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Journal of Pharmaceutical and Biomedical Analysis
34 (2004) 19–26
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Validated specific HPLC methods for determination of prazosin, terazosin and doxazosin in the presence of degradation products formed under ICH-recommended stress conditions

Monika Bakshi, Tina Ojha, Saranjit Singh*

Department of Pharmaceutical Analysis, National Institute of Pharmaceutical Education and Research, Sector 67, S.A.S. Nagar, Punjab 160 062, India

Received 28 March 2003; received in revised form 29 July 2003; accepted 7 August 2003

Abstract

The present paper describes development of stability-indicating high-performance liquid chromatographic (HPLC) assay methods for three alpha-adrenergic-blocker drug substances, namely, prazosin, terazosin and doxazosin, in the presence of degradation products generated from forced decomposition studies. Resolution of drugs from degradation products was obtained using a reversed-phase C-18 column using water/acetonitrile/methanol/glacial acetic acid/diethylamine (25:35:40:1:0.017) as mobile phase for prazosin and terazosin and acetonitrile/water/glacial acetic acid/diethylamine (65:35:1:0.02) for doxazosin. The detection was done at 254 nm. The methods were validated with respect to linearity, precision, accuracy, specificity and robustness.

Keywords: Prazosin; Doxazosin; Terazosin; Stability-indicating methods; HPLC

1. Introduction

The current International Conference on Harmonisation (ICH) guidelines require that analysis of stability test samples should be done by using stability-indicating assay methods (SIAMs), developed and validated after stress testing on the drug under a variety of conditions, including hydrolysis (at various pH's), oxidation, photolysis and thermal degradation [1]. Unfortunately, this route to development of stability-indicating assays is not found in most of the stability-indicating methods reported in the literature [2]. Therefore, there exists a necessity to subject the drugs to stress studies and establish stability-indicating assay methods based on analysis of stressed samples.

In our laboratory, we are actively engaged in stress studies and development of SIAMs following the current ICH guidelines [2–5]. In one of our recent publications, we reported stress decomposition behaviour of prazosin, terazosin and doxazosin [5]. All the three drugs are selective α_1-adrenergic blockers, used in the treatment of hypertension and benign prostatic hyperplasia. Their structures are shown in

* Corresponding author. Tel.: +91-172-214682; fax: +91-172-214692.
E-mail address: ssingh@niper.ac.in (S. Singh).

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doi:10.1016/j.japna.2003.08.009

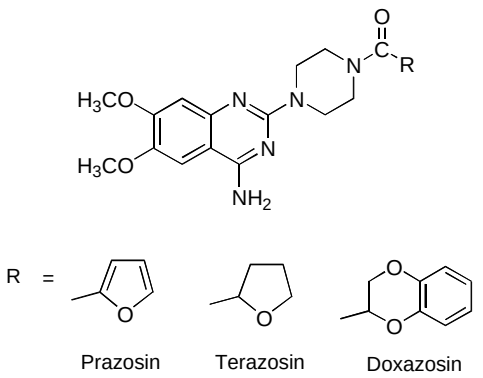

Fig. 1. Structures of drugs.

Fig. 1. In this paper, we report further on the development of validated stability-indicating HPLC assay methods for the same three drugs.

There is no such earlier report in literature that involves establishment of stability-indicating assays for these drugs through the stress test route, although a number of reports exist, in general, on their analysis. For example, automated coulometric [6], spectrophotometric and HPLC [7] methods have been employed for assay of pure prazosin. Differential pulse polarographic [8,9], spectrophotometric [10–14], fluorometric [10], spectrofluorometric [13], voltammetric [14], colorimetric [15] and HPLC [16–18] methods have been proposed for determination of the same drug in different dosage forms.

