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Quantification of Piperine by TLC Densitometer Method and Forced Degradation Study in a Classical Ayurvedic Formulation-Trikatu Churna

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Authors' contributions

This work was carried out in collaboration among all authors. Authors SHA and SA has designed the research protocol, authors AQ and AA performed the experimental work and Author MT performed statistical analysis and draft the manuscript. All author approved the final manuscript.

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ABSTRACT

Developed a thin layer chromatography (TLC) method for the quantification of piperine in *Trikatu Churna* formulation it is a major and important ingredient in the formulation. TLC methods for the determination of piperine in the *Trikatu churna* along with its raw materials have been developed, as per the ICH guideline. The developed method has been validated, experimented with parameters like linearity, accuracy, the limit of detection, the limit of quantification, inter-day and intra-day assay precision, repeatability of measurement, and repeatability of a sample application. Performed a degradation study in different conditions. Prepared a calibration curve in the concentration range (100-800 ng/spot) with correlation coefficients r^2 (0.997) with Rf value 0.46 ± 0.03. The Limit of detection (LOD) and Limit of quantification (LOQ) value has 100 ng and 329 ng, respectively. The maximum degradation found in the bench top (98%) follow with an acidic condition (86%), almost

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similar in the range of condition basic, oxidation and wet (75-77%), and a minimum in dry heat condition (49%). The freeze-thaw stability study, the accelerated stability study, the real-time stability study result of these conditions almost is the same range (92-99%).

Keywords: TLC; validation; ICH guideline; LOD; LOQ; Trikatu Churna.

1. INTRODUCTION

The fruits of black and long peppers, *P. Nigrum* and *P. Longum*, belonging to the family Piperaceae have piperine as a major constituent [1]. Adulteration in aromatic plants directly affects their medicinal and economic importance. Authentication of the medicinal in quality, as well as the quantity of the drugs can be done by the different analytical methods. Conventionally medicines are obtained from medicinal plants, minerals, and organic matter, the herbal drugs are produced from medicinal plants only. Globally, a plant as a source of medicines has been used from ancient times in the health care system and it growing very rapidly [2]. Unsound food habits and lifestyles produce so many diseases like cancer, diabetes, heart problems, depression, and many others. Allopathic medicines have so many disadvantages in that situations require alternative therapy to good health for all. Herbal drugs are the best choice to overcome the problems that produce in the same [3]. The development of herbal drugs requires guidelines for its preparation; globally International Regulatory Cooperation for Herbal Medicines (IRCH) is an authority that comes under the WHO guideline. The objective of this authority is to encourage the use of safe herbal medicines by sharing valuable information. Countries like Armenia, Australia, Argentina, Darussalam, Canada, Brazil, Brunei, Chile, China, Cuba, Ghana, Hungary, Japan, Malaysia, India, Indonesia, Italy, Pakistan, Peru, Portugal, Republic of Korea, Saudi Arabia, Singapore, UAE, UK, United Republic of Tanzania, and USA are members of this authority.

In quality control, standardization is a process by which prepare a set of standards, constant parameters, definitive quality and quantitative values that assure the quality, efficacy, safety, and reproducibility. Different analytical techniques are available, but modern chromatography methods of identification, separation, and quantification of such active components are very valuable for the quality control of herbal drugs [4]. Testing of new drug substances performed under the ICH guideline entitled stability testing, formulation requires studying force degradation to elucidate the inherent stability characteristics of the active substance [5]. In developed as well as developing countries, the quality drug is a major problem, where adulterated medicines spread in the market rapidly, which harms the health of the people. Validation is very important step to check the quality of the medicines, it is very necessary to follow the ICH guideline when a new method developed in the laboratory for the estimation of drugs in the formulation and herbal extract, It is way by which the production of quality drugs can be checked and confirm their rational use [6,7].

To cure the disease like asthma, cough and cold, tuberculosis, fever, indigestion, chronic rhinitis/sinusitis and other inflammatory and respiratory disorders from ancient times by Ayurveda formulation, available different types of powder (churna) such as *Trikatu churna, Sitopaladi churna, Hingavastaka churna, Avipattikara churna, Sringyadi churna, and Talisadya churna* [8]. From research it has been confirmed, piperine an alkaloid has IUPAC name (1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-petadienyl] piperidine) and molecular formula $C_{17}H_{19}NO_3$ is an active constituent in the ayurvedic formulation. This herbal constituent's origin from the *Piperaceae* family, of plants *Piper longum* (pipli), *Piper nigrum* (peppercorn), *Piper chaba* (Pipli), etc. [9,10,11,12]. The role of the piperine is to increase the bioavailability of other nutritive substances including β-carotene, curcumin, selenium, pyridoxine, glucose, and amino acids [13,14]. The purpose of this research was to find out the quantity of piperine in the formulation and its stability.

