ejpmr, 2025, 12(7), 109-118

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN (O): 2394-3211

ISSN (P): 3051-2573

COMPARATIVE STUDY OF LEVETIRACETAM IN- VITRO DRUG RELEASE IN VARIOUS BRANDS OF PR TABLETS BY RP-HPLC

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Article Received on 03/05/2025 **Article Revised on 27/05/2025** **Article Accepted on 16/06/2025**

ABSTRACT

This research aims to assess and compare the in vitro drug release profiles of various brands of Levetiracetam prolonged release tablets utilizing reverse phase high-performance liquid chromatography (RP-HPLC). Levetiracetam PR tablets from different manufacturers were selected for in vitro drug release studies conducted in phosphate buffer at pH 6.0, with a mobile phase consisting of 10:90 acetonitrile and buffer. The released drug was collected, separated through chromatography, and detected using RP-HPLC. The drug release rates were calculated and compared among the different brands purchased from the market. The findings indicated a significant variation in drug release rates among the various brands of levetiracetam PR tablets, with some brands falling below the limits set by the USP monograph for controlled drug release, while others exceeded these limits. This study underscores the critical role of in vitro drug release testing in ensuring therapeutic equivalence. The results suggest that not all brands demonstrate similar drug release profiles, which may influence therapeutic efficacy and safety. RP-HPLC serves as a sensitive and selective method for comparing drug release from prolonged release formulations, thereby supporting formulation development and quality control.

KEYWORDS: This study underscores the critical role of in vitro drug release testing in ensuring therapeutic equivalence.

INTRODUCTION

Levetiracetam is a novel antiepileptic drug used to treat partial, myoclonic, and tonic-clonic seizures. In 2000, the FDA approved the use of the oral formulation as adjunctive therapy for the treatment of focal seizures, myoclonic seizures, and primary generalized seizures. In addition, the FDA approved intravenous levetiracetam (LEV) in 2006 for use in patients older than 15 years as adjunctive anticonvulsant therapy when the oral formulation is not tolerated. In Europe, it is approved for treating partial seizures as a single agent and as an add-on treatment for partial seizures, tonic-clonic seizures, and myoclonic seizures.[1] epilepsy is a group of disorder characterized by two or more unprovoked seizures. The estimated average prevalence of epilepsy in US is 6.8 per 1000, Europe is 5.5 per 1000, and Asia is 1.5 to 14 per 1000 people respectively. The older or first generation antiepileptic drugs like phenytoin, carbamazepine and sodium valproate are widely used but they have increased risk of adverse reactions and drug interactions.[2]

Due to the pharmacokinetic characteristics of LEV, including a wide reference range and low side-effect rate, therapeutic drug monitoring (TDM) of second-generation AEDs is not performed routinely in clinical settings.[3]

The antiepileptic drug (AED) levetiracetam was approved in November 1999 as addon therapy for the treatment of partial-onset seizures in adults based on its safety profile as well as its effectiveness. This review summarizes the safety and tolerability data from recently published phase III levetiracetam studies[4–5] that led to its approval and from additional data provided by the manufacturer.[6]

MATERIAL AND METHODS

The Levetiracetam peak should not exceed 2% relative standard deviation or five replicate injections. The tailing factor for the peak should not be more than 2%. To prepare the solution, accurately weigh and transfer sodium hydroxide, potassium dihydrogen phosphate, sodium hydroxide, buffer solution, mobile phase, and reference solution. For each tablet, withdraw 10 ml of the solution into a test tube, replace it with 10 ml of dissolution media, and filter it through a 0.45 nylon filter. The process involves placing 900 medium buffers into six dissolution bowls, equilibrating them at 37°C, and transferring one tablet to each bawl. At the end of 1, 2, 4, and 8 hours, a 15 ml aliquot (10ml Sample + 4 ml Rinse) is withdrawn from each bawl and replaced with the same amount of dissolution media. The solution is diluted to 20ml with mobile phase and injected into the system. The amount of Levetiracetam released at the end of 1, 2, 4, and 8 hours is calculated.

