SPECIAL GUEST EDITOR SECTION

Reversed-Phase HPLC Analysis of Levetiracetam in Tablets Using Monolithic and Conventional C18 Silica Columns

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Development and validation of an RP-HPLC method for determination of levetiracetam in pharmaceutical tablets is described. The separation and quantification of levetiracetam and caffeine (internal standard) were performed using a single analytical procedure with two different types of stationary phases, conventional Phenomenex Gemini C18 (100 4.6 mm, 5 m) and Merck Chromolith Performance RP18e (100 4.6 mm, macropore size 2 mm, micropore size 13 nm) monolithic silica. Five-microliter aliquots of samples were injected into the system and eluted using water–acetonitrile (90 + 10, v/v) mobile phase pumped at the rate of 1 mL/min. The analyte peaks were detected at 200 nm using a diode array detector with adequate resolution. Validation studies were performed using the method recommended by the International Conference on Harmonization, the *U.S. Pharmacopeia*, and AOAC INTERNATIONAL, which includes accuracy, precision, range, limits, robustness, and system suitability parameters. Levetiracetam and caffeine were detected in about 7 min using the conventional column, whereas less than 5 min was required when the monolithic column was used. Calibration plots had r values close to unity in the range of 0.8–8.0 g/mL. Assay of levetiracetam in a tablet formulation was demonstrated as an application to real samples.

Levetiracetam, (S)- -ethyl-2-oxo-1-pyrrolidineacetamide (1), is a relatively new, promising antiepileptic compound marketed by UCB S.A. (Braine-l'Alleud, Belgium) under the brand name of Keppra™, which was approved by the U.S. Food and Drug Administration in 1999 (2). The chemical structure of levetiracetam is shown in Figure 1; its molecular formula is $C_8H_{14}N_2O_2$, and it has a molecular weight of 170.21 g/mol. The exact mechanism by which levetiracetam exerts its antiepileptic action is unknown (3).

To date, several methods for the determination of levetiracetam were used in analytical and bioanalytical studies. The reported techniques utilized for the determination of levetiracetam include HPLC, GC, capillary electrophoresis, and microemulsion electrokinetic chromatography (MEKC). HPLC with UV detection (4–12) and diode array detection (13–16) were mainly preferred by researchers, including studies on the stability of levetiracetam (15) and sample cleanup protocols (16). In addition to UV-based detection techniques, MS (17–19) and electrochemical detectors (20) were also used to trace levetiracetam signals. GC was another technique used in levetiracetam assays, coupled to MS (21, 22) or nitrogen-phosphorus (23–27) detectors. GC (8), MEKC (28), and capillary electrophoresis (29) were rarely used for assays. Within the techniques used to determine levetiracetam levels, HPLC/MS (17–19) and GC/MS (21, 22) are superior to other methods because of unique capabilities such as ion-trap, multiple, and selected-reaction monitoring that allow better specificity and lower quantification limits.

In addition to various methods used to determine levetiracetam, different extraction and cleanup protocols were also reported in the studies. Liquid–liquid extraction (4, 5, 7, 9, 10, 14, 24) and SPE (8, 17, 19, 21, 22) were used in several methods. In general, the methods reported in the literature suggest use of SPE, multistep pretreatment applications, or expensive instrumentation to determine levetiracetam levels. These approaches sometimes lead to analytical assays that are not economically feasible for routine use in pharmacokinetic and pharmaceutical studies.

In order to develop robust, simple, and fast assays, utilization of novel and improved analytical instrumentation or equipment is a key point in today's applications. One of the easiest ways to improve analysis efficiency is utilization of novel stationary phases, such as columns packed with sub-2 m, monolithic, polymeric, or composite materials. The columns with narrow-bore or sub-2 m particles have many advantages, e.g., higher column efficiency and lower analysis time. However, specially designed liquid chromatographs are necessary for rapid and acceptable separations. Monolithic columns override pressure problems with their unique structure, which contains a single monolithic silica rod in the

Guest edited as a special report on "New and Improved HPLC Methods for Drug Formulations and Clinical Analysis" by Danica Agbaba and Joseph Sherma.

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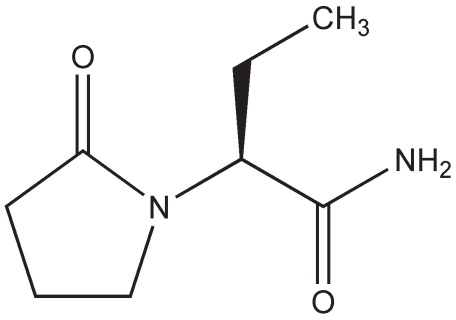


Figure 1. Chemical structure of levetiracetam.

column, making efficiency comparable to conventional C18 columns with remarkable pressure drops (30–32). Three mL/min or faster mobile phase flow rates through monolithic columns are common in pharmaceutical analyses with ordinary HPLC systems (33–36). Columns packed with novel composite or polymeric materials are another choice, especially for wide-pH-range applications and efficient separations.

