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DETERMINATION OF PIPERINE CONTENT IN PEPPER BY USING DIFFERENT ORGANIC SOLVENTS: THROUGH HPLC

Genetics

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ABSTRACT

Chromatography can be described as a mass transfer process involving adsorption using a nonpolar stationary phase and a mobile polar phase titrating through the column. The active component of the column, the sorbent or the stationary phase , is typically a granular material made of solid particles (e.g. silica, polymers, etc.,). The component of the sample mixture are separated from each other by means of mobile phase and different degrees of interaction with the sorbent particles based on their relative polarity. In the present study we have extracted piperine from grounded pepper using different chemicals such as petroleum ether, acetone and methanol. Petroleum ether extraction showed higher piperine content of 9.12% than methanol and acetone 3.15% and 3.37% respectively.

KEYWORDS

stationary phase, mobile phase, piperine, petroleum ether, acetone and methanol.

INTRODUCTION

High performance liquid chromatography is a chromatographic technique used to separate a mixture of components in a analytical chemistry and biochemistry with the purpose of identifying, quatifying or purifying the individual components of the mixture before the invention of HPLC Chemists had chromatography at their disposal, and column chromatography was time consuming.

To speed up a classic column chromatography, chemists would have to use a short column for separation, however this lead to poor separation of molecular components held within solution. The basic setup of a classic column chromatography would include the column that varied in internal diameter from 10nm to 50nm and column lengths of 50-500cm. The column was then packed with the stationary phase ranging particle size from 150-200mm thick. Chemists, wanting to speed the separation process up, first experimented with the introduction of a vacuum source or a high pressure source. However, they found with the increased negative or positive pressure, the column length would have to be increase linearly in order to acquire a valid separation that could be used for analytical data with a confidence level .Chemists realized that with the development of pressurized systems, reducing the particle size would increase the efficiency. It was not until the late 60s that chemists and industrial engineering process acquired adequate technology and manufacturing techniques to develop a smaller grained stationary phase that would be cohesive with a pressurized system. Today, HPLC has many uses including medical, legal, research (e.g. separating the components of a complex biological sample, or of similar synthetic chemicals from each other).

Black pepper belongs to the family piperaceae. It is cultivated for its fruit which is usually dried and used as spice. It was discovered in 1819 by Hans Christian Orsted, who isolated it from the fruits of *Piper nigrum*, the source plant of both the black and white pepper grains. Anderson first hydrolysed piperine by alkalis into a base and an acid, which were later named piperidine and piperic acid respectively. The alkaloid was first synthesized by the action of piperoyl chlorid on piperidine. The pungency of piperine is caused by activation of the heat and acidity sensing TRPV ion channel TRPV1 and TRPA1 on pain sensing nerve cells .The full mechanism of piperines bio-availability-enhancing abilities is unknown but it has been found to inhibit human CYP3A4 and para-glycoprotein, enzymes important for the metabolism and transport of xenobiotics and metabolites. In animal studies, piperine also inhibited other CYP450 enzymes important for drug metabolism . Piperine has been shown to dramatically increase the bioavilability of curcumin in humans.

MATERIALS AND METHODS

Standard solution and different solvent extract for methanol, acetone and petroleum ether were prepared using standard chromatographic conditions for all the solvents

A. PREPARATION OF STANDARD SOLUTION: Accurately weighed about 20mg of standard in 50ml of volumetric flask. To this 30ml of methanol was added and sonicated for about 5 to 10minutes, allowed it to cool under room temperature, made up with the same solvent and mixed it thoroughly. Then transferred 1ml of resulting solution into a 10ml volumetric flask with mobile phase and mix it thoroughly. Later it was filtered using 0.45µm nylon membrane and the filtrate was collected in a HPLC vial and was loaded for injection.

B. STANDARD CHROMATOGRAPHIC CONDITIONS:

Column	Venusil XBP C18(l) 5µg, 150A°
Detector	UV-visible
Wave length	342nm
Flow rate	1.0ml/min
Mobile phase	solvent: Water(70:30)
Injection volume	20µl
Column temperature	30°C

C. SAMPLE PREPARATION FROM METHANOL: Weighed accurately about 375mg to 425mg of pepper powder to 50ml flask, 25ml methanol was added and sonicated for 15 min, then it was made up with the same solvent . Later 1ml from this solution was added to 25ml flask, diluted with diluents (methanol : water, 70:30) filtered then followed by injection.

