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Molecules of benidipine: experimental and theoretical investigation

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In the search for a plausible mechanism for the photocatalytic degradation reaction of BEN, DFT reactivity descriptors were employed to have information about the most susceptible sites for hydroxyl radical attack. Subsequently, the lowest energy status was found out through geometric optimization via Gaussian 09 programme. This study aims to determine the most probable path for the product distribution of transition state complexes and OH radical interaction in gas phase and aqueous media. With the aim to determine the intermediates occurring at the reaction of transition state complexes degradation, geometric optimization of the reactant and transition status complexes were realized through semiempirical AM1 and PM3, ab initio Hartree-Fock HF/3-21G, HF/6-31G* and Density Functional Theory (DFT) methods. Based on the Quantum mechanical calculation, all probable rate constants of reaction paths were calculated by using Transition State Theory (TST). The impact of water solvent was investigated by using COSMO as the solvation model.

Keywords: benidipine; DFT; COSMO; Gaussian 09; hydroxyl radical.

1. INTRODUCTION

Benidipine HCl (BEN), (±)-(R')-3-[(R')-1-benzyl-3-piperidyl] methyl 1,4 dihydro-2,6-dimethyl-4-(m-nitrophenyl)-3,5-pyridenecarboxylate hydrochloride is a dihydropyridine-derived calcium channel blocker with general properties similar to those of nifedipine. It has relatively high vascular selectivity and is expected to show protective effects on vascular endothelial cells. It is used orally in the management of hypertension and angina pectoris [1,2]. In this Benidipine molecule. Various analytical procedures have been reported for the determination of BEN. They include cyclic voltammetry, liquid chromatography [3], tandem mass spectrometry, capillary column gas chromatography [4], negative ion chemical ionization mass spectrometry [5] and spectrophotometric methods [6]. Tipre and Vavia developed validated stability indicating HPLC (A), HPTLC (B) and spectrophotometric (C) method for the estimation of nitrendipine and the stability indicating capability of the assays were proved using forced degradation, by exposing drug solution to sunlight, acidic and alkaline medium [7]. Canavesi et al., were examined validation and development of a stability-indicating LC–UV method for the determination of pantethine (PAN) and its degradation product based on a forced degradation study. The method showed linearity for PAN (0.4–1.2 mg mL^{-1}), methyl-p-hydroxybenzoate (MHB), propyl-p-hydroxybenzoate (PHB) (0.4–1.2 μg mL^{-1}) and in the this work the chemical stability of PAN was for the first time established through a forced degradation study followed by liquid chromatography tandem mass spectrometry investigation showing the formation of three degradation products of PAN (PD1, PD2 and POx) arising from hydrolytic, thermal and oxidative stresses. PD1 (2.5–100 μg mL^{-1}); the precision, determined in terms of intra-day and inter-day precision, expressed as RSDs, were in the ranges 0.4–1.2 and 0.7–1.4, respectively. The method demonstrated to be accurate and robust; indeed the average recoveries were 100.2, 99.9, and 100.0% for PAN, MHB and PHB, respectively, and 99.9% for PD1 [8]. A rapid and gradient high-performance liquid chromatography combined with quadrupole time-of-flight electrospray ionization tandem mass spectrometry (LC/Q-TOF–ESI-MS/MS) method has been developed for the identification and structural characterization of stressed degradation products of tamsulosin. The drug was degraded significantly under hydrolytic (base and neutral), thermal, oxidative and photolytic conditions, while it was stable to acid hydrolytic stress conditions by Namdev et al. [9] Kamila et al were examined a simple, sensitive and accurate UV spectrophotometric method was developed for the assay of nebivolol hydrochloride in raw material and tablets. The absorbance was measured at 282 nm for nebivolol hydrochloride tablet solution. The linearity range was found to be 5–50 μg/mL for the drug [10]. The International Conference on Harmonization (ICH) guideline entitled 'stability testing of new drug substances and products' requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance [11]. In the present analytical work, we have developed and compared new, simple and reproducible stability indicating spectrophotometric

