



JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

Development and Validation of Analytical Method for Estimation of Leucovorin Calcium in It's Marketed Dosage Form

Patel Namrata C., Sakhreliya Bhumika, Patel Mandev B.

Department of Quality Assurance, A-One college of Pharmacy, Anasan, Ahmedabad, Gujarat, India

ABSTRACT:

RP-HPLC method for estimation of Leucovorin Calcium In a tablet formulation. An isocratic reversed phase HPLC method has been developed for the determination of Leucovorin Calcium on a C18Phenomenex (250 x 4.6 mm) column using a mobile phase consisting of 0.00 5M tetrabutyl ammonium phosphate buffer pH 6.6 (70:30,v/v) at a flow rate of 1 mL/min and the detection was carried out at 292 nm. Retention times of Leucovorin Calcium was found to be 3.99 min. The method was validated with respect to specificity, linearity, accuracy, precision, ruggedness and robustness. This method is simple, precise, and sensitive and is applicable for simultaneous quantification of Leucovorin Calcium in a tablet formulation

KEYWORDS: isocratic, reverse phase, validation, RPHPLC, Leucovorin Calcium

Article history:

Received 10 April 2015

Revised 14 Jan 2016

Accepted 16 Feb 2016

Available online 01 March 2016

Citation:

Patel N. C., Sakhreliya B. Patel M. B.
Development and Validation of Analytical
Method for Estimation of Leucovorin
Calcium in It's Marketed Dosage Form. *J
Pharm Sci Bioscientific Res.* 2016
6(2):207-210

INTRODUCTION:

Leucovorin Calcium is a Anti-cancer drug which is a anti –metabolites analog Leucovorin is an active metabolite of folic acid and an essential coenzyme for nucleic acid synthesis. Leucovorin can be used to selectively “rescue” cells from the adverse effects of methotrexate or to increase the efficacy of fluorouracil. Methotrexate inhibits nucleic acid synthesis by blocking the activation of folic acid. Leucovorin is folic acid in its active (reduced) form, so it allows nucleic acid synthesis to proceed even in the presence of methotrexate. Leucovorin can also compete with methotrexate for the same transport processes into the cell.¹ Leucovorin is usually administered 24 hours after methotrexate so that it does not interfere with the therapeutic effect of methotrexate. Leucovorin can also be used in overdose situations; it should be administered as soon as possible. Leucovorin Calcium is official in United Pharmacopoeia².

MATERIALS AND METHODS:

Apparatus

High performance liquid chromatography including aThermoseparation instrument equipped with rhynodyne manual sampler, UV detector, C18Phenomenex column having dimensions 4.6mm x 250mm was used.

Materials and reagents

Leucovorin Calcium was obtained as a gift sample from Active Ingredients Smarth pharma Pvt.Ltd, Mubai

*For Correspondence:

Patel Namrata C.

Student of Quality Assurance,

A-One college of Pharmacy, Anasan,
Ahmedabad, Gujarat, India.

(www.jpsbr.org)

Tablets containing 15 mg Recovorin was procured from local market. HPLC grade methanol, water used was purchased from S.D. Fine Chemicals (Mumbai, India).

Chromatographic condition

A mobile phase consisted of Water :ACN phosphate buffer pH-6.6 (30:70, v/v) was pumped at a flow rate of 1 mL/min. The elution was monitored at 292 nm and the injection volume was 20 μ L.

The validation of the method was done following the ICH guidelines.

Preparation of mobile phase and standard solutions

Standard solutions of 5-15 μ g/ml of Leucovorin calcium was injected in column with 20 μ l micro-syringe. The chromatogram was run for appropriate minutes with mobile phase :water:acetonitrile (30:70) . The detection was carried out at wavelength 292 nm. The chromatogram was stopped after separation achieved completely. Data related to peak like area, height, retention time, resolution etc were recorded using softwar Take 1 mL from the leucovorin calcium stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by mobile phase which was used in particular trials.

Analysis of marketed formulation.

Take Crushed Tablet powder equivalent to Leucovorin calcium 10 mg of was transferred to a 100 ml volumetric flask, add 60 mL of mobile phase and transferred to 10 ml volumetric flask and made up volume up to the mark with mobile phase. The solution was filtered through Whatman filter paper no. 42 and first few drops of filtrate were discarded. 10 ml of this solution was diluted to 100 ml with mobile phase. The solution was injected 10 μ L. The areas of resulting peak were measured at 292 nm.

Validation of the method

Specificity.-Specificity of the method was studied by injecting blank, standard, placebo and sample solutions.

