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Development of chewable tablet of *Trikatu churna* and standardization by densitometry

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Trikatu is a well known polyherbal powder form formulation in *Ayurveda* consisting of *Piper longum* L. fruit, *Piper nigrum* L. fruit and *Zingiber officinale* Rosc. rhizome in equal amounts, i.e., 1:1:1 ratio. It is prescribed for cold, fever, asthma, cough, respiratory problems and treatment of digestive disorders. The aim of the present study was to develop and validate a densitometric method for the identification and quantification of piperine and 6-gingerol in the crude drug *Trikatu* as the markers for quality of raw materials and to develop a well characterized formulation of *Trikatu* as a chewable tablet.

Keywords: *Piper longum*, *Piper nigrum*, *Zingiber officinale*, *Trikatu*, Densitometry, HPTLC
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Ayurveda is the oldest medical science that describes various aspects of life including abnormalities and their treatment practiced in India since ancient times. The increasing awareness of *Ayurvedic* medicines is acknowledged by World Health Organization (WHO 2003), which has defined traditional medicine as "Health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination, to treat, diagnose and prevent illness or maintain well-being[1]." The *Ayurvedic* or traditional preparations comprise of medicinal plants, animals and minerals. On the basis of *Charak Samhita* (4th chapter of *Sutrasthan*), five varieties of pharmaceutical preparations (*Panch Kashaya Kalpana*) such as *swaras* (expressed juice), *kalka* (paste form), *kvatha* (decoction), *shita* (cold infusion) and *phanta* (hot infusion) are primarily found[2]. Later on *kalka* was further modified to *churna*, *vati* or pills. These are the basic dosage forms of all *Ayurvedic* medicines. Different dosage form such as *churna, vati, gutika, awaleha, asavas-arishtas, ghritas* and *tailas* have been also described in ancient *Ayurvedic* classics[2,3]. On the basis of available ancient literature, more than 30 dosage forms are found and frequently prescribed by physicians. *Kalka* (paste) dosage form is first prepared, followed by *svarasa* extraction and preparation of *vati*.

In ancient era *Ayurvedic* physicians used to handle all the work regarding the preparation of a formulation right from procurement of authentic drug, preparing the formulation and dispensing to the patients. In the present time, all *Ayurvedic* physicians depend on the marketed products manufactured by *Ayurvedic* pharmacies. Standardization, stability studies, safety evaluation and quality control are essential for herbal formulations. Besides this, other improvements are also required to enhance their utility such as those in terms of bioavailability, dosages-regimen, popularity and patient acceptance. Some problems such as bitter and astringent taste and unpleasant flavors are associated with solid dosage forms especially with *churna* and *vati*. The shelf life for prepared *churna* and *vati* dosage form is very less due to which *Ayurvedic* pharmacies generally manufacture small batch sizes. Sometimes, a few formulations are so voluminous and create problems during the oral administration. All these factors result in poor patient compliance and hence reduce the contribution of TM to healthcare in modern times[4,5,6]. *Trikatu* is derived from two words *Tri* and *Katu*, meaning three and pungent in taste. All three ingredients of this formulation are pungent in taste.

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Despite its pungent taste *Trikatu* is very famous, effective and popular formulation in *Ayurveda*. It contains a mixture of equal amounts of *Sunthi* (*Zingiber officinale* Rosc.), *Kali Marich* |(*Piper nigrum* L.) and Pepper (*Piper longum* L.). Other synonyms of *trikatu* are *trayushna, vyosh* and *katutrik. Trikatu churna* dosage form is frequently prescribed by physicians for treating cough, asthma, respiratory problems, fever, cold and amelioration of digestive disorders[2,3,7,8,9,10,11].

Objectives

The present study was designed to develop a more acceptable tablet from *Trikatu churna* and its standardization by densitometry.

Methodology

Chemicals, materials, and solutions

Analytical grade hexane, diethyl ether and methanol were purchased from CDH Laboratory Reagents, India. Fruits of *Piper longum* and *Piper nigrum* and rhizome of *Zingiber officinale* were purchased from Sec-26 Chandigarh market, specimen voucher numbers are NIP-NPM-CD-166, NIP-NPM-CD-165 and NIP-NPM-CD-164, respectively and were authenticated by Dr AS Sandhu (Department of Natural Products, NIPER, SAS Nagar, Mohali, Punjab). The standards piperine and 6-gingerol were available in our lab from previous work.

