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STABILITY INDICATING HPLC-UV METHOD FOR DETERMINATION OF DAPOXETINE HCl IN PHARMACEUTICAL PRODUCT

KAI BIN LIEW and KOK KHIANG PEH*

School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia

Abstract: A stability-indicating HPLC-UV method for the determination of dapoxetine hydrochloride in solution and pharmaceutical product was developed. The mobile phase was composed of acetonitrile and 0.2 M ammonium acetate buffer at 50 : 50 ratio. The chromatographic parameters, theoretical plates (N), tailing factor (T), capacity factor (k') and peak asymmetry factor (As) were calculated. Stress degradation studies, namely, acid, alkali, oxidation, heat and UV light, were performed. The analyte was eluted at 5.8 min using gradient system at a flow rate of 1.5 mL/min. The theoretical plates count was > 2000, tailing factor < 1.54, capacity factor > 5.38 and peak asymmetry factor was < 1.10. The method was linear from 1 to 40 µg/mL with a correlation coefficient of 0.9994. The intraday precision and accuracy values were 0.14–1.54% and 0.63–1.83%, respectively. On the other hand, the interday precision and accuracy results were 0.49–1.83% and 1.15–1.85%, respectively. The drug solution was stable at ambient room temperature (26°C) for 48 h. Dapoxetine HCl was found susceptible to oxidation and degraded slightly under acid and alkali conditions but was stable under UV light and heat. No interference from tablet excipients and degradation products was found. Hence, the method can be employed as a stability-indicating method for the determination of dapoxetine HCl in pharmaceutical products.

Keywords: dapoxetine HCl, stress degradation study, stability indicating HPLC-UV method

Dapoxetine hydrochloride (dapoxetine HCl), (1S)-N,N-dimethyl-3-naphthalen-1-yloxy-1-phenylpropan-1-amine hydrochloride, belongs to a pharmacological group called selective serotonin reuptake inhibitors (1). The chemical structure of dapoxetine HCl is shown in Figure 1. Dapoxetine HCl is used in the treatment of anxiety disorder and depression. It was also reported to delay ejaculation in men during sexual relationship and patented for premature ejaculation (2).

Up-to-date, there are only a few publications on quantification of dapoxetine HCl. Giri et al. (1) reported a HPLC-UV method for simultaneous quantification of tadalafil and dapoxetine in drug solution. The linearity of dapoxetine HCl only covered the range of 0.75–12 µg/mL, which might not be sufficiently sensitive to detect dapoxetine HCl in dissolution study. Chandran et al. (3) reported a RP-HPLC method to simultaneously quantify tadalafil and dapoxetine HCl but dapoxetine HCl was eluted at 11.4 min, which was too long. The UV spectrophotometric method reported by Amin et al. (4) and thin layer high performance liquid chromatography reported by Pandya et al. (5) were not selective and specific.

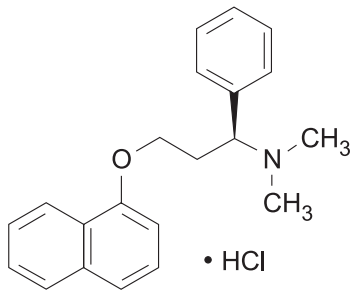

Figure 1. Chemical structure of dapoxetine HCl

Hitherto, the reported methods did not carry out suitability tests and stress degradation study on dapoxetine HCl solution and tablets. The environmental factors such as temperature, humidity, and light can be detrimental to active pharmaceutical ingredient (6) and its quality might be greatly compromised by the changes in the environmental factors (7). International Conference on Harmonization (ICH) guideline on Stability Testing of New Drug Substances and Products requires stress tests to elucidate the inherent stability characteristics of the

* Corresponding author: e-mail: kkpeh@usm.my; kkpehken@gmail.com; phone: 604-6532257; fax: 604-6570017

active pharmaceutical ingredient. Stability-indicating methods have received considerable attention for the determination of drugs (8).

The aim of this study was to develop and validate a simple, rapid and reproducible stability-indicating RP-HPLC-UV method to quantify dapoxetine HCl in drug solution and a pharmaceutical product, Priligy® tablets.

