Arabian Journal of Chemistry (2013) 6, 369–378

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

UV-absorption and fluorimetric methods for the determination of alprazolam in pharmaceutical formulation

Ayaz Mohd, Aftab Aslam Parwaz Khan, Shaista Bano, K.S. Siddiqi *

Department of Chemistry, Aligarh Muslim University, Aligarh 202002, India

Received 12 May 2010; accepted 14 October 2010
Available online 20 October 2010

KEYWORDS

Alprazoalm;
UV-absorption;
Fluorescence quenching;
Pharmaceutical formulation

Abstract The development of UV and fluorescence spectrophotometric methods for the quantitative determination of alprazolam in dosage forms using As(III)–SDS system. The two simple and sensitive, spectrophotometric and spectrofluorimetric methods were developed for the determination of alprazolam (ALP) in tablets. These methods are based on formation of ALP–As(III) complex in the presence of SDS. The UV-spectrum of 30% methanolic solution of ALP (5×10^{-5} M) at pH 6.5 (Mclivaine buffer) was run between 200 and 380 nm. The absorption spectrum of ALP exhibits two peaks with a $\lambda_{max.}$ at 255 nm and a weak band at 325 nm. When the spectra of the drug were run at varying pH in the region 200–380 nm, one isosbestic point at 290 nm was observed, which indicated the presence of two ionic conditions in solution. The complex exhibited an absorption maximum at 265 nm and emission peak at 520 nm with respect to the excitation wavelength of 325 nm. The spectrophotometric method was found to be linear in 8.0–17.0 μg ml^{-1} range with detection limit of 13.520 μg ml^{-1}, while 0.05–9.5 μg ml^{-1} range was with detection limit of 1.048×10^{-2} μg ml^{-1} by spectrofluorimetric method. The mean percentage recovery of the added quantity was found to be 99.54 (spectrophotometric method) and 100.22 (spectrofluorimetric method) and the %RSD are lower than 0.478 and 0.296 determined spectrophotomerically and spectrofluorimtrically, respectively. This indicates that the proposed method is accurate. The apparent ionization constant of ALP was found to be 9.29. The spectra, experimental conditions were set followed by determination stoichiometry, stability constant and thermodynamic parameters of the As(III), Co(II), Ni(II), and Zn(II) complexes with ALP at pH 6.5. The proposed methods have been successfully applied to the assay of ALP in tablets and the results were statistically evaluated.

* Corresponding author. Tel.: +91 9319788197/9837284930.
E-mail addresses: ks_siddiqi@yahoo.co.in, aizi_pasha@yahoo.com (K.S. Siddiqi).

1878-5352

Peer-review under responsibility of King Saud University.
doi:10.1016/j.arabjc.2010.10.013

Production and hosting by Elsevier

1. Introduction

Alprazolam (ALP) {8-chloro-1-methyl-6-phenyl-4H-s-triazolo(4,3-a)(1,4)benzodiazepine} (Fig. 1) belongs to the class of benzodiazepine with anxiolytic, muscle relaxant, anticonvulsant properties which is generally used as a hypnotic and as a tranquilizer (Abernethy et al., 1983; Dawson et al., 1984; Maitra et al., 2007). It is most frequently prescribed in the therapy of anxiety as being relatively safe with mild side effects. It

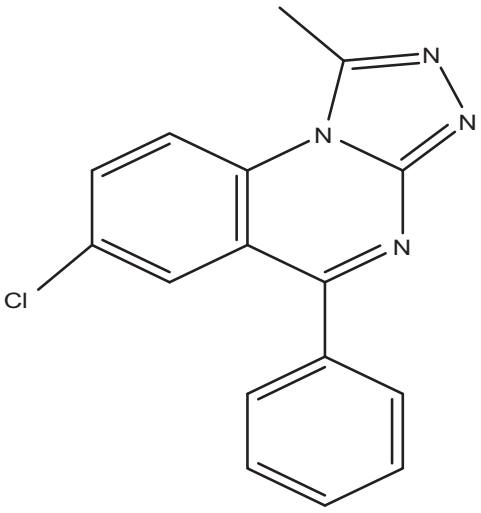

Figure 1 Chemical structure of alprazolam (ALP).

has no appreciable solubility in water at physiological pH. It is rapidly and completely absorbed after oral administration, with peak levels in plasma occurring within 1–2 h after oral administration (Greenblatt and Wright, 1993). The predominant metabolites in human plasma are α′-hydroxy alprazolam, 4-hydroxyalprazolam and α′-benzophenone. The pharmacological activity of α′-hydroxyalprazolam and 4-hydroxyalprazolam is about 60% and 20% less than that of ALP, respectively, and the benzophenone is essentially inactive (Sethy and Harris, 1982; Andrisano et al., 1999). ALP was found to be highly photolabile and special care should be taken to avoid light exposure during its storage and handling. Drug photostability constitutes an important current subject of investigation because the photodegradation process can result in the loss of potency of the drug and also in adverse effects due to the formation of minor toxic degradation products (Albini and Fasani, 1998; Moore and Tonnesen, 1996). A large number of analytical and pharmacological techniques for the determination of some benzodiazepines and their metabolites have been reported (Salem et al., 2002, 2004; Uddin et al., 2008; Walash et al., 1994) especially in biological fluid and pharmaceutical formulations (Lozano et al., 2004).

Since there is no report on the use of metal ion as analytical reagent for the determination of ALP, we have developed a highly sensitive spectrophotometric and fluorescence quenching methods for its determination in tablets by complex formation with As(III) in presence of sodium dodecyl sulfate (SDS). The complexation of Co(II), Ni(II), and Zn(II) with ALP has also been investigated by spectrophotometry and fluorescence spectroscopy although the interaction between ALP and these metal ions can cause fluorescence quenching. The binding mechanism, binding constant and binding sites can be obtained using fluorescence quenching study of ALP in presence of these metal ions. The stoichiometry of the drug to metal ions was determined by continuous variation method and fluorescence quenching. The absorption spectra of the drug were run in the pH range 2.25–10.50 to find isosbestic point which indicates the presence of different ionic species formed in solution. The stability constant and thermodynamic parameters of the process were also evaluated.

2. Experimental

2.1. Equipments

The absorption spectra were obtained with Elico-SL-169 double beam UV–visible spectrophotometer. Fluorescence emission spectra were scanned with Hitachi-F-2500FL-spetrophotometer. All potentiometric measurements were done with Elico-Li-120 pH meter.

