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A STABILITY INDICATING RP-LC METHOD FOR THE DETERMINATION OF STRYCHNINE IN *KRIMIMUDGARA RASA*

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Keywords:

Strychnine, Polyherbal formulation, Stability indicating, Reversed-Phase Liquid Chromatography, *Krimimudgara rasa*

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ABSTRACT: A reliable, rapid, simple and accurate high-performance liquid chromatography method with UV detection was developed for the simultaneous quantitative determination of strychnine in a polyherbal formulation prepared from *Nux Vomica*. *Krimimudgara rasa*, a herbal dosage form containing *Strychnos nux vomica*, *Apium graveolens, Embellia ribes, Butea monosperma* in combination. Separation of the strychnine from its major and minor degradation products was successfully achieved on a reversed-phase C-18 column using (250 mm × 4.6 mm ID, 5 µm particle size), with isocratic elution using a mixture of Methanol: KH_2PO_4 buffer solution (10 mmol, adjusted to pH 3 with orthophosphoric acid) (50:50 v/v) at flow rate 0.7 ml/min at 254 nm. The method was validated concerning linearity, precision, accuracy, system suitability and robustness. The responses were linear in the drug concentration range of 1 - 10 µg/ml. The percent recoveries were in the range between 98 - 101% from a mixture of degradation products. The utility of the procedure was verified by its application to a marketed formulation that was subjected to accelerated degradation studies. The method could distinctly separate the drug and degradation products. The products formed in marketed tablet dosage form were similar to those formed during stress studies.

INTRODUCTION: India has an ancient heritage of traditional medicine, which comprises of Ayurvedic, Siddha and Unani systems of medicines. Ayurveda is accepted to be one of the oldest treatises on the traditional medicinal system. It is presumed that Ayurvedic knowledge is given by God of a different world and also supposed to be more effective in certain cases than modern therapies [1].

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The World Health Organization has recognized the importance of traditional medicine and has created strategies, guidelines and standards for botanical medicines. Every herbal formulation must be standardized as per WHO guidelines. It is necessary to develop methods for rapid, precise and accurate identification and estimation of active constituents to bring out the consistency of important constituents in the formulations [1].

Some of the important parameters are stability testing, safety assessment, specific therapeutic activity, analysis and estimation of the active constituents in crude drugs as raw materials and finished products. The objective of WHO guidelines is to define basic criteria for the evaluation of quality, safety, and efficacy of herbal

medicines and therefore to assist national regulatory authorities, scientific organizations and manufacturers to undertake an assessment of the documentation in respect of such products [2]. Stress degradation stability studies of drug molecule include, the study of different stress conditions such as, hydrolysis at lower and higher pH, neutral hydrolysis, photolysis, oxidation of drug and analyzing the degradation by different analytical methods.

Because of biological nature, the presence of other phytoconstituent and other factors, such as pH, temperature, moisture, microbes, active constituents of formulation, are more prone to degradation and the degraded products may be toxic to the health of patients. In the case of toxic drugs, degraded products may prove to be more toxic. Hence, stability indicating assay method development is important for herbal preparations[3-5].

The development of stability indicating assay method thus will help in establishing the inherent stability of the drug, which in turn will assure to detect changes in identity, purity & potency of the product on exposure to various conditions. Stress testing is important in developing strategies in ICH guidelines (Q1A [R2]), and it is carried out in more severe conditions than accelerated studies [6, 7]. In the present study, stability indicating HPLC method was developed for the determination of strychnine in Ayurvedic formulation *Krimimudgara* Rasa (KMR) [8].

