

Simultaneous Determination of Piracetam and its Four Impurities by RP-HPLC with UV Detection

M. Saeed Arayne^{1,*}, Najma Sultana², Farhan Ahmed Siddiqui¹, Agha Zeeshan Mirza¹, Faiza Qureshi¹, and M. Hashim Zuberi¹

¹Department of Chemistry, University of Karachi, Karachi-75270, Pakistan and ²Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Karachi, Karachi

Abstract

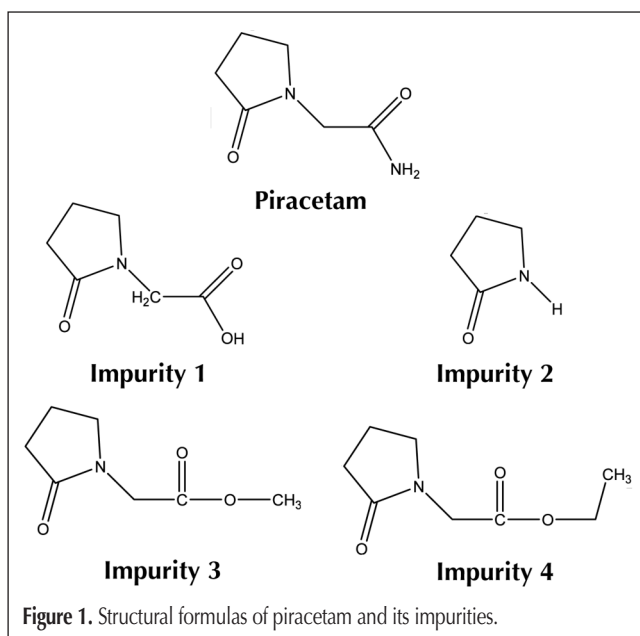
A simple and rapid high-performance liquid chromatographic method for the separation and determination of piracetam and its four impurities, 2-oxopyrrolidin-1-yl)acetic acid, pyrrolidin-2-one, methyl (2-oxopyrrolidin-1-yl)acetate, and ethyl (2-oxopyrrolidin-1-yl)acetate, was developed. The separation was achieved on a reversed-phase C₁₈ Nucleosil column (25 cm × 0.46 cm, 10 μm). The mobile phase is composed of an aqueous solution containing 0.2 g/L of triethyl amine–acetonitrile (85:15, v/v). The pH of the mobile phase was adjusted to 6.5 with phosphoric acid at a flow rate of 1 mL/min at ambient temperature and UV detection at 205 nm. The developed method was found to give good separation between the pure drug and its four related substance. The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 50–10,000 ng/mL, 25–10,000 ng/mL, 45–10,000 ng/mL, 34–10,000 ng/mL, and 55–10,000 ng/mL, respectively, with $r^2 = 0.9999$. The method was validated for precision, accuracy, ruggedness, and recovery. The minimum quantifiable amounts were found to be 50 ng/mL of piracetam, 25 ng/mL of 2-oxopyrrolidin-1-yl)acetic acid, 45 ng/mL of pyrrolidin-2-one, 34 ng/mL of methyl (2-oxopyrrolidin-1-yl)acetate, and 55 ng/mL of ethyl (2-oxopyrrolidin-1-yl)acetate. Statistical analysis proves that the method is reproducible and selective for the estimation of piracetam as well as its related substance. As the method could effectively separate the drug from the related substances, it can be employed as a stability-indicating one. The proposed method shows high efficiency, allowing the separation of the main component piracetam from other impurities.

Introduction

Piracetam (2-oxo-pyrrolidin-1-yl)-acetamide is a nootropic psychopharmacological agent having a variety of physiological effects that may result from the restoration of cell membrane fluidity. It is used for the treatment of patients suffering from pathological, neurosensitive, and cognitive deficits, brain-organic psychosyndromes (e.g., primary degenerative dementia), vertigo, and myoclonus of cortical origin (1,2). Piracetam is absorbed almost completely and quickly, and the highest level in

plasma is reached within 30–90 min after oral administration. Until yet, no metabolite of piracetam has been discovered. After 30 h, piracetam gets eliminated in urine, and the tiny proportion of the dosage excreted with urine is independent of the dose administered.

