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ORIGINAL ARTICLE

Establishment of inherent stability on piracetam by UPLC/HPLC and development of a validated stability-indicating method

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Abstract A novel comparative force degradation UPLC assay method was developed and validated for Piracetam and its degradation products. Piracetam was subjected to acid (5 M HCl), neutral (water) and alkaline (0.5 M NaOH) hydrolytic conditions at 80 °C, as well as to oxidative decomposition (H_2O_2) at room temperature. Photolytic studies were carried out by exposing this drug into sunlight (60,000–70,000 lux) for 2 d. Additionally, the solid drug was subjected to 50 °C for 60 days in a hot air oven for thermal degradation. The UPLC chromatographic separation was performed on Acquity UPLC BEH C18 column (1.7 μm, 2.1 mm × 150 mm) using isocratic mode (ACN:water, 25:75 v/v) at a flow rate of 0.15 mL min^{-1} and HPLC chromatographic separation was achieved on phenomenex C18 using isocratic mode (ACN:10 mM ammonium acetate, pH 5.0, 20:80 v/v) at a flow rate of 0.9 mL/min. Piracetam was found to degrade only in the base and shows stable behavior under all stress conditions. The UPLC and HPLC linearity of the proposed method was investigated in the range of 10–50 μg mL^{-1}. The r^2 value of UPLC and HPLC was found to be 0.999 and 0.999, respectively. Method detection limit (MDL) and Method quantification limit (MQL) were found to be 0.180 μg $mL^{-1}$and 1.10 μg mL^{-1} for UPLC and 0.500 μg $mL^{-1}$and 1.700 μg mL^{-1} for HPLC respectively. The %RSD values for intra-day and inter-day precision were < 1.2%, confirming that the method was sufficiently precise. The validation studies were carried out fulfilling ICH requirements. The developed method was simple, fast, accurate and precise and hence could be applied for routine quality control analysis of Piracetam in solid dosage forms.

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1. Introduction

The International Conference on Harmonization (ICH) for drug stability test guideline Q1A (R2) requires that the analysis of stability samples should be done through the use of validated stability-indicating analytical methods. It also recommends carrying out of stress testing on this drug substance to establish its

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inherent stability characteristics and to support the suitability of the proposed analytical procedure (ICH, 2003; Junwal et al., 2012; Dong, 2006; http://pubs.acs.org/subscribe/journals/tcaw/10/i09/html/09dong.html; Singh et al., 2000; Sahu et al., 2010, 2011). With the objective of reducing analysis time and maintaining constant efficiency, there has been a substantial focus on high-speed chromatographic separations. Recently, a commercially available innovative ultra performance liquid chromatography (UPLC) has proven to be one of the most promising developments in the area of fast chromatographic separations. The comparative reverse phase chromatographic stability indicating assay method was developed using UPLC and HPLC for Piracetam bulk drug. Piracetam is a nootropic drug. It shares the same 2-oxo-pyrrolidone base structure with 2-oxo-pyrrolidine carboxylic acid (pyroglutamate). Piracetam is a cyclic derivative of GABA. It is one of the groups of racetams. Piracetam is prescribed by doctors for some conditions, mainly myoclonus, but is used off-label for a much wider range of applications like aging, alcoholism, alzheimer's and senile dementia, depression and anxiety, schizophrenia, closed craniocerebral trauma, preventive for breath-holding spells etc. The chemical name of piracetam is 2-oxo-1-pyrrolidineacetamide (Fig. 1) (http://en.wikipedia.org/wiki/Piracetam; The Merck Index, 2001).

