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Chromatographic fingerprint analysis of piperine in polyherbal and marketed formulation by HPTLC and GC-MS methods

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Abstract:

The standardization of polyherbomineral formulation (PHF) is significant with regard to accessthe quality of natural medicines. The current research study highlights the chromatographic fingerprint investigation of piperine in PHF by employing GC-MS and HPTLC. PHF contain piperinewhich is utilized to take care of cough and cold. It was prepared from the mixture of Zingiber officinalis (Ginger), Piper nigrum (Kali mirch), Piper longum (Pipali), Terminalia belerica (Bahera), Terminalia chebula (Harde), Cuminumcyminum (Jira), Piper retrofractum (Chavya), Emblica officinalis (Amla), Coriander sativum (Dhaniya), sulphur, mercury, abharakbhasam and lohbhasam. The methanolic extract of both PHF and market formulation (MF) were subjected to HPTLC and GC-MS chromatographic analysis. HPTLC chromatogram finger printing of piperine in PHF demonstrated Rf values at 0.49 which was found in MF (Rf 0.47) and in standard marker (Rf 0.43). The piperine phytoconstituent present in both MF and PHF were investigated and recognized by GC-MS analysis, Thin layer chromatography (TLC), Fourier transformer infra-red (FTIR) spectroscopy and phytochemical tests. HPTLC finger printing, GC-MS analysis, FTIR and phytochemical screening tests of PHF may be useful in discriminating the species, affirm the existence of piperine phytoconstituen and act as a biochemical marker for polyherbomineral formulation. The consequence of these acquired parameters could serve as diagnostic tools to assist the regulatory authorities, scientific manufacturers and organizations for authentication and growing standard polyherbomineral formulation of high efficacy.

Key-words:

Piperine, Polyherbomineral formulation, Phytomarker, market formulation, Gas Chromatography-Mass Spectroscopy, High Performance Thin Layer Chromatography

Introduction

Since traditional time, the health of human beings has been of utmost importance and market of all commodities has become global in the present era. Health pertaining marketing products have been active and prepared at distinct divisions of the globe and marketed all over the world. The necessity of standardization assures the supply of consistency of product in almost whole environment of the globe (1). WHO hooks up and aids ministry of health in endowing mechanisms for the launching of typical plant remedies into prime medical care programs, in examining efficacy and safety, and assuring sufficient resources in quality control of raw materials and manufacturing of products (2).

In order to establish the necessary framework for control of quality, safety and therapeutics effectiveness of Ayurvedic herbal formulation, there is need for standardization of manufacturing

procedures and suitable analytical techniques. Among these techniques, GC-MS and HPTLC are extensively employed to create referral fingerprints of PHF against which MF and raw substances can be analyzed and assay the final products (3,4). The finger print technique delivers the means for suitable identification, because it is specifically suited for comparison of PHF, atest based upon fingerprints in contrast to MF. From the profile of phytoconstituents, a number of phytomarkers can be selected which might be employed to further reveal the quality of the PHF. GC-MS and HPTLC have been employed for quantitative estimation of smart phytomarkers(5).

The control of quality of herbal formulation is very much tedious in contrast to synthetic drugs due to chemical complexity of herbal constituents which are responsible for pharmacological action. It is tedious to completely evaluate and identify all these compounds because Ayurvedic herbal formulations consist of hundreds of species-specific and unique substances. It is also complicated to identify accurately which usually play vital role in remedial action since these substances generally function synergistically in eliciting the therapeutic outcomes (6).

Hence, it is difficult to maintain the consistent quality from batch to batch in Ayurvedic herbal formulations because necessity and serious attention is a challenging conditional task currently. Now a days, significant initiatives have been established to control the quality of herbs along with Ayurvedic formulation through employing qualitative fingerprinting tools and/or quantitative techniques (7,8).

So, the present studies evolve to evaluate PHF and marketed formulation (MF) employing HPTLC and GC-MS techniques. PHFwas prepared from admixture of number of herbs and minerals such as Zingiber officinalis (Ginger), Piper nigrum (Kali mirch), Piper longum(Pipali), Terminalia belerica (Bahera), Terminalia chebula (Harde), Cuminum cyminum (Jira), Piper retrofractum (Chavya), Emblica officinalis (Amla), Coriander sativum (Dhaniya), sulphur, mercury, abharak bhasam and loh bhasam. HPTLC study of extract of PHF and MF were investigated to access the phytomarker and make certain relationship by contrasting their chromatogram. GC-MS analysis was also performed for investigation and identification of phytomarkers in PHF and MF.

