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Determination of nimesulide in pharmaceutical and biological samples by a spectrophotometric method assisted with the partial least square method

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Abstract A simple and sensitive spectrophotometric method for the determination of nimesulide in bulk, in pharmaceutical dosage form, and in biological fluids was developed. The method is based on the reduction of the nitro group of nimesulide by zinc and hydrochloric acid followed by diazotization, and coupling with orcinol in basic medium to form a stable chromophore, which absorbs at 465 nm. The method showed a good linearity in the range 0.4–4.0 μg mL^{-1}. Partial least square modeling as a powerful multivariate statistical tool is also applied, compiled, and compared for determination of nimesulide. The experimental matrix for the partial least square calibration method was designed with 24 samples. The cross-validation was used for selecting the number of factors. The root mean square error prediction (RMSEP) and the relative error of prediction (REP %) were 0.089 and 3.95, respectively. The developed method is free from the interference of common excipients used in pharmaceutical dosages. The method was also used for the determination of nimesulide in pharmaceutical dosages as well as in human serum and urine samples.

Keywords Partial least square · Relative error of prediction · Root mean square error prediction · Spectrophotometric · Nimesulide · Orcinol

Introduction

Nimesulide is chemically known as *N*-[4-nitro-2-phenoxyphenyl] methanesulphonamide. It is used in pharmaceutical formulations for anti-inflammatory activity [1], and is a non-steroidal anti-inflammatory drug. The drug is a selective inhibitor of the prostaglandins synthesis enzyme, cyclooxygenase. Nimesulide also provides

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a better activity profile and high therapeutic index [2]. Clinically, studies have established the analgesic, anti-inflammatory and antipyretic effectiveness of nimesulide in the treatment of a variety of painful inflammatory conditions including those associated with fractures, soft tissue injury, post-operative trauma, sports injuries, ear, nose, and throat disorder, dental surgery, thrombophlebitis, and upper respiratory inflammations. Nimesulide therapy is characterized by a rapid onset of analgesic and symptomatic relief in pain where a significant difference in clinical efficacy between active treatments was observed [3]. Various methods have been developed for the determination of nimesulide. They include potentiometric titration [4], liquid chromatography [5–7], HPLC [8–10], capillary electrophoresis [11], voltammetry [12], and UV–visible spectrophotometry [13–21]. However, these methods are generally complex in nature and need expensive instruments and ultra pre-solvents. On the other hand, analysis of the clinical samples demands simple and fast analytical methods, and therefore finding an alternative analytical procedure or technique is crucial. Spectrophotometry combined with chemometric methods will be a simple analytical method for quantitative analysis.

PLS is a quantitative spectral decomposition technique that is closely related to principal component regression (PCR). However, in PLS, the decomposition is performed in a slightly different fashion. Instead of first decomposing the spectral matrix into a set of eigenvectors and scores, and regressing them against the concentrations as a separate step (as done in PCR), PLS actually uses the concentration information during the decomposition process. Thus, the eigenvectors and scores calculated using PLS is quite different from those of PCR. The main idea of PLS is to get as much concentration information as possible into the first few loading vectors. There are actually two versions of the PLS algorithm, PLS1 and PLS2. The differences between these methods are subtle but have very important effects on the results. In PLS1, a separate set of scores and loading vectors is calculated for each constituent of interest. In this case, the separate set of scores and loading vectors are specially tuned for each constituent and, therefore, should give a more accurate prediction than PCR and PLS2.

The present paper describes a simple and sensitive spectrophotometric method for the determination of nimesulide by its reduction, followed by diazotization coupling of reduced nimesulide with orcinol as a univariate method and PLS used as a multivariate method. The proposed method has been validated and applied to the determination of nimesulide in bulk, in pharmaceutical formulations, and in biological fluids.

Experimental

Apparatus

A Systronic spectrophotometer was used for absorbance measurement. The pH measurements were made with a Systronic digital pH-meter (model-335). The PLS program (for calibration-prediction and experimental design) of PLS-Toolbox (Eigenvector) was used.

Stock and reagent solution

Pharmaceutical grade nimesulide, sodium nitrite, and orcinol came from Aldrich. All other reagents and solvents were of analytical grade commercial dosage forms obtained from the local market. A 1,000 μg mL^{-1} stock solution of the drug was prepared by dissolving 100 mg of nimesulide in 200 mL methanol and then diluting with water up to the mark in a 100-mL volumetric flask. Then, 0.1 % (w/v) sodium nitrite aqueous solution, 0.1 % orcinol solution was prepared in double-distilled water, and 2 M sodium hydroxide and 3 % (w/v) sulfamic acid solution was also prepared in double-distilled water.