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methods for the estimation of BEN. The stability indicating capability of the assays is proved using forced degradation, by exposing drug solution to acidic, alkaline and oxidative medium. The aim of this work was to develop stability methods for estimation of percentage degradation of BEN. Therefore in the present investigation, an attempt has been made to develop an accurate, simple and an economic UV method for the estimation of BEN in tablet formulation and validated for accuracy, linearity and stability to forced degradation studies according to the prescribed procedures mentioned in ICH guidelines. Modern pharmaceuticals consist of small organic molecules that are moderately water soluble as well as lipophilic to be biologically active. Among the groups of pharmaceutical compounds of greatest environmental interest are antibiotics due to their extensive use for the treatment of bacterial infections in the whole world. The most dangerous effect of antibiotics is the development of multi-resistant bacterial strains that can no longer be treated with the presently known drugs [12]. After the administration, antibiotics are absorbed, metabolized and finally excreted from the body as unchanged or as metabolites. Metabolism reactions introduce functional groups that are attached to drug molecules to yield polar and hydrophilic molecules that can be readily excreted in urine and feces [13,14]. Thus, they subsequently enter into sewage treatment plants. However, polar antibiotics are not completely removed in sewage treatment plants. As a result, they are often present in effluents from sewage treatment plants as well as in surface and groundwater, where they have to be removed.

2. EXPERIMENTAL

2.1. Instrument and materials

The instruments used were Shimadzu 2600 double-beam UV/Visible spectrophotometer. All chemicals and reagents used were of analytical grade. BENwas kindly provided by Deva (Istanbul, Turkey). Coniel tablets (4 mg) (Deva, Tekirdag, TR) was purchased from a local pharmacy.

2.2. Methods

2.2.1. UV Spectrophotometric method of BEN. In our previous studies, we have developed and validated the UV spectrophotometric method of BEN. This method was used to study the stress degraded behavior of BEN.

2.2.2. Preparation of standard stock solution. A stock solution of BEN was prepared in methanol at 1 mg mL^{-1}. This stock solution was further diluted with methanol to obtain working solutions of 100 µg mL^{-1}.

2.2.3. Preparation of calibration curve. Aliquots of 1-3.5 mL portion of stock solutions were transferred to series of 10 mL volumetric flasks, and the volume made up to mark with methanol. Solutions were scanned in the range of 200- 800 nm against blank. The absorption maxima values were found at 357 nm against blank. The calibration curve was plotted.

Forced degradation studies

A stock solution containing 1 mg BEN in 10 mL methanol was prepared. This solution was used for forced degradation to provide an indication of the stability-indicating property and specificity of the proposed method. In all degradation studies, the absorbance of the drug was measured at 357 nm and the amount of degraded drug was calculated.

2.2.4. Acid and base induced degradation. Acid decomposition studies were performed by using 0.1 mol L^{-1}, 0.5 mol L^{-1}, and 2 mol L^{-1} HCl, and basic decomposition studies were performed by using 0.1 mol L^{-1}, 0.5 mol L^{-1} and 2 mol L^{-1} NaOH. The concentration of BEN was 10 µg mL^{-1} for both acid and base degradation studies. The studies were carried out at 70ºC in water bath within 2, 4, and 6 h.

2.2.5. Oxidative degradation. To study hydrogen peroxide-induced degradation, initial studies were performed in 30% hydrogen peroxide at room temperature for 22 h. For the spectroscopic studies, the resultant solutions were scanned between 800 and 200 nm and the absorbance was taken. The studies were carried out at room temperature within 2, 4, and 6 h in dark.

2.2.6. Neutral hydrolysis. To study the degradation behavior of BEN under neutral conditions, it was dissolved in distilled water and the solution was kept for 2, 4 and 6 h.