KMR is an Ayurvedic formulation containing herbs and minerals, which indicate digestive disorders, mentioned in Ayurvedic Formulary of India [9, 10, 11]. The medicine comprises the herbs *Strychnos nux-vomica*, *Apium graveolens, Embellia ribes, Butea monosperma* [6]. Strychnine is the marker compound of *Strychnos nux-vomica.*

Nux vomica seeds contain indole alkaloids like strychnine and brucine [12]. Strychnine is stranding-10-one, and brucine is 2,3-dimethoxystrychnidin-10-one. These alkaloids are physiologically more active than the minor alkaloids which include a-colubrine, b-colubrine, icajine, 3-methoxyicajine, proto-strychnine, vomicine, novacine, N-oxystrychnine, pseudo-strychnine and iso-strychnine [13].

Ayurveda has mentioned the therapeutic uses of *Nux vomica* seeds in the treatment of digestive impairment, muscle spasm, cramps, ascariasis and also has the CNS stimulant activity. It also has analgesic, stimulant activity and also useful in impotence, spermatorrhea, and sexual frigidity of women. It is used as a nervine tonic and aphrodisiac [14].

Some HPLC methods have been developed for the separation of strychnine from formulations and mixtures. Hiruntad *et al*., (1997) has developed a quantitative analysis method for the determination of strychnine and brucine in the seeds, root, stem, and leaves of *Strychnos species* [15]. YH Jiang *et al*., (2002) have reported an HPLC method for determination of strychnine and brucine in *Semen strychni* and its processed products [16]. Perumal *et al*., (2016) have developed the methods for phytochemical determination of strychnine [17]. Several other chromatographic and spectroscopic methods have also been developed for the isolation and analysis of strychnine [18, 19, 20, 21, 22]. However, no studies related to the stability indicating assay method have been reported. Thus, we report stability indicating assay method for strychnine in KMR by reversed phase HPLC method.

MATERIALS AND METHODS:

Instrumentation: The HPLC system (Jassco 2000) consisted of Jasco PU 2080 plus pump, a manual Rheodyne injector with 20 µl fixed loop and Jasco UV 2075 detector. The column used for the separation of constituents was HiQ Sil (C-18, 5µm; 4.6 × 250 mm). The chromatographic data were recorded and processed using a BORWIN 2000. Degassing of the mobile phase was done by sonication in an ultrasonic bath.

Materials: Standard Strychnine was purchased from Sigma Aldrich Pvt. Ltd., Bangalore. The Ayurvedic medicine, KMR was procured from a local drug store. Methanol and water of HPLC grade were purchased from Qualigens. All other solvents and reagents used for the present studies were of analytical grade. For filtration of mobile phase Nylon membrane filter paper of pore size, 0.45 µm (Pall Life sciences) was used.

Chromatographic Conditions: [25] Chromatographic estimations were performed on reverse phase

chromatography using HiQ Sil C-18 column. Analysis and separation of Strychnine were performed with a mobile phase consisting of Methanol: KH_2PO_4 buffer solution (10 mmol, adjusted to pH 3 with orthophosphoric acid) (50:50 v/v) at flow rate 0.7 ml/min in an isocratic system. Absorbance maxima for Strychnine were found to be 254 nm, which was selected for the detection of Strychnine in the present study. The mobile phase was freshly prepared every day. The components of the mobile phase were filtered through a 0.45 µm to remove any particulate matter. The components were mixed in an appropriate ratio, and the mixture was sonicated to remove the dissolved gases. The column was saturated with the mobile phase until 30 ml of solution passes through column before use.

Preparation of Standard Solution: Approximately 5 mg of standard strychnine was weighed in a volumetric flask and dissolved in methanol to obtain a stock solution of 1000 µg/ml. Aliquots of the standard were further diluted to obtain solutions in the range of 1-10 µg/ml with methanol.

Calibration Curve: Appropriate aliquots from the standard solution were transferred to a series of 10 ml volumetric flask, and the volume was made up to mark with methanol to produce the concentration of marker in the range from 1-10 µg/ml.

