An experimental approach on analysis of pytoconstituents in the methanolic extract of stem of *Adenium obesum* with the help of LC-MS technique

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ABSTRACT:

Chromatography is a technique for separation that uses a stationary and mobile phase to extract specific compounds from mixtures. Chromatography's discovery is a seminal moment in the field of biomedical research. The principles of adsorption, partition, ion exchange, molecular exclusion, affinity, and chirality serve as the foundation for chromatographic separation. Liquid Chromatography-Mass Spectrometry (LC-MS) is an extremely sensitive and specific analytical method. It is a very strong tool. Liquid chromatography (LC) and mass spectrometry (MS) are combined to form LC-MS. Liquid chromatography's physical separation capabilities are combined with mass spectrometry and mass analysis to create LC-MS, an analytical chemistry technique. Liquid chromatography assembly, ion generating unit/ionization source, mass analyzer, and mass spectrometric data collection are the main components of the LC-MS. In the biomedical sciences, pharmacokinetic analysis, genetic analysis, and structure elucidation are the most frequent uses of LC-MS.

1. INTRODUCTION:

Liquid chromatographers favor Liquid Chromatography/Mass Spectrometry (LC-MS) because of its rapid development. A method called liquid chromatography-mass spectrometry combines mass spectrometry with liquid chromatography (also known as HPLC). It is an analytical chemistry method that combines mass spectrometry's mass analysis skills with liquid chromatography's physical separation capabilities. Components can be separated using liquid chromatography (LC), and sample eluents from LC are subsequently transferred to mass spectrometry (MS) for component detection, identification, and mass determination in the presence of other components. Pharmaceutical drug components, intermediates, and related molecules are determined using LC-MS for both quantitative and qualitative purposes. [1] In vitro dissolution, bioequivalence, bioavailability, and metabolite investigations are the main applications of LC-MS. Moreover, forensic labs, the food industry, agrochemical, and fundamental research all employ LC-MS.

For the qualitative and quantitative examination of drug compounds, drug products, and biological samples, laboratories frequently use LC-MS. It has been consistently utilized in drug development at many phases, such as bio-affinity screening, impurity identification, peptide mapping, glycoprotein mapping, natural products de replication, metabolic stability screening, and metabolite identification in addition to in vitro drug screening. Nowadays, LC-MS is used successfully for routine analysis in a variety of fields, such as doping control, clinical and forensic toxicology, and therapeutic drug monitoring (TDM).[2]

A broad classification of the several techniques used in quantitative analysis is as follows:

1. Chemical/classical Method (Titrimetric, Volumetric, and Gravimetric method)
2. Instrumental Technique (GC, HPLC, Spectrophotometry, Polarography)

The analytical method known as liquid chromatography-mass spectrometry, or HPLC-MS, blends the mass spectrometry's mass analysis skills with the physical separation powers of liquid chromatography (or HPLC). LC-MS is a potent method with excellent selectivity and sensitivity that is employed in numerous applications. It is the most widely utilized method in the field of bio analysis and is regularly employed in pharmacokinetic investigations of medicines. In pharmacognosy, particularly in the area of molecular Pharmacognosy, LC-MS is very useful in determining the differences between the constituents in phenotypic cloning. [3]

Mass Spectrometry and Liquid Chromatography High-Pressure Liquid Chromatography-Mass Spectrometry (HPLC-MS), sometimes known as Liquid Chromatography-Mass Spectrometry (LC-MS), is an analytical method that combines sensitive and targeted mass spectrum detection with high resolution chromatographic separation. This comprises capillary electrophoresis (CE)-MS, capillary electro chromatography (CEC)-MS, and high-performance liquid chromatography (HPLC)-MS. [4]

Mass spectrometry and gas chromatography were combined for the first time in a 1958 study, and the method became commercially available in 1967. The 1980s saw the significant advancement in chromatography history of the combination of LC and MS. In LC-MS, mass spectrometry aids in the structure clarification and elemental composition analysis of a material.

1.1 Principle of LC-MS The LC-MS method use an HPLC to separate the different components of a mixture, followed by ionization and separation of the ions based on their mass/charge ratio. The ions are then separated and routed to a photo or electron multiplier tube detector, which recognizes and quantifies each ion. The ion source is a critical component in any MS study since it assists in the efficient creation of ions for analysis. Ion sources for ionizing intact molecules include APCI (Atmospheric Pressure Chemical Ionization), ESI (Electron spray Ionization), and others. The chemical type of the analyse of interest, polar or non-polar, also influences the choice of ion source. Because the examination is done at the molecular level, the key advantages of this technique include sensitivity, specificity, and accuracy. The structural features of the analyse can also be deduced. A typical LC-MS system uses an interface (ionization source) to combine HPLC and MS. Following LC separation of the sample, the separated sample species are sprayed into an atmospheric pressure ion source to undergo gas phase conversion into ions.