The mechanisms by which levetiracetam exerts its antiepileptic effects are not clearly defined. However, the most relevant mechanism of action is believed to be binding to a unique synaptic vesicle protein 2A (SV2A). SV2A protein is a part of secretory vesicle membranes that mediates calcium-dependent vesicular neurotransmitter release. The binding of levetiracetam to SV2A appears to decrease the rate of vesicle release.[7]

The mechanism of action of levetiracetam is different from first-generation and other second- generation anti-epileptic drugs (AEDs).[8]

It does not work by the three classic routes of other AEDs: sodium channel modulation, low- voltage-activated (T-type) calcium channel modulation, or direct gamma-aminobutyric acid (GABA) facilitation.[8]

In the other study, patients initiated treatment at the full dose level (levetiracetam 2,000 or 4,000 mg/day) (5). Patients were generally seen every 4 weeks during the studies.[4–6]

To evaluate the effect of concomitant AEDs, patients were separated into four groups of (1) LEV with neither VPA (broad-spectrum inhibitor of drug-metabolizing enzymes).[11]

All chemicals used were of analytical grade. Double distilled water was used throughout the experiments. Laboratory glassware was kept in a 10% v/v nitric acid solution overnight and rinsed with de-ionized water and dried in a dust-free environment before used.

2.1.1 Chemicals used

Table no.2.1: Chemical Used.

S. No.	Chemical	Grade
1	Potassium Dihydrogen Orthophosphate	AR Grade
2	Potassium hydrogen Orthophosphate	AR Grade
3	Potassium Hydroxide	AR Grade
4	Acetonitrile	HPLC Grade
5	Orthophosphoric Acid	AR Grade
6	Levetiracetam	IPRS

Levetiracetam pharmaceutical formulations were purchased from local drug stores. The levetiracetam tablets used in the dissolution test and for profile comparison were.

2.1.2 Apparatus used.

Table no 2.3: Apparatus used.

S.No.	Apparatus	Company/Model
1	Dissolution Apparatus	LAB INDIA DS 8000
2	HPLC	ThermoScientific Ultimate3000 UHPLC
3	pH Meter	Mettler Toledo
4	Sonicator	Spectro Lab
5	Nylon Filter	Nupore

2.1 METHODOLOGY

2.1.1 Initialisation of the RP-HPLC method

Dissolution tests were performed on levetiracetam Prolonged Release and Sustained Release Tablets according to the method provided by Indian Pharmacopoeia Commission.

2.1.2 *Dissolution parameters*

Table no 2.4: Dissolution Parameters.

DISSOLUTION PARAMETERS	
APPARATUS	Basket

DISSOLUTION MEDIUM	Phosphate buffer(ph-6.0)
MEDIA VOLUME	900 ml
ROTATION SPEED	100 rpm
SAMPLING INTERVALS	1 Hr, 2 Hr, 4 Hr, 8 Hr
LIMITS	At 1 Hr - 25% - 45% At 2 Hr - 45% - 65%, At 4 Hr - 60% -85%, At 8 Hr - NLT 80

System Suitability Parameters

The relative standard deviation for five replicate injections of the standard solution corresponding to the Levetiracetam peak must not exceed 2%. Additionally, the tailing factor for the Levetiracetam peak should also remain within a limit of 2%.

Preparation of 1N Sodium Hydroxide

Accurately weighed and transferred 4.142 g of sodium hydroxide into a 100 ml volumetricflask. Added 50 ml of Milli-Q water to dissolve and made up the volume to 100 ml. Marked, mixed well, and sonicated.

Preparation of dissolution media

Accurately weighed and transferred 54.435 g of potassium dihydrogen phosphate and theappropriate amount of sodium hydroxide into a media preparation bucket. Then added 8000 ml of DM water, mixed well, and adjusted the pH to 6.00 with 1 N sodium hydroxide solution.

Preparation of Buffer Solution

Accurately weighed and transferred 2.825 g of disodium hydrogen orthophosphate anhydrous into a 2000 ml mobile phase bottle. Added 2000 ml of Milli-Q water, mixed well, and sonicated. Then adjusted the pH to 3.5 with orthophosphoric acid and filtered it through a 0.45 µm membrane filter**.**

Preparation of Mobile Phase

Took 1800 ml of buffer solution and 200 ml of acetonitrile into a 2000 ml mobile phase bottle, mixed well, and sonicated.

Preparation of Reference Solution

Accurately weighed and transferred 17 mg of Levetiracetam IPRS into a 10 ml volumetric flask. Added 5 ml of Milli-Q water to dissolve it and made up the volume to 10 ml with Milli-Q water, mixed well, and sonicated.

