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Research Article

DETERMINATION OF SIBUTRAMINE ADULTERATED IN HERBAL SLIMMING PRODUCTS USING TLC DENSITOMETRIC METHOD

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ABSTRACT

Determination of sibutramine adulterated in herbal slimming product using thin layer chromatography (TLC) densitometric method with TLC silica gel 60 F_{254} aluminium plate as stationary phase and mixture of toluen-diethylamine (10:0.3) as mobile phase has been developed. The calibration curve in the concentration range of 0.50 to 5.00µg/spot showed good linier relationship (r^2 = 0.9986). The limit of detection and quantitation (LOD and LOQ) were 217.5ng and 724.9ng/spot, respectively. The method gave satisfactory specificity, linierity, precision and accuracy validation criteria and was applied for determination of sibutramine in herbal slimming products obtained from several drugstrores in Depok City, West Java, Indonesia. Results of the determination showed that six of seven samples analyzed were detected containing sibutramine HCl with the concentration of 2.45-26.24mg in a single dosage of slimming herbal products.

Key words: Sibutramine HCl; Herbal Slimming Products; TLC, Densitometry; Validation Method.

INTRODUCTION

Herbal medicines play an important role as an alternative of synthetic drugs to improve people health in developing countries and increasingly popular in developed countries. This is mainly because of the general perceptions that they are safer than synthetic chemical drugs. Herbal medicines are traditional medicines containing active ingredients of plants, or other plant materials, or combinations thereof. But it is a fact that there are many herbal medicines adulterated with synthetic chemical drugs to enhance their efficacy of respective products in the claimed indications. The adulterations can cause a significant public health risks, especially after cosuming them in the long term (Haneef, 2013; Monika, 2011).

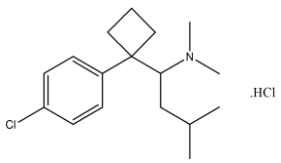


Figure1. Chemical structure of sibutramine HCl

The adulteration practice violates the laws of many countries, but the presence of undeclare synthetic chemical drugs in the herbal products are still often found, including in the herbal slimming products (Departemen Kesehatan RI, 1994; WHO, 2005; Carvalho *et al*., 2011; BPOM RI, 2014; Khazan *et al.,* 2014). The most common synthetic chemical drugs adulterated in herbal slimming product is sibutramine (Figure 1) (Phattanawasin, 2012; HAAD, 2012). This drug is a serotonine and noradrenaline reuptake inhibitors, causing an increase in the synaptic concentrations of these neurotransmitters, which then leads to the subsequent activation of α-adrenoceptors, β-adrenoceptors and serotonin receptor 2A and 2C subtypes (5-HT2A/2C). These interactions produce an eventual increase in satiety and energy expenditure, with a subsequent decreased body weight. However, since the year of 2010, sibutramine was withdrawn from the global market because of its unacceptable risk/benefit ratio. Sibutramine proved to have harmful side effects such as psychosis, hepatitis, arrhythmias and other cardiovascular diseases (Canadian, 2002; Oberholtzer, 2014). Those taking herbal slimming products containing undeclared sibutramine may be suffered from the above side effects ranging from headache to serious cardiovascular diseases, depending on the amount of drug consumed (Daglioglu and Akcan, 2012; Phattanawasin *et al*., 2012;

Chen *et al*., 2010). In order to protect comsumer risks, screening and determination of sibutramine in the herbal slimming products available in the market is very important. For this purpose, several chromatographic methods have been reported, such as HPLC (Kanan *et al*., 2009; Ancuceanu *et al*., 2013; Khazan *et al*., 2014), LC-MS/MS (Boguzs *et al*., 2006; Aliburnu *et al*, 2012) and TLC/HPTLC-densitometry (Phattanawasin *et al*., 2012; Mathon *et al*., 2014; Aliburnu *et al*, 2012). TLC allows for greater flexibility in choice of chromatographic system (Adamovics, 1997). The method is simpler, more rapid and lower cost rather than HPLC and LC-MS/MS.

