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ORIGINAL ARTICLE

Development and validation of RP-HPLC Method for Isolongifolene

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ABSTRACT
The aim of the present work was carried out to develop an RP-HPLC method for simultaneous analysis of Isolongifolene. Chromatographic system was optimized and isolongifolene was well separated using thermohypersil C_{18} (250X4.6mm, 5micron) reverse phase column at a flow rate 1ml/min with detection wavelength 247nm. The mobile phase consisting of water, acetonitrile, methanol and acetic acid in the ratio of 25:55:20:0.3 respectively. Finally the pH was adjusted to 5.5using acetonitrile. This method was developed and validated in accordance with ICH guidelines. The retention time and % RSD of isolongifolene was found to be 4.04 mins, 0.38 respectively. Further the method was validated for the parameters like linearity, accuracy, and precision and system suitability. The linearity of the developed method was validated over a different concentration ranging from 25-150%. The correlation coefficient value (R^2) was 0.999. The precision was tested over six times using same dilution and the RSD value was found to be 0.14. Validation revealed the method is accurate, precise, and suitable and it can be routinely used for characterization of isolongifolene.
Keywords : Isolongifolene; Sesquiterpene; RP-HPLC; ICH; %RSD.

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INTRODUCTION

Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases. Medicinal plants have played a key role in world health [1]. In recent years there has been a growing interest in the therapeutic use of herbal medicines or phytopharmaceutical products [2,3].

Murraya koenigii. (Linn) Spreng is a tropical tree of the family Rutaceae, which is native to India. Their properties include much value as an anti-diabetic [4,5,6,7], antioxidant, antimicrobial [8,9,10], anti inflammatory [11], hepatoprotective [12], anti hypercholesterolemic [13,14], as well as efficient against colon carcinogenesis. A Sesquiterpene compound named isolongifolene is found in *Murraya koenigii.* Isolongifolene has the molecular formula $C_{15}H_{24,}$ molecular weight 204.356g/mol. The structure of isolongifolene is shown in fig.1.

HPLC is one of the most widely used analytical techniques today, among the different chromatographic procedure, due to the significant evolution in liquid chromatographic instrument, providing superior qualitative and quantitative results [15]. Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use [16].

A stability-indicating RP-HPLC method for the analysis of isolongifolene has been developed and validated. In this paper, we proposed an RP-HPLC method for the assay of isolongifolene and it has been demonstrated to be accurate, linear, precise, and therefore the method is suitable for routine analysis of isolongifolene.

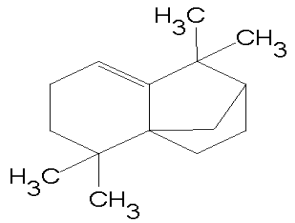

Fig .1. Structure of Isolongifolene

MATERIALS AND METHODS

Isolongifolene was purchased from sigma Aldrich. HPLC grade water, ethanol, methanol, acetic acid, and acetonitrile used were of analytical grade. All the chemicals were procured from Rankem and sigma Aldrich.

Instrumentation and Chromatographic Conditions

Analysis was carried out using shimadzu RP-HPLC system spectra series p4000. HPLC system equipped with SCM 1000 degasser, Spectra system UV-2000 detector Spectra system AS 3000 auto sampler thermohypersil C_{18} column. The chromatographic and the integrated data were recorded using ChromQuest software.

Preparation of standard and sample dilution

Stock solution was prepared by dissolving 0.1g of isolongifolene in 25ml of ethanol and methanol (50:50). Working solutions were prepared by diluting stock solution and it has the concentration of 0.98g/ml as an internal standard. Sample was injected by using auto sampler and Sample was eluted at a flow rate of 1ml/min at 30°C. The chromatogram was recorded and monitored at 247nm.

Mobile Phase

The mobile phase consisted of water: acetonitrile: methanol: acetic acid in the ratio of 25:55:20:0.3 and pH of the mobile phase was adjusted to 5.5 using acetonitrile. The mobile phase was filtered through whattman filter paper no 41 and degassed.

Optimization of HPLC method

The RP-HPLC procedure was optimized to develop a suitable analytic method for isolongifolene. Preliminary experiments were carried out to optimize the parameter for the development of method i.e. column, wavelength, mobile phase concentration, and flow rate. These parameters were optimized to determine the best chromatographic conditions for the separation of isolongifolene in chromatogram. It was observed that developed method of isolongifolene could be determined simultaneously with no mobile phase interaction, good separation, sensitivity and consistent base line.

Method validation

The proposed method was validated according to the ICH guidelines [17]. The developed method was validated by determining the parameters like precision, accuracy, linearity, system suitability. The correlation coefficient and Percentage relative standard deviation values were calculated.