Accurately pipetted out 3.0 ml of the solution into a 10 ml volumetric flask and made up the volume to 10 ml with the dissolution media, mixing well.

2.1.2.1 Preparation of Test Solution

For 1 hour) Accurately withdraw 10 ml of the solution into a test tube and replaced that 10 ml of the solution with 10 ml of dissolution media. Filtered it through a 0.45 µm nylon filter and followed this procedure for all 6 tablets**.**

For 2 hours) Accurately withdraw 10 ml of the solution into a test tube and replaced that 10 ml of the solution with 10 ml of dissolution media. Filtered it through a 0.45 µm nylon filter and followed this procedure for all 6 tablets**.**

For 4 hours) Accurately withdraw 10 ml of the solution into a test tube and replaced that 10 ml of the solution with 10 ml of dissolution media. Filtered it through a 0.45 µm nylon filter and followed this procedure for all 6 tablets**.**

For 8 hours) Accurately withdraw 10 ml of the solution into a test tube and replaced that 10 ml of the solution with 10 ml of dissolution media. Filtered it through a 0.45 µm nylon filter and followed this procedure for all 6 tablets**.**

2.1.3 Procedure

900 ml of medium buffers were placed into each of six dissolution bowls, and the temperature was set to 37°C to equilibrate. One Levetiracetam tablet was transferred into each dissolution bottle. The basket was lowered into the bowls, and the rotation speed of the basket was set at 100 rpm. At the end of 1, 2, 4, and 8 hours, a 14 ml aliquot (10 ml sample + 4 ml rinse) was withdrawn from each bowl, and the withdrawn solution was replaced with the same amount of dissolution media. The aliquots were filtered through 0.45 µm nylon filters. Five ml of the solution was dilutedto 20 ml with the mobile phase. The solution was injected into the system under the previously described operating conditions. The amount of Levetiracetam released at the end of 1, 2, 4, and 8 hours was calculated**.**

RESULT AND CONCLUTION

F1 and F2 data of levetiracetam PR tablets

Study result		
Marketer	**Difference factor(f1)**	**Similarity factor(f2)**
Intas	20.10905	47.43171

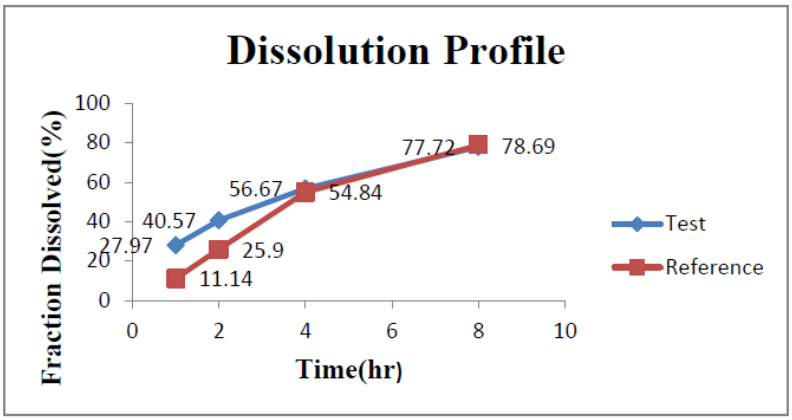
Lupin	22.73553	45.36627
Torrent	19.69279	49.37495
La Renon	31.59407	41.54613
Arinna	19.07135	53.63941
Abbott	39.16281	37.90813
Alkem	47.04813	33.79903
Cipla	30.92572	42.68744

GRAPH AND TABLE OF VARIOUS BRAND OF TABLETS

1. (INTASPHARMA/V2300644(500mg))

Table NO 3.3: Results for dissolution profile of sample A1 at different time intervals.

