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A Sensitive Dissolution Test Method for the Development and Validation of Levetiracetam Tablets by Reverse Phase-HPLC Technique

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Abstract: This study describes the development and validation of dissolution tests for levetiracetam tablets using a reverse phase high performance liquid chromatography method. The appropriate conditions were determinate after testing *sink* conditions; dissolution medium, agitation intensity and the USP apparatus, paddle was applied. The best dissolution conditions tested, for the products in each respective pharmaceutical dosage form were applied to evaluate the dissolution profiles. The parameters of difference factor, similar factor, and dissolution efficacy were employed. Optimal conditions to carry out the dissolution tests were 900 mL of purified water as dissolution medium, paddle at 50 rotations per minute (rpm) stirring speed for tablets and detection was carried out at 217 nm. The retention time of levetiracetam was found to be 3.87 minutes. The comparison of the obtained dissolution profiles of tablets, obtained from three different batches (A, B and C) of 1000 mg levetiracetam was performed and the results showed no significant difference among the products. The developed and validated dissolution tests satisfactorily describes the time-course of the drug release. The obtained results provided adequate dissolution profiles. The HPLC method was validated to meet requirements for a global regulatory filing and also to quantify levetiracetam tablets from the dissolution tests.
Keywords: Dissolution profile, Dissolution efficiency, Anti-epileptic agent, RP-HPLC, Validation.

Introduction

Levetiracetam is a single enantiomer of (-)-(s) - ethyl-2-oxo-1-pyrrolidine acetamide[1] (Figure 1). Levetiracetam is used in combination with other medications to treat certain types of seizures in people with epilepsy. Levetiracetam is in a class of medications called anticonvulsants and it works by decreasing abnormal excitement in the brain. Levetiracetam can prevent myoclonic jerks and generalized epileptiform activity in patients with photosensitive epilepsy whose mechanism of action is thought to involve binding of the synaptic vesicle protein SV2A, a protein involved in neurotransmitter vesicle exocytosis[2]. There is no monograph of this drug in any pharmacopoeia. Moreover, the literature presents few methods related to the quality control of Levetiracetam, mainly in its pharmaceutical dosage forms. The dissolution test has emerged as a valuable quality control tool to assess batch-to-batch product release performance and to assure the physiological

availability of the drug[3]. Its significance is based on the fact that for a drug to be absorbed and available on the systemic circulation, it must previously be solubilized[4].

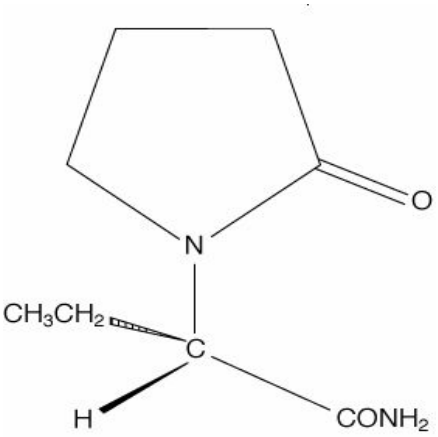

Figure 1: Chemical structure of levetiracetam

Levetiracetam distributes well into saliva, with salivary levetiracetam concentrations being on average slightly higher than serum concentrations in patients receiving chronic levetiracetam therapy. Salivary levetiracetam concentrations correlate well with those in serum, which makes saliva an alternative sample to perform therapeutic drug monitoring. Levetiracetam has been determined in biological fluids by HPLC with mass spectrometry detection[5-7], gas chromatography with mass spectrometry detection[8], and impurity determination of levetiracetam using capillary electro chromatography[9]. The separation and quantitation of levetiracetam from other antiepileptic drugs was realized using microemulsion electrokinetic chromatography[10] and HPLC methods with ultraviolet detection[11-14]. There is no dissolution tests describe in literature for levetiracetam in pharmaceutical dosage forms.

