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Original Research Paper

Analytical Method Development and Validation of Withaferin A and Piperine in Capsule Dosage Form

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Abstract:

HPLC method has been developed for simultaneous estimation of Withaferin A and Piperine in capsule dosage form. The method is uncomplicated, precise, accurate and robust. The separation was executed by Phenomenex C-18 (250 × 4.6 mm, 5µm particle size) column and Acetonitrile: water (70: 30 %v/v) as a mobile phase with the flow rate 1ml/min. Detection was carried out at wavelength of 230 nm. The retention time for Withaferin A and Piperine were found to be 3.77 and 4.55 respectively. Linearity complied for range of 5-15 µg/ml for Withaferin A and 25-75 µg/ml for Piperine. The accuracy of the method was judged by recovery studies. The developed method was validated as per the ICH guidelines parameters like linearity, precision, accuracy, robustness, LOD and LOQ. The results were validated as per ICH Q2 R1 guideline and were adequate. This method can be successfully employed for simultaneous estimation of Withaferin A and Piperine in bulk drugs and formulation

Keywords: Withaferin A, Piperine, HPLC, ICH.

Introduction:

HPLC is an useful tool for determining active constituents, adulterants present in herbal drugs. It emerged as a powerful analytical technique used to separate, Identify and quantify component of interest.[1] Now a day's HPLC is a choice of method for analysis of herbal drugs. In the present study attempt is made to estimate the Withaferin A and Piperine in marketed formulation**.** Withaferin A is first isolated from Ashwagandha (Withania somnifera) and it is first member of the withanolides. Withaferin A (4β, 27-dihydroxy-1-oxo-5β, 6β-epoxywitha-2-24-dienolide) is a poly functional steroidal lactone with 28 carbon atoms based on ergostane framework.[2] It is a steroidal lactone which binds to and inhibits vimentin. It is a potent inhibitor of angiogenesis and also it is inhibits both NF-κB and Sp1 Transcription factor activity. Withaferin A also down regulates VEGE gene expression and affect calcium signalling. Structure of withaferin A is shown in figure no I. It has Anti-cancer Activity, Anti-inflammatory activity, Anti-angiogenesis activity, Chemo preventive activity, Radio sensitizing activity.[3] Piperine is isolated from black papper and lone

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papper and along with its isomer chavicine. It is an alkaloid.[4] Piperine is a N-acylpiperidine that is piperidine substituted by a (1E,3E)-1-(1,3-benzodioxol-5-yl)-5-oxopenta-1,3-dien-5-yl group at the nitrogen atom. Piperine has shown anti-depression like activity and cognitive enhancing effects in rats, anti-inflammatory and anti-arthritic effects in human interleukin-1beta-stimulated fibroblast-like synoviocytes, anti-angiogenic activities, anticonvulsant, anticarcinogenic.[5,6] Withaferin A and Piperine combination is use in Neuro muscular debility, Tension, Anxiety, Stress, Parkinson's disease, Alzheimer's disease, Tuberculosis, Immuno-compromised conditions, Rejuvenation therapy, cardiovascular diseases, Improvement in sperm count and motility in male, helps reduce platelet aggregation, maintain normal cholesterol levels.[7,8] Structure of Piperine is shown in figure no II. Literature review revels that numbers of analytical development methods available of Withaferin A and Piperine in their individual dosage form or in combination with other dosage forms but no HPLC method is developed for combination of Withaferin A and Piperine.

MATERIAL AND METHOD:

Chemicals:

Standard withaferin A and Piperine was procured from aum laboratory, Ahmadabad, Gujarat. HPLC grade Acetonitrile, methanol and HPLC grade Water were purchased from Merck (India) Ltd., Mumbai, India. All other chemicals are of analytical grade from S.D.Fine Chemical Ltd., Worli, India. Capsule dosage form (Neuro Tip) containing 40 mg of Withaferin A and 25 mg of piperine was procured from Indian market manufactured by Maximaa Proyurveda.