The purpose of this research reported here was to develop a new, alternative, simpler, lower operating cost and validated TLC-densitometric method for screening and determination of sibutramine in herbal slimming products available in the market, which was useful to small laboratories or local health authorities.

MATERIAL AND METHODS

Chemicals

Sibutramine HCl RS (IPRS), methanol (Merck), toluene (Merck), n-hexane (Mallinckrodt), diethylamine (Merck), dragendorff reagent and 7 (seven) samples of various brands of herbal slimming products (AR, SU, LN, PL, SL, LK, LM). All reagents and chemicals were analytical grade.

Standar solution

A working standard solution with concentration of 0.5mg sibutramine HCl per mL was obtained by diluting 1.0mL stock solution (5.0mg sibutramine HCl per mL) with methanol to 10.0mL.

Sample solution

Sample solution was prepared as follows: a half of single dosage of homogenous sample was accurately weighed, placed in flask, added 5mL methanol, stirried and sonicated for 15min, followed by addition of methanol to the volume of 10.0mL and standing for 1h so as insoluble part precipitate. The soluble part of the sample was used as sampel solution.

Instrumentation and analytical conditions

TLC analysis was performed on aluminium TLC plates coated with silica gel 60 F_{254} with 250μm thicknes (E.Merck, Darmstandt, Germany). TLC plate was cut to the size of 10cm x 3-8cm depend on the number of samples/standard solution to be analyzed. Samples/standard solution were applied to the plate as spot using Nanomat completed with 2uL capilary tube (Camag, Swiszerland). The distance between each spot was 1.0cm. The plates was developed to a distance of about 7cm using a mixture of toluen-diethylamine (10:0.3v/v) as mobile phase in a twin-through glass chamber which had been pre-saturated with mobile phase vapours. Densitometric scanning was performed at 227nm with Camag® TLC Scanner III in reflectance–absorbance mode and operated by CATS software. The slit dimensions were 8mm×0.4mm and the scanning speed was 10mm s^{-1}.

Method validation

Validation of the analytical method was performed according to ICH Q2 guidelines (2005), including specificity, linearity, limit of detection (LOD), limit of quantitation (LOQ), precision and accuracy:

Specificity

The specificity of the TLC method was determined by comparing TLC densitometric chromatogram of blank sample (sample identified free of sibutramine), sibutramine HCl standard and sample addition (blank sample added sibutramine HCl standard). The method meets the specific criteria when the chromatogram peak of the sibutramine spot from sample addition was not interferenced by other components of the sample.

Linearity

The linearity was evaluated by determining the correlation coefficient (r^2) of linear regression analysis ($y=bx+a$) of calibration curve contructed between peak area and drug concentration in the range of 0.50-5:00μg/spot.

