RP-HPLC analytical method development and optimization for quantification of donepezil hydrochloride in orally disintegrating tablet

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Abstract: An easy, fast and validated RV-HPLC method was invented to quantify donepezil hydrochloride in drug solution and orally disintegrating tablet. The separation was carried out using reversed phase C-18 column (Agilent Eclipse Plus C-18) with UV detection at 268 nm. Method optimization was tested using various composition of organic solvent. The mobile phase comprised of phosphate buffer (0.01M), methanol and acetonitrile (50:30:20, v/v) adjusted to pH 2.7 with phosphoric acid (80%) was found as the optimum mobile phase. The method showed intraday precision and accuracy in the range of 0.24% to -1.83% and -1.83% to 1.99% respectively, while interday precision and accuracy ranged between 1.41% to 1.81% and 0.11% to 1.90% respectively. The standard calibration curve was linear from 0.125 µg/mL to 16 µg/mL, with correlation coefficient of 0.9997±0.00016. The drug solution was stable under room temperature at least for 6 hours. System suitability studies were done. The average plate count was > 2000, tailing factor <1, and capacity factor of 3.30. The retention time was 5.6 min. The HPLC method was used to assay donepezil hydrochloride in tablet and dissolution study of in-house manufactured donepezil orally disintegrating tablet and original Aricept.

Keywords: RP-HPLC, donepezil hydrochloride, dissolution study, assay.

INTRODUCTION

Donepezil hydrochloride (Donepezil HCl) is presented as a white powder with crystalline structure. It can be dissolved in methanol, water and glacial acetic acid (Pappa *et al*., 2002). Donepezil HCl is generally known as E2020 in the pharmacological literature. Its empirical formula is $C_{24}H_{29}NO_3HCl$. It has a molecular weight of 415.96. Its melting point is 218-220˚C. There are a few published works to quantify donepezil hydrochloride in drug solution. Kumar *et al*. reported a method using keystone phenyl reverse phase column with mobile phase of methanol, 0.02 M phosphate buffer and triethylamine at ratio of 60:40:0.5 v/v (Kumar *et al*., 2011). The retention time was 7.05 min. Pappa *et al* reported a method using C18 reverse phase column with mobile phase of methanol, 0.02M phosphate buffer and triethylamine at ratio of 50:50:0.5 v/v (Pappa *et al*., 2002). The retention time was 9.0 min. Bobbarala *et al* published his work using C-18 reverse phase column with mobile phase of Acetate buffer and acetonitrile at ratio of 50:50 v/v (Bobbarala *et al*., 2010). However the method was only linear within the range of 12.5 to 75 µg/mL.

The objective of the study was to develop a HPLC method with short retention time, high sensitivity and applicable for quantification of donepezil hydrochloride in drug solution and dissolution study. Optimization was done using various combination of organic solvent and buffer at different ratio. The method was then applied to assay donepezil hydrochloride in orally disintegrating tablet and dissolution study.

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METHODS

Materials

Donepezil hydrochloride was obtained from Ind-Swift Laboratory Limited (Chandigarh, India). Perchloric acid, potassium dihydrogen phosphate, phosphoric acid, HPLC-grade acetronitrile and chloroform were purchased from Merck (New Jersey, USA). HPLC-grade methanol was obtained from J.T. Baker (Philipsburg, USA).

Instrumentation

The HPLC system was comprised of a Shimadzu (VP series, Kyoto, Japan) pump (LC-10AT vp/FCV-10AL-vp) with solvent cabinet, an auto-injector (SIL-10AD vp), UV/VIS detector (SPD-20AD vp), and a computer software (VP-CLASS).

Chromatography condition

The separation was carried out using a reversed phase C-18 Agilent Eclipse Plus column (250 x 4.6 mm ID, 5µm) (Agilent, USA) fitted with analytical guard column (Zorbax Eclipse Plus) packed with replaceable C-18 cartridge (12.5 X 4.6 mm ID, 5 µm). The flow rate was set at 1 ml/min and detection wavelength of 268 nm was used. Sample of 80 µl was injected onto the column.