General procedure

Preparation of calibration curve

Nimesulide solution was treated with 10 mL of 1 M HCl and 0.25 gm of zinc dust and heated at 45 °C for 15 min. The solution was filtered and the residue was washed with 3 × 5 mL portions of methanol and diluted with methanol stepwise to prepare the working solution.

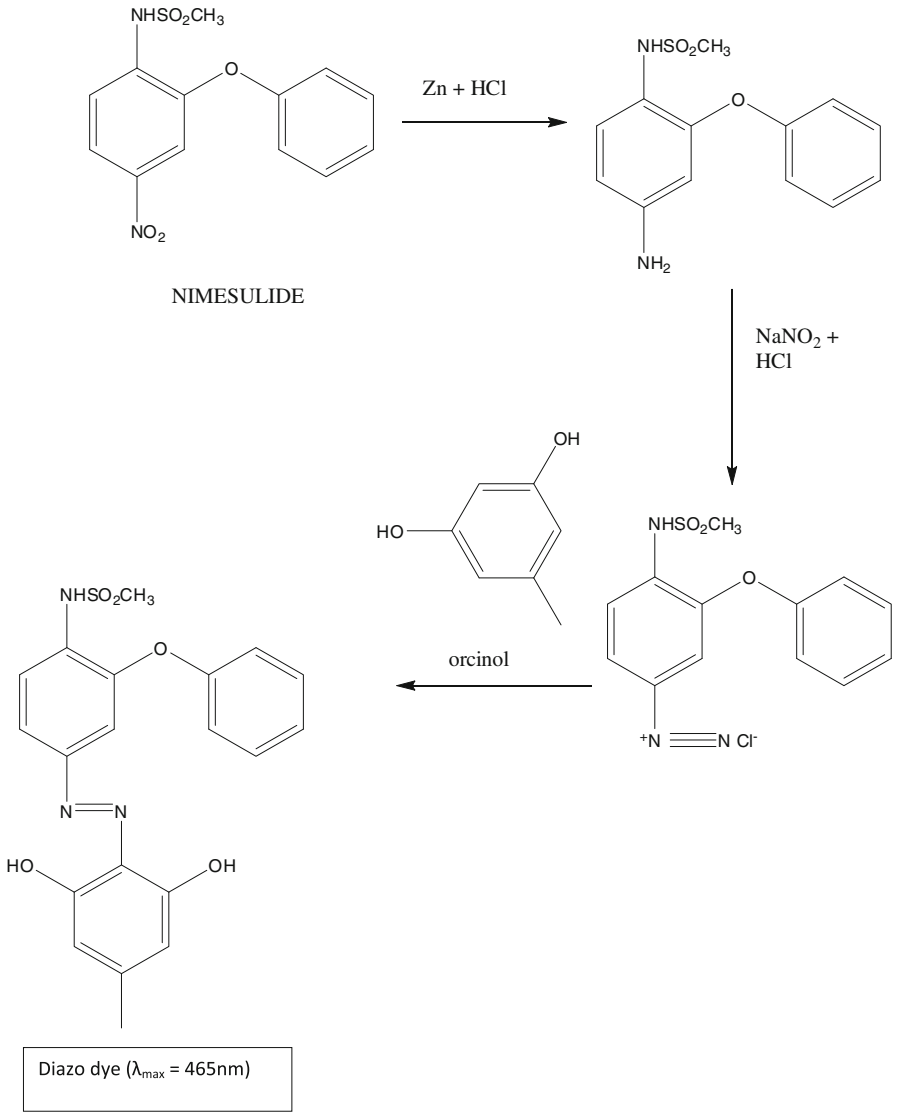
Aliquots of a reduced standard nimesulide solution (0.4–4.0 μg mL^{-1}) were placed in a series of 25-mL calibrated tubes. Then, 1 mL of 5 M HCl and 0.5 mL of sodium nitrite solution were added successively and allowed to stand for 5 min with occasional shaking in an ice bath. Excess nitrite was removed by addition of 1 mL of sulfamic acid, then 1 mL of orcinol solution was added. An orange-yellow solution was obtained after addition of 1 mL of 2 M sodium hydroxide solution. The solution was made up to the mark with distilled water and the absorbance was measured at 465 nm against a reagent blank, which gave negligible absorbance at this wavelength.

