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Development of Method and Validation for Related Substances in Pemetrexed Injection formulation With HPLC

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Abstract

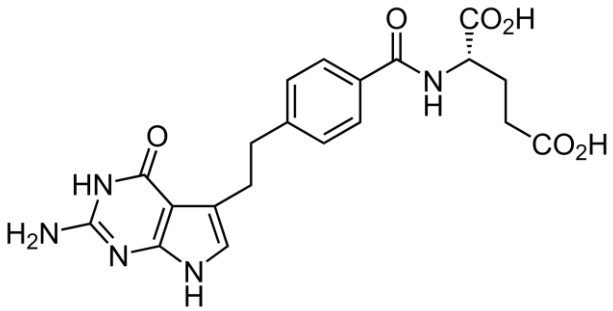
A validated HPLC method was developed for the determination of Pemetrexed (PMD) in pharmaceutical formulation. Isocratic elution at a flow rate of 1.00ml/min is employed on waters X bridge C18 50 mm × 4.6mm, 3.5µ Column, or equivalent. As a mobile phase 90 volumes of buffer solution and 10 volumes of Acetonitrile. Acetonitrile is utilized as mobile phase B. UV detection wavelength is set at 230nm. Injected sample is 5.0µl. Run time is 16 minutes to Sample, Blank, Placebo, System suitability along with 60minutes to diluted Regular. Approximate retention time is identified to PMD is ± 2.4 minutes. % R.S.D PMD is identified. Mean Percentage recovery to PMD is identified that within specification limit. This work is validated by using rules and regulations given by ICH. Therefore, proposed HPLC process should successfully applied to routine quality control analysis formulations. This process developed is simple also is better to that of different processes those are reported in earlier literature. Values permits application for proposed stability indicating HPLC methodology in syrup dose forms.

Keywords: RP-HPLC Refractive index detector, PMD, flow rate, column, ICH Guidelines, USP reference.

1. Introduction:

The molecular formulae for Pemetrexed (PMD) is C20H21N5O6. The IUPAC name for this PMD is (2S)-2-{[4-[2-(2-amino-4-oxo-1,7-dihydropyrrolo[2,3-d]pyrimidin-5-yl)ethyl]benzoyl]amino}pentanedioic acid. PMD is used to treat malignant pleural mesothelioma, thin layer of tissue which covers so many organs, in association with cisplatin[1] to patients those diseases are either unresectable nor which may not otherwise

candidates to surgery which may be curative.[2] PMD is generally recommended in association with carboplatin as well as pembrolizumab to base -line treatment to well advanced non-small cell lung cancer.[3][4] Patients which are recommended for taking folic acid along with vitamin B_{12} supplement even if levels were normal whenever they were over therapy of PMD.[5] As per Suresh Kumar Agrawal et.al.,[6] PMD is chromatographed over Inertsil C18 column with diameters as (250×4.6mm, 5µm) in a mobile phase containing of 35v/v and 65v/v of acetonitrile as well as buffer as2.76gm sodium dihydrogen orthophosphate along with 2.00gm sulphonic acid sodium salt in 1000.00ml milli-Q-water. 3.0 is the pH adjusted using OPA. 254nm is the absorbance. 25ºC is column oven temperature. 20µL injection volume. 10min. is runtime. Linearity is 12.50µg/ml - 37.50µg/ml. 1.0 correlation coefficient. S. Hemchand et.al., [7] PMD is utilized to treat malignant pleural mesothelioma as well as lung cancer. A simple stability providing RP-HPLC process is developed as well as validated to determine disodium of Pemetrexed. 27°C is the ideal temperature used for Hypersil BDS C18 100 x 4.6mm, 3µm. Mobile phase mixture consisting as 0.02M sodium dihydrogen phosphate by 0.1% HCOOH. 3.8 is pH by dilute sodium hydroxide: Acetonitrile as 40 v/v 60 v/v with pH as 3.8. 1.20mL/min is rate of flow. Methanol as 1v/v, water as 1v/v is used as diluent finally, eluted substances are observed at 240nm. 0.50µg/ml -1500µg/ml by linear regression equation y = 20588x - 9294.1 (R_2=0.9999). Ankit D. Patel et.al., [8] used Kromasil C18 (250 × 4.6mm, 5 µm) column. Mobile phase as 20mM dibasic phosphate buffer 88v/v. pH is adjusted as 6.50 by OPA and acetonitrile 12v/v. Wave length is 225nm. By considering all the reviews author has proposed this developed method is successfully applied for estimating amount of PMD injection in formulations.



