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Simultaneous identification of caffeine¸ acetaminophen, sildenafilcitrate, tadalafil and vardenafil HCl in aphrodisiac traditional herbal medicines by Thin Layer Chromatography-Densitometry

Reni Septiani[1] and Sophi Damayanti[2]

[1]National Agency of Drug and Food Control of the Republic of Indonesia, Jl. Percetakan Negara No 23, Jakarta 10560, Indonesia
[2]Pharmacochemistry Research Group, School of Pharmacy Bandung Institute of Technology, Labtek VII, Ganesha 10 Bandung 40132, Indonesia

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ABSTRACT

Thin Layer Chromatography (TLC)-Densitometry has been developed and validated for the identification of caffeine, acetaminophen, sildenafil citrate, tadalafil and vardenafil HCl in traditional medicine aphrodisiac simultaneously. Liquid-liquid extraction method was used for sample preparation and then continued by TLC-Densitometry using TLC-Scanner-4 CAMAG. The method used silica gel 60 F_{254} as the stationary phase. Mobile Phase for TLC was ethyl acetate, n-propanol, NH_3 25%(45:5:1). Rf values ofcaffeine, acetaminophen, sildenafil citrate, tadalafil and vardenafil HCl and tadalafil were 0.57, 0.69, 0.27, 0.77 and 0.42, respectively. Resolution value (R) of caffeine-acetaminophen, acetaminophen-tadalafil, sildenafil citrate-vardenafil HCl, and vardenafil HCl-caffeine was obtained in a value of 2.0, 1.5, 2.4 and 2.4, respectively. This method have limits of detection (LOD) of reference standards for caffeine, acetaminophen, sildenafil citrate, tadalafil and vardenafil HCl were 0.1475, 0.2273, 0.1346, 0.1951 and 0.2047 µg/g spot whereas LOD of spiking were 0.3350, 0.3817, 0.1716, 0.3031 and 0.2659 µg/g spot,respectively.

Keywords: Traditional Herbal Medicines, Aphrodisiac, TLC-Densitometry, Liquid-liquid extraction, Validation

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INTRODUCTION

The utilization of traditional herbal medicines has increased in society due to its nature origin that is considered safe. However, chemicals adulteration in traditional medicines is also increasing. One of traditional medicine claim revealed that it contains compound for aphrodisiac. Chemicals adulteration in traditional medicines of aphrodisiac turned out quite rapidly from year 2005-2013. Chemicals that have been reported used in traditional medicines are caffeine, acetaminophen, sildenafil citrate, tadalafil and vardenafil HCl[(1,2,3)]. Caffeine acts as a stimulant of the central nervouswhereas sildenafil citrate, tadalafil and vardenafil are used for the treatment of erectile dysfunctionAcetaminophen acts as analgesic and used to treat headache as a side effect of sildenafil citrate[(4,5,6,7,8)].

Research in simultaneous aphrodisiac chemicals analysis that have been reported are determination of sildenafil, tadalafil, vardenafil HCl and its analogue in herbal medicine using TLC and HPLC-PDA-MS[(5)]. Simultaneous determination of sildenafil, tadalafil, vardenafil, stimulants (caffeine and ephedrine) and anorectic drugs were also reported by LC-HMRS[(9)]. However, simultaneously determination of caffeine, acetaminophen, sildenafil citrate, tadalafil, and vardenafil HCl in aphrosidiac traditional medicine have not been reported.

In this paper, we reported a TLC-Densitometry method that is simple, selective and sensitive to identify the presence of caffeine, acetaminophen, sildenafil citrate, tadalafil, and vardenafil HCl, in traditional medicines of aphrodisiac. The method has been validated according to USP36 guidelines[10].

MATERIALS AND METHODS

Camag TLC System

Camag TLC system consists of Automatic TLC Sampler (ATS), Automatic Development Chamber2 (ADC 2) a twin trough chamber, Spectrodensitometer and Visualizer.

Materials and Reagents

Reference standards used were caffeine, acetaminophen, sildenafil citrate and tadalafil, which obtained from National Agency of Drug and Food Control of the Republic of Indonesia (NAFDC) and vardenafil HCl from the European Pharmacopoeia. Plate of silica gel was GF254 (Merck). Sample used are blank traditional and traditional medicines that are included in the list of NAFDC public warning.

