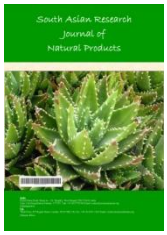


South Asian Research Journal of Natural Products

2(3): 135-143, 2019; Article no.SARJNP.50919

Method Development and Validation for the Estimation of Valerenic Acid Content in Valerian Tablets Using RP-HPLC Method

M. Sahiti[1] and B. M. Gurupadayya[1*]

[1]Department of Pharmaceutical Chemistry, JSS College of Pharmacy, JSS Academy of Higher Education and Research, Shivarathreeswara Nagar, Mysuru-570015, India.

Authors' contributions

This work was carried out in collaboration between both authors. Author MS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author BMG managed the literature searches and analyses of the study. Both authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Stefano Manfredini, Professor of Pharmaceutical Chemistry and Toxicology, Department of Life Sciences and Biotechnology, University of Ferrara, Italy.
(2) Elmarie Van Der Watt, Department of Soil, Crop and Climate Sciences, University of the Free State, South Africa.
(3) Dildar Ahmed, Professor, Department of Chemistry, Forman Christian College University Lahore, Pakistan.
Reviewers:
(1) Manohar V. Lokhande, Sathaye College, India.
(2) Syed Umer Jan, University of Balochistan, Pakistan.
Complete Peer review History: http://www.sdiarticle4.com/review-history/50919

Original Research Article

Received 29 June 2019
Accepted 01 September 2019
Published 03 October 2019

ABSTRACT

Valerian (*Valeriana officinalis*) has been used from so long as a traditional medicine since it has therapeutic effects such as enhances sleep, acts as an anxiolytic for nervous unrest etc. For determining the dose of herbal formulations and to estimate the quality of dosage form, we need to develop an effective reproducible validated method for the determination of the bioactive molecules essential for the sedative activity of the valerian tablets. So, the primary objective of the present work was to develop an efficient and sensitive method for the determination of valerenic acid content in valerian tablets using reverse-phase high-performance liquid chromatography technique. Since it is RP-HPLC method the column used was Phenomenex C18 Luna® 5 µm (250 x 4.6mm) column. The LC system used was Shimadzu UFLC, Nexera series utilizing a Photo-diode Array detector (SPD-M20A) detector and the software used was LC Solutions. The mobile phase used is

**Corresponding author: Email: bmgurupadayya@jssuni.edu.in;*

acetonitrile: Ortho-phosphoric -acid in the ratio of 97:3 v/v. Ortho-phosphoric acid (gradient mode) employed was of 5% solution with a pH of 3.5. The chromatographic conditions employed are 1ml/min flow rate and absorbances were observed at 220 nm. The selected chromatographic conditions were found to give effective separation of Valerenic acid at 4.4±0.2 min. Validation was carried out for linearity, system suitability, accuracy, precision (intra-day and inter-day), and robustness, the limit of detection and limit of quantification which were found to be within acceptable limits according to ICH Guidelines. Thus, the method proposed was found to be accurate, precise, reproducible and analyte-specific. In this work, based on the observations made from the literature review a relatively best suitable method was developed for the estimation of valerenic acid in valerian tablets with shortened retention time along with better resolution thus, reducing the wastage of solvents and the time of analysis.

Keywords: RP-HPLC; Valeriana officinalis; valerenic acid; validation.

1. INTRODUCTION

The perennial herb valerian comprises of iridoids also called as valepotriates. Its essential oil has multipart of non-volatile cyclopentane sesquiterpenes which are called as valerenic acid (and its derivatives). This valerenic acid is considered to have a remarkable influence on the cerebrospinal system, as a tranquillizer and sedative in cases of nervous disturbance and unrest, neuralgia and anxiety [1,2]. For determining the dose of herbal formulations and to estimate the quality of dosage form, we need to develop an effective reproducible validated method for the determination of the bioactive molecules essential for the sedative activity of the valerian tablets. So, the primary objective of the present work was to develop an efficient and sensitive method for the determination of valerenic acid content in valerian tablets using reverse-phase high-performance liquid chromatography technique. There were some assay methods by HPTLC and HPLC, but they have a greater retention time and complex gradient elution method. These days, consideration has fixated on valerenic acid and its derivatives as vital sedative components in *Valeriana officinalis*. Pulverized valerian comprises not less than 0.3% of volatile oil (a sesqui-terpenoid constituent of the essential oil) and not below 0.04% of valerenic acid. Subsequently, the safety of the valepotriates has been cross-examined. So, it is considered that until there is better evidence, the best option is to use water-soluble extracts standardized for valerenic acid content [3].