Instrumentation:

Analysis was performed on HPLC instrument equipped with PDA detector and Phenomenex C-18 (250 × 4.6 mm, 5µm particle size) column with Millennium software (Waters).Other instruments used for study were, UV visible Spectrophotometer(Analytical Technologies), Ultra-sonicator (PEI), Digital Weighing balance (Shimadzu), FTIR (Analytical Technologies).

Method development:

Preparation of Standard solution for Withaferin A and Piperine:

Accurately weighed equal amounts 25 mg of Withaferin A and Piperine working standards were transferred into two 25 mL volumetric flasks. 20 ml of methanolis added to each flask, sonicated and made up the volume to 25 mL with diluents to 1000µg/ml of standard stock solution.

Preparation of working Standard solution for Withaferin A and Piperine:

From the stock solution (1000µg/ml),of Withferine A an accurately measured 0.5, 0.75, 1.0, 1.25, and 1.5ml was transfer into separate 100ml volumetric flask and final volume was adjusted with methanol up to mark to prepare 5-15µg/ml solution of Withferine A. From the stock solution (1000µg/ml), of Pipeerine an accurately measured 2.5, 3.75, 5.0, 6.25, and 7.5ml was transfer into separate 100ml volumetric flask and final volume was adjusted with methanol up to mark to prepare 25-75µg/ml solution of Piperine.

Chromatographic conditions used for the method:[9,10]

Phenomenex C-18 (250 × 4.6 mm, 5µm particle size) column was used for the separation with mobile phase composition Acetonitrile: water (70: 30 %v/v) , flow rate 1 ml/min, run time 8 minutes. Detection of drugs was carried out at 230nm.

Preparation of sample solution:

Twenty capsules were taken and weight powder equivalent to 40mg Ashwagandha and 25mg Pippali was transferred to test-tube and add 20ml of methanol in test-tube. For extraction put it in heating water bath for 1 hr, cool it and filtered it. These filtrates take in 25 ml volumetric flask and make up 25 ml with methanol. Above solution was injected into HPLC system.

Method Validation:[11,12,13]

Linearity

The linearity was evaluated by linear regression analysis. The calibration curve was obtained with concentrations of 5-10µg/ml for Withaferin A and 25-50µg/ml for Piperine as shown in fig. III and IV.

Precision

The precision of the procedure was determined by repeatability (intraday). Intraday precision was evaluated by assaying same concentration and during the same day. Repeatability of sample measurement was carried out in six different sample preparations from same homogenous blend of sample. Another replicate determination on three different days to estimate interday precision.

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To a pre analysed sample solution, a definite concentration of standard drug was added and recovery was studied. A 80%, 100% and 120% of pure drug solutions were added to the pre analyzed samples.

Analysis of Laboratory mixture

The response of sample solution was measured at 230nm using HPLC. The amount of Withaferin A and piperine were determined by regression equation.

Results and discussion:

The mobile phase consisting of Acetonitrile: water (70: 30 %v/v) at 1mL/min flow rate which gave sharp, well-resolved peak with minimum tailing factor for Withaferin A and piperine. The retention time for Withaferin A and piperine were 3.77 and 4.55 respectively as shown in fig. V. Overlay

Chromatogram of Withaferin A and Piperine is shown in figure no VI. The calibration curve for Withaferin A and piperine was found to be linear over the range of 5-15 µg/ml and 25-75 µg/ml respectively. The data of regression analysis of the calibration curves is shown in Table I. The LOD for Withaferin A and piperine was found to be 0.78 µg/ml and 2.38 µg/ml respectively. The LOQ for Withaferin A and piperine was found to be 4.28µg/ml and 09.99µg/ml respectively. The results for recovery study are summarized in Table II. The results for system suitability test parameters are summarized in Table III. The Summary of validation parameters for analysis of Withaferin A and piperine were shown in Table IV.