EXPERIMENTAL

Materials

Dapoxetine hydrochloride was a free sample from Rakshit Drugs PVT LTD. (India). Ammonium acetate, potassium dihydrogen phosphate, HPLC-grade acetronitrile, analytical grade hydrochloric acid, sodium hydroxide and analytical grade hydrogen peroxide solution were purchased from Merck (USA). Priligy® tablets (Janssen-Cilag, Italy) were purchased from a local pharmacy.

Instrumentation

The HPLC system comprised of a Shimadzu (VP series, Kyoto, Japan) pump (LC-20AT vp) with solvent cabinet, a degasser (DGU-20A_3), a column oven (CTO-10S VP), an auto-injector (SIL-20A HT VP), a UV/VIS detector (SPD-20A vp), and a computer software (LC-Solution VP).

Chromatographic condition

The separation was carried out using a Synchronize (Thermo Scientific, USA) C-18 column (150 × 4.6 mm ID, 5 µm). The column temperature was set at 30°C. The detection wavelength was 240 nm. Sample of 25 µL was injected onto the column.

Mobile phase optimization and elution system

Different compositions of mobile phase were studied to determine the optimum mobile phase for good resolution and short elution time. Two elution methods were studied, namely, isocratic and gradient systems.

For isocratic elution system, various compositions of mobile phase studied are shown in Table 1. The 0.2 M buffer solution was prepared by weighing 15.42 g of ammonium acetate and dissolving in 1 L of distilled water. The buffer solution was mixed with acetonitrile, accordingly. The mobile phase was filtered through 0.45 µm nylon membrane filter (Whatman, UK) under vaccum using a filtration set.

Table 1. Various compositions of mobile phase used for isocratic system.

Acetonitrile : 0.2 M ammonium acetate buffer (v/v)	pH	Flow rate (mL/min)
30 : 70	7.20	1.2
30 : 70	6.00	1.2
30 : 70	5.00	1.2
50 : 50	7.40	1.2
70 : 30	7.70	1.5
90 : 10	7.90	1.5

Table 2. Various composition of mobile phase and time programme for gradient system.

Time (min)	Acetonitrile : 0.2 M ammonium acetate buffer (v/v)		
	Trial 1	Trial 2	Trial 3
0.00	50 : 50	50 : 50	50 : 50
0.50	50 : 50	50 : 50	50 : 50
2.50	60 : 40	75 : 25	90 : 10
6.50	60 : 40	75 : 25	90 : 10
6.51	50 : 50	50 : 50	50 : 50
8.00	50 : 50	50 : 50	50 : 50

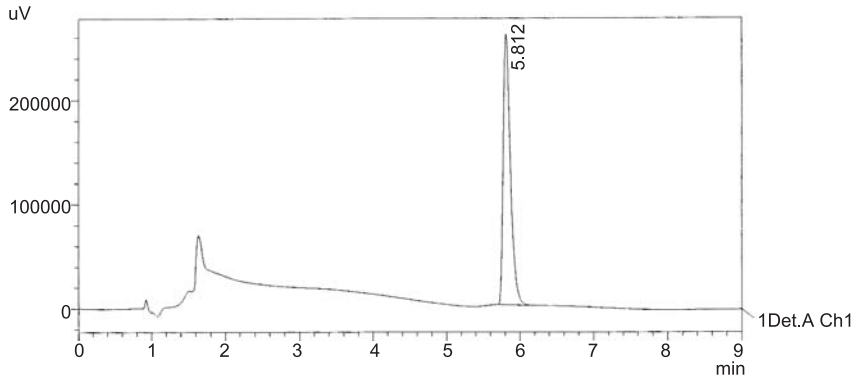

Figure 2. Chromatogram of 40 μg/mL dapoxetine HCl solution (RT = 5.812 min)

