. Naproxen is official in The Merk Index [2], Martindale [3], IP [4], BP [5], and USP [6]. Naproxen is a NSAID commonly used for the reduction of pain, fever, inflammation and stiffness caused by conditions such as osteoarthritis, kidney stones, rheumatoid arthritis, gout, ankylosing spondylitis, menstrual cramps, tendonitis and primary dysmenorrhea. It works by inhibiting both the COX-1 and COX-2 enzymes [4]. A tablet formulation containing 375 mg of naproxen and 20 mg of esomeprozole magenisum trihydrate has recently approved for the relief of signs and symptoms of osteoarthritis, rheumatoid arthritis and ankylosing spondylitis and to decrease the risk of developing gastric ulcers in patients at risk of developing NSAID associated gastric ulcers. Literature review revealed

that many analytical methods such as UV spectrophotometric [10-15], TLC [16], GC [17] and HPLC[18, 19] methods were reported for determination of esomeprazole individually in pharmaceutical dosage form. However, analytical methods like UV spectrophotometry[20], HPLC [21] and HPTLC[22] were reported for determination of naproxen. Different analytical methods were developed for the simultaneous estimation of esomeprazole magnesium and naproxen in dosage form involving UV spectrophotometric methods [23-30] such as simultaneous equation method [23-29], absorption ratio [23], zero crossing first derivative spectrophotometry [24] and ratio derivative spectrophotometry [24]. RP-HPLC methods [30-34] as well as RP-UPLC methods [35, 36] were reported for the simultaneous estimation of esomeprazole magnesium and naproxen in their combined tablets.

Objectives of the study: In this study, simultaneous estimation of esomeprazole magenisum trihydrate and naproxen in their synthetic mixtures was performed using CLS, PCR and PLS calibration techniques applied to the HPLC data set at multiwavelengths using DAD. These combined numerical methods with HPLC were denoted as HPLC-CLS, HPLC-PCR and HPLC-PLS. As a comparative method, classic HPLC was used to analyze the same samples. For a statistical comparison, *t*-test and *F*-test were applied to the obtained results. Multichromotographic data obtained by DAD in one run reduces the number of injections and saves time and reagents. HPLC-chemometric techniques using DAD eliminate the errors of single regression equations based on single wavelength (chromatographic area errors coming from injection, instrumental and experimental environment fluctuations), do not require tedious validation steps and provide reliable results with high sensitivity, accuracy and robustness as well as high purity assessment via DAD empowered by PCR and PLS.

MATERIALS AND METHODS

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. Separation and quantitation were carried out on Inertsil ® C18 Column 5µm (4.6 x 250mm). 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 (293, 295, 297, 299 and 301 nm). CLS, PCR, and PLS analyses were carried out using the Chemometrics Toolbox 3.02 software [37] for use with MATLAB 6.

Materials: Esomeprazole magenisum trihydrate (100.15%) was supplied by Sigma Company for pharmaceutical industries, Egypt; by importing from Disto Pharmaceuticals PVT Ltd., Cherlapally, Hyderabad. Naproxen (99.60%) and dicofenac sodium (99.91%) were kindly supplied by Rameda for pharmaceutical industries and diagnostic reagents, 6th of October City, Egypt.

Pharmaceutical preparation: Vimovo ® tablet contains 20 mg of esomeprazole magnesium trihydrate and 375mg of naproxen. Vimovo® approved by US-FDA in 2011 produced by Astrazeneca Ltd. It is not available in Egypt. Laboratory prepared synthetic mixture was used in this study.

Reagents: Acetonitile (HPLC grade) was used. Anhydrous sodium acetate and glacial acetic acid were of analytical grade. Acetate buffer (20 mM) was prepared using anhydrous sodium acetate and distilled water, adjusted to pH 4.2 using glacial acetic acid (HPLC grade) then filtered through a membrane filter 0.22µm and degassed using sonication.

Standard solutions and calibrations: Esomeprazole magenisum trihydrate (ESO), naproxen (NAP), and diclofenac sodium (DIC) (internal standard) were weighed (50 mg each) and transferred to three separate 50 ml volumetric flasks and dissolved in 20 ml of methanol and make up the volume up to the mark with mobile phase. Aliquots from the stock solutions of each drug were appropriately diluted with mobile phase to obtain working standard of 50µg/ml of esomeprazole magenisum trihydrate, 250 µg/ml of naproxen and 50µg/ml ketoprofen.

Construction of calibration matrices and curves: A training set of ten synthetic mixture solutions in different combinations containing 3-12µg/ml (ESO) and 50-150µg/ml (NAP) was used to develop the chemometric calibrations. A validation set containing ten synthetic binary mixtures in the range of 4-10 µg/ml and 60-130µg/ml for (ESO) and (NAP), respectively, was prepared using the above stock solutions. Triplicate 20 µl injections were made for each solution and chromatographed under the specified conditions using 20 µg/ml diclofenac sodium as internal standard (IS).

For HPLC-multivariate analysis purpose, the ratio of peak areas was calculated to be manipulated by the Chemometrics Toolbox 3.02 software [37] for use with MATLAB 6. For classical HPLC purpose, the ratio of peak areas of the training mixtures were plotted versus the concentrations.

Conditions: A good chromatographic separation between the two drugs with diclofenac sodium as an internal standard (IS) was achieved using Inertsil® C18 Column 5µm (4.6 x 250mm) and a mobile phase containing 20 mM acetate buffer (pH=4.2) and acetonitrile [35 : 65, v/v] at flow rate 1.5 ml/ min at ambient temperature.

For chemometric -HPLC methods: Quantitation based on peak area was achieved using DAD at different five wavelengths (293, 295, 297, 299 and 301 nm).

For classical HPLC method: Quantitation based on peak area was achieved using DAD at 299 nm.

