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STABILITY SIGNALLING THE DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS FOR THE HPLC-BASED DETERMINATION OF RELATED COMPOUNDS IN THE PERAPANEL

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Abstract:

The goal of the presented work is to develop and validate the chromatographic methods for determination of process as well as degradation related impurities in API's. It also provides the valuable information about the product stability. In research work the forced degradation or stress testing is undertaken to demonstrate specificity when developing stability-indicating methods. These studies also provide information about the degradation pathways and degradation products that could form during storage. Forced degradation studies may help facilitate pharmaceutical development as well in areas such as formulation development, manufacturing, and packaging, better understanding of the potential liabilities of the drug molecule chemistry, and the resolution of stability related problems. 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 and Robustness

1. INTRODUCTION

Most of the active pharmaceutical ingredients are produced by organic chemical synthesis. Various components, including residual solvents, trace amounts of inorganic and organic components can be generated during such process. Impurities in pharmaceuticals are the unwanted chemicals that remain with the active pharmaceutical ingredients or develop during formulation, or upon aging of both API and formulated APIs to medicines. 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].

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 resonance spectroscopy and chromatographic techniques TLC, HPLC, GC, LC-MS and GC-MS [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.

This mobile phase is then forced through an immiscible stationary phase, which is fixed in a column or on a solid surface. The two phases are chosen so that the components of the sample distribute themselves between the mobile phase and stationary phases to varying degree. The components which are strongly held by the stationary phase moves slowly whereas the components which are weakly held by the stationary phase moves faster and migrates to detector. Due to these differences in migration rates, sample components separate into discrete bands or zones, which can be analyzed quantitatively and qualitatively.

The classification of chromatographic methods is based on the type of mobile phase, type of stationary phases and the type of equilibria involved in the transfer of components between phases. Based on the above-mentioned criteria, there are three general categories of chromatography: gas chromatography, liquid chromatography, and supercritical fluid chromatography. In GC, LC, and SFC techniques gases, liquids, and supercritical fluids, respectively are the mobile phases.

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.

High Performance Liquid Chromatography: HPLC

High Performance Liquid Chromatography HPLC [9] is a physical separation technique conducted in the liquid phase in which a sample is separated into its constituent components by distributing between the mobile phase and a stationary phase sorbent packed inside a column. An online detector monitors the concentration of each separated component in the column effluent and generates a chromatogram.

HPLC is now one of the most powerful techniques in analytical chemistry and it has been most widely used for the quantitative analysis of pharmaceuticals, biomolecules, polymers, and other organic compounds.

Stationary Phases in RP-HPLC

Selecting an appropriate stationary phase can also help to improve the efficiency of method development in HPLC analysis. The stationary phase can be a solid, a liquid, or a bonded phase i.e., stationary phase chemically bonded to a support that is used for the separation. The most

common columns are packed with silica particles. The silica is the most widely used as the base material for bonded phases [10]. The alkyl silanes materials are most widely used as bonded phases which are coated on a packing materialconsistingof3-10μmporoussilicaparticles [11].Polymethyl methacrylate poly-styrene-divinyl benzene, methacrylate, hydroxyethyl methacrylate alumina, carbon, and other polymeric and inorganic materials are also used as base materials. The particles may be regular or irregular in shape. Spherical particles are more popular, as are the smaller particle sizes usually about 3, 5, or 7 μm . The particle size plays a vital role on separation. Larger particles will generate less system pressure and smaller particles will generate more pressure.

Stationary phases used in Research work:

The non-polar (C2, C8, and C18) and polar stationary phases (Cyano and Phenyl) used in HPLC method development for the research work. The non-polar hydro carbonaceous moieties of low polarity are ethyl (or butyl), octyl (C8), and octadecyl (C18) linear alkanes. The octadecyl packing is used when maximum retention is required and has unique selectivity[12]. By contrast, the ethyl group is used in applications that involve very strongly retained solutes. Octyl packings are a good compromise for the separation of samples with wide-ranging polarities.