Preparation of mobile phase

The mobile phase was prepared using ethyl acetate, n-propanol, and NH_3 25% (45:5:1 v/v).

Plate activation

TLC plate were eluted with methanol, dried in an oven 120°C for 30 minutes, cooled and stored in a desiccator[13].

Preparation of single and the mixture standard solutions

Single and mixture standard solutions were made by dissolving references standards in methanol to obtain a concentration of 0.5 mg/mL for each reference.

TLC conditions

Samples and standards were applied using ATS-4 on a commercial 20 cm x 10 cm pre-coated TLC Silica gel GF254. The application conditions were: spray gas: air; application volume: 5µl; bandwidth: 5 mm; distance between tracks: 10 mm; distance from bottom: 10 mm. Elution was carried out using the ADC-2 with twin trough chamber. The ADC conditions: pre-drying: 5 minutes, saturation: 30 minutes, pre-conditioning: 1 minute, eluation distance: 7.5 cm and post drying: 15 minutes.

Liquid-liquid extraction

Liquid-liquid extraction method was used for sample preparation. At first, one gram sample was added with 5 mg each reference standards then put in a 250 mL Erlenmeyer and added 50mL of methanol, shaken for 60 minutes, filtered and evaporated with a rotary evaporator at 60°C. The methanol extract was added with 20 mL of water and 1N NaOH to pH 12, shaken for 30 min and extracted with 20 mL of ethyl acetate. Ethyl acetate extract was then collected, evaporated at 60°C and diluted with 10 mL of methanol.

Preparation of Calibration Curve

The calibration curve obtained by diluting standard and sample addition solution with analyte to obtain concentration 1.5 mg/mL of each solution. 1.2, 1.4, 1.6, 1.8, and 2.0 mL were then diluted by methanol to 5mL, respectively.

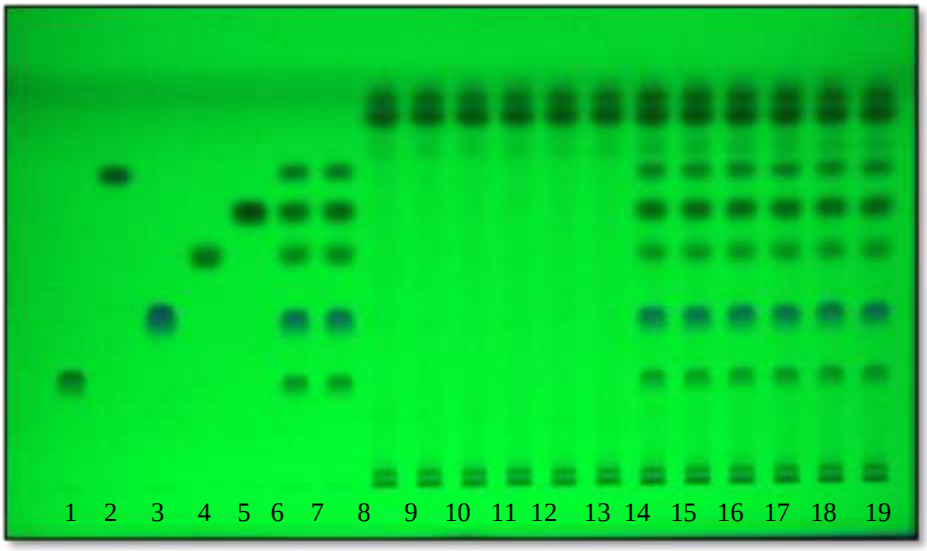
Identification of chemical drugs on Commercial Traditional Medicines

Several packs of commercial traditional medicine for aphrodisiac were homogenized and weighed then extracted by liquid-liquid extraction method. The extract obtained was evaporated and diluted with methanol, then was applied to the TLC plate and waseluted with ethyl acetate-n propanol-NH_3 25% (45:5:1). The TLC plates were measured by TLC Scanner-4 CAMAG.

