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Development And Validation Of Method For The Estimation Of Withanolide A From Herbal Oral Thin Film

Safeena Sheikh
Unijules Life Sciences Ltd
MIDC Area, Kalmeshwar, Nagpur , India
Suhail Asghar
Unijules Life Sciences Ltd
MIDC Area, Kalmeshwar, Nagpur , India
Showkat Ahmad
Unijules Life Sciences Ltd
MIDC Area, Kalmeshwar, Nagpur , India

Abstract:
A rapid and simple high performance thin layer chromatographic method has been developed for the standardization and quantification of withanolide A from an herbal oral thin film (OTF). HPTLC profile was developed using Toluene: Ethyl acetate: Formic acid (8:4:0.6) as a mobile phase and evaluated on the basis of different parameters. The Rf values of withanolide A was found to be 0.23. The total peak areas of the withanolide A and the corresponding peak areas of different formulations were compared and withanolide A were estimated as 2.25mcg. The limit of detection and limit of quantification was found to be 30ng/spot and 80ng/spot. The percent recovery was found to be well within the limit of 98 to 102%.The present study rationalizes the use of different withanolide A formulations profiles for ascertaining the identity, purity and strength of the different withanolide A formulations and also for generating data which may be use in setting up of these herbal formulations by evaluating these formulations on the basis of various parameters. . The method was found to be simple, reliable, accurate and precise in accordance with ICH guidelines.

Keywords: *Ashwagandha, Withanolide A, OTF, Mouth dissolving film, method development, validation, densitometry, Quantification, withanolide A and chromatography.*

1.Introduction

Ashwagandha, Withania somnifera Dunal has been used for thousands of years as a rejuvenating treatment. It is one of the best health tonics and restorative agents that have been used to exhaustion stress [2] induced fatigue and insomnia. Ashwagandha has many significant benefits, but is best known for its powerful adaptogenic properties, meaning that it helps mind and body adapt better to stress. It nourishes the nerves and improves nerve function to help you maintain calm during stressful situations. It is also good for people who do physical labor or exercise a lot, to help the body adapt to physical stress. It nourishes all the body tissues. Ashwagandha is used to rejuvenate the nervous system [1] ,relieve insomnia, and counteract stress without the drowsy after effects of sedatives.

Withanolides [9] are a group of naturally occurring oxygenated ergostane type steroids, having lactone in side chain and 2-en-1-one system in the ring. Withanolides are present in medicinal plants of Solanaceae family. Withanolide A is a steroidal lactone present in dried roots of Withania somnifera Dunal.

Literature survey reveals the method for the quantitative estimation of Withanolide D using HPLC [4] and [7] from the herb and its extracts. Also HPTLC [6][11] method for the simultaneous estimation of Withanolide A and Bacoside A from spansules. But no method describes the estimation of Withanolides A from mouth dissolving film (OTF).

The aim of this investigation was to develop and validate the estimation of Withanolides A marker in mouth dissolving thin film (OTF). The system can be utilized successfully to investigate presence of marker in different species of Withania somnifera plant part as well in market formulations.

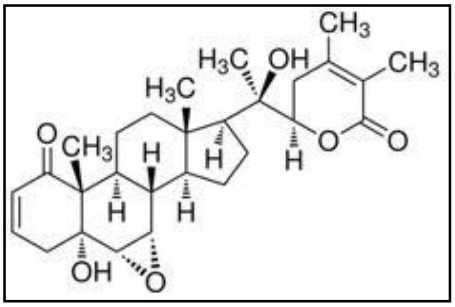

Figure 1: Chemical structure of Withanolide A

2.Experimental

2.1.Reagent and Chemicals

The solvents (Toluene, Methanol and Formic acid) were of analytical laboratory grade. The standard of withanolide A was purchased from Natural Remedies Bangalore.

2.2.Preparation Of The Standard Solution

Transfer an accurately weighed 10.0mg of standard withanolide A in 10ml of volumetric flask, dissolved the content in the mixture Chloroform: methanol (9:1) by shaking and made the volume up to the mark with. Take 1.0ml from the above stock solution and dilute to get the final concentration of 100.0mcg/ml in methanol.

