VOLUME - 13, ISSUE - 11, NOVEMBER - 2024 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper | Pharmaceutical Science

NEW VALIDATED METHOD FOR THE ESTIMATION OF WARFARIN SODIUM IN PHARMACEUTICAL DOSAGE FORM BY VISIBLE SPECTROSCOPY

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ABSTRACT A simple, sensitive, rapid and accurate analytical method has been developed for the estimation of warfarin sodium in pharmaceutical dosage form. Here the method was based on reaction between warfarin sodium and 2,4 Dinitro phenyl hydrazine (2,4 DNP) involving the formation of orange coloured complex which showed a linearity range of 30 to 600 μg/ml at the max of 450 nm. The coloured complex attained stability at 10 min. The coloured complex was stable for more than 2 hours 30 min. The concentration of reagent (2,4 DNP) used in this study is 2% and the volume of 2,4 DNP is 2ml. The method was validated based on ICH guidelines. Accuracy of this method is 99.4%. The %RSD of Precision and Ruggedness were found to be 1.4 & 1.6 respectively. Detection Limit and Quantification Limits are 8.25μg/ml and 25 μg/ml respectively. Hence this method is useful for the routine analysis of warfarin sodium.

KEYWORDS : Warfarin sodium, 2,4 di nitro phenyl hydrazine, colorimetry.

INTRODUCTION

Colorimetry or visible Spectroscopy is the measurement of the wavelength and the intensity of electromagnetic radiation in the visible region of the spectrum[1,14]. It is used extensively for the identification and determination of concentration of substances that absorb light. The sodium salt form of warfarin, a coumarin and a vitamin K antagonist, with anticoagulant activity[23]. Warfarin sodium inhibits both vitamin K and vitamin K epoxide reductases, thereby interfering with the cyclic interconversion of vitamin K epoxide to its reduced form, vitamin KH2[25]. Colorimetric methods using 2,4 Dinitro phenyl hydrazine were not published[22]. So the present study was aimed to develop a new method for the estimation of warfarin sodium using 2,4 Dinitro phenyl hydrazine[24].

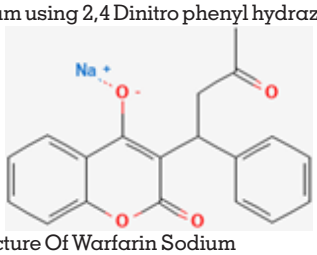

Figure 1. Structure Of Warfarin Sodium

MATERIALS

Warfarin sodium, 2,4-Di Nitrophenyl Hydrazine (2,4 DNP), Distilled water, weighing balance and Colorimeter (Cl157).

Experimental

Solvent Selection:

Solubility studies were conducted to the Warfarin sodium with various solvents.

Preparation Of Standard Stock Solution:

Standard warfarin sodium of 100mg was weighed and transferred to a100ml volumetric flask and dissolved in 25ml of ethanol. The flask was shaken and volume was made up to the mark with ethanol to give a solution containing 1000μg/ml (stock solution)[4].

Selection Of Reagent:

Reagent was selected based on the chemical structure of Warfarin sodium (analyte). According to the functional groups in the structure, reagent was selected. Linearity of the drug was checked with reagents like bromothymol blue, 2,4 DNP[23].

Determination Of Maximum Absorbance Wavelength Of 10 μg/ml solution:

Stock solution of warfarin sodium was further diluted with distilled water to get concentration of 10 μg/ml and 2 ml of reagent was added. Absorbance was checked at various wavelengths[11].

Selection Of Volume Of Reagent: Different volumes of reagents were checked[15].

Selection Of Concentration Of Reagent: Various concentrations of reagents were checked with drug solution.

Stability Of The Coloured Complex: The time required for achieving stability of coloured complex was measured[17].

Selection Of Analytical Concentration Range: From standard stock solution of warfarin sodium, appropriate aliquots 0.7, 0.8, 0.9, 1, 2, 3 ,4, 5, 6 ml respectively were pipetted out in 10ml volumetric flasks and make up with distilled water to obtain working standard solutions of concentration range 30-600μg/ml. Absorbance for these solutions were measured at 450nm[18].

Construction Of Calibration Curve: From standard warfarin sodium stock solution, concentrations of 100, 200, 300, 400, 500, 600 μg/ml were prepared. Absorbance value of each solution against blank were measured at 450nm. From the absorbance values and concentration values, calibration curve was constructed. Regression equation and correlation coefficient (R^2) were determined[19].

Assay Procedure for the Determination of Warfarin Sodium: Twenty tablets were weighed and individual tablet weight was determined. Label claim of warfarin sodium is 5 mg. Drug equivalent to 100 mg was taken in a 100 ml volumetric flask from tablet powder and made up the volume with ethanol which is a sample stock solution. 20 μg/ml solution was prepared from sample stock solution. Its absorbance was noted at 450 nm. Amount of drug in the tablet is calculated by Regression equation method[20].

Validation

Linearity: From standard stock solution of warfarin sodium, appropriate aliquots of 0.3, 0.4, 0.5, 0.6, 0.7,0.8,0.9, 1, 2, 3, 4, 5, 6ml respectively were pipetted out in 10ml volumetric flasks and make up with distilled water to obtain working standard solutions of concentrations from 30-600μg/ml. Absorbance for these solutions were measured at 450 nm. These concentrations were showing linear values. From that absorbance value, Regression equation and correlation coefficient (R^2) are determined[8].