More than a few components of the plant could probably subsidize to the outcome. Out of all the other components, Valerenic acid is believed to be partially accountable for the sedative effects. Valerenic acid turns as a subtype-selective $GABA_A$ receptor positive allosteric modulator via a binding site in the transmembrane domain at the $\beta^+\alpha^-$ interface. It acts as a partial agonist at the $5\text{-}HT_{5A}$ receptor, which is a serotonin receptor subtype present in the suprachiasmatic nucleus. It is a very minute region in the brain which is in authority for the sleep-wake cycle [4,5]. Nevertheless, there are some side-effects which are reported such as headache, mental dullness, depression, dizziness, hepatotoxicity on continuous use of valerian products with LD50 amount i.e., 15,000mg/kg and 3300 mg/kg when given through oral and IP respectively [6].

Powdered methanolic extracts of air-dried *V. officinalis* and *V. jatamansi* rhizomes were analyzed using HPTLC where valerenic acid was found to be present in greater amounts in the former with 0.43% and later being only 0.12%N [7]. It was found that there is 13.2% and 10% minimum of isovalerenic acid and 3-methyl valerenic acid in some of the valerian species. These were analyzed by hydro distillation then by GC-MS analysis [8].

Valerenic acid and its hydroxyl and acetoxy derivatives (three characteristic compounds for the species) were separated using capillary electrophoresis. This was achieved using 40mM phosphate buffer at pH 8.5, 10% isopropanol as organic modifier at 17.5kv voltage and 35°C applied temperature [9]. Some valerian pharmaceutical products like tablets, capsules, drops etc were used to evaluate the content of valerenic acid which was found to be around 0.03 to 2.8%. This method used HPLC reverse phase method where the products were macerated in methanol then the extract was analyzed by TLC and HPLC [10]. Various species of valerian were evaluated for the major volatile components using GC/MS method which also involved supercritical fluid extraction

technique. This was compared with the hydro distillation extracted which resulted in the conclusion that the amount of the volatile matter differed depending on the method of extraction and the conditions employed [11]. Usage of HPTLC method for the separation of the constituents of valerian has been reported many times for the quantification of valerenic acid using silica gel with a linear ascending development method [12,13]. In most of the cases, spots were noticed at 254 and 366nm. Then exposure was made with the help of HCl- acetic acid reagent followed by anisaldehyde- sulphuric acid reagent spray visualized under UV [14].

Changes in the number of sesquiterpene acids were documented independently of the environmental influences. It was recognized that there was a significant (80%) degradation of the three valerenic acids depending on the storage conditions which indicates that valerenic acids are sensitive to the external conditions [15]. So far, a maximum of sixty-five components of the essential oil was identified with gas chromatography-mass spectrometry using chemometric resolution [16]. It was stated that using higher processing temperatures helps in enhancement of extraction rate but at the same time, it induces light degradation of the valerian constituents [17]. Valerenic acid and the corresponding constituents were evaluated by HPLC with a C18 KROMSSILE (250 x 4.6 mm) column and a mobile phase comprising of methanol and an orthophosphoric acid solution (0.5% v/v in water) in ratio of 75: 25 at a constant flow rate of 1 ml/ min[18].

More than 30 commercial valerian formulations (which are existing in Australia) were studied by HPLC to detect valepotriates and their derivatives. The concentration of valerenic acid and its derivatives ranged from < 0.01 to 6.32 mg/g. Bokstaller, et al. issued an RP-HPLC analytical method which used ODS25 µm (300 x 2.5 mm) and 30 min gradient elution program with 6% acetonitrile, photodiode array detection at 221nm for determination of valerenic acid [19]. It can also be synthesized using some key transformations by stereoselective reduction of a 3,4 disubstituted cyclohexane and a microwave-assisted Wittig reaction [20].

In the present work, a literature review was done for a relatively suitable method which could be developed for the analysis of valerenic acid in valerian tablets was developed with shortening the retention time along, with better resolution thus reducing the wastage of solvents and time of analysis.