2. MATERIALS AND METHODOLOGY:

Table: 1 List of drug and possible impurities used in HPLC:

S.NO	DRUG/Possible impurities	MANUFACTURER
1	PERAMPANEL	Hetero Drugs limited
2	PPP	Hetero Drugs limited
3	BPP	Hetero Drugs limited
4	PPD	Hetero Drugs limited

EQUIPMENTS AND CHEMICALS

S.NO	Name of the instrument	MAKE	MODEL	SOFTWARE
1	HPLC	Waters	E2695 & 2487 dual λ absorbance detector	Empower@3 software
2	Weighing balance	Sartorius	BSA2245-CW	NA
3	pH meter	Lab India	AD102U	NA
4	Sonicator	Enertech	SE60US	NA

Table: 2 list of chemicals used in HPLC

Chemicals and reagents	Manufacturer	Grade
Potassium dihydrogen phosphate	Merck	AR Grade
Phosphoric acid (solid)	Merck	GR Grade
Orthophosphoric acid(˜88%w/w)	Merck	AR Grade
Potassium hydroxide	Merck	AR Grade
Sodium hydroxide pellets	Merck	GR Grade
Acetonitrile	JT Baker	HPLC Grade
Water		Milli-Q grade

Vol. 21, No. 1, (2024)
ISSN: 1005-0930

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.

LIMIT OF QUANTIFICATION (LOQ): The LOQ for imp-1, imp-2 and perampanel were estimated at a signal to noise ratio of 3:1 and 10:1, respectively by injecting a serious of diluted solutions at known concentrations.

Linearity: The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of the analyte in the sample. Linearity of test solutions for related substances was prepared by diluting stock solution to required concentrations. The solutions were prepared at nine concentration levels to the impurities specification level of 0.15% 0.02 %, 0.04 %, 0.05 %, 0.075 %, 0.1 %, 0.125 %,
0.15 %, 0.2 % and 0.25 %. Linearity test solutions for the assay method were prepared from perampanel stock solution at six concentration levels from 25 to 150 % of assay analyte concentration 25, 50, 75, 100, 125 and 150 µg/mL.

The calibration curve was drawn by plotting peak areas of imp- 1, imp- 2 and perampanel versus its corresponding concentrations. Linearity test was performed for three consecutive days in the same concentration range for related substances and assay method. The linearity was evaluated by linear regression analysis, which was calculated by least square regression method. The correlation coefficient, slope and y - intercept of the calibration curve were obtained from the calibration graph.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. To determine the robustness of the development method, experimental conditions were deliberately changed and the resolution between perampanel and its impurities and their tailing factors were also studied. The fixed flow rate of mobile phase was 1 mL/min. To study the effect of the flow rate on the resolution, flow was changed by ± 0.1 units i.e. 0.9 and 1.1 mL/min. The effect of column temperature on resolution was studied by making small changes in column temperature ± 5°C of the fixed temperature of 35°C . 30°C

3. RESULTS AND DISCUSSION

Determination Of Working Wavelength: Maximum absorbance for all impurities determined by UV absorbance spectrophotometer and observed that all impurities have maximum absorbance at 220nm

Vol. 21, No. 1, (2024)
ISSN: 1005-0930

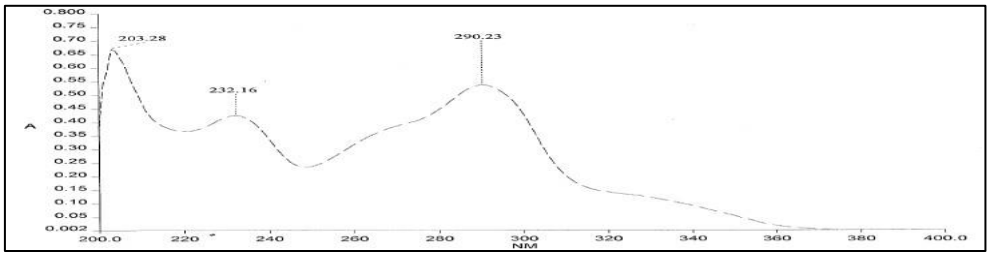

Fig. 1 PPP UV-VIS SPECTRUM (Des bromo pyridine impurity)

