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DEVELOPMENT AND VALIDATION OF LEUCOVORIN CALCIUM BY UV SPECTROPHOTOMETRIC METHOD

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Abstract

A simple, accurate, rapid and reproducible UV-Spectroscopy method has been developed and validated for the estimation of Leucovorin Calcium in Pharmaceutical dosage form. The absorbance of drug was scanned in range of 200-400 nm and maximum absorbance observed at 286 nm wavelength using Water as a blank. The range of linearity was found to be 3-15 $\mu\text{g/mL}$ with the linearity equation is $Y = 0.044x + 0.105$ and correlation coefficient is 0.998. Developed method was validated according to the ICH Q2 (R1) guidelines. The % RSD values for Interday and Intraday were found to be less than 2%. And % Recovery was in between 98.45-100.96%. LOD and LOQ were found to be 0.225 $\mu\text{g/mL}$ and 0.683 $\mu\text{g/mL}$ respectively. The result concluded that the developed method is accurate, precise and reproducible as per ICH guideline. Hence it can be conveniently adopted for routine quality control analysis of the drug.

Keywords: Method development, Validation, Leucovorin Calcium, Spectrophotometry method.

Introduction

Leucovorin Calcium is 2S)-2-[[4-({[(6S)-2-amino-5-formyl-4-oxo-1,4,5,6,7,8-hexahydro-pteridin-6-yl] methyl} amino) phenyl] formamido]pentanedioic acid with molecular formula of $\text{C}_{20}\text{H}_{23}\text{CaN}_7\text{O}_7$ and molecular weight 511.51 Da^[1,2]. As Leucovorin is a derivative of folic acid, it can be used to increase levels of folic acid under conditions favoring folic acid inhibition (following treatment of folic acid antagonists such as methotrexate). Leucovorin enhances the activity of fluorouracil by stabilizing the bond of the active metabolite (5-FdUMP) to the enzyme thymidylate synthetase. Leucovorin Calcium is used in colorectal cancer. Colorectal Cancer is a term used for cancer that starts in the colon or the rectum. These cancers can also be referred to separately as colon cancer or rectal cancer, depending on where they start^[3,4]. Literature survey revealed only one UV^[5] method is available for estimation of

Leucovorin Calcium in Pharmaceutical Dosage form and various HPLC^[6-11], HPLC-ECD^[12], methods were reported for the estimation of Leucovorin Calcium for pharmaceutical dosage form. Aim of Present work was to develop simple, precise, accurate and economic spectrophotometric method for estimation for estimation of Leucovorin Calcium in Pharmaceutical dosage form. The proposed method was optimized and validated in accordance with International conference on Harmonization (ICH guideline).

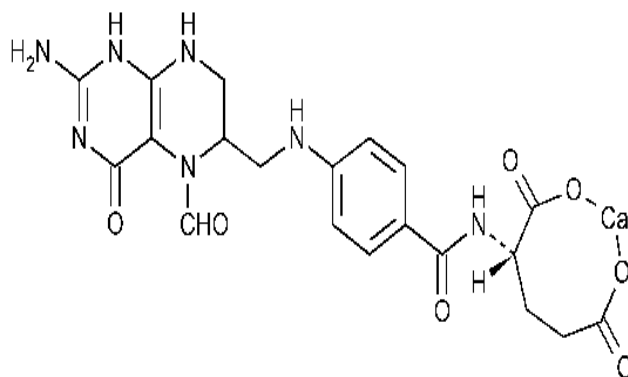


Figure 1: Structure of Leucovorin Calcium.

Material and Methods

Instrumentation

Spectrophotometric measurements were performed on Shimadzu UV –visible double beam spectrophotometer (Model-1800). All weighing were done on electronic analytical balance (Wensar Dab220).

Chemicals and Reagents

Leucovorin Calcium and Leucovorin Calcium for Injection were gifted by Zydus Cadila pharmaceutical company in Ahmedabad, India.

Selection of Solvent:

Leucovorin Calcium is freely soluble in Water and Sodium Hydroxide, sparingly soluble in Methanol, and Ethanol. Water is selected as solvent as it is economic and readily available so it is selected for further determination of Leucovorin Calcium.

