RESEARCH ARTICLE

A Correlative Dissolution Study of Oral Drug Delivery Systems through Reverse Phase-HPLC Analysis

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ABSTRACT

Oral drug administration is essential for treating diverse diseases due to its acceptable route, non-invasiveness, minimal off-target side effects, versatility, and painless patient delivery. However, multiple drug accessibility is increasing currently but there is often a deficiency of indication in developing procedures with assessment of two diverse medicinal formulations. Oral administration of a novel antiepileptic drug, levetiracetam in grouping with either L-dopa unaccompanied or L-dopa/ Ropinirole treatments was associated with dyskinesia. Altered shooting patterns of neurons relating to a compulsive harmonization process may back to the origin of dyskinesia. The current study aims to develop a dissolution technique and validate the antiparkinsonian drug-ropinirole and antiepileptic drug-levetiracetam tablets by RP-HPLC, which plays a major part in pharmaceutical formulations. The optimal dissolution conditions were experienced for the products respective to the pharmaceutical formulation and applied to assess the dissolution profiles. Ideal settings for the dissolution method were 500 mL of pH of 4.0 citrate buffer, 50 rpm and 250 nm for ropinirole tablets and 900 mL of distilled water, the rotation speed of paddle 50 rpm and 217 nm for levetiracetam tablets, then 3.84 and 3.87 minutes were set as the retention time of ropinirole and levetiracetam tablets, respectively. Technique optimization and validation process helps to reduce the bioequivalence of both dosage forms. The current study ensues detailed information on the drug release, even if there is a change in pH medium, filter, rpm, instrumentation, etc. Focusing on current pharmaceutical sciences, the proposed correlative dissolution study coupled with RP-HPLC analysis could offer a holistic insight into the performance of future oral drug delivery systems.

Keywords: Levetiracetam, Ropinirole, RP-HPLC, Dissolution efficiency, Method validation.

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INTRODUCTION

Dyskinesias denotes to a movement illness of a patient after Parkinson's disease (PD), causes uncontrolled shakes of part of a body or entire body in idiosyncratic patterns, tics or tremors, chorea, myoclonus, and dystonia often develops an acute exposure.[1] Typically, dyskinesias is a side effect of long-term levodopa treatment also termed levodopa-induces dyskinesia (LID), whilst it may also be caused by taking antipsychotic mediations called tardive dyskinesia (TD)[2]. Typically, most of the drugs undergo extensive first-pass metabolism, which may result in low bioavailability of drug. In addition, a high dose of drugs is given to patients per day several times, which may lead to an intricate medication process, increase the risk of morbidity and impaired drug absorption by PD patients due to gastrointestinal dysfunctions.[3] Indeed, such complications can be eliminated by developing an oral medicament, optimization of dissolution and validation.

Smith Kline Beecham developed ropinirole to treat PD and improve disease symptoms like stiffness, slowness and shaking.[4] Ropinirole is a non-ergolinic dopaminergic agonist with a strong affinity towards D2 and D3 receptors, indicating a monotherapy at the earlier stage of PD or as adjunctive therapy to levodopa.[5] The absorption of medicine from the drugs is proportionate to the release in GI for its biological activity. Since the first two stages are serious, dissolution by *in-vitro* stretches the calculation by *in-vivo* performance.[6] The measurement of ropinirole (Figure 1) under different situations is assessed by liquid chromatographic technique. A sequential administration of levodopa-ropinirole for 25 patients with PD in random order and 150% of DOPA alone. The dyskinesia time curves of both levodopa-ropinirole drugs resulted in more prolonged end-phase dyskinesia compared with L-DOPA alone.[7]

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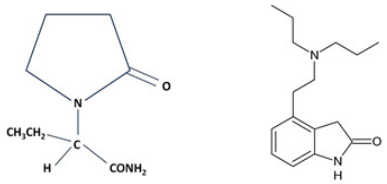

Figure 1: Chemical structure of ropinirole and levetiracetam

Levetiracetam (LEV), (S)-2-(2-oxopyrrolidin-1-yl) butanamide) is a second-generation antiepileptic drug approved by the FDA and an anticonvulsant drug used to treat partial, tonic-clonic and myoclonic seizures[8] (Figure 1). Levetiracetam prevents myoclonic jerks and general epilepsy in patients having photosensitive epilepsy.[9]