According to the chromatographic performances afforded by small particles, the latter can be used for two main objectives. First, small particles can be used to perform fast and ultrafast analyses since a good efficiency can be maintained with short columns and at high flow rates. Second, a high resolution can be generated with longer columns, close to the optimal flow-rate. UPLC has been evaluated in terms of practical gains in speed and efficiency that can be achieved compared with current HPLC systems. Acquity bridge ethylene hybrid (BEH) 1.7-μm columns are characterized by high optimum velocities (~0.37 cm s^{-1}) and low minimum plate heights (~4.4 μm) for well-retained compounds (k = 3.6). A slightly higher-than-expected C-term measured from the experimental Knox plots is ascribed to a residual temperature effect under non-ideal adiabatic conditions, lower packing efficiency, and extra-column band broadening. The combination of high optimum flow rates and shorter column lengths enables gains in speed of factors of approximately 4.3 and 3.5 in comparison with 5-μm and 3.5-μm particles, respectively, without sacrificing efficiency. From the various kinetic plots constructed according to the method of poppe are evident that the use of

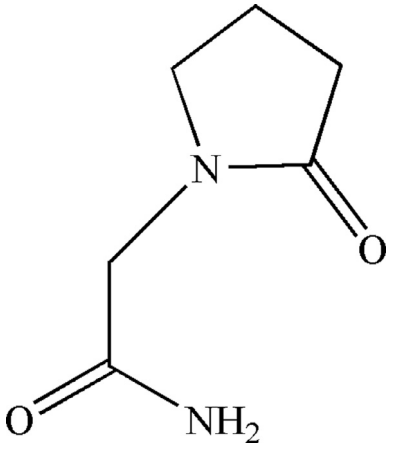

Figure 1 Structure of Piracetam.

UPLC instrumentation and columns has advantages in terms of speed for analyses requiring up to ~80,000 theoretical plates. UPLC seems to be the possibility to extend and elaborate the utility of chromatography. In conclusion, the main benefits of this relatively new approach of chromatography are: increased sensitivity, rapid analysis through the use of a novel separation material of a very fine particle size, decreased operation cost, less solvent consumption and increased sample throughput. (Nguyen et al., 2006; Mazzeo et al., 2005; De Villiers et al., 2006; Wren and Tchelitcheff, 2006).

In the literature, a few methods have been reported for the estimation of Piracetam by spectrophotometric methods (Bhowmick et al., 2010), HPLC (El-Saharty, 2008; Augustin and Imre, 2007), LC-MS/MS (http://pubs.rsc.org; Xianqin et al., 2010). The major objective of the present work is to develop stress degradation studies of Piracetam under different ICH recommended stress conditions, and to establish a validated stability-indicating UPLC/HPLC method for reducing analysis time and solvents. So far to our knowledge there was no method that has yet been reported for comparative UPLC/HPLC method on the development of stability-indicating assay method for this drug.

2. Experimental

2.1. Chemicals

Piracetam was obtained as a gratis sample from jubilant organosys (Noida, India). Analytical reagent (AR) Sodium hydroxide and hydrogen peroxide were purchased from S.D. Fine-chem. Hydrochloric acid and acetonitrile (99.8%) was from Merck India (Mumbai). All other chemicals were of analytical grade.

2.2. Instrumentation

2.2.1. Ultra performance liquid chromatography (UPLC)

UPLC was performed using a Waters Acquity system equipped with a binary solvent delivery pump, an auto sampler and a PDA detector. The chromatographic separation was performed using a Waters Acquity BEH 150 × 2.1 mm, 1.7 μm, C18 column. The mobile phase containing a mixture of acetonitrile (ACN) and water in the ratio of 25:75 (v/v) at a flow rate of 0.15 mL min^{-1} was used. The detection was obtained at a wavelength of 210 nm. The injection volume was 2 μL; mobile phase was used as a diluent while the column was maintained at 30 °C. Forced degradation studies were carried out with a photo diode array detector.

2.2.2. High performance liquid chromatography (HPLC)

The HPLC system used for chromatographic development was shimadzu, separation module with a PDA detector. HPLC system (Shimadzu, Japan) consisted of a LC-10AT VP pump, a SPD-10AVP, PDA detector, a phenomenex C18 (250 mm × 4.6 mm, 5 μm) column, a Phenomenex, HPLC guard cartridge system and a Class LC10/M10A software. Mobile phase consisting of acetonitrile and buffer (10 mM ammonium acetate, pH 5.0) in the ratio of 20:80 (v/v) in an isocratic mode with the flow rate of 0.9 mL min^{-1} was employed at ambient temperature. The injection volume was 20 μL while the detector was set at 210 nm.