Preparation of Standard Solutions

Preparation of Standard Stock Solution of Leucovorin Calcium (100µg/mL) :

Accurately weighed quantity of Leucovorin Calcium 0.1 gm was transferred to 100 mL of volumetric flask, dissolved in 50 ml of Water and diluted up to mark with Water to give a solution having strength of (1000µg/mL).

Preparation of Working Standard Solution of Leucovorin Calcium

From that above Standard stock solution of Leucovorin Calcium pipette out 1 mL and transferred into 10 mL of volumetric flask and make up the volume upto mark with Water to produce a stock solution having strength of (100 μ g/mL).

Preparation of Standard Stock Solution of Leucovorin Calcium for Injection (100 μ g/mL)

Reconstitute the 500mg/vial with 50mL of water (10000 μ g/mL). From 1000 μ g/mL of solution pipette out 5 mL and transferred into 50 mL of Volumetric flask and make up the volume with water up to mark to give a solution having strength of 1000ppm. From the above solution (1000 μ g/mL) pipette out 1 mL of solution into 10mL of Volumetric flask and make up the volume with water up to the mark to give a solution having strength of 100 μ g/mL

Selection of Analytical Wavelength: To determine wavelength for measurement, Standard solution of Leucovorin Calcium (15 μ g/mL) scanned in range of 200-400 nm and maximum absorbance obtained at 286 nm so 286 nm was selected as wavelength for further determination of Leucovorin Calcium.

Preparation of Calibration Curve

Calibration Curve for Leucovorin Calcium:

Calibration curve for Leucovorin Calcium consists of different concentrations of standard Leucovorin Calcium solution ranging from 3-15 μ g/mL. The solutions were prepared by pipetting out 0.3, 0.6, 0.9, 1.2 and 1.5 ml of the working standard solution of Leucovorin Calcium (100 μ g/mL) into series of 10 ml volumetric flasks and the volume was adjusted to mark with water. The absorbance of the solutions was measured at 286 nm against Water as a blank.

Method Validation ^[13]

The method was validated as per ICH Q2(R1) guidelines.

Linearity (n=5)

The linearity of the method is its ability to bring out the test results which are directly proportional to the concentration of an analyte present in the sample. The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 3-15 μ g/mL of Leucovorin Calcium (n = 5).The calibration curve of absorbance vs. respective concentration was plotted and correlation coefficient and regression line equations for Leucovorin Calcium were calculated.

Precision:

(A) Repeatability (n=6): Aliquots of 0.9 ml of working standard Leucovorin Calcium (100 µg/mL) were transferred to a 10 ml volumetric flask. The volume was adjusted up to mark with Water to get 9µg/mL solution of Leucovorin Calcium. The absorbance of solution was measured six times and % RSD was calculated.

(B) Intraday precision

Aliquots of 0.3, 0.9, and 1.5 mL of working standard Leucovorin Calcium (100 µg/mL) were transferred to a series of 10 ml volumetric flask. The volume was adjusted up to mark with Water to get 3, 9 and 15µg/mL solution of Leucovorin Calcium. Solution was analyzed 3 times on the same day and % RSD was calculated.

(C) Interday Precision

Aliquots of 0.3, 0.9, and 1.5 mL of working standard Leucovorin Calcium (100 µg/ml) were transferred to a series of 10 mL volumetric flask. The volume was adjusted up to mark with Water to get 3, 9 and 15µg/mL solution of Leucovorin Calcium. Solution was analyzed 3 times on the 3 different days and % RSD was calculated.

Limit of Detection and Limit of quantification:

LOD and LOQ were determined from the linearity data.

$$\text{LOD} = 3.3 \times \sigma/S$$

$$\text{LOQ} = 10 \times \sigma/S$$

Where, σ = the standard deviation of the Intercept of the 5 calibration curve and S = slope of the calibration curve.

