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### Determination of Dexamethasone and Prednisolone Adulterated in Herbal Medicines Using Thin-layer Chromatography

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#### ABSTRACT

Thin-layer chromatography (TLC) is a qualitative, fast, and inexpensive method for screening of steroid adulteration. The purpose of this study was to determine steroids including dexamethasone and prednisolone adulterated in herbal medicines available in Thailand using TLC. Percentages of steroid adulterated samples from 2007 to 2011 were 28.57, 10.39, 3.33, 4.41, and 6.95 %, respectively. The present study indicated that steroid adulterated herbal medicines in Thailand tend to increase and then decrease over the period 2007 to 2011. Most of herbal medicines adulterated with steroids were pills and powder especially for treatment of arthritis and muscle pain. Steroids can cause serious side effects such as Cushings syndrome, osteoporosis, and peptic ulcer. Therefore, people should be aware of steroid adulteration in herbal medicines.

**Keywords:** Dexamethasone, Prednisolone, Steroids, Thin-layer chromatography

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## **INTRODUCTION**

Herbal medicines in dosage forms of powder and pills have long been an integral part of Thai culture and practices over the past several decades. They are usually composed of a numbers of medicinal plants and plant extracts. Herbal medicines are used to treat a variety of diseases such as rheumatism, muscle pain, allergy, infection, hypertension, obesity, and diabetes [1,2]. However, the increasing use of herbal medicines in Thailand has led to increasing concern regarding their safety. World Health Organization (WHO) and The Thai Ministry of Public Health issued the guidelines and acts concerning safe use of herbal medicines [3,4]. The safety issues related to adulteration of steroids in herbal medicines. The adulteration of herbal medicines with steroids sometimes occurred since some producers and herbalists do not have appropriate education and ethics [5]. Screening methods based on thin-layer chromatography (TLC) were recommended for quality control of herbal medicines [6].

Dexamethasone and prednisolone are steroidal anti-inflammatory drugs, widely used to treat a variety of diseases such as allergy, asthma, arthritis, autoimmune diseases, ulcerative colitis, and to prevent organ transplant rejection [7]. However oral steroids can cause many adverse effects such as Cushings syndrome, osteoporosis, cataract, muscle weakness, peptic ulcer, and liver toxicity [8]. Therefore, the adulteration of herbal medicines with steroids can cause serious adverse effects and put consumers at risk [9]. In our previous work [10] we presented the TLC method for determination of steroids adulterated in some herbal medicines. In analytical practice TLC is still commonly applied as the method for qualitative and quantitative analysis as well as screening of steroids in biological and pharmaceutical samples [1,11]. This work is a continuation of our previous research focusing on development of TLC for simple separation and analysis of steroids adulterated in herbal medicines available in Thailand from 2007 to 2011. The objective of this paper is to establish the detection capability of TLC towards dexamethasone and prednisolone in herbal medicines. The data obtained could be used for the quality control systems to ensure the safety of herbal medicines in Thailand.

## **MATERAILS AND METHODS**

### **Materials and Reagents**

The dexamethasone reference standard was obtained from the Department of Medical Sciences, Ministry of Public Health in Thailand. The prednisolone reference standard was of British Pharmacopoeia (BP)/ The United State Pharmacopoeia (USP) standard. All solvents and reagents were of analytical reagent grade. Dichloromethane, methanol, cyclohexane, and ethyl acetate were supplied from Merck (Darmstadt, Germany). The tetrazolium blue chloride was purchased from Sigma-Aldrich (St. Louis, MO). A solution of 1% w/v tetrazolium blue chloride was prepared immediately in methanol prior to use. TLC Silica gel 60 F254 aluminum sheets were purchased from Merck (Darmstadt, Germany). Whatman filter papers No.1 were products of GE Healthcare (UK).

## **Herbal Medicine Samples**

A total of 335 herbal medicine samples including declared, undeclared, and unlabelled drugs such as pills, tablets, capsules, powder, and tea bags were obtained from many consumers in Thailand, from March 2007 to February 2011. A blind numbering system was applied to the samples. Each sample was powdered with a mortar and pestle and transferred into a plastic bag. All pulverized samples were protected from light and kept in a desiccator at room temperature until analysis. All samples were treated in an identical manner.

## **Preparation of Standard and Sample Solutions**

A stock standard solution containing a reference standard of steroids at 0.5 mg/mL was prepared in dichloromethane. A working solution at 0.1 mg/mL was prepared by dilution of the stock solution in dichloromethane. For preparation of sample solutions, one gram of each pulverized sample was extracted with 5 mL of dichloromethane. The mixture was mixed thoroughly by vortexing and sonication to improve the dissolution of extract. The supernatant was collected and filtered through a Whatman filter paper. The filtrate was collected for TLC analysis. All solutions were stored at -20 °C prior to use.