Procedure for the assay of dosage forms

The tablet formulations were purchased from a local market. Twenty tablets were powdered and mixed thoroughly, and an amount equivalent to 100 mg nimesulide was then dissolved in methanol, filtered, and then diluted with methanol up to 100 mL. Appropriate aliquots of the solution were taken and the recommended procedure, above, was followed.

Results and discussion

A new spectrophotometric method has been developed for the determination of nimesulide. The method depends upon diazotization of reduced nimesulide followed by coupling with orcinol in basic medium due to which an orange-yellow dye is formed (Scheme 1). The dye has an absorption maximum at 465 nm (Fig. 1).



Scheme 1 Color reaction

Effect of varying reaction conditions

The variation of one variable at a time optimized the reaction conditions.

Effect of reagent concentration

For reduction of the nimesulide molecule, the use of 10 mL of 1 M hydrochloric acid and 0.25 gm of zinc dust were found optimum. It was found that 0.5 mL of sodium

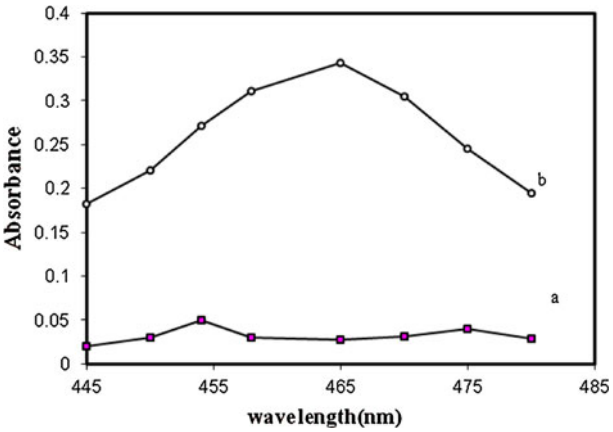


Fig. 1 Absorption spectra of **a** blank solution: 0.5 mL of 0.1 %(w/v) sodium nitrite, 1 mL of 0.1 %(w/v) orcinol, 1 mL of 3 % (w/v) sulfamic acid, and 1 mL of 2 M NaOH solution; **b** sample solution: nimesulide 1.6 μg mL^{-1} with blank solution in 25-mL volumetric flask and diluted to volume with distilled water

nitrite and 1 mL of orcinol were required for maximum color intensity. Excess amounts of nitrite caused no effect on the absorbance value as the addition of sulfamic acid solution removed excess nitrite. There was no significant change in the absorbance even if a large excess of orcinol was taken. An amount of 1 mL of 2 M NaOH was required for full color development; excess NaOH decreases the intensity of the color. Amounts of 0.5–5.0 mL of sulfamic acid do not affect the intensity of the dye so 1 mL of sulfamic acid was added for removal of excess nitrite.

Effect of pH, time and temperature

The reduction time of 15 min was sufficient to yield maximum absorbance. Since diazotization of 2 min or more gave the same results after addition of orcinol, it required 5 min for complete color development.

The effect of temperature on reduction, diazotization, and coupling was studied and the effect of temperature on the reduction rate was studied at various temperature ranges. The reduction rate was slow below 35 °C while it was instantaneous at temperatures above 35 °C, hence 45 °C was selected for reduction. Diazotization at 0–5 °C gave maximum color intensity whereas coupling rate below 10 °C was slow, hence room temperature was selected for coupling.

The effect of pH on the reaction was studied, and maximum intensity was found at pH range 10–11, hence 1 mL of 2 M NaOH was used in the study.

Method validation

Univariate calibration

The absorbance versus concentration was plotted and a linear correlation was found (Fig. 2). Beer's law was obeyed over the concentration range, the molar

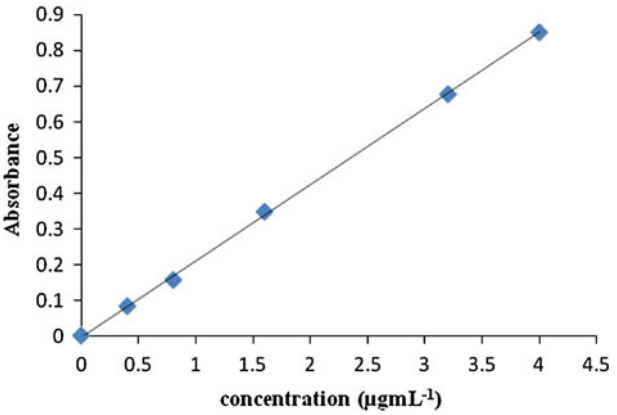


Fig. 2 Calibration graph

absorptivit,y and Sandell's sensitivity given in Table 1. The limits of detection (LOD), limit of quantification (LOQ), regression equation, and correlation coefficient values are given in Table 1, as are the LOD and LOQ calculated according to the current ICH guidelines [22].

