HOSTED BY

ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Floral research http://dx.doi.org/10.1016/j.apjtb.2015.11.006

Quantitative determination of vitexin in *Passiflora foetida* Linn. leaves using HPTLC

Aussavashai Shuayprom[1], Donruedee Sanguansermsri[2,3], Phanchana Sanguansermsri[1], Ian Hamilton Fraser[4], Nalin Wongkattiya[5*]

[1]*Department of Biochemistry, Faculty of Medical Science, Naresuan University, Phitsanulok 65000, Thailand*

[2]*Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Phitsanulok 65000, Thailand*

[3]*Centre of Excellence in Medical Biotechnology, Faculty of Medical Science, Naresuan University, Phitsanulok 65000, Thailand*

[4]*School of Chemistry, Monash University, Clayton 3800, Victoria, Australia*

[5]*Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai 50290, Thailand*

ARTICLE INFO

Article history:
Received 25 Sep 2015
Received in revised form 14 Oct,
2nd revised form 3 Nov,
3rd revised form 10 Nov 2015
Accepted 25 Nov 2015
Available online 2 Jan 2016

Keywords:
HPTLC
Thin layer chromatography
Vitexin
Passiflora foetida Linn.

ABSTRACT

Objective: To establish a simple, rapid, precise and accurate high performance thin layer chromatography (HPTLC) method with densitometric detection for the determination of vitexin in *Passiflora foetida* Linn. (*P. foetida*).

Methods: Ethanolic extract of the plant leaf powder was used for the experimental work. Separation was performed on silica gel 60 F_{254} HPTLC plates with ethyl acetate: methanol: distilled water: formic acid in the proportion of 50:2:3:6 (v/v), as the mobile phase. The determination was carried out using the densitometric absorbance mode at 340 nm.

Results: Vitexin response was linear over the range of 2.5–17.5 μg/mL with a correlation coefficient of 0.996. Intraday and interday precision studies showed the relative SD was < 3%. Accuracy of the method was determined and the average recovery was 100.3%. The limit of quantitation and limit of detection were 0.879 and 0.290 μg/mL, respectively. The contents of vitexin in *P. foetida* leaf extracts were within the range of 0.030%–0.310%.

Conclusions: The method was evaluated for sensitivity, accuracy, precision and reproducibility. Each analysis by HPTLC is less expensive than current methods. This method is suitable for routine quality control of raw material of the leaves of *P. foetida* extract and its products.

1. Introduction

Passiflora foetida Linn. (*P. foetida*) (family Passifloraceae) is native to South America, which has spread to tropical regions around the world, including Thailand. The leaves of this plant are also utilized as traditional medicine for the treatment of hysteria, fever, ear infections, emmenagogue, asthma, insomnia and skin disease [1–6]. The ethanolic extracts of the leaves of *P. foetida* display remarkable activity against *Pseudomonas putida*, *Vibrio cholerae*, *Shigella flexneri* and *Streptococcus pyogenes*. A further study conducted by Mohanasundari *et al*. did not show the active compound present in the crude extract of *P. foetida* [7]. The major phytochemical constituents of *P. foetida* have several active constituents like hydrocyanic acid, groups of flavonoids, harman alkaloids, passifloricins, polyketides, α-pyrones and vitexin [2,8,9]. Vitexin has been reported to have antioxidant, anti-inflammatory, anti-thyroid, anti-arteriosclerotic, antihypertensive and antihepatotoxic properties [10–23]. Levels of vitexin in different plants extracts have been determined by numerous techniques, including spectroscopic and chromatographic methods. High performance thin layer chromatography (HPTLC), coupled with densitometric

*Corresponding author: Nalin Wongkattiya, Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai 50290, Thailand.
Tel: +66 877880432
E-mail: nalin.wongkattiya@gmail.com

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

detection, is among the various methods reported for determination of vitexin [24–30]. HPTLC has advantages because of high sample throughput at low operating cost, easy sample preparation, short analysis time and use of a small quantity of mobile phase unlike high performance liquid chromatography. The major advantage of HPTLC is that it is both a qualitative and a quantitative technique [31–37]. The objective of this work was to develop a quick, economical HPTLC method for vitexin determination in the ethanolic extract of leaves of *P. foetida*. The proposed method was validated in compliance with International Conference on Harmonization guidelines [38].

2. Materials and methods

2.1. Apparatus

A Camag HPTLC system equipped with an automatic thin layer chromatography (TLC) sampler 4 (ATS 4, connected to a nitrogen tank), TLC scanner 3 (winCATS version 1.3.4), and UV cabinet (Reprostar 3) and automatic developing chamber was used for the analysis.

