ejpmr, 2024, 11(6), 594-598

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

ELECTROANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF FOLIC ACID AND FERROUS ASCORBATE FROM PHARMACEUTICAL FORMULATION

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Article Received on 26/04/2024 **Article Revised on 16/05/2024** **Article Accepted on 06/06/2024**

ABSTRACT

A simple, specific, accurate, precise and reproducible method has been developed and validated for the simultaneous determination of Folic acid and Ferrous Ascorbate using Differential Pulse Polarography (DPP) technique. Quantification of Folic Acid and Ferrous Ascorbate was done in Acetate buffer pH 4.7 respectively using 1M KCl as a supporting electrolyte. Both Folic Acid and Ferrous Ascorbate exhibit reduction cathodic peak in given respective pH with peak potential (Ep) as -0.85V for Folic Acid and -1.281V for Ferrous Ascorbate vs. S.C.E. Linearity of the method was found to be in the concentration range of 0.99 µg/mL to 6.54 µg/mL for FA and 1.98 µg/mL to 13.08 µg/mL for FEAS with correlation coefficient greater than 0.999 for both the analytes The parameters were used for method validation are linearity; accuracy, precision, LOD and LOQ. Proposed method was successfully applied for routine quality control analysis and simultaneous determination Folic Acid and Ferrous Ascorbate in combined drug formulation.

KEYWORDS: Differential Pulse Polarography (DPP), Folic Acid (FA), Ferrous Ascorbate (FeAs), Acetate buffer.

INTRODUCTION[1-8]

Vitamins are organic compounds which occur in very small quantities in food but are very important to life for specific regulatory functions and the maintenance of life and normal growth. Vitamins provide proper metabolism, ensure good health and protect against certain diseases. Vitamins are classified according to their solubility in water or fats. The fat-soluble vitamins are A, D, E, and K; the B complex and C vitamins are water soluble.

Minerals
(Macroelements or microelements)

It is a group of chemical elements that are needed in minute quantities for the proper growth, development, and physiology of an organism. Minerals can be classified into macroelements and microelements. Macroelements are those elements that are needed in relatively large amounts by the body and micro elements are those that are needed in small 'trace' amounts. Some macroelements that are needed by the body are calcium, phosphorous, magnesium, sodium, potassium, chlorine and sulfur. The micro elements needed by the body are iron, manganese, copper, zinc, cobalt, chromium.

OBJECTIVE

The present study gives a simple, rapid, efficient, reliable and economic method for the simultaneous determination of Folic Acid and Ferrous Ascorbate in pharmaceutical formulations using Differential Pulse Polarography technique. The proposed method has been validated as per ICH guidelines

MATERIALS AND METHODS (EXPERIMENTAL)[11-12]

Introduction to workstation

Electrochemical workstation- PG STAT 30 with 663 VA Electrode stand (Metrohm)
It is made up of three electrode system namely-

1) Hanging Mercury Drop electrode (HMDE) as the working electrode
2) Saturated calomel electrode as the reference electrode
3) Platinum electrode as the counter electrode

The pH measurements were made with Euiptrances model No. 610.

Reagents
Standard Folic Acid and Ferrous Ascorbate was obtained from local pharmaceutical company. All the solutions were prepared in double distilled water. All the reagents use were of AR grade.

Preparation of acetate buffer (pH-4.7)
1.36 g of sodium acetate monohydrate and 0.57 mL (570µL) of glacial acetic acid was transferred to a 100 mL standard flask. Sufficient amount of distilled water was added till a homogeneous mixture was formed and finally it was diluted with distilled water upto the mark

Analytical method development
Preparation of standard solution
Preparation of 1000 µg/mL of stock solution of standard FA (STD A)
100 mg of FA was accurately weighed and transferred into a 100 mL standard flask, dissolved in minimum amount of distilled water and diluted up to the mark with distilled water.

Preparation of 1000 µg/mL of stock solution of standard FEAS (STD B)
100 mg of FEAS was accurately weighed and transferred into a 100 mL standard flask, dissolved in minimum amount of distilled water and diluted up to the mark with distilled water.