2.2. Material and methods

Double distilled water was used throughout. Pure ALP (Sigma Laboratories Ltd., India) and tablets from Morepen Labs Ltd. (India), sodium hydroxide, arsenic oxide and metal chloride (Merck Ltd., Mumbai, India) and HCl (Ranbaxy Fine Chem. Ltd., India) were used as received.

2.3. Preparation of standard solutions

The stock solution (1×10^{-2} M) of ALP was prepared by dissolving 0.3088 g of ALP in 30% aqueous methanol, metal salt solution (1×10^{-2} M) and sodium dodecyl sulfate (1×10^{-4} M) were prepared in water.

2.4. Procedure for commercial tablets

Thirty tablets (1.5 mg of ALP each) were accurately weighed, finely powdered and quantity of equivalent to 25 mg was extracted by shaking with 25 ml of 30% aqueous methanolic solution followed by three extractions each with 15 ml of 30% aqueous methanol. It was filtered by Whatman filter paper No. 42 and made up to 100 ml by the same solvent.

2.5. Spectrophotometric measurements

The solution containing 8.0–17.0 μg ml^{-1} of drug was transferred into a series of 10 ml volumetric flask. One milliliter

Table 1 Statistical data of calibration graph for the determination of ALP using proposed methods.

Parameters	Spectrophotometric method	Spectrofluorimetric method
λ_{max} (nm)	255	λ_{em} = 520 λ_{ex} = 325
Bear's law limit (μg ml^{-1})	8.0–17.0	0.05–9.5
Slope (m)	0.362	178.17
Intercept (b)	−2.694	280.16
SD of residual ($S_{y/x}$)	0.970	72.142
SD of intercept (S_a)	1.113×10^{-2}	0.566
SD of slope (S_b)	1.482	8.434
Correlation coefficient (r)	0.9875	0.9978
Variance $(S_{y/x})^2$	0.106	0.752
Limit of detection (μg ml^{-1})	13.520	1.048×10^{-2}
Limit of quantification (μg ml^{-1})	40.970	3.178×10^{-3}

Number of standard samples, n = 7.

(5×10^{-5} M) of As(III) solution, 1.5 ml SDS and 1 ml Mclivaine buffer of pH 6.5 were added. The mixture was then made up to the mark. The absorbance of the resulting solution was measured at specific wavelength (nm) cited in Table 1 against reagent blank.

2.6. Spectrofluorimetric measurements

Fluorescence emission spectra were measured at 25 °C at optimized excitation wavelength. To an aliquot of the solution containing 0.05–9.5 μg ml^{-1} of ALP were added 1 ml (2×10^{-9} M) of As(III) solution, 1.5 ml SDS and 1 ml Mclivaine buffer (pH 6.5). The fluorescence intensity of solution was measured at 520 nm with the excitation at 325 nm against a reagent blank prepared with the reagent concentration but not with ALP.

2.7. Determination of stoichiometries binding constant and binding sites

The stoichiometries, binding constant and binding sites were determined by spectrophotometric and spectrofluorimetric methods.

2.7.1. Spectrophotometric method

Solutions of equimolar concentration (5×10^{-5} M) of ALP and metal ions were prepared. In order to calculate the apparent ionization constant of the drug, its pH was adjusted between 2.25 and 10.50 by adding sodium hydroxide and hydrochloric acid (1×10^{-1} and 1×10^{-2} M, respectively). The absorption spectra were recorded 30 min after sample preparation in the range of 200–380 nm. The binding constant and ratio of metal to ALP was determined by Job's method in the presence of 1.5 ml SDS (1×10^{-4} M) and 1 ml Mclivaine buffer (pH 6.5).

2.7.2. Spectrofluorimetric method

Solution of the ALP (2.4×10^{-8} M) and those of metal ions (0.2×10^{-8}–1.2×10^{-8} M) were prepared. To prepare dilute solutions, an aliquot of stock was transferred into a 10 ml volumetric flask and made up to the mark with distilled water. Spectra were recorded 30 min after the sample preparation in the optimum wavelength range of 480–560 nm and at optimum excitation wavelength of 325 nm.

3. Results and discussion

3.1. Spectrophotometric measurements

The UV-spectrum of 30% methanolic solution of ALP (12.0 μg ml^{-1}) at pH 6.5 (Mclivaine buffer) was run between 200 and 380 nm. It exhibits two peaks at 255 nm and a weak band at 325 nm (Fig. 2). Since the first peak is very strong it was selected for further studies.

The Beer's law was validated from 8.0 to 17.0 μg ml^{-1} for ALP. The spectrophotometric method is based on complexation of ALP with As(III)–SDS system under mild acidic condition. Under the experimental condition described the linear regression equation: $A = 0.362X - 2.694$ ($r = 0.9875$), where A is the absorbance and X is the concentration in μg/ml. The apparent molar absorptivity was 3.956×10^4 l/mol cm. The

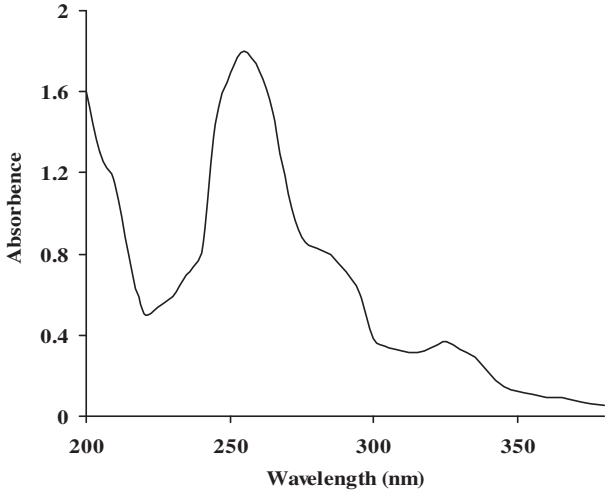

Figure 2 Absorption spectrum of ALP (5×10^{-5} M) at pH 6.50 at 25 °C.

detection limit and the limit of quantification determined with the acceptable accuracy and precision were 13.520 and 1.048×10^{-2} μg ml^{-1}, respectively.

The low detection and quantification limits showed negligible scatter of the points with respect to the line of regression. For optimization of the reaction condition of As(III) with ALP, several factors have been studied carefully. Concerning the effect of pH and buffer, it was found that maximum absorbance was achieved at pH 6.5 in Mclivaine buffer (Fig. 3). Other buffers, such as borate and acetate buffers having the same pH were studied and compared with Mclivaine buffer. Mclivaine buffer was found to be superior to phosphate and borate buffers, since it has the highest absorbance value. This is due to the hydrolysis of metal to metal hydroxide in other buffers (Miyano et al., 1985). It was found that concentration of 5×10^{-5} M of As(III) and 1×10^{-4} M of SDS has maximum absorbance.