Dissolution test has emerged in the pharmaceutical field as a very important tool to characterize drug product performance. Therefore the dissolution studies are used not only to assess batch-to-batch consistency of drug release from solid dosage forms, but they are also essential in several stages of formulation development, for screening and proper assessment of different formulations. Moreover, the *in vitro* dissolution studies are relevant to predict *in vivo* performance of a drug release and have been used as a tool to estimate bioavailability of the drug[15-17]. This way, the aim of this work is to present the development and validation of dissolution tests and HPLC method to the quantitation of levetiracetam tablets in routine quality control and from the dissolution tests, as well as to evaluate the dissolution profiles for tablets. Parameters to set up the dissolution test should be researched and defined for drugs that do not possess official monographs[18]. For this reason, there is a crescent number of works describing the development of dissolution test for deflazacort, diacerhein and rupatadine[19-21].

Experimental

Instrumentation

The dissolution tests were performed in a Vankel (VK7025) auto sampler (VK8000) dissolution test system, multi-bath (n=6), in accordance with the United States Pharmacopeia (USP) general methods[18]. A Shimadzu Prominence HPLC system (Shimadzu, Kyoto, Japan) equipped with a SCL-10Avp system controller, LC-10 ADvp pump, DGU-14A degasser, CTO-10Avp column oven, SIL-10ADvp autosampler and a SPD-M10Avp photodiode array (PDA) detector was used. Detector was set at 217 nm and peak areas were integrated automatically by computer using a Shimadzu Class VP V 6.14 software program. The stationary phase was a Prontosil C18-EPS, column (150 mm x 4.6 mm i.d., with a particle size of 5 µm and pore size of 120 Å, Stuttgart, Germany by Bischoff Chromatography). A security guard holder was used to protect the analytical column. The Shimadzu Prominence HPLC system was operated isocratically at ambient temperature. The mobile phase was prepared by mixing 1.37 gms of potassium dihydrogen orthophosphate and 0.61 gms of sodium 1-heptane sulphonate in 1000 mL purified water (pH adjusted to 2.8 with orthophosphoric acid) and acetonitrile (90:10, v/v). The injection volume was 10 µl with the run time 8 minutes. The mobile phase was filtered using a 0.45 µm membrane filter (Milipore, Milford, MA) and degassed with helium. The mobile phase flow rate was 1.2 mL per minute. Elico pH analyzer (Model: Elico LII20) was used to determine the pH of all solutions.

Materials and reagents

Levetiracetam reference substance (Batch no: LVMU100002, Potency: 99.4%) was obtained from Orchid Chemicals and Pharmaceuticals Ltd., India whereas the pharmaceutical formulations containing Levetiracetam 1000 mg (Product A (reference)-Keppra®, Teva Pharmaceuticals, USA) and the products B and C were obtained from Orchid HealthCare Ltd., India. All reagents and solvents used were analytical grade. For all the analysis, ultrapure water was purified using an Elix 3 coupled to a Milli-Q Gradient A10 system (Millipore, Bedford, USA). Purified water, 0.01 M HCl of pH 2.0, pH 2.1 simulated gastric fluid pH 4.5 sodium acetate and pH

6.8 sodium phosphate buffer solutions were prepared according to USP Pharmacopoeia[18].

Dissolution test conditions

Levetiracetam *sink* conditions were determined in different solvents. The solubility of the drug was tested using an amount of Levetiracetam equivalent a three times of the dose in the pharmaceutical formulation in 900 mL of Purified water, Hydrochloric acid 0.01 M, pH 2.1 simulated gastric fluid buffer, acetate buffer pH 4.5 and phosphate buffer pH 6.8. Then, all the dissolution medium was chosen to be tested in the drug release percent. Thus, stirring speeds of 50 rpm and 75 rpm for tablets were tested. For dissolution tests, 900 mL of each medium were deaerated in ultrasonic bath for 15 minutes and maintained at 37 ± 0.5 °C and USP apparatus, paddle were used for tablet dissolution. The test time was set on 60 minutes[22].

HPLC

Preparation of standard solution

The standard solution was prepared using an amount of levetiracetam reference standard about 111 mg was transferred to a 100 mL volumetric flask and it is dissolved and diluted with dissolution medium obtaining the final concentration of 1.11 mg mL^{-1}. The solution was filtered in a 0.45 µm membrane filter before the injection into the column.

Dissolution tests and HPLC validation

The dissolution tests were validated to levetiracetam tablets through the determination of specificity, linearity, intermediate precision, accuracy, robustness and solutions stability according to USP and ICH guidelines[18, 23].

