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Quantitative analysis of paeoniflorin, geniposide and glycyrrhizin in Jing-Jieh-Lian-Chyau-Tong by high performance liquid chromatography

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藥物食品分析
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荆芥連翹湯中paeoniflorin, geniposide及glycyrrhizin成分之高效液相層析定量方法之研究

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摘　要

本研究應用高效液相層析法探討荆芥連翹湯中芍藥之芍藥苷(paeoniflorin)、梔子之梔子苷(geniposide)、甘草之甘草甜素(glycyrrhizin)等的分析方法，其分析條件爲：(1)paeoniflorin、geniposide—層析管柱：GL Sciences INC Inertsil 5 ODS-2, 250mm × 4.6 mm I.D. 5μm.移動相：水．乙腈(85:15)，檢測波長：UV 254 nm.(2)glycyrrhizin—層析管柱：Merck 50734 Lichrospher 100 RP-18 125mm × 4 mm I.D. 5 μm. 移動相：1%醋酸：乙腈(65:35)。檢測波長：UV 254nm，各成分之檢量線顯示良好之線性關係，且paeoniflorin，geniposide及glycyrrhizin三者之添加回收率分別爲100.0%、99.6%及99.9%，顯示有良好之精確性。另分析三家市售之濃縮製劑其paeoniflorin、geniposide及glycyrrhizin之最高最低差分別爲2.22、4.75及4.62倍。而標準湯劑經減壓濃縮、冷凍乾燥及噴霧乾燥等方法處理上述三種成分含量幾不受影響。

前　言

芍藥、梔子、甘草均爲中藥製劑常用之配伍藥材，有關以成分爲其品質評價之報告較爲常見[1－7]，而於其製劑中同時定量三種成分之報告則較少被提出。本研究希望以一簡單之抽提法及簡便、快速之檢驗法分離分析中藥濃縮製劑—荆芥連翹湯中此三種藥材之主成分paeoniflorin (圖一)，geniposide (圖二)及glycyrrhizin (圖三)。選擇此三種成分爲指標成分乃因其各爲芍藥、梔子、甘草之主要療效成分[8－11]，水可溶，於水溶液及醇水溶液中均相當穩定[3,12,13]；再者三者在紫外光波長254nm處均有吸收。[1－7]假如能以同一分析條件同時分離三指標成分爲最理想，否則亦希望能以最簡單之分析條件作最快速之分離及定量，以因應例行性檢驗工作之方便性。本實驗將利用探討之分析方法對市售荆芥連翹湯製劑予以分析，以確定其適用性。並探討經減壓濃縮、冷凍乾燥、噴霧乾燥等不同製程之濃縮製品，其paeoniflorin，geniposide及glycyrrhizin之含量差異，以了解中藥濃縮製劑不同濃縮製程其成分含量是否因而有所影響。

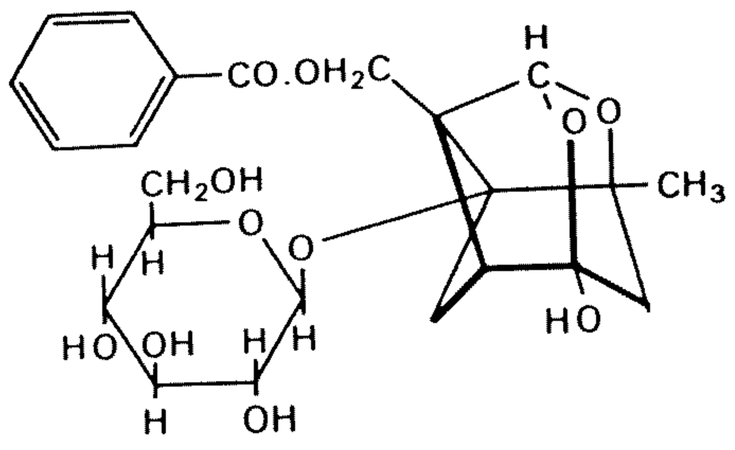

Figure 1. Structure of paeoniflorin

材料與方法

一、材料

㈠處方及原料生藥

荆芥連翹湯：當歸1.5g、芍藥1.5g、川芎1.5g、黃芩1.5g、梔子1.5g、連翹1.5g、防風1.5g、荆芥1.5g、枳殼1.5g、桔梗2.0g、白芷2.0g、柴胡2.0g、甘草1.5g、地

