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Full Length Research Paper

Simultaneous determination of trans-caryophyllene and piperine for detection of exhausted drugs in an ayurvedic formulation

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Admixture of exhausted drugs is one of the major menace faced by the herbal industry. This paper presents a new, simple, selective and precise high performance thin-layer chromatographic (HPTLC) method, which is validated to determine the quantities of trans-caryophyllene and piperine content in an Ayurvedic formulation Trikatu churna. The method employed HPTLC aluminum plates precoated with silica-gel 60F-254 as stationary phase. The solvent system consists of toluene: ethyl acetate (9:3 v/v) and was found to give compact spots for trans-caryophyllene (R_f value of 0.33) and piperine (R_f value of 0.26). Analysis of these compounds was carried out in the absorbance mode at 254 nm. The calibration curves were linear in the range of (1 to 12 µg). The method is simple, specific, rapid and reliable for simultaneous determination of trans-caryophyllene and piperine in Trikatu churna, which can speed up detection of exhausted drugs.

Key words: Adulteration, trans-caryophyllene, piperine, trikatuchurna, validation.

INTRODUCTION

Eighty percent of people in developing countries rely on alternative medicine, of these, a significant proportion of population are experiencing an alarming danger due to impurities/adulterations herbal medicine (Liang, 2004). This creates a challenge in establishing quality control standards for raw materials and the standardization of finished herbal drugs (Kumar, 2011; Kumar and Bhatnagar, 2006).

This difficulty has been acknowledged in the draft of a Strategic Plan for Regional Traditional Medicine of the World Health Organization (WHO, 2001). Trikatuchurna is an Ayurvedic formulation used in traditional medicine as digestive, carminative, expectorant, febrifuge and anti-periodic. The formulation contains *Zingiberofficinale* 1 part, *Piper nigrum* 1 part and *P. longum* 1 part each (Anonymous, 2000).

The main active constituent reported in these ingredients are trans-caryophyllene and piperine. Piperine has many activities (Siddiqui et al., 1997; Stohr et al., 2001). An amide from fruits of *P. nigrum* has also been isolated (Siddiqui, 1997). The active constituents of a genuine drug are removed by using different methods without many morphological changes, constituting an exhausted drug. In exhausted drugs, the contents of these chemicals would be significantly low. This paper gives a new-reliable method for simultaneous estimation of the main active constituents.

MATERIALS AND METHODS

The samples were collected from the physicians of Dhanbad, Bokaro and Ranchi (India). Pure Trans-caryophyllene standard and pure piperine standard was obtained (Sigma Aldrich, Kolkatta, India) and used without further purification, certified to contain 99.98% (w/w). HPTLC pre-coated plates, silica gel Merck 60, F 254, 10 × 10 cm were used (Merck, Darmstadt, Germany). All chemicals and solvents were of analytical grade and used as obtained.

Development of the optimum mobile phase for estimation of trans-caryophyllene and Piperine

Thin layer chromatography (TLC) procedure was optimized

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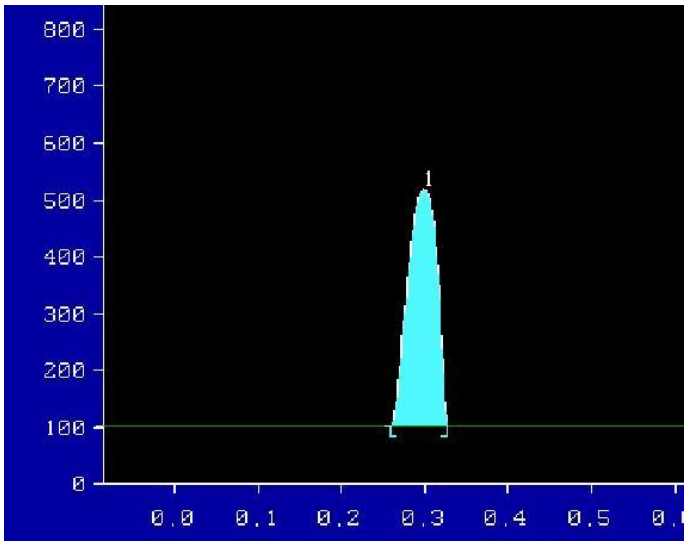

Figure 1. Typical chromatogram of piperine.

estimation of trans-caryophyllene and piperine. Initially, toluene: ethyl acetate in varying ratios was tried till sharp and well defined peaks were obtained. The time required for saturation was estimated for good resolution was also estimated.

