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Development of fingerprints for an ayurvedic formulation Ajmodadi churna, via piperine estimation by UV-Spectrophotometry.

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Abstract
A simple and reproducible UV-spectrophotometric method for the quantitative determination of piperine in Ajmodadi churna (AJC) were developed and validated in the present work. The parameters linearity, precision , accuracy, limit of detection ,limit of quantification and standard error were studies according to international conference on harmonization guidelines. In this present study a new, simple, rapid, sensitive, precise and economic spectrophotometric method in ultraviolet region has been developed for the determination of piperine in market and laboratory herbal formulation of Ajmodadi churna. Which were procured and purchased respectively from the local market and they were evaluated as per Indian Pharmacopoeia and WHO guidelines. Piperine has the maximum wavelength at 342.5 nm and hence the UV spectrophotometric method was performed at 342.5 nm. The samples were prepared in methanol and methods obey Beers law in concentration ranges employed for evaluation. The content of piperine in ayurvedic formulation was determined. The result of the present study revealed that all six batches of Ajmodadi churna were found in close proximity. The result of analysis has been validated statistically and recovery studies confirmed the accuracy of the proposed method. Hence the proposed method can be used for the reliable quantification of Piperine in crude drug and its herbal formulation.

Key Words
Finger printing, piperine, ajmodadi churna, UV-Spectrophotometer.

Introduction
The formulation of Ajmodadi churna (AJC) is well known ayurvedic formulation is official in Ayurvedic Formulary of India, traditionally used for abdominal pain, carminative, antispasmodic, and helps in all painful conditions like sciatica and stiffness[4].Though it is very popular medicine, no established parameters for this drug studies have been performed yet. This paper reports on instrumental methods for ensuring the Identity, Potency, Purity, Safety and efficacy of the ajmodadi churna. This paper includes the investigation of establishment of UV-spectrophotometric analysis methods for different samples of Ajmodadi churna (AJC) and comparison studies between marketed formulations as AJC-A, AJC-B and AJC-C and lab formulations as AJC-I, AJC-II, AJC-III, they were evaluated as per Indian Pharmacopoeia and WHO guidelines[3]. Spectroscopic studies were carried out to develop the spectrum of the formulation and validated by overlain and linearity study.

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The results of all batches were found in close proximity with each other. The methods used for determination of piperine in Ajmodadi churna found to be precise, reproducible and can be considered for routine quality control and finger printing of the formulation. The present study is an attempt to develop the fingerprinting method for Ajmodadi churna (AJC) by spectrophotometric determination using Piperine as a standard[2], which is an important and major content in formulation. The developed spectroscopic fingerprints can be used as a standard and piperine can be used as a possible marker compound for fingerprinting of AJC.

Material and Methods
Procurement of crude drug
Ajmodadi churna (AJC) consist of 12 ingredients viz; *Apium graveoens, Embellia ribes, Saindhava lavana, Cedrus deodara, Anethum souia, Pipper longum (Pippali), Plumbago zeylanica, Pipper longum linn (Pippalimula), Terminalia chebula retz, Piper nigrum linn.(Marica)*[1]*, Zingiber officinalis, Ipomoea petaloidea.* All these 12 ingredients were procured from local market and identified

morphologically, microscopically and compared with standard Pharmacopoeial monograph[6,7,8]. Samples of crude drugs were also authenticated by Department of Botany, Dr. H. S. Gour Vishwavidyalaya, Sagar (M.P.)

Preparation of the formulation AJC

Ajmodadi churna (AJC) contains piper species (fruit and root of piper longum, and piper nigrum) as main ingredients .Ayurvedic formulary of India has given the specification for the composition of AJC, it should contain piper species as a major ingredient apart from different herbs and salts. Three marketed formulation of Ajmodadi churna (designated as AJC-A, AJC-B and AJC-C) and three laboratory batches were prepared in local laboratory and were named as AJC-I AJC-II, AJC-III. Were procured for the present study.

Chemicals

All the chemicals and solvents were used of A.R. grade; standard piperine (98%) was procured from Lancaster (England).

Preparations of piperine extract of Ajmodadi churna (AJC)

Reflux the powdered AJC (1gm) with 60 ml methanol for 1hr. filter the extract and re-reflux. The marc left with 40 ml of methanol for 1 hour. Filter and combine the filtrate. Concentrate the methanol extract under vacuum till the semisolid mass was obtained. Dissolve the residue in 75 ml methanol and filter through sintered glass funnel 9G-2) by vacuum filtration assembly. The filtrate was centrifuged at 2000 rpm for 20 minutes, the supernatant was collected in 100 ml volumetric flask and volume was made with methanol[9,10]. The same procedure was performed for each batch of AJC and separately powdered fruits of piper longum (pippli) and 100 ml of their piperine extract were prepared. The same procedure was performed for each batch of Ajmodadi churna and separately powderd fruits of piper longum (Pippli), piper nigrum (Marica) and piper longum (Pipplimula) and solution (100ml) of their piperine extract were prepared.

