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Research Article

DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR THE ESTIMATION OF AQUEOUS EXTRACT OF *FAGONIA SCHWEINFURTHII* HADIDI

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ABSTRACT
A simple, rapid, accurate, precise, and economic spectrophotometric method for aqueous extract of *Fagonia schweinfurthii* hadidi have been developed. Aqueous extract of plant was obtained from decoction process of coarse powder of whole plant. Aqueous extract of plant shows absorbance maximum at 265 nm when Phosphate Buffer of pH 6.8 and 0.1N HCl used as solvent system. Calibration curves were constructed in phosphate buffer pH 6.8 and 0.1 N HCl. Beer's law was obeyed in the concentration range of 25-350 µg/ml. In phosphate buffer pH 6.8 the equations and R^2 obtained, were y = 0.0025x - 0.0083 and R^2 = 0.9995 respectively and in 0.1N HCl the equations and R^2 obtained were y = 0.0024x + 0.0181 and R^2 = 0.9987 respectively. Validation of developed method was done according to I.C.H. guideline.
Keywords: Dhamasa, *Fagonia schweinfurthii* hadidi, Zygophyllaceae

INTRODUCTION

Herbal medicines are prepared from materials of plant origin which are prone to contamination, deterioration, variation in composition and level of active constituents due to variation in climatic conditions. Also variation in the chemical profile of the herbal formulations is due to the factors like growing, harvesting, storage and drying processes. Therefore quality control of herbal medicines offers a host of problems. It is very important that a system of standardization is established for every plant medicine available in the market because the scope for variation in different batches of medicine is enormous.[1,2] Plant *Fagonia schweinfurthii* hadidi belonging to family Zygophyllaceae is widely distributed in deserts and dry areas of India, Pakistan to tropical Africa. It is commonly known as dhamasa and dhamasia.[3,4] Traditionally, the plant has been used to cure a number of ailments by the people living in desert region such as skin eruptions, in heal sores, skin diseases, anti-pyretic, in pain relief, ear infection, venereal diseases, etc. many other diseases.[5,6] There is no reported UV Visible method for estimation of aqueous extract which is necessary in the development of suitable formulations for this drug. Hence, in present study the simple UV spectroscopic method was developed for direct estimation of aqueous extract of *Fagonia schweinfurthii* hadidi. The assay validation of calibration curve was carried out as per ICHQ2A guidelines. In validation procedure, calibration curve prepared in phosphate buffer of pH 6.8 was run in triplicate for three days to determine intra-day and inter-day variations.

MATERIALS AND METHODS

Plant material

The plant was collected from Jodhpur region of Rajasthan, India and was authentified from Botanical Survey of India, Jodhpur, Rajasthan (India). Voucher specimens (No. JNU/PH/2013/Fs/F3) and herbarium sheet was kept in the institute for further references.

Preparation of aqueous extracts

Fresh plant of *Fagonia schweinfurthii* hadidi was shade dried and grounded to prepare a moderately coarse powder. The extraction was carried out by decoction method with water at 40˚C. The extract was filtered through a filter paper, and the filtrate was dried. The crude extract was stored in a desiccator.[7]

Analytical Method Development

Preparation of stock solution

100 mg of extract was dissolved in 100 ml of different solvents. This 1000 µg/ml solution was serves as a standard stock solution.

Determination of maximum absorbance (λ_{max}) of extract

The solutions of different ppm were prepared in different solvents of 6.8 pH buffer and 0.1 N HCl. The resulting solutions were scanned under UV spectrophotometer in range 200 to 400 nm for the determination of λ_{max}.

Solution Stability study

For the solubility study 200 µg/ml solution of extract was prepared from stock solution and absorbance was recorded for 6 hours at an interval 2 h.

Construction of calibration curve

Calibration curve were established with eight dilutions of standard prepared from stock solution at concentration range from 25 to 350 µg/ml.

Validation of developed method according to I.C.H. guidelines

Following parameters were taken into consideration for validation of developed method.