Linearity

Linearity of the developed HPLC method was assayed to detect the ability of the procedure. The obtained results are directly proportional to the concentration of the analyte. The linearity was evaluated at six different concentrations ranging from 25-150%. The area at each concentration is calculated and calibration curve was plotted against concentration. The correlation co-efficient (R^2) was calculated and recorded.

Precision

The precision of the developed method was assayed by six replicate aliquot samples and relative standard deviation value was calculated.

Accuracy

Accuracy was evaluated by injecting the isolongifolene about three replicates and spiked by four different concentrations ranging 110-130%. The recovery percentage was determined. Average recoveries were calibrated by the formula recovery (%) = {(amount found – original amount)/amount spiked} x 100.

System Suitability

System suitability was performed by taking peak area and retention time of the five replicate injections and the % RSD was not more than 2.0.

RESULTS AND DISCUSSION

The simple and sensitive RP-HPLC method was optimized for parameters like mobile phase, column and detection wavelength. Since a fixed wavelength detector was used. The optimum wavelength for detecting

isolongifolene was found to be 247nm. Thermohypersil C_{18} column was used (250X4.6mm; 5micron). The separation using this column was good. It was found that the mobile phase containing water, acetonitrile, methanol and acetic acid at a flow rate of 1ml/min gave optimum and adequate peak separation. The retention time and percentage relative standard deviation (%RSD) was observed 4.04 and 0.38 respectively. According to ICH guideline the %RSD value was found to be <2. So the developed method is acceptable and further the method was validated.

Linearity was studied by preparing standard solution at different concentration levels. The linearity graph was shown in fig 2. The regression equation was found to be y=38,582.344X+12,169.8929 with R^2=0.9998.

System suitability was determined by five repeated injections. The peak area and retention time were determined. It was shown in table 1. There was no interfering peak found in the chromatogram and the %RSD was found 0.38 it shows that it is less than 2.0.

Accuracy was performed at three levels spiking of 10, 20 and 30% of standard concentration. The recovery percentage was 110-130%. Accuracy of isolongifolene was given in table 2. The results indicate that the method enables accurate estimation of isolongifolene.

The precision of the system was tested by six dilutions of the sample, the retention time (t_R) and peak area were determined. The RSD in precision studies was found to be 0.14 for isolongifolene. It was concluded that the analytical technique showed good repeatability.

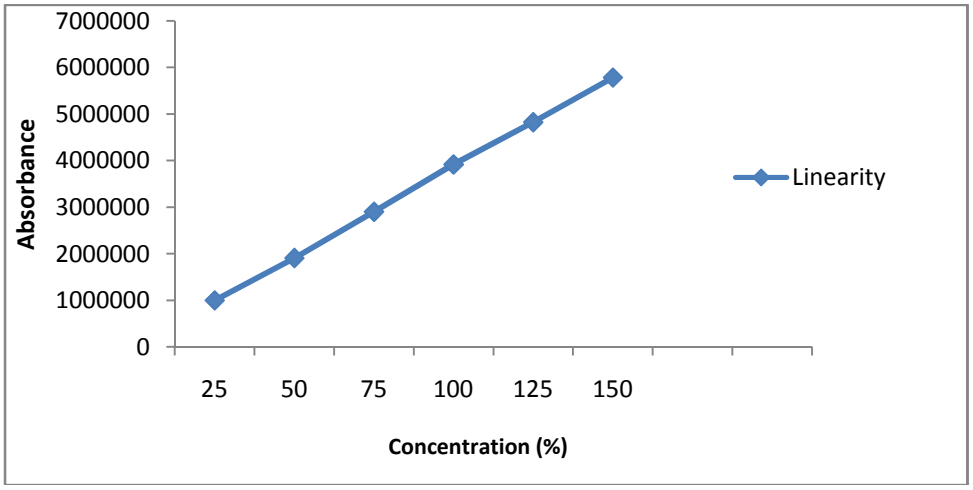

Fig.2.Linearity of Isolongifolene

Concentration	Sample Area	Retention time
0.10g	4014196	4.040
	4015089	4.042
	4044651	4.037
	4041178	4.033
	4015335	4.032
AVERAGE :	4026089.8	
SD :	15414	
RSD :	0.38	

Table 1. System suitability of isolongifolene

S.NO	Concentration % of spiked level	% Recovery
1	100	100.07
2	110	109.90
3	120	120.13
4	130	130.12

Table 2. Accuracy of isolongifolene

CONCLUSION

The newly developed RP-HPLC method was found to be simple, precise, rapid and sensitive for routine determination of isolongifolene. Hence, sesquiterpenoids has so many properties like antioxidant,

antimicrobial, anticancer activity so this method could be recommended for analysis of isolongifolene for further quality assurance and formulations.

