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METHOD VALIDATION OF RELATED COMPOUNDS IN THE *PERAMPANEL* BY USING HPLC

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Abstract:

Simply rapid, precise, sensitive and reproducible High Performance Liquid Chromatography Method has been developed for the quantitative analysis of perampanel and its impurities was achieved on WatersAlliance-e2695, by using Zorbax SB C18(150x4.6mm,3.5µ) column and the mobile phase containing Water & Acetonitrile in the ratio of (1:1) v/v. The flow rate was 0.8 ml/min; detection was carried out by absorption at 220nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for perampanel and its impurities were NLT2000 and NMT2 respectively. %Relative standard deviation of peak areas of measurements always <2.0. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate& robust method for Quantitatively analysis of perampanel and its impurities and study of its stability.

Keywords: HPLC, Perampanel, Impurity-1, Impurity-2.

1. INTRODUCTION:

Active Pharmaceutical Ingredient is a substance intended to be used in the manufacture of a drug product and is responsible for eliciting the desired pharmacological activity [1]. Such substances are generally called drug substances and used to formulate the drug product which are consumed by the patients. The role of analytical chemist in the pharmaceutical industry plays an extremely important role in developing analytical methods that ensure the safety, efficacy, purity, stability and overall quality of the API and formulated drug products[2]. The presence of these unwanted chemicals even in small amounts may influence the efficacy and safety of the pharmaceutical products. Hence impurity profiling is a very important document and hence gaining critical attention from regulatory authorities. The impurity profile can be defined as “A description of the identified and unidentified impurities present in a new drug substance” [3].

The different pharmacopoeias, such as the European pharmacopoeia British Pharmacopoeia (BP) and the United States Pharmacopoeia are slowly incorporating limits to the tolerance levels of impurities present in the APIs or formulations [4]. The International Conference on Harmonization is a triplicate body of United States, European Union and Japan. The ICH Steering Committee includes observers from WHO World Health Organization, Health Canada and the European Free Trade Association [5]. ICH has published guidelines for impurities present in new drug substances, products and residual solvents. According to ICH guidelines for impurities in new drug products, the drug with a maximum dose of less than 1g/day

the identification threshold for unknown impurity is 0.10%. Between 1- 2 g/day the identification threshold is 0.1-0.05% and above 2 g/day it is0.05%[6].

Analytical methods/techniques used for impurity profiling study:

Identification, characterization and quantitative determination of impurities (and degradation products) in APIs and pharmaceutical formulations is one of the most important activities in modern pharmaceutical analysis. The reason for the increased importance of this area is that unidentified, potentially toxic impurities are health hazardous and in order to increase the safety of drug therapy, impurities should be identified and determined by selective methods.

The separation, identification and determination of impurities to lowest possible level in drug substance are done by various techniques. The separation techniques include various chromatographic techniques. These techniques are based on the separation of a mixture of species in a sample due to differential migration. Now-a-days, impurity profiling studies in drug substances are carried out by different spectroscopic Mass spectrometry and Nuclear magnetic [8]. The application of the techniques is dependent on the nature of impurities and API's. Quantification of impurities in drug substances is a need of pharmaceutical industry. In all chromatographic separations the sample is dissolved in mobile phase, which may be a gas, liquid, or a supercritical fluid.

In the present work, analytical chemistry research conducted to develop proper analytical methods for analyzing raw materials, intermediates, in-process checks, and impurity profiling while developing the process for the preparation of API's followed their validation The impurity profiling study of active pharmaceutical ingredient is carried out by GC and LC methods. Generally, the LC and GC techniques are mostly used for impurity profiling studies in drug substances because of simplicity, easy availability, less expensive and readily adapted to quantitative analysis.

