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Rapid detection of the metformin illegally added of in TCM and health food by TLC-IR

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Abstract: Based on TLC-IR, the study established an effective method for rapid detection of metformin illegally added in hypoglycemic traditional Chinese medicine and health food products. 12 batches of hypoglycemic traditional Chinese medicine and health products were purchased in the pharmacy, which were produced by different manufacturers. TLC was used to separate metformin and phenformin for preliminary identification from. IR was applied to further identification and HPLC method was used to verify the experimental results of TLC-IR. TLC developing solvents was petroleum ether-methanol-glacial acetic acid (5:12:0.5) and the stationary phase was silica gel prefabricated GF_{254} plate. IR used KBr pellet pressing method with a resolution of $4cm^{-1}$ and scanned 64 times. The column for HPLC analysis was SinoChrom ODS-BP 5 μm (4.6mm *250mm) and the injection volume was 20μL. The detection wavelength was 234nm. The flow rate was $1ml \cdot min^{-1}$. Metformin and phenformin were significantly separated under the TLC condition. Joint identification by TLC-IR, none of metformin and phenformin were identified in the hypoglycemic traditional Chinese medicine. Phenformin was detected in two kinds of health products while metformin was identified in one kind of health food. The result of HPLC was consist with TLC-IR. The established TLC-IR method was simple, rapid and selective, which was suit to apply in the identification of metformin illegally added in hypoglycemic traditional Chinese medicine and health food products.

Keywords: TLC, IR, Traditional Chinese Medicine and hypoglycemic health products, biguanides

INTRODUCTION

Diabetes is a complex disorder of endocrine disorders, often accompanied by severe complications (Anonymous 2017). At present, many traditional Chinese medicines (TCMs) are used to treat diabetes mellitus, many of which have well efficacy and low side effects, such as Dan-Shen (*Radix Salviae Miltiorrhizae*) (Lee *et al.*, 2016), Gegen Qinlian Decoction (Xu *et al.*, 2015), Liu-Wei-Di-Huang-Wan (Hsu *et al*., 2014), Jiang Tangning (Sun *et al.*, 2013) and berberine (Lan *et al.*, 2015). Many manufacturers add similar chemical synthetic drugs in the TCM to get benefits. The one of common illegal additions is biguanides. While the patient is unwitting and taking a large amount of this kind of "pure" TCM or health care products, it will seriously threaten the safety of its own life (Sun *et al.*, 2010). The common side effects contain gastrointestinal side effects (nausea and vomiting) (Dujic *et al.*, 2016) and diarrhea (Ji *et al.*, 2015). Therefore, it is necessary to establish a sensitive and special analytical method for the identification of the chemical drugs mixed illegally in the antidiabetic and health care products of Chinese medicine.

At present, usually use TLC-HPLC, HPLC, HPLC-DAD, LC-MS/MS and HPLC-MS/MS to detect guanidine chemicals in China (Zhang *et al.*, 2007; Chinese Pharmacopoeia Commission 2015; Ganming 2009; Zeng *et al.,* 2006; Zhang *et al.,* 2007; Dong *et al.*, 2005). Recently, Raman is applied in fast detection for the institute of medicine and laboratory (Penido *et al.*, 2016). However, within the limitation of the instrument and function, some drug inspection units, especially at the grass-roots level (county level and below) do not have enough conditions for the detection of this kind of chemical drugs (Fu 2016) Based on the relevant literature, the study established the TLC-IR method that was devoted to the discovery of biguanide hypoglycemic compositions which were illegally added in the TCM and health foods (Li *et al.*, 2013). Then, HPLC was a way to detect the results of established methods, which verified the established methods (Wen 2013). Finally, it performed drug sampling work by screening two kinds biguanides illegally added in the TCMs and health products. The study is aimed to give a TLC quick test box for the grassroots drug control unit, which provided technical support for the supervision department.

MATERIALS AND METHODS

Instruments and reagents

Waters E2695 high-performance liquid chromatograph, Waters E2487 type analysis and preparation of high-performance liquid chromatography system (Waters group company, USA), NICOLET 380 FT-IR spectrometer (Thermo Fisher Scientific, USA), YOKO ultraviolet

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analyzer, XS105 electronic balance leveling (Mettler Toledo, Switzerland).

