

利用高效液相層析法篩檢中藥製劑中摻加 之風濕鎮痛類西藥成分

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摘 要

本研究利用附光二極體陣列檢出器之高效液相層析儀篩檢十七種經常於風濕鎮痛類中藥製劑中檢出之西藥成分,包括十種非類固醇類鎮痛消炎劑,acetaminophen, aminopyrine, buctin, ethoxybenzamide, indomethacin, ketoprofen, mefenamic acid, phenylbutazone, piroxicam及salicylamide;二種類固醇,dexamethasone及prednisolone;二種肌肉鬆弛劑,chlormezanone及chlorzoxazone;利尿劑,hydrochlorothiazide;鎮靜安眠劑,diazepam;中樞神經興奮劑,caffeine等六類西藥成分。本法使用C18逆相層析管柱,移動相為乙腈及0.1%醋酸,利用梯度沖提,分析上述成分,效果良好,優於薄層層析法。

本研究分析82年8月至9月,由地方衛生局消費者服務中心送驗之風濕鎮痛中藥製劑三種sample A、B及C,結果sample A檢出mefenamic acid, hydrochlorothiazide, chlorzoxazone, diazepam及caffeine西藥成分。sample B檢出mefenamic acid, indomethacin, salicylamide及hydrochlorothiazide西藥成分。sample C檢出acetaminophen, indomethacin, ethoxybenzamide, hydrochlorothiazide, chlorzoxazone, diazepam及caffeine西藥成分。

前 言

中藥製劑摻加西藥之檢驗本局向極重視,為本局檢驗之重點工作。同時亦藉行政單位之積極追蹤取締,以及藥事法⁽¹⁾規定對於偽藥案件處以七年以下有期徒刑之重刑外,於判決確定時其工廠登記證以及其所持所有之藥品許可證均予以註銷,罰則甚重,由近年來檢驗結果顯示⁽²⁾,合法藥廠產製之包裝產品已甚少檢出摻加核准外之西藥成分。惟非藥廠產製之中藥,即一般坊間之不肖中醫診所、中藥房、非醫療專業人員、國術館、青草店、地攤等供應之中藥,則時有檢出摻加西藥成分之情形。

行政院衛生署為保護消費者及用藥安全之考慮,中藥摻加西藥在台灣地區均未被允許,故檢驗上只限於定性,即檢出與否即可。為因應龐大之檢

體數量常採薄層層析法及紫外光分光光譜法^(3,4)進行篩檢,惟此項檢驗之檢出對象雖為西藥,但檢體之基質均為中藥,然中藥之成分複雜以及遇有摻加多種西藥成分,或構造式類似之同類成分,或摻加西藥成分之量甚少時,以薄層層析板短距離的展開,其分離效果仍屬有限,為此常需採用多種溶媒系,多次或多次不同方向展開,再刮取比對其最大最小之紫外光吸收圖譜而耗時。因此,探討一種可同時分析多種西藥成分之方法,對於篩檢該類製劑則更趨簡單迅速。

據文獻報告應用高效液相層析法分析多種非類固醇消炎鎮痛劑,如Kearns等⁽⁵⁾報告血液中ibuprofen等藥物之分析;Omile等⁽⁶⁾報告血液中indomethacin, ketoprofen, mefenamic acid, ibuprofen, acetylsalicylic acid, salicylic acid, naproxen, fenoprofen, flurbiprofen, diflunisal等十種成

分分析,橋本等⁽⁷⁾報告中藥製劑摻加 dexamethasone, acetaminophen, buctin, phenylbutazone 及 indomethacin 等西藥成分之定量。因此,如能以高效液相層析儀分離,並藉光二極體陣列檢出器予以確認是否屬單一成分應屬可行。

本研究就治療風濕鎮痛類中藥選擇平素檢出頻率較高之西藥為對象加以探討,利用附光二極體陣列檢出器之高效液相層析儀篩檢十七種經常於風濕鎮痛類中藥製劑中檢出之西藥成分,包括十種非類固醇類鎮痛消炎劑,acetaminophen, aminopyrine, buctin, ethoxybenzamide, indomethacin, ketoprofen, mefenamic acid, phenylbutazone, piroxicam 及 salicylamide; 二種類固醇, dexamethasone 及 prednisolone; 二種肌肉鬆弛劑, chlormezanone 及 chlorzoxazone; 利尿劑, hydrochlorothiazide; 鎮靜安眠劑, diazepam; 中樞神經興奮劑, caffeine 等六類西藥成分。