Piracetam is a white powder and is freely soluble in water, soluble in alcohol, and slightly soluble in methylene chloride (3). It possesses no fluorescent properties and has low absorption in UV radiation with a maximum peak of absorption at 197 nm. The chemical structure of the molecule presented in Figure 1 can be predicted as a flexible molecule due to a variety of hydrogen bonds giving conformational polymorphs (4). In order to characterize piracetam, the purity of the active substance can be checked by several techniques (5,6). The determination of piracetam by fourier transform infrared spectroscopy (FTIR) (6) doesn't merit much application as it could not determine all four known impurities but only one (pyrrolidine acetic acid) for synthesis control only. The other method that employs thin-layer chromatography (TLC) (5) does separate piracetam and its four impurities in 45 min but could not be used for the quantitative determination in pharmaceutical formulations. Many high-performance liquid chromatography (HPLC) separation methods



*Author to whom correspondence should be addressed: e-mail msarayne@gmail.com.

were developed for quantification of piracetam from medicine mixtures (7–15). Capillary electrophoretic methods are also reported, which offer very similar results (migration times and limits of quantification) to that of HPLC ones (retention times and lower limits of quantification) (16,17). HPLC methods were developed for determination of piracetam in plasma using different parameters and liquid chromatography devices. Starting with a simple determination of piracetam, after protein precipitation, a quantification limit of 1 µg/mL was obtained by using a mobile phase of perchloric acid in a short SpheriSorb S5 amino column and detection at 200 nm (18). Other methods (8–15) applied extraction (liquid–liquid and solid-phase extraction) using isocratic elution, similar UV detection but having different compositions of mobile phases and flow rates. The quantification limits were within 3–4 µg/mL yet with different retention times differed significantly from 3.3 (14) to 6 min (12).

The objective of this research was to develop and validate a simple, reliable, rapid, economical, and sensitive method for quantitative determination of piracetam and its related substances, degraded products, and impurities in pharmaceutical formulation and in raw form. The method can be used for quantification of piracetam with its impurities in pharmaceutical formulation and to validate the procedures in order to demonstrate its good analytical and statistical parameters with respect to the pharmacokinetic and bioequivalence studies regulations (19–21).

The major advantage of the proposed methods is that four impurities and piracetam can be determined on a single chromatographic system without changing the chromatographic system, which would be suitable for routine use in quality control with regards to the wide range of concentration in plasma samples and large number of samples needed to be analyzed in a short time.

Table I. Linearity, Accuracy, and Precision of Piracetam and its Impurities By the Proposed Method

Drug	Added conc. (ng/mL)	Found conc. (ng/mL)	% Recovery (mean)	% RSD*	
				intra-day	inter-day
Piracetam	50	48.46	96.92	2.687	3.12
	100	98.16	98.16	0.775	1.29
	250	243.19	97.28	0.854	1.05
	500	489.44	97.89	3.617	2.55
	1000	991.13	99.11	1.075	2.17
	5000	5000.00	100.00	2.771	1.95
	10000	9990.03	99.90	2.434	3.46
Impurity 1	25	24.32	97.26	2.669	3.14
	50	49.22	98.43	0.770	2.10
	100	99.55	99.55	1.498	2.60
	500	493.23	98.65	0.936	1.70
	1000	996.73	99.67	1.943	1.50
	5000	5000.00	100.00	1.855	0.94
	10000	10153.82	101.54	2.095	2.50
Impurity 2	45	44.46	98.81	3.005	2.10
	90	89.05	98.94	1.266	0.88
	250	249.42	99.77	2.157	3.10
	500	496.78	99.36	2.251	2.30
	1000	1003.11	100.31	1.272	1.40
	5000	5000.00	100.00	2.414	2.20
	10000	10103.38	101.03	1.356	2.60
Impurity 3	34	32.97	96.96	1.632	2.20
	80	78.29	97.86	2.349	2.90
	250	248.68	99.47	0.856	1.50
	500	492.83	98.57	1.478	2.10
	1000	993.58	99.36	1.276	2.18
	5000	5000.00	100.00	1.034	1.24
	10000	10009.24	100.09	0.949	1.62
Impurity 4	55	53.20	96.73	2.841	2.05
	100	97.22	97.22	2.332	3.14
	250	250.71	100.28	0.864	1.58
	500	498.88	99.78	1.117	1.25
	1000	995.04	99.50	1.411	0.94
	5000	5000.00	100.00	1.096	2.28
	10000	10046.00	100.46	0.799	1.66