Pemetrexed

2. EXPERIMENTAL

LC 20AT pump and UV-Visible detector with flexible wavelength programme and Rheodyne injector are used in this present problem. waters 50mm × 4.6 mm, 3.5µ, or similar is used to this chromatography analysis. With the help of Loba ultrasonic bath sonicator mobile phase containing the gas was separated. Methanol is AR grade and water collected and used. 12.00ml orthophosphoric acid in 1000mL of triply pure water. Mobile Phase A &B are prepared by 90 volumes of buffer solution and 10 volumes of Acetonitrile. And Mobile Phase B as Acetonitrile.

Preparation of Solutions:

Authors are prepared different solutions used for this present investigation which includes PMD Regular Stock; Sample solution (100mg/vial); Sample solution (500mg/vial) along with placebo

3. METHOD DEVELOPMENT

For100ppm solution of PMD by using UV spectrophotometer the spectrum in Acetonitrile recorded separately. Peak of wavelength 230nm showed spectra of PMD. Required separation along with peak appearances are identified on waters X bridge C18 50 mm × 4.6 mm, 3.5µ Column, or equivalent. PMD is generally blended by transferring exactly 12 mL of Orthophosphoric acid in 1000 mL distilled water is added in required proportion. Acetonitrile is used as a mobile phase B in alternated volumes. This revealed that most suitable of all other compositions. Peak of chromatography measured is best when compared with others; this is nearly freed to tailing and proven that most suitable of all combinations. For optimum separation, in order to measure the course of the reaction in between 0.50 – 1.50 mL/min is applied. From the observation of the reaction 1.0mL/min flow rate is acceptable for effective separation of analyte.

4. VALIDATION OF PROPOSED METHOD AND REQUIREMENTS

4.2 System Suitability:

In order check whether system is working properly or not, in order to provide very accurate and more precise parameters of SS were fixed. Injected Blank (as 1 injection), Regular solution (as 6 injections) in chromatography also recorded different chromatograms. By using below values, finally it is decided that proposed method is more suitable for validation of method. Obtained results are tabulated in table.1.

	Regular Solution	PMD Results
Tailing factor	Pemetrexed	1.2
% RSD	Pemetrexed	0.1

Table 1: System suitability results

4.3 Specificity:

Carried out this experiment by passing Blank, placebo, regular solution, Impurity-1, Impurity-2, Sample solution and spiked sample solution (Sample along with impurity) into chromatographic system also documented different retention times. There should be no interference due to different peaks obtained for Blank (diluent), Placebo, Impurity-1 and Impurity-2 may not obstruct peak PMD and each other. With the help of obtained values, at last it is finalized that there is no obstruction due to Blank (Diluent), Placebo, Impurity-1, Impurity-2, regular at retention time peak of PMD. Obtained values were tabulated in table.2.

Solution		Retention Time
Blank (Diluent)		--
Regular solution	Pemetrexed	5.1
Placebo preparation		-
Sample preparation	Pemetrexed	5.0
Impurity-1		10.1
Impurity-2		12.2
	Pemetrexed	5.0
	Impurity-1	10.1
	Impurity-2	12.2

Table 2: Specificity results

Stressed Condition Studies:

Authors are studied various forced degradation studies using Specificity by Stressed condition for these prepared the sample solution, p;acebo, acid stressed specimen as 1.0N HCl; Alkali Stressed sample as 1.0N NaOH 3.0% w/v Hydrogen Peroxide Stressed sample as 3.0%w/v H_2O_2; Neutral Stressed sample; UV light exposed sample; Photo stability and sunlight exposed sample; Thermal Stressed - Dry heat sample; Acid Stressed specimen as 5.0N HCl; Alkali Stressed sample as 5.0N NaOH; 1.0% w/v Hydrogen Peroxide Stressed sample as 1.0%w/v H_2O_2. Finally authors are concluded that overall degradation products if any should be well separated from PMD Peak and each other. Under the different stress conditions, product has degraded and finally obtained mass balance of Gemcitabine. Finally, sample is found that it is undergoing degrading in alkali within 4 hrs condition. In various solutions like peroxide, neutral, alkali along with acidic conditions PMD peak may slightly undergoes degradation. Anyhow various unknown impurities, known impurities and different degradation impurity peaks were subjected to separation from PMD peak. PMD peak is purer and this is finalized by Empower software. Hence, Assay process is considered as more specific & stability indicating.

4.4 Precision:

System Precision

The Retention time **(**RT) and area for total 6 determinations were calculated along with that % RSD may also calculated. Recorded % RSD for RT and response of peak for PMD from regular preparation. It is evident that from obtained data RT and peak responses are same which may be supported by RSD. (Less than 1.0% and less than 2.0% respectively). The relative regular deviation (%RSD) of RT for PMD which is achieved for total 6 injections for diluted regular solution is equal to NMT 1.0. Relative regular deviation (%RSD) for PMD achieved from total 6 injections diluted regular solution is NMT 2.0. From results obtained, finally it is given as a conclusion that retention time & area responses were consistent those are supported by using relative regular deviation. Due to this reason, it is finalized as SP parameters satisfies requirement for validation. Obtained results are tabulated in Table 3.

Pemetrexed for Injection		
Injection No.	**Retention Time (Min)**	**Area Response**
1	5.162	1471406
2	5.204	1471155
3	5.210	1471622
4	5.225	1474107
5	5.249	1472366
6	5.242	1472985
Mean	5.215	1472274
%RSD	**0.6**	**0.1**

Table 3: System Precision results

Method Precision:

This parameter is analogous sample for individual group is subjected to analysis to about 6 times. Provides information that this parameter is providing regular values for individual group or not. PMD to about six times of a same group and calculated for Assay values of Pemetrexed for 6 determinations is NMT 2.0.

Intermediate Precision:

In method precision a analogous of one group is analyzed for total six reduplications. Parameter gives information related to dependable values for a single group. Analyzed sample of PMD injection for total six reduplications by this procedure and calculated percentage assay of PMD. Same process is repeated to no.of times with help of various instruments, different column on different days. Calculated % of assay compared values obtained in method precision along with Intermediate Precision. Calculated % RSD for total 12 determinations. %RSD calculated for assay for total 6 determinations is NMT 2.0. The %RSD calculated for assay for total 6 determinations is NMT 2.0. strength. %RSD of 12 Determination for 100mg/vial is 0.5 for 500mg/vial is 0.7

Stability in analytical Solution:

This is calculated stability with help of injecting regular, sample preparation along at specified range in regular intervals by day wise with help of room temperature(25°C) and 5°C. % Difference to PMD in regular is within ± 2.0. Regular solution is stable for 38 hours at 5°C (% difference is -0.5). Sample

Solutions of 100mg/vial & 500mg/vial is stable for 38 hours at 5°C (difference in % -0.2 & -0.2). Regular solution is balanced for 38 hours at 25°C (difference in % is -0.8). Solutions of 100mg/vial & 500mg/vial is stable for 38 hours at 5°C (difference in % -0.6 & 0.2).

4.5 Linearity:

In the range between 50% and 150% linearity of PMD is measured with working concentration and covered minimum five ranges from 80% to 120%. Performed linearity by using PMD. Noted area Performed precision at maximum ranges by passing solution about 6 times into chromatographic system. Plotted a graph of PMD, as strength (PPM). Drawn a graph by taking PPM on X-axis along with area response on Y-axis. For this case authors are constructed linearity regular as well as PMD Linearity stock solution. By using these samples coefficient correlation along with coefficient regression values are calculated and they are shown in table from Table. Correlation coefficient and R square are 1.000 intercept should be with in limit of ± 5.0 of response at 100% range. Precision at Lower & higher ranges %RSD is NMT 5.0. Values obtained are reported in Table 4, related graphs for this analysis are shown in represented from Fig. **2.**

