ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF PIPERINE BY GAS-LIQUID CHROMATOGRAPHY

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DOI: 10.31838/ecb/2023.12.si6.091

ABSTRACT

In this study, a novel, rapid, simple, sensitive, and low-cost method was developed and validated by gas chromatography (GC) for the quantification of piperine. The GC analysis was performed on a capillary column [Column Rtx-5 (30 mm × 0.25 mm ID × 0.25 µm)], at a consistent flow rate of 1.3 ml/min and an injector temperature of 250 °C, nitrogen is utilized as the transporter gas with a split proportion of 50 with the flame ionization detection (FID). The injection volume was 10µl, and the run time was 20 minutes with a 16.7 retention time. All examinations were performed utilizing chemicals of analytical grade. The technique was validated according to the International Conference on Harmonization (ICH) guidelines for linearity, specificity, precision, the limit of detection (LOD), and the limit of quantification (LOQ). The standardization curve showed respectable linearity in the 50-150(ppm) series with a correlation coefficient of 0.999. The relative standard deviation (RSD) is not more than 2%. The method precision and intermediate precision were also within the acceptable range of RSD < 2%. The LOD and LOQ were 3.39% and, 9.99% respectively. The method was specific for piperine and did not show any interference from other components in the samples.

Key points: *Gas chromatography, LOD, LOQ, Piperine.*

INTRODUCTION

Piperine is an organic compound that belongs to the class of alkaloids, which are nitrogen-containing substances with biological activity. Piperine was first isolated in 1819 by Danish chemist Hans Christian Ørsted, who obtained a yellow crystalline substance with the molecular formula $C_{17}H_{19}NO_3$ and a melting point of 128-130°C. The structure of piperine was later determined to be (2E,4E)-5-(benzo[d] [1,3] dioxol-5-yl)1-(piperidin-1-yl) penta-2,4-dien-1-

one, according to the IUPAC nomenclature. Piperine enhances the bioavailability and absorption of drugs [1]. When coupled with other pure bioactive compounds, piperine becomes an excellent biomarker. Their derivatives, or pharmacological compounds, indicate that they can be used as medicinal compounds [2].

Piperine can be used as a treatment for patients with diabetes mellitus (DM2), stoutness, rheumatic disorder, Kahler disease, mouth cancer, lobular carcinoma, metabolic syndrome, hyperpiesis, idiopathic parkinsonism, dementia disease, cerebral stroke, coronary thrombosis, nephrosis, inflammatory diseases, and rhinoplasty disease [3].

It has been recognized as a safe substance by the US FDA [4]. However, more research is needed to fully understand its effects and potential risks for developing better and safer therapies. Piperine has many possible medical applications, but its mechanisms of action and new uses need to be further explored [5]. In order to account for both major and small components of piperine, a straightforward and trustworthy method for gas chromatography (GC) in combination with a flame ionization detector (GC-FID) was developed [6]. However, only volatile and thermally stable compounds can be analyzed by GC instruments [7]. There were no such methods developed for the piperine drug. According to a comprehensive review, only a few analytical techniques were available for the analysis of piperine in API and capsule dosage forms.

The gas chromatography method was developed by Noyer, I., et al using a perkin elmer autosystem chromatogram equipped with a fid detector with a run time of 35 minutes and reported retention was 20min using a capillary apolar column BPI (50m×0. 22mmI.D,0.25μm) where hydrogen is used as carrier gas with a flow rate of 1ml/min, split at 25ml/min, injector temp. 300℃ and the detector temperature was also 300℃ [8].

Vazquez-Martinez, Juan, et al presented a method of gas chromatograph model 7890A coupled to a quadrupole triple-axis detector with electronic impact ionization using capillary column DB-1MSUI (60m×250μm×0.25μm), helium is used as a carrier gas and reported retention time was 40.815 with the injection volume of 1μL was injected in splitless mode, injection temperature was 250℃ [9].

Another method was developed by the Mohammed jihadi Ghaidaa, at el, using helium as a carrier gas with 1ml/min flow rate and the reported retention time was 22.31 having a run time of 30min [10].

Some animal studies had been done using the drug piperine with the gas chromatography method. Praveen B. et al, 2015 determine and explain the pharmacokinetic profile of sodium valproate by using piperine with gas chromatography [11].

This study employed a GC method that is simple, accurate, and precise, and that follows the ISO recommendation, to analyze piperine in black pepper. This process is suitable for routine investigation of piperine by a quality regulator research laboratory [12], as it has several advantages over other scientific techniques such as Ultraviolet-Visible Spectroscopy, Gas Chromatography-FID, and Hplc. The method's routine features, such as linearity, (LOD), (LOQ), and precision, were evaluated and then found to meet the acceptance criteria.