2. MATERIALS AND METHODS

2.1 Plants

The crude drugs (fruits) comprising *Piper longum* (pippali), fruits of *Piper nigrum* (black paper), and rhizome of *Zingiber officinale* (shunt). Crude drug purchased from the local market, Kharibaoli, old Delhi, The crude drugs were dried in the shade, powdered to coarse and were kept in air-tight containers individually, away from moisture. The powdered drugs were

standardized for the following parameters (microscopy, foreign organic matter, organoleptic properties).

2.2 Chemical, Solvents and Reagents

Piperine was procured in analytical grade, LR grade solvents from "Chemical labs" were used, and for another laboratory, either LR grade or its equivalent grade solvents were used. For thin-layer chromatography, pre-coated silica gel plates were used. Solvent distilled water is used for the solution and also, for another purpose.

2.3 Chromatographic Methods

2.3.1 Thin Layer Chromatography (TLC)

In the laboratory made TLC plates prepared, used silica gel G (0.3 mm thickness), activating them for 30 mins at 110ºC. The quantitative analysis by thin-layer chromatography and fingerprint profiling were done using pre-coated silica gel TLC plates (E. Merck) of 200 micrometre thickness after developing an appropriate solvent system, the number and position of bands in the plate were observed under ultraviolet light. Various compositions of the solvent system were tried for developing the fingerprint profiles. CAMAG TLC scanner was used for analytical studies. The data was altered and processed through software CAMAG Win cats 3.0. The samples were applied using CAMAG automatic TLC was sampled by TLC sampler four and were developed in CAMAG twin through the chamber using appropriate solvents. The plates were dried, and images of TLC chromatography were taken under 284 nm UV light using CAMAG Reporter scanner. Peaks were recorded. The amount of the desired component was calculated from the standard of the respective marker.

2.3.2 Method development

TLC fingerprint profile - The standard solution prepared by dissolving 1mg of piperine in ethanol and making volume up to 1ml. Sample application - A uniform volume of 1, 2, 3, 4, 5, 6, 7, 8µl of standard and 4µl test solution were applied separately using CAMAG automatic TLC sampler in the form of a 6 mm band on pre-coated silica gel 60F254 TLC plates of uniform thickness of 200 µm.

Development of the plate and visualization: The plate was developed in the appropriate solvent system to a distance of 80% of the plate in a twin trough chamber with a pre-saturation time of 10 min. The plate was air-dried and image of TLC chromatogram was taken under 254 nm ultraviolet light using CAMAG Reporter.

2.3.3 Quantitative estimation of Piperine in *Trikatu Churna* formulation

TLC plates – Pre-coated plates of silica gel 60F254 with a uniform thickness of 200µm. Solvent system–Toluene: Ethyl acetate: Formic acid (7: 2: 1).Scanning wavelength–350 nm. Preparation of sample – prepared as described under section.

2.3.4 Method validation

After the development of new method further it was validated as per ICH guidelines carried out an experiment for linearity range, precision, accuracy as recovery, limits of detection (LOD) and limits of quantification (LOQ) [15,16,17].

2.3.5 Stress degradation studies

The stability of the drug substance or drug product defined as its extent to be remaining within the established specifications to maintain its identity, strength, quality, quantity, and purity throughout the retest or expiration period. In the drug development process, its role is significant. The purpose of the stability study is to give the authentication for the quality and purity of drug substance or drug product. Condition like temperature, humidity, oxidation, light, pH and moisture, etc. drug substance or drug product must be stable. The pharmaceutical formulation must have experimented with stability profile at accelerated temperature. The result of the study beneficial to forecast the authentic shelf life at room temperature by adopting certain assumptions [18].

The purpose of the stability is to know about the stability of the drug substance in the dosage form. Establish degradation pathways of the active compound to interpret the structure of the degradation product, to decide the intrinsic stability of a drug substance in the dosage form. The exact mechanism of the degradation by hydrolysis, oxidation, and photolysis shall be beneficial to know the chemical properties of drug formulation further it possible to prepare a more stable formulation, minimize the unstability problems in the formulation. From stability data, the expiry date, retesting period and storage conditions of the active drug established. Many

guidelines are exits for the stability testing of the pharmaceutical formulation [19, 20].