2.2.7. Procedure for tablets. Ten tablets of BEN were accurately weighed and the average weight of tablet was calculated. The tablets were crushed well to a fine powder. A portion of the powder equivalent to 100 mg BEN was transferred into a 100 mL calibrated flask and dissolved in 50 mL of methanol. The contents of the flask were sonicated for 30 minutes and then completed to volume methanol. The contents were mixed well and filtered; the first portion of the filtrate was rejected. An aliquot was used for the determination of each drug according to the procedure mentioned above. This solution was prepared three times and the absorbance of each solution was determined at 357 nm. All determinations were conducted in triplicate.

2.2.8. Method validation. The accuracy and precision of the assay, as well linearity of the calibration curve, were determined. The absorbance

of each solution was determined at 357 nm. The standard graph was plotted by taking concentration of drug on x-axis and absorbance on y-axis. All determinations were conducted in triplicate.

3. COMPUTATIONAL SET-UP

Geometry optimization of the reactants, the product radicals, pre-reactive and transition state complexes were performed with the DFT method within the Gaussian 09 package [15]. DFT methods use the exact electron density to calculate molecular properties and energies, taking electron correlation into account. They do not suffer from spin contamination and this feature makes them suitable for calculations involving open-shell systems. The DFT calculations were carried out by the hybrid B3LYP functional, which combines HF and Becke exchange termswith the Lee–Yang–Parr correlation functional.

Choice of the basis set is very important in such calculations. Based on these results, optimizations in the present study were performed at the B3LYP/6-31G(d) level. The forming C–O bonds in the addition paths and the H–O bond in the abstraction path were chosen as the reaction coordinates in the determination of the transition states. Ground-state and transition-state structures were confirmed by frequency analyses at the same level. Transition structures were characterized by having one imaginary frequency that belonged to the reaction coordinate, corresponding to a first-order saddle point. Zero-point vibrational energies (ZPEs) were calculated at the B3LYP/6-31G(d) level [16].

4. RESULTS AND DISCUSSION

The overlay graph of 10 µg mL^{-1} BEN is shown in Fig.1.

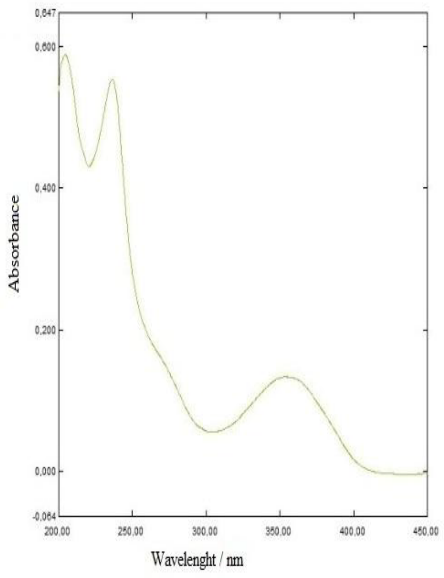

Fig 1. The overlay graph of 10 µg/mL BEN.

4.1. Linearity and calibration curve

The linearity range was found to be 10-35 µg mL^{-1}. The absorbance of each solution was determined at 357 nm. The calibration curve is shown in Fig. 2 and Table 1. The equations of the calibration curves are as in the following:

$$y = 0.0165x\text{-}0.0053 \ (r^2 = 0.999)$$

where y is absorbance intensity and x is BEN concentration (µg mL^{-1}).

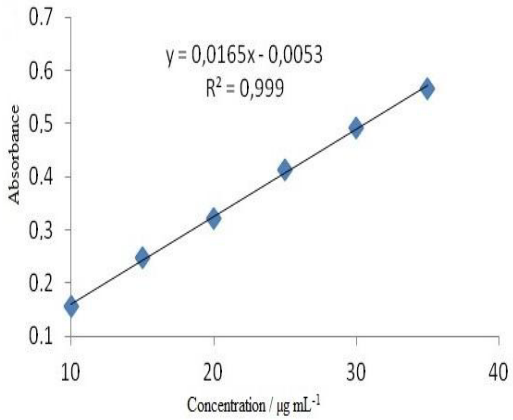

Fig.2. Calibration graph of BEN hydrochloride.