D. SAMPLE PREPARATION FROM ACETONE : Piperine was extracted from pepper using acetone. Therefore, approx. 6g grounded black pepper was exactly weighed in a suitable flask and 50ml acetone were added followed by 30 min ultrasonic bath at 60°C. After a total extraction time at 6O°C of about 120minutes, the powder was sedimented and the supernatant was collected. While caring that no precipitation occurs in the sample 600µl water were added per 1ml extract. After filteration through a 0.45µm filter, the solution was ready for injection to the HPLC system respectively the purification via preparative HPLC.

E. SAMPLE PREPARATION FROM PETROLEUM ETHER: 5g of pepper powder was extracted by soxhlet method at 90°C using petroleum ether(40°C to 60°C) for about 2hours.Then it is filtered and distilled under reduced pressure. After, the preparation of petroleum ether extract, the sample was again extracted using methanol at 90°C

for about an 1hour.Then it is centrifuged, sonicated for about 1hour and filtered through 0.45µm nylon membrane .The filtrate was collected in a HPLC vial and was loaded for injection.

F. Calculation Of Retention Time And Area Using Standard Deviation And Relative Standard To Find Out Piprine Content .

i. Calculation For Stanadard Deviation:

SD =$\sqrt{\sum}(X-x)^2/(n-1)$ where X is average retention time, x is trial retention time and n is number of trials.

ii.Calculation For Relative Standard:

RSD% = SD/AVG*100 where SD is standard deviation, AVG is average retention time.

iii.Calculation of Piperine Content (%):

Piperine content=A2A1 × W1V1 × V2W2 × P100 × (100-LOD1)(100-LOD2) ×100=…….. %

Piperine content= sample area/standard area*standard weight/dilution* dilution/sample weight*purity of standard/100

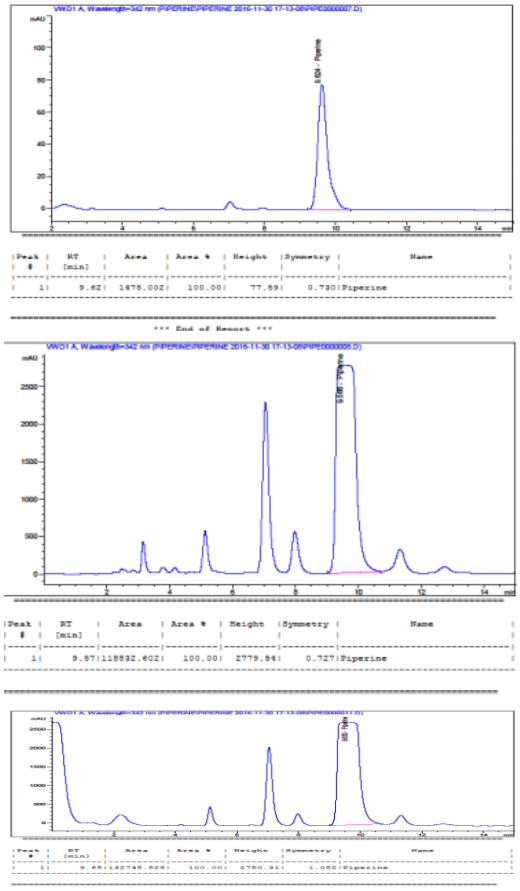
RESULT

In the present study, it was observed that average retention time , area for piperine content and piperine percentage:

Methanol was 9.56 min(trial 1=9.50 and trial 2= 9.62) and 1457.079 mAU(trial 1=1439.156 and trial 2=1475.002) and 3.15% .

Acetone 9.54 min(trial 1=9.57 and trial 2= 9.51) and 116609.9105 mAU(trial 1=118832.602 and trial 2=114387.219) and 3.37% .

Petroleum ether 9.625 min(trial 1=9.60 and trial 2= 9.65) and 130951.293 mAU(trial 1=129153.758 and trial 2=132748.828) and 9.12% respectively.



DISCUSSION

Jansz et al (1983) method of determination of piperine in Sri Lanka pepper provides a bank of data illustrating high levels of piperine in Sri Lanka pepper- generally in the range of 7-15% as against 2-7% of the commercial Indian, Malaysian and other varieties by direct uv method. This paper introduces a new technique of piperine assay based on TLC-UV densitometry which produces nearly identical results to the already known TLC-UV spectrophotometric method.

Compared to Jansz et al(1983) method the piperine percentage obtained was more through petroleum ether method than that of the other solvents such as methanol, acetone by HPLC.

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