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Separation of Isoalantolactone and Alantolactone in *Inula racemosa* Root by RP-HPLC

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ABSTRACT

*A simple reversed phase high performance liquid chromatographic method has been developed, for the separation of Isoalantolactone (**1**) and Alantolactone (**2**) in the root, Inula racemosa (Pushkarmool). Resolution of 2.20 has been achieved for Alantolactone and Isoalantolactone. Linearity, with respect to the peak area of the constituents and their respective concentration was established using the regression equation, giving co-relation coefficient (r^2) as 0.997 and 0.999 for **1** and **2** respectively. Repeatability (n=5) reached relative standard deviation values (R.S.D.) of 0.99% and 1.7% for the peak areas of **1** and **2**, respectively.*

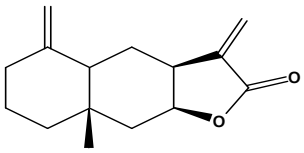
Keywords*:* RP-HPLC, Isoalantolactone, Alantolactone, *Inula racemosa.*

INTRODUCTION

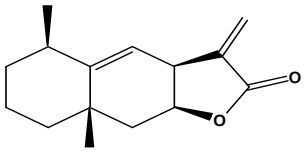
Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological activities, higher safety margins and lesser costs [1]. The lack of standardization and quality control for medicinal plants is still being problem [2]. For quality control of herbal products, high performance liquid chromatography (HPLC) is a popular method for the analysis of herbal medicines because it is accurate, precise and not limited by the volatility or stability of the sample compounds [3].The major advantage of TLC is that several samples can be run simultaneously using a small quantity of mobile phase- unlike HPLC - thus reducing the analysis time and cost per analysis [4].

Inula racemosa Hook f. of Compositae family, found in temperate and western alpine regions of Himalayas, is commonly known as Puskarmool in Indian Ayurvedic system of medicine [5] and as Tumuxiang in China [6]. Traditionally, the root of this plant is used to treat ailments like allergic skin disorders, cough, dyspnoea, dysmenorrhoea and heart diseases [7]. The roots are also reported to possess antifungal, anthelmentic, and hypolipidemic properties [8] and found to

be beneficial in histamine induced bronchospasm [9]. In the traditional Chinese system of medicines, it is used as an anti-microbial agent for nearly a thousand years [10]. The healing medicinal properties are exhibited due to the presence of sesquiterpene lactones, mainly Isoalantolactone (**1**) and Alantolactone (**2**). These lactones are also reported to exhibit good cytotoxic activity against the K562 human leukemia cell line [11].



Isoalantolactone (1)



Alantolactone (2)

Due to the complex nature of herbal matrix, separation of the pair of positional isomers, **1** and **2**, present in *Inula racemosa* roots was found to be very difficult up to now, gas chromatography (GC) [12], thin layer chromatography (TLC) [13], capillary methods are reported for the separation and quantification of **1** and **2**. However, most of these methods have limitations either due to the preparatory steps involved or time taken for analysis. Considering HPLC as a powerful tool in the analysis of complex matrices, a new simple and efficient method is developed on HPLC, to resolve the peaks of **1** and **2**. The method is also found suitable for routine estimation of the compounds **1** and **2** in the root. The method developed and presented here also provides a simple and rapid separation of the two isomers in methanolic extracts of the roots of *Inula racemosa*.

MATERIALS AND METHODS

Instruments

Reversed phase high performance liquid chromatography was carried out on a Shimadzu system, equipped with a pump (LC-10AT$_{VP)}$, Diode array detector (SPD-M 10A$_{VP)}$, auto-injector (SIL – 10 AD$_{VP)}$, Column oven (CTO-10 AS$_{VP}$), and system controller (LC-10A$_{vp)}$. Separation was carried out using a Zorbax C-18 column (150 mm x 4.6mm, particle size 5 µm). The temperature of the column oven was kept at 25°C±1°C. The data acquisition was done on Shimadzu software Class-VP. Sample was filtered through milli-Q nylon syringe filters of 0.45µ pore size before injecting to increase the life of the column so that the method can be used for routine and continuous analysis of raw materials, to check adulteration in the root.

Plant Material

Roots of *Inula racemosa* were procured from Agronomy Department and authenticated by the botanist of Ayurvedic Department. The reference standards of **1** and **2** used for identification were isolated and characterized in-house using Mass, ^{1}H and ^{13}C NMR spectroscopic techniques and by comparing the data with the reported by literature [14]. Methanol (AR Merck grade) was used as solvent for extraction of root. Acetonitrile (HPLC Merck grade) and 0.1M Ammonium acetate (AR Merck grade) solution in water (milli-Q) were used in the composition of the mobile phase.

Standard Preparation
Standard stock solutions of **1** and **2**, of concentration 1000 ppm, were prepared in methanol. Further the stock solutions were diluted to different concentrations using methanol, as and when required.

