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The ICH guidance in practice: stress decomposition studies on three piperazinyl quinazoline adrenergic receptor-blocking agents and comparison of their degradation behaviour

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Abstract

Stress degradation studies were carried out on three piperazinyl quinazoline α_1-adrenergic receptor blockers, viz. prazosin, terazosin, and doxazosin, following the conditions prescribed in the parent drug stability testing guideline (Q1AR) issued by International Conference on Harmonization (ICH). All drugs showed significant decomposition at 80 °C in acidic conditions (0.1 M HCl) and complete degradation in alkaline conditions (0.1 M NaOH). Under both these conditions, 2-piperazinyl-6,7-dimethoxy-4-aminoquinazoline was formed as a major decomposition product in all three drugs. The degradation pattern under ICH-prescribed photolytic conditions in liquid and solid states was also similar for all the drugs. The light exposure resulted in the formation of a cluster of degradation products. No degradation was observed in neutral and oxidative conditions. In solid state, all drugs were stable at 50 °C in a 1-month study. In alkaline conditions, the order of sensitivity to degradation of the three drugs was doxazosin > terazosin > prazosin, while the same was terazosin > doxazosin > prazosin under acidic conditions. Mechanistic explanation is provided for the variable behaviour of decomposition.

Keywords: Prazosin; Terazosin; Doxazosin; Degradation behaviour; Stress studies

1. Introduction

Stress testing is a part of development strategy under the International Conference on Harmonization (ICH) requirements and is carried out under more severe conditions than accelerated studies. These studies serve to give information on drug's inherent stability and help in validation of the analytical methods to be used in stability studies [1–3]. The parent drug stability guideline (Q1AR) requires that stress testing of drug substance should include the effect of elevated temperature, humidity, light, and oxidising agents, as

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well as the susceptibility across a range of pH values [4].

Accordingly, the major aim of this study was to put ICH recommendations into practice by subjecting three piperazinyl quinazoline α_1-adrenergic receptor blockers, viz. prazosin, terazosin, and doxazosin (Fig. 1) to the variety of suggested stress test conditions. The endeavour was to compare the nature and the overall rate of degradation, and to propose mechanistic explanation to variable behaviour of decomposition, if any. In literature, information on stability and degradation aspects is available only for terazosin, which is reported to degrade to 2-piperazinyl-6,7-dimethoxy-4-aminoquinazoline and tetrahydrofuroic acid [5]. The drug is shown to be relatively stable under thermal and photochemical stress conditions, and in water at room temperature. It degrades rapidly in weak acid, water, and weak base at elevated temperatures.

2. Experimental

2.1. Materials

Terazosin hydrochloride was supplied gratis by Intas Pharmaceuticals Ltd. (Matoda, India). Prazosin hydrochloride BP and doxazosin mesylate were received gratis from Sun Pharmaceutical Industries Ltd. (Baroda, India). The drugs were used without further purification. Acetonitrile (HPLC grade) was purchased from Mallinckrodt Baker, Inc. (Paris, KY). All other chemicals were of analytical reagent grade. Ultra-pure water was obtained from an ELGA (Bucks, UK) water purification unit.

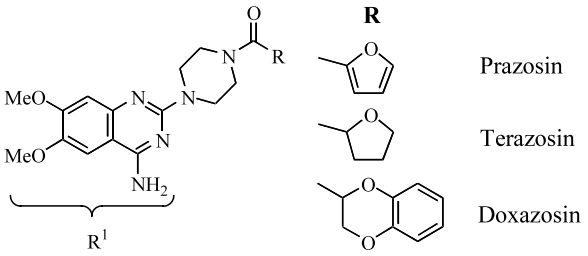


Fig. 1. Structures of the drugs.