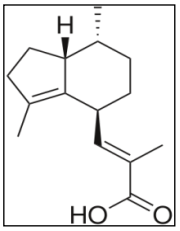


Fig. 1. Chemical structure of valerenic acid

Fig. 2. Valerian plant and root (Source of valerenic acid)

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Valerian root extract pure standard from Sigma Aldrich. Valerian tablets from commercially available formulation available in the market called by the name Tagara. Other solvents and chemicals used in the experimental process are analytical and HPLC grade.

2.2 Instrumentation

The LC system used was Shimadzu UFLC, Nexera series utilizing a Photo-diode Array detector (SPD-M20A) detector achieves a 0.4 x 10^{-5} AU noise level with an online degassing unit and an autosampler (SIL 30AC) featuring a pressure tolerance of 130 Mpa, solvent delivery unit (LC-30AD). Separation of the analyte was achieved by using Phenomenex C18 Luna® 5 µm C18(2) 100 Å (250 x 4.6mm) reverse phase column.

2.3 Chromatographic Conditions

The separation was carried out using the mobile phase involving of acetonitrile (HPLC grade) and 5% orthophosphoric acid solution in water with Ph of 3.5 (97:3, v/v) which was degassed, and membrane filtered before running through the column. Phenomenex C18 Luna® 5 µm C18 (2)

100 Å (250 x 4.6mm) column was utilized and found adequate for better separation of the analytes. The column temperature was maintained at 25ºC and each injection volume was 10 µl. The wavelength used to measure the absorbance is at 220 nm with a flow rate of 0.8 ml/min and the run time was set at 10 min. The peak identification was done by the reference of the literature review done which states that the two main constituents of valerian are isovaleric acid and valerenic acid among other less prevalent components. On a polar mobile phase interaction isovalerenic acid being more polar has a greater affinity and elutes first that the valerenic acid. Isovaleric acid has less activity pharmacologically and it is mainly responsible only for the smell of the drug whereas, valerenic acid is responsible for the sedative effect though it is present in a very lower amount than the former. So, the elution of the peak at 3.5minutes is isovaleric acid and the peak at 4.4±0.2 min minutes corresponds to valerenic acid.

2.4 Standard Preparation

The crude extract of valerian (625 mg) was dissolved in 50ml of methanol to prepare 100 ppm solution of extract and standard. It is then sonicated for 20minutes (with the lid closed) and then filtered to remove the insoluble particles. The standard solution was subsequently diluted to prepare different concentrations (5, 10, 25, 50, 75ppm) of standard solutions. All aliquots were filtered through Whatman's syringe filters (NYL 0.45 µm) before transferring to vials for analysis.

2.5 Sample Preparation

The tablets from commercial formulation are chosen for the analysis as the test. An average of four tablets was taken and was crushed properly using a motor-pestle. 250 mg of tablet powder is mixed in 50 ml methanol and sonicated for 20 minutes, filtered and an aliquot from that solution is taken in the vial which is tested for analysis.

2.6 Calibration Curve

The calibration curve was plotted by analyzing five different concentrations of valerian standard solution ranging from 5 to 75ppm. It was established using linear regression analysis of the peak area against the respective concentration of valerenic acid (Fig. 3). The quantification of Valerenic acid in the chosen formulation was enumerated regarding the calibration curve.

3. METHOD VALIDATION

The RP-HPLC method developed was validated for system suitability, method specificity, limits of detection and quantification, accuracy, precision, method robustness, LOD and LOQ. The tests for the above method validation parameters were done based on ICH guidelines.

3.1 System Suitability

Usually, system suitability parameters are useful in assessing the equipment, electronics, analytical operations and how good a sample is analyzed which in turn constituting of an integral system that is evaluated. So, the parameters that are considered are peak area or the response, tailing factor, number of theoretical plates, capacity and resolution factor which are individually checked for % RSD and average (Table 1). It is done by analyzing the six replicates of a reference standard solution (of 25ppm concentration).

3.2 Specificity

It is useful to understand the capability of the selected method to able to segregate and differentiate the required analyte of interest from the other counterparts. This is useful for apt quantification of the analyte. Hence, specificity is sometimes said to be 100% selectivity of an analyte. For this, an assay is performed to know the content of the analyte in the herbal sample. The chromatogram of the valerian extract showed a peak for iso valerenic acid at 2.9±0.2 min and the peak for valerenic acid at 4.4±0.2 min. Both these components do not interfere with each other in analysis, therefore there is no peak merging that is observed with valerenic acid. The chromatogram peaks are well resolved which indicates that the method is specific.