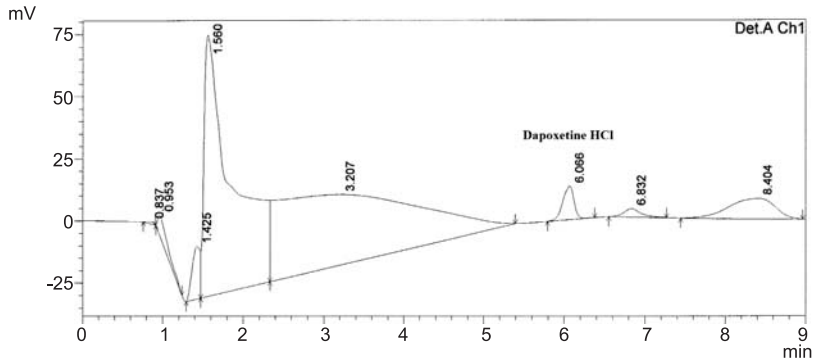

Figure 3a. Chromatogram of drug solution (zero hour) in oxidative degradation

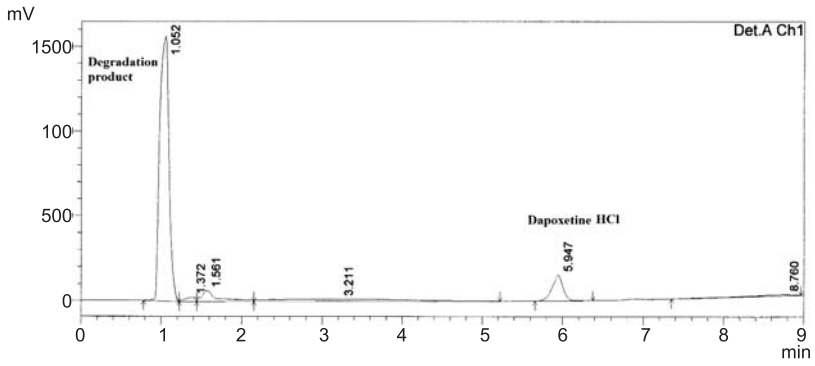

Figure 3b. Chromatogram of drug solution (after 3 h) in oxidative degradation study

The filtered mixture was degassed using a ultrasonicator for 15 min.

On the other hand, for gradient elution system, acetonitrile and 0.2 M buffer solution was run according to the time program presented in Table 2.

Preparation of stock standard solution

Forty milligrams of dapoxetine HCl working standard was weighed and transferred to a 100 mL volumetric flask and dissolved in 50 mL of mobile phase. The volumetric flask was shaken using ultra-

sonicator for 5 min. The solution was diluted to volume with mobile phase. The stock standard solution had a concentration of 400 µg/mL of dapoxetine hydrochloride.

Preparation of working standard solution

Two and a half milliliters of stock standard solution (400 µg/mL) was pipetted into 10 mL volumetric flasks and diluted to final volume with mobile phase and mixed well. This working standard solution had a concentration of 100 µg/mL of dapoxetine HCl.

Preparation of standard drug solutions for standard calibration curve

Samples of 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 and 4.0 mL of the working standard solution were pipetted into seven separate 10 mL volumetric flasks and diluted to the final volume with mobile phase and mixed well. These seven standard solutions had concentrations of 1, 2, 4, 8, 16, 32 and 40 µg/mL of dapoxetine HCl.

Preparation of quality control standard solutions

Three quality control standard solutions at 3 (low), 20 (medium) and 30 (high) µg/mL were prepared and used in method validation. Samples of 0.3, 2.0 and 3.0 mL of the working standard solution were pipetted into three separate 10 mL volumetric flasks and diluted to the final volume with mobile phase and mixed well. These three quality control standard solutions had concentrations of 3, 20 and 30 µg/mL of dapoxetine HCl.

System suitability study

The chromatographic parameters, such as: theoretical plates (N), tailing factor (T), capacity factor (k') and peak asymmetry factor (As), were calculated. The value of tailing factor should be not more than 2.0 (9). Peak asymmetry factor (As) is the simplest way of measuring the degree of peak distortion (skew). The peak asymmetry was determined at 10% peak height. For a tailed peak, As > 1. For a fronted peak, As < 1. For a symmetric peak, As = 1. Recommended acceptance criteria for asymmetry factor is between 0.9 to 1.1 (10, 11). Capacity factor (k') is an indicator of efficiency of a column to retain sample molecule during an isocratic separation. The literature proposed the acceptable k' value ranges 2–10 (11).

Linearity

Few concentrations of calibration standard namely: 1, 2, 4, 8, 16, 32 and 40 µg/mL were prepared using the stock solution described above. The standard calibration curve was constructed using peak area *versus* known concentrations of dapoxetine HCl. The linear regression line was used to determine the linearity and concentration of the samples. The linearity of dapoxetine HCl was conducted using six sets of the calibration standards.