Materials and Methods

Procurement of Crude Herbal Drugs : After checking, confirmation and authentication from Department of Botany, BabuShivnath Agrawal (BSA) PG College, Mathura, U.P., India, crude herbs were purchased from regional market, Mathura, U.P. and developed thepolyherbomineral formulation.The chemicals employed were of analytical grade in the experiment.

Preparation of PolyherbomineralFormulation (PHF): PHF was produced according to the method specified in Ayurvedic Sarsangrah(9,10). Individually all ingredients were powdered and transferred through mesh (#80). Separately amount of every active powder ingredient was analyzed and blended in stipulated proportion so as to achieve uniform homogeneous mixture of PHF.

Development of PHF and MF extracts : According to standard typical techniques of Ayurvedic Pharmacopoeia of India (11,12), PHF and MF (Marketed Formulation) extraction were accomplished. The extracts were produced in large quantity and gathered by using the same technique. In sterile container the extracts were preserved and stored in refrigerator till further investigation.

Phytochemical Screening Test of Piperine : Phytochemical screening of PHF and MF extracts were used for the identification of piperine alkaloid. Prepare standard solution by dissolving 50 mg of extracts and piperine in 50 ml of 95% ethanol separately and shake well. The extracts and piperine were screened for various secondary metabolites by Mayer's test, Dragendroff'stest, Hager's test and Wagner's test.

Thin Layer Chromatography (TLC) study :

Test Sample: PHF, MF and piperine were added in 10ml methanol separately, and subsequently heated for 10 min, then filter and evaporated the filtrate up to 3 ml.

Identification of piperine in PHF and MF by TLC : Each sample (10µl) was spotted on precoated Silica gel-G aluminium plates of uniform thickness of 0.5mm as a stationary phase. TLC was produced by employing a blend of distinct solvents; Toluene: Ethyl acetate (7:3) as a mobile phase. The development was ceasedas the solvent entrance progressed about 75 percent. UV Fluorescence light was utilized as a visualizing agent after drying the plates in air for the detection of piperine. The presence of piperine in LPF and MF formulations was diagnosed when compared in contrast to spot of standard piperine phytomarker. The spots were marked and Rf (Retardation factor) value was calculated by using following equation(11,13). The experiment was performed in triplicate for reproducibility of results.

Rf = Dsolute/Dsolvent

Where, Dsolute - Distance travelled by solute; Dsolvent- Distance travelled by solvent

Fourier Transformer Infra-Red Spectroscopy (F.T.I.R.) Study : Thephytoconstituents present in a plant are specific in nature and generally do not occur in other plant. The phytoconstituent such as piperine alkaloid [Fig. 1]has specific absorption of light in infra-red region due to their different functional groups. The absorption peaks and finger print region is specific for a phytoconstituent and cannot match with another. From Infra-Red spectrum analysis, absorption peak or finger print region of PHF and MF was investigated and thepurity and presence of phytomarkerpiperine alkaloid was confirmed.

Solvent was evaporated to dryness on water bath (Accumax Equipment India Ltd., New Delhi, India) and I.R. spectrum was recorded using F.T.I.R. Spectrophotometer (Shimadzu, Japan) in the frequency range between 400-4000 cm-1 and resolution (4 cm-1) was obtainedas scanning range between wave number (cm-1) and % Transmittance.A disc of KBr (200mg) was preparedwith 2mg sample. Infrared spectrum of PHF and MF gave information about the group present in that particular compound. Therefore, I.R. spectrums of PHF and MF were compared with respect to I.R. of piperine (14) as a standard. The experiments were performed in triplicate manner to check the reproducibility.

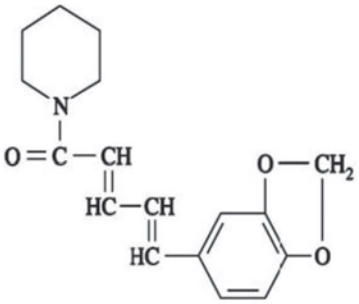


Fig. 1. Structure of Piperine.

