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Fluorimetric Investigation of Host-Guest Complexation of Nizatidine with β-Cyclodextrin and its Analytical Application

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ABSTRACT

The supramolecular interaction of nizatidine (NIZ) and β-cyclodextrin (β-CD) has been studied by spectrofluorimetry. The results showed that β-CD reacted with NIZ to form an inclusion complex.The NIZ-β-CD complex formed a host-guest complex in 1:1 stoichiometryand inclusion constant ($K = 3.15\times10^2$ L mol^{-1}) and was ascertained by the typical double reciprocal plots. Furthermore, the thermodynamic parameters (ΔH°, ΔS° and ΔG°) associated with the inclusion process were also determined. Based on the significant enhancement of the fluorescence intensity of NIZ produced through complex formation, a simple, accurate, rapid and highly sensitive spectrofluorometric method for the determination of NIZ in aqueous solution in the presence of β-CD was developed. The measurement of relative fluorescence intensity was carried with excitation at 314 nm.

The factors affecting the inclusion complex formation were studied and optimized. Under the optimum reaction conditions, linear relationships with good correlation coefficients (0.999) were in the concentration range of 0.04-0.1μg $mL^{-1}$for spectrofluorimetry. The limit of detection (LOD) was found to be1.27×10^{-3} μgmL^{-1}.The proposed method was successfully applied to the analysis of NIZ pharmaceutical dosage formulations with good accuracy; the recovery percentages ranged from 95.4–106.85%.

Key word: Nizatidine, inclusion complex; β-cyclodextrin; Spectrophotometry; Spectrofluorimetry.

1. INTRODUCTION

Cyclodextrins (CDs) are water-soluble cyclic oligosaccharides composed of six (α–), seven (β–) and eight (γ–) units of D-(+)-glucopyranose arranged in a truncated cone shape structure. The hydrophobic cavity of CDs can host a large variety of organic and inorganic compounds of suitable size. The formation of an inclusion complex greatly affects the physical chemical properties of the guest molecules, such as solubility, chemical reactivity and the spectroscopic and electrochemical properties, and most of these effects can be utilized in many fields including pharmaceutical industry (to improve the solubility, stability and bioavailability of pharmaceuticals, as a carriers of active substances in biological systems and to retard the release of active substances from the pharmaceutical matrix) and various branches of analytical chemistry [1–3]. From an analytical point of view the formation of inclusion complexes allows, to improve fluorescence intensity [4–9] and induce chiral separation in capillary electrophoresis [10-12]. Analysts have used this property of CDs, and a lot of methods based on the fluorescence of inclusion complexes with CDs have been proposed for the determination of several pharmaceutical drugs, pesticides, and metal ions [13, 14].

The histamine H_2 receptor antagonists (H_2-RAs) are among the most effective anti-secretory drugs available for management of acid peptic diseases. This class of drugs was developed with the aim of producing agents for the treatment of peptic ulcer [15]. H_2-RAs include cimetidine (CIM), famotidine (FAM), nizatidine(NIZ) and ranitidine hydrochloride (RAN).

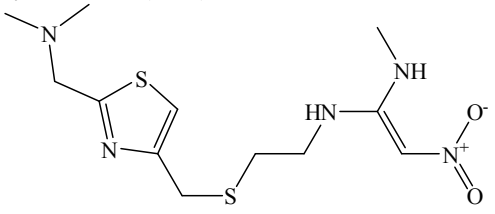

Fig. 1. Chemical structure of nizatidine (NIZ).

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NIZ (Fig.1) is N-[2[[[2-[(dimethylamino)methyl]-4-thiazolyl] methyl]thio]ethyl]-N-methyl-2-nitro-1,1-ethenediamine, is a specific potent H_2 receptor antagonist. Unlike cimetidine, which contains an imidazole ring structure, or ranitidine, which contains a furan ring structure, NIZ has a thiazolyl ring structure. Nizatidine is more potent than cimetidine for inhibition of gastric acid secretion induced by various stimuli. However, NIZ lacks cimetidine's anti-androgenic and hepatic microsomal enzyme inhibiting effects [16,17]. NIZ has been used in the treatment of duodenal ulceration.