Solutions and sample standard preparation

For calibration curve of Piperine and 6-gingerol

Piperine

Different concentrations of piperine in methanol were prepared to obtain calibration curve. These concentrations range from 0.01 - 0.06 mg/mL. To obtain 0.01 mg/mL concentration, approximately 5 mg of weighed piperine was diluted accordingly to make concentration of 1 mg/mL. Then 1 mL was withdrawn and diluted to 10 mL to obtain concentration of 0.1 mg/mL. Furthermore, 1 mL from this solution was diluted to 10 mL resulting in concentration of 0.01 mg/mL. Similarly other dilutions were obtained.

6-gingerol

Different concentrations of 6-gingerol in methanol were prepared to obtain calibration curve. These concentrations ranged from 0.05 mg/mL to 0.35 mg/mL. A concentration of 0.05 mg/mL was obtained by serial dilution of a solution of concentration 5 mg/mL. The other concentrations were obtained similarly by serial dilution of separately weighed samples.

The standard solutions were prepared with the aim to keep the applied volume constant (2 µl) with the change of concentration. The standard concentrations for calibration curve ranged from 20-140 ng/spot and 100-700 ng/spot for piperine and 6-gingerol, respectively.

High performance thin layer chromatography

Pre-coated silica gel HPTLC plates (20 cm × 20 cm, G F_{254} Merck, Germany) were used for analysis. The standards and samples were applied as 6-mm-wide bands at 12 mm from the bottom and 15 mm from both sides using Hamilton microliter (100 µL) syringe and CAMAG Linomat 5 applicator under flow of N_2 gas with dosage speed 200 nL/s. After sample application, the plates were developed in a CAMAG twin trough glass tank pre-saturated with the mobile phase (10 ml) *n-hexane:diethyl ether* (6.2:3.8) for 20 min. The plate was developed twice in ascending mode up to 9 cm. The developed plates were dried using a hair dryer for 20 s and observed under CAMAG UV cabinet. To determine the λ_{max} of piperine and 6-gingerol, developed plates were scanned from 200-400 nm using spectrum mode. The developed plate was scanned at 286 and 345 nm using remission-absorption mode by CAMAG TLC scanner III equipped with win-CATS software (CAMAG) under the conditions as follows: slit width 4 mm × 0.3 mm, scanning speed 20 mm/s, and data resolution 100 mm/step. The identification of 6-gingerol and piperine was confirmed by superimposing the UV spectra of the samples and standards. For quantitative analysis, calibration curve was obtained by application of standard solutions of seven different increasing concentrations on HPTLC plate, and each concentration was spotted thrice. Peak areas were graphed against the amount of standards spotted.

Formulation of *Trikatu* tablet

The unit formula used for preparing chewable tablets of *Trikatu* is given in Table 1. *Trikatu* tablets were prepared using wet granulation method. In wet granulation method, powders were mixed with excipients and compressed into tablets using 10 mm punch with single punch machine. The average weight of tablets was 700 ± 8 mg.

All three ingredients were cleaned manually, dried at 40 ºC for 8 hrs, pulverized into powder form and

Table 1—Unit formula of chewable tablets for *Trikatu*

Sl no.	Ingredients	Quantity (mg)	Role
1	*Piper longum* L. fruit, *Piper nigrum* L. fruit and *Zingiber officinale* Rosc. rhizome	375	Active ingredient
2	Lactose	60	Diluent
3	Micro crystalline cellulose (Avicel PH101)	127.5	Diluent
4	Sucrose	30	Sweetener
5	Poly vinyl pyrrolidone (PVP K30)	70	Binder
6	Stevia	10	Sweetener
7	Purified Talc	5	Glidant
8	Colloidal silicon dioxide (Aerosil 200)	10	Glidant and dessicant
9	Flavour Vanilla	10	Flavor
10	Magnesium stearate	2.5	Lubricant

sieved through British standard (BSS) 85 No. sieve. Three ingredients were mixed together for 20 min using a double cone blender (Karnavati, Gujarat) to get a homogeneous *Trikatu* powder. Weighed quantity of lactose (30 gm), microcrystalline cellulose (63.75 gm), sucrose (15 gm) were passed through BSS # 40 sieve and mixed with *Trikatu* powder (187.5 gm) for 15 min using a double cone blender for complete mixing. Binder solution was prepared by dissolving PVP in demineralized water by stirring to obtain a clear solution. This binder solution was added into the dry mix blend to generate a wet dough mass. Wet dough was passed through BSS # 18 sieve, to obtain granules. The latter were dried in a fluidized bed dryer (RETSCH, Germany) at inlet air temperature of 50-60 ºC. Remaining ingredients "talc, Magnesium stearate, Aerosil 200, Stevia and Flavour Vanilla" were mixed with dry granules in double cone blender. The mixture was converted into tablets using Mini Press (RIMEK, Minipress 2MT, Karnavati, Gujarat).

