ISSN 2581 – 4303

Available Online at www.ijpba.info
International Journal of Pharmaceutical & Biological Archives 2018; 9(3):73-77

RESEARCH ARTICLE

Validated High-performance Liquid Chromatography Method for Degradation Study of Ursodeoxycholic Acid and Silymarin

Imran A. Sheikh[1]*, Manoj Charde[2], Abhilasha Mittal[1]

[1]*Department of Pharmacy, NIMS Institute of Pharmacy, NIMS University, Jaipur, Rajasthan, India,* [2]*Department of Pharmacy, Government College of Pharmacy, Karad, Maharashtra, India*

Received: 30 May 2018; Revised: 30 June 2018; Accepted: 01 August 2018

ABSTRACT

A novel and basic reversed-phase liquid chromatographic strategy has been set up for the determination of ursodeoxycholic corrosive and silymarin and studies its degradation pattern in pharmaceutical dosage forms. Ursodeoxycholic acid and silymarin are used to control Type 2 diabetes. The proposed work was performed on Young Lin (S.K) isocratic System UV Detector C18 column (150 mm × 4.6 mm). A mixture of potassium phosphate, mobile phase in this method with a flow rate of 0.7 mL/min (UV detection at 203 nm) and the method was validated as per the ICH guidelines. Forced degradation studies were performed by exposing the drug ursodeoxycholic acid and silymarin to acidic, alkaline, oxidation, and thermal stress degradations. The proposed reversed-phase-high-performance liquid chromatography technique was observed to be powerful and particular, and this strategy is reasonable for the measure of pharmaceutical dose frames and in addition kinetic examinations.

Keywords: Reversed-phase-high-performance liquid chromatography, silymarin, stability indicating, ursodeoxycholic acid, validation.

INTRODUCTION

The drug Ursodeoxycholic acid (1) decreases cholesterol absorption and is cast-off to melt (cholesterol) gallstones in patients who want a substitute to surgery. If the patient halts taking the drug the gallstones tend to reoccur if the situation that gave rise to their formation does not change. For these reasons, it has not supplanted surgical handling by cholecystectomy. Also discard to discharge itching in intrahepatic cholestasis of pregnancy. Silymarin [2,3] has cytoprotection activities due to its antioxidant activity and radical scavenging. The possible known mechanisms of action of silymarin protection are blockade and adjustment of cell transporters, p-glycoprotein, estrogenic and nuclear receptors.

***Corresponding Author:**
Imran A. Sheikh
E-mail: iaspharma@gmail.com

EXPERIMENTAL

Chemicals and reagents

Ursodeoxycholic acid and silymarin were provided from Merck Laboratories Ltd. High-performance liquid chromatography (HPLC) grade potassium phosphate buffer pH - 3.2 with orthophosphoric acid, acetonitrile, and sodium hydroxide was procured from Merck Ltd. High pure water was prepared using Millipore Milli Q plus purification system.

HPLC instrumentation and conditions[4-6]

A HPLC framework, with LC arrangements information and the isocratic framework was utilized to develop method and further subsequent analysis. The data were recorded using Autochro-3000 solutions software. The sample separation was performed on a Shimadzu Primesil C18 (4.6 mm × 150 mm) with the mobile phase

