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NEW SPECTROPHOTOMETRIC METHODS FOR THE ESTIMATION OF *SILYMARIN* IN PURE AND PHARMACEUTICAL DOSAGE FORMS

T. Sathish, U. Kiran Babu, K. Kishore Kumar, N. Pratap[1], and M. Krishna Prasad

Center for Biotechnology, Department of Chemical Engineering,
Andhra University, Visakhapatnam - 530 003 (India)
[1]Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam - 530 003 (India)

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ABSTRACT

Silymarin, as the plant active ingredient of milk thirsle a polyphenolic antioxidant flavanoids, protecting the liver cells membrane and facilitate the recovery of the impaired liver cells. Very few analytical methods have been reported for the determination of Silymarin Estimation of Silymarin with (MBTH & CAS) in pure & pharmaceutical dosage form (Method-I), with $fecl_3$ and 2-2 Bipyrdine (Method – II) by using Elico – uv – Visible- spectrophotometer model SL-150. The concentration range 5-40 mg/ml was obeying beer-lambert's law, at 520 nm in Method – I and concentration range 5- 25 mg/ml was obeying beer-lambert's law at 525 nm in Method – II. In case of pharmaceutical dosage forms, the silymarin percent content was found to be 99.50 in both the methods. The proposed methods are applicable for the assay of silymarin in both pure and pharmaceutical dosage forms.

Keywords: Silymarin, MBTH (3-Methyl Benzthia-zolinone-2-Hydrozone), 2-2,Bipyridine-$FeCl_3$ and CAS(Cerric Ammonium Sulphate)

INTRODUCTION

Silymarin, as the plant active ingredient of Milk Thistle has a consolidating effect on the liver cell membrane by protecting the liver from harmful effects and facilitating the recovery of the impaired liver cells. Very few analytical methods have been reported so far for the determination of silymarin, which include

1. TLS spectrophotometry[1]
2. HPLC[2]
3. TLC – Photodensitometry[3]
4. Colorimetric method[4]
5. TLC[5]
6. Liquid Chromotography[6]

Silymarin contains silybin, isosilybin, silydianin and silychristin. These are usually analysed by HPLC and capillaryzone electrophoresis method[7].

Silymarin compounds are extracted from Milk Thistle using hot water extraction[8] and using organic solvents[9]. Silymarin protects liver injury by inhibiting phosphatidylcholine synthesis or stimulation of hepatic RNA and protein synthesis[10]. Silymarin is found to be a polyphenolic antioxidant flavanoid, inhibits azoxymethane - induced colon carcinogensis in male rats[11].

MATERIAL AND METHODS

Silymarin pure sample was obtained from Micro labs, Thirubavanai – Pondicherry, India as a gift sample, Silymar® (Silymarin 140 mg) capsules were purchased from German Remedies Ltd., Mumbai - India. $FeCl_3$ & 2,2-Bipyridine were purchased from Qualigens Fine Chemicals-Mumbai, India. All the other chemicals used were of analytical grade.

Method - I

Estimation of Silymarin with 3-Methyl Benzthia - zolinone - 2 - Hydrozone (MBTH) & Cerric Ammonium Sulphate (CAS): Preparation of standard silymarin solution - stock solution (1 mg/ml): Accurately weighed 100 mg pure sample of Silymarin was dissolved in methanol and made up to 100 ml with methanol. It gives 1 mg/ml solution. From the above stock solution further dulutions are made with distilled water to get a working standard solution of 100 mg/ml.

Preparation of the reagents

Preparation of MBTH solution (0.2% W/V)

Accurately weighed 0.2 gms of MBTH was dissolved with little quantity of distilled water in 100 ml volumetric flask and made up to the volume with distilled water.

Preparation of CAS solution (1% W/V):

Accurately weighed 1.0 gram of CAS was dissolved with 0.72 M H_2SO_4 in a 100 ml volumetric flask, made up to the volume with H_2SO_4.

Procedure: 1 ml of standard Silymarin solution (100mg/ml), 1ml of MBTH and 1 ml of CAS were taken in a 10 ml volumetric flask and set aside for 5 minutes and made up to the volume with distilled water. Blank solution was prepared same as above except sample. There was a change in colour between blank and sample solution (blood red). The absorbance was measured against corresponding reagent blank by changing the wavelength from 500-550nm and maximum absorbance (λ_{max}) was found to be at 520 nm.