Mobile phase optimization

Few compositions of mobile phase were tested to determine the optimum mobile phase with good resolution, small tailing factor and short retention time. table 1 listed the composition of mobile phase. 1.75 g of potassium dihydrogen phosphate was weighed and dissolved in 1 L of distilled water. Known volume of buffer solution was taken out to mix with organic phase according to the ratio stated in table 1. The mobile phase was stirred using a magnetic stirrer for 15 minutes. The pH of the mobile phase was adjusted using phosphoric acid (80%). The mobile phase was then filtered thru Whatman nylon membrane filters 0.45 µm using a filtration set. The filtered mixture was degassed using a sonicator for 15 minutes.

Table 1: Composition of mobile phase for optimization

No.	Composition	Ratio	pH
1	0.01 M Phosphate buffer : Methanol	70:30	3.0
2	0.01 M Phosphate buffer : Methanol	70:30	2.7
3.	0.01 M Phosphate buffer : Methanol	50:50	2.7
4	0.01 M Phosphate buffer : Methanol : Acetonitrile	50:40:10	2.7
5	0.01 M Phosphate buffer : Methanol : Acetonitrile	50:30:20	2.7
6	0.01 M Phosphate buffer : Methanol : Acetonitrile	50:20:30	2.7

Preparation of stock solution

10 mg of donepezil HCl working standard was weighed and transferred to a volumetric flask with 100mL volume. 50 mL of mobile phase was added into volumetric flask and shaken using ultrasonic vibrator for 5 min. The solution was diluted with mobile phase to volume and mixed well. The stock standard solution had concentration of 100 µg/mL of donepezil hydrochloride.

Preparation of calibration standards

The stock solution that described above was diluted with mobile phase to prepare calibration standards of 0.125, 1, 4, 8, 16 µg/mL. The standard calibration curve was constructed using peak height versus known concentrations of donepezil HCl. The resulting regression line data were used to determine the concentration of the samples. The linearity of donepezil HCl was conducted using six set of the calibration standards.

Specificity

Specificity was determined by injecting six (6) placebo samples, followed by six (6) samples of LOQ. Placebo samples were prepared by dissolving blank orally disintegrating tablet in mobile phase. There should be no peak found at the retention time of the analyte in the placebo samples.

Precision and accuracy

Three quality control standard solutions at concentrations of 0.5, 10 and 14 µg/mL and LOQ (0.125 µg/mL) were prepared to determine the method precision and accuracy. For intra-day precision and accuracy, six sets of samples at the concentration stated above were assayed on the same day. For inter-day precision and accuracy, six replicates of each standard solution concentration were injected over six consecutive days, with one standard curve on each day. The coefficient of variation (%CV) was determined to assess the precision of the assay. The coefficient of variation was derived from the following equation:

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

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

$$\%\ Bias = \frac{Calculated\ concentration - Cstd}{Cstd} \times 100\%$$

Where C_{std} = the concentration of standard solution

Limit of detection (LOD)

LOD was determined by injecting successively lower concentrations until a signal to noise ratio of 3:1 was obtained.

Limit of quantification (LOQ)

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

Stock solution stability

Stock solution stability was determined by keeping stock solutions of donepezil hydrochloride at concentration of 100 µg/ml for 6 hours. The instrumental responses at six hours were compared with that of fresh samples at zero hour.

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 number of theoretical plates (N) which is used to describe the quality of chromatographic column was determined from the following equation: $N = 16\ (t/W)^2$
Where t = retention time of analyte and W = width of analyte peak

The tailing factor (T) was determined from the following equation: $T = W_{0.05} / 2f$
Where $W_{0.05}$ = Width of peak at 5% height and f = distance from the peak maximum to the leading edge of the peak. The value of not more than 2.0 is acceptable (USP 2003)

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. The peak asymmetry factor was determined by the following equation: As = b /a

Where b is the distance between the peak maximum point and the latest eluting porting of the curve, and a is the distance between the peak maximum point and the earliest eluting portion of the curve (Paul, 2000). Recommended acceptance criteria for asymmetry factor is between 0.9 to 1.1 (Snyder *et al*., 1997).

Capacity factor (k') is an indicator of efficiency of a column to retain sample molecule during an isocratic separation. Literature proposed the acceptable k' value ranges from 2-10 (Snyder *et al.*, 1997). The capacity factor was determined by the following equation:

$$K' = (TR - T_O) / T_O$$

Where TR is the analyte retention time and To is the non-retained substance retention time.