Recently, the dose adjustment of levetiracetam drug using physiology-based pharmacokinetics (PBPK) modeling for treating epilepsy (i.e. brain dysfunction) in pregnant women to maintain or protect therapeutic concentration which may lead to seizures. The study reported a PBPK model using plasma concentration and pharmacokinetics parameters to optimize the regimen dose of levetiracetam drug.[10] Similarly, the antidyskinetic activity of levetiracetam potentiate amantadine against levodopa (L-DOPA)-induced dyskinesia. As a result, the combination of levetiracetam and amantadine revealed an increase in antidyskinetic effect compared to monotherapy to minimize the adverse effect in Parkinson's disease patients.[11]

Using UV detection, the estimation of levetiracetam from other antiepileptic drugs was analyzed by RP HPLC.[12] Levetiracetam, an anticonvulsant drug administered along with levodopa alone or ropinirole/L-dopa reduces the neuronal harmonization of epilepsy which was studied in the marmoset model-MPTP.[13] The current study encompasses a correlation of validated dissolution methods using RP-HPLC analysis for ropinirole and levetiracetam tablets.

MATERIALS AND METHODS

Reagents and Materials

All reagents and materials used for analyzing dissolution parameters and validation of the method for ropinirole and levetiracetam are listed in Table 1.

Instrumentation

The instruments used for dissolution tests are mentioned in Table 2. The mobile phase used for ropinirole estimation was 70:30% v/v of pH 6.5 potassium dihydrogen orthophosphate and acetonitrile, 1.2 mL/min is flow rate, detection at 250 nm using PDA detector. In 100 µL injection volume is fixed for a sample and standard solution. Likewise, the mobile phase used for quantification of levetiracetam was made by adding 0.60 g of sodium 1 heptane sulphonate and 1.35 gm of potassium dihydrogen phosphate in 1-liter of water, mixed well and pH was adjusted to 2.8 with orthophosphoric acid and acetonitrile in the ratio 90:10% v/v. The flow rate is 1.2 mL/min and the injection volume is 10 µL. Both the mobile phase solutions were filtered through 0.45 µ filter paper.

Table 1: Materials and reagents used for dissolution method analysis

Materials used	*Ropinirole*	*Levetiracetam*
Reference standard	99.65% purity	99.4% purity
Tablets	Requip®, Glaxosmithkline, India	Keppra®, Teva Pharmaceuticals, USA
HPLC grade reagents	Hydrochloric acid, acetonitrile, sodium acetate and monobasic potassium phosphate	Millipore purified water

Optimized Dissolution Parameters

The optimized dissolution parameters for the analysis of ropinirole and levetiracetam tablets are mentioned in Table 3.

Standard Solution

In 50.01 mg of ropinirole reference substance was weighed and transferred to a 50 mL flask, which was dissolved and diluted to volume with methanol with a concentration of 1-mg/mL. Further, the stock solution was diluted to 10 µg/mL with dissolution medium and stored at 2 to 8°C. In the same way, 111 mg of levetiracetam reference standard was weighed and transferred to a 100 mL flask which was dissolved and diluted to a concentration of 1.11 mg/mL. Before injecting into the column, it was filtered through a 0.45 µ membrane filter. The chromatogram of ropinirole and levetiracetam standard in their corresponding dissolution medium is shown in Figures 2a and b.

Dissolution Tests and HPLC Validation

The following parameters are validated for the dissolution methods of both tablets.[14]

Specificity

Samples containing only a placebo were prepared to measure the specificity through the HPLC method. For ropinirole samples, 500 mL medium was taken and stirred at 50 rpm for an hour. For levetiracetam samples 900 mL medium was taken and stirred at 50 rpm for 45 minutes. The medium's volume change reflects the concentration of the corresponding samples.[15,16]

Linearity

Six ropinirole reference substances, concentrations in the 1 to 30 µg/mL range and levetiracetam standard substance in the 0.25 to 1.75 mg/mL range were made in their corresponding dissolution medium. To measure detector response, triplicates of standard solutions were injected and analyzed by HPLC. The least square method is employed for the determination of linearity.[17]

Precision

Six tablets of ropinirole and levetiracetam were subjected to a dissolution test for repeatability on the same day and two different days. Different HPLC instrument (Agilent 1200 series LC system) is also used for the determination of precision.[18]

Accuracy

Accuracy estimation was done by taking the placebo solution with known concentration of standard using dissolution

Table 2: Instrumentation used for the analysis ropinirole and levetiracetam tablets

Instruments used	*Ropinirole*	*Levetiracetam*
Disslution apparatus	Vankel multi bath (n=6)	C18 EPS Prontosil 150 x 4.6 mm
HPLC system	Shimadzu Prominence	
Detector	PDA SPD-M10 AVP	
Software program	Shimadzu Class VP V 6.14	
pH analyzer	Elico LII20	
HPLC Column	CTO-10AVP	

Table 3: Dissolution parameters followed for analysis of tablets

Dissolution parameters	*Ropinirole*	*Levetiracetam*
Dissolution medium	Purified water, 0.01 M HCl, pH 4.0 citrate buffer, pH 4.5 acetate buffer and pH 6.8 phosphate buffer	
Volume of media (mL)	500	900
Wavelength (nm)	250	217
Temperature (°C)	37 ± 0.5	
RPM	50,75	
Time (minutes)	60	

medium at 80, 100 and 120% assays of ropinirole and levetiracetam. The concentration of samples was calculated accordingly. Triplicate injections were made and injected into HPLC.