MATERIAL AND METHODS

Chemicals and reagents

We used chemicals with high purity levels for all analyses. These chemicals were methanol and acetonitrile, both of HPLC grade, which is suitable for gas chromatography applications. Piperine Standard was a generous gift from Sandeep Arora at Chitkara University.

INSTRUMENT: SHIMADZU (GC-2010 PLUS) was equipped with the FID1 detector.

Selection of the solvent:

The ability of piperine to dissolve in different solvents was examined. Among the solvents tested, acetonitrile showed a high solubility for piperine. Therefore, acetonitrile was selected as the most suitable solvent and diluent for the purpose of this study.

Sample Preparation of standard drug

A standard solution of 100 ppm was prepared by dissolving 1 milligram of piperine in acetonitrile. The piperine was accurately weighed and transferred to a 10 ml volumetric flask that had been thoroughly cleaned and dried beforehand. The flask was then filled up to the mark with acetonitrile and mixed well.

Sample preparation of the marketed formulation

6640mg of the marketed formulation of piperine, which was available in capsule form, was taken into the 50ml volumetric flask, dissolved with the acetonitrile solvent, and made up the volume with the solvent.

Protocol for GC-FID1

The following protocol is used: With a split ratio of 50.0, nitrogen is used as the carrier gas in column Rtx-5 (30 mm 0.25 mm ID 0.25 m) at a constant flow rate of 1.3 ml/min and an injector temperature of 250 °C.

A 0.2-minute equilibration interval is included in the oven temperature setting of 50°C. The GC runs for 20 minutes.

METHOD VALIDATION: In order to validate the method, many validation parameters were determined, such as specificity, linearity range, precision, the limit of detection (LOD), and the limit of quantitation (LOQ).

Specificity

The analytical method was evaluated for specificity by injecting a blank and reference solutions. The chromatogram of the blank solution did not show any peak, indicating no interference from other components. The chromatogram of the reference solution showed a clear peak corresponding to the analyte of interest.

Linearity

If an analytical method is linear, the output is directly proportional to the concentration (or amount) of the analyte in the sample. The test outcomes, assuming the process is linear, are directly or via a precise mathematical transformation proportionate to the analyte concentration in samples falling within a given range. Seven concentration levels between 50% and 150% of the piperine in its commercial formulation were used to determine the linearity. The calibration curve's linearity was plotted by comparing the concentration to the corresponding peak area comparing the concentration to the corresponding peak area, and the linearity of the calibration curve was plotted. The slope value and r^2 values for the correlation coefficient were calculated [13].

Precision

A way to describe how consistent two measurements are is precision. Precision studies include method precision and intermediate precision. These were determined by testing sample solutions on the same day and on different days.

System Precision: Using the proposed method, we analyzed six repeated measurements of a piperine standard solution. We evaluated the precision of the method by calculating the percentage relative standard deviation (% RSD) of the peak area. The method was considered acceptable if the % RSD was less than 2%.

Method Precision: The marketed formulation of piperine was used to prepare the samples. Then, six samples were analyzed in replicates.

Intermediate Precision: The marketed formulation of piperine was used to prepare the samples. The next day, six samples were subjected to analysis by different analysts in replicates.

LOD (Limit of detection) and LOQ (Limit of quantification)

The limit of detection (LOD) is the smallest amount of an analyte that can be reliably detected but not necessarily quantified. The limit of quantification (LOQ) is the smallest amount of an analyte that can be reliably measured with a given level of accuracy and precision. To calculate the LOD and LOQ using the calibration curve method, we need to use the following formula: LOD = 3.3σ / S and LOQ = 10σ / S, where σ is the standard deviation of the response and S is the slope of the calibration curve [13].

RESULT AND DISCUSSION

Method validation

In this study, the following method validation parameters were examined.

Specificity

The retention time was 16.7 at which the sharp peak of the standard drug was obtained. No interference was found between the blank and standard solution as shown in Figures 1(a) and 1(b).