Linearity of Pemetrexed (50% to 150%)		
Sl. No	Conc.in PPM	Area Response
1	51.2250	720375
2	81.9600	1142968
3	92.2051	1283661
4	102.4501	1421600
5	112.6951	1564011
6	117.8176	1616144
7	156.2363	2174944
Slope	13789.009	
Intercept	10032.170	
%Intercept	0.71	
Correlation Coefficient	1.000	

Table 4: Linearity values to PMD

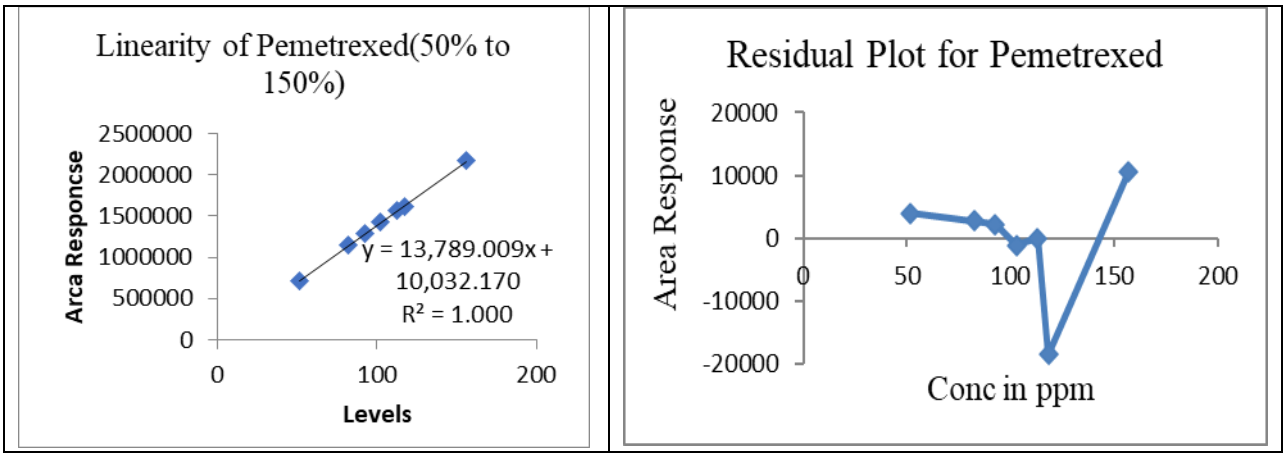


Figure: 2 Linearity and residual plot for PMD

From statistical treatment of linearity data of PMD, straight among 50% range to 150% specification limit. Correlation along withregression coefficient are more to value 0.998. P value is more than 0.7. Also, basis is with in minimum and maximum limit of 95% certainty range which provides high degree of certainty to value got for intercept. Intercept is with in ± 2% with response area at 100% range.

4.6 Accuracy:

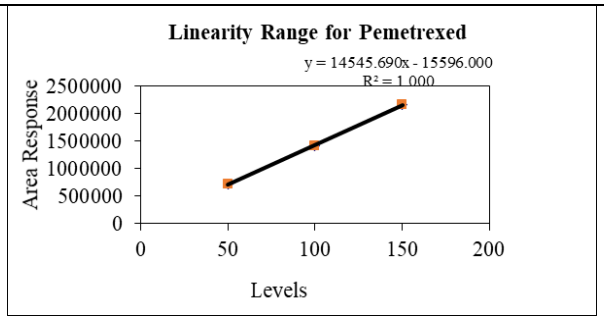
Analyzed these samples in three times. From results, calculated accuracy and range parameters. Single along with mean recovery for every range lies in 50% to 150% for Known impurity. Individual along with mean recovery for each and every range lies in 80.0% to 120.0% for PMD. Accuracy results are tabulated from Table 5.