Resolution is calculated by the equation below:

$$Rs = 2 (RTa - RTb) / (Wa + Wb)$$

where RTa and RTb are the retention times of peak a and b and Wa and Wb are the widths at the baseline of the peaks.

Assay of donepezil HCl in ODT formulation.

Orally Disintegrating tablet (ODT) formulations containing donepezil hydrochloride was developed using crospovidone as superdisintegrant and table ttose (agglomerated lactose monohydrate) as filler. Ten ODTs were weighed and crushed using mortar and pestle. The powder was mixed well and a portion of powder weight equivalent to the mean weight of ten ODTs (containing donepezil HCl equivalent to 10 mg) was taken and dissolved in a 500 mL volumetric flask with mobile phase. The solution was subjected to sonication for 30 minutes. 12.5 mL was drawn out and was diluted with mobile phase to 25 mL in a volumetric flask to give a drug concentration of 10 µg/mL. 80 µL sample was injected into the HPLC system.

DISSOLUTION STUDY

The drug dissolution study was carried out in 900 mL of 0.1 M HCl at 37.0 ± 0.5˚C, using USP paddle method at a stirring speed of 50 rpm. At preset time intervals of 1, 3, 5, 10, 20 and 30 min, 3 mL of samples were withdrawn and immediately replaced with an equal volume of fresh dissolution medium. The samples were filtered through 0.45 µm membrane filter and the amount of drug released was determined using HPLC method. Six ODT were analysed for each formulation. Six samples of Aricept® were analysed as reference product.

RESULTS

Mobile phase optimization

The result of mobile phase optimization is presented table 2. Mobile phase with composition of 0.01 M Phosphate buffer : Methanol : Acetonitrile at 50:30:20 v/v, pH 2.7 was found as the most optimum mobile phase to quantify donepezil hydrochloride in drug solution with retention time of 5.4 min.

Table 2: Result of mobile phase optimization

No.	Composition	Result
1	0.01 M Phosphate buffer : Methanol at 70:30, pH 3.0	Separation was very poor
2	0.01 M Phosphate buffer : Methanol at 70:30, pH2.7	Separation slightly improved.
3.	0.01 M Phosphate buffer : Methanol at 50:50, pH 2.7	Separation improved, retention time at 7.0 – 8.0 min
4	0.01 M Phosphate buffer : Methanol : Acetonitrile at 50:40:10, pH 2.7	Good separation, retention time at 6.0 – 7.0 min
5	0.01 M Phosphate buffer : Methanol : Acetonitrile at 50:30:20, pH 2.7	Good separation, retention time at 5.5 – 6.0 min.
6	0.01 M Phosphate buffer : Methanol : Acetonitrile at 50:20:30, pH 2.7	Back tailing, retention time at 5.0 – 5.5 min.

Linearity and reproducibility

The standard calibration curve exhibited an excellent linearity and a good correlation coefficient over the given range of 0.125 – 16 µg/mL of donepezil hydrochloride. The mean linear regression equation from six calibration curves was y = 9965.5 (± 247.35) x + 1699.4 (± 82.68), (x = donepezil concentration, y = average peak height) with a correlation coefficient of 0.9997 (0.00016) as given in fig. 1. The six standard calibration curves were injected over six days to test the reproducibility of the method. The results are presented in table 3.

Table 3: Result of six calibration standard curves N = 6

No.	Slope	Interception	R^2
1	9558.2	1852.2	0.9999
2	9900.1	1730.5	0.9998
3	9927.5	1665.4	0.9997
4	9980.6	1649.1	0.9997
5	10137	1674.4	0.9995
6	10289	1624.9	0.9995
Average	9965.40	1699.42	0.9997
SD	247.35	82.68	0.00016