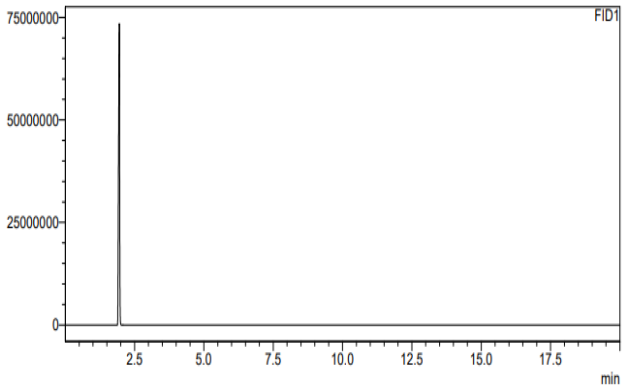

Fig.1(a) Blank

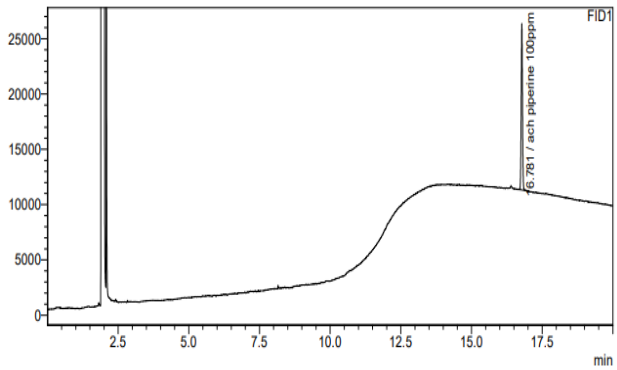

Fig.1(b) Standard

Linearity

The calibration curve between peak regions of different concentrations and their nominal concentrations is depicted in the figure. By injecting 7 different concentrations in duplicate, ranging from 50% to 150%, the linearity of the method was assessed. Over 0.999 was the correlation coefficient (r^2). It was determined that the relative standard deviation percentage (% RSD) was 0.37%. The slope and correlation coefficient r^2 values were calculated as shown in **Table 1** and the calibration curve of piperine is shown in fig. 2.

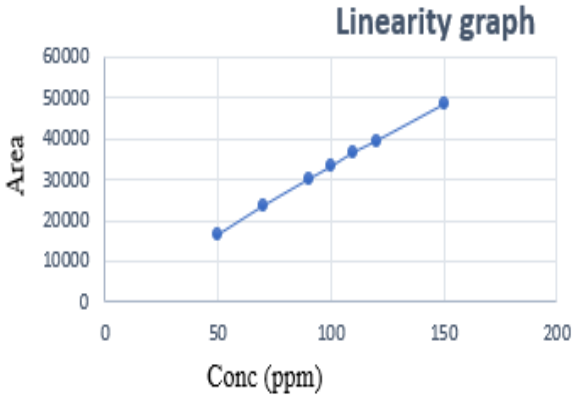

Fig. 2: Calibration curve of piperine

Table 1: Retention time and the area of the linearity standard concentration in ppm

Standard conc. (ppm)	Retention time	Area
50	16.707	16447

70	16.707	23482
90	16.710	29959
100	16.712	33126
110	16.706	36585
120	16.710	39380
150	16.708	48345
Slope	**0.003**	
Intercept	**-3.08**	
Correlation coefficient(r^2)	**0.999**	

Precision

The reproducibility of precision was assessed. The same piperine samples were made in six replicates, each of which was then independently examined as shown in **Fig 3**. The table illustrates the values that were obtained. The average piperine content and the percentage relative standard deviation (% RSD) values were calculated. The method was shown to have good precision, with a six-replicate average value of piperine and a percentage relative standard deviation (% RSD) of 1.52% shown in **Table 2**. The values of method precision and intermediate precision were also calculated as shown in **Table 3** and **Fig. 4** shows the chromatogram of the marketed formulation.

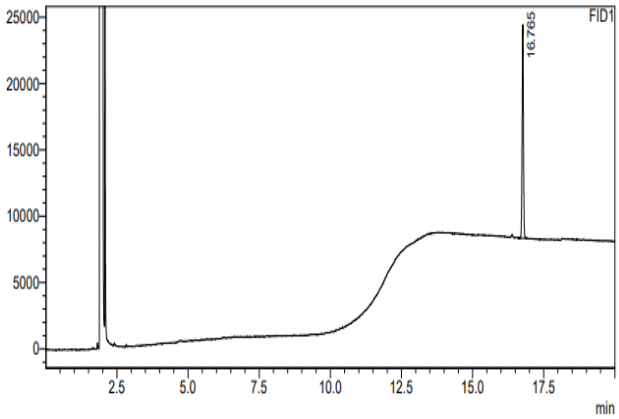

Fig. 3: Standard of piperine

SYSTEM PRECISION	
SAMPLE NO.	**AREA**
Sample 1	52335
Sample 2	52701
Sample 3	52970
Sample 4	54692
Sample 5	53090
Sample 6	53250
Mean	**53173**
Standard deviation	**810.73**
%RSD	**1.52**

Table 2: Mean, standard deviation, and %relative standard deviation of the system precision with the area.