The accuracy of an analytical method expresses the closeness between the reference value and the found value [22]. Accuracy was evaluated as the percentage relative error between the measured concentrations and the taken concentration for nimesulide. The precision of the method was calculated in terms of the intermediate precision (intra-day and inter-day) [23]. Three different concentrations of nimesulide (within the working limits) were analyzed in seven replicates during the same day and on five consecutive days. The SD and RSD values of intra-day and inter-day studies showed that the precision was good (Table 2).

Table 1 Analytical and regression parameters for spectrophotometric determination

Parameters	Results ($n = 6$)
λ_{max} (nm)	465
Beer's law limits (µg mL^{-1})	0.4–4.0
Molar absorptivity (L mol^{-1} m^{-1})	0.616×10^6
Sandell's sensitivity (µg cm^{-2})	0.005
Limit of detection (µg mL^{-1})	0.134
Limit of quantification (µg mL^{-1})	0.407
Regression equation ($Y = bX^* + a$)	
Slop (b)	1.213212
Standard deviation of slop (S_b)	0.003698
Intercept (a)	−0.00249
Standard deviation of intercept (S_a)	0.001048
Variance (S_a^2)	1.098×10^{-6}
Correlation coefficient	0.9998

*Concentration of sample in micro gram per mL

Table 2 Inter-day and intra-day precision and accuracy studies

Nimesulide taken (μg mL^{-1})	Intra-day ($n = 7$)			Inter-day ($n = 5$)		
	Found[a]	Precision[b]	Accuracy[c]	Found	Precision	Accuracy
1	1.01	1.69	1.43	1.01	1.49	1.16
2	2.03	0.84	1.78	2.03	1.02	1.5
3	3.04	0.80	1.33	3.03	0.78	1.16

[a] Mean value of five determinations

[b] Relative standard deviation (%)

[c] Bias %: [(found − taken)/taken] × 100

Multivariate calibration

Multivariate calibration methods such as PLS require a suitable experimental design of the standard belonging to the calibration set in order to provide good predictions. Two sets of standard solutions were prepared. The calibration set contained 16 standard solutions (Table 3), while the prediction set contained eight test mixtures (Table 4).

Selection of optimum number of factors

To select the number of factors in the PLS algorithm, in order to model the system without over-fitting the concentration data, a cross-validation method, leaving out one sample at a time, was used [24–27]. Given the set of 16 calibration spectra, the

Table 3 Concentration data of the calibration set for nimesulide

Solution nimesulide	No. (μg mL^{-1})
1	0.4
2	0.5
3	0.75
4	1.0
5	1.25
6	1.5
7	1.75
8	2.0
9	2.25
10	2.5
11	2.75
12	3.0
13	3.25
14	3.5
15	3.75
16	4.0

Table 4 Concentration data for the prediction set and their predicted values for nimesulide

Solution no.	Nimesulide (μg mL^{-1})		
	Added (μg mL^{-1})	Found (μg mL^{-1})	Error (%)
1	0.7	0.704	0.56
2	1.2	1.23	2.39
3	1.5	1.46	−2.65
4	1.9	1.93	2.05
5	2.3	2.31	0.49
6	2.8	2.78	−0.51
7	3.4	3.56	4.92
8	4.3	4.14	−3.58

