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HPLC Analysis of Emodin in Serum, Herbs and Chinese Herbal Prescriptions

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ABSTRACT

Rapid and simple methods for the analysis of emodin in serum, herbs—Da Huang(大黃),Huu Jahng(虎杖),Her Shoou U(何首烏), Jyuer Ming Tzyy(決明子)and four Chinese herbal prescriptions—Tiaowei Chengqi Tang(調胃承氣湯), Xiao Chengqi Tang(小承氣湯), Taoren Chengqi Tang(桃仁承氣湯), Da Chengqi Tang(大承氣湯) were developed by application of reversed phase HPLC system. The HPLC conditions include a RP-18 column ; elution with the mixture of MeCN and 2% HOAc; detection at 280 nm and using 2-methylanthraquinone as the internal standard. We observed that the optimum elution ratio(MeCN: 2 % HOAc) and flow rates for serum samples are 73:27 and 0.9 ml/min, respectively, while those for herbs and Chinese herbal preparations are 55:45 and 1.5 ml/min, respectively. The retention times of emodin/internal standard are 6.4 min/9.1 min and 8.3 min/13.0 min, respectively, for these two elution conditions. The established HPLC methods are accurate and precise. They could be uselful in pharmacokinetic study of emodin, quality evaluation of herbs containing emodin and quality control of Chinese herbal preparations including Da Huang as their component.

Key words: emodin, HPLC, serum, herbs

INTRODUCTION

Emodin is an active constituent of many herbs used in Chinese medicine, e.g. Da Huang(大黃), Huu Jahng(虎杖), Her Shoou U(何首烏)and Jyuer Ming Tzyy(決明子). Pharmacological studies have recently demonstrated that emodin possesses anticancer[1], antibacterial[2], diuretic[3], vasorelaxant and immunosuppressive effects[4,5].

HPLC analysis of emodin had been done mainly on Da huang[6], some rhubarb preparations[7], urines[8] collected from volunteers taking rhubarb preparation and serum[9] of rabbit injected with emodin. The objective of this study was to develop a general, sensitive and expedient HPLC method of emodin assay for pharmacokinetic study and for quality control of concentrated type Chinese herbal prescriptions containing Da Huang.

MATERIALS AND METHODS

I. *Chemicals and Reagents*

LC grade acetonitrile (BDH, England) ;

pure water (Pure water products RO-50HP, PURITE, England); emodin standard (ROTH, Germany); 2-methylanthraquinone (WAKO, Japan) recrystallized from EtOH.

II. *Crude Drugs and Herbal Prescriptions*

Da Huang (rhizomes of *Rheum officinale*), Huu Jahng (rhizomes of *Polygonum cuspidatum*), Her Shoou U (rhizomes of *Polygonum multiflorum*) and Jyuer Ming Tzyy (seeds of *Cassia tora*) were purchased from five Chinese drug stores in Taichung and were pulverized. Herbal prescriptions were filled by a licensed pharmacist according to the formulas in Yi Fang Jir Jiee(醫方集解). Four Chinese herbal preparations were prepared as follows :Da Chengqi Tang(大承氣湯)—Rhei rhizoma(大黃) 2.0g, Magnoliae cortex(厚朴) 3.0g; Aurantii fructus immaturus(枳實) 3.0g, Niter(芒硝) 3.0g; Xiao Chengqi Tang(小承氣湯)—Rhei rhizoma 2.0g, Magnoliae cortex 1.0g, Aurantii fructus immaturus 1.5g ; Tiaowei Chengqi Tang(調胃承氣湯)—Rhei rhizoma 2.0g , Niter 2.0g , Glycyrrhizae radix(甘草)1.5g ; Taoren Chengqi Tang(桃仁承氣湯)—Rhei rhizoma 2.0g, Niter 1.0g, Glycyrrhizae radix 1.0g, Persicae semen(桃仁)2.0g, Cinnamomi ramulus(桂枝)1.0g.