After that, the ions are sorted by mass to charge ratio using the mass analyzer. A detector counts the ions that emerge from the mass analyzer and may also magnify the signal that is produced by each ion. The outcome is the creation of a mass spectrum, which is a plot of the ion signal as a function of the mass-to-charge ratio and is used to ascertain a sample's elemental or isotopic makeup, as well as the masses of particles and molecules, and to clarify the molecular structures. [5]

1.2 Requirement of LC-MS HPLC is typically the LC utilized in LC-MS. In HPLC, the normal phase mode or reverse phase mode of adsorption serves as the separation principle. With polar stationary phase and non-polar solvent/mobile phase, the normal phase constricts, while with non-polar stationary phase and polar solvent/mobile phase, the reverse phase constricts. The normal phase mode is not frequently employed in biological research and is not recommended for use in pharmaceutical applications because the majority of drugs are polar and take longer to elute and detect. There are numerous medicinal applications for reverse phase mode. Reverse phase columns include C18, C8, C4, and octadecylsilane. [6]

1.3 LC-MS Instrumentation Pump, mixing unit (solvent degassing system), injector (manual/auto), guard column, analytical columns, detectors, recorder, and integrators are the main components of an HPLC device.

An LC unit's fundamental parts are as follows:

(1) Pump: this part supplies the mobile phase at the necessary flow rate,

(2) The samples are injected by the auto sampler.

(3) Column – for sample separation.

(4) Detector - for the analysis of the separated components of a sample;

The instrumentation for an LCMS system consists of the following:

(1) An LC unit;

(2) An interface connecting the LC and MS;

(3) An ion source that ionizes samples (such as an API unit)

(4) An ion guide (an electrostatic lens that effectively inserts the produced ions into the MS)

(5) A mass analyzer unit that uses mass-to-charge (m/z) to separate the ions; (6) A detection device that finds the separated ions.

(6) A detector unit that detects the separated ions.

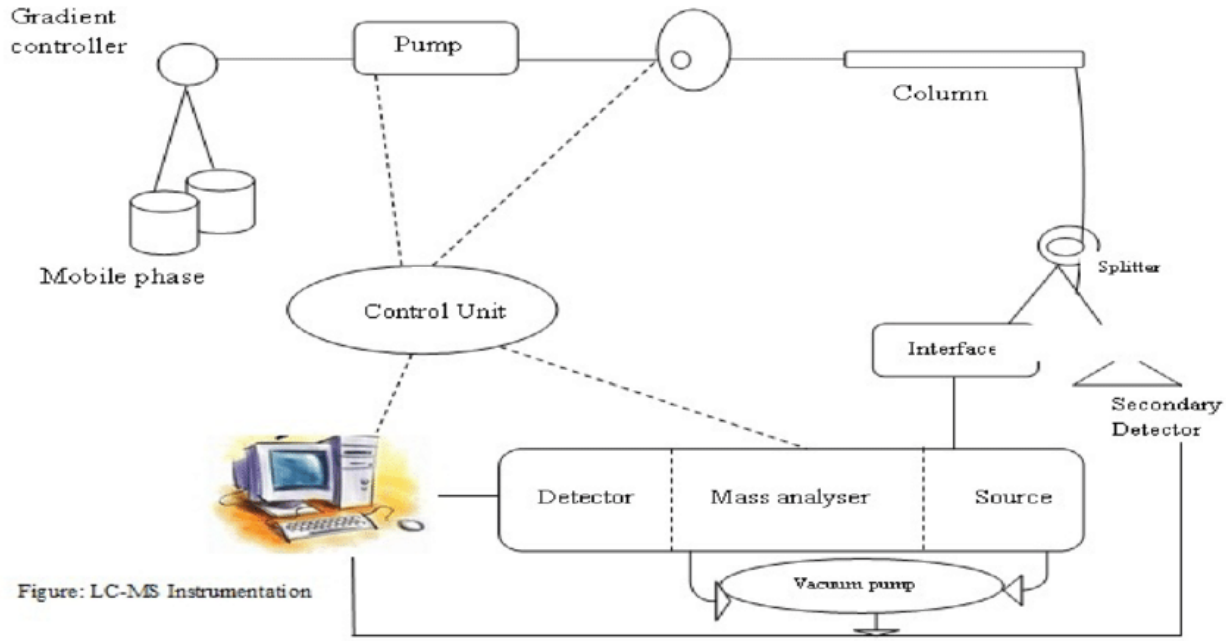

Figure: LC-MS Instrumentation

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1.4 Procedure: The analyte's solid physical or chemical characteristics, such as mass-to-charge ratios, refractive index, or light absorption or emission, are what cause the mobile phase to move through the column and detector. A peak or signal whose peak height or area (intensity) equals the whole quantity of the component present in the sample is what is recorded for this response. Retention time is the amount of time the analyte "sees" by the detector.