Detection and quantitation limit

LOD and LOQ determined using data of standard deviation of the response and the

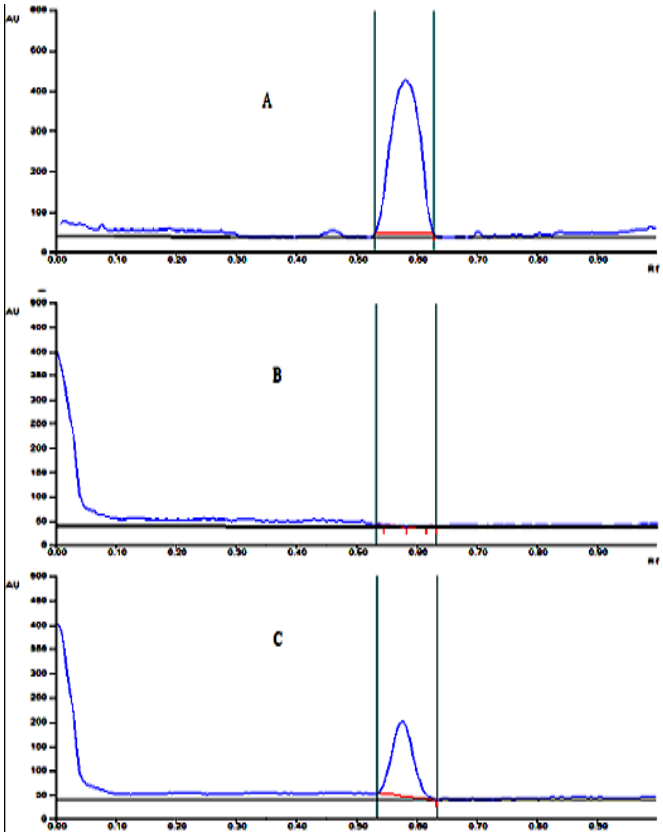


Figure 2. TLC densitometric chromatogram for specificity test. (A) Sibutramine HCl standard, (B) blank sample (free of sibutramine HCl), and (C) sample spiked with sibutramine HCl.

slope of the calibration curve. LOD and LOQ calculated using the equation of 3.3 σ/S and 10 σ/S, respectively. σ is the standard deviation of the y-intercept of the regression line. S is the slope of the calibration curve. The LOD and LOQ were confirmed by analyzing the spot of sibutramine HCl with the LOD and LOQ concentration. The detection limit is expressed in ng/spot.

Precision

The precision was performed by repeatability and intermediate presicion. Repeatability (intraday precision) was evaluated by determining the amount of standard sibutamine HCl at three different concentration levels (1, 2 and 3µg/spot) in triplicate. Intermediate precision was evaluated by repeating the determination in triplicate on two consecutive days. The relative standard deviation (RSD) values for repeatability and intermediate precision were calculated.

Accuracy

The accuracy was determined by adding known amount of standard sibutramine HCl to blank sample (sample identified free of the sibutramine HCl) to give concentrations of 1, 2 and 3µg/spot and was analyzed by the proposed methods. The experiment was conducted in triplicate. A mean percent recovery was calculated.

Analysis of sibutramine in herbal slimming product

The sample solution and standard solution was spotted on the same plate, eluted and analyzed by the proposed methods. Identification of sibutramine in the samples was performed by comparing the similarity of Rf, UV spectrum and colour (after spraying with Dragendorff reagent) of the spot from sample with those from a standard. The amount of sibutramine HCl was calculated from peak area of the spot obtained before

Tabel I. Intraday and intermediate precision study

Sibutramine HCl concentration (µg/spot)	Intraday precision*)	Intermediate precision*)
1	1.47	1.65
2	1.43	1.54
3	1.03	1.26

*) Relative standard deviation (% RSD, n=3)

Tabel II. Recovery Study (Accuracy)

Sibutramine HCl added (µg/spot)	Recovery (%)	Average recovery (%)
1	99.57±1.46	
2	100.32±1.43	99.70 ± 1,22
3	99.20±1.02	

Tabel III. The content of sibutramine HCl in herbal slimming products

Sample Code	Dosage form	Amount ± SD (mg/single dosage)
AR	Capsule	9.83±0.03
SU	Capsule	2.45±0.01
DI	Capsule	26.24±0.01
PL	Pil	ND*)
SL	Capsule	20.47±0.19
LK	Capsule	15.97±0.11
LM	Capsule	3.43±0.01

*) ND = not detected

spraying with Dragendorff reagent. Each sample was analyzed in triplicate. The results obtained were expressed as mean of sibutramine HCl content (mg per one single dose).

RESULTS AND DISCUSSION

Sibutramine is the most commonly added illegally into herbal slimming products. Several papers have reported the application of TLC and HPTLC-densitometric method for the screening and determination of sibutramine adulterated in herbal slimming products. However, the method need to be further developed to obtain simpler and lower operating cost method.

In our study, the chromatographic system used was aluminum TLC plates coated with silica gel 60 F254 as stationary phase and mixture of two solvens as mobile phase. The chormatographic system was simpler and lower cost than that reported earlier. A mixture of toluene-diethylamine (10:0.3v/v) resulted a fairly compact spot with Rf of 0.58±0.02 and short elution time. Spraying the TLC plate with Dragendorff reagent obtained orange coloured spot observed corresponding to sibutramine HCl.

