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Kinetic spectrophotometric method for the determination of silymarin in pharmaceutical formulations using potassium permanganate as oxidant

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A new simple and sensitive kinetic spectrophotometric method for the determination of silymarin in pure form and in pharmaceutical formulations is described. The method is based on the oxidation of the drug with potassium permanganate at pH 7.0 ± 0.2. The reaction is followed spectrophotometrically by measuring the decrease in the absorbance at 530 nm. The calibration graph is linear in the range of 18–50 $\mu g \cdot ml^{-1}$. The method has been successfully applied to the determination of silymarin in pharmaceutical formulations. Statistical comparison of the results with the reference method shows excellent agreement and indicates no significant difference in accuracy and precision.

1. Introduction

Silymarin, 3,5,7-trihydroxy-2-[3-(4-hydroxy-3-methoxy phenyl)-2-(hydroxy-methyl)-1,4-benzodioxan-6-yl]-4-chromanone, is an important antihepatotoxic drug which is obtained from the dried fruits of *Silybum marianum* and widely used for the treatment of hepatic disorders (Cavalieri 1974; Salmi and Sarna 1982; Ferenci et al. 1989; Hruby 1983). The drug is officially listed in Martindale The Extra Pharmacopoeia (Martindale 1996).

The assay procedure listed in the monograph of the Italian pharmacopoeia describes the UV-Vis spectrophotometric method (Farmacopoeia 1985) for determination of the drug in bulk and pharmaceutical formulations. Several other methods have been employed to determine silymarin in biological specimens and/or pharmaceutical formulations such as high performance liquid chromatography (Martinelli et al. 1991; Marcher and Kikuta 1993; Rickling et al. 1995; Tittel and Wagner 1977; Tittel and Wagner 1978; Quercia et al. 1980; Quercia et al. 1983; Steinigen 1983; Law and Das 1987; Tomov et al. 1985; Pashankov 1988; Nikolov 1990; Kurkin 1996; Wang et al. 1998), thin layer chromatography (Lin 1982; Abdel-Salam 1982; Vanhaelen and Vanhaelen-Fastre 1983; Heinz et al. 1985; Wu and Wang 1989; Lin et al. 1998; Szilarjtes-Obeiglo and Kowalesioska 1984), high performance thin layer chromatography (Funk et al. 1987), potentiometric titration (Koerbl et al. 1983), diffuse-reflectance fourier transform infrared spectroscopy (Martinez et al. 1991) and UV spectrophotometry (Lan et al. 1983; Belikov 1985).

In the literature only few spectrophotometric methods have been reported. The drug content in pharmaceutical formulations was determined (Zarapkar et al. 2000), based on the formation of a coloured complex of the drug with 2,4-dinitrophenyl hydrazine in the presence of tetramethyl amine hydroxide. Quantitative determinations are done at 490 nm. Another spectrophotometric method for the estimation of silymarin was based on its reaction with diazotized sulfanilic acid in alkaline medium to form an orange-red coloured chromogen which absorbed maximally at 460 nm (Rajasekaran 1997). Reddy et al. (2001) have reported two spectrophotometric methods for silymarin determination. The first method was based on the oxidation of the drug by Fe(III) and estimating the reduced Fe(II) with 1,10-phenanthroline at 510 nm. The other method involved the formation of a blue complex on treatment with Folin-Ciocalteau reagent in the presence of NaOH and subsequent determination was done at 740 nm. These methods are somewhat tedious and time consuming. Therefore, we developed a fast, low cost and selective method for routine analysis of silymarin in bulk and drug formulations.

The extent of oxidation of organic substrate by potassium permanganate depends on the pH of the medium. The heptavalent manganese changes to Mn(VI) in alkaline medium while in neutral and acidic medium the permanganate is further reduced forming ultimately Mn(II). This behaviour of permanganate has been exploited to develop a kinetic spectrophotometric method for assay of nifedipine in drug formulations (Rahman and Azmi 1999).

The present communication describes a method for the assay of silymarin in bulk and pharmaceutical formulations. The method is based on the oxidation of silymarin by potassium permanganate at pH 7.0 ± 0.2 and the course of the reaction was followed by measuring a decrease in absorbance at 530 nm.