Several methods have been reported for the analysis of NIZ, these include; spectrophotometric [18-21], coulometric [22] and HPLC [23–28], potentiometric titration [29] and differential pulse polarographic [30] have been described the determination of NIZ in pharmaceutical formulations. Among these reported methods, there is no spectrofluorometric method for NIZ determination.

To best of our knowledge, there is no report dealing with analytical application of cyclodextrins for determination of NIZ in pharmaceutical formulation. This paper describes a simple, rapid, and sensitive spectrofluorometric method, for the determination of NIZ in pharmaceutical formulation.

2. EXPERIMENTAL

2.1. Chemical and reagent

Pure nizatidine (NIZ) reference standard was obtained from Jamjoom Pharmaceuticals (Jeddah, Saudi Arabia) and used as working standard. Fizit® capsules (150 mg of NIZ) were supplied from Jamjoom pharmaceuticals, Jeddah, Saudi Arabia. β-Cyclodextrin was supplied by Janssen Chimica (Beerse, Belgium).Doubly distilled water was used to prepare all solutions in this study.

2.2. Instruments and apparatus

Fluorescence spectra and intensity measurements were made on a Shimadzu RF-1501 spectrofluorimeter (Japan) equippedwith a 150W xenon lamp. Slit widths for both monochromators were set at 10 nm. All of the spectrophotometric measurements were made with a double beam UV1800 ultraviolet-visible spectrophotometer provided with matched 1-cm quartz cell (Shimadzu Japan). pH meter model HI 255 (Hanna Instruments, Mumbai, India) was used for pH measurements and thermostat LAUDA Ecoline model RE220 (Gaithersburg, MD) was used.

2.3. Stock and standard solutions

A 100 μg mL^{-1} stock standard solution of NIZ was prepared daily by dissolving 2.5 mg of NIZ in distilled water and diluted to the mark in a 25 mL volumetric flask with water.

2.4. Buffer solutions

Stock solution of Britton- Robinson buffer solutionwas prepared by adding 0.04 mol L^{-1}acetic acid, 0.04 mol L^{-1} phosphoric acid and 0.04 mol L^{-1} boric acid) and appropriate amount of 0.2 mol L^{-1} of sodium hydroxide was added to prepare solution of pH range from 3 -13.

2.5. Procedure

2.5.1. **Spectrofluorimetric method**

Into a 10 mL volumetric flask, solutions were added in the following order: 1.0 mL (1.0 μg mL^{-1}) of NIZ, 2.5 mL (pH 10) of Britton-Robinson buffer solution and appropriate amount of 15.78 % β-CD. The mixture was heated in thermostat for 15 minutes (60 °C) then the solution was diluted to the mark with distilled water. The fluorescence intensity of NIZ-β-CD was measured at $\lambda_{ex}/\lambda_{em}$ = 314 nm/454 nm.

2.5.2Spectrofluorimetric determination of stoichiometry and inclusion constant

1.0 mL of 6.0×10^{-5}molL^{-1} NIZ and 1.0mL pH 10.0of BR buffer solution were added to a volumetric flask, then the varied amounts of β-CD (0.0, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 mL of 5.0×10^{-3}molL^{-1}) were added sequentially. The mixture was diluted to 10.0mL with water and heated for 15 minutes in thermostat 60°C. The fluorescence spectra were measured.

3. RESULTS AND DISCUSSION

3.1. Emission spectra

The spectral characteristics of NIZ were studied and the result showed that the wavelengths of maximum emission of NIZ at pH 10.0 were 454 nm Fig. 2. When β-CD was added into the NIZ solution, the wavelength of maximum of emission did not change but the fluorescence intensity dramatically increased. This can be rationalized by having NIZ enters the hydrophobic cavity of β-CD and binding takes place through non-covalent bonding van der Waals forces and hydrogen bonding. In the cavity, the degree of motion freedom of NIZ molecule is reduced, thus the cavity can shield the excited signal of NIZ from quenching by quencher in the aqueous solution. So the fluorescence intensity increased when the NIZ-β-CD inclusion complex was produced.

