MINISTRY OF EDUCATION AND TRAINING
HANOI UNIVERSITY OF PHARMACY

SOCIALIST REPUBLIC OF VIETNAM
Indepedence – Freedom - Happiness

DISSERTATION ABSTRACT

1. Introduction

Name of candidate: Dao Thi Cam Minh

Topic of dissertation: *Method development for analysis of synthetic drugs illegally adulterated in herbal products*

***Specialization*:** Drug quality control and Toxicology

Code number: 62720410

Name of academic institute: Hanoi University of Pharmacy

2. Summary

2.1. Objectives

- Developing qualitative and quantitative methods for analgesic, anti-inflammatory, hypoglycemic, phosphodiesterase type 5 inhibitors illegally adulterated in herbal products by liquid chromatography - tandem mass spectrometry (LC-MS / MS).

- Developing methods of rapid identification of synthetic drugs in herbal products using high-performance thin-layer chromatography (HPTLC) and surface-enhanced Raman scattering (SERS).

- Applying these developed methods to determination of illegal synthetic drugs adulterated in herbal preparations in Vietnam.

2.2. Materials and methods

2.2.1. Materials and equipments

- National reference standards of betamethasone (100.42%), dexamethasone acetate (99.6%), hydrocortisone acetate (99.5%), prednisolone (99.49%), prednisone (99.19%), paracetamol (100.04%), piroxicam (99.49%), indomethacine (99.7%), ketoprofen (99.54%), glibenclamide (100.10%), glipizide (99.17%), glimepiride (99.49%), gliclazide (99.53%), sildenafil citrate (98.28%), and tadalafil hydrocloride (97.65%) were purchased from National

Institute of Drugs Quality control of Vietnam; whereas European Pharmacopeial reference standard of vardenafil hydrocloride (100.0%) was obtained from European Directorate for the Quality of Medicines and HealthCare (EDQM). Herbal medicines were purchased at Vietnamese Pharmacopoeial grade; chemical substances and solvents were purchased at analytical grade or LC-MS grade.

- LC-MS/MS analysis was implemented on an EVOQ QubeTM Triple Quadrupole LC-MS system (Bruker, USA), with Ultra C18 (Restek, USA) column (100mmx 2.1 mm; 1.9 μm); precolumn (10mm x 4mm; 3 μm).
- Raman spectra were recorded with a LabRam HR-Raman (Horiba Jobin Yvon S.A.S, France) with input laser of 632.8 nm and CCD camera.
- An HPTLC system (CAMAG, Switzerland) CAT No 027.6200 was used for repeatable sampling, reproducible development conditions with temperature and humidity control; System was controlled by WinCAT software and TLCscanner scan thin plates, record chromatographic images, and process image data on computer. Common laboratory equipment such as micropipet, precision glass pipette, centrifuge tube.

2.2.2. Samples

- Test samples were 184 traditional and herbal products, dietary supplements; including 119 samples for supporting treatment of osteoarthritis; 41 samples for treatment of diabetes and stabilized blood sugar; 24 samples for treatment of erectile dysfunction in men in Vietnam. These products are purchased online, through internet, and directly from pharmacies.
- 9 placebo samples were prepared at Traphaco company, from major representative herbal medicines and excipients with presented clearly in Vietnamese Pharmacopoeia 5^{th} editon (2018) and official books for traditional medine.

2.2.3. Methods

- Developing qualitative and quantitative methods of synthetic drugs adulterated in traditional herbal products using LC-MS/MS: investigating extraction

process; developing procedures for identification and quantification of 16 synthetic drugs in traditional/herbal preparations; method validation according to guidance of AOAC 2016 and ICH 2005 on 9 placebo matrices.

- Development of rapid identification method of 16 synthetic drugs in herbal preparations using HPTLC: screening and selection of chromatographic conditions; method validation on 9 placebo matrices according to AOAC 2016 and ICH 2005.

- Development of rapid and simple detection method of sildenafil in herbal products by thin layer chromatography combined with surface-enhanced Raman scattering (TLC - SERS): screening of SERS conditions; method validation according to the guidance of AOAC 2016 and ICH 2005 on 3 placebo matrices (NP1, NP2, NP3).

