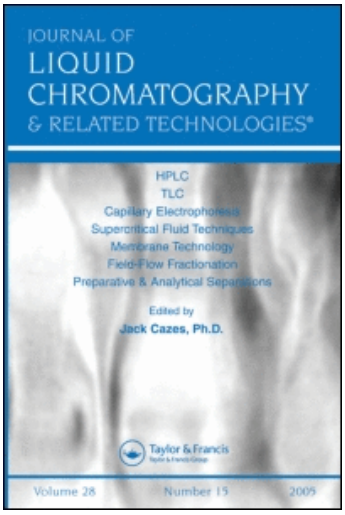

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

HPTLC METHOD FOR QUANTIFICATION OF VALERENIC ACID IN AYURVEDIC DRUG *JATAMANSI* AND ITS SUBSTITUTES

Amit Srivastava[a]; Shashi Shankar Tiwari[a]; Sharad Srivastava[a]; A. K. S. Rawat[a]
[a] Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute, Lucknow, India

Online publication date: 19 November 2010

To cite this Article Srivastava, Amit , Tiwari, Shashi Shankar , Srivastava, Sharad and Rawat, A. K. S.(2010) 'HPTLC METHOD FOR QUANTIFICATION OF VALERENIC ACID IN AYURVEDIC DRUG *JATAMANSI* AND ITS SUBSTITUTES', Journal of Liquid Chromatography & Related Technologies, 33: 18, 1679 — 1688
To link to this Article: DOI: 10.1080/10826076.2010.519250
URL: http://dx.doi.org/10.1080/10826076.2010.519250

Journal of Liquid Chromatography & Related Technologies, 33:1679–1688, 2010

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826076.2010.519250

Taylor & Francis
Taylor & Francis Group

HPTLC METHOD FOR QUANTIFICATION OF VALERENIC ACID IN AYURVEDIC DRUG *JATAMANSI* AND ITS SUBSTITUTES

Amit Srivastava, Shashi Shankar Tiwari, Sharad Srivastava, and A. K. S. Rawat

Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute, Lucknow, India

□ *Objective of the present study was quantification of valerenic acid in rhizome of three plant species which is generally traded under the name of* Jatamansi. *A simple, rapid, cost-effective and accurate high performance thin layer chromatographic method has been developed for quantification of valerenic acid in* Valeriana jatamansi, Nardostachys jatamansi, *and* Selinum vaginatum, *which is one of the stable compounds and designated as a key marker compound. Separation and quantification of valerenic acid was achieved by HPTLC using ternary mobile phase of toluene: ethyl acetate: formic acid (80:20:5 v/v/v) on precoated silica gel 60F_{254} aluminum plate and densitometric determination was carried out in λ_{280} absorption-reflectance UV mode by deuterium lamp.*

Keywords HPTLC, *Nardostachys jatamansi*, *Selinum vaginatum*, valerenic acid, *Valeriana jatamansi*

INTRODUCTION

Over thousands of years, herbal medicines have been used as a therapeutic agent and practiced by physicians of indigenous systems. Jatamansi botanically equated to *Nardostachys jatamansi*, is an important drug of Indian medicinal systems and used for nervous headache, excitement, menopausal symptoms, flatulence, epilepsy, fungal disease, hyperlipidemia, and intestinal colic.[1,2] In combination with cold water, the oil is considered to be effective against nausea, stomachache, flatulence, liver problems, jaundice, kidney complaints, insomnia, and headache.[3–8] Rhizomes of some other species such as *Valeriana jatamansi* and *Selinum vaginatum*, being rich in essential oil contents, are sold in the herbal drug market

Address correspondence to A. K. S. Rawat, Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute, Lucknow 226001, India. E-mail: pharmacognosy1@rediffmail.com