## **Thin-layer Chromatography (TLC)**

Extraction solutions of samples and standard solutions of dexamethasone and prednisolone (20 µL) were separately spotted onto 20x20 cm TLC plates containing a 250-µm thick silica gel stationary phase. A TLC plate was then placed in a closed chamber containing a layer of mobile phase for approximately 1 h. Mobile phase A consisted of dichloromethane : methanol (9:1, v/v), and mobile phase B consisted of cyclohexane : ethyl acetate (1:3, v/v). When the mobile phase reached to the solvent front, the TLC plate was removed and then dried at room temperature. The resulting TLC pattern was viewed under short-wave (254 nm) ultraviolet light. Steroids which absorb shortwave UV at 254 nm were detected due to quenching. Then the plate was sprayed with 10% w/v sodium hydroxide solution and 1% w/v methanolic tetrazolium blue solution (freshly prepared), respectively. After heating, the plate was visualized the violet spots of steroid compounds under daylight. The  $R_f$  values of steroid compounds were calculated ( $R_f = \text{distance moved by the eluted steroid compound} / \text{distance moved by the solvent front}$ ).

## **Validation Method**

The procedure of validation was modified according to the ICH Guideline Q2B [12-14]. For validation of identity test, matrix components were developed simultaneously side-by-side to the reference standards of dexamethasone and prednisolone on the same TLC plate. The observed standard deviation of the  $R_f$  values was determined after observing under UV light at 254 nm and spraying with tetrazolium blue, respectively. The limit of detection (LOD) could be performed using reference standards of dexamethasone and prednisolone in appropriate concentration.

## RESULTS AND DISCUSSION

For the qualitative identity test, there were no other spots detected on similar  $R_f$  values of the steroids investigated when the spots were observed under 254-nm UV light and reacted with tetrazolium blue, respectively. The  $R_f$  values of dexamethasone and prednisolone obtained from mobile phase A were 0.54 and 0.49, respectively while those obtained from mobile phase B were 0.30 and 0.19, respectively. The LOD was found to be 0.5 mg/mL for dexamethasone and prednisolone. Described procedure was applied for determination of various samples of herbal medicines, available in Thailand. Adulteration of steroids in samples was detected at a higher concentration than the LOD.

Samples of herbal medicines in this study claimed to treat various diseases such as asthma, diabetes, obesity, rheumatism, pain, and paralysis as well as to improve health. Three hundred and thirty-five samples were collected from consumers in five parts (12 provinces) of Thailand during March 2007 to February 2011. Steroids adulterated in samples were detected at quenching and violet spots on TLC plates using 254-nm UV light and tetrazolium blue spraying solution, respectively. The  $R_f$  values of dexamethasone and prednisolone adulterated in samples were similar to those of steroid standards. Using a spiking technique and comparing the TLC patterns of spots with those of the steroid standards were found to be adulterated with dexamethasone and prednisolone.

Table 1 showed a total of 335 samples from 2007 to 2011 (28, 77, 90, 68, and 72 samples, respectively). From 2007 to 2011, the percentages of samples adulterated with steroids were 28.57, 10.39, 3.33, 4.41, and 6.95 %, respectively while those of unadulterated with steroids were 71.43, 89.61, 96.67, 95.59, and 93.06 %, respectively. The percentages of dexamethasone adulterated samples from 2007 to 2011 were 7.14, 2.60, 2.22, 1.47, and 4.17 %, respectively. The percentages of prednisolone adulterated samples from 2007 to 2011 were 14.29, 5.19, 0, 2.94, and 2.78 %, respectively. The percentages of dexamethasone and prednisolone adulterated samples from 2007 to 2011 were 7.14, 2.60, 1.11, 0, and 0 %, respectively. Table 2 showed the adulteration of dexamethasone and prednisolone in a variety of herbal medicine samples. In most cases, samples unadulterated with steroids were found in the dosage forms of tablets and tea bags. Even more dangerous was detection of dexamethasone or prednisolone in herbal pills or powder, used to cure arthritis, muscle pain, paralysis, and asthma. The most dangerous was detection of both dexamethasone and prednisolone in herbal pills or powder, used in the treatment of arthritis, muscle pain, and asthma. From 2007 to 2011, the samples obtained from Nakhon Pathom province were found to be the most steroid adulteration, with 51.85 % of total 27 adulterated samples as shown in Table 2.