材料與方法

一、檢體

82年8月至9月,由桃園縣,台北市及彰化縣衛生局消費者服務中心送驗治療風濕鎮痛類之未知名中藥丸三種,代號分別為sample A、B及C。

二、試藥

acetaminophen, buctin, caffeine, chlorzoxazone, hydrochlorothiazide, indomethacin, ketoprofen, mefenamic acid, phenylbutazone, piroxicam 及 salicylamide 購自美國SIGMA公司; dexamethasone 及 prednisolone 購自日本NACALAI公司; aminopyrine, chlormezanone, diazepam 及 ethoxybenzamide 均為製劑原料,乙腈(LC級)購自愛爾蘭Labscan limited公司,醋酸購自皓峰公司,乙醇購自公賣局。

三、儀器

使用日立廠牌附光二極體陣列檢出器之高效液相層析儀(HITACHI L-6200 Intelligent Pump with HITACHI L-3000 Photo Diode Array Detector),所用條件如下:層析管為Cosmosil 5C18 AR 5 μ m, 15 cm $\times$ 4.6 mm I.D.,移動相為乙腈及水(含0.1%醋酸),以最初為含10%乙腈,最終為100%,30分鐘線性梯度沖提,流速為1.0 ml/min,

波長為235 nm。

四、方法

對照標準品配製:分別稱取適量之acetaminophen, aminopyrine, buctin, caffeine, chlormezanone, chlorzoxazone, dexamethasone, diazepam, ethoxybenzamide, hydrochlorothiazide, indomethacin, ketoprofen, mefenamic acid, phenylbutazone, prednisolone, piroxicam 及 salicylamide 約10 mg,加乙醇溶解並稀釋使成0.04 mg/ml。

檢品之調製:稱取中藥丸檢體約1g,剪碎,加少量水待崩散後,加乙醇25 ml經超音波振盪30分後過濾,濾液加乙醇稀釋供作檢液。

結果與討論

就本局以往受理主述治療風濕鎮痛類中藥製劑檢體,其所摻加之西藥成分種類,一般包括非類固醇類之鎮痛消炎劑如acetaminophen,利尿劑如hydrochlorothiazide,類固醇如prednisolone,肌肉鬆弛劑如chlorzoxazone,安眠鎮靜劑如diazepam