The columns used in this study, Gemini C18 and Chromolith Performance RP18e, were chosen for the reasons described above. Gemini C18 is packed with a wide-range, pH-stable, high-density composite material, whereas a highly porous monolithic silica rod clad to the column shell is used in Chromolith Performance RP18e. A literature search indicated that there is no previous study reported in which these columns were used in levetiracetam assays. On this basis, a single HPLC method carried out using two different types of stationary phases was developed and validated according to the International Conference on Harmonization (ICH; 37), *U.S. Pharmacopeia* (USP; 38), and AOAC INTERNATIONAL (39) recommendations, and it was applied to levetiracetam tablets assay. In addition, robustness of the proposed method was improved by utilization of caffeine as an internal standard (IS). The analytical procedure is advantageous for method transfer between laboratories due to its simplicity.

Experimental

Materials

(**a**) *Levetiracetam*.—99.9% purity, provided by Tocris Bioscience (Ellisville, MO). ^{1}H NMR and mass spectra were consistent with authentic reference material, and it was used without further treatment.

(**b**) *Caffeine*.—Anhydrous with claimed purity of 99.0%, provided by Fluka (Darmstadt, Germany) and used without further treatment.

(**c**) *Tablet formulation of levetiracetam*.—Labeled as "Keppra 500 mg Film Tablet," provided by UCB S.A. with a label claim of 500 mg levetiracetam.

Reagents

(**a**) *Acetonitrile*.—Chromasolv for HPLC, far UV grade, Riedel-de Haen, purchased from Sigma-Aldrich Lab GmbH (Seelze, Germany).

(**b**) *Milli-Q water*.—Purified with a Milli-Q Synthesis A10 system from Millipore SAS (Molsheim, France).

Instruments

(**a**) *Liquid chromatograph*.—Prominence HPLC series chromatograph consisting of a DGU-20A5 online degasser, LC-20AT pump equipped with low-pressure gradient unit, SIL-20A autosampler, CTO-10ASVP column oven, CBM-20A system controller, SPD-M20A diode array detector (DAD), and LCSolutions 1.11 SP1 software (Shimadzu, Kyoto, Japan).

(**b**) *Multipoint stirrer*.—RO 15 P; Ika-Werke GmbH (Staufen, Germany).

(**c**) *Centrifuge*.—EBA 20; Andreas Hettich GmbH & Co. KG (Tuttlingen, Germany).

(**d**) *Ultrasonic bath*.—RK 100H Sonorex; Bandelin (Berlin, Germany).

Chromatographic Parameters

Water–acetonitrile (90 + 10, v/v) was used as the mobile phase. Chromatographic analyses were performed on two different columns: a conventional C18 column (Gemini C18 100 4.60 mm id, 5 m composite particle with 110 Å pore size; Phenomenex, Torrance, CA) and a monolithic silica column (Chromolith Performance RP18e 100 4.6 mm id, 2 mm macropore and 13 nm micropore size; Merck KGaA, Darmstadt, Germany). The column oven temperature was 40 C, flow rate 1.0 mL/min, and injection volume 5 L. The DAD was set at 200 nm with a spectral recording range between 190 and 380 nm.

Standard Solution Preparation

(**a**) *Levetiracetam stock solution*.—An accurately weighed 10.0 mg portion of levetiracetam standard was transferred to a 50 mL volumetric flask, and water was added to dissolve the substance by sonication; the contents of the flask were diluted to volume with water. The resulting levetiracetam concentration was 200 g/mL. The final solution was diluted to 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6, 4.0, and 8.0 g/mL concentration levels for validation studies. Solutions were found to be stable at least 30 days when kept at 5 C and away from daylight.

(**b**) *Caffeine stock solution*.—An accurately weighed 10.1 mg portion of anhydrous caffeine standard was transferred to a 100 mL volumetric flask, and water was added to dissolve the substance by sonication; the contents of the flask were diluted to volume with water to obtain a concentration of 101 g/mL caffeine. The stock solution was found to be stable at least 30 days when kept at 5 C and away from daylight.