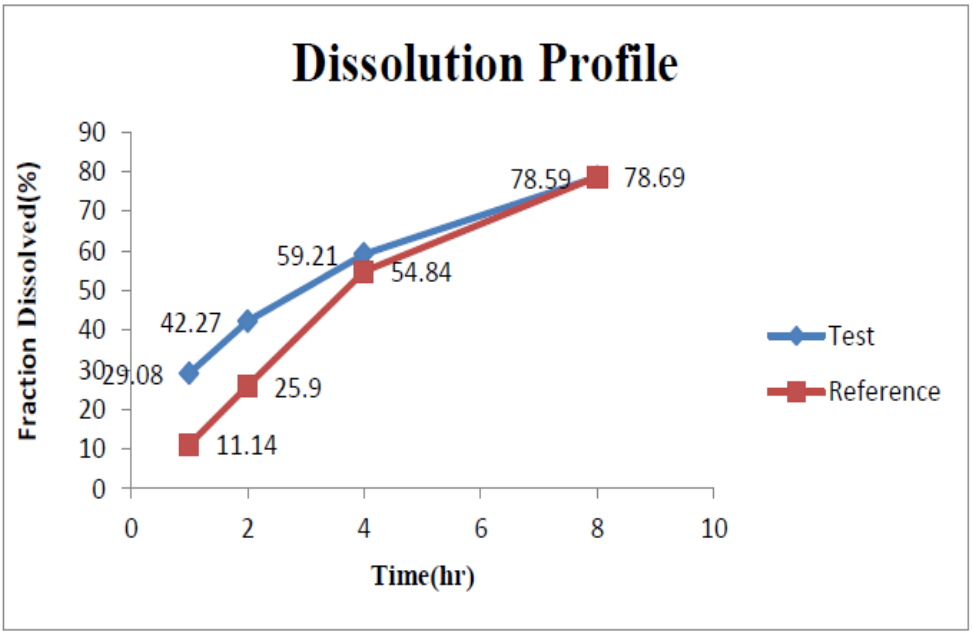
TEST READING	%at 1hr (25-45)	%at 2hr (45-65	%at 4 hr (60-80)	%at8hr NLT 80
TEST-1	26.84	39.19	54.94	72.54
TEST-2	28.23	41.26	57.68	75.42
TEST-3	29.68	42.35	59.44	77.72
TEST-4	28.50	41.31	57.34	76.98
TEST-5	27.14	39.60	54.68	73.38
TEST-6	27.14	39.73	55.91	76.35
Content(max)	**29.68**	**42.35**	**59.44**	**77.72**
Content(min)	**26.84**	**39.19**	**54.68**	**72.54**
Average % Label Claim dissolved	**27.97**	**40.57**	**56.67**	**77.72**



2. (LUPIN/J304512(500mg))

Table No 3.4: Results for dissolution profile of sample A2 at different time intervals.

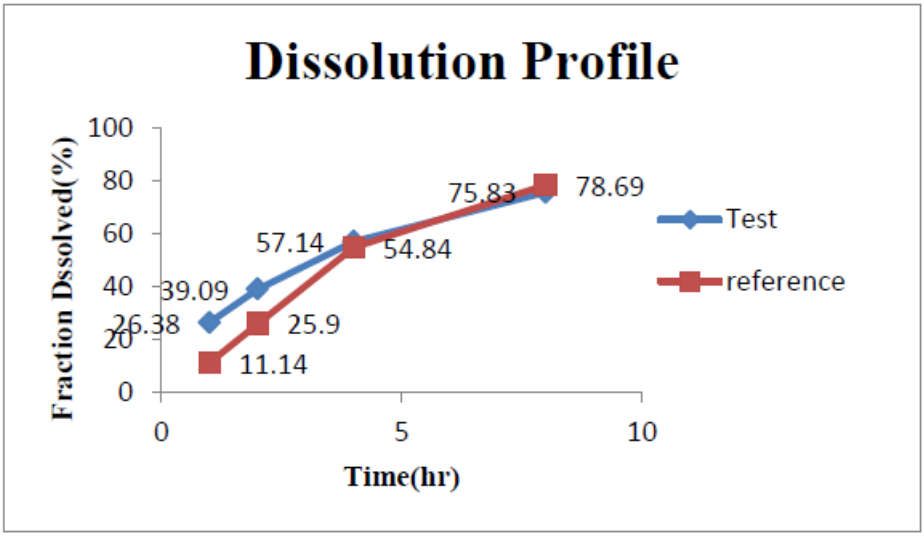
TEST READING	%at 1hr (25-45)	%at 2hr (45-65	%at 4 hr (60-80)	%ar8hr NLT 80
TEST-1	28.87	41.95	58.83	77.56
TEST-2	29.38	42,67	5874	79.70
TEST-3	29.12	42.75	59.85	79.14
TEST-4	28.19	41,01	57.93	78.54
TEST-5	29.10	42.10	59.03	78.14
TEST-6	29.80	43.14	60.87	78.47
Content(max)	**29.80**	**43.14**	**60.87**	**79.70**
Content(min)	**28.19**	**41.01**	**57.21**	**77.56**
Average % Label Claim dissolved	**29.08**	**42.27**	**59.21**	**78.59**



3. TORRENT/2PE5J004(500mg))

Table No 3.5: Results for dissolution profile of sample A3 at different time intervals.