Table 1. Optical characteristics and statistical data.

Parameters	Value
Linearity (µg mL^{-1})	10-35
Regression equation	y = 0.0165x – 0.0053
Slope	0.0165
Slope ± SD	0.0165 ± 0.00092
Intercept	0.0053
Intercept ± SD	0.0053± 0.00743
LOD (µg mL^{-1})	1.56
LOQ (µg mL^{-1})	4.69

4.2 .Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were calculated according to the current ICH guidelines [17]. LOD and LOQ were calculated. Linearity range, regression equation, correlation coefficient, LOD and LOQ (n=3) from the equation of (standard deviation of intercept)/(slope of regression equation) by multiplying 3.3 and 10, respectively. LOD and LOQ values were calculated as 1.56 and 4.69 µg mL^{-1}, respectively.

4.3. Precision and accuracy

Accuracy and precision of the assay was determined by intra-day and inter-day for 3 consecutive days. Three different concentrations of BEN were analyzed in six independent series in the same day (intra-day precision) and 3 consecutive days (inter-day precision). The accuracy and precision of the method was expressed by relative mean error (RME %) and by relative standard deviation (RSD %), respectively. Intra- and inter-day precision and accuracy were calculated by measuring the amount of BEN in three times of 30 µg mL^{-1} concentration levels. The intra-day accuracy ranged from 1.42 % and the inter-day accuracy ranged from 2.62%. The intra-day precision ranged from 0.7, and the inter-day precision ranged from 1.4%.

4.4. Pharmaceutical application

The proposed method was successfully applied to pharmaceutical preparation (Table 2). The determination of BEN tablets, Coniel 4 mg were analyzed at 357 nm against blank. From the calibration curve, concentration of drug in percentage was calculated. The recovery was found to be 100±0.05 %.

Table 2. Analysis of benidipin hydrochloride in tablets.

Tablet	Labeled claim (mg)	Amount found (mg)	Assay ± SD%	RSD %
	4.00	4.00	100 ± 0.05	0.88

4.5. Forced degradation studies

4.5.1. Acid degradation. The acid degradation was performed using 0.1 mol L^{-1}, 0.5 mol L^{-1}, and 2 mol L^{-1} HCl. The overlay graph of 10 µg mL^{-1} acid-degraded BEN is shown in Fig.3. BEN is degraded within 2, 4 and 6 h. The percentage of degraded drug was calculated. The results are shown in Tables 3-5.

4.5.2. Alkaline degradation. The alkaline degradation was performed using 0.1 N, 0.5 N, and 2 N NaOH. The overlay graph of 10 µg/mL alkaline-degraded BEN is shown in Fig.3. BEN is degraded within 2, 4 and 6 h. The percentage of degraded drug was calculated. The results are shown in Table 6-8.

4.5.3. Neutral degradation. The neutral degradation was performed. The overlay graph of neutral-degraded BEN is shown in Fig. 3. BEN is degraded within 2, 4 and 6 h. The percentage of degraded drug was calculated. The results are shown in Table 9.

Table 3. Acid degradation (0.1 mol L^{-1} HCl) of Benidipine hydrochloride

Name	Absorbance	Concentration mg	Degradation %
Analyte at 2 h	0.168	10.5	0
Analyte at 4 h	0.154	9.65	3.5
Analyte at 6 h	0.150	9.41	5.9

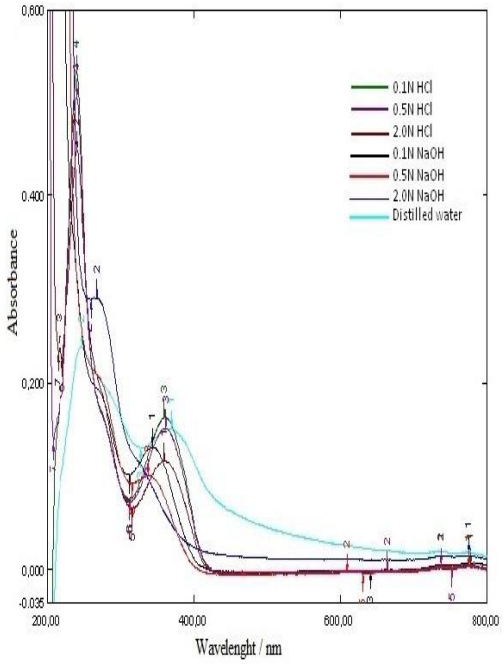

Fig.3. Overlay graphs of BEN hydrochloride degraded (acidic, alkaline degradation) within 6 h.