- Application of analytical methods of LC-MS/MS, HPTLC and TLC-SERS in 184 herbal products; preliminary assessment of the situation of the synthetic drugs adulterated illegally in the herbal product in Vietnam.

2.3. The principal results and conclusion

2.3.1. Developing 3 LC-MS/MS qualitative and quantitative methods for 16 synthetic drugs adulterated in herbal products for supporting treatment of osteoarthritis, diabetes and ecretile dysfunction.

- 16 synthetic drugs including paracetamol, piroxicam, indomethacine, ketoprofen, betamethasone, dexamethasone acetate, hydrocortisone acetate, prednisolone, prednisone, glibenclamide, gliclazide, glimepiride, glipizide, sildenafil, tadalafil and vardenafil. The sample was finely grounded, homogenzed, ultrasonic extracted with methanol and appropriately diluted. The chromatogrphic system includes Restek Ultra II C18 column (100 mm × 2.1 mm; 1.9 µm), mobile phase with acetonitrile and water containing 0.1% formic acid in gradient mode, detection by MS/MS with ESI (+) ; selected ions (mother ion → quantitative ion / qualitative ion).

- Method validation according to the guidance of AOAC and ICH on 9 placebo matrices with high specificity and limit of detection (LOD) from 0.076 to 1.300 ng/ml.

2.3.2. Developing 4 new rapid HPTLC detection methods for synthetic drugs adulteration and a qualitative TLC-SERS method for sildenafil adulteration in herbal preparations

- HPTLC method was developed for fast detection of 16 synthetic drugs in samples with ultrasonic extraction by methanol, appropriately diluted if needed. Introduce 5 µl on silica gel 60 F_{254} TLC plate. Mobile phase of chloroform - ethyl acetate - ethanol (8.5: 1: 0.5; v/v/v) was used for glucocorticoids separation, n-hexane: ethyl acetate: acetic ;acide (6: 2,5: 1,5; v/v/v) for NSAIDs; n-butyl acetate - formic acid (100: 0.5; v/v) for hypoglycemic drugs and ethyl acetate - isopropanol - 25% ammonia (45: 5: 2.6; v/v/v) for PDE-5 inhibitors. Method validation was done on 9 placebo matrices, with LOD of 20-188 ng/spot.

- A detection method for sildenafil was established with TLC - SERS with sample extraction with methanol, mobile phase including ethylacetate - isopropanol - 25% ammonia (45: 5: 2.6; v/v/v). TLC spot equivalent to the retardation factor (R_f) of sildenafil was marked under UV illumination at 254 nm in UV cabinat, then 1.5 µl of silver colloid was applied directly on the marked spot on TLC plate. Finally, SERS spectrum was recorded with input beam of 632.8 nm during 60 seconds. SERS measurement was optimized using double coffee-ring effect, thus sensitivity was improved with LOD of 2 ng/trace.

2.3.3. Application of developed methods on real herbal preparations

- HPTLC methods: analyzing 184 samples of herbal preparations, of which 25 samples were detecpositive for paracetamol, indomethacine, dexamethasone acetate, glibenclamide, sildenafil, tadalafil, and vardenafil.

- LC-MS/MS methods: among 184 samples of traditional medicine products, 25 samples were found adulterated synthetic drugs: 9 adulterated with paracetamol (31.21-1318.62 mg / dose), 1 with indomethacine (5, 64 mg / dose), 3 samples

with dexamethasone acetate (0.44-0.95 mg / dose), 8 samples with glibenclamide (0.14-2.16 mg / dose), 5 samples with sildenafil (15.58 - 70.00 mg / dose), vardenafil (10.10 mg / dose), and tadalafil (12.34-17.17 mg / dose).

- TLC-SERS method: 5 sildenafil-positive samples were detected in 24 samples for supporting treatment of erectile dyfunctions. The same results were obtained by LC-MS/MS analysis.

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The scientific advisors **Doctoral candidate**

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