Silica gel GF254 thin plate (Qingdao marine chemical plant), specification: 50mm x 100mm, thickness: 0.25mm. Standard metformin (Batch number: 100664-200602, National Institutes for Food and Drug control), standard phenformin (Batch number: 100922-201001, National Institutes for Food and Drug control). The hypoglycemic Chinese Medicines were totally seven kinds of 12 batches from different pharmaceutical companies. Purchased them at a drugstore. Mark the TCM into 1 to 7 The over-the-counter (OTC, No. 1~4) were Jiangtangning capsules (21020001), Jiangtangning capsules (090702), Tangniaole capsule (201102051; 20120107) and Xiaokejiangtang capsules (20120101; 20120102) in turn. Hypoglycemic health care products (No. 5~7) successively were Yuanhengyitai (20111107 and 20120321), Milaidan (20120215 and 20111201) and Zangtangping (20111206 and 20120409). The chemical solution, such as petroleum ether, methanol and glacial acetic acid were analytical reagent.

Preparation of the control solution

Precise weighing metformin and phenformin control 0.1g, respectively. Then the solution of 1mg·ml^{-1} was prepared by adding anhydrous ethanol.

Preparation of the test product solution

Take the capsules and the content is equivalent to one times the oral dose (capsule shell spare), tablets or granules, grinding, adding anhydrous ethanol 10mL (80W, 60kHz), ultrasonic treatment 15min, filtration, the filtrate transpose volumetric flask, dilute to the10 mL as the test solution.

TLC method

Point the reference solution and the test solution 5 μLin the same thin layer plate of silica gel GF254, respectively. The developing solvent was petroleum ether-methanol-glacial acetic acid (5:12:0.5). After starting out, then we dried the plate. Placed it at 254nm under UV light view.

IR method

IR was used to determine the results of TLC. Firstly, take separately TLC plate of two control solution and No. 4~6 test solution spots to place in the test tube. Next, we added anhydrous ethanol and ultrasonic treatment (80W, 60kHz). The following was filtration. The next step was to wave the filtrate and kept the solid in the dryer. Using KBr compression method to detect the spectrum. Scanning range was in 400~4000nm.

Chromatographic conditions

The mobile phase was set methanol-water-phosphoric acid (17:83:0.2). The chromatographic column was Sino Chrom ODS-BP 5μm (4.6mm *250mm). The detection wavelength was 234nm. The flow rate was 1ml·min^{-1}. The column temperature was room temperature and the injection volume was 20μL.

Preparation of control product solution and sample solution preparation for HPLC

Weighed standard metformin and phenformin 0.5mg and taken them into the 10mL volumetric flask, respectively. Simultaneously, the solution of 1mg·ml^{-1} was prepared by adding mobile phase.

Accurately weighed 3.0g of 6 kinds of TCMs and health products. Consisted of the preparation of control solution, separately took the solid powder into the 10mL volumetric flask and added the mobile phase to the scale. Kept being ultrasonic for 15min, filtering. Finally, make mobile phase dilute to the scale, shaken to obtain the sample solution.

RESULTS

Thin layer chromatography

Under the selected TLC conditions, the detection limit of metformin and phenformin was 0.4μg. The separation degree R was >1.5 and the separation efficiency was superior. The color and location of the dominant spots in the test solution (No. 5 and No.6 solution) were consistent with the color and location of the main spots in the control solution of the phenformin. The color and location of the dominant spots in the No.7 solution was consistent with the main spots in the control solution of the metformin (fig. 1). The TCM medicine (No.1~3) did not detect spots consistent with two reference substances. The results suggested that the three hypoglycemic health care products had been illegally added.

(1) No. 7

(2) No. 5

(3) No. 6

(4) Metformin

(5) Phenformin

Fig. 1: The positive results of TLC

The identifications of infrared spectrophotometry (IR)

The infrared absorption spectrum displayed in the paper

(figs. 2-4). In the spectrum, No.7 scraping TLC extract and infrared control of metformin spectrum the spots obtained by the TLC of No.7 were consistent with the infrared spectra of metformin (fig. 4) The expansion vibration of N-H bond appeared at 3500~3300cm^{-1}, and the bending vibration in the N-H bond surface occurred at 1650~1550cm^{-1} and the C=N bond expansion vibration appeared at about 1600cm^{-1}. The infrared spectrum illustrated that both the spots extract of No. 5 and No. 6 had the consistent characteristic peaks with the phenformin. The benzene skeleton C-H stretching vibration in 3100~3000 on cm^{-1}, C-H with benzene ring bending vibration under 1000 cm^{-1}, the same with the standard spectrum consistent with phenformin.