- **Acid stability-**0.1N HCl added to the mixture of 2gm of the powdered drug was extracted with a hydroalcoholic solution for 2 hrs. then filtered. The filtrate is concentrate to dryness and weighed. The sample made by taking 0.1mg of *Trikatu Churna* extract in 1ml ethanol. After 18 hrs. of sample preparation analysis done.
- **Basic stability-** 4N NaOH added to the mixture of 2gm of the powdered drug (pH-11) which was further extracted with a hydroalcoholic solution for 2 hrs. then filtered. The filtrate is concentrate to dryness and weighed. The sample made by taking 0.1 mg of *Trikatu Churna* extract in 1ml ethanol. After 18 hrs. Of sample preparation analysis done.
- **Oxidation stability-**1ml (3% v/v) hydrogen peroxide added to the mixture of 2gm of powdered drug which further extracted with a 70% hydroalcoholic solution for 2 hrs. Filtered, concentrate to dryness and weighed. The analysis performed after 18 hrs. Of sample preparation.
- **Wet heat stability**- 2 gm of the powdered drug refluxed with a hydroalcoholic solution on a water bath for 3 hrs. at 70ºC. The solution was filtered, concentrates to dryness and weighed. After 18 hrs. Of sample preparation analysis done.
- **Dry heat stability-**2 gm of the powdered drug was kept in a hot air oven at 80ºC for 4 hrs. then refluxed on a water bath for 2 hrs. The solution was filtered concentrate to dryness and weighed. After 18 hrs. Of sample preparation analysis done.
- **Bench top stability**- 2 gm powdered drug extracted with a hydroalcoholic solution for 2 hrs. at 70ºC. The extract 'was filtered, concentrates to dryness and weighed. The residue reconstituted with ethanol (10 ml). The prepared sample kept at room temperature for at least 24 hrs. After 18hrs of sample preparation analysis done
- **Stock solution stability**-2 gm of the powdered drug extracted with a 100 ml hydroalcoholic solution for 2 hrs. at 70ºC. The extract was filtered, concentrated to dryness and weighed. The residue reconstituted with ethanol and volume was made up to 10 ml. The stock solution is then frozen at -20ºC for 7 days and subsequently kept for 6 hrs. at room temperature. After 18 hrs. Of sample preparation analysis done.
- **Freeze-thaw stability**- 2 gm of the powdered drug was refluxed with a 100ml hydro alcoholic solution for 2 hrs. The extract was filtered, concentrates to dryness and weighed. The residue is reconstituted with ethanol (10 ml). From these three concentrations of the high, medium, and low is prepared. The volume is made up to 5 ml. These samples are frozen at -20ºC for 24 hrs. and thawed unassisted for the next 24 hrs. This cycle was repeated 3 times before analysis. The analysis is done after 18 hrs. Of sample preparation.
- **Accelerated stability study** - The study of the drug substance in the formulation of its chemical degradation or physical change using over stress storage conditions as a part of the formal stability studies. The conditions provided are temperature 40ºC and relative humidity provided is 75% RH [21].
- **Real-time stability testing**- This test was performed to know about product degradation under recommended storage conditions for a longer duration of time. The temperature in this varies between 15-30ºC [22].

3. RESULTS AND DISCUSSION

3.1 Standard Solution

Prepared a stock solution of piperine (1mg/ml) dissolving accurately weighed 1mg in 1ml of ethanol and making the volume up to 1 ml. CAMAG automatic sampler was then commanded to place the spots of 1 µl 2 µl 3 µl 4 µl 5 µl 6 µl 7 µl and 8 µl from the stock solution of piperine containing 1 µg, 2 µg, 3 µg, 4 µg, and 5µg 6 µg 7 µg and 8 µg of piperine respectively.

3.2 Calibration Curve

Applied 6 µl of the standard solutions using CAMAG automatic sampler, on a pre-washed, silica gel 60F254 plates. Development of the plate in the solvent system to a distance of 80% of the plate in a CAMAG twin trough chamber with a saturation time of 10 min. The plate was then scanned at 350 nm in the CAMAG TLC scanner. The peak areas for different concentrations were recorded. The curve of piperine was prepared by plotting mean AUC vs the concentration of piperine (Range-100-800 ng/spot, y= 0.028x+28.72 & r^2= 0.997) and Rf

value -0.46. The calibration curve (Fig. 1). Picture showing spots of Trikatu Churna extract (track no.9) in comparison with piperine track no. 1-8 (Fig. 2). Denstiogram showing various peaks of standard piperine and extract of *Trikatu Churna* formulation along with a peak treated with base (Fig. 3) & Standard Piperine (Rf= 0.46) (Fig. 4).