Specificity

Specificity study any possible bias was determined by comparison of results of any value obtained in presence of excipients with any value obtained without excipients. From

stock solution, a working standard solution was prepared and analyzed without addition of excipients. A small quantity of an excipients mixture (containing 100 mg of lactose, 5 mg of Aspartame, 10 mg of crospovidone and 10 mg SSG) were added to pre analyzed stock solution of 1000 µg/ml. It was kept in Ultra sonicator for 15 minutes and then filtered through Whatman filter paper No. 44. From stock solution, a working standard solution was prepared (200 µg/ml) and analyzed with addition of excipients. The absorbance of filtrates was determined at λ_{max} against the reference solvent. Assay bias was evaluated by calculating % agreements;

$$\% \text{ Agreement} = T_P/T_A$$

There, T_P = Test result in the presence of excipients,
T_A = Test result in the absence of excipients

The results of specificity study are shown in Table.

Linearity
Graphical method (by visual examination) was used for the determination of linearity.

Precision
Repeatability
The precision of method was assessed by carrying out six replicate determination of extract at a concentration of 200 µg/ml in solvents.

Intraday precision
For Intraday precision of the method, solution of extract were prepared at three concentration levels 160, 200, 240 (µg/ml) each in triplicate. These solutions were analyzed three times within one day at different time interval.

Interday precision
For Interday precision of the method, solutions were prepared at three concentration levels 160, 200, 240 (µg/ml) each in triplicate. These solutions were analyzed for three consecutive days.

Linearity range
It is the interval in which the response is directly proportional to the concentration between the upper and lower levels including the level (which is generally ± 5 % of the intercept having slope equal to zero).

Limit of Detection (LOD)
The limit of detection was calculated by using following equation

$$LOD = 3.3\ \sigma /S$$

Where, σ is noise estimate and is the standard deviation (SD) of the blank responses and S is the slope of calibration curve of extract.

Blank sample was analyzed at λ_{max} for six times and responses were recorded.

Limit of Quantification (LOQ)
The limit of Quantification was calculated by using following equation

$$LOQ = 10\ \sigma /S$$

Where, σ is noise estimate and is the standard deviation (SD) of the blank responses and S is the slope of calibration curve.

Blank sample was analyzed at λ_{max} for six times and responses were recorded.

RESULTS AND DISCUSSION

Determination of maximum absorbance (λ_{max}) of extract
Maximum absorbance (λ_{max}) of extract was observed in three solvent system 6.8 pH buffer, 0.1 N HCl. Scanned graph are given in following Figures and comparative λ_{max} are given in Table 1. (Figure 1 & 2)
For all the analytical work 265 nm was selected as λ_{max}.

Solution Stability study
The results of stability study in different solvent system 6.8 pH buffer and 0.1 N HCl are shown in Table 2.
Data of stability study shows that the extract was found to be stable in both solvent systems.

Construction of calibration curve in 0.1 N HCl
Calibration curves were constructed in phosphate buffer pH 6.8, and 0.1 N HCl. Beer's law was obeyed in the concentration range of 25-350 µg/ml. In phosphate buffer (pH 6.8) the linearity was obtained between concentration range 25-350 µg/ml. The equations and R^2 obtained were y = 0.0025x - 0.0083 and R^2 = 0.9995 respectively. In 0.1N HCl the linearity was obtained between concentration range 25-350 µg/ml. The equations and R^2 obtained were y = 0.0024x + 0.0181 and R^2 = 0.9987 respectively. (Table 3, Figure 3)

Validation of the developed method
Specificity
The results obtained for the specificity study in 6.8 pH buffers are given in following Table 4. The result shows that there is negligible changes in absorbance have been observed by addition of excipients.

Specificity
The results obtained for the specificity study in 0.1 N HCl are given in following Table 5. The result shows that there is negligible changes in absorbance have been observed by addition of excipients.
Average agreement in 6.8 pH buffer and 0.1 N HCl were found to be 100.02 and 100.14 respectively. Specificity study shows that good agreement with result, indicating that the excipients did not interfere with the analysis. (Table 6 & 7)
The method shows that good repeatability that was demonstrated by RSD of lower than 0.6 %. The RSD was found to be within acceptance limit < 2.0 %.