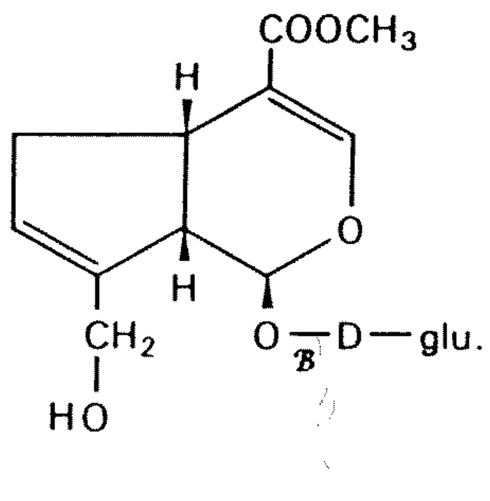

Figure 2. Structure of geniposide

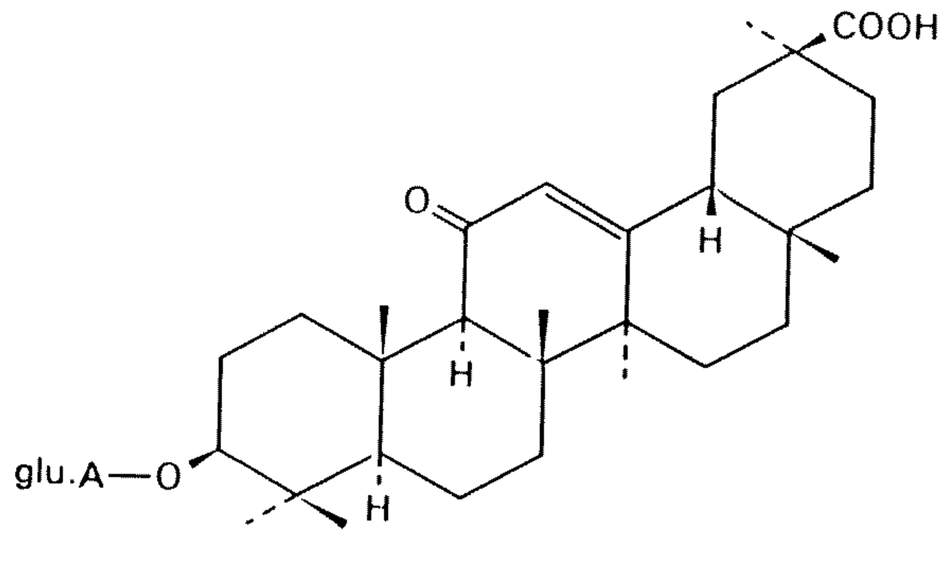

Figure 3. Structure of glycyrrhizin

黃1.5g、黃連1.5g、黃柏1.5g、薄荷1.5g。

㈡市售濃縮製劑：含芍藥、梔子、甘草之荊芥連翹湯(三家不同廠商產品)

㈢標準品、內部標準品與溶媒：

1.對照用標準品：paeoniflorin, geniposide及glycyrrhizin(購自日本半井公司)

2.內部標準品：p-hydroxybenzoic acid methyl ester (methylparaben), p-hydroxybenzoic acid butyl ester (butylparaben)(購自美國Sigma公司)

3.溶媒：乙腈、甲醇(LC級，Labscan limited)醋酸(LC級，皓峰)

二、實驗方法

㈠標準品溶液之配製

分別精確稱取paeoniflorin，geniposide，glycyrrhizin對照用標準品5 mg，以適量之70%甲醇溶解後，再定容至10 ml，即爲paeoniflorin，geniposide，glycyrrhizin標準溶液。

㈡內部標準品溶液之配製

分別精確稱取methlparaben，butylparaben對照用標準品10 mg，以適量之70%甲醇溶解後，再定容至100 ml，即爲methylparaben，butylparaben內部標準溶液。

㈢檢量線之製作

1.paeoniflorin及geniposide

精確量取二.㈠及二.㈡之paeoniflorin，geniposide標準溶液及methylparaben內部標準溶液適量，以70%甲醇稀釋調配成一系列濃度之溶液依序爲：paeoniflorin 0.025，0.050，0.100，0.200 mg/ml; geniposide 0.020，0.040，0.060，0.100 mg/ml及methylparaben 0.030 mg/ml，分別取上述不同濃度之標準溶液10 μl注入高效液相層析儀分析。以各標準品與內部標準品波峰面積比爲X軸，標準品之濃度爲Y軸，作圖並求出檢量線之線性迴歸方程式(Y＝mX＋b)及相關係數。

2.glycyrrhizin

精確量取二.㈠及二.㈡之glycyrrhizin標準溶液及butylparaben內部標準溶液適量，以70%甲醇稀釋調配成一系列濃度之溶液依序爲：glycyrrhizin 0.016，0.040，0.100，0.150 mg/ml及butylparaben 0.025 mg/ml，分別取上述不同濃度之標準溶液10 μl注入高效液相層析儀分析。檢量線之製作與上述1.同。

㈣高效液相層析法

1.高效液相層析系統

溶媒輸送系統：WATERS 510 HPLC Pump.