Forced Degradation Studies of Standard Drug Solution: [6, 25] A standard stock solution of Strychnine (1 mg/ml) was prepared by dissolving 10 mg of standard strychnine in 10 ml methanol. From the above stock solution, 1 ml was diluted up to 10 ml with distilled water, 2N HCl, 1N NaOH and to 1 ml stock solution, 1 ml of 6% H_2O_2 was added, and volume was made up to 10 ml with methanol to achieve the concentration of 100 µg/ml strychnine. The above solutions in water, 1N HCl, 1N NaOH, and 6% H_2O_2 were heated at 80 ºC for 12 and 24 h the above drug solutions were also kept at room temperature and exposed to sunlight for 24 h in separate volumetric flasks. The solid drug was spread 1mm thick in a petri-plate and exposed to sunlight for the 24 h. Solid sample was also placed in a desiccator containing saturated NaCl solution (75% relative humidity at 30 ºC) for degradation in humid conditions. Samples were withdrawn periodically and were neutralized and diluted with methanol to obtain concentrations of 10 µg/ml of strychnine. Samples were collected at different time intervals and neutralized for further analysis.

Analysis of the Marketed Formulation:

Stress Degradation Studies of formulation KMR: 3 g of the formulation was moistened with water at pH 9 and kept overnight. This mixture was transferred to separating funnel and shaken with chloroform until complete extraction of alkaloids was effected. The chloroform layer was allowed to evaporate, and the residue was used for further studies.

Conditions used for the degradation of standard strychnine were also applied on the formulation extract, and developed method was used for the estimation of strychnine in the formulation. 10 mg of the alkaloidal fraction was dissolved in 10 ml of methanol. 1 ml of this solution was taken in a volumetric flask, and 1 ml of 2N HCl, 1N NaOH, 6% H_2O_2 was added. To this mixture, methanol was added to make up the volume 10 ml. To the 1ml of the working solution, water was added, and volume was made up to 10 ml. These solutions were kept at room temperature, in sunlight, and at 80 ºC. Alkaloidal extracts were also exposed to dry heat at 80 ºC and sunlight for 48 h and 24 h respectively and exposed to humidity for 7 days. Samples were collected at different time intervals and neutralized for further analysis.

The resulting solutions were analyzed as the degraded sample and using the same chromatographic conditions.

Method Validation: The method developed for analysis of strychnine was validated for linearity, accuracy, precision, robustness, detection and quantification limits as per ICH guidelines [7, 24].

Calibration (Linearity): Linearity was studied by injecting 6 different concentrations (1-10 µg/ml) of standard marker compound and plotting the graph of concentration versus peak area of the respective chromatogram.

Precision and Stability: The precision was validated by injecting three concentrations of marker compound (intraday 3 injections in a day and inter day 3 injection over 3 days).

Limit of Detection and limit of Quantification: LOD and LOQ of the method were determined by *k* SD/s, where *k* is the constant (3 for LOD and 10 for LOQ), SD is the standard deviation of the analytical signal, and s is the slope of concentration/response graph.

Robustness: Robustness of the proposed method was evaluated by keeping the chromatographic conditions constant, except for the followings changes:

1) The detection wavelength was changed from 254 nm to 252 nm and 256 nm.

2) Solvent Brand of methanol used was of E-Merck, Qualigens and SD Fine chemicals.

Accuracy: Accuracy was evaluated by fortifying the sample solution of the formulation with three different levels of the standard solution of strychnine (80%, 100%, and 120%) and calculating the percent recovery from the differences between the peak areas obtained for the fortified and unfortified solutions

System Suitability: A system suitability test was performed for the method before the validation runs. The parameters evaluated are capacity factor, separation factor, HETP, asymmetry, and resolution.

RESULTS AND DISCUSSION: A simple HPLC method was developed for the determination of Strychnine in KMR. To optimize the proposed HPLC method, all of the experimental conditions were investigated [23]. From the above mobile phases tried, mobile phase containing potassium dihydrogen phosphate buffer (10 mmol, pH 3 maintained with the help of orthophosphoric acid): methanol (50:50) was selected, as it shows sharp peak and significant reproducible retention time at 254 nm as detection wavelength with a flow rate of 0.7 ml/min **Fig. 1**.