Robustness and ruggedness

The parameter robustness of an analytical technique remains unvaried in deliberate differences in conditions used in the analysis. It delivers a consistent signal in all analyses. Test samples of both tablets were filtered through different filters and centrifuged. All the samples were tested for the release of ropinirole and levetiracetam from the dosage form.[19] Two different analysts were involved in the analysis of the ruggedness of the dissolution method.[20]

Dissolution method

Using the above finalized dissolution parameters, both the tablets were examined. In 10 mL of sample were withdrawn from each vessel of the dissolution apparatus and the same volume of dissolution medium added into the flask at the time

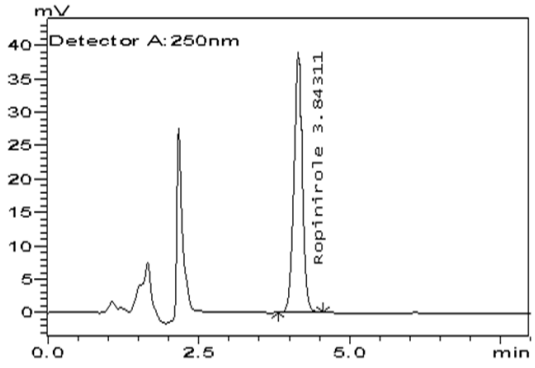

Figure 2a: Chromatogram of ropinirole standard in pH 4.0 citrate buffer

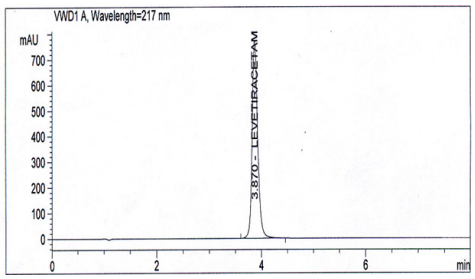

Figure 2b: Chromatogram of levetiracetam standard in purified water

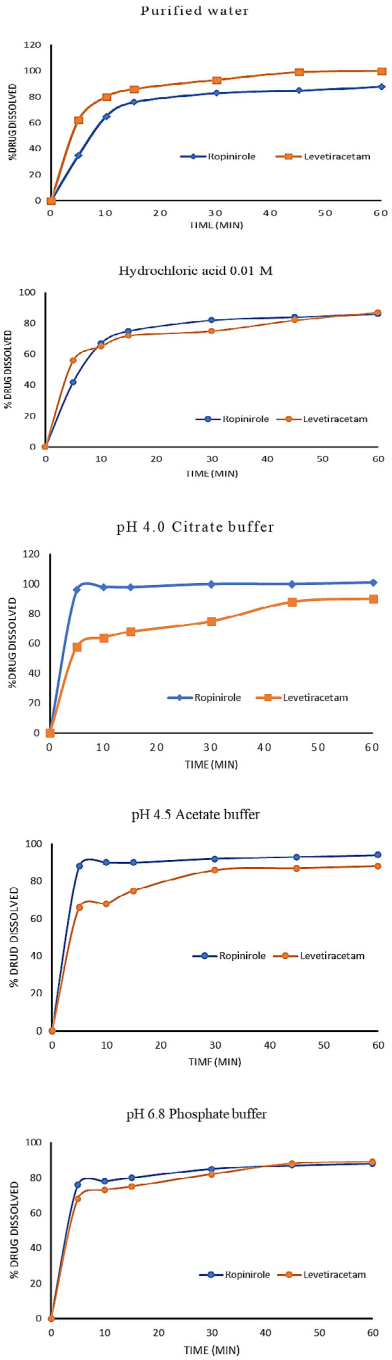

Figure 3: Parametric influence of different media on dissolution percentage

Table 4: Effect of dissolution medium of drug release percentage

Parameter	*%Drug release ± %RSD*	
Dissolution medium	*Ropinirole (%)*	*Levetiracetam (%)*
pH 4.0 citrate buffer	99.97 ± 0.98	94.42 ± 1.06
Hydrochloric acid 0.01 M	86.32 ± 1.02	87.15 ± 1.26
pH 4.5 acetate buffer	94.82 ± 0.88	88.75 ± 1.32
pH 6.8 phosphate buffer	88.34 ± 1.04	89.114 ± 0.38
Purified water	88.65 ± 0.96	100.65 ± 0.46

intervals of 5, 10, 15, 30, 45 and 60 minutes. The withdrawn samples were filtered and injected into HPLC to estimate the amount of ropinirole and levetiracetam.[21,22]