2. METHODOLOGY:

PREPARATION OF SOLUTIONS

BUFFER PREPARATION: Dissolved 1.36 gm of solid Potassium dihydrogen phosphate in 1000 ml of water. Adjusted pH to 7.0 ± 0.05 with Potassium Hydroxide solution. Filtered through 0.45µm membrane filter. Solvent A: Buffer. Solvent B: Prepared a mixture of water and acetonitrile in the ratio of 20:80 v/v

PREPARATION OF MIX SOLUTION: Weigh accurately each 5.0 mg of PPD, PPP,BPP and of PMP reference standards into a 5 ml volumetric flask Dissolve and dilute to the volume with diluent and mix.

PREPARATION OF REFERENCE STOCK SOLUTION: Weigh accurately each 3.0 mg of PPP,BPP and 2.0 mg of PMP reference standard into a 100 ml of VF Dissolve and dilute to the volume with diluent and mix

PREPARATION OF SYSTEM SUITABILITY SOLUTION: Weigh accurately about 10.0 mg of PMP reference standard into a 20 ml volumetric Add 1.0 mL of reference stock solution dissolve and dilute to the volume with diluent and mix.

PREPARATION OF REFERENCE SOLUTION: Take 1.0 mL reference stock solution into a 20 ml volumetric flask dissolve and dilute to the volume with diluent and mix. Preparation of Test solution: Weigh accurately about 10.0 mg of test sample into a 10 mL volumetric flask, dissolve and dilute to the volume with diluent and mix.

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DETERMINATION OF WORKING WAVELENGTH: Maximum absorbance for all impurities determined by UV absorbance spectrophotometer and observed that all impurities

have maximum absorbance at 220nm

3. RESULTS AND DISCUSSION

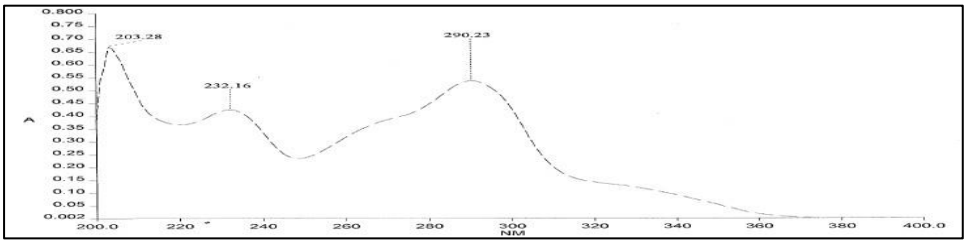

Fig. 1 PPP UV-VIS SPECTRUM (Des bromo pyridine impurity)

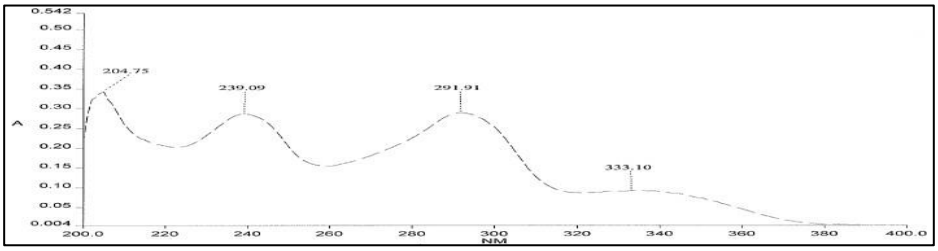

Fig. 2. BPP UV-VISSPECTRUM (Bromo pyridine)

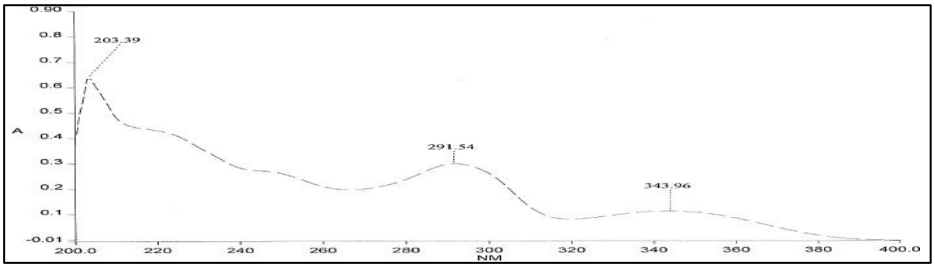

Fig. 3 PMP UV-VIS SPECTRUM (Perampanel)