Table 4. Acid degradation (0.5 mol L^{-1} HCl) of Benidipine hydrochloride

Name	Absorbance	Concentration mg	Degradation %
Analyte at 2 h	0.159	9.96	0.4
Analyte at 4 h	0.152	9.53	4.7
Analyte at 6 h	0.145	9.11	8.9

This proved that there is degradation of BEN under heat conditions. The proposed method has good reproducibility, accuracy and revealed that the commonly used excipients and additives in formulation were not interfering and the drug is stable to acid and oxidative treatments. The method can be adopted for routine quality control.

Table 5. Acid degradation (2 mol L^{-1} HCl) of Benidipine hydrochloride

Name	Absorbance	Concentration mg	Degradation %
Analyte at 2 h	0.137	8.62	13.8
Analyte at 4 h	0.122	7.72	22.8
Analyte at 6 h	0.099	6.32	36.8

4.5.4. Oxidative degradation. Oxidative degradation was performed by using 30% hydrogen peroxide. BEN is degraded within 22 h. The percentage of degraded drug was calculated. The results are shown in Table 10. Fig 4 shows overlay graph of BEN degraded (oxidative) within 22 h at room temperature in dark. This proved that there is degradation of BEN under heat conditions. The proposed method has good reproducibility, accuracy and revealed that the commonly used excipients and additives in formulation were not interfering and the drug is stable to acid and oxidative treatments. The method can be adopted for routine quality control.

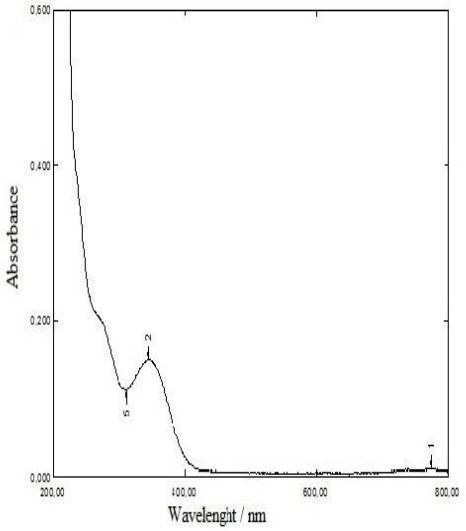

Fig.4. Overlay graph of BEN hydrochloride degraded (oxidative) within 22 h at room temperature.

Table 6. Alkaline degradation (0.1 mol L^{-1} NaOH) of Benidipine hydrochloride

Name	Absorbance	Concentration mg	Degradation %
Analyte at 2 h	0.147	9.23	7.7
Analyte at 4 h	0.141	8.87	11.3
nalyte at 6 h	0.130	8.20	18.0

4.6. Theoretical prediction of the degradation mechanism

In the search for a plausible mechanism for the photocatalytic degradation reaction of BEN, DFT reactivity descriptors were employed to have information about the most susceptible sites for hydroxyl radical attack. Fig. 5 shows the optimized structure of BEN molecule and the numbering system that is used throughout the calculations. Three main competing reaction pathways shown in Fig. 6 were determined by selecting the specific sites of BEN molecule, on the basis of their softness values being close to that of the •OH radical. The predicted mechanism was confirmed by comparison with the experimental results on simple structures reported in the literature, as explained below. The lowest-energy structure is the most stable structure. Statements in this fragmentation took place both experimental and theoretical, as seen from the Gibbs free energy values of Table 11 and support it.