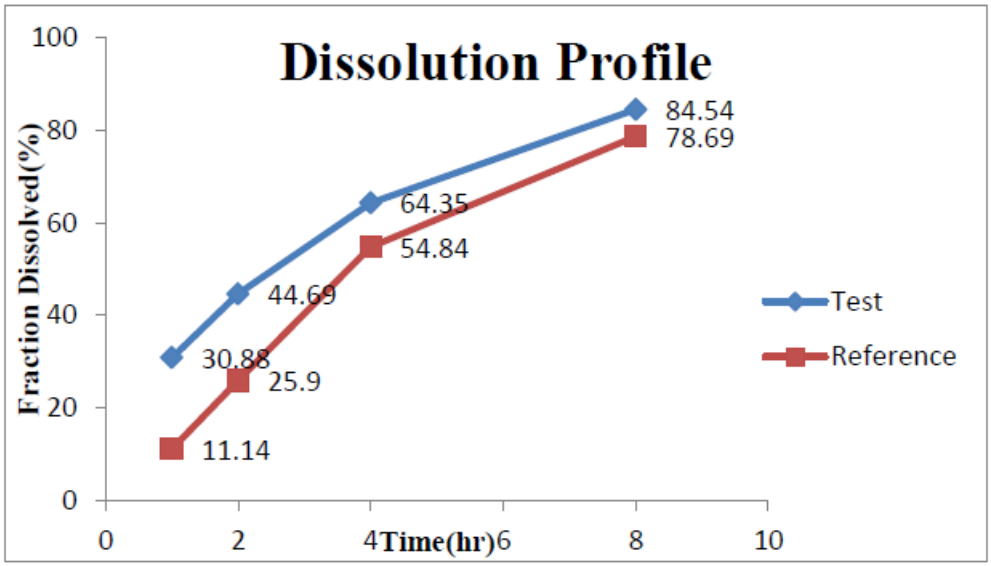
TEST READING	%at 1hr (25-45)	%at 2hr (45-65	%at 4 hr (60-80)	%ar8hr NLT 80
TEST-1	31.14	46.79	67.29	90.21
TEST-2	0.13	1.60	3.48	5.28
TEST-3	31.21	44.31	66.51	87.83
TEST-4	30.08	47.45	69.74	91.59
TEST-5	30.83	45.93	66.95	89.27
TEST-6	32.88	48.43	68.84	90.80
Content(max)	**32.88**	**48.43**	**69.74**	**91.59**
Content(min)	**0.13**	**1.60**	**3.48**	**5.48**
Average % Label Claim dissolved	**26.38**	**39.09**	**57.14**	**75.83**



4. (LARERON/D222109(1000mg))

Table No 3.6: Results for dissolution profile of sample A4 at different time intervals.

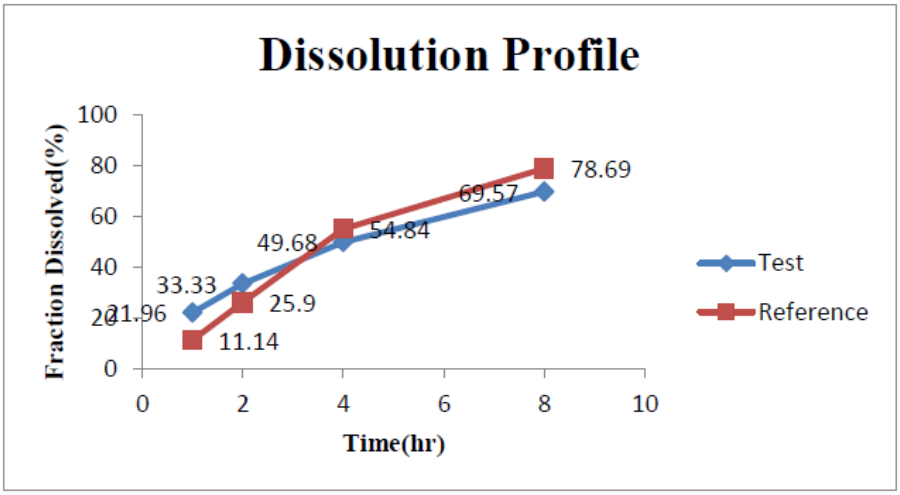
TEST READING	%at 1hr (33-53)	%at 2hr (45-65)	%at 4 hr (65-85)	%ar8hr NLT 80
TEST-1	30.91	45.23	65.20	83.80
TEST-2	31.14	45.33	65.03	85.84
TEST-3	30.91	44.61	65.48	85.62
TEST-4	30.60	44.55	64.54	85.29
TEST-5	31.12	44.69	63.68	84.66
TEST-6	30.61	43.73	62.17	82.00
Content(max)	**31.14**	**45.33**	**65.48**	**85.84**
Content(min)	**30.60**	**43.73**	**62.17**	**82.00**
Average % Label Claim dissolved	**30.88**	**44.69**	**64.35**	**84.54**