III. *Instrumentation*

The HPLC apparatus was equipped with two pumps (LC-6AD, Shimadzu, Japan), monitored by an SLC-6B controller, a photodiode array detector (SPD-M6A, Shimadzu, Japan) and a data processor (Acer 1116SX) with an NEC color printer. The RP -18 column (Merck 50983 Lichrospher 100, 5 μm, 25cm × 4mm) was equipped with a guard column (Merck 50957 Lichrospher 100, 5 μm).

IV. *Mobile phase*

The mobile phase consisted of acetonitrile and 2% acetic acid (73:27 and 55:45 for serum and herbal samples, respectively).

V. *Internal standard*

An internal standard solution was prepared by dissolving 2-methylanthraquinone in small amount of methanol and diluting with acetonitrile to the desired concentration (15.6 μg/ml).

VI. *Standard Solution*

Emodin was dissolved in a small amount of methanol and diluted with acetonitrile to prepare various stock solutions of proper concentrations.

(I). For serum analysis, emodin solutions (20 μl) were mixed with blank serum (180 μl) and internal standard solution (600 μl) to afford calibrators in concentrations of 65.00, 16.25, 8.13, 4.06, 2.03 and 0.51 μg/ml.

(II). For herbal analysis, emodin solutions (20 μl) were mixed with water (180 μl) ,methanol (300 μl) and internal standard solution (300 μl) to afford calibrators in concentrations of 65.00, 32.50 , 16.30, 8.15, 4.06 and 2.03 μg/ml.

VII. *Sample Preparations*

(I). Serum sample (200 μl) was mixed with internal standard solutions (600 μl) , vortexed for 10 sec and then centrifuged at 15,000 rpm for 5 min. The supernatant was decanted and concentrated by blowing N_2 gas, then was forzen at -20°C for later analysis.

(II). Each herb (2.0 g) was extracted with methanol (25 ml) in an ultrasonic bath at 50°C for 4 hrs and filtered into a volumetric flask to which methanol was added to 20 ml. This sample solution (200 μl) was mixed with methanol (300 μl) and the internal standard solution (300 μl), vortexed for 1 min and then centrifuged at 15,000 rpm for 5 min. The supernatant was ready for HPLC analysis or diluted with acetonitrile if necessary.

(III). To each herbal prescription, water (25 ml) was added and gently boiled under reflux for 2 hrs with a mantle heater, then filtered and enou-

gh water added to 20 ml. Decoctions were prepared in triplicate for each presciption. Sample solution (200 μl) was mixed with methanol (300 μl) and internal standard solution (300 μl), vortexed for 1 min and then centrifuged at 15,000 rpm for 5 min. The supernatant was ready for HPLC analysis.

VIII. *Chromatographic conditions*

The optimum flow rates were 0.9 ml/min for serum and 1.5 ml/min for herbal samples. The detection wavelength was 280 nm. The column was at ambient temperature.

IX. *Statistical analysis*

Tuckey's method was used for statistical comparison. Significant level was set at 0.05.

RESULTS AND DISCUSSION

Figure 1A and 1B showed chromatograms of emodin and 2-methylanthraquinone (internal standard) in serum samples. The calibration curve for emodin in serum was linear (r = 0.9998) over the concentration range 0.51-65.00 μg/ml. The recoveries were found quantitative. The stability of emodin in serum during storage at -20°C for 21 days was found satisfactory. The reproducibility was measured from three runs within a day and three runs in three different days with samples spiked to 65.0, 2.0 and 0.5 μg /ml of emodin. The coefficients of variation for intraday runs were 0.68%, 2.24% and 2.51%, while for interday runs were 2.99%, 3.94% and 3.97%, respectively. The detection limit was 20 ng/ml which was lower than that of the method reported recently[9]. Therefore, while using our method to study the pharmacokinetics of emodin, the serum concentrations could be successfully monitored for longer duration to get better data fitting. The application of this method in a study of emodin pharmacokinetics in rabbits will be reported later elsewhere.