紫外線檢出器：SHIMADZU SPD-M6A Detector.

數據處理系統：訊華實驗室數據處理系統

記錄器：EPSON LQ-1000

2.高效液相層析條件

(1)荊芥連翹湯中芍藥之paeoniflorin及梔子之geniposide分析條件爲：

層析管：GL Sciences INC. Inertsil 5 ODS-2, 250 mm × 4.6 mm I.D. 5 μm

移動相：水：乙腈(85:15)

注入量：10 μl

流速：1.0 ml/min

檢測波長：UV 254nm

(2)荊芥連翹湯中甘草之glycyrrhizin分析條件爲：

層析管：Merck 50734 Lichrospher 100 RP-18 125 mm × 4 mm I.D. 5 μm

移動相：1%醋酸：乙腈(65:35)

注入量：10 μl

流速：1.0 ml/min

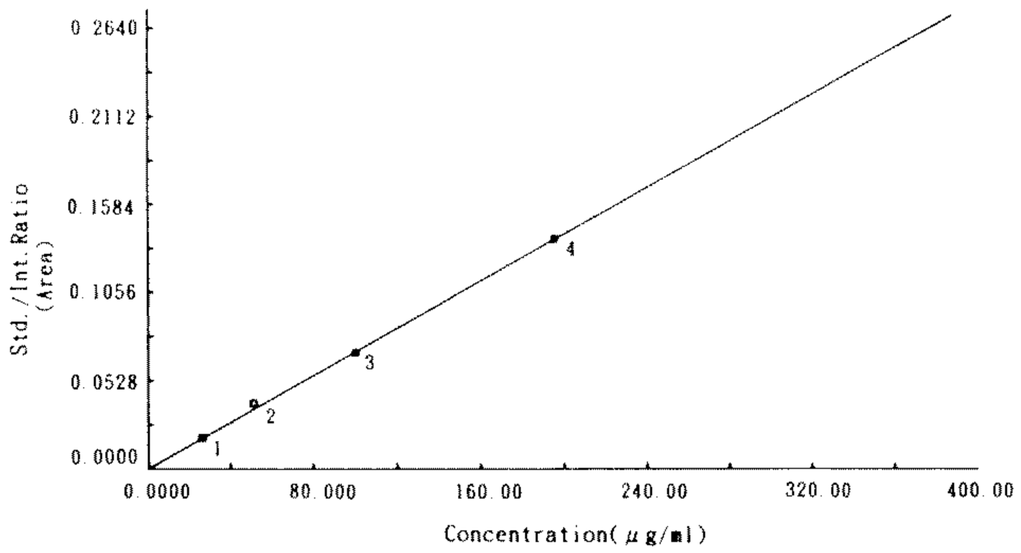

Figure 4. Calibration curve of paeoniflorin

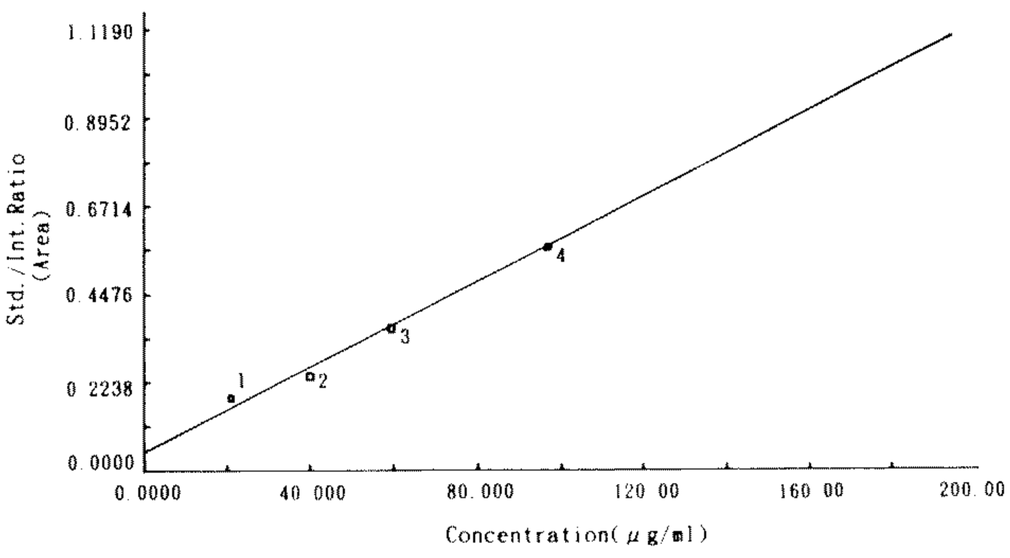

Figure 5. Calibration curve of geniposide

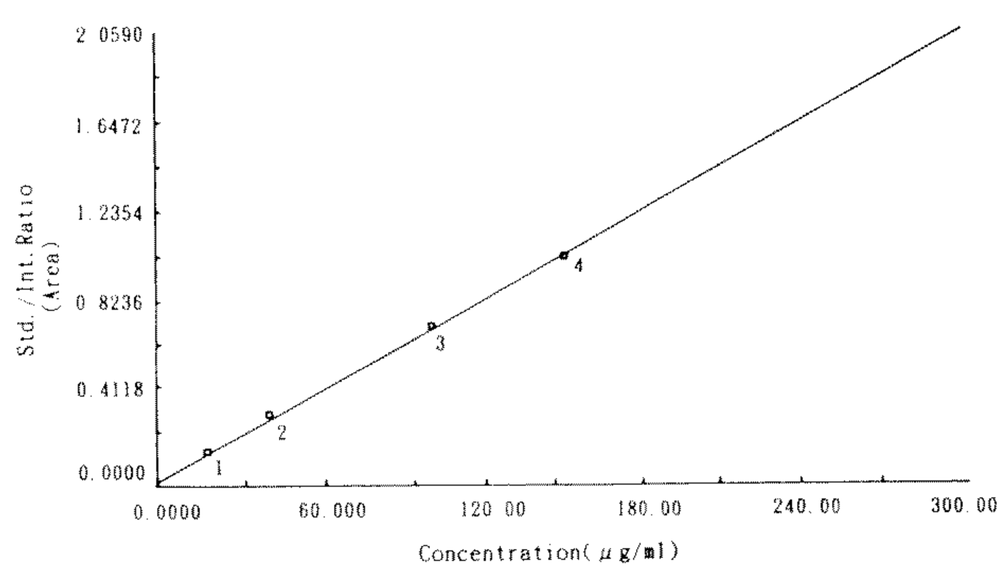

Figure 6. Calibration curve of glycyrrhizin

檢測波長：UV 254nm

(五)檢品溶液之調製與測定

1.荊芥連翹湯標準湯劑檢液之調製

稱取一(一)所列處方之一日量，加二十倍量的水，煮沸三十分鐘以上，至煎煮液爲原加水量之一半，用兩層紗布趁熱過濾，取濾液配製成70%甲醇溶液，定容至50 ml，並加入內部標準品溶液，使其含0.030 mg/ml methylparaben或含0.025 mg/ml butylparaben之溶液，供作檢液。