3.3 Validation

First in laboratory estimate the value for a limit of detection (LOD) and limit of quantification (LOQ) of piperine, found 100 ng and 329 ng / ml respectively. It is a good value and further performed the other validation parameter. Calculate the precision value as intraday and inter-day precision. Analysis of standard drugs done on the same day for the determination of Intra-day precision and carried out analysis at three different days for the determination of Inter-day precision value. The % RSD was found to be ≤ 2 for both inter-day and intra-day precision. Performed experiment for the determination repeatability of sample application and repeatability of measurement. Spotting 10 ml of drug solution, six times for determining the value of Repeatability of the sample application, analysis of peak area done the % RSD (0.968) and determined Repeatability of measurement found % RSD (0.422). The complete validation parameters shown in [Table 1].

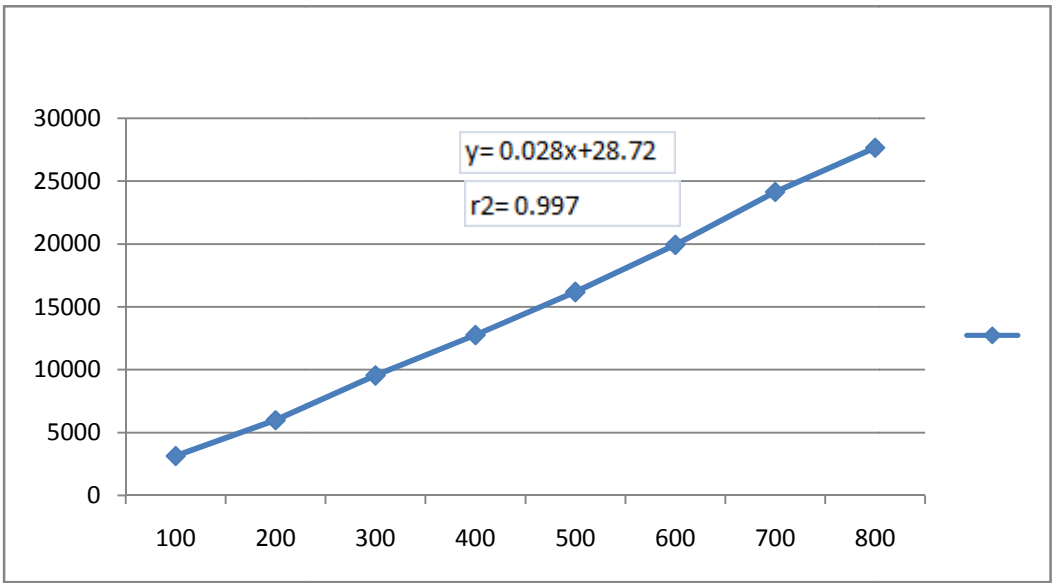

Fig. 1. Calibration curve

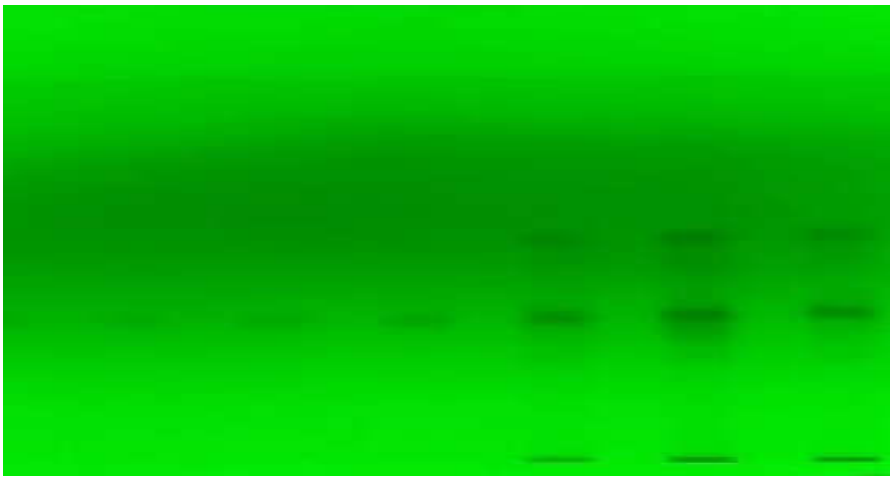

Fig. 2. Picture showing spots of Trikatu Churna extract (track no.9) in comparison with piperine track no. 1-8