TRIAL-1

TYPICAL CHROMOTOGRAPHIC CONDITIONS:

HPLC **:** A liquid Chromatograph equipped with variable wavelength detector.
Column **:** Luna Amine, 250×4.6mm, 5µm
Wavelength : 220nm
Flow rate : 0.8mL/minute
Temperature **:** 30ºC **Injection volume**: 10µl
Run time : 60 minutes
Diluent **:** water : Acetonitrile 80:20v/v

Table 1 Results for trial-1 chromatogram

Time (min)	Solution-A % v/v	Solution-B % v/v
0.01	95	5
5.00	95	5
45.00	5	95
50.00	5	95
55.00	95	5
60.00	95	5

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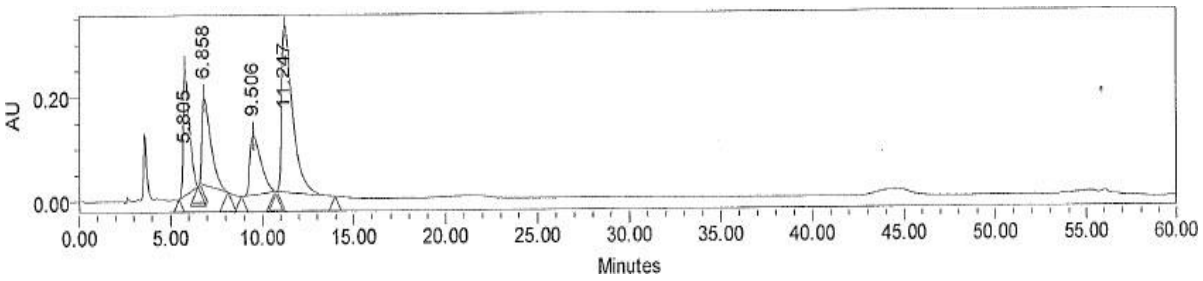

Figure 4 chromatogram of TRIAL-1

OBSERVATION: The resolution between PPD and PPP impurities is not good. All peaks are broad. Recommended to change the column and buffer to improve the resolution and peak shape.

TRIAL-2

TYPICAL CHROMATOGRAPHIC CONDITIONS:

HPLC : A liquid chromatograph equipped with variable wavelength detector.
Column : Zorbax SB Phenyl, 150 x 4.6mm, 5µm
Injection **:** 10 µL
Volume Run time : 60 minutes
Wavelength : 220nm
Flow rate : 0.8mL/minutes
Temperature : 30ºC
Diluent : Water : Acetonitrile 80:20 (v/v)

Table-2 Gradient programme

Time(min)	Solution-A % v/v	Solution-B % v/v
0.01	95	5
5.00	95	5
45.00	5	95
50.00	5	95
55.00	95	5
60.00	95	5