2.荊芥連翹湯標準劑空白對照檢液之調製

(1)芍藥、梔子：

稱取一(一)所列處方，但不含芍藥、梔子兩味之一日量藥材，加二十倍量的水，煮沸三十分鐘以上，至煎煮液爲原加水量之一半，用兩層紗布趁熱過濾，取濾液配製成70%甲醇溶液，定容至50 ml，並加入內部標準品溶液，使其含0.030 mg/ml methylparaben供作檢液。

(2)甘草：

稱取一(一)所列處方，但不含甘草之一日量藥材，加二十倍量的水，煮沸三十分鐘以上，至煎煮液爲原加水量之一半，用兩層紗布趁熱過濾，取濾液配製成70%甲醇溶液，定容至50 ml，並加入內部標準溶液使其含butylparaben爲0.025 mg/ml供作檢液。

3.荊芥連翹湯市售製劑檢液之調製

取三家不同廠牌之濃縮製劑1日量，各加70%甲醇35 ml，以超音波振盪器抽取30分鐘後過濾，以70%甲醇定容至50 ml，在定容過程中同時加入內部標準溶液，以下列不同方法配製：

(1)荊芥連翹湯：加入methylparaben內部標準溶液，使其含量爲0.030 mg/ml。

(2)荊芥連翹湯：加入butylparaben內部標準溶液，使其含量爲0.025 mg/ml。

4.荊芥連翹湯市售製劑添加paeoniflorin, geniposide及glycyrrhizin之檢液調製：取一家市售製劑檢品一日量，加70%甲醇65 ml，置於超音波振盪器抽提30分鐘後過濾，以70%甲醇定容至100 ml。

(1)paeoniflorin之添加：精確稱定paeoniflorin對照用標準品0.4 mg, 0.8 mg, 1.6 mg, 2.5 mg, 3.0 mg分別置於適當容量瓶中，加入適量之上述濾液，定容至適量，定容過程中同時加入methylparaben內部標準溶液，使其含量爲0.030 mg/ml。