Concerning the emodin assay in herbs and herbal prescriptions, previous reports used external standard method[6,7]. Our method found 2-methylanthraquinone was a proper internal standard. The chromatograms shown in Figure 2A-2D were obtained from Da Huang, Huu Jahng, Her Shoou U and Jyuer Ming Tzyy, respectively. Figure 3A-3D showed the chromatograms of four Chinese herbal prescriptions — Tiaowei Chengqi Tang(調胃承氣湯), Xiao Chengqi Tang(小承氣湯), Taoren Chengqi Tang(桃仁承氣湯) and Da Chengqi Tang(大承氣湯), respectively. The calibration curve was linear (r = 0.9998) over the concentration range 2.03-65.00 μg/ml. The precision was evaluated from three runs within a day and three runs in three

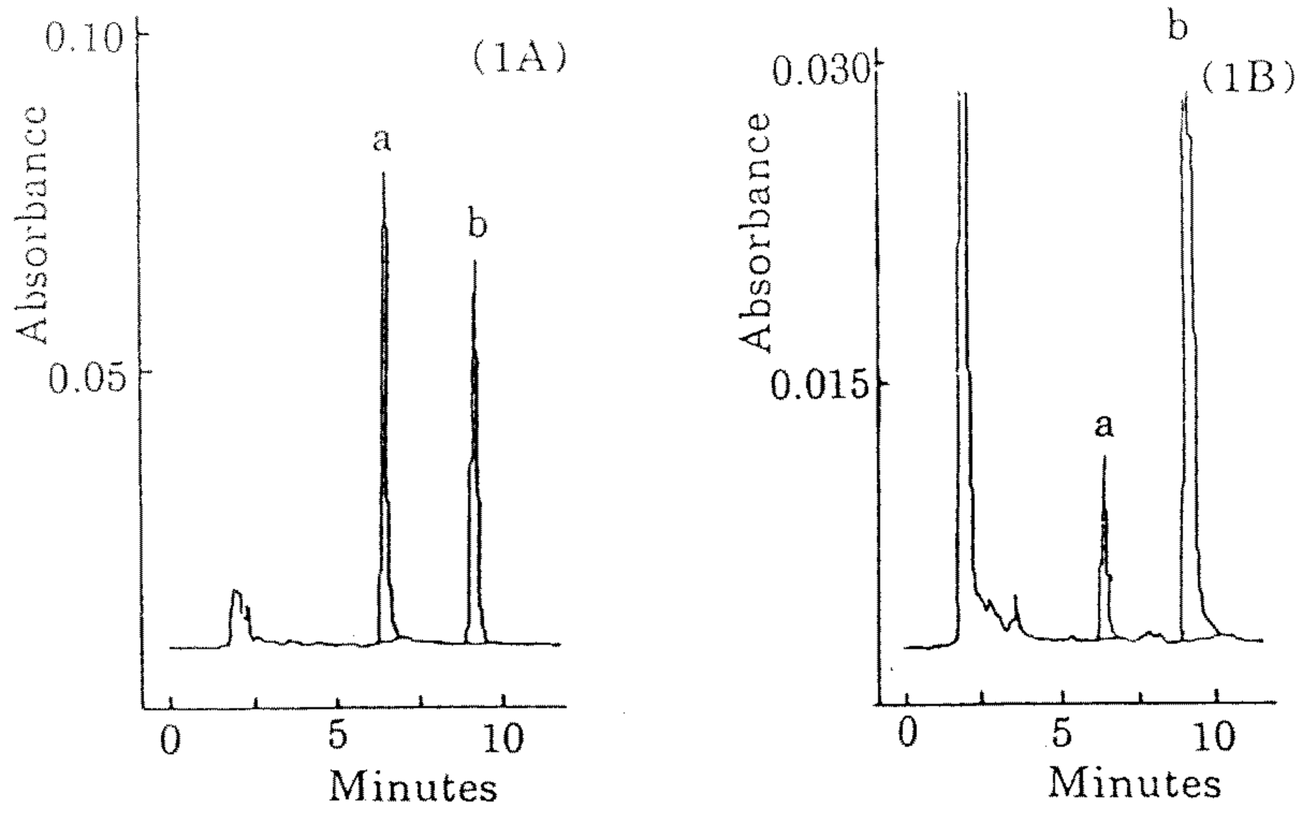

Figure 1A-1B :Chromatograms of Emodin in Serum (1A : serum spiked with emodin, 1B : rabbit serum sample withdrawn after taking emodin) a : emodin, b : internal standard