High Performance Thin Layer Chromatography (HPTLC) Study

Instrumentation:

Application mode: CAMAG Linomat IV – Applicator
Filter system: Whatman filter paper (No.41)
Chromatographic conditions:
Stationary phase: Precoatedaluminium silica gel F254 plate MERCK-TLC/HPTLC
Mobile phase: Diethyl ether :Ethyl acetate : Benzene (10:30:60)
Application on Y axis
Start position: 1cm
Development on Y axis
Band length:6 mm
Chamber saturation time:30 minutes
Development mode:CAMAG TLC Twin Trough Chamber
End position:90mm from plate base
Derivatization mode: CAMAG – Dip tank for about 1minute
Visualization: 366nm, Visible, 254nm, (After spray of Anisaldehyde Sulphuric acid reagent)
Drying mode, temp. &Time:Preheated at 100 ± 5°C (TLC Plate Heater)

Procedure

HPTLC study of methanolic extracts of PHF and MF was performed along with the standard marker as an active constituent to ensure the presence of piperinephytoconstituent in PHF and MF. For HPTLC, each sample (2g) was extracted using methanol (25 ml) for 25 minutes on boiling water bath three times successively employing 25 ml fresh methanol and concentrated after filtration. All samples of extracts and standard were spotted on pre-coated plate (10cm×10cm with 250µm thickness) of silica gel aluminium 60F-254 employing sample applicator (CamagLinomat IV) and Hamilton syringe of 100µl. Ten millimeter from the bottom and 10 mm apart, all samples of band length (6mm) were spotted employing nitrogen aspirator at a constant application rate (15nl/s).TLC plates were dried subsequently in a current of an air dryer.The densitometric scanning was takenin the absorbance/reflectance mode on Camag TLC scanner III.

HPTLC fingerprinting: Estimation of phytoconstituents in extracts : Piperine phytoconstituent was confirmed in the methanolic extract by HPTLC technique. The standard solution (1mg/ml) was prepared separately by misciblizing standard Piperine 10 mg (Sigma Aldrich, USA) in methanol (10 ml) and sample solutions of PHF and MF (1%w/v) were prepared by dissolving extracts 100 mg in 10 ml of respective solvent. Camag HPTLC system (Switzerland) was employed for analyzing the sampleswhich was equippedfor applying the samples with a sample applicator device Linomat IV, twin trough liner development chamber, Camag Scanner III attached with integration software CATS4.06 (Switzerland) and precoated aluminium silica gel F254 plate of Merck. Standard marker (Piperine) 5 µl of 1mg/ml, PHF and MF 5 µl of 10 mg/mlsolutions of extracts were placed respectively as band width (6 mm) from the edge (about 10 mm) of HPTLC plate employing applicator (CamagLinomat IV). Benzene: Ethyl acetate: Diethyl ether (60:30:10) solvent system as mobile phase was applied for investigation of Piperine. The chromatograms were developed and scanned at 254nm, 366nm and white remission using TLC scanner(15-22). For recognition and quality judgment of the formulation, HPTLC fingerprint can be utilized competently.

Gas Chromatography-Mass Spectrometry (GC-MS) study :

GC-MS analysis was performed employing an injector (Agilent7683 Bauto) which is coupled with a selective detector (5975 C VL Agilent mass) gas chromatography Agilent Technologies, (Santa Clara, CA) 7890A. Sample injection volume (1 µl) was employed and set at scanning rate of 2.86 scans per second. The flow rate (0.7mL/min) of carrier gas (helium grade 5) was maintained in GC and operated split less mode, 10 psi a column head pressure. On the basis of electron impact (EI) function, mass spectrometer was handled by applying 70 eV ofionization voltage and temperature 230°C. GC injector was maintained at 250°C; transmit line at 280°C. Temperature system comprised of preliminary temperature which was bring up to 250°C at a rate of 30°C/min and maintained at 70°C for 1min preceded bykeeping at 250°C for 30 min. It was maintained in 40-400 m/z scan range and attainsthe recorded mass spectra by subtraction of background and takes mean of at least five scans. The collection of retention data and chromatographic separation had been departed on a column of 30 m×0.25 mm-i.d.,which has layer of 0.25µm 100% dimethyl polysiloxane (Rtx-1) which was procured from Restek Corporation, Bellefonte, PA (23). The amount of each component was calculated in term of relative percentage by comparing its average peak area to the total areas.

