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Solid-phase Extraction and High-performance Liquid Chromatographic Analysis of Prednisone Adulterated in a Foreign Herbal Medicine

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ABSTRACT

Prednisone was detected in a herbal medicine from Southeast Asia using thin layer chromatography (TLC) and ultraviolet spectroscopy (UV). After treating with solid-phase extraction (SPE), the sample was further assayed by high-performance liquid chromatography (HPLC). The sample solution was achieved with a silica gel cartridge conditioned with methanol and chloroform in sequence, and then eluted with dichloromethane-isopropanol (6:4, v/v). Separation was conducted with an Inertsil ODS-80A reversed-phase column using isocratic elution with acetonitrile and water (3:7, v/v) as mobile phase. Fludrocortisone acetate was used as an internal standard and detection wavelength was at 240 nm. Calibration curve of standard prednisone was constructed in the range of 10.0-200.0 μg/mL. The content of adulterated prednisone was 5.5 mg/21g sample.

Key words: HPLC, prednisone, solid-phase extraction, herbal medicine, adulterant

INTRODUCTION

Over the years, adulterants have been detected in some herbal medicines referred to our laboratories from various sources, and the results have been reported each year[1-4]. The first island-wide monitoring of prohibited adulteration of herbal medicines through hospital pharmacies, was carried out in 1992[5]. Steroids, including prednisolone, betamethasone and dexamethasone, were ranked as the 25 most frequent adulterants. Therefore, there is a strong demanded for detecting steroid adulterants in herbal medicines. Although during the past four years, the adulteration of steroids in herbal medicines has been rare[1-4], an herbal medicine named "JAMU JAWA ALSI", made in Banyumas, Indonesia, was recently found to contain prednisone. This herbal medicine is used to treat backaches, tooth pain, impotence, stress, physical weakness and apoplexy. For consumer's safety, we analyzed the adulterants in this medicine with solid-phase extraction (SPE) and high-performance liquid chromatography (HPLC).

SPE has recently become popular for sample preparation due to the advantages of fast speed, minimal amount of solvents, and a wide range selection of adsorbents. Our previous studies have established an SPE method for the pretreatment of adulterated TCM[6-8]. Furthermore, a combination of SPE and HPLC for the extraction and determination of eight steroids including betamethasone, cortisone acetate, dexamethasone, hydrocortisone acetate, methylprednisolone, prednisolone, prednisone and triamcinolone in herbal medicine[9] has been reported in previous studies and applied to this experiment to achieve good results.

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MATERIALS AND METHODS

I. *Reagents and Materials*

Prednisone and fludrocortisone acetate were purchased from Sigma (St. Louis, MO, USA). Acetonitrile, chloroform, dichloromethane and methanol from Labscan (Dublin, Ireland) were LC grade. Ultrapure distilled water with a resistance greater than 18 MΩ was used. Ethanol (Taiwan Tobacco & Wine Monopoly Bureau, ROC) was ChP. grade. The silica gel SPE cartridge was obtained from Varian (Harbor City, CA, USA).

The sample was a yellow-brown powder named "JAMU JAWA ALSI" and was obtained from Dr. Ching-Yao Chuang (Hwayo Tech & Lab Co., Ltd).

II. *TLC Screen Test*

A small portion of sample (1 g) was extracted with ethanol (4 mL). The extract was developed by TLC with 1 mm thickness silica gel plates ($60F_{254}$, E. Merck) and detected with UV lamp. A mixture of ethyl acetate and ether (4/1, v/v) was used as the mobile phase.

III. *UV Confirmation*

A spot of the sample corresponding to prednisone standard (R_f: 0.50) was scraped out from the TLC plate developed as above and dissolved in ethanol (3 mL). The ethanol solution was filtered and processed for UV scanning. Ultraviolet spectra were recorded on a Hitachi U- 3210 spectrophotometer.

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VI. *HPLC Apparatus and Conditions*

HPLC was conducted with a Hitachi L-6200 intelligent pump with a Hitachi L-3000 photodiode array detector and a Shimadzu SIL-9A auto-injector. An Inertsil ODS-80A (250 × 4.6 mm I.D.) column was used. The mobile phase consisted of acetonitrile and water (3:7, v/v). The flow rate was 1.2 mL/min and detection was carried out at 240 nm.

V. *Preparation of Standard Solutions*

An appropriate volume (0.5 mL) of internal standard solution (fludrocortisone acetate, 1.0 mg/mL) was added to an ethanol solution containing an accurate amount of prednisone to give various concentrations within the range of 10.0-200.0 μg/mL. A calibration graph was plotted subsequently to linear regression analysis of the peak area ratios versus concentration.

VI. *Preparation of Sample Extract*

A two gram sample of powder was accurately weighed and extracted with chloroform two times (15 and 10 mL, successively) at 30˚C, each for 30 min in an ultrasonic bath, filtered, and made to 25 mL with chloroform.

VII. *Sample Clean-up by SPE*

The sample solution (5 mL) was loaded onto a SPE column (SiOH, 500 mg, 3 mL column volume), which had been preconditioned before use by sequentially passing a series of solvents consisting of 5 mL of methanol and then 5 mL of chloroform, and eluted with chloroform-isopropanol (6: 4, v/v, 10 mL). The eluate was collected and concentrated under reduced pressure to dryness. Finally, the residue was dissolved in 8 mL of ethanol and transferred to a 10 mL volumetric flask to which 0.5 mL of fludrocortisone acetate solution (1.0 mg/mL) was added and made up to 10 mL with ethanol. This solution was filtered through a 0.45 μm membrane before HPLC analysis.