5. ARINNA/29AU02(1000mg))

Table No 3.7: Results for dissolution profile of sample A5 at different time intervals.

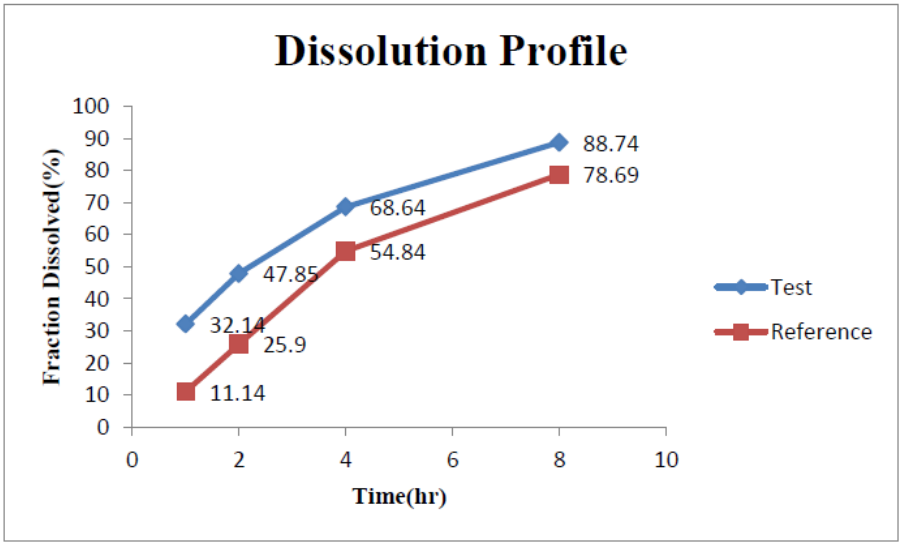
TEST READING	%at 1hr (33-53)	%at 2hr (45-65)	%at 4 hr (65-85)	%at 8hr NLT 80
TEST-1	22.25	33.63	49.82	70.20
TEST-2	22.41	34.03	49.75	69.74
TEST-3	22.11	33.67	50.39	71.66
TEST-4	20.85	32.56	48.75	66.54
TEST-5	21.75	32.37	48.71	69.37
TEST-6	22.38	33.70	50.65	69.93
Content(max)	**22.41**	**37.30**	**50.65**	**71.66**
Content(min)	**20.85**	**32.37**	**48.71**	**66.54**
Average % Label Claim dissolved	**21.96**	**33.33**	**49.68**	**69.57**



6. ABB0TT/PCH0398(500mg)

Table No 3.8: Results for dissolution profile of sample A6 at different time intervals.

