RAPID HIGH RESOLUTION ACCURATE MASS MULTI-CLASS MULTI-RESIDUE SCREENING METHOD FOR ASHWAGANDHA (WITHANIA SOMNIFERA) PRODUCTS

Praveen Kumar Sharma ; Prasanth Joseph; Manoj G Pillai; Neha Bhasin; Umesh Pawa; Andre Schreiber
AB SCIEX,121, Udyog Vihar , Ph-IV , Gurgaon , India ; AB SCIEX, 71 Four Valley Drive, Concord, ON, L4K 4V8 Canada

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ABSTRACT

Medicinal plant materials may contain pesticide residues and chemical contaminants which accumulate as a result of agricultural practices or the presence of a wide variety of illegal drug adulterants which is a matter of public concern. Effective analytical methods are required for the screening and identification for these contaminants to ensure the quality of herbal products. High resolution mass spectrometry was employed for multi-class multi-residue screening of herbal products of Ashwagandha (*Withania somnifera*). Single injection workflow using TOF-MS survey scan followed by dependent TOF-MS/MS scans was used for the simultaneous screening, identification and confirmation of residues.

INTRODUCTION

Botanical supplements or Herbal remedy products are one among the fastest growing market worldwide. According to the WHO, more than 80 % of the world's population relies on traditional medicine for their primary healthcare needs. The safety and quality of botanical supplements and finished herbal products have become a major concern for health authorities and Regulatory bodies like World Health Organization[1] and European Medicine Agency etc. Therefore, effective analytical methods are required for the screening and identification for these contaminants in order to ensure the quality of herbal products.

In the present study high resolution mass spectrometry was employed for multi-class multi-residue screening in herbal products of Ashwagandha. Ashwagandha (*Withania somnifera*) Indian ginseng, poison gooseberry,-or winter cherry, is a plant in the Solanacea family. An herb commonly used in Ayurvedic medicine, which is the traditional health care system of India and used to cure multiple ailments [2].

A generic sample extraction protocol with minimal LC separation was employed. Single injection workflow was used and Screening of compounds was done using the accurate mass TOF MS data. In addition to this, high resolution TOF MS/MS data was used as an additional confirmation tool to identify detected compounds by automated searching against mass spectral library. Mass spectral library contains MS/MS spectra of over 2400 chemicals, including pesticides, mycotoxins, veterinary drugs, pharmaceuticals, drugs of abuse, etc.

MATERIALS AND METHODS

Sample Preparation:

Twelve commercially available ashwagandha herbal products procured locally were used in this study. A generic extraction protocol using Methanol as extraction solvent was used. 1000 mg of each samples were extracted with 10 mL of methanol, stirring at room temperature overnight. The extract was centrifuged at 3500 rpm for 10 minutes to remove any particulates. The supernatant was concentrated to 1 ml using Nitrogen concentrator and finally dissolved in MeOH:H_2O (90:10) prior to injection.

HPLC Conditions:

A Shimadzu UFLCXR LC system with an Phenomenex Luna C18 150 x 4.6 mm 3 µm column at 40°C with a gradient of eluent A water (0.1% Acetic Acid) and eluent B Acetonitrile (0.1% Acetic Acid) was used at a flow rate of 500µL/min. The injection volume was set to 50µL.

MS/MS Conditions:

AB SCIEX TripleTOF® 4600 LC/MS/MS system was operated with the DuoSpray™ ion source operated in electrospray ionization (ESI). The APCI probe of the source was used for fully automatic mass calibration using the Calibrant Delivery System. CDS injects a calibration solution matching polarity of ionization and calibrates the mass axis of the TripleTOF® system in all scan functions used (MS or MS/MS).

The combination of TOF-MS and MS/MS with Information Dependent Acquisition (IDA) in a single method, additionally, allows the identification of non-targeted and unexpected compounds through powerful software tools [3]. The data were processed in MultiQuant™ software version 2.1 and PeakView® software version 1.2 with the XIC Manager®[4].

Figure 1. Ashwagandha plant and products.

RESULTS

Preliminary screening results indicate the presence of wide variety of compounds ranging from pesticides, mycotoxins, pharmaceuticals drugs and Pharmaceutical and personal care products in various samples. Screening of twelve Ashwagandha samples indicate presence of pesticides such as Kersoxim Methyl, Dodemorph and Cyprodinil. Pharmaceutical and personal care products (PPCP) like Adenine, Norethandrolone and Hymecromone were also detected. For isobaric compounds, along with TOF MS data, TOF MS/MS was used for high confidence compound identification. For example, Betaxolol and Butamirate have same elemental formula and mass accuracy. However theoretical fragments of Betaxolol show more match with generated high resolution MS/MS data.