3.2. Spectrofluorimetric method

The fluorescence emission spectra were run between 480 and 560 nm at optimum excitation wavelength of 325 nm. The

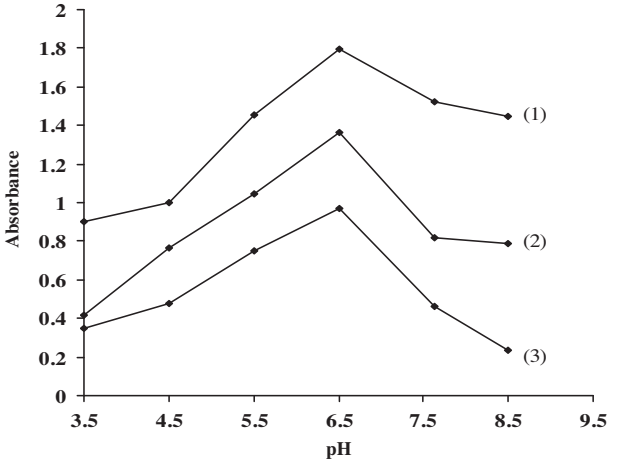

Figure 3 Effect of pH on complex formation of ALP–As(III)–SDS system: (1) Mclivaine, (2) BR and (3) acetate buffer.

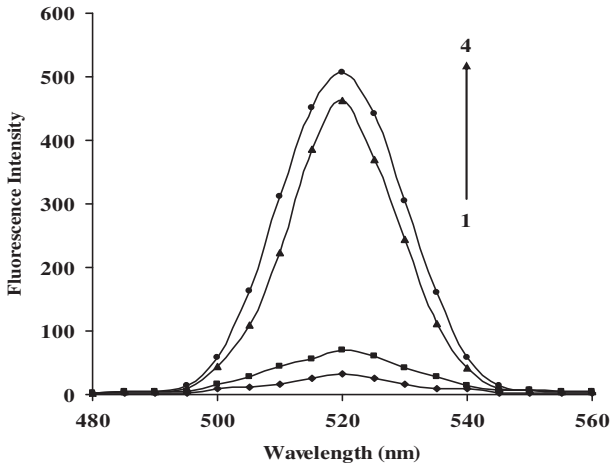

Figure 4 Fluorescence emission spectra: (1) blank, (2) ALP, (3) ALP–AS(III)–SDS system, (4) ALP–SDS system.

spectra of blank, ALP and ALP–As(III)–SDS system are shown in Fig. 4. It is observed that maximum emission occurs at 520 nm with no significant change in peak position. The fluorescence intensity of ALP was enhanced in the presence of SDS but quenched when AS(III) was added to it. Considering the non-interference effect, stability and lower value of blank signal the excited wavelength of 325 nm was selected for recording emission spectra (λ_{em} = 520 nm) in the subsequent experiment. A linear correlation was obtained between the fluorescence intensity and concentration of ALP in the range 0.05–9.5 μg ml^{-1} and the correlation coefficient was not less than 0.9944. The concentrations of different samples of ALP in pharmaceutical formulation were calculated from the regression equation: $F = 178.17X + 280.16$ (X is the concentration of ALP in μg ml^{-1} and F is the fluorescence intensity of ALP–As(III)–SDS system at 520 nm with the excitation at 325 nm). The maximum fluorescence intensity were obtained at pH 6.5 with 0.6×10^{-5} M of As(III) and 1×10^{-4} M of SDS.

3.3. Optimum conditions for the reaction

3.3.1. Effect of the acidity

The influences of different kinds of buffer solutions, such as Mclivaine, BR and HCl–NaAc on the reaction system were tested. The results showed that Mclivaine buffer of pH 6.5 was better than other buffers. Optimum pH range for the reaction was 5.0–7.0. Thus, 1.0 ml of Mclivaine's buffer of pH 6.5 was taken in each case.

3.3.2. Effect of the SDS concentration

The results showed that when the concentration of SDS was 1×10^{-4} M, the values of absorbance and fluorescence intensity reached the maximum. Without enough SDS, the reaction was incomplete but when SDS is in excess, the absorbance and fluorescence intensity would decrease because of the formation of SDS dimer by self-aggregation.

3.3.3. Effect of ionic strength

The effect of ionic strength on the intensities of ΔI was studied. The experimental results showed that it had little effect on spectrum, if NaCl concentration was less than 5×10^{-2} M. Therefore, the ion-association reaction should be under a low ionic strength condition. The effect also indicated that the electrostatic interaction was a very important factor in this ion-association.

3.4. Analytical performance

3.4.1. Validation of the proposed methods

The validity of the method for linearity, specificity, accuracy, repeatability and precision according to recommendations were tested (http://www.fda.gov/eder/guidance/4252fnl.pdf (accessed September 1, 2004)). The results are shown in Table 1. The limits of detection (LOD) were determined by establishing the minimum level at which the analyte can be reliably detected. The LOQ and LOD were calculated according to the following equation:

$$\mathrm{LOQ} = 10\sigma/S \quad (1)$$

$$\mathrm{LOD} = 3.3\sigma/S \quad (2)$$

where σ is standard deviation of the intercept of regression line and S is the slope of calibration curve. The proposed methods were evaluated for the accuracy as percent relative error (%Er) and the precision as percent relative standard deviation (%RSD) (Table 2). The results of determination of ALP in comparison with other method (Nudelman and Gallardo Cabrera, 2002) are listed in Tables 3 and 4.

3.4.2. Accuracy and precision

The reproducibility or precision of the method was evaluated by statistical analysis of the regression data regarding standard deviation of the residuals ($S_{y/x}$), the intercept (S_a) and the slope

Table 2 Accuracy and precision for the determination of ALP using proposed methods.

Method	Added amount (μg ml^{-1})	Found ± SD[a]	%Er[b]	%RSD[c]	% Recovery
Spectrophotometric	9.0	$8.898 \pm 4.261 \times 10^{-2}$	1.146	0.478	98.86
	12.0	$11.96 \pm 5.344 \times 10^{-2}$	0.317	0.446	99.68
	15.0	$15.02 \pm 1.166 \times 10^{-2}$	0.146	0.077	100.1
Spectrofluoremetric	1.0	$0.99 \pm 0.989 \times 10^{-2}$	0.140	0.990	99.86
	3.0	$3.02 \pm 0.894 \times 10^{-2}$	0.662	0.296	100.6
	7.0	$7.01 \pm 0.748 \times 10^{-2}$	0.256	0.106	100.2

[a] Mean ± SD for five deteterminations.
[b] Percentage of relative error.
[c] Percentage relative standard deviation.