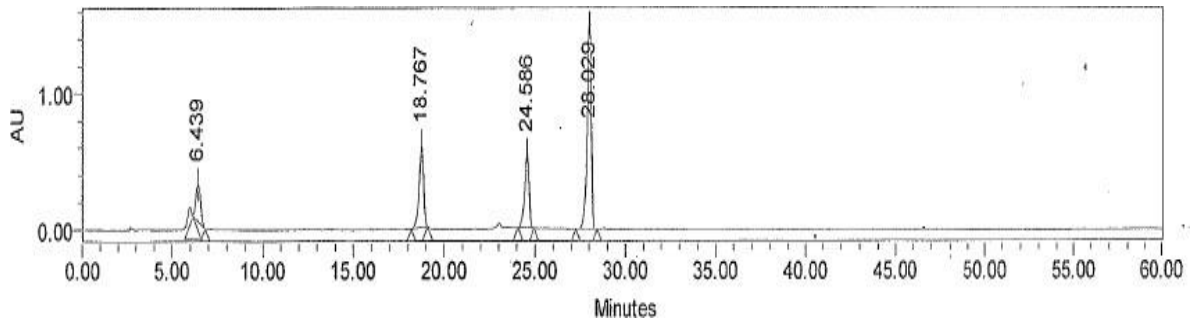

Fig. 5 Chromatogram of TRIAL-2

OBSERVATION: There is good separation between all impurities. Peak fronting observed for all impurities. To improve the peak shape recommended to change the buffer pH and column

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OPTIMIZED METHOD CHROMATOGRAPHIC CONDITIONS:

Column : Zorbax SB Phenyl, 150 x 4.6 mm, 5.0 µm or equivalent (Make: Agilent, Part No:883952-701)
Flow rate : 0.8 ml/minute Detector wavelength: 220 nm
Injection volume : 10 µL Column temperature: 30 ºC
Auto sampler temp : 5ºC
Elution mode : Gradient
Run time : 55 minutes
Diluent : Prepare a mixture of water and acetonitrile in the ratio (1:1v/v)

Table 3 Gradient programme

Time(min)	Solution-A % v/v	Solution-B % v/v
0.01	60	40
5.00	60	40
15.0	30	70
45.0	10	90
50.0	60	40
55.0	60	40

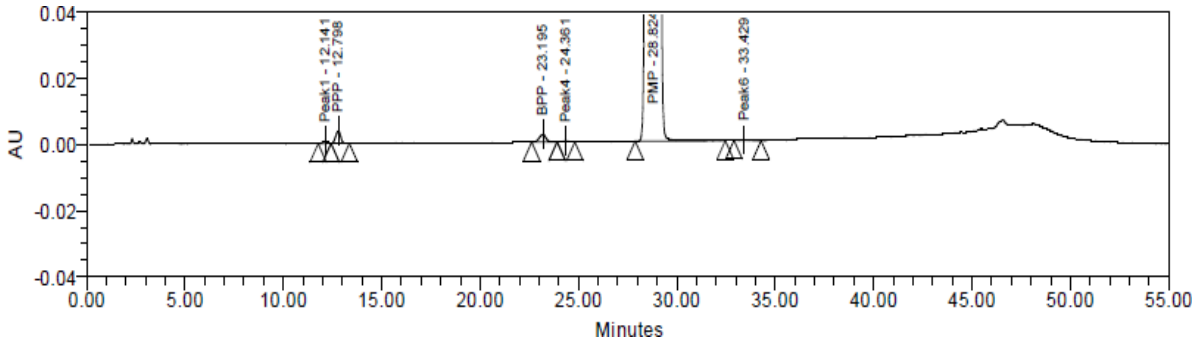

Fig. 6 Reference Solution chromatogram:

S.NO	RT	AREA	HEIGHT	%AREA	USP RESOLUTION	RT RATIO	NAME
1	12.141	11743	709	0.02		0.42	Peak 1
2	12.798	64765	3748	0.11	1.40	0.44	ppp
3	23.195	52918	2260	0.09	18.74	0.80	bpp
4	24.361	7071	308	0.01	1.84	0.85	Peak4
5	28.824	584921	214640	99.75	6.69	1.00	Pmp

