

Gas Chromatography- Mass Spectroscopy Analysis of Ashokarishta w.s.r. to Metabolomics

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Abstract

Herbal products have been identified to be the source of many active substances. They are widely used in multiple conditions. Ashokarishta is a poly herbal formulation consisting of 15 drugs, and being practiced in multiple gynecological disorders. Metabolomics provides relevant data of secondary metabolites and help in the interpretation of genomic and proteomic data in drug discovery. Mass spectroscopy has proven to be a valuable analytical tool for the identification of secondary metabolites. Gas chromatography (GC) is the widely used technique, as it offers very high resolution. The GC method is mainly useful for identification of small structures and volatile molecules. The paper focuses on the use of metabolic identification of the phyto chemicals in Ashokarishta through Gas chromatography - Mass spectroscopy.

Keywords

Ashokarishta, Metabolic analysis, Mass spectroscopy, Gas chromatography.

Introduction

Metabolite identification¹ plays a critical role in the phases during drug development. Drug metabolites can contribute to efficacy, toxicity, and drug-drug interaction. Thus, the correct identification of metabolites is essential to understanding the behavior of drugs in humans. Metabolite profiling is the measurement of the levels of a metabolite set in a sample. Mass spectroscopy (MS) is a rapidly growing technology for the comprehensive profiling of small molecules (metabolites) and proteins².

GC/MS³ is an analytical method that separates different components with respective spectral output. The step involved is vaporization, separation, and then analysis of compounds. The retention time is helpful in the differentiation of compounds. Quantification of the compound is based on the size of the peak.

Ashokarishta^{4,5} is a poly herbal formulation explained in Ayurvedic Classics. It is widely used in Gynecological conditions like Raktapradara (Dysfunctional Uterine Bleeding), Pida (Pain), Shopha (Swelling), Poly Cystic Ovarian Syndrome (PCOS)⁶, Anartva (Amenorrhea)⁷, Stree Vandyatva (Female infertility)^{8,9}, Swetapradara (Leucorrhea)¹⁰, Grabhashaya Granthi (Uterine fibroids)^{11,12}, Garbhasrava Vandyatva (Repeated Abortion)¹³,

Arthava Nivrutti Vikara (Menopause syndrome)¹⁴ and Gabashaya Brmsha (Uterine prolapse).

Aim & Objective

To explore the Metabolites of Poly herbal formulation Ashokarishta through GCMS.

Materials Required

Instrument Model

7890 A GC with 5975C with triple axis detector.

Column

DB 5MS 30 m x 0.250mm Diameter x 0.25 Micro Meter Thickness

Chemicals

Ashokarishta, Dichloromethane, Helium gas.

Equipment

Separating funnel, Nylon 13 mm 0.2um syringe filter.

Database

NIST -08 SPECTRAL DATA

Methodology

Sample Preparation

10g sample extracted with 50ml dichloromethane in a separating funnel, collect DCM layer and evaporated at room temperature and residue reconstituted with 1ml DCM, filter through a syringe filter (Nylon 13 mm 0.2um) and injected to GCMS.

Gas Chromatography - Mass Spectroscopy Analysis

GC-MS analysis was carried out on a

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7890 A GC with 5975C with triple-axis detector which includes automated sample preparation. The column measuring 30m x 0.25 mm diameter x 0.25-micrometer thickness. The carrier gas used was Helium gas with a split ratio of 1:10 at a flow rate of 1 mL/min. Analysis was performed by injecting 1 µL of the sample. The injector temperature was maintained at 280°C (constant). The column oven temperature-programmed as initially 60oC for 3 mins, then increased to 240oC for 5 mins and then increased to 280oC for 20 mins. The total runtime was 20 mins. The analysis was performed in the EI (electron impact) mode with 70 eV of ionization energy.

Data Acquisition

Data was evaluated using total ion count (TIC) for compound identification and quantification.

Data Identification

The compounds were identified after comparing the spectral configurations obtained with that of the available mass spectral database (NIST -08 SPECTRAL DATA).

Results

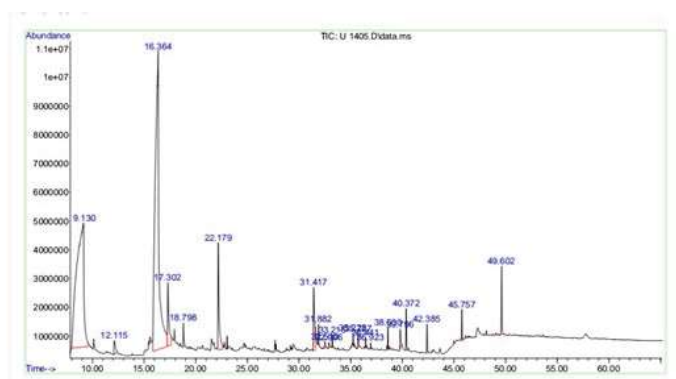
The peaks compared with NIST-08 SPECTRAL DATA and the compounds were identified. The peaks of the identified compounds of Ashokarishta are given in figure1. The detailed tabulations of GC-MS analysis of the extracts are given in Table 1.