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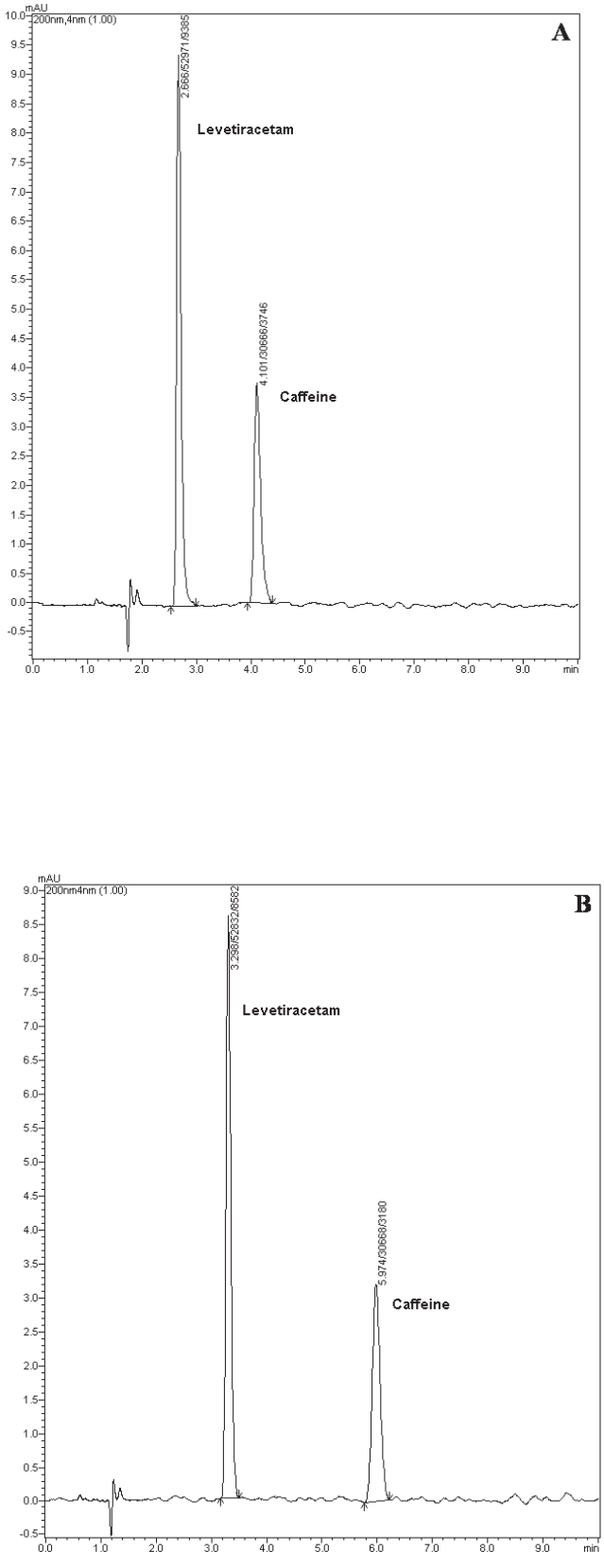


Figure 2. Typical chromatograms of levetiracetam (4 g/mL) and caffeine (1 g/mL) standards using (A) Chromolith Performance RP18e and (B) Gemini C18 columns.

Tablet Sample Preparation

Thirty tablets were used for the assay. Each tablet was transferred to a separate 100 mL volumetric flask, and 50 mL water was added. The contents of the flask were stirred using a magnetic stirrer for 20 min. Subsequently, the flask was spiked with IS, filled up to volume with water, and the contents sonicated for 10 min. Then a 10 mL portion of the mixture was removed and centrifuged at 6000 rpm for 10 min to precipitate insoluble excipients. Three milliliters of the supernatant were filtered through a 0.20 m PVDF filter. The estimated levetiracetam concentration of the final solution was 5 mg/mL, and this solution was diluted to the working range using water.

Method Validation

(**a**) *Specificity*.—ICH recommends use of a second analytical procedure to compare the test results of samples that possibly contain impurities or degradation products. According to the statement, possible effects of levetiracetam impurities and pharmaceutical excipients on the assay could be assessed by comparing two chromatograms acquired by using both HPLC columns. So, the results obtained from one column were used to compare to the other column to determine specificity. The chromatograms and peaks of interest were examined for signals to confirm that a positive or negative response was not obtained. In addition, peak purities were tested using the DAD to show that the analyte and IS peaks were not attributable to more than one compound.

(**b**) *Linearity and range*.—The linearity plot was prepared covering 10 concentration levels of levetiracetam (0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6, 4.0, and 8.0 g/mL). The range of the method corresponded to 40–400% of the estimated concentration of the test solutions. Each solution was analyzed using both of the columns, injected in triplicate, and the average values were accepted as representative. Linearity was evaluated by linear regression analysis with intraday and interday replicates.