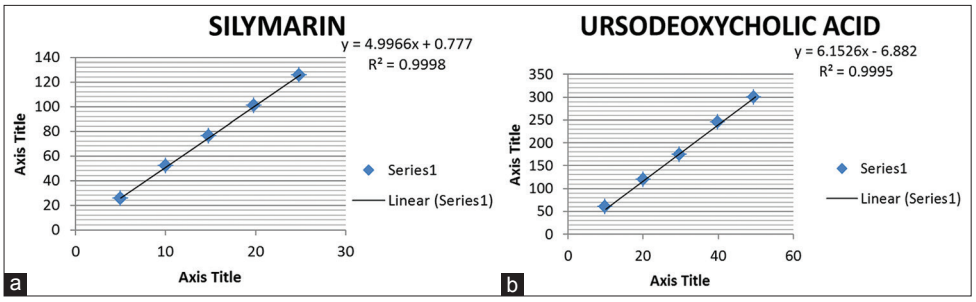

Figure 1: (a and b) Linearity curve for ursodeoxycholic acid and silymarin

Table 1: Assay data of proposed RP-HPLC method

Method	Concentration of silymarin and ursodeoxycholic acid (mcg/mL)	Peak area	Amount found (mg) (%)
RP-HPLC method for SILY	25	758.56	12.03 (12.03)
	25	758.53	12.05 (15.03)
	25	75.56	25.03 (12.03)
	25	75.23	26.03 (16.23)
	25	75.26	15.2 (12.26)
		Mean	12.02 (14.25)
		SD	0.01 (1.001)
		%RSD	0.55 (25.26)
RP-HPLC method for URSO	50	43.569	26.23 (26.23)
	50	43.569	26.45 (12.03)
	50	43.265	26.35 (12.05)
	50	43.256	26.35 (15.02)
	50	43.215	26.20 (18.56)
		Mean	26.15 (16.02)
		SD	0.02 (18.02)
		%RSD	0.23 (1.023)

RP-HPLC: Reversed-phase-high-performance liquid chromatography

Table 2: Results of force degradation studies of silymarin and ursodeoxycholic acid

Degradation parameter	SILY amount found	URO amount found
Alkali DEG 0.1 N NaOH - after 1 hr 2+20 mcg	13.35	18.35
Acid DEG 0.1 N HCl- after 1 h 2+20 mcg	39.43	22.39
Acid DEG 0.1 N HCl- after 2 h 2+20 mcg	84.79	29.84
Acid DEG 0.1 N HCl- after 3 h 2+20 mcg	19.11	29.01
3% H202 DEG 0.1N after 1 h 2+20 mcg	20.37	32.47
Neutral 0.1 N after 1 h 2+20 mcg	22.35	20.60
H_2O study after 2 h 2 + 20 mcg	28.75	22.71

consisting of acetonitrile and potassium phosphate buffer pH-3.2 with a ratio of 40:60 (v/v) at ambient temperature. The flow rate was kept at 0.7 mL/min, and the determination wavelength was 225 nm. Retention time for silymarin 2.55 min and ursodeoxycholic acid was 3.40 min [Table 1].

Mobile phase

Mix 700 mL of acetonitrile to the buffer, the mobile phase was sonicated for 15 min and then it was filtered through 0.45 µm membrane filter paper. chromatogram was recorded. The procedure was repeated for the sample solution [Figure 2].

Standard solution[4,7,8]

The standard was dissolved with mobile phase to 5 mg/mL. The test samples were dissolved with mobile phase. With the optimized chromatographic conditions, a steady baseline was recorded, the standard solution was injected, and the

chromatogram was recorded. The procedure was repeated for the sample solution.

Forced degradation studies

Ursodeoxycholic acid and silymarin were allowed to hydrolyze in different strengths of base and acid (0.1 N) and hydrogen peroxide (0.1 N). The combination was studied for its neutral degradation. Further, it is important to note that from the chromatograms [Figure 3], it is evident that although the degraded peaks are observed. The combination ursodeoxycholic acid and silymarin are stable under the applied stress conditions such as acid, base, oxidative, neutral and degradation states [Figure 3 and Table 2].

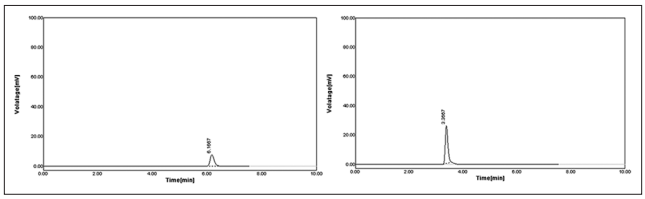

Figure 2: Chromatogram of ursodeoxycholic acid and silymarin

Linearity

The calibration curve showed good linearity in the range of 10–50 mg/mL for ursodeoxycholic acid and 1–5 mg/mL for sil. The combination of Ursodeoxycholic acid and Silymarin with RSD - 0.95 [Figure 1]. A typical calibration curve has the regression equation of Y=103.0X+123 R^2 = 0.999 for ursodeoxycholic acid and Y=85.97X–3.638 R^2 = 0.999 for silymarin.