3.2. Optimization of experimental variables

In order to optimize the reaction conditions between the NIZ and β-CD the following parameters were investigated pH of the buffer, the volume of the buffer, reaction time, and temperature and β-CD concentration.

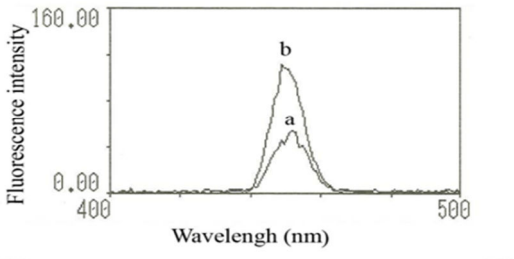

Fig. 2. Emission spectra of NIZ (a) of NIZ in the absence of β-CD. Emission spectrum (b) of NIZ in the presence of β-CD. Concentration of NIZ : 1.0 μg mL^{-1}. λ_{em} = 454 nm.

3.2.1. Effect of pH on the fluorescence intensity

The effect of pH on the fluorescence intensity of the NIZ-β-CD complex was studied in the pH range 3 – 13, the solutions were prepared as described in the general procedure and the obtained results are presented in Fig. 3. It was found that for NIZ-β-CD complex the fluorescence intensity in strong acidic and slightly alkaline media both wavelength and emission intensity are different for ionized and non-ionized forms of the NIZ. In acidic media protonation occur on several sites on the NIZ molecule such as the nitro group, amine nitrogen in both secondary and tertiary amine, and nitrogen in a thiazole ring. Accordingly, acid dissociation constant for the excited molecule for some species differ in their ground state, therefore analytical procedure based on fluorescence frequently requires close

control of pH, At pH 10 the additional resonance forms lead to a more stable first excited state. Beyond these pH values any further increase in the pH caused a gradual decrease in the fluorescence intensity.

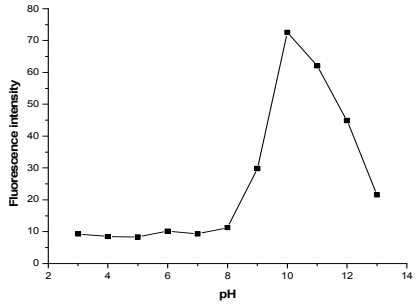

Fig. 3. Influence of the pH on the fluorescence intensity of NIZ and NIZ- β -CD complex; [NIZ] = 3.016×10^{-6} mol L^{-1}, and [β-CD] = 7.0×10^{-3} mol L^{-1}, λ_{ex} = 314 nm, λ_{em} = 454 nm. Temperature 25.0 °C; reaction time: 15 minutes.

3.2.2. Effect of β-CD Concentration

When various concentrations of β-CD solution were added to a fixed concentration of NIZ, 2.0 mL of 15.78 % of β-CD solution was found give the highest fluorescence intensity (Fig. 4). With the increase of β-CD concentration, the fluorescence intensity of NIZ gradually enhanced until the stable inclusion complex is formed at about 15.78 %, above which a decreases in peak value is observed. Therefore, a concentration of 15.78 % was adopted as the most suitable for analytical purpose.

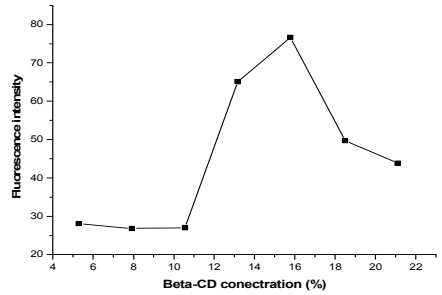

Fig. 4. Effect of β-CD concentration on the fluorescence intensity of inclusion complex.

3.2.2. Influence the volume of buffer solution

The effect of the volume of the Britton-Robinson buffer at pH 10 on NIZ was also studied. It was found that increasing the buffer volume resulting in a subsequent increase fluorescence intensity up to 2.5 mL, after which fluorescence intensity remained constant. A volume of 2.5 BR buffer at pH 10 was recorded as optimum in this study (data not shown).