High performance thin layer chromatography (HPTLC) instrumentation

The chromatographic estimation was performed by spotting standards and extracted samples on precoated silica gel aluminum plate 60F-254 (10 × 10 cm, with 250 µm thickness, E. Merck, Darmstadt, Germany, supplied by Anchrom Technologies, Mumbai, India) using a Camag Linomat IV sample applicator (Camag, Muttenz, Switzerland) and a 100 µl Hamilton syringe. Optimized mobile phase was used for development of plates.

Purity of spot in chromatogram

The spot obtained on the chromatogram were analysed at wavelength 260 and 341 nm for piperine and trans-caryophyllene at three points in the standard as well as in sample that is, in the start point to middle, middle and finally in the middle to end.

Validation

The method was validated according to the ICH guidelines (ICH, 1994, 1996). All the results were expressed as percentages, where n represents the number of values (Wu et al., 2004). For the statistical analysis, Excel 2003 (Microsoft Office) was used. A 5% level of significance was selected (Stohr et al., 2001).

Sensitivity

The sensitivity of the method was determined with respect to limit of detection (LOD),limit of quantitation (LOQ), linearity range and correlation coefficient. Solutions containing 1 to 12 µg of piperine and trans-caryophyllene were spotted on TLC plate. The LOD was calculated as 3 times the noise level and LOQ was calculated as 10 times the noise level.

Accuracy (recovery study)

Recovery of piperine and trans-caryophyllene were determined by spiking piperine and trans-caryophyllene in drug free formulation to obtain three different concentrations covering the low, medium and higher ranges of the calibration curve. The samples were then extracted and analyzed. The recovery was calculated by comparing the resultant peak heights/peak areas with those obtained from pure standards in toluene at the same concentrations.

Precision

Different amount of piperine and trans-caryophyllene covering low, medium and higher ranges of the calibration curve were spotted on the TLC plate. These spots were analyzed by using above described HPTLC method. Five micro liter aliquots of sample containing 10 µg of piperine and five micro liter aliquots of sample containing 22.5 ng of trans-caryophyllene were analyzed according to the proposed method. In order to control the scanner parameters, one spot was analyzed several times. By spotting and analyzing the same amount several times (n = 3) the precision of the automatic spotting device and the derivatization technique was evaluated.

Robustness of the method

By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different composition of toluene: ethyl acetate (9:3 and 9:2.5 v/v) was tried at two different concentration levels.

Calibration plot of trans-caryophyllene and piperine

A stock solution of trans-caryophyllene and piperine 0.046 and1 mg/ml, respectively were prepared in toluene. Different volumes of stock solution 1, 5, 10 and 12 µl were spotted in triplicate on TLC plate to obtain concentrations of 1, 2, 5, 12 µg per spot of trans-caryophyllene and piperine, respectively. The data of peak height/area versus drug concentration were treated by linear least-square regression.

Analysis of trans-caryophyllene and piperine in formulation

To determine the content of piperine and trans-caryophyllene in the formulation, 5 g of powder was extracted with toluene. To ensure complete extraction of the drug, it was sonicated for 15 min and volume was made up to 30 ml. The resulting solution was centrifuged at 3000 rpm for 5 min and supernatant was analyzed for drug content. The filtered solution was applied on TLC plate and then trans-caryophyllene and piperine was estimated by optimised method. Analysis was repeated in triplicate (Kumar and Patra, 2010; Kumar et al., 2010).

RESULTS AND DISCUSSION

Optimization of the mobile phase

The mobile phase toluene: ethyl acetate (9:3 v/v) gave good resolution with R_f value of 0.26 and 0.33 for piperine and trans-caryophyllene. (Figures 1 and 2). Well-defined spots were obtained when the chamber was saturated

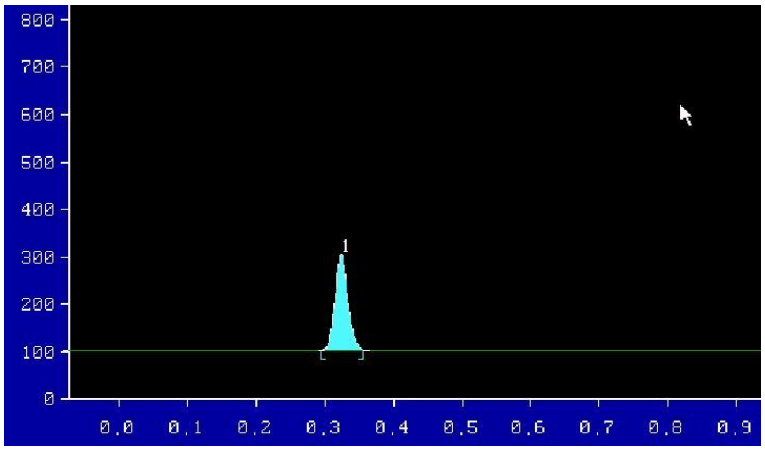

Figure 2. Typical chromatogram of t-caryophyllene.