LOD data for Aqueous extract in 6.8 pH buffer and 0.1 N HCl (Table 8 & 9)

LOQ data for Aqueous extract in 6.8 pH buffer and in 0.1 N HCl (Table 10, 11 & 12)

Intraday and Interday precision
In the study of the data intra-day which has conducted at three different time on the solution having the concentration value 80 %, 100 % and 120 % of the 200 ppm. (Table 13 & 14)
In the study of the data inter-day which has conducted on the solution having the concentration value 80 %, 100 % and 120 % of the target concentration, at three different days. (Table 15 & 16)
Intraday and Interday studies were shown that the proposed method is precise.

Table 1: λ_{max} in different solvents

S. No.	Solvent	λ_{max} (nm)
1.	6.8 pH buffer	265
2.	0.1 N HCl	265

Table 2: Stability study in different solvent system 6.8 pH buffer and 0.1 N HCl

S. No.	Time (h)	Absorbance (at 265 nm)	
		6.8 pH buffer	0.1 N HCl
1.	0	0.493	0.498
2.	2	0.489	0.492
3.	4	0.492	0.498
4.	6	0.491	0.496

Table 3: Calibration curve in 6.8 pH buffer and 0.1 N HCl

S. No.	Conc. (µg/ml)	Absorbance (in 6.8 pH buffer)	Absorbance (in 0.1 N HCl)
1.	25	0.051	0.071
2.	50	0.114	0.159
3.	100	0.236	0.250
4.	150	0.355	0.368
5.	200	0.496	0.489
6.	250	0.609	0.615
7.	300	0.730	0.738
8.	350	0.844	0.858

Table 4: Specificity study in 6.8 pH buffer

S. No.	Conc. (µg/ml)	Before addition of Excipients		After addition of Excipients		% Agreement
		Absorbance*	Conc. (µg/ml)	Absorbance*	Conc. (µg/ml)	
1.	200	0.493	200.52	0.493	200.52	100.00
2.	200	0.492	200.12	0.491	199.72	99.86
3.	200	0.492	200.12	0.492	200.12	100.00
4.	200	0.491	199.72	0.492	200.12	100.20
5.	200	0.492	200.12	0.491	199.72	99.86
6.	200	0.491	199.72	0.492	200.12	100.20
Mean						100.02

*Mean of triplicate

Table 5: Specificity study in 0.1 N HCl

S. No.	Conc. (µg/ml)	Before addition of Excipients		After addition of Excipients		% Agreement
		Absorbance*	Conc. (µg/ml)	Absorbance*	Conc. (µg/ml)	
1.	200	0.494	198.29	0.496	199.12	100.41
2.	200	0.497	199.54	0.494	198.29	99.37
3.	200	0.498	199.95	0.498	199.95	100.00
4.	200	0.498	199.95	0.501	201.2	100.62
5.	200	0.498	199.95	0.499	200.51	100.28
6.	200	0.496	199.12	0.497	199.54	100.21
Mean						100.14

*Mean of triplicate

Table 6: Repeatability data of Aqueous extract in 6.8 pH buffer

S. No.	Conc. (µg/ml)	Absorbance	Found Conc. (µg/ml)	Mean ± S.D.	% RSD
1.	200	0.493	200.52	200.32 ± 0.41	0.204
2.	200	0.491	199.72		
3.	200	0.492	200.12		
4.	200	0.493	200.52		
5.	200	0.492	200.12		
6.	200	0.494	200.92		

Table 7: Repeatability data of Aqueous extract in 0.1 N HCl

S. No.	Conc. (µg/ml)	Absorbance	Found Conc. (µg/ml)	Mean ± S.D.	% RSD
1.	200	0.499	200.57	199.91 ± 1.14	0.570
2.	200	0.494	198.29		
3.	200	0.496	199.12		
4.	200	0.502	201.62		
5.	200	0.498	199.95		
6.	200	0.498	199.95		