Precision

The results of system precision (% RSD = 0.97) for ursodeoxycholic acid and silymarin method precision are found within the prescribed limit of ICH guidelines [Table 3].

Intra-assay and inter-assay

The intra- and inter-day variation of the method was carried out and high values of mean assay and low values of standard deviation and percentage RSD within a day and day-to-day variations for

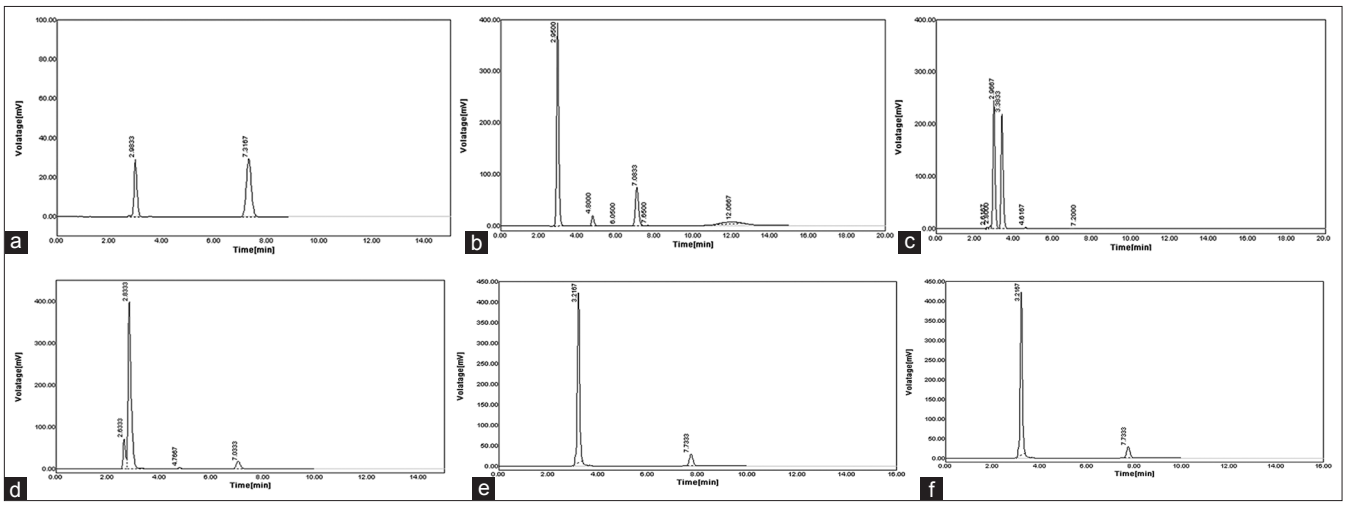

Figure 3: Chromatograms of (a) acid degraded sample, (b) hydrolyzed degraded (c,d) alkali degradation, (e) neutral degradation, and (f) photolysis degradation

Table 3: Precision data of proposed RP-HPLC method

Drug	Concentration (µg/mL)	Intra-day precision		Inter-day precision	
		Mean ± SD	%Amount found	Mean ± SD	%Amount found
SILY	10	423.16 ± 12.56	14.25	426.16 ± 12.23	10.08
	15	420.36 ± 12.45	15.26	419.30 ± 23.20	10.26
	20	415.23 ± 23.56	3.25	416.20 ± 25.23	10.45
URSO	20	256.23 ± 25.8	12.56	257.23 ± 25.12	15.26
	30	115.23 ± 12.03	0.12	116.20 ± 12.25	18.25
	40	116.02 ± 15.20	1.025	117.02 ± 12.26	30.2