The method showed a good specificity. There was not observed any interference from the sample components and others at Rf of sibutramine HCl (Figure 2). The linear regression analysis of the calibration curve between the amount of sibutramine HCl and peak area showed good linear relationship over the concentration range of 0.5-5µg/spot ($r^2 \geq 0.9986$) (Figure 3). The LOD and LOQ values obtained were at 217.5ng and 724.9ng/spot, respectively.

The precisions of the method were found to be satisfactory as the RSD values determined by repeatability and intermediate precision studies were all less than 2.0% as shown in table I. The accuracy of the method was determined from the recovery studies.

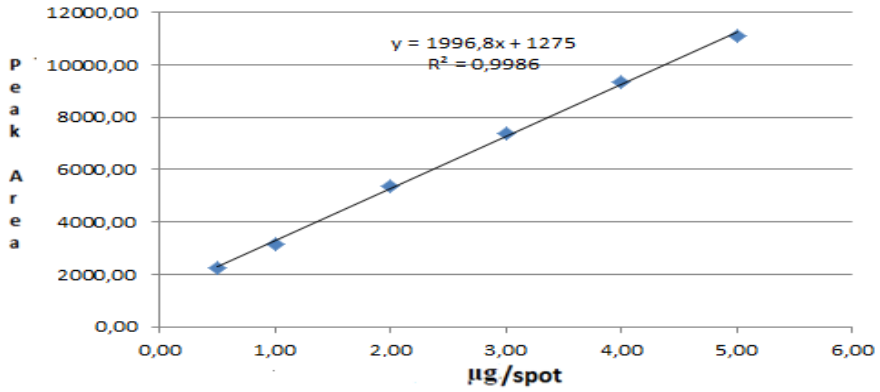
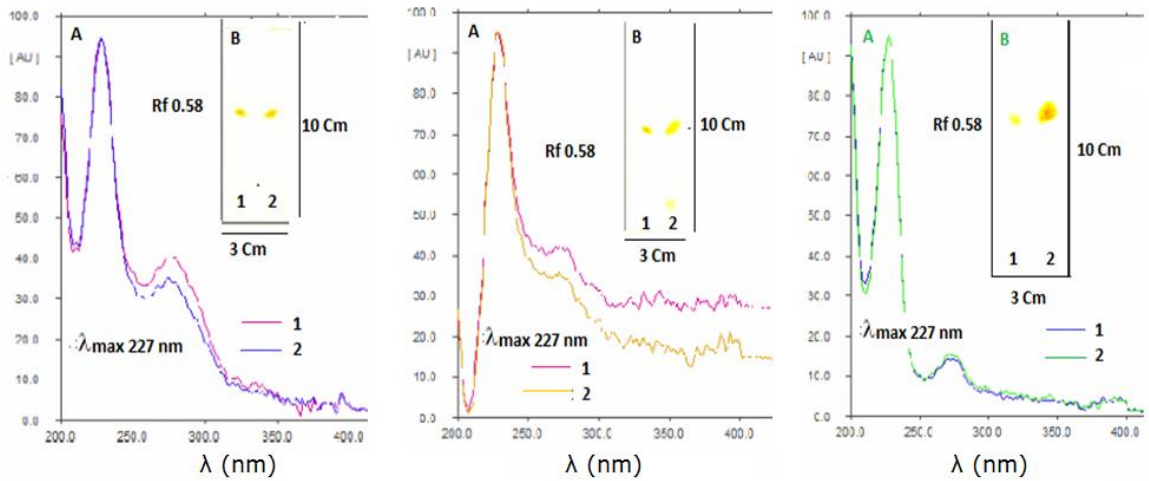
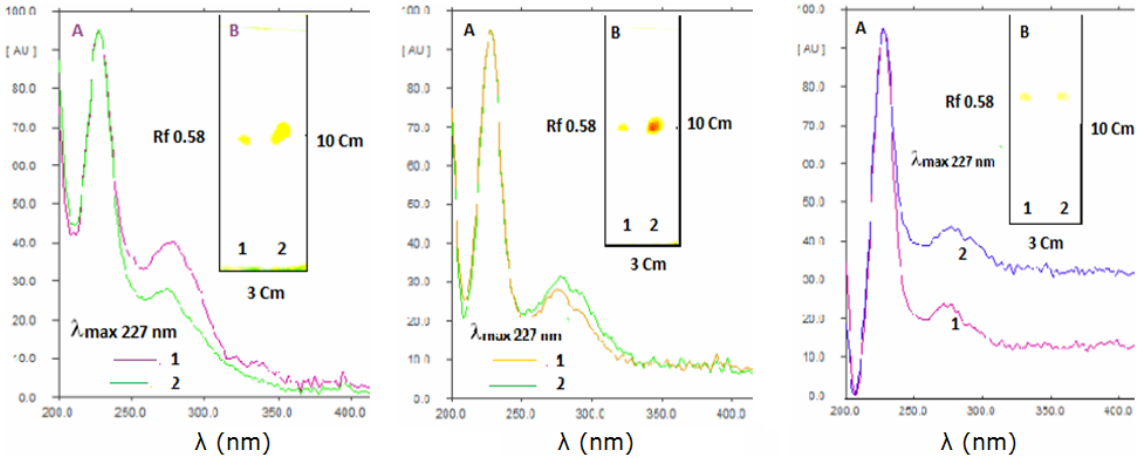


