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Silymarin as a Phytopharmaceutical: Quantitative Estimation of the Isolated Compounds Using HPLC and HPTLC

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ABSTRACT

Background and Aims: Central Drug Standards and Control Organization (CDSCO) have introduced a new class of drugs called phytopharmaceutical, in the year 2014. It includes purified and standard fraction with a minimum of four bio-active compounds of an extract of a medicinal plant. Thus, we have isolated four flavonolignans from the silymarin extract.

Methods: The four flavanolignans, silychristin, silybin A, silybin B, and mixture of isosilybin A and B were isolated by using semi-prep HPLC. The isolated biomarkers are stereoisomers of each other thus we have qualitatively characterized them by their optical rotation. Validated sensitive and highly selective HPLC method was developed for the simultaneous quantitative assessment of the isolated compounds. The developed method has been validated and proved to meet the requirements delineated by ICH guidelines for the linearity, accuracy, precision, robustness, and specificity. Additionally, quantitative assessment of the marker compounds in the extract was also done by the HPTLC method reported in the ICMR book. The RP-HPLC separation was achieved on C18 LiChrospher100 (250x4.6mm, 5 µm particle size) using a mobile phase of methanol: water (45:55 v/v) at a flow rate of 1.0 mL/min. The injection volume was 10 µL and elute was analyzed with a UV detector set at a wavelength of 288 nm.

Results: The calibration curve of all the components was observed to be linear in the range of 10-120 µg/ml with all the parameters reported in table 1. The estimated percentage of silychristin, silybin A, silybin B, and mixture of isosilybin A and B was found to be 12.64%, 8.71, 8.71 and 10.02%, respectively in the extract using HPLC. Using HPTLC the concentration of silychristin, micture of silybin A and B, and mixture of isosilybin A and B was found to be 11%, 15.5% and 9.9%, respectively.

Conclusions: The proposed method was compared statistically with reported HPLC method with no significant difference regarding accuracy and precision. Thus the phytopharmacetical containing minimum four bioactive compounds has been succesfully analyzed.

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Table 1: Validation parameters of the isolated components by HPLC

HPLC Method Validation					
Parameters		Silychristin	Silibin A	Silybin B	Iso silibin A and B
Rt		5.85	16.24	19.05	27.31 and 30.44
Linearity		20-120 ug/mL	20-120 ug/mL	20-120 ug/mL	20-120 ug/mL
Regression equation		y=8224x-19838	y=9357x-65779	y=11063x-74738	y=11118x+2014
R square		0.997	0.996	0.997	0.996
Slop		8224	9357	11063	11118
Intercept		19838	65779	74738	2014
LOD		6.7 ug/mL	6.3 ug/mL	6.3 ug/mL	8.7 ug/mL
LOQ		22.9 ug/mL	21.1 ug/mL	21.1 ug/mL	22 ug/mL
Precision Parameters	Intra day (%RSD)	1.02	1.21	0.58	0.41
	Inter day	1.35	1.39	1.47	0.85
Purity (%)		99.25	99.85	99.85	85.73
Accuracy at 60 ug/mL (%)		96.95	96.29	96.18	94.90