Table 3 Result for determination of ALP in pharmaceutical samples.

Method	Sample	Label claim (mg per tablet)	Found ± SD	%RSD	% Recovery
Spectrophotometric	Alpaz 1.0 SR	1.0	0.997 ± 0.013	1.319	99.76
	Alpaz 1.5 SR	1.5	1.508 ± 0.017	1.186	100.5
Spectrofluorometric	Alpaz 1.0 SR	1.0	1.003 ± 0.015	1.518	100.4
	Alpaz 1.5 SR	1.5	1.504 ± 0.013	0.892	100.2

Table 4 Comparison of sensitivities of developed methods with reported method for the determination of ALP.

Methods	Linearity	LOD	Recovery	%RSD	Literature
Spectrofluorimetric	$0.8–0.9 \times 10^{-3}$ M	$\geqslant 10^{-5}$ M	101.4–108.3	2.59–5.92	Nudelman and Gallardo Cabrera (2002)
Specrophotmetric	8.0–17.0 μg ml^{-1}	1.048×10^{-2} μg ml^{-1}	98.86–100.1	0.077–0.478	This work
Spectrofluorimetric	0.05–9.5 μg ml^{-1}	3.178×10^{-3} μg ml^{-1}	99.86–100.6	0.106–.296	This work

(S_b). The small values of the figures point out the low scattering of the calibration graph and high precision.

3.4.2.1. Accuracy. To test the accuracy of the proposed method a certain amount of ALP was assayed by the proposed method. The mean percentage recovery of the added quantity was found to be 99.54 (spectrophotometric method) and 100.22 (spectrofluorimetric method). The results indicate that the proposed method gives accurate results.

3.4.2.2. Precision. The precision assay was done by both these methods. An amount of 9, 12, 15 μg ml^{-1} of ALP for spectrophotometric and 1, 3, 7 μg ml^{-1} for spectrofluorimetric method were taken, respectively. The results (Table 2) indicate that the %RSD value for precision are lower than 0.478 and 0.296 determined spectrophotomerically and spectrofluorimetrically, respectively.

3.4.3. Repeatability

To test the reproducibility of the proposed method, five replicate analysis were done. The mean percentage recovery was found to be 99.54 (spectrophotometric method) and 100.22 (spectrofluorimetric method), respectively.

3.4.4. Specificity

The specificity of the method was verified by checking any interference encountered by the excipients of tablets. Lactose which is frequently co-formulated with ALP did not interfere with the proposed method.

3.4.5. Robustness of the method

The robustness of the method adopted is demonstrated by the consistency of the absorbance with the deliberate minor changes in the experiment, such as volume of As(III) 1.0 ± 0.1 ml and volume of SDS 1.5 ± 0.1 ml. These minor changes did not affect the absorbance of the reaction product.

3.5. Determination of stoichiometry, binding constant and binding sites

3.5.1. Spectrophotometric method

Ratio of the two reacting components was determined by Job's method (Job, 1928; Siddiqi et al., 2009). Different volumes of equimolar concentration (5×10^{-5} M) of both the drug and metal ions were taken in all possible ratios and absorbance were measured at 265 nm, pH 6.5 and t = 25 and 35 °C. The Job's curves at different temperatures showed the formation of 2:1 (drug–metal) complexes (Fig. 5A–D). The obtained curves have a marked maximum at a molar ratio of 1:0.666 and show the presence of mononuclear complexes. The stability constant of the complexes was calculated by the following equation:

$$K = \frac{A/A_{ex}C_X}{(C_M - A/A_{ex}C_X)(C_L - nA/A_{ex}C_X)^n} \quad (3)$$

where K is the binding constant of the metal chelate formed in solution, M is metal, L is ligand, $n = X/(1 - X)$ where X is the mole fraction of the ligand at maximum absorption. A/A_{ex} is the ratio of the observed absorbance to that indicated by the tangent for the same wavelength. C_X, C_M and C_L are the limiting concentration, metal ion and the ligand concentrations, respectively. The stability constant of complex between ALP and metal ions is fairly high. The log K increases with increase in temperature as shown in Table 5.

3.5.1.1. Calculation of apparent ionization constant. When the spectra of the drug were run at varying pH in the region of 200–380 nm one isosbestic point (Fig. 6), at 290 nm was observed which indicated the presence of two ionic species in solution (Park et al., 2000; Siddiqi et al., 2009). The apparent ionization constant (pK'_a) of the drug was calculated (9.29) by the following equation:

$$pK'_a = pH + \log\{(A_I - A_M)/(A - A_M)\} \quad (4)$$

where A_I = absorbance of drug in basic medium, A_M = absorbance of drug in acidic medium, A = absorbance of drug in aqueous medium.

3.5.2. Spectrofluorimetric method

The fluorescence emission spectra of the pure drug scanned in 480–560 nm range (at excitation wavelength of 325 nm) are markedly different from its UV spectrum (Fig. 7A–D) in the presence of concentrations of metal ions. Although, the fluorescence intensity of ALP–SDS system decreases steadily with increasing concentration of metal ions, the emission maxima remain unchanged. As there was no significant λ_{em} shift with the addition of metal ions, it indicated that metal ion can quench intrinsic fluorescence of ALP and that the interaction between ALP and metal ion indeed existed without inducing any conformational change in it under the experimental

