



# Development and Validation of a Rapid RP-HPLC Method for the Determination of Pemetrexed in Lyophilized Powder for Injection

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## ABSTRACT

An economic, rapid and versatile RP-HPLC method for the determination of pemetrexed, an effective antifolate in lyophilized powder for endovascular injection, was developed employing a Luna C18 Column (250 × 4.6 mm; 5 µm), and spectrophotometric detection (analytical wavelength at 254 nm). A column oven temperature of 25°C and a mobile phase composed of ACN: Sodium dihydrogen orthophosphate buffer of ratio 30 v/v:70 v/v with pH adjusted to 4. The flow rate was 0.8 ml/min in isocratic elution with a 10:1 of injection volume of sample. The method validation was performed in accord with the ICH guidelines, considering parameters as accuracy, precision, linearity, robustness, limits of detection and quantification. The linear dynamic range was between 80 µg/ml and 120 µg/ml with a correlation coefficient of 0.999 and a runtime of 10 minutes. This propose method stable after 10 hrs. At 25°C proving to be reliable a such it can be used in the determination of Pemetrexede in Pharmaceutical formulation for injectable.

**Keywords:** HPLC; Lyophilized powder; Pemetrexed; Antifolate; Pharmaceutical formulations.

## INTRODUCTION

Pemetrexede disodium (N-[4-[2-[2-Amino-4,7-dihydro-4-oxo-3H-pyrrolo[2,3-d]pyrimidine-5-yl ethyl]benzo-yl]-L-glutamic acid) is a multi-target novel antifolate of synthetic origin, extensively used as chemotherapeutic agent for the treatment of pleural mesothelioma, Non-Small Cell Lung Cancer (NSCLC) breast cancer, cervical cancer and colorectal cancer (Figure 1) [1-5]. This is formulated as a sterile lyophilized powder for intravenous application, a reconstituted with water is required prior to its administration. Pemetrexed limits cell proliferation through the interruption of folate dependent metabolic processes indispensable for cell growth. Despite the effectiveness of this drug it's use is not without side effects some of which include chest pains, unusual weakness, chills, fever, nausea and pale skin [6]. The versatility of Liquid chromatography makes it the most used for routine analysis in biological, pharmaceutical and food industries [7]. Few analytical methods are available for the quantification of pemetrexed in pharmaceutical lyophilized powder for injectable. In general, this methods employing,

isocratic and gradient HPLC coupled with reverse phase columns and spectrophotometric methods for the determination of pemetrexed in bulk pharmaceutical products [8-11]. On the other hand, HPLC and LC-MS methods are also currently accessible for the quantification of pemetrexed in biological fluids [12-15]. Analytical methods will be efficient for determining the purity and identity of drugs. It is paramount, efficient methods are developed not only to safeguard the optimization of resources, but rather their aptness towards their intended use [16]. The development and the validation of an analytical method is constant and evolves comparatively with the progress of the drug. Therefore, is methods purpose to reflect the phase of a giving drugs growth. Clearly, a method ought to meet all regulatory guidelines as well as be simple and robust [17-19]. Furthermore, method ought to fully evaluate to establish its technical soundness, a requirement that varies within the regulatory registration procedure. In general, it affords the analyst a clear understanding into the behavior of the designed method making it possible to institute performance limits [20]. The aim of this work is focused on acquiring results with high

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reproducibility, precision and in the least possible time, with little margin for error, RP-HPLC technique was chosen for the development of a simple, rapid and economic method for the quantification of pemetrexed in injectable powder.

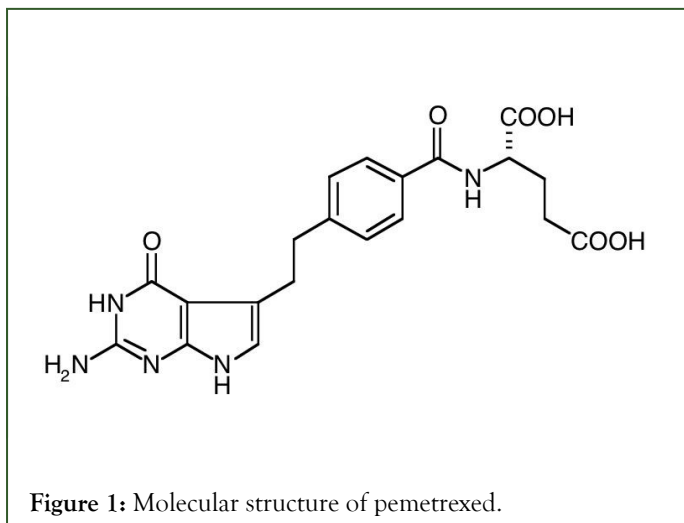


Figure 1: Molecular structure of pemetrexed.