Precision and accuracy

The results of precision and accuracy are shown in table

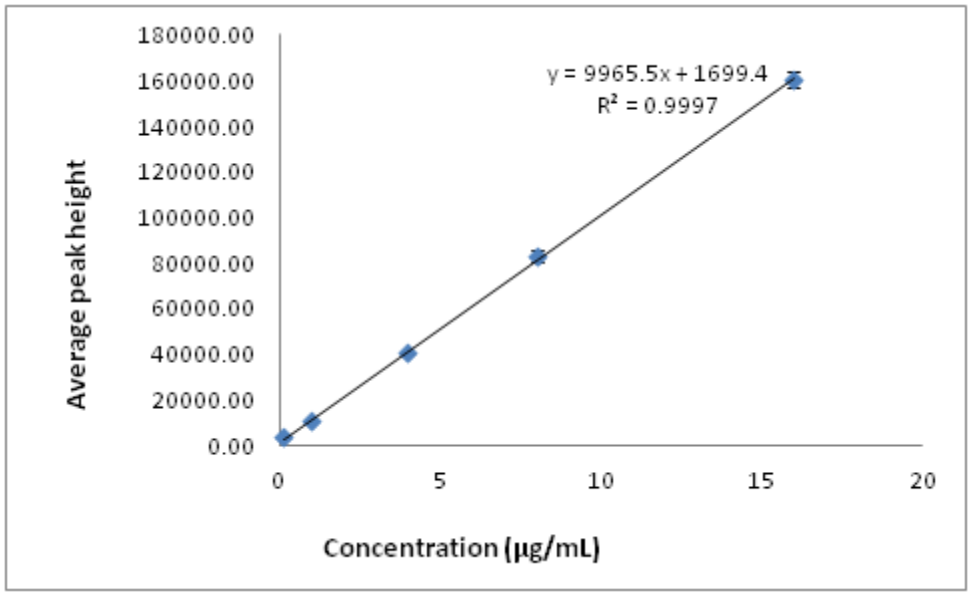

Fig. 1: Calibration standard curve ranged from 0.125-16 ug/mL donepezil hydrochloride in drug solution. The results is expressed as mean ± SD (N=6).

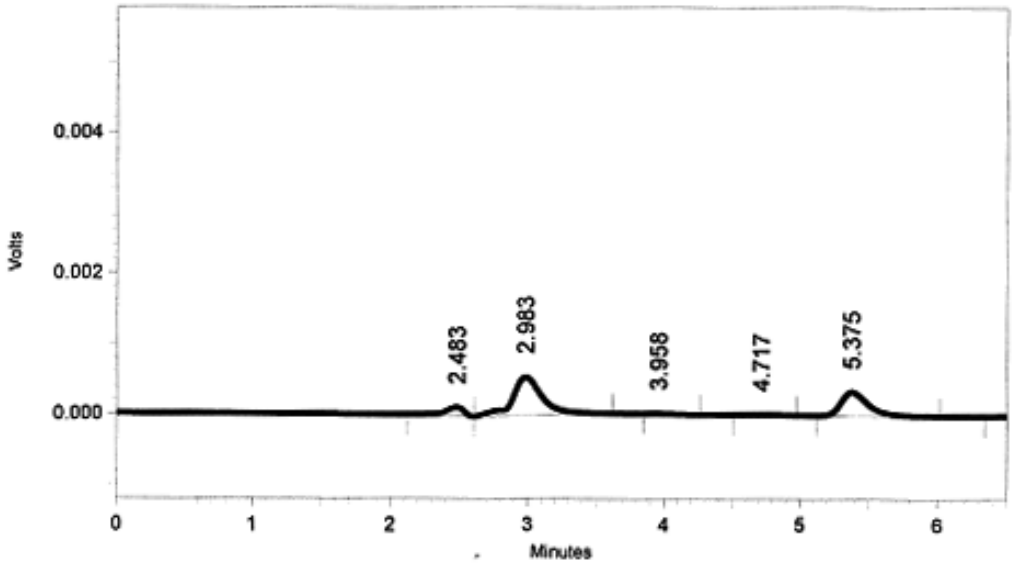

Fig. 2: Chromatogram of donepezil hydrochloride at 0.125 µg/mL (retention time : 5.375)

4. The chromatogram of donepezil hydrochloride 0.125 µg/mL and 14 µg/mL standard solution are presented in fig. 2 and 3. Precision and accuracy were tested at four concentrations namely LOQ, 0.5 µg/mL, 10 µg/mL and 14 µg/mL. Intra-day precision was in the range of 0.24 - 1.83% whereas intra-day accuracy was in the range of -1.83-1.99%. Inter-day precision and accuracy was in the range of 1.41-1.81% and 0.11-1.90% respectively. The results were within the ± 2% range recommended by USP guideline. Hence the method indicates good method precision and accuracy.

