Available on line www.jocpr.com

Journal of Chemical and Pharmaceutical Research

ISSN No: 0975-7384
CODEN(USA): JCPRC5

J. Chem. Pharm. Res., 2011, 3(2):605-616

A kinetic method for the determination of diazepam based on ligand-exchange reaction

Snežana S. Mitić[1], Aleksandra N. Pavlović[1*], Snežana B.Tošić[1], Emilija T. Pecev[1], Milan N. Mitić[1], Ružica J. Micić[2]

[1]Faculty of Sciences and Mathematics, Department of Chemistry, University of Niš, Višegradska 33, P.O.Box 224, 18000 Niš, Serbia
[2]Faculty of Sciences and Mathematics, University of Priština, Lole Ribara 29, 38220 Kosovska Mitrovica

ABSTRACT

New kinetic-spectrophotometric method has been developed to determine diazepam (DZP) in pharmaceutical formulations and human control serum. The method is based on ligand-exchange reaction. The reaction was followed spectrophotometrically by measuring the rate of change of absorbance at 425 nm in ethanolic sodium hydroxide solution. The optimum operating conditions regarding concentration of reagents and temperature were established. The initial-rate method is adopted for constructing the calibration curve, which was found to be linear over the concentration range 0.28-3.70 µg mL^{-1}. The optimized conditions yielded a theoretical detection limit of 0.14 µg mL^{-1} based on the $3S_b$ criterion. The interference effects of certain drugs, foreign ions and amino acids upon the reaction rate were studied in order to assess the selectivity of the method. The method described in this work was validated following the analytical performance parameters required by the USP-27/NF-22. Statistical Students t-test and F-test have been used and satisfactory results were obtained.

Keywords: diazepam; ligand-exchange reaction; kinetic spectrophotometry; validation; pharmaceutical preparations

INTRODUCTION

Diazepam (DZP), first marketed as Valium by Hoffmann-La Roche, is a widely prescribed benzodiazepemine in the world for the past 40 years. It is effective for the symptomatic relief of tension and states of anxiety as well as for the treatment and prevention of seizures. It may also be used in some surgical procedures to induce amnesia [1]. It is inexpensive, widely available in

developing countries and effective when given by the intravenous or rectal routes. The acute toxity of diazepam is a result of its central nervous depressant effect. The drug is notably more toxic by the intravenous route than by other modes of administration.

The various analytical methods such as spectrophotometry [2-5], HPLC [6-13], thin layer chromatography [14], gas chromatography [15-17], polarography [18-20], fluorimetry [2,21,22], IR spectrometry [23], solid-phase microextraction (SPME) [24] and capillary zone electrophoresis (CZE) [25,26] have been reported for the determination of DZP. However, as far we know, there is not any kinetic-spectrophotometric method for the determination of DZP related in the literature. Spectrophotometry is the technique of choice even today due to its inherent simplicity. It is frequently used in the laboratories of the developing countries to overcome a variety of analytical problems. Also, the wide use of diazepam necessitates a rapid, reliable and sensitive method for its quantitation.

The present work describes a kinetic method for the determination of DZP in commercial pharmaceutical preparations and human control serum. The method is based on ligand-exchange reaction. This type of the reactions is quite recent and has thus been studied less. The procedure is easier to execute and requires less sample handling than methods currently described in the literature. Using methods such as HPLC, insoluble additives should be removed to prevent the columns from becoming blocked. For the GLC methods, chemical derivatization is essential. Fluorimetric methods are simple, accurate and precise, but these methods have not been widely used in practice because the species that can be detected are limited. Also, with this technique, fluorescent species should be generated either by thermal heating in acidic solvent, photochemical degradation, or by derivatization with phthaldehyde and fluorescamine. All these methods are time consuming, complex, occasionally suffering from lack of selectivity and require procedures that may increase the amount of the hydrolyzed product during manipulation of the sample.

EXPERIMENTAL SECTION

Apparatus

The reaction rate was monitored spectrophotometrically. The absorbance of the solution was measured at the wavelength of 425 nm. The readings were done on a Perkin-Elmer Lambda 15 UV/Vis spectrophotometer, connected to a thermo-circulating bath.

A model 1200 Agilent Technologies was used for HPLC analysis. The analytical column was C_{18} (Zorbax, 5μm, 250x4.6 mm).

A Julabo MP-5A model thermostatic bath (operating in a temperature range 20.00-60.00 ± 0.02 $^{\circ}$C) was used to control the reaction temperature at 22.00 ± 0.02 $^{\circ}$C.

