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COMMUNICATION

Dissolution Test for Silymarin Tablets and Capsules

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ABSTRACT

Silybine (SBN), isosilybine (ISBN), silycristine (SCN), silydianine (SDN), and taxifoline (TXF) are the main active flavonoids commonly found in the dried fruits of Silybum marianum, *Gaertner (Compositae). Concentrations of these compounds, except TXF, are usually expressed together as silymarin content. This paper describes a simple dissolution test developed to estimate silymarin (Sl) in pharmaceutical formulations. Five commercial products were tested using this new method (including tablets, sugar tablets, and capsules): two from Argentina, one from Brazil, one from Spain, and one from Italy. Results demonstrated that, provided the dosage form disintegrates, amounts dissolved range from 50 to 90% of the labeled value. Products were analyzed by high performance liquid chromatography (HPLC) and UV spectrophotometry.*

KEY WORDS: Dissolution test; Sylimarin; Tablets; Capsules HPLC; UV.

INTRODUCTION

Silybine (SBN), isosilybine (ISBN), silycristine (SCN), silydianine (SDN), and taxifoline (TXF) (Fig. 1) are the main active flavonoids commonly found in the dried fruits of *Silybum marianum*, Gaertner (Compositae). Concentrations of these compounds, except TXF, are usually expressed together as silymarin content (1). Silymarin (Sl) itself is a well known cytoprotective agent proven to be effective in several lines of research (2–9). The flavonoid is

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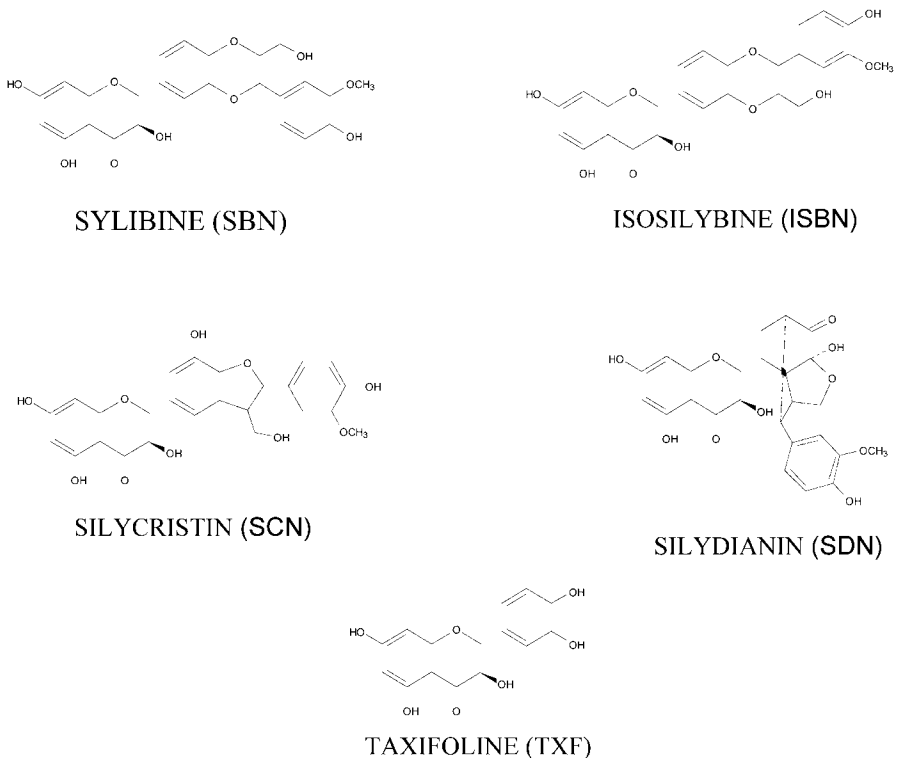

Figure 1. Chemical structure of Silymarin flavonoids.

used in clinical treatment of several hepatopathies in which degenerative necrosis and functional impairment are involved.

Although Sl has been incorporated to the pharmacopoeias in many countries, little is known about the biochemical basis of its mechanism of action. The drug is generally formulated in tablets and capsules thus dissolution testing is a good tool to evaluate pharmacotechnical quality by lot.