Forced Degradation Studies:

Acid Degradation: When subjected to acid hydrolysis, it was observed that strychnine is slightly susceptible to acidic hydrolysis. There were four minor degradation peaks at 4.9, 6.1, 6.7 and 9.9 min **Fig. 2**.

Alkali Degradation: Strychnine was also susceptible to alkali hydrolysis, and it was observed that 20% of strychnine was degraded in 3 h of refluxing at 80 °C. The degradation peak was observed at 4.9, 6.1 and 11.6 min **Fig. 3**.

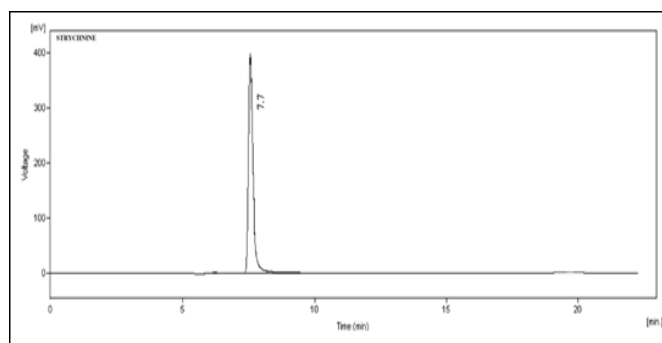

FIG. 1: CHROMATOGRAM OF STANDARD STRYCHNINE (100 µg/ml)

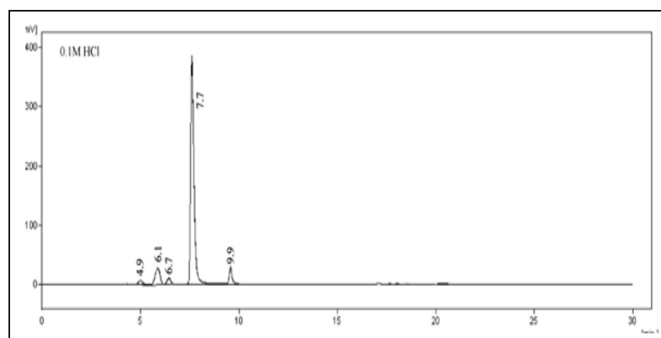

FIG. 2: CHROMATOGRAPHIC SEPARATION OF STRYCHNINE IN STRESSED SAMPLE OF STANDARD SUBJECTED TO ACID HYDROLYSIS

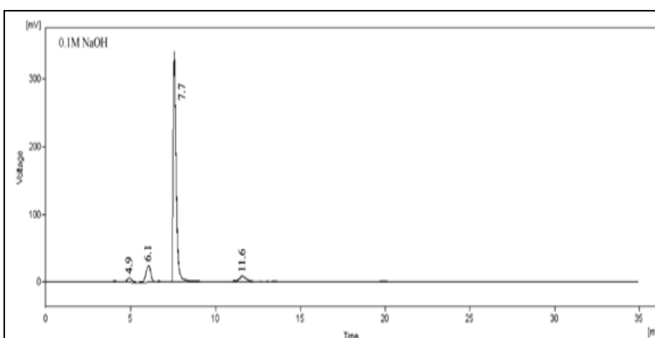

FIG. 3: CHROMATOGRAPHIC SEPARATION OF STRYCHNINE IN STRESSED SAMPLE OF STANDARD SUBJECTED TO ALKALI HYDROLYSIS

Neutral Degradation: Strychnine was slightly susceptible to neutral hydrolysis, and 30% of the drug was degraded after refluxing it for 24 h in water. Degradation peak was observed at 4.9 and 9.9 min **Fig. 4**.

Oxidative Degradation: Strychnine undergoes oxidative hydrolysis and degradation peak was detected at 4.9, 6.1, 6.3 min **Fig. 5**.