2.2. Instrumentation

Precision water baths equipped with MV controller (Julabo, Seelbach, Germany) and a dry bath (Barnstead/Thermolyne, Iowa, USA) were used in decomposition studies. Photostability studies were conducted in a stability chamber (KBF 240, Binder, Germany) equipped with an illumination bank consisting of a combination of two black-light UV lamps (OSRAM L73) and four white fluorescent lamps (OSRAM L20) in accordance with option 2 of ICH guideline Q1B [6]. The chamber was set at 40 °C and 75% RH. Both UV and visible lamps were put on simultaneously. The samples were placed at a distance of 9 in. from the light bank. The overall illumination at the point of placement was 7000 lx fluorescent light and 0.3 W m^{-2} UV light. The samples were exposed for a total period of 30 days. Thermal stability studies were performed in dry air oven (NSW Limited, India) set at 50 °C.

The HPLC system (all equipment from Waters, Milford, USA) consisted of a 600E pump, a 996 photo-diode array (PDA) detector, a 717 auto-injector, and a degasser module. The data were acquired and processed by use of Millennium software ver. 3.2.

LC–MS studies were carried out on Finnigan Mat LCQ ion-trap equipment. The LC part consisted of a P4000 pump, an AS3000 autosampler, a UV6000LP PDA detector, a SCM1000 degasser (all equipment was from Spectrasystem, USA) and 5 μm Waters Spherisorb ODS2 column (250 mm × 4.6 mm i.d.). The mass determinations were made in positive ESI mode in the mass range 150–500.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DPX 300 spectrometer in $CDCl_3$ containing a small quantity of MeOD. Chemical shifts are reported in ppm (δ) value relative to tetramethylsilane as internal standard for ^{1}H NMR and in ppm relative to $CDCl_3$ for ^{13}C NMR. FT-IR spectra were determined in a KBr disc with NICOLET IMPACT 410 spectrometer.

2.3. Decomposition studies

All stress decomposition studies were performed at an initial drug concentration of 1 mg ml^{-1} in solutions containing 30% dimethyl sulfoxide. Acid decomposition studies were carried out at 80 °C in a solution made using 0.1 M HCl. The period for reactions was 32 h for terazosin and 90 h for prazosin and doxazosin. Alkaline degradation studies were performed in 0.1 M NaOH and the solutions were heated at 80 °C up to 3 h. For study under neutral conditions, solutions made with water were heated at 80 °C for 240 h. Oxidative studies were done in 3% H_2O_2 for 24 h at room temperature and also at peroxide concentration of 30% for 48 h. Photolytic studies in solution were performed in 0.1 M NaOH, 0.1 M HCl, and water up to 30 days. Photolytic studies in solid state were performed by keeping each of the drugs in Petri plates in a thin layer of 1 mm thickness for 3 months. Suitable controls were maintained under dark conditions. Solid samples were exposed to dry heat at 50 °C for 30 days and also to accelerated conditions of 40 °C and 75% RH for 3 months. Samples were withdrawn at suitable time intervals and subjected to HPLC analysis, after suitable dilution.

2.4. Kinetic studies

To determine the relative rate of decomposition of the three drugs, kinetics studies were carried out at a drug concentration of 1 mg ml^{-1} at 80 °C in 0.1 M HCl and 0.1 M NaOH. The samples were withdrawn at different times and subjected to HPLC analysis. The studies were done in triplicate.

2.5. HPLC analysis

Separations were achieved on a 5 μm Waters Spherisorb ODS2 column (250 mm × 4.6 mm i.d.) using acetonitrile:water:acetic acid:diethyl amine (65:35:1:0.2) in an isocratic mode. The mobile phase was filtered through 0.45 μm nylon membrane and degassed before use. The flow rate was kept constant at 1 ml min^{-1}. The injection volume was 5 μl and the detection wavelength was 254 nm.