Methods for the analysis of prazosin in biological samples include HPLC [18–28], densitometry [29], voltammetry [14], spectrophotometry [14] and radio-receptor assay [30]. An HPLC method has been reported for determination of terazosin and its impurities [31]. A fluorometric method was developed for assay of the drug from the tablets [32]. Also, several HPLC methods have been reported for its determination in biological fluids [25,33–36]. Two stability-indicating methods have been reported recently for doxazosin mesylate [37]. The first method involves the use of first-derivative spectrophotometry, while the second encompasses quantitative densitometric evaluation by TLC. The methods were found to be applicable in determining doxazosin in laboratory-prepared mixtures containing up to 90% degradation products formed in alkali. The chromatographic resolution of racemic doxazosin using reversed-phase HPLC with the chargeable cyclodextrins has been reported [38]. Methods involving pulse polarography [39–41], voltammetry [42–45], HPTLC [46], spectrophotometry [44] and flow injection analysis using UV-detection [47] have been reported for the determination of this drug in pharmaceutical preparations. Voltammetric [42–44], spectrophotometric [44], and liquid chromatographic [48,49] methods have been employed for the analysis of doxazosin in biological samples.

Among the three drugs, only prazosin is listed in official compendia, and the monographs in BP [50] and USP [51] suggest use of HPLC methods for drug assay.

2. Experimental

2.1. Materials

Prazosin hydrochloride and doxazosin mesylate were supplied by Sun Pharmaceutical Industries Ltd. (Baroda, India). Terazosin hydrochloride was procured from Intas Pharmaceuticals Ltd. (Matoda, India). All drugs were used as received. Sodium hydroxide was purchased from Ranbaxy Laboratories (S.A.S. Nagar, India) and hydrochloric acid was procured from LOBA Chemie Pvt. Ltd. (Mumbai, India). Hydrogen peroxide was procured from s.d. Fine-chem Ltd. (Boisar, India). HPLC-grade methanol was purchased from Mallinckrodt Baker Inc. (Paris, USA) and acetonitrile of the same grade was purchased from J.T. Baker (Xalostoc, Mexico). All other chemicals were of analytical reagent grade. Ultra-pure water was obtained from ELGA (Bucks, UK) water purification unit.

2.2. Instrumentation

The HPLC system consisted of a 600E pump, a 996 photo-diode array (PDA) detector, a 717 autoinjector, and a degasser module; data were acquired and processed using a Millennium software ver. 2.1 (all from Waters, Milford, USA). Robustness testing of methods was done on another HPLC system, equipped with an LC-10ATVP pump, an SPD-10AVVP UV-Vis dual-wavelength detector, an SIL-10ADVP autoinjector, and a DGU-14A degasser module; data were acquired and processed using a CLASS-VP software (all

from Shimadzu, Kyoto, Japan). The chromatographic separations were carried out on Spherisorb (Waters, Milford, USA) C-18 columns (250 mm × 4.6 mm i.d., with a particle size of 5 μm).

2.3. Generation of stress samples for establishment of stability-indicating assay

The stress samples were generated in the same manner as given in our earlier report on the degradation behaviour of the three drugs [5]. The reactions were carried out at a drug concentration of 1 mg ml^{-1}. The stress conditions were as follows:

(i) Stress study under hydrolytic condition
Acidic hydrolysis: drug solution in 0.1N HCl was exposed at 80 °C for 90 h; neutral hydrolysis: drug solution in water at 80 °C for 10 days; alkaline hydrolysis: drug solution in 0.1N NaOH at 80 °C for 4 h for prazosin and terazosin and 30 min for doxazosin.

(ii) Stress study under oxidative condition
Drug solutions in 3 and 30% H_2O_2 were stored at room temperature for 6 and 24 h, respectively.

(iii) Stress study under light
Drug solutions in 0.1N HCl, 0.1N NaOH and water were exposed to ICH-recommended light conditions [5] according to option 2 [52] at 40 °C for 7 days.

(iv) Thermal stress
Bulk drug was subjected to dry heat at 50 °C for 3 months.

2.4. Separation studies

The initial analyses of different stressed samples for all the three drugs were performed on an HPLC system using a C-18 column and a mobile phase composed of acetonitrile/water/glacial/acetic acid/diethylamine (65:35:1:0.02). It was filtered through 0.45 μm nylon membrane and degassed before use. The injection volume was 5 μl, and the mobile-phase flow rate was 1 ml min^{-1}. The detections were carried out at 254 nm.

2.5. Optimization studies

The separations of drugs from their degradation products were optimised by varying the ratio and/or nature of organic modifier. Trials were also made by modifying the concentration of diethylamine (DEA) and acetic acid.