2.2. Reagents and materials

Ten samples of *P. foetida* were collected from areas where it has colonized in Thailand during January 2013. The samples were identified by comparison with the plant specimens in the Herbarium Section, Forestry Department, Bangkok, Thailand. Solvents were obtained from Merck (Darmstadt, Germany) and were of analytical grades. Standard vitexin (95% pure) was obtained from Sigma–Aldrich Chemie (Steinheim, Germany). Precoated silica gel 60 F_{254} HPTLC plates were procured from Merck (Darmstadt, Germany).

2.3. Preparation of standard stock solution

A vitexin (100 μg/mL) stock solution was prepared with methanol to give a series of working standard solutions with concentrations of 2.5, 5.0, 10.0, 12.5 and 17.5 μg/mL.

2.4. Sample preparation

The leaves of *P. foetida* were separated from the stems, washed thoroughly and dried in an oven at 50 °C. The dried samples were ground to powder. The powder was extracted with ethanol (3 × 10 mL), filtered and concentrated under vacuum to obtain the crude extract. A total of 200 mg of the accurately weighed extract was transferred to a 10 mL volumetric flask. Methanol was added to the volumetric flask (final concentration of 20 000 μg/mL).

2.5. Mobile phase

The mobile phase was comprised of ethyl acetate: methanol: distilled water: formic acid in the proportion of 50:2:3:6 (v/v).

2.6. Method validation

The analytical method was validated for linearity, precision, accuracy, limit of detection (LOD) and limit of quantitation (LOQ) according to the International Conference on Harmonization guidelines.

2.7. Linearity

Standard vitexin solutions from above were used for the determination experiment using silica gel 60 F_{254} HPTLC plates with a mobile phase of ethyl acetate: methanol: distilled water: formic acid (50:2:3:6, v/v). A total of 10 μL of each standard solution was applied to the plate as per the method of Kumar *et al*. [37]. A wavelength of 340 nm (λ_{max}) was used for quantitation. This was repeated in triplicate and the mean was used for calculation.

2.8. Precision

The precision of the method was studied by analyzing standard solutions of vitexin (5 and 10 μg/mL) after application on TLC plate ($n = 6$) on the same day for intraday precision and on 3 different days for interday precision by proposed method. The results were expressed as percentage of relative SD (%RSD).

2.9. Accuracy

Accuracy of the method was studied by performing recovery studies at 3 levels of vitexin reference standard added to the samples. Three different volumes (500, 750 and 1 000 μL) of standard solution (containing 5 μg/mL vitexin in methanol) were added to the sample solution (20 000 μg/mL) and analyzed by the densitometric HPTLC method.

2.10. LOD and LOQ

A plot of experimental concentration (μg/mL) against SD was performed to reflect a linear correlation and the value of the SD on the y-axis intercept was obtained. This intercept value was then multiplied by a factor of 3 to give the LOD or by 10 to give the LOQ.

2.11. Quantification of vitexin content in the leaves of *P. foetida* extract

Ten microliters of the plant leaf extract was applied to the HPTLC plate in triplicate and run as per the determination experiment above. The amount of vitexin in the extracted samples was then determined using linearity equation.

3. Results

The method developed was a normal phase HPTLC method adapted from Kumar using silica gel 60 F_{254} stationary phase precoated on aluminum sheet for the analysis [37]. Mobile phase used was ethyl acetate: methanol: distilled water: formic acid in the proportion of 50:2:3:6 (v/v), which gave good separation of vitexin ($R_f = 0.70$) from the other phytochemicals of *P. foetida*. Concentrations of vitexin in leaf extracts from various sites were in the range of 0.030%–0.310% (Table 1).

Identity of vitexin was confirmed by overlay of spectrum chromatograms obtained with the Camag TLC scanner. The vitexin from the plant extract was compared with the vitexin standard. The detector response of vitexin showed linearity over

Table 1
Vitexin contents in *P. foetida*. %.