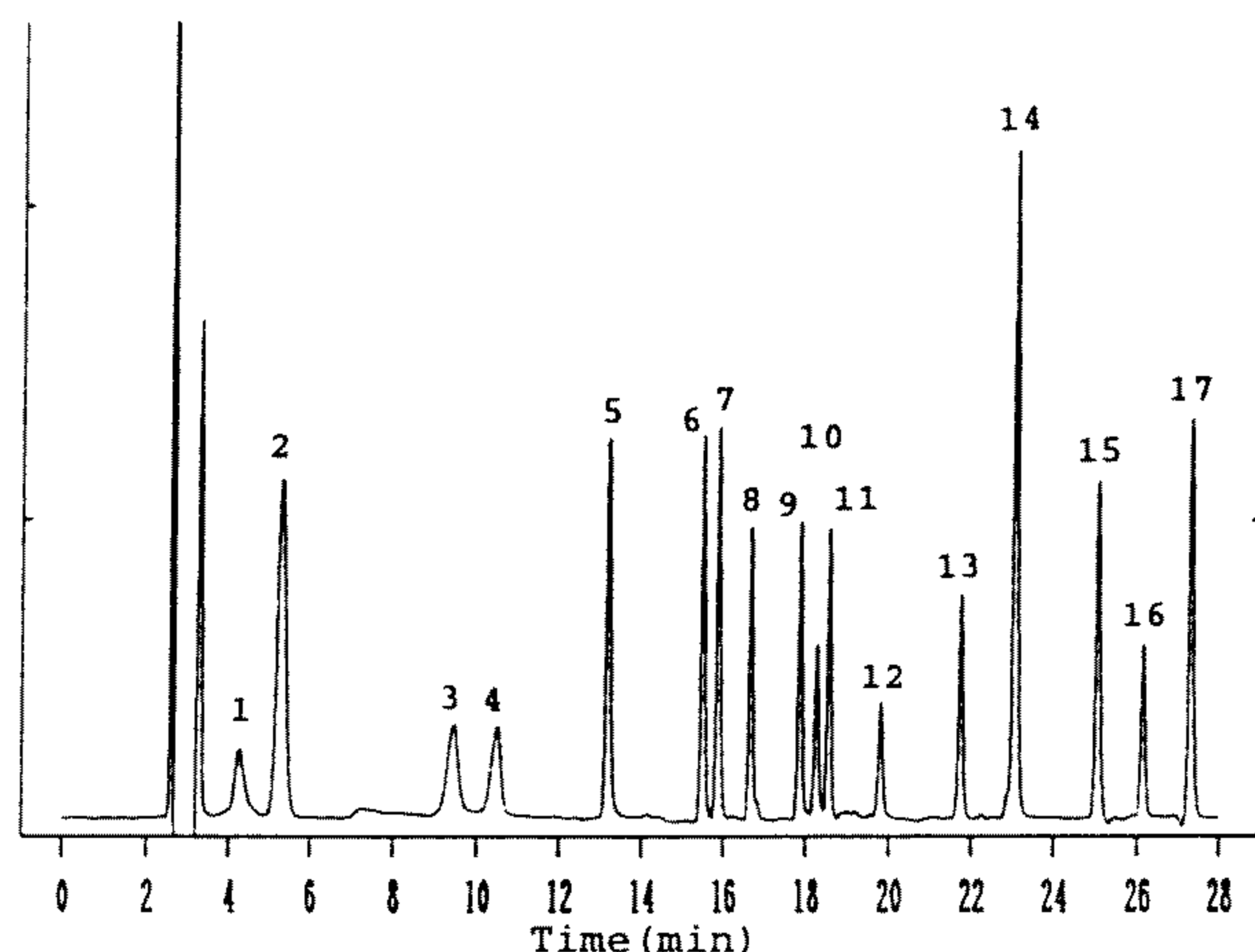


Figure 1. HPLC chromatogram obtained with a standard ethanolic solution containing a mixture of 17 synthetic drugs at detector wavelength of 235 nm. Each peak corresponds to 400 ng. Peak identities: (1) aminopyrine; (2) acetaminophen; (3) caffeine; (4) hydrochlorothiazide; (5) salicylamide; (6) buctin; (7) ethoxybenzamide; (8) prednisolone; (9) chlormezanone; (10) chlorzoxazone; (11) dexamethasone; (12) piroxicam; (13) ketoprofen; (14) diazepam; (15) indomethacin; (16) phenylbutazone; (17) mefenamic acid.