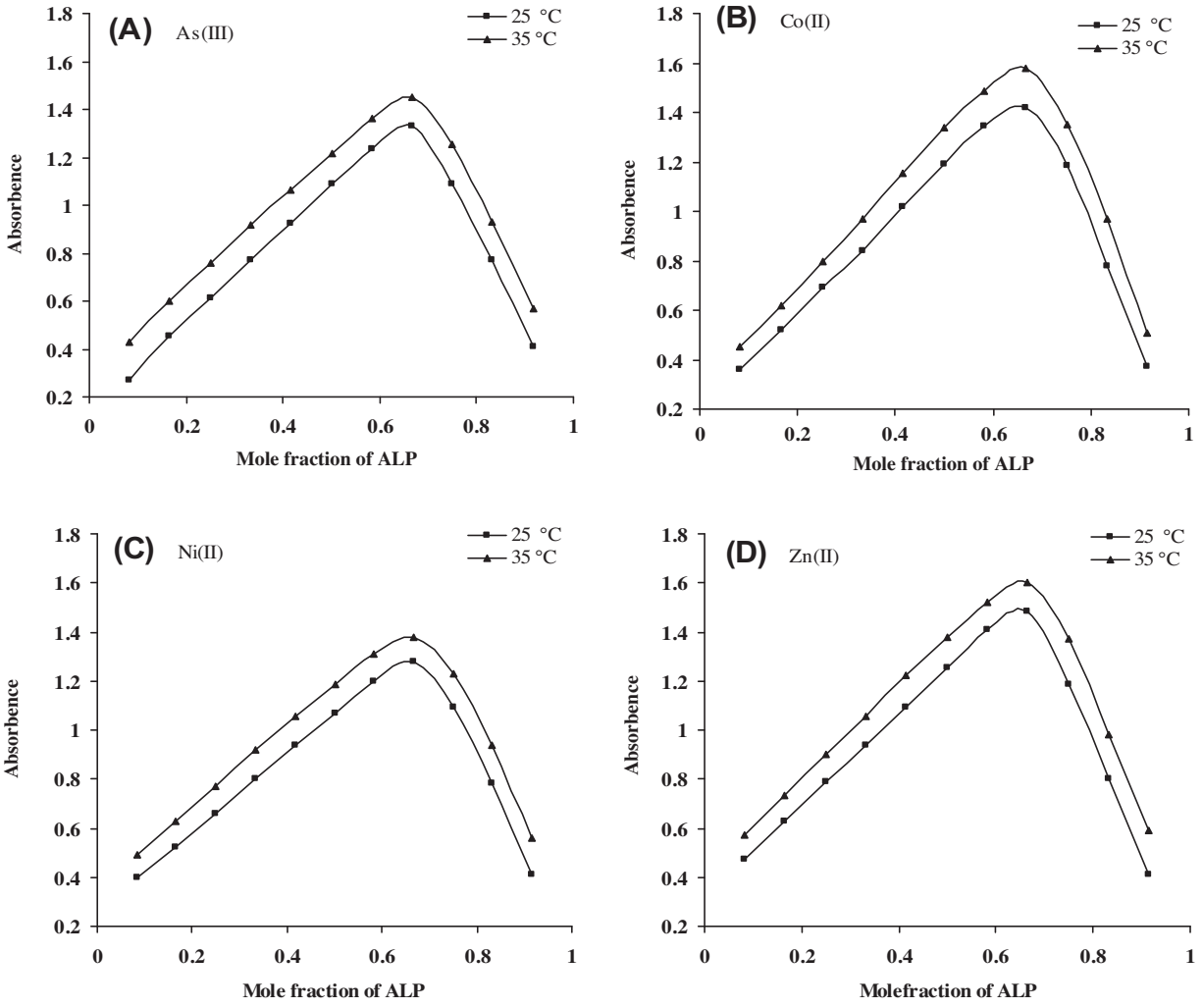

Figure 5 Continuous variation curves of complexes of ALP with (A) As(III), (B) Co(II), (C) Ni(II) and (D) Zn(II) at 25 and 35 °C.

Table 5 Stability constant and thermodynamic parameters of ALP complexes.

Metal ions	log K		$(-\Delta G)$ (kJ mol^{-1})		(ΔH) (kJ mol^{-1})	(ΔS) (kJ mol^{-1} K^{-1})	
	25 °C	35 °C	25 °C	35 °C		25 °C	35 °C
As(III)	13.399	14.897	76.452	87.852	2.509	0.264	0.293
Co(II)	13.265	14.731	75.688	86.734	2.456	0.262	0.289
Ni(II)	13.145	14.659	75.003	86.448	2.541	0.260	0.288
Zn(II)	13.098	14.416	74.735	85.015	2.208	0.258	0.283

condition. Quenching can occur by a variety of molecular interactions, viz. excited state reactions, molecular rearrangement, energy transfer, ground state complex formation (static quenching) and collisional or dynamic quenching. Static and dynamic quenching can be distinguished from their dependence on temperature and excited state life time. Dynamic quenching is diffusion controlled because the quencher must diffuse to the fluorophore during the life time of the excited state. If the K_{SV} decreased with increased temperature it may be concluded that the quenching process is static rather than dynamic (Guo et al., 2004; Wang et al., 2007). Static quenching refers to the existence of a sphere of effective quenching or the formation of a ground state non-fluorescent complex, whereas collisional or dynamic quenching involves the collision and subsequent formation of a transient complex between an excited state fluorophore and a ground state quencher. The excited state complex dissociates upon radiative and non-radiative deactivation. In order to confirm the quenching

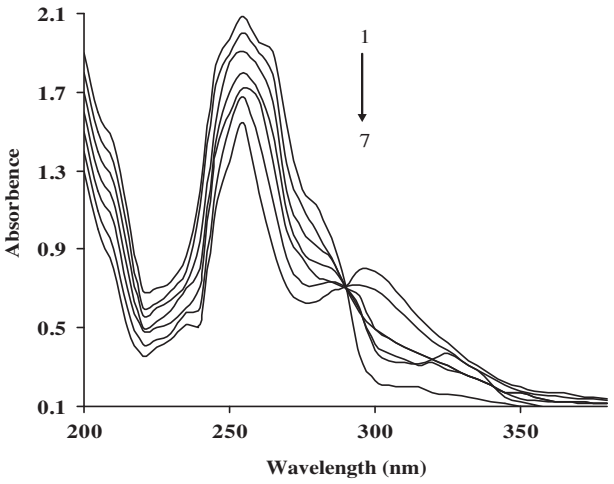

Figure 6 Absorption spectra of ALP (5×10^{-5} M) in (2.25–10.50) pH range (1–7: 2.25, 3.50, 5.0, 6.50, 7.50, 8.50, 10.50).

mechanism the procedure of fluorescence quenching was first assumed to be dynamic. For dynamic quenching, the mechanism can be described by the Stern–Volmer equation (Lakowicz, 1999):

$$F_0/F = 1 + K_{SV}[Q] \quad (5)$$

where F_0 and F are the fluorescence intensities in the absence and presence of the quencher, respectively. K_{SV} is the dynamic quenching constant (Table 6). Fig. 8A–D displays the Stern–Volmer plots of quenching of the ALP by metal ions at different temperatures. For static quenching, the relationship between intensity and the concentration of quencher can be described by the binding constant formula (Feng et al., 1996; Xu et al., 1997);

$$\log(F_0 - F)/F = \log K + n \log[Q] \quad (6)$$

where K is the binding constant and n is the number of binding sites per ALP. Fig. 9A–D show double-logarithm curve and Table 6 shows the corresponding calculated