Set	Levels (in%) (About)	Mean Area Response	*mg added	mg Added (Actual)	mg recovered	% recovery	Mean % Recovery	% RSD
1	50	679949	2.690	2.4479	2.4324	**99.4**		
2	50	681764	2.700	2.4570	2.4389	**99.3**		
3	50	683793	2.704	2.4606	2.4462	**99.4**		
1	100	1397080	5.508	5.0123	4.9979	**99.7**		
2	100	1393494	5.501	5.0059	4.9851	**99.6**		
3	100	1399737	5.508	5.0123	5.0074	**99.9**		
1	150	2079942	8.227	7.4866	7.4408	**99.4**		
2	150	2085830	8.235	7.4939	7.4618	**99.6**		
3	150	2085284	8.241	7.4993	7.4599	**99.5**		
%RSD for 3x3 Levels							**0.2**	

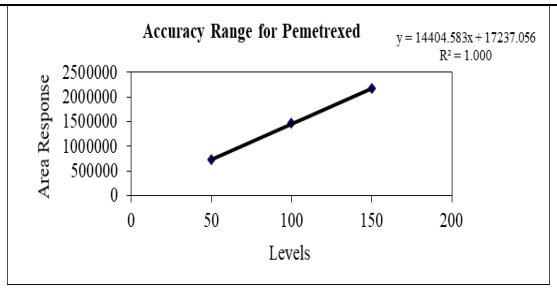
Table 5: Recovery ranges

4.7 Range:

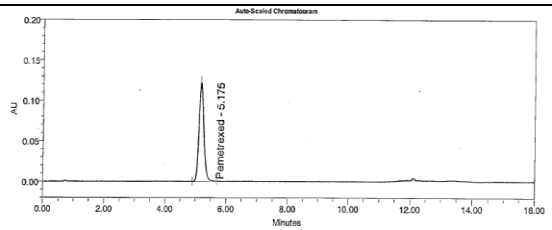
%RSD obtained for all accuracy range determinations is NMT 2.0. Correlation also regression coefficient are NLT 0.998 for Linearity along with accuracy range parameter. Linearity with accuracy range graphs represented from Fig 2.6 to 2.7. Linearity and accuracy range tabulated from Table. Finally concluded that range of method is from 50% to 150% of target strength for PMD, obtained graphs are reported in Fig.4.

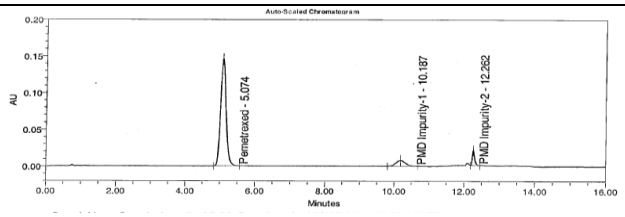


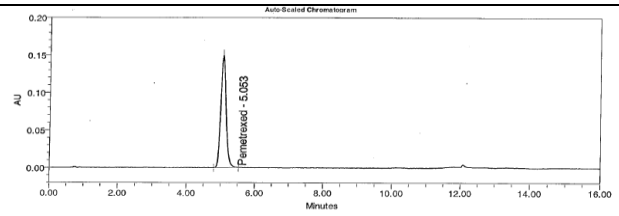
Linearity range of PMD



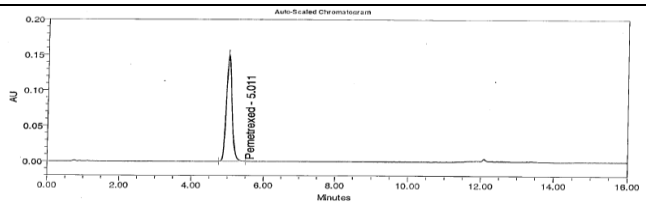
Accuracy range of PMD







1.0N HCl Stressed sample



Thermal Stressed sample

Fig:4 Accuracy curves

4.8 Robustness:

Changed column temperature to in and around 5°C. SS parameters obeyed for all conditions. Total impurities which are known, taken off to each other along with PMD peak in sample spiked with impurities.