TEST READING	%at 1hr (25-45)	%at 2hr (45-65	%at 4 hr (60-80)	%at 8hr NLT 80
TEST-1	32.26	48.59	68.00	86.79
TEST-2	30.67	46.36	67.11	86.39
TEST-3	32.95	49.10	69.08	89.64
TEST-4	31.67	47.33	68.10	88.98
TEST-5	32.14	46.78	68.74	90.69
TEST-6	33.15	48.93	70.82	89.95
Content(max)	**33.15**	**49.10**	**70.82**	**90.69**
Content(min)	**30.67**	**46.36**	**67.11**	**86.39**
Average % Label Claim dissolved	**32.14**	**47.85**	**68.64**	**88.74**

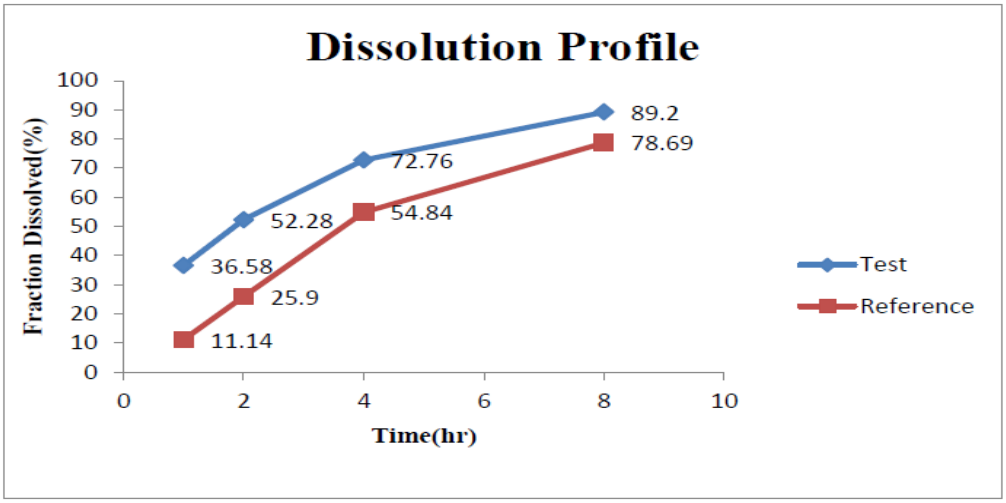


7. ALKEM/22443294(500mg)

Table NO 3.9 Results for dissolution profile of sample A7 at different time intervals.

TEST READING	%at 1hr (25-45)	%at 2hr (45-65	%at 4 hr (60-80)	%at 8hr NLT 80
TEST-1	35.46	51.32	71.28	86.67
TEST-2	36.67	52.54	73.06	89.24
TEST-3	36.51	51.76	72.82	90.97
TEST-4	37.08	53.17	72.50	9047

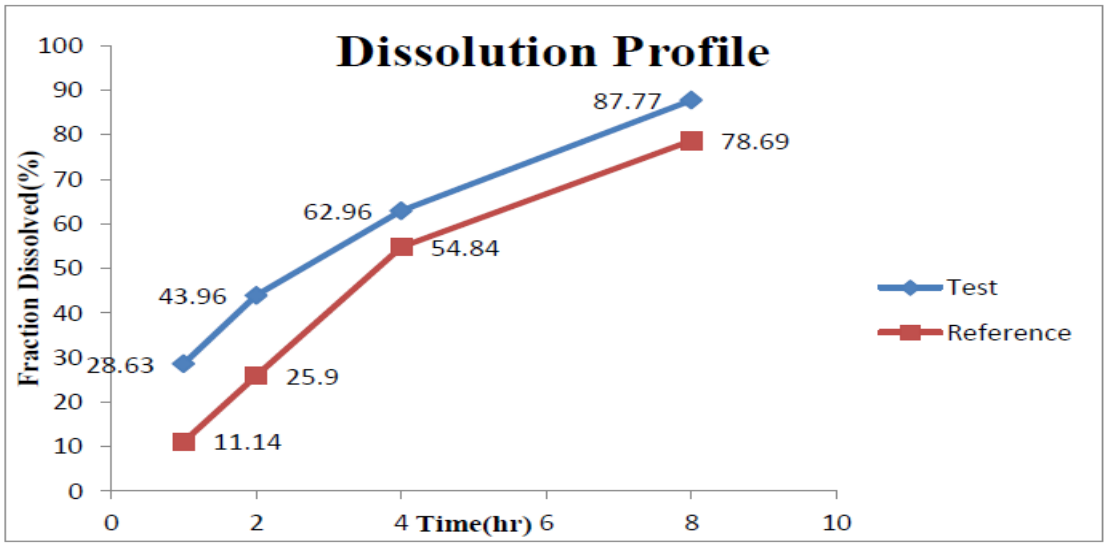
TEST-5	36.07	50.50	71.88	87.64
TEST-6	3769	54.41	75.04	90.18
Content(max)	**37.69**	**54.41**	**75.04**	**90.97**
Content(min)	**35.46**	**50.50**	**71.28**	**86.67**
Average % Label Claim dissolved	**36.58**	**52.28**	**72.76**	**89.20**