Sample preparation : In screw cap vials, add a weighing amount (1g) of eachPHF and MF extract separately and 10 ml methanol then kept aside for 12 hrs after sonication for 60 min.

Identification of components : The identification and interpretation of the compounds was achieved by employing in-built National Institute Standard and Technology (NIST) library data bank to deal with greater than 62,000 patterns. Compare the mass spectrum of the unfamiliar test compound

against the spectrum of the reference compound recorded in the main library. The name, percentage peak area and retention time (RT) value, and structure of the components were confirmed.

Result and Discussion

The various specific phytochemical tests gave positive result [Table 1] for the identification of piperine in LPF and MF. Piperine alkaloid showed specific color when extracts of PHF and MF reacted with Dragendroff's, Mayer's, Wagner's and Hager's reagents.

TLC profile of PHF and MF were developed. PHF and MF showed Rf value of 4.7 and 0.48 which was near to Rf value 4.7 of piperine[Fig. 2], that indicated the presence of piperinephytomarker in PHF and MF respectively.

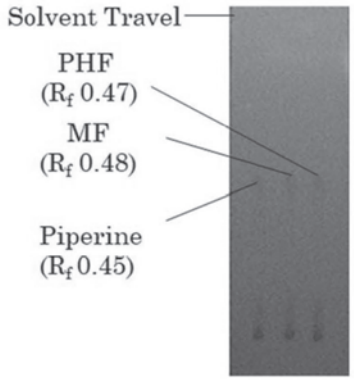


Fig. 2: Rf value of PHF, MF and pure piperinephytomarker.

FTIR spectrum of pure piperine exhibited various bandswhich appeared at 2865.12 cm-1 attributed to symmetric CH_2 stretching and at 2923.88 cm-1 asymmetric CH_2 stretching respectively. The band at 3070.32cm-1 indicated aromatic C-H stretching and at2923.88 cm-1 indicated aliphatic C-H stretching.Aromatic stretching of C=C and CO-N stretching showed at 1620.08 cm-1 and 1450.37cm-1 respectively. Peaks at 1027.99 cm-1 belongs to symmetric while at 1244.00 cm-1 belongs to asymmetric =C-O-C stretching and 1450.37 cm-1 showed CH2 bending and at 852.48 cm-1 showed C-O stretching. The out-of-plane phenyl C-H bending was observed at 837.05 cm-1 while 1120.16 cm-1 indicated in-plane C-H bending, results showed in [Fig. 3] (14). The piperine phytoconstituent was found to be present in both PHF and MF thus it reveals good relationship between them.

HPTLC analysis of PHF and MF extracts were performed to assure the presence of piperineas well as relationship between them. In Fig. 4 HPTLC fingerprint of extracts of PHF, MF formulation and standard (piperine) are depicted. Rf values 0.49, 0.47 and 0.43 were detected in chromatogram of extracts of PHF, MF and standard piperine respectively, as showed in [Fig 4-5]. It was noticed that the chromatogram of the PHF coordinated accurately with that of the MF. Thus, HPTLC study confirmed good correlation between both PHF and MF extracts and confirmed the presence ofpiperine (24).

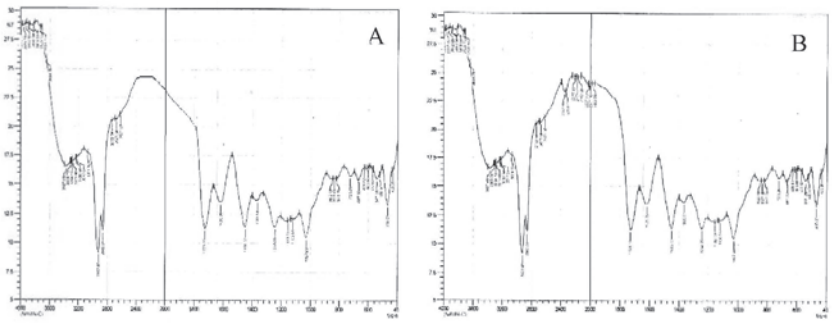
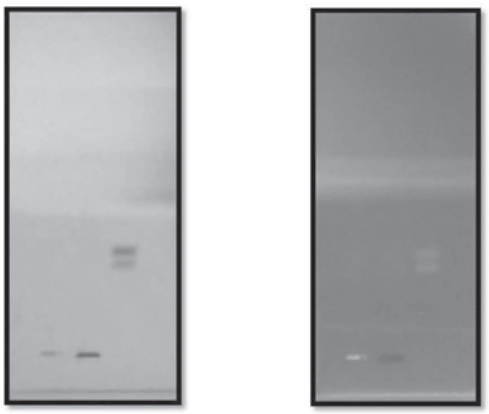


