



Population Pharmacokinetic Analysis of Silymarin Bioavailability in Rats

I. Locatelli¹, B. Perissutti², D. Voinovich², A. Mrhar¹, I. Grabnar¹

¹University of Ljubljana, Faculty of Pharmacy, Slovenia

²University of Trieste, Department of Pharmaceutical Sciences, Italy

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igor.locatelli@ffa.uni-lj.si

iztok.grabnar@ffa.uni-lj.si

INTRODUCTION

Silymarin, an extract of the seeds of *Silybum marianum* L. (milk thistle), is used for treatment of liver diseases. It contains several active flavonolignans; the major are silybin, which is a mixture of diastereomers A and B, and silychristin. Low bioavailability of silybin and silychristin is related to low water solubility of these compounds. In order to enhance the solubility, *S. marianum* dry extract was coground with two polymers: crospovidone (PVP-CL) and carboxymethylcellulose (Ac-Di-Sol) in 1:3 dry extract-to-polymer weight ratios [1].

In this study, population PK analysis was used to investigate the relative bioavailability of silybin A, silybin B, and silychristin for the coground systems in rats.

Study design

The amount of silybin A, silybin B, and silychristin in *S. marianum* dry extract was 17%, 16%, and 21%, respectively. Two dose levels of the dry extract (50 and 200 mg/kg), one dose level for Ac-Di-Sol coground system (50 mg/kg) and three dose levels for PVP-CL coground system (50, 100, and 200 mg/kg) were administered to altogether 88 rats. Single blood samples per animal were drawn at 0.5, 1, 2, and 4 h post dose. In low dose dry extract group and in Ac-Di-Sol group five animals per sampling time were applied, while in other groups three animals were used. A new HPLC-UV method was developed for quantification of the three components of interest [1].

Pharmacokinetic analysis

The plasma concentrations were modelled separately for each of the compounds measured. The following PK models were compared:

- one compartment model with first order absorption rate considering the influence of the coground polymers on relative bioavailability,

$$F1 = \theta_{Ac-Di-Sol}^{Ac-Di-Sol=1} \cdot \theta_{PVP-CL}^{PVP-CL=1}$$

- the influence of dose level on relative bioavailability was added,

$$F1 = \theta_{Ac-Di-Sol}^{Ac-Di-Sol=1} \cdot \theta_{PVP-CL}^{PVP-CL=1} \cdot \left(\frac{Dose}{max(Dose)} \right)^{\theta_{Dose}}$$

- Michaelis-Menten elimination kinetics was integrated in addition to the influence of the coground polymers and dose level on relative bioavailability.

Population pharmacokinetic (PK) analysis was performed using NONMEM V1 (level 2) and PsN (version 2.3). First-order conditional method with interaction (FOCEI) was applied for parameters estimation. The interanimal variability on volume of distribution (V/F) and plasma clearance (CL/F) was evaluated using exponential model. Proportional residual error for the quantified concentrations was fixed to the estimated precision of the analytical method. Concentration levels below lower limit of quantification (LLOQ) were replaced with LLOQ/2 values. The proportional residual error for these values was fixed to 25% [2]. The portion of concentration values below LLOQ were 11%, 0, and 9% for silybin A, silybin B, and silychristin, respectively.

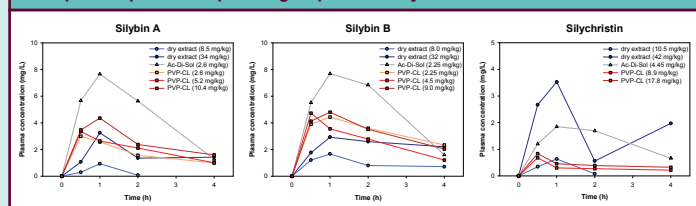
Analytical method validation parameters.

