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Lc-Ms Analysis Of Pleurotus Citrinopileatus (Golden Oyster Mushroom) Fruiting Body Extracts After Treatment With Acetone

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BACKGROUND AND OBJECTIVE

Fruiting bodies of Pleurotuscitrinopileatus represent a natural reservoir of bioactive molecules [cardial glycosides, phenols, steriods, terpenoids and proteins] that can used for therapeutic purposes. This work was conducted to evaluate the biocompounds in the acetone treated golden oyster mushroom extracts.

Methods: Herein, the acetone treated golden oyster mushroom extracts is subjected to Liquid chromatography-Mass Spectrometry (LC-MS) analysis quantitatively.

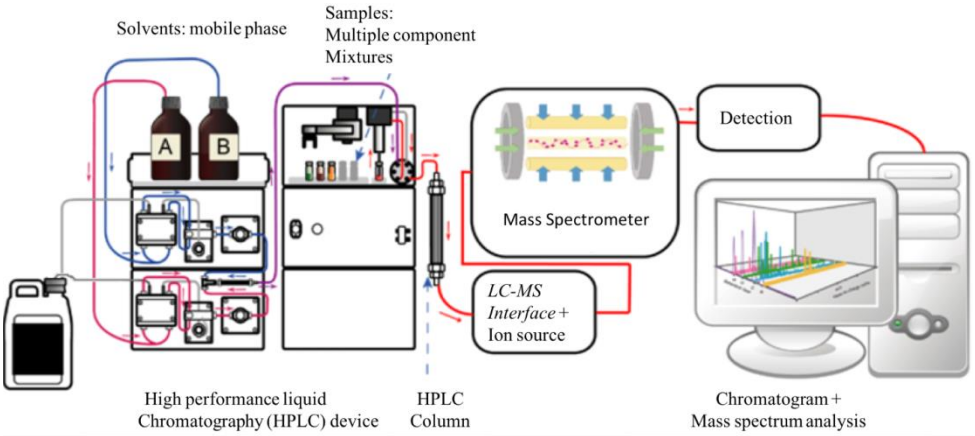
Results: Bioactive compounds present in the treated sample (Pc-A-1) is relatively compared with that of control (pc-a-1-ve) to know the impact of the treatment on the concentration of the compound of interest in this quantitative LC-MSexperiment.The chromatogram with relative abundance % in Y-axis against retention time (min) in X-axis is discussed further.

Novelty: LC-MS is a hyphenated analytical technique which is a combination of liquid chromatography (LC) and mass spectrometry (MS) where separation and quantitation of the components can be done; relative atomic and molecular masses can be performed simultaneously. It is used in pharmacokinetics, bioavailability-bioequivalence studies, metabolite, forensic studies and for determination of assays of drug substances.

Keywords: Absorbance, bioactive compounds, liquid chromatography, mass sepctrometry, oyster mushroom extracts.

I. INTRODUCTION

Liquid chromatography Mass spectrometry (LC-MS) is basically a hyphenated coupling analysis techniques which was conducted both off-line and on-line. The off-line coupling involved fraction collection, evaporation of solvent, and transfer of analytes to the MS using probes. Off-line analyte treatment process was time consuming and there was an inherent risk of sample contamination. Rapidly, it was realized that the analysis of complex mixtures would require the development of a fully automated on-line coupling solution in LC-MS. This method has applications in analysis of food, pesticides, drug development, pharmacokinetics, proteomics and metabolomics study.[1]



Herein, we are using Q-Exactive Plus Biopharma-High Resolution Orbitrap Liquid Chromatograph Mass spectrometer to extract the raw sample datasets.

LC-MS features are defined by special characteristic combinations of retention time and mass-to-charge (m/z) ratio. Associated with the chromatographic peak of a feature is a peal area/intensity, which serves as a relative measure of the abundance of the compound producing the feature. These parameters represent a fingerprint of biocompounds in a given sample in our case the acetone extracts of golden oyster mushroom.

II. MATERIALS AND METHODS

TREATED SAMPLE [PC-A-1]

Acetone extract of the dry fruiting body of golden oyster mushroom [dry powder form]

CONTROL SAMPLE [PC-A-1-VE]

INSTRUMENT AND SOFTWARE DETAILS

Instrument used: Q-Exactive Plus Biopharma, Thermo Scientific

Data Acquisition Software: Thermo Scientific Xcalibur, Version 4.2.28.14

Data Processing Software: Compound Discoverer 2.1 SP1

Column details---Hypersil Gold 3micron 100 x 2.1 MM (Thermo Scientific).