Preparation of synthetic mixture: US-FDA had approved a formulation (VIMOVO ®) containing 375 mg Naproxen and 20 mg esomeprazole. Referring to Preparation of synthetic mixture [38], Formula per tablet contains [27]: Naproxen - 375 mg, Esomeprazole - 20 mg and the excipients: microcrystalline cellulose, starch paste, talc and magnesium stearate. An amount of the tablet mass equivalent to one tablet content 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.

RESULTS AND DISCUSSION

Method Development and Optimization: The HPLC method depended on reversed - phase separation using a 250 mm x 4.6 mm (i.d.) Inertsil®C18 column (5µm particle size). The method has been optimized after studying the effect of different parameters on the separation. Several mobile phase systems with other chromatographic conditions were tested for experimental optimization. The mobile phase was chosen after several trials with acetonitrile and acetate buffer solutions in various proportions and at different pH. A mobile phase consisting of 20 mM acetate buffer (pH=4.2) and acetonitrile [35: 65, v/v] at flow rate 1.5 ml/min with injection volume 20 ml was found to be suitable for separation and determination of ESO and NAP in their binary and synthetic mixtures using DIC as internal standard (Figs. 3 and 4) in a short run time with good system suitability parameters (Table 1) calculated by Chromeleon® 7.1 Chromatography Data System software. Chromatographic separation was carried out at ambient temperature. The retention times at a flow rate of 1.5 ml/min were found to be 2.233± 0.011 min for ESO (I), 3.257 ± 0.016 min for NAP(II) and 4.521 ± 0.022 for IS for ten replicates. Run time was found to be less than 5 min. Chromatograms corresponding to the training set (Table2) in the range of 3-12µg/ml (ESO) and 50-150µg/ml (NAP) were used to develop the chemometric calibrations using 20 µg/ml (DIC) as IS. A validation set (Table 3) containing ten synthetic binary mixtures in the range of 4-10 µg/ml and 60-130µg/ml for (ESO) and (NAP), respectively, was prepared. Triplicate 20 µl injections were made for each solution and chromatographed under the specified conditions using 20 µg/ml (DIC) as IS by diode array detector using a five wavelength set; 293, 295, 297, 299 and 301nm, as shown in Fig.3. Introduction of multiwavelength DAD to the HPLC systems make possible, simultaneous multi-detection of samples at multiwavelengths. 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 esomeprazole magenisum trihydrate and naproxen. Data 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. The obtained multiwavelength detections produce different peak area information about qualitative and quantitative properties of the analyzed compounds. For a comparison of these HPLC-chemometric calibrations, a mixture of the subjected drugs was analyzed by the classic HPLC method using a single wavelength detection response. The experimental results of the HPLC-chemometric calibration methods were compared with each other, as well as with those obtained by classic-HPLC method. In the present study, the main advantage of multivariate calibration techniques based on the multivariate HPLC data is the elimination of errors of single regression equation based on single wavelength resulting from sample injection and experimental environment that may affect the peak area. Therefore, HPLC-chemometric calibration permits the removal of errors and residuals of calibration of classic HPLC using single wavelength detection. Thus, the

sensitivity, accuracy and precision of the HPLC-chemometric calibrations are higher than that provided by the classic- HPLC method. In addition, HPLC-chemometric methods provide high purity assessment via DAD empowered by PCR and PLS. Multichromatographic data obtained in one run by DAD, reduces the number of injections and saves time and reagents. Implementation of the multivariate calibration algorithms is applied in the following section.

Processing of HPLC Data: A training set consisting of the mixture solution in the concentration range of 3-12µg/ml (ESO) and 50-150µg/ml (NAP) with 20µg/ml IS was prepared. The ratio of peak areas for the training set was obtained at a five wavelength set (293, 295, 297, 299 and 301nm) and at corresponding retention time for each drug. The HPLC data set corresponding to the training mixtures is given in Table 4. The chemometric calibration techniques, CLS, PCR, and PLS, were applied to the prepared training set and its measured HPLC data set. The amount of esomeprazole magenisum trihydrate and naproxen in synthetic mixtures was determined by the HPLC-chemometric calibrations.

HPLC- chemometric techniques

HPLC-CLS Approach: The coefficient matrix (*K*) was calculated using the linear equation system based on the relationship between the peak area data and training set (Table 4). By replacing the coefficient matrix (*K*) into the linear equation system, HPLC-CLS calibration was obtained. The prediction of an unknown concentration of ESO and NAP in their binary and synthetic mixtures 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 [37] for use with MATLAB 6.

HPLC-PCR Approach: The HPLC-PCR calibration was constructed using the PCR algorithm. In this case, the square matrix of peak area data was obtained by decomposition of peak area ratio values. Linear correlation between the training set and decomposed peak area ratio values was used to obtain the HPLC-PCR calibration. This procedure was applied individually for ESO and NAP, respectively. The obtained HPLC-PCR calibration was subjected to the determination of both drugs in the synthetic mixtures. The data given in Table 4 were used for HPLC-PCR calibration. Chemometrics Toolbox 3.02 software [37] for use with MATLAB 6 was used for both the calculation of calibration and data treatment.

HPLC-PLS Approach: PLS calibration algorithm was applied to HPLC data summarized in Table 4. 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 ratio data and concentration set. The amount of ESO and NAP in their binary and synthetic mixtures was determined using the HPLC-PLS calibration. The mathematical treatments have been performed by means of the Chemometrics Toolbox 3.02 software [37] for use with MATLAB 6.

In order to validate the developed calibrations, an independent set of validation synthetic mixtures containing esomeprazole magenisum trihydrate and naproxen in the different compositions given in Table 3, was prepared and analyzed. The mean percentage recoveries, standard deviations (S.D.) and relative standard deviations (R.S.D.) are indicated in Table 5. The results indicate the high accuracy and precision of the developed HPLC-chemometric methods.