The solutions were thermostated at 22.00 ± 0.02 $^{\circ}$C before the beginning of the reaction.

Reagents

Stock solution (1.0×10^{-3} mol L^{-1}) of diazepam was prepared in absolute ethanol from pharmaceutical 99.92 % certified products, kindly provided by a pharmaceutical laboratory Galenika, a.d., Belgrade, Serbia. Diazepam solution was stored at 4ºC.

Sodium hydroxide solution (NaOH, 1.0 mol L^{-1}) was prepared from NaOH (Merck).

1-nitroso-2-naphthol solution (1.0×10^{-3} mol L^{-1}) (Merck) was prepared by dissolving a known amount in 5 ml absolute ethanol and diluting it with water (total volume 50 mL).

Stock solution of copper(II) (1.6×10^{-3} mol L^{-1}) was prepared by dissolving $CuCl_2\times2H_2O$ (Merck) in water. A working solution (1.6×10^{-4} mol L^{-1}) was obtained by appropriately diluting the stock copper solution with water.

Ionic strength was kept constant at 0.1 by adding the appropriate amount of NaCl solution (mol L^{-1})
Analytical grade chemicals and deionised water (MicroMed high purity water system, TKA Wasseraufbereitungssysteme GmbH) were used for the preparation of all solutions. All the glassware used was washed with aqueous HCl (1:1) and then thoroughly rinsed with running, distilled water, and then finally with deionised water.

General Procedure
In order to obtain good mechanical and thermal stability, the instruments were run for 10 min before the first measurement. The reaction was carried out in the following way. In the reaction - mixture vessel with four compartments, the solution of 1-nitroso-2-naphthol was placed in one compartment, sodium hydroxide in the second, diazepam in the third, copper(II), electrolyte for the ionic strength and ethanol (total volume 10 mL) in the fourth compartment.

The vessel was thermostated at 22.00 ± 0.02 °C and the reaction was initiated by mixing. The reaction solution was put into a cell, and the absorbance at 425 nm was measured spectrophotometrically every 30 s over a period of 5-6 min after mixing against the reagent blank prepared similarly. The rate of the reaction at different concentrations of each of the reactants was obtained by measuring the slope of the linear part of the kinetic curves to the absorbance-time plot (from Beer`s law $A = \varepsilon \cdot l \cdot c$, $dA/dt = \varepsilon \cdot l \cdot dc/dt$, $dc/dt = \frac{dA/dt}{\varepsilon \cdot l}$, $slope = dA/dt$, $rate = dc/dt$). The calibration graph was constructed by plotting the slope of the linear part of the kinetic curve, versus concentration of DZP (c_{DZP}, μg mL^{-1}).

Procedure for tablets
A total of ten tablets of each one of the different used pharmaceutical preparations containing DZP were weighed and finely powdered using a mortar and pestle. An accurately weighed portion of the resulting powder, equivalent to 2 mg of DZP, was dissolved in 25.0 mL of ethanol. Then it was centrifuged at 3500 rpm for 5 minute and filtered through a 0.45 μm membrane filter (Millipore) directly in a 50.0 mL volumetric flask and filled up to a volume with ethanol to obtain a solution whose expected DZP concentration was 40.0 μg mL^{-1}.

Into a 50.0 mL volumetric flask, 4 rectal solutions (appropriate amount of diazepam 20 mg) were transferred and filled up to a volume with ethanol. Then, 2.50 mL of this solution was transferred into a 25.0 mL volumetric flask and added ethanol to obtain a solution whose expected DZP concentration was 40.0 μg mL^{-1}.

Aliquots of these solutions were transferred into vessels covering the concentration range listed in Table 4.

In all cases it was assumed that the actual content of the tablet corresponds to that reported by the manufacturing laboratories.

Serum sample preparation

Human lyophilised control serum (Lyotrol N) was used. Serum sample was spiked at one concentration level listed in Table 5. To 0.5 mL of serum, the appropriate amount of the stock solution of DZP (1.0 mg mL^{-1}) and 25 mL of ethanol was added and after brief vortex mixing it was centrifuged for 5 min at 3000 rpm to deposit the protein precipitate. The separated supernatant was collected in a 50.00 mL standard volumetric flask and filled up to the mark with the same solvent. Serum sample was contained 200.0 μg mL^{-1} of DZP. Aliquots of this solution were transferred into vessels covering the concentration range listed in Table 5. For kinetic determination, Fe^{3+} ions were masked by adding the appropriate amount of F^- ions (1×10^{-4} g mL^{-1}). For HPLC determination, aliquots of DZP solution were transferred in a 10.00 mL volumetric flask, evaporated to dryness in a water bath, the residue was reconstituted with mobile phase and 10 μL was transferred into glass vial for automatic injection into the HPLC system.