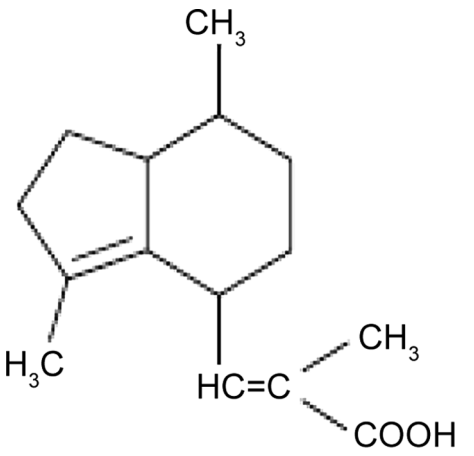

FIGURE 1 Structure of Valerenic acid.

by the same vernacular name.[9] Valerenic acid (Figure 1) is a sesquiterpenoid and key marker of Valerian plant used in CNS activity[10] and also has been demonstrated to be both a subtype-selective $GABA_A$ agonist[11,12] acting mainly at $\alpha1\beta2$ and $\alpha1\beta3$ subtypes and also a $5HT_{5A}$ partial agonist.[13] Valerenic acid has also been reported to be an NF-κB inhibitor and may be partly responsible for anti-inflammatory action of the valerian plant.[14] Although there is one report on quantitative estimation of valerenic acid through High Performance Liquid Chromatography (HPLC) with mobile phase Methanol: 0.5% phosphoric acid (80:20),[15] but there is no validated HPTLC method reported so far. In the present study, we used a new HPTLC method for quantification of valerenic acid in rhizome of three plant species, i.e., *Valeriana jatamansi, Nardostachys jatamansi*, and *Selinum vaginatum*, which is generally traded under the name of *Jatamansi*. This was done to provide a cost effective, economic, and reproducible method for the estimation of valerenic acid. The present study was also aimed for quantification of valerenic acid in *Valeriana jatamansi, Nardostachys jatamansi*, and *Selinum vaginatum* species for their authenticity, identity, validity, quality, and batch to batch consistency in the pharmaceutical industry that is not yet reported. This study will also be useful to check adulteration and substitution in commercial samples.

EXPERIMENTAL

Reagent and Chemicals

All chemicals were laboratory grade and solvents were analytical grade, HPTLC plates Silica higlachrosep Nano UV $60F_{254}$ [20 cm × 10 cm] were

procured from s d fine-chem. Limited, Mumbai, India. The Valeriana root kit was procured from Chromadex, CA, USA which contains Valerenic acid (ASB-00022150-010) and Valerian root BRM (ASB-0003400-005).

Plant Material

Rhizomes of *Nardostachys jatamansi, Valeriana jatamansi*, and *Selinum vaginatum* were collected from the Munsiayari region, Dist. Pitthoragarh, state Uttarakhand, India and authenticated by Dr. A. K. S. Rawat, Head, Pharmacognosy and Ethnopharmacology division, National Botanical Research Institute, Lucknow, India. Voucher specimens (Nos. 262501NJ, 262502VJ, 262503SV) were deposited in a herbarium, the National Botanical Research Institute, Lucknow, India.

Preparation of Crude Extract

Accurately weighed 2.0 gm of the coarse powder of *Nardostachys jatamansi* (NJ-I), *Valeriana jatamansi* (VJ-I), *Selinum vaginatum* (SV-I), and Valerian root BRM (VRC-I) were extracted separately with methanol (4×25 mL) under reflux (30 min each time) on water bath. The combined extracts were filtered, concentrated on a freeze drier, and prepared in 10 mg/mL solution with an analytical grade methanol.

Preparation of Standard Solution

A stock solution of valerenic acid (1 mg/mL) was prepared by dissolving 1 mg of accurately weighed valerenic acid in 1 mL methanol, and a further working solution of 100 µg/mL was prepared by adding 900 µL analytical grade methanol into the 100 µL of stock solution of Valerenic acid.