(2)geniposide之添加：精確稱定geniposide對照用標準品2.0 mg, 3.0 mg, 3.5 mg, 4.0 mg, 5.0 mg，同4.(1)法調製之。

(3)glycyrrhizin之添加：精確稱定glycyrrhizin對照用標準品1.0 mg, 2.0 mg, 2.5 mg, 3.0 mg, 3.5 mg，同4.(1)法調製之。使butylparaben內部標準溶液含量爲0.025 mg/ml。

5.荊芥連翹湯不同濃縮製程產品之檢液調製：

分別取(五)1.法自行調製標準湯製適量，分別以冷凍乾燥、噴霧乾燥及減壓濃縮法濃縮後再依(五)4.市售製劑檢液調製法調製。

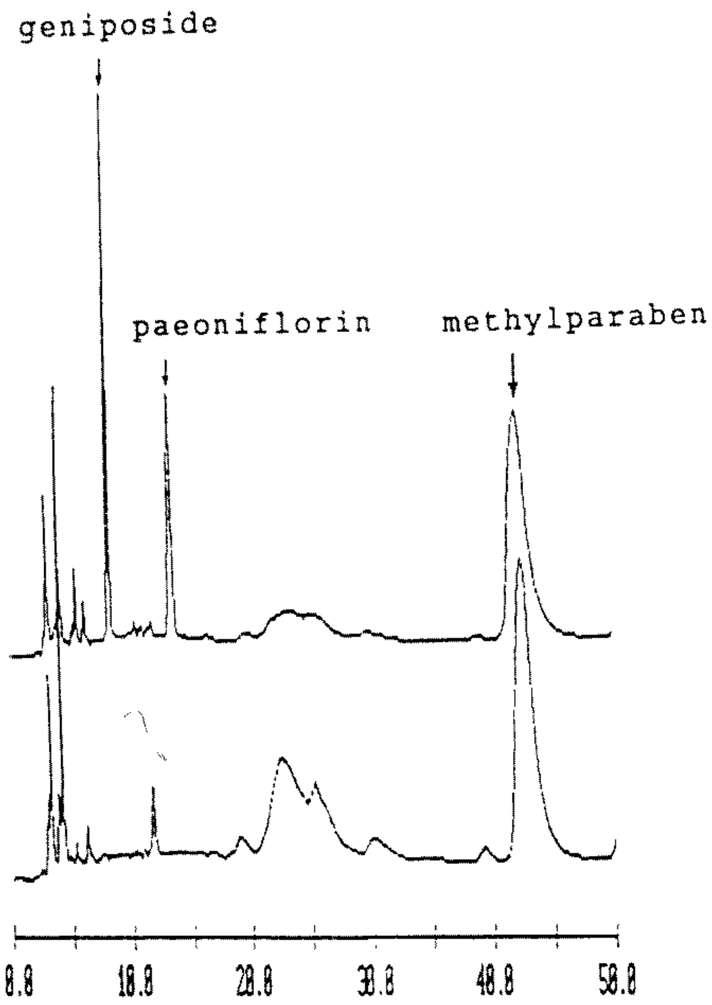

Figure 7. Chromatograms of Jing-Jieh-Lian-Chyau-Tong and Jing-Jieh-Lian-Chyau-Tong without Paeoniae Radix and Gardeniae Fructus.