* RSD = Relative standard deviation, where $n = 5$ for both intra- and inter-day.

Experimental

Materials and reagents

All reagents were of analytical reagent-grade. Potassium dihydrogen orthophosphate, phosphoric acid triethyl amine, sodium sulfate, sodium octane sulfonate, and HPLC-grade acetonitrile were purchased from Merck (Darmstadt, Germany). Distilled and deionized water was used throughout the study. Piracetam and impurities were obtained from UCB (Brussels, Belgium). Nootropil 800 mg tablets were purchased from a local market.

Instrumentation

The development and validation of the assay was performed on an HPLC system consisting of an LC-10 AT VP Shimadzu (Kyoto, Japan) pump and SPD-10AV VP Shimadzu UV visible detector. A Nucleosil C₁₈ column (25 cm × 0.46 cm, 10 µm) was used for separation. The chromatographic and integrated data were recorded using a CBM-102 communication Bus Module Shimadzu on a P-IV PC loaded with Class-GC software.

Chromatography

The mobile phase consisted of an aqueous solution containing acetonitrile–0.2 g/L solution of triethyl amine (15:85) with pH 6.5 adjusted with phosphoric acid. The flow rate was 1 mL/min. The detections were made at 205 nm.

Standard solutions preparation

Accurately weighed 10 mg of piracetam and its impurities were individually transferred to 100-mL volumetric flasks separately, and appropriate dilution was made with the mobile phase. The resulting solutions were sonicated for 25 min and filtered through membrane filter. Final concentrations were 100.0 µg/mL. Aliquots of each solution were accordingly diluted with same solvent in order to obtain solutions with final concentrations of all drugs. All solutions were prepared fresh each day.

Calibration curves

Seven different concentration levels (50, 100, 250, 500, 1000,

5000, and 10,000 ng/mL) for piracetam; (25, 50, 100, 500, 1000, 5000, and 10,000 ng/mL) for 2-oxopyrrolidin-1-yl)acetic acid (impurity 1); (45, 90, 250, 500, 1000, 5000, and 10,000 ng/mL) for pyrrolidin-2-one (impurity 2); (34, 80, 250, 500, 1000, 5000, and 10,000 ng/mL) for methyl (2-oxopyrrolidin-1-yl)acetate (impurity 3); and (55, 100, 250, 500, 1000, 5000, and 10,000 ng/mL) for ethyl (2-oxopyrrolidin-1-yl)acetate (impurity 4) were obtained for each standard solution. Each solution was injected in the chromatographic system ($n = 5$), and mean values of peak areas were plotted against concentrations. The curves were adjusted by linear regression with the least mean squares method (22,23).

Method validation

The method was validated by using the ICH guideline (24). The selectivity, limits of detection and quantification, linearity, precision, and accuracy were determined. Determination was carried out using tablet formulation. The reversed-phase (RP)-HPLC method that was presented has been proved successfully in separation and simultaneous determination of all five compounds. The developed method was validated for linearity, accuracy, precision, recovery, limit of detection, limit of quantification, and ruggedness.