Accuracy

The accuracy of the method was determined by calculating recovery of Leucovorin Calcium by the standard addition method. Aliquots of 0.48, 0.6, and 0.72 mL of working standard Leucovorin Calcium(100 µg/mL) were added at 80, 100 and 120 % level to pre-analyzed 0.6 ml (6µg/mL) sample solutions of Leucovorin Calcium. The volume was adjusted up to mark with water to get 10.8, 12 and 13.2µg/mL solution of Leucovorin Calcium.

Results and Discussion

A reliable method was developed for estimation of Leucovorin Calcium in by UV Spectrophotometry. Standard solution of Leucovorin Calcium (15µg/mL) was scan in range of 200-400 nm maximum absorbance observed at 286 nm so 286 nm was selected as wavelength for further determination of Leucovorin Calcium (Figure 2). Beers law was obeyed in

concentration range of 3-15 $\mu\text{g}/\text{mL}$ for Leucovorin Calcium. The Regression Equation of Leucovorin Calcium was found to $Y=0.044x+0.105$ (Figure 3). The Correlation Co-efficient of Leucovorin Calcium was found 0.998 (Figure3). The mean % recoveries were found to be in the range of 98.45-100.96 % (Table 4). The %RSD of Repeatability (9 $\mu\text{g}/\text{ml}$) is 0.489 (Table 2). The %RSD of Intraday of 3, 9, 15 $\mu\text{g}/\text{mL}$ of Leucovorin Calcium on same day is less than 2 (Table 3) and %RSD of Interday of 3, 9, 15 $\mu\text{g}/\text{mL}$ of Leucovorin Calcium on 3 different days is less than 2 (Table 4). The LOD and LOQ were 0.225 $\mu\text{g}/\text{mL}$ and 0.683 $\mu\text{g}/\text{mL}$ of Leucovorin Calcium. The proposed method was precise, accurate and reproducible and acceptable recovery of the analyte, which can be applied for the analysis of Leucovorin Calcium.

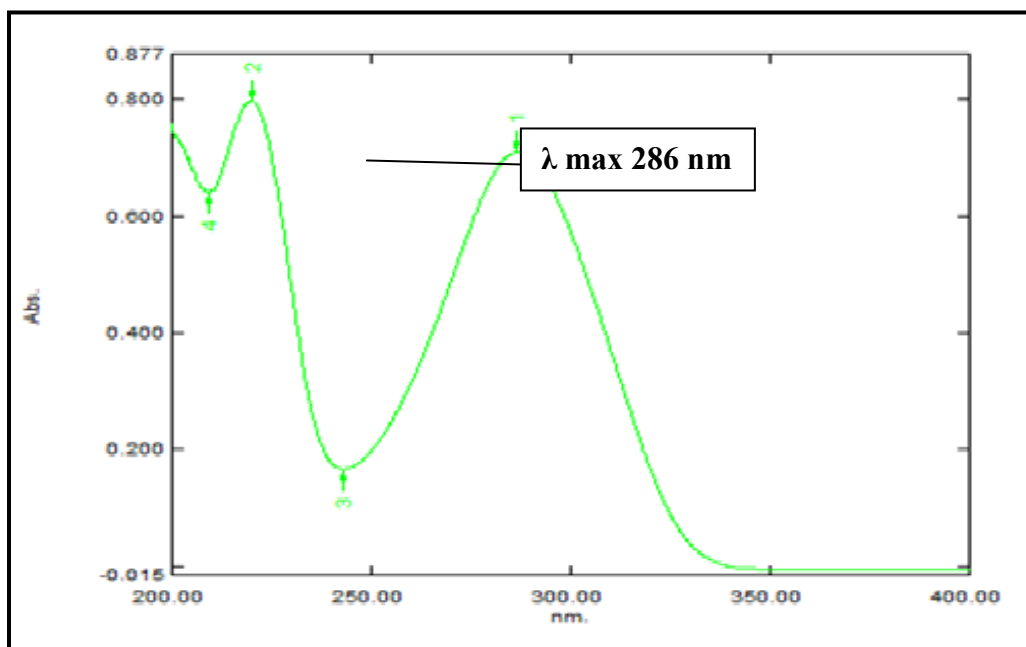


Figure 2: UV Spectra of Leucovorin Calcium (15 $\mu\text{g}/\text{ml}$).