Thermal Degradation: It was observed that strychnine is very stable to thermal degradation when exposed to dry heat at 80 ºC for 24 h. Three minor peaks observed at 4.1, 4.9 and 6.0 min **Fig. 6**.

Photo Degradation: It was observed that strychnine is susceptible to photodegradation and 40 % of the drug was degraded in 24 h. Degradation peak was observed at retention time 4.1, 4.9, 6.1 and 8.3 min **Fig. 7**.

Humidity Degradation: It was observed that the strychnine was very stable in humid condition, as there is no extra peak and no change in the peak area.

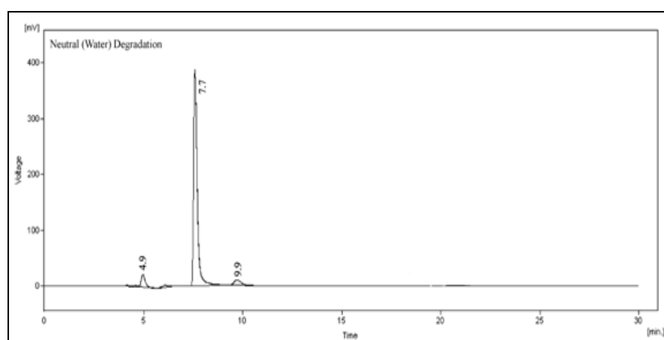

FIG. 4: CHROMATOGRAPHIC SEPARATION OF STRYCHNINE IN STRESSED SAMPLE OF STANDARD SUBJECTED TO NEUTRAL HYDROLYSIS

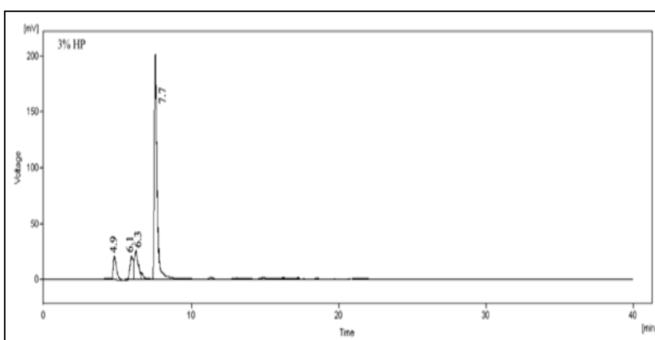

FIG. 5: CHROMATOGRAPHIC SEPARATION OF STRYCHNINE IN STRESSED SAMPLE OF STANDARD SUBJECTED TO OXIDATIVE HYDROLYSIS

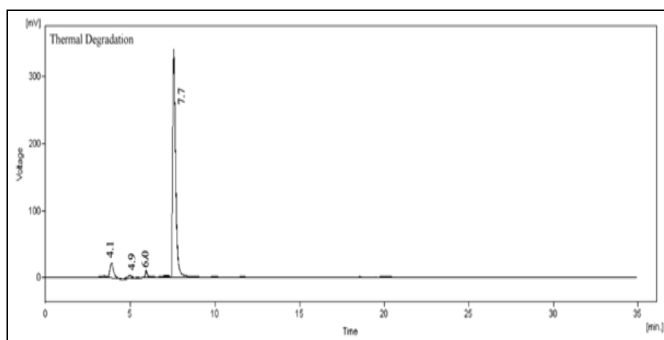

FIG. 6: CHROMATOGRAPHIC SEPARATION OF STRYCHNINE IN STRESSED SAMPLE OF STANDARD SUBJECTED TO THERMAL DEGRADATION

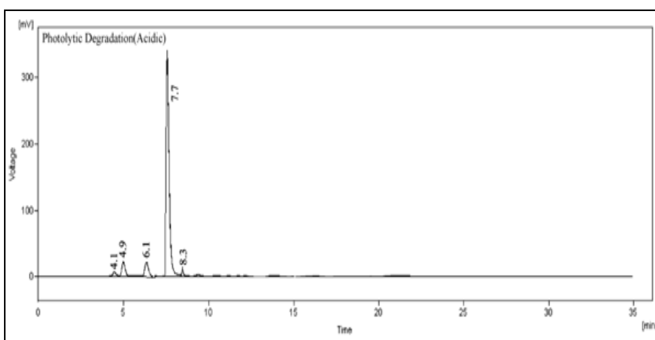

FIG. 7: CHROMATOGRAPHIC SEPARATION OF STRYCHNINE IN STRESSED SAMPLE OF STANDARD SUBJECTED TO PHOTOLYTIC DEGRADATION

Method Validation:

Calibration Curve (Linearity): Linear regression analysis confirms that the r^2 values for strychnine were 0.998 (by area) and 0.997 (by height), confirming the linear relationship between the concentration of the drug and area under the curve and height of the peak. The calibration curves constructed for the marker were linear over the concentration range of 1-10 µg/ml **Table 1**.

Precision and Stability: The precision result of the solution at the three concentrations is presented in **Table 2**, and it is seen that the RSD values of retention time were less than 1%, while the RSD values of peak area were less than 3% both for intra-day assay and inter-day assay precision **Table 2**.