Linearity

Stock solution of piracetam and its related substances (100 µg/mL) were prepared individually in mobile phase. A series of

standard curves were prepared over a concentration range of 50, 100, 250, 500, 1000, 5000, and 10,000 ng/mL for piracetam; 25, 50, 100, 500, 1000, 5000, and 10,000 ng/mL for impurity 1; 45, 90, 250, 500, 1000, 5000, and 10,000 ng/mL for impurity 2; 34, 80, 250, 500, 1000, 5000, and 10,000 ng/mL for impurity 3; and 55, 100, 250, 500, 1000, 5000, and 10,000 ng/mL for impurity 4 (Table I). The data of peak area versus drug concentration was treated by linear least square regression analysis. The standard curves were evaluated for intra-day and inter-day reproducibility. Linear regression characteristics of proposed method are given in Table II.

Precision and accuracy

Nootropil tablet solution mixtures containing known amounts of piracetam and its impurities were prepared and analyzed by HPLC. The accuracy of the method was checked for three different concentrations. It was found that these additions were accurately reflected in their peak areas. All estimations were repeated thrice, and the amounts of recovered drug were calculated (Table III). The precision of the method was determined as relative standard deviation (RSD) on replicate injections ($n = 5$) of a standard solution as shown in Table III.

Limit of detection and limit of quantification

In order to estimate the limit of detection and limit of quantification, mobile phase was injected six times, and the noise level was determined. The limit of detection was calculated to be three times the noise value and ten times the noise, which gave limit of quantification, and was also cross-checked by formulas given below (Table IV).

$$\text{LOD} = \frac{3.3\sigma}{S} \text{ and } \text{LOQ} = \frac{10\sigma}{S}$$

where σ is the standard deviation of the lowest standard concentration and S is the slope of the standard curve. Analysis of impurities in pharmaceutical formulation is indicated in Table V, and all the results were found in accordance to acceptable limits.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. In order to study the robustness of the proposed method, intentional modifications in pH values of the mobile phase was made. It was observed that in every engaged condition, the chromatographic parameters were in accordance with the established value (25). A change of ± 0.2 unit of pH of the mobile phase (approximately 6.3–6.7) and variation influenced by different lots of the same type of column had no considerable impact on chromatographic performance.

	Piracetam	Impurity 1	Impurity 2	Impurity 3	Impurity 4
r^2	0.9999	0.9999	0.9999	0.9999	0.9999
Intercept	06.45	233.73	121.09	371.83	1032.7
Slope	40.60	44.94	29.75	47.91	83.16

	Added conc. (ng/mL)	Recovered conc. (ng/mL)	% Recovery	%RSD (n = 5)
Piracetam	800	810.2	101.25	1.56
	1000	987.8	98.78	0.98
	1200	1208	100.7	1.27
Impurity 1	800	813.4	101.67	2.2
	1000	991.2	99.12	1.4
	1200	1187.2	98.93	1.7
Impurity 2	800	792.7	99.09	0.84
	1000	1002.2	100.22	0.71
	1200	1221.2	101.77	1.7
Impurity 3	800	808.4	101.05	0.64
	1000	1012.7	101.27	2.14
	1200	1213.8	101.15	1.27
Impurity 4	800	814.7	101.84	1.34
	1000	1024.1	102.41	1.8
	1200	1209.7	100.8	0.96

	Impurity 1	Impurity 2	Impurity 3	Impurity 4	Piracetam
LOQ (ng/mL)	3.12	14.84	3.92	16.06	1.42
LOD (ng/mL)	0.93	4.45	1.17	4.82	0.42

Tailing (symmetry) factor (T) and number of theoretical plates (N) were calculated by formulas: $T = W/(2W_a)$ and $N = 5.545(t_R/W_{1/2})^2$, where W is the peak width at 5% height from baseline, W_a the peak front edge width at the same height, and $W_{1/2}$ is the peak width at half height.