2. Investigations, results and discussion

The oxidation of organic compounds by permanganate is dependent upon the pH of the medium. During the course of the reaction the valence of manganese changes and intermediate ions have been suggested as participating oxidants. But which species have the main role as potential oxidants depends on the nature of the substrate and pH of

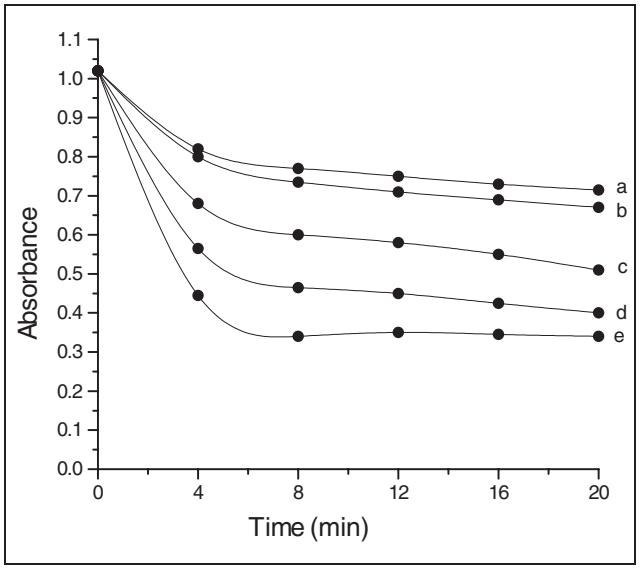


Fig. 1: Absorbance-time curves: $KMnO_4$, 4.8×10^{-4} M and silymarin (a) 3.731×10^{-5} M; (b) 4.146×10^{-5} M; (c) 6.219×10^{-5}; (d) 8.292×10^{-5} M; (e) 10.365×10^{-5} M

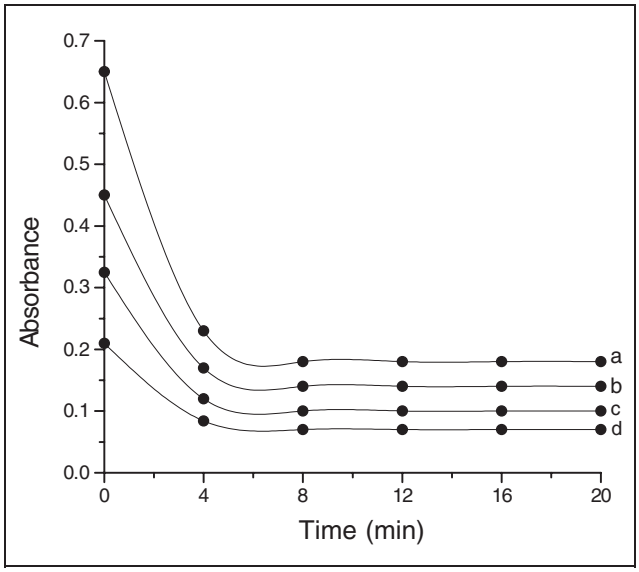


Fig. 2: Absorbance-time curves: silymarin, 10.365×10^{-5} M and $KMnO_4$ (a) 3.00×10^{-4} M; (b) 2.10×10^{-4} M; (c) 1.50×10^{-4} M; (d) 9.00×10^{-5} M

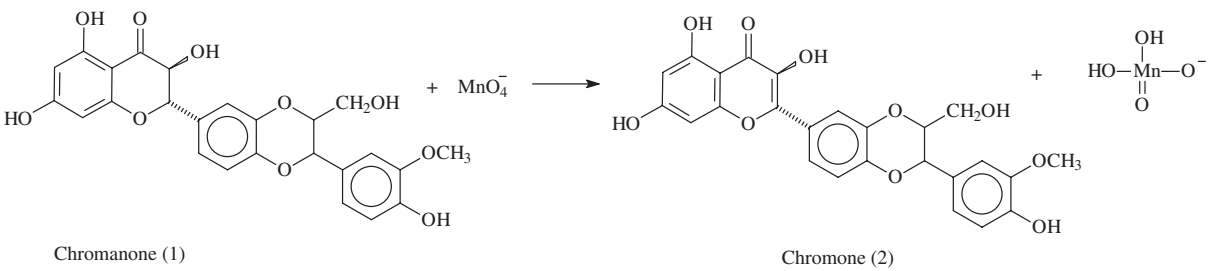
the medium. The oxidation of silymarin by potassium permanganate at pH 7.0 ± 0.2 was followed spectrophotometrically at 530 nm. The absorbance-time curves are shown in Figs. 1 and 2, which are all sigmoid in nature throughout the entire range and therefore, the initial rates of reaction were determined from the slope of the initial tangent to the absorbance-time curves. The order with respect to permanganate was determined by studying the reaction at different initial concentrations of permanganate with fixed silymarin concentration. Keeping the silymarin concentration higher than that of the oxidant, the plot of initial rate [-d[A]/dt] against initial absorbance was linear passing through the origin suggested that the order of reaction with respect to permanganate at the start is one. The order with respect to silymarin was evaluated from the measurement of the rates at several concentrations of silymarin but at fixed concentration of $KMnO_4$ which was also found to be one. The first order dependence on both permanganate and silymarin in the initial stages leads to the simple rate expression:

$$\text{Rate} = K[\text{silymarin}]\,[MnO_4^-] \qquad (1)$$

Silymarin is a naturally occurring aryl chromanone(1) related to flavanone. The flavanones are 2,3-dihydro derivatives of flavones, which are readily interconvertible. Though the basic skeleton of flavanone and flavone is the same, the key which distinguishes one structural type from another is the oxidation level of the various carbons in the heterocyclic ring. The flavones are in a high state of oxidation whereas flavanones are in low state of oxidation. The oxidation of flavanone to flavone with a variety of reagents such as selenium dioxide (Sammel 1979) or triphenyl methyl carbonium ion (Schonberg and Schutz 1960) has been reported. In this study, the same mechanism seems to be involved. The oxidation of silymarin by permanganate takes place at pH 7.0 ± 02 leading to the formation of chromone(2). Thus, the dehydrogenation is effected by permanganate and consequently disproportionation between immediate oxidation states of manganese results in the formation of MnO_2. However no precipitation of MnO_2 was observed under study time period. The reaction mechanism is presented in the Scheme.

Under the optimum experimental conditions, a calibration curve was constructed by plotting logarithm of initial rate of reaction ($\log \nu$) versus logarithm of silymarin concentration ($\log C$), which showed a linear relationship over silymarin concentration range of $18-50\ \mu g \cdot ml^{-1}$. The linear regression analysis using the method of least square was made to evaluate the slope, intercept and correlation coefficient. The linear regression equation and correlation coefficient are $\log \nu = 1.024 \log C + 3.235$ and $r = 0.9997$ which indicates an excellent linearity. The confidence limits for the slope of the line of regression and intercept were computed using the relation $b \pm tS_b$ and $a \pm tS_a$ (Miller 1991), at 95% confidence level and found to be $1.024 \pm 3.96 \times 10^{-2}$ and $3.235 \pm 1.67 \times 10^{-1}$, respectively. This indicated the high reproducibility of the kinetic method proposed. Linearity was also evaluated (Torrado et al. 1994) from the relative standard deviation of the slope of the calibration line and found to be 1.215.

Scheme 1



Chromanone (1)

Chromone (2)

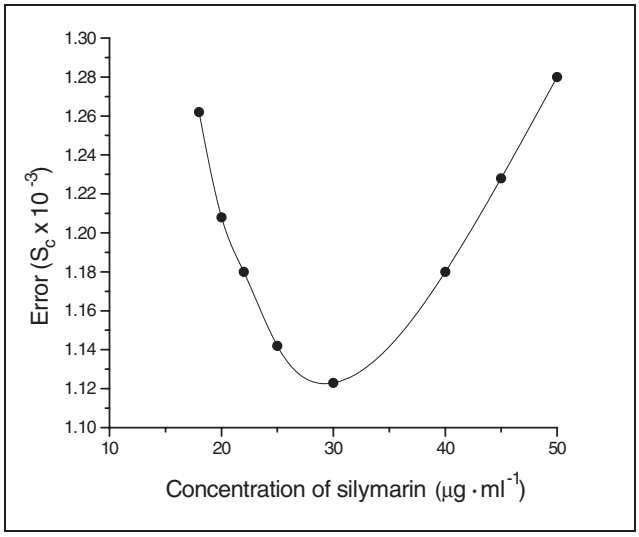


Fig. 3: Error in the determination of the concentration of silymarin using statistical analysis of standard calibration data

The limit of detection was established using the equation (Morelli 1983, Nalimov 1963):

$$\text{Limit of detection} = \left(S_o^2 \times \frac{n-2}{n-1}\right)^{1/2} \frac{t}{b} \qquad (2)$$

where n is the number of standard samples, t is the value of student's t for n−2 degrees of freedom at 95% confidence level and S_o^2 = variance. The variance and detection limit were calculated to be 1.100×10^{-6} μg · ml^{-1} and 2.823×10^{-3} μg · ml^{-1} respectively, which confirmed negligible scattering of experimental data points around the line of regression and good sensitivity of the method.