Study of the effect of volume of MBTH and CAS

In order to find out the effect of volume of MBTH and CAS, each 1 ml of standard Silymarin solution was placed in five 10 ml volumetric flasks and to it 0.5, 1.0, 1.5, 2.0, 2.5 ml of MBTH and 1 ml of CAS were added to the above flasks and set aside for 5 minutes. The corresponding blank was prepared except sample as above. The volume was made upto 10 ml and the absorbance was measured against corresponding reagent blank and similar procedure was carried out to find out CAS volume effect. The maximum absorbance (λ_{max}) was observed with 1.5 ml MBTH and 1.5ml of CAS reagent.

Preparation of standard graph for pure silymarin with MBTH and CAS

Procedure: Aliquots of working standard solution of Silymarin ranging from 0.5 to 4.0 ml were transferred in a series of 10 ml volumetric flasks, to that 1.5 ml of MBTH and 1.5 ml of CAS were successively added and the final volume was brought to 10 ml with distilled water, the absorbance of blood red coloured samples were measured at 520 nm, against reagent blank.

RESULTS

The concentration range 5-40 mg/ml was obeying Beer-Lambert's Law and the calibration curve was linear.

Analysis of silymarin in formulation

Preparation of sample solution of silymarin

Twenty capsules of silymarin were taken and the contents were poured into a mortar and made into fine powder. From that the weight equivalent to 100 mg of Silymarin was taken into 100 ml volumetric flask and it was dissolved in methanol and adjusted to 100 ml with methanol in order to get 1 mg/ml solution. The above stock solution, further dilution was made with distilled water to get a working sample solution of 100 mg/ml.

Procedure: 2 ml of sample solution (100 mg/ml) was taken into a 10 ml volumetric flask and to it 1.5 ml of MBTH, 1.5 ml of CAS were added and made up to the volume with distilled water. The absorbance of the solution was measured at 520 nm. Amount of the drug in sample was calculated from the calibration curve and the results were recorded in Table-1.

Recovery studies:Procedure: To 1 ml and 2 ml of sample solutions (100 mg/ml) were taken into 10 ml volumetric flasks separately and 1 ml and 2 ml of standard drug solution were added respectively. To the above flasks add 1.5 ml of MBTH and 1.5 ml of CAS and made up the volume with distilled water. Blank solution was prepared as above except sample. The absorbance was measured against

Table - 1: Estimation of Silymarin in Formulation

Labelled amount of Silymarin (mg)	Estimated amount of Silymarin (mg)	Percentage amount of Silymarin (mg)
140	139.80	99.50

* The above results were average of 3 determinations

Table - 2: Data for the validation of proposed method

Amount of Silymarin added (µg)	Amount of Silymarin found (µg)	Percentage recovery of Silymarin
200.0	196.0	98.00
400.0	392.0	98.00

corresponding reagent blank. The readings were shown in Table -2.

The percentage recovery of Silymarin with method-II (Ferric-Chloride and 2,2-Bipyridine) is 98.0.

Method - II

Estimation of Silymarin with $FeCl_3$ and 2,2-Bipyridine

Preparation of standard Silymarin solution:

From the stock solution (in method –I) further dilutions were made with distilled water to get a working standard solution of 100 mg/ml.

Preparation of reagents :

Preparation of Ferric Chloride solution (0.0033 M)

Accurately weighed 0.0535 gm of Ferric Chloride was dissolved with little quantity of distilled water in 100 ml volumetric flask and made up to the volume with distilled water.

Preparation of 2,2 Bipyridine (0.01 M)

Accurately weighed 0.347 gm of 2,2 Bipyridine was dissolved with small quantity of distilled water in 100 ml volumetric flask, made up to the volume with distilled water.

Procedure

1 ml of standard Silymarin solution, 1 ml of Ferric Chloride and 1 ml of 2,2 Bipyridine were taken in 10 ml volumetric flask and set aside for 5 minutes and made up to the volume with distilled water. Blank solution was prepared same as above except sample. There was a change in colour between blank and sample solution (blood red). The absorbance against corresponding reagent blank was measured against the corresponding reagent blank by changing the wavelength from 500-550 nm, the absorbance maximum (λ_{max}) was found to be at 525 nm.

Study of the Effect of Volume of Ferric Chloride and 2,2-Bipyridine

Procedure

Each 1 ml of standard Silymarin solution was placed in five 10 ml volumetric flasks and 0.5, 1.0, 1.5, 2.0, 2.5 ml of $FeCl_3$ and 1 ml of 2,2 Bipyridine were added to above flasks and set aside for 5 minutes. Blank was prepared except sample as above. The volume was made up to 10 ml and the absorbance was measured against corresponding reagent blank. Similar procedure was carried out to find out the effect of volume of 2,2-Bipyridine.

The maximum absorbance was observed with 0.5 ml $FeCl_3$ and 2.0 ml of 2,2-Bipyridine reagent.