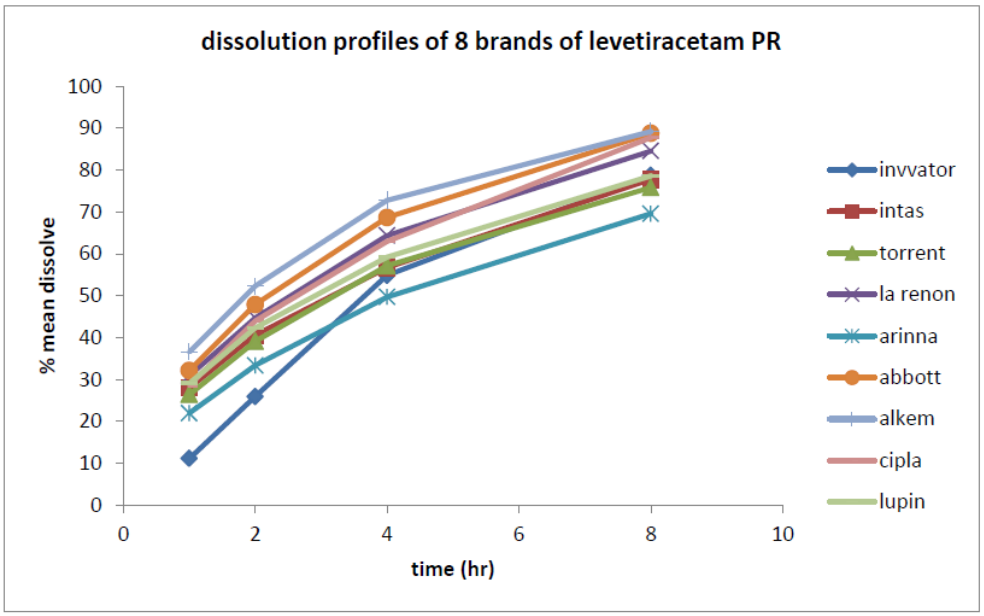
8. CIPLA/AFB23W87(750mg)

Table NO 3.10 Results for dissolution profile of sample A8 at different time intervals.

TEST READING	%at 1hr (33-53)	%at 2hr (45-65)	%at 4 hr (65-85)	%ar8hr NLT 80
TEST-1	27.73	43.40	57.72	88.23
TEST-2	28.56	44.20	64.23	89.27
TEST-3	28.68	44.16	64.26	87.61
TEST-4	28.57	43.64	63.32	86.12
TEST-5	28.90	44.01	63.91	87.50
TEST-6	29.36	44.36	64.30	87.90
Content(max)	**29.36**	**44.36**	**64.30**	**89.27**
Content(min)	**27.73**	**43.40**	**57.72**	**86.12**
Average % Label Claim dissolved	**28.63**	**43.96**	**62.96**	**87.77**



COMBINED GRAPH OF VARIOUS BRANDS OF TABLETS



CUMULATIVE DRUG RELEASE PROFILE OF ALL BRANDS

Brands	% at 1hr	% at 2 hr	% at 4 hr	% at 8 hr
Innovator	11.14	25.9	54.84	78.79
A1	27.97	40.57	56.67	77.72
A2	29.08	42.27	59.21	78.59
A3	26.38	39.09	57.14	75.83
A4	30.88	44.69	64.35	84.54
A5	21.96	33.33	49.68	69.57
A6	32.14	47.85	68.64	88.74
A7	36.58	52.28	72.76	89.20
A8	28.63	43.96	62.96	87.77

Cumulative Drug Release profile of all brands

2 CONCLUSIONS

1. There is considerable variability in the in vitro drug release profiles of levetiracetam PR tablets produced by different manufacturers.
2. Brand A demonstrated the highest release rate, whereas Brand F exhibited the lowest release rate.
3. This study underscores the necessity of conducting in vitro drug release assessments to ensure therapeutic equivalence among various brands of levetiracetam PR tablets.
4. The results imply that both healthcare professionals and patients should remain cognizant of the potential discrepancies in drug release profiles among the different brands of levetiracetam PR tablets.

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