The prepared *Trikatu* was evaluated for hardness, friability, weight variation, disintegration and *in vitro* dissolution studies. The hardness of tablets was determined using Electrolab hardness tester and the friability of tablets was determined using (Friabilator EF-2W, Electrolab). The disintegration test was carried out using Disintegration Tester (USP) ED-2AL, Electrolab and their mean disintegration time was calculated. *In vitro* dissolution studies were carried out on Dissolution Tester (USP) TDT-06L, Electrolab and Temperature Controller ETC-11L, Electrlab) USP II (paddle type). Percentage of piperine and 6-gingerol release in 0.1 N HCl (900 mL) media at 100 rpm was calculated. Dissolution studies were carried out up to 40 min. Samples (5 ml) were withdrawn at specified time intervals and percent drug release was calculated.

Validation of HPTLC method

The protocol of Ferenczi-Fodor *et al*. was followed for the validation of the analytical method developed for precision, LOD and LOQ, specificity and accuracy[12,13].

Precision

Instrumental precision was measured by replicate (n = 6) applications of same piperine and 6-gingerol solution. Intra-day assay precision was assessed by analysis of replicate (n = 3) applications of freshly prepared standard solution of same concentration on same day at different time whereas, inter-day precision was assessed by analysis of replicate (n = 3) applications of standard solution of the same concentration on three different days. The results are expressed in terms of % RSD[14].

Limit of detection and limit of quantification

Limit of detection and limit of quantification was determined by serial dilution of the standard solutions of piperine and 6-gingerol. On the basis of signal to noise ratio, LOD was determined as S/N of 3:1 and LOQ as S/N of 10:1.

Specificity

The specificity of the method was ascertained by analyzing standard piperine and 6-gingerol in the formulation and respective extracts on TLC plates. The spots of piperine and 6-gingerol on TLC plates were well separated from each other and other compounds in extracts and formulation. The R_f and UV overlay spectra were also comparable.

Accuracy

The accuracy of the method was measured by performing recovery experiments at 3 different levels (50 %, 100 % and 150 % addition of standard piperine and 6-gingerol) using the standard addition method. The known amounts of standards were added to the standardized extract. The values of % recovery for piperine and 6-gingerol were calculated using the formula:

Recovery (%) = 100 × (amount found–original amount)/amount spiked,

RSD (%) = (SD/mean) × 100 %.

Quantification of piperine and 6-gingerol in tablet and *churna*

To determine the content of markers in the *Trikatu* tablet, one tablet was crushed, powdered and extracted with 50 ml of methanol in triplicate. The solvent was evaporated under reduced pressure and finally diluted with methanol to make the volume 50 mL. The resulting solution was centrifuged at 4000 rpm for 5 min. and the supernatant was analyzed for piperine and 6-gingerol content. Similar extraction procedure was adopted to analyze *Trikatu Churna*. A further dilution was required for above prepared samples if the peak areas of standards were above the highest linear limit of calibration curves. The spot of both standards (piperine and 6-gingerol) were observed in the extract and quantification was done using calibration curve of respective standards (Figs. 1, 2a&b).