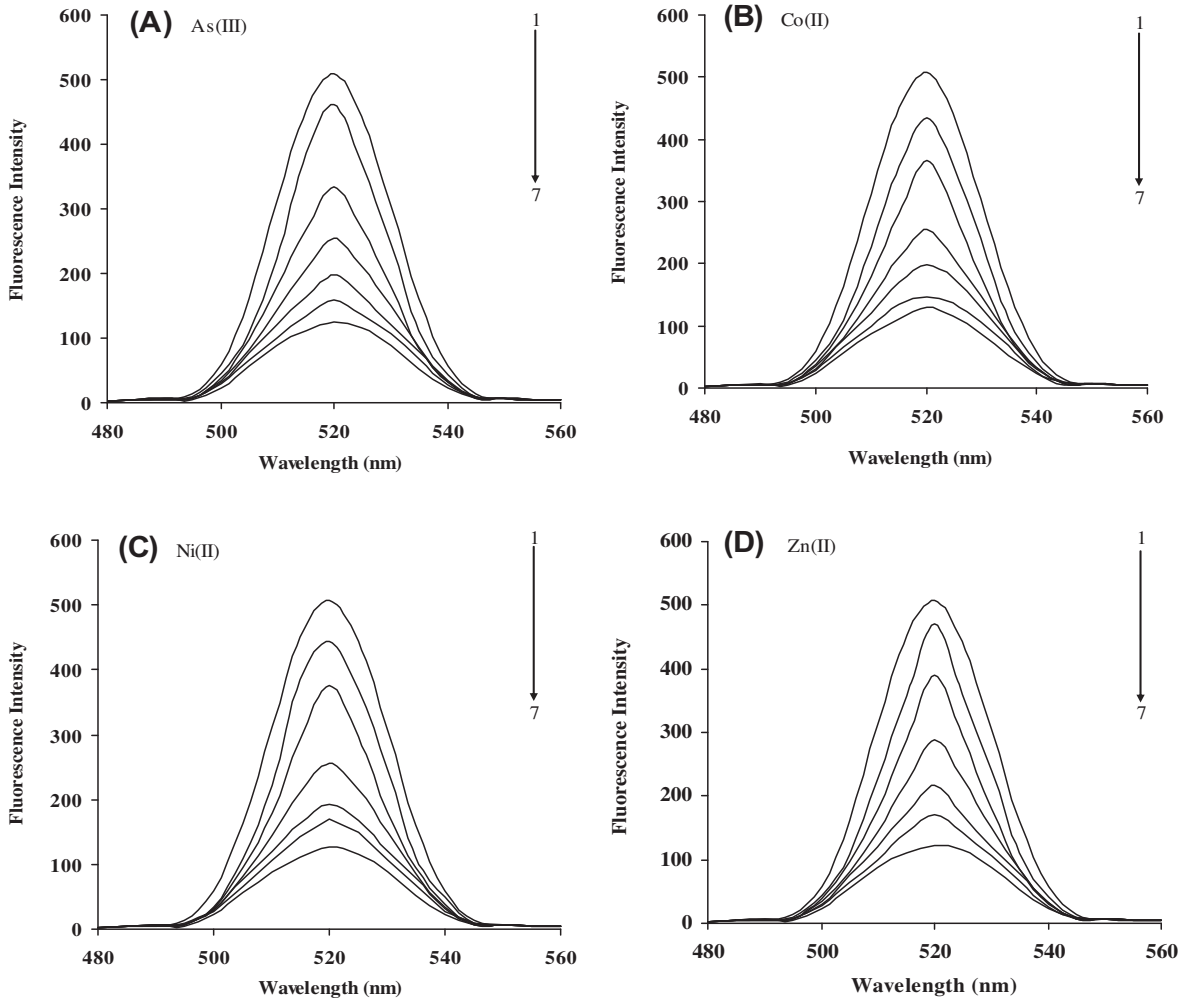

Figure 7 Fluorescence quenching spectrum (at λ_{ex} 325 nm) of ALP with (A) As(III), (B) Co(II), (C) Ni(II) and (D) Zn(II) at 25 °C, (1) 2.4×10^{-8} M ALP, from (2) to 7:0.2×10^{-8}, 0.4×10^{-8}, 0.6×10^{-8}, 0.8×10^{-8}, 1.0×10^{-8}, 1.2×10^{-8} M of metal ions.

Table 6 Stern–Volmer constant (K_{SV}), binding constant (log K), binding site (n) and regression coefficient (r) at 25 and 35 °C.

Metal ions	K_{SV} (mol^{-1})		log K		n		r	
	25 °C	35 °C	25 °C	35 °C	25 °C	35 °C	25 °C	35 °C
As(III)	2.98×10^8	2.90×10^8	13.419	14.922	1.874	2.029	0.9944	0.9920
Co(II)	3.21×10^8	2.92×10^8	13.360	14.826	1.848	1.986	0.9860	0.9974
Ni(II)	2.89×10^8	2.82×10^8	13.305	14.787	1.812	1.924	0.9866	0.9906
Zn(II)	3.11×10^8	3.02×10^8	13.102	14.505	1.828	1.957	0.9852	0.9968