From values those are obtained, it is finalized that this method is robust towards small variations possible in this process. Results tabulated in table. 6

Evidence perception		Theoretical plates	Tailing factor	%RSD
Original Condition		5431	1.2	0.1
	-0.2 ml/min	5871	1.2	0.1
	+0.2 ml/min	5083	1.2	0.1
	-5°C	5559	1.2	0.1
	+5°C	5366	1.2	0.0
	-5nm	5445	1.2	0.1
	+5nm	5441	1.2	0.4
	-2.0%	5246	1.2	0.1
	+2.0%	4966	1.0	0.1

Table 6: Results for Robustness

5. RESULTS AND DISCUSSION

Upgraded various RP-HPLC parameters, different mobile phase configurations are verified and tested. A satisfactory segregation with good peak symmetry is measured with the configurations of both Mobile phase A and B. By transferring 12.00ml of orthophosphoric acid in 1000mL of WFI is treated as a mobile phase A and Acetonitrile treated as mobile phase B. 1.00mL/min flow proved the better resolution and peak shape than other mixtures. Column used in this measurement is waters X bridge (50 × 4.6) mm, 3.5µ, or proportionate. Specificity(Stressed condition) the peaks of Blank (diluent), Placebo, Impurity-1 and Impurity-2 may not interfere with PMD Peak and each other. There is no interference of Blank (diluent), Placebo, & known Impurities peaks with PMD Peak. Degradation products if any are well separated from PMD Peak and each other purity angle less than peak threshold as per empower software. For, System suitability the Tailing factor is about 2.0. Plate count not less than 2000. Obtained results are respectively. Peak tailing of PMD in regular injection may not be maximum than2.0 with %RSD for total 6 replicated injections of regular may not be maximum to value 2.0. %RSD of the retention time for PMD peak measured from total 6 injections of diluted solution may not be maximum to value 1.0 and %RSD area of PMD peak response measured for total 6 injections of diluted regular solution is NMT 2.0 respectively. %RSD of Assay for 6 determinations is 0.08 & 0.16. %RSD for total 12 measurements (Method Precision & Intermediate Precision) is NMT 2.0 which is identified as 0.56 & 0.6. % difference in sample solution obtained between initial and after specified period is

-0.6 & 0.2 at 25 °C. Estimated HPLC method of related substance in drug product PMD injection is validated as per ICH guidelines. Proposed process found as specific. Method is also indicating as evidenced by stress conditions.

1. References

1. Manegold C (August 2003). "Pemetrexed (Alimta, MTA, multitargeted antifolate, LY231514) for malignant pleural mesothelioma". Semin. Oncol. 30 (4 Suppl 10): 32–6. doi:10.1016/S0093-7754(03)00283-5. PMID 12917819.
2. National Cancer Institute: FDA Approval for Pemetrexed Disodium
3. Ettinger DS et al. NCCN Clinical Practice Guidelines in Oncology: Non-small Cell Lung Cancer V.2.2009 available from www.nccn.org
4. Gandhi, Leena; Rodríguez-Abreu, Delvys; et.al.,. "Pembrolizumab plus Chemotherapy in Metastatic Non–Small-Cell Lung Cancer". New England Journal of Medicine. 2018; 378 (22): 2078–2092.
5. Hazarika M, White RM, Johnson JR, Pazdur R (2004). "FDA drug approval summaries:pemetrexed(Alimta)". *Oncologist. 9 (5):482–8.* doi:10.1634/theoncologist.9-5-482. PMID 15477632.
6. Suresh Kumar Agrawal, Devendra Singh Rathore; "Development And Validation Of Pemetrexed By Rp-Hplc Method In Bulk Drug And Pharmaceutical Dosage Forms"; Indian Journal of Research in Pharmacy and Biotechnology Volume 1(4) July-August 2013 Page 537-542.
7. S. Hemchand; R. Ravi Chandra Babu; Mukthinuthalapati Mathrusri Annapurna; A new validated stability-indicating gradient RP-HPLC method for the determination of pemetrexed disodium and its process related substances; 2019; 9(3-s):588-610; DOI https://doi.org/10.22270/jddt.v9i3-s.2918;
8. Ankit D. Patel, Shalin K. Parikh, Dr. D. J. Sen and Dr. C. N. Patel; "Development and validation of high performance liquid chromatographic and uv spectrophotometric method for estimation of pemetrexed disodium in bulk drug and pharmaceutical formulation"; Int. J. Drug Dev. & Res., April-June 2011, 3 (2): 301-307.