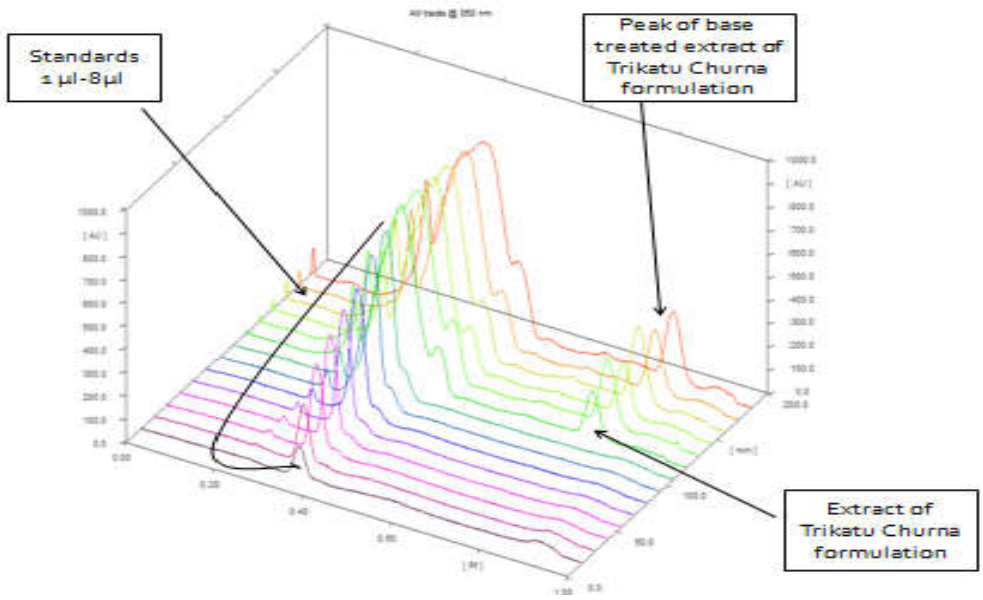

Fig. 3. Denstiogram showing various peaks of standard piperine and extract of *Trikatu Churna* formulation along with a peak treated with base

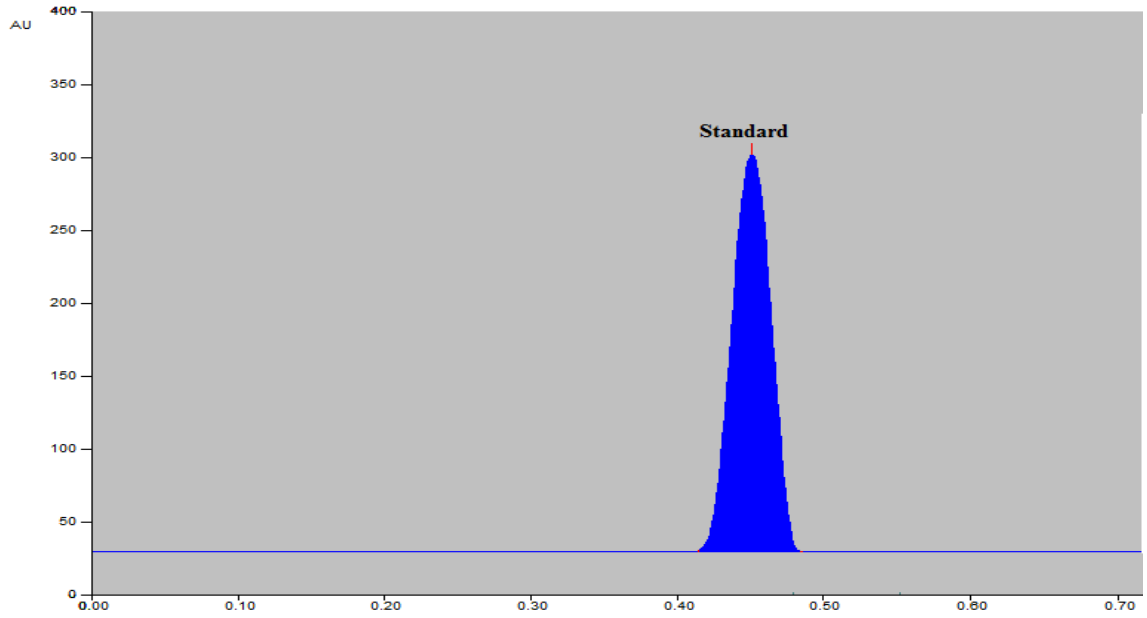

Fig. 4. Standard piperine (R_f= 0.46)

3.4 Stability Studies

3.4.1 Estimation of piperine in dried fruit in Trikatu Churna by densitometry

For stability study, the extract of *Trikatu Churna* was prepared with coarse powder in 100ml of ethanol at 70ºC for 2 hrs, and the sample was analyzed by TLC densitometry. The percentage content of piperine was calculated from the standard curve by considering the area under the curve. The content of piperine in the coarse powder of *Trikatu Churna* was found to be an indifferent condition.