High Performance Thin Layer Chromatography

HPTLC was performed on 20 cm × 10 cm TLC glass plates pre coated with 200-µm layer thickness of silica gel $60F_{254}$ (s d fine-chem. Limited, Mumbai, India). Samples were applied as 6 mm band width using Camag 100 microlitre sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland) under a flow of N_2 gas. The Linear ascending development was carried out with Toluene: Ethyl acetate: Glacial acetic acid [80:20:5 v/v/v] for valerenic acid as mobile phase in a Camag glass twin trough chamber (20×10 cm). The chamber was previously saturated with mobile phase vapor for 10 min at room temperature ($25 \pm 2°C$), $50\% \pm 2$ relative humidity and plates were developed at distance of approximately 80 mm from the point of application. After development, plates were dried

through an air drier and scanning was performed using a Camag TLC Scanner 3 at λ_{280} nm in UV absorbance mode for valerenic acid operated by win CATS Software (Version 3.2.1). The slit dimensions were 5 mm × 0.45 mm and the scanning speed was 100 mm/s.

RESULT AND DISCUSSION

The TLC Separation Optimization at different compositions of the mobile phase was tested and the desired resolution of valerenic acid with symmetrical and reproducible peaks was achieved in all samples by using a ternary mobile phase of Toluene:Ethyl acetate:Glacial acetic acid [80:20:5 v/v/v]. The chamber saturation period was 10 min and approximately 20 min was required for the development of the TLC plate. A band of Valerenic acid was observed at R_f 0.55 in standard track. as Additionally, the same R_f in samples tracks confirmed the presence of valerenic acid in all samples. A comparison of the spectral characteristics of the peaks for standard valerenic acid and that of the sample revealed the identity of Valerenic acid present in the sample.

Method Validation

The HPTLC method was developed for valerenic acid and validated in terms of precision, accuracy, recovery, and robustness; LOD and LOQ were checked as per ICH guidelines.[16,17] Instrumental precision was checked by repeated scanning of the same spot 100, 750, and 1250 ng five times and the results were expressed as % RSD. Method precision was studied by analyzing the standards 100, 750, and 1250 ng/spot under the same analytical procedure and lab conditions on the same day (intra- day precision) and on different days (inter-day precision) and the results were expressed as % RSD. Accuracy of the method was tested by performing the recovery studies of the pre-analyzed sample with standard at five different levels in each samples and result were expressed as % recovery and % RSD.

Calibration for Valerenic Acid

A stock solution of 1000 µg/mL of valerenic acid was prepared in methanol, and dilution was performed to obtain a solution of 100 µg/ mL, which was used for further analysis. Different volumes of diluted solution [1.0, 2.5, 5.0, 7.5, 10.0, and 12.5 µL] were applied on a TLC plate to furnish 100–1250 ng/spot of Valerenic acid standard (Figure 2). Peak area data and the corresponding amounts were treated by linear least square regression analysis (Table 1).

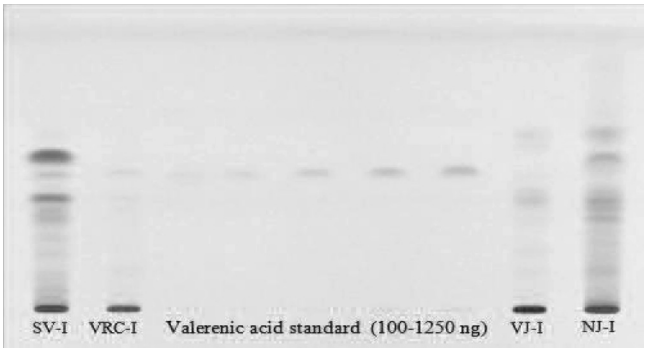

FIGURE 2 CCD image of TLC plate of SV-I, VRC-I, VJ-I, NJ-I, and Valerenic acid standard.

Quantification of Valerenic Acid in Test Sample

10 μL methanolic extract of *Nardostachys jatamansi* (NJ-I), *Valeriana jatamansi* (VJ-I), *Selinum vaginatum* (SV-I), and Valerian root BRM (VRC-I) samples were applied through Linomat 5 applicator on a TLC plate and developed and scanned at λ_{280} UV absorption mode. Peak area was recorded in each sample and the amount of valerenic acid was calculated using the calibration plot (Figure 2). Peak purity test of Valerenic acid was done by comparing its UV–visible spectra in standard and sample track (Figure 3).