Calibration curve (Linearity of HPLC method)

The linearity for Leucovorin calcium was assessed by analysis of standard solution in range of 5-15 μ g/ml respectively.

5,7.5,10,12.5,15 ml solutions were pipette out from the Stock solution of Leucovorin calcium (100 μ g/ml) transfer to 100 ml volumetric flask and make up with mobile phase to obtain 5,7.5,10,12.5,15 μ g/ml for Leucovorin calcium respectively In term of slope, intercept and correlation co-efficient value. The graph of peak area obtained verses respective concentration was plotted.

Accuracy (% recovery).-Accuracy of the method was studied by recovery experiments using standard addition method at three different levels (80%, 100% and 120%). The known amounts of standard solutions containing Leucovorin calcium added to prequantified sample solutions to reach the 80,100 and 120 % levels. These samples were analyzed by injecting the sample solution and recovery was calculated.

Precision (Repeatability)

Precision of the assay method was demonstrated by injecting six different sample solutions containing 10 μ g/mL Leucovorin calcium and RSD of mean assay value was calculated

Intermediate Precision (Ruggedness)

Intermediate Precision of the method was demonstrated by carrying out the experiment on different day, by different analyst and on different instrument using different C-18 column.

Robustness:

Robustness of the method was demonstrated by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from 0.2 mL/min to -0.2 mL/min. The composition of mobile phase was changed From Water :ACN phosphate buffer pH-0.2 (30:70, v/v). The solutions for robustness study were applied on the column in triplicate and the responses were determined.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

LOD and LOQ of Leucovorin Calcium calculated using the following equations as per International Conference on Harmonization (ICH) guidelines.

$$\text{LOD} = 3.3 \times \sigma/S \quad \text{LOQ} = 10 \times \sigma/S$$

Where, σ = Standard deviation of response , S = Slope of regression equation.

RESULT AND DISCUSSIONS:

Several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was obtained by using the mobile phase containing Water :ACN (30:70, v/v)phosphate buffer pH- 6.6 Quantification was achieved with UV detection at 292 nm based on peak area. A representative chromatogram is shown in Figure 1.

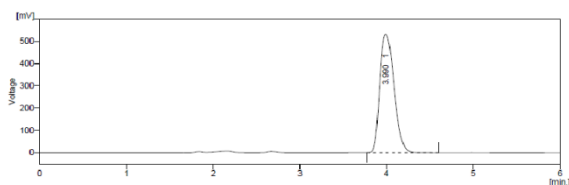


Figure 1. chromatogram for Leucovorin Calcium

System suitability tests were carried out on freshly prepared standard solutions (n = 6) containing Leucovorin Calcium. System suitability parameters obtained with 20 L injection volume are summarized in Table 1.

Table 1: System suitability test parameters for Leucovorin Calcium

Parameter	LC
Theoretical plate	2518
Tailing factor	1.58
Resolution	-
Correlation coefficient (r2)	1
Retention time (min.)	3.99

Specificity studies indicated that there is no interference from excipients, impurities and degradation products and assured that the peak response was due to Leucovorin Calcium only.

Linearity regression data is summarized in Table 2 which shows a good linear relationship between concentration and peak areas over a concentration range of 5-15 µg/ml) The correlation coefficient (R2) was found to be 0.9993 for Leucovorin calcium

The limit of detection was found to be 0.58 µg/ml for Leucovorin calcium. The limit of quantification was found to be 1.76 µg/ml for Leucovorin calcium. These values indicate that the method is sensitive.

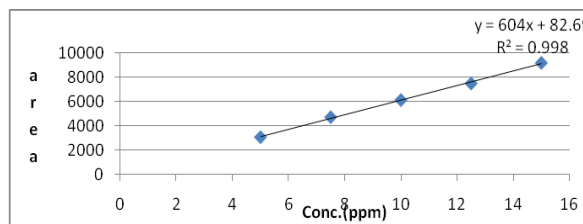


Figure 2: Calibration curve diagram for Leucovorin calcium

Table 2: Regression analysis of the calibration curves for Leucovorin

Parameters	Leucovorin Calcium
Detection wavelength	292 nm
Linearity (µg/ml)	0.999
Regression equation	Y=604x+82.69
Slope (m)	0.079
Intercept (c)	106.33

In the precision studies, RSD of mean assay values was found to be 0.50 for Leucovorin calcium. These values indicate that the repeatability of this method is satisfactory.

Intermediate precision (Ruggedness) study reveals that the method is rugged with RSD values of 0.53 for Leucovorin calcium. Accuracy studies indicate that the mean percent recovery of the added standard drug to be 100.81 for Leucovorin calcium respectively.