Preparation of standard solution of piperine

An accurately weighed piperine (100gm) was dissolved in methanol and volume was made up to 100 ml with methanol in volumetric flask. 2 ml of this solution was diluted with methanol up to 100 ml in volumetric flask to give 20 µgm/ml piperine solutions[9,10].

Experimental

Calibration curve from standard solution of piperine was prepared and with the help of this curve the content of piperine from Ajmodadi churna was estimated. The method was validated for precision and accuracy.

Calibration curve of piperine in AJC

A series of calibration 10 ml volumetric flask were taken and appropriate aliquots of the working standard solution of piperine were withdrawn and diluted up to 10 ml with methanol. The absorbance was measured at absorption maxima 342.5 nm, against the reagent blank prepared in similar manner without the piperine. The absorption maxima and Beer's law limit were recorded and data that prove the linearity and obey Beer's law limit were noted. The linear correlation between these concentrations (x-axis) and absorbance(y-axis) were graphically presented and slope(b), intercept(a), and correlation coefficient (r^2) were calculated for the linear equation (Y=bx+a) by regression using the methanol of the least square, Table1 Figure1.

Estimation of piperine in AJC:

The appropriate aliquots from piperine extract of each batch of Ajmodadi churna and separately powderd fruits of piper longum (Pippli) ,piper nigrum (Marica) and piper longum (Pipplimula) were withdrawn in 10ml volumetric flask separately absorbance for aliquots of each was noted at 342.5 nm. The corresponding concentration of piperine against respective absorbance value was determines using the piperine calibration curve. The statistical analysis for checking uniformity in batches is also performed below (Table-2).

Precision and accuracy in AJC

The method was validated for precision and accuracy by performing the recovery studies at two levels by adding known amount of piperine extract of Ajmodadi churna, of which the piperine content have been estimated previously. The data were obtained and recovery was calculated (Table-3).

Data of recovery study (Mean% ± SD, n=3) of AJC: (Table-3).

Results and Discussion

Piperine obeys Beer Lambert' law in concentration range 10-50µg/ml at the λ_{max} 342.5 nm. The correlation coefficient (r^2) was calculated where the (r^2) value 0.9961 indicates the good linearity between the concentration and absorbance.

The estimation of piperine in Ajmodadi churna (laboratory batch AJC-I, AJC-II and AJC-III and marketed formulations AJC-A, AJC-B, and AJC-C) and powderd fruits of piper longum (Pippli), piper nigrum (Marica) and piper longum (Pipplimula) was carried out separately. The concentration of piperine present in raw material was found to be 1.45±0.34 w/w in piper longum fruit, 1.63±0.21 w/w in piper longum root (pipplimula) and 3.97±0.16 w/w in piper nigrum fruit (marica). The concentration of piperine in different batches of AJC (laboratory batches AJC-I, AJC-II and AJC-III) was found to be 0.18±0.001%, 0.20±0.003%, and 0.13±0.006. In marketed formulations AJC-A, AJC-B, and AJC-C was found to be 0.16±0.002%, 0.19±0.006%, and 0.17±0.005% respectively (Table-2).

In order to obtain precision and accuracy the recovery study were performed at three levels by adding known amount of piperine with preanalysed sample of piperine in Ajmodadi churna (AJC). The experiment was repeated Six Times at both level (Table-3) and result shows 99.48%, 99.73%, and 99.61% recovery of piperine at all the level with mean value 99.60%, which prove reproducibility of the result. This shows significant precision of methods at 95% confidence level. The % relative standard deviation (%RSD) value was found to be 0.36, 0.32, and 0.34, with mean 0.34 at all the level while the standard error was 0.3118, 0.3695 and 0.4965 with Mean 0.3926 respectively. From the data it is obvious that the present method of UV-Spectrophotometric fingerprinting determination of Piperine is simple, precise, accurate, and suitable for routine analysis of Piperine in Ajmodadi churna (AJC).

Conclusion

A simple rapid and accurate UV- spectrophotometric method for the estimation of piperine in Ajmodadi churna could be used as a valuable analytical tool in routine analysis, to check the batch to batch variations. The method was showed excellent sensitivity, reproducibility, accuracy and repeatability, which is evidenced by low percentage relative standard deviation. The result obtained in recovery studies were indicating that there is no interference from the excipients used. Hence it is suggested that the proposed UV-Spectrophotometric method can be effectively for the routine analysis of piperine in Ajmodadi churna and its crude drugs. In general, pharmaceutical development of finger printing provide a certain assurance of batch uniformity and integrity of the product manufactured. Estimation of piperine can be used as one of the appropriate analytical markers for the finger printing.