RESULTS AND DISCUSSION

Standard prednisone showed a dark spot at R_f values of 0.50 under UV 254 nm, sample showed a spot at R_f 0.50 corresponding to prednisone. Both spots of sample and standard prednisone displayed the same UV spectra and the same UV λ_{max} at 238 nm.

In this study, we used an SPE and HPLC combination method for the determination of prednisone adulterated in herbal medicine extract. Figure 1A shows a chromatogram of the sample extract after treatment with SPE, in which the retention times of prednisone and internal standard fludrocortisone acetate, were 6.3 and 25.8 min, respectively. Figure 1B shows a chromatogram of the sample extract without SPE treatment. Although the presence of interference in herbal medicine did not obviously interfere with the identification of prednisone, those interferences could be moved efficiently with SPE.

The content of prednisone was calculated with a calibration graph: peak-area ratio, y, vs. concentration, x, μg/mL was obtained over the range of 10.0-200.0 μg/mL for prednisone. The regression equation of the curve and its correlation coefficient (r) was calculated as: y = 0.025 x – 0.008 (r = 0.9999).

A known amount of prednisone was added to herbal medicine, and the overall recovery was estimated by the standard addition method. As shown in Table 1, the recovery of predisone is greater than 92%. The result was similar to a previous study[9]. There is good agreement between the theoretical and experimental prednisone concentrations.

From above, a 21.0 g sample powder of a pack was

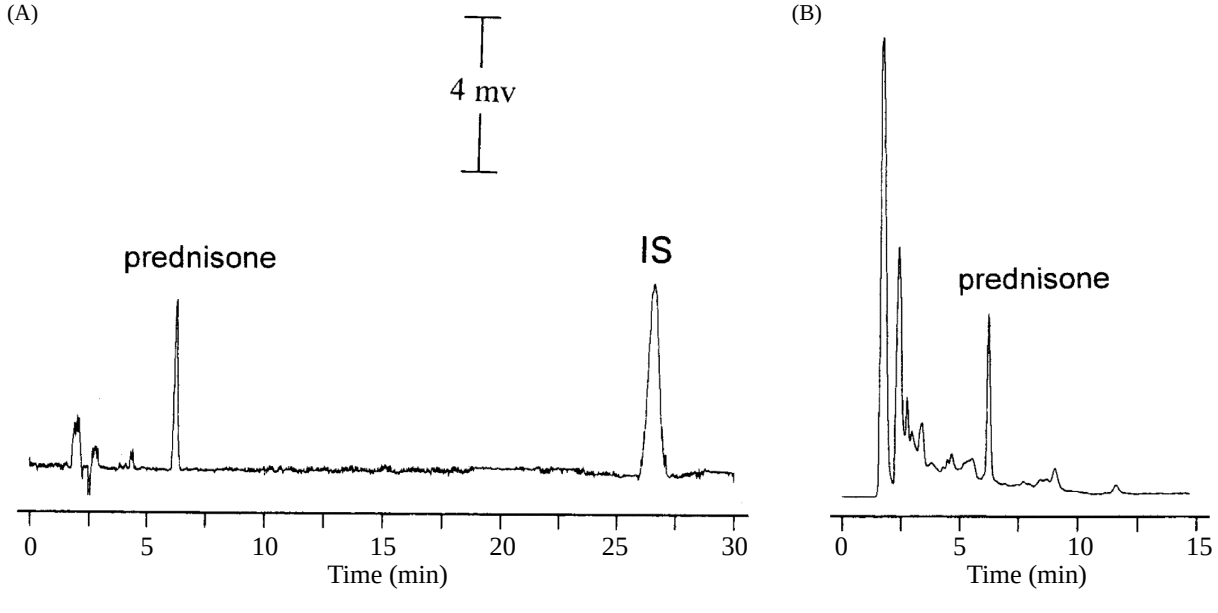

Figure 1. Chromatogram of herbal medicine (10.7 μg/mL) after (A) and before (B) SPE treatment. IS = fludrocortisone acetate.

Table 1. Recovery of prednisone added to herbal medicine

Prednisone (mg per 1.0 g)		
Added	Found	Recovery (%)
0	0.27	–
0.5	0.48	92.1
1.0	0.95	93.7
2.0	1.92	95.6

adulterated with 5.5 mg prednisone, and the content of prednisone in the sample was 0.027%. Because this dose is not massive, patients might unwittingly be hurt by the chemical drug, which was not labeled as part of the ingredients. Therefore, it is important to use a fast and efficient method to detect this adulterant which was first found in our study.

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中草藥中摻加Prednisone之固相萃取與高效液相層析定量分析

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摘　要

一種來自東南亞的中草藥檢體，以薄層層析及紫外分光光度計檢驗出含prednisone成分，經固相萃取管處理後，再以高效液相層析法定量。固相萃取管依序以甲醇及氯仿先活化，繼用二氯甲烷及異丙醇(6:4, v/v)沖提檢體。高效液相層析採用逆相層析管柱（Inertsil ODS-80A），沖提液為乙腈與水（3:7, v/v），檢測波長為240 nm，內部標準品為fludrocortisone acetate。Prednisone檢量線之濃度範圍為10.0-200.0 μg/mL。確效結果顯示精密度、準確度及回收率均良好。以上述方法定量分析檢出每包檢體（約21公克）含prednisone 5.5毫克。

關鍵詞：Prednisone，固相萃取，高效液相層析，中藥製劑，中藥摻加西藥之檢驗