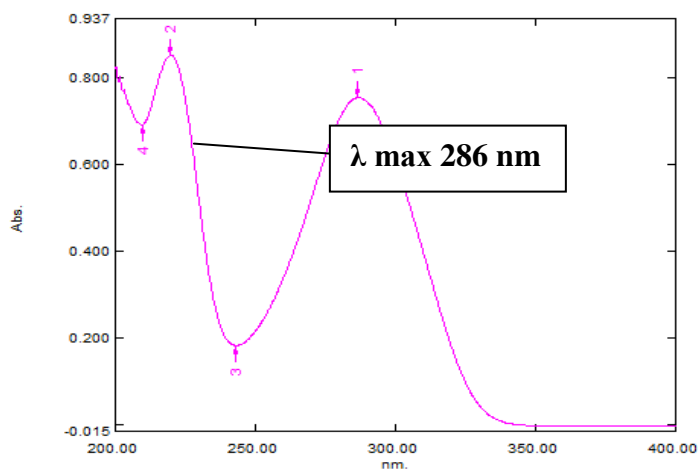


Figure 3: UV Spectra of Leucovorin Calcium for Injection (15 $\mu\text{g}/\text{mL}$).

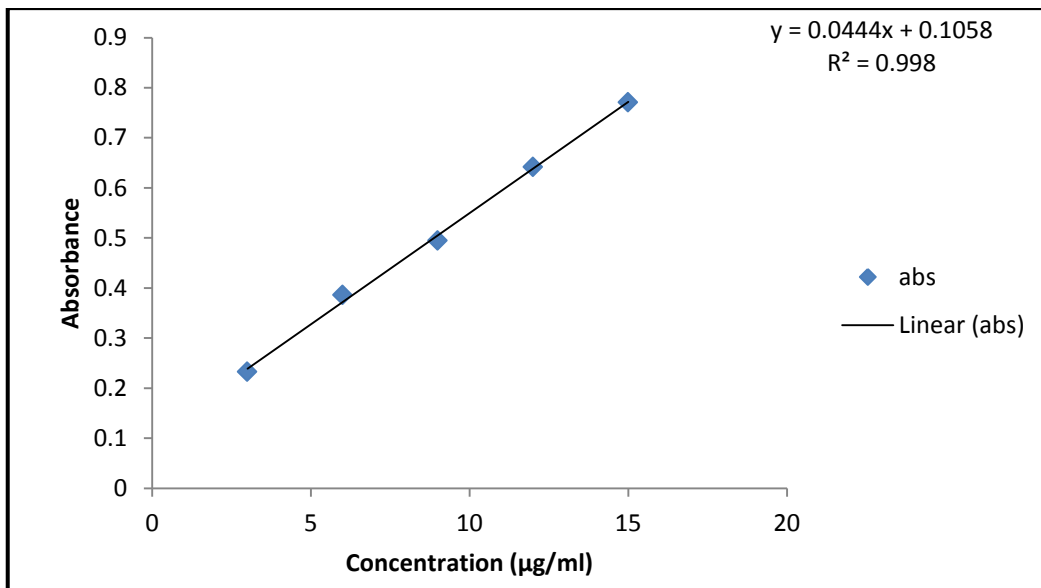


Figure 4: Calibration curve of Leucovorin Calcium at 286 nm.