REFERENCES

1. Calixto, J.B. (2000) Efficacy, Safety, Quality control, marketing and regulatory guidelines for herbal medicines. *Brazillian Journal of medical and Biological research*., 179-189.
2. Sombra, L.L., G´omez M.R, Olsina R, Martınez L.D., and. Silva M.F. (2005). Comparative study between capillary electrophoresis and high performance liquid chromatography in 'guarana' based phytopharmaceuticals. Journal of Pharmaceutical and Biomedical Analysis., 36 (5), 989–994.
3. Mesbah, M.K., Khalifa, S.I., El-Gindy, A., Tawfik, K.A. (2005). HPLC determination of certain flavonoids and terpene lactones in selected Ginkgo biloba L. *Phytopharmaceuticals*., Farmaco, 60: 6-7.
4. Arulselvan, P., Senthilkumar., G.P., Sathish Kumar, D., Subramanian, S. (2006). Anti-diabetic effect of Murraya koenigii leaves on streptozotocin induced diabetic rats. *Pharmazie*., 61(10): 874–7.
5. Yadav, S., Vats, V., Dhunnoo, Y., Grover, J.K. (2001). Hypoglycemic and anti hyperglycemic activity of Murraya koenigii leaves in diabetic rats. *J Ethnopharmacol*., 82:111.
6. Vinuthan, M.K., Girish Kumar,V., Ravindra, J.P., Narayana, K. (2004). Effect of extracts of Murraya koenigii leaves on the levels of blood glucose and plasma insulin in alloxan induced diabetic rats. *Indian J Physiol Pharmacol*., 48: 348.
7. Achyut, N.K., Gupta, R.K., Watal, G. (2005). Hypoglycemic effects of Murraya koenigii on normal and alloxan-diabetic rabbits*. J. Ethnopharmacology*., 97: 247.
8. Arulselvan, P., Subramanian, S.P. (2007). Beneficial effects of Murraya koenigii leaves on antioxidant defence system and ultra structural changes of pancreatic beta-cells in experimental diabetes in rats. *Chem Biol Interact*.,165 (2) : 155–64.
9. Baliga, M.S., Jagetia, G.C., Rao, S.K., Babu, K. (2003). Evaluation of nitric oxide scavenging activity of certain spices invitro: a preliminary study. *Nahrung*., 47 (4): 261.
10. Abhishek Mathur, V.K., Dua, Prasad G.B.K.S. (2010). Antimicrobial Activity of Leaf Extracts of Murraya Koenigii against Aerobic Bacteria Associated with Bovine Mastitis. *Int. J. of Chemical Environmental and Pharmaceutical Research*.,1(1): 12-16
11. Muthumani, P., Venkatraman, S., Ramseshu, K.V., Meera, R., Devi, P., Kameswari, B., Eswarapriya, B. (2009). Pharmacological studies of anticancer, anti inflammatory activities of Murraya Koenigii (Linn) Spreng in experimental animals. *J. Pharm. Sci., & Res*. 1(3): 137-141.
12. Pande, M., Gupta, SPBN, Pathak, A. (2009). Hepatoprotective activity of Murraya Koenigii Linn. Bark. *Journal of Herbal Medicine and Toxicology*., 3(1): 69-71.
13. Iyer, U.M., Mani, U.V.(1990). Studies on the effect of curry leaves supplementation (Murraya koenigii) on lipid profile, glycated proteins and amino acids in non-insulin dependent diabetic patients. *Plants and Food in Human Nutrition*.,40:275.
14. Khan, B.A., Abraham, A., Leelamma, S. (1996). Murraya koenigii and Brassica juncea alterations on lipid profile in 1-2 dimethylhydrazine induced colon carcinogenesis. *Investigational New Drugs*.,14: 365.
15. Srikanth Inturi, RaviKanth Inturi, Venkatesh, G. (2011). A Validated Novel RP-HPLC method development for the estimation of Palonosetron Hydrochloride in bulk and softule dosage forms**.** *Der Pharmacia Sinica*., 2 (5): 223-234.
16. Anand Shrivastava, Anup K Chakraborty, Sujit K Rambhade, Umesh K Patil. (2011). Development of validated RP-HPLC method for determination of letrozole in bulk and its pharmaceutical dosage forms . *Der Pharmacia Sinica*., 2 (2):263-269.
17. Guideline on Validation of Analytical Procedure-Methodology. (1996). International Conference on Harmonization, Geneva, Switzer- land.