RP-HPLC: Reversed-phase-high-performance liquid chromatography

Table 4: Robustness study of silymarin (a) and ursodeoxycholic acid (b)

a

Flow rate = 0.9 mL						**Flow rate = 1.1 mL**	
S. No.	**Concentration**	**µg/mL**	**Area**		**S. No.**	**Concentration µg/mL**	**Area**
1		25	3465.988		1	25	3526.394
2		25	3509.685		2	25	3569.155
		Mean	3487.84			Mean	3547.77
		SD	30.90			SD	30.24
		%RSD	0.89			%RSD	0.85
Mobile phase volume			68%+32			72 + 28	
	S. No.	**Concentration**	**µg/mL**	**Area**	**S. No.**	**Concentration µg/mL**	**Area**
	1		10	1145.874	1	10	1268.041
	2		10	1163.548	2	10	1296.357
			Mean	1154.7		Mean	1282.20
			SD	12.50		SD	20.02
			%RSD	1.08		%RSD	1.56
Wavelength change			272 nm			271 nm	
	S. No.	**Concentration**	**µg/mL**	**Area**	**S. No.**	**Concentration µg/mL**	**Area**
	1		25	3645.248	1	25	3526.258
	2		25	3612.287	2	25	3598.56
			Mean	3628.8		Mean	3562.41
			SD	23.31		SD	51.13
			%RSD	0.64		%RSD	1.44

b

Flow rate = 0.9 mL			**Flow rate = 1.1 mL**	
S. No.	**Concentration µg/ml**	**Area**	**Concentration µg/mL**	**Area**
1	50	2572.09	50	2346.867
2	50	2516.389	50	2316.395
	Mean	2544.24	Mean	2331.63
	SD	39.39	SD	21.55
	%RSD	1.55	%RSD	0.92
Mobile phase volume			68%+32	
	Concentration			
S. No.		**Concentration µg/mL**	**Area**	**Concentration µg/mL**	**Area**
1		20	1125.951	20	1168.041
2		20	1101.525	20	1196.357
		Mean	1113.7	Mean	1182.20
		SD	17.27	SD	20.02
		%RSD	1.55	%RSD	1.69
Wavelength change	Concentration		272 nm		271 nm
S. No.		**Concentration µg/mL**	**Area**	**Concentration µg/mL**	**Area**
1		50	3159.607	50	3031.136
2		50	3112.287	50	3001.294
		Mean	3135.9	Mean	3016.21
		SD	33.46	SD	21.10
		%RSD	1.07	%RSD	0.70

ursodeoxycholic acid and silymarin revealed that the proposed method is precise in Table 3.

Method robustness

Influence of small changes in chromatographic conditions such as change in flow rate (10%), organic content in mobile phase (2%), wavelength of detection (5%), and pH of buffer in mobile phase (0.2%) studied to determine the robustness of the method is also in favor [Table 4] of the developed RP-HPLC.

Limit of detection (LOD) and limit of quantification (LOQ)

The minimum concentration level at which the analyte can be reliably detected (LOD) and quantified (LOQ) were found to be for ursodeoxycholic acid 0.3 and 0.93 and silymarin- 0.11 and 0.34, respectively.

RESULTS AND DISCUSSION

The present investigation gives an account of strength showing examine of combination ursodeoxycholic corrosive and silymarin in the nearness of degraded items by HPLC. In this strategy, isocratic elution technique was chosen for examination of the mix. Since, it gave a better baseline detachment and peak width, which is reasonable for routine investigation of the blend. The created technique was approved according to the ICH rules.

Stability testing shapes a critical piece of procedure of drug product advancement. The reason for stability testing is to give prove on how the drug quality substance shifts with time under the impact of different ecological factors, for example, temperature, stickiness, and light, and empowers suggestions of capacity conditions, retest periods, and time span of usability to be set up.

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