Table 1. The Amount of Emodin (μg) in Methanol Extract of Crude Drugs (1 gm each)

Drug store	DH	HJ	HSW	JMT
A	1657.4	12882.3	57.0	32.3
B	1874.4	12587.4	49.2	33.6
C	1684.3	10495.7	93.9	39.2
D	1555.1	8183.7	61.8	57.3
E	1221.8	11788.7	29.5	62.1
mean	1598.7	11187.6	58.3	44.8
S.D.	240.3	1916.7	23.4	13.9

DH:Da Huang(大黃) HSW:Her Shoou U(何首烏)
HJ:Huu Jahng(虎杖) JMT:Jyuer Ming Tzyy(決明子)

Table 2. The Amount of Dissolved Emodin in Decoctions of Four Chinese Herbal Prescriptions

	A	B	C	D
*amount of emodin(μg)	51.2	142.1	200.9	92.8
	72.9	104.3	166.9	85.3
	67.4	95.9	199.5	67.9
mean	63.8	114.1	189.1	82.0
S.D.	11.3	24.6	19.2	12.9

A:Da Chengqi Tang(大承氣湯)
B:Xiao Chengqi Tang(小承氣湯)
C:Tiaowei Chengqi Tang(調胃承氣湯)
D:Taoren Chengqi Tang(桃仁承氣湯)
*calculation based upon one gram of Da Huang(大黃).