Compound	LOD [mg/L]	LLOQ [mg/L]	Precision (RSD)
Silybin A	0.024	0.096	4.8 %
Silybin B	0.026	0.104	8.6 %
Silychristin	0.010	0.050	12 %

LOD, limit of detection; LLOQ, limit of quantification; RSD, relative standard deviation.

RESULTS

Mean plasma profiles of pooled groups of rats by dose and formulation.

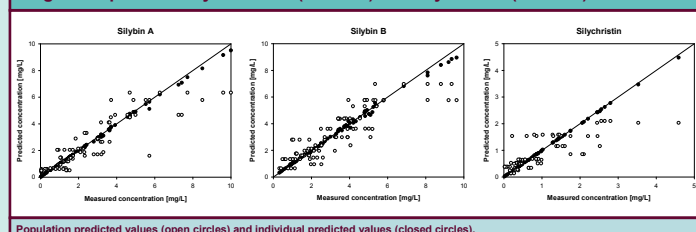


Model 1	Silybin A	Silybin B	Silychristin			
Parameter	Estimate	Std. error	Estimate	Std. error	Estimate	Std. error
θ_{KA}	2.21	1.34	2.67	11	4.54	3.19
$\theta_{CL/F}$	5.46	0.83	1.8	2.37	4.77	1.22
$\theta_{V/F}$	10.9	3.7	6.14	10.8	16.3	2.96
$\theta_{Ac-Di-Sol}$	41.4	8.91	23.4	5.16	7.12	1.11
θ_{PVP-CL}	10	1.38	7.9	1.31	-0.759	0.108
$\omega_{CL/F}$	0.334	0.141	0.405	1.05	0.763	0.401
$\omega_{V/F}$	0.414	0.0927	0.296	0.185	0.207	0.0635
Residual error	0.048	fixed	0.086	fixed	0.12	fixed

Model 2	Silybin A	Silybin B	Silychristin			
Parameter	Estimate	Std. error	Estimate	Std. error	Estimate	Std. error
θ_{KA}	2.52	1.28	1.92	0.491	4.4	3.05
$\theta_{CL/F}$	6.19	1.16	3.47	0.458	4.25	1.22
$\theta_{V/F}$	14.7	4.11	9.02	1.44	13.7	2.99
$\theta_{Ac-Di-Sol}$	20.1	5.53	6.16	0.993	9.89	2.4
θ_{PVP-CL}	5.93	1.20	3.18	0.400	0.851	0.121
θ_{Dose}	-0.368	0.118	-0.722	0.0695	0.212	0.142
$\omega_{CL/F}$	0.422	0.165	0.253	0.0862	0.67	0.365
$\omega_{V/F}$	0.214	0.063	0.561	0.0234	0.205	0.0573
Residual error	0.048	fixed	0.086	fixed	0.12	fixed
ΔOFV	-10.8		-84.9		-2.7	

The bioavailability of the studied compounds is formulation dependent. The bioavailability for silybin A in B was also inversely related to the dose administered. The Michaelis-Menten elimination kinetics (Model 3) failed to significantly reduce the objective function value (OFV) for all of the components.

Diagnostic plots for silybin A in B (model 2) and silychristin (model 1).



Population predicted values (open circles) and individual predicted values (closed circles).

CONCLUSION

The bioavailability of silymarin components was 6 to 20-fold higher in Ac-Di-Sol coground system compared to the dry extract. However, lower bioavailability can be expected with application of higher doses of silymarin.

REFERENCES

[1] Voinovich D, Perissutti B, Magarotto L, et al. Solid state mechanochemical simultaneous activation of the constituents of the *Silybum marianum* phytoextract with crosslinked polymers. *J Pharm Sci* 2009 98(1) 215-28.

[2] Hing JP, Woolfrey SG, Greenslade D, et al. Analysis of toxicokinetic data using NONMEM: impact of quantification limit and replacement strategies for censored data. *J Pharmacokinetic Pharmacodyn* 2001 28(5) 465-79.