Comparative Method

HPLC was used as a parallel method [9]. Diazepam was detected and quantified on a 250x4.6 mm Zorbax C_{18} (5μm) analytical column operating at room temperature. The mobile phase was a mix of acetonitrile-water, 30:70 (by vol). The eluate was monitored at 254 nm. Injection of the samples (20 μL) was performed using an autosampler. Flow rate was 1 mL min^{-1}.

RESULTS AND DISCUSSION

Mechanism of the Reaction

Diazepam shows complexing ability with Cu(II). The complex agrees with the empiric formula $[Cu(DZP)_2]^{2+}$, $[Cu(DZP)_2]X_2$ $(X = Cl, Br)$. The IR and electronic spectroscopy suggest an octahedral symmetry around metal ions (coordination via the nitrogen atoms 1,4 of the diazepemine ring) [27]. Its chelate copper complex is more stable than that formed with R(NO)OH. The reaction moves to the right and DZP was determined by monitoring the rate of appearance of 1-nitroso-2-naphthol in basic medium at 425 nm.

$$Cu^{2+} + 2Cl^- + 2R(NO)OH \rightarrow Cu[R(NO)O]_2 + 2Cl^-$$

$$Cu[R(NO)O]_2 + 2Cl^- + 2DZP \rightarrow [Cu(DZP)_2]Cl_2 + 2R(NO)OH$$

The plot of the reaction as a function of the diazepam concentration is a straight line which can be used as a calibration graph.

Kinetic studies

A tangent method was used for the processing of the kinetic data. The rate of the reaction was obtained by measuring the slope of the linear part of the kinetic curves to the absorbance-time plot (slope = dA/dt).

Effect of variables

In order to determine the lowest possible determinable concentration of diazepam, the conditions needed to be optimized. Therefore, the dependence of the rate of reactions on the concentration of each of the reactants was determined.

The dependence of the reaction rate on the alkalinity of the solution (Fig. 1) shows a maximum at the concentration of sodium hydroxide of 1.5×10^{-2} mol L^{-1} and this concentration was selected for further work.

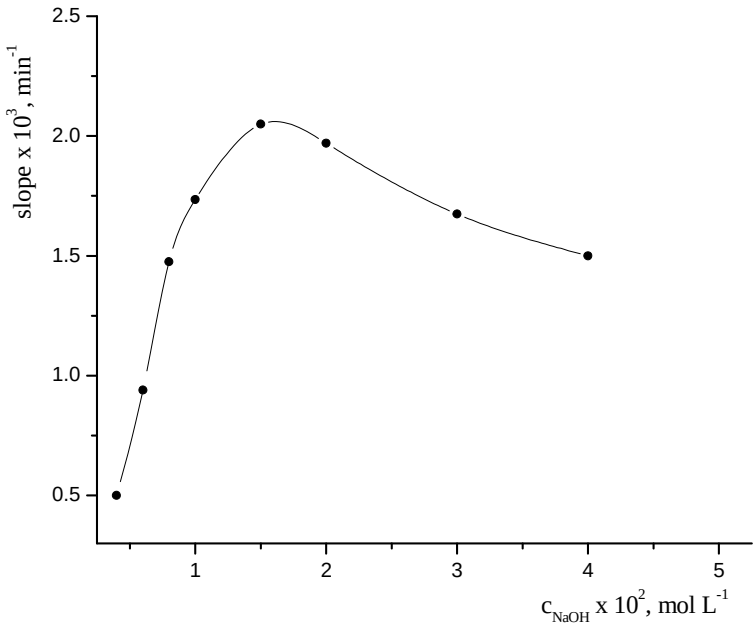

Figure 1. Effect of sodium hydroxide concentration on the slope of the absorbance-time curve. Initial concentrations: $c_{R(NO)OH}$ = 1.0×10^{-5} mol L^{-1}, $c_{Cu(II)}$ = 1.6×10^{-5} mol L^{-1}, c_{DZP} = 1.0×10^{-5} mol L^{-1}, c_{NaCl} = 0.1 mol L^{-1}, t = 22.00 ± 0.02 °C

The effect of the concentration of 1-nitroso-2-naphthol on the rate of reaction was studied in the range 0.3-1.5×10^{-5} mol L^{-1} (Fig 2). It can be seen that the reaction rate increases linearly with increasing of the1-nitroso-2-naphthol concentration from 0.3-1.0×10^{-5} mol L^{-1} and become constant at 1.0×10^{-5} mol L^{-1}. Thus, a concentration of 1.2×10^{-5} mol L^{-1} was chosen as the optimum concentration.