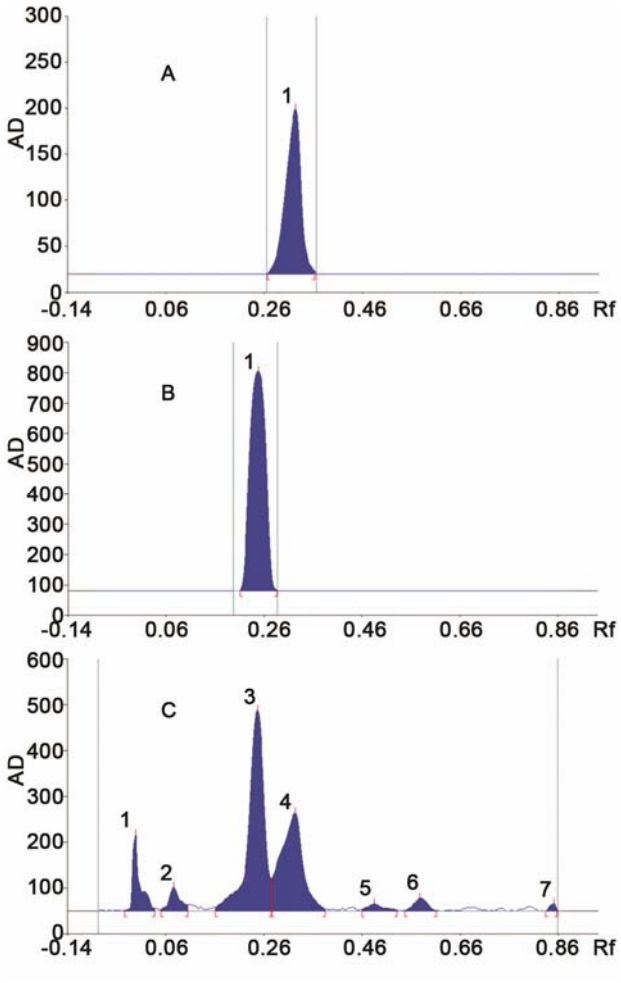

Fig. 1—Chromatogram showing the resolved peaks of piperine and 6-gingerol in the extract a) 6-gingerol, b) piperine standard, c) *Trikatu churna*.

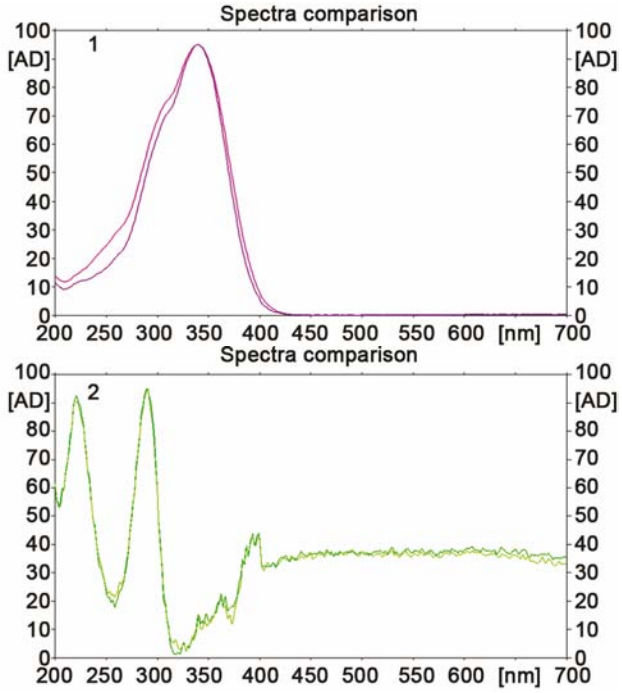

Fig. 2—(a): Overlaid UV absorption spectra of piperine and 6-gingerol in the sample. 1 = piperine, 2 = 6-gingerol

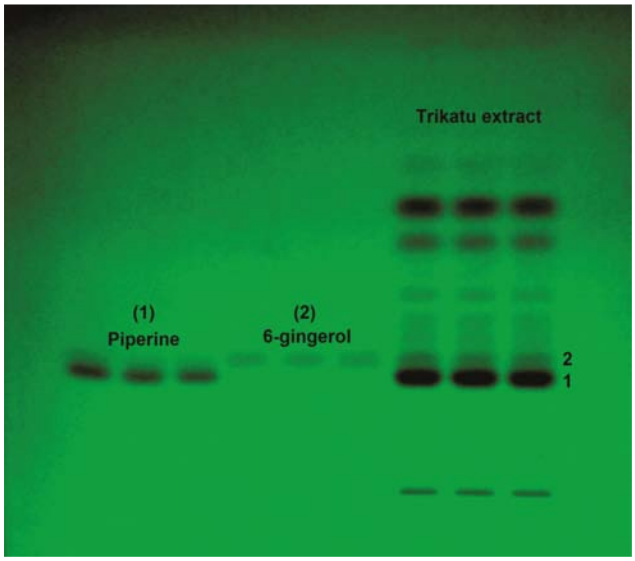

Fig. 2—(b): HPTLC–densitometric chromatogram of *Trikatu* extract and standards piperine, 6-gingerol.