2.3.Preparation Of Sample Solution

Take the average weight of the ginger OTF and transfer an accurately weighed 700.0mg of OTF in 250.0ml round bottom flask containing 50.0ml methanol and allow refluxing the content for about 30.0minnute; it was cooled and filter. The remaining residues refluxed again with 30.0ml methanol for 20.0minutes; filter and combine the washing. Then again reflux the remaining residue with 20.0ml of methanol, cool and filter. Combine the filtrate and evaporate it on water bath till complete dryness. Dissolve the residue in 5.0ml of methanol.

2.4.Instrumentation And Chromatographic Condition

Chromatography was performed on Aluminium backed silica gel $60F_{254}$, 2µl and 4µl of methanolic solutions of samples and standard withanolide A of known concentrations were applied to he plate of size (20X10cm) with 250µm thickness;(Merck") using a *CAMAG* Linomat 5 sample applicator (Switzerland) under a flow of nitrogen; positioned 10mm from the bottom and 15mm from the side of the plate; The sample were spotted in the form of bands with a *CAMAG* 100µl sample syringe (Hamilton, Bondouz, Switzerland) syringe. The linear ascending development was carried out in 20cmX 10cm twin trough chamber using Toluene: Ethyl acetate: Formic Acid (8: 4:0.6v/v/v) as mobile phase. After development, plates were dried on *Camag* TLC plate heater III and the densitometric scanning was performed using a

Camag TLC scanner 3 at λmax 235nm in the reflectance-absorbance mode for withanolide A operated by winCATS Software (version 1.4.3). The slit dimensions were 4x 0.45mm and the scanning speed was 100mm/s.

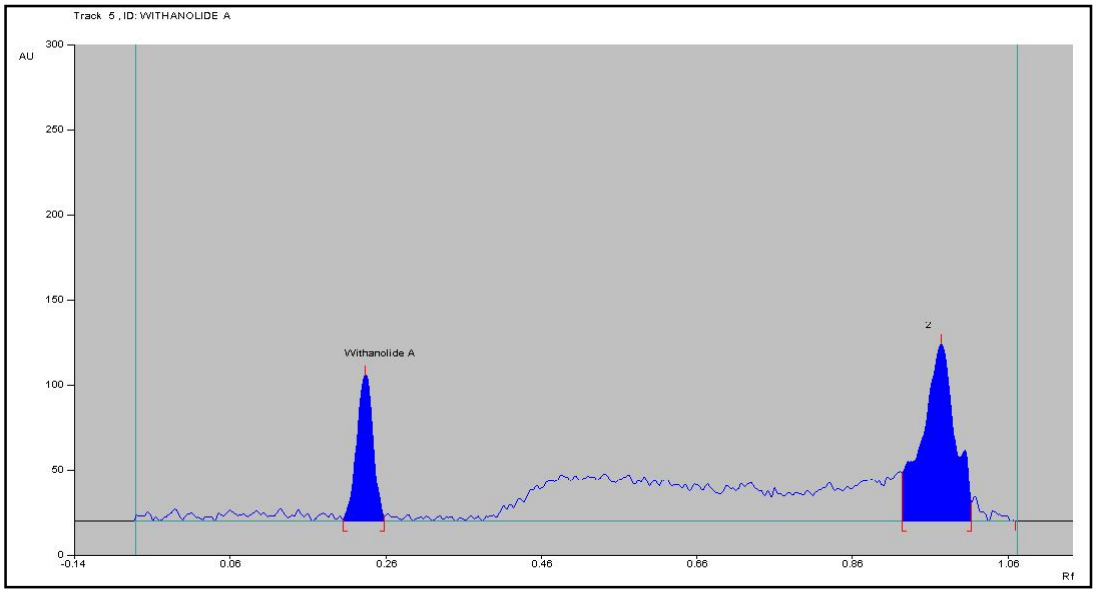

Figure 2: Densitogram of Standard Withanolide A after scanning under 235nm. (Rf=0.23).