The correlation between slope and the Cu(II) concentration is given on Fig. 3. The influence of the concentration of Cu(II) on the rate of reaction examined in the range of 0.6-4.4×10^{-5} mol L^{-1}. The reaction rate increased with increasing the concentration of Cu(II) from 0.6-2.5×10^{-5} mol L^{-1} and becomes constant at higher concentrations than 2.5×10^{-5} mol L^{-1}. Cu(II) concentration of 3.2×10^{-5} mol L^{-1}, this was recommended for the determination process.

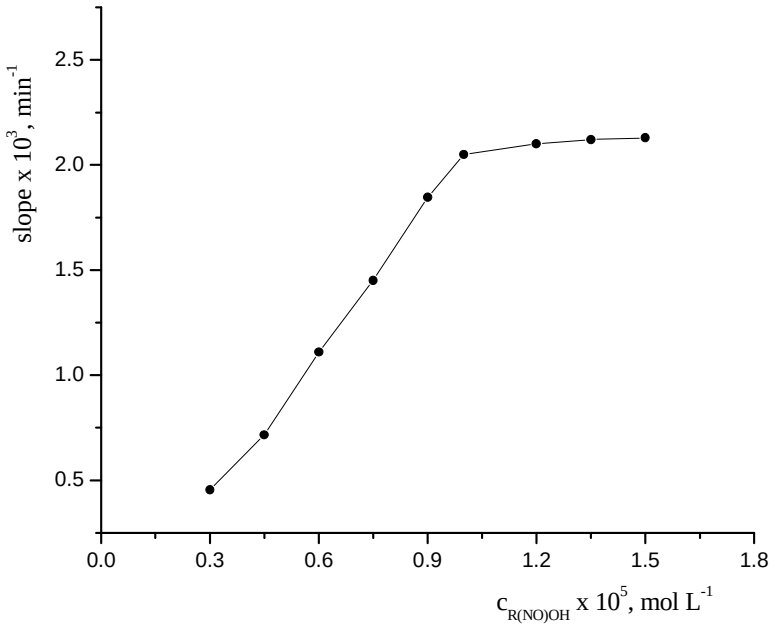

Figure 2. Effect of 1-nitroso-2-naphthol concentration on the slope of the absorbance-time curve. Initial concentrations: C_{NaOH} = 1.5×10^{-2} mol L^{-1}, $C_{Cu(II)}$ = 1.6×10^{-5} mol L^{-1}, C_{DZP} = 1.0×10^{-5} mol L^{-1}, C_{NaCl} = 0.1 mol L^{-1}, t = 22 ± 0.02 °C

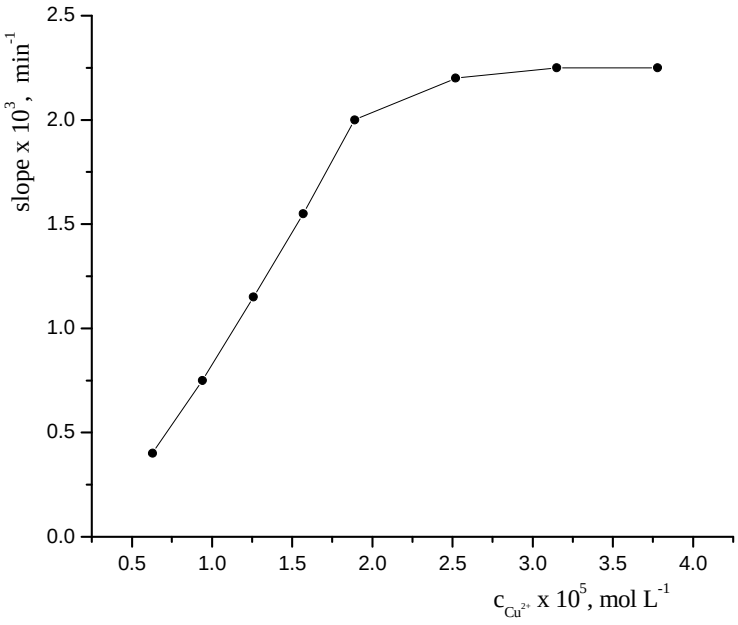

Figure 3. Effect of copper(II) concentration on the slope of the absorbance-time curve. Initial concentrations: C_{NaOH} = 1.5×10^{-2} mol L^{-1}, $C_{R(NO)OH}$ = 1.2×10^{-5} mol L^{-1}, C_{DZP} = 1.0×10^{-5} mol L^{-1}, C_{NaCl} = 0.1 mol L^{-1}, t = 22 ± 0.02 °C

