ISSN 0976 – 3333

Available Online at www.ijpba.info.

International Journal of Pharmaceutical & Biological Archives 2010; 1(2):183 – 186

ORIGINAL RESEARCH ARTICLE

HPLC Estimation of Withaferin-A and Boswellic Acid in Formulated Gels.

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Received 18 April 2010; Revised 3 May 2010; Accepted 5 May 2010

ABSTRACT

Withania somnifera and Boswelia serrata are important drugs of many ayurvedic preparations. The interactions of the ingredients in the process of formulations are unimaginable. Hence, the attempt made in order to assess the concentration of Boswellic acid and Withaferin A in the formulation. 2% W/W each of Methanolic Extracts of *Withania somnifera* and *Boswellia serrata* were prepared, incorporated in gel formulations using different polymers and estimated the content of withaferin A & Boswellic Acid in the formulations using HPLC method. The result shown 95-105% of expected Withaferin A and 96-104% of expected Boswellic acid in formulations

Key Words: Withania somnifera, Boswellia serrata, Withaferin A, Boswellic acid.

INTRODUCTION

Traditional medicine from all the ancient civilizations has come upfront during the last decade, throughout the world as pressing need for the alternatives is mounting [1]. There is some overlap in the herbal and Ayurvedic medicines. Effort is on to provide the scientific validity to these medicines by applying the methodology of modern medicine. Withania somnifera (L.) Dunal. (Solanaceae) is a valued herb, upto 1.5m high shrub with ovate leaves and greenish-yellow flowers can be found in western India, and is locally known as Ashwagandha [2]. The root is known to possess CNS depressant properties [3], some of its CNS effects including antistress, antianziety and CNS inhibitory properties [4]. Boswellia serrata belonging to family Frankincense is a large tree that grows in the dry and hilly parts of India [5]. The gum resin of Boswellia serrata, commonly known as Salai guggal, has been traditionally used in the Ayurvedic medicine of India for a variety of inflammatory disease [6]. The Boswellia serrata herb reported to possess potent anti-inflammatory activity and lower ulcerogenic index [7].

Withania somnifera and Boswellia serrata are important drugs in many ayurvedic antiarthritic formulations. The antiarthritic activity of the both the drugs are well established by many scientific works. Very less attempts were made to estimate the active constituents in the formulations containing these drugs. In the present study efficacious and stable oleogels containing methanolic extracts of Withania somnifera and Bosewellia Serrata were prepared and evaluated using HPLC method.

EXPERIMENTAL

Materials: Standards **Withaferin A** and **Boswellic Acid** obtained from Natural Remedies Ltd. Veerasandra, Bangalore-100. The Crude drugs Ashwagandha and Shallakki were purchased from local market at Avenue Road, Bangalore. Ashwagandha roots were authenticated by Regional Research Institute, Ashoka Pillar, Bangalore. Boswellia gum resin authenticated from FRLHT, Bangalore. Solvent Methanol of A.R and HPLC grade purchased from E-Merck for extraction and Analysis.

Sample Extraction: 600gm each of Withania somnifera and Boswellia serrata gum resins were powdered and extracted with methanol separately on soxhlet for 4 hours. Filtered, Evaporated the solvent and collected the extracts, dried and used for the preparation of gels.

Preparation of Gels: Different polymers are used to prepare the gels viz. HPMC 15cps, HPMC K4M, Sodium CMC MVP, Carbopal 934, PEG 400 and PEG 4000 The formulations are as shown in **Table 1**.

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PREPARATION OF STANDARD AND SAMPLES FOR HPLC ANALYSIS

Standard: Dissolved 10mg of Withaferin A working standard in 50ml of methanol HPLC grade. Further diluted 1ml of this solution to 50ml using methanol HPLC grade.

Sample: 1gm of the samples were accurately weighed and dissolved in 50ml of methanol HPLC grade. Further diluted 1ml of this solution to 50ml using methanol HPLC grade.

Procedure: Inject 20µl of standard and sample and record the chromatogram, calculated the content of Withaferin A of the samples in comparison with standard.

Table 1: Formulation of gels.

Ingredients	Formulation 1	Formulation 2	Formulation 3	Formulation 4
Polymer	NaCMC 4%	HPMC 15cps 4%	HPMC K4M 4%	CARBOPAL934 4%
Boswellia Extract	2%	2%	2%	2%
Withania Extract	2%	2%	2%	2%
Propylene glycol	25%	25%	25%	25%
Glycerol	5%	5%	5%	5%
Benzalkonium chloride	0.1%	0.1%	0.1%	0.1%
Triethanolamine	----	----	----	Q.S to neutral pH
Water Q.S	100%	100%	100%	100%

ANALYTICAL METHOD

HPLC estimation of Withaferin A & Boswellic acid performed on Shimadzu 10AS HPLC system, equipped with UV detector. For estimation of withaferin A, Lichosorb C18 RP column (250X 4.6mm, 5µm particle size) and the mobile phase with mixture of Acetonitrile: Methanol: ortho phosphoric acid (55:45:1) were used at flow rate of 1.2ml/min and monitored at the detection wavelength of 224nm.For Boswellic acid, Lichosorb C18 RP column (250X 4.6mm, 5µm particle size) and the mobile phase with mixture of methanol: Acetonitrile: Water (90:0.5:9.5) were used at flow rate of 1.0ml/min and monitored at the detection wavelength of 247nm.Since the method is obtained from official book, Indian Pharmacopoeia only system suitability tests were carried and precision recovery studies and accuracy studies are left out. Peak of Withaferin A & Boswellic acid identified by using primary standard. Quantification done by taking the values of Methanolic Extract of Withania somnifera and Methanolic Extract of Boswellic Acid as 100%, which were standardized using working standards of Boswellic acid and Withaferin A.

