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Analysis of Marketed Herbal Formulations Containing *Withania somnifera* for its Withanolides content by HPLC Technique

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ABSTRACT

Standardization is the process where we can make sure about the quality of the product; broadly it covers the qualitative and quantitative part of analysis. Qualitative analysis mainly covers the identification of the component present in a particular compound, whereas the quantitative analysis covers the estimation of the components, which are, present in that particular product**.** Standardization refers to the process of delivering a product with a specified minimum level of one or more plant constituents. In some cases this is accomplished by measuring the level of a chemical in a crude herbal extract and establishing a standard amount of that chemical for future production. The standardization of herbal formulations has become very essential as there is increase in the demand and frequent usage of the herbal formulations by the people. Monoherbal formulations containing *Withania somnifera* extract were collected from the commercial market and standardized for their withanolides content by High Performance Liquid Chromatographic Technique (HPLC). The total peak area of standard (withanolides) and the corresponding peak area of samples were compared and the amount present in it was calculated. The results reveal that there are lot of variations between the samples and the content of withanolides is not uniform. The present study indicates the necessity of development of analytical procedures for all herbal formulations available in the market to ensure the quality and efficacy of the products.

Keywords: *Withania somnifera*, HPLC Analysis, Withanolides, Content variation

INTRODUCTION

Plants contain several hundred constituents and some of them are present at very low concentrations. In spite of the modern chemical analytical procedures available, only rarely do phytochemical investigations succeed in isolating and characterizing all secondary metabolites present in the plant extract. Apart from this, plant constituents vary considerably depending on several factors that impair the quality control of phytotherapeutic agents. Quality control and standardization of herbal medicines involve several steps. However, the source and quality of raw materials play a pivotal role in guaranteeing the quality and stability of herbal preparations. Other

factors such as the use of fresh plants, temperature, light exposure, water availability, nutrients, period and time of collection, method of collecting, drying, packing, storage and transportation of raw material, age and part of the plant collected, etc., can greatly affect the quality and consequently the therapeutic value of herbal medicines. Some plant constituents are heat labile and the plants containing them need to be dried at low temperatures[1].

The idea of standardization is to establish consistent potency and to control the full spectrum of bioactive chemical constituents naturally occurring in medicinal plants from batch to batch. This is complicated by the complex chemical group of plants and the difficulty in obtaining the pure materials needed to compare and measure the amounts of any one particular compound in a plant mixture[2].

The aim of the present study is to determine the content variations of monoherbal formulations containing *Withania somnifera* available in the commercial market. For our study we have selected withanolides as analytical marker present in the Ashwagandha for the HPLC analysis. The tablet and capsule forms of different brands of marketed monoherbal formulations of Ashwagandha were selected and standardized for their withanolides content by HPLC Technique.

MATERIALS AND METHODS

Sample collection

The tablet and capsules forms of different brands of five monoherbal formulations containing *Withania somnifera* were collected from various community pharmacies and given name as A to E and used for the study.

Standard Preparation

Prepared 0.1mg/ml concentration of withanolides (Withanoside IV, Withanoside V, withaferin A, 12-Deoxwithastramonolide, Withanolide A & Withanolide B) in HPLC grade methanol and used as standard solution.

Sample Preparation

Prepared 40mg/ml concentration of Ashwagandha powder in HPLC grade methanol and used as sample solution.

CHROMATOGRAPHIC CONDITIONS

Solvent A

Dissolved 0.136gm of anhydrous potassium dihydrogen orthophosphate (KH2PO4) in 900ml of HPLC grade water and added 0.5ml of ortho phosphoric acid.Added water to the above to make up the volume upto 1000ml. The above solution was filtered through 0.45µ membrane and degasses it in a sonicater for 3 minutes[3-6].

Solvent B

Acetonitrile solution.