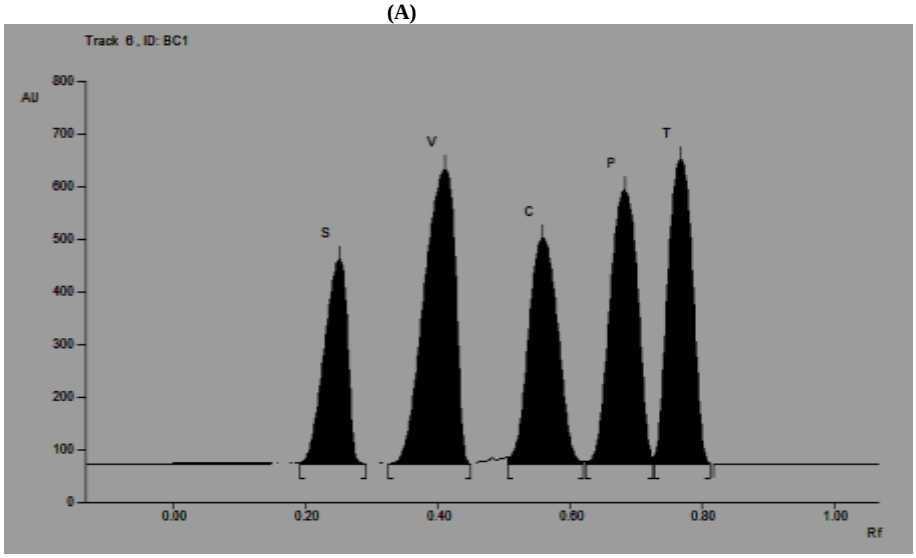
RESULTS AND DISCUSSION

The developed method was validated as per USP 36 guidelines[10] and parameters were evaluated for specificity and limit detection. The optimization of mobile phase has resulted Rf values of caffeine, acetaminophen, sildenafil citrate, tadalafil and vardenafil HCl and tadalafil were 0.57, 0.69, 0.27, 0.77 and 0.42, respectively. Blank traditional medicine has been tested and showed no chemicals and drugs. It has a composition of 0.8 g *Eurycoma longifoliae, Retrofracti Piperis* 0.96 g, *Rhizoma Zingiberis* 0.64 g, 0.64 g *Caryophlis* and the other ingredients up to 7 g. Optimation of liquid-liquid extraction method has been used for sample preparation and was combined with an

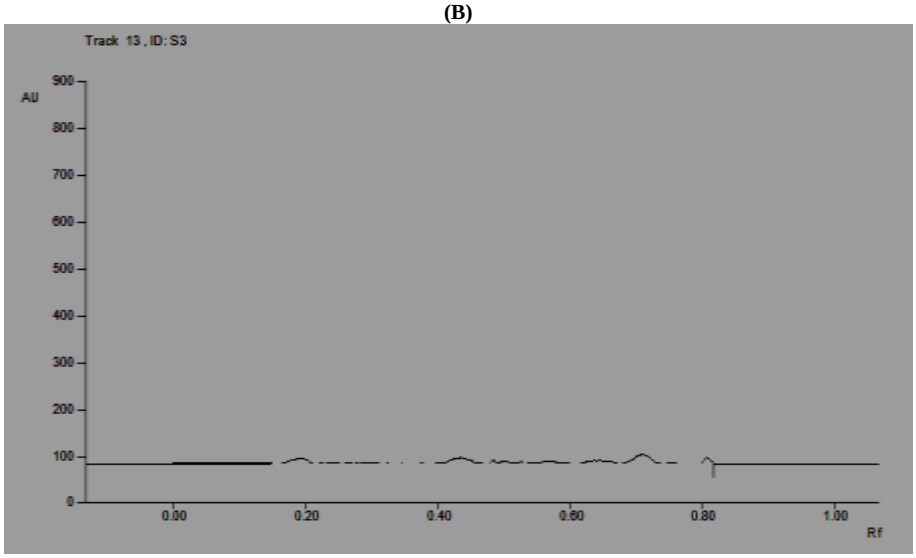
optimized mobile phase. It showed no impurities that interfered on TLC plates and densitogram (Figure 1). Moreover, it showed that the method was specific.