Table 1. Linear regression data for the calibration curve of t-caryophyllene.

Linearity range (ng/spot)	r^2 ± SEM	Slope ± SEM	Intercept × 10 ± SEM
4.5-54	0.999±0.0034	13.134±0.332	12.933±0.301

Table 2. Linear regression data for the calibration curve of Piperine..

Linearity range (µg/spot)	r^2 ± SEM	Slope ± SEM	Intercept × 10 ± SEM
1-12	0.998±0.004	102.69±3.32	8.021±0.331

Table 3. Accuracy and precision of t-caryophyllene in the assay.

Amount of spotted t-caryophyllene (ng)	Amount detected (mean ± SD, n = 3) (ng)	Accuracy (%)
4.5	4.333±0.153	96.28
22.5	21.933±0.603	97.48
45	43.180±1.830	95.95

with mobile phase for 15 min at room temperature.

Purity of spot in chromatogram

The purity of each spot of t-caryophyllene and piperine which is scanned at wave length 341 and 260 nm, respectively with value of r (S, M) within the range 0.998 to 0.999 and r (M, E) within the range 0.997 to 0.998

Validation

Sensitivity

Under the experimental conditions employed, the lowest amount of t-caryophyllene and piperine which could be detected (LOD) was found to be 0.101 ng/spot and 0.0102 µg/spot, and the lowest amount of drug which could be quantified (LOQ) was found to be 1.01 ng/spot 0.102 µg/spot, respectively. The calibration curve was found to be linear in the range of 4.5 to 54 ng and 1 to 12 µg for t-caryophyllene and piperine, respectively. Peak height and concentration was subjected to least-square linear regression analysis to calculate the calibration equation and correlation coefficients. The regression data as shown in (Tables 1 and 2) shows a good linear relationship over the concentration range studied.

Accuracy (recovery study)

Results showed high extraction efficiency of t-caryophyllene and piperine from formulation components. The recovery of t-caryophyllene ranged from 98.76 to 95.83%, average of 97% and of piperine ranged from 97.66 to 96.93%, average of 97%. This confirms that the proposed method can be used for determination of t-caryophyllene and piperine in the formulation (Tables 3 and 4).

Table 4. Accuracy and precision of piperine in the assay.

Amount of spotted Piperine (µg)	Amount detected (mean ± SD, n = 3) (ng)	Accuracy (%)
1	0.933±0.153	93.30
5	4.833±0.603	96.66
10	9.180±1.830	91.80

Table 5. Robustness of the method for estimation of t-caryophyllene (n = 3).

Amount ng/spot	Mobile phase composition	
	Toluene: ethyl acetate (9:3 v/v), RSD (%)	Toluene: ethyl acetate (9:2 v/v), RSD (%)
4.5	0.79	0.78
54	0.69	0.83

Table 6. Robustness of the method for estimation of piperine (n = 3).

Amount µg/spot	Mobile phase composition	
	Toluene: ethyl acetate (9:3 v/v), RSD (%)	Toluene: ethyl acetate (9:2 v/v), RSD (%)
5	0.74	0.72
10	0.89	0.73

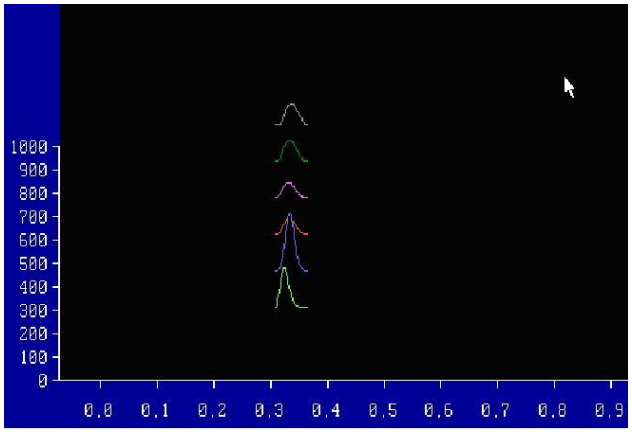

Figure 3. t-Caryophyllene in all tracks

Robustness of the method

The low values of RSD (%) obtained in t-caryophyllene and piperine after introducing small changes in mobile phase composition indicated there was no significant variation in the slope values (ANOVA, P > 0.05) (Tables 5 and 6).