Table No. 1 Gradient conditions

TIME (min)	Buffer-Concentration (Solvent a)	Acetonitrile Concentration- (solvent b)
0.01	95.0	5.0
18.0	55.0	45.0
25.0	20.0	80.0
28.0	20.0	80.0
35.0	55.0	45.0
40.0	95.0	5.0
45.0	95.0	5.0

Column	:	Hibar, Prepacked column, LiChrosher 100, RP-18e (5µm) (Merck) Phenomenex – Luna 5µ C-18(2) SIZE: 250×4.60mm
Detector	:	Photo diode array detector & UV Detector
Wave length	:	227nm
Flow rate	:	1.5ml/min
Injection volume:		20µl

RESULT AND DISCUSSION

The HPLC analyses of different monoherbal marketed formulations were carried out for the quantitative estimation of withanolides, the active principle present in *Withania somnifera*. In the present study the monoherbal formulations were selected in different dosage forms like tablets and capsules from various community pharmacies in the market. The results are tabulated in Table No: 3 and Fig No. 1-6.

Table No. 2 Retention Time of Standard and Samples

Name of Withanolides	STD RT	Retention Time of Samples				
		A	B	C	D	E
Withanoside IV	15.677	15.670	15.666	15.666	15.708	15.674
Withanoside V	19.871	19.871	19.866	19.862	NIL	19.859
Withaferin A	20.479	20.469	20.466	20.312	NIL	20.467
12-Deoxwithastramonolide	21.532	21.601	21.601	20.466	NIL	21.603
Withanolide A	22.328	22.305	22.321	22.320	NIL	22.296
Withanolide B	25.744	25.971	25.762	25.743	NIL	25.818

Table No. 3 Results of HPLC Analysis of herbal formulations

Sl. No	Brand Name	State of Ashwagandha	% Content of Total Withnolides
1	SAMPLE A	Extract	0.53
2	SAMPLE B	Extract	3.33
3	SAMPLE C	Root Powder	0.66
4	SAMPLE D	Extract	0.17
5	SAMPLE E	Extract	3..81