Results

HPTLC fingerprint patterns have been developed for tablet of *Trikatu* herbal formulation. Piperine and 6-gingerol were quantified densitometrically using HPTLC [*n*-hexane: diethyl ether (6.2:4.8 *v/v* twice run]. The R_f value for piperine and 6-gingerol were 0.24 & 0.32, respectively.

Densitometric quantification of piperine and 6-gingerol

There is no report of quantification of piperine and 6-gingerol in the extracts and prepared tablet of *Trikatu* by HPTLC. Hence, we first formulated the tablet for better patient compliance and then developed a simple and precise method for quantitation of the marker compounds. The mobile phase consisting of *n*-hexane:diethyl ether (6.2:4.8 v/v twice run) gave better, sharp and well define spots. The linear regression equation for piperine and 6-gingerol were y = 58.01 x + 3195.8 and y = 1.614 x + 983.82, respectively. The linearity of calibration curves and adherence of the system to Beer's law were validated by high value of correlation coefficient and a low vaule (< 2 %) of the SD for the intercept. The developed HPTLC densitometric method was validated in terms of precision, repeatability, and accuracy (Table 2). The linearity range for piperine and 6-gingerol were 20–120 ng/spot and 1000-7000 ng/spot. The measurement of the peak area levels showed low values of % RSD, 0.46 & 0.67 for inter-day and 0.93 & 1.21 intra-day measurement for piperine and 6-gingerol, respectively. The % RSD below 0.5 for repeatability of measurement and % RSD less than 1.20 for repeatability of application for piperine and 6-gingerol suggested the excellent precision of the method (Table 2). The limits of detection (LOD) and quantification (LOQ) were 6, 260 and 19.6, 858 ng, respectively which indicate the good sensitivity for piperine and adequate sensitivity of the method for 6-gingerol (Table 2). Results from recovery studies, listed in Table 3, were within the acceptable limits (97.5 – 101.6 %), indicating that the accuracy of the method was good. The contents of piperine and 6-gingerol in *Trikatu churna* and tablet as determined using TLC densitometric methods were shown in Table 4.

Preparation and evaluation of *Trikatu* tablet

The prepared granules were compressed into tablet by direct compression method. *Trikatu* tablet was evaluated for different pharmaceutical parameters mentioned in Indian Pharmacopoeia. Results are given in Table 5 which showed that the parameters for prepared *Trikatu* tablet were within IP limits.

Discussion

A validated HPTLC method was developed for quantitation of piperine and 6-gingerol in *Trikatu Churna*. The method was validated in terms of precision, repeatability and accuracy as discussed below.

Linearity

The linearity range for piperine and 6-gingerol were 20–120 ng/spot and 1000-7000 ng/spot. The correlation coefficients of 0.999 validated good linearity. The linear regression line for piperine and

Table 2—HPTLC method validation parameters for the quantification of piperine and 6-gingerol

	Piperine	6-Gingerol
R_f	0.24	0.32
λ_{max}	345	286
Range of calibration curve (ng/band)	20-120	100–700
Regression equation	y = 58.01x + 3195.82	y = 1.614x + 983.86
r^2	0.9974	0.9969
LOD (ng/band)	6	26
LOQ (ng/band)	19.6	85
Repeatability of application[a]	0.54	1.18
Repeatability of measurement[a]	0.23	0.39
Intraday precision[a]	0.46	0.67
Interdayprecision[a]	0.93	1.21
Specificity	Specific	Specific

[a]result expressed as % RSD

Table 3—Recovery study of piperine and 6-gingerol in *Trikatu churna*

Compunnd	Amount present in matrix (ng/band)	Amount added (ng/band)	Amount found (mean, n= 3, ng/band)	Recovery (%) ± RSD
Piperine	112	52	163	98.1 ± 2.12
	112	120	234	101.5 ± 2.87
	112	156	264	97.4 ± 1.36
6-gingerol	1052	550	1588	97.5 ± 3.98
	1052	1020	2050	97.8 ± 2.63
	1052	1596	2644	99.7 ± 3.55

Table 4—Piperine and 6-gingerol content (mg/g) in *Trikatu churna* and tablet

Compound	*Trikatu churna*	*Trikatu* tablet
Piperine	23.8 ± 1.9	10.6 ± 0.8
6-gingerol	8.2 ± 1.1	3.3 ± 0.5

Table 5—Evaluation of pharmaceutical parameters

Sr. no.	Parameters	API	Observed value
1	Hardness	5 - 8 kg	Average 6.08 kg
2	Weight variation	5 %	2 %
3	Friability	0.5 %	0.18 %
4	Disintegration	Uncoated 30 min	Average 6 min. 30 sec
5	Dissolution	-	≥ 75 % dissolves before 30 min

6-gingerol were y = 58.01x + 3195.8 and y = 1.614x + 983.82, respectively. The linear regression of standard curve was determined as $R^2 = 0.9974$ and $R^2 = 0.9969$, respectively which is adequate for quantification of analyte in an unknown sample (Table 2).