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References

1. Mukherjee PK. Quality Control of Herbal Drug. Ist edition, New Delhi: Eastern Publishers (Business Horizontal Ltd.) 2002; 03-30,184-219.
2. Jain V, Saraf Swarnlata, Saraf S. Standardization of Triphala Churna: Spectrophotometric approach. Asian J of Chem 2007; 19(2):1406-10.
3. Kokate CK, Purohit AP, Khandelwal KR. Pharmacognosy, 29th edition, New Delhi: Nirali Prakashan 2002;550-9.
4. The Ayurvedic Formulary of India, Part-I, II nd edition, New Delhi: Govt. of India, Ministry of Health and Family Planning, Depart. of Health 2003, 103-19.
5. Pharmacoepical standards for Ayurvedic Formulations. Central Council for Research in Ayurvedic and Siddha, New Delhi: Ministry of Health and Family Welfare 1987; 112-23.
6. Quality Standards of Indian Medicinal Plants. Volume I & II, New Delhi: Indian Council of Medicinal Research 2003;95,168
7. Indian Herbal Pharmacopoeia, Volume II, Mumbai: Indian drug Manu-facturing Association and Regional Research Laboratory Jammu 1999; 90-3,126-62.
8. World Health Organization, Quality Control Methods For Medicinal Plants Materials, Geneva: 1998, 1-15.
9. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments. 9th edition, New Delhi, Nirali Prakashan 2002; 152.
10. Jain V, Saraf Swarnlata, Saraf S. Spectrophotometric Determination of Piperine in Trikatu Churna: An Ayurvedic Formulation Asian J. of Chem 2007; 19 (7):5331-5.

Table 1: Validation parameters of piperine in AJC (Mean% ± SD, n=3).

S.No.	parameters	value
1	Absorption maxima	342.5nm
2	Beer's law limit	10-50ug/ml
3	Regression equation(y=bx+a)	0.013x+0
4	Intercept(a)	0
5	Slope(b)	0.013
6	Correlation coefficients(r2)	0.9961
7	Precision (n=6, % RSD)	2.33
8	Accuracy (%)	99.43
9	LOD microgm/ml	0.063
10	LOQ microgm/ml	0.071

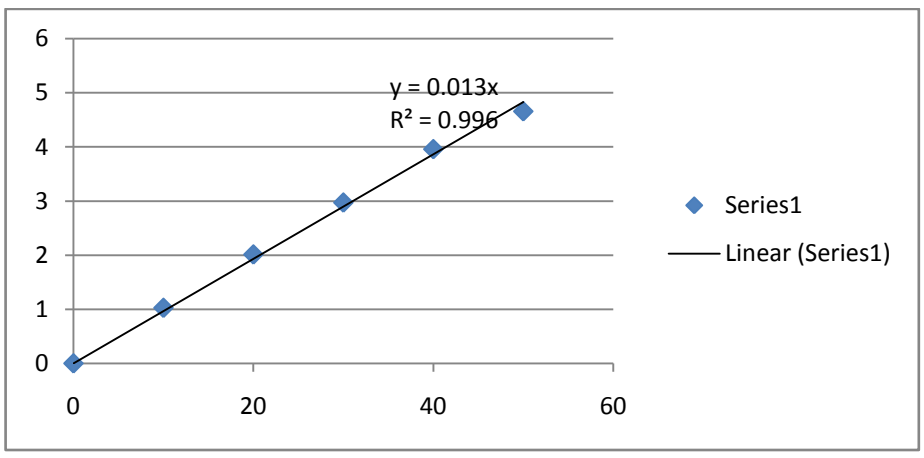
Table 2: uniformity in batches.

S.No.	Name		Piperine content %w/w
	piper longum(pippli) piper longum(pipplimula) piper nigrum(marica)		1.45±0.34 1.63±0.21 3.97±0.16
01	AJMODADI CHURNA	AJC-I	0.18±0.001
02		AJC-II	0.20±0.003
03		AJC-III	0.13±0.006
04		AJC-A	0.16±0.002
05		AJC-B	0.19±0.006
06		AJC-C	0.17±0.005

Table 3: Data of recovery study (Mean% ± SD, n=3) of AJC.

S.No.	Amount of piperine(µgm/ml)			RSD%	SE	Recovery%
	In sample	added	estimated			
01	100	50	149.22±0.54	0.36	0.3118	99.48
02	100	100	199.47±0.64	0.32	0.3695	99.73
03	100	150	249.03±0.86	0.34	0.4965	99.61
Mean				0.34	0.3926	99.60

Figure 1: Calibration curve of piperine in AJC.



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