Table 4: Result of intra-day and inter-day precision and accuracy (N = 6)

Conc. (µg/mL)	Intraday		Interday	
	Precision	Accuracy	Precision	Accuracy
0.125	1.83	-1.83	1.41	0.54
0.5	1.21	-1.00	1.66	1.90
10	0.24	1.99	1.81	0.34
14	1.03	1.67	1.73	0.11

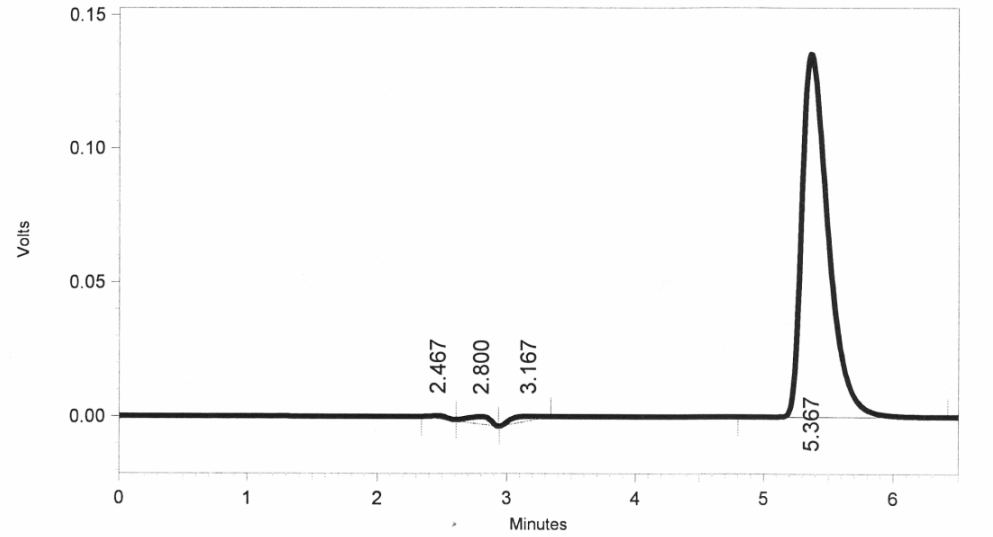

Fig. 3: Chromatogram of donepezil hydrochloride at 14 µg/mL (retention time : 5.367 min).

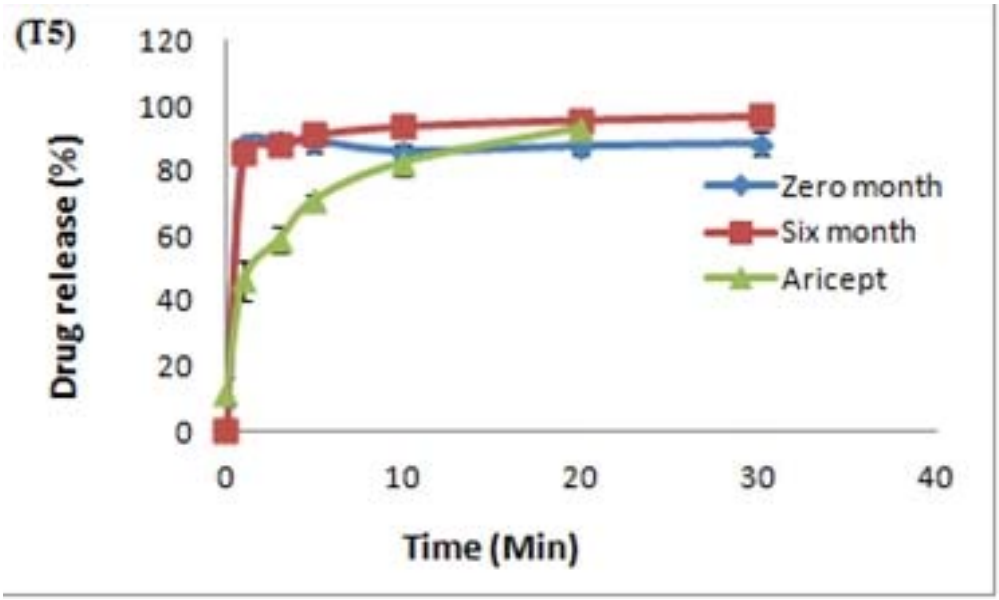

Fig. 4: Dissolution profile of Aricept, formulation T5 at zero month and after six month stability.