3.3 Limits of Detection (LOD) and Limit of Quantification (LOQ)

The LOD (lowest amount of the analyte that could be detected by a certain method) and LOQ (lowest amount of the analyte that can be detected) were measured by calculating the standard deviation (σ of the response and the slope(S) of the linear equation. The following formulas were used to determine the LOD and LOQ.

LOD =3.3 **σ**/S
LOQ =10 **σ**/S

Where,

σ= standard deviation of the response,
S = slope of the calibration curve (Table 2).

The results of the limit of detection and limit of quantification are tabulated in Table 3.

3.4 Accuracy

It is the closeness of a measured value with the true value. We usually conduct a recovery study of reference standard was performed. Accuracy test includes analyzing a sample with known concentrations then we compare the practically measured value with the theoretical value (Table 4). Here, three different concentration ranges are selected with two determinations for each (n=2). Recovery study is expressed as % RSD from mean recovery.

3.5 Precision

Precision is the repeatability factor of a method which is done by multiple sampling of the homogenous sample (25 ppm) at least six times (Table 5). It is indirectly is the reproducibility of the entire analytical method under normal operating conditions. The same sample is repeated for six times on the same day for intra-day precision and six successive days for inter-day precision (n=6). The precision is then expressed as mean and %RSD.

Table 1. System suitability parameters for valerian (n=6)

Injection number	Rt	Response	No. of theoretical plates	Tailing factor	Resolution factor	Capacity factor
1	4.26	1878682	6089.5	0.968	8.2	4.409
2	4.29	1898327	6092.1	0.976	8.32	4.623
3	4.31	1917462	6201.8	0.967	7.89	4.515
4	4.25	1895034	5979.2	0.99	8.01	4.223
5	4.32	1901234	6050.7	0.955	8.19	4.129
6	4.24	1950524	5934.7	0.957	8.09	4.317
Mean		1906877.6	6058	0.96	8.11	4.369
%RSD		1.29	1.55	1.33	1.88	0.18

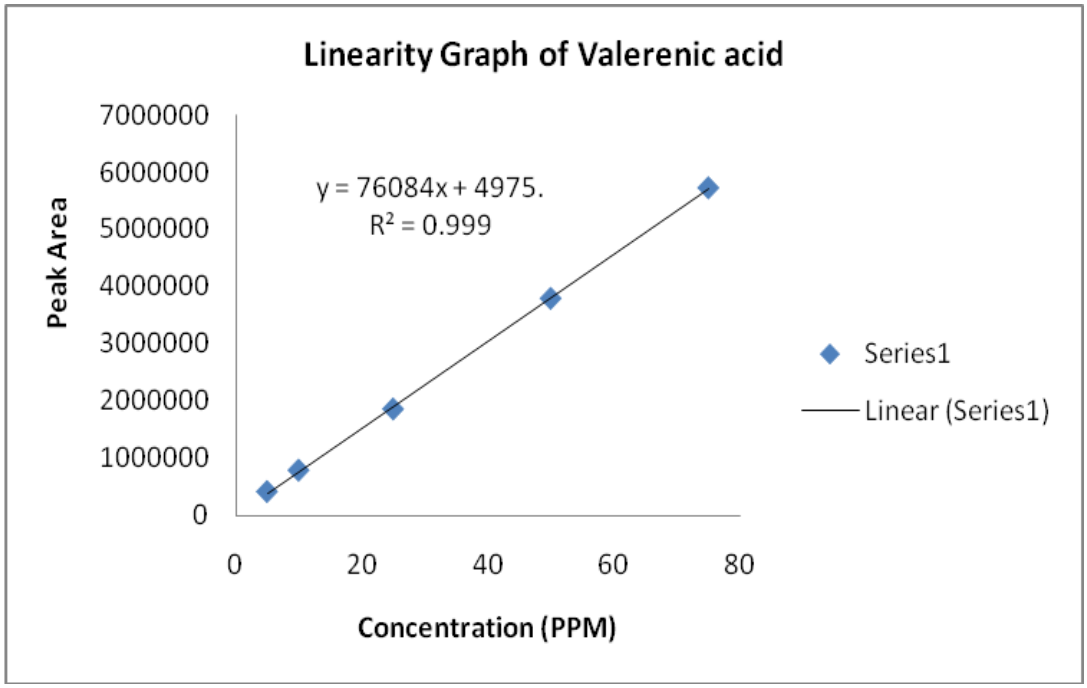


Fig. 3. Linearity calibration graph

Table 2. Results of linearity

S. No	Concentration (ppm)	Area under curve
1	5	409188
2	10	784216
3	25	1856924
4	50	3795003
5	75	5733387
	Correlation coefficient	0.99
	The standard deviation of the slope	37011.76