Specificity

The dissolution tests specificity was evaluated by preparing samples of each placebo of the commercial formulation of tablets. These samples were transferred to separate vessels with 900 mL of the dissolution medium and stirred for 1 hour at 150 rpm using the respective method apparatus. The interference of the excipients of each formulation was evaluated by UV and HPLC. The evaluation of the HPLC method specificity was performed by preparing placebo tablets containing the same excipients of the commercial products. The solutions were prepared using the same procedure described for the sample solutions and injected three times.

Linearity

In order to assess the linearity of the method, seven doses of the reference substance (0.25; 0.50; 1.00; 1.25; 1.50 and 1.75 mg mL^{-1}) were used at HPLC method for the standard curves. The calculation of regression line was employed by the method of least squares.

Precision

The evaluation of the intermediate precision of the dissolution tests was performed using a well-characterized lot of the drug product of tight content uniformity and compared with the results of the dissolution tests. According to USP 28[18], the content uniformity was evaluated assaying ten tablets individually and calculating the content of levetiracetam of each one. For the HPLC method, the repeatability (between equipments) and intermediate precision (inter-assay) were determined by assaying samples of tablets, at the concentration (1.11 mg mL^{-1}), under the same experimental conditions as described earlier, during the same day and in three different days, respectively. The intermediate precision (inter-assay) was evaluated by comparing the assays on these two different days. The relative standard deviation (RSD) was determined.

Accuracy

The accuracy was evaluated by adding known amounts of the reference substance to the placebo sample in the dissolution medium at 80, 100 and 120% of the nominal assay of levetiracetam, corresponding to the concentrations of 0.88, 1.10 and 1.32 mg/mL, respectively. The accuracy was calculated as the percentage of the drug recovered from the formulation matrix and also expressed as the percentage relative error (bias %) between the measured mean concentrations and added concentrations. Each concentration was prepared in duplicate and was injected in triplicate.

Robustness and ruggedness

The robustness was tested by changing the following parameters of the HPLC method (one by one): mobile phase proportion – it was used pH 2.8 potassium dihydrogen orthophosphate buffer and acetonitrile (90:10, v/v) mobile phase; Change in pH of buffer – it was used pH 2.4 and pH 4.6; stationary phase – it was used a Develosil octa decyl silane UG (150 x 4.6 mm, 5 µm, Nomura Chemical Co Ltd., USA) and another liquid chromatograph–the quantitation was performed in a Agilent 1200 series, USA. Ruggedness of the method is determined by carrying out the analysis by two different analysts using the final parameters set for the dissolution analysis.

Solutions stability

The solutions stability was analyzed over a specified period of time, verifying the response of the sample solution stored at room temperature.

Dissolution profiles

The dissolution profiles were obtained after the determination of the best dissolution condition tests. Aliquots of 10 mL were withdrawn of each vessel and the same volume of the dissolution medium was replaced to maintain a constant total volume. The time intervals selected were 5, 10, 15, 30, 45, and 60 minutes. Twelve samples were assayed for each dissolution profile. The withdrawn samples were filtered through 0.45 µm membrane filter and injected (1.1 mg mL^{-1}) to HPLC system for quantification at 217 nm.

Release dissolution profiles comparison

The dissolution profiles were compared through the calculation of dissolution efficiency (DE) and model-independent simple method. The DE was calculated from the area under the dissolution curve at time t_i (measured using the trapezoidal rule) and expressed as a percent area of the rectangle described by 100% dissolution in the same time.

The model-independent simple method includes the difference factor (f_1) and the similarity factor (f_2). The f_1 factor measures the percent error between two curves over all time points. The percent error is zero when the test and drug reference profiles are identical and increase proportionally with the dissimilarity between the two dissolution profiles.

The f_2 factor is a logarithmic transformation of the sum-squared error of differences between the test and the reference products over all time points. This factor is 100 when the test and reference profiles are identical and tends to 0 as the dissimilarity increases. Two dissolution profiles are declared similar if f_1 is between 0 and 15 and if f_2 is between 50 and 100[24, 25] and it is calculated by using the following equations (1) and (2).

$$f_1 = [\{\sum_{t=1}^{n} |R_t - T_t| / \sum_{t=1}^{n} R_t\} x100] \quad \text{..... (1)}$$

$$f_2 = 50 \log[\{1 + 1/n \sum_{t=1}^{n} (R_t - T_t)^2\}^{-0.5} x100] \quad \text{..... (2)}$$

Where Rt and Tt are the percent drug dissolved at each time point for the reference and test products, respectively; n is the number of dissolution sample times and t is the time points for collecting dissolution samples.