2.6. Validation of the developed methods

A similar method validation protocol was followed for all the three drugs. Linearity of the methods was established by triplicate injections of solutions containing drugs in the range of 50–500 μg ml^{-1}. The linearity plots were constructed and the acceptable fit to the linear regression was demonstrated by construction of residual plots and evaluation of sum of squares of residuals (SS^2_{res}). A *t*-test was also performed using SigmaStat ver. 2.0 (Jandel Scientific GmbH, Erkrath, Germany).

To determine intra-day precision, six injections of three different concentrations (50, 200 and 500 μg ml^{-1}) were given on the same day and the values of relative standard deviation were calculated. These studies were repeated with different weightings on different days to determine inter-day precision. For determination of accuracy, stress samples of conditions under which significant degradation was observed were mixed and the solution was fortified with three known concentrations of each drug substance, and the recovery of the added drug substance was determined. Specificity of the method towards the drug was established through determination of purity of the drug peak in a mixture of stressed samples through study of purity plots using a PDA detector. The resolution factor of the drug peak from the nearest resolving peak was also determined. Resolution of the drug in a mixture of stressed samples was studied by performing the analyses on a different chromatographic system on a different day to establish robustness of the method.

3. Results and discussion

3.1. Development of optimized stability-indicating methods

The mobile phase used in initial studies in this investigation (acetonitrile/water/glacial acetic acid/diethylamine (65:35:1:0.02)) was the same as employed in our previous study on stress testing of the