The effect of temperature on the reaction rate was studied at 19, 22, 25, 28 and 31°C (Fig. 4). The absorbance-time curves obtained at these temperatures indicated the temperature dependence of the reaction rate. The rate for different concentrations of DZP at each temperature was calculated and utilized for plotting the calibration curve. It was found that the calibration graph obtained at 22°C possessed good linearity and is recommended that the determination can be carried out at 22°C.

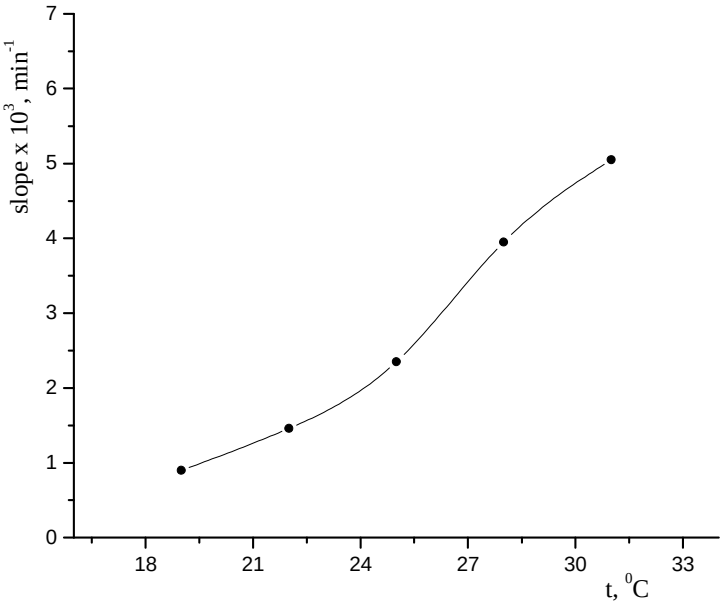

Figure 4. Effect of the temperature on the slope of the absorbance-time curve. Initial concentrations: c_{NaOH} = 1.5×10^{-2} mol L^{-1}, $c_{R(NO)OH}$ = 1.2×10^{-5} mol L^{-1}, $c_{Cu(II)}$ = 3.2×10^{-5} mol L^{-1}, c_{DZP} = 0.7×10^{-5} mol L^{-1}, c_{NaCl} = 0.1 mol L^{-1}

For the validation process the following parameters were characterized: linearity and range; limit of detection; repeatability; percent recoveries; precision; selectivity [28].

The least squares' equation (y = bx + a, where b and a are slope and intercept, respectively) for the calibration graph and correlation coefficient (r) [29] for the determination of DZP in the interval 0.28 to 3.70 µg mL^{-1} under the optimal reaction conditions ($c_{R(NO)OH}$ =1.2×10^{-5} mol L^{-1}, c_{NaOH} =1.5×10^{-2} mol L^{-1}, $c_{Cu(II)}$ = 3.2×10^{-5} mol L^{-1}, t = 22.00 ± 0.02 °C) were calculated:

$$tg\alpha \cdot 10^3 = 0.53509 \cdot c_{DZP} + 0.37589 \qquad r = 0.9984$$

where *slope* is the slope of the linear part of the kinetic curve to the absorbance-time plot ($slope = dA/dt = \varepsilon \cdot l \cdot dc/dt$, Beer's law) and c_{DZP} is the diazepam concentration expressed in µg mL^{-1}.

The variance (S_0^2) of the calibration line was evaluated to be 6.2x10^{-10} (µgmL^{-1})^2. The low value of variance indicated negligible scattering of the experimental data points around the line of regression.Quantitative parameters of the analysis are given in Table 1.