Methods commonly used for dissolution testing present many difficulties because of its poor water solubility. The only technique found in the literature was developed by Schulz and co-workers (10), who use USP Apparatus 2, pH 7.5 buffer as dissolution medium for 1 hr at 37°C in 4-L vessels. In this work, the apparatus used was a DT6 (Erweka, Heusenstamm, Germany).

Here we describe the development of a new dissolution test method for SI tablets and capsules with UV-spectrophotometer detection, using 1-L vessels and 0.5% sodium lauryl sulfate as a dissolution medium. Although higher concentrations of sodium lauryl sulfate may be used as dissolution media, in this case, greater concentrations hinder UV measurement. Five products, two from Argentine, one from Brazil, one from Spain, and one from Italy, were tested using this new method. To check the proportion of each component (SBN, ISBN, SCN, SDN, and TXF), commercial products were analyzed using high-performance liquid chromatography (HPLC).

EXPERIMENTAL PROCEDURES

Instrumentation

The dissolution apparatus was an Alycar (Argentina) device. The UV spectrophotometer was a Variant Cary 1. The HPLC system consisted of a dual piston reciprocating pump (model KNK-500 G), a UV-Vis detector (model KNK-029-757), an integrator (model SP 4600) (all from Konik, Barcelona, Spain) and a Rheodyne injector (model 7125, California, USA).

Materials, Reagents, and Chemicals

Laboratorios Schwabe S.A.C.I. (Argentina) donated the authentic working standard for Sl (Indena, Analysis Certificate 85936, batch 23734/M/11). Solvents were HPLC grade. Water HPLC grade was obtained by

Table 1.

List of Products

Product (in mg)	Origin	Expiration Date
Sugar tablets 70	Argentina	3/00
Tablets 150	Argentina	2/00
Sugar tablets 70	Brazil	2/01
Sugar tablets 70	Italy	7/02
Capsules 150	Spain	6/01

distillation and passed through a 0.45-μm membrane filter. Five commercial formulations were used (Table 1).

Dissolution Conditions

USP Apparatus 2 (Paddle), dissolution medium consisted of 0.5% sodium lauryl sulfate, 900 mL; rate, 100 rpm; time sampling, 15, 30, 45, and 60 min.

Spectrophotometric Conditions

A standard stock solution of Sl, 1.7 mg/mL, was prepared in methanol. The stock was suitably diluted with 0.05% sodium lauryl sulfate to reach a final concentration of 17 μg/mL. Samples were diluted in water at a concentration of 17 μg/mL and measured at a wavelength of maximum absorbance at about 288 nm in 1-cm cells, using 0.05% sodium lauryl sulfate as blank.

Preparation of Solutions Used for UV-Spectrophotometer Assay Validation

For the study of Sl response linearity, six solutions were prepared in 0.05% sodium lauryl sulfate at concentrations ranging from 5.9 to 39 μg/mL. System precision was evaluated by performing five consecutive measurements of the standard and of two commercial products.

Chromatographic Conditions

The experiment was performed on a Hewlett Packard LiChroCART® 250 × 4 mm HPLC LiChrospher® 100 RP-18 Cartridge (5 μm). The mobile phase consisted of water, methanol, and acetic acid (50:50:1). The mobile phase was filtered through a Micron Separations N04SP0-4700 nylon membrane (pore size, 0.45 μm) and degassed before use. Chromatography was performed at room temperature using a 1.0 mL/min flow rate and 20-min run time. The column was used at room temperature. Detector sensitivity was set at 0.05 a.u.f.s. and eluents monitored at 285 nm. The volume of each injection was 50 μL. Before injecting solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the system.

Working Standard Solution

A standard solution of Sl, 33 μg/mL (as SBN), was prepared in methanol.

Sample Solution

Twenty tablets were weighed and crushed to fine powder. Powdered samples equivalent to 33 mg of Sl were placed in a 100-mL volumetric flask. Methanol (70 mL) was added and the flask kept in an ultrasonic bath for 5 min. The extraction process used moderate sonication (5 min) to avoid exposing samples to heat generated from prolonged treatment. The mixture was then diluted to 100 mL with methanol, thoroughly mixed, and filtered through Whatman no. 42 paper. The sample solution was obtained by diluting the sample stock solution with methanol to give a concentration of 33 μg/mL.