Figure 3. Calibration curve between peak area and concentration.

Figure 4. UV spectrum of the chromatogram spot (A) and TLC chromatogram after spraying with Dragendorff reagent.



Sampel AR Sampel SU Sampel DI



Sampel SL Sampel LK Sampel LM

Mean recoveries obtained from blank sample addition to give 1, 2 and 3 µg/spot sibutramine HCl were 99.57±1.57%; 100.32±2.06%, and 99.20±1.24%, repectively (Table II).

Qualitative analysis of seven samples found six samples containing sibutramine (Figure 4). Spots from the six samples had similarity in Rf, UV spectrum and colour with those from a sibutramine HCl standard. Results of the determination of sibutramine HCl concentration in the samples showed that the slimming products contain 2.45-26.24mg sibutramine HCl per single dosage of slimming products (Table III). The size and colour intensity of the spots were in line with the amount of sibutramine HCl (Figure 4B, Table III). The results illustrated the alarming public health situation, particularly since sibutramine increases the risk of cardiovascular events.

CONCLUSIONS

The above proposed TLC-densitometric method for the determination of sibutramine adulterated in herbal slimming product was successfully developed. The method gave satisfactory specificity, linearity, precision and accuracy validation criteria, was simpler and lower operating cost, could therefore be useful to small laboratory or local health authorities to conduct safety surveillance of herbal slimming products.

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REFERENCES

Adamovics JA. and Eschbach JC., 1997, Planar Chromatography, in *Chromatographic Analysis of Pharmaceuticals*, (Edited by Adamovics, JA), Marcel Dekker, New York., p. 57-77.

Ariburnu E., Uludag M.F., Yalcinkaya H. and Yesilada E., 2012. Comparative determination of sibutramine as an adulterant in natural slimming products by HPLC and HPTLC densitometry. *J. Pharm. and Biomed. Anal.*, 64– 65: 77– 81

Ancuceanu R., Dinu M. and Arama C., 2013. Weight loss food supplements: Adulteration and multiple quality issues in two products of Chinese origin. *Farmacia*, vol. 61, 1, 28-44

Badan Pengawas Obat dan Makanan (BPOM) RI, 2014. Hasil Pengawasan Obat Tradisional Mengandung Bahan Kimia Obat. http://www.pom.go.id/new/index.php/view/pers/242/Hasil-Pengawasan--Obat-Tradisional-Mengandung-Bahan-Kimia-Obat.html

Bogusz MJ., Hassan H., Enazi E., Ibrahim Z., Tufail M. and Bohrer D., 2006. Application of LC–ESIMS–MS for detection of synthetic adulterants in herbal remedies. *J. Pharm. Biomed. Anal.* 41. 554–564.

