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Comparative estimation of withanolides content from *Withania somnifera* obtained from commercial market in the form of tablets and capsules by HPLC technique

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ABSTRACT

Herbal formulations are increasingly sought out as medicinal products, dietary supplements and cosmetics in recent years and can be easily bought over the counter at pharmacy stores or health food shops. Herbal medicines originated firstly in the Asian countries before spreading to the West and today they are being seen as therapeutic agents for several chronic diseases. Hence standardization is important parameters to check the quality of herbal products.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, Analytical markers, Content variation

INTRODUCTION

Herbal drugs are a finished product which is labeled depending upon the ingredients contained in them. Active ingredients like the underground or aerial part of the plant or plant material or a combination of both is contained in herbal medicines. Herbal medicine products in the form of dietary supplements are taken to promote one's health and wellness. However, it is incorrect to ingest them without a prescription as some can lead to health problems of a different sort, some may interfere with other drugs or some may not be very effective. Standardization of herbal products and dietary supplements is very important for evaluating the drug quality based on the strength of their active principles. 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	15.666	15.712	15.638	15.673	15.666
Withanoside V	19.871	19.859	19.886	NIL	19.860	19.859
Withaferin A	20.479	20.460	20.322	NIL	20.469	20.466
12-Deoxwithastramonolide	21.532	21.520	21.725	NIL	21.526	21.598
Withanolide A	22.328	22.317	22.317	NIL	22.321	22.321
Withanolide B	25.744	25.739	25.736	NIL	25.744	25.741

Table No. 3 Results of HPLC Analysis of herbal formulations

Sl. No	Brand Name	State of Ashwagandha	% Content of total Withanolides
1	SAMPLE A	Extract	0.25
2	SAMPLE B	Extract	0.18
3	SAMPLE C	Root Powder	NIL
4	SAMPLE D	Extract	0.40
5	SAMPLE E	Extract	2.12

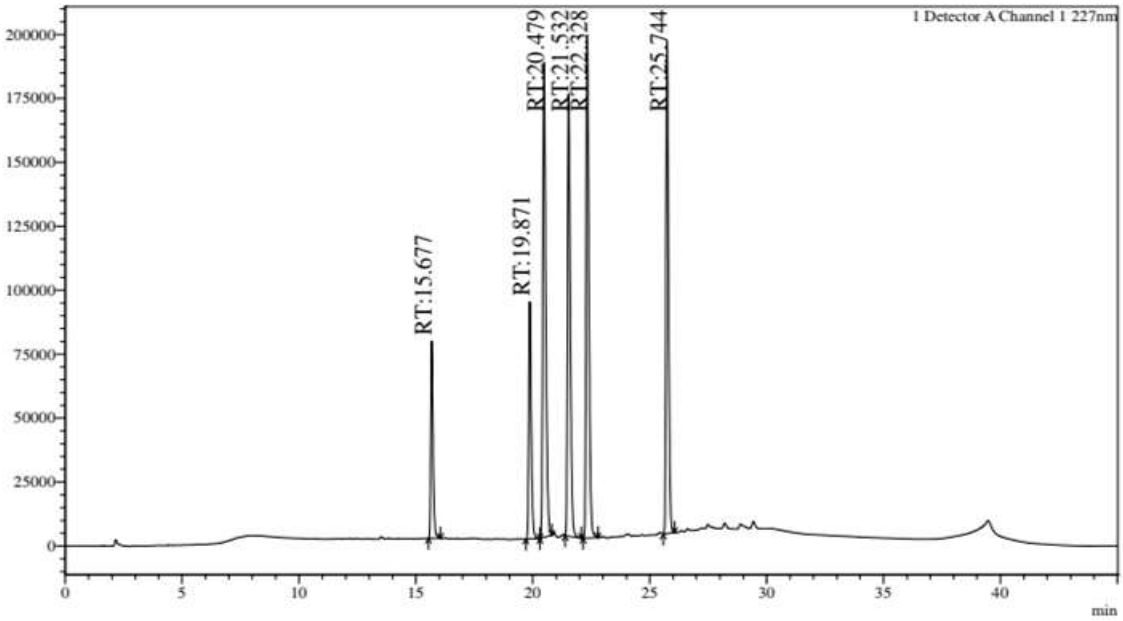

Fig.No. 1. HPLC Chromatogram of Standard Withanolides.