Calibration curves

The data of peak height versus drug concentration were treated by linear least-square regression. The calibration curves of piperine, and trans-caryophyllene shows a good linear relationship over the concentration range 1 to 12 µg per spot for piperine and 4.5 to 54 ng/spot for trans-caryophyllene (n = 3). No significant difference was observed in the slopes of standard curve.

Analysis of trans-caryophyllene and piperine in formulation

A single spot at R_f 0.33 and 0.26 were observed in the chromatogram of the toluene extract of formulation. There was no interference from other constituents in the formulation. The t-caryophyllene and piperine content were found to be 4.1 µg/gm of Trikatu churna, with a SEM of 0.053 and 1.992 mg/g of Trikatuchurna, with a SEM of 0.083, respectively (Figures 3 and 4).

Conclusion

The method described is sensitive, precise and rapid, and involves single sample step sample preparation and number of samples can be analyzed within a short time for routine analysis in quality control laboratory. Thus, exhausted drugs can be detected within a short time and quality of herbal formulation can be maintained. Lack of proper standardization procedure will risk the use of traditional medicine (Kumar and Bhatnagar, 2006).

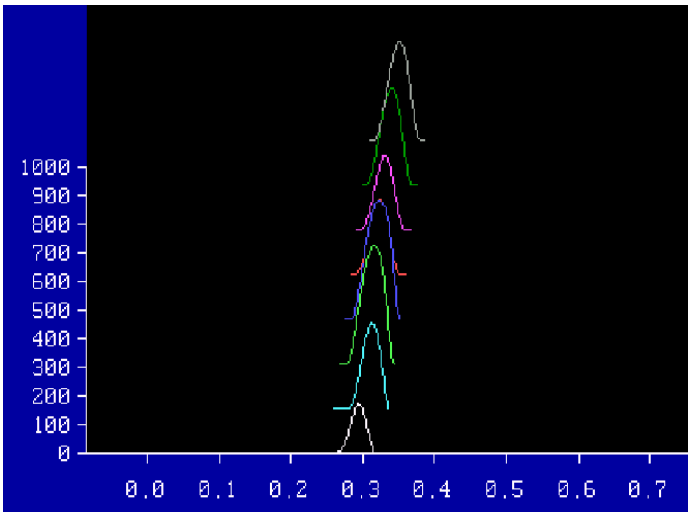

Figure 4. Piperine in all tracks.

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REFERENCES

Anonymous (2000). Formulary of Siddha Medicine, The Indian Medical Practitioner's Co-operative Pharmacy and Stores Ltd., Chennai p.511.

ICH (1994). Text on Validation of Analytical Procedures. ICH Harmonized Tripartite Guideline.

ICH (1996). Guidance for industry Q2B validation of analytical procedures: methodology, US Department of Health and Human Services, Food and Drug Administration. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use.

Kumar JK, Patra KC (2010). A Validated HPTLC Method for Simultaneous Analysis of Eugenol and Piperine in a Siddha Formulation. J. Planar Chromatogr. 23(4):293-297.

Liang YZ, Xie PS, Chan K (2004). Quality control of herbal medicines. J. Chromatogr. B, Anal. Technol. Biomed. Life Sci. 812(1-2):53-70.

Siddiqui BS, Begum S, Gulzar T, Noor FF (1997). An Amide from fruits of *Piper nigrum*. Phytochemistry 45:1617-1619.

Stohr JR, Xiaso PG, Bauer R (2001). Constituents of Chinese piper species and their inhibitory activity on prostaglandin and leukotriene biosynthesis in vitro. Journal of Ethanopharmacology 75:133–139.

World Health Organization (WHO) (2001). A Draft Regional Strategy for Traditional Medicine. WPR/RC52/7: In Western Pacific. WHO Regional Committee, 52nd. Session Brunei Darussalam, 10–14 September.

Wu S, Sun C, Pei S, Lu Y, Pan Y (2004). Preparative isolation and purification of amides from the fruits of *Piper longum* L by upright counter-current chromatography and reversed-phase liquid chromatography. J. Chromatogr. A 1040:193-204.