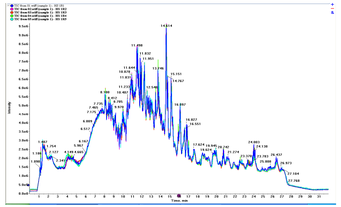

Figure 2. Overlaid representative chromatograms of five replicates .

Fifty percentages of the samples showed the presence of mycotoxin. Several drugs including illegal drugs which are used as hypnotic, neurotransmitters, local anaesthetics, analgesics, tranquilisers were found. In addition to this few of the steroids and antibiotic drug products were also found to be present. Hymecromone, an orally active narcotic analgesic and antitussive was found in two of the samples. Hydromorphone and Morphein were detected in three of the samples where as 3, 4-Methylenedioxyamphetamine and betaine were found in four samples.

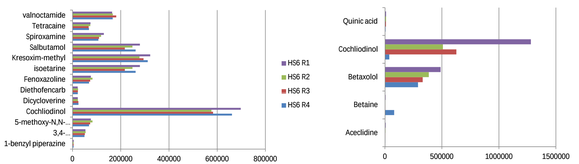

Figure 3. Presence of common compounds in various extracts .

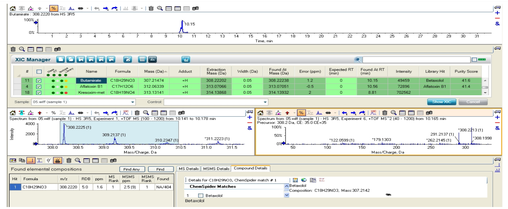

Figure 4. Identification of Betaxolol / Butamirate using TOF-MS and TOF-MS/MS

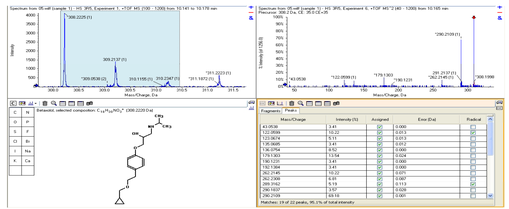

Figure 5. Confirmation of Betaxolol using High resolution MS/MS data.

Present study also confirms that same workflow can be used for identification and confirmation of totally unknown compounds present in herbal samples.

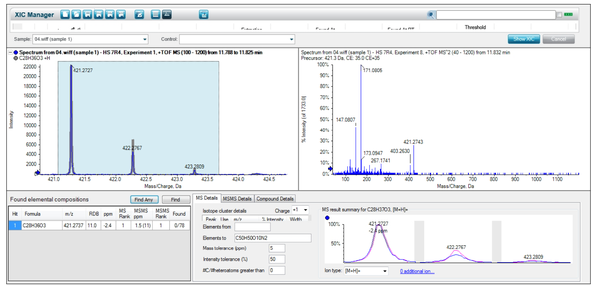

Figure 6. Formula finder shows the elemental composition as C28H36O3

For example, based on TOF-MS and MS/MS data, software calculates elemental formula as C28H36O3 for *m/z*: 420.2664 in positive ionisation mode. Predicted formula was automatically searched against chemical database and identified the compound as Tingenone. Final confirmation was done by comparing theoretical fragments to the obtained TOF -MS/MS of Tingenone.

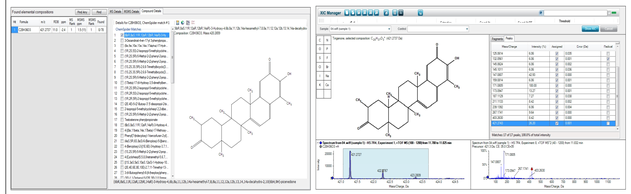

Figure 7. Theoretical fragments of Tingenone matching with the obtained TOF MS/MS with 100% matching

CONCLUSIONS

The workflow used for the screening, identification and confirmation of chemical contaminants can also be used for identification and confirmation of totally unknown compounds present in the herbal samples. Three confirmatory steps were followed in order to confidently report unknowns. Based on the TOF MS and simultaneously generated high resolution MS/MS data, software empirically calculates potential molecular formulas for the detected compound. Predicted formula was automatically searched against chemical database. Final confirmation was done by comparing the high resolution TOF MS/MS data to the theoretical fragments of the identified compound. Moreover, the new simplified sample preparation in combination with high resolution LC, and sensitive full scan detection allows detecting chemical contaminants faster and less labor-intensive and time-saving.

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TRADEMARKS/LICENSING