RESULTS AND DISCUSSION

The separation of Withaferin A and Boswellic Acids in formulations can be attained under optimized conditions. By changing Mobile phase systems interference of Boswellic Acid in estimation of Withaferin A and vice-versa could be avoided. Withaferin A peak eluted at about 14.2minutes, which was considered for standardization purposes. Withaferin A could be baseline separated from other compounds of similar polarity in 30 minutes.Similarly, in case of Boswellic Acid no interference found at the Retention time of Boswellic acid. Any other modification made in detection wavelength or changes in mobile phase constitution were not advantageous. The HPLC conditions were slightly changed as the separation had to be performed at room temperature and a flow rate of 1.0ml did not allow a sensitive detection of the compounds. Hence, flow rate altered to 1.2ml, which enabled the good separation of Withaferin A. Whereas for Boswellic Acid 1.0ml/min flow rate was sufficient for good separation.No indication of impurities could be found in reference peaks used for identification purpose (**Fig.1 & 7**). Area under curve of Working Standard peaks considered for the purpose of standardization (**Fig.2, 8**).The analysis of Withaferin A in formulated gels indicated that the interaction of withaferin A with other ingredients was negligible (Fig.3-6, Fig.9-12). The gel shows the presence of about 95-105% of the amount of Withaferin A in them against 100% of expected value. (**Table2**)Similarly, the content of Boswellic Acid found to be within the acceptable range. The gels show the presence of 96-104% of the expected amount of Boswellic acid. (**Table. 2**)

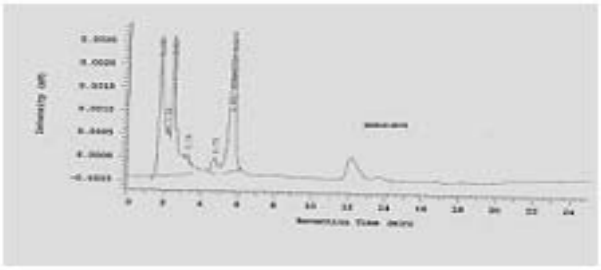

Fig.1: Boswellic acid RS

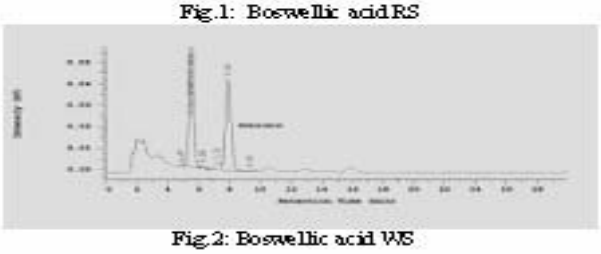

Fig.2: Boswellic acid WS

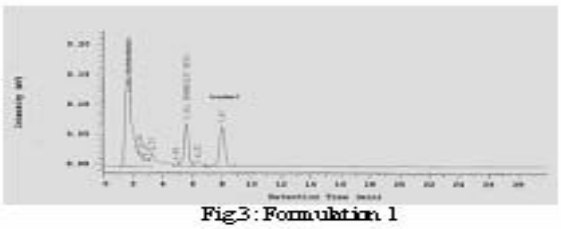

Fig.3: Formulation 1

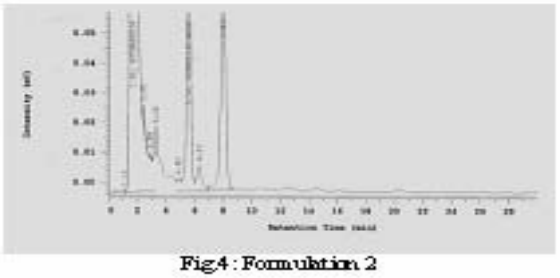

Fig.4: Formulation 2

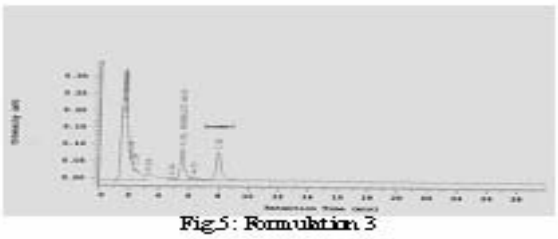

Fig.5: Formulation 3

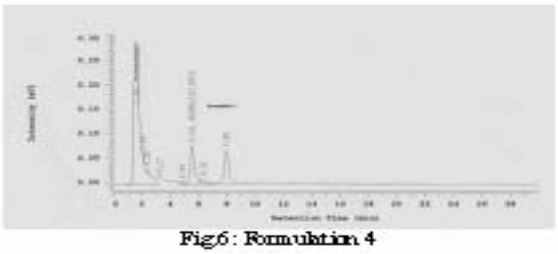

Fig.6: Formulation 4

Fig.1 & 2. HPLC Chromatogram of Boswellic acid RS and Working Standard, 3. Chromatogram of Formulation1, 4. Chromatogram of Formulation 2. 5.Chromatogram of Formulation 3, 6. Chromatogram of Formulation4.