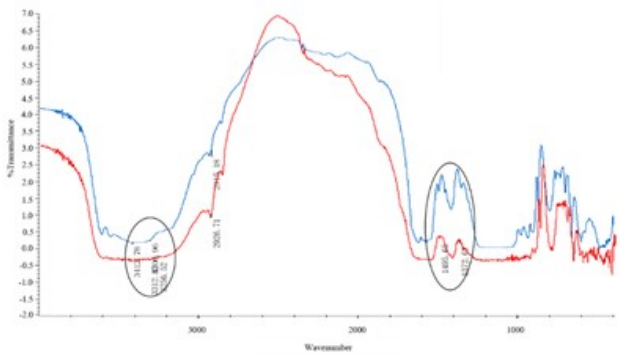

Fig. 2: IR spectrum comparison of extract from TLC spots in phenformin (blue) and No.5 (red)

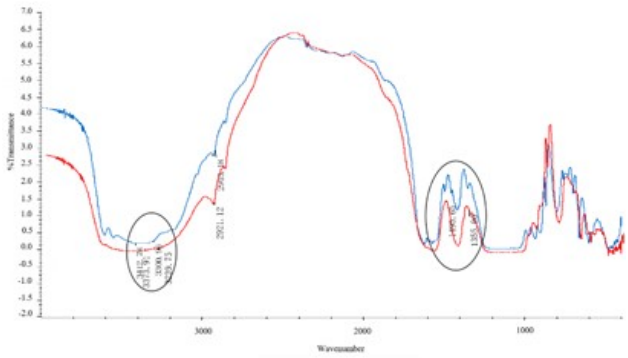

Fig. 3: IR spectrum comparison of extract from TLC spots in phenformin (blue) and No.6 (red)

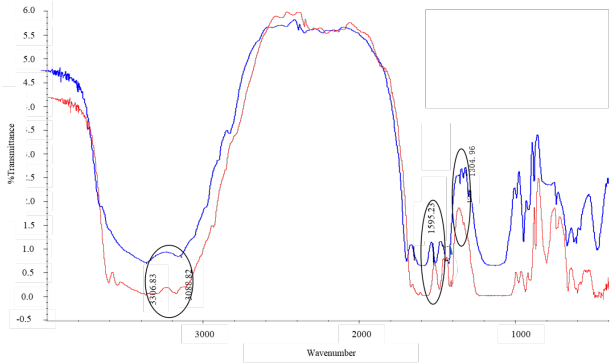

Fig. 4: IR spectrum comparison of extract from TLC spots in metformin (blue) and No.7 (red).

The results of methodological verification

According to the chromatography conditions, the theoretical plate number of phenformin peak was 8773.65 and the theoretical plate number of metformin peaks was 12426.43. Under the chromatography conditions, the theoretical plate number of both metformin and phenformin was not less than 2000 and the separation degree was >1.5. There was a significant chromatography peak at the retention time of metformin in No.7 solution and reference solution, which was illegally added metformin. It was significant that chromatographic peak existed in the retention time of phenformin in the chromatogram of No.5 and No.6, which was the determination of the addition of phenformin. The results showed in the figs. 5-8. Besides, the study was also used simulation experiment to confirm the TLC-IR method. The results showed in table 1.