2.2.3. Others

pH of the mobile phase was checked on a microprocessor water proof pH tester (pH tester 20, Eutech Instruments, Oakton, USA). The overall illumination at the point of placement of samples was 6000 lux, which was tested using a calibrated lux meter (Lutron LX-102 digital light meter, Marcucci S.P.A, vignate, Milan). Thermal stability study was performed in a hot air oven (Oven universal with thermotech thermostat TIC-4000N, S.M. Industries, New Delhi, India).

2.3. Degradation studies

Stress studies were performed under conditions of dry heat (thermal studies), hydrolysis (acidic, alkaline and neutral), oxidation, and photolysis, as mentioned in ICH Q1A (R2) (1–4). The approach suggested by Singh and Bakshi was adopted for these studies. A minimum of four samples were generated for every stress condition, viz., blank solution stored under normal conditions, the blank subjected to stress in the same manner as this drug (Piracetam), a zero time sample containing this drug (which was stored under normal conditions), and this drug solution subjected to stress treatment. Hydrolytic decomposition of Piracetam was conducted at 80 °C in 5 M HCl, water, and 0.5 M NaOH at a drug concentration of 2 mg mL^{-1} until sufficient degradation (~20% of the initial amount) of this drug was achieved. For oxidative stress studies, Piracetam was dissolved at a concentration of 3 mg mL^{-1} in 30% H_2O_2 and kept for two days at room temperature. Photolytic studies of the dry drug and this drug in solution in acetonitrile at a concentration of 2 mg mL^{-1} were performed by exposure to sunlight during the daytime (60,000–70,000 lux) for 2 d.

2.4. Chromatography and development of a stability-indicating method

UPLC was performed with a binary solvent delivery pump, an auto sampler and a PDA detector of Acquity UPLC system manufactured by Waters Corporation; Milford, Massachusetts, USA; data were acquired and processed using Empower software. An initial literature search revealed that some reported HPLC methods for Piracetam were developed on either C8 or C18 columns, using different temperature conditions. Peak shapes were not persuasive, and there was substantial tailing. So, attempts were made to develop a simple method on an advanced BEH C18 column, with possible lowering of retention time at 30 °C column temperature. Separations were achieved using isocratic elution. The mobile phase was filtered through 0.22 μm PTFE membranes and had degassed. The injection volume was 2 μL and the mobile phase flow rate was kept constant at 0.15 mL min^{-1}. The detection wavelength was 210 nm; PDA analysis was conducted to study the behavior at other wavelengths. First, UPLC studies were performed on all reaction solutions individually and then on a mixture of degraded drug solutions. Different conditions, for example pH, mobile phase composition, and column temperature were varied to obtain a reasonable separation between this drug and the degradation products. Methanol was avoided during the study because of its significant absorption at the detection wavelength of this drug between 210 and 215 nm. Acquity UPLC BEH C18 column (1.7 μm, 2.1 mm × 150 mm) stainless steel analytical column was used as stationary phase. In order to determine the method is stability indicating, forced degradation studies were conducted on Piracetam powder. The analysis was carried out by UPLC with a PDA detector at a wavelength of 210. Two microliters of each of forced degradation samples was injected at regular intervals.

HPLC chromatographic analysis was performed at ambient temperature on a Phenomenex (C-18) analytical column with a mobile phase composed of buffer (10 mM Amm. Acetate buffer pH 5.0): acetonitrile (80:20, v/v) and was isocratically eluted at a flow rate of 0.9 mL min^{-1}. A small sample volume of 20 μL was used for each sample run, being injected into the HPLC system. The chromatogram was monitored with UV detection at a wavelength of 210 nm.