Statistical analysis: The key step in factor space analysis is determining how many factors to be used in the PCR and PLS calibrations. Only those factors that contain analytical information must be kept. The discarded factors should contain only noise [39, 40]. 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 [39, 41] 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
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 [39]. 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 [39]. To develop the HPLC-PCR model for esomeprazole magenisum trihydrate (ESO), the following indicator functions have been used to select the optimum number of factors: PCAREV (as shown in Fig. 5), PCRCROSS and PCRPRESS. A rank of one factor was found to be the

optimum system rank according to all the studied indicators. To develop the HPLC-PLS model for esomeprazole magenisum trihydrate (ESO), a rank of one factor was also found to be the optimum system rank according to PLSCRS (Fig. 6) and PLSPRS indicators. To develop the HPLC-PCR model for naproxen (NAP), the following indicator functions have been used to select the optimum number of factors: PCAREV, PCRCROSS and PCRPRESS (as shown in Fig. 7). A rank of one factor was found to be the optimum system rank according to all the studied indicators. To develop the HPLC-PLS model for naporoxen (NAP), a rank of one factor was also found to be the optimum system rank according to PLSCRS and PLSPRS (Fig. 8) indicators.

According to the studied indicators, the HPLC-PCR and HPLC-PLS models were constructed using one factor 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) [39, 42]. The MSEP and RMSEP characterize both the accuracy and the precision of prediction [42].

$$SEP= [\sum^{n}_{i=1} (C_i^{Predicted} - C_i^{True})^2 / n-1]^{½}$$

$$MSEP= \sum^{n}_{i=1} (C_i^{Predicted} - C_i^{True})^2 / n$$

$$RMSEP= [\sum^{n}_{i=1} (C_i^{Predicted} - C_i^{True})^2 / n]^{½}$$

$$s^2 = \sum^{n}_{i=1} (C_i^{Predicted} - C_i^{True} - bias)^2 / n-1$$

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 6. The small values of the calculated validation diagnostics indicate the negligible error of prediction and the high predictive ability of the proposed methods.

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 [40]. 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 6. The absence of bias was proved by determining the confidence limits for the intercept, a, and the slope, b, at the 95% significance level [43]. The upper and lower confidence limits are shown in Table 6. For ESO and NAP, 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.

Analysis of synthetic mixture: The proposed CLS, PCR and PLS methods were applied to the simultaneous determination of esomeprazole magenisum trihydrate (ESO) and naproxen (NAP) in synthetic mixture. Three replicates were determined. Satisfactory results were obtained for each compound in good agreement with label claim (Table 7).

Classic HPLC Method: In the classic HPLC technique, the ratio of peak area of analyte to IS was plotted against the concentration for each drug. At wavelengths of 293, 295, 297, 299 and 301nm. Five linear regression equations for each drug were obtained from the HPLC data given in Table 4. All linear regression equations and their statistical parameters are presented in Table 8. The correlation coefficients of regression equations were found to be higher than 0.999. At a specific wavelength of 299 nm, a linear equation giving successful results for each drug was selected from Table 8. At the subject wavelength point, the calibration equations gave us good linearity for esomeprazole magenisum trihydrate (ESO) and naproxen (NAP). The developed classical HPLC system was applied to the simultaneous determination of esomeprazole magenisum trihydrate (ESO) and naproxen (NAP) in their binary mixtures (Table 9).

Method Validation

The method was validated as per ICH guidelines [44].

Linearity and Range: Linearity of HPLC detector response for determination of ESO and NAP was evaluated by analyzing a series of standard solutions of different concentrations of each compound. Calibration graphs established for standards containing 4-12µg/ml for ESO and 50-150 µg/ml for NAP at 299 nm for ESO and NAP, respectively. Regression data of the calibration graphs are given in Table 10. The good linearity of the calibration graphs and negligible scatter of the experimental points is clearly evident by the values of the correlation coefficients and the standard deviations around the slope and the intercept (Table 10).

Accuracy: It was carried out to determine the suitability and reliability of the proposed method. Accuracy of the method was determined by calculating the mean percentage recovery of triplicate determination for ESO and NAP at three concentrations within the linearity range. The mean percentage recoveries were found to be 99.617 ± 0.973 % and 100.494± 1.073% for ESO and NAP, respectively.

Specificity: According to ICH document for specificity [44], the method is specific when the results are unaffected by the presence of the dosage form excipients, so the above results demonstrated the specificity of the method. Furthermore, the specificity of the proposed HPLC method was confirmed by comparing the chromatograms of standards and test solutions. The average retention times ± standard deviation for ESO and NAP in the synthetic mixtures were found to be 2.232 ± 0.052 (2.233± 0.011 min for standard ESO), and 3.263± 0.071 (3.257 ± 0.016 min for standard NAP), respectively.

Precision: Repeatability (intra-day precision) was determined by calculating the relative standard deviations (% RSD) for triplicate determinations of three different test concentrations of ESO and NAP within the linearity range in the same day. Intermediate (inter-day) precision was calculated by the relative standard deviations (%RSD) by triplicate determinations of ESO and NAP at three concentrations within the range of the linearity on three different days. The relative standard deviations were found to be less than 2% for ESO and NAP (Table11). The accuracy and precision were furthermore confirmed by comparing the results obtained for the assay of synthetic mixtures using the developed HPLC method to those of the studied HPLC-chemometrics methods. The results obtained were statistically analyzed and compared using t-test and F-test. At 95% confidence level, the difference in the mean percentage recovery (t-test) or in variance (F-test) was not statistically significant (Table 13). Therefore, there is no significant difference between the methods with regard to accuracy (t-test) and precision (F-test).

Detection and Quantitation limits: According to ICH recommendations [44], the approach based on the S.D. of the response and the slope of calibration curve was used for determining of detection and quantitation limits. The theoretical values were assessed practically and are given in Table 10.

***Robustness*:** Variation of pH of the buffer used in the mobile phase by ± 0.2 pH unit as well as variation of methanol % in the mobile phase by ± 4% did not give significant effect on the chromatographic separation.

Stability: The synthetic binary mixture of ESO and NAP in mobile phase was found to be stable for 24 hours when kept at room temperature and 3 days when stored refrigerated at 5 ^{o}C based upon 98% recovery limit.