Table 1: Metabolites identified in the Ashokarishta by GC-MS Peak Report TIC.

Sl.No	R. Time	IUPAC Name	Chemical Formula
1.	9.130	Ethanol, 2,2'-oxybis	C4H10O3
2.	12.115	Phenylethyl Alcohol	C8H10O
3.	16.364	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	C6H6O3
4.	17.302	Benzenemethanol, 4-(1-methylethyl)-	C10H14O
5.	18.798	Eugenol	C10H12O2
6.	22.179	2-Nonyl-1-ol, diethyl acetal	C13H24O2
7.	22.179	8,8-Dimethoxy-1,4-dioxaspiro[4.5]deca-6,9-diene	C10H14O4
8.	31.417	Tricyclo[4.4.0.0(2,7)]dec-8-ene-3-methanol, $\alpha,\beta,\gamma,\delta$ -tetramethyl-, stereoisomer	C15H24O
9.	31.417	Ledene alcohol	C15H24O
10.	31.882	Hexadecanoic acid, methyl ester	C17H34O2
11.	32.510	Hexadecanoic acid, ethyl ester	C18H36O2
12.	32.906	3-Heptyn-2-one, 5-cyclopentyl-6-hydroxy-6-methyl-5-(1-methylethyl)-	C16H26O2
13.	32.906	1,2-Benzenedicarboxylic acid, butyl methyl ester	C13H16O4
14.	33.215	1,3,3-Trimethyl-2-(2-methyl-cyclopropyl)-cyclohexene	C13H22
15.	33.215	Preg-4-en-3-one, 12,17-dihydroxy-20-nitrilo	C20H27NO3
16.	35.228	8-Octadecenoic acid, methyl ester	C19H36O2
17.	35.228	9-Octadecenoic acid, methyl ester, (E)-	C19H36O2
18.	35.727	Octadecanoic acid, methyl ester	C19H38O2
19.	36.441	Ethyl Oleate	C20H38O2
20.	36.923	Octadecanoic acid, ethyl ester	C20H40O2
21.	36.923	Methyl 17-methyl-octadecanoate	C20H40O2
22.	38.600	1-Octanamine, N,N-dioctyl	C24H51N
23.	38.600	Tris(2-ethylhexyl)amine	C24H51N
24.	39.796	1,4-Benzenediamine, N-(1,3-dimethylbutyl)-N'-phenyl	C18H24N2
25.	40.372	Hexanedioic acid, bis(2-ethylhexyl) ester	C22H42O4
26.	42.385	2-Phenylethylamine, N,N-dinonyl	C26H47N

27.	42.835, 49.602	Dinonylneopentylamine	C23H49N
28.	45.757	Benzenamine, 4-octyl-N-(4-octylphenyl)-	C28H43N
29.	45.757	1-Picryl-2,2-bis(p-(1,1,3,3-tetramethylbutyl)phenyl)hydrazyl radical	C34H44N5O6
30.	49.602	Isonipectic acid, N-isobutoxycarbonyl-, undecyl ester	C22H41NO4

Fig1: Chromatograph of GC/MS analysis of Ashokarishta



Discussion

Metabolites are responsible for the therapeutic activity of medicinal herbs. Ayurvedic formulations are polyherbal and used in multiple diseases. Hence, the identification of bioactive compounds responsible for the action is the need of the hour.

The previous analytical studies on Ashokarishta proved the presence of Gallic acid, water-soluble vitamins, Rutin, Protocatechuic acids, and major groups like phenols, flavonoids, saponins, etc with different analytical techniques. Only 3-5 maximum compounds can be identified with these techniques.

The GC is mainly used for the liquid dosage form. It is mainly used in the identification of volatile compounds. The GC-MS also works on the same principle and is widely used for Asava-Arishta preparations. This method aid in the identification of more compounds present in Ashokarishta. A total of 30 compounds have been identified by comparing the spectrum obtained with the database. These compounds are having different therapeutical actions like anti-inflammatory, antitumor, anti-cancer activity, anti-microbial, etc.,

Conclusion

Mass Spectroscopy is the technique of choice in metabolomics because of its high sensitivity and wide range of covered metabolites. GC-MS is often used to qualitatively and quantitatively determine organic compound purity and stability and to identify components in a mixture. This technique is sensitive, possesses a large linear range. It acts as a tool for quality evaluation and scientific validation of herbal products.

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