System Suitability Test

Linearity and Detection Limit

Linearity was checked by applying standard solutions of valerenic acid at six different concentrations. The calibration curve was drawn in the concentration range of 100–1250 ng/spot. The equation for the calibration curve of valerenic acid is $Y = 34.9 + 2.14x$ and the correlation coefficient of the calibration plot was 0.998. Results of regression analysis on the calibration curve and quantification range are described in Table 2a.

TABLE 1 Calibration Data of Valerenic Acid Standard

S. No.	Sample Concentration (in ng)	Peak Area [AU]
1	100	225.3
2	250	568.4
3	500	1153.1
4	750	1652.0
5	1000	2099.5
6	1250	2735.2

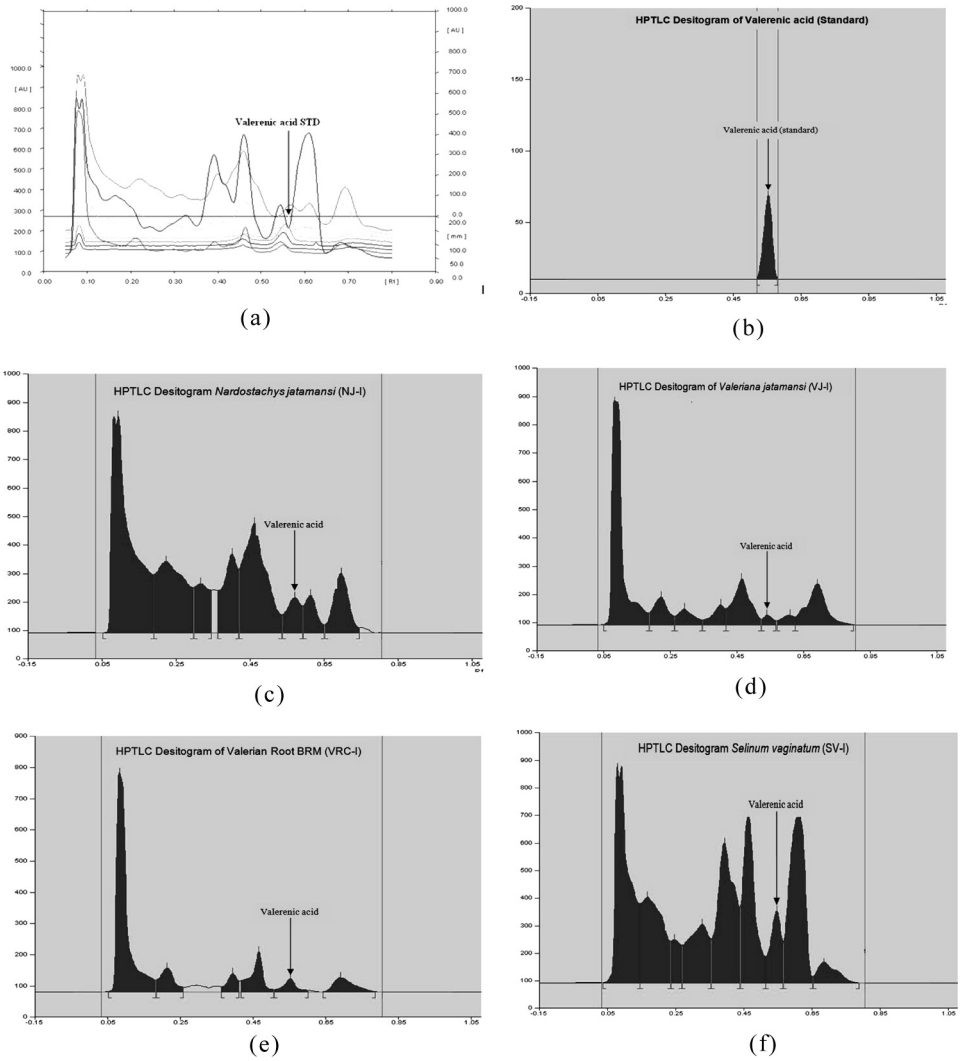

FIGURE 3 (a) Three dimensional chromatogram of standard track and sample tracks; (b) Peak response of Valerenic acid STD; (c) Peak response of *Nardostachys jatamansi* (NJ-I) sample; (d) Peak response of *Valeriana jatamansi* (VJ-I) sample; (e) Peak response of Valerian root BRM (VRC-I); (f) Peak response of *Selinum vaginatum* (SV-I) sample.