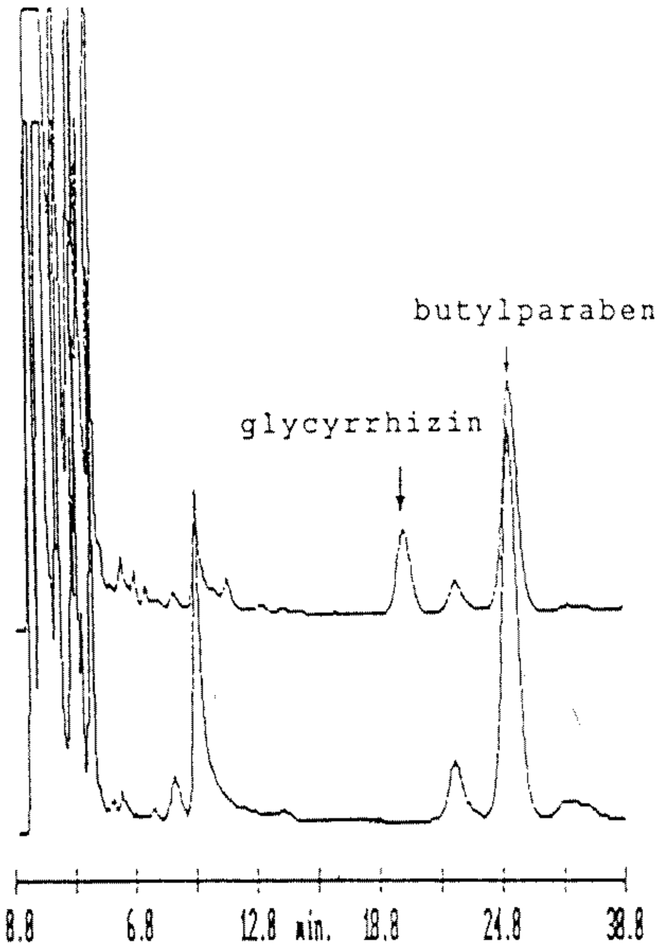

Figure 8. Chromatograms of Jing-Jieh-Lian-Chyau-Tong and Jing-Jieh-Lian-Chyau-Tong without Glycyrrhizae Radix.

6.指標成分之測定:取以上待測溶液適量,經微孔過濾器(孔徑約0.45μm)過濾,取此濾液依二㈣2.高效液相層析條件測定,並由檢量線求得各指標成分之含量。

結果與討論

一、檢量線

以paeoniflorin,geniposide及glycyrrhizin對照用標準品與內部標準品波峰面積比爲X軸,標準品濃度爲Y軸,作圖求出之檢量線如圖四、五、六。線性迴歸方程式及相關係數(r)分別爲:

paeoniflorin Y＝1.522X-1.082E-3,(r＝0.9997)
geniposide Y＝0.197X-1.023E-2,(r＝0.9957)
glycyrrhizin Y＝0.147X-2.080E-3,(r＝0.9999)

顯示paeoniflorin在濃度0.025 mg/ml至0.200 mg/ml範圍內,geniposide濃度在0.02 mg/ml至0.10 mg/ml範圍內及glycyrrhizin在濃度0.016 mg/ml至0.150 mg/ml範圍內均呈良好之線性關係。

二、標準湯劑中指標成分之分析

本研究首先嘗試能否以一分析條件同時分離三種成分,結果發現僅paeoniflorin及geniposide可在相同條件下被分離,而甘草之glycyrrhizin則因極性與其它成分相差甚多,移動相溶媒配比及層析管柱均不同,乃另法分離。以實驗方法二.㈣.(2).1.所列單液法同時進行荆芥連翹湯之自配標準湯劑中paeoniflorin,geiposide之分析及二.㈣.2.(2)所列方法分析glycyrrhizin,兩者之高效液相層析圖譜如圖七、八所示,其滯留時間分別約爲8分、14分、19分。且與空白對照湯劑比對,同一滯留時間處無其他干擾成分出現,並以3D-HPLC檢視後,確認三者均爲單一波峰,且methylparaben及butylparaben內部標準品出現處亦無其他干擾之波峰。

三、市售製劑之分析

同上述之分析條件,分析市售三家廠商出品之荆芥連翹湯之濃縮製劑,探討本方法的適用性,其結果如圖九所示,三種不同廠牌之濃縮製劑各於約8分、14分、19分出現與標準湯劑相同之波峰,顯示以標準湯劑之分析方法應用於市售濃縮製劑雖三種市售製劑其藥材組成與分量與自配標準湯劑不盡相同,但三種指標成分出現處均無干擾。分析標準湯劑及市售製劑中paeoniflorin,geniposide,gly-

cyrrhizin成分，並計算其含量結果，如表1所示。各成分之標準偏差值(S.D.)及變異係數值(C.V.)均在2%以下。以本分析法作爲市售荆芥連翹湯中paeoniflorin，geniposide及glycyrrhizin成分之品質評價應尚屬簡便且顯示良好之精確性。市售產品其三種指標成分含量與本局自配標準湯劑有所差異，另不同廠牌間之差異頗大，A、B兩廠之paeoniflorin，geniposide含量相近，而B廠之glycyrrhizin含量較低僅爲C廠之22%，C廠則三種指標成分含量均高於其他兩廠，此差異可能由於使用之藥材與製程不同所致。

四、添加回收實驗

以一系列量之指標成分對照用標準品添加至市售濃縮製劑中，測其添加回收率，其結果如表2所示，paeoniflorin，geniposide，glycyrrhizin三者分別爲100.01%、99.57%及99.88%，各成分所測得值之標準偏差值及變異係數值均在1%以下，顯示本方法具有良好之精確度。