3.2.3. Effect of Reaction Temperature and Time

The effect of temperature on the reaction was also studied by varying the temperature from 20 °C to 80 °C for NIZ. The inclusion complex does not form at room temperature but the highest fluorescence intensity is obtained at 60 °C for 15 min. The optimum temperature 60 °C and at temperature above 60 °C , an inclusion–dissociation mechanism takes place in which the inclusion process becomes entropically unfavourable and the inclusion complex may dissociate by increasing the temperature, which is reflected by the reduction of the fluorescence intensity and quantum reaction yield. This can be ascribed to an increase in the kinetic energy of the molecules and hence the probability of their colliding; as a result, radiationless deactivation through the internal conversion prevailed and the fluorescence quantum efficiency decreased as shown in Figure 5.

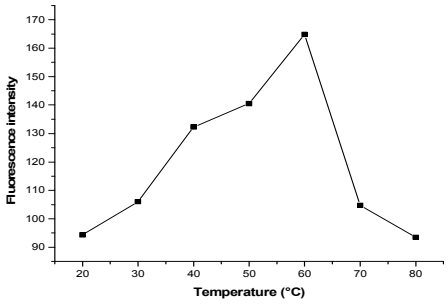

Fig. 5. Influence of the temperature on the fluorescence intensity of NIZ and NIZ- β -CD complex;[β-CD] = 7.0×10^{-3} mol L^{-1}, λ_{ex} = 314 nm, λ_{em} = 454 nm. Both excitation and emission slits widths were set at 10 nm.

3.5. Spectrofluorimetric determination of stoichiometry and inclusion constant

The stoichiometry and apparent association constant of the inclusion complex were studied under the established experimental condition: assuming that the composition of the complex was 1:1, the following expression can be written

$$\text{NIZ} + \text{beta-CD} \rightleftharpoons \text{beta-CD-NIZ} \quad (1)$$

The apparent association constant of the complex (*K*) is given by:

$$K = [\text{beta-CD-NIZ}] / [\text{NIZ}]\,[\text{beta-CD}] \quad (2)$$

where [β-CD], [NIZ] and [β-CD-NIZ] are equilibrium concentrations. The apparent association constant value for the inclusion complex can be determined bythe typical double reciprocal (or Benesi–Hildebrand) plots:

$$\frac{1}{(F - F_0)} = \frac{1}{(F_\infty - F_0)\,K\,[\beta\text{-CD}]_0} + \frac{1}{F_\infty - F_0} \quad (3)$$

where $[\beta\text{-CD}]_0$ denotes the initial β-CD concentration; F_0 the fluorescence intensity of NIZ in the absence of β-CD; F_∞ the fluorescence intensity when all of the NIZ moleculesare essentially complexed with β-CD; and F the observed fluorescence intensity at each β-CD concentration tested. When a plot of $1/F - F_0$ vs. $1/[\beta\text{-CD}]$ is constructed (Fig. 6) straight line is obtained which indicative of a 1:1 stoichiometry for β-CD-NIZ complex. Good linear relationship (r = 0.9987) is observed in Fig. 6. On the other hand, assuming the stoichiometry of the inclusion complex was 1:2, the following expression is obtained:

$$\frac{1}{(F - F_0)} = \frac{1}{(F_\infty - F_0)\,K'\,([\beta\text{-CD}]_0)^2} + \frac{1}{F_\infty - F_0} \quad (4)$$

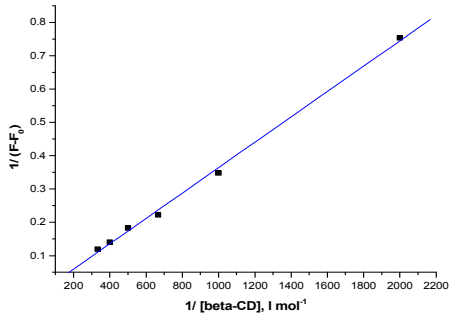

Fig. 6. Plot of $1/(F - F_0)$ vs. $1/[\beta\text{-CD}]$ of NIZ-β-CD complex; [NIZ] = 2 µg ml^{-1}.