According to the data of robustness test study, we proposed criteria for system suitability test (tailing factors < 2 , numbers of theoretical plates > 4000), and repeatability (RSD) of five replicates must not be more than 1.5 (peak area for replicate analysis). It is used to verify that the resolution and repeatability of the system are adequate for the analysis of the intended drug.

Analysis of the pharmaceutical formulation

To determine the content of piracetam from the Nootropil tablet label claim (piracetam 800 mg per tablet), 20 tablets equivalent to 10 mg of piracetam were powdered, weighed accurately, and transferred to a 100-mL volumetric flask. Mobile phase was used for extraction. To ensure complete extraction of the drug, it was sonicated for 30 min, and the solution was made up to 100 mL. The resulting solution was filtered, and the supernatant was diluted to obtain the desired concentration with the mobile phase and injected. The analysis was repeated in triplicate. A placebo tablet was also subjected to the same extraction process as discussed earlier before injection. The possibility of excipient interference in the analysis was studied.

Results and Discussion

Optimization of solvent system

Varying compositions of acetonitrile–0.2 g/L solution of triethyl amine at pH 6.5 viz. 25:75, 40:60, 50:50, 75:25, and 15:85 (v/v) were evaluated as mobile phase in order to achieve good resolution between the drug and its impurities.

A common cause of peak tailing in RP-HPLC is the secondary retention that occurs when an ion exchange interaction takes place between a positively charged solute (amine) and an acidic

silanol on the surface of silica stationary phase support particles. Acidic or neutral compounds are not affected, and some basic compounds are more adversely affected than others. Triethyl amine is commonly added to mobile phases for this purpose. Triethyl amine interacts strongly with silanols and inhibits them from interacting with amines in your sample.

Acetonitrile–0.2 g/L solution of triethyl amine at pH 6.5 in the proportion 15:85 (v/v) was found to be a suitable solvent system. When 50:50 (v/v) was employed as the mobile phase, poor resolution was obtained between the drug and its degradation product.

The effect of sodium sulfate in mobile phase was also checked. It is observed that sodium sulfate has the most important effect on chromatographic behavior. This effect is negative for the five separations, which means that an increase of the amount of sodium sulfate will decrease the resolution of the peak pairs studied. The effect of sodium 1-octanesulfonate in mobile phase was also studied and has a positive effect on the resolution of the impurities, which means that an increase of the amount of ion pairing agent improves resolution. Initially, the experiment was carried out with ion pairing before testing both sodium-1-octanesulfonate and triethyl amine. Later, triethyl amine was selected for method validation.

Figure 1 shows the molecular structures of piracetam and its structurally related impurities impurity 1, impurity 2, impurity 3, and impurity 4 studied in the present investigation. All these

Table V. Analysis of Impurities of Piracetam in Nootropil Tablet (800 mg)

Sample	Impurity 1 (%recovery)	Impurity 2 (%recovery)	Impurity 3 (%recovery)	Impurity 4 (%recovery)
1	0.050	0.040	–	0.04
2	0.030	0.036	0.01	–
3	–	–	–	–
4	–	0.004	0.02	–
5	0.007	–	0.05	–