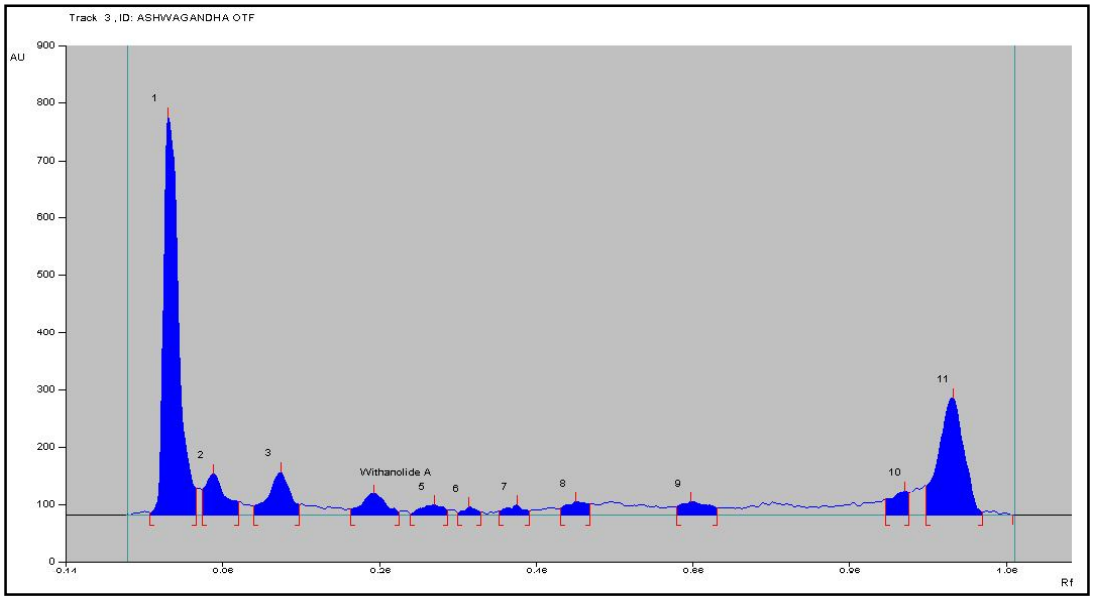

Figure 3: Densitogram of Sample OTF A after scanning under 235nm. (Rf=0.25)

3.Method Validation [8]

An HPTLC method was developed for the quantification of withanolide A in pharmaceutical oral thin film and validated in terms of precision, accuracy, recovery and robustness, LOD and LOQ were checked as per ICH guidelines. Instrumental precision was checked by repeated scanning of the same spots five times and the results were expressed as % RSD. Method precision was studied by analyzing the standards under the same analytical procedure and laboratory conditions on the same day (intra-day precision) and on different days (inter-day). The results are expressed as % RSD. Accuracy of the method was tested by performing the recovery studies of the pre-analyzed sample with standard at three different levels in each sample and the results are expressed as percentage recovery and % RSD.

4.LINEARITY

Linearity was determined in the range 80 to 120% of working concentration of standard. The peak area responses were plotted against the corresponding concentrations and r2 values were calculated.

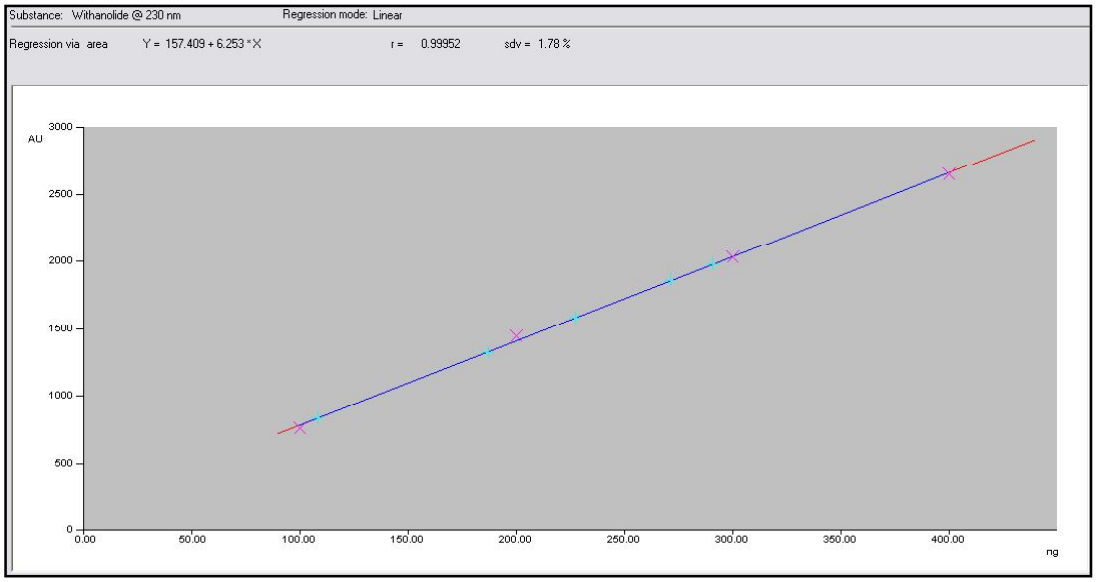

Figure 4: Showing the linearity of withanolide A from 100 – 400 ng / spot

5.Precision

5.1.System Precision

Six replicate injections of standard solution at the concentration of 0.1µg/µl were spotted by using Linomat V semi automatic sample applicator. The percentage relative standard deviations (% RSD) in each case were calculated.