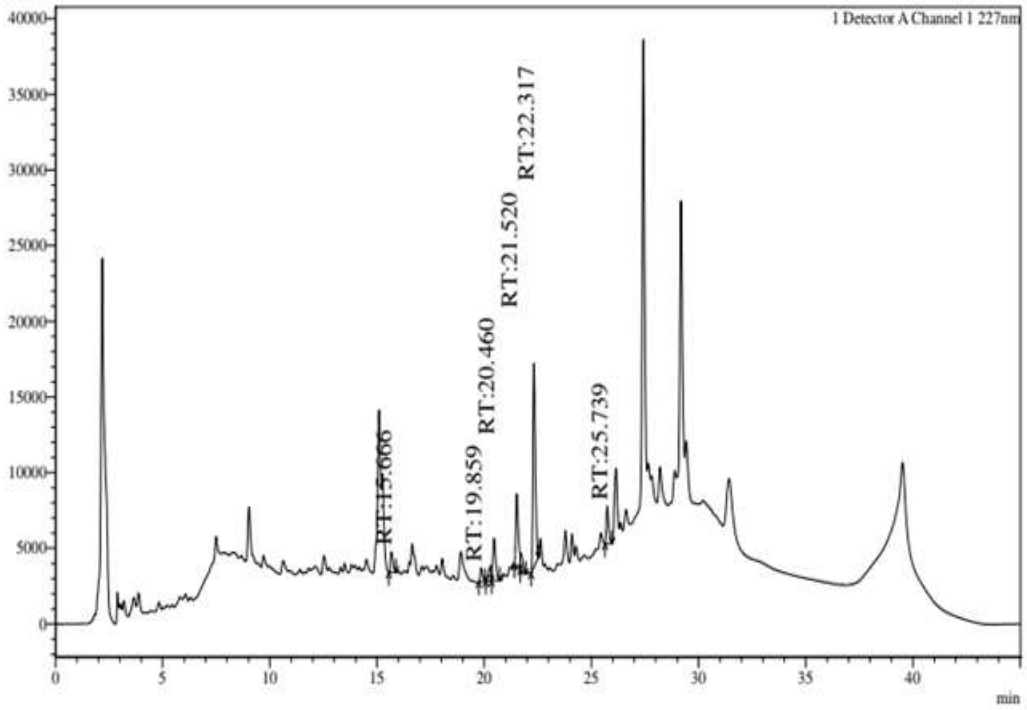

Fig.No. 2. HPLC Chromatogram of SAMPLE – A.

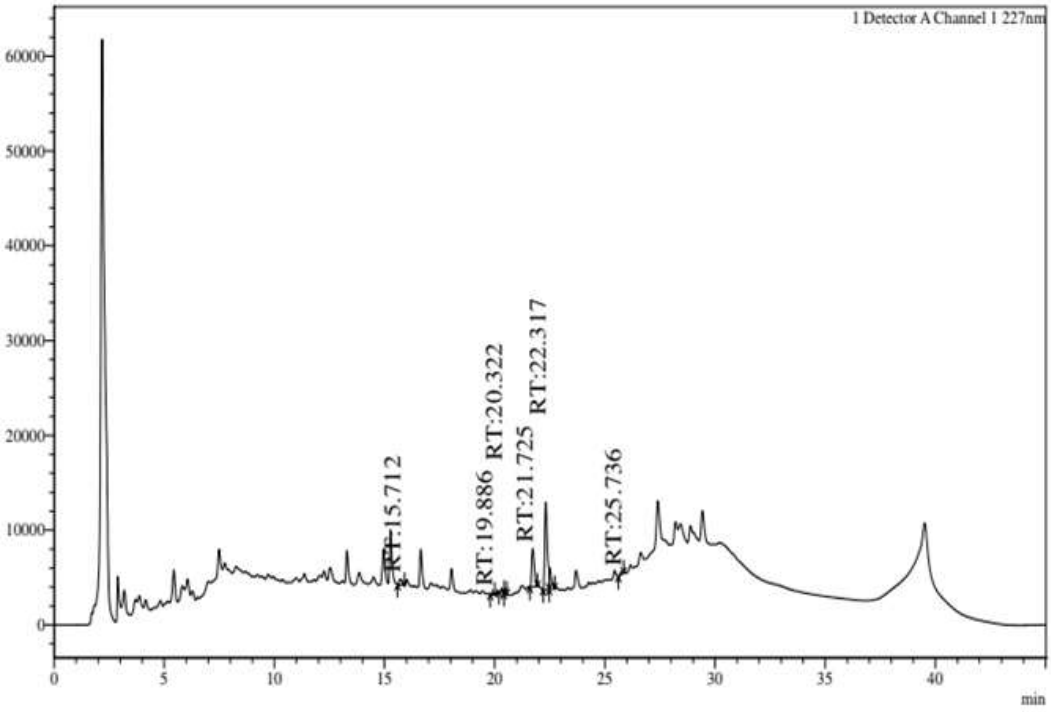

Fig.No. 3. HPLC Chromatogram of SAMPLE – B.