Procedure

Solutions were prepared on a weight basis and volumetric flasks used as suitable containers to minimize solvent evaporation. Quantitation was accomplished using an external standard method. Each solution was injected in triplicate.

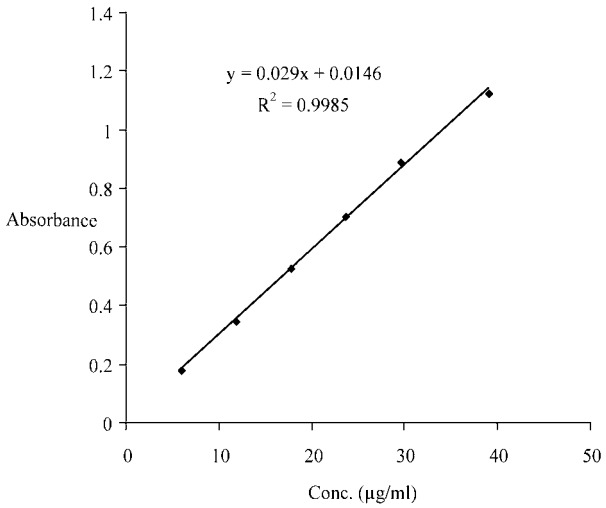

Figure 2. Linearity.

Table 2.

HPLC and UV Assay

	Percentage by HPLC					
Product	TXF	SCN	SDN	SBN	ISBN	Percentage by UV
Standard	5.39	26.90	6.48	49.93	11.29	
S1	4.09	13.32	6.12	69.16	7.36	104.4
S2	5.39	23.87	6.42	52.86	11.44	106.2
S3	5.66	24.17	3.29	56.03	9.84	108.5
S4	4.79	22.08	5.16	56.32	11.65	70.6
S5	4.33	25.05	4.43	54.14	12.01	94.2

RESULTS AND DISCUSSION

Linearity

Six solutions containing Sl at concentrations ranging from 5.9 to 39 μg/mL were analyzed. The regression line equation calculated by least-squares method was $Y = 2.90 \times 10^{-2}X + 1.46 \times 10^{-2}$ and had a coefficient of correlation $r = 0.9993$; intercept values were not significantly different from zero ($p = 0.05$).

Microsoft Excel software was used to plot peak areas versus micrograms measured (Fig. 2).

Precision

Precision was considered at one level of ICH recommendation (11): repeatability evaluated by performing five consecutive measurements of standard and two commercial products yielded an RSD of 0.4, 0.5, and 0.3% each.

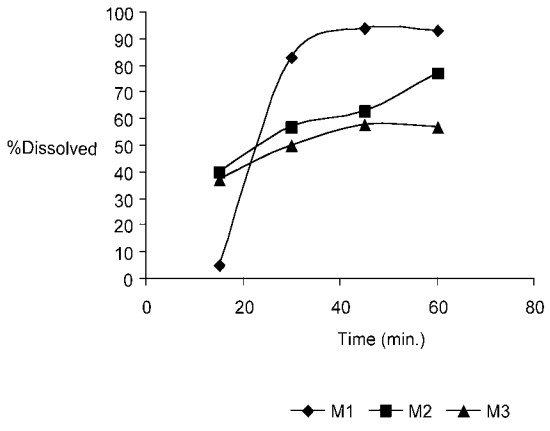

Figure 3. Dissolution profiles.

Dissolution Test

Individual commercial products were not identified in Table 2 and Figure 3, but their order was randomized. To compute the dissolution rate, results of percentage of drug release versus time were plotted (Fig. 3). Two products failed to disintegrate at these experimental conditions.

CONCLUSIONS

The UV method provides a linear response, is precise, and may be used for quality assessment and to study uniformity of content. Although it has not yet been correlated in vivo, the dissolution test may be used as a pharmacotechnical quality indicator.

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