五、各種不同濃縮方法對指標成分之影響

探討荆芥連翹湯經不同濃縮處理方式，對製劑中指標成分含量之影響，將標準湯劑分別以減壓濃縮法(1)、冷凍乾燥法(2)、噴霧乾燥法(3)等製備成濃縮粉末或浸膏，再予以分析其指標成分，結果如表3所示。(1)、(2)、(3)法與標準湯劑其平均含量相差分別爲 paeoniflorin:-5.3%，-6.8%，-1.0%；geniposide: -0.4%，-1.2%，-0.9%; glycyrrhizin: -7.3%。-1.8%，-1.2%。由以上數據顯示經不同濃縮方法濃縮，各成分有少許之流失但並不顯著，其原因可能係一旦經濃縮爲浸膏再加溶媒，並不完全溶解所致，以上可作爲中藥濃縮製劑製程之參考。

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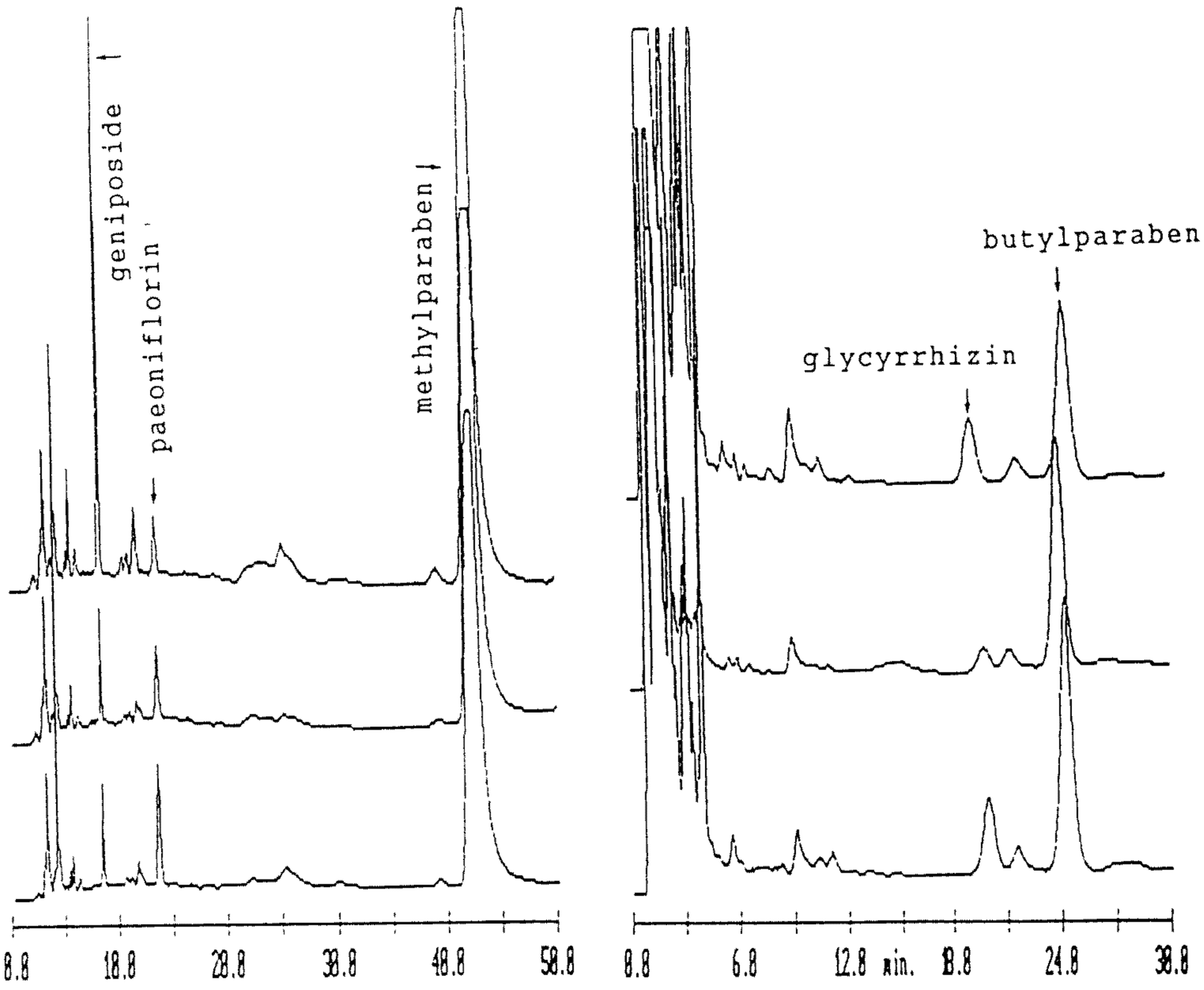

Figure 9. Chromatograms of three different commercial preparations of Jing-Jieh-Lian-Chyau-Tong.

