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Stability-Indicating HPTLC Determination of Piperine in Bulk Drug and Pharmaceutical Formulations

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A simple, selective, precise and stability-indicating high performance thin layer chromatography method of analysis of piperine both as bulk drug and in formulations was developed and validated. The method employed TLC aluminum plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of toluene: ethylacetate :: 93:9. This system was found to give compact spots of piperine (R_f value of 0.10 ± 0.02). Densitometric analysis of piperine was carried out in absorbance mode at 254 nm.The linear regression analysis data for the calibration plots showed good linear relationship with r = 0.000000 with respect to peak height in concentration range of 3-4 µg per spot. The mean value ± SD of slope and intercept were 0.03, 0.1, respectively. Piperine was subjected to acid and alkali hydrolysis, oxidation and photodegradation. The drug undergoes degradation under acidic, basic, light and oxidation conditions. This indicates that the drug is susceptible to acid, base hydrolysis, oxidation and photo-oxidation. Statistical analysis proves that the method is repeatable, selective and accurate for estimation of said drug. As the method could effectively separate the drug from its degradation products, it can be employed as stability-indicating one.

Key Words: Piperine, HPTLC determination.

INTRODUCTION

Piperine (1-[5-(1,3-benzodioxol-5yl)-1-oxo 2,4 pentadienyl piperine] (Fig. 1) is a yellow coloured alkaloidal pigment from the fruits of *Piper nigrum* Linn (family: Piperaceae)[1]. It has been used to give relief from stomach pain, cold, cough, indigestion since ancient medication. It acts on human vanilloid receptor TRPV1 using whole cell patch clamp electro-

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physiology. It produces a clear agonist activity at the human TRPV1 receptor. These TRPV1 mediated effects of piperine have effect on gastrointestinal function. It also inhibits human P-glycoprotein and CYP3A4[2-4].

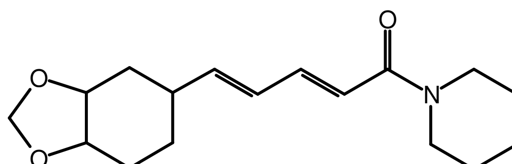

Fig. 1. Structure of piperine

Piperine is unstable at basic pH and undergoes alkaline hydrolysis in alkali/higher pH solution. Hydrolytic decomposition is reported even in *in vitro* physiological condition (isotonic phosphate buffer, pH 7.25-7.00). It undergoes photodegradation when exposed to light in solution as well as in solid form[5-7]. Various methods are available for the analysis of piperine in the literature like UV, HPLC, TLC and HPTLC[8,9], but there are very few reports on analytical methods for the estimation of piperine in bulk and its dosage form. Moreover none of them is a stability-indicating method. The International Conference on Harmonization (ICH) guideline entitled 'stability testing of new drug substance and products' requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance. Susceptibility to oxidation is one of the required tests. Also, the hydrolytic and photolytic stability are required. An ideal stability-indicating method is one that quantifies the drug *per se* and also resolves its degradation products. Nowadays HPTLC is becoming a routine analytical technique due to its advantages[10,11]. The major advantage of HPTLC is that several samples can be run simultaneously using small quantity of mobile phase like HPLC, thus lowering analysis. Mobile phase having pH 8 and above can be employed. Suspensions' dirty or turbid samples can be directly applied. It facilitates repeated detection (scanning) of chromatogram with the same or different parameters. Simultaneous assay of several components in a multi-component formulation is possible. The aim of this work is to develop an accurate, specific, repeatable and stability-indicating method for the determination of piperine in the presence of its degradation products and related impurities as ICH guidelines.

EXPERIMENTAL

Piperine was obtained from Regional Research Laboratory, Jammu. All chemicals and reagents were of analytical grade and purchased from Merck Chemicals, India.

The samples were spotted in the form of bands of width 3 mm with a Camag microlitres syringe on precoated silica gel aluminum plate 60F-254 (20 cm × 10 cm with 0.2 mm thickness (E. Merck, Germany) using a Camag LinomatV (Switzerland). A constant application rate of 2 mm/s was employed and space between two bands was 5.0 mm. The slit dimension was kept at 4 mm × 0.1 mm and 20 mm/s scanning speed was employed. The mobile phase consisted of toluene:ethylacetate (93:9). Linear ascending development was carried out in twin trough glass chamber saturated with the mobile phase. The optimized chamber saturation time for mobile phase was 10 min at room temperature. The length of chromatogram run was 10 mm. Subsequent to the development of TLC plate were dried in a current of air. Densitometric scanning was performed on Camag TLC scanner IV in the absorbance mode of 254 nm. The source of radiation utilized was deuterium.

Limit of detection and limit of quantification: In order to determine detection and quantification limits, piperine concentrations in the lower part of the linear range of calibration curve were used. Piperine solutions of 100, 300, 400 ng/μL were prepared and applied in triplicate (1 μL each). The amount of piperine by spot *versus* average response (peak area) was graphed and the equation for this curve was determined, thereby obtaining an estimate of the target response (ybl). The ybl value corresponds to the intersection of the curve. Subsequently, a second curve was graphed showing the amount of piperine by spot *versus* standard deviation for target (sbl), which corresponds to the intersection of this curve. Detection and quantification limits were calculated by means of the equation

$$\text{Detection limit} = (ybl + 3sbl)/b$$

$$\text{Quantification limit} = (ybl + 10sbl)/b$$

where b corresponds to the slope obtained in the linearity study of the method.

Forced degradation of piperine

Acid and base induced degradation: 50 mg piperine and complex equivalent to 50 mg of piperine were separately dissolved in 50 mL of methanolic solution of 1 M HCl and 1 M NaOH. These mixtures were refluxed for 6 h in darkness in order to exclude the possible degradative effect of light. The resultant solutions were diluted 10 times and applied on the TLC plate in triplicate (2 μL each, *i.e.* 200 mg per spot).

Hydrogen peroxide-induced degradation: To 25 mL of methanolic solutions of piperine and complex (piperine equivalent to 2 mg/mL), 25 mL of hydrogen peroxide (30 % v/v) were added separately. The solutions were heated in boiling water bath for 6 h to remove completely the excess of hydrogen peroxide. The resultant solutions were diluted appropriately and applied (2 μL each) on TLC plate in triplicate (200 mg per spot).

Photochemical degradation product: The 50 mg pf piperine and complex equivalent to 50 mg of piperine were separately dissolved in 50 mL of methanol and exposed to direct sunlight and UV chamber at 254 nm for 24 h. The resultant solutions were diluted appropriately and applied on TLC plate (200 mg per spot).

In all degradation studies, the average peak area of piperine after application (200 mg per spot) of three replicates was obtained.

RESULTS AND DISCUSSION

Development of the optimum mobile phase: TLC procedure was optimized with a view to develop a stability-indicating method. Initially, toluene:ethylacetate (93:9 v/v) gave good resolution with R_f value of 0.10 for piperine. Well defined spots were obtained when the chamber was saturated with the mobile phase for 15 min at room temperature.

Conclusion

The developed HPTLC technique is precise, specific, accurate and stability-indicating method. Statistical analysis proves that the method is repeatable and selective for the analysis of piperine as bulk drug and in pharmaceutical formulations. The method can be used to determine the purity of the drug available from various sources by detecting related impurities. It may be extended to study the degradation kinetics of piperine and for its estimation in plasma and other biological fluids. As the method separates the drug from its degradation products, it can be employed as a stability-indicating one.

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