Preparation of Standard Graph for pure Silymarin with Ferric Chloride and 2,2 Bipyridine

Procedure

Aliquots of working standard solution Silymarin ranging from 0.5 to 2.5 ml were transferred in a series of 10 ml volumetric flasks, to that 0.5 ml of $FeCl_3$ and 2.0 ml of 2,2 Bipyridine were successively added and final volume was made up to 10 ml with distilled water. The absorbance of red coloured samples were measured at 525 nm against reagent blank. The concentration range obeying Beer-Lambert's Law was 5-25 mg/ml.

Analysis of Silymarin Formulation

From the stock solution (in method I), further dilutions were made with distilled water to get a working sample solution of 100 mg/ml.

Procedure

2ml of sample solution (100 mg/ml) was taken into a 10 ml volumetric flask and to it 0.5 ml of $FeCl_3$, 2.0 ml of 2,2 - Bipyridine were added and

Table - 3: Estimation of Silymarin in Formulation

Labelled amount of Silymarin (mg)	Estimated amount of Silymarin (mg)	Percentage amount of Silymarin
140	139.80	99.50

* The above results were average of 3 determinations

Table - 4: Data for the validation of proposed method

Amount of Silymarin (µg)	Amount of Silymarin found (µg)	Percentage recovery of Silymarin
200.0	195.0	97.50
400.0	390.2	97.55

Table - 5: Optical characteristics and precision

Parameters	Method - I	Method - II
Beer's Law limit (µg/ml)	5-40	5-25
Sandeller's sensitivity (µg/cm²/0.001 absorbance unit)	0.05524	0.03125
Molar extinction coefficient (1 $mole^{-1}$, cm^{-1})	0.8732×10^4	1.5438×10^4
% Relative standard deviation	0.5096	0.5585
% Range of error		
0.05 confidence limit	± 0.4273	± 0.4670
0.01 confidence limit	± 0.6313	± 0.6909
Correlation coefficient	0.9961	0.9998
Regression equation (Y*)		
Slope (a)	0.0014	0.0045
Intercept (b)	0.0815	0.0032

Y* = b + aC, where "C" is concentration in µg/ml and Y is absorbance unit.

made up to the volume with distilled water. The absorbance of solution was measured at 525 nm. Amount of the drug in sample was calculated from the calibration curve and the results were recorded in Table - 3.

Recovery Studies

Procedure

To 1 ml and 2 ml of sample solutions (100 mg/ml) were taken into 10 ml volumetric flasks separately and 1 ml and 2 ml of standard drug solution were added respectively. to the above flasks add 0.5 ml of Ferric Chloride and 2.0 ml of 2,2 - Bipyridine and made up the volume with distilled water. Blank solution was prepared as above except sample. The absorbance was measured against corresponding reagent blank. The readings were shown in Table - 4.

The percentage recovery range of Silymarin with method II (Ferric-Chloride and 2,2 - Bipyridine) is 97.5 - 98.0.

RESULTS & DISCUSSION

Two simple Spectrophotometric methods were developed for Silymarin. In method I, the reaction is based on the oxidative coupling between the drug and MBTH in presence of CAS and formed blood red coloured complex. In method II, it is based on formation of blood red coloured complex with Ferric Chloride and 2,2 - Bipyridine. The colour exhibit absorption maxima at 520 nm and 525 nm for method I and method II respectively. The colour has been found to be stable and it is sufficient to carryout analysis. After a thorough study of effect of different volumes of reagent, optimum volume was fixed to get maximum sensitivity in estimation of drugs.

Beer-Lambert's Law was found to be obeyed in the concentration range of 5 to 40 mg/ml and 5 to 25 mg/ml for Method I and Method II respectively. The satisfactory low value of percentage relative standard deviation and percent range of error (0.05 and 0.01 level confidence limits) indicated that the proposed methods were quite simple and economical. An excellent correlation was found between absorbance and concentration with the value of 0.9961 (Method I) and 0.9998 (Method II). Intercept value is 0.08145 (Method I) and 0.0045 (Method II). Slope was found to be 0.0014 (Method I) and 0.0032 (Method II).

The percentage recovery and labeled claim were satisfiable in formulation. In order to confirm the validity, reliability and suitability of the proposed method, the recovery methods conducted by adding known concentration of standard drug to previously analysed formulation and the mixture was analysed as per the calibration curve. The recovery studies carried out gave satisfactory results in the range of 97.5% - 98.0% approximately and it was the indication of non-interference of excipients in determination of the drug.

Conclusion

The presently reported two Spectrophotometric methods were simple, economical, rapid, convenient, accurate, sensitive and reproducible. Therefore, it is suitable for routine analysis of Silymarin in bulk drug and formulations.

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