## MATERIALS AND METHODS

### Chemicals reagents and solutions

USP pemetrexed disodium heptahydrate of 78.3% purity, orthophosphoric acid, acetonitrile were obtained from LAS do Brasil Ltd and sodium dihydrogen orthophosphate was acquired from Merck. The medicament, lyophilized Pemetrexed disodium powder for injectable samples used for the study were provided by ChemicalTech Ltd.

### Buffer preparation

2.76 mg of sodium dihydrogen orthophosphate was weighed, and further diluted with 800 ml of HPLC grade water after which the pH of the resulting solution was adjusted to 4 and then completed to the 1000 ml with the same diluent and passed through filter of 0.22  $\mu$ m porosity.

### Mobile phase and diluent solution

A mobile phase, consisting of acetonitrile and buffer was prepared in the ratio of 30 v/v:70 v/v and then filtered and degassed, this solution was as well a diluent for samples and standard.

### Preparation of standard solution

About 35.1 mg of pemetrexed disodium heptahydrate was accurately weighed, (equivalent to 27.7 mg of pemetrexed disodium) and transferred into a 25 ml volumetric flask dissolved and diluted to mark with water of HPLC grade. After which 2 ml of the resulting solution was then transferred to 20 ml volumetric flask and completed to the mark with the diluent.

### Preparation of sample solution

A vial of the 500 mg presentation of pemetrexed was weighed,

after which the contents of vial was reconstituted with 20 ml of HPLC grade water, transferred to 200 ml volumetric flask and the volume was completed to the mark with the same solvent. 2 ml of that previous solution was then transferred to a 100 ml volumetric flask and volume completed to the mark with the diluent solution.

### Equipment

The HPLC systems (Rigol L3000 HPLC) consisting of a Rigol L3500 dual-wavelength absorbance detector with a Rigol L3240 quaternary pump. The system operating on the data acquisition, monitoring and processing software system empowered by Clarity. A pH meter model H2221 (Hanna), an ultrasound Bath of model S120 (Elmas) and an analytical balance of model aux 220 (Shimadzu) were used for the development of the method.

### Chromatographic conditions

Samples separation was performed using Luna C18 Column (250  $\times$  4.6 mm; 5  $\mu$ m), at a flow rate of 0.8 ml/min in isocratic elution, column temperature maintained at 25°C  $\pm$  2°C, and a detection wavelength 254 nm. The programed run time was 10 mins with an injection volume 10  $\mu$ l

## RESULTS AND DISCUSSIONS

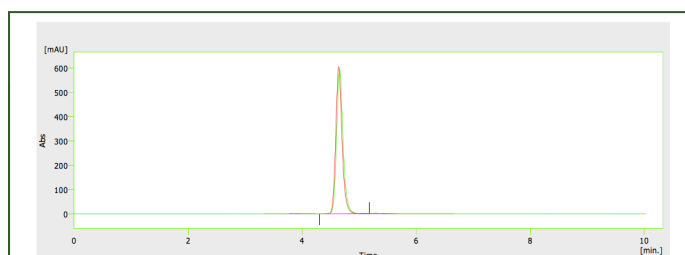
Multiple tests involving the use of diluents consisting of water, acetonitrile, and orthophosphate buffer solution of pH 3.5-6 and a mobile phase of the same solvents were performed and not significant variations were observed. The resulting optimized conditions selected were a C18 Column (250  $\times$  4.6 mm; 5  $\mu$ m), a mobile phase containing sodium dihydrogen orthophosphate buffer pH 4 and acetonitrile in the proportion of 70:30 v/v respectively, in isocratic elution mode. Furthermore, the validation is in accordance with ICH guidelines as well the ANVISA directives for analytical procedures [17-19]. Elected parameters adopted for the validation of this method were, specificity, Linearity, accuracy, precision, Limit of Quantification (LoQ), Limit of Detection (LoD) and robustness.