3. Results and discussion

3.1. Degradation behaviour

Fig. 2 shows the changes observed during degradation of the three drugs in acid and alkali in comparison to the initial sample. It is evident from the study of Fig. 2(a)–(c) that terazosin decomposes both in acid and alkali to a product, which appears right to the drug peak, at retention time of around 20 min. Correlating the behaviour with known degradation chemistry of conversion of terazosin to 2-piperazinyl-6,7-dimethoxy-4-aminoquinazoline [5], it was assumed that the product peak was due to this compound. To confirm the same, samples were subjected to LC–MS studies and a mass of 290.4 was obtained in positive ESI mode. It strongly indicated that it was the same compound; however, for further confirmation, the product was isolated from the reaction mixture and was subjected to FT-IR and NMR studies. The spectra obtained were compared with those of the drug. In the FT-IR spectra for the product, there was disappearance of absorption due to amido carbonyl at 1660 cm^{-1}, which was prominently present in the FT-IR for the pure drug, indicating cleavage at the amido bond. The same was also substantiated by disappearance of a triplet at 8.466 ppm in ^{1}H NMR and a singlet at 170.634 ppm in the ^{13}C NMR spectra for the product as compared with the pure drug.

It was expected that the same chemistry would be followed during degradation of prazosin and doxazosin, because of their structural similarity, e.g., all of them contain 4′-acylated-2-piperazinyl-4-aminoquinazoline moiety and differ only in acyl side chain (Fig. 1). As shown in the chromatograms (d–f) and (g–i) in Fig. 2, a peak appears at the same location around 20 min in case of prazosin and doxazosin, respectively, similar to the one obtained in terazosin (chromatograms a–c in Fig. 2). The formation of 2-piperazinyl-6,7-dimethoxy-4-aminoquinazoline was also confirmed in these two cases through LC–MS studies, as same mass value of 290 was obtained.

The chromatograms e and f in Fig. 2 show that another product with retention time around 2.8 min was formed during degradation of prazosin