Table 8: LOD data for Aqueous extract in 6.8 pH buffer

S. No.	Absorbance value for blank	S.D	LOD (µg/ml)
1.	0.001	0.000861	1.077
2.	0.002		
3.	0.002		
4.	0.001		
5.	0.003		
6.	0.001		

Table 9: LOD data for Aqueous extract in 0.1 N HCl

S. No.	Absorbance value for blank	S.D	LOD (µg/ml)
1.	0.001	0.000516	0.7095
2.	0.002		
3.	0.002		
4.	0.001		
5.	0.001		
6.	0.001		

Table 10: LOQ data for Aqueous extract in 6.8 pH buffer

S. No.	Absorbance value for blank	S.D	LOQ (µg/ml)
1.	0.001	0.000816	3.26
2.	0.002		
3.	0.002		
4.	0.001		
5.	0.003		
6.	0.001		

Table 11: LOQ data for Aqueous extract in 0.1 N HCl

S. No.	Absorbance value for blank	S.D	LOQ (µg/ml)
1.	0.001	0.000516	2.15
2.	0.002		
3.	0.002		
4.	0.001		
5.	0.001		
6.	0.001		

Table 12: Range data for Aqueous extract

S. No.	Parameters	In 6.8 pH buffer	In 0.1 N HCl
1.	Working Range	3.25 µg/ml to 350 µg/ml	2.15 µg/ml to 350 µg/ml
2.	Linearity Range	25 µg/ml to 350 µg/ml	25 µg/ml to 350 µg/ml

Table 13: Intraday precision data of Aqueous extract in 6.8 pH buffer

S. No.	Intraday	Actual Conc. (µg/ml)	Absorbance	Found Conc. (µg/ml)
1.	0 h	160	0.396	161.72
		200	0.493	200.52
		240	0.600	243.32
2.	2 h	160	0.396	161.32
		200	0.489	198.92
		240	0.598	242.52
3.	4 h	160	0.395	161.32
		200	0.492	200.12
		240	0.601	243.72

Table 14: Intraday precision data of Aqueous extract in 0.1 N HCl

S. No.	Intraday	Actual Conc. (µg/ml)	Absorbance	Found Conc. (µg/ml)
1.	0 h	160	0.388	154.12
		200	0.498	199.95
		240	0.605	244.54
2.	2 h	160	0.380	151.00
		200	0.492	197.45
		240	0.601	242.87
3.	4 h	160	0.381	151.10
		200	0.496	199.12
		240	0.601	242.87

Table 15: Interday precision data of Aqueous extract in 6.8 pH buffer

S. No.	Interday	Actual Conc. (µg/ml)	Absorbance	Found Conc. (µg/ml)
1.	I day	160	0.396	161.72
		200	0.493	200.52
		240	0.600	243.32
2.	II day	160	0.395	161.32
		200	0.495	201.32
		240	0.597	242.12
3.	III day	160	0.394	160.32
		200	0.495	201.32
		240	0.695	242.52

Table 16: Interday precision data of Aqueous extract in 0.1 N HCl

S. No.	Interday	Actual Conc. (µg/ml)	Absorbance	Found Conc. (µg/ml)
1.	I day	160	0.384	152.45
		200	0.499	200.37
		240	0.605	244.54
2.	II day	160	0.379	150.37
		200	0.498	199.95
		240	0.602	243.29
3.	III day	160	0.380	151.00
		200	0.500	200.79
		240	0.605	244.54