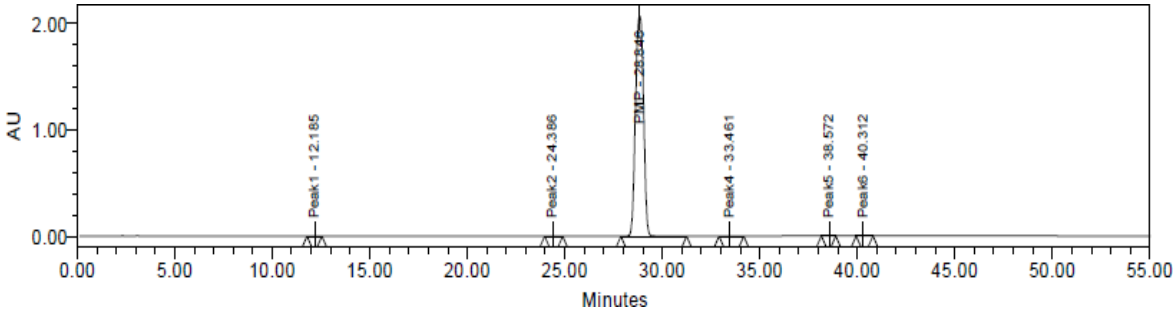

Fig. 7 Test Sample Chromatogram

Table 4 Results of Test sample chromatogram

S.NO	RT	AREA	HEIGHT	%AREA	USP RESOLUTION	RT RATIO	NAME
1	12.185	15894	864	0.03		0.42	Peak1
2	24.386	8952	362	0.02	21.18	0.85	Peak2
3	28.846	59167	2065285	99.93	6.45	1.00	PMP
4	33.461	7142	241	0.01	6.16	1.16	Peak4
5	38.572	5105	228	0.01	7.42	1.34	Peak5

Observations: All the Perampanel possible process impurities are well separated in this method with good resolution. The resolution between BPP and Perampanel is more than **2.0.** The USP tailing factor for all peaks is less than 2.0. The detection wave length was optimized by using the Diode array detector and it is found that Perampanel and related impurities have maximum response at 220nm wave length

Table 5 Results of Optimized Method Chromatogram

S.NO	Name of the compound	~ RT (Minutes	~ RRT
1	PPP	12.80	0.44
2	BPP	23.20	0.80
3	PMP	28.82	1.00

METHOD VALIDATION: Method Validation was carried out as per ICH guidelines

System suitability: Established system suitability / system precision by injecting standard solution for six times and calculated % RSD for peak area of all impurities.

Table 7 system suitability parameters

S.No	Area Counts PPP	Area counts BPP	Area counts PMP
1	45860	39411	49798
2	44408	38943	47825
3	44329	38592	47681
4	43517	37788	46560
5	42563	37121	45280
6	42428	36997	45689
Average	43851	38142	47139
St dev	1293.9	993.3	1657.0
% RSD	3.0	2.6	3.5

Observation: The %RSD for PPP is3.0, BPP is 2.6 and PMP

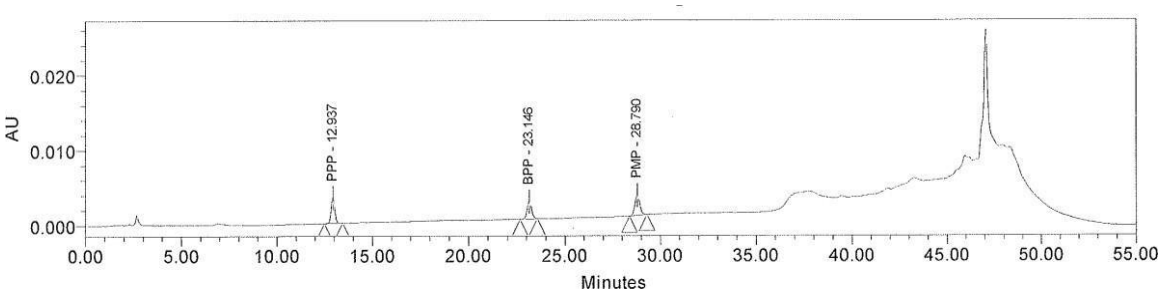

Fig. 8 Chromatogram of system suitability

Table 8 peak results of system suitability

S.No.	RT	AREA	HEIGHT	%AREA	USP RESOLUTION	RT RATIO	NAME
1	12.937	45860	3397	33.95		0.45	PPP
2	23.146	39411	2347	29.18	25.11	0.80	BPP
3	28.790	49798	2728	36.87	11.98	1.00	PMP
SUM		135069					