Collection site (province)	Dry weight of vitexin
Bangkok	0.190 ± 0.004
Nakhon Pathom	0.030 ± 0.001
Ayutthaya	0.290 ± 0.006
Kanchanaburi	0.080 ± 0.004
Nonthaburi	0.240 ± 0.007
Pathum Thani	0.210 ± 0.020
Nakhon Nayok	0.290 ± 0.003
Rayong	0.310 ± 0.005
Chanthaburi	0.250 ± 0.003
Phrae	0.220 ± 0.002

Vitexin contents were presented as w/w of the dried *P. foetida* leaf, as obtained from 3 determinations. All values were expressed as mean ± SD.

the range of 2.5–17.5 μg/mL with a correlation coefficient of 0.996 (Table 2). The overlay of UV spectrum of standard vitexin and vitexin in plant extract was shown in Figure 1. HPTLC chromatograms of standard vitexin and vitexin in the plant extract were shown in Figure 2.

Instrument precision and interday and intraday precision were measured to determine the overall precision of the method. Percentage of RSD was found to be < 3% (Table 3).

Vitexin at various concentrations was determined by the TLC-densitometric method. The percent recovery at 3 different levels of vitexin was found to be 98.3%, 103.0% and 99.7%, with a mean value of 100.3% (Table 4).

Table 2
Method validation parameters for the quantification of vitexin by the proposed TLC-densitometric method.

Parameters	Results
Range of linearity (μg/mL)	2.5–17.5
Regression of equation	$y = 462.68x + 748.65$
Correlation coefficient (r^2)	0.996
LOQ (μg/mL)	0.879
LOD (μg/mL)	0.290

x: The amount of vitexin in μg/mL; *y*: The peak area at 340 nm.

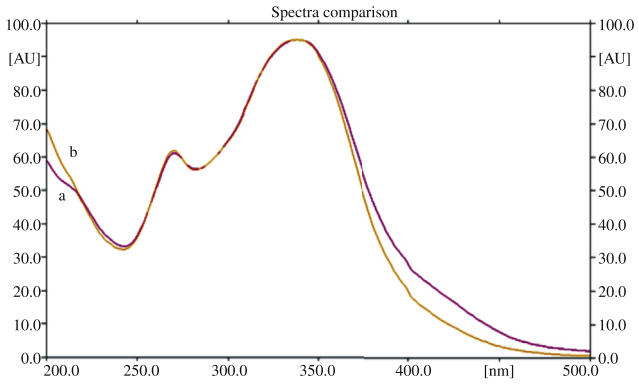

Figure 1. Overlay UV absorption spectra.
a: Vitexin standard; b: Vitexin in the sample extract of the leaves of *P. foetida*.

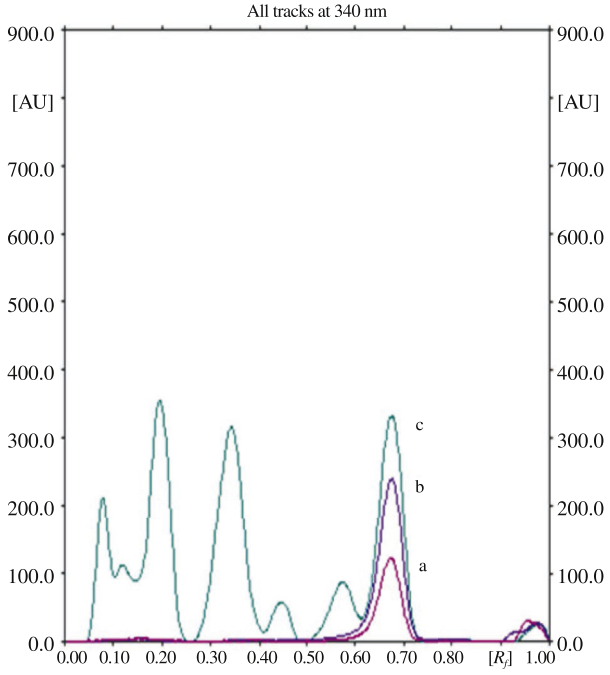

Figure 2. HPTLC chromatograms.
a: Standard vitexin (2.5 μg/mL); b: Standard vitexin (5 μg/mL); c: Vitexin in the ethanolic extract of leaves of *P. foetida*.

Table 3
Interday and intraday precision for quantification of vitexin determination by proposed TLC-densitometric method[a]. %.

Concentrations (μg/mL)	Interday precision	Intraday precision
5	2.93	1.85
10	0.05	0.94

[a]: Percentage of RSD (n = 6).

Table 4
Accuracy determined for the TLC-densitometric method.

Sample no.	Vitexin added (μg)	Theoretical content (μg)	Experimental content (μg)	Recovery (%)	RSD (%)
1	2.50	17.52	17.2 ± 0.2	98.3 ± 0.2	0.87
2	3.75	18.77	19.3 ± 0.3	103.0 ± 2.0	1.70
3	5.00	20.02	19.9 ± 0.1	99.7 ± 0.6	0.63

Values were presented as means ± SD (n = 3). Theoretical content: Calculated vitexin content in sample after spiking.