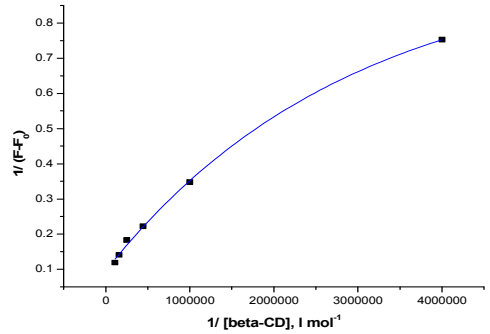

Fig. 7. Plot of $1/(F - F_0)$ vs. $1/[\beta\text{-CD}]^2$ of NIZ-β-CD complex; [NIZ] = 2 µg ml^{-1}.

When making a plot of $1/F - F_0$ against $1/([\beta\text{-CD}]_0)^2$, nonlinear relationship is obtained (Fig. 7), which indicated that the stoichiometry of the inclusion complex is not 1:2. These confirmed that β-CD and NIZ formed host–guest complex in 1:1 stoichiometry [31-34]. The inclusion constant (K) was calculated to be 3.15×10^2 Lmol^{-1}.

3.6. Inclusion complex thermodynamics

The thermodynamic parameters (ΔH°, ΔS° and ΔG°) for the formation of inclusion complex were determined from temperature dependence of apparent association constants, by using classical van't Hoff equation ($\ln K = -\Delta H°/RT + \Delta S°/R$), and plotting ln K versus $1/T$ [35, 36] The corresponding enthalpy and entropy can be obtained from the slope and intercept, respectively, which indicate the marked tendency of NIZ to complex with β-CD. ΔG◦ was obtained according to the equation: ΔG◦ =ΔH◦ −TΔS◦. The results are shown in Table 1.The stability constant of the complexes of NIZ with the β-CD at different temperature (303, 313,323 and 333 K) are shown in Table 1. We noted that the association constant for NIZ-β-CD increases as the temperature rises.

Thermodynamic parameters were calculated based on the temperature dependence of the association constant for NIZ-β-CD binding. The thermodynamic parameters such as the enthalpy changes (Δ*H)* and entropy changes (Δ*S)* of the binding reaction are important to confirm the force of interactions of NIZ with β-CD.

Table 1. Thermodynamic parameters

	Temperature (K)			
	303	313	323	333
K[a]	92.8	127.5	217.2	315.0
ΔG° [b] (kJ mol^{-1})	-31.38	-32.42	-33.46	-34.49
ΔH° [c] (kJ mol^{-1})				0.0035
ΔS° [d] (J mol^{-1} K^{-1})				103.59

[a] Apparent formation constant(K), [b] Standard free energy(ΔG°)
[c] Enthalpy(ΔH°), [d] Entropy(ΔS°).

Four driving forces for the inclusion of CDs with substrates were proposed, including hydrogen bonding between the hydroxyl groups of CDs and the NIZ molecules, van der Waals interactions between host and guest molecules, hydrophobic interaction, and the release of 'high-energy water' molecules from the cavities of β-CDs to the bulk water. Hydrophobic interaction essentially involves favourable positive entropy together with a slightly positive enthalpy change, whereas the other forces involve negative Δ*H* and Δ*S* [37]. Upon complexation both positiveenthalpic changes and positive entropic values are obtained, indicating that this inclusion ismainly entropically driven. Apparently, when NIZ is free in solution, it seems to have astrong interaction with its solvent shell. Upon binding, this solvent shell is brokenup, leadingto the partly unfavourableenthalpic change. As discussed above, ΔG◦ obtained are negative (Table 1), which indicated that the inclusion process proceeded spontaneously at experimental temperature. The positive ΔH◦ together with positive ΔS◦ suggested that the inclusion process is an enthalpy controlled process in the case of the NIZ and NIZ-CD complex.