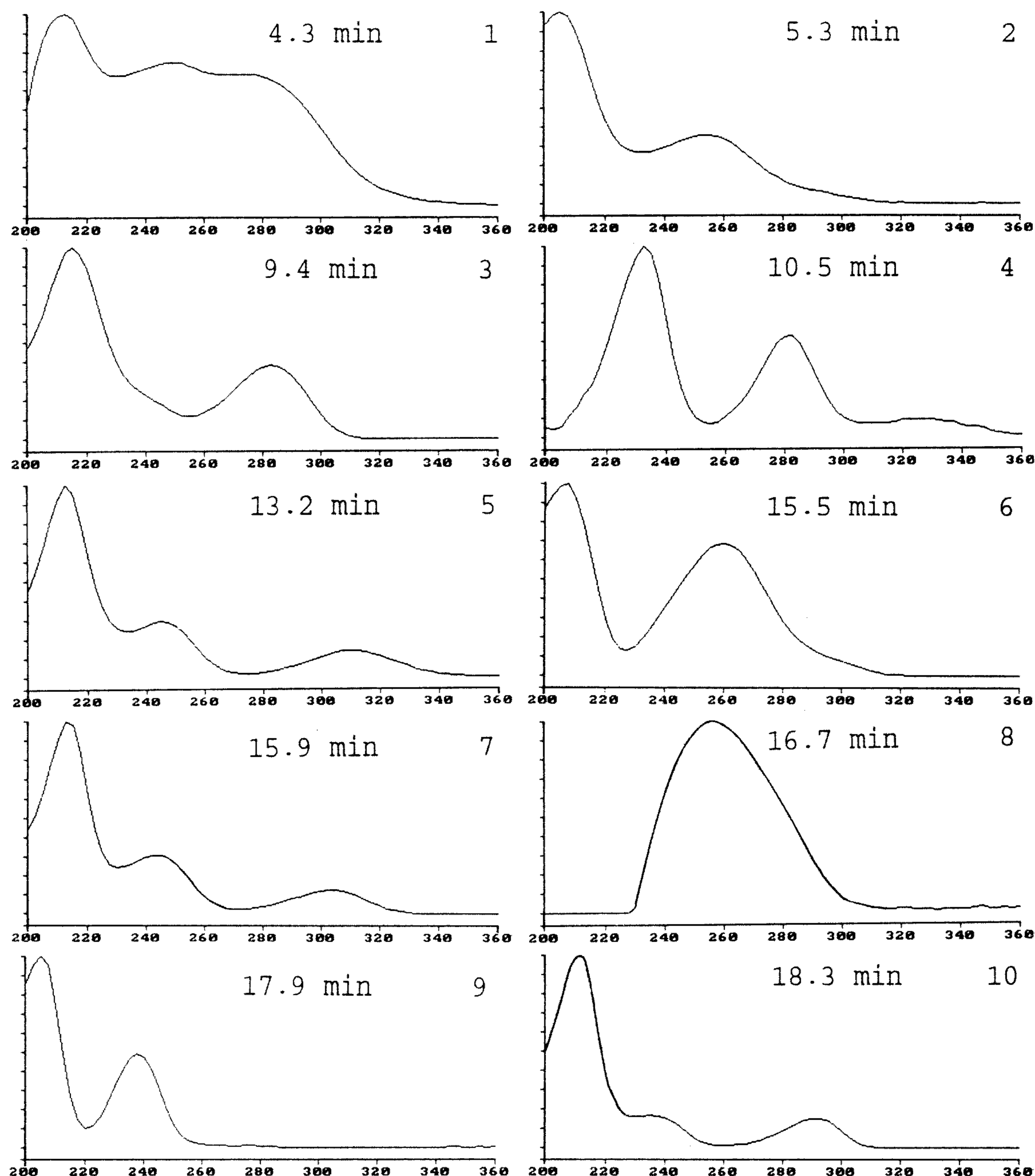


Figure 2. UV spectra of standards. Peak identity: same as Figure 1.

及中樞神經興奮劑如caffeine等六類成分。且通常一種檢體常摻加二類以上之西藥成分，甚有摻加七、八種西藥成分之情形。由於複方之中藥製劑本身即含有複雜之成分，因此單以薄層層析法展開後，用紫外光燈（波長254 nm及366 nm）與呈色劑偵測，實不易正確快速地篩檢是類中藥製劑中之西藥成分。

本研究所建立之高效液相層析法，分析17種西藥成分，較薄層層析法所費之分析時間短，不需如

薄層層析法經展開，刮取層析板上之成分後，再以紫外光分光光度器分析。選用之移動相溶媒組成單純，配製容易。選用之235 nm波長，篩檢之各成分波峰顯著，且輔以光二極體陣列檢出器檢視波峰之成分，即可快速篩檢中藥中之西藥成分。