Table 1 Quantitative parameters of the analysis

Parameters	
Calibration range / µg mL^{-1}	0.28 – 3.70 n = 7
Regression equation	$tg\alpha \cdot 10^3 = 0.53509 \cdot c_{DZP} + 0.37589$
(Slope ± SD)×10^3	0.53509 ± 0.00828
(Intercept ± SD)×10^3	0.37589 ± 0.019
Correlation coefficient, r	$r = 0.9984$
Variance ($S_0 \times 10^3$)^2 / (µg mL^{-1})^2	6.2×10^{-4}
Detection limit / µg mL^{-1}	0.14

The following kinetic equation for the reaction was deduced on the basis of the graphic correlations obtained.

$$rate = k \cdot c_{NaOH} \cdot c_{DZP}$$

k- constant proportional to the rate constant of the reaction

The equation is valid for the following concentrations: R(NO)OH (1.0-1.5)×10^{-5} mol L^{-1}, NaOH (0.4-1.5)×10^{-2} mol L^{-1}, Cu(II) (2.5-4.4)×10^{-5} mol L^{-1}, DZP 0.28-3.70 µg mL^{-1}.

The limits of detection (LOD) [30] was evaluated using the following equation:

$$LOD = 3.3 \times S_0 / b$$

where S_0 is the residual standard deviation of the calibration line, b is the slope of the calibration line (analytical sensitivity) and found to be 0.14 µg mL^{-1}.

The precision and accuracy of the above system were studied by performing the experiment 5 times for different concentrations of diazepam. The results of accuracy and precision of the recommended procedure are given in Table 2.

Table 2 Accuracy and precision of the determination of DZP

Taken µg mL^{-1}	Found[a] $\bar{x} \pm \overline{SD}$, µg mL^{-1}	RSD[b] (%)	$(\bar{x} - \mu) / \mu \cdot 100$[c]
0.28	0.30 ± 0.01	4.93	7.14
1.99	1.97 ± 0.06	3.23	-1.01
3.70	3.67 ± 0.08	2.39	-0.81

[a)]Mean and standard deviation of five determinations and 95 % confidence, [b)]relative standard deviation, [c)]accuracy of the method

The effect of temperature on reaction rate is well known and important in understanding the various activation parameters of the reaction products. In order to evaluate the apparent activation parameters, the reaction rate was studied at 19, 22, 25, 28 and 31°C at c_{NaOH} = 1.5×10^{-}

^{2} mol L^{-1}, $c_{R(NO)OH}$ = 1.2×10^{-5} mol L^{-1}, $c_{Cu(II)}$ = 3.2×10^{-5} mol L^{-1}, c_{DZP} = 0.7×10^{-5} mol L^{-1}. Arrhenius curve was constructed by plotting log k versus 1/T and found to be linear with coefficient of correlation, r = 0.9997. Activation energy (Ea) can be calculated from the slope (-Ea/2.303R) and found to be 99.14 ± 0.08 kJ mol^{-1}. The ability to predict changes in the rate of the reaction is based on a knowladge of the value of the activation energy. According to Hammond [31] and Smith [32], most reactions which have observable rates at ordinary temperatures have activation energies of 15-30 kcal mol^{-1} (62.76-125.52 kJ mol^{-1}).A value of 99.14 kJ mol^{-1} is in accordance with this postulate, indicated that the reaction is feasible.

Interference studies

To assess the selectivity of the method, the interference of those species accompanying DZP in pharmaceuticals was studied. The tolerance limits (expressed as w/w ratio), for the species studied on the determination of 1.99 µg mL^{-1} of DZP were given in Table 3.

Table 3 Tolerance ratio for foreign species in the determination of 1.99 µg mL^{-1} of diazepam

Foreign species	I[a] (%)	Tolerance level (µg mL^{-1} interferent/ µg mL^{-1} DZP)
citric acid	10 – 15	10^2
fructose, glucose, lactose, B_1, B_6, B_{12}, F^-	5 - 10	
mannitol, sorbitol, stearic acid, Li^+, K^+, $C_2O_4^{2-}$	< 5	
Ca^{2+}, Mg^{2+}, Zn^{2+}	5 – 10	10
Met, Tyr, Trp	< 5	
Phe, Asp, Lys	5 -10	1
His, Arg, Gly, Ala, Ser	interference	1
Fe^{3+} [b)]	interference	1

[a)] Interference coefficient, $I = (c^{\circ}_{DZP} - c_{DZP}) / c^{\circ}_{DZP}$

c°_{DZP} and c_{DZP} are measured concentrations of DZP without and with the interfering species

[b)]Masking with F^- ions

As can be seen, the usual ingredients of powdery drugs (fructose, glucose, lactose), some of the amino acids (Phe, Asp, Met, Tyr, Trp, Lys), will not interfere with the method, because the amounts tolerated are much higher than those usually present in pharamceuticals. It also should be noted that a higher tolerance level exists to the presence of vitamins B_1, B_6 and B_{12}. Ions Ca^{2+}, Mg^{2+}, Zn^{2+} interfere when present in approximately 10-fold excesses. Amino acids (His, Arg, Ala, Ser, Gly) interfere with the method. More severe intrference was observed for Fe^{3+} ion. No interference was found when including up to 100-fold mannitol, sorbitol, stearic acid, citric acid, F^- and $C_2O_4^{2-}$ anions.