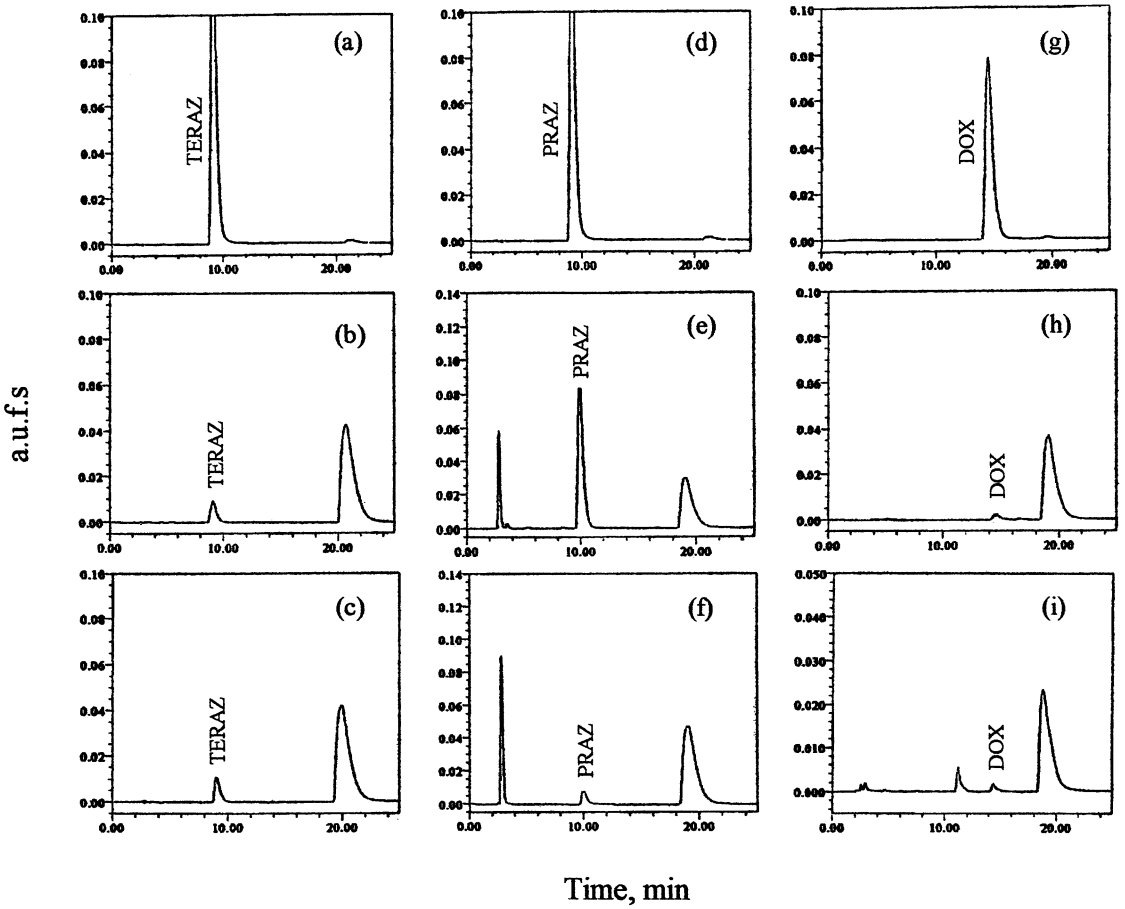


Fig. 2. Chromatograms showing decomposition of terazosin (TERAZ), prazosin (PRAZ), and doxazosin (DOX) in acid and alkali at 80 °C. Key: (a) terazosin control, (b) terazosin in 0.1 M HCl after 32 h, (c) terazosin in 0.1 M NaOH after 1.5 h, (d) prazosin control, (e) prazosin in 0.1 M HCl after 90 h, (f) prazosin in 0.1 M NaOH after 2 h, (g) doxazosin control, (h) doxazosin in 0.1 M HCl after 90 h, and (i) doxazosin in 0.1 M NaOH after 16 min.

under acid and alkali conditions. This peak is ascribed to 2-furoic acid, the leaving group for this drug. In comparison to prazosin, no peak for the leaving group was observed in terazosin, as shown from the chromatograms a–c in Fig. 2. Perhaps it is so because tetrahydrofuroic acid, the leaving group decomposes further to tetrahydrofuran and carbon dioxide [5]. In comparison, 2-furoic acid is a relatively stable acid [7] and shows up during HPLC analysis (chromatograms e and f, Fig. 2). Fig. 2(i) shows that in case of doxazosin, a similar new peak is formed around 11 min during decomposition in alkaline conditions. Considering parallelism with other drugs, the peak in this case may be ascribed to the leaving group. It seems that the product is stable in alkali, but decomposes further to non-chromophoric product(s) in acid, as no similar peak was observed under the latter conditions (Fig. 2(h)).

All drugs were found to be stable in neutral environment. No degradation was observed even after heating the drugs for 10 days at 80 °C in water.

Representative chromatograms showing photolytic decomposition of prazosin in acid, neutral, and alkali conditions are shown in chromatograms b, d, and f, respectively, in Fig. 3. The corresponding dark controls are shown in chromatograms a,