Specificity: Each known impurity solution and Perampanel standard solution was prepared individually at target concentration of the test sample. A solution of all known impurities spiked with the Perampanel test sample (Blend solution) was also prepared. All these solutions weyzed using the PDA detector as per the HPLC method.

Table: 9 PPP solution chromatogram

Component	Retention time	
	Blend solution	Individual solution
PPP	12.10	12.14
BPP	22.75	22.76
PMP	28.37	28.35

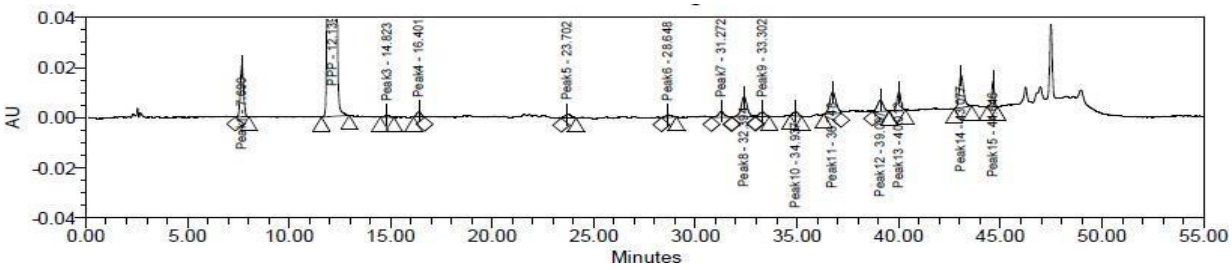

Fig.9. PPP solution chromatogram

S.NO	RT	AREA	HEIGHT	%AREA	USP RESOLUTION	RT RATIO	NAME
1	7.690	231788	20515	0.62		0.63	Peak1
2	12.139	359089	268229	96.54	13.59	1.00	PPP
3	14.823	13460	800	0.04	7.27	1.22	Peak3
4	16.401	34153	2268	0.09	3.80	1.35	Peak4

BPP SOLUTION CHROMATOGRAM:

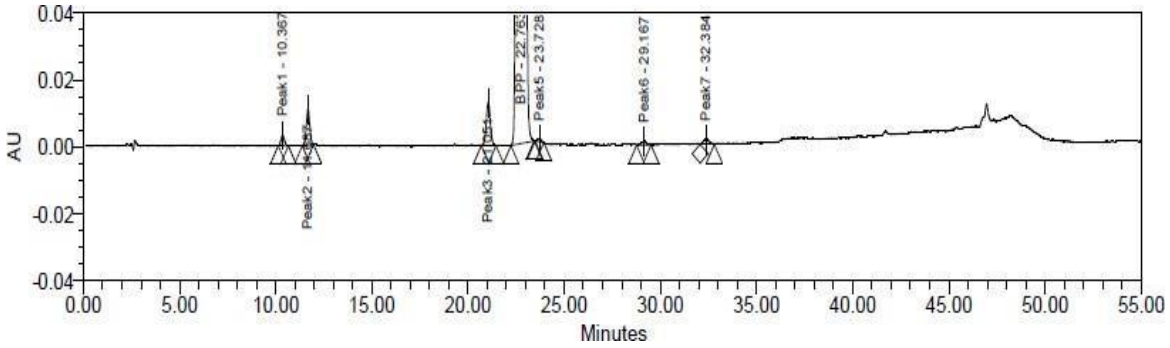

Fig. 10 BPP solution chromatogram

Table 11 results of BPP solution chromatogram

S.NO	RT	AREA	HEIGHT	%AREA	USP RESOLUTION	RT RATIO	NAME
1	10.367	38080	3050	0.09		0.46	Peak1
2	11.687	10589	10589	0.32	3.87	0.51	Peak2
3	21.051	12785	12785	0.47	25.16	0.92	Peak3
4	22.763	251567	251567	98.97	4.08	1.00	BPP