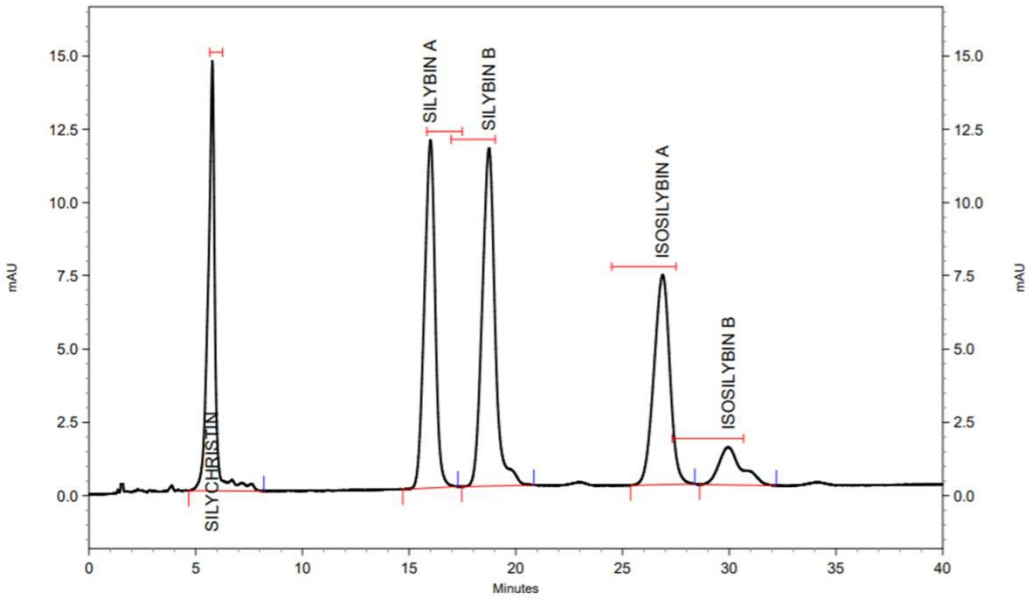

Fig. 1: Chromatogram of the prepared Phytopharmaceutical drug

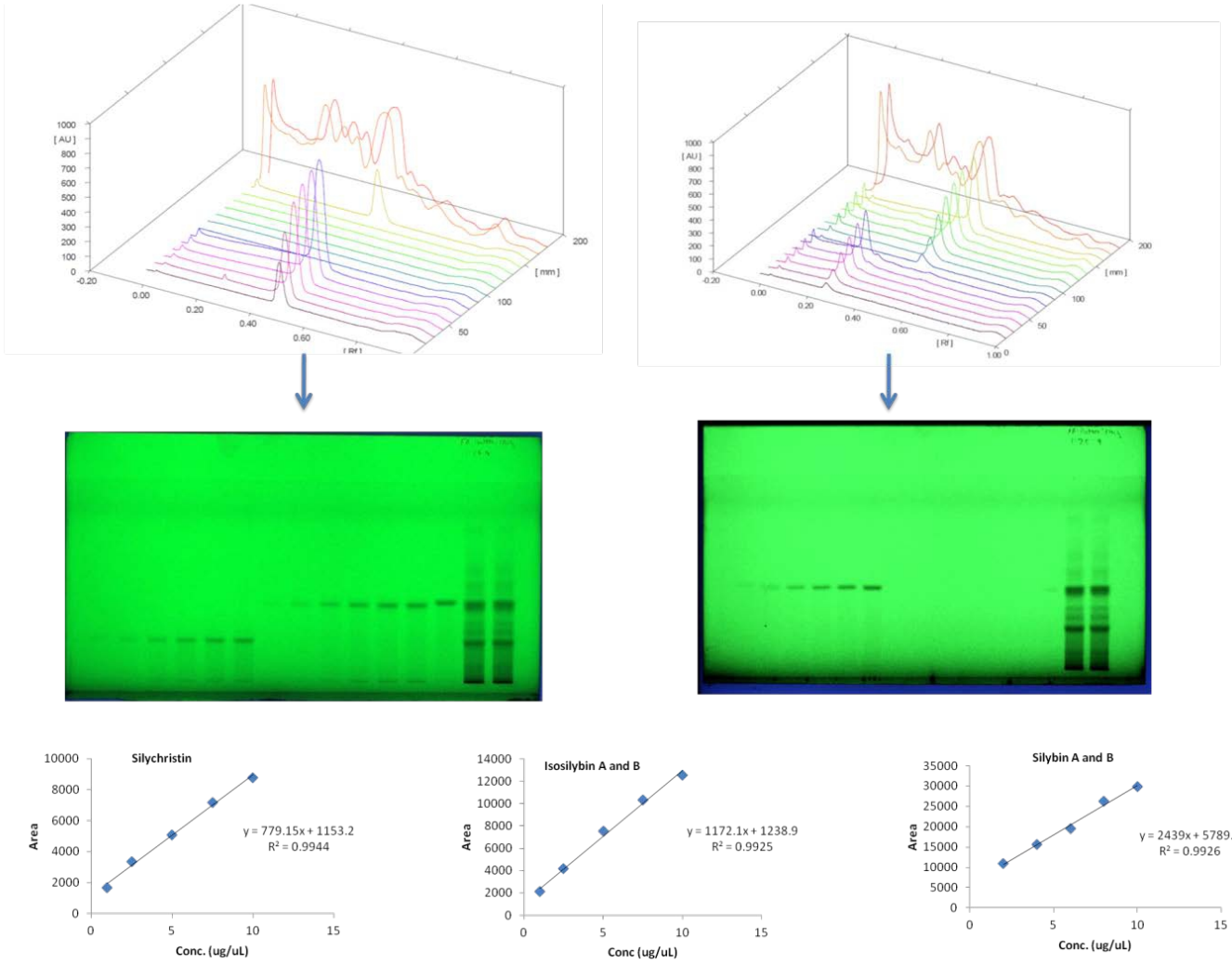

Fig. 2: HPTLC chromatogram of the isolated components

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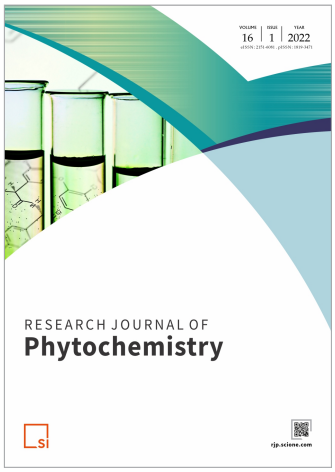

Aims & Scope

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