Fig.3. FTIR spectrum of (A) PHF; (B) MF.



(A) (B)

Fig. 4. HPTLC chromatogram of (A) at 254 nm contains PHF, MF and standard piperine and (B) at 366 nm containsPHF, MF and Standard Piperine.

The presence of phytoconstituent in the PHF and MF was also recognized by GC-MS method. From [Fig. 6-7], the chromatogram of PHF and MF showed the presence of several peaks but piperine peak (10.88% peak area) in PHF extract was found to be at 20.668 retention time and in MF extract, piperine peak (2.38% peak area) was found to be at 20.609 retention time. The substances relating to peaks were investigated by accessing data of the NIST library of peaks and mass spectra of the peaks with those reported in literature. The piperine phytoconstituent was found to be present in both PHF and MF extract thus proving good relationship between them (25).

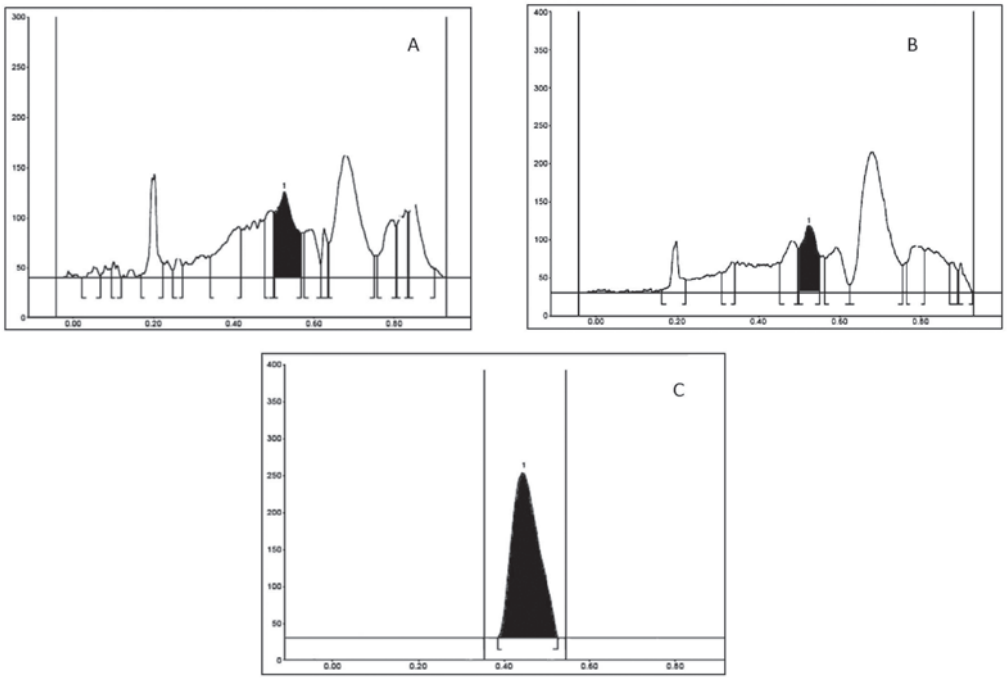


Fig. 5.HPTLC chromatogram of (A) PHF, (B) MF and (C) Standard Piperine.