Applicability of the Proposed Method

The proposed method was applied to the determination of diazepam in three pharmaceutical formulations and human control serum using the direct calibration curve. They were treated as described in the Experimental section. As can be seen in Table 4, the results obtained by this method are in accordance with the HPLC method. Also, good recovery was observed in the case of serum sample (Table 5), indicating that the constituents of the human control serum do not interfere (Fe^{3+} ions were masked with F^- and the protein precipitate was deposited) in any way with the detection of diazepam. Therefore, the proposed method could be used for the determination of diazepam in serum samples. The results of the proposed method were statistically compared with those of the HPLC method using a point hypothesis test [33,34]. Statistical analysis of the results (Tables 4 and 5) showed that calculated F- and t- values at 95 % confidence levels are less than the theoretical ones, confirming no significant differences between the performance of the proposed and the HPLC method.

Table 4 Determination of diazepam by the kinetic and HPLC method

Pharmaceutical Preparation	Taken µg mL^{-1}	DZP found by the proposed method[a)] $\bar{x} \pm \overline{SD}$, µg mL^{-1}	RSD[a)] %	Recovery[a)] %	HPLC[a)] $\bar{x} \pm \overline{SD}$, µg mL^{-1}	F-value[b)]	t-value[b)]
Diazepam[c)]	1.42	1.39 ± 0.06	4.09	97.89	1.36 ± 0.07	1.62	0.730
Bensedin®[d)]	2.56	2.51 ± 0.03	1.21	98.05	2.48 ± 0.05	2.81	1.129
Diazepam[e)]	3.13	3.09 ± 0.04	1.30	98.72	3.06 ± 0.06	2.01	0.968

[a)]Data are based on the average obtained from five determinations
[b)]Theoretical F-value (v_1=4, v_2=4) and t-value (v=8) at 95 % confidence level are 6.39 and 2.306, respectively
[c)]Rectal solution (from Pharmacy Belgrade, Serbia) containg 5 mg of diazepam in 2.5 mL of solution
[d)]Tablets (from Galenika a.d., Belgrade, Serbia) containing diazepam 2 mg and excip.
[e)]Tablets (from Panfarma d.o.o., Belgrade, Serbia) containing diazepam 2 mg and excip

Table 5 Determination of diazepam in human control serum ("Lytorol N", bioMérieux® sa, France) by standard addition method

Proposed method µg mL^{-1}		RSD[a)] %	Recovery[a)] %	HPLC[a)] $\bar{x} \pm \overline{SD}$, µg mL^{-1}	F-value[b)]	t-value[b)]
Added	Found[a)] $\bar{x} \pm \overline{SD}$					
0.85	0.83 ± 0.03	3.81	97.65	0.80 ± 0.02	2.35	1.757

[a)]Data are based on the average obtained from five determinations
[b)]Theoretical F-value (v_1=4, v_2=4) and t-value (v=8) at 95 % confidence level are 6.39 and 2.306, respectively

CONCLUSION

In conclusion, the proposed kinetic-spectrophotometric method for the determination of DZP in pharmaceutical samples and human control serum reported in this work is simple, rapid, inexpensive, thus being very appropriate for routine quality control analyses of active drug in the laboratories of hospitals, pharmaceutical industries and research institutions. Also, is very suitable for developing countries. Statistical comparison of the results with the HPLC method shows good agreement and indicates no significant difference in accuracy and precision.

Acknowledgement
This research was supported by grant number 142015 from the Serbian Ministry of Science and Environmental Protection. The authors are grateful for the financial support provided by this Ministry.