Results and Discussion

The *sink* condition tested shows that levetiracetam bulk is soluble in Purified water, HCl 0.01 M, pH 2.1 SGF buffer, acetate buffer pH 4.5 and phosphate buffer pH 6.8. Then, dissolution test for levetiracetam tablets (product B) are performed using these dissolution medium at the stirring speed of 50 rpm using paddle apparatus, to investigate the drug release in different dissolution medium (Figure 2). The result shows that purified water is the best dissolution medium, since it provides greater stability and highest drug release percent.

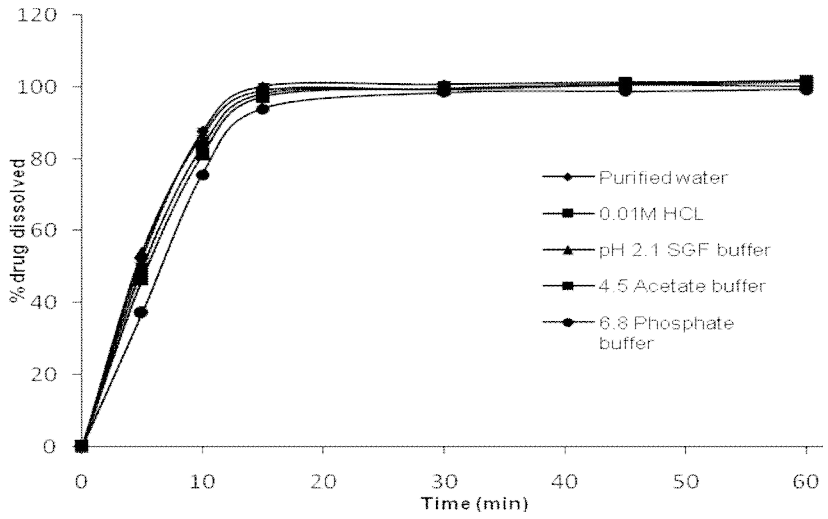

Figure 2. Dissolution profiles in different media (DPDM) of levetiracetam tablets using 900 mL medium with paddle apparatus at stirring rate of 50 rpm

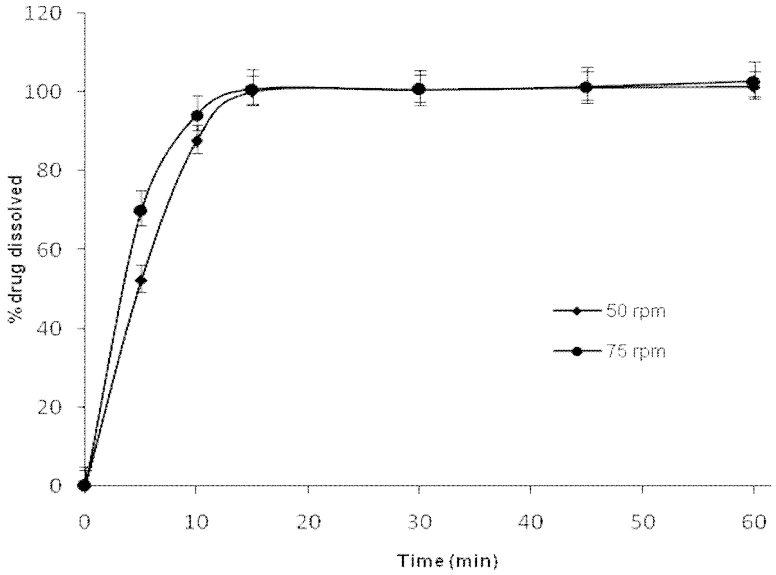

Figure 3. Dissolution profile in purified water at a stirring rate of 50 rpm and 75 rpm