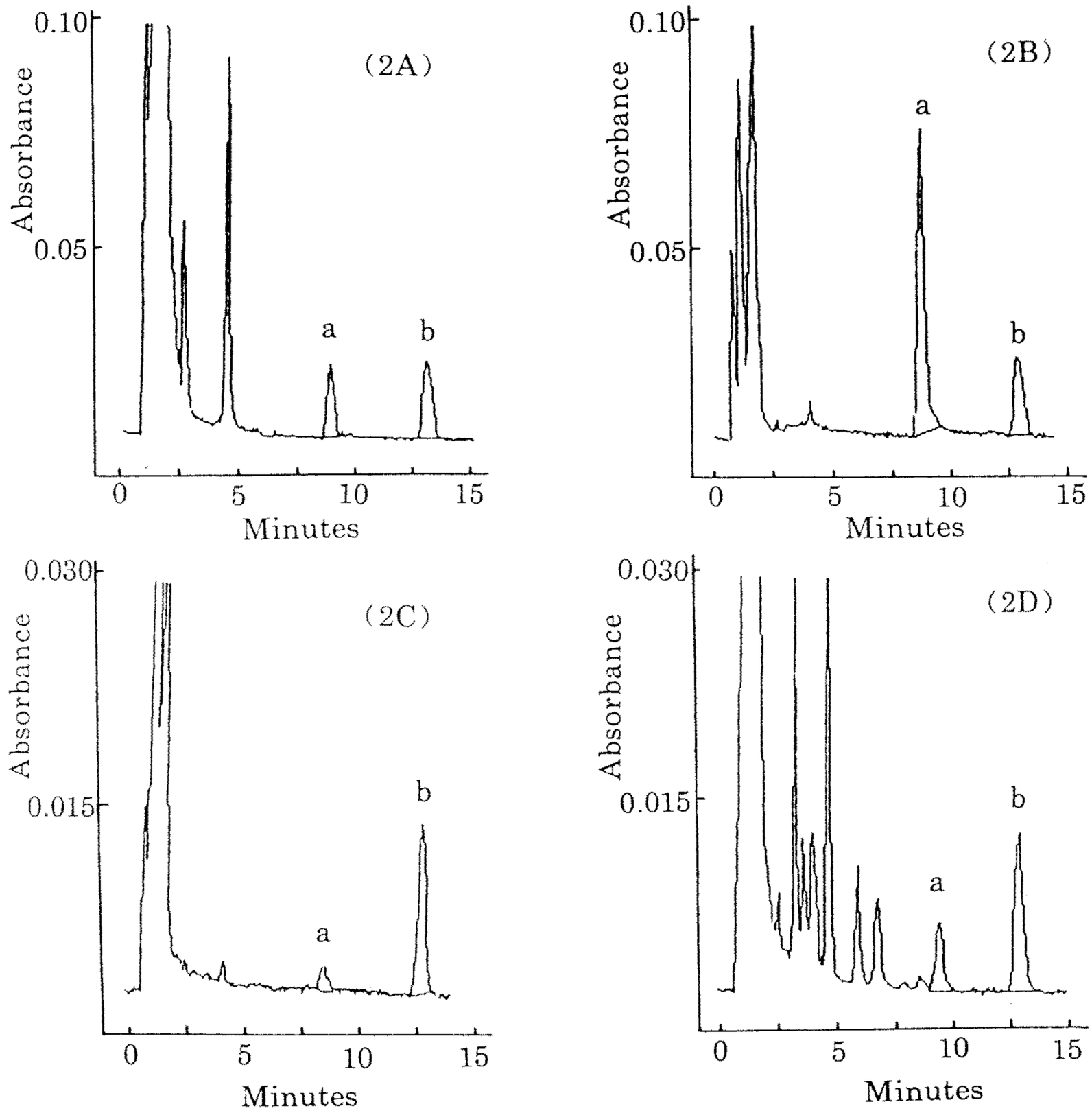

Figure 2A-2D : Chromatograms of Herbs (a : emodin, b : internal standard) 2A : Da Huang(大黃),2B : Huu Jahng(虎杖),2C : Her Shoou U(何首烏),2D : Jyuer Ming Tzyy(決明子)