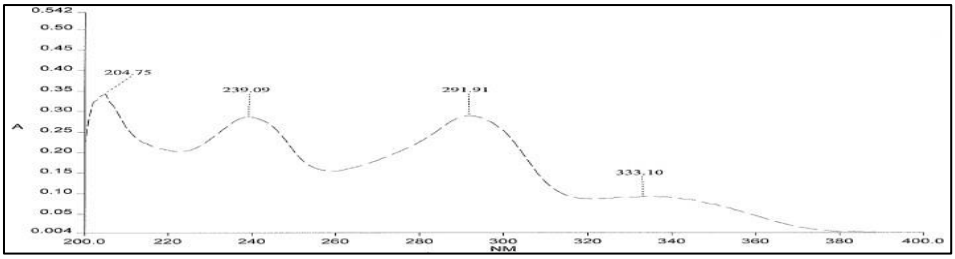

Fig. 2 BPP UV-VISSPECTRUM (Bromo pyridine)

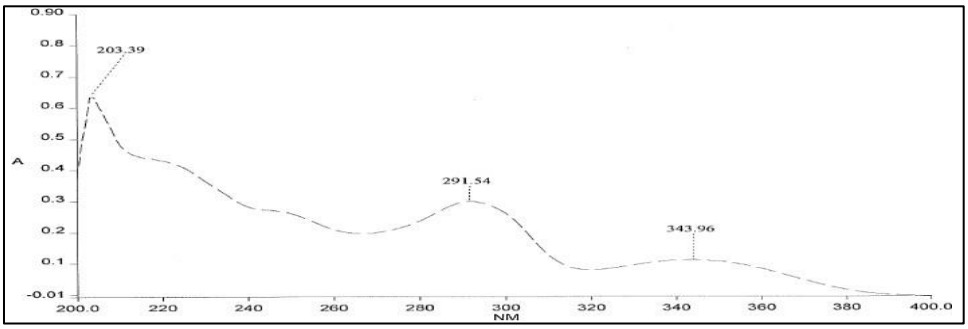

Fig. 3 PMP UV-VIS SPECTRUM (Perampanel)

Linearity

Linearity study was conducted for all Known impurities and Perampanel standard in the range of QL level to 150% level. Correlation coefficient values for all impurities were derived from respective linearity graph and the results are given below.

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Table 3 Linearity for PPP:

Level	Concentration in%(X-axis)	Area
1	0.0002	7955
2	0.0008	32350
3	0.0011	49304
4	0.0015	63716

Vol. 21, No. 1, (2024)
ISSN: 1005-0930

5	0.0019	81248
6	0.0023	100492
Correlation coefficient		**0.9993**
Intercept		**-937**
% Y Intercept		**-0.93**

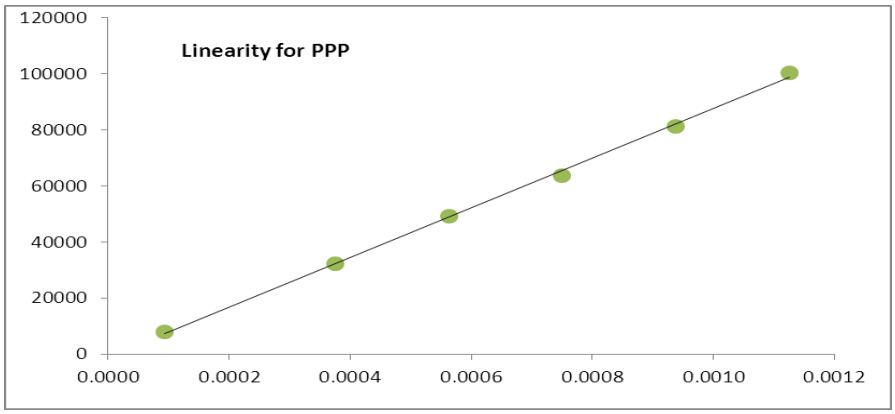

Fig.4 Calibration curve for PPP

Table 4 Linearity for BPP:

Level	Concentration in% (X axis)	Area
1	0.0003	10319
2	0.0008	24915
3	0.0012	39443
4	0.0015	51646
5	0.0019	63436
6	0.0023	80629
Correlation coefficient		**0.9986**
Intercept		**-1063**
% Y Intercept		**-1.32**

Vol. 21, No. 1, (2024)
ISSN: 1005-0930

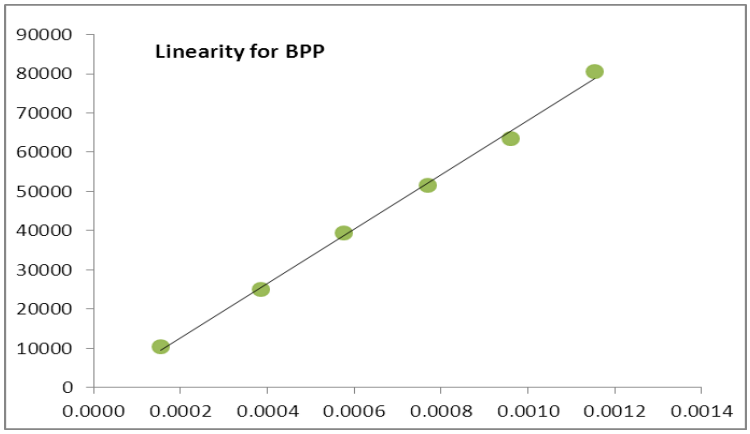

Fig. 5 Calibration curve for BPP

Table 5 Linearity for PMP:

Level	Concentration in %(X- axis)	Area
1	0.0002	21391
2	0.0005	39189
3	0.0008	51953
4	0.0010	67474
5	0.0013	84059
6	0.0016	106003
Correlation coefficient		**0.9946**
Intercept		**8309**
% Y Intercept		**7.84**

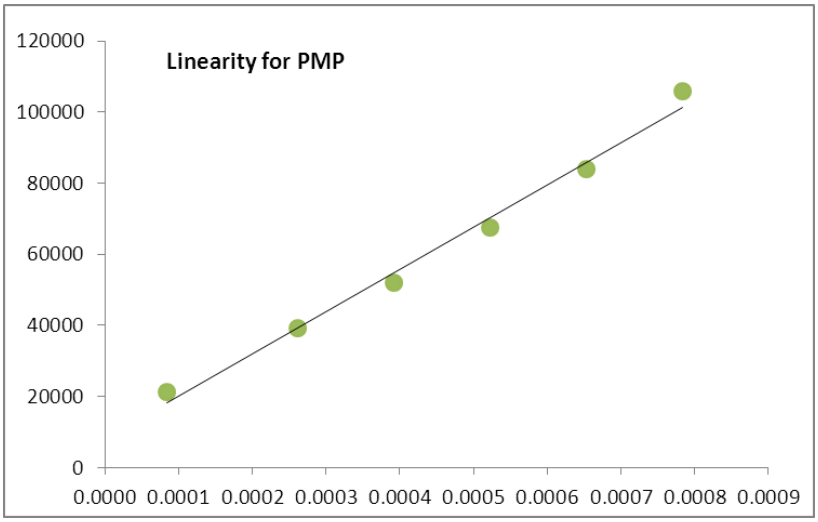

Fig.6 calibration curve for PMP

Observation: 1. Correlation coefficient value for each component was within limit. (More than 0.99)

Robustness: Ph Variation: Perampanel test sample Spiked with all above impurities was taken and analyzed at two different Ph conditions pH 2.8 and Ph 3.2.