RESULTS AND DISCUSSION

The dissolution parameters confirmed that ropinirole and levetiracetam are freely soluble in purified water, 0.01 M hydrochloric acid, citrate buffer pH 4.0, acetate buffer pH 4.5 and phosphate buffer pH 6.8. The drug release percentages of ropinirole and levetiracetam tablets are presented in Figure 3. The drug release profile of both tablets in different mediums is presented in Table 4. It is observed that pH 4.0 citrate buffer is the best medium with maximum release percent for ropinirole tablets. In the same way, levetiracetam tablets are also assessed and the best dissolution medium with extreme release is observed in purified water.

The dissolution test was performed using the paddle method with 50 and 75 rpm for both tablets. The percentage drug release of tablets is compared for both rpm. Less than 0.05 significant level in student t-test is observed and there is no clear-cut variation in the drug release percentage for both tablets. Since the *p-value* obtained for tablets is larger than the demarcated significant value.

It is suggested that the stirring speed of the paddle at 50 rpm is fixed for both tablets (Figure 4). A study of piracetam capsules, levetiracetam and brivaracetam tablets using RP-HPLC method coupled with UV detection in the presence of other drugs as green technique is examined.[23] A novel gradient using the HPLC technique is developed to analyze the Ropinirole drug.[24] In this regard, the current study reports a reverse phase-HPLC analytical method is developed and validated for the dissolution of the combination of dual drug ropinirole and levetiracetam tablets. The specificity of the dissolution trial is estimated through the analysis of placebo using the RP-HPLC. The specificity test by the HPLC method proved that the placebo does not affect standard ropinirole and levetiracetam peaks (Figures 5a and b).

Standard curves for ropinirole and levetiracetam were raised to assess the linearity which shows respectable linearity with 1 to 30 µg/mL for ropinirole and 0.25 to 1.75 mg/mL for levetiracetam. Linear regression method of analysis was used to test the linearity with an observed correlation coefficient of 0.999 for ropinirole and 0.998 for levetiracetam (Figures 6a and b). No significant deviation with a *p-value* less than 0.05 was observed for both tablets.

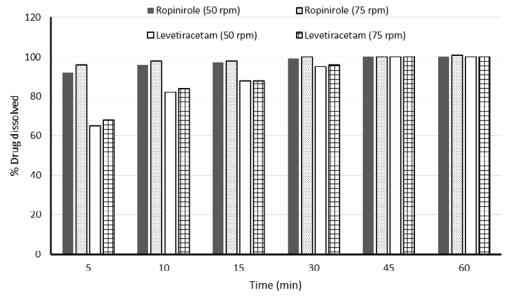

Figure 4: Dissolution efficiency of ropinirole and levetiracetam tablets using paddle speed at 50 and 75 rpm