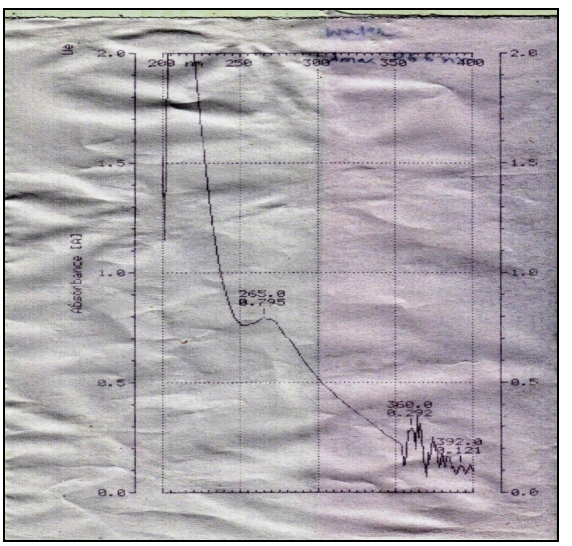


Figure 1: Maximum absorbance (λ_{max}) of extract in 6.8 pH buffer

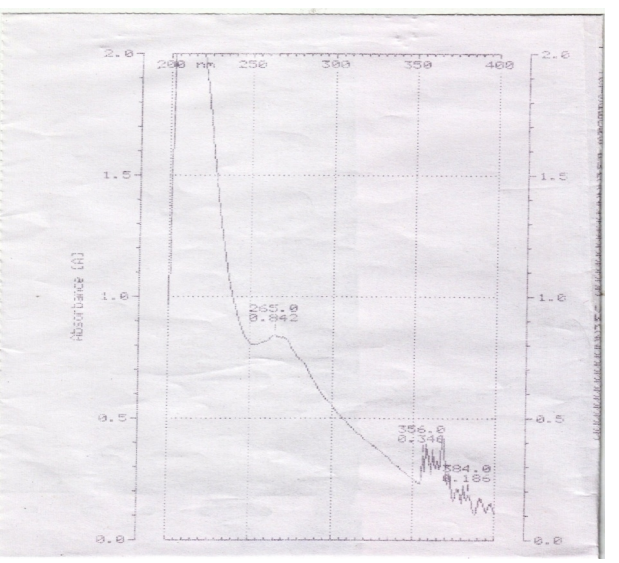
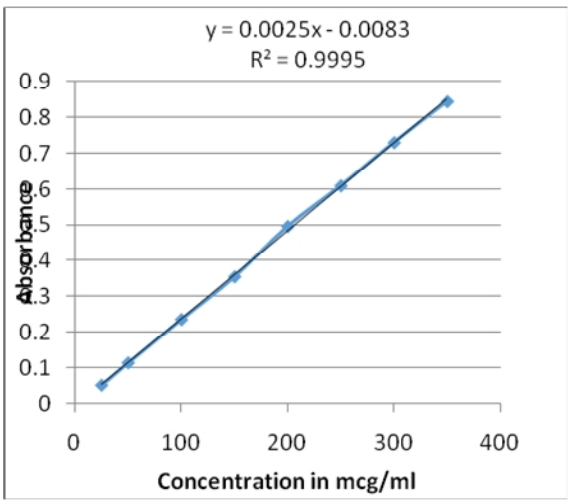


Figure 2: Maximum absorbance (λ_{max}) of extract in 0.1 N HCl



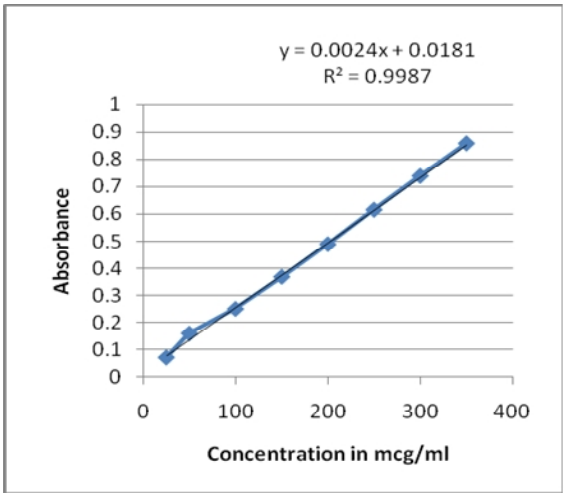


Figure 3: Calibration curve in 6.8 pH buffer and 0.1N HCl

CONCLUSION

On the basis of validation study the results of developed method was found accurate, precise and reproducible. Therefore developed method can be used for routine quality control analysis of *Fagonia schweinfurthii* hadidi.

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