Table 1. Contents of three marker substances in standard decoction of Jing-Jieh-Lian-Chyau-Tong and three different commercial preparations.

marker substance	paeoniflorin		geniposide		glycyrrhizin	
content / sample	mean±S.D.* (mg/g)	C.V.(%)	mean±S.D.* (mg/g)	C.V.(%)	mean±S.D.* (mg/g)	C.V.(%)
standard decoction	21.88±0.24	1.11	49.60±0.31	0.61	31.29±0.26	0.83
commercial preparation (A)	16.08±0.08	0.49	18.06±0.30	1.62	26.95±0.15	0.55
commercial preparation (B)	14.27±0.10	0.70	16.01±0.14	0.84	7.46±0.14	1.88
commercial preparation (C)	31.72±0.10	0.31	76.05±0.18	0.23	34.43±0.09	0.26

*n=3

Table 2. Recoveries of paeoniflorin、geniposide and glycyrrhizin in Jing-Jieh-Lian-Chyau-Tong

marker substance	amount of added(mg)	amount of recovery(mg)	No. of injection	percentage of recovery(%)	mean±S.D.	C.V.(%)
paeonifiorin	0.402	0.403	3	100.24	100.01±0.26	0.25
	0.804	0.801	3	99.63		
	1.616	1.618	3	100.12		
	2.500	2.505	3	100.20		
	3.000	2.996	3	99.87		
geniposide	2.005	2.005	3	100.00	99.57±0.73	0.73
	3.008	3.016	3	99.93		
	3.509	3.515	3	100.17		
	4.010	3.985	3	99.38		
	5.013	4.932	3	98.38		
glycyrrhizin	1.002	1.004	3	100.20	99.88±0.54	0.54
	2.004	1.990	3	99.30		
	2.505	2.488	3	99.32		
	3.006	3.021	3	100.50		
	3.507	3.510	3	100.09		

Table 3. Comparison between the contents of the three marker substances in Jing-Jieh-Lian-Chyau-Tong with different concentration methods.

marker substance	paeoniflorin		geniposide		glycyrrhizin	
content / sample	mean±S.D.* (mg/g)	C.V.(%)	mean±S.D.* (mg/g)	C.V.(%)	mean±S.D.* (mg/g)	C.V.(%)
decoction without concentration	21.88±0.23	1.04	49.60±0.33	0.66	31.29±0.14	0.43
concentration under reduced pressure (45°C)	20.72±0.14	0.68	49.39±0.31	0.62	29.01±0.29	0.78
lyophilization	20.39±0.20	0.99	49.02±0.29	0.59	30.72±0.26	0.85
spray drying	21.67±0.25	1.15	49.14±0.22	0.44	30.91±0.19	0.62

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Quantitative Analysis of Paeoniflorin, Geniposide and Glycyrrhizin in Jing-Jieh-Lian-Chyau-Tong by High Performance Liquid Chromatography

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ABSTRACT

High Performance Liquid Chromatographic methods for determination of paeoniflorin in Pasoniae Radix, geniposide in Gardenia Radix and glycyrrhizin in Glycyrrhizae Radix were developed for the quality control of traditional Chinese medicinal prescriptions Jing-Jieh-Lian-Chyau-Tong. Samples were extracted with 70% methanol by ultrasonic shaking for 30 minutes. The extract was separated by Inertsil 5 ODS-2 column with water-acetonitrile (85:15) as the mobile phase at a flow-rate of 1.0 ml/min. for paeoniflorin and geniposide, while for glycyrrhizin, it was separated by Lichrospher 100 RP-18 column with 1% acetic acid-acetonitrile (65:35) as the mobile phase at a flow-rate of 1.0 ml/min. The detecting wavelength was 254 nm with methylparaben and butylparaben used as the internal standards respectively. The calibration curves were linear in the range of 0.025-0.200 mg/ml for paeoniflorin; 0.02-0.10 mg/ml for geniposide and 0.016-0.150 mg/ml for glycyrrhizin. The recovery for each constituent from this prescription was 100.0% for paeoniflorin; 99.6% for geniposide and 99.9% for glycyrrhizin and the coefficient of variation was lower than 1.0%.

The contents of these preparation constituents were not affected by treatment with concentration under reduced pressure, spray-drying or lyophilization respectively. The difference between the highest and lowest content of paeoniflorin, geniposide and glycyrrhizin were 4.2, 4.8 and 4.6-fold respectively.

Key Words : Paeoniflorin, Geniposide, Glycyrrhizin, HPLC.