Preparation of combined stock solution of standard FA and standard FEAS containing 100 µg/mL and 200 µg/mL of FA and FEAS respectively (STD C)
10 cm^3 of the (Std A) and 20 cm^3 of the (Std B) solution was taken in 100 cm^3 of volumetric flask and diluted up to the mark with distilled water.

Proposed voltammetric method
An aliquot of 20cm3 made up of 18 mL Sodium Acetate Buffer pH 4.7 + 2 mL of 1M KCl as a supporting electrolyte was placed in the dry and clean voltammetric cell. Then it was purged with highly pure nitrogen gas for 180s. A negatively directed DP scans between the potential 0.0 V to -2.0 V vs. S.C.E was applied. The operational parameters were as follows: 1] Scan rate- 0.015 V s-1. 2] Pulse amplitude- 0.05V After recording a polarogram of blank, aliquots of (1mL) the required standard solution was added from the standard stock solution. Resulted polarograms were recorded under the optimum experimental conditions. Peak currents were recorded. Calibration curve was prepared by plotting peak current versus concentration of Folic Acid and Ferrous Ascorbate applied. The results were shown in **[Table.1]**

Preparation of sample solution
Ten tablets of drug sample were weighed and powdered. The amount of the powdered sample, equivalent to 1.5 mg of FA and 100 mg of FEAS. 0.920 g of sample was weighed and transferred to a 100 cm^3 volumetric flask containing distilled water. The solution was shaken for 5 minutes and then made up to the mark. The solution so obtained is 15 µg/mL of FA and 1000 µg/mL of FEAS and named as sample I respectively.

Analytical method validation[9-10]
System suitability
System suitability tests are used to ensure reproducibility of the equipment. The test was carried out by recording polarogram for FA (1.9670 µg/ml) and for FEAS (3.9212 µg/ml with five replicates and the mean was used for the whole calculations. The % RSD was found to be 0.82 for FA and 0.31 for FEAS, which was acceptable as it is less than 2%.

Specificity
The specificity of method was confirmed by observing the polarograms of both the combined standard solution and the drug sample solutions. The polarograms obtained from the drugs sample solution were found to be identical to those obtained for standard solution. The addition of the standard solution to the drug sample solution did not change the characteristics of differential pulse polarogram. This gives the validity of method for the determination of both drugs from combined pharmaceutical formulation.

Linearity and Range
The linearity for FA and FEAS were observed simultaneously by addition of standard solution. A good linearity was achieved in the concentration ranges of **0.9900 µg/mL to 7.4074 µg/mL of FA and 1.9801 µg/mL to 14.8148 µg/mL of FEAS.** The calibration curves were constructed with concentration (C) against peak current (Ip). The slope, Intercept, regression equation and correlation coefficient for the tinidazole was obtained is given in **(Table 1).**

Limit of Detection and Limit of quantitation
The limit of detection (LOD) and the limit of quantification (LOQ) were determined by signal to noise ratio of 3:1 and 10:1 respectively. The limit of detection and limit of quantification were found to be **0.11**µg/mL and **0.98** µg/mL for FA and the limit of detection and limit of quantification were found to be **0.97**µg/mL and **1.98** µg/mL for FEAS.

Intraday and Interday precison
The intra-day and inter-day precision was used to study the variability of the method. It was checked by

recording the polarograms of standard solutions of FA and FEAS i.e. whole concentration ranges (0.9900 µg/ml to 6.5420 µg/ml for FA and 1.9801 µg/ml to 13.0841 µg/ml for FEAS) both at intra-day (five times within 24 hour) and inter-day (two times each. during 3 days intervals) to check the precision. The mean % RSD for intra-day and inter-day precision for FA found to be 1.01% and 0.93% and for FEAS it was 0.53% and 1.76%, respectively.