Specificity

There was no peak found at the retention time of the analyte in the placebo samples.

LOD and LOQ

The LOD was 0.03 µg/mL. The LOQ was 0.125 µg/mL with inter-day precision and accuracy of 1.41% and 0.54% respectively.

Stock solution stability

The percentage of donepezil hydrochloride remaining after six hours kept under room temperature was 101.06%. The result suggests that the stock solution is stable at least for 6 hours under room temperature.

System suitability

The result of theoretical plates (N), tailing factor (T), peak asymmetry factor (As) and capacity factor (k') of the method at three QC concentrations are shown in table 5. The result was satisfactory. The average theoretical plate is > 2000. The analyte peak had a slight back tailing, but both tailing factor (< 2) and peak asymmetry factor (0.9-1.1) still met the stated requirement in USP 26. The capacity factor falls in the ideal range which is 2-10 (Synder *et al*., 1997).

Table 5: Result of system suitability tested at three different concentrations: 0.5, 10 and 14 µg/mL. The result is expressed as mean ± SD, (N = 6)

Parameter	Donepezil hydrochloride		
	0.5 µg/mL	10 µg/mL	14 µg/mL
Theorectical plates	2292± 77.23	2707± 13.37	2155± 145.9
Tailing factor	0.61± 0.09	0.73± 0.07	0.73± 0.03
Peak Asymmetry factor	1.10± 0.13	1.10± 0.03	1.09± 0.05
Capacity factor	3.30± 0.01	3.31± 0.01	3.33± 0.01
Resolution	2.38± 0.12	2.49± 0.09	2.43± 0.07

Table 6: Result of assay of donepezil hydrochloride in ODT formulations, N =6.

Formu-lation	Condition	% compared to actual	SD	AV
T1	Zero month	94.53	1.93	9.72
	After stability	94.85	1.42	8.27
T2	Zero month	95.70	2.11	8.95
	After stability	93.47	2.15	11.25
T3	Zero month	94.73	1.81	9.24
	After stability	94.38	1.38	8.66
T4	Zero month	95.17	1.47	8.06
	After stability	96.85	1.98	7.51
T5	Zero month	95.60	1.25	7.15
	After stability	97.00	1.02	5.25

DISCUSSION

Application of HPLC method

Assay of Donepezil Hydrochloride in ODT formulation

The validated HPLC method was applied to assay donepezil hydrochloride content in the ODT formulations. table 6 presents the assay content of donepezil hydrochloride in all ODT formulations. The donepezil hydrochloride content in all formulations ranged from 92.41% to 97.00% of the theoretical concentration, with relative standard deviations ranged from 0.86% to 4.17%. The drug content for donepezil hydrochloride tablet should be within the range of 90% to 110% (USP, 2010). Hence all the formulations passed the stated requirement. Noted that the drug content in the ODT formulations after stability study still fell within the ranged recommended by USP31. Donepezil hydrochloride was stable in the ODT formulations without significant degradation. Moreover, the acceptance value (AV) was found to be in the range of 5.25 to 13.38%, which was also within the 15% limit of the uniformity of dosage units for Japanese Pharmacopoeia 15 (2006).

DISSOLUTION STUDY

The HPLC method was also used to quantify the drug release percentage during the dissolution study. For Formulation T5 80% of the drug was released in 1 minute. Formulation T4 released 80% of the drug within 3 minutes while T3 in 5 minutes, whereas formulation T1 and T2 achieved 80% of the drug concentration within 10 minutes. The original Aricept tablet released 80% of the drug in 20 minutes. The ODT formulations were found more superior in drug release profile than the original Aricept tablet. The dissolution profile of formulation T5 and Aricept is presented in fig 4. However, it was noted that there was a significant delayed in drug released for all formulations beside formulation T5 after six month stability study. Formulation T4 released 80% of the drug content in 5 minutes whereas formulation T3 and T2 released 80% of drug content within 10 minutes. Formulations T1 showed most significant delayed, which was 20 minutes.

CONCLUSION

It can be concluded that the newly developed HPLC method is accurate, precise, sensitive and reproducible for the assay of donepezil HCl in pharmaceutical dosage form. The method was validated by system suitability parameter and linear within the range of 0.125-16 µg/mL.

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