2.5. Validation of the method

The method was validated in accordance with ICH guidelines Q2 (R1). To establish linearity and range, a stock solution containing 1 mg mL^{-1} drug in acetonitrile was diluted to yield solutions in the concentration range of 10–50 μg mL^{-1}. The solutions were injected in triplicate using water–acetonitrile as mobile phase and keeping the injection volume constant (2 μL). To assess precision, six injections of five different concentrations (10, 20, 30, 40, and 50 μg mL^{-1}) were made on the same day and intraday precision was determined as relative standard deviation. These studies were also repeated on different days to determine interday precision. Accuracy was evaluated by fortifying a mixture of decomposed reaction solutions with five known concentrations of this drug and recovery of the added drug was evaluated. The specificity of the method for this drug was established by a study of the resolution factor of this drug peak from the nearest other peak. Overall selectivity was established by the determination of purity for each degradation product peak by the use of PDA detector. Robustness was assessed by changing the composition of mobile phase. Method detection limit (MDL) and method quantification limit (MQL) were determined experimentally, by an analysis of samples spiked with decreasing concentrations of the analytes. MDL was defined as the smallest amount of an analyte that can be reliably detected or differentiated from the background for a particular matrix (by a specific method). MQL was calculated as the smallest amount of an analyte that can be reliably quantified with a certain degree of reliability within a particular matrix (by a specific method). In chromatography methods, the limits are often set based on the ratio between the analyte signal and the baseline noise (for example, MDL = Height/Noise ratio of 3, MQL = Height/Noise ratio of 10).

3. Results and discussion

3.1. UPLC and HPLC studies on the stressed solutions

The forced-degradation study shows that Piracetam degraded under alkali stress condition. The specificity and selectivity of the method with the samples under these stresses were demonstrated through the evaluation of R_T, RR_T, resolution, and purity data for all peaks in the chromatograms (http://www.chem.agilent.com/Library/posters/Public/ASMS_2011_MP_181.pdf; Singh and Bakshi, 2002; Singh et al., 2006). Piracetam did not degrade under acid, oxidative, thermal & photolytic stress conditions. In a mixture of solution, only

Table 1 Retention times and relative retention times of various peaks.

Peaks	UPLC		HPLC	
	Retention time (R_T)	Relative retention time (RR_T)	Retention time (R_T)	Relative retention time (RR_T)
Deg01	2.05	0.576	2.75	0.370
Piracetam	3.56	1.000	7.44	1.000

R_T, retention time (min); RR_T, relative retention time.

one degradation product was formed. The retention times (R_T) and relative retention times (RR_T) of this drug and the degradation products are given in Table 1 for UPLC and HPLC. This drug and degradation products carry the notations Deg01 and Piracetam in accordance with the sequence in which the peaks appeared from left to right on UPLC and HPLC chromatogram (mixture of stressed sample) (Fig. 2). Ultra-performance liquid chromatography (UPLC) is a new category of separation technique based upon well-established principles of liquid chromatography, which utilizes sub-1.7 μ particles for a stationary phase. These particles operate at elevated mobile phase linear velocities to affect a dramatic increase in resolution, sensitivity and speed of analysis.

3.2. Development and optimization of the method

3.2.1. UPLC

The best separation was achieved on the same column at 30 °C using the mobile phase acetonitrile: water (70:30) in an isocratic mode. The flow rate was kept at 0.15 mL min^{-1} at a constant volume of 2 μL, and the detection wavelength was 210 nm. UPLC studies on Piracetam under different stress conditions suggested the following degradation behaviors:

Piracetam gradually degraded with time on heating at 80 °C in 0.5 M NaOH after 10 h. The drug showed ~25% degradation in 0.5 M NaOH at 80 °C. A new peak emerged at Rt 2.05 min along with the drug peak at Rt 3.56 (RR_T 1.00). In a mixture of the stressed samples (Fig. 2(a)), the degradation product at RR_T 0.576 (with the normal drug peak at RR_T 1.0) pertained to Deg01. The drug was stable to acidic stress, and it was observed that the drug was not degraded by heating at 80 °C in 5 M HCl for 24 h. the drug shows stable behavior at neutral hydrolysis on heating with water at 80 °C for two days. No degradation was observed on exposure of the drug to 30% H_2O_2 for five days, showing that it was stable against oxidative stress. The drug was also stable during exposure to direct sunlight (~60,000–70,000 lux) for two days. Finally, there was no significant degradation of solid piracetam on