Analysis of synthetic mixtures: The proposed HPLC method was applied to the simultaneous determination of ESO and NAP in their synthetic mixtures. Three replicates were determined. Satisfactory results were obtained for each drug (Table 12). The assay results of the proposed HPLC-CLS, HPLC-PCR and HPLC-PLS methods were compared to those of the proposed classical HPLC method. Statistical comparison between the results was performed with regards to accuracy and precision using student's t-test and F-test at 95% confidence level (Table 13). The calculated values did not exceed the tabulated (theoretical) values, indicating that there is no significant difference between the methods compared. The results obtained by the HPLC-CLS method are not significantly different from those obtained by the factor based methods (HPLC-PCR and HPLC-PLS). This confirmed that the excipients in the dosage form did not show any interference.

CONCLUSION

In this study, three HPLC-chemometric multivariate approaches (HPLC-CLS, HPLC-PCR and HPLC-PLS) and a new validated classic HPLC method were developed and applied to the simultaneous determination of esomeprazole magenisum trihydrate and naproxen in their synthetic mixtures. The experimental results obtained from HPLC-chemometric calibrations were compared with those obtained by a classic HPLC method. There is no significant difference between the results of the methods compared. However; the developed HPLC-chemometric techniques save the time as multichromotographic data based on DAD responses obtained in one run thus reduces the number of injections, saves time and reagents as well as being self-validated multivariate tools of analysis and do not require tedious validation steps. Furthermore, HPLC-chemometric techniques using DAD 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 purity assessment via DAD empowered by PCR and PLS techniques.

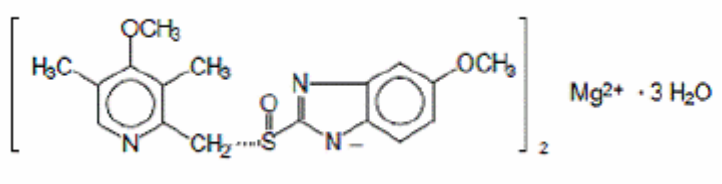

Fig. 1: Chemical structure of esomeprazole magenisum trihydrate.

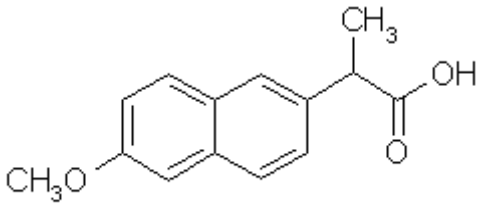

Fig 2: Chemical structure of naproxen.

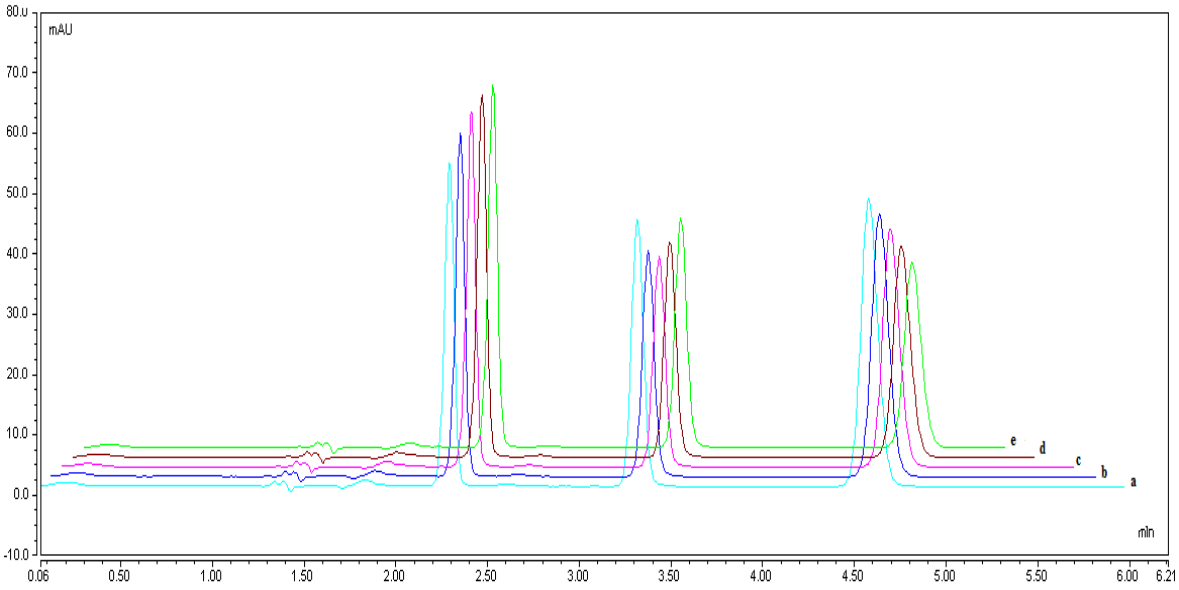

Fig.3: HPLC chromatograms of 20 µl injection of a mixture in training set containing 10 µg/ml ESO (I) and 100 µg/ml NAP (II) using 20 µg/ml DIC (IS) at five different wavelengths (293 (a) , 295(b) , 297(c), 299(d) and 301(e) nm).