Specificity

Specificity of the method was determined by analysis of valerenic acid standard and in *Nardostachys jatamansi* (NJ-I), *Valeriana jatamansi* (VJ-I), *Selinum vaginatum* (SV-I), and Valerian root BRM (VRC-I) samples. The spot for valerenic acid in the samples was confirmed by comparing the R_f and spectra of the spot with that of the standard. The peak purity of valerenic

TABLE 2 Method Validation Data for Quantification of Valerenic Acid by Using Proposed HPTLC Densitometric Method Using Under λ_{280} UV Detection Mode

(2.a) Linearity regression of Valerenic acid

Sl. No.	Parameters	Results
1	R_f	0.55
2	Dynamic range (ng spot^{-1})	100–1250 ng
3	Equation	$Y = 34.90 + 2.14x$
4	Slope	2.14
5	Intercept	34.90
6	Limit of detection	30 ng
7	Limit of quantification	100 ng
8	Linearity (correlation coefficient)	0.998
9	Specificity	Specific
10	Robustness	Robust

(2.b) Precision studies

Concentration (ng spot^{-1}) Sample Amount	Instrumental Precision (% RSD)	Method Precision (% RSD)	
		Intra-Day	Inter-Day
100	0.603	1.670	1.893
750	1.325	1.721	1.645
1250	2.029	1.432	1.543

(2.c) Percentage amount of Valerenic acid present in samples

Sl. No.	Sample Name	Valerenic Acid Percentage Present (%)
1.	*Selinum vaginatum* (SV-I)	0.9151
2.	Valerian root BRM(VRC-I)	0.2794
3.	*Valeriana jatamansi* (VJ-I)	0.1067
4.	*Nardostachys jatamansi* (NJ-I)	0.3136

(2.d) Result and statistical data for recovery studies of Valerenic acid

Sl. No.	Amount of Valerenic acid present in the sample (in ng)	Amount of Valerenic acid added (in ng)	Amount of Valerenic acid found (in ng)	Recovery (%)	Range	(%) RSD
Selinum vaginatum (SV-I) samples						
1	915	0	923	100.80	99.26	1.949
2	915	500	1422	100.40	(99.22–101.00)	
3	915	1000	1875	97.91		
4	915	1500	2440	101.00		
5	915	2000	2805	96.22		
Valerian root BRM(VRC-I) samples						
1	279	0	285	102.10	100.40	2.670
2	279	100	360	94.98	(94.98–102.20)	
3	279	200	490	102.2		
4	279	300	565	97.58		
5	279	400	670	98.67		

(*Continued*)

TABLE 2 Continued

(2.d) Result and statistical data for recovery studies of Valerenic acid

Sl. No.	Amount of Valerenic acid present in the sample (in ng)	Amount of Valerenic acid added (in ng)	Amount of Valerenic acid found (in ng)	Recovery (%)	Range	(%) RSD
Valeriana jatamansi (VJ-I) samples						
1	106	0	110	103.70	99.78	2.323
2	106	50	148	94.87	(93.92–103.70)	
3	106	75	170	93.92		
4	106	100	200	97.08		
5	106	125	230	99.56		
Nardostachys jatamansi (NJ-I) samples						
1	313	0	321	102.50	99.51	0.810
2	313	200	511	99.61	(98.03–102.50)	
3	313	300	601	98.04		
4	313	400	699	98.03		
5	313	500	808	99.38		

acid was assessed by comparing the spectra at three different levels, (i.e., peak start, peak apex, and peak end positions of the spot) for obtaining the best analytical result.