- Stress Degradation Studies
- Freeze-Thaw Stability Study
- Accelerated Stability Study
- Real-Time Stability Study

Carried out experiments in a different condition to the stability of the piperine, 0.566 % piperine present in the formulation, in the different conditions the percentage reduced mention in the table. Reduction in the percentage with respect to the extract of *Trikatu Churna* formulation of a different condition like acidic, basic, oxidation, wet heat, dry heat, and bench-top stability study result in [Table 2] & Chromatogram-Peak of different stress degradation study (Fig. 5). The result of the Freeze-Thaw Stability Accelerated Stability Study and Real-Time Stability Study in [Table 3] and Chromatogram (Fig. 6).

Table 1. Validation parameter

Parameter	Value
Rf	0.46±0.03
Linearity (ng/spot)	100-800 ng
Correlation coefficients r^2	0.997
LOQ (ng /spot)	329 ng
LOD (ng /spot)	100ng
Precision (%RSD)	
Inter day	0.69
Intra day	0.57
Recovery Studies	
Accuracy(%RSD)	0.405
SE	0.46
Recovery%	99.18
Repeatability of sample application	0.968
Repeatability of measurements	0.422

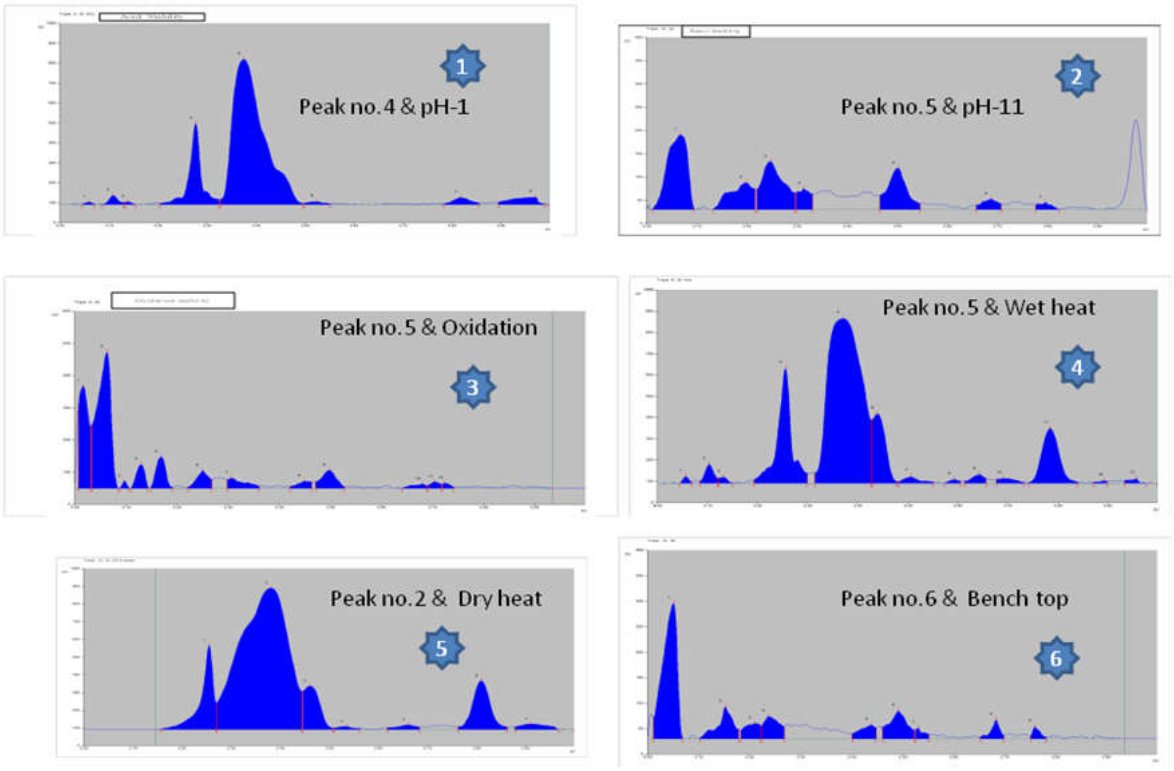

Fig. 5. Peaks of different stress degradation study (1-Acidic, 2-Basic, 3-Oxidatio, 4-Wet heat, 5-dry heat and 6-bench top