一、對照標準品之分析

對照標準品溶液以具光二極體陣列檢出器之

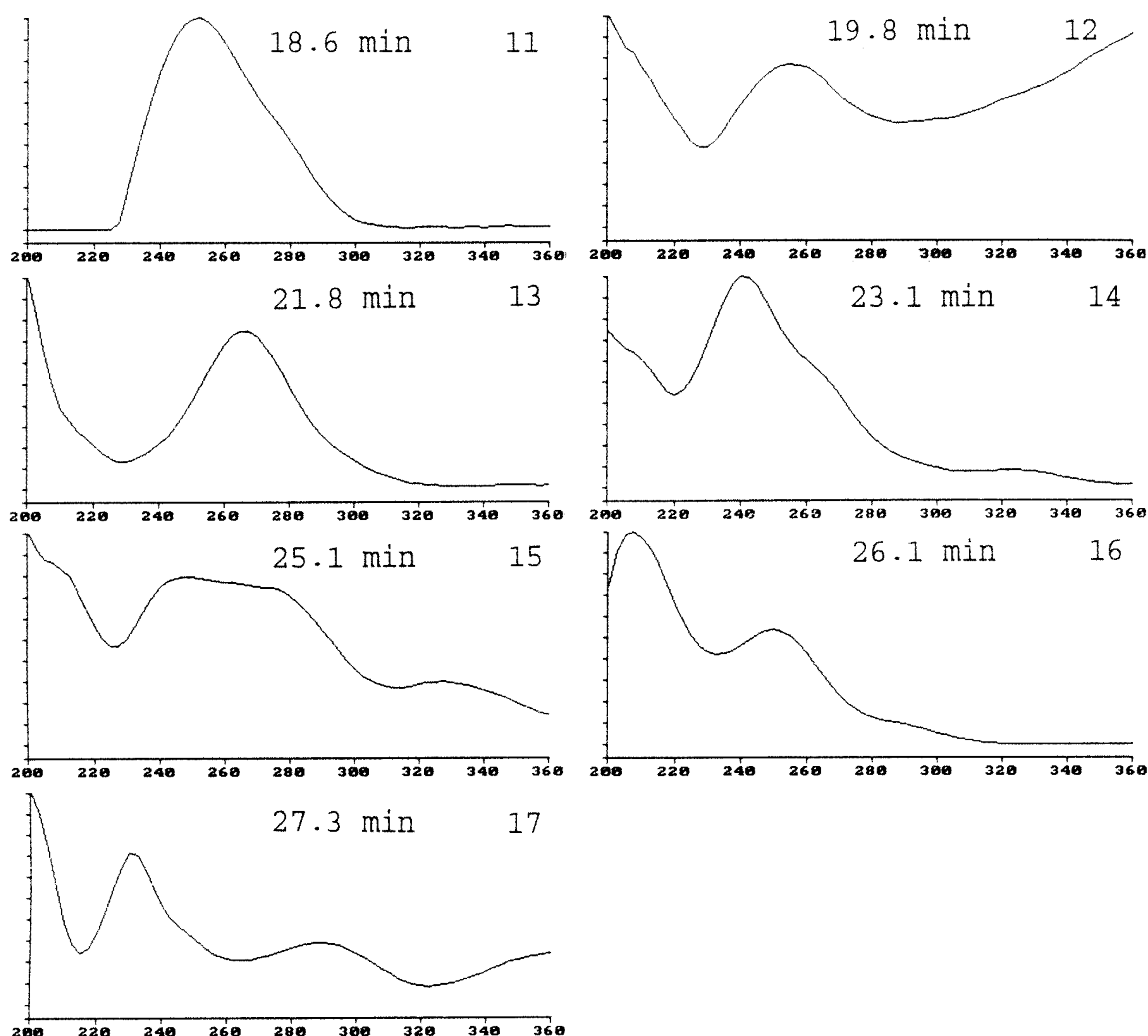


Figure 2. UV spectra of standards. Peak identity: same as Figure 1.

高效液相層析儀, 依材料與方法所載之條件分析, 其層析圖譜如Figure 1.所示, 各成分之紫外光譜如Figure 2.所示, 上述17種成分在30分鐘內即可分離, 各成分之滯留時間依序為aminopyrine: 4.3 min; acetaminophen: 5.3min; caffeine: 9.4min; hydrochlorothiazide: 10.5min; salicylamide: 13.2 min; buccetin: 15.5min; ethoxybenzamide: 15.9 min; prednisolone: 16.7min; chlormezanone: 17.9 min; chlorzoxazone: 18.3min; dexamethasone: 18.6min; piroxicam: 19.8min; ketoprofen: 21.8min; diazepam: 23.1min; indomethacin: 25.1min; phenylbutazone: 26.1min; mefenamic acid: 27.3min.

二、未知名中藥丸之分析

三種檢體之檢液以HPLC分析, 經由滯留時間及各波峰之紫外光譜圖比對, 結果sample A如Figure 3所示, 檢出 mefenamic acid, hydrochlorothiazide, chlorzoxazone, diazepam及caffeine; sample B如Figure 4所示, 檢出 mefenamic acid, indomethacin, salicylamide 及 hydrochlorothiazide; sample C如Figure 5所示, 檢出 acetaminophen, indomethacin, ethoxybenzamide, hydrochlorothiazide, chlorzoxazone, diazepam 及 caffeine。層析圖譜上之其他波峰均經紫外光二極體陣列檢出器檢視加以確認, 均非為本實驗探討之西藥成分。其中sample B及C均各含有三種鎮痛劑, sample A及C均含有藥理作用相反之中樞神經興奮劑caffeine及抑制劑diazepam, 三種檢體均檢