Precision Studies

Instrumental precision was checked by repeated scanning of the same spots (100, 750, and 1250 ng/spot) of standard valerenic acid five times and the RSD values were 0.603, 1.325, and 2.029, respectively. To determine the precision of the developed assay, 100, 750, and 1250 ng/spot of the valerenic acid standard was analyzed five times within the same day to determine the intra-day variability and the % RSD values were 1.670, 1.721, and 1.432 for 100, 750, and 1250 ng/spot, respectively. Similarly the inter-day precision was tested on the same concentration levels on consecutive days and the % RSD values were 1.893, 1.721, and 1.543, respectively (Table 2b).

Sample Analysis and Recovery Studies

Developed TLC method was subsequently applied for the analysis and quantification of valerenic acid in methanolic extract of *Nardostachys jatamansi* (NJ-I), *Valeriana jatamansi* (VJ-I), *Selinum vaginatum* (SV-I), and Valerian root BRM (VRC-I); the free valerenic acid content in the roots by this proposed method was found to be 0.9151 for SV-I, 0.2794 for VRC-I, 0.1067 for VJ-I, and 0.3136 for NJ-I (Table 2c, Figure 4). The examination of recovery rates of pure valerenic acid in pre-analyzed samples and

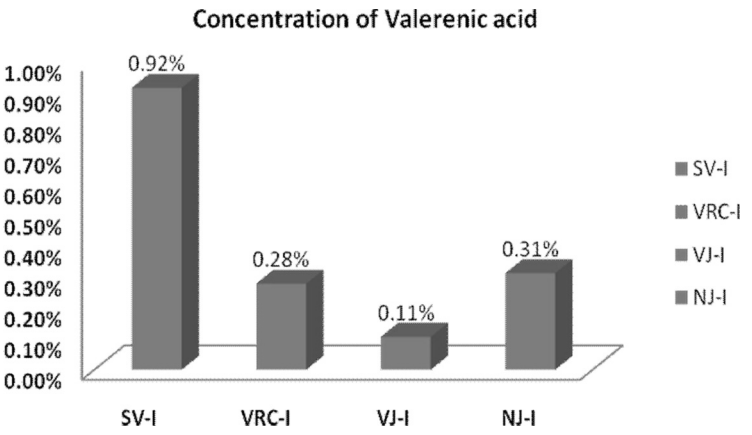

FIGURE 4 Concentration of free Valerenic acid standard in *Selinum vaginatum* (SV-I), Valerian root BRM (VRC-I), *Valeriana jatamansi* (VJ-I), and *Nardostachys jatamansi* (NJ-I) samples.

quantitative analysis was performed. The recovery ranges were obtained 99.22–101.0 for SV-I; 94.98–107.5 for VRC-I; 93.92–106.7 for VJ-I; and 98.03–102.5 for NJ-I samples (Table 2d).

CONCLUSION

The HPTLC method developed here for the quantification of valerenic acid in *Nardostachys jatamansi, Valeriana jatamansi, Selinum vaginatum*, and in Valerian root BRM is simple, rapid, cost-effective, and easily adaptable for the screening and quantitative determination than any other HPTLC technique. This may be utilized by pharmaceutical industries for quality control and batch to batch consistency of crude drug.

ACKNOWLEDGEMENT

The authors are thankful to the Director, National Botanical Research Institute, Lucknow, India for provision of the facilities to conduct the research work.

REFERENCES

1. Parikh, A.; Jadhav, V. M. Development of Validated HPTLC Method for Quantification of Jatamansone in Jatamansi Oil. *J. Pharm. Res.* **2009**, *2* (5), 975–977. doi: jpronline.info/article/viewFile/361/316
2. Ali, S.; Ansari, K. A.; Jafry, M. A.; Kabeer, G. *Nardostachys jatamansi* protects Against Liver Damage Induced by Thioacetamide in Rats. *J. Ethnopharmacol.* **2000**, *71*, 359–363.