The error (Sc) in the determination of a given concentration of silymarin (C) was established using the equation (Morelli 1987):

$$S_c = \frac{S_0}{b}\left[1 + \frac{1}{n} + \frac{(y-\overline{y})^2}{b^2(\sum C^2 - n\overline{C}^2)}\right]^{1/2} \qquad (3)$$

Where $\overline{y}$ and $\overline{C}$ are average ordinate and abscissa values, respectively, for n standard samples. Fig. 3 shows the graph of Sc versus the concentration of silymarin. It is apparent from the graph that the error is reached minimum when the actual initial rate was equal to the average initial rate in the calibration graph.

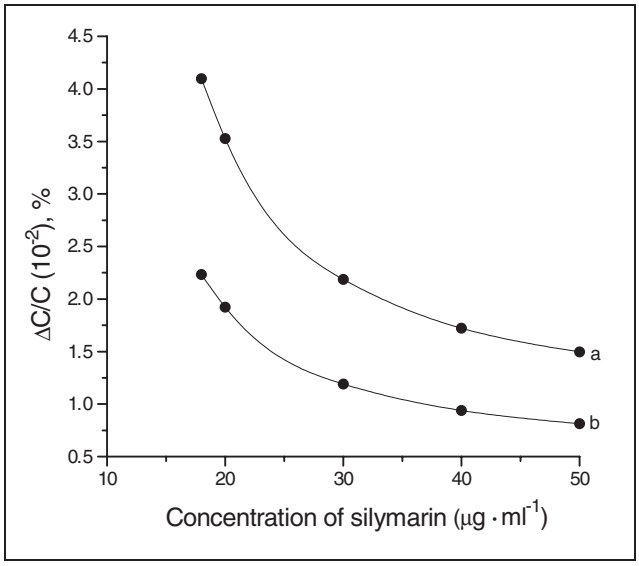


Fig. 4: Variation of the confidence limit at (a) p = 0.05 and (b) p = 0.01 level of significance in the form of uncertainty percentage on the concentration of silymarin

Table 1: Precision test of the proposed kinetic method

Sample No.	Initial rate of reaction (mol · l^{-1} · min^{-1})	Amount found μg per 10 ml
1	11.4×10^{-2}	402
2	11.5×10^{-2}	404
3	11.3×10^{-2}	400
4	11.3×10^{-2}	399
5	11.5×10^{-2}	406
6	11.5×10^{-2}	405
7	11.2×10^{-2}	396
8	11.4×10^{-2}	401
9	11.3×10^{-2}	398
10	11.2×10^{-2}	397

Mean percent recovery = 100%
Relative standard deviation = 0.8%

The minimum error of 1.123×10^{-3} μg · ml^{-1} was found in the determination of about 30 μg · ml^{-1} silymarin. The value of Sc also allows establishing the confidence limit at the selected level of significance for the determination of unknown concentrations by using the relation, $C_i \pm tS_c$. The results are shown graphically (Fig. 4) in the form of percent uncertainty $\left(\frac{tS_c}{C_i} \times 100\right)$ on the concentration at 95% and 99% confidence levels. This is a useful way of expressing the confidence limits because the relative uncertainty can be estimated directly on the concentration over the full range of the concentration tested.

The reproducibility was established for ten independent analyses of solution containing 0.40 mg silymarin. The analytical results obtained from the investigation are summarized in Table 1. The relative standard deviation and mean percent recovery were 0.87% and 100.3%, respectively and considered to be very satisfactory. The validity of the proposed kinetic method was checked by applying standard addition method and the results are reported in Table 2.

