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Quantitative determination of sibutramine in adulterated herbal slimming formulations by TLC-image analysis method

Panadda Phattanawasin [a,*], Uthai Sotanaphun [a], Tasamaporn Sukwattanasinit [a], Jariya Akkarawaranthorn [b], Sarunyaporn Kitchaiya [b]

[a] Faculty of Pharmacy, Silpakorn University, Nakhon Pathom, Thailand
[b] Regional Medical Sciences Center 3 (Chonburi), Department of Medical Sciences, Ministry of Public Health, Chonburi, Thailand

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ABSTRACT

A simple thin layer chromatographic (TLC)-image analysis method was developed for rapid determination and quantitation of sibutramine hydrochloride (SH) adulterated in herbal slimming products. Chromatographic separation of SH was achieved on a silica gel 60 F_{254} TLC plate, using toluene-*n*-hexane-diethylamine (9:1:0.3, v/v/v) as the mobile phase and Dragendorff reagent as spot detection. Image analysis of the scanned TLC plate was performed to quantify the amount of SH. The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 1–6 μg/spot. The limits of detection and quantitation were 190 and 634 ng/spot, respectively. The method gave satisfactory specificity, precision, accuracy, robustness and was applied for determination of SH in herbal formulations. The contents of SH in adulterated samples determined by the TLC-image analysis and TLC-densitometry were also compared.

1. Introduction

Overweight and obesity have been recognized as one of the serious worldwide problems leading to the search for a number of weight-loss medicines and especially the use of alternative treatments based on herbal formulations. The demand for slimming formulations and dietary supplements originated from purely natural substances is prominently increasing due to the belief that these natural products have been regarded as being harmless and do not cause harmful side effects as synthetic chemical drugs [1,2].

From the increasing popularity all over the world, the market share of herbal slimming products has been highly competitive. As a result, some manufacturers committed illegal adulterations of synthetic anorexics in order to improve the efficacy of their products for weight loss. Sibutramine hydrochloride (SH) has been one of the most commonly adulterated anorexic drugs found in herbal slimming formulations [3–6]. In many countries including Thailand, SH was withdrawn from the market due to an associated risk of serious cardiovascular events such as nonfatal heart attack and nonfatal stroke [7]. Without adequate quality control, those taking herbal slimming formulations with the presence of undeclared sibutramine may be suffered from several unpredictable implications ranging from headache, vertigo, numbness to serious cardiovascular effects, depending on the amount of drug consumed [8,9]. In order to protect consumer risks, a simple, rapid and low-cost method for screening and quantitative analysis of the adulterated sibutramine in numerous brands of herbal slimming products available in the market is of importance for local authorities related to public health control and surveillance.

Several methods including HPLC [5,8,10], LC–MS/MS [11,12], capillary electrophoresis [13] and infrared spectroscopy [14] for identification and quantitative determination of SH in pharmaceutical formulations and herbal products have been reported. Even though the major advantages of these methods have been claimed for their being highly sensitive and specific, the analytical instruments are quite costly and expertise is usually required. Unlike those, the use of simple and inexpensive TLC method can overcome these drawbacks and being more accessible to many local authorities and small laboratories. Furthermore, based on a combination with simple computer technology and image analysis software, TLC-image analysis method has been developed and applied for quantitative assay with good accuracy and precision [15].

Therefore, the aim of this study was to develop an economic, accurate, reproducible and convenient TLC-image analysis method for rapid determination and quantitative analysis of illegal

* Corresponding author at: Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000, Thailand.
Tel.: +66 89 787 6710; fax: +66 34 255 801.
E-mail addresses: ypanadda@su.ac.th, p.phattanawasin@yahoo.com (P. Phattanawasin).

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adulterant, sibutramine, in herbal slimming formulations. The proposed method was validated in compliance with ICH guidelines and compared with the TLC-densitometry.

2. Materials and methods

2.1. Standards and samples

Standard sibutramine hydrochloride (SH), phentermine, fenfuramine and methamphetamine (purity > 99%) were obtained from Regional Medical Sciences Center 3, Chonburi, Thailand. Twenty products of herbal slimming formulations were purchased from local vendors, drugstores and *via* Internet. All reagents and chemicals were analytical grade.

2.2. Preparation of standard and sample solution

A working standard solution of SH (0.5 mg mL^{-1}) was prepared from a stock solution (5.0 mg mL^{-1}) in methanol. For preparation of sample solutions, S1–S20, the weighed content of one single dose (one cachet or one capsule) of each herbal formulation was placed in an Erlenmeyer flask and sonicated with 20 mL MeOH for 15 min. S10 and S19 solutions were further diluted twofold since they were highly adulterated with SH. All sample solutions were used for TLC assay.

2.3. Chromatographic conditions

TLC analysis was performed on TLC silica gel 60 F_{254} plastic plates (20 cm × 10 cm with 250 μm thickness, Merck, Germany). A standard or sample solution was applied as a 5 mm band onto a TLC plate using a TLC sampler Nanomat 4 (Camag, Switzerland) and a 2 μL capillary tube (Camag, Switzerland). The distance between each band was 1.0 cm. The plate was developed to a distance of 7.0 cm in a TLC chamber previously saturated with toluene-*n*-hexane-diethylamine (9:1:0.3, v/v/v) for 30 min.

2.4. TLC-image analysis method

For the determination of SH by TLC-image analysis method, the developed TLC plate under the chromatographic condition as above was air-dried at room temperature, then, dipped into Dragendorff reagent for 6 s using Immersione Device 3 (Camag, Switzerland). After 5 min, the plate was dipped again in the reagent for another 6 s to enhance the color and dried for 10 min before scanning by a digital scanner (Hewlett Packard SCAN Jet 3500C) at a resolution of 200 dpi. The color image of the plate was saved as a joint photographic experts group (JPEG) file. The JPEG image was resized and cropped according to the plate dimension at 20 cm × 10 cm and saved at a resolution of 40 pixels cm^{-1} for image analysis of SH content by Sorbfil TLC Videodensitometer software (Sorbpolymer, Russia).