3. Subashini, R.; Yogeeta, S.; Gnanapragasam, A.; Devaki, T. Protective Effect of *Nardostachys jatamansi* on Oxidative Injury and Cellular Abnormalities During Doxorubicin-Induced Cardiac Damage in Rats. *J. Pharm. Pharmacol.* **2006**, *58*, 257–262.
4. Subashini, R.; Ragavendran, B.; Gnanapragasam, A.; Yogeeta, S. K.; Devaki, T. Biochemical Study on the Protective Potential of *Nardostachys jatamansi* Extract on Lipid Profile and Lipid Metabolizing Enzymes in Doxorubicin Intoxicated Rats. *Pharmazie.* **2007**, *62*, 382–387.
5. Kniebel, B. Therapy of Depression in the Clinic. A Multicenter Double-Blind Comparison of Herbal Extracts from Valerian Roots and Roses of Sharon with the Standard Antidepressant Amitryptiline [in German]. *Ther. Erfahrungen.* **1988**, *64*, 689–696.
6. Chatterjee, B.; Basak, U.; Datta, J.; Banerji, A.; Neuman, T., Prange *Nardostachys jatamansi* DC. Potential Herb with CNS effects. *Cheminform* **2005**, *36*, 17.
7. Shakir, A.; Khursheed, A. Ansari.; Jafry, M. A.; Kabeer, H.; Diwakar, G. *Nardostachys jatamansi* Protects Against Liver Damage Induced by Thioacetamide in Rats. *J. Ethnopharm.* **2007**, *1*, 359–363.
8. Ebrahim, Z. H.; Radjabian, T.; Ekhteraei, T. S.; Bashiri, S. Z.; Niknam, V.; Zarrei, M. Quantification of Valerenic Acid and its Derivatives in Some Species of *Valeriana 1* and *Centranthus longiflorus* Stev. *Asian J. Plant Sci.* **2008**, *7* (2), 195–200.
9. Seshadri, T. R.; Sood, M. S. Chemical Comparison of the Roots of *Selinum Vaginatum* and *Nardostachys Jatamansi. Phytochem.* **1967**, *6* (3), 445–446.
10. Hendriks, H.; Bos, R.; Woerdenbag, H. J.; Koster, A. S. Central Nervous Depressant Activity of Valerenic Acid in the Mouse. *Planta Med.* **1985**, *51*, 28–31.
11. Benke, D.; Barberis, A.; Kopp, S.; Altmann, K. H.; Schubiger, M.; Vogt, K. E.; Rudolph, U.; Möhler, H. GABA A Receptors as in Vivo Substrate for the Anxiolytic Action of Valerenic Acid, a Major Constituent of Valerian Root Extracts. *Neuropharmacol.* **2009**, *56* (1), 174–181.
12. Khom, S.; Baburin, I.; Timin, E.; Hohaus, A.; Trauner, G.; Kopp, B.; Hering, S. Valerenic Acid Potentiates and Inhibits GABAA Receptors: Molecular Mechanism and Subunit Specificity. *Neuropharmacol.* **2007**, *53* (1), 178–187.
13. Dietz, B. M.; Mahady, G. B.; Pauli, G. F.; Farnsworth, N. R. Valerian Extract and Valerenic Acid are Partial Agonists of the 5-HT5a receptor *in vitro. Mol. Brain Res.* **2005**, *138* (2), 191–197.
14. Jacobo-Herrera, N. J.; Vartiainen, N.; Bremner, P.; Gibbons, S.; Koistinaho, J.; Heinrich, M. $F_{-k}B$ Modulators from *Valeriana officinalis. Phytotherapy Res.* **2006**, *10*, 917–919.
15. Upton, R. *Valerian Root-Valeriana Officinalis Analytical, Quality Control and Therapeutic Monograph*, American Herbal Pharmacopeia: Santa Cruz, CA, USA, April 1999.
16. ICH Guideline Q2R1, *Validation of Analytical Procedures: Text and Methodology* (November 1996/2005), Geneva, Switzerland.
17. Bos, R.; Woerdenbag, H. J.; Hendriks, H.; Zwaving, J. H.; De Smet, P. A. G. M.; Tittel, G., Wilkstrom, H. V.; Scheffer, J. J. C. *Phytochem. Anal.* **1996**, *7*, 143–151.