Assay

The developed Polarographic method was used for determination of Folic Acid and Ferrous Ascorbate from different brands of formulations. The sample working solutions were analyzed by the developed method described above. Polarograms were recorded under the optimum experimental conditions. Assay studies were carried out at three different levels. The percentage assay at three different levels for Folic Acid and Ferrous Ascorbate was found to be from 98.00 % to 102.00 %. The results were shown in. **(Table 3)**

Robustness

The robustness of the method was examined by the consistency of peak height and peak shape with the deliberately small changes in the experimental parameter. It is a measure of its capacity to retain 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 proposed method, the following variations were made in the analytical method- 1] Scan rate by ± 0.5 mVs^{-1}. 2] Pulse amplitude ± 1.0 mV

These parameters were deliberately changed one at a time and the effect of these changes on the assay studies was carried out. The proposed method was found to be robust.

Accuracy (Recovery)

The recovery was used to evaluate the accuracy of the method. Accuracy of the method was determined using the standard addition method. A fixed volume of standard Folic Acid and Ferrous Ascorbate solution was mixed with different concentrations of pre-analyzed sample solutions and mixtures were analyzed by proposed method. The percent recovery was determined at different levels. The results were shown in **[Table 4]**

RESULT AND DISSCUSSION

In the present study quantification of Folic Acid and Ferrous Ascorbate have been done from the formulations using Differential Pulse Polarography technique. The developed method was validated as per the ICH guidelines. But before the method development and subsequent validation, optimization of the conditions for the analyte was done i.e. pH, supporting electrolyte and also the parameters i.e. 1] scan rate 2] Pulse amplitude has been studied. During optimization of the conditions, the polarographic response of Rosiglitazone Maleate and Metformin Hydrochloride in different buffer solutions have been studied i.e. Acetate, Phosphate and Britton-Robinson Buffer. Britton-Robinson buffer was prepared by mixing 0.04M Boric acid, 0.04M Phosphoric acid and 0.04M Glacial acetic acid. Further pH was adjusted with 1M NaOH. In the Britton-Robinson Buffer the whole pH range i.e. pH 2.0 to pH 10.0 has been studied.

As the pH was shifted from acidic to basic there is change in peak potential was observed. Finally, Sodium Acetate Buffer of pH 4.7 was chosen as the best, due to good separation of both the analytes, more uniform peak shape, less tailing, less broadening of peak, normal base line starts and regression analysis. The KCl used as a supporting electrolyte. With KCl more uniform and sharper peaks were observed. Pulse amplitude of 50mV was chosen as optimum as there is loss of resolution at high pulse amplitude.

The Differential Pulse polarograms of Folic Acid and Ferrous Ascorbate were recorded at various scan rates. At higher scan rate than $15mVs^{-1}$ the width of peak increases, its height decreases and peak shape was distorted. At slower scan rate than $15mVs^{-1}$ uniform peak shape and peak height was small as compared to that of higher scan rate than $15mVs^{-1}$, so a scan rate of $15mVs^{-1}$ was chosen as a best for the analysis. The height of peak increase gradually with concentration of norfloxacin and tinidazole and the response of peak current $\mathbf{i_p}$ as function of concentration is linear.

No significant interference was observed from excipients commonly used in the formulation i.e. glucose, sucrose, starch, magnesium stearate or talc powder.

Table 1: Optimum Conditions and Parameters for the polarographic determination of FA and FEAS.

Parameters	Values	
	Folic Acid (FA)	Ferrous Ascorbate (FEAS)
Solvent	Distilled Water	Distilled Water
Optimum PH	Sodium Acetate Buffer pH 4.7	Sodium Acetate Buffer pH 4.7
Supporting Electrolyte	1M KCl	1M KCl
Peak Potentials	-0.85V	-1.281V
Scan rate (mVs-1)	15 mVs-1	15 mVs-1

Table 2: Method Validation Parameters For determination of FA AND FEAS.