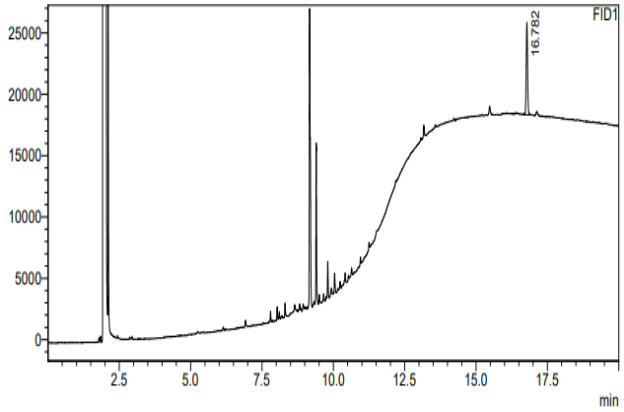

Fig. 4: Marketed Formulation

Table 3. Method precision v/s intermediate precision

METHOD PRECISION		**INTERMEDIATE PRECISION**	
RESULT		RESULT	
Sample 1	100.09	Sample 1	101.24
Sample 2	98.32	Sample 2	97.43
Sample 3	102.61	Sample 3	97.31
Sample 4	96.11	Sample 4	97.29
Sample 5	97.10	Sample 5	98.40
Sample 6	101.41	Sample 6	101.80
Mean	**99.27**	**Mean**	**98.91**
Standard deviation	**2.53**	**Standard deviation**	**2.07**
%RSD	**2.54**	**%RSD**	**2.09**
%Relative difference		**0.36%**	

Limit of detection (LOD) and Limit of quantification (LOQ)

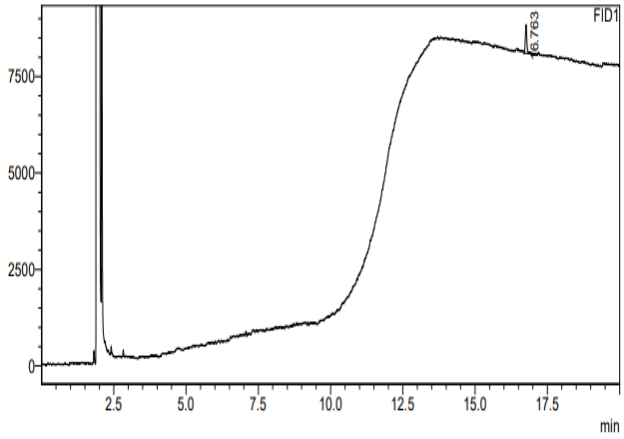

Fig. 5(a): Chromatogram of LOQ

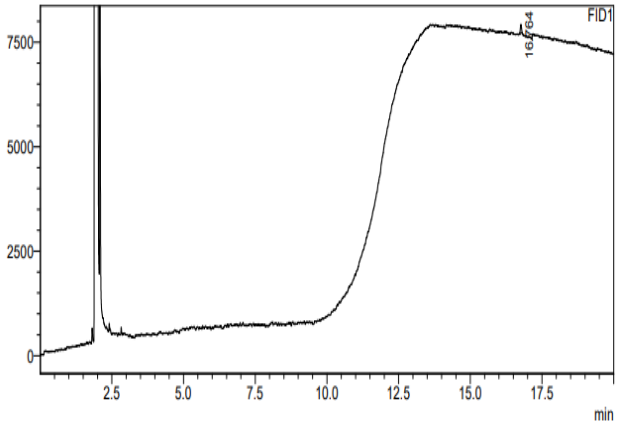

Fig. 5(b): Chromatogram of LOD

Using gas chromatography equipment, the method limit of detection and method limit of quantification were established in this experiment. The signal-to-noise ratios of 3 and 10, respectively, were used to calculate LOD and LOQ. The technique LOQ was found to be 9.99% in accordance with **Table 4**, whereas the method LOD was found to be 3.39% and shown in the chromatogram also in Fig. 5(a) and 5(b). To calculate the data, just the instrument's system was employed.

Table 4: Area and the signal noise of LOD

AND LOQ

	Area	**S/N**
LOQ	2632	9.99%
LOD	592	3.39%

CONCLUSION

To summarize, gas chromatography is a widely used analytical technique that separates and measures volatile compounds in a mixture. We have improved the gas chromatography method by reducing the analysis time and extracting more information from a sample. The main factor in current piperine GC analysis is the partition coefficient, which determines how fast each compound travels through the column. The column is a narrow tube filled with a stationary phase, usually a viscous liquid, and a mobile phase, usually an inert gas. The compounds are injected as a vapor into the column and carried by the mobile phase. The detector at the end of the column records the elution time, peak height, peak width, and peak area of each compound. These parameters can be used to calculate the repeatability, linearity, LOD, and LOQ of the method. The compounds must be thermally stable and sufficiently volatile to be analyzed by GC, which limits its applicability to some substances.

ACKNOWLEDGEMENT

The KIET Group of Institutions in Delhi-NCR, Ghaziabad, and Prof. (Dr.) K. Nagarajan, Principal of the KIET School of Pharmacy, is appreciated by the authors for their support during the research process.

CONFLICT OF INTEREST

There is no conflict of interest, according to the paper's authors.

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