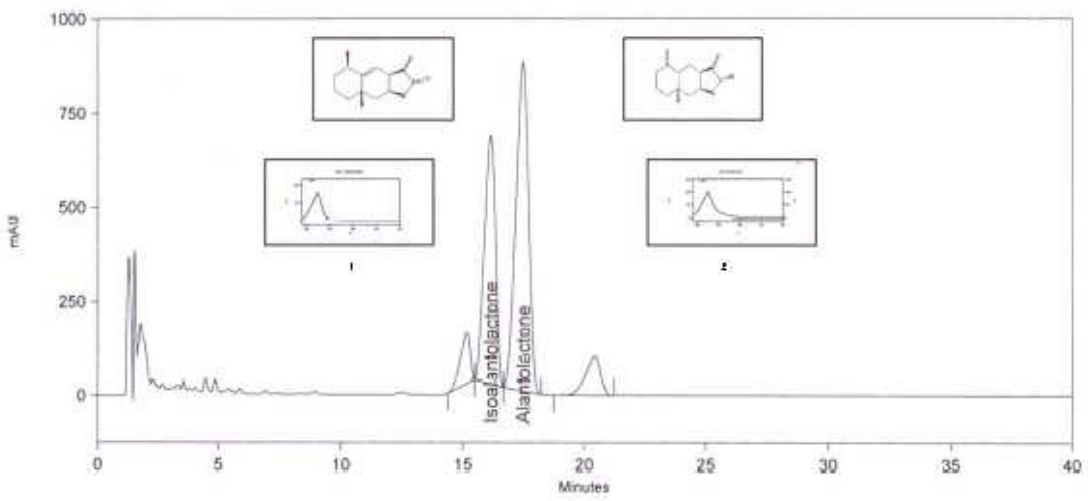
Sample Preparation
A 5.0g amount of fine powder (grounded and passed through a 40 mesh BS sieve) of *Inula racemosa* root was extracted using methanol (100ml) on a sonicator for 4 hr. An aliquot of 1ml from the methanolic extract was filtered through a 0.45µ nylon syringe filter and analyzed.

Mobile phase
Acetonitrile and 0.1 M Ammonium acetate in water in ratio of 1:1 were used for the study.

RESULTS AND DISCUSSION

The major sesquiterpene lactones **1** and **2** of *Inula racemosa* root could not be separated under isocratic/gradient RP-HPLC conditions using methanol and water or acetonitrile and water, as mobile phase. In order to achieve this separation, the analysis was carried out under an isocratic flow of mobile phase prepared using Acetonitrile and aqueous 0.1M Ammonium acetate mixture in a ratio of 1:1.

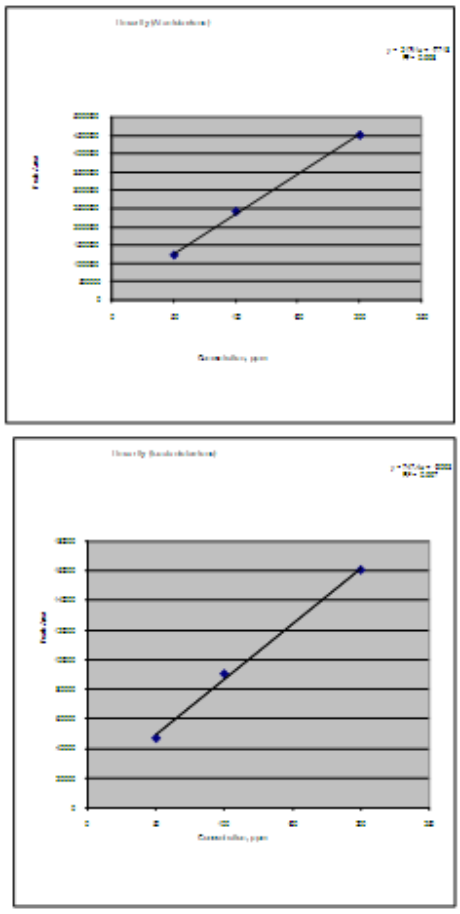
To establish the completion of extraction of the two isomers in methanol in 4 hr, the solution was further extracted for 3 hr in a sonicator. There was no difference in the peak area of the two isomers in the injections made after 4 hr and 7 hr respectively.



Chromatogram showing the separation of the Isoalantolactone (1) and Alantolactone (2) in *Inula racemosa* root

The Reproducibility of the method was established by extraction of *Inula racemosa* root powder in duplicate. The RSD in the resolution between the peaks of **1** and **2** of the duplicate preparation was found to be 1.15%.

The repeatability of the method was established by making five injections of a mixture of **1** and **2**. RSD for the peak area obtained from the five replicate injections of **1** was 0.99 %, and that for **2** was 1.7%.



The linearity relationship between the concentration of the isomers and the respective peak area was plotted over a concentration range of 50 ppm to 400 ppm. The correlation coefficients obtained for the two isomers were 0.997 for **1** and 0.9989 for **2**, indicating an excellent curve.

The peak purity index for **1** and **2** in the root of *Inula racemosa* was found to be 0.99999 for both the isomers, which rules out the co-elution of any other compound from the root matrix.The values for all the parameters have been summarized in **Table 1**.

Table 1

Compound	Repeatability (%RSD) (n=5)	Linearity (r^2)	3 point peak purity	Single point threshold	Peak purity index	Resolution
1	0.99	0.997	0.99404	0.99998	0.99999	2.20
2	1.7	0.999	0.99036	0.99998	0.99999	

CONCLUSION

The two sesquiterpene lactones present in the roots of *Inula racemosa*, namely Isoalantolactone (**1**) and Alantolactone (**2**) were separated with a resolution of 2.20, using RP-HPLC technique. The proposed chromatographic system was found suitable for effective separation and quantitation of with good resolution, peak shapes and minimal tailing [15].The various studied parameters demonstrate that it is a simple and rapid method for separation and identification of two isomers in the *Inula racemosa* roots, which can be used as a standardized method for the routine testing of raw material and quality control. The separation of two isomers obtained, makes the method suitable for quantification of **1** and **2** in the *Inula racemosa* root.

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