(**c**) *Accuracy*.—Recovery experiments were performed in order to determine assay accuracy. Preanalyzed tablet samples were spiked with a known amount of levetiracetam solution at three different levels corresponding to 50, 100, and 150% of the assay concentration. Three sets were prepared for each concentration level. Spiked samples were reanalyzed, and mean recovery was calculated.

(**d**) *Precision*.—Precision studies included intraday and interday (intermediate) precision experiments. Precision was evaluated by analyzing the standard solution eight times using the proposed methods on the same and 3 different days. The standard solution was chosen to be at 100% of the concentration of the test solution. Results were analyzed statistically including mean, SD, RSD, and confidence interval at the 95% confidence level.

(**e**) *LOD and LOQ*.—LOD and LOQ were determined according to ICH recommendations based on the SD of the response and the slope. The ratio of the SD of *y*-intercepts of regression lines ( ) to the slope was multiplied by 3.3 for

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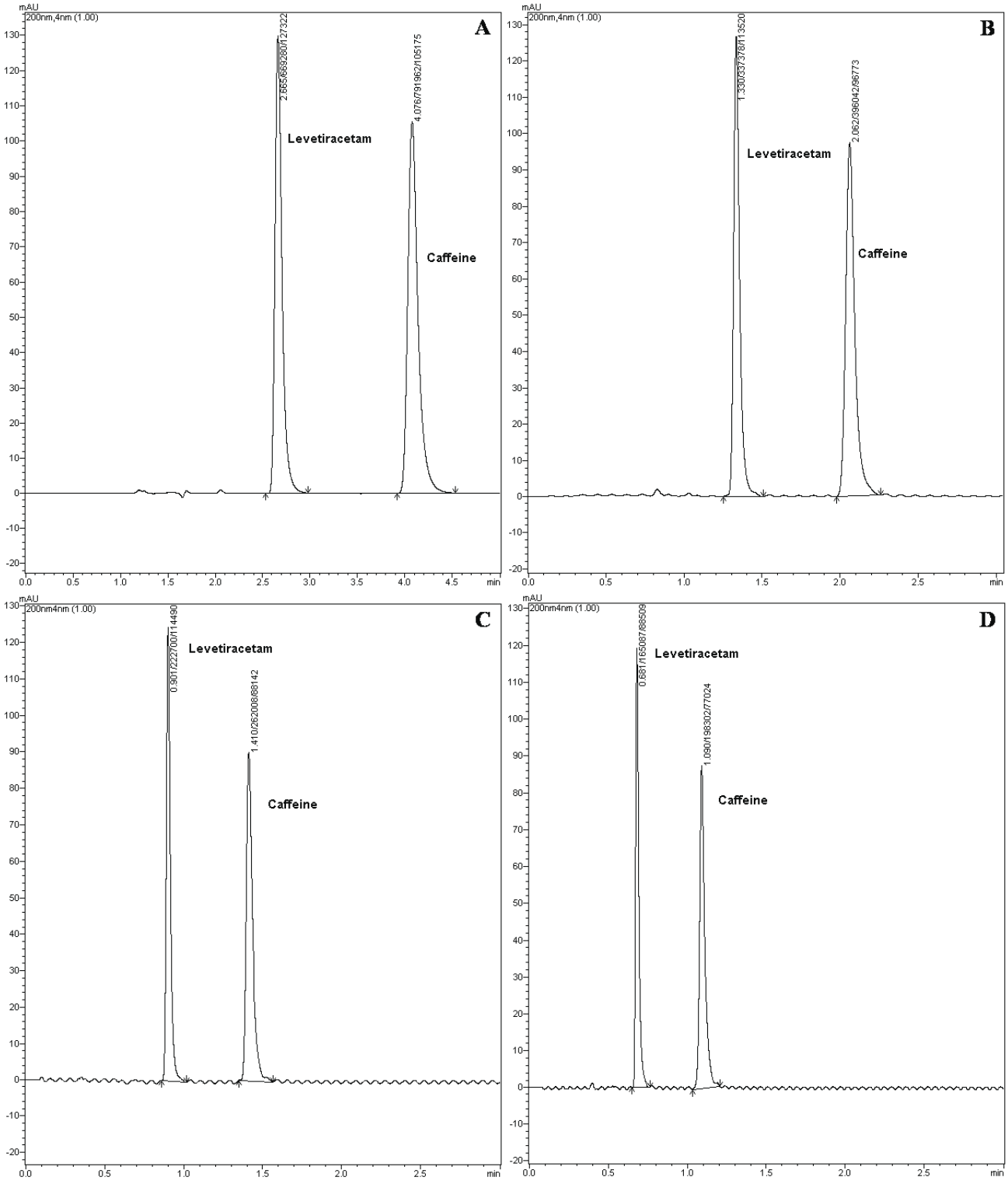


Figure 3. Effect of flow rate on retention of levetiracetam and caffeine on a Chromolith Performance RP18e column. Flow rates are (A) 1 mL/min, (B) 2 mL/min, (C) 3 mL/min, and (D) 4 mL/min.