Conclusion:

A rapid, simple, sensitive, accurate, precise and linear HPLC method has been developed and validated for simultaneous estimation of Withaferin A and Piperine in capsule dosage form. All chromatographic conditions were optimized by observing conditions producing best peak results. The proposed method was validated as per ICH guideline. Because of importance of short retention time in routine analysis of drug, the current method sounds to be applicable as quality control tool for simultaneous estimation of Withaferin A and Piperine in capsule dosage form.

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Parameters	Result	
	Withaferin A	Piperine
Linearity Range (µg/ml)	5-15	25-75
Slope	52705	48892
Intercept	12576	63548
Retention Time (min)	3.77	4.55
Correlation Coefficient (R2)	0.999	0.999

Table I: Data of regression analysis of the calibration curves

	Conc.		Conc.	%
r u g		o r c . o f		

	Formu (µg/ml)	Standar d added (µ g / m l)	Recov	Recover y ± S D *
With afer in A	5	4	3.97	99.52±
	5	5	4.98	99.84±
	5	6	6.01	100.18±
	25	20	19.96	99.83±
iperine	25	25	24.95	99.84±
	25	30	29.96	99.90±

*= Average of three determination

Table II : Data of recovery study for Withaferin A and piperine

Parameters	Withaferin A (10µg/ml)	Piperine (50µg/ml)	Acceptance criteria
Retention time (min)	3.77	4.52	------
Tailing factor	1.15	1.5	≤2
Theoretical plates	3044	4388	≥2000
Resolution	2.74		≥2

Table III: Results of system suitability parameters.

Sr No.	Parameter	P-HPLC method	
		Withaferin A	Piperin
1	Linearity and Range (µg/ml)	5-15	25-75
2	Slope	52705	48892
3	Intercept	12576	63548
4	Correlation coefficient (r^2)	0.999	0.999
5	LOD (µg/ml)	0.78	2.38
6	LOQ (µg/ml)	4.28	12.99
7	Precision (% RSD)		
	Intra-day (n=6)	1.01	0.1
	Inter-day (n=6)	1.25	0.57
8	Accuracy(% Recovery) (n=3)	99.52-100.18	99.83-
9	Robustness(% RSD)		
	Flow 0.	0.12	0.027
	(ml/min 1.	0.07	0.08

Table IV: Summary of validation parameters for analysis of Withaferin A and piperine**Figure Legends**

Fig. I: A structure of Withaferin A
Fig. II : A structure of Piperine.
Fig. III: Calibration curve of Withaferine A
Fig. IV: Calibration curve of Piperine
Fig. V Chromatogram of Withaferin A and Piperine
Fig. V Chromatogram of Withaferin A and Piperine