Table 7. Alkaline degradation (0.5 mol L^{-1} NaOH) of Benidipine hydrochloride

Name	Absorbance	Concentration mg	Degradation %
Analyte at 2 h	0.133	8.38	16.2
Analyte at 4 h	0.113	7.17	28.3
Analyte at 6 h	0.095	6.02	39.2

Table 8. Alkaline degradation (2 mol L^{-1} NaOH) of Benidipine hydrochloride

Name	Absorbance	Concentration mg	Degradation %
Analyte at 2 h	0.114	7.23	27.7
Analyte at 4 h	0.082	5.29	47.1
nalyte at 6 h	0.057	3.78	62.2

Table 9. Neutral degradation of Benidipine hydrochloride

Name	Absorbance	Concentration mg	Degradation %
Analyte at 2 h	0.114	10.5	0
Analyte at 4 h	0.082	10.3	0
Analyte at 6 h	0.057	9.84	1.6

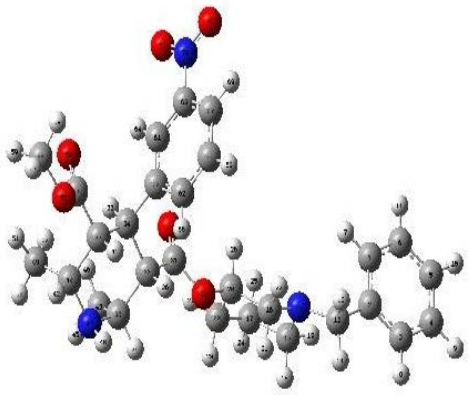

Fig. 5. Optimized structure of BEN and the numbering system (gray, carbon;red, oxygen; blue, nitrogen; white, hydrogen).

Table 10. Oxidative degradation (30% hydrogen peroxide) of Benidipine hydrochloride

Name	Absorbance	Concentration mg	Degradation %
Analyte at 22 h	0.144	9.05	9.5

Three main competing reaction pathways shown in Fig. 6 were determined by selecting the specific sites of BEN molecule, on the basis of their softness values being close to that of the •OH radical. The predicted mechanism was confirmed by comparison with the experimental results on simple structures reported in the literature, as explained below. The lowest-energy structure is the most stable structure. Statements in this fragmentation took place both experimental and theoretical, as seen from the Gibbs free energy values of Table 11 and support it.

Table 11. Constant energy, enthalpy and Gibbs free energy values according to the DFT method.

Molecules	Energy (kcal mol^{-1})	Enthalpy (kcal mol^{-1})	Gibbs free energy (kcal mol^{-1})
Benidipine	-1069012.515	-1068609.154	-1068676.829
F1	-926021.799	-925648.345	-925709.257
F2	-742038.567	-741798.939	-741847.870
F3	-327727.700	-327551.055	-327582.649
F4	-72611.831	-72692.532	-72709.473

