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HPLC In Standardization Of Withaferin - A In *Withania Somnifera* Root Extract

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ABSTRACT

HPLC is a popular method of analysis because it is easy to learn and use and is not limited by the volatility or stability of the sample compound. By virtue of its ability to simultaneously separate, identify and analyze the complex mixture of organic substances, HPLC will be highly useful for the analysis of herbal drugs. In the present communication we report the HPLC analysis of withaferin-A from methanolic extract of *Withania somnifera*, a popular adaptogenic in the western world. The proposed HPLC method was found to be simple, feasible and easily reproducible method.

KEYWORDS

HPLC;
Withaferin – A;
Withania somnifera.

INTRODUCTION

Withania somnifera Dunal (Family: Solanaceae) an ancient herb which is commonly known as Ashwagandha, an ayurvedic medicinal plant. This plant is distributed in the northern parts of India, Mediterranean region and in Africa[1]. It is reported to have several pharmacological effects including anthelmintic, narcotic, radiosensitizer, antistressor, adaptogenic and cardioprotective effects[2–5]. The roots are used in cough, dropsy, rheumatism and gynecological disorders[6]. It is official herb in Indian pharmacopoeia (1985) and Indian herbal pharmacopoeia (1998). The major constituents of this plant include alkaloids, steroidal lactones, glycowithanolides and sitoinosides. Total alkaloids, withanolide and withaferin-A content along with proximate analysis may be used to compare different samples. Recently this herb has been introduced in west, where it has received a very positive response from scientific community. The products of ashwagandha are available throughout the world as dietary supplements. It is being referred as "Indian Ginseng" and is finding a unique place in the diet as

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functional foods to reduce the incidence of various diseases. Hence there is a need to standardize and analyze the contents of *Withania somnifera* in general and withaferin-A in particular. The objective of this paper is to develop a system for the accurate, rapid, efficient and reproducible method for the estimation of withaferin-A in the methanol extract of the roots of *Withania somnifera.*

EXPERIMENTAL

Materials and Methods

Withania somnifera roots and withaferin-A (standard) were obtained as gratis samples from Natural Remedies India Pvt. Ltd., Bangalore. Solvents used in HPLC system were of HPLC grade and other reagents were of analytical grade.

Extraction

Dried roots of *Withania somnifera* (100 g) were powdered and defatted with petroleum ether (500 ml), followed by extraction with methanol for 4 h. The methanol extract was refluxed for another 4 h and concentrated using rotary evaporator.

Sample preparation

About 25 mg of the extract was dissolved in 10 mL of methanol and diluted suitably followed by filtration through a 0.45 μm membrane filter. The sample solutions were injected in triplicate for the analysis.

Calibration curve for withaferin-A

A series of standard solution of withaferin-A (0.02, 0.04, 0.08, 0.16, 0.24, 0.32 and 0.40 mg/ml) were prepared by diluting the stock solution (2.0 mg/ml) with methanol. All of the solutions were filtered through 0.45μm membrane filter, and a 20μL was injected in to a chromatograph in triplicate. A calibration curve was constructed by plotting the peak-area against concentration of withaferin-A.

Instrumentation

The HPLC system consisted of a SHIMADZU LC- 10 AD pump, an SPD-10A ultraviolet – visible detector, a 20μL rheodyne injector, and an LC workstation for data collection, all operated at room temperature. The separation column was one filled with Hypersil ODS C18 (25 X 4.6 mm i.d., 5 μm particle size). The mobile phase consisted of Acetonitrile and water (50:50). The flow rate was 1 ml/ min, and the wavelength set at 220 nm.

RESULTS AND DISCUSSION

Standardization using marker compound is one of the best methods of standardizing herbs and herbal preparations[7]. Withaferin-A has been used as biomarker for standardization purpose. The methanol extract of the roots of *Withania somnifera* was subjected to reverse phase HPLC analysis to de-

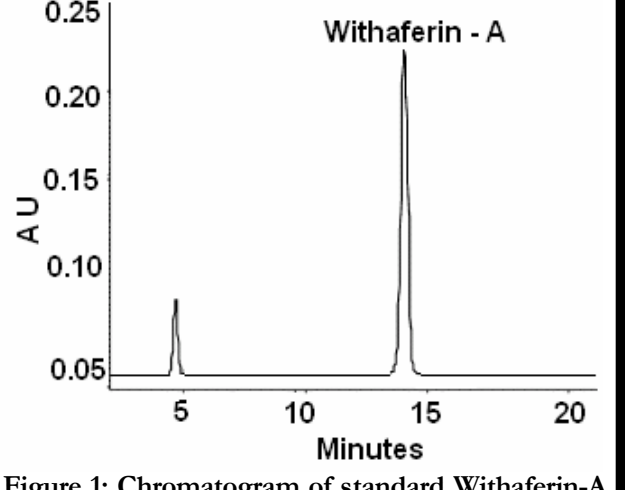

Figure 1: Chromatogram of standard Withaferin-A

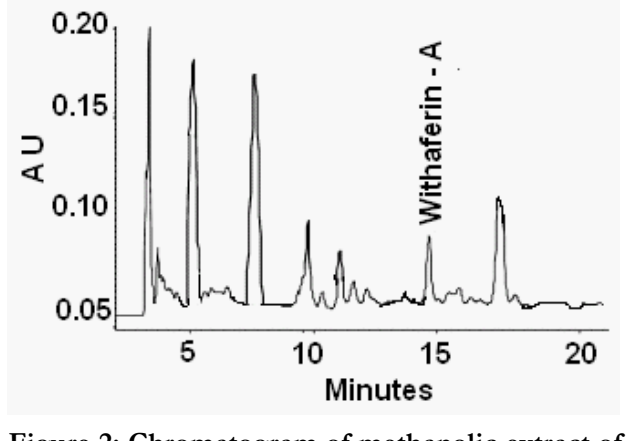

Figure 2: Chromatogram of methanolic extract of the roots of *Withania somnifera*

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termine the Withaferin-A content. A typical chromatogram of standard withaferin - A is shown in figure 1. The calibration curve of the area versus the concentration was linear (r^2 = 0.9999; n=7). The linear range for the determination of withaferin-A was 0.02 - 0.04 mg/ml. The RSD was found to be not more than 2.0%. Withaferin-A could be baseline separated in less than 20 min, and was assessed for the content after complete separation to the baseline, and has been shown in the chromatogram (Figure 2). The retention time of standard withaferin-A was found to be 14.5 min which, was also found at the similar retention time in sample extract at a + 0f 0.5 min. The content of withaferin-A in the methanolic root extract was found to be 1.29 g/100 gm.

CONCLUSION

The method described here represents a simple and easily reproducible HPLC method for the estimation of withaferin-A. The proposed methodology will be helpful for various analytical purposes.

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