3.4. Validation of the Methods

3.4.1. Linearity and Limit of Detection

In the two proposed methods, linear plot (n =7) with good correlation coefficients were obtained in concentration range 1-8 µg mL^{-1}, 0.04-0.1µg mL^{-1} for NIZ for spectrophotometric and spectrofluormetric, respectively (Table 2). The limits of detection (LOD) and quantification (LOQ) were determined using the formula:

LOD or LOQ=KS. Da/b, where K=3.3 for LOD and 10 for LOQ, S. Da is the standard deviation of the intercept, and b is the slope [38]. The LODs value for NIZ are 1.27×10^{-3}µg mL^{-1}and 0.256and LOQs are 3.85×10^{-3}µg mL^{-1}and0.776µg mL^{-1}for spectrofluorimetric and spectrophotometric methods respectively.

Table 2

Parameter	Spectrofluorometric method	Spectrophotometric method
Linear range (μg mL^{-1})	0.04-0.1	1-8
Molar a bsorptivity, ε , ($L\ mol^{-1}\ cm^{-1}$)	1.227×10^{8}	4.344×10^{4}
Correlation coefficient(r)	0.9998	0.9994
Limit of detection (μg mL^{-1})	1.27×10^{-3}	0.256
Limit quantification (μg mL^{-1})	3.85×10^{-3}	0.776
Slope	247.62	0.12312
Intercept	0.746	0.02104

3.4.2. Accuracy and Precision

The accuracy and precision of the proposed method were determined at three concentration levels of NIZ by analyzing three replicate samples of each concentration. The relative standard deviations (RSD) for the results did not exceed 2% as shown in Table 3 indicating the high reproducibility of the results and the precision of the method. This good level of precision was suitable for quality control analysis of NIZ pharmaceutical formulations.

Table 3 Recovery of the methods

Method	Drug	Sample Content (μg mL^{-1})	Added (μg mL^{-1})	Found (μg mL^{-1})	Recovery (% ± RSD)*
Method 1	NIZ	1.0	1.0	1.928	96.40 ± 1.85%
		1.0	4.0	5.116	102.32 ±1.69%
		1.0	6.0	7.260	102.32 ±1.69%
Method 2	NIZ	0.01	0.03	0.0397	99.25 ± 0.18 %
		0.01	0.04	0.0511	102.20 ± 1.80 %
		0.01	0.08	0.0511	100.89 ± 0.41 %

* Recovery was calculated as the amount found/amount taken × 100. Values are mean for three determinations. Method 1 spectrophotometric and Method 2 spectrofluorometric

Table 4 Determination of nizatidine in their pharmaceutical dosage formulations using spectrophotometric and spectrofluorometric methods.

Method	Drug	Pharmaceutical Product	Percentage ± SDa *
Method 1	NIZ	150 mg NIZ / capsule	99.40 ± 0.002
Method 2	NIZ	150 mg of NIZ /capsule	102.60 ± 0.272

*Values are mean of five determinations

Method 1 spectrophotometric and Method 2 spectrofluorometric

3.4.3. Selectivity

The effect of the presence of common excipients such as; starch, magnesium stearate, iron oxide yellow, titanium dioxide and gelatin was studied. It was found that no interference was introduced by any of them.

3.5. Applications of the methods

The proposed method was applied to the pharmaceutical formulations of NIZ. The results are shown in Table 4, Indicate the high accuracy of the proposed method for the determination of the studied drug.The proposed method has the advantage of being virtually free from interferences by excipients. The percentages were 99.40±0.002, 102.28±0.272 for the spectrophometric andspectrofluormetric methods respectively as shown in Table 4.

4. CONCLUSIONS

Simple, sensitive and selective spectrophotometric and spectrofluorometric methods were developed for the determination of NIZ. The proposed methods were applied for the analysis of NIZ in its pharmaceutical formulations and have comparable analytical performances. The formulation excipients did not cause any potential interference. The proposed methods could be used for routine quality control studies.

REFERENCES

[1] Mosinger J, Tomankova V, Nemcova I, Zyka J. Cyclodextrins in analytical chemistry. Anal Lett., 2001;34:1979–2004.

[2] Szejtli J. Introduction and general overview of cyclodextrin chemistry. Chem Rev 1998;98: 1743–53.

[3] Szejtli J, Osa T (Eds.) (1996). Comprehensive Supramolecular Chemistry, vol. 3, Pergamon, Oxford.