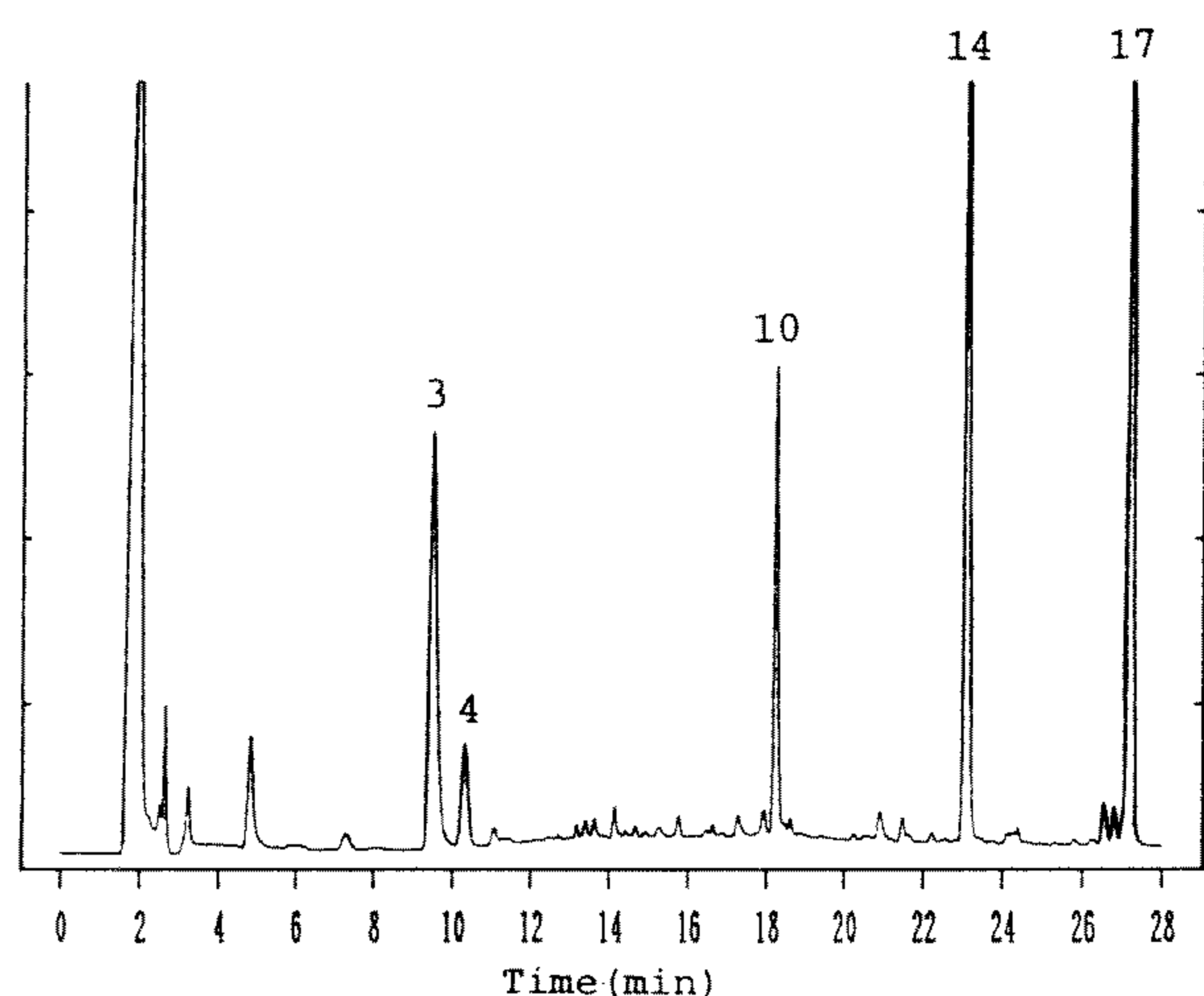


Figure 3. HPLC chromatogram of sample A. Peak identity: same as Figure 1.