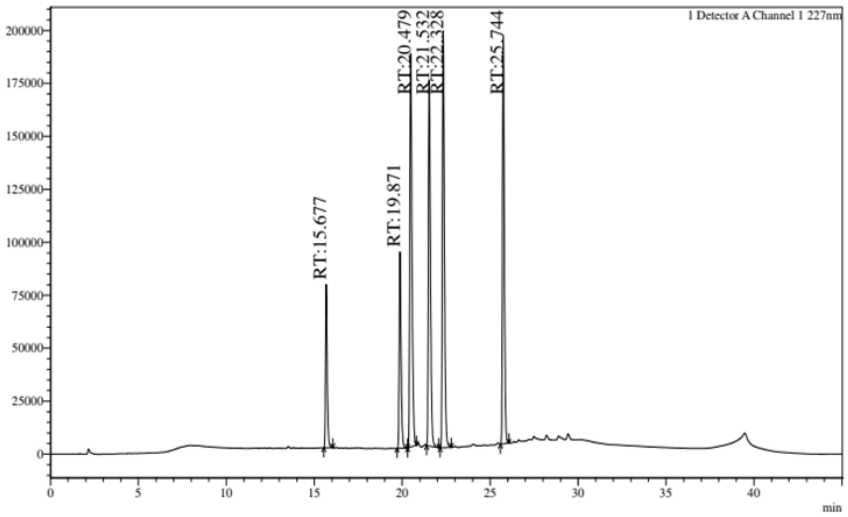

Fig. No. 1. HPLC Chromatogram of Standard Withanolide

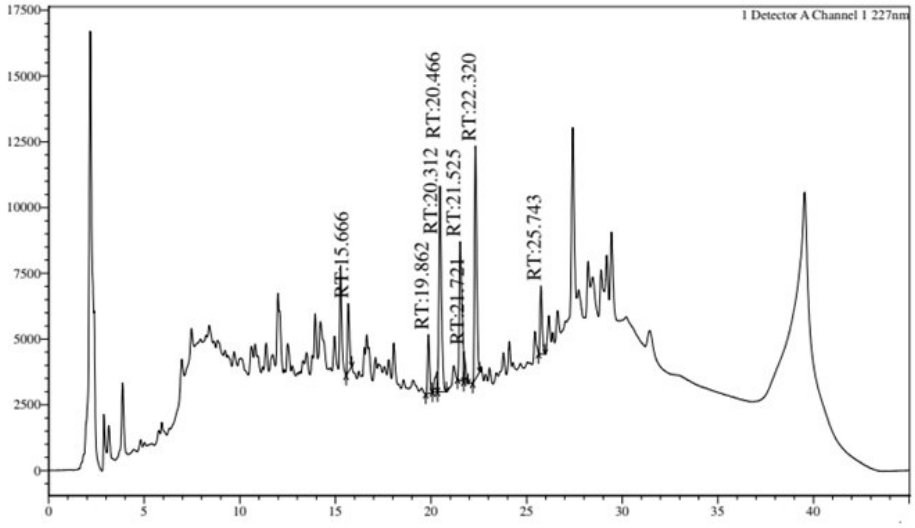

Fig. No. 2. HPLC Chromatogram of SAMPLE – A.