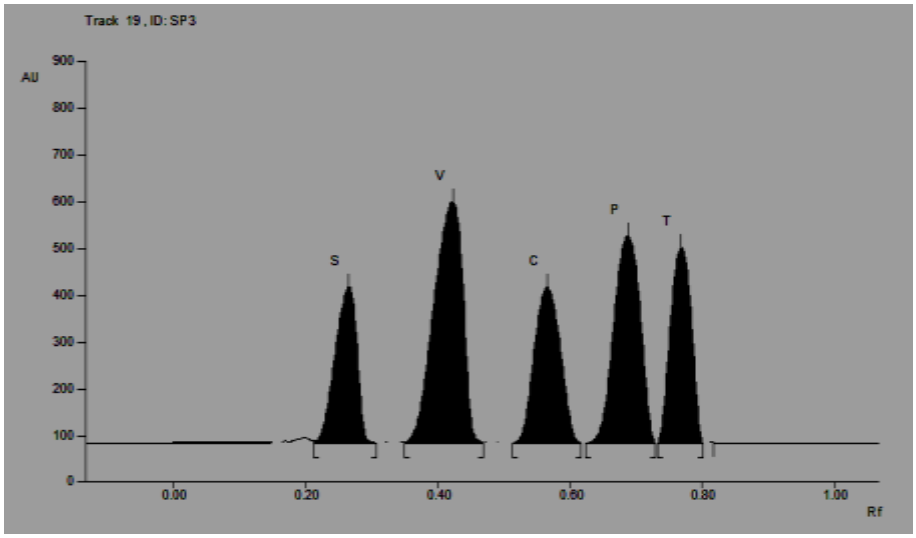
(A)



(B)



(C)

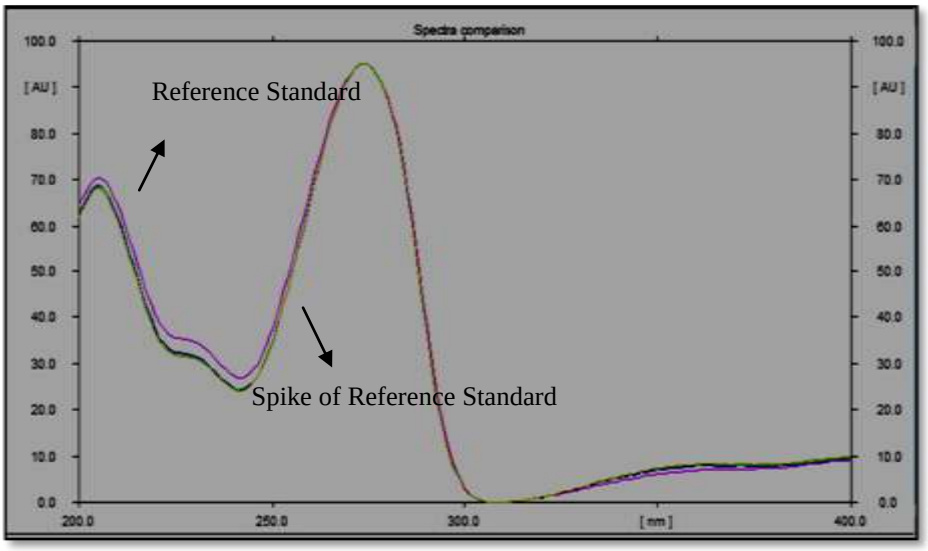


(D)

