Separation, Identification and Estimation of Piperine as Major Constituent from Black Pepper, by Thin Layer Chromatography Coupled with GC-MS

DELIA NICA-BADEA*
Constantin Brancusi University of Targu - Jiu, Faculty of Health and Behavioural Sciences of Targu- Jiu, 75B Calea Bucuresti, 210135, Targu-Jiu, Romania

Black pepper is one of the most important spices, rich in aromatic and medicinal ingredients known for organoleptic properties and uses in food, medicine and cosmetics. The paper addresses the extraction, separation, identification and estimation of the major component, piperine from black pepper, as evidenced in the literature between 17.000 to 90.000 ppm. The active principle piperine was extracted and concentrated from black pepper powder using different solvents: alcohol, chloroform and petroleum ether. Identification was performed using plates Polygram Sil G / UV 254 Macherey Nagel, mobile phase: toluene - ethyl acetate (70: 30, v / v), UV examination (254, 366 nm). In order to observe the influence of the mobile phase in the separation, they have different volume of the extract was applied on the plate chromatography using a mixture of different mobile phases. Identified spots were extracted and measured gas chromatograph, Hewlett Packard 5890 equipped with mass detector MS 5972. Quantitative estimation of piperine from Piper technique performed by HPTLC, silica gel plates (Polygram Sill G / UV 254 Macherey Nagel) Mobile phase: toluene - ethyl acetate (70: 30, v / v), record the chromatograms were assessed densitometrically. The calibration curve is linear in the 10-60 mg. L-^{1} (Y = 77.857 X - 253.57 R2 = 0.9908), the method can be used to determine the content of active ingredients pepper, food (mustard, spices), and pharmaceutical preparations.

Keyword: piperine, separation, identification, assessment, TLC / GC / MS

Piperine is the naturally occurring alkaloid that gives the spice, black pepper its characteristic biting taste. The large number of volatile and semivolatile compounds present in black pepper are responsible for the unique properties odoriferous and flavour, components having health promoting properties (table 1). The pharmacological, toxicological and clinical applications and general uses black pepper: analgesic; antipyretic; rubefacient; inhibits lipopolysaccharide induced inflammatory responses; intermittent fever, colic, dysentery, worms and piles; cures cold cough, dyspnoea, diseases of the throat (improves breathing, reduce cough); improves appetite; increases digestive power; antimicrobial activity; a protective impact upon key liver enzymes (improves breathing, reduce cough); antioxidant (prevent DNA damage, cells, control,oxidative stress); mutagenic and carcinogenic properties; spice, culinary applications (improves food quality); preservatives (prevent food spoilage); cosmetic industry (improves beauty); natural insecticide [1-3].

Table 1
ANTIOXIDANT ACTIVE CHEMICALS ISOLATED FROM BLACK PEPPER

Active Ingredients	Quantitative Estimation
Ascorbic-acid	0–10 ppm
Beta-carotene	0.114–0.128 ppm
Camphene	
Carvacrol	
Eugenol	
Gammaterpinene	
Lauric-acid	400–447 ppm
Linalyl-acetate	
methyl-eugenol	
myrcene	
myristic-acid	700–782 ppm
myristicin	
palmitic-acid	12.200–13.633 ppm
piperine	17.000–90.000 ppm
terpinen-4-ol	
ubiquinone	

Pungens pepper has been worth in 1821 for the two components and piperine piperanina. Historically, the pepper used to treat diseases has been such as piperine apparently has the ability to increase thermogenesis. Extraction, isolation and purification of alkaloids from the mixture of pepper followed by TLC analysis, ^{1}HNMR, ^{13}CNMR, MS, IR confirms the data related to the synthesis, structure and composition of piperine, piperidine, piperittinei as major components of black pepper. Piperine, a major alkaloid of black pepper that has been studied today finds multiple uses for food, medicines, cosmetics, pharmacological / toxicological. [1, 4-6]. Recent medical studies have shown piperine to be very helpful in increasing the absorption of certain vitamins such as Selenium, Vitamin B and Beta-Carotene [7].

From this point of view, analysis and identification of tissue extraction compounds cause a variety of techniques for sample preparation. The methods available for the identification and quantification of curcumin and piperine alone in herbal raw material include HPLC, HPTLC and spectrophotometric method [7-8, 9-12]. Piperine in rat plasma was determined using the technique of high-performance liquid chromatography [13]. The presence in human body fluids is measured using liquids chromatography methods [14].