Table 3. LOD and LOQ of valerenic acid

Parameter	Values (ppm)
Limit of detection (LOD)	1.6
Limit of quantification (LOQ)	4.8

3.6 Robustness

Robustness is the degree of capability of a process parameter to stay undisturbed by small but careful changes in the experimental parameters. This indicates a method's extent of reliability during normal use. In this case, it is studied by analyzing the standard solution of Valerenic acid under critical modifications in mobile phase ratio, detection wavelength, flow rate and column temperature to determine their effect on Rt, peak response and the recovery. Later %RSD of Rt and peak area response and the percentage of mean recovery are calculated. The mobile phase ratio selected to test the robustness is acetonitrile: OPA (5%) in the proportion of 90:10, with a flow rate of 1.3ml/min. The absorbance was checked at 254nm with the column temperature at 35°C.

4. RESULTS AND DISCUSSION

HPLC is an exceptional, adaptable, common and familiar means for qualitative and quantitative estimation of herbal products against their corresponding biologically active molecules concerning quality and batch-to-batch reproducibility. In the current RP-HPLC analysis, the chromatogram of the valerian commercial formulation exhibited a peak for valerenic acid at tR 4.45min which was analogous with the standard valerenic acid (tR, 4.4±0.2 min) (Fig. 4b, 4c). The calibration plot of standard valerenic acid showed decent linearity relationship (Fig. 3) in the stated concentration range (5 to 75 ppm) with a correlation coefficient (r^2) being 0.99 (Table 2).

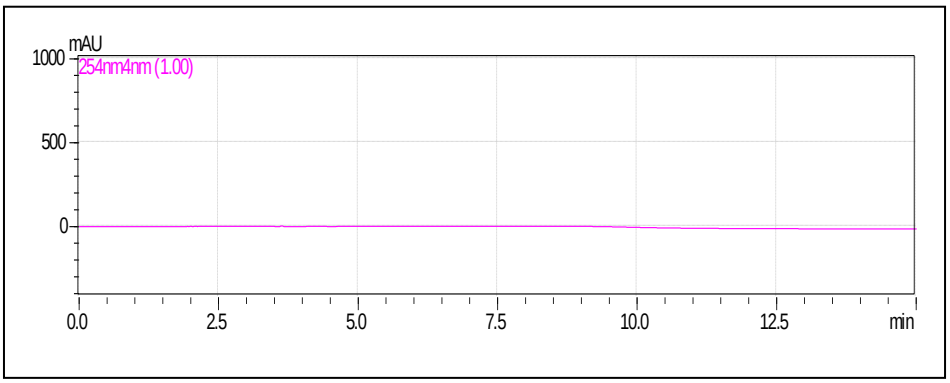
The concentration of valerenic acid in the valerian tablet was found to be 39.16 ppm (or µg/ml) and the quantity was found to be 1.95 mg per tablet. Fig. 4b, 4c demonstrated the clear separation of valerenic acid with adequate peak resolution and there were no peaks at tR range of 4.4 min which shows that the method is selective for valerenic acid. The outcomes of system suitability constraints were given in Table 1 and % RSD of the parameters were found to be less than 2% signifying the system suitability of the method. The LOD and LOQ for valerenic acid were found to be 1.6 ppm and 4.8 ppm, respectively.