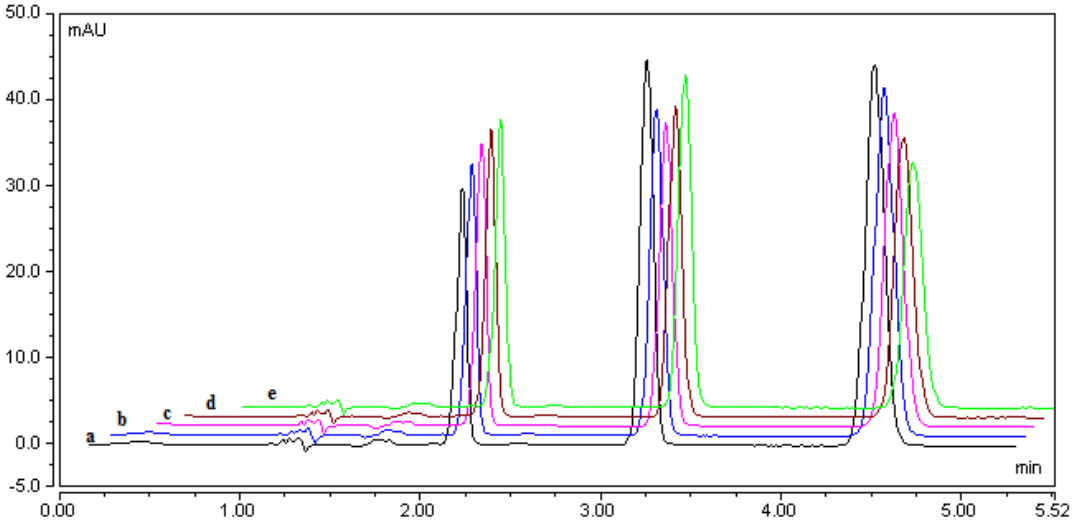

Fig. 4: HPLC chromatograms of 20 µl injection of synthetic solution of ESO and NAP in presence of 20 µg/ml DIC (IS) at five different wavelengths (293, 295nm, 297nm, 299nm and 301nm).

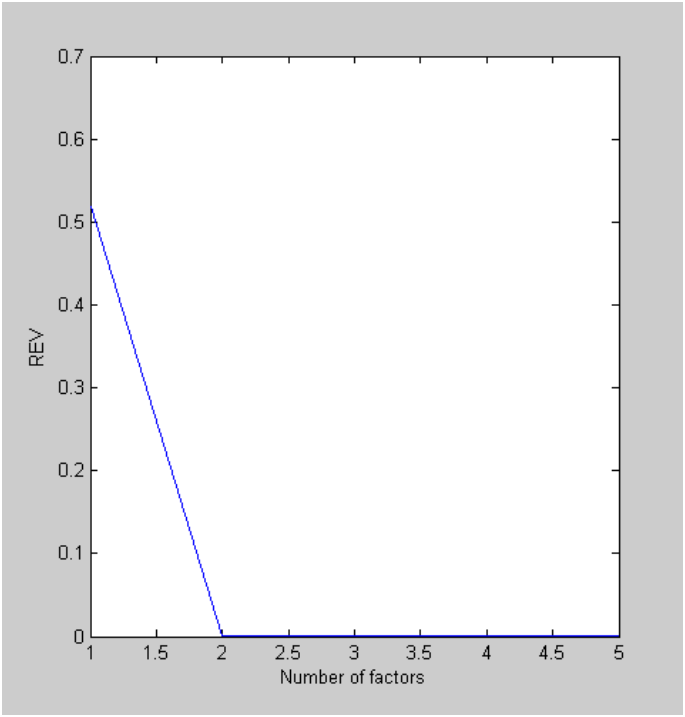

Fig. 5: Reduced eigenvalues (REV) versus number of factors for the PCR model of ESO.

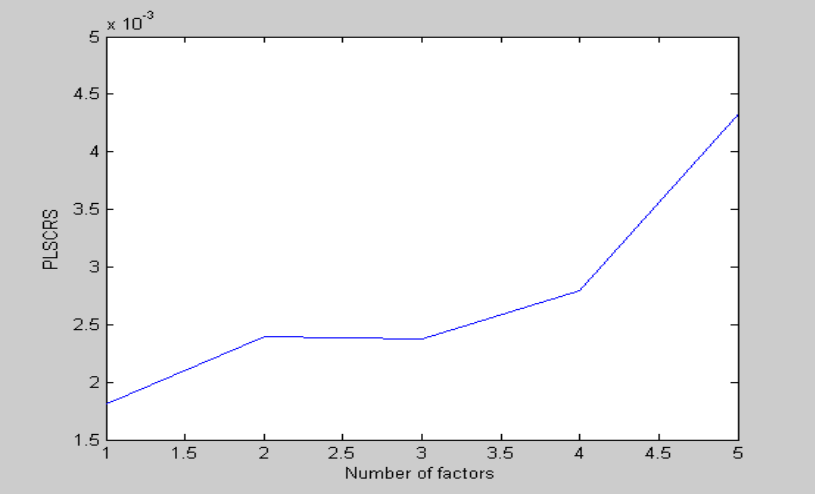

Fig.6: PLSCRS versus number of factors for the PLS model of ESO.

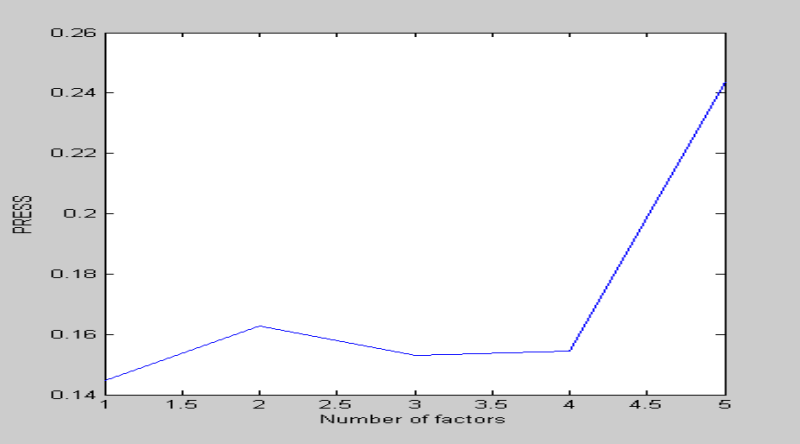

Fig.7: PRESS versus number of factors for the PCR model of naproxen.