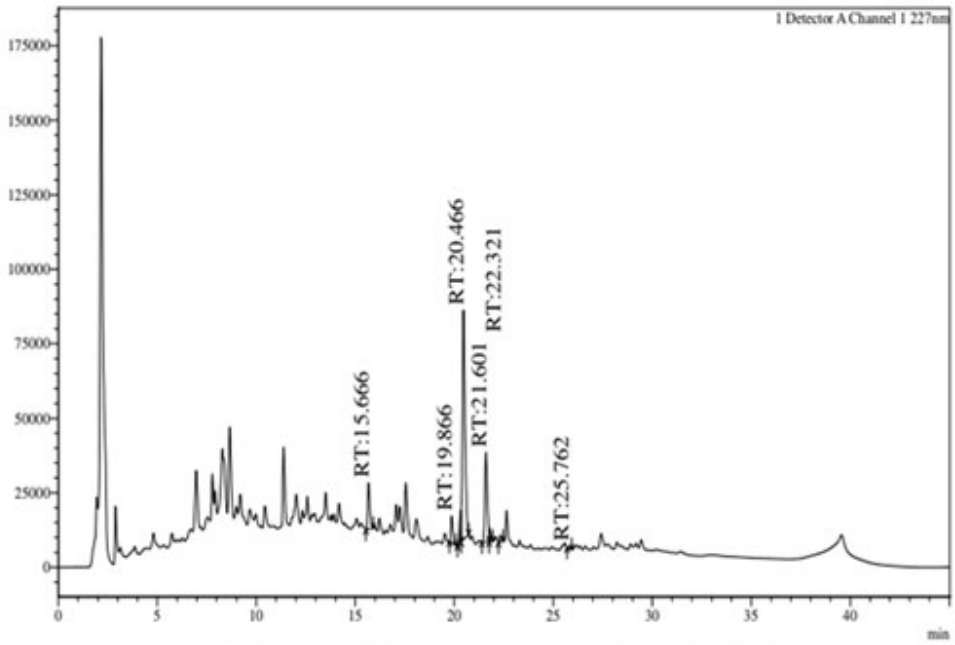

Fig. No. 3. HPLC Chromatogram of SAMPLE – B

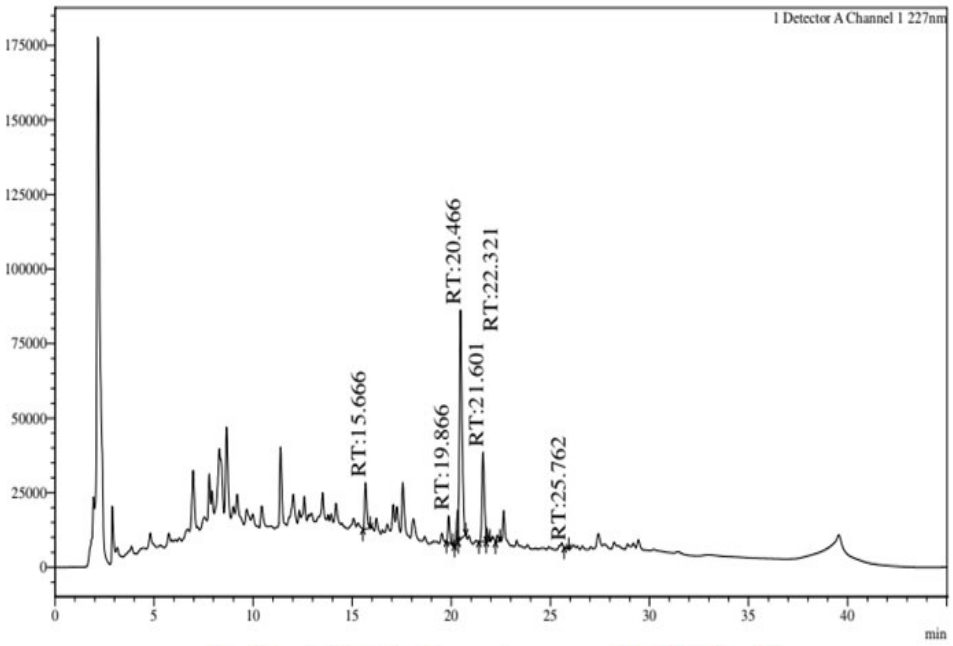

Fig. No. 3. HPLC Chromatogram of SAMPLE – B

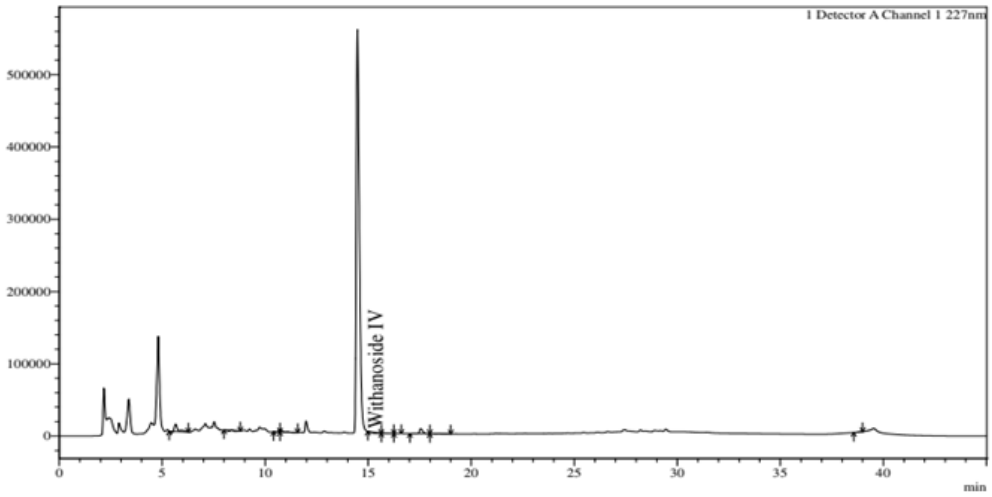

Fig.No. 4. HPLC Chromatogram of SAMPLE – C.

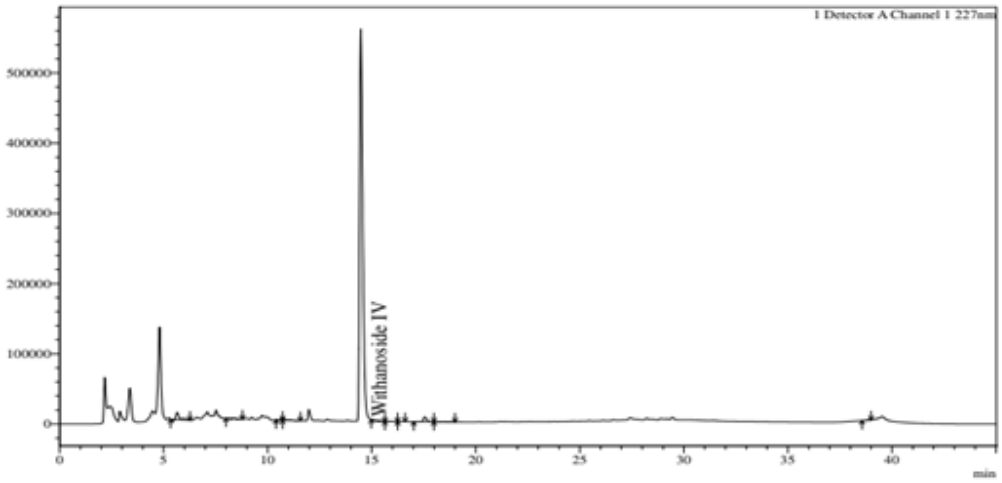

Fig.No. 5. HPLC Chromatogram of SAMPLE –D.