PLS calibration were performed and, using this calibration, the concentration of the compounds in those left out during calibration was predicted. This process was repeated 16 times until each calibration sample had been left out once. The predicted concentration of the compounds in each sample was compared with the known concentration of the compound in this reference sample, and the prediction error sum of squares (PRESS) was calculated. The PRESS was calculated in the same manner each time a new factor was added to the PLS models. The maximum number of factors used to calculate the optimum PRESS was selected as 9 (half the number of standards plus one). One reasonable choice for the optimum number of factors would be that number which yielded the minimum PRESS. However, using the number of factors that yields a minimum PRESS, h*, leads to over-fitting. A better criterion for selecting the optimum number of factors involves the comparison of PRESS from models with fewer than h* factors. The *F* statistic was used to determine significance. Haalad and Thomas [24] empirically determined that the *F* ratio probability of 0.75 is a good choice. So, we also selected the optimum number of factors for the PRESS value of the *F* ratio probability, which drops below 0.75. The PRESS value has minimum value when the number of factors is 3 for nimesulide. Therefore, this number of latent variables was selected as the optimum number of factors for PLS1 model building. The results obtained by applying the PLS1 algorithm to the prediction samples are listed in Table 4. The percentage errors were also quite acceptable, as they never exceeded 5 %.

Statistical parameters

For the constructed model, four general statistical parameters were selected to evaluate the prediction ability of the model for determination of nimesulide. For this the predicted concentrations of each sample in calibration step were compared with the actual concentrations. The first statistical parameter is the root mean square difference (RMSD). This parameter is an expression of the average error in the analysis for each component in training samples. The RMSD was obtained by the following formula:

$$\mathrm{RMSD} = \left[\frac{1}{n}\sum_{i=1}^{n}\left(c_i' - c_i\right)^2\right]^{0.5}$$

The second statistical parameter was the relative error of prediction (REP) that shows the predictive ability of each component, and is calculated as

$$\mathrm{REP}\,(\%) = \frac{100}{\bar{c}}\left[\frac{1}{n}\sum_{i=1}^{n}\left(c_i' - c_i\right)^2\right]^{0.5}$$

The predictive applicability of a regression model is described in various ways. The most general expression is the standard error of prediction (SEP) and the standard error of calibration denoted by SEC which is given in the following formula:

$$\mathrm{SEP}\ (\mathrm{SEC}) = \left[\frac{\sum_{i=1}^{n}\left(c_i' - c_i\right)^2}{n-1}\right]^{0.5}$$

The square of the correlation coefficient (R^2), which is indicated as the quality fit among all the data to a straight line, is calculated for the checking of each calibration, and is calculated as:

$$R^2 = \frac{\left[\sum_{i=1}^{n}\left(c_i' - \bar{c}\right)^2\right]}{\left[\sum_{i=1}^{n}\left(c_i - \bar{c}\right)^2\right]}$$

where c_i is the actual concentration of the analyte in the sample i, c_i' the predicted concentration of the analyte in the sample i, c the mean of true concentration in the prediction set, and n the total number of samples used in the prediction set. The statistical results are summarized in Table 5.

Effect of foreign species

The effects of common excipients, such as talc, glucose, dextrose, etc., commonly used in pharmaceutical preparations were investigated under the optimal conditions. An amount in 1,000-fold excess of that used in pharmaceutical preparations was added in 0.1 μg mL^{-1} nimesulide solution and no effect due to these excipients was found under the proposed experimental conditions.

Table 5 Statistical parameters obtained by applying the PLS method to the synthetic mixtures

Parameter	PLS
RMSD	0.08945
REP %	3.9534
SEP	0.0894
R^2	0.975
PRESS	0.309
No. of factors	3

Table 6 Results of assay of tablets by the proposed methods and statistical evaluation

Tablet brand name	Nominal amount (mg/tab)	Literature method [4]	Proposed methods	
			Spectrophotometric method	PLS method
Nice	100	98.84 ± 0.25	99.06 ± 0.23	99.1 ± 0.22
			1.385*	1.514*
			1.173**	1.110**
Nisulide	100	98.66 ± 0.24	98.9 ± 0.25	98.9 ± 0.24
			1.897*	1.545*
			1.098**	1.077**
Nimulide	100	99.1 ± 0.27	99.44 ± 0.27	99.5 ± 0.21
			1.976*	2.28*
			1.027**	1.045**

Mean value of five determinations

Tabulated t value at the 95 % confidence level is 2.78; tabulated F value at the 95 % confidence level is 6.39

*t value

**F value

Application

Analysis of pharmaceutical preparations

The applicability of the proposed method for the assay of different pharmaceutical formulations containing nimesulide was examined for tablets. For this, 20 tablets were crushed and a drug equivalent to 100 mg was weighed accurately and dissolved in methanol, then diluted up to the mark with distilled water. The concentration was determined by applying the proposed method. The results were statistically compared with those obtained by the official method [4], and using Student's t test and F test were found not to differ significantly. The results summarized in Table 6.