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Table 1. Effect of flow rate on the retention time of levetiracetam and caffeine

Flow rate, mL/min	Back-pressure, bar	Retention time, min	
		Levetiracetam	Caffeine
1	30	2.665	4.076
2	60	1.330	2.062
3	90	0.910	1.401
4	120	0.681	1.090

LOD and by 10 for LOQ. Slope and were calculated from pooled data obtained from linearity experiments. In addition to the calculation method, evaluation of LOD and LOQ based on S/N was also used to confirm the values. S/N was taken as 3.3 for LOD and 10 for LOQ.

(**f**) *Robustness*.—To determine robustness, some instrumental conditions that have a major effect on the retention were purposely altered by 2%, one at a time. Test solutions were analyzed with the following variations: ±0.02 mL/min change in flow rate, ±0.2% change in acetonitrile ratio in the mobile phase, and ±1.8 C change in the column temperature. Relative changes in peak intensity, retention time, and number of theoretical plates were assessed, and test results of both columns were compared with each other to provide additional information on robustness.

(**g**) *System suitability testing*.—As an essential part of HPLC method development, system suitability parameters were also investigated to control the performance of the HPLC instrument. Column efficiency (N), resolution between levetiracetam and caffeine peaks (R_s), asymmetry factor (A_s), tailing factor (T), and separation factor ( ) were calculated according to USP recommendations.

Results and Discussion

The aim of this study was to develop a novel and validated HPLC method in which monolithic and conventional C18 silica columns were utilized for routine assays of levetiracetam tablet formulations. Selection of suitable analytical conditions was the first step of the method development. Acetonitrile and methanol were principally preferred because they are found in many QC laboratories. Due to the relatively low hydrophobic (log P = –0.64) and highly water-soluble (1.04 g/mL) nature of levetiracetam, detection occurred in 10 min when the organic solvent ratio was kept between 5 and 15%. The absorbance maximum of the analyte was found to be between 190 and 200 nm in a supplementary UV spectrophotometric study (relevant data are not given here). Lack of chromophore groups on the levetiracetam molecule results in low absorbance in the UV region and, as expected, high signal intensity of levetiracetam was seen at 200 nm with DAD. Addition of buffer was not considered due to the closeness of the detection wavelength to the far UV region, in which many buffer reagents absorb UV light and give baseline noise that causes significant increases in LOD and LOQ. Also, peak enhancers such as triethylamine or octylamine were not used because adequate peak symmetry was obtained. Acetonitrile was preferred to methanol because of a lower UV cutoff value (190 nm) and viscosity than methanol, which allows better mass transfer and lower noise and back-pressure. The detector signal acquisition frequency was set at 40 Hz to be allow observation of more representative signals for intracolumn interactions.

Although both columns allow higher flow rates, 1 mL/min was chosen; however, flow rate was increased up to 4 mL/min for the Chromolith Performance RP18e column to test the chromatographic behavior of the analytes and performance limitations of the method. Effect of column temperature on the retention of analytes was also investigated by changing temperature between 25 and 40 C; 45 C was the upper limit for the monolithic column stability. Therefore, 40 C was chosen as the "safe" temperature, and it had the benefits of decreased viscosity of the mobile phase and higher mass transfer and sharper peaks.

To enhance robustness of the developed method, an IS was utilized. Solubility, absorptivity, and hydrophobicity properties of some compounds for this purpose were examined. Caffeine was considered as a suitable candidate because it has physicochemical properties similar to levetiracetam. Caffeine has a log P value of –0.23, water solubility of 2.17 g/mL, and UV absorbance maximum at about 210 nm. Initial studies indicated that retention of caffeine was close to levetiracetam; thus, it was chosen as the IS.

In light of the foregoing findings, assay conditions were as follows: mobile phase water–acetonitrile (90 + 10, v/v) pumped at 1 mL/min flow rate, column temperature 40 C, and levetiracetam and caffeine detection at 200 nm. Analysis of levetiracetam and caffeine standards with the specified conditions using Chromolith and Gemini columns gave the chromatogram shown in Figure 2A and B, respectively. As seen in Figure 2, the levetiracetam and caffeine peaks were well separated; respective retention times on the Chromolith Performance RP18e column were 2.67 ± 0.1 and 4.03 ± 0.1 min, and 3.30 ± 0.1 and 5.95 ± 0.1 min on the Gemini C18 column.