The applicability of the proposed method for the determination of silymarin in dosage forms was examined by analyzing marketed products; the results were compared to those obtained by the reference method (Rajasekaran et al. 1997) and are summarized in Table 3. The performance of the proposed method was judged by calculating t- and F-values (Christian 1986). At 95% confidence level judged, the calculated t- and F-values do not exceed the theoretical values indicating no significant difference between the proposed kinetic method and the reference method.

Table 2: Determination of silymarin in pharmaceutical formulations by the standard addition method

Preparation	Amount taken (μg · ml^{-1})	Amount added (μg · ml^{-1})	Total amount found (μg · ml^{-1})	Recovery (%)	RSD (%)[a]
Silybin-70	10	10	20.0	100.1	0.35
(tablet)	20	20	39.9	99.9	0.34
Silvia-70	10	10	20.0	100.1	0.47
(tablet)	20	20	40.0	100.0	0.22
Limarin-70	10	10	20.0	100.1	0.58
(capsule)	20	20	40.0	100.2	0.46
Sivylar-70	10	10	20.0	100.1	0.57
(capsule)	20	20	40.0	100.0	0.22
Levalon-70	10	10	20.0	100.2	0.68
(capsule)	20	20	39.9	99.9	0.34

[a] Three independent analyses.

Table 3: Determination of silymarin in dosage forms by the kinetic method proposed and by the reference method

Preparation	Labelled amount) (mg/tablet or capsule)	Proposed kinetic method			Reference method			$t^b_{calc.}$	$F^c_{calc.}$
		Recovery (%)	SD	RSD (%)[a]	Recovery (%)	SD	RSD (%)[a]		
Silybin (tablet)	70	100.0	0.13	0.33	99.8	0.09	0.23	0.452	2.010
Silvia (tablet)	70	100.1	0.09	0.23	100.1	0.06	0.15	0.205	2.340
Limarin (capsule)	70	100.0	0.09	0.24	99.6	0.13	0.32	1.147	1.751
Sivylar (capsule)	70	100.1	0.12	0.30	100.0	0.06	0.15	0.227	3.940
Levalon (capsule)	70	99.9	0.05	0.13	100.3	0.10	0.26	1.268	3.830

[a] Five independent analyses.
[b] Theoretical value for t at 95% confidence level is 2.776.
[c] Theoretical value for F at 95% confidence level is 6.39.

In conclusion, the proposed method is fast, simple and sensitive for the determination of silymarin in bulk and commercial formulations with good accuracy and precision. The sensitivity is very high and compares favourably with that of other known methods and, can therefore be successfully applied as an alternative to the existing methods.

3. Experimental

3.1. Apparatus

The absorbance was measured on a spectronic 20 D^+ spectrophotometer (Milton Roy, USA) with 1 cm glass cells.

3.2. Reagents

All chemicals used were of analytical or pharmaceutical grade. Potassium permanganate, 0.003M was freshly prepared by dissolving 47.41 mg of potassium permanganate in 100 ml of doubly distilled water. The apparent purity of the potassium permanganate solution was checked titrimetrically (Vogel 2002).

3.3. Test solution

Silymarin (1 mg · ml^{-1}) (Micro Labs, Bangalore, India) was dissloved in methanol.

3.4. Procedure

3.4.1. Silymarin

Aliquots of 0.18–0.50 ml of silymarin standard test solution were pipetted into a series of 10 ml standard volumetric flasks. To each standard flask 1.6 ml of 0.003 M potassium permanganate was added and then diluted to volume with doubly distilled water at 30 ± 1 °C. After mixing, reaction mixture was immediately transferred to a spectrophotometric cell and the decrease in absorbance was recorded as a function of time for 20 min at 530 nm. The initial rate of the reaction (ν) at different concentrations was obtained from the slope of the tangent to the absorbance-time curve. The calibration curve was constructed by plotting the logarithm of the initial rate of reaction (log ν) versus logarithm of the concentration of the silymarin (log C).

3.4.2. Silymarin in pharmaceutical formulations

An accurately weighed quantity of the mixed contents of five capsules or five powdered tablets, equivalent to 50 mg of the drug, was extracted into 50 ml chloroform with shaking and the residue was filtered using Whatman No. 42 filter paper. The filtrate was evaporated to dryness and the residue was taken up with methanol and transferred to a 50 ml standard volumetric flask, diluting to volume. The assay was completed following the recommended procedure.

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