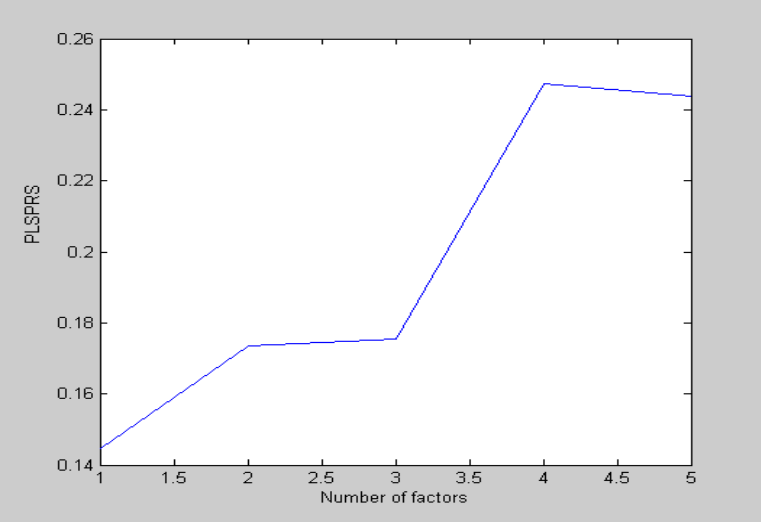

Fig. 8: PLSPRS versus number of factors for the PLS model of naproxen.

Table 1: System suitability parameters for the proposed chromatographic separation

Drug	Retention Time (min)	Capacity Factor k	Selectivity α	Resolution R	Asymmetry Factor A_s	Plate Count N
ESO	2.233	0.86	1.03	…..	0.88	7128
NAP	3.257	1.71	1.96	8.33	0.81	8605
DIC	4.521	2.77	3.11	7.51	1.03	8421

Table 2: Mixtures of esomeprazole magenisum trihydrate and naproxen used in the training set

Mixture no.	ESO (µg/ml)	NAP (µg/ml)
1	3	65
2	4	75
3	5	130
4	6	50
5	7	110
6	8	150
7	9	80
8	10	100
9	11	60
10	12	70

Table 3: Mixtures of esomeprazole magenisum trihydrate and naproxen used in the validation set

Mixture no.	ESO (µg/ml)	NAP (µg/ml)
1	7	120
2	8	130
3	6	60
4	4	75
5	5	90
6	6	112.5
7	8	80
8	9	95
9	7	100
10	10	110

Table 4: The HPLC data corresponding to the training set

Training	**The ratio of peak areas (ESO/ IS)**					**The ratio of peak areas (NAP/ IS)**				
Mixture no.	293	295	297	299	301	293	295	297	299	301
1	0.17875	0.22887	0.25585	0.30062	0.35566	0.41657	0.38722	0.40264	0.45912	0.57565
2	0.24485	0.29707	0.35207	0.40122	0.45483	0.47834	0.45192	0.46327	0.52844	0.65979
3	0.31458	0.37128	0.43833	0.50384	0.57087	0.82751	0.78181	0.79948	0.91536	1.14898
4	0.38202	0.44041	0.52491	0.60124	0.69131	0.31718	0.30892	0.31655	0.35013	0.44014
5	0.45048	0.52257	0.61504	0.69999	0.79975	0.69681	0.66385	0.67492	0.77271	0.96979
6	0.50739	0.60949	0.70071	0.80316	0.89865	0.94104	0.90615	0.93811	1.05341	1.31556
7	0.57126	0.67522	0.79097	0.90548	1.01782	0.50416	0.48101	0.49667	0.56911	0.70319
8	0.62469	0.75271	0.86551	0.99103	1.13337	0.64358	0.60188	0.61615	0.70506	0.87954
9	0.69206	0.82531	0.95131	1.08834	1.24413	0.38405	0.35441	0.38096	0.42878	0.52445
10	0.75271	0.90911	1.04982	1.20068	1.37683	0.44947	0.41922	0.43157	0.49658	0.60685

Table 5: Assay results of esomeprazole magenisum trihydrate (ESO) and naproxen (NAP) combinations in synthetic mixtures (validation mixtures) by the proposed HPLC-chemometrics methods

Validation mixture no.	Found %					
	ESO			NAP		
	CLS	PCR	PLS	CLS	PCR	PLS
1	100.16	100.15	100.15	100.58	100.57	100.57
2	99.94	99.93	99.93	100.10	100.10	100.10
3	100.65	100.64	100.64	101.06	101.06	101.06
4	98.57	98.56	98.56	100.07	100.06	100.06
5	100.08	100.08	100.08	99.37	99.36	99.36
6	100.14	100.13	100.13	99.95	99.95	99.95
7	100.21	100.21	100.21	100.32	100.32	100.32
8	99.89	99.89	99.89	99.72	99.72	99.72
9	100.13	100.13	100.13	100.34	100.34	100.34
10	99.99	99.98	99.98	99.77	99.77	99.77
Mean	99.976	99.970	99.97	100.128	100.125	100.125
± S.D.	0.536	0.537	0.537	0.478	0.479	0.479
R.S.D.%	0.536	0.537	0.537	0.478	0.479	0.479

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

Table 6: Statistical parameters of the validation set mixtures of esomeprazole magenisum trihydrate (ESO) and naproxen (NAP) using the proposed HPLC - chemometrics methods

Method	SEP	MSEP	RMSEP	s^2	a	Lower* 95%	Upper* 95%	b	Lower** 95%	Upper** 95%	r
ESO											
CLS	0.0246	0.0005	0.0234	0.0006	-0.0233	-0.0998	0.0532	1.0035	0.9929	1.0141	0.99991
PCR	0.0246	0.0005	0.0234	0.0006	-0.0233	-0.0999	0.0532	1.0035	0.9929	1.0141	0.99992
PLS	0.0246	0.0005	0.0234	0.0006	-0.0233	-0.0999	0.0532	1.0035	0.9929	1.0141	0.99992
NAP											
CLS	0.4010	0.1447	0.3804	0.1458	0.1171	-1.3228	1.5570	0.9998	0.9853	1.0143	0.99984
PCR	0.4009	0.1446	0.3803	0.1484	0.1171	-1.3228	1.5571	0.9998	0.9853	1.0143	0.99984
PLS	0.4009	0.1446	0.3803	0.1484	0.1171	-1.3228	1.5571	0.9998	0.98539	1.0143	0.99984