5.2.Intermediate precision or inter-day precision

Method precision was studied by analyzing the standards under the same analytical procedure and laboratory conditions on the same day (intra-day precision) and on different days (inter-day).

The intermediate or inter-day precision of the method was determined by six replicate analysis of withanolide A from sample, as per the proposed method by different analyst on same day and on different days. The average drug content and the %RSD were calculated in each case.

Repeatability Data (Intra-day)			**Intermediate Precision (Interday)**		
Actual Concentration (ng/µl)	**Concentration obtained(ng/µl)**	**% RSD**	**Actual Concentration (µg/µl)**	**Concentration obtained(µg/µl)**	**% RSD**
200	200.02	0.27	200	198.86	0.29
200	200.02		200	199.56	
200	199.06		200	200.01	
400	405.01	0.68	400	401.23	0.17
400	403.03		400	400.45	
400	399.59		400	399.85	
600	599.23	0.89	600	598.99	0.36
600	605.00		600	598.56	
600	610.01		600	602.55	

Table 1: Precision Data

6.LOD and LOQ

The limit of detection and limit of quantification was found to be 30ng/spot and 80ng/spot.

7.Accuracy

Recovery studies were performed by standard addition method at three levels i.e. 80%, 100%, 120%. Known amount of standard was added to preanalyzed sample and they were subjected to proposed HPTLC method. Result of recovery study is shown in table.

S.No.	Withanolide A in sample (ng)	Std. added (ng)	Total added conc.	Found conc.	% Recovery
1.	250	80	200	200.13	100.06
2.	250	80	200	201.98	100.99
3.	250	80	200	198.09	99.04
4.	250	100	250	250.95	100.38
5.	250	100	250	253.11	101.24
6.	250	100	250	254.00	101.60
7.	250	120	300	302.89	100.96
8.	250	120	300	301.99	100.66
9.	250	120	300	305.00	101.66

Table 2: Recovery Data

8.Robustness

To check the method robustness by small changes in the mobile phase composition, the effect on the result was examined. Mobile phases having different ratio of solvents, i.e. Toluene: ethyl acetate: formic acid 8:4:0.6, 8.0:4.2:0.4, 7.8:4.2:0.6 and 7.7:4.3:0.6 v/v/v were tested. The volume of mobile phase and duration of the saturation investigated were 10±2ml (8, 10, 12ml) and (6, 8 and 10min) respectively. The plates were activated at 60±5ºC for 2, 5 and 7minues before chromatography.

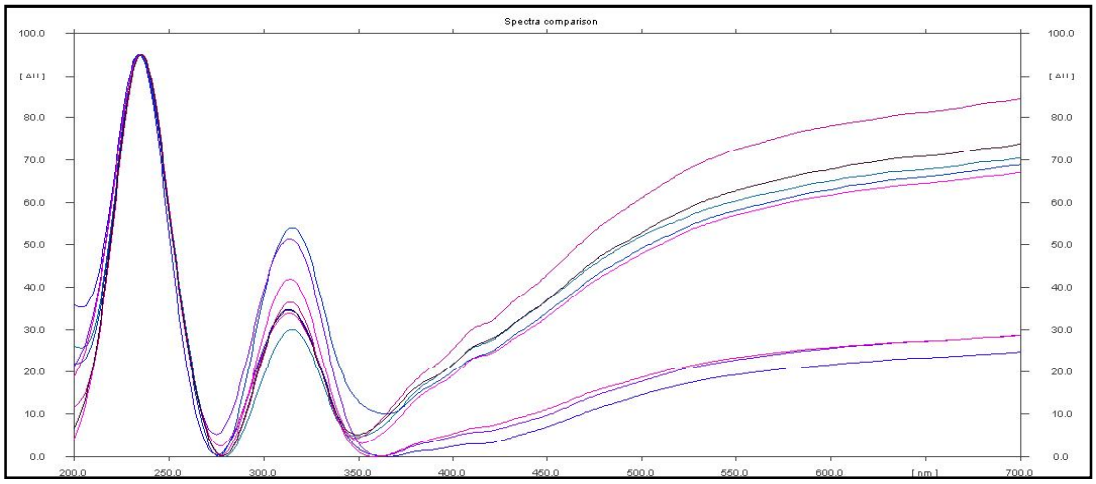

Figure 5: Shows spectral scan of withanolide A from all the tracks showing presence.