TABLE 1: LINEAR REGRESSION DATA FOR THE CALIBRATION CURVES (n=3)

Parameter	Values
Retention time, min	7.7 ± 0.2
Detection wavelength, nm	254
Limit of detection, LOD, µg/mL	0.0684
Limit of quantification, LOQ, µg/mL	0.02
Linearity range, µg/mL	1-10
Correlation coefficient (height)	0.997
Correlation coefficient (area)	0.998
Regression equation (height)	Y= 0.4225x-1.823
Regression equation (area)	Y=6.754x – 24.054

TABLE 2: INTRADAY AND INTERDAY PRECISION OF THE DEVELOPED METHOD (n=6)

Concentrations (µg/mL)	Intraday				Interday			
	Retention time		Peak Area		Retention time		Peak Area	
	Mean	RSD[a], %	Mean	RSD[a], %	Mean	RSD[a], %	Mean	RSD[a], %
1	7.69	0.24	58.22	2.21	7.72	0.15	59.37	1.98
4	7.71	0.12	234.4	0.92	7.69	0.26	234.4	1.11
10	7.74	0.18	631.47	1.63	7.68	0.32	631.47	1.45

Limit of Detection and Limit of Quantification: LOD was found to be 0.02 µg/ml, and LOQ was found to be 0.0684 µg/ml respectively for strychnine.

Robustness: Robustness of the proposed method was evaluated by making some changes and the results are given in **Table 3**.

TABLE 3: ROBUSTNESS OF THE METHOD (n=6)

Chromatographic Change		Recovery, % ± SD
Factor	Level	Strychnine
	methanol	
E-Merck	1	98.56 ± 0.45
SD Fine Chemicals	2	99.18 ± 0.69
Qualigens	3	98.17 ± 1.28
	Detection wavelength	
252	-1	99.23 ± 0.27
254	0	98.51 ± 1.21
256	+1	98.97 ± 1.04

Accuracy: The recovery of the investigated components ranged from 97.5 - 98.42% **Table 4**. It was known from the recovery tests that the developed methods manifested the reliability and accuracy for the measurement of the strychnine.

System Suitability: A system suitability test was performed to evaluate the chromatographic parameters **Table 5**.