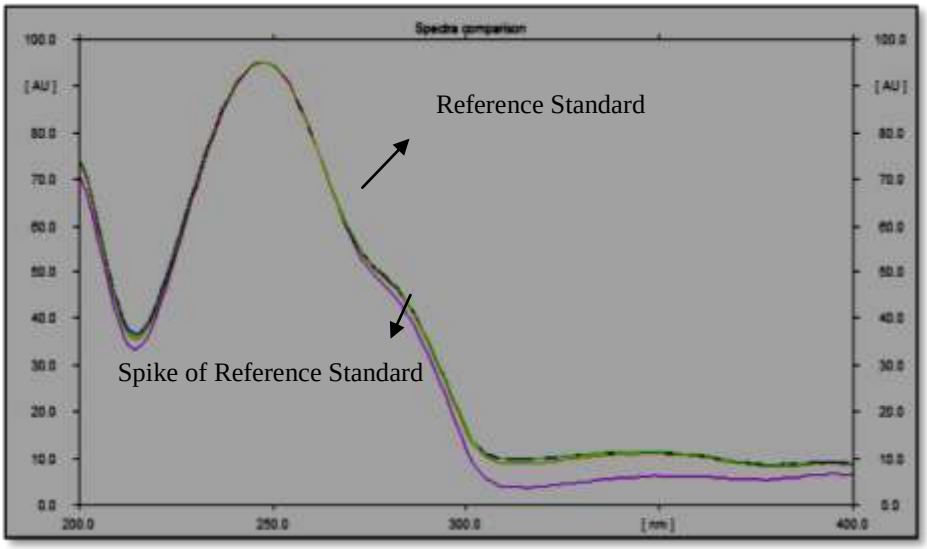
Figure 1 Densitogram of Specifity Test (A) (1) Sildenafil, (2) Tadalafil, (3) Vardenafil, (4) Caffeine, (5) Acetaminophen, (6-7) Mixture standards, (8-13) Blank Traditional Medicine and (14-19) Traditional Medicine Spike with reference standards, (B) Mixture standards (C) Blank traditional medicine, and (D) Traditional Medicine Spike with reference standards.
Note: (S) Sildenafil Sitrat, (V) Vardenafil, (C) Caffein, (P) Acetaminophen dan (T) Tadalafil.

In the analytical methods involving chromatography, selectivity was determined by calculating the resolution (R)[12]. Resolution value of the five analytes was greater than 1.5 and fulfilled the requirement[13]. Resolution value (R) of caffeine-acetaminophen, acetaminophen-tadalafil, sildenafil citrate-vardenafil HCl, and vardenafil HCl-caffeine was obtained in a value of 2.0, 1.5, 2.4 and 2.4, respectively.

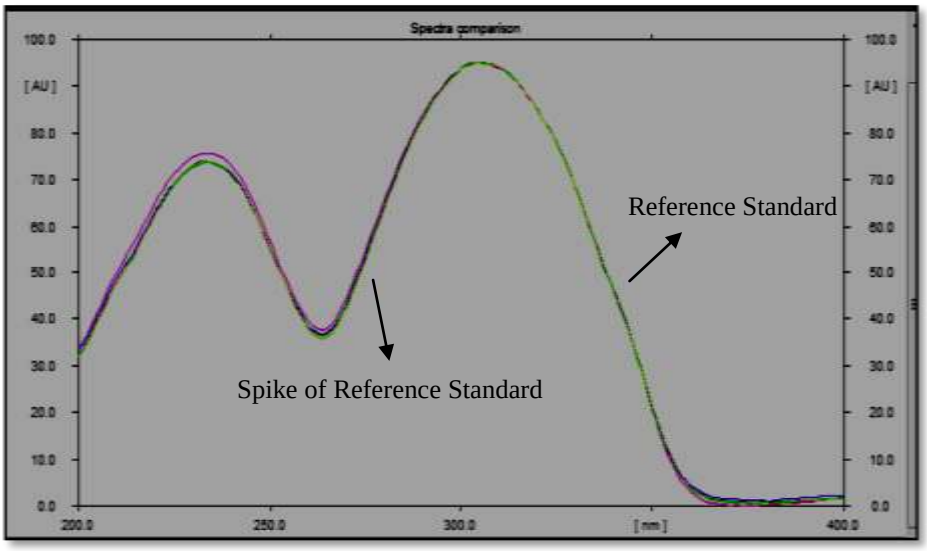
Unique chromatogram and spectrumof maximum wavelength of each analyte proved that densitometry method was selective for the separation of the five analytes (Figure 2). The wavelength of each analyte were 274nm (caffeine), 248nm (acetaminophen), 233 and 304 nm (sildenafil citrate), 236 nm and 290 nm (tadalafil), and 251 nm (vardenafil HCl).