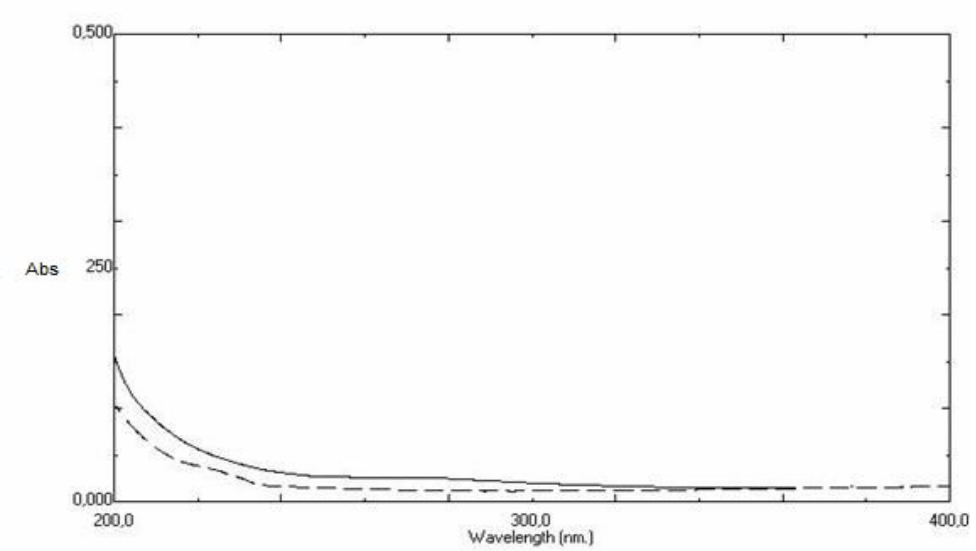

Figure 4. Absorbance vs wavelength specificity of levetiracetam tablets from the dissolution test by UV

For tablets, the paddle method is frequently used at 50 or 75 rpm[22]. Thus, stirring speeds 50 rpm and 75 rpm for product B (Figure 3) is tested. The statistical student's *t* test at 0.05 significance level is applied to compare the drug release percent (DR %), using 50 or 75 rpm for tablets. The P-values presented for tablets are greater than the delineated significance level, indicating that there is no statistically significant difference between the drug release percent and suggested that any of the stirring speed can be used, for products A, B and C. However, it is observed that stirring speed of 75 rpm presents high drug release percent until 30 minutes. Thus, the stirring speed of 50 rpm for tablets is chosen.

The reversed-phase liquid chromatography method is developed and validated for dissolution of levetiracetam tablets. The validation analytical parameters described in the guidelines[18, 23] are evaluated. The type of method and its respective use determine which parameters shall be evaluated and also it is the responsibility of the analyst to select the parameters considered relevant for each method.

The specificity of the dissolution test is evaluated through the analysis of placebo tablets from a dissolution test using the HPLC and UV methods. The analysis by UV shows that the excipients from tablets absorbed at 220 nm (Figure 4), which characterize interference in the analysis. So, the UV method cannot be use to quantify levetiracetam tablets from the dissolution tests.