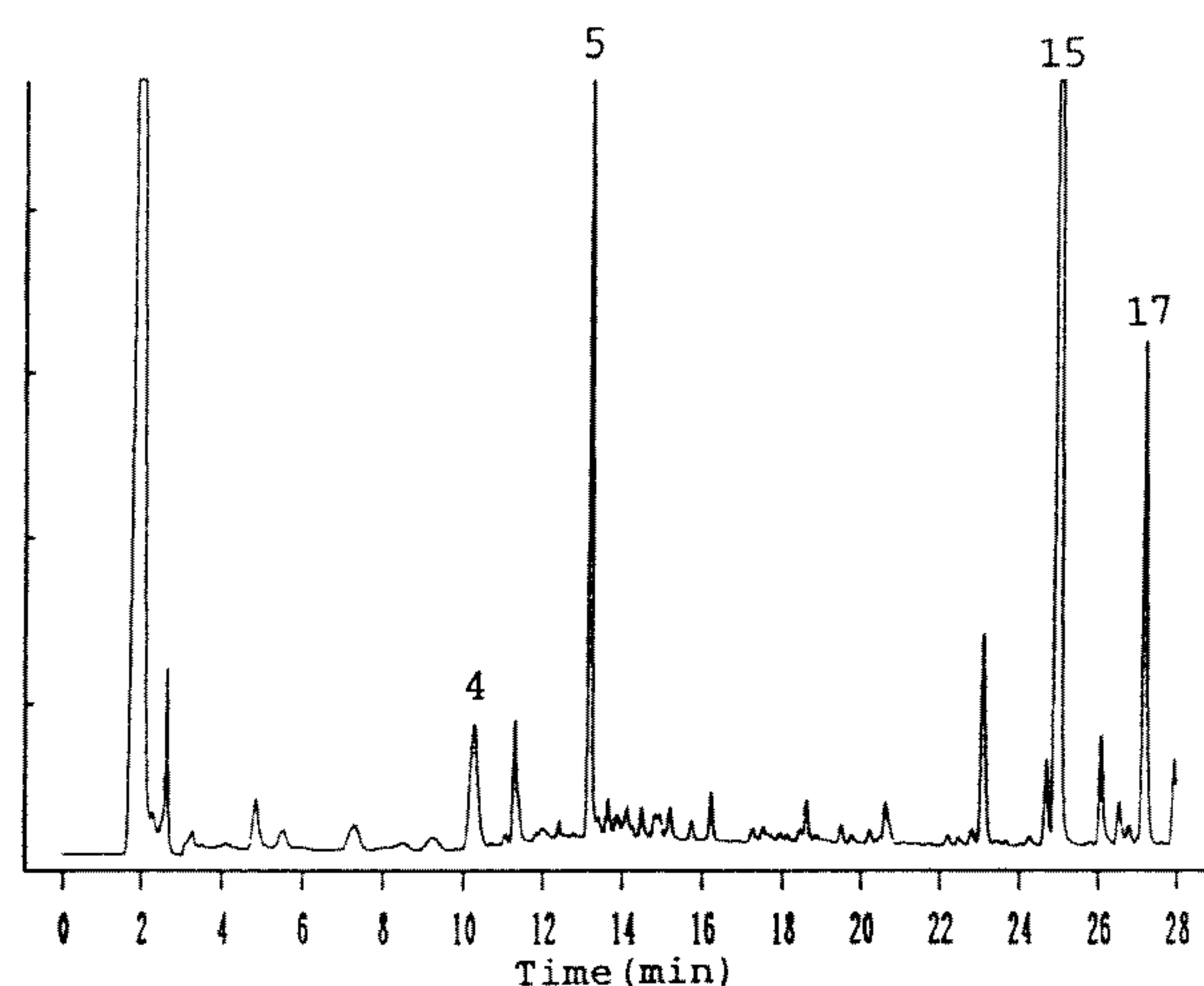


Figure 4. HPLC chromatogram of sample B. Peak identity: same as Figure 1.