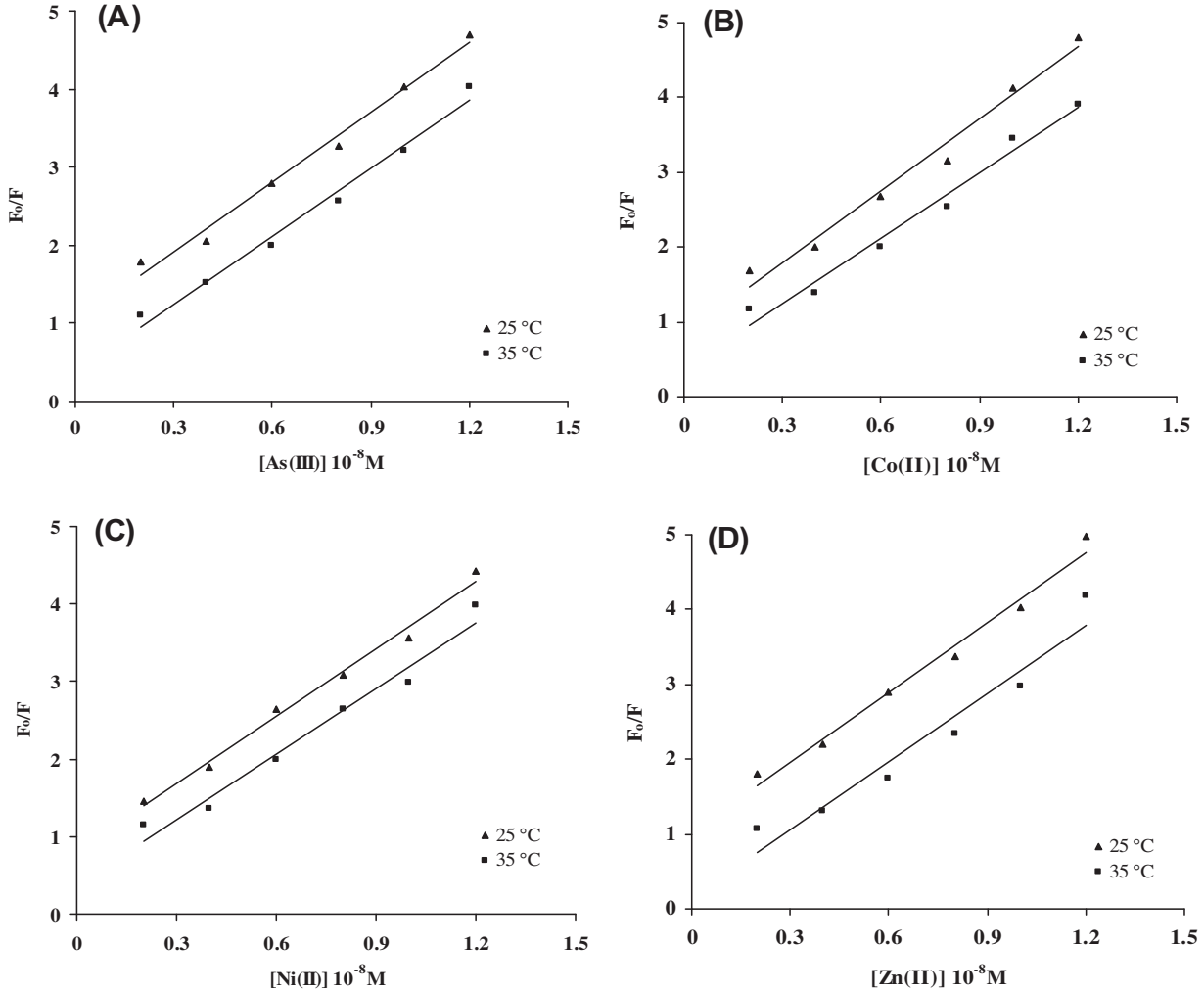

Figure 8 Stern–Volmer plots of ALP with (A) As(III), (B) Co(II), (C) Ni(II) and (D) Zn(II) at 25 and 35 °C.

results. The linear correlation coefficients of all the curves are larger than 0.9852, suggesting interaction of metal ions with ALP which agrees well with the site-binding model underlying Eq. (6).

3.5.3. Thermodynamic parameters and nature of binding forces
Considering the dependence of the binding constant on the temperature a thermodynamic process was considered to be responsible for this interaction and analyzed in order to further characterize the forces acting between ALP and metal ions. The thermodynamic parameters enthalpy (ΔH), entropy (ΔS), and free energy (ΔG) are the main evidences to determine the binding mode. If the temperature does not vary significantly, the enthalpy changes (ΔH) can be regarded as constant. The ΔG, ΔH and ΔS can be estimated from the following standard equations:

$$\Delta G = -2.303RT\log K = \Delta H - T\Delta S \quad (7)$$

$$\log K_2/K_1 = [1/T_1 - 1/T_2]\Delta H/2.303R \quad (8)$$

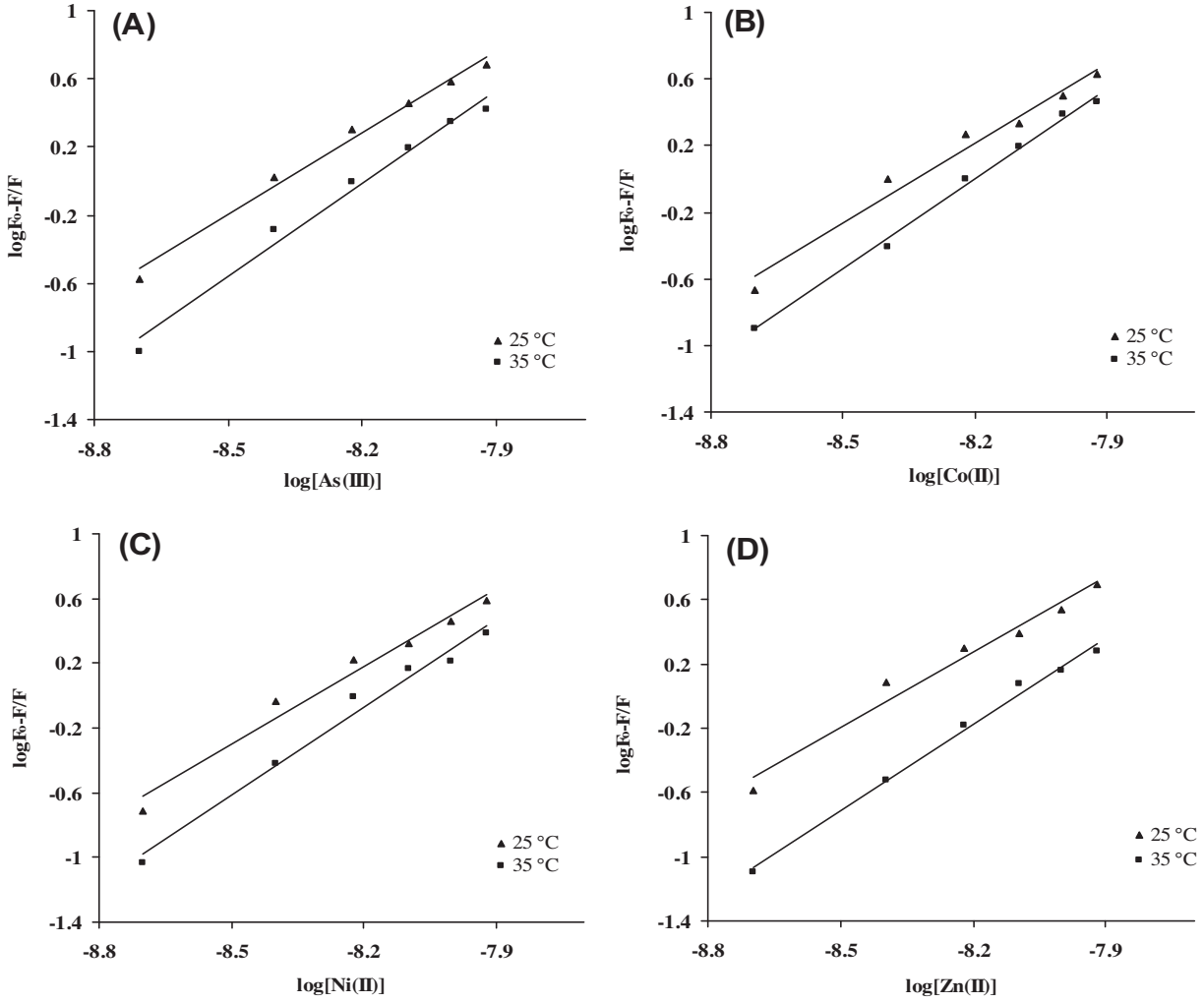

Figure 9 Double reciprocal plots of ALP with (A) As(II), (B) Co(II), (C) Ni(II) and (D) Zn(II) at 25 and 35 °C.

Table 7 Thermodynamic parameters at 25 and 35 °C.

Metal ions	$-\Delta G$ (kJ mol^{-1})		ΔH (kJ mol^{-1})	ΔS (kJ mol^{-1} K^{-1})	
	25 °C	35 °C		25 °C	35 °C
As(III)	76.566	88.126	2.518	0.265	0.294
Co(II)	76.230	87.433	2.054	0.262	0.290
Ni(II)	75.916	87.203	2.483	0.263	0.291
Zn(II)	74.775	85.540	2.350	0.258	0.285

The thermodynamic parameters for the interaction of metal ions with ALP are shown in Tables 5 and 7. The negative value of ΔG means that the interaction was spontaneous while the +ve ΔS value is characteristic of chelation. It occurs because the water molecules that are normally arranged in an orderly fashion around the drug and metal ions have acquired a random configuration as a result of chelation. It indicates a gain in configurational entropy (Calvin and Melchior, 1948). The +ve value of ΔH indicated that the processes were endothermic and binding between metal ions and ALP is mainly ΔS-driven, with little contribution from the enthalpy factor.

4. Conclusion

The proposed methods are simple and sensitive. These do not require any pretreatment of the drug and tedious extraction procedure. The methods have wider linear range with good accuracy and precision. The proposed methods are accurate and precise. It can be extended for routine analysis of ALP in pharmaceutical formulation, hospital and research laboratory. Since the stability increases in the presence of the metal ions the drug may be absorbed with traces of essential elements.

References

Abernethy, D.R., Greenblatt, D.J., Divoll, M., Moschitto, L.J., Harmatz, J.S., Shader, R.I., 1983. Psychopharmacology 80, 275.

Albini, A., Fasani, E., 1998. In: Albini, A., Fasani, E. (Eds.). Royal Society of Chemistry, Cambridge, pp. 1–73.

Andrisano, V.R., Gotti, A., Leoni, Cavrini, V., 1999. J. Pharm. Biomed. Anal. 21, 851.

Calvin, M., Melchior, N.C., 1948. J. Am. Chem. Soc. 70, 3270.

Dawson, G.W., Tue, S.G., Brogden, R.N., 1984. Drugs 27, 132.

Feng, X.Z., Jin, R.X., Qu, Y., Whe, X., 1996. Chem. J. Chin. Univ. 17, 866.

Greenblatt, D.J., Wright, C.E., 1993. Clin. Pharmacokinet. 24, 453.

Guidance for Industry Bioanalytical Method Validation. US Department of Health and Human Services, Food and Drug Administration, Center for Drug - Evaluation and Research, Rockville, MD, May 2001. <http://www.fda.gov/eder/guidance/4252fnl.pdf> (accessed September 1, 2004).

Guo, M., Zou, J.W., Yi, P.G., Shang, Z.C., Hu, G.X., Yu, Q.S., 2004. Anal. Sci. 20, 465.

Job, P., 1928. Ann. Chim. 9, 113.

Lakowicz, J.R., 1999. Principles of Fluorescence Spectroscopy, second ed. Plenum Press, New York, p. 698.

Lozano, P.P., Montoya, E.G., Orriols, A., Minarro, M., Tico, J.R., Negre, J.M.S., 2004. J. Pharm. Biomed. Anal. 34, 979.

Maitra, S., Saha, B., Santra, C.R., Mukharjee, A., Goswami, S., Chanda, P.K., Karmakar, P., 2007. Int. J. Biol. Macromol. 41, 23.

Miyano, H., Oka, T.T., Imai, K., 1985. Anal. Chim. Acta 170, 81.

Moore, D.E., Tonnesen, H.H. (Eds.), 1996. Taylor and Francis, London. pp. 9–38.

Nudelman, N.S., Gallardo Cabrera, C., 2002. J. Pharm. Biomed. Anal. 30, 887.

Park, H.R., Chung, K.Y., Lee, H.C., Lee, J.K., Bark, K.M., 2000. Bull. Korean Chem. Soc. 21, 849.

Salem, A.A., Barsoum, B.N., Saad, G.R., Izake, E.L., 2002. J. Electroanal. Chem. 536, 1.

Salem, A.A., Barsoum, B.N., Saad, G.R., Izake, E.L., 2004. Spectrochim. Acta Part A: Mol. Biomol. Spectr. 60, 771.

Sethy, V.H., Harris, D.W., 1982. J. Pharm. Pharmacol. 34, 115.

Siddiqi, K.S., Mohd, A., Khan, A.A.P., Bano, S., 2009. J. Korean Chem. Soc. 53, 152.

Uddin, M.N., Samanidou, V.F., Papadoyannis, I.N., 2008. J. Liq. Chromatogr. Relat. Technol. 31, 1258.

Walash, M.I., Belal, F., Metwally, M.E., Hefnawy, M.M., 1994. J. Pharm. Biomed. Anal. 12, 1417.

Wang, C., Wu, Q.H., Li, C.R., Wang, Z., Ma, J.J., Zang, X.H., Qin, N.X., 2007. Anal. Sci. 23, 429.

Xu, Y., Shen, H.X., Huan, H.G., 1997. Anal. Chem. 25, 419.