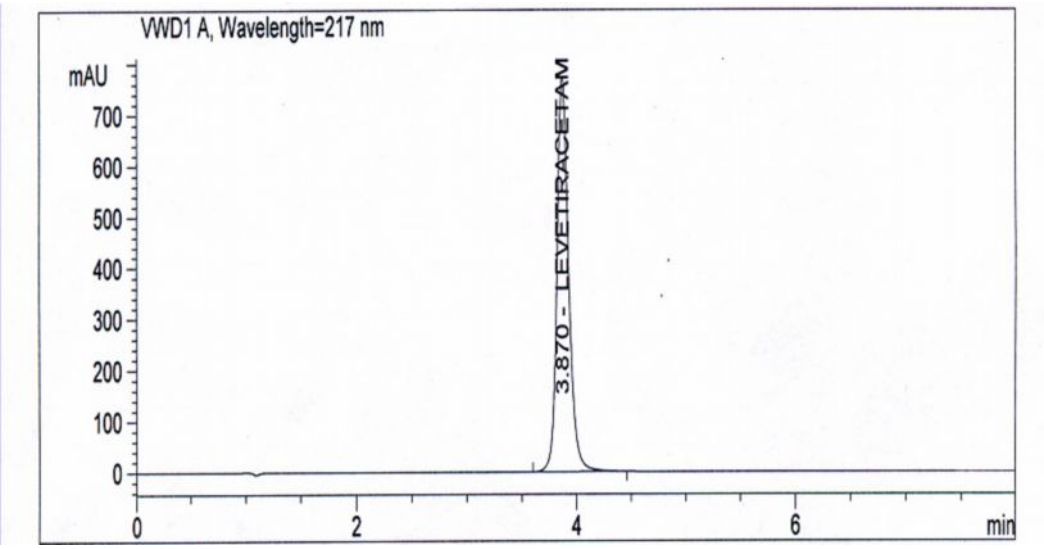

Figure 5. Chromatogram of levetiracetam standard in purified water showing λ_{max} at 217 nm

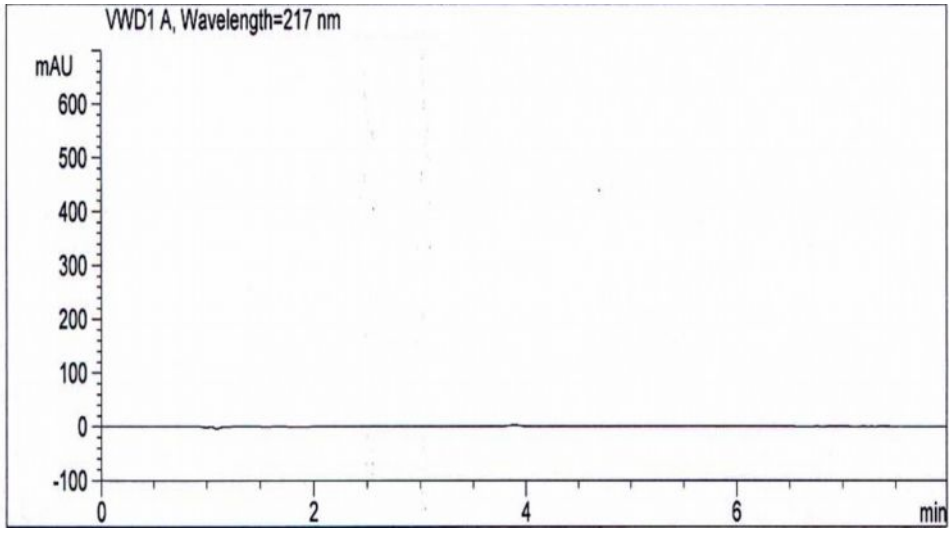

Figure 6. Chromatogram of placebo solution measured at 217 nm

The specificity test by HPLC demonstrated that the excipients from tablets do not interfere the standard levetiracetam peak (Figure 5). Thus, the HPLC method is useful to quantify levetiracetam in pharmaceutical formulation from the dissolution tests. The chromatogram obtained through the injection of the placebo solution does not present any other peak in the same retention time (3.87 minutes) of levetiracetam standard (Figure 6). The chromatographic peak purity tool is used in order to verify the purity. This tool works analyzing the peak and given a value between 0 and 1. The obtained value is 0.9999; this result shows that the analyzed peak was only levetiracetam, without interference. Thus, it is proved that the peak at 3.87 minute is not suffering interference of any excipients from the formulation.

To assess the linearity, three standard curves for levetiracetam were constructed, plotting concentrations (µg/mL) versus absolute area (mV s) and showed good linearity on the 0.25-1.75 mg mL^{-1} range. The representative linear equation is y=1E+06x—2916, where x is concentration and y is the peak absolute area. The correlation coefficient was r^2 = 0.999, indicating good linearity (Figure 7). The data are validated by means of the analysis of variance, which demonstrated significant linear regression and no significant linearity deviation ($p< 0.05$).