*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.*

Table 7: Determination of esomeprazole magenisum trihydrate (ESO) and naproxen (NAP) in their synthetic mixture by the developed HPLC- chemometrics methods

	Percentage found*					
Sample	**ESO**			**NAP**		
	HPLC - CLS	HPLC - PCR	HPLC - PLS	HPLC - CLS	HPLC - PCR	HPLC - PLS
1	98.97	98.97	98.97	101.41	101.41	101.41
2	99.66	99.66	99.66	99.37	99.37	99.37
3	100.65	100.64	100.64	99.68	99.68	99.68
Mean	99.76	99.757	99.757	100.153	100.153	100.153
± S.D.	0.840	0.839	0.839	1.099	1.099	1.099
%R.S.D.	0.842	0.841	0.841	1.098	1.098	1.098

Table 8: The calculated calibration straight lines and its statistical parameters

Drug	Wavelength (nm)	Regression equation	r	S_a	S_b	S_r
ESO	293	Y=0.0635 C+ 0.0041	0.99953	0.00551	0.0006858	0.006229
	295	Y=0.0759 C+ 0.0058	0.99973	0.00493	0.0006137	0.005574
	297	Y=0.0870 C+ 0.0017	0.99982	0.00477	0.0005942	0.005397
	299	Y=0.0992 C+ 0.0058	0.99987	0.00459	0.0005715	0.005191
	301	Y=0.1128 C+ 0.0085	0.99972	0.00757	0.0009372	0.008512
NAP	293	Y=0.0063 C+0.0083	0.99973	0.00488	5.1815E-05	0.005061
	295	Y=0.006 C+0.0022	0.99981	0.00402	4.2641E-05	0.004165
	297	Y=0.0062 C+0.0040	0.99960	0.00579	6.1438E-05	0.006001
	299	Y=0.007 C+0.0056	0.99993	0.00281	2.9799E-05	0.002911
	301	Y=0.0088 C+0.0023	0.99989	0.00416	4.4203E-05	0.004317

Y, peak area ratio ; C,concentration (µg/ml); r, correlation coefficient; S_a, standard error of intercept; S_b, standard error of slope; S_r, standard error of regression constant.

Table 9: Assay results of ESO and NAP combinations in their binary mixtures (validation mixtures) by the proposed classic HPLC method

Mixtures		**Recovery (%)**	
Added (µg/ml)			
ESO	**NAP**	**ESO**	**NAP**
7	120	99.9	100.52
8	130	98.75	100.33
6	60	100.22	101.47
4	75	97.86	99.33
5	90	99.36	100.68
6	112.5	100.37	100.03
8	80	99.19	101.33
9	95	100.09	99.08
7	100	100.09	100.92
10	110	99.1	99.94
Mean		99.493	100.363
± S.D.		0.793	0.789
R.S.D.%		0.797	0.786

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

Table 10: Characteristic parameters of the calibration equations for the proposed classic HPLC method for the simultaneous determination of ESO and NAP

Parameters	ESO	NAP
Calibration range (µg/ml)	3-12	50-150
Detection limit (µg/ml)	0.451	8.564
Quantitation limit (µg/ml)	1.368	25.95
Regression equation(Y) [a]: Slope (b)	0.995453	0.993828
Standard deviation of the slope (S_b)	0.00914	0.010444
Intercept (a)	-0.00118	0.89204
Standard deviation of the intercept (S_a)	0.06591	1.03817
Residual standard deviation $S_{Y/X}$	0.06282	0.76688
Correlation coefficient (r)	0.99966	0.99956

[a] $Y = a + bC$, where C is the concentration of compound in µg/ml and Y is the peak area.

Table 11: Evaluation of the precision the proposed classic HPLC method for the determination of ESO and NAP combinations in their binary mixtures

Parameters	Concentration taken (µg/mL)					
	ESO			NAP		
	5	7	10	90	120	110
	Intra-day					
***Mean %recovery**	100.40	98.86	99.98	99.97	100.05	100.14
%R.S.D	1.035	1.287	1.049	1.374	1.225	0.545
	Inter-day					
***Mean %recovery**	101.05	99.83	100.46	100.41	100.71	100.86
%R.S.D	1.140	1.371	1.241	1.414	1.34	0.96

* The mean % recovery is calculated from three determinations of each concentration.

Table 12: Determination of ESO and NAP in their laboratory prepared mixtures by the developed classic HPLC method

	Percentage found*	
Sample	**ESO**	**NAP**
1	98.88	101.76
2	99.65	100.25
3	100.95	99.80
Mean	99.827	100.603
± S.D.	1.046	1.027
%R.S.D	1.048	1.021

Table 13: Collective table comparing the proposed HPLC-chemometric and classic HPLC methods used for the determination of ESO and NAP in their laboratory prepared synthetic mixtures

Parameter	HPLC-CLS	HPLC-PCR	HPLC-PLS	Classic HPLC
ESO				
Mean [a] **± S.D.**	99.760 ± 0.840	99.757 ±0.839	99.757 ±0.839	99.827 ±1.046
%R.S.D.	0.842	0.841	0.841	1.048
t	0.08641	0.08654	0.08654	(2.776) [b]
F	1.551	1.554	1.554	(19) [b]
NAP				
Mean [a] **± S.D.**	100.153 ±1.099	100.153 ±1.099	100.153 ±1.099	100.603 ±1.027
%R.S.D.	1.098	1.098	1.098	1.021
t	0.518	0.518	0.518	(2.776) [b]
F	1.145	1.145	1.145	(19) [b]

[a] Mean percentage found of five different concentration levels.
[b] Theoretical values for t (0.05) and F (0.05).

