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Study on the plasma protein binding rate of *Schisandra* lignans based on the LC-IT-TOF/MS technique with relative quantitative analysis

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[ABSTRACT] The main objective of the current study was to develop a universal method for a protein binding assay of complicated herbal components, and to investigate the possible relationship between compound polarity and protein binding using *Schisadra* lignans as an example. Firstly, the rat, dog and human plasma were spiked with three different concentrations of *Schisandra chinensis* extract (SLE), and ultramicrofiltration was used to obtain the unbound ingredients. Secondly, thirty-one *Schisandra* lignans in total plasma and ultrafiltered fluid were measured by LC-IT-TOFMS. Lastly, a relative exposure approach, which entailed calculating the relative concentrations of each *Schisandra* lignan from the corresponding calibration equation created from the calibration samples spiked with the stock solution of SLE, was applied in order to overcome the absence of authentic standards. The results showed that *Schisandra* lignans exhibited a high capability to bind with plasma protein, furthermore, the protein binding ratio of the lignan components increased proportionally with their individual chromatographic retention time, which indicated that the ratio of protein binding of lignans might increase accordingly with decreasing polarity. This study suggested that the compound polarity might be an important factor affecting the plasma protein binding of herbal components.

[KEY WORDS] *Schisandra chinensis* extract; *Schisandra* lignans; Protein binding ratio; LC-IT-TOF/MS

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1 Introduction

Plasma protein binding of drugs, referring to the percentage of drug bound with plasma protein, is a significant parameter in pharmacokinetics (PK) studies as the extent of plasma-bound drug influences the distribution, metabolism and excretion of the drug *in vivo* [1-2]. Usually, the less bound a drug is, the more efficiently it can traverse cell membranes and diffuse [3-4]. Moreover, the efficiency of a drug may also be affected by the degree to which it binds to the proteins within the blood plasma. Therefore, plasma protein binding is an important parameter in order to understand the PK and pharmacodynamic (PD) properties of a drug.

It has been well-known that PK research is an indispensible part in the whole pipeline of new drug discovery. Similarly, elucidation of the PK profile of a herbal medicine is critical for its modernization, as it provides important information for explaining and predicting their efficacy, toxicity, and the widely reported herb-drug interactions [5-8]. However, pharmacokinetic research for herbal medicines is a formidable task, and still in its infancy largely due to complicated components and the absence of authentic standards. For the same reason, characterization of the plasma protein binding of complicated herbal components represents a challenging task and, to date, there have been few studies reported on the protein binding ratio of herbal medicine.

In the present study, a generally applicable approach was proposed to conduct a protein binding study for herbal medi-

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cines based on ultramicrofiltration and LC-IT-TOFMS analysis. *Schisandra chinensis* (Turcz.) K. Koch extract (SLE), the active components from *Schisandra* fruits (Schisandraceae), are widely used in Chinese medicine for hepatoprotection and anti-oxidation, was taken as the model drug in this study [9-11]. As was verified in previous studies, LC-IT-TOFMS provided a very powerful tool for qualitative and quantitative determination of complicated herbal components [12-14]. In addition, to overcome the obstacle of the absence of authentic standards, a relative exposure approach was developed by calculating the relative concentrations of each component from the corresponding calibration equation created from the calibration samples spiked with the stock solution of herbal medicine, and its validity was verified by absolute quantitation results. In our previous study [12-16],

Table 1 The structure and retention time of *Schisandra* lignans in SLE

No.	R_t/ min	R_1	R_2	R_3	R_4	R_5	R_6	Type I	Structures
3	10.4	OH	CH_3	CH_3	CH_3	CH_3	CH_3	Gomisin T-ol	
8	21	H	CH_3	CH_3	CH_3	CH_3	H	Gomisin J	
23	35.7	H	CH_3	CH_3	CH_3	CH_3	CH3	Gomisin K1	
24/25	36.9	CH_2		CH3	H	CH_3	CH_3	Gomisin M1	
24/25	37	CH_2		H	CH_3	CH_3	CH_3	Gomisin M2	
27	38	CH_3	CH_3	H	CH_3	CH_2		Gomisin L1	
28	38.5	H	CH_3	CH_3	CH_3	CH_2		Gomisin L2	
29	39.2	CH_3	CH_3	CH_3	CH_3	CH_3	CH_3	Schizandrin A	
30	42.3	CH_2		CH_3	CH_3	CH_3	CH_3	γ-Schizandrin	
31	42.9	CH_3	CH_3	CH_3	CH_3	CH_2		Schizandrin B	
		R_1	**R_2**	**R_3**	**R_4**	**R_5**	**R_6**	**Type II**	
6	17.0	CH_3	CH_3	H	H	CH_3	OH	Schizandrol A	
10	23.5	CH_2		H	H	CH_3	OH	Schizandrol B	
19	32.9	CH_2		H	OH	CH_3	H	Gomisin O	
20	33.2	CH_2		OH	H	CH_3	H	Epigomisin O	
4	11.8							Isoschizandrin	
		R_1	**R_2**	**R_3**	**R_4**	**R_5**	**R_6**	**Type III**	
1	7.7	CH_2		CH_3	H	H	OBz	Gomisin G1	
2	9.2	CH_3	CH_3	H	OH	H	H	Gomisin H	
5	15.2	H	CH_3	CH_3	H	H	OH	Gomisin	
9	22.2	CH_3	CH_3	OTig	OH	H	H	Tigloylgomisin H	
12	25	CH_3	CH_3	OAng	OH	H	H	Angeloylgomisin H	
22	34.1	CH_2		CH_3	H	OH	OTig	TigloylgomisinP	
26	37.2	CH_2		CH_3	H	OH	OAng	Angeloylgomisin P	
		R_1	**R_2**	**R_3**	**R_4**	**R_5**	**R_6**	**Type IV**	
11	24.6	CH_3	CH_3	CH_2		OAng	OH	Schisantherin B	
13	25.8	CH_2		CH_3	CH_3	OBz	OH	Gomisin G	
21	33.4	CH_3	CH_3	CH_2		OBz	OH	Schisantherin A	
14	25.8	CH_3	CH_3	CH_3	CH_3	OAng	OH	Angeloylgomisin Q	
15	27.8	CH_2		CH_2		OH	H	Gomisin R	
16	29.1	CH_3	CH_3	CH_2		OTig	OH	Schisantherin C	
18	32.8	CH_2		CH_3	CH_3	OAng	OH	Gomisin F	
		R_1	**R_2**					**Type V**	
7	19.8	OH	CH_3					Gomisin D	
17	29.8