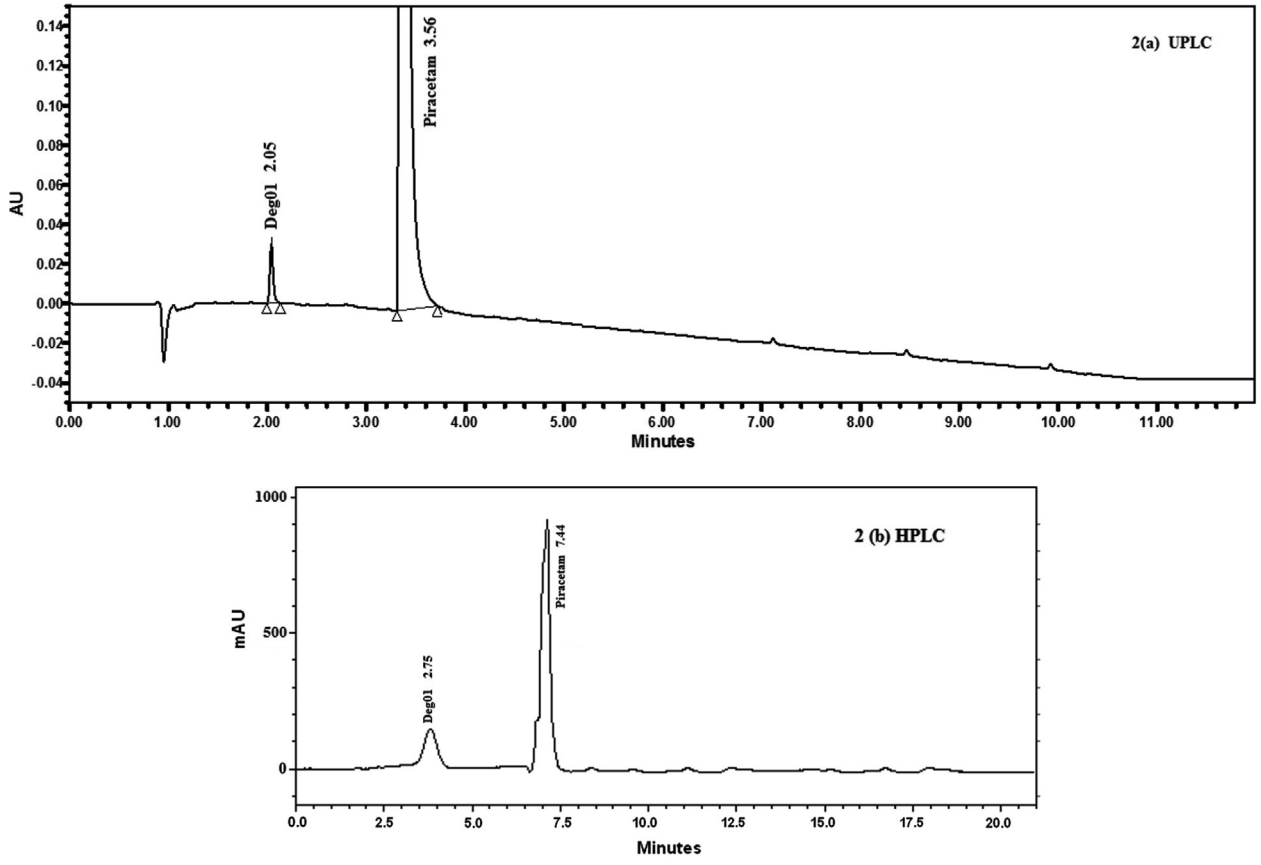

Figure 2 Chromatogram showing separation of Piracetam and its degradation products in a mixture of stressed samples by (a) UPLC and (b) HPLC.