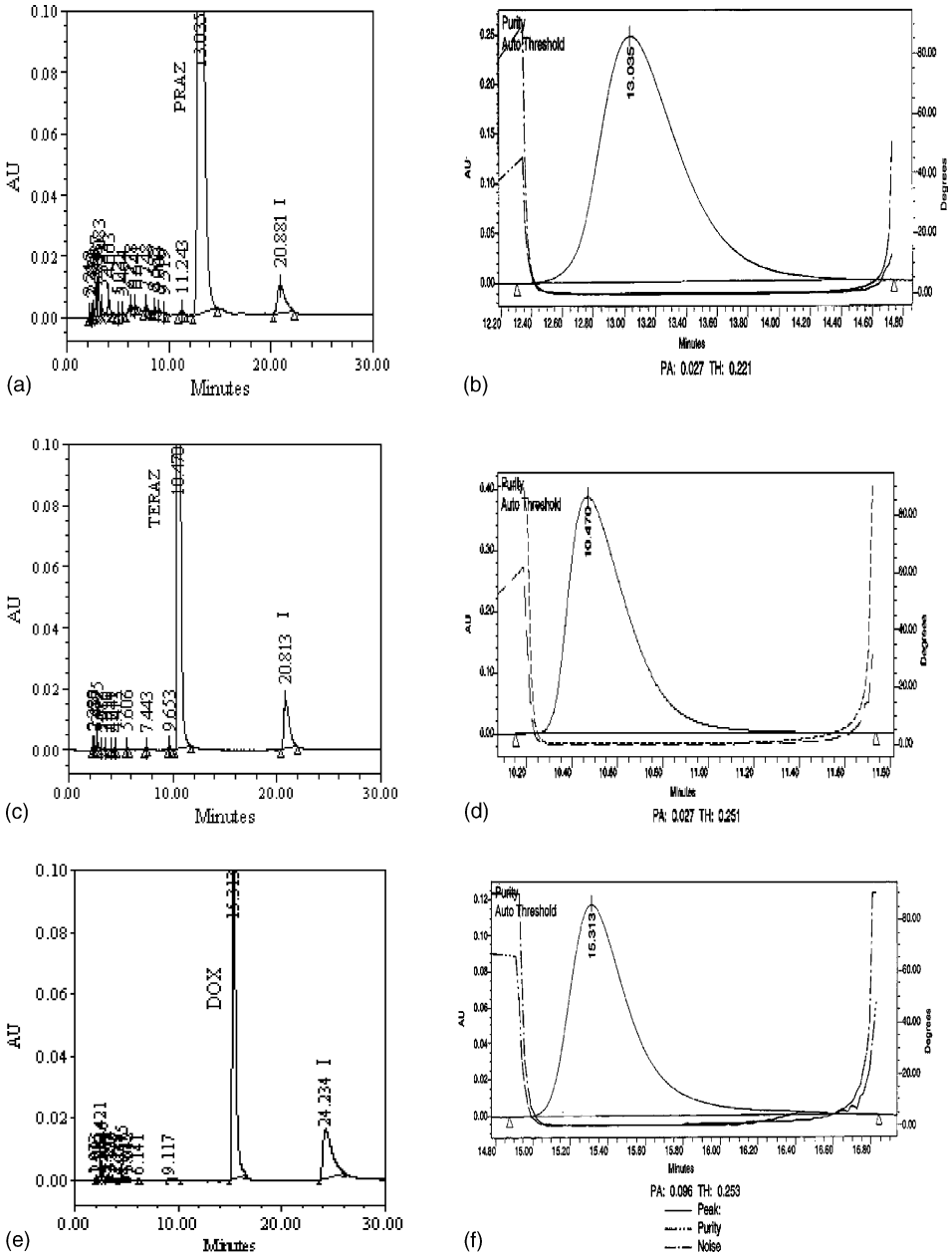

Fig. 2. Chromatograms showing separation of (a) prazosin (PRAZ), (c) terazosin (TERAZ) and (e) doxazosin (DOX) from degradation products in a mixture of respective stressed samples. Key – I: major degradation product in 0.1N HCl and 0.1N NaOH. The peaks shown in retention time periods of 2–11.2 min are the photolytic degradation products. (b), (d) and (f) depict the purity plots for prazosin, terazosin and doxazosin, respectively, in a mixture of stressed samples. Key for (b), (d) and (f): (—) peak, (– – –) purity and (– - – -) noise.

same drugs [5]. It was a slight modification of the one suggested in USP [51] for the assay of prazosin (methanol/water/glacial acetic acid/diethylamine (70:30:1:0.02)). However, when the method in the present study was extended to mixtures of stressed samples with 40–95% drug degradation, the resolution between the degradation products (generated under different stress conditions) and the drug peak was not satisfactory, particularly for prazosin and terazosin. Therefore, further modifications were made till a satisfactory resolution was achieved. The optimization was done by varying the concentration of glacial acetic acid and DEA. With the decrease in concentration of DEA or glacial acetic acid, there was a drastic increase in the retention time of the drug and the peak showed severe tailing. On increasing the concentration of acetic acid or DEA, the drug peak started eluting very early in the chromatogram and there was loss of resolution between the drug and degradation products. Even on varying the percentage of acetonitrile or replacing acetonitrile with methanol, no improvement in resolution was seen. Finally, combination of methanol and acetonitrile was tried and it was found that good resolution was obtained with 35% acetonitrile, 40% methanol and 25% water. Further trials revealed that resolution improved when DEA concentration was slightly decreased.

The mobile phase that showed acceptable separation of prazosin and terazosin from their respective degradation products was water/acetonitrile/methanol/glacial acetic acid/DEA (25:35:40:1:0.017). All other conditions were same as those employed in the initial study. The behaviour of separation is depicted in Fig. 2a and c. In case of doxazosin, it was possible to resolve the drug from degradation products with the initial method, as shown in Fig. 2e.

3.2. Validation of the developed stability-indicating methods

The method was validated for parameters such as linearity, precision, accuracy, specificity and robustness.

3.2.1. Linearity and range

Table 1 lists the mean HPLC area responses for prazosin, terazosin and doxazosin at different concentrations. The linearity plots are shown in Fig. 3. As shown, the responses for the drugs were strictly linear ($r^2 > 0.99$) in the concentration range of 50–500 μg ml^{-1}. Fig. 3 also includes residual plots corresponding to each linearity plot, indicating random distribution of residuals. The values of sum of squares of residuals (SS^2_{res}) for the plots b, d and f in Fig. 3 were 254.98, 89.10 and 123.74, respectively. The *t*-test ($P < 0.05$) also confirmed that there was no statistically significant difference in the predicted and observed values.

3.2.2. Precision

Table 2 gives the intra-day precision values of measured concentrations of prazosin, terazosin and doxazosin, as calculated from linearity plots. Similar mean measured concentration values for inter-day studies are given in Table 3. In both situations, the R.S.D. values are <1 and <2%, respectively, demonstrating that the methods were precise.