An advantage of the highly porous structure of monolithic stationary phases was that higher flow rates were easily tested to observe the chromatographic behavior of levetiracetam and caffeine. Flow rate of the mobile phase was increased up to 4 mL/min, and effects on the retention time and back-pressure were evaluated. The back-pressure did not exceed 120 bars in all tests, and detection of levetiracetam occurred at 0.681 min; the caffeine peak had a retention time of 1.090 min, earlier than under standard conditions. The relevant chromatograms and data are shown in Figure 3 and Table 1, respectively.

Although higher flow rates were possible, the HPLC instrument showed a significant increase in noise, especially above 3 mL/min, that made quantification inefficient at low

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Table 2. System suitability values calculated for the levetiracetam peak under the specified conditions

Parameter	Chromolith Performance RP18e	Gemini C18 column	Recommended value
Retention time, min	2.66 ± 0.1	3.31 ± 0.1	NA
Asymmetry (A_s)	1.26	1.10	0.95 < *x* < 1.5
Tailing (*T*)	1.42	1.11	<2
Selectivity ( )	2.59	2.26	>1
Resolution (R_s)	5.68	9.14	>2
Theoretical plates (N)	7999.7	10725.4	>2000

concentrations. Thus, the 1 mL/min flow rate was unchanged so as to be able to compare performance of the two columns under the same analysis conditions.

As an integral part of the analytical procedure, system suitability parameters were tested prior to injection of standards to ensure that the system was performing adequately for the assay. These parameters were evaluated according to recommendations of ICH (37), USP (38), and AOAC INTERNATIONAL (39), including number of theoretical plates, resolution, asymmetry, tailing factor, and separation factor. Results of the system suitability tests were in agreement with the specified values. Relevant data are given in Table 2. Following the system suitability tests, the proposed method was validated for both of the columns according to official recommendations.

The specificity of the method was determined by checking signals of possible interferences in the assay solutions. Peaks of the claimed pharmaceutical excipients or possible impurities were not observed close to the levetiracetam and caffeine peaks. In addition, peak purities of levetiracetam and caffeine were checked, and results confirmed that analyte peaks were not attributable to more than one compound in all of the analyzed standards and test samples. Peak purity data are shown in Table 3.

Linearity was evaluated by linear regression analysis using 10 different concentrations of levetiracetam. The ratio of levetiracetam peak area to caffeine peak area versus concentration was used for quantification. Signals were found to be directly proportional to concentrations of the calibration solutions and obeyed Beer's law in the range of 0.8–8.0 g/mL. The regression equations were:

$$y = 0.4229x + 0.02959 \ (r = 0.9998)$$

for the Chromolith Performance RP18e column and

$$y = 0.4222x + 0.02980 \ (r = 0.9997)$$

for the Gemini C18 column, where *x* is the concentration in g/mL and *y* is the peak area ratio. Statistical data related to linearity studies are shown in Table 4.

The proposed methods were tested for accuracy in the working range. Accuracy was interpreted as the closeness of assay results to the values that are accepted as conventional true values (37). Independent recovery experiments with the standard addition method were employed using nine determinations over three different concentrations covering the range. Preanalyzed tablet samples were reanalyzed after spiking with known amounts of levetiracetam solutions. The mean recovery of levetiracetam was found between 99.37 ± 1.28% and 100.11 ± 1.26% for the two columns. Data obtained from the accuracy studies are shown in Table 5.

Reproducibility of the methods was investigated in terms of intraday (repeatability) and interday (intermediate) precision. Method transfer from the Gemini C18 to the Chromolith Performance RP18e column did not cause any change in precision results; thus, reproducibility was not investigated separately since the method was found to be highly precise for both columns. Using a general statistical approach, SD, RSD, and confidence interval were calculated. The overall RSD of the methods was between 1.008–0.994 for repeatability and 1.039–1.032 for intermediate precision studies. Precision data are shown in Table 4.

LOD and LOQ were determined according to ICH recommendations, based on the SD of the response ( ) and the slope. The ratio of the SD of *y*-intercepts of regression plots ( ) to the slope was multiplied by 3.3 for LOD and by 10 for LOQ. As an additional confirmation, these values were recalculated based on S/N, which was equal to 3.3 for LOD and 10 for LOQ. As a result, LOD and LOQ of the method for the Chromolith Performance RP18e column were calculated to be 0.047 and 0.144 g/mL, and they were 0.049 and 0.150 g/mL for the Gemini column, respectively. These values were considered to be excellent when compared to previous studies on levetiracetam. This improvement can be explained by the very low noise of the assays (about 250–500 mAU), which was the result of acetonitrile usage in mobile phase, high absorptivity of levetiracetam at 200 nm,