9.Result

In linearity study, the graphical data proves that the withanolide A has linearity in the range of 100ng to 400ng with the r^2 values 0.999.

In system precision study, the % RSD for withanolide A was found to be 1.03%. The % RSD observed on the replicate indicates the precision of the system.

The % RSD for repeatability and intermediate precision was found to be 0.27, 0.68, 0.89 and 0.29, 0.17, 0.36. There is no significant difference by same analyst with same instrument on same day or different day. Therefore the intermediate or inter- day precision of the method can be considered to be acceptable.

In accuracy or recovery studies, the results are shown in table 1. The overall % recovery and % RSD in marketed ginger OTF indicated that there is no significant difference in percentage of recovery. The accuracy of the method considered acceptable as it was well within 98 to 102%.

In robustness or system suitability study, there was no significant impact on the % RSD. The results of robustness study also indicated that the method is robust and is unaffected by small variations in the chromatographic condition.

10.Discussion

The HPTLC methods are significant methods for the quality assurance of withanolide A from oral thin film and its different formulations. HPTLC has emerged as a routine technique due to its advantages of low operating costs, high sample out put and the need for the minimum sample preparation. The major advantage of HPTLC is that the several

samples can be run simultaneously using a small quantity of mobile phase unlike LC thus reducing the analysis time and cost per analysis and also the contamination and choke up of the columns by the plant constituents. Hence, the method was developed for withanolide A in herbal oral thin film is simple, reliable, accurate and precise. The method was validated and found to be suitable for routine analysis of the withanolide A and its formulations individually or in combine formulations.

11.Conclusion

The proposed HPTLC method is simple, rapid, specific and accurate. This assay can be performed without any special pre-treatment. Method validation proved that it is selective and reproducible. A method can be applied for other formulations too.

12.Reference

1. Tohda C, Kuboyama T, Komatsu K. Search for natural products related to regeneration of the neuronal network. Neurosignals. 2005; 14(1-2):34-45.
2. Singh N, Nath R, Lata A, et al. Withania somnifera (ashwagandha), a rejuvenating herbal drug which enhances survival during stress (an adaptogen). Int J Crude Drug Res 1982; 20:29-35.
3. M.A. Weiner, J. Weiner. Ashwagandha (India ginseng). In: Herbs that Heal. Quantum Books, Mill Valley, CA; 70–72 (1994).
4. M. Ganzera, M.I. Choudhary, I.A. Khan. Quantitative HPLC analysis of withanolides in Withania somnifera. Fitoterapia 74(1-2): 68-76 (2003).
5. S.K. Bhattacharya, A.V. Muruganandam. Adaptogenic activity of Withania somnifera: an experimental study using a rat model of chronic stress. Pharmacol. Biochem. Behav. 75(3): 547-555 (2003)
6. Archana R, Mathur S. Quantitative determination of withaferine A, Total withanolides and alkaloid in *Withania somnifera* by TLC densitometry. J Med Arom Plant Sci 1998; 18:788-9.
7. Shaila D, Kulkarni SM. Determination of Withaferine A in two Withania species by RP-HPLC method. Indian J Pharm Sci 2006; 68:253-6.
8. ICH guidelines Q2A, Text on Validation of Analytical Procedure, International Conference of harmonization, Geneva 1994; 11:1-5.
9. Mukherjee PK. Quality control of Herbal drugs. 1 st Ed. New Delhi: Business Horizons; 2002. p. 57.
10. Ali M, Shuaib M. Withanolides from the stem bark of Withania somnifera. Phytochemistry 1997; 44:1163-8.
11. PB Shinde, PD Aragade, MR Agrawal, UA Deokate, SS Khadabadi Simultaneous determination of withanolide A and bacoside A in spansules by high-performance thin-layer chromatography. Indian journal of pharmaceutical sciences 2011: 73: 2: 240-243