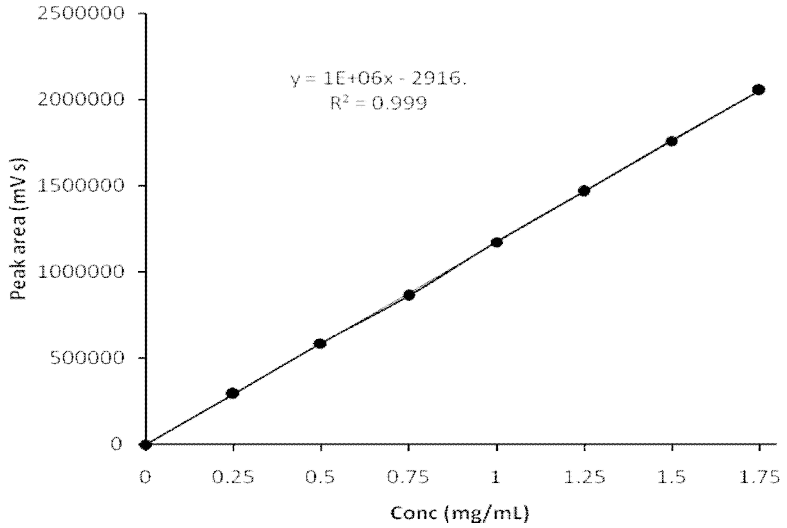

Figure 7. Linearity/calibration curve of levetiracetam

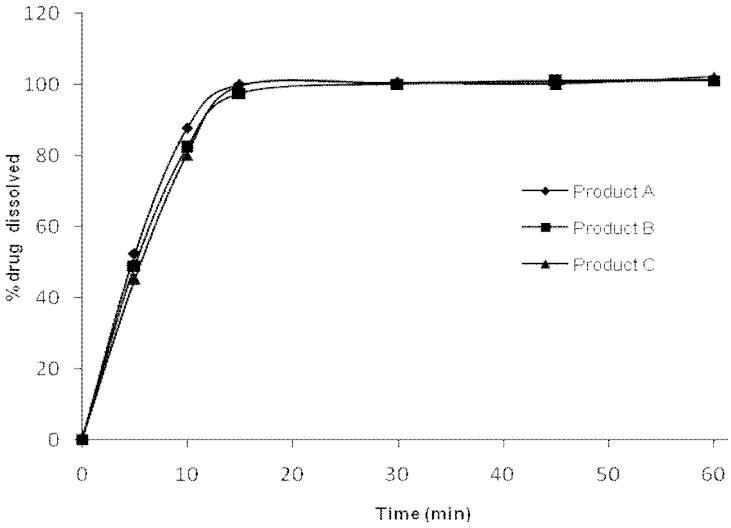

Figure 8. Mean dissolution profiles (*n*=6) of three batches (A, B, and C) in purified water at 37°C using paddle at 50 rpm

Table 1. Repeatability and intermediate precision of the dissolution method

Samples	% levetiracetam released			
	Inter-day precision		Between-equipments	
	Day 1	Day 2	HPLC A	HPLC B
1	99.8	100.1	99.8	99.1
2	100.4	99.7	100.4	100.5
3	100.1	100.2	100.1	99.6
4	100.6	100.3	100.6	100.4
5	101.2	101.1	101.2	100.2
6	101.4	101.3	101.4	101.4
Mean	100.6	100.5	100.7	100.2
% RSD[a]	0.10	0.10	0.14	0.13
Student's *t*-test		*t* calculated[b]		*p* value
Inter-days		0.37		0.71
Between equipments		0.93		0.37

[a]RSD = Relative standard deviation [b]*t* critic for $p = 0.05$ (2.23)

Table 2. Results from accuracy as recovery studies (n = 3) for levetiracetam tablets

Sample	Amount of reference (mg/mL)		Recovery (%)	*Mean (%)*	[a]*RSD (%)*
	Added	Recovered			
R1	0.88	0.879	99.89		
R2	1.10	1.112	101.09	100.40	0.21
R3	1.32	1.323	100.23		

[a]Relative standard deviation

Table 3. Ruggedness of levetiracetam in tablet dosage form

Samples	**% levetiracetam released**	
	Analyst 1	**Analyst 2**
1	100.1	99.5
2	99.7	100.6
3	100.2	100.3
4	100.3	98.9
5	101.1	99.8
6	101.3	99.2
Mean	100.5	99.7
[a]% RSD	0.1	0.1
[b]Student's *t*-test	t=2.00	p=0.07

[a]RSD = Relative standard deviation
[b]t critic for p = 0.05 (2.23)

Table 4: Comparison of levetiracetam tablet dissolution profiles through the dissolution efficiency (DE), difference factor (f_1) and the similarity factor (f_2).