High-Performance Thin Layer Chromatography - HPTLC is one of the most convenient techniques for the analysis

* *email: nicabadeadelia@yahoo.com.;Tel.: +40762684144*

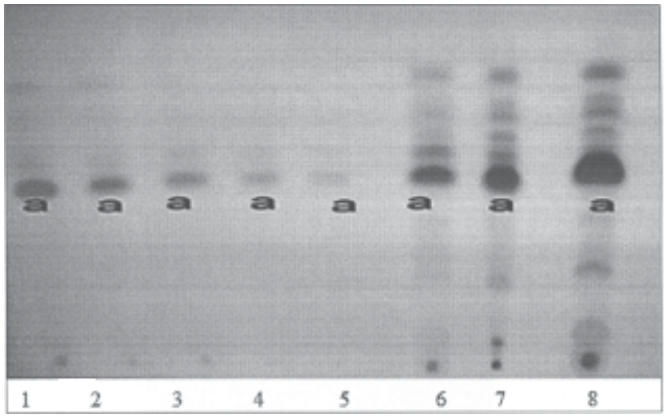

Fig. 1. Chromatogram separate piperine Polygram silica gel plates Sil G / UV 254 Macherey Nagel of standards (1-5) and extracts of pepper (6-8). Mobile phase toluene-ethyl acetate (70:30 v / v). Extracts: 1-5 piperine standards in ethyl alcohol, 6 - ethyl alcohol extracts of pepper; 7 - pepper extracts in chloroform, 8 - pepper extracts in petroleum ether

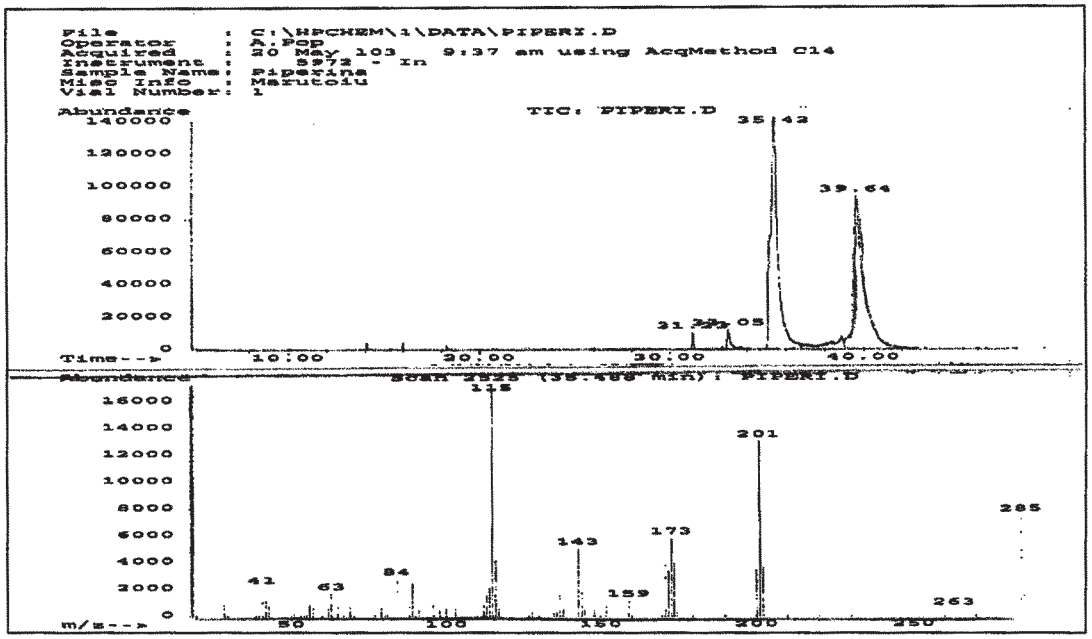

Fig. 2. Chromatogram and mass spectrum of piperine

of herbal products ayurvedic. Quantification of active ingredients punguente chromatographic techniques coupled with mass spectrometry in plant food products highlight the characteristics of this method [15-19].