3.2.3. Accuracy

The respective HPLC area responses from the accuracy determination study are shown in Table 4. Good recoveries were obtained for each concentration, confirming that the method was accurate.

Table 1
Data for prazosin, terazosin and doxazosin from linearity studies ($n = 3$)

Concentration (μg ml^{-1})	Mean peak area ± S.D., R.S.D. (%)		
	Prazosin	Terazosin	Doxazosin
50	1960747 ± 14142.14, 0.721	1002024 ± 7075.31, 0.706	1151268 ± 4683.17, 0.407
100	4286506 ± 1081.87, 0.025	1930046 ± 28298.41, 1.466	2125202 ± 14819.54, 0.697
200	8133049 ± 57279.18, 0.704	4373262 ± 71290.51, 1.630	4138503 ± 38876.02, 0.939
250	10384361 ± 58041.45, 0.559	5625855 ± 49522.93, 0.875	5336654 ± 42426.41, 0.795
400	17274350 ± 119634, 0.693	9057058 ± 141421.36, 1.562	8025727 ± 136822.3, 1.705
500	22278872 ± 90109.45, 0.405	11212788 ± 94752.31, 0.845	10129289 ± 113137.1, 1.117

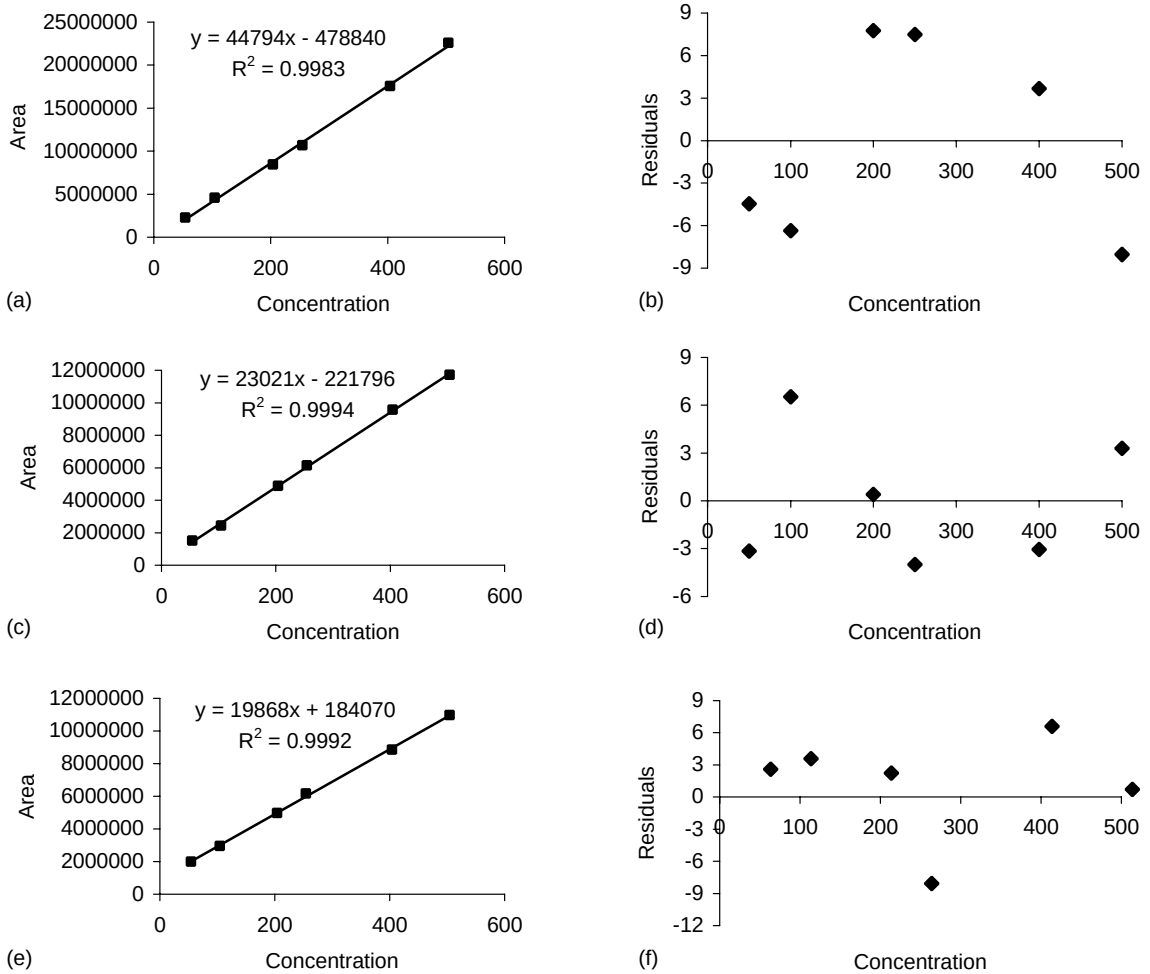

Fig. 3. Linearity plots for (a) prazosin, (c) terazosin and (e) doxazosin. The corresponding residual plots for the three drugs are shown in (b), (d) and (f), respectively.

3.2.4. Specificity

The purity plots for the drug peaks in a mixture of stressed samples are shown for the three drugs in Fig. 2b, d and f, respectively. In each case, the purity angle (PA) was less than purity threshold (TH), indicating the absence of any co-eluting peak. The resolution factor for the drug peaks in the mixture of degradation solutions was ~2 from the nearest resolving peak.

Table 2
Intra-day precision studies ($n = 6$)

Spiked concentration ($\mu g\,ml^{-1}$)	Measured concentration ± S.D. ($\mu g\,ml^{-1}$), R.S.D. (%)		
	Prazosin	Terazosin	Doxazosin
50	50.041 ± 0.344, 0.686	46.505 ± 0.249, 0.535	52.713 ± 0.223, 0.423
200	202.360 ± 0.531, 0.262	199.285 ± 0.766, 0.385	201.118 ± 2.658, 1.322
500	501.879 ± 3.397, 0.677	503.469 ± 0.615, 0.122	498.481 ± 1.267, 0.254

Table 3