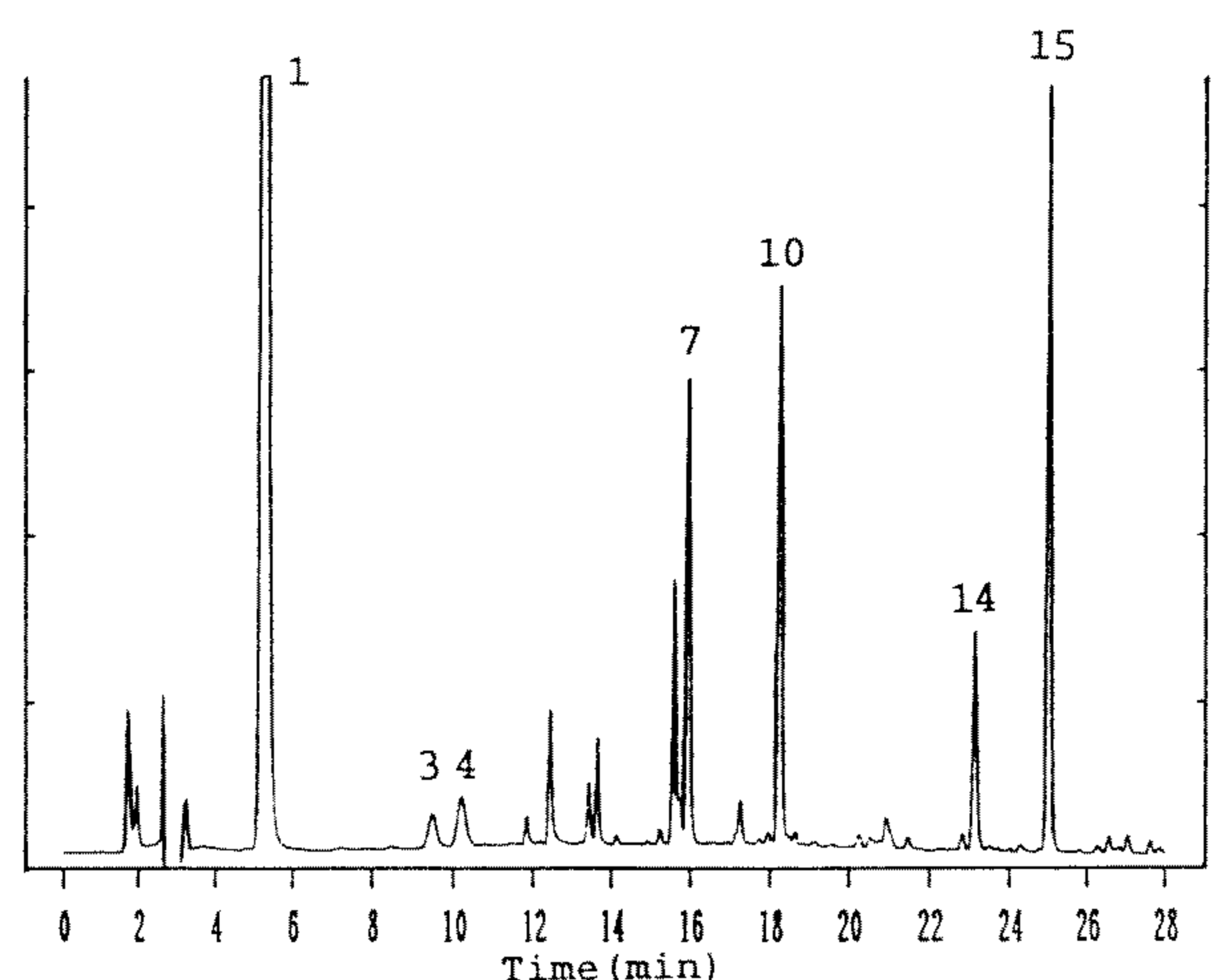


Figure 5. HPLC chromatogram of sample C. Peak identity: same as Figure 1.

出hydrochlorothiazide, 惟均未檢出類固醇成分。風濕鎮痛類中藥中摻加之西藥成分之篩檢, 一般使用乙酸乙酯: 氯仿(1:1)及乙醚: 乙酸乙酯(1:4)二種溶媒作展開溶媒之薄層層析法分離, 就sample A而言, diazepam及chlorzoxazone於紫外光燈254 nm下之吸光度小, 因此, 在薄層層析板上之色點不明顯, 需配合呈色劑呈色, 且diazepam因摻加量少(常用劑量為2-5 mg), 色點較不明顯, 與中藥成分亦有干擾, 常需用上述二種溶媒作二次不同方向展開。就sample B而言, salicylamide與mefenamic

acid以乙醚: 乙酸乙酯(1:4)展開, 二者色點重疊, Rf值分別為0.81及0.82, 需用乙酸乙酯: 氯仿(1:1)展開。就sample C而言, diazepam及chlorzoxazone, 亦有如sample A之情形; acetaminophen與indomethacin, 以乙醚: 乙酸乙酯(1:4)展開, 色點甚為接近, Rf值分別為0.52及0.55, indomethacin及caffeine, 以乙酸乙酯: 氯仿(1:1)展開, 色點甚為接近, Rf值分別為0.15及0.18; 因此, 欲分離acetaminophen需用乙酸乙酯: 氯仿(1:1), 欲分離caffeine需用乙醚: 乙酸乙酯(1:4), 而分離indomethacin, 需用二種溶媒, 作二次不同方向展開, 惟因acetaminophen之相對摻加量甚大, 其與indomethacin之分離更加困難。因此, 使用薄層層析法分析是類中藥, 經常遭遇上述種種困難。而本法使用C18逆相層析管柱, 以梯度沖提, 可一次分離十七種化性(極性)差異頗大之西藥成分, 移動相由最初10%乙腈(高極性)至100%乙腈(低極性), 可使層析管柱得到較快之條件化, 波長設定235 nm, 各成分之波峰明顯, 可以得到很好的分離效果, 應用於中藥製劑之分析, 結果亦佳。

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Screening Chemical Drugs Used to Adulterate in Rheumatic and Analgesic Traditional Chinese Medicine by HPLC-DAD

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ABSTRACT

High-performance liquid chromatography with photodiode array detector (DAD) was developed for the identification of adulterants in rheumatic and analgesic Chinese medicine. A study was performed by using a gradient elution with acetonitrile and 0.1% acetic acid solution on a C-18 column. This method was applied to simultaneously screen 17 synthetic drugs adulterated in traditional Chinese medicine within half an hour. The drugs belonged to six pharmacological categories: antipyretic analgesics, glucocorticoids, diuretics, CNS stimulants, muscle relaxants and sedatives. They included acetaminophen, aminopyrine, buccetin, ethoxybenzamide, indomethacin, ketoprofen, mefenamic acid, phenylbutazone, piroxicam, salicylamide, dexamethasone, prednisolone, chlormezanone, chlorzoxazone, hydrochlorothiazide, diazepam and

caffeine. This method can provide higher resolution and greater efficiency than thin-layer chromatography for screening adulterated synthetic drugs. Three traditional Chinese medicines sold by the dealer of Chinese natural drugs that were collected by the consumer centers of local health bureaus from August, 1993 to September, 1993 and all were examined by this method. Five synthetic drugs, mefenamic acid, hydrochlorothiazide, chlorzoxazone, diazepam and caffeine, four synthetic drugs, mefenamic acid, indomethacin, salicylamide and hydrochlorothiazide, and seven synthetic drugs, acetaminophen, indomethacin, ethoxybenzamide, hydrochlorothiazide, chlorzoxazone, diazepam and caffeine were found from Sample A, B and C, respectively.

Key Words : HPLC-DAD, Traditional Chinese medicine, Adulterant, Rheumatic and Analgesic.