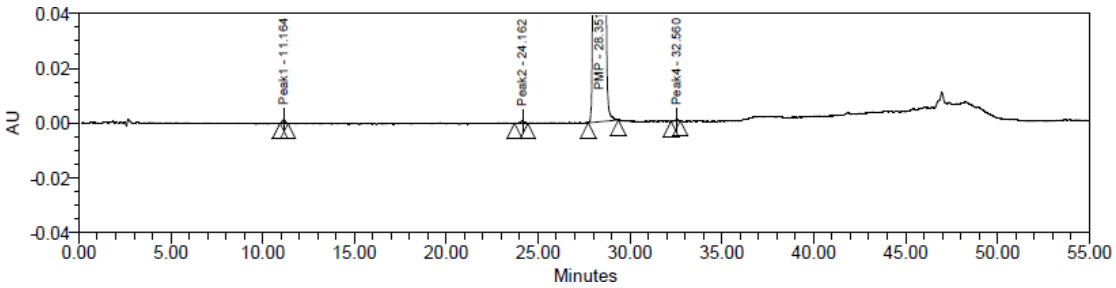

Fig. 11 PMP solution chromatogram

Table 12. Results of PMP solution chromatogram

S.NO	RT	AREA	HEIGHT	%AREA	USP RESOLUTION	RT RATIO	NAME
1	11.164	16468	1405	0.02		0.39	Peak1
2	24.162	15477	842	0.02	28.42	0.85	Peak2
3	28.351	77466	4195427	99.95	7.95	1.00	pmp
4	32.560	5844	453	0.01	10.91	1.15	Peak4

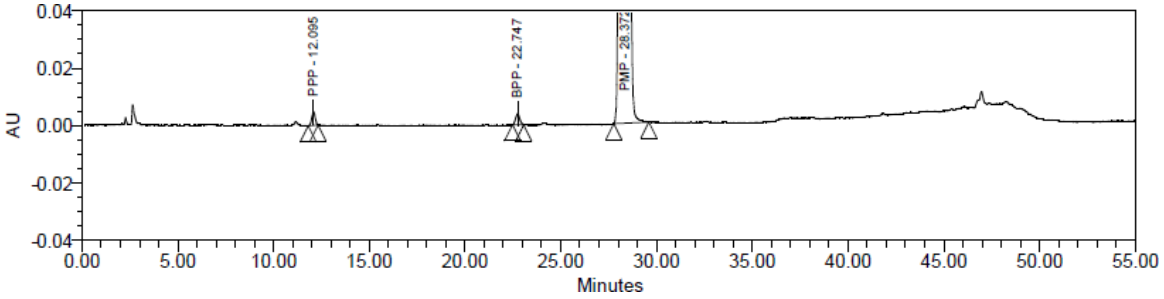

Fig. 12 PMP/Blend solution chromatogram

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Table 13. results of PMP /blend solution chromatogram

S.NO	RT	AREA	HEIGHT	%AREA	USP RESOLUTION	RT RATIO	NAME
1	12.095	58592	4683	0.08		0.43	PPP
2	22.747	58162	3819	0.08	26.96	0.80	BPP
3	28.372	71191006	3919261	99.84	12.75	1.00	PMP
sum		71307759					

Observations and interference:

No interference was observed due to the blank at the retention time of Perampanel and it's known impurities peaks. The elution order and retention times obtained from individual solution and blend solution were comparable. Peak purity passed for all impurities obtained from individual solutions. It is observed that the proposed method is specific and capable to separate all the impurities.