4. Discussion

Quantitative determination of vitexin in *P. foetida* was performed by HPTLC with densitometric detection. Tools used such as sample applicator, densitometer and chromatogram evaluation with electronic image acquisition, make HPTLC to be an important analytical technique. Various steps of HPTLC were run by fully automated machines but method development requires primary knowledge of TLC. Generally, there are two ways of sample application on HPTLC plate: contact application and spray-on technique. Contact application of sample causes irregular distribution of the sample and spots may be broad and asymmetric after development [39]. In this study, sample application using the spray-on technique (automatic TLC

sampler 4) produced narrow bands to ensure the best resolution of the samples.

The mobile phase for HPTLC was selected on the basis of the analyst's experience. In this study, vitexin was successfully separated with a mobile phase of ethyl acetate: methanol: distilled water: formic acid (50:2:3:6) which provided vitexin R_f of 0.70. Previous works on HPTLC determination of flavonoids and phenolic acid reported similar mobile phase systems of ethyl acetate: acetic acid: formic acid: distilled water (100:11:11:26) [40,41].

The linearity and sensitivity shown by this method was shown to be superior to that of reverse phase high performance liquid chromatography with detection limits, an order of magnitude lower using HPTLC. The response for vitexin in this study was shown to be linear over the range of 2.5–17.5 μg/mL compared to reverse phase high performance liquid chromatography that gave the range of 25–500 μg/mL [9].

Percentage of RSD of interday and intraday precision for quantification of vitexin determination by TLC-densitometric method in this study was found to be < 3%. This suggests that the proposed method is highly precise and reproducible for vitexin [38].

The present method provides a lower LOD than current methods. It is practical for routine analysis and could be used for pharmaceutical quality control of raw materials for regulatory purposes. The percent recovery for the method proposed here is comparable with previous methods [9]. The percentage of RSD and correlation coefficient values indicate a high reproducibility of the method and the proposed method is specific for determination of vitexin from a single plant species. The pattern produced is characteristic of *P. foetida* and can be readily profiled.

A TLC densitometric method was validated in terms of linearity, accuracy, precision, LOD and LOQ. This method offers high degree of sensitivity, economic and rapid analysis combined with single-step sample preparation. Simultaneously, a large number of samples along with the standard can be analyzed in one TLC plate and solvent requirement is negligible, thus making it far less expensive when compared to high performance liquid chromatography. In addition, it requires very small amount of sample and can detect active principle concentration at the nanogram level. The proposed method is simple, precise, accurate and sensitive and can be used for routine quality control of *P. foetida*, and standardizing the vitexin.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Puricelli L, Dell'Aica I, Sartor L, Garbisa S, Caniato R. Preliminary evaluation of inhibition of matrix-metalloprotease MMP-2 and MMP-9 by *Passiflora edulis* and *P. foetida* aqueous extracts. *Fitoterapia* 2003; **74**(3): 302-4.

[2] Dhawan K, Dhawan S, Sharma A. Passiflora: a review update. *J Ethnopharmacol* 2004; **94**(1): 1-23.

[3] Arung ET, Kusuma IW, Christy EO, Shimizu K, Kondo R. Evaluation of medicinal plants from Central Kalimantan for anti-melanogenesis. *J Nat Med* 2009; **63**(4): 473-80.

[4] Chivapat S, Bunjob M, Shuaoprom A, Bansidhi J, Chavalittumrong P, Rangsripipat A, et al. Chronic toxicity of *Passiflora foetida* L. extract. *Int J Appl Res Nat Prod* 2011; **4**(2): 24-31.

[5] Sasikala V, Saravanan S, Parimelazhagan T. Analgesic and anti-inflammatory activities of *Passiflora foetida* L. *Asian Pac J Trop Med* 2011; **4**(8): 600-3.

[6] Joseph Asir P, Priyanga S, Hemmalakshmi S, Devaki K. *In vitro* free radical scavenging activity and secondary metabolites in *Passiflora foetida* L. *Asian J Pharm Res Health Care* 2014; **6**(2): 3-11.

[7] Mohanasundari C, Natarajan D, Srinivasan K, Umamaheswari S, Ramachandran A. Antibacterial properties of *Passiflora foetida* L. – a common exotic medicinal plant. *Afr J Biotechnol* 2007; **6**(23): 2650-3.