Table 2 Data for Piracetam from linearity studies ($n = 6$).

UPLC				HPLC			
Concentration (μg mL^{-1})	AUC	±SD	RSD (%)	Concentration (μg mL^{-1})	AUC	±SD	RSD (%)
10	53163.00	50.402	0.095	10	1875032.7	17315.0	0.923
20	107907.00	80.829	0.075	20	3570996.0	11523.4	0.323
30	153736.64	625.167	0.407	30	5458691.7	5756.8	0.105
40	208638.00	555.698	0.266	40	7159961.3	34627.7	0.484
50	259296.99	601.775	0.232	50	9059744.7	34816.7	0.384

SD, standard deviation; RSD, relative standard deviation; AUC, area under curve.

Table 3 Data of intra-day and inter-day precision studies ($n = 4$).

Actual concentration (μg mL^{-1})	Intra-day			Inter-day		
	Measured concentration	±SD	RSD (%)	Measured concentration	±SD	RSD (%)
UPLC						
10	9.958	0.030	0.304	9.9627	0.0866	0.8691
20	20.173	0.084	0.417	20.1067	0.1557	0.7742
30	30.059	0.210	0.700	29.7853	0.1622	0.5445
40	40.246	0.467	1.160	39.9067	0.2068	0.5183
50	50.044	0.314	0.628	49.8867	0.1881	0.3771
HPLC						
10	9.934	0.051	0.514	9.9670	0.0260	0.2609
20	20.123	0.150	0.746	20.1995	0.0705	0.3490
30	30.022	0.187	0.622	30.0030	0.1870	0.6233
40	40.129	0.407	1.015	40.1035	0.3965	0.9887
50	49.977	0.257	0.514	49.9455	0.2645	0.5296

SD, standard deviation; RSD, relative standard deviation.

exposure to dry heat at 50 °C for two months, indicating that the drug was stable to thermal stress.

3.2.2. HPLC

As UPLC, the same degradation product appeared in HPLC as shown in Fig. 2(b). In alkali, the drug was found to decompose gradually, similar to the behavior observed in UPLC. The drug was degraded (~25% degradation) in 0.5 M NaOH at 80 °C at 10 h, and one peak was generated, at Rt 2.75. In a mixture of stressed samples the degradation product appeared at RR_T 0.576, the retention time of Deg01. On heating at 80 °C in 5 M HCl for 24 h, the height of the drug peak was not decreased, which shows that the drug was stable in acidic stress conditions. Similarly, the drug shows stable behavior against the oxidative, thermal and photolytic stress conditions.

3.3. Validation of developed stability-indicating method

The response for this drug was strictly linear in the concentration range between 10 and 50 μg mL^{-1}. The linearity study data are given in Table 2.

Regression equation

$$Y = 5129X + 2648 \quad (\text{UPLC } r^2 = 0.999, \text{intercept} = 2648)$$

$$Y = 17958X + 37368 \quad (\text{HPLC } r^2 = 0.999, \text{intercept} = 37368)$$

$$Y = \text{AUC}, \quad X = \text{conc. in } \mu\text{g mL}^{-1}$$

The data obtained from precision experiments are given in Table 3 for intra and inter day precision studies. The %RSD. values for intraday precision study and for interday study were < 1.0% confirming that the method was sufficiently precise. Excellent recoveries were made at each added concentration shown in Table 4. Fig. 2(a) and (b) shows that the method was sufficiently specific to this drug. The USP resolution factor for this drug peak was > 2 from the nearest resolving peak. The method was found to be robust by varying the composition of mobile phase as shown in Table 5. Good separations were always achieved, indicating that the method remained selective for all components under the tested conditions as shown in Fig. 2(a) and (b). The influence of retention time for different degradation products (UPLC/HPLC) has been depicted in Fig. 2.