Inter-day precision studies ($n = 3$)

Spiked concentration ($\mu g\,ml^{-1}$)	Measured concentration ± S.D. ($\mu g\,ml^{-1}$), R.S.D. (%)		
	Prazosin	Terazosin	Doxazosin
50	49.484 ± 0.755, 1.525	46.274 ± 0.829, 1.791	48.621 ± 0.829, 1.707
200	202.105 ± 3.168, 1.567	200.465 ± 3.167, 1.578	199.289 ± 3.799, 1.907
500	503.137 ± 4.989, 0.992	502.612 ± 0.405, 0.122	499.532 ± 2.643, 0.529

Table 4
Recovery studies ($n = 3$)

Spiked concentration ($\mu g\,ml^{-1}$)	Prazosin		Terazosin		Doxazosin	
	Measured concentration ± S.D. ($\mu g\,ml^{-1}$), R.S.D. (%)	Recovery (%)	Measured concentration ± S.D. ($\mu g\,ml^{-1}$), R.S.D. (%)	Recovery (%)	Measured concentration ± S.D. ($\mu g\,ml^{-1}$), R.S.D. (%)	Recovery (%)
50	50.815 ± 0.078, 0.153	101.63	50.82 ± 0.113, 0.223	101.64	50.958 ± 0.996, 1.954	101.92
200	201.825 ± 3.036, 1.504	100.91	203.298 ± 1.177, 0.579	100.65	200.2 ± 3.175, 1.586	100.10
500	506.867 ± 6.915, 1.364	101.37	505.7 ± 2.858, 0.565	101.32	504.283 ± 2.776, 0.550	100.87

3.2.5. Robustness

Resolution of the drug in a mixture of stressed samples was found to be similar to that shown in Fig. 2a, c and e, when studies were performed on a different chromatographic system on different days, indicating that the methods had sufficient robustness.

4. Conclusion

Validated stability-indicating HPLC methods were developed for prazosin, terazosin and doxazosin after subjecting them to stress testing under ICH-recommended conditions. These were validated for linearity and precision in the studied concentration range. The recovery of the drugs was essentially quantitative. The methods were found to be 'specific' to the drugs, as the peaks of the degradation products did not interfere with the drug peaks [2]. Thus the proposed methods can be employed for the assay of the three drugs in the stability samples.

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