Robustness study signified that the results of the method remained unaffected by small, deliberate changes in the flow rate and mobile phase composition. The RSD of mean Assay values was found to be 0.50 for Leucovorin calcium with a flow rate of 1.0 mL/min . respectively with mobile phase composition of containing Water :ACN (30:70, v/v)phosphate buffer pH- 6.6 for Leucovorin calcium. Results obtained for various validation parameters are summarized in Table 2.

The assay results obtained by using the proposed method for the analysis of marketed tablet formulation containing Recoverin 15 mg tablet was in good agreement with the labeled amounts of Leucovorin calcium . The average contents of Leucovorin calcium was 15 mg per tablet (98.79) per tablet respectively.

Parameter	LC
Wavelength for estimation	292nm
Linearity range ($\mu\text{g/ml}$)	5 – 15
Regression equation	$Y = 604x + 82.69$
Correlation coefficient (r^2)	0.998
Retention time (min.)	3.99
Accuracy (n=3)	100.82 ± 0.43
Assay (n=6)	99.04 ± 0.50
LOD ($\mu\text{g/ml}$)	0.58
LOQ ($\mu\text{g/ml}$)	1.76

CONCLUSION:

The proposed RP- HPLC method is accurate, precise, sensitive, selective and rapid for the determination of Leucovorin calcium in a tablet formulation

ACKNOWLEDGEMENT:

The authors are grateful to Smarth Pharmaceutical Pvt Limited, Mumbai (India) for gift sample of Leucovorin calcium

REFERENCES:

1. "Leucovorin calcium Drug Profile", February 2013, <http://www.drugbank.ca/drugs/DB01120>
2. United States Pharmacopoeia-30, National Formulary-25, Asian edition the United State Pharmacopoeia, convention Rockville, MD, 2007, pp 499.
3. Ling-yun HE and Gao-yun HU, "Determination of Leucovorin calcium and its related substances by RP-HPLC." West China J. Pharma. Sci. 2011
4. Haripriya M, Antony N and Jayasekhar P "Development and validation of UV spectrophotometric method for the simultaneous estimation of Leucovorin and Acetoaminophen in tablet dosage form." Int. J. Pharma and Pharmaceutical Sci. 2013, 3(1), 343-348.
5. Abdullah NS, Hassan MA and Hassan RO, "Spectrophotometric Determination of Leucovorin Pharmaceutical Formulations Using Different Order Derivative Methods." Arab. J. Chem. 2014, 1-20.
6. Haque AM, Kumar PK, Kumar PT and Diwan PV, "Simultaneous Estimation of Leucovorin calcium & 5-Methyltetrahydrofolate as Bulk and In Tablet Dosage Form Using HPLC." Int. Org. Sci. Res. J. Pharma. Bio. Sci. 2012, 1(4), 20-23.
7. Sueware BH, Tapkir AS, Jadhav SB and Chaudhari PD, "Development and validation of Stability indicating method for estimation of Leucovorin calcium in bulk and tablet dosage form." Der Pharma letter 2013, 5(1), 149-154.
8. O.Wallece "Introduction to Leucovorin calcium", February 2014, <http://www.wisegeek.com/what-is-Leucovorin-calcium.html>
9. Willard HH., Merritt LL., Dean JA and Settle FA. Instrumental Methods of Analysis; 7th Edn; CBS Publication and Distributors Pvt. Ltd, 2008, pp 524-28.
10. 1 ICH, Q2A, harmonized tripartite guideline, text on validation of analytical procedures, IFPMA, in proceeding of international conference on harmonization, Geneva; March 1994.
11. ICH, Q2B, harmonized tripartite guideline, text on validation of analytical procedures, IFPMA, in proceeding of international conference on harmonization, Geneva; March 1996.
12. ICH, "Q2R1: Validation of Analytical Procedures: Text And Methodology. Availability," Federal Register 1997; 62(96), 27463–27467.
13. "Introduction to Anti Cancer" <http://www.americanhealthassociation.com>
14. "Introduction to Anti Cancer" <http://www.faqs.org/health/Sick-V2/Anti-cancer.html>
15. Rang H P., Dale M M., and Ritter J M. In Pharmacology; 4th Edn; New York, Churchill Livingston, 1999, pp 257,462.
16. Tripathi K D. In Essential of Medical Pharmacology; 4th Edn; Jaypee brothers, New Delhi, 2001, pp 412,589-591
17. Shereen Skola, "Introduction of Leucovorin calcium", January 2014, <http://www.wisegeek.com/what-is-Leucovorin-calcium.html>