Table 4. Results of accuracy (n=6)

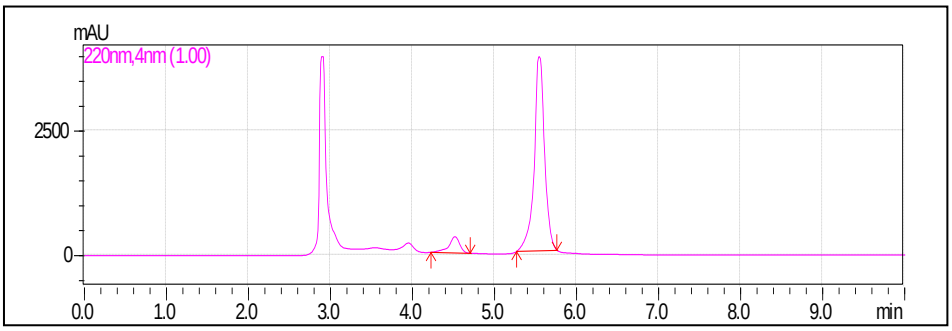
S. No	Concentration of valerenic acid (ppm)	Area under curve	Actual content of valerenic acid	Recovery (%)
1	5	383265	4.88	97.6
	5	383249	4.88	97.6
2	10	774329	10.02	102
	10	781004	9.93	99.3
3	50	3758415	49.41	98.8
	50	3755517	49.37	98.7
			STDEV	1.62
			Average	99
			%RSD	1.63

Table 5. Results of precision (n=6)

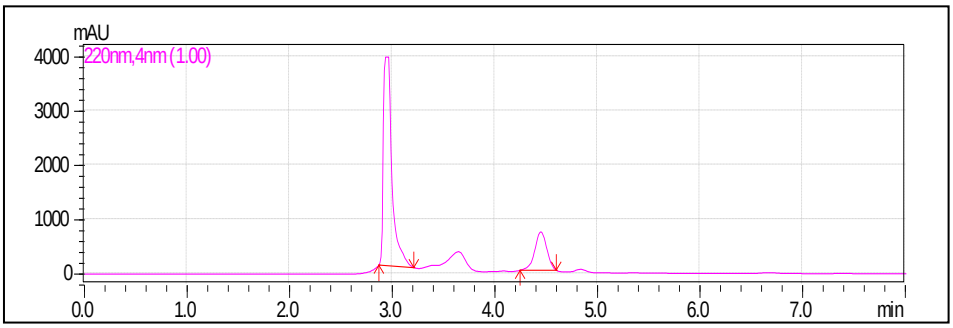
S. No	Concentration (ppm)	Intraday precision (AUC)	Inter-day precision (AUC)
1	25	1878682	1892004
2	25	1898327	1897397
3	25	1917462	1892234
4	25	1895034	1892675
5	25	1901234	1949200
6	25	1950524	1891334
	STDEV	24727.56	22994.31
	Average	1906877.16	1902474
	%RSD	1.29	1.20



(a)



(b)



(c)

Fig. 4. RP-HPLC chromatograms (a) Blank, (b) standard valerian extract (c) Valerian formulation

Table 6.1. Results of robustness linearity

S. No	Retention time	Concentration (ppm)	Area under curve
1	4.2	5	64189
2	4.2	10	110822
3	4.3	25	269135
4	4.2	50	501577
5	4.2	75	780045
		Correlation coefficient	0.99

Table 6.2. Results of robustness accuracy

S. No	Parameter tested	Concentration (ppm)	Peak area	Concentration measured	%Recovery
1		5	56123	4.85	97%
2		5	56450	4.85	97%
3	Accuracy	25	259473	24.76	98.96%
4		25	256892	24.48	97.96
5		75	749505	72.74	96.98%
				Mean recovery	97%

The mean recovery of valerenic acid at various concentrations was 99 ± 1.62% which showed the good accuracy of this method (Table 4). Inter-day and intra-day precision results are represented in Table 5. The % RSD of inter- and intra-day analysis of standard and extract were found to be lesser than 2% with good repeatability in the retention time. There was no noteworthy change in the inter- and intra-day analysis shows that the proposed method is appropriate for the analysis of valerenic acid in herbal medicine. The outcomes of method robustness were given in Table 6.1, 6.2, 6.3 and no significant disparity in retention time (tR), peak area response and recovery of valerenic acid were detected under modified conditions which indicate the proposed method is robust.

5. CONCLUSION

In conclusion, the anticipated method is very apt for assaying the valerenic acid in valerian tablet formulations for routine quality control analysis because of the excellent validation results. Based on the parameters such as linearity, precision, accuracy, LOD and LOQ, we were able to state that the method and the chromatographic conditions used are very reliable. Also due to low retention time and good resolution in the separation of valerenic acid from other constituents makes it a very decent method for the estimation of valerenic acid in the herbal drug formulations.