REFERENCES

[1] HR Mattson; DM Woodbury; JK Penry; RP Schmidt. Antiepileptic Drug, 1 st Edition, Raven Press, New York, **1982**; 497.
[2] A El-Brashy; FA Aly; F Belal. *Microchim. Acta*, **1993,** 110(1-3), 55-60.
[3] AA Salem; BN Barsoum; EL Izake. *Spectrochim. Acta, Part A*, **2004**, 60(4), 771-780.
[4] SM Khalile; MA Zayed; SM Abd Allah. *Egypt. J. Chem*., **2004**, 47(1), 1-16.
[5] S Liawruangrath; J Makchit; B Liawruangrath, *Anal. Sci.*, **2006**, 22(1), 127-130.
[6] K Chiba; H Horii; T Chiba; Y Kato; T Hirano; T Ishizaki. *J. Chromatogr. B*, **1995**, 668(1), 77-84.
[7] MJ Koenigbauer; MA Curtis. *J. Chromatogr. B*, **1988**, 427, 277-285.
[8] A El-Mahjoub; C Staub. *J. Pharm. Biomed. Anal.*, **2000**, 23(2-3), 447-458.
[9] C Ferreyra; C Ortiz. *J. Pharm. Biomed. Anal.*, **2001**, 25(3-4), 493-499.
[10] SN Muchohi; BR Ogutu; CRJC Newton; GO Kokwaro. *J. Chromatogr. B*, **2001,** 761(2), 255-259.
[11] Z Liu; J Short; A Rose; S Ren; N Contel; S Grossman; S Unger. *J. Pharm. Biomed. Anal.*, **2001**, 26, 321-330.
[12] S Diallo; E Bugni; F Senhadj-Raoul; S Gasdeblay; D Marot; MC Dessalles; G Mahuzier. *Talanta*, **2001**, 55(4), 721-732.
[13] W Hanpitakpong; V Banmairuroi; B Kamanikom; A Choemung; K Na-Bangchang. *J. Pharm. Biomed. Anal*., **2004**, 36(4), 871-876.
[14] M Bakavoli; M Kaykhaii. *J. Pharm. Biomed. Anal*., **2003**, 31(6),1185-1189.
[15] L Wang; H Zhao; Y Qiu; Z Zhou. *J. Chromatogr. A*, **2006**, 1136(1), 99-105.
[16] M Pujadas; S Pichini; E Civit; E Santamarina; K Perez; R De la Torre. *J. Pharm. Biomed. Anal.*, **2007**, 44(2), 594-601.
[17] R Cordero; S Paterson. *J. Chromatogr. B*, **2007**, 850(1-2), 423-431.
[18] N Thuaud; B Sebille; MH Livertoux; J BessiereJ. *J. Chromatogr. A*, **1983**, 282, 509-518.
[19] MM Correia dos Santos; V Famila; ML Simoes Goncalves. *Anal. Bioanal. Chem.*, **2002,** 374(6), 1074-1081.
[20] W Guo; H Lin; L Liu; J Song. *J. Pharm. Biomed. Anal.*, **2004**, 34(5), 1137-1144.
[21] RS Guerrero; CG Benito; JM Calatayud. *J. Pharm. Biomed. Anal.*, **1993**, 11(11-12)**,** 1357-1360.
[22] J Dolejsova; P Solich; ChK Polydorou; MA Koupparis; CE Efstathiou; *J. Pharm. Biomed. Anal.*, **1999**, 20, 357-362.
[23] J Moros; S Garrigues; M De la Guardia. *J. Pharm. Biomed. Anal.*, **2007**, 43(4), 1277-1282.
[24] A Es-haghi; X Zhang; FM Musteata; H Bagheri; J Pawliszyn. *The Analyst*, **2007**, 132(7), 672-678.
[25] S Furlanetto; S Orlandini; G Massolini; MT Faucci; E La Porta; S Pinzauti. *The Analyst*, **2001**, 126(10), 1700-1706.
[26] MSA Pardo; M Steppe; MFM Tavares; ERM Kedor-Hackmann; MIRM Santoro. *J. Pharm. Biomed. Anal.*, **2005**, 37(2), 273-279.

[27] C Preti C; G Tosi. *J. Inorg. Nucl. Chem.*, **1979**, 41(2), 263-266.
[28] The United States Pharmacopoeia USP-27/NF-22, Authority of the United States Pharmacopoeia Convention, Inc., Rockville, MD; **2004**.
[29] JN Miller. *Analyst*, **1991**, 116(1), 3-14.
[30] J Ermer. *J. Pharm. Biomed. Anal.*, **2001**, 24(5-6), 755-767.
[31] GS Hammond. *J. Chem. Phys.*, **1955**, 77(2), 334-338.
[32] IWM Smith. *Chem. Soc. Rev.*, **2008**, 37(4), 812-826.
[33] C Hartmann; J Smeyers-Verbeke; W Penninckx; YV Heyden; P Vankeerberghen; DL Massart. *Anal. Chem.*, **1995**, 67(24), 4491-4499.
[34] DA Skoog; DM West; FJ Holler. *Fundamentals of Analytical Chemistry*, 7th Edition, Saunders College Publishing, Philadelphia, **1996**; 11.