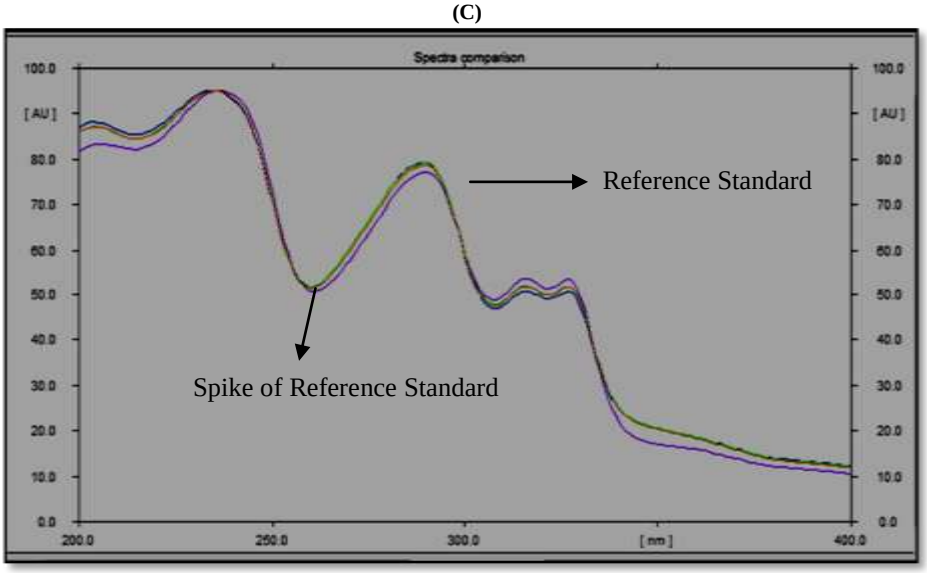
(A)


Spectra comparison
Reference Standard
Spike of Reference Standard
[AU]
[nm]

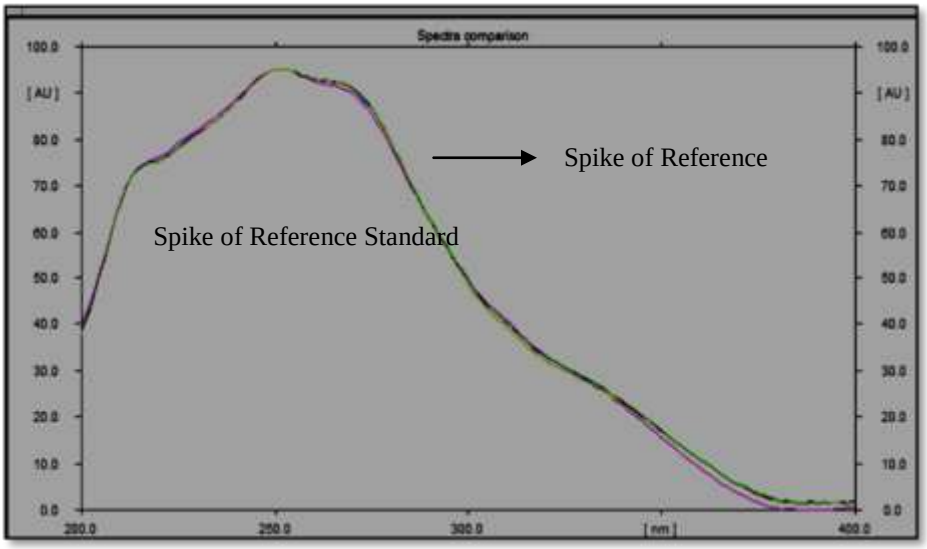
(B)


Spectra comparison
Reference Standard
Spike of Reference Standard
[AU]
[nm]

(C)


Spectra comparison
Reference Standard
Spike of Reference Standard
[AU]
[nm]

(D)



(E)

Figure2 Chromatogram spectrum: (A) Caffeine, (B) Acetaminophen, (C) Sildenafil Citrate, (D) Tadalafil and(E) VardenafilHCl

The limit of detection was done using the experimental method using a calibration curve between concentration and area. Concentration of the solution was obtained from standard solutions and dilutions of spiking into five levels of concentration (371 – 641 µg/mL) and each concentration was measured by value of the area. The limit of detection can be expressed as 3.3 σ/S[(14)].