TABLE 4: RECOVERY STUDY OF STRYCHNINE ADDED TO THE PRE-ANALYZED SAMPLES USING PROPOSED METHODS (n = 3)

The quantity added, %	Total Quantity present	Amount quantity found	Recovery (%)	% RSD
0	0.121	0.118	97.5	0.62
80	0.2178	0.2143	98.39	0.83
100	0.242	0.238	98.34	0.49
120	0.2662	0.262	98.42	0.66

TABLE 5: RESULTS OF SYSTEM SUITABILITY PARAMETERS OBTAINED FROM LC METHOD

S. no.	Parameter	Strychnine
1	Retention time, min	7.7
2	Capacity factor (K´)	6.254
3	Separation factor (α)	1.437
4	Efficiency/length (t.p/m)	123587
5	HETP, mm	0.046
6	Resolution (Rs)	5.47
7	Asymmetry (As)	1.06

Application of Developed Method in Formulation: The developed method was applied for the determination of strychnine in *Krimimudgara rasa*. Degraded samples of the formulation showed the degradation behavior as strychnine with extra peaks of other components present in formulation **Fig. 8**. Strychnine was estimated in the formulation. Results of strychnine estimation in the formulation are given in **Table 6**.

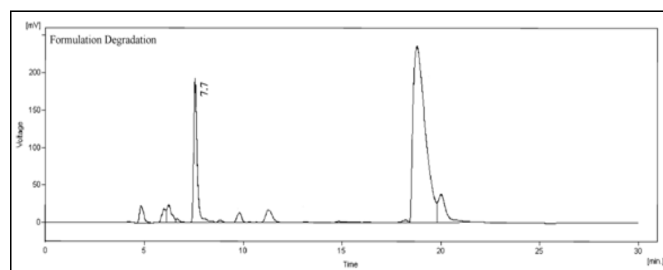

FIG. 8: CHROMATOGRAPHIC SEPARATION OF STRYCHNINE IN DEGRADED FORMULATION

TABLE 6: RESULT OF ASSAY PERFORMED FOR FORMULATION, KMR

Formulation	Strychnine(mg) ± SD
Lab prepared Ayurvedic formulation (100 mg)	0.121 ± 0.06
Marketed Ayurvedic formulation (100 mg)	0.059 ± 0.0082

CONCLUSION: The stability indicating assay method reported is a simple, rapid and reliable method for estimation of strychnine in polyherbal formulations. Since, proprietary Ayurvedic medicines containing *Nux vomica* seeds are becoming increasingly more popular as a medicine used in the global market, methods for standardization of those medicines are in demand. So, it is highly recommended that the determination of these alkaloids in the proprietary Ayurvedic medicines must be done as a routine measurement, provide a safe application to patients in clinics, and good manufacturing practices.

To establish the potentiality of Ayurvedic medicine, research needs to be conducted on different disciplines of Ayurveda to meet the requirement of the society. This can be done by standardization of materials, methods, and measures for preparation, preservation, presentation, and administration of Ayurveda drugs. Thus, the rationale and judicious use of modern scientific methods pertain to the development of Ayurveda.

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CONFLICT OF INTEREST: There is no conflict of interest in the work presented in the manuscript.

REFERENCES:

1. Darshan S and Patwardhan B: AYUSH for New India: Vision and strategy. Journal of Ayurvedic and Integrative Medicine 2017; 8(3): 137-139.
2. Moreira DL, Teixeira SS, Monteiro MH, De-Oliveira DX and Paumgartten FR: Traditional use and safety of herbal medicines. Revista Brasileria de Farmacognostica 2014; 24: 248-257.
3. Rai PD and Rajput SJ: Biological evaluation of polyherbal ayurvedic cardiotonic preparation "*Mahamrutyunjaya rasa*". Evidance based Complementary and Alternative Medicine 2011; 1-11.
4. Mukherjee PK: Quality control herbal drugs: An approach to the evaluation of botanicals, Business Horizons, Edition 1st, 2002.
5. Jaiswal Y, Liang Z and Zhao Z: Botanical drugs in Ayurveda and Traditional Chinese Medicine. Journal of Ethnopharmacology 2016, 194: 245-259.
6. ICH, Q1A (R2) Stability testing of new drug substances and products in International Conference on Harmonization, Geneva Switzerland 2003.
7. ICH, Q2B Validation of Analytical Procedures: Methodology. International Conference on harmonization, Geneva, Switzerland 1996.
8. Ayurved Sarsangrah, Shri Baidyanath Ayurved Bhab Ltd., Edition 1st, 2014: 237.
9. The Ayurvedic Formulary of India, published by Ministry of health and family welfare, Dept. of Indian system of medicine and homeopathy, New Delhi, Edition 2nd, Part-1, 362.
10. The Ayurvedic Formulary of India, published by Ministry of health and family welfare, Dept. of Indian system of medicine and homeopathy, New Delhi Edition 2nd, Part-1, 348.
11. The Ayurvedic Formulary of India, published by Ministry of health and family welfare, Dept. of Indian system of medicine and homeopathy, New Delhi, Edition 2nd, Part-1, 255.
12. Chopra RN, Nayar SL and Chopra IC: Glossary of Indian Medicinal Plants. National Institute of Science Communication and Information Resources. Council of Scientific and Industrial Research 1956; 122.
13. Duddukuri GR, Brahmam AN and Rao DN: Suppressive effect of *Strychnos nux-vomica* on induction of ovalbumin-specific IgE antibody response in mice. Indian J Biochem Biophys 2008; 45: 341-344.
14. Nadkarni KM: Indian Materia Medica. Popular Prakashan, Mumbai, Edition 3rd, 1954; 2: 1179.
15. Hiruntad Y, Palanuvej C and Ruangrungsi N: Pharmacognostic specification and quantitative analysis of strychnine and brucine in *Strychnos nux-vomica* seeds. 2016; 14(1): 21-29.
16. Jiang YH, Yang WL and Gong QF: Method for determination of strychnine and brucine in semen strychnine and its processed products. China Journal of Chinese Materia Medica 2002; 27: 899-901.
17. Perumal B, Velmani S, Santhosh C, Vetrivel C and Maruthupandian A: Phytochemical and medicinal properties on *Strychnos-nux-vomica* (L). Journal of Advanced Applied Scientific Research 2016; 1(3): 96-99.
18. Pawar RP: Isolation and characterization of alkaloids of *Nux-vomica* by instrumental technique. J of Medicinal Chemistry and Drug Discovery 2017; 2(3): 54-61.
19. Hashim A, Mohammed R, Umar D, Vaswani RV and Bahija B: A reverse phased high-pressure liquid chromatographic method for the estimation of a poisonous matter in *Strychnos nux-vomica*. J Adv Pharm Technol Res 2015; 6(3): 108-113.
20. Chen J: Pharmacological evaluation of total alkaloids from *Nux vomica*: effect of reducing strychnine contents. Molecules 2014; 19: 4395-4408.
21. Tiana JX, Penga C, Xua L, Tiana Y and Zhang ZH: *In-vitro* metabolism study of *Strychnos* alkaloids using High-Performance Liquid Chromatography combined with hybrid ion trap/time-of-flight Mass Spectrometry. Biomedical Chromatography 2013; 27: 775-783.
22. Hashim A: A Reverse Phased High-Pressure Liquid Chromatographic method for the estimation of a poisonous matter in *Strychnos nux-vomica*. Journal of Advanced Pharmaceutical Technology & Res 2015; 6(3): 108-113.
23. Hosseini M, Malekpour H, Khoshnoud R and Abbasinia H: Fatal cases of strychnine ingestion referred to Fars

Legal Medicine Organization; Autopsy findings and Analytical methods used in strychnine detection. Trends in Pharmaceutical Sciences 2017; 3(3): 209-214.

24. Snyder LR, Kirkland JJ and Glajch JL: Practical HPLC Method Development, Wiley Interscience, New York, Edition 2nd, 1997.
25. Rai P, Pathak A and Rajput SJ: Stability indicating RP-LC method for the determination of Aconitine and piperine in a polyherbal formulation. J AOAC Int 2009; 92(4): 1044-54.

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