Experimental part

For the analysis of piperine extractions were made from three samples of black pepper with different solvents. Were extracted each time 10 g of finely divided plant product with 100 mL ethyl alcohol, chloroform and petroleum ether. This was done in glass bottles fitted with ground-glass stoppers where the material was shaken for 30 min every day for 10 days. Resulting from the extraction solutions were concentrated to dryness rotovapor, then extracted material was dissolved in 20 mL ethyl alcohol to 25 μL flask with a dish washing solutions plus ethanol concentration up to the mark. The pure piperine were made standard solutions in ethyl alcohol in concentrations 0.129%, 0.1032%, 0.0774%, 0.0516% and 0.0258%. Volume of 10 mL / spot of Polygram silica gel plates Sil G / UV 254 Macherey Nagel. The plates were developed in unsaturated chambers chromatography using as mobile phase mixture toluene - ethyl acetate (70: 30, v / v) normal. Viewing was done by examination under UV light (254, 366 nm) using a Camag lamp or iodine vapor exposure (fig. 1). To observe the influence of mobile phase on separation were applied to different volumes of alcoholic extract (10, 7, 5 and 3 μL), and 10 μL of chloroform and ether extracts oil in the same chromatographic plates, using as mobile phase mixture: benzene - ethyl (70: 30, v / v) toluene-ethyl acetate (70: 30, v / v), hexane - ethyl acetate (70: 30, v / v) and toluene-chloroform - acetate ethyl (40: 30: 30, v / v). The spots indentified as containing piperine have been cut from the plates visualized with iodine vapour and organic compound was extracted in petroleum ether. The extract was analyzed using a gas - Hewlett Packard 5890 chromatograph equipped with an MS 5972 mass detector.

Working conditions: May MP-MS column (30 x 0.32 x 0.25), helium carrier gas, temperature program of 60^0 C, 3 ^{0}C / min, transfer line temperature 250 ^{0}C.

Results and discussions

The spots (a), (chromatogram in fig. 1) contained in standard and pepper extracts was analyzed by gas chromatography coupled with mass spectrometry (MS), (fig. 2). For mass spectra identification to separate the component spectra of the library computer database (Wiley 275 Library). This is the same as the library data, confirming that the product present in extracts and identified by standard is piperine. Analyzing the chromatogram in figure1, we can see that change then in the case of the concentration and intensity spots (spots 1-5). Also, solvent extraction using petroleum ether the amount of the organic substance extracted from black pepper is higher than in the case of ethyl alcohol and chloroform. This follows from the intensity and size of spots in positions 6-8.

By using mixtures benzene - ethyl acetate (70: 30 v / v), and toluene - ethyl acetate (70: 30 v / v), spots containing piperine is located about midway of the migration of the eluent. In this way the components that migrate before or after piperine are distributed on the same distance as the top five components appear and the bottom four components. When using mixtures of hexane - ethyl

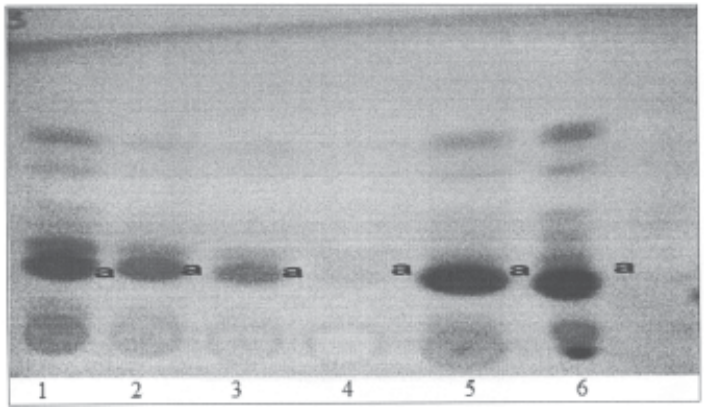

Fig. 3. Chromatogram of piperine (a) separate the alcoholic extracts (1-4) in chloroform (5) and petroleum ether (6). Silica gel plates Polygram Sil G / UV 254 Macherey - Nagel. Mobile phase: hexane - ethyl acetate (70: 30: 30, v / v)

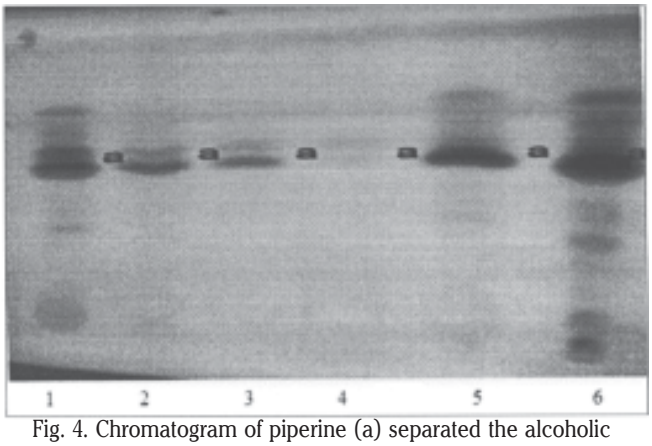

Fig. 4. Chromatogram of piperine (a) separated the alcoholic extracts (1-4) in chloroform (5) and petroleum ether (6). Silica gel plates Polygram Sil G / UV 254 Macherey - Nagel.Mobile phase: toluene - chloroform - ethyl acetate (40:30:30, v / v).