Reference standard calibration curve has better correlation coefficient and the detection limitis smaller than spike of reference standard, it is caused by the influence of the sample matrix effects (Table 1).

Table1.Limit of Detection of Reference and Spike Standard

Analyte	Reference standard		Spike of reference standard	
	r	µg/g spot	r	µg/g spot
Caffeine	0.9996	0.1475	0.9971	0.3350
Acetaminophen	0.9989	0.2273	0.9964	0.3817
Sildenafil citrate	0.9996	0.1346	0.9993	0.1716
Tadalafil	0.9920	0.1951	0.9977	0.3031
Vardenafil HCl	0.9991	0.2047	0.9983	0.2659

The analytical method for the determination of chemical drugs contained in the traditional medicine of solid dosage has been tested on nine traditional medicines with claims aphrosidiac which includes a list of traditional medicines containing chemicals drugs based on data from a public warning NADFC (Table 2).

Table 2. Result Analysis for Traditional Medicines

Sample Code	Resulted
A, B, E	Caffein, acetaminophen,sildenafil citrate
C	Caffeine, sildenafil citrate
D	Tadalafil
F,G, H	Sildenafil sitrat
I	Acetaminophen,sildenafil citrate

CONCLUSION

Simultaneous analysis method of caffeine, acetaminophen, sildenafil citrate, tadalafil and vardenafil using TLC-densitometry has been proved selective, sensitive, simple and applicable for determination of the addition of chemicals drugs for traditional medicine aphrosidiac solid dosage form.

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REFERENCES

[1] NADFC, *Public Warning 2001 –2010*, **2010.**
[2] NADFC, *Public Warning No. HM.03.05.1.43.09.12.6081*, **2012**
[3] NADFC, *Public Warning No. HM.03.05.1.43.11.13.4940*, **2013**
[4] British Pharmacopoeia **I-II**, Medicines and Healthcare Products Regulatory Agency, The Stationery Office, London, **2009**, 897-901
[5] Y. Cai, T.G. Cai, Y. Shi, X.L. Cheng, L.Y. Ma, S.C. Ma, R.C. Lin, W. Feng, *Journal of Liquid Chromatography & Related Technologies***, 2010,**33**,** 1287-1306
[6] H. Goker; M. Coskun, G.A. Kulcigil, In S. Dhanarasu, Dr (Ed.), *Chromatographic and Its Aplications*, InTech, Rijeka – Croatia, **2012,** 51-52. ISBN: 978-953-51-0357-8.
[7] M. Mahmoudian, *Iranian Journal of Pharmacology & Therapeutics*, **2005**, 4(2), 72-75
[8] S. Sing, B. Prasad, A.A, Savaliya, R.P.Shah, V.M. Gohil, A. Kaur. *Trends in Analytical Chemistry*, **2009**, 28 (1), 13-17
[9] S.S. Rossi, S. Odoardi, E. Castrignano, G. Serpelloni, M. Chiarotti., *Journal of Pharmaceutical and Biomedical Analysis*, **2014**.
[10] The United State Pharmacopeia. The United States Pharmacopoeial Convention, **2013,** 36(1), 987
[11] E.H. Deinstrop. *Applied Thin-Layer Chromatography, Best Practice and Avoidance Mistake, Second, Revised and Enlarge Edition*, Wiley-VCH Verlag GmbH & Co. KgaA, German, **2007,** 43.
[12] Harmita. *Majalah Ilmu Kefarmasian*, **2004,** I(3), 117-113
[13] J. Ermer, In J. Ermer, J. H..Mcb, Miller (Eds) *Method, Validation in Pharmaceutical Analysis*, WILEY-VCH Verlag GmbH, & Co. KGaA, Weinheim, **2005**, 15
[14] *Text On Validation Of Analytical Procedur*, ICH Harmonized Tripartit Guideline (**1994**)