REFERENCES:

1. http://www.rxlist.com/vimovo-drug.htm.
2. O'Neil M, Heckelmann PE. The Merck's Index, An Encyclopedia of Chemicals, Drugs and Biologicals. 14 th ed., USA; Merck Research Laboratory: 2006, pp. 6417, 8997.
3. Sean CS. The complete drug reference Martindale. 34 th ed., London; Pharmaceutical Press: 2005, pp. 65-66.
4. Indian Pharmacopoeia, Vol-II, Govt. of India, Indian Pharmacopoeial Commission. Ghaziabad; Ministry of Health and Family Welfare: 2010, pp. 1754-1758, 2173-2175.
5. British Pharmacopoeia, Vol- II, The Department of Health. London; The Stationary Office: 2009, pp. 1427-1430, 1962-1966.
6. United State Pharmacopoeia. 31 th ed., Vol- III, Rockville; USP Convention: 2008, pp. 2760-2763, 3310-3313.
7. Scott LJ, Dunn CJ, Mallarkey G, Sharpe M. Drugs, 2002; 62(10): 1503- 1508.
8. Trivedi PD, Maheshwari DG. Int J ChemTech Res, 2010; 2(3): 1598-1605.
9. Hassan-Alin M, Andersson T, Bredberg E, Röhss K. Eur J Clin Pharmacol, 2000; 56(9): 665–670.
10. Moreno MA, Torrado S, Lastres JL. Pharm Biomed Anal, 1999; 21: 291–298.
11. Ozaltin N, Kocer A. J Pharm Biomed Anal, 1997; 16: 337–342.
12. Patil SS, Dhabale PN, Kuchekar B. Asian J Research Chem, 2009; 2(2):154-156.
13. Basavaiah K, Anil Kumar UR. Indian J Chem Technol, 2007; 14(6): 611-615.
14. Devi OZ, Vasavaiah K, Vinay KB. Chem industry chem engineering quarterly, 2010; 16(1): 97-102.
15. Basavaiah K, Rajendraprasad N, Tharpa K, Anilkumar UR, Hiriyanna SG, Vinay KB. J Mex Chem Soc, 2009; 53(1): 34-40.
16. Dogrukol AK, Tunalier Z, Tuncel M. Pharmazie, 1998; 53(4): 272–273.
17. Petersen KU, Schmutzler W. Detsche Apotheker Zeitung, 1999; 139: 68–69.
18. Sluggett GW, Stong JD, Adams JH, Zhao Z. J Pharm Biomed Anal, 2001; 25(3): 357–361.

19. Johnson DA, Roach AC, Carlsson AS, KarlssonAA, Behr DE. Pharmacotherapy, 2003; 23(6): 731–734.
20. Warmorkar V, Santosh P, Manjunth SY. J Pharm Res, 2011; 4(8): 2633-2635.
21. Toshi H, Shozo M, Ikea K. J Chromatogr B, 1997; 692(2): 375-388.
22. Mouiay H, Nadia A, Hocine C. J Assoc Anal Chem Inter, 2000; 83(6): 1489-1492.
23. Jain NA, Lohiya RT, Umekar MJ. Int J Pharm Sci Res, 2011; 2(5): 130-134.
24. Sojitra C, Pathak A, Rajput S. Int J Chem Tech Res, 2012; 4(2): 602-608.
25. Mahaparale SP, Mahaparale PR, Gonjari ID. Int J Res Develop Pharm Life Sci, 2013; 2(2): 377-382.
26. Patel M, Vohra J, Kakadiya J, SHAH KH. Res Bio Sci, 2012; 1(2): 274-286.
27. Vetrichelvan T,.Suresh J. Int J Pharm Res, 2012; 1(1): 1-3.
28. Mahaparale SP, Mahaparale PR, Gonjari ID. Pharma Utility, 2012; 6(3): 1-9.
29. Jain N, Kulkarni S, Jain DK, Jain SK. Acta Poloniae Pharm Drug Res, 2012; 69(6): 1195-1199.
30. Vani P, Kottapalli KS. Int J Pharm.Technol, 2013; 3(4): 3446-3455.
31. Kumar SA, Manidipa Debnath, Seshagiri Rao JVLN. Int J Pharm Sci Res, 2013; 4(8): 2988-2999.
32. Sojitra C, Rajput S. Int J Pharm Pharm Sci, 2012; 4(3): 533-537.
33. Jain DK, Jain N, Charde R. Pharm Methods, 2011; 2(3): 167–172.
34. Ampati S, Lagishetti S, Bairi AG. Int J Pharm, 2014; 4(3): 95-104.
35. Bhavyasri K, Balaram MV, Nageswarao R, Rambabu D, Ajitha M. J Chem Pharm Res, 2013; 5(12): 1230-1236.
36. Rao TM, Prabhakar T, Sankar GG, Naidu PVL. Int J Pharm Sci, 2013; 3(2): 205-210.
37. Kramer R. Chemometrics TOOLBOX for use with MATLAB. Natick; The Math Works. Inc: 1995.
38. Rowe RC, Sheskey PJ, Weller PJ. Hand book of Pharmaceutical Excipients. 4th ed., USA; Pharmaceutical Press and The American Pharmaceutical Association: 2003, pp.108, 354, 603, 641.
39. Kramer R. Chemometric Techniques in Quantitative Analysis. New York; Marcel Dekker Inc,: 1998, pp.51-159.
40. Salinas F, Berzas-Nevado JJ, Espinosa-Mansilla A. Talanta, 1990; 37(3): 347-351.
41. Espinosa-Mansilla A, Salinas F, De Orbe Paya I. Anal Chim Acta, 1995; 313(1):103–112.
42. Kenneth KB, Randy JP, Seasholtz MB. Chemometrics A Practical Guide. New York; John Wiley and Sons Inc: (1998).
43. Miller JN, MillerJC. Statistics and Chemometrics for Analytical Chemistry. 4 th ed., England; Pearson Education Limited: 2000, pp. 127, 128.
44. The European Agency for the Evaluation of Medical Products, ICH Topic Q2B Note for Guidance on Validation of Analytical Procedures, Methodology GPMP/ICH/28 1/95, 1996.