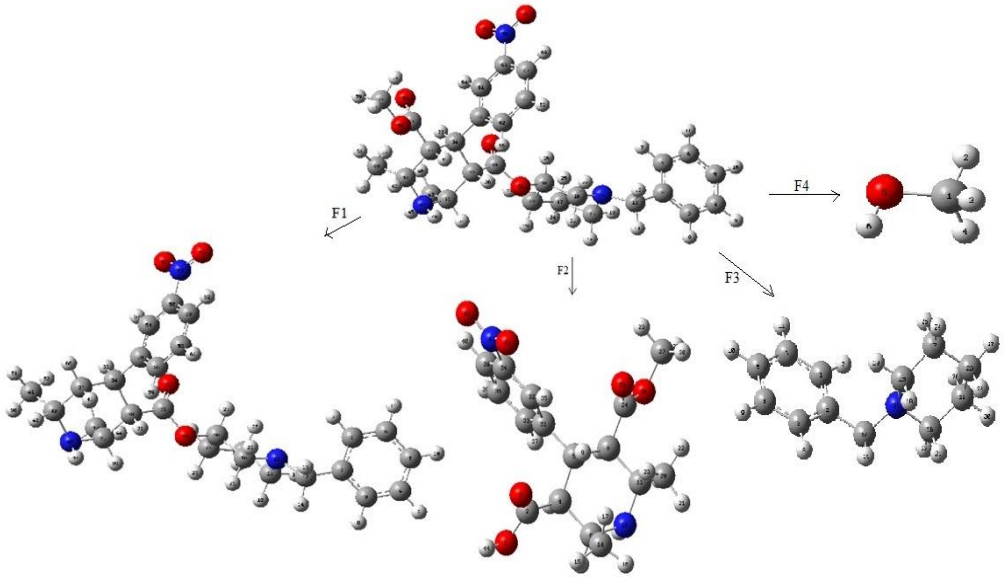

Fig. 6. Possible pathways for the photocatalytic degradation of BEN.

5. CONCLUSIONS

Stress degradation study of BEN was done using UV spectrophotometric method. The method was found to be simple and cost effective. It was found that BEN was degraded in acidic, alkaline and oxidative conditions. This method also provides quantification of BEN in presence of degraded products. Degradation of BEN was predicted to occur through intramolecular F1, F2, F3 and F4 ring cleavages followed by subsequent reactions with •OH radicals transforming the fragments into smaller species such as NO_3^- and NH_4^+.

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REFERENCES

1. S.C. Sweetman Martindale, The Extra Pharmacopoeia, thirty five ed. in: Pharm Press, 2007..
2. K.D. Sanborn, Physicians Desk Reference, sixty one ed. in: Med. Eco. Co., Montvale, 2007.
3. N. Karadas, S. Sanli, M. Gumustas, S.A. Ozkan, *J. Pharm. Biomed. Anal*., **66,** 116 (2012).
4. W. Kang, H.Y. Yun, K.H. Liu, K.I. Kwon, J.G. Shin, *J. Chromatogr.* B., **805,** 311 (2004).
5. H. Magara, H. Kobayashi, S.J. Kobayashi, *J. Chromatogr.*, **617,** 59 (1993).
6. I. Singhvi, S.C. Chaturvedi, *Ind. J. Pharm. Sci*.,**61,** 190 (1999).
7. D.N. Tipre, P.R.Vavia, *J. Pharm. Biomed. Anal*., **24,** 705 (2001).
8. R. Canavesi, S. Aprile, E. Varese, G. Grosa, *J. Pharm. Biomed. Anal*., **97,** 14 (2014).
9. D. Namdev, R.M. Borkar, B. Raju, P.D. Kalariya, V.T. Rahangdale, S. Gananadhamu, R. Srinivas, *J. Pharm. Biomed. Anal*., **88,** 245 (2014).
10. M.M. Kamila, N. Mondal, L.K. Ghosh, B.K. Gupta, *Pharmazie.*, **62,** 486 (2007).
11. ICH, Stability Testing of New Drug Substances and Products, in: International Conference on Harmonization, 1993.
12. M. Addamo, V. Augugliaro, A.D. Paola, E. Garcia-Lopez, V. Loddo, G. Marci, L. Palmisano, *J. Appl. Electrochem.,* **765,** 35 (2005).
13. M. Rabiet, A. Togola, F. Brissaud, J.L. Seidel, H. Budzinski, F. Elbaz-Poulichet, *Environ. Sci.Technol.*, **40,** 5282 (2006).
14. K. Ikehata, N.J. Naghashkar, M.G. El-Din, *Ozone: Sci. Eng*., **28,** 353 (2006).
15. Gaussian 09, Revision B.04, in: *Gaussian, Inc.*, 2009.
16. A. Hatipoglu, D. Vione, Y. Yalçın, C. Minero, Z. Çınar, *J. Photochem.Photobiol. A: Chemistry*, **215,** 59 (2010).
17. International Conference on Harmonization, ICH Guideline, Q2R1, in: Validation of Analytical Procedure: Text and Methodology, 2005.