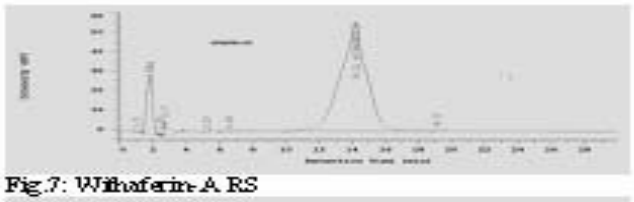

Fig.7: Withaferin A RS

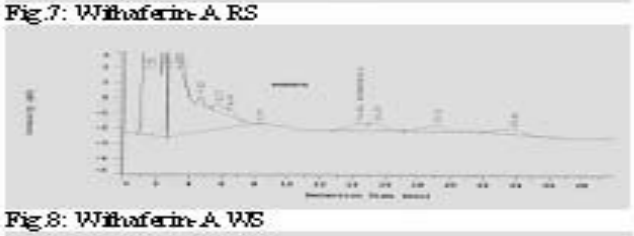

Fig.8: Withaferin A WS

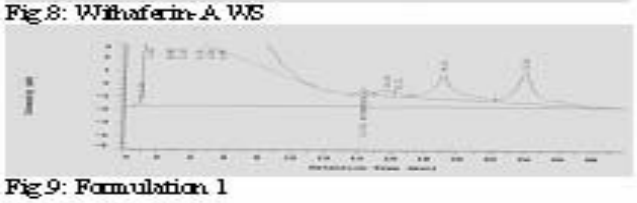

Fig.9: Formulation 1

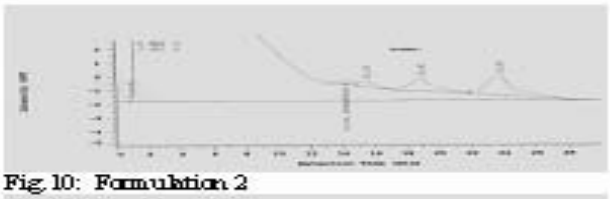

Fig.10: Formulation 2

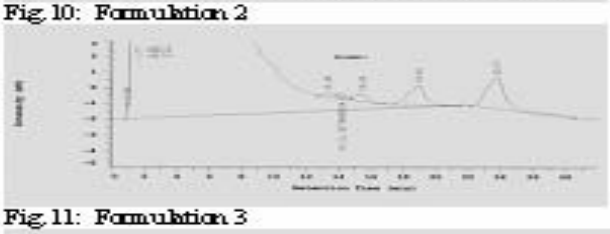

Fig.11: Formulation 3

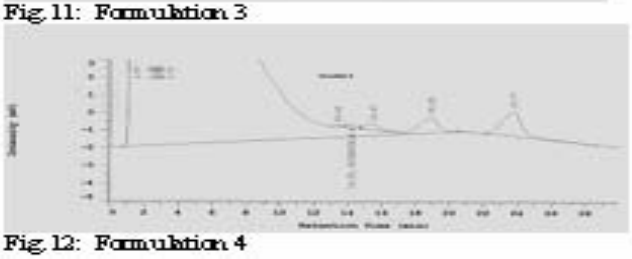

Fig.12: Formulation 4

Fig.7 & 8. HPLC Chromatogram of Withaferin A RS and Working Standard, 9. Chromatogram of Formulation1, 10. Chromatogram of Formulation 2. 11.Chromatogram of Formulation 3, 12. Chromatogram of Formulation4.

Table2: HPLC results of Boswellic Acid and Withaferin A.

Formulation	Boswellic acid in %	*Withaferin A in %*
Formulation 1	102.22	95.28
Formulation 2	103.84	103.91
Formulation 3	103.34	101.90
Formulation 4	102.25	102.46
Formulation 5	96.45	101.67
Formulation 6	100.93	103.41
Formulation 7	96.97	104.11
Formulation 8	103.63	104.92
Formulation 9	100.94	101.16

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ACKNOWLEDGMENTS

The authors Rajendra.C.E is thankful to M/S Natural remedies Pvt. Ltd. for providing gift samples of Withaferin A & Boswellic acid reference standard and Working standard. In addition, the co-operation of Superintendent, Drugs Testing Laboratory, Drugs Control Department was important to carry out the HPLC Estimations