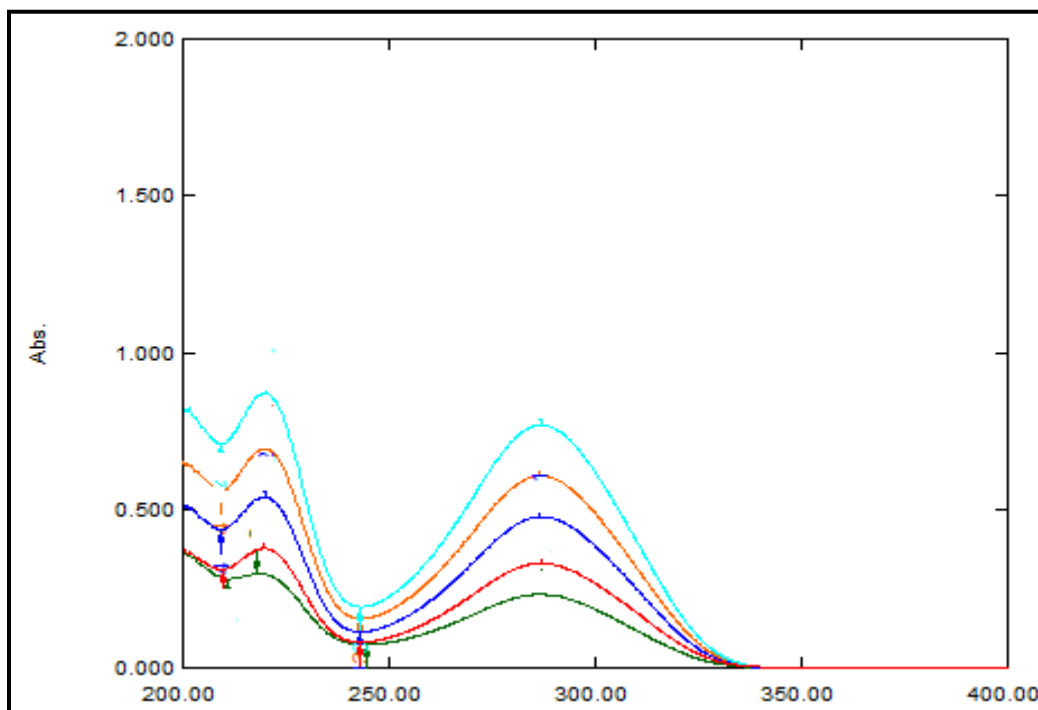


Figure 5: Overlain spectra of Leucovorin Calcium (3-15µg/mL)

Table-1: Linearity data of Leucovorin Calcium (286 nm).

Con (µg/mL)	Mean absorbance ±SD (n=5)	%RSD
3	0.236±0.00233	0.99
6	0.360±0.00384	0.98

9	0.494±0.00256	0.51
12	0.046±0.00390	0.61
15	0.774±0.00278	0.35
Regression Equation		Y=0.044x+0.105
Slope		0.105
Intercept		0.044
R ²		0.998

Table-2: Repeatability data of Leucovorin Calcium.

Con (µg/mL)	Mean absorbance ±SD (n=6)	% RSD
9	0.4946±0.002422	0.49

Table-3: Intraday Precision data of Leucovorin Calcium.

Con (µg/mL)	Mean absorbance ±SD (n=3)	% RSD
3	0.233667±0.000943	0.40
9	0.489333±0.00419	0.85
15	0.776667±0.003682	0.47

Table-4: Inter day Precision data of Leucovorin Calcium.

Con (µg/mL)	Mean absorbance ±SD (n=3)	% RSD
3	0.228667±0.001886	0.84
9	0.488667±0.005312	1.08
15	0.765333±0.005312	0.69

Table-5: Accuracy data of Leucovorin Calcium.

Level (%)	Amount taken ($\mu\text{g/mL}$)	Amount added ($\mu\text{g/mL}$)	Total concentration ($\mu\text{g/mL}$)	Recovered Concentration ($\mu\text{g/mL}$)	% Recovery	% Recovery \pm SD (n=3)
80	6	4	10	10.88	100.77	100.77 \pm 0.045
100	6	6	12	12.11	100.96	100.96 \pm 0.080
120	6	7.2	13.2	13.05	98.45	98.45 \pm 0.117

Table-6: Assay Study Parameter.

Label claim($\mu\text{g/mL}$)	Amount found ($\mu\text{g/mL}$)	% Assay \pm SD (n=3)
10	10.13	101.59 \pm 0.0028

Conclusion

The proposed Spectrophotometric method was found to be simple, accurate and precise for determination of Leucovorin Calcium in Pharmaceutical Dosage form . The method utilizes easily available and cheap solvent for analysis of Leucovorin Calcium hence, the method is economic for estimation of Leucovorin Calcium. Hence it can be conveniently adopted for routine quality control analysis of the drug.

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