Table 6: pH Variation Results:

Name of the Impurity	InitialpH3.0 results (%)	Ph2.8 results (%)	Variation	Ph3.2 Content%	Variation
PPP	0.12	0.12	0.00	0.13	0.01
BPP	0.10	0.10	0.01	0.10	0.00
MSUI	0.02	0.02	0.00	0.02	0.00
TI	0.26	0.27	0.01	0.29	0.03

Observation: Initial results and pH variation results are comparable.

a. **Temperature Variation**:

Perampanel test sample Spiked with all above impurities was taken and analyzed at two different temperatures (38 ºC and 42 ºC).

Table 7. Temperature Variation Results:

Name of the Impurity	Initial40ºC results (%)	38 ºC results (%)	Variation	ºCresults (%)	Variation
PPP	0.12	0.11	0.01	0.13	0.01
BPP	0.10	0.10	0.00	0.10	0.00
MSUI	0.02	0.02	0.00	0.02	0.00
TI	0.26	0.25	0.01	0.28	0.02

Observation: Initial results and temperature variation results are comparable.

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
Test solution stability	Initial	-	0.11%	0.09%	±30.0% Specification limit
	After 12hrs	-	0.11%	0.09%	
	Linearity (µg/ml)	0.0002-0.0016	0.0003-0.0023	0.0002-0.0016	

Vol. 21, No. 1, (2024)
ISSN: 1005-0930

Linearity	Correlation coefficient®	0.9946	0.9993	0.9986	MT 0.99
	Intercept	8309	-937	-1063	
	%Y Intercept	7.84	-0.93	-1.32	

% 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%

Robustness(%RSD)	**PPP**	**BPP**	**Limits**
Initial pH3.0	0.1	0.10	%RSD NMT 5.0%
pH 2.8	0.12	0.10	
pH variation	0.01	0.00	
Initial Temperature	0.12	0.10	
Temperature 38°C	0.11	0.10	
Variation	0.01	0.00	

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 method for determining related substances in the perampanel using HPLC was devised that is specific, selective, accurate, precise, robust, robust, and stable. After numerous trials, this approach was refined since it allowed for the separation of all linked chemicals found in the drug compounds. Zorbax SB Phenyl, 150×4.6mm5.0µm, gradient pump mode, flow rate of 0.8 ml/min, injection volume of 10µl, and run time of 55 minutes were the parameters of the column utilised in the optimised technique. Perampanel's retention time of roughly 28.8 minutes was verified by comparing it to other common medications.

All of the characteristics, including LOQ, linearity, range, robustness, etc., were used to validate this approach. All of the parameters' findings fell inside the acceptable range. This approach was particular because all associated chemicals' retention durations were verified by injecting each individual impurity individually, and there were no additional interference peaks at those times for any related substances in the drug substances. The correlation coefficient for all

related chemicals and drug compounds was NLT 0.990, indicating that this approach of determining related substances was linear. Because the associated chemicals and drug material were recovered between 80 and 120 percent as specified, this procedure was accurate. Because the percentage RSD for any related chemical should be less than 10.0% under all specific conditions, this approach of determining related substances was precise.

The following variables, which had no effect on the procedure, were used to test the robustness: pH fluctuation, temperature variation, column lot variation, wavelength variation, and flow rate variation. It is sensitive as a result.The forced degradation circumstances under a variety of stress conditions, including acid, base, peroxide, temperature, humidity, and photolytic conditions, demonstrated the stability of this approach. It may be concluded that under all conditions, there were no interference-degraded peaks at their retention durations of any linked chemicals. In all these changed conditions, the composition of mobile phase remained constant.

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