[8] Echeverri F, Arango V, Quiñones W, Torres F, Escobar G, Rosero Y, et al. Passifloricins, polyketides alpha-pyrones from *Passiflora foetida* resin. *Phytochemistry* 2001; **56**(8): 881-5.

[9] Pongpan N, Luanratana O, Suntornsuk L. Rapid reversed-phase high performance liquid chromatography for vitexin analysis and fingerprint of *Passiflora foetida*. *Curr Sci* 2007; **93**(3): 378-82.

[10] Choi HJ, Eun JS, Kim BG, Kim SY, Jeon H, Soh Y. Vitexin, an HIF-1alpha inhibitor, has anti-metastatic potential in PC12 cells. *Mol Cells* 2006; **22**(3): 291-9.

[11] Picerno P, Mencherini T, Della Loggia R, Meloni M, Sanogo R, Aquino RP. An extract of *Lannea microcarpa*: composition, activity and evaluation of cutaneous irritation in cell cultures and reconstituted human epidermis. *J Pharm Pharmacol* 2006; **58**(7): 981-8.

[12] Kim J, Lee I, Seo J, Jung M, Kim Y, Yim N, et al. Vitexin, orientin and other flavonoids from *Spirodela polyrhiza* inhibit adipogenesis in 3T3–L1 cells. *Phytother Res* 2010; **24**(10): 1543-8.

[13] Sahreen S, Khan MR, Khan RA. Hepatoprotective effects of methanol extract of *Carissa opaca* leaves on CCl_4-induced damage in rat. *BMC Complement Altern Med* 2011; **11**: 48.

[14] Lee CY, Chien YS, Chiu TH, Huang WW, Lu CC, Chiang JH, et al. Apoptosis triggered by vitexin in U937 human leukemia cells via a mitochondrial signaling pathway. *Oncol Rep* 2012; **28**(5): 1883-8.

[15] Yao Y, Cheng XZ, Wang LX, Wang SH, Ren G. Major phenolic compounds, antioxidant capacity and antidiabetic potential of rice bean (*Vigna umbellata* L.) in China. *Int J Mol Sci* 2012; **13**(3): 2707-16.

[16] Abbasi E, Nassiri-Asl M, Sheikhi M, Shafiee M. Effects of vitexin on scopolamine-induced memory impairment in rats. *Chin J Physiol* 2013; **56**(3): 184-9.

[17] Demir Özkay U, Can OD. Anti-nociceptive effect of vitexin mediated by the opioid system in mice. *Pharmacol Biochem Behav* 2013; **109**: 23-30.

[18] Lu CC, Xu YQ, Wu JC, Hang PZ, Wang Y, Wang C, et al. Vitexin protects against cardiac hypertrophy via inhibiting calcineurin and CaMKII signaling pathways. *Naunyn Schmiedebergs Arch Pharmacol* 2013; **386**(8): 747-55.

[19] Simpson MJ, Hjelmqvist D, López-Alarcón C, Karamehmedovic N, Minehan TG, Yepremyan A, et al. Anti-peroxyl radical quality and antibacterial properties of rooibos infusions and their pure glycosylated polyphenolic constituents. *Molecules* 2013; **18**(9): 11264-80.

[20] Yang SH, Liao PH, Pan YF, Chen SL, Chou SS, Chou MY. The novel p53-dependent metastatic and apoptotic pathway induced by vitexin in human oral cancer OC2 cells. *Phytother Res* 2013; **27**(8): 1154-61.

[21] Sahreen S, Khan MR, Khan RA. Comprehensive assessment of phenolics and antiradical potential of *Rumex hastatus* D. Don. roots. *BMC Complement Altern Med* 2014; **14**: 47.

[22] Yang L, Yang ZM, Zhang N, Tian Z, Liu SB, Zhao MG. Neuroprotective effects of vitexin by inhibition of NMDA receptors in primary cultures of mouse cerebral cortical neurons. *Mol Cell Biochem* 2014; **386**(1–2): 251-8.

[23] Yang ZB, Tan B, Li TB, Lou Z, Jiang JL, Zhou YJ, et al. Protective effect of vitexin compound B-1 against hypoxia/reoxygenation-induced injury in differentiated PC12 cells via NADPH oxidase inhibition. *Naunyn Schmiedebergs Arch Pharmacol* 2014; **387**(9): 861-71.