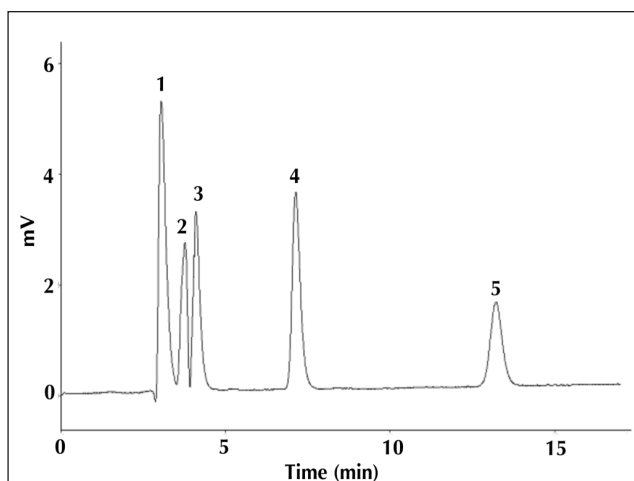


Figure 2. Representative chromatogram of piracetam and its four impurities. Peak 1 = impurity 1, peak 2 = piracetam, peak 3 = impurity 2, peak 4 = impurity 3, peak 5 = impurity 4.

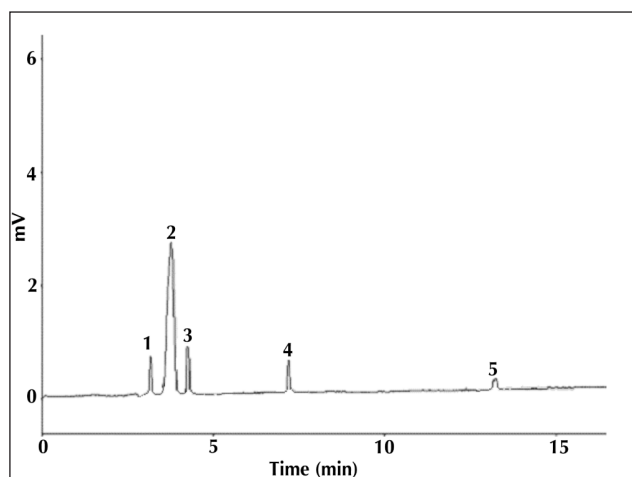


Figure 3. Representative chromatogram of piracetam (using NOOTROPIL tablet) and addition its four impurities. Peak 1 = impurity 1, peak 2 = piracetam, peak 3 = impurity 2, peak 4 = impurity 3, peak 5 = impurity 4.

materials were subjected to separation by RP-HPLC. The separation and resolution were found to be pH-dependent. Due care was given to the pH of the mobile phase while standardizing the HPLC conditions. pH values between 6 and 7 were found to be the optimum values for good separation. Acetonitrile was used as an organic solvent modifier to improve the separation. The effect of concentration of acetonitrile and temperature of the column on resolution was also studied. In the optimized condition, column temperature was set 40°C, but in this condition the piracetam peak was merged with a neighboring peak, so validation was conducted at room temperature 25°C. The chromatographic separation was also found to be dependent on the concentration of triethyl amine aqueous solution and the best separation was found to be optimum with acetonitrile–0.2 g/L aqueous solution of triethyl amine (15:85, v/v). The retention time of piracetam obtained 3.6 min, and its impurity 1, 2, 3, and 4 were obtained at 3, 4, 7, and 13.2 min, respectively. A typical chromatogram of a synthetic mixture containing piracetam and its impurities is shown in Figure 2. Two sample chromatograms (using Nootopil tablet), one with the addition of impurities and the other without any impurities are presented in Figure 3–4, respectively. The peaks were identified by injecting and comparing the retention times with those of authentic standards. Reproducible peak shapes were obtained under the optimum conditions. It can be clearly seen that the shapes of these peaks are undistorted. Therefore, the symmetry C_{18} column was preferred over the other column, and it provided better resolution of the peaks with symmetry and reproducibility.