Precision

The agreement among individual test results from repeated analyses was performed at three different levels, inter day and intraday precision, precision of application and precision of measurement. Intraday precision (within day) was determined by performing repeated analyses on same day, same equipment, same analyst and identical reagents for short interval of time. The % RSD for piperine and 6-gingerol (0.46 & 0.67 % respectively) indicated the satisfactory precision (Table 2) of the method.

Inter day precision (between days) was determined performing analyses on three or more consecutive days, on same equipment. The % RSD for piperine and 6-gingerol were 0.93 & 1.21 % respectively, which indicate the adequate level of precision (Table 2).

The results of precision of application were followed by the measurement of peak area based on six applications. This test provides information about the variation caused during sample preparation, sample application, and evaluation of result within a short period of time. The obtained % RSD for piperine and 6-gingerol were 0.54 & 1.18, indicating the satisfactory level of repeatability (Table 2).

Limits of detection (LOD) and quantification (LOQ)

The limit of detection is described as the lowest concentration of an analyte in a sample that can be detected. LOD is a limit test that specifies whether an analyte is above or below a certain assessment. The lowest concentration of an analyte in a sample that can be quantitated with acceptable precision and accuracy under the stated set conditions of the developed method is the limit of quantitation. For determination of LOD and LOQ using serial dilution method, sample solutions along with blank solvent were applied in decreasing quantities. After development, using peak heights against the applied quantities of the substances, a graph was constructed. LOD and LOQ are calculated based on the signal-to-noise ratio. The limits of detection (LOD) were 6 and 260 ng and LOD were 19.6 and 858 ng, respectively for piperine and 6-gingerol. This indicated that method has good sensitivity for piperine and an adequate sensitivity for 6-gingerol (Table 2).

Accuracy

Accuracy expresses the closeness of agreement between the theoretical and experimental results and was assessed after spiking with 50, 100, and 150 % of standards. The expected recovery depends on sample processing procedure and sample matrix. Results from recovery studies, listed in Table 3, were within acceptable limits (97.5 – 101.6 %), indicating the accuracy of method was good.

Specificity

Ensuring that the peak response is due to a single component only is indicative of the specificity of the method. Single peak chromatogram is generally not an indication of specificity. Wincats software was used to ascertain the specificity of the method by peak purity profiling recording the UV absorption spectrum at several points across each peak by the TLC scanner. The method was specific for both piperine and 6-gingerol as shown in Figs. 1, 2 a&b.

Chewable tablet from *Trikatu Churna* was formulated by using direct compression method. The tablets were standardized w.r.t. contents of piperine and 6-gingerol by the developed HPTLC method (Table 4). The quantities found in the *churna* and tablets corresponded well to their reported percentages in the raw materials. The tablets were evaluated for pharmacopeial parameter like hardness, weight variability, friability, disintegration and dissolution and all these observed values were found to be in acceptable limits (Table 5).

Traditional significance of the study

Trikatu is traditionally used in powder dosage form in various diseases such as cold, fever, asthma, cough, respiratory problems and treatment of digestive disorders. However, its use poses challenges in patient compliance because of its extremely pungent taste. We have developed a chewable tablet of *Trikatu* with improved organoleptic properties. This shall enhance its ease of administration and patient compliance. Additionally, the densitometry method for verification of marker compound shall enable quality control of the active raw materials.

Conclusion

Trikatu churna is a very popular, effective Ayurvedic formulation and recommended by physicians in various diseases but due to pungent taste its compliance is poor. So, it was decided to develop its chewable tablet with organoleptic improvement,

enhanced shelf life and better compliance of patients, and avoid dose variation. HPTLC method was developed and validated, for rapid–routine quality control analysis and quantitative estimation of piperine and 6-gingerol present in the extracts and tablet. This work provides guidance to researchers to develop and standardize new dosage forms with improved organoleptic properties from classical formulations.

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