[24] Pereira CA, Yariwake JH, Lanças FM, Wauters JN, Tits M, Angenot L. A HPTLC densitometric determination of flavonoids from *Passiflora alata, P. edulis, P. incarnata* and *P. caerulea* and comparison with HPLC method. *Phytochem Anal* 2004; **15**(4): 241-8.

[25] Ringl A, Prinz S, Huefner A, Kurzmann M, Kopp B. Chemosystematic value of flavonoids from *Crataegus x macrocarpa* (Rosaceae) with special emphasis on (*R*)- and (*S*)-eriodictyol-7-*O*-glucuronide and luteolin-7-*O*-glucuronide. *Chem Biodivers* 2007; **4**(2): 154-62.

[26] Zhang Y, Tie X, Bao B, Wu X, Zhang Y. Metabolism of flavone *C*-glucosides and *p*-coumaric acid from antioxidant of bamboo leaves (AOB) in rats. *Br J Nutr* 2007; **97**(3): 484-94.

[27] Wang J, de Yue Y, Jiang H, Tang F. Rapid screening for flavone *C*-glycosides in the leaves of different species of bamboo and simultaneous quantitation of four marker compounds by HPLC-UV/DAD. *Int J Anal Chem* 2012; **2012**: 205101.

[28] Zucolotto SM, Fagundes C, Reginatto FH, Ramos FA, Castellanos L, Duque C, et al. Analysis of *C*-glycosyl flavonoids from South American *Passiflora* species by HPLC-DAD and HPLC-MS. *Phytochem Anal* 2012; **23**(3): 232-9.

[29] da Silva Morrone M, de Assis AM, da Rocha RF, Gasparotto J, Gazola AC, Costa GM, et al. *Passiflora manicata* (Juss.) aqueous leaf extract protects against reactive oxygen species and protein glycation *in vitro* and *ex vivo* models. *Food Chem Toxicol* 2013; **60**: 45-51.

[30] Yan C, Liu H, Lin L. Simultaneous determination of vitexin and isovitexin in rat plasma after oral administration of *Santalum album* L. leaves extract by liquid chromatography tandem mass spectrometry. *Biomed Chromatogr* 2013; **27**(2): 228-32.

[31] Reich E, Widmer V. Plant analysis 2008–planar chromatography. *Planta Med* 2009; **75**(7): 711-8.

[32] Sherma J. Review of HPTLC in drug analysis: 1996–2009. *J AOAC Int* 2010; **93**(3): 754-64.

[33] Attimarad M, Ahmed KK, Aldhubaib BE, Harsha S. High-performance thin layer chromatography: a powerful analytical technique in pharmaceutical drug discovery. *Pharm Methods* 2011; **2**(2): 71-5.

[34] Kaale E, Risha P, Layloff T. TLC for pharmaceutical analysis in resource limited countries. *J Chromatogr A* 2011; **1218**(19): 2732-6.

[35] Shewiyo DH, Kaale E, Risha PG, Dejaegher B, Smeyers-Verbeke J, Vander Heyden Y. HPTLC methods to assay active ingredients in pharmaceutical formulations: a review of the method development and validation steps. *J Pharm Biomed Anal* 2012; **66**: 11-23.

[36] Kaale E, Manyanga V, Makori N, Jenkins D, Michael Hope S, Layloff T. High-performance thin layer chromatography to assess pharmaceutical product quality. *Trop Med Int Health* 2014; **19**(6): 747-51.

[37] Kumar KA, Shetty SR, Narasu L. HPTLC method development and validation for determination of rutin in flavanoidal fraction of *Hibiscus micranthus* Linn. *E J Chem* 2011; **8**(3): 1444-50.

[38] Renger B, Végh Z, Ferenczi-Fodor K. Validation of thin layer and high performance thin layer chromatographic methods. *J Chromatogr A* 2011; **1218**(19): 2712-21.

[39] CAMAG. Instruments, tools and concepts for HPTLC. Switzerland: CAMAG; 2015. [Online] Available from: http://www.camag.com [Accessed on 25th September, 2015]

[40] Vundać VB, Maleš Z, Plazibat M, Golja P, Cetina-Čižmek B. HPTLC determination of flavonoids and phenolic acids in some Croatian *Stachys* taxa. *J Planar Chromatogr Mod TLC* 2005; **18**: 269-73.

[41] Amanzadeh Y, Khanavi M, Khatamsaz M, Rajabi A, Ebrahimi SES. High-performance thin-layer chromatographic fingerprints of flavonoids and phenol carboxylic acids for standardization of Iranian species of the genus *Crataegus* L. *Iran J Pharm Sci* 2007; **3**(3): 143-52.