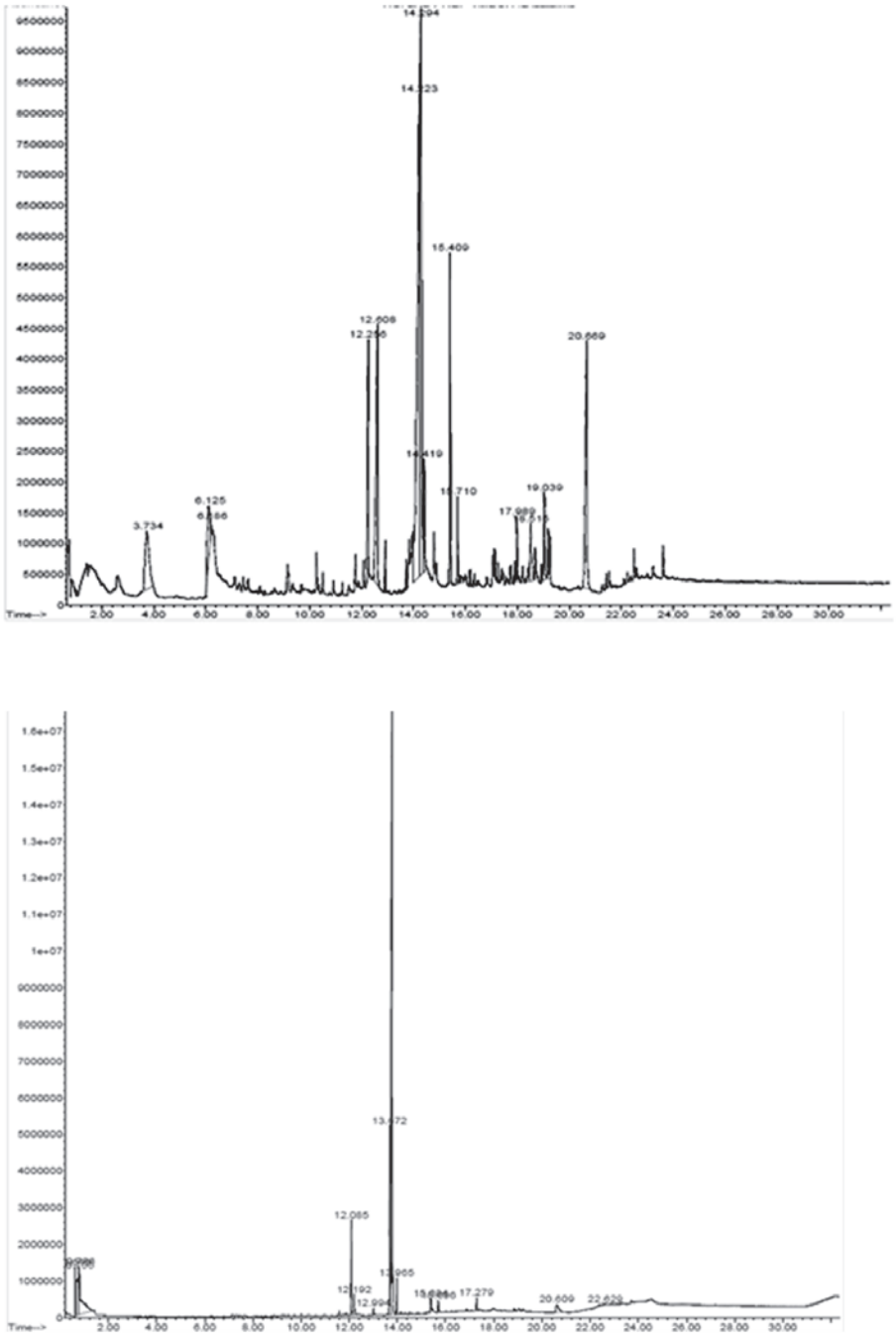


Fig. 6. Gas Chromatography-Mass Spectroscopyspectrum of (A) PHFand (B) MF.