Test Solution Stability

Perampanel test sample spiked with impurities was taken and analyzed for solution stability at about 12hrs and results are mentioned below table. Observation: Test solution was stable up to 12hrs at room temperature. Acceptance criteria: The variation of content (%) of each impurity obtained from solution stability study and initial result (fresh sample) should be meet ± 30.0% of the specification limit.

Name of the Impurity	Initial Fresh results%	After12hrsResults%	Variation
PPP	0.11	0.11	0.00
BPP	0.09	0.09	0.00
MSUI	0.02	0.02	0.00
TI	0.25	0.24	0.01

Accuracy of the method was proved by checking the % recovery of each impurity in test solution, spiked with each impurity at QL level, 100% level and 150% level.

TABLE 14 ACCURACY:

% of Recovery	Accuracy at		
	QL Level	100% Level	150% Level
PPP	107.4	99.6	99.7
BPP	85.1	101.3	100.7

Observation: The % recovery of all impurities was within the limit. Acceptance criteria: The % Recovery should be between 80 to 120 for QL level to 150% level.

4. SUMMARY AND CONCLUSION

	PARAMETERS	Perampanel	PPP	BPP	LIMITS
System suitability parameters	USP resolution	6.69	1.40	18.74	NLT 2.0
	Test Sample Chromatogram	6.45	6.16	21.18	
	USP tailing	1.00	0.44	0.80	NMT 2.0
	%RSD	3.5	3.0	2.6	NMT 5.0
Specificity	Specificity	No interference peaks observed at their retention time	No interference peaks observed at their retention time	No interference peaks observed at their retention time	No Interference Peaks were observed at their retention times when compared to that of their individual standard retention times
Test solution stability	Initial	-	0.11%	0.09%	±30.0% Specification limit
	After 12hrs	-	0.11%	0.09%	

% of Recovery	Accuracy at			
	QL Level	100% Level	150% Level	LIMITS
PPP	107.4	99.6	99.7	80-120
BPP	85.1	101.3	100.7	

Presicion parameters	PPP	BPP	LIMITS
Method Presicion	0.12%	0.10%	NMT 10%
Intermediate Precision	0.12%	0.10%	NMT 10%

FORCED DEGRADATION STUDIES	
Acid degradation	NOT DETECTED
Base degradation	NOT DETECTED
Peroxide degradation	NOT DETECTED
Thermal degradation	NOT DETECTED
Photolytic degradation	NOT DETECTED
Humidity degradation	NOT DETECTED

5. CONCLUSION

A Specific, Selective, Accuracy, Precise, rugged, Robust and Stability indicating method was developed for the determination of related compounds in the perampanel by using HPLC.

This method was optimized after many trails because in this all related substances were well separated present in the drug substances. For the optimized method the used column was ZorbaxSBPhenyl,150×4.6mm5.0µm in gradient pump mode with flow rate was 0.8ml/min and injection volume was 10µl with run time was 55min.The retention time of perampanel is about 28.8min which was confirmed by comparing with other standard drug. This method was validated by using all the parameters like specificity, accuracy, precision, LOD etc. The results obtained in all parameters were within the acceptance criteria.

This method was specific, because there were no other interference peaks at their retention times of all related substances in the drug substances and the retention times for all related substances were confirmed by injecting the all individual impurities separately. This method was linear for the determination of related substances because the correlation coefficient for all the related substances and drug substances was NLT 0.990.This method was accurate because the related substances and drug substance were recovered inbetween80 to 120%according to specification.

This method was precise for the determination of related substances because for all precise conditions the %RSD should be less than 10.0%for all related substance. This method was stable, it was confirmed by forced degradation conditions under various stress conditions like Acid, Base, Peroxide, Thermal, Humidity, Photolytic conditions. In all the conditions there were no any interference degraded peaks at their retention times of all related substances the hence conclude.

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