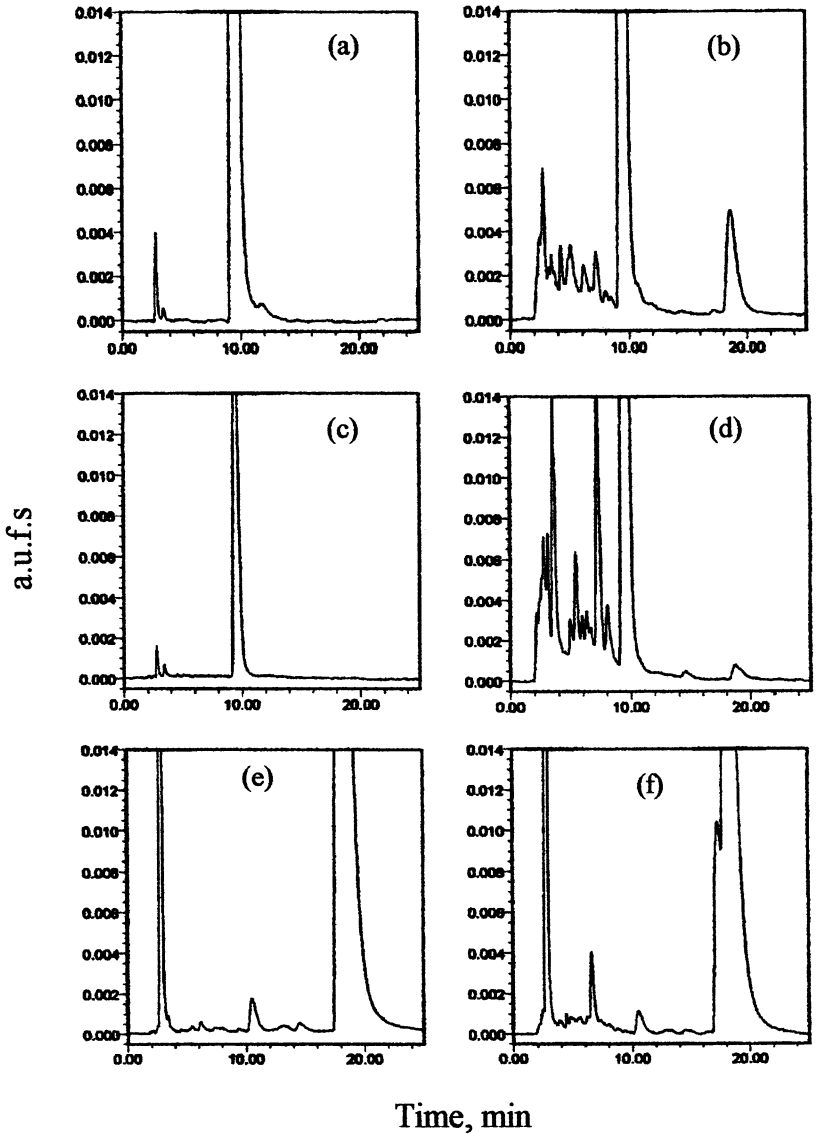


Fig. 3. Typical example of decomposition of prazosin under photolytic conditions at 40 °C for 7 days. Other drugs showed almost similar behaviour. Key: (a) dark control in 0.1 M HCl, (b) exposed sample in 0.1 M HCl, (c) dark control in water, (d) exposed sample in water, (e) dark control in 0.1 M NaOH, and (f) exposed sample in 0.1 M NaOH.

c, and e in the same figure. Evidently, a cluster of products is formed on left to the drug peak on exposure of solutions to light in acid and neutral conditions, with overall decomposition being higher in neutral conditions. The cluster is not formed in alkali, perhaps due to rapid loss of the drug due to hydrolysis, before light influences the decomposition. This is evident from comparison of chromatogram f in Fig. 3 with chromatogram f in Fig. 2, where the peak due to drug at around 10 min is almost lost with the formation of hydrolytic product at around 20 min. The differential extent or nature of degradation in acid, neutral, and alkali conditions was also exposed from the physical change in colour of the reacting solutions. While the acid solutions were pale yellow, neutral

solutions were reddish (due to higher decomposition), and alkali solutions remained colourless on exposure to light up to 7 days. Overall, a similar degradation pattern was observed for terazosin and doxazosin.

On exposure of drugs to light in solid state, there was change in colour of all the three drugs from white to orange yellow. The chromatographic study of the exposed samples again showed formation of multiple products. Overall, the extent of degradation in solid state under photolytic conditions was much lesser than when in solution.

All drugs were found to be stable to oxidative stress. The reason perhaps is the absence of functional group prone to oxidation in the structures of these drugs (Fig. 1). The drugs were also stable in the solid state when exposed to thermal stress (dry heat at 50 °C) and under accelerated conditions of 40 °C, 75% RH.

3.2. Relative rates of degradation in acid and alkali

Kinetics studies were carried out in acid and alkali by following the fall in drug with time. The plots of log percent remaining versus time are shown in Fig. 4. As evident, strict straight-line behaviour ($r^2 > 0.98$) was obtained for all the three drugs indicating that the reactions followed pseudo-first-order kinetics. Rate constants were determined from the slopes and the calculated values were 0.0096, 0.0999, and 0.0424 h^{-1} in 0.1 M HCl, and 0.9898, 1.75, and 15.705 h^{-1} in 0.1 M NaOH for prazosin, terazosin, and doxazosin, respectively. Apparently, two inferences can be made out from these rate constant values. First is that hydrolysis for all the three drugs is overall faster in alkaline solutions than in acidic solutions. Second, the order of sensitivity to hydrolysis of the three drugs is terazosin > doxazosin > prazosin and doxazosin > terazosin > prazosin in acidic and alkaline conditions, respectively.