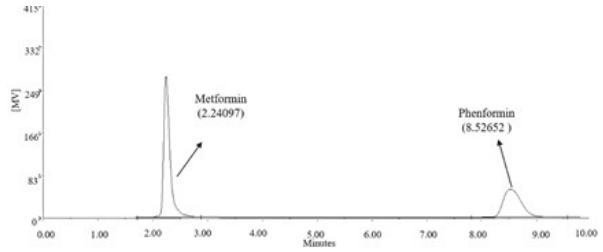

Fig. 5: The HPLC chromatogram of standard metformin and phenformin

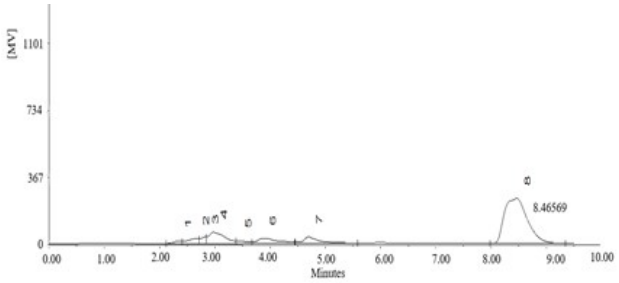

Fig. 6: The HPLC chromatogram of No.5

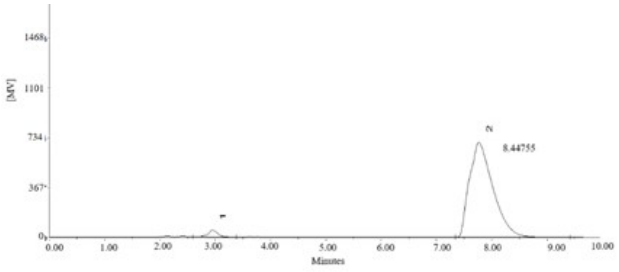

Fig. 7: The HPLC chromatogram of No.6

DISCUSSION

The established TLC method can identify the metformin and metformin illegally added in hypoglycemic TCMs and health care products. In the experiment, the anhydrous ethanol was chosen as the solvent; and use the petroleum ether and methanol as the developing solvent.

Table 1: Experimental data of three samples and simulated addition experiment

	No. 5	No.5 added reference	No. 6	No.6 added reference	No.7	No.7 added reference
Retention time (min)	8.46569	8.45569	8.44755	8.42755	2.36839	2.36839
Height (mv)	246.74	380.69	710.22	716.69	1969.25	1981.32
Peak area (mv.sec)	7054.16	11052.56	22550.30	22613.50	26522.08	26634.21

Both reagents and the convenient operation avoided using toxic solvents, such as tri-chloromethane (Isaac *et al*., 2015), benzene (Minciullo *et al*., 2014). The established method can decrease the toxicity of the operator.

The advantages of IR (Larkin 2011) were wide application range, strong characteristic, protruding integrity and a large amount of information as well as high sensitivity and high accuracy. Additionally, it has different preparation methods for solid, liquid and gas. Simultaneously, the operation of IR is easy and convenient. The extract of metformin and phenformin hydrochloride disturbed due to the excipients in the TCMs and health-food. After purification by ethanol, it can remove most materials and eliminate interference of excipient. Therefore, it directly compared with the referential chromatogram or infrared spectra of standard samples.

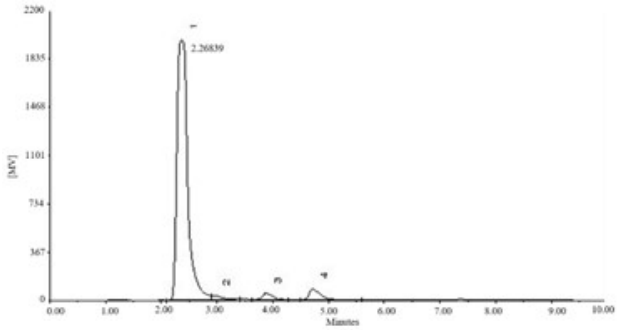

Fig. 8: The HPLC chromatogram of No.7

The paper established the method of HPLC with high specificity to detect metformin and phenformin. The chromatographic peaks was the illegally addition in the sample solution, which were coincided with the retention time of reference solution. Each illegal compound can meet separation, which means the TLC-IR method was suited to rapid detection of metformin and phenformin in the TCMs and health food. Considering the need for future counterfeiting and the dissolution of the compounds, methanol was selected as a solvent to set up an isometric HPLC system in this experiment. The simulated experiment by adding a standard solution to check whether the sample is added illegally by observing whether the peak area increased at the retention time of the main peak of the detection component. In the HPLC validation experiment, the reference substance was added to the test products and the increase of peak area was used to further verify the illegal addition of the guanidine compounds.

CONCLUSION

In this paper, we established a TLC system to detect the components of biguanide by screening TLC conditions, and the TLC results were verified by IR and HPLC. The TLC was highly specific to the qualitative identification of biguanide (metformin and dimethyl metformin) that illegally added in Chinese patent medicine and healthcare products. It is simple and quick to work. It is suitable for the supervision department and daily self-examination. It also provides the technical reference for the study of the rapid detection method for the illegal addition of Chinese traditional medicine and traditional Chinese medicine health products.

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