Table 3. Peak purity profiles of the analytes found by diode array detection

	Peak purity	
Column	Levetiracetam	Caffeine
Chromolith Performance RP18e	0.999999	1
Gemini C18	1	1

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Table 4. Results of linearity, accuracy, and precision studies

Validation parameter	Chromolith Performance RP18e	Gemini C18
Linearity range, g/mL	0.8–8.0	0.8–8.0
Slope ± SD (intraday, *n* = 10)	0.4264 ± 0.001957	0.4257 ± 0.002172
Intercept ± SD (intraday, *n* = 10)	0.02121 ± 0.006930	0.02144 ± 0.007694
Regression coefficient (intraday, *n* = 10)	0.9999	0.9999
Slope ± SD (interday, *n* = 30)	0.4229 ± 0.001725	0.4222 ± 0.001794
Intercept ± SD (interday, *n* = 30)	0.02959 ± 0.006108	0.02980 ± 0.006353
Regression coefficient (interday, *n* = 30)	0.9998	0.9997
Repeatability (intraday, mean, *n* = 8)	0.8624	0.8647
Repeatability (intraday, RSD, *n* = 8), %	1.008	0.994
Repeatability (intraday, SE, *n* = 8)	0.002412	0.002383
Repeatability (intraday, CI, *n* = 8)[a]	0.006024	0.005956
Repeatability (interday, mean, *n* = 24)	0.8627	0.8650
Repeatability (interday, RSD, *n* = 24), %	1.039	1.032
Repeatability (interday, SE, *n* = 24)	0.001831	0.001823
Repeatability (interday, CI, *n* = 24)	0.003586	0.003571

[a] CI = Confidence interval at a 95% confidence level.

Table 5. Statistical evaluation of accuracy studies conducted using both columns

Added, g/mL	Found, g/mL	Recovery, %	Mean recovery, %	RSD, %
		Chromolith Performance RP18e		
0.8	0.79	98.73 ± 1.19	99.37 ± 1.28	1.29
1.6	1.60	99.70 ± 0.62		
2.4	2.39	99.67 ± 1.96		
		Gemini C18		
0.8	0.80	99.87 ± 1.42	100.11 ± 1.26	1.26
1.6	1.60	99.83 ± 1.35		
2.4	2.42	100.64 ± 1.37		

Table 6. Summary of results obtained from robustness studies

	Chromolith Performance RP18e			Gemini C18		
Robust condition	Assay, %	Retention time, min	N	Assay, %	Retention time, min	N
Flow rate (0.98 mL/min)	100.18	2.719	7951	100.22	3.376	10790
Flow rate (1.02 mL/min)	99.80	2.614	8124	99.79	3.243	10700
Water–acetonitrile (90.2 + 9.8, v/v)	100.11	2.713	8000	100.16	3.306	10822
Water–acetonitrile (89.8 + 10.2, v/v)	99.93	2.608	8098	99.89	3.315	10677
Oven temperature, 40.8 C	100.06	2.645	8124	100.10	3.275	10735
Oven temperature, 39.2 C	99.97	2.691	8100	99.92	3.347	10706

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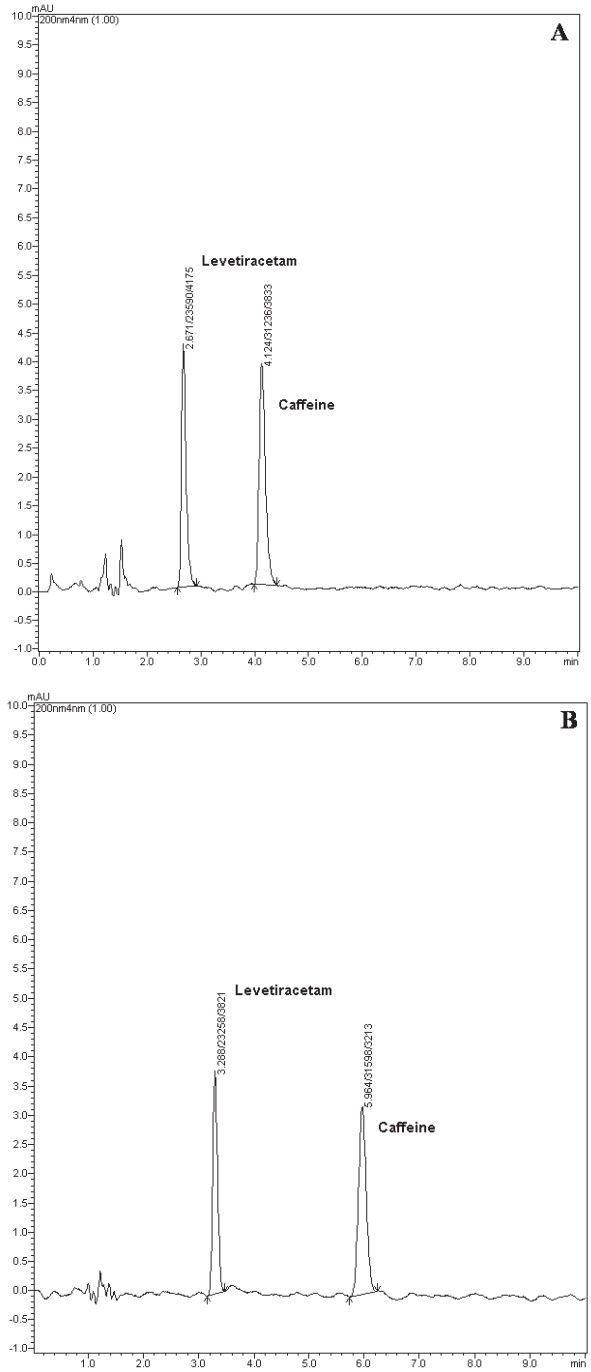