Table 2. Degradation study

Sample	AUC	Estimated content of piperine in spotted volume (ng)	Piperine content in the dried residue (mg)	% Piperine found in the coarse powder	% degraded (% 0.566 Piperine in formulation extract)
Acidic	12188.8	9.2	1.50	0.075	86.74
Basic	21972.1	16.89	2.75	0.137	75.79
Oxidation	23024.3	17.72	2.894	0.144	75.26
Wet heat	20298.5	15.57	2.5431	0.127	77.03
Dry heat	4974.1	3.513	5.76	0.288	49.11
Bench top	2304	1.43	5.23	0.011	98.23

Table 3. Freeze-thaw, accelerated stability and real-time stability study

Sample	AUC	Estimated content of piperine in spotted volume (ng)	Piperine content in the dried residue (mg)	% Piperine found in the coarse powder	% degraded (% 0.566 Piperine in formulation extract)
Freeze-Thaw Stability					
Freeze-thaw first dilution	6769	4.92	0.8	0.04	92.93
Freeze-thaw second dilution	5334	3.81	0.62	0.031	94.52
Accelerated Stability Study					
3 month	6412.7	4.662	0.76	0.038	93.28
6 month	4762.7	3.365	0.54	0.027	95.22
Real-Time Stability Study					
3 month	2411.7	1.517	0.24	0.012	97.87
6 month	1201.8	0.565	0.092	0.005	99.11

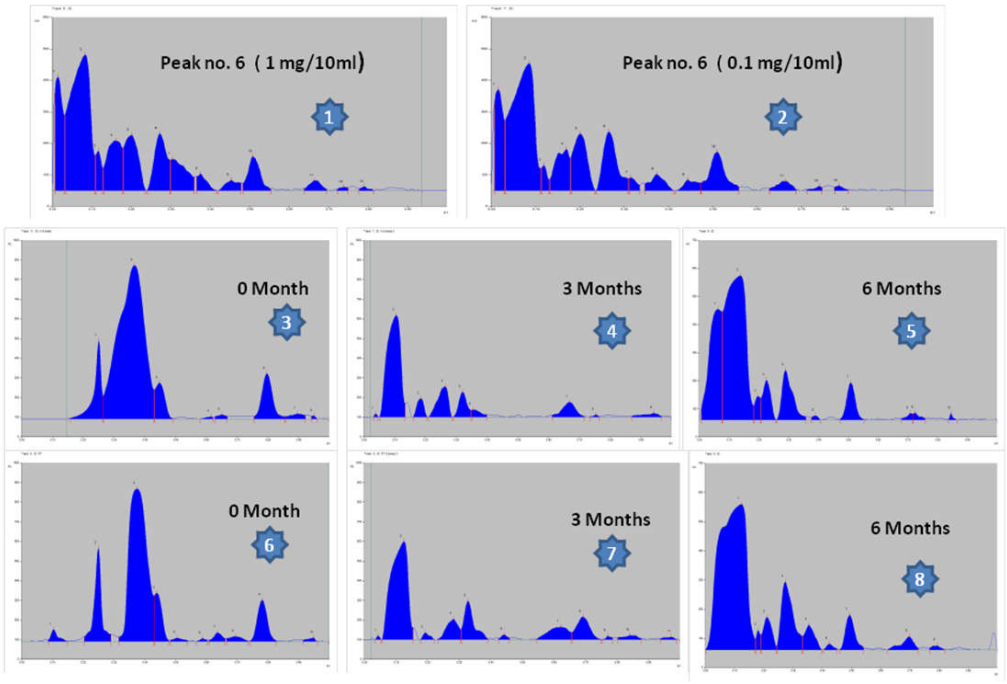

Fig. 6. Freeze-Thaw Peak (1&2), Accelerated Stability Peak (3, 4 & 5) and Real-Time Peak (6, 7 & 8)

4. CONCLUSION

Stability acts as a symbol of quality, purity, and efficacy in herbal drugs and drug products. Stability in itself is a very important criterion, without which it is difficult to ensure a drug product's safety and efficacy. The attempt was made to carry out stability studies on Trikatu Churna formulation herbal drug industry both in India as well as other countries.

The evaluation of the stability studies has been carried out by both comparative fingerprints chromatograms and quantitative analysis of different markers evaluated by TLC densitometry. In a comparative fingerprint profile, the comparison was established after different stability studies and compared with reference extract. The condition leading to degradation was noted for the drug. The change in contents of piperine for Trikatu Churna was further analyzed by TLC densitometry. The percentage reduction in the contents of the respective marker was calculated after the various stability studies and compared with the initial values.