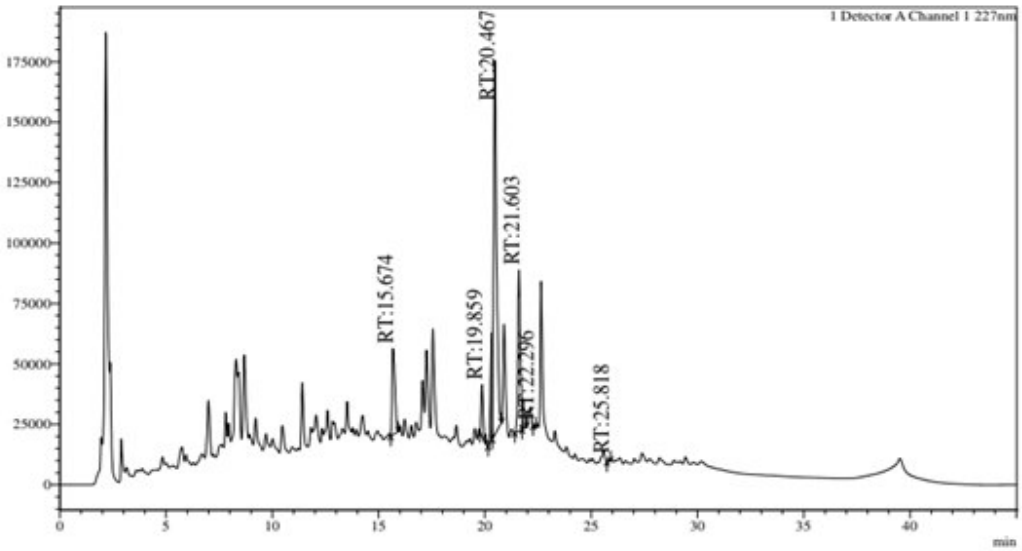

Fig.No. 6. HPLC Chromatogram of SAMPLE – E.

The monoherbal formulations of Ashwagandha containing the label claim 450mg, 500mg, 300mg, 300mg and 500mg of *Withania somnifera* were taken for analysis. The retention time of the standard withanolides were matching with corresponding samples and confirmed the presence of withanolides except Sample D. In sample D only Withanoside IV has been reported and remaining withanolides were not detected (Table No.2). The content of withanolides was estimated by comparing the peak area of standard and the respective samples. The amount of withanolides was found to be 0.53%w/w, 3.33%w/w, 0.66%w/w, 0.17%w/w and 3.81%w/w from the sample A, B, C, D and E respectively. From the results it was reveals that the content of total withanolides is high in SAMPLE D with 3.81% followed by SAMPLE B with 3.33% and Less in all three remaining samples.

SUMMARY AND CONCLUSION

The method of extraction and contamination with microorganisms, heavy metals, pesticides, etc., can interfere with the quality, safety and efficacy of herbal drugs[7]. For these reasons, pharmaceutical companies prefer to use cultivated plants instead of wild-harvested plants because they show smaller variation in their constituents. Furthermore and certainly more relevant, when

medicinal plants are produced by cultivation, the main secondary metabolites can be monitored and this permits definition of the best period for harvesting.

High performance liquid chromatography (HPLC) is the method of choice for checking peak purity of new chemical entities, monitoring reaction changes is in synthetic procedures or scale up, evaluating new formulations and carrying out quality control / assurance of the final drug products[8-9]. The Goal of HPLC method is to try & separate, quantify the main drug, any reaction impurities, all available synthetic intermediates and any degrades. High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantify the compounds that are present in any sample that can be dissolved in a liquid. HPLC is the most accurate analytical methods widely used for the quantitative as well as qualitative analysis of drug product and used for determining drug product stability[10]. Hence we selected the standardization of herbal products containing withanolides by HPLC technique. From the results it was concluded that a lot of variations in the total withanolides content in the selected five products containing ashwagandha and it indicates that the need of analytical procedure to test all herbal formulations available in the commercial market as like allopathic medicines to ensure the uniform quality.

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