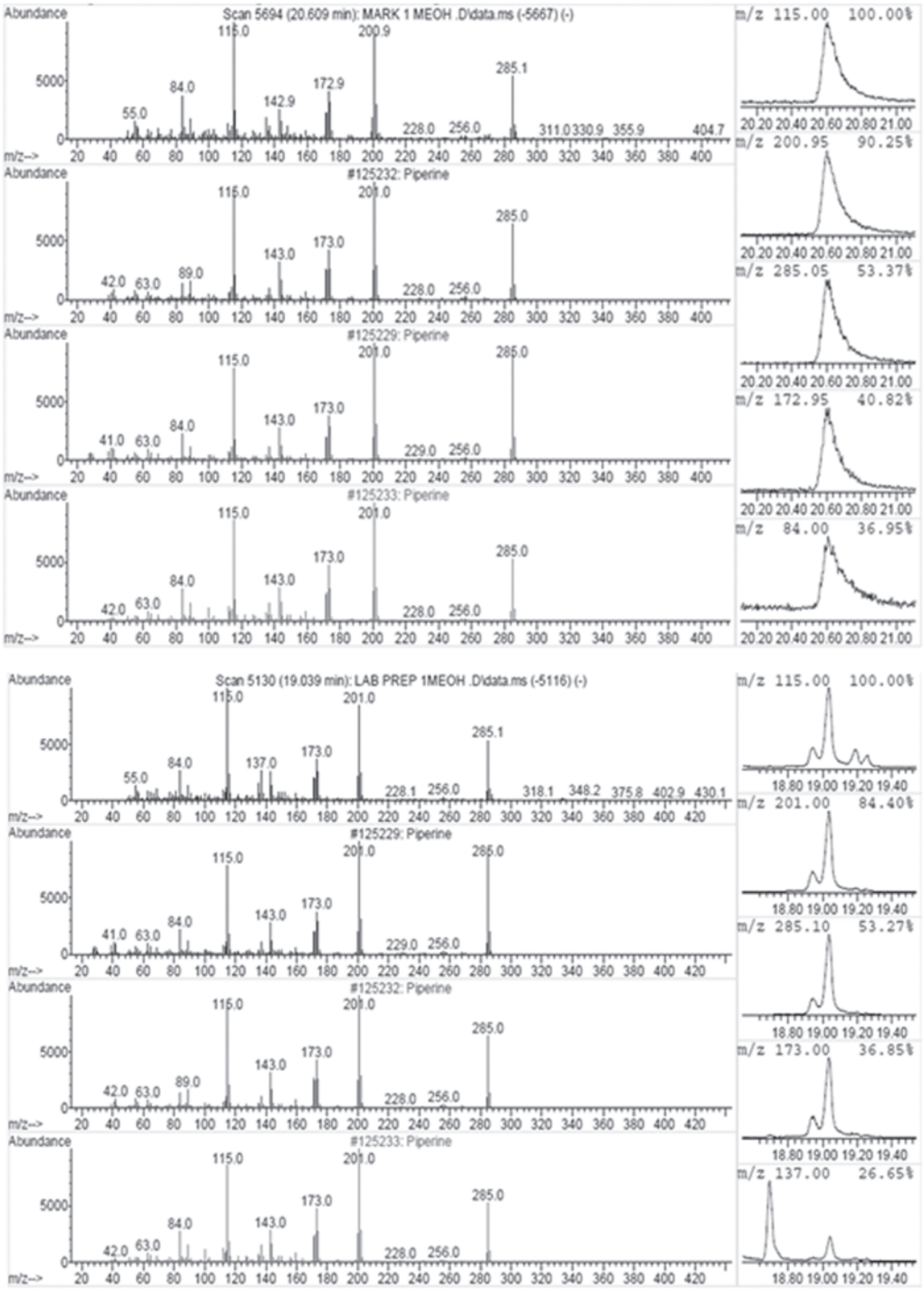


Fig. 7. Mass spectrum of (A) MF and (B) PHF.

Table 1. Various specific Phytochemical tests for identification of piperine in PHF and MF.

Test/Reagent	Colour	MF	PHF	Piperine
Wagner's reagent	Reddish brown precipitate	+	+	+
Hager's reagent	Yellow precipitate	+	+	+
Mayer's reagent	Dull white precipitate	+	+	+
Dragendroff's reagent	Orange red precipitate	+	+	+

'+': Presence, '-': Absence, MF: Marketed Formulation, PHF: Polyherbomineral Formulation

Conclusions

Phytochemical screening, TLC, FTIR, HPTLC finger printing and GC-MS analysis of poly herbomineral formulation (PHF) may be useful in discriminating the species, affirm the existence of phytoconstituents such as piperine whichact as a phytomarker for polyherbomineral formulation. The consequence of assessment of these tests could serve as analyzing tool to assist thescientific manufacturers, regulatory bodies, and organizations for authentication and growing standard traditional polyherbomineral formulation (PHF) of outstanding quality and therapeutic efficacy.

Conflict of interest

The author has no conflict of interest on this article.

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