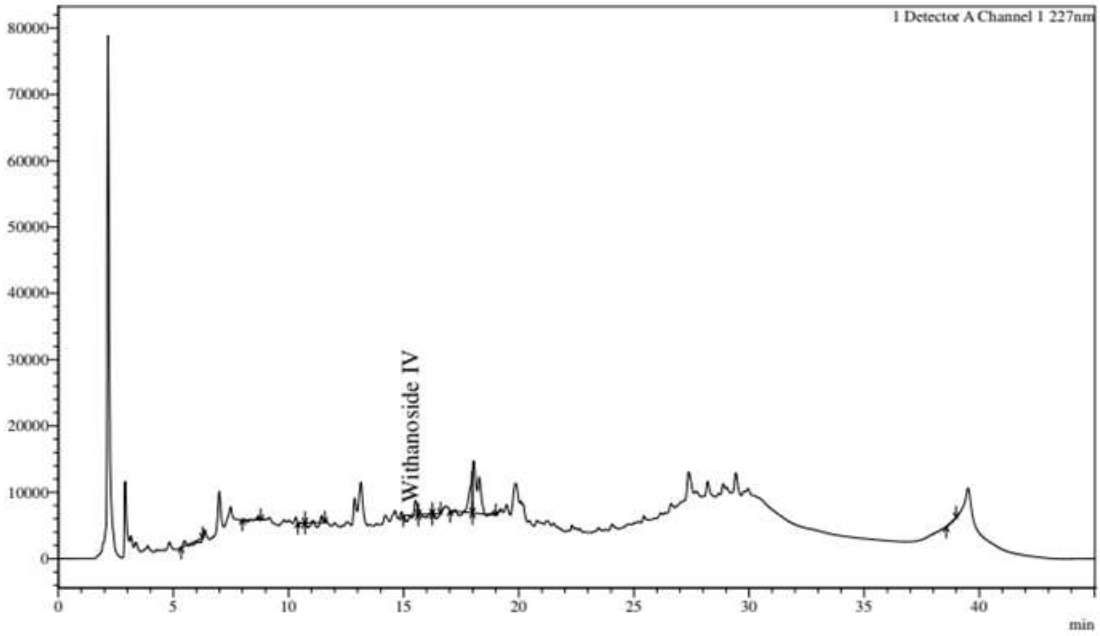

Fig.No. 4. HPLC Chromatogram of SAMPLE – C.