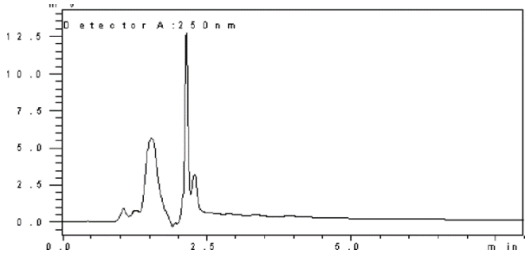

Figure 5a: Chromatogram of ropinirole placebo solution

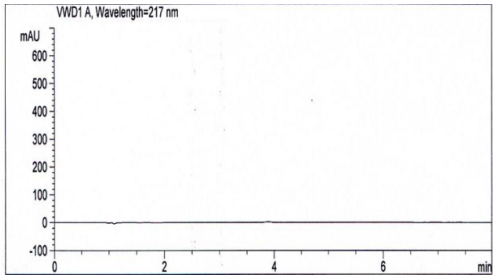

Figure 5b: Chromatogram of levetiracetam placebo solution

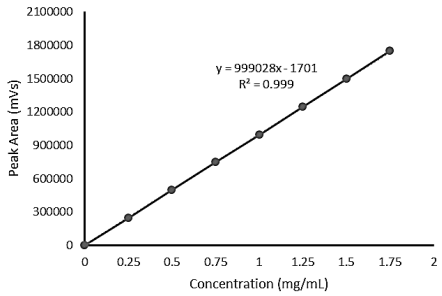

Figure 6a: Linearity of ropinirole

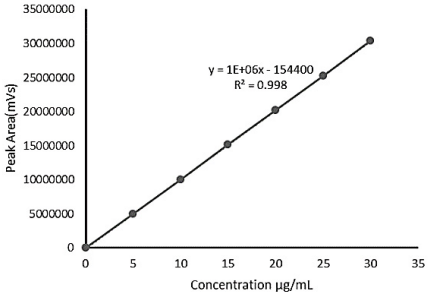

Figure 6b: Linearity of levetiracetam

Table 5: Exactness of the methodology

Samples	*%release of ropinirole*				*%release of levetiracetam*			
	Inter day precision		*Between equipment*		*Inter day precision*		*Between equipment*	
	First day	*Second day*	*HPLC A*	*HPLC B*	*First day*	*Second day*	*HPLC 1*	*HPLC 2*
1	102.2	100.3	102.2	100.3	100.6	100.3	100.6	100.4
2	103.1	101.1	103.1	100.2	101.2	101.1	101.2	100.2
3	103.4	101.3	103.4	101.1	101.4	101.3	101.4	101.4
4	97.8	95.1	97.8	94.3	99.8	100.1	99.8	99.1
5	100.2	98.2	100.2	97.5	100.4	99.7	100.4	100.5
6	101.1	100.2	101.1	99.6	100.1	100.2	100.1	99.6
Mean%	101.3	99.4	101.3	98.8	100.6	100.5	100.7	100.2
RSD%	0.35	0.39	0.35	1.02	0.10	0.10	0.14	0.13

Student's *t*-test

	Ropinirole		Levetiracetam	
T value for inter days	0.57	0.21	0.37	0.93
p-value Between equipment	0.86	0.63	0.71	0.37

Table 6: Accuracy values of recovery study

	Sample	*Amount of reference standard (mg/mL)*		*%Recovery*	*Average%*	*RSD%*
		Added	*Recovered*			
Ropinirole RS	R1	0.008	0.0079	101.26	101.03	1.01
	R2	0.010	0.0099	101.01		
	R3	0.012	0.0119	100.84		
Levetiracetam RS	R1	0.88	0.879	101.14	99.77	0.99
	R2	1.09	1.102	99.09		
	R3	1.22	1.231	99.10		

The six samples were analyzed for inter-day and between equipment performance to assess the intermediate precision. The mean percentage drug release of dissolution for inter-day precision was 99.4% of ropinirole and 100.5% of levetiracetam. The mean percentage drug release of dissolution between equipment precision was 101.3% ropinirole 100.7% levetiracetam. The percentage of drug release with RSD values for intermediate precision or exactness is represented in Table 5.

Accuracy is estimated by multiple-level accuracy test using three different standard solutions in triplicate in three separate levels comprising 0.008, 0.010 and 0.012 mg/mL of ropinirole and 0.88, 1.09 and 1.22 mg/mL of levetiracetam. The average recovery values obtained are 101.03% with an RSD of 1.01% for ropinirole and 99.77% with an RSD of 0.99% for levetiracetam. The observed accuracy values are mentioned in Table 6.

The test solution of both tablets was centrifuged and filtered through 0.1, 0.2 and 0.45 µ cellulose acetate filters and Whatman filters. The solutions of blank, standard, centrifuged sample and filtered samples were examined by LC method. The percentage of drug release was estimated and it is found within the limit of RSD 1.06%.

Two different analysts perform dissolution and estimate the drug release percentage. The values obtained are within the limit of % RSD and the t-test by students is used for statistical analysis with non-significant differences ($p>0.05$).

CONCLUSION

A simple dissolution method for ropinirole and levetiracetam tablets was developed and validated. The dissolution medium volume is 500 mL pH 4.0 citrate buffer and 900 mL purified water, temperature 37°C, paddle speed 50 rpm and the run time of dissolution test is 60 mins gives the appropriate value for both ropinirole and levetiracetam tablets. The dissolution method is validated using the HPLC method and it is optimal for both the tablets, which contributes to replacing many bioavailability studies during the formulation of dosage forms. In clinical applications, the administration of two different classes of drugs is common nowadays. The administration of levetiracetam, an anticonvulsant drug also used for the treatment of dyskinesia, which originated due to the presence of ropinirole induced to perform the present study. Eventually, the correlative approach of the current study elucidated a valuable insight into dissolution behavior coupled with RP-HPLC

analysis of oral drug delivery systems, leading to improved formulation design and therapeutic efficacy.

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