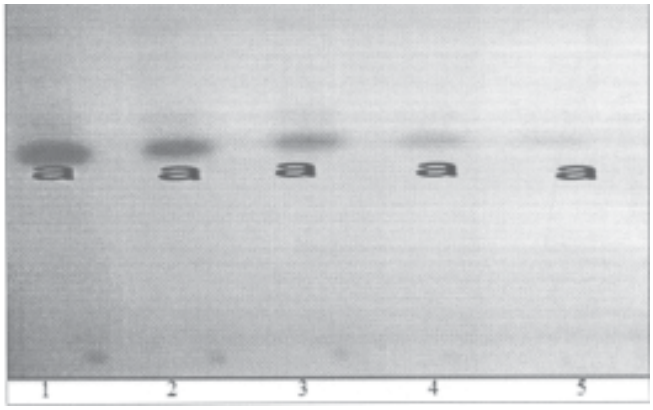

Fig. 5. Chromatogram of standard solutions of piperine: 1 to 0.129%, from 2 to 0.1032%, from 3 to 0.0774%, from 4 to 0.0516%, from 5 to 0.0258%. Polygram Sil G / UV 254 Macherey - Nagel. Mobile phase: toluene - ethyl acetate (70: 30, v / v)

acetate (70: 30 v / v) (fig. 3) or toluene - chloroform - ethyl acetate (40: 30: 30) (fig. 4) is close to the start of piperine in other components are well separated, the distance between spots is quite high. In the case of the use of the second mixture, toluene - ethyl acetate chloroform, piperine is located at the top of the plate and components migrating as it is well separated at greater distance from the other. If use of the second mixture, toluene - chloroform, ethyl acetate, piperine is in the top plate and the members who migrate after it is well separated and more distant from others. The chromatogram of figure 2 appears a bit with retention time 39.64 minutes, which seems to be piperanina, which could not be identified due to lack of spectrum and standard data library device.

Quantitative assessment of food piperine exciting is using standards of piperine on silica gel plates Polygram

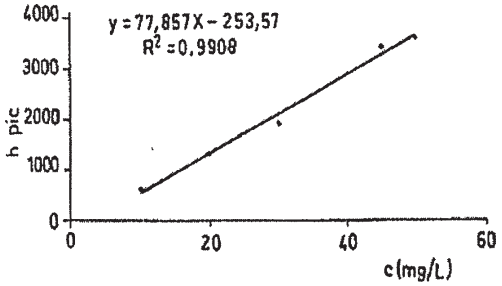

Fig. 6. Piperine calibration curve, recorded with pouch type densitometer.

Sil G / UV 254 Macherey Nagel, the results being shown in figures 5 and 6 recorded by the standard densitometer Desaga.

Calibration graph was found to be linear over the concentration range 20- 60 mg/L. The peak area and concentration was subject to least square linear regression analysis to calculate the calibration equation Y= 77.857 X - 253.57 and regression coefficient (R^2) was 0.9908.

Conclusions

Black pepper is a natural spice that can provide multiple nutritional and health benefits. Piperine, a major alkaloid of black pepper, is evaluated by instrumental analytical techniques (TLC/GC/MS), through multiple uses for food, medicines, cosmetics, pharmacological and toxicological effects. The proposed method Thyn Layer Chromatography coupled gas chromatography with mass detector for extraction separation and identification of piperine black pepper lead to rapid, accurate and sensitive method requiring less amount of solvent. The best extraction solvent is petroleum ether and toluene mixture as mobile phase - ethyl acetate (70: 30: 30, v / v). When you want to identify and isolate compounds distributed between piperine and front spot using mobile phase hexane - ethyl acetate (70: 30, v / v) and those between home and piperine mobile phase toluene - chloroform - ethyl acetate (40: 30: 30, v / v). Quantitative assessment of piperine stimulating the different foods (mustard, spices) meet analytical thin layer chromatography, the calibration curve was linear in the 10-60 mg.L^{-1}. The results obtained with the HPTLC method were compared with the results using the spectrophotometric and spectrofluorimetric pharmacopoeial methods.

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