Precision and accuracy

Three quality control standard solutions at 1 (LOQ), 3, 20 and 30 µg/mL, were prepared to determine the method precision and accuracy. For intra-day precision and accuracy, six sets of standard solutions were assayed on the same day. For inter-day precision and accuracy, six sets of standard solutions were injected over six consecutive days, with one standard calibration curve injected on each day. The coefficient of variation (% CV) was calculated for the precision of the assay, using the following equation:

$$CV\ (\%) = \frac{Standard\ deviation}{Mean\ volume} \times 100\%$$

Table 3. Results of system suitability at LOQ and three quality control samples. The mean ± SD, n = 6.

Parameter	Dapoxetine HCl (µg/mL)			
	1.0	**3.0**	**20.0**	**30.0**
Theorectical plates	18706.10 ± 662.89	16880.03 ± 1019.93	16721.05 ± 1650.51	16877.40 ± 1399.83
Tailing factor	1.33 ± 0.04	1.52 ± 0.10	1.54 ± 0.16	1.51 ± 0.14
Peak asymmetry factor	1.08 ± 0.13	1.09 ± 0.03	1.10 ± 0.05	1.08 ± 0.04
Capacity factor	5.43 ± 0.01	5.40 ± 0.03	5.39 ± 0.03	5.38 ± 0.03
Resolution	25.88 ± 1.65	24.34 ± 1.02	25.37 ± 0.79	25.55 ± 0.67
Height equivalent to the theoretical plate (HETP)	8.03 ± 0.29	8.91 ± 0.52	9.04 ± 0.83	8.94 ± 0.72

Table 4. Results of six standard calibration curves.

Set	Slope	Intercept	R^2
1	45502.03	5079.47	0.9995
2	44652.70	4475.19	0.9995
3	44889.85	4752.52	0.9994
4	44982.31	5550.40	0.9992
5	45053.13	1792.50	0.9992
6	45056.47	3381.20	0.9997
Mean	45022.75	4171.88	0.9994
SD	278.57	1374.03	0.0002

The accuracy was presented as the relative percentage error (% bias) of calculated concentration of the samples. The accuracy was computed using the following equation:

$$\text{Accuracy} = \frac{(\text{Calculated concentration} - C_{std})}{C_{std}} \times 100\%$$

where C_{std} = the concentration of standard solution.

Limit of quantification (LOQ)

The LOQ was the lowest point of concentration in the calibration curve. Acceptance criteria were RSD of 2% for precision and accuracy of 2%.

Limit of detection (LOD)

The LOD value was determined by injecting samples successively until a concentration at a signal to noise ratio of 3:1 was obtained.

Stock solution stability

The stock standard solution of dapoxetine hydrochloride (400 µg/mL) was kept at ambient room temperature (26°C, 65% RH). Sample was injected and collected at 6 and 48 h. The instrumental responses at 6 and 48 h were compared with fresh samples.

Stress degradation studies

Dapoxetine HCL solution of 0.35 mg/mL was prepared by weighing 35 mg of dapoxetine HCl powder (equivalent to 30 mg of dapoxetine) and dissolving it in a 100 mL volumetric flask. On the other hand, ten tablets of Priligy® were crushed with mortar and pestle. Powder with weight equivalent to the mean weight of ten tablets (containing 35 mg of dapoxetine HCl equivalent to 30 mg of dapoxetine) was taken and dissolved in a 100 mL volumetric flask. The mixture of 0.2 M phosphate buffer and acetonitrile (50 : 50, v/v) was used as solvent.

Acid degradation study

One milliliter of the sample solution was transferred into a 10 mL volumetric flask. Two sets of flasks for each study were prepared. Three milliliters of 3 M HCl was added into each of the flask. For the first set, 3 mL of 3 M NaOH was added immediately to neutralize the solution and adjusted to volume. It served as the zero hour sample. Twenty five microliters of the solution was injected into HPLC apparatus. Another set of flasks was left on the bench under room temperature (28°C, 60% RH) and the same neutralization procedure was performed after 3 h.

Alkali degradation study

One milliliter of the sample solution was transferred into a 10 mL volumetric flask. Two sets of flasks for each study were prepared. Three milliliters of 3 M NaOH was added into each of the flask. For the first set, 3 mL of 3 M HCl was added immediately to neutralize the solution and adjusted to volume. It served as the zero hour sample. Twenty five microliters of the solution was injected into HPLC apparatus. Another set of flasks was left on the bench under room temperature (28°C, 60% RH) and the same neutralization procedure was performed after 3 h.