Figure 4. Assay chromatograms of levetiracetam tablet formulation carried out by using (A) Chromolith Performance RP18e and (B) Gemini C18 columns. Levetiracetam concentration was about 2.5 g/mL.

and avoiding the use of buffers to maintain pH. Literature searching showed that 205 nm (7, 9, 15), 210 nm (13), and 214 nm (28, 29) wavelengths were mainly preferred in UV-based detections of levetiracetam. Method development studies showed that levetiracetam absorbs UV light 25% less at 205 nm, 50% less at 210 nm, and 65% less at 214 nm when compared to 200 nm. High signal intensity and low determination limits were associated with high absorptivity of levetiracetam at 200 nm.

Table 7. Assay results of Keppra 500 g Film Tablet[a]

	Chromolith Performance RP18e	Gemini C18
Minimum	495.6	495.7
Maximum	504.6	504.8
Mean (n = 30)	499.6	499.1
SD	2.602	2.625
RSD, %	0.520	0.526
SE	0.475	0.4793
Confidence interval[b]	0.939	0.939

[a] n = 30.
[b] 95% Confidence level.

As a measure of the ability of the developed method's performance to remain unaffected by small but deliberate variations in method parameters, robustness studies were performed. Typical sources of variation in HPLC analyses include flow rate, mobile phase composition, and column temperature, which frequently affect assay results in method transfers. These parameters were altered by 2% to assess the deviations from initial values. It was found that results obtained using the altered conditions were similar to the results using the initial conditions, so it was accepted that the variations were not distorting the methodology. Robustness data are shown in Table 6.

At the final stage of the experimental studies, the proposed methods were applied for the assay of levetiracetam in a film tablet formulation. There were no interferences with the levetiracetam and caffeine peaks observed in the assay of Keppra 500 g Film Tablet. Typical chromatograms for both columns are shown in Figure 4. Each sample was injected into the system triplicate, and average values were treated statistically. Related data are given in Table 7. On the whole, the developed method was successfully applied to the real samples and gave relatively good performance for both columns.

Conclusions

The proposed methodology showed better performance when compared to many of the methods developed in previous studies. A main advantage was the cost-effectiveness of the instrumentation, chemicals, and other apparatus used in sample preparation and analysis, which are generally found in many QC laboratories. The method did not require SPE, additional sample cleanup, buffer preparation, extra column washing, or gradient elution, which lowered the cost of analysis and made it simple and fast. Simplicity of the sample preparation step allows very high recovery due to low analyte loss. Although performance of the proposed method cannot be evaluated as being much better than HPLC/MS (17–19) and GC/MS (21, 22) methods, it offers practical and robust results that can be acquired within a couple of minutes. Total analysis

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time was under 5 min for the monolithic column, which is fast compared to previous methods (7, 9, 13, 15, 16, 21, 22, 28, 29). However, Matar, Jain et al., and Guo et al. reported better analysis times for levetiracetam using HPLC/MS/MS (17–19).

LOD and LOQ values of the method were better than almost all of the previously published studies, including HPLC/MS and GC/MS methods. These values were reported between 0.1–0.5 g/mL in the earlier studies. When the Chromolith Performance RP18e column was used at a 4 mL/min flow rate, the end of an HPLC run could be shifted easily to under 1.2 min without compromising resolution, with a back pressure of about 120 bar. These improvements make the method preferable for levetiracetam assays.

As a general conclusion, the method proposed was developed, adequately validated, and tested for its intended purpose. The results of our study indicate that the analytical method is fast and robust for determination of levetiracetam levels in film tablets for QC laboratories.

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