Parameters	Values	
	FA	FEAS
System suitability (n=5) %RSD	0.82%	0.31%
Linearity range (µg/ml)	0.99 to 6.54	1.98 to 13.08
Slope (m) [a)]	8.0762	75.88
Intercept(c) [a)]	2.2538	204.85
Correlation coefficient (R^2)	0.9997	0.9995
LOD (µg/ml)	0.11µg mL^{-1}	0.99 µg mL^{-1}
LOQ (µg/ml)	0.98 µg mL^{-1}	1.81 µg mL^{-1}
Intraday precision (n=5)	1.08%	0.97%
Interday precision (n=5)	0.93%	1.98%
Assay	98% to 102%	98% to 102%
Recovery	98% to 102%	98% to 102%

a) Of the equation y = mx + c, where y is peak area, m is the slope, x is the Concentration and c is the intercept

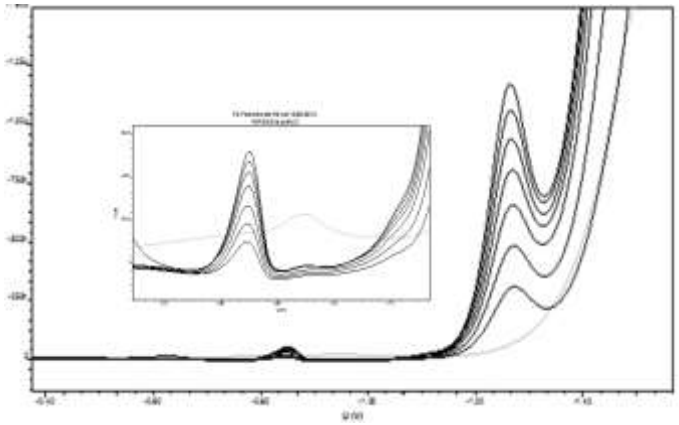
Table 3: Results of Assay Studies for FA and FEAS.

Brand Name	Ferrogen-Xt (Intas pharma)	
A.P.I	Folic Acid	Ferrous Ascorbate
Labelled Claim(mg)	1.5	100
Drug found in mg	1.52	99.75
%RSD	0.55	0.27
%Assay	101.3	99.75

Table 4: Results of Recovery Studies for FA and FEAS.

Analyte	Level	RSD (%) (n = 6)	Recovery (%)	
			Minimum	Maximum
Folic Acid	40%	0.6	101.1	101.5
	100%	1.1	100.2	100.4
	140%	0.9	99.1	99.1
Range			99.1	101.5
Ferrous Ascorbate	40%	0.5	101.1	102.0
	100%	0.8	99.2	99.6
	140%	1.3	98.1	98.3
Range			98.1	102.0

Poarograms of Fa and Feas



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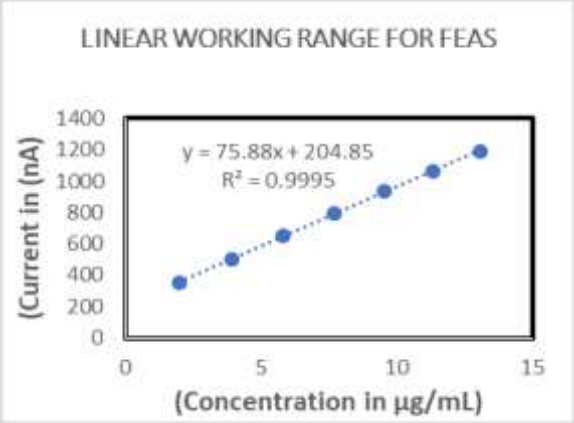

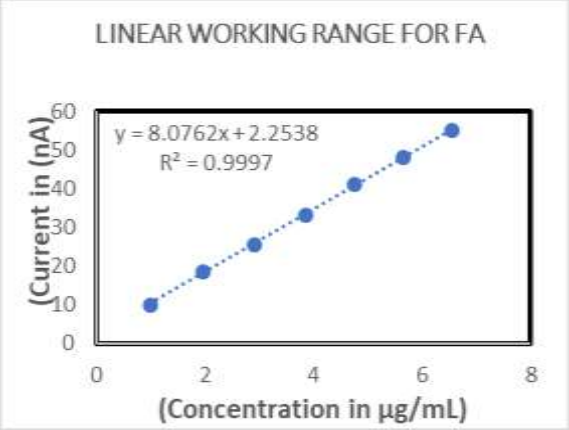

ACEKNOWLEDGEMENT

We thank to our Department of Chemistry St. Xavier's College and Ramnarain Ruia College for providing us all the Necessary instrumentation facilities and their technical assistance.

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