Oxidative (H_2O_2) degradation

One milliliter of the sample solution was transferred into a 10 mL volumetric flask. Two sets of flasks for each study were prepared. Three milliliters of 35% H_2O_2 was added into each flask. For the first set, the solution was adjusted to volume and 25 µL of the solution was injected into HPLC column immediately. It served as zero hour sample. Another set of flasks was left on the bench under room temperature (28°C, 65% RH) and the same procedure was performed after 3 h.

Heat degradation study

One milliliter of the sample solution was transferred into a 10 mL volumetric flask. Two sets of flasks were prepared. For the first set, the solution was adjusted to volume and 25 µL of the solution was injected into HPLC column immediately. It served as the zero hour sample. Another set of flasks was heated in water bath at 80°C and the samples were injected after heating for 2 h.

UV light degradation

One milliliter of the sample solution was transferred into a 10 mL volumetric flask. Two sets of flasks were prepared. For the first set, the solution was adjusted to volume and 25 µL of the solution was injected into HPLC column immediately. It served as the zero hour sample. Another set of flasks was stored in UV cabinet (254 nm) and the samples were injected after 24 h.

Assay of dapoxetine HCl in pharmaceutical tablet

The HPLC-UV method was applied to determine dapoxetine HCl content of Priligy®, a commercial immediate release dapoxetine HCl tablets. Ten tablets were weighed and crushed using mortar and pestle. The powder was mixed well. Powder with weight equivalent to the mean weight of ten tablets (containing 30 mg of dapoxetine) was taken and dissolved in a 10 mL volumetric flask with mobile phase. The solution was subjected to sonication for 15 min. The sample of 0.1 mL was drawn and diluted with mobile phase to 10 mL in a volumetric flask to give a drug concentration of 30 µg/mL. The sample of 25 µL was injected into the HPLC system.

RESULTS

System suitability

The result of theoretical plates number (N), tailing factor (T), peak asymmetry factor (As), capacity factor (k'), resolution and height equivalent to theoretical plate (HETP) at three QC concentrations and LOQ are shown in Table 3. The average theoretical plate was > 2000. Both tailing factor (< 2) and peak asymmetry factor (0.9–1.1) met the requirement stated in USP 34. The capacity factor was in the ideal range between 2 and 10 (11).

Linearity

The standard calibration curve exhibited an excellent linearity and a good correlation coefficient over the given range of 1–40 µg/mL of dapoxetine HCl. The mean linear regression equation from six calibration curves was, $y = 45022.75 (\pm 278.57) x + 4171.88 (\pm 1374.03)$, (x = dapoxetine concentration, y = average peak area), with a correlation coefficient of 0.9994 (0.0002) as given in Figure 4. The six standard calibration curves were injected over six days to test the reproducibility of the method. The results are presented in Table 4.

Precision and accuracy

The results of precision and accuracy are shown in Table 5. Precision and accuracy were tested at four concentrations, namely, LOQ, 3, 20 and 30 µg/mL. Intra-day precision was in the range of 0.14–1.54% whereas intra-day accuracy was in the range of 0.63–1.83%. Inter-day precision and accuracy were in the range of 0.49–1.83% and 1.15–1.85%, respectively. The results were within the ± 2% range recommended by USP guidelines. Hence, the method indicates good precision and accuracy.

Specificity

There was no peak found at the retention time of the analyte in the blank solution. The results from the stress testing studies indicated that the method was highly specific for dapoxetine HCl. The degradation products were completely resolved from the parent compound.

Table 5. Result of intra-day and inter-day precision and accuracy. The result is presented as the mean, n = 6.

Conc. (µg/mL)	Intra-day		Inter-day	
	Precision (% CV)	Accuracy (% bias)	Precision (% CV)	Accuracy (% bias)
1	1.38	1.83	1.83	1.63
3	1.54	0.63	0.89	1.15
20	0.14	1.55	0.49	1.51
30	0.20	1.81	0.98	1.85

Table 6. Results of stress degradation studies. The mean ± SD, n = 3.