Conclusion

Determination of nimesulide was based on its reduction followed by diazotization coupling as a univariate and a multivariate calibration method, and PLS1 modeling is presented as established. The method is very simple and has satisfactory prediction ability for the real samples. The results showed the approximate superiority of the PLS1 method over the diazotization method. The method has acceptable detection limits, and sensitivity and reproducibility are in the margin of these types of studies.

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References

1. A. Singh, P. Singh, V.K. Kapoor, *Analytical Profiles of Drug Substances and Excipients*, vol. 28 (Academic, New Jersey, 2001), p. 201
2. R. Davis, R.N. Brogden, Nimesulide: an update of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy. Drugs **48**(3), 431–454 (1994)
3. A. Ward, R.N. Brogden, Nimesulide: a preliminary review of its pharmacological properties and therapeutic efficacy in inflammation and pain states. Drugs **36**, 732 (1988)
4. British Pharmacopoeia (HM Stationary Office, London, 2002), p. 1216
5. P. Kovarikova, M. Mokry, J. Klimes, J. Pharm. Biomed. Anal. **31**(4), 827–832 (2003)
6. P. Ferrario, M. Bianchi, J. Chromatogr. B Analyst. Technol. Biomed. Life Sci. **785**(2), 227–236 (2003)
7. V.B. Patravale, S. D'Souza, Y. Patravale, J. Pharm. Biomed. Anal. **25**(3–4), 685–688 (2001)
8. B.S. Nagaralli, J. Seetharamappa, B.G. Gowda, M.B. Melwanki, J. Anal. Chem. **58**(8), 873–875 (2003)
9. H. Nimje, S.P. Wate, D.P. Dharkar, R. Razdan, Indian J. Pharm. Sci. **69**(2), 281–283 (2007)
10. D.J. Jaworowicz, M.T. Filipowski, K.M.K. Boje, J. Chrom. B **723**, 293–299 (1999)
11. A. Macia, F. Borrull, M. Calull, C. Aguilar, Trends Anal. Chem. **26**(2), 133–153 (2007)
12. A. Alvarez-Lueje, P. Vasquez, L.J. Nunez-Vergara, J.A. Squella, Electroanalysis **9**(15), 1209–1213 (2005)
13. O.S. Kamalapurkar, Y. Harikrishna, East. Pharm. **40**(478), 145–146 (1997)
14. X. Chen, Zhongguo Xiandai Yingyong Yaoxue **24**(2), 151–153 (2007)
15. S. Chandran, S. Saggar, K.P. Priya, R.N. Saha, Drug Dev. Ind. Pharm. **26**(2), 229–234 (2000)
16. T.M.T. Nguyen, D.T. Nguyen, Tap chi Duoc Hoc **4**, 16–18 (2003)
17. M. Florea, C.M. Monciu, A.M. Laura, B.L. Gabriela, *Farmacia,* vol LVI, (2008), p. 6
18. C.S.R. Lakshmi, M.N. Reddy, Mikrochim. Acta **132**, 1–6 (1999)
19. M.A. Khan, M.M. Karim, S.M. Wabaidur, S.O. Jin, S.H. Lee, Appl. Chem. **11**(2), 453–456 (2007)
20. P. Nagaraja, H.R. Arun Kumar, R.A. Vasantha, H.S. Yathirajan, Oxid. Commun. **26**(1), 116–120 (2003)
21. P. Nagaraja, H.R. Arun Kumar, R.A. Vasantha, H.S. Yathirajan, J. Pharm. Biomed. Anal. **29**(1–2), 277–282 (2002)
22. International Conference on Hormonisation of Technical Requirements for registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R1), complementary Guideline on Methodology dated 06 November 1996, incorporated in November 2005, London
23. G.A. Shabir, J. Chromatogr. A **987**, 57–66 (2003)
24. D.M. Haalad, E.V. Thomas, Anal. Chem. **60**, 1193 (1988)
25. J. Ghasemi, A. Niazi, Microchem. J. **68**, 1–11 (2001)
26. J. Ghasemi, D.E. Mohammadi, Microchem. J. **71**, 1–8 (2002)
27. J. Ghasemi, Sh Ahmedi, K. Torkestani, Anal. Chim. Acta **487**, 181–188 (2003)