[4] Manzoori JL, Amjadi M. Spctrofluorometric study of host–guest complexation of ibuprofen with β-cyclodextrin and its analytical application.Spectrochim. Acta, Part A 2003;59:909–16.

[5] Manzoori JL, Amjadi M.Spectrofluorimetric and cyclodextrin enhanced spectrofluorimetric methods for the determination of naphazoline in nasal and eye drops. Indian J Chem 2003; 42:2988–92.

[6] BerzasNevado JJ, Murillo Pulgarín JA, Gómez Laguna MA.Spectrofluorimetric study of the β-cyclodextrin: vitamin K3 complex and determination of vitamin K3. Talanta2001; 53:951–9.

[7] Tang B, Ma L, Ma C.Spectrofluorimetric study of the β-cyclodextrin rubidate complex and determination of rubidate by β-CD-enhanced fluorimetry.Talanta2002;L58:841–8
[8] Archontaki HA, Vertzoni MV, Athanassiou-Malaki MH.Study on the inclusion complexes of bromazepam with β- and β-hydroxypropylcyclodextrins. J Pharm Biomed Anal2002;28:761–9.
[9] Xiliang G, Yu Y, Guoyan Z, Guomei Z, Jianbin C, Shaomin S. Study on inclusion interaction of piroxicamwith β-cyclodextrin derivatives.Spectrochim.Acta, Part A2003;59:3379–86.
[10] Elbashir AA, Suliman FEO, Saad B, Aboul-Enein H.Y.Capillary electrophoretic separation and computational modeling of inclusion complexes of β-cyclodextrin and 18-crown-6 ether with primaquine and quinocide.BiomedChromatogr.,2010;24:393-8.
[11] Elbashir AA, Suliman FO. Computational modeling of capillary electrophoretic behavior of primary amines using dual system of 18-crown-6 and β-cyclodextrin.) J.Chromatogr. A2011;1218:5344-51.
[12] Elbashir AA, Saad B, Ali ASM, Saleh MI, Aboul-Enein HY.Enantioselective analysis of primaquine and its impurity quinocide by capillary electrophoresis. BiomedChromatogr.,2009;23:295-301.
[13] Marquez JC, Hernandez M, Garcia Sanchez F. Enhanced Spectrofluorimetric Determination of the Pesticide Warfarin by Means of the Inclusion Complex With β-Cyclodextrin. Analyst, 1990;115:1003-5.
[14] Elbashir AA, Dsugi NFA, Mohmed TOM, Aboul-Enein HY. Spectrofluorometric analytical applications of cyclodextrins, DOI 10.1002/bio.2504, Luminescence, 2013.
[15] Monson PL, Mueller RA, Breese GR. Principles of Pharmacology, Chapman and Hall, l064.(1995).
[16] Klotz U, Dammann HG, Gottlieb WR, Walter TA,Keohane P. Nizatidine (300 mg nocte) does not interfere with diazepam pharmacokinetics in man.Brit J, ClinPharmacol1987; 23:105-6.
[17] Klotz U, Gottieb WR, Keohane PP, Dammann HG.Nocturnal doses of ranitidine and nizatidine do not affect the disposition of diazepam.J. ClinPharmacol.,1987 *;27*: 210-2.
[18] El-Yazbi FA, Gazy AA, Mahgoub H, El-Sayed MA, Youssef RM.Spectrophotometric and titrimetric determination of nizatidine in capsules, J. Pharm. Biomed. Anal.2003*;* 31**:**1027–34.
[19] Hassan EM, and Belal F. Kinetic spectrophotometric determination of nizatidine and ranitidine in pharmaceutical preparations, J. Pharm. Biomed.Anal.*2002;*27**:**31–8.
[20] El-Gendy AE, El-Bardicy MG, Loutfy HM, El-Tarras MF.Stability indicating method for the determination of nizatidine using 3-methyl-2-benzothiazolinone hydrazone, Spectrosc.Lett.2001;34:221– 34.
[21] Darwish IA, Hussein SA, Mahmoud AM, Hassan AI. A sensitive spectrophotometric method for the determination of H 2-receptor antagonists by means of N-bromosuccinimide and p-aminophenol**.** Acta Pharmaceutica*,* 2008*;*58:87-97.