Various stress degradation studies, the Bench top conditions caused maximum (98%) degradation and minimum in dry heat (49%) condition. In accelerated stability study real-time stability study testing showed almost the same level of degradation during 3 months with no further significant change during the next 3 month (6-month study)

The study is likely to be immense value for the herbal drug industry with respect to usage of crude drug and formulation development based on any of the three plants worked in this document.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mittal R, Gupta RL. *In vitro* antioxidant activity of piperine. Meth Find Exp. Clin. Pharmac 2000;22(5):271–274.
2. Seth SD, Sharma B. Medicinal plants in India. Indian Journal of Medical Research 2004;120(1):9-11.
3. WHO, Quality control methods for medicinal plant materials, Geneva. 1998;1-15.
4. Poole SK, Poole CF. Thin-layer chromatographic method for the determination of the principal polar aromatic flavor compounds of the cinnamons of commerce. Analyst. 1994;119:113–120.
5. Taleuzzaman M, Imam SS, Gilani SJ. Quantitative Determination of thymoquinone in *Nigella Sativa* and its nano-formulation using validated stability-indicating HPTLC densiometric method. International Current Pharmaceutical Journal, September 2017;6(10):53-60.
6. Farah MH. Olsson S, Bate J, Lindquist M, Edwards R, Simmonds MS et al. Botanical nomenclature in pharmacovigilance and a recommendation for standardization. Drug Saf. 2006;29:1023.
7. WHO. Guidelines for assessing the quality of herbal medicines with reference to contaminants and residues. Geneva: World Health Organization; 2007.
8. The Ayurvedic Pharmacopeia of India. New Delhi: Ministry of Health and Family Welfare, Dept. of AYUSH; 2007.
9. Pathak N, Khandelwal S. Cytoprotective and immunomodulating properties of piperine on murine splenocytes: an in vitro study. Eur J Pharmacol 2007; 576:160-70.
10. Singh A, Duggal S. Piperine: review of advances in pharmacology. Int. J. Pharm Sci Nanotech 2009;2:615-20.
11. Srinivasan K. Black pepper and its pungent principle-piperine. A review of diverse physiological effect. Cri Rev Food Sci Nutr. 2007;47:735-48.
12. Ahmad N, Fazal H, Abbasi BH, Farooq S, Ali M, Khan MA. Biological role of Piper nigrum L. (black pepper): a review. Asian Pac. J. Trop. Biomed 2012: S1945-53.
13. Majeed M, Badmeev V, Rajendran R. Use of piperine as a bioavailability enhancer. United States Patent No.5. 972. 1999. 382.
14. Patil U, Singh A, Chakraborty A. Role of piperine as a bioavailability enhancer. Int J Recent Adv Pharma Res. 2011;4:16e23.
15. Sai Bindu NH, Rubesh Kumar S, Duganath N, Devanna N. Extraction and screening of trimyristin in the seeds of *Myristica fragnans* and in poly herbal formulations by spectroscopic and chromatographic techniques. Int. J. Uni. Pharm. Bio. Sci. 2013;2:569-581.
16. Gilani SJ, Imam SS, Ahmed A, Chauhan S, Mirza MA, Taleuzzaman M. Formulation and evaluation of *Thymoquinone niosomes*: Application of

developed and validated RP-HPLC method in delivery system. Drug Dev. Ind. Pharm. 2019;45(11):1799-1806.
17. Taleuzzaman M, Jahangir MA, Gilani SJ. Quantification and Identification of Bioactive Eugenol in Myristica fragrens Seed Using Validated High Performance Thin Layer Chromatographic. Pharmaceutical Analytica Acta. 2017;8:563.
18. Bankoti K, Rana MS. Bharadwaj MK. Accelerated stability study of herbal capsules. IOSR Journal of Pharmacy. 2012;2(5):1-6.
19. Blessy M, Patel RD, Prajesh N, Prajapati Y, Agrawal K. Development of Forced Degradation and Stability Indicating Studies of drugs-A Review. J Pharm Anal. 2014;4(3):159-165.
20. Sengupta P, Chatterje B, Tekade RK. Current regulatory requirements and practical approaches for stability analysis of pharmaceutical products: A Comprehensive Review. Int J Pharm. 2018;543(1-2):328-344.
21. Guidance for Industry Dissolution Testing of Immediate Release Solid Oral Dosage Forms. U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER); 1997.
22. Aneesh TP, Rajasekaran A. Stress degradation studies and development and validation of RP-HPLC method for the estimation of Asenapine Maleate. Intern. J. of Pharmacy and Pharmaceu. Sci. 2012;4(4):1-4.

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