The MDL and MQL were found to be 0.180 μg mL^{-1}and 1.10 μg mL^{-1} for UPLC and 0.500 μg mL^{-1} and 1.700 μg mL^{-1}respectively for HPLC.

3.4. Comparative study on chromatographic performance

A comparative data on the chromatographic performance of HPLC (isocratic) and UPLC (isocratic) have been obtained by injecting a mixture of stressed solution of Piracetam (30 μg mL^{-1}). It is observed that the elution time of pure Piracetam and degradation products in UPLC was reduced

Table 4 Recovery data for Piracetam spiked into a mixture of stressed samples ($n = 4$).

Concentration (μg mL^{-1})	Calculated spiked concentration	± S.D.	RSD. (%)	Recovery (%)
UPLC				
10	9.889	0.055	0.557	98.893
20	20.527	0.457	2.226	102.635
30	29.792	0.101	0.340	99.307
40	39.740	0.556	1.399	99.351
50	49.714	0.619	1.244	99.428
HPLC				
10	9.978	0.061	0.616	99.777
20	20.130	0.125	0.620	100.650
30	29.942	0.132	0.442	99.807
40	39.976	0.233	0.583	99.939
50	49.934	0.219	0.439	99.867

SD, standard deviation; RSD, relative standard deviation.

Table 5 Data for robustness.

Concentration (μg mL^{-1})	Acetonitrile: H_2O (22:78 v/v)			Acetonitrile: H_2O (27:73 v/v)		
	Measured concentration	± SD	RSD (%)	Measured concentration	± SD	RSD (%)
UPLC (n = 3)						
10	9.944	0.047	0.469	9.948	0.042	0.422
20	20.173	0.084	0.416	20.123	0.150	0.746
30	30.055	0.207	0.690	30.025	0.191	0.636
40	40.252	0.472	1.173	40.122	0.398	0.992
50	50.010	0.293	0.585	50.010	0.287	0.575
HPLC(n = 3)						
	Acetonitrile: 10 mM Amm. acetate (**17:83 v/v**)			Acetonitrile: 10 mM Amm. acetate (**22:78 v/v**)		
10	9.938	0.048	0.482	9.941	0.045	0.456
20	20.090	0.159	0.790	20.163	0.122	0.606
30	29.989	0.150	0.499	30.022	0.184	0.613
40	39.989	0.246	0.615	40.122	0.427	1.064
50	49.964	0.247	0.495	49.964	0.247	0.495

UPLC, ultra performace liquid chromatography; HPLC, high performance liquid chromatography; *n*, number of replicates.

by 6-fold compared to that of isocratic mode HPLC. The resolution and theoretical plates obtained for Piracetam and degradation products in UPLC showed comparatively a better separation efficiency than HPLC.

4. Conclusions

The validated stability-indicating method was established for the analysis of Piracetam in the presence of its degradation products as per ICH recommendations. In the present work, UPLC and HPLC methods were used to assess degradation products' peaks during a stress testing analysis of Piracetam drug substance. The newly developed UPLC method for the separation of different degradation products along with the pure drug of Piracetam was found to be capable of giving faster retention times while still maintaining satisfactory resolution than that achieved with conventional HPLC. The drug is only degrading in alkaline conditions confirmed by the emerging of single degradation product in a mixture of stress samples. The specificity and selectivity of the method with the samples under these stresses were demonstrated through the evaluation of R_T, RR_T, resolution, and purity data for all peaks in the chromatograms using PDA detector. Piracetam did not degrade on acidic, neutral, thermal & photolytic stresses. The method was fully validated showing satisfactory data for all the parameters tested. This method exhibited an excellent performance in terms of sensitivity and speed. The method proved to be simple, accurate, precise, specific and selective. It is hoped that the developed method is stability indicating which can be used for the impurity testing and assay determination in the routine analysis of production samples as well as to analyze stability samples.

Conflict of interest

The authors declare that they have no conflict of interest.

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