3.3. Mechanistic explanation to observed degradation behaviour in acid and alkali

The acid/base-catalysed degradation (hydrolytic cleavage) of the three drugs can be presumed to occur via two competitive pathways: (a) through

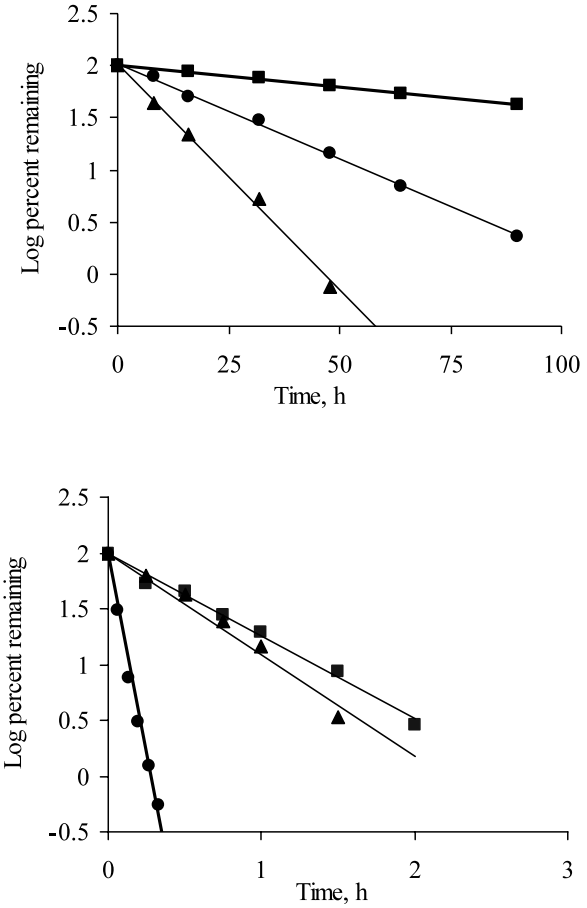
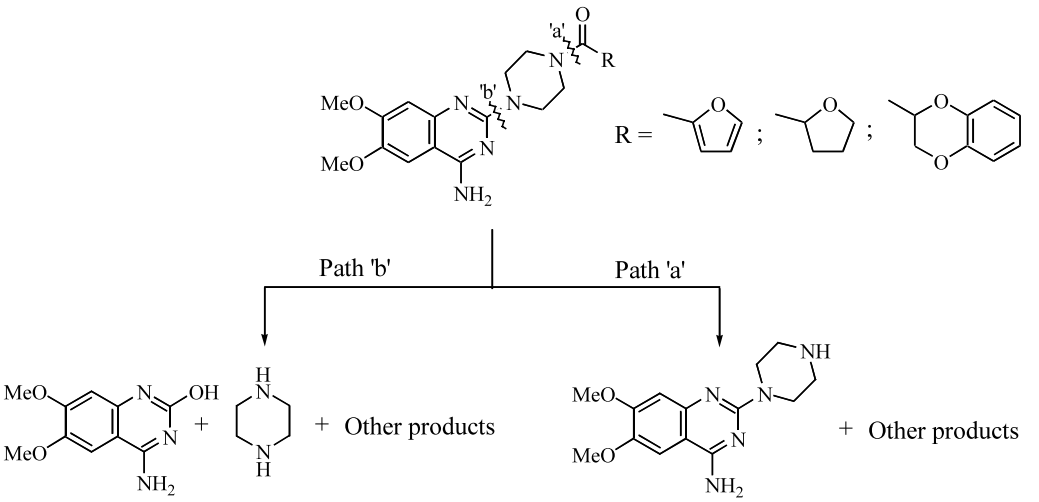