Parameter	Product A (reference)	Product B	Product C
DE	91.7	90.2	88.5
f_1*	1.80		-
f_2*	95.45		-

* calculated between products A and B

The intermediate precision of the dissolution tests is verified through the comparison of the results of uniformity of content and the percentage drug release. The mean values found for the uniformity of content of product A, B and C tablets are 100.1% (RSD = 1.20), 99.5% (RSD = 1.04), and 101.6% (RSD = 1.31), respectively. The drug release percent are 101.4%, for product A, 101.1% for product B and 102.3% for product C (Figure 8). In all tests, almost all drugs are dissolved and the results show the good precision of the dissolution tests. The experimental values obtained for the determination of levetiracetam in samples are presented in Table 1. The low relative standard deviation (% RSD) of 0.10 (inter-day precision), and 0.13 (between equipments precision) shows the good precision of the method.

The accuracy is assessed from three replicate determinations of three different samples containing 0.88, 1.10 and 1.32 mg/mL of levetiracetam, giving concentrations respectively of 0.879, 1.112 and 1.323 mg/mL. The recoveries obtained with a mean value of 100.40% and bias lower than 0.21%, demonstrated that method is accurate for its intended use (Table 2).

The robustness of the method evaluated by changing the mobile phase proportion, pH 2.8 potassium dihydrogen ortho phosphate buffer and acetonitrile (85:15, v/v) demonstrated an increase on the retention time of the drug. The use of pH 2.4 decreases in retention time. The method is robust with these two modifications. When pH 4.6 is used, the retention time is about 4.3 minutes, but the peak becomes wide, probably because in this pH the drug may be in an ionized form. The effect of using Develosil octa decyl silane UG (150 x 4.6 mm, 5 mm, Nomura Chemical Co Ltd., USA) as stationary phase has increased the retention time in two minutes. Even so, the method is robust. The last experiment is the quantitation in another liquid chromatograph (Agilent 1200 series, USA) where the retention time is a little high (about 4.12 minutes), but it is possible to quantify the drug satisfactorily, confirming the robustness of the method. At that rate, it is possible to demonstrate that the developed method is robust with all the changes employed, except for the use of pH 4.6 in the mobile phase.

Ruggedness

Ruggedness of the method is determined by carrying out the analysis by two different analysts and the respective dissolution values are indicated by % RSD and statistical analysis was also done by using student's *t*-test, showing non-significant difference (p>0.05), as shown in Table 3.

The stability test of the solutions shows that levetiracetam is stable in purified water at least 24 hours at room temperature and this way it can be analyzed with precision during the dissolution assay.

The comparison of the dissolution profiles for the different products is well established. The results of dissolution efficiency (DE), difference factor (f_1) and the similarity factor (f_2) are presented in Table 4 for levetiracetam tablets. Since product A is the reference

brand, the factors f_1 and f_2 are calculated between product A and B. Two dissolution profiles are declared similar if f_1 is between 0 and 15 and if f_2 is between 50 and 100. The results of f_1 and f_2, 1.80 and 95.45, respectively, for the comparison of product A and B, showed that the profiles are almost similar. The dissolution efficiency is calculated for all products. The analysis of variance of the DE values shows that the profiles are almost similar for all the three products.

Typical acceptance criteria for the amount of drug dissolved are in the range of 75% to 80% dissolved in 30 minutes. Acceptance criteria including test times are usually established on the basis of an evaluation of the dissolution profile data[26]. In this article, it is observed that for all products a dissolution of 80% /15 minutes. So, this acceptance criterion is utilized.

Conclusion

In this work, a simple dissolution method is developed and validated dissolution tests and evaluated dissolution profiles for levetiracetam tablets. The use of 900 mL of purified water at 37 °C, paddle at the stirring speed of 50 rpm apparatus for tablets and 60 minutes of test provides satisfactory results for all products. The comparison of the obtained dissolution profile is realized by DE and the factors f_1 and f_2 and show that the profiles are almost similar for three products A, B and C. However, for all products the drug delivery is satisfactory, since at least 70% is dissolved in 30 minutes. The HPLC method is validated to the routine quality control of levetiracetam in tablets and is satisfactory in the quantitation of levetiracetam tablets from the dissolution tests contributing to assure the therapeutic efficacy of the drug. The UV method cannot be used, since it lacks specificity.

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