Canadian Medical Association, 2002. Obesity Drug Sibutramine (Meridia): Hypertension and Cardiac Arrhythmias. *Can. Adverse Reaction Newsletter*, 1307-1308.

Carvalho LM., Martini M., Moreira APL., de Lima APLS., Correia D., Falca T., Garcia SC., de Bairros AV., do Nascimento PC. and Bohrer D., 2011, Presence of synthetic pharmaceuticals as adulterants in slimming phytotherapeutic formulations and their analytical determination. *For. Sci. In.*, 204, 6–12

Chen SP., Tang MH., Ng SW., Poon WT., Chan AY. and Mak TW., 2010. Psychosis associated with usage of herbal slimming products adulterated with sibutramine: a case series. *Clin. Toxicol.*, 48(8): 832-8.

Daglioglu N and Akcan R., 2012. Sibutramine, chinese herbal drug, clinical medicine, forensic toxicology . *Nobel Med*, 8(1): 100-102

Departemen Kesehatan Republik Indonesia, 1994. *Kodifikasi Peraturan Perundang-undangan Obat Tradisional*. Direktorat Jendral Pengawasan Obat dan Makanan: Departemen Kesehatan Republik Indonesia.

Haneef J., Shaharyar M., Husain A., Rashid M., Mishra R., Siddiqueb N.A. and Pal M., 2013. Analytical methods for the detection of undeclared synthetic drugs in traditional herbal medicines as adulterants. *Drug Test. Analysis*, 5, 607–613

Health Authority Abu Dhabi (HAAD), 2012. Adulterated Herbal Weight Reduction

http://www.haad.ae/haad/tabid/58/ctl/Details/Mid/417/ItemID/262/Default.aspx

ICH guidelines, 2005. *International Conference on Harmonisation Guidelines on Validation of Analytical Procedure: Text and Methodology Q2 (R1)*, Geneva, 1-8.

Kanan S., Abu-Yousef LA., Gunasekar C., Abdo N. and Narasimhan S., 2009. Detection and quantification of synthetic drugs in herbal slimming formula. *Eur. J. Sci.* Res. 34. 348–357.

Khazan M., Hedayati M., Kobarfard F., Askari S. and Azizi F., 2014. Identification and Determination of Synthetic Pharmaceuticals as Adulterants in Eight Common Herbal Weight Loss Supplements . *Iran Red Crescent Med J.*, 16(3): 15344.

Mathon C., Ankli A., Reich E., Bieri S. and Christen P., 2014. Screening and determination of sibutramine in adulterated herbal slimming supplements by hptlc-uv densitometry. *Food Addit Contam. Part A. Chem Anal Control Expo Risk Assess.*, 31(1):15-20.

Monika H., Munish G., Jyoti S., Sandeep S. and Sneha D., 2011. A Recent Update on Undeclared Chemicals in Herbal Formulations, *JPBMS,* 9 (03), 1-4.

Oberholzer HM., Bester MJ., van der Schoor C. and Venter C., 2014. Weight Loss Products Adulterated with Sibutramine: A Focused Review of Associated Risks. *J. Endocrinol Diabetes Obes., 2(4): 1061-7*

Phattanawasin P., Uthai S., Tasamaporn S., Jariya A. and Sarunyaporn K., 2012. Quantitative Determination of Sibutramine in Adulterated Herbal Slimming Formulations by TLC-Image Analysis Method. *For. Sci. In.*, 219, 96-100.

World Health Organization (WHO), 2005. *National policy on traditional medicine and regulation of herbal medicines.* Report of a WHO Global Survey, Geneva.