Fig. 4. Kinetics plots for decomposition of drugs in 0.1 M HCl (a) and 0.1 M NaOH (b) at 80 °C. Key: (■) prazosin, (▲) terazosin, and (●) doxazosin.

scission of the amide bond (a) involving tetrahedral mechanism and/or (b) through scission 'b' involving S_NAr mechanism (Scheme 1). However, the presence of the OMe and NH_2 groups in the quinazoline moiety should make it electronically rich and disfavour the competitive aromatic nucleophilic substitution of the piperizine moiety in path b. Therefore, path a should prevail, which explains the formation of 2-piperazinyl-6,7-dimethoxy-4-aminoquinazoline as the major product during acid and alkali degradation of the three drugs.

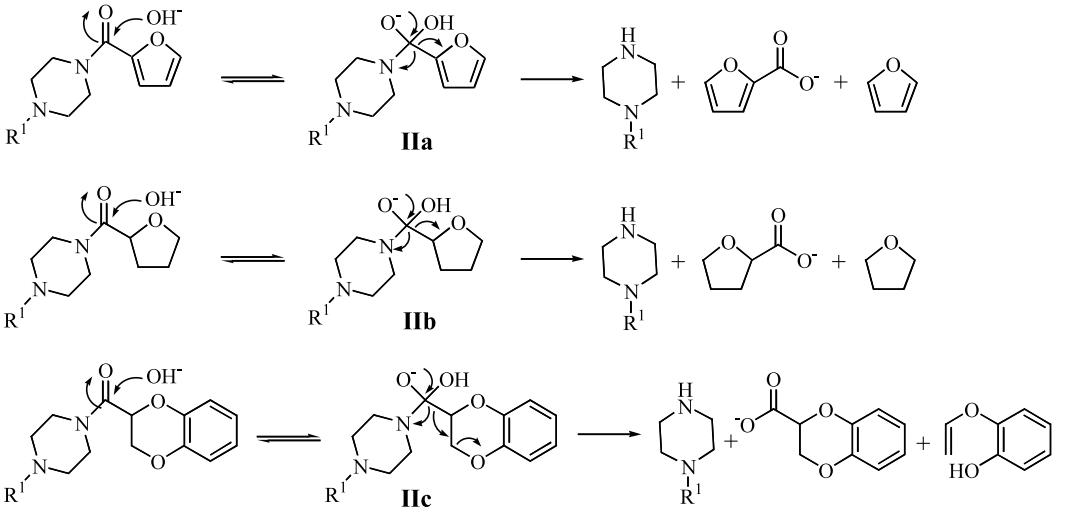
The overall higher rate of hydrolytic degradation of all the drugs under alkaline conditions, as compared with that under acidic conditions may



Scheme 1.

be explained by better nucleophilicity of HO^- compared with that of H_2O [8]. Generally, the hydrolysis of carbonyl substrates via $B_{AC}2$ mechanism is a preferable pathway compared with the $A_{AC}2$ mechanism.

An explanation to the order of sensitivity of the three drugs under basic conditions (doxazosin > terazosin > prazosin) is provided by considering that the hydrolytic cleavage of the drugs proceeds via tetrahedral intermediates, **IIa**, **IIb**, and **IIc**, respectively, for prazosin, terazosin, and doxazosin (Scheme 2), arising from the nucleophilic attack at the carbonyl carbon. The resonance interaction of the carbonyl group with the furan moiety makes the carbonyl carbon of prazosin less electrophilic, accounting for its slower rate of hydrolysis compared with that of doxazosin and terazosin. While tetrahedral intermediates **IIa**, **IIb**, and **IIc** are usually expected to undergo elimination of the piperizinyl moiety, an alternate path of degradation may be the elimination of furan, tetrahydrofuran, and benzodioxan moieties, re-



Scheme 2.