All calibration curves showed linearity over a concentration range. The correlation coefficients (r^2) obtained with linear regression of curve were above 0.9999 for every impurity and piracetam. Linearity data show the concentration interval of studied piracetam in which the intensity of the detector response is proportional to the concentration of the analyzed substance. The theoretically determined values of quantitation limits were crossed checked by actual analysis of these concen-

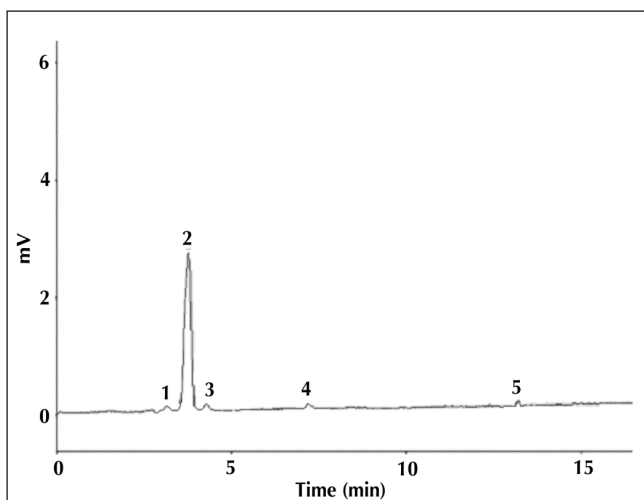


Figure 4. Representative chromatogram of piracetam (using NOOTROPIL tablet) impurities. Peak 1 = impurity 1, peak 2 = piracetam, peak 3 = impurity 2, peak 4 = impurity 3, peak 5 = impurity 4.

trations using proposed methods. The consequent RSD amongst these values are within acceptable limits (< 3.5%). Table II shows the statistical treated linear regression data of piracetam and all impurities.

The RSD values were less than 3.5%, indicating precision of the method. The recovery values obtained were between 98.78–101.25% for piracetam, 99.12–101.67% for impurity 1, 99.09–101.77% for impurity 2, 101.05–101.27% for impurity 3, and 100.8–102.41% for impurity 4, as shown in Table III, confirming accuracy of the proposed method. The percentage of recovery results is also presented in Table I. The excipients present in pharmaceutical dosage forms do not interfere in the analysis. The results prove specificity of the proposed methods. Table V shows the analysis of all impurities in dosage formulation.

A linearity relationship was evaluated across the range of analytical procedure. The linearity was performed over the range of concentration 50, 100, 250, 500, 1000, 5000, and 10,000 ng/mL for piracetam; 25, 50, 100, 500, 1000, 5000, and 10,000 ng/mL for impurity 1; 45, 90, 250, 500, 1000, 5000, and 10,000 ng/mL for impurity 2; 34, 80, 250, 500, 1000, 5000, and 10,000 ng/mL for impurity 3; and 55, 100, 250, 500, 1000, 5000, and 10,000 ng/mL for impurity 4. The regression line was calculated as $y = Bx + D$, where x was the drug concentration (ng/mL) and y was the response (peak area expressed as AUC). D is the intercept, and B is slope of regression line as mention in Table II. The calibration curve was obtained using the linear least squares regression procedure; representative linear regression data of five different analytes is shown in Table II. The correlation coefficient (r^2) value is 0.9999.

Stability of piracetam solution

The stability of the piracetam solutions during time was investigated. A solution of 10,000 ng/mL of piracetam was analyzed at intervals of 0, 24, 48, and 72 h after storing the samples at room temperature. It was found that up to 72 h the chromatographic peak area of piracetam was not significantly changed. It can be concluded that piracetam solution was stable at least for 72 h after its preparations. This solution was also analyzed in the HPLC system at 2, 4, 6, and 8 days when stored at -20°C , and no significant change was found when compared with freshly prepared samples.

Conclusion

An improved LC method was developed for the analysis of piracetam with its impurities. This method does not only allows complete separation of piracetam and its impurities but could also be used for the quantitative analysis of these five components in bulk drug as well as in dosage formulations up to less than 17 ng/mL, as our method is selective and would not allow interference of the excipients. The developed HPLC technique is precise, specific, and accurate. The statistical analysis proves that the method is reproducible and selective for the estimation of piracetam with its impurities in bulk drug and pharmaceutical formulations.

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