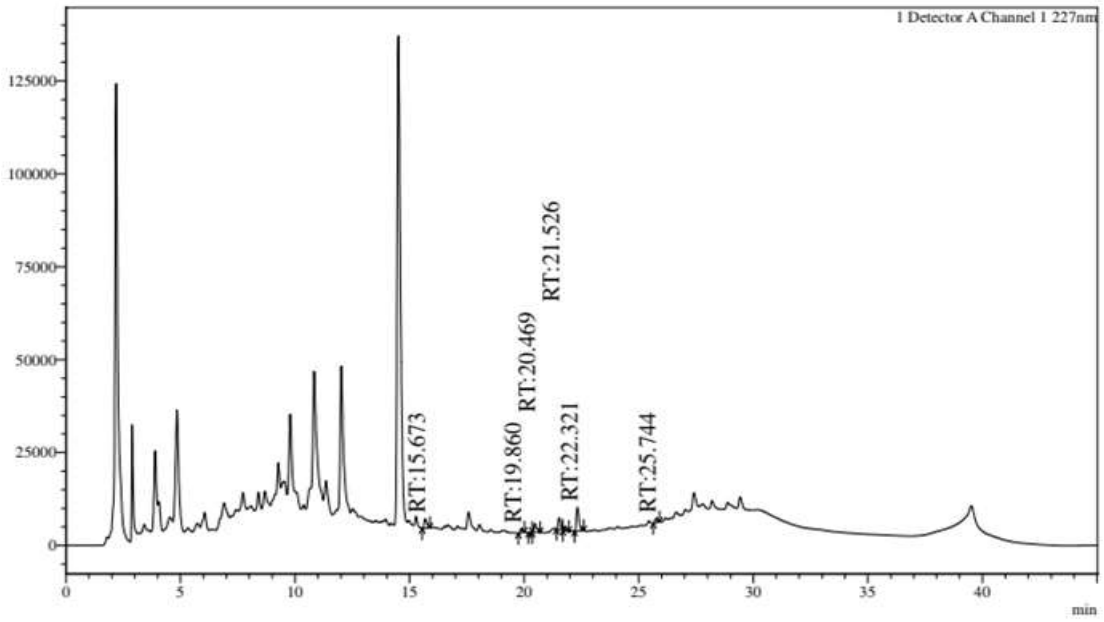

Fig.No. 5. HPLC Chromatogram of SAMPLE – D.

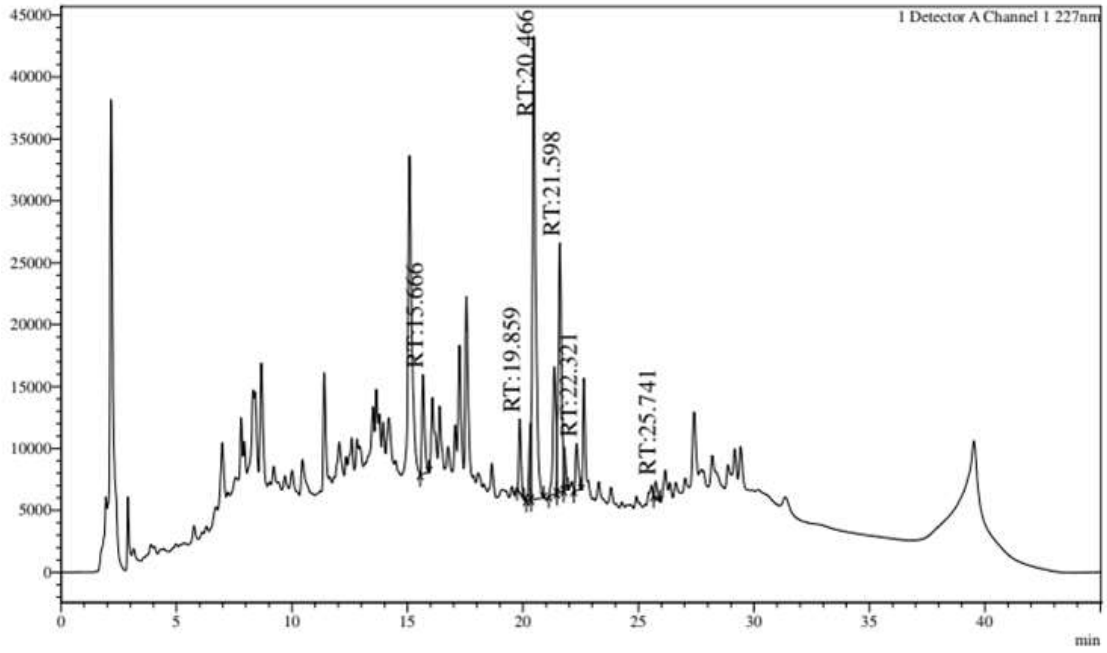

Fig.No. 6. HPLC Chromatogram of SAMPLE – E.

The monoherbal formulations of Ashwagandha containing the label claim 250mg, 850mg, 500mg, 250mg 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 C. In sample C 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 3.81%w/w, 0.25%w/w, 0.18%w/w, and 0.53%w/w from the sample A, B, D and E respectively. From the results it was reveals that the content of total withanolides is high in SAMPLE A

with 3.81% and Less in B, D and E. Sample C did not reported any amount of withanolides.

SUMMARY AND CONCLUSION

Greater numbers of people are moving towards use of herbal medicines in today's times, in view of the shortcomings of modern medicine which are becoming known each day. It is the absolute responsibility of the regulatory authorities in this regard to guarantee availability of pure, safe, potent and effective herbal medicines to the consumer. Various quality standards as laid down in formularies, pharmacopoeias or manufacturing operation are followed through statutory imposed good manufacturing practices by regulatory authorities. However, in case of herbal medicines it is difficult to attain a standard quality profile on account of the inherent variability of the plant constituents. Batch to batch differences begin from the raw material collection stage itself due to lack of any reference standard for identification. Further, stages of storage and processing add on to the variations.

High Performance Liquid Chromatography 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.

ACKNOWLEDGEMENT

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