All determinations indicated that herbal medicines are sometimes intentionally adulterated with dexamethasone and/or prednisolone to achieve desired action especially for treatment of arthritis, muscle pain, paralysis, and asthma. Furthermore, dexamethasone and/or prednisolone adulteration was often found in herbal pills and powder. Therefore, people should be aware of consuming herbal medicines assumingly herbal plants. Nowadays, Thailand's Food and Drug Administration issued a warning to the public on illegal herbal medicines that contain an undeclared steroids, dexamethasone and prednisolone.

**Table 1.** Steroids found in herbal medicines.

Steroids detected	Year									
	2007		2008		2009		2010		2011	
	No. of samples	%								
Dexamethasone	2	7.14	2	2.60	2	2.22	1	1.47	3	4.17
Prednisolone	4	14.29	4	5.19	0	0	2	2.94	2	2.78
Dexamethasone and Prednisolone	2	7.14	2	2.60	1	1.11	0	0	0	0
Adulterated	8	28.57	8	10.39	3	3.33	3	4.41	5	6.95
Unadulterated	20	71.43	69	89.61	87	96.67	65	95.59	67	93.06
Total samples	28	100	77	100	90	100	68	100	72	100

**Table 2.** Adulteration of dexamethasone and prednisolone in herbal medicines.

Year	Name of sample	Type of sample	Suggested use	Sample source	Steroids detected
2007	Undeclared drugs	Extract	No data	No data	Dexamethasone
	Undeclared drugs	Pills	No data	No data	Dexamethasone
	Unlabelled drugs	Powder	Against arthritis	Nakhon Pathom	Prednisolone
	Undeclared drugs	Powder	No data	No data	Prednisolone
	Unlabelled drugs	Pills	Against arthritis	Nakhon Pathom	Prednisolone
	Undeclared drugs	Pills	No data	No data	Prednisolone
	Undeclared drugs	Powder	No data	No data	Dexamethasone and Prednisolone
2008	Unlabelled drugs	Capsules	Against arthritis	Nakhon Pathom	Dexamethasone
	Unlabelled drugs	Powder	Against arthritis and muscle pain	Nakhon Pathom	Dexamethasone
	Kasaisen	Pills	Against asthma	Nakhon Pathom	Prednisolone
	Undeclared drugs	Pills	No data	No data	Prednisolone
	Unlabelled drugs	Pills	Against arthritis, muscle pain and paralysis	Bangkok	Prednisolone
	Unlabelled drugs	Pills	Against arthritis and muscle pain	Sakaeo	Prednisolone
	Unlabelled drugs	Powder	Against arthritis and muscle pain	Nakhon Pathom	Dexamethasone and Prednisolone
2009	Unlabelled drugs	Pill	Against asthma	Nakhon Pathom	Dexamethasone and Prednisolone
	Pradong	Suspension	Against arthritis and muscle pain	Nakhon Pathom	Dexamethasone
	Kasaisen	Suspension	Against arthritis and muscle pain	Nakhon Pathom	Dexamethasone
2010	Unlabelled drugs	Powder	No data	Nakhon Pathom	Dexamethasone and Prednisolone
	Unlabelled drugs	Powder	Against arthritis and muscle pain	Nakhon Pathom	Dexamethasone
	Unlabelled drugs	Pills	Against arthritis, muscle pain and paralysis	Supanburi	Prednisolone
2011	Unlabelled drugs	Pills	No data	Nakhon Pathom	Prednisolone
	Unlabelled drugs	Pills	No data	Phuket	Dexamethasone
	Unlabelled drugs	Pills	No data	Phitsanulok	Dexamethasone
	Unlabelled drugs	Pills	No data	Nakhon Pathom	Dexamethasone
	Unlabelled drugs	Pills	No data	Nakhon Pathom	Prednisolone
Unlabelled drugs	Pills	No data	Songkhla	Prednisolone	

### CONCLUSION

A simple and reliable TLC method using 254-nm UV light and tetrazolium blue spraying reagent was developed for rapid qualitative analysis of steroids adulterated in various dosage forms of herbal medicines. Steroids including dexamethasone and prednisolone adulterated in herbal medicines were simultaneously determined by the developed TLC method. This method can be used as an economic method for routine quality control of herbal medicines. From 2007 to 2011, the percentage of samples adulterated



with steroids in Thailand was 8.06 % of total 335 samples. Therefore, consumers should be aware that herbal medicines adulterated with steroids are harmful to human health.

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