Accuracy: Weighed accurately tablet powder equivalent to 100mg and transferred in to 100ml volumetric flask and warfarin sodium was extracted in 10ml distilled water. The solution was then filtered and the filtrate was then made up to 100 ml with distilled water to get 1000μg/ml concentration. This solution was further diluted to get concentration of

10μg/ml. To keep an additional check on accuracy of developed assay method, analytical recovery experiments were performed. The different solutions of different concentrations like 16, 20 and 24μg/ml were prepared in case of both pure drug solution and the formulation extract solution and these solutions were subjected to analysis by above developed method [9].

Precision: Precision of methods was studied as intraday and inter day. Intra-day study was performed by analysing the three different concentrations of drug for three times in the same day. Inter-day Precision was performed by analysing three different concentrations of drug for three days in a week. Three different concentrations were LQC, MQC, HQC [10].

Ruggedness: Absorbance values were taken by two analysts with the same instrument and with the two instruments by the same analyst [11].

RESULTS AND DISCUSSION

Solvent Selection: It was found that Warfarin sodium was freely soluble in ethanol[5].

Selection Of Reagent: There is good linearity of drug with 2,4-Dinitrophenyl hydrazine and the colour of the complex is orange[6].

Figure 2. Orange Coloured Complex

Determination Of Maximum Absorbance Wavelength Of 10 μg/ml solution:

It was found that 450 nm is the maximum absorbance wavelength[7].

Table 1. Selection Of Concentration Of Reagent

S.NO	Concentration of reagent	R^2
1	1%	0.963
2	2%	0.999
3	3%	0.983
4	4%	0.992
5	5%	0.973

Table 2. Effect Of Time On The Linearity Of Coloured Complex

S.NO	Time(min)	R^2
1	0	0.972
2	5	0.984
3	10	0.990
4	15	0.990
5	20	0.990

Table 3. Effect Of Volume Of Reagent On The Linearity Of Coloured Complex

S NO	Volume(ml)	R^2
1	1	0.962
2	2	0.999
3	3	0.987
4	4	0.967

Selection Of Analytical Concentration Range: The concentrations in the analytical concentration range were showing linear values[16].

Construction Of Calibration Curve:

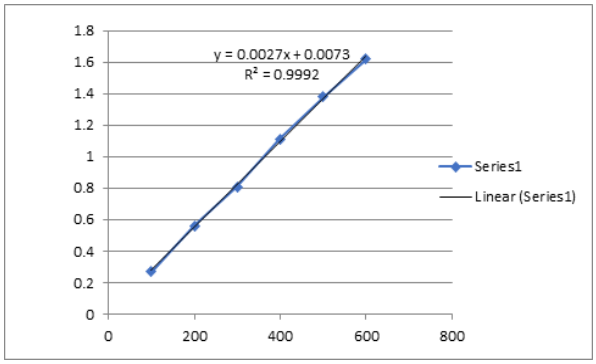

Figure 3. Calibration Curve Of Warfarin Sodium

Assay For The Determination Of Warfarin Sodium:

Table 4. Assay Of Warfarin Sodium

Drug	Lable claim	Amount found	%Recovery	%RSD
Warfarin sodium	5mg	4.95 mg	99	1.95

Table 5. Linearity Data Of Warfarin Sodium

	Concentration(μg/ml)	Absorbance ± SD	%RSD
1	30	0.76 ± 0.0052	1.90
2	40	0.77 ± 0.0041	1.97
3	50	0.80 ± 0.0058	0.87
4	60	0.81 ± 0.005	1.82
5	70	0.86 ± 0.0065	1.34
6	80	0.98 ± 0.0082	0.895
7	90	0.98 ± 0.0075	0.98
8	100	1.06 ± 0.0049	0.096
9	200	1.07 ± 0.0072	1.53
6	80	0.98 ± 0.0082	0.895
7	90	0.98 ± 0.0075	0.98
8	100	1.06 ± 0.0049	0.096
9	200	1.07 ± 0.0072	1.53

Accuracy

Table 6: Data Of Recovery Studies

S. No	Name of the drug	Amount of sample (μg/ml)	Recovery level	Amount of drug added (μg/ml)	Total amount found (μg/ml) ±SD	% Recovery	% RSD
1	Warfarin sodium	20	80% 100% 120%	16 20 24	35.8± 39.7± 43.9±	99.4 99.2 99.7	1.9 1.9 1.8

Precision

Table 7. Precision Data Of Warfarin Sodium

S.NO	Sample	Intra day precision %RSD	Inter day precision %RSD
1	LQC	1.9	1.86
2	MQC	1.72	1.989
3	HQC	0.87	0.957

Table 8. Ruggedness Data

S.No	Sample (Warfarin sodium)	Different analysts %RSD	Different Instruments %RSD
1	LQC	1.5	1.46
2	MQC	1.86	1.67
3	HQC	1.56	1.23

Table 9. Data Of Regression And Analytical Parameters

S. No	Parameter	Result
1	Maximum absorbance wavelength (nm)	450
2	Molar absorptivity (L/m mol.cm)	4.9382
3	Range (μg/ml)	30-600

4	Sandell's sensitivity ($\mu g/cm^2$)	0.37
5	Limit of detection (μg/ml)	8.25
6	Limit of quantification (μg/ml)	25
7	Regression equation	Y = 0.002x + 0.007
8	Slope	0.002
9	Intercept	0.007
10	Correlation coefficient	0.999

CONCLUSION

The proposed method is simple, rapid, linear, accurate, precise, reproducible and Cost effective. It can be used for the determination of Warfarin sodium in pharmaceutical dosage form and for routine analysis of Warfarin sodium.

Conflict Of Interest

There is no conflict about this research work.

Acknowledgement

Thankful to the authorities of Sri Krishnadevaraya University College of Pharmaceutical Sciences, Ananthapuramu for providing laboratory facilities to carry out work and thankful to Sequent Scientific Limited for providing gift sample (Warfarin sodium).

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