[22] Nikolic K, Bogavac M, Stankovic B. J. Coulometric determination of nizatidine.J.Pharm Biomed Anal.,1995;13:683-5.
[23] Tracqui A, Kintz P, Mangin P.Determination of nizatidine and two of its main metabolites in human serum using high-performance liquid chromatography J. Chromatogr. Biomed. Appl. 1990;94: 369-76.
[24] Carlucci G. High-performance liquid chromatographic assay for nizatidine, a new H2 blocker, in human plasma and urine using disposable solid-phase extraction columns .J. Chromatogr.Biomed.Appl. 1990; 90: 490-494.
[25] Tracqui A, Kintz P, Kreissig P, Mangin P, Lugnier AA, Chaumont A. J.HPLC-BESTIMMUNG VON NIZATIDIN IN SERUM UND URIN | [HPLC determinationofnizatidine in serumandurine].Fresenius Z. Antal. Chem. 1988; 332: 468-9.
[26] Ho C, Huang H.-M, Hsu S.-Y., Shaw C.-Y, Chang B.-L.Simultaneous high-performance liquid chromatographic analysis for famotidine, ranitidine HCl, cimetidine, and nizatidine in commercial products, Drug Dev. Ind. Pharm. 1999; 25: 379–85.
[27] Imre S, Vlase L, Leucuţa SE.HPLC method for quantification of nizatidine and its N-desmethylmetabolite in human plasma**.** *Revue Roumaine de Chimie*2007*;*52:261-6.
[28] Youssef RM.Validated stability-indicating methods for the determination of nizatidine in the presence of its sulfoxide derivative**.** J. AOACInt 2008; 91:73-82.
[29] Koricanac Z, Jovanovic T, Stankovic B.Determination of nizatidine in pharmaceutical formulations by potentiometric titration.Pharmazie 1995;50: 151-2
[30] Kapetanovic V, Milovanovic L, Vhadmirov S.Differential pulse polarographic determination of nizatidine in pharmaceutical formulations. Farmaco II1994;49: 377-9.
[31] Tang B, Jia BX, Shi SF.Flow injection spectrofluorimetric study of the supramolecular interaction between β-cyclodextrin and dequalinium chloride and its analytical application.Spectrochim.Acta, Part A**2005;**61:2203–09.
[32] Maafi M, Laassis B, Aaron J.-J., Mahedero MC, De La Peña AM, Salinas, F.Photochemically induced fluorescence investigation of a β-cyclodextrin: Azure A inclusion complex and determination of analytical parametersJ. Incl. Phenom. 1995;22:235-47.
[33] Connors KA.Binding Constants: The Measurement of Molecular Complex Stability, Wiley, New York, 1987.
[34] Catena GC, Bright FV.Thermodynamic study on the effects of beta-cyclodextrin inclusion with anilinonaphthalenesulfonates, Anal.Chem.1989;61:905–9.
[35] Rekharsky MV, Inoue Y. Complexation Thermodynamics of Cyclodextrins. Chem Rev1998;98:1875–1918.
[36] Inoue Y, Hakushi T, Liu Y, Tong LH, Shen BJ, Jin DS.Thermodynamics of molecular recognition by cyclodextrins. 1. Calorimetric titration of inclusion complexation of naphthalenesulfonates with. alpha-, beta-, and gamma-cyclodextrins: enthalpy–entropy compensation.J Am ChemSoc1993;115:475–81.
[37] Zhang Q, Jiang Z, Guo Y, Li R.Complexation study of brilliant cresyl blue with β-cyclodextrin and its derivatives by UV-vis and fluorospectrometry.**S**pectrochim.Acta, Part A 2008;69:65–70.
[38] Validation of analytical procedure: Text and Methodology. International Conference on Harmonization Q2(R1), 2005:1450. Accessed at: http://www.bioforum.org.il/Uploads/Editor/karen/q2_r1_step4.pdf