The image file was opened with the software. Equal width of each track line was set and the evaluation of the chosen track to measure peak area was performed by Process Track command using for each line method option at the values of width and height at 3 and 30, respectively, and noise filtering at 7. The software evaluated a SH band in each track on a TLC image on the assumption that the size and the intensity of a band (in relation to the plate background intensity) depended on the quantity of a substance in the band. A chromatogram was constructed on the deviation of track intensity from background intensity. R_f and peak area of SH were determined.

2.5. TLC-densitometric method

For TLC-densitometric analysis, the air-dried TLC plate developed under the chromatographic condition as above was scanned by using a Camag TLC scanner II with Camag CATS 3.1 software in the absorbance mode at 225 nm. The slit dimension was 5 mm × 6 mm and the scanning speed was 4 mm/s.

2.6. Method validation

The specificity of the TLC methods was determined by comparing an R_f value of SH in a sample with that of standard SH and in relation to interferences from other components in herbal formulations. A TLC analysis of SH and synthetic compounds, e.g., phentermine, fenfuramine, and methamphetamine, was performed. The peak purity of SH was also determined by TLC-densitometry. The spectra at three different regions of the sibutramine peak, i.e., peak start (S), peak apex (M) and peak end (E) were compared.

For calibration curve, different aliquots (2–12 μL) of standard solutions at 0.5 mg mL^{-1} were applied on TLC plates using the Nanomat 4 with a 2 μL capillary tube to give the concentrations of 1, 2, 3, 4, 5 and 6 μg/spot of SH. The plate was subjected to the TLC-image analysis and TLC-densitometric methods as described above. The calibration curve was constructed between peak area and drug concentration in μg/spot and the calibration data were subjected to least-squares regression analysis.

In order to determine limit of detection (LOD) and limit of quantitation (LOQ), the linear regression data constructed from the lower concentration range (1, 2 and 3 μg/spot) was used [16]. The LOD and LOQ were determined from the formulae 3 SD/S and 10 SD/S where SD was the standard deviation (SD) of the intercept and S corresponded to the mean value of the slope.

The repeatability (intra-day precision) and the intermediate precision were performed by determining the amount of standard SH at three different concentration levels (2, 3 and 4 μg/spot) in triplicate. The intermediate precision was determined on two consecutive days. The relative standard deviation (RSD) values for repeatability and intermediate precision were calculated.

Robustness of the TLC methods was studied by introducing small changes in the mobile phase compositions, e.g., toluene-*n*-hexane-diethylamine (9:1:0.3, v/v/v), (9.1:0.9:0.3, v/v/v), (8.9:1.1:0.3, v/v/v) and by varying the time from spotting to chromatography and from chromatography to scanning or image capturing for 15 min. A concentration of 3.0 μg/spot was employed. Each variation was performed in triplicate. The RSD values of the peak areas were calculated for all variations.

The accuracy was performed in term of recovery studies using a standard addition method. A sample spiked with known amount of standard SH to give the additional concentrations of 1, 2 and 3 μg/spot was analyzed by the proposed methods. The experiment was conducted in triplicate. A mean percent recovery was calculated.

2.7. Analysis of SH in herbal slimming samples

A 6 μL of each sample solution was spotted on TLC plates and analyzed by the methods described above. The amount of adulterated SH in the samples was analyzed by the TLC-image analysis and TLC-densitometry. Each sample was analyzed in triplicate. The results obtained were expressed as mean of SH content (mg per one single dose).

2.8. Statistical analysis

The mean values of SH contents in one single dose assayed by the TLC-image analysis and TLC-densitometric methods were tested by paired *t*-test at 95% confidence level.

3. Results and discussion

3.1. Method optimization and method validation

The chromatographic condition for determination of adulterated SH in herbal products was attempted on the most common used silica gel 60 F_{254} TLC plates. Among various mobile phases, the solvent system consisting of toluene-*n*-hexane-diethylamine (9:1:0.3, v/v/v) gave a narrow band of SH at an R_f value of 0.46 ± 0.02 but weakly detected under UV at 254 nm. The intensity of spot detection was enhanced by dipping the plate in Dragendorff reagent. The orange-colored band corresponding to SH was clearly observed and well separated from other components in the samples. The specificity of the TLC method was ensured by comparing an R_f value of SH in the samples with that of standard SH. A TLC analysis of SH compared with synthetic compounds, e.g., phentermine, fenfuramine, and methamphetamine was also performed. The data showed that, the tertiary amine SH produced the strongest positive orange color whereas the other synthetic compounds, belonging to a group of primary and secondary amines, were weakly or not detectable with Dragendorff reagent and none of the compounds were detected at the same R_f value as SH (Fig. 1).

Furthermore, no other interferences in the samples were observed at the R_f value of SH. There were no any orange-colored bands corresponding to the R_f value of SH found in the non-adulterated samples, suggesting that other compounds found in the herbal samples did not interfere with the identification (Fig. 2). Peak purity of SH in each adulterated sample was also assessed from TLC-densitometry by comparing spectra at the start (S), apex (M) and the end (E) of the peak obtained from the band, i.e., r(S, M) > 0.999 and r(M, E) > 0.999.

The digital images of scanned TLC plates were analyzed by Sorbfil TLC Videodensitometer (Fig. 3). The calibration curves between peak area and concentration were obtained from TLC-image analysis and TLC-densitometric method. The polynomial regression data showed good linear relationship over the concentration range of 1–6 μg/spot from both methods