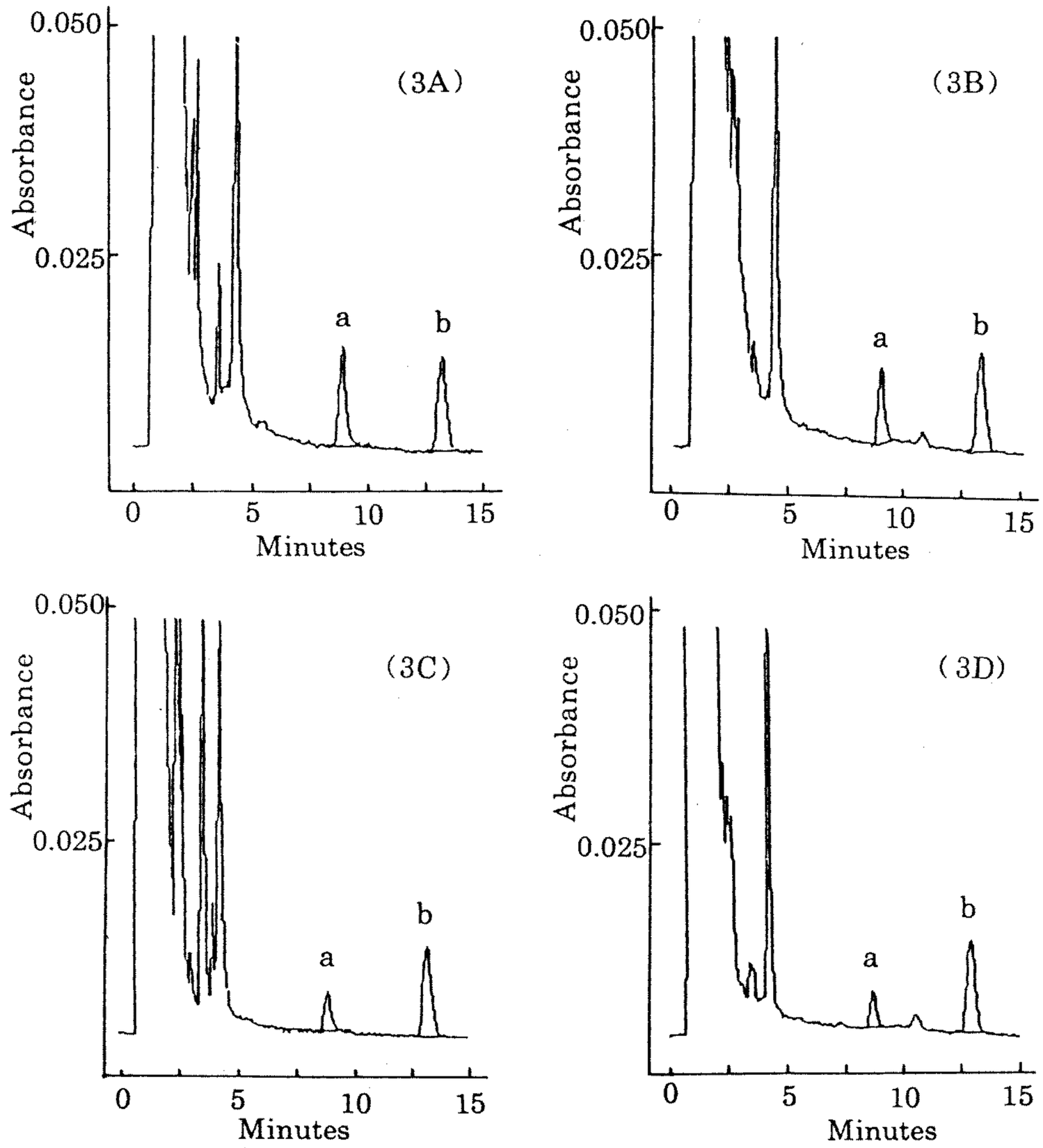

Figure 3A-3D : Chromatograms of Chinese Herbal Prescriptions (a : emodin, b : internal standard)
3A : Tiaowei Chengqi Tang(調胃承氣湯),3B : Xiao Chengqi Tang,(小承氣湯),3C : Taoren Chengqi Tang(桃仁承氣湯),3D : Da Chengqi Tang(大承氣湯)

different days with samples spiked to 65.0, 32.5 and 4.06 μg/ml. The coefficients of variation for intraday runs were 1.41%, 1.66% and 2.97%, while for interday runs were 1.10%, 3.85% and 4.37%, respectively. Application of the proposed method to herbs and herbal prescriptions provided the results given in Table 1 and Table 2. The peak purity of emodin was monitored with a photodiode-array detector, showing no interference within the peak. The results indicated that among the four herbs, Huu Jahng is abundant in emodin, while Jyuer Ming Tzyy and Her Shoou U are the poor sources. We also found that while the weight of Da Huang in the four entitled Chinese herbal prescriptions was identical, the amounts of emodin detected were found significantly variant among the different prescriptions. Statistical analysis of the results obtained from triplicate decoctions indicated the dissolved emodin in Tiaowei Chenqi Tang (調胃承氣湯) is significantly higher than that in the other three prescriptions and the dissolved emodin in Xiao Chengqi Tang (小承氣湯) is significantly higher than that in Da Chengqi Tang (大承氣湯). These phenomena indicated the amount of dissolved emodin was complicately influenced by other ingredients existing in the prescription.

The method established in this study can be applicable to quality evaluation of emodin-con-

taining crude drugs and concerntrated type Chinese herbal prescriptions.

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大黃素在血清、藥材及中藥方劑中之高效層析定量

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摘　要

大黃素爲中藥大黃、虎杖、何首烏及決明子的活性成分之一。本研究利用高效液相層析法建立了大黃素在血清、中藥材(大黃、虎杖、何首烏、決明子)及四種中藥方劑(調胃承氣湯,小承氣湯,桃仁承氣湯及大承氣湯)中之快速簡便定量法,內標準選用2-甲基蒽醌。分離管柱採用RP-18。移動相溶媒爲氯甲烷及2%醋酸水溶液,其最佳比例用於血清試樣爲73:27,用於中藥試樣爲55:45。移動相流速分別爲0.9毫升/分及1.5毫升/分,檢測波長爲280 nm,大黃素/2-甲基蒽醌於血清試樣中的滯留時間爲6.4分/9.1分,於中藥試樣中的滯留時間爲8.3分/13.0分。此高效液相層析定量法精確性頗佳。本研究結果可應用於大黃素的藥物動態學研究、含大黃素中藥材之規格化及含大黃、虎杖、何首烏及決明子等中藥材的中藥方劑之品質管制。