ACKNOWLEDGEMENT

The authors are grateful to the Principal, JSS College of Pharmacy, Mysore, India for providing all the necessary facilities and chemicals for conducting the research work and for extending the constant assistance and support.

COMPETING INTERESTS

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

REFERENCES

1. Mills S, Bone K. Principles and practice of phytotherapy. Modern Herbal Medicine. Churchill Livingstone; 2000.
2. Rotblatt M. American herbal pharma-copoeia and therapeutic compendia: analytical, quality control and therapeutic monographs. The Journal of Alternative and Complementary Medicine. 2000;6(1): 95-6.

3. Pizzorno JE, Murray MT. textbook of natural medicine-e-book. Elsevier Health Sciences; 2012.
4. Dietz BM, Mahady GB, Pauli GF, Farnsworth NR. Valerian extract and valerenic acid are partial agonists of the 5-HT5a receptor *in vitro*. Molecular Brain Research. 2005;138(2):191-7.
5. Taibi DM, Landis CA. Valerian and other CAM botanicals in treatment of sleep disturbances. In Complementary and Alternative Therapies and the Aging Population. Academic Press. 2009;57-81.
6. Robinson L. Herbal Monograph: *Valeriana officinalis*; 2012.
7. Singh N, Gupta AP, Singh B, Kaul VK. Quantification of valerenic acid in *Valeriana jatamansi* and *Valeriana officinalis* by HPTLC. Chromatographia. 2006;63(3-4):209-13.
8. Pavlovic M, Kovacevic N, Tzakou O, Couladis M. The essential oil of *Valeriana officinalis* L. sl growing wild in Western Serbia. Journal of Essential Oil Research. 2004;16(5):397-9.
9. Mikell JR, Ganzera M, Khan IA. Analysis of sesquiterpenes in *Valeriana officinalis* by capillary electrophoresis. Die Pharmazie. 2001;56(12):946-8.
10. Ghafari S, Esmaeili S, Aref H, Naghibi F, Mosaddegh M. Qualitative and quantitative analysis of some brands of valerian pharmaceutical products. Studies on Ethno-Medicine. 2009;3(1):61-4.
11. Safaralie A, Fatemi S, Sefidkon F. Essential oil composition of *Valeriana officinalis* L. roots cultivated in Iran: Comparative analysis between super-critical CO2 extraction and hydro-distillation. Journal of Chromatography A. 2008;1180(1-2):159-64.
12. Srivastava A, Tiwari SS, Srivastava S, Rawat AK. HPTLC method for quantification of valerenic acid in Ayurvedic drug Jatamansi and its substitutes. Journal of liquid chromatography & related technologies. 2010;33(18):1679-88.
13. Ebrahimzadeh H, rajabian T, Ekhteraei TS, Bashiri SZ, Niknam V, Zareei M. Quantification of valerenic acid and its derivatives in some species of Valeriana l. and *Centranthus longiflorus* stev.
14. Hassan E, Tayebeh R, Samaneh ET, Zeinalabedin BS, Vahid N, Mehdi Z. Quantification of Valerenic acid and its derivatives in some species of Valeriana L and Centranthus longiflorus Stev. Asian Journal of Plant Sciences. 2008;7(2):195-200.
15. Goppel M, Franz G. Stability control of valerian ground material and extracts: A new HPLC-method for the routine quantification of valerenic acids and lignans. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2004; 59(6):446-52.
16. Asadollahi-Baboli M. Comprehensive analysis of *Valeriana officinalis* L. essential oil using GC-MS coupled with integrated chemometric resolution techniques. International Journal of Food Properties. 2015;18(3):597-607.
17. Boyadzhiev L, Kancheva D, Gourdon C, Metcheva D. Extraction of valerenic acids from valerian (*Valeriana officinalis* L.) rhizomes. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2004; 59(9):727-8.
18. Torrado JJ. In vitro release of valerenic and hydroxyvalerenic acids from valerian tablets. Die Pharmazie. An International Journal of Pharmaceutical Sciences. 2003; 58(9):636-8.
19. Shohet D, Wills RB, Stuart DL. Valepotriates and valerenic acids in commercial preparations of valerian available in Australia. Die Pharmazie. 2001;56(11):860-3.
20. Kopp S, Schweizer WB, Altmann KH. Total synthesis of valerenic acid. Synlett. 2009; 11:1769-72.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/50919