### System suitability

Both sample and standard solutions were within the intervals of 0, 2, 4, and 10 hours for the verification of how storage of prepared samples could be relied upon. Both solutions showed stability, confirmed by the 99% recovery obtained, suggesting stability of pemetrexed at room temperature.

### Selectivity/ Specificity

In the Figure 2, showing chromatograms of diluent, pemetrexed standard and sample obtained by the proposed method. The superposition of sample and standard is practically total and the diluent (mobile phase) not present variation in the base line. No other signals are observed for the retention time of analyte and the method is satisfactory for the proposed application.



**Figure 2:** Overlay: chromatograms of diluent, pemetrexed standard and sample obtained by the proposed method.

### Linearity

The method proved to be linear meeting the acceptance criteria within the range of 80% (80 µg/ml) to 120% (120 µg/ml) of the expected concentration of sample. The equation of regression was  $Y = (37.5 + 0.6) + (214.6 + 65.0) X$ , with a correlation coefficient ( $r^2$ ) of 0.99883 and %RSD less than 0.1% for all levels of concentration.

### Precision

This test was performed through reproducibility were six replicate injections of both sample and standard solution of 100 µg/ml concentration where performed and their standard deviation, were checked for both analysts on different days, results are showing in Table 1. Intraday samples recorded %RSD=0.01 for each analyst. A inter day %RSD considering all the data of analyst was 1.8%. The acceptance criteria is that %RSD<2, then the precision of the method was considering satisfactory.

Samples	Analyst 1	Analyst 2
	Area	Area
1	4016145	4154747
2	4016914	4154214
3	4016018	4154341
4	4016228	4154928
5	4016483	4154590
6	4016995	4155315
Mean	4016464	4154689
RSD%intra-days	0.01	0.01
Acceptance criteria	DPR ≤ 2,0%	
RSD%inter-days	1.8	
F cal	1.0399	
F critical	5.0503	

**Table 1:** Studies of precision: Reproducibility and intermediary precision.

### Limit of detection and quantification

Were determinate considering: Limit of Detection (LoD)=3 S/N and Limit of Quantification (LoQ)= 10 S/N, where, S is the standard deviation of intercept and m the pendent of the analytical curve. The LoD by the method was 0.008 µg/ml and the LoQ determined by the method was 0.028 µg/ml, this values are acceptable for the application

### Robustness

The robustness was estimated by deliberately varying of four parameters of the method, the mobile phase pH, change in column, flow rate and proportion of mobile phase (Table 2). In accord with ICH (18) the method is robust if %RSD<2%. The method proved robust because all variables tested were between the determined limits, with the highest RSD being 0.7%.

Variable	%RSD	
	Standard	sample
Change in flow rate to 0.7 mL/min.	0.1	0.1
Change in flow rate to 0.9 mL/min	0.3	0.3
Change in column temperature-10%	0.3	0.1
Change in column temperature+10%	0.7	0.2
Change in column Batch number	0.1	0.2
Change in proportion of the mobile phase-10%	0.1	0.1
Change in proportion of the mobile phase +10%	0	0.3

**Table 2:** Results for robustness.

### Accuracy

Method accuracy was determined by recovery for all the three levels of concentrations (80%, 100%, 120%) within the specified interval and in triplicates (Table 3). The mean recovery observed for all injections varies between 101.23% to 101.3%, with a % RSD 0.01, this recovery is accepted for the criteria because is greater to 98% and less than 102%.

Injections	Recovery	Recovery	Recovery
	80%	100%	120%
1	101,3	101,3	101,4

2	101,3	101,4	101,1
3	101,3	101,2	101,2
Mean%	101.3	101.3	101.23
%RSD	0.01		
Acceptance criteria	$98 \leq RC \leq 102\%$		

**Table 3:** Results for accuracy.

## CONCLUSION

The developed method is unique, rapid, precise, and highly robust as confirmed by this validation. Offering superior recovery and adequate selectivity with no interference at the acknowledged retention time. The method proves to be suitable for the determination of pemetrexed present in lyophilized powder for injection, encouraging its application in routine physicochemical quality control analysis directed towards safeguarding its use.

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