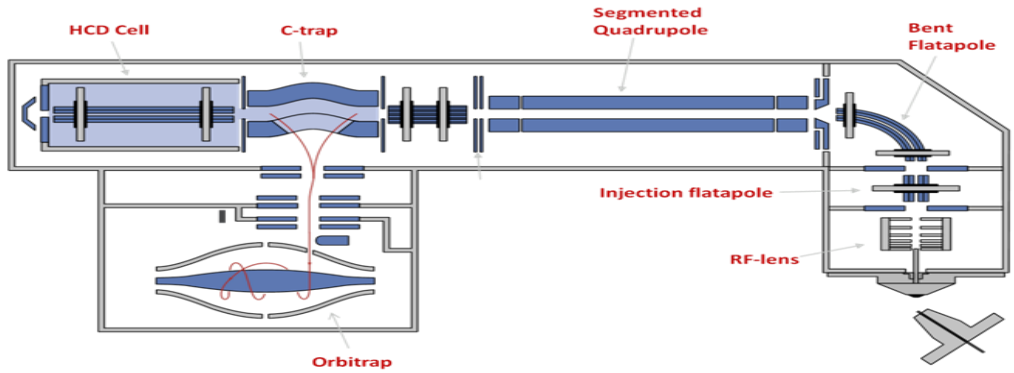
SOLVENTS USED

Solvent A: 0.1% formic acid in milliq water, Solvent B: Methanol

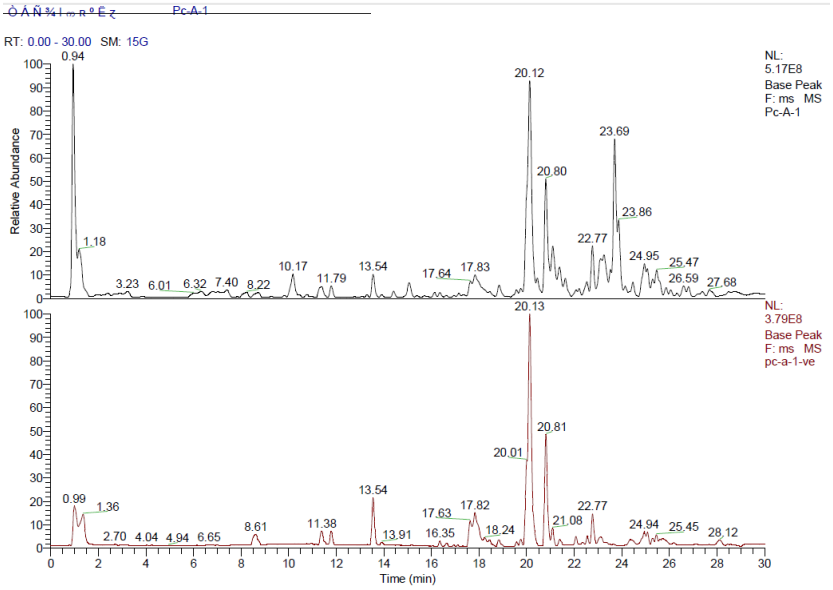
The total run time for the analysis was 30 minutes with positive polarity. The Q Exactive was operated in the data dependent mode, collecting a full MS scan from 100-1500m/z at 70K resolution and an AGC target of $1e^6$. The most abundant ions per scan were selected for MS/MS at 17.5K resolution and AGC target of $8.00e^3$ and intensity threshold of 1.6K. Maximum fill times were 50msec and 60msec for MS and MS/MS scans respectively with a dynamic exclusion of 10sec. Normalized collision energy (NCE) was varied from 12–44 for the peptide standard experiments. Pc-A-1 sample was analyzed at 25, 30 and 40 NCE and at 30 with 25% stepped energy which effectively combines fragmentation at energies of 25, 30 and 40.

WORKING PRINCIPLE OF Q-EXACTIVE PLUS BIOPHARMA LC-MS

The orbitrap is an ion trap mass analyzer that consists of two outer electrodes and a central electrode, which enable it to act as both an analyzer and detector. Ions enetering the Orbitrap are captured through the "electrodynamic squeezing", after which they oscillate around the central electrode and in between the two outer electrodes. Different ions oscillate at different frequencies, resulting in their separation. By measuring the oscillation frequencies induced by ions on the outer electrodes, the mass spectra of the ions are acquired using image current detection. LC-MS ia a technique which combines high performance liquid chromatography (HPLC), a powerful analytical separation technique with mass spectroscopy, a powerful analysis and detection technique. There are two common atmospheric pressure ionization (API) LC/MS process: Electrospray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI). Both are soft ionization technique. Q Exactive Plus provides fast, sensitive, relaible and confident detection and identification of compounds in complex mixture while maintaining full compatibility with UHPLC and fast chromatography. The HCD collision cell adds more functionality to the bench top system by providing ion fragmentation while maintaining high resolution, accurate mass and high sensitivity.[1]



III. RESULTS AND DISCUSSION



Herein, we are comparing the retention time (the time taken for a solute to pass through a chromatography column) to the relative abundance percentage of ions (the time taken for a solute to pass through a chromatography column). Nicotonic acid (niacin supplement),Ergosterol peroxide, 1-Linoleoyl-sn-glycero-3-phosphocholine are a few compounds found in Pc-A-1 sample which has a relative absorbance of 22%, 24.5% and 35% respectively. Ergosterol peroxide is found in the control sample [pc-a-1-ve] as well [t_R=22.77].

Ergosterol based compounds are found in golden oyster mushroom extracts. It is estimated that P. citrinopileatus (common name Golden oyster mushroom) contained the highest amount of ergothionine (822.1±20.6 $mgkg^{-1}$) dry sample.[2]

IV. CONCLUSION

LC-MS analysis has given us the biocompounds present in the acetone treated mushroom extracts which has helped us understand the usage of golden oyster mushroom in the cancer treatment, a possible drug assay method to help us with the further study against HEPG2 cell line.[3]

ACKNOWLEDGMENT

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