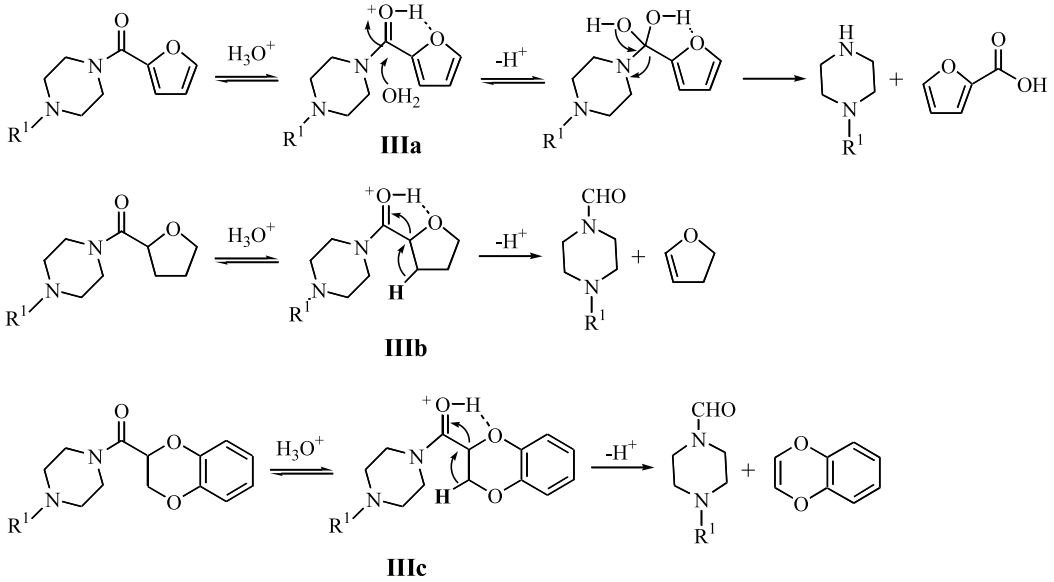
spectively, from **IIa**, **IIb**, and **IIc**. While the relatively poor leaving group properties of the 2-furyl and 2-tetrahydrofuryl anions make this alternate decomposition of **IIa** and **IIb** less likely, the 1,2 elimination of the benzodioxan anion (leading to the formation of allyl catechol) may be the driving force for the alternate decomposition of **IIc**. It thus explains the higher rate of hydrolysis of doxazosin compared with that of terazosin.

The relative hydrolytic decomposition under acidic conditions, viz. terazosin > doxazosin > prazosin can be explained by considering the intermediates **IIIa**, **IIIb**, and **IIIc** during the reaction process (Scheme 3). While the nucleophilic attack of H_2O involving the tetrahedral intermediate should be the preferred decomposition of **IIIa**, the decomposition of **IIIb**, and **IIIc** should take place via the elimination of β-hydrogen of the tetrahydrofuran and benzodioxan moieties, respectively. As the nucleophilic attack on **IIIa** occurs reversibly but the β-elimination from **IIIb** and **IIIc** takes place irreversibly, the rate of decomposition of prazosin should be slower than that of doxazosin and terazosin under acidic conditions. However, the presence of oxygen atom adjacent to β-hydrogen in **IIIc** should make the elimination from **IIIc** slower than that of **IIIb** accounting for the fact that under acidic condition terazosin undergoes decomposition at a faster rate than that of doxazosin.

4. Conclusions

This is a typical study in which stress studies were conducted on three members of a class of compounds following the conditions suggested in the ICH guideline Q1AR [4]. The study highlights that by following the ICH requirements of stress testing, good information on the decomposition behaviour of drugs can be obtained, in addition to what one gets normally by drug decomposition under a few selected conditions [3].

The report describes additional information on degradation behaviour of terazosin than what is known in literature. Information on degradation behaviour is also provided on prazosin and doxazosin, where no earlier literature report exists. It is found that all three congeners follow the same decomposition behaviour under all conditions. Wide differences, however, exist in their decomposition rates in acid and alkali, indicating a strong effect of side attachment on the lysis of



Scheme 3.

the amidic bond. The same is well explained through the proposed mechanisms. The drugs are found to be mildly unstable in photolytic conditions in both solution and solid state, yielding multiple products. However, the drugs remain stable under neutral and oxidative conditions in solution and under thermal stress in solid state.

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