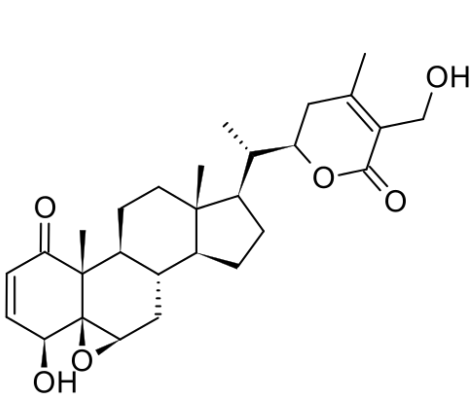


Fig. I: A structure of Withaferin A

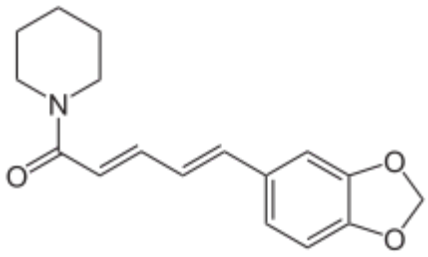
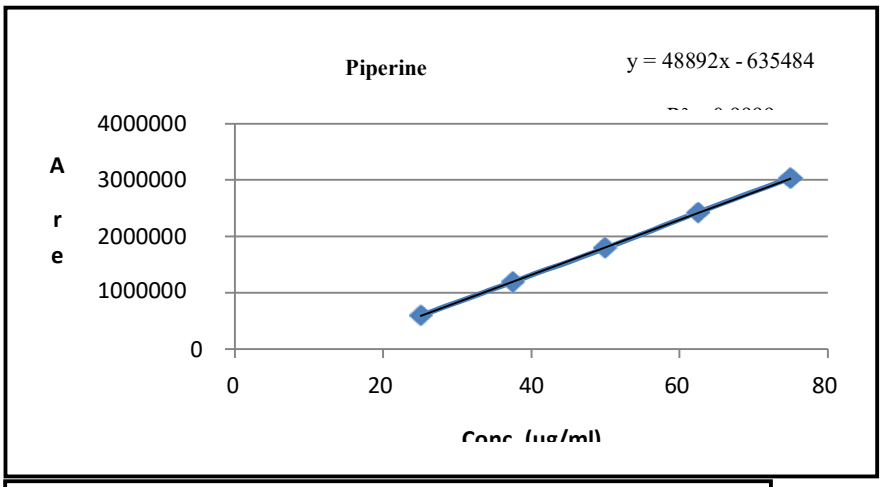


Fig. II : A structure of Piperine.



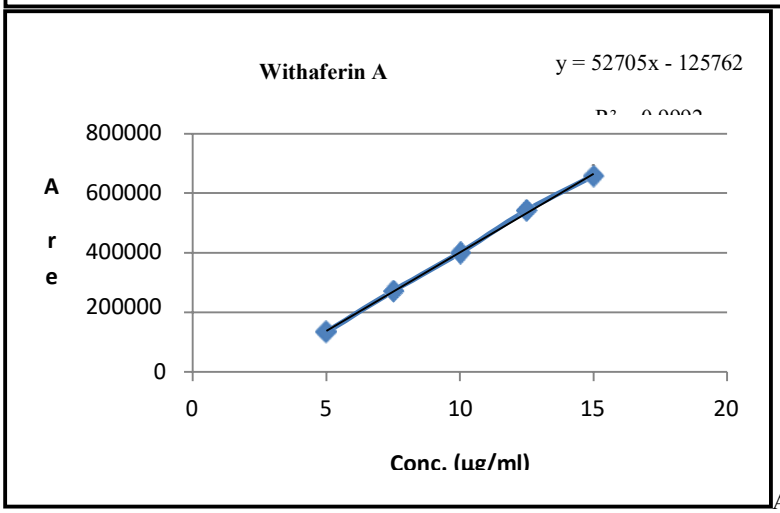


Fig. III: Calibration curve of Withaferine A

Fig. IV: Calibration curve of Piperine

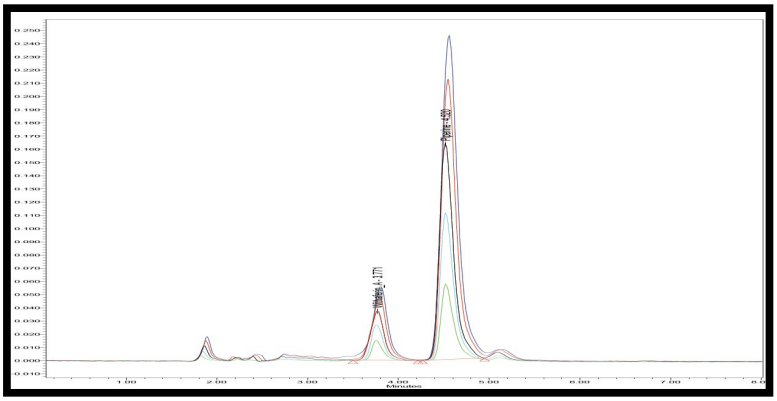


Fig. V Chromatogram of Withaferin A and Piperine

Fig. VI Overlay Chromatogram of Withaferin A and Piperine