Parameters		Exposure time (h)	
		Assay at 0 h (%)	Assay after 3 h (%)
Acid hydrolysis	In drug solution	99.87 ± 0.15	95.18 ± 1.34
	In tablet formulation	101.00 ± 0.03	93.51 ± 0.05
Alkali hydrolysis	In drug solution	99.25 ± 1.28	97.57 ± 0.75
	in tablet formulation	100.48 ± 0.05	98.40 ± 0.75
H_2O_2 Oxidation	In drug solution	83.58 ± 2.47	5.88 ± 3.59
	In tablet formulation	81.05 ± 1.45	1.56 ± 0.96
Heat degradation	In drug solution	100.45 ± 0.29	101.53 ± 0.52
	In tablet formulation	99.38 ± 0.05	101.85 ± 3.50
UV degradation	In drug solution	100.08 ± 1.34	101.94 ± 0.88
	In tablet formulation	101.97 ± 3.67	105.28 ± 5.24

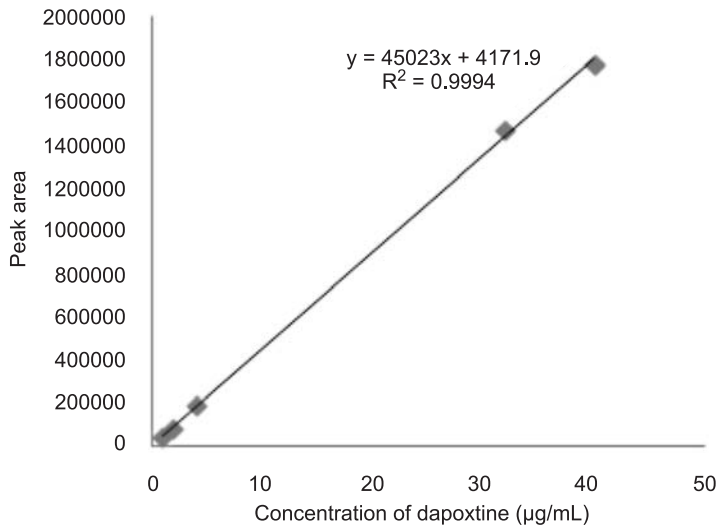

Figure 4. Mean standard calibration curve. The mean ± SD (n = 6)

LOQ and LOD

The LOQ was 1 µg/mL with inter-day precision and accuracy of 1.83 and 1.63%, respectively. The LOD was 0.01 µg/mL.

Stock solution stability

The percentage of dapoxetine HCl remained after 6 and 48 h storage at ambient room temperature was 99.70 and 99.32%, respectively. The results suggest that the stock solution was stable for 48 h when kept at ambient room temperature.

Assay of dapoxetine content in tablet

The result of assay content of Prilligy tablets calculated in this study was within 98–102%.

DISCUSSION

Initially, Synchronize C-18 analytical column with 250 mm length was used. However, the elution time of the analyte was too long for all the compositions of acetonitrile and 0.2 M ammonium acetate buffer studied, ranging from 20 to 30 min. At ace-

tonitrile content of 90%, an elution time of 20 min was obtained, which was too long. Although a shorter Synchronize C-18 column with 150 mm length was used, the elution time could only be shortened to 16 min. The elution time was considered too long and unsuitable for analysis of large quantity of samples. As such, gradient elution system was studied.

It was found that gradient elution is able to produce a relatively higher peak height in a shorter operation cycle when compared with isocratic elution (12). Figure 2 shows the chromatogram of 40 µg/mL dapoxetine HCl in drug solution separated using gradient elution system. The composition of mobile phase of trial 3 was selected based on the shortest elution time. The retention time was 5.8 min. This method was used for method validation procedure and subsequent tests. The method was validated and reproducible.

Susceptibility to oxidation, hydrolytic, and photolytic stability are required by the ICH guideline (8). An ideal stability-indicating method is one that can quantify the standard drug alone and also resolve its degradation products (13). The chromatograms of oxidative degradation study in drug solution at zero hour and 3 hours are presented in Figures 3a and 3b, respectively. The method was able to separate both analyte and degradation product peak that was eluted at 1.05 min. The results of acid, alkali, oxidation, heat and UV degradation are shown in Table 6, respectively. Dapoxetine HCl was found easily oxidized by hydrogen peroxide even just exposed to it for 5 min (from preparation to injection). The assay of drug dropped slightly in acid and alkali condition. Dapoxetine HCl was found stable in UV light and heat.

CONCLUSION

It can be concluded that a stability indicating HPLC-UV method for determination of dapoxetine HCl in tablets was successfully developed. The method was rapid, simple, precise, sensitive and reproducible. The method can also be used to assay dapoxetine HCl in other pharmaceutical dosage forms such as capsules.

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