The uniqueness of an analytic can be verified by comparing its relative tension to that of a recognized standard, making sure blank samples exhibit no detectable signal, and observing that the signal increases predictably with concentration. Even though this is not an exact way to identify compounds, it is useful when there is little information available about a sample. While numerous detectors with varying sensitivity and technology have been used with liquid chromatography (LC) to analyze various sample types, the mass spectrometer (MS) has become a highly effective, sensitive, and versatile detector that provides enhanced efficiency, selectivity, and sensitivity. [7]

The LC eluent transporting the analyses cannot flow directly into the mass spectrometer, despite the fact that several detector types allow for flow-through analysis. Through an interface (or ion source), the mass spectrometers and the LC MS system are connected, and LC is run at standard back pressures. A vacuum is used to operate the mass spectrometer detector. Heat and voltage are applied to the solvent to cause it to ionize or evaporate as the column eluent runs. The next step is to introduce the charged analytic molecules into the interface; this is crucial since only ionized particles can be detected and measured by the mass spectrometer.

The technique is known as atmospheric pressure ionization, or API (atmospheric pressure ionization), and the interface is referred to as the API source because the analytic ions are produced there at atmospheric pressure. The two sources that are most frequently employed in liquid chromatography-mass spectrometry are atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI). Analyze ions are attracted to magnetic and electric fields within the MS. By altering the applied fields, the ions' flight trajectories are changed, resulting in their separation from one another according to their mass-to-charge values. After the separation, the ions might accumulate and be found by different mass detectors. The electron multiplier is the mass detector that is most frequently employed.[8] Secondary electrons are emitted when the unattached ions come into contact with the electron-multiplier's (a dynode) surface. By passing these secondary electrons through a series of dynodes, they are multiplied. At every given moment in time, the ion intensities in the mass spectrometer are monitored and linked to the amplified current generated by the secondary electron flow. In order to distinguish between mass-to-charge ratios, the integrated software tracks and modulates rapid changes in the hardware. The reported mass-to-charge ratios are based on the electromagnetic fluctuation in each of the quadruples as ions approach the detector. [9]

2.0: Material & methods: Numerous factors and parameters must be considered and adjusted while setting up an LC-MS test. Giving general conditions is challenging because they largely depend on the characteristics of the analytic and the LC separation. Analyses need to be optimized separately. While published procedures provide a useful foundation, test performance and ideal circumstances might fluctuate significantly between various instruments and sample matrices. The assay conditions and the instrument being used have a significant impact on sensitivity. Mass spectrometer sensitivity is constantly being increased by instrument makers, who typically provide a variety of models with varying sensitivity levels.

For this reason, determining whether an instrument has the sensitivity required to reach the intended limits of detection is crucial. The best ionization technique and polarity will be determined in large part by the characteristics of the analytic. Derivatization is another way to improve the detection of particular analyses. [10]

Method Condition

LC conditions		
Eluent:	A: 0.1% formic acid in water B: 0.1% formic acid in acetonitrile	
Injection volume:	10 µL	
Flow rate:	0.5 mL/min	
Gradient:	Time (min)	% B
	0.0	5
	0.5	5
	1.0	40
	1.5	60
	2.5	95
	6.0	95
	6.1	10
Stop time:	6.2 min	
Post run:	2.5 min	
Max pump pressure:	400 bar	
Needle wash:	Flush port with 75:25 acetonitrile:water for 10 s	
Overlapped injection:	Disabled	
Automatic delay volume reduction:	No	
ES source parameters		
Ionization mode:	Positive	
Capillary voltage:	3,600 V	
Drying gas flow:	7 L/min	
Drying gas temperature:	350 °C	
Nebulizer gas:	40 psi	
Sheath gas flow:	9 L/min	
Sheath gas temperature:	350 °C	
Nozzle voltage:	0 V	
MS parameters		
Scan type:	MRM	
Delta EMV:	(+) 300 V	

Sample Preparation

LCMSMS Sample Preparation:

- Sample Weight 50 mg (approx.)
- Extracted in N-Hexane 10 ml for 24 hrs. in a Soxhlet apparatus.
- Sample was air dried and extracted with methanol 10 ml.
- Sample drying at 40 degrees onto rotatory evaporator
- Make up with 1 ml methanol
- Inject into LCMSMS

3.0: Applications of LC-MS technique

3.1: Uses of LC-MS technique in the assessment of microbial infections:

Effective management of emerging infectious illnesses necessitates the use of quick and accurate diagnostic techniques. Because human body fluids reflect patients' clinical status and may be collected through minimally intrusive sampling procedures, they are appealing clinical samples for identifying diagnostic targets. Viral, bacterial, and parasitic infections can thrive in bodily fluids. Consequently, in an effort to identify diagnostic markers that are pathogen- or human-originated, contemporary clinical proteomics techniques have concentrated on bodily fluids. In this regard, state-of-the-art liquid chromatography–mass spectrometry (LC-MS)–based proteomics has been utilized; it is regarded as one of the most selective and sensitive proteomics methods. [11]

The ongoing coronavirus disease of 2019 pandemic serves as stark reminder of the serious hazards that infectious diseases pose to international health. Despite the availability of numerous therapies, infectious diseases continue to rank among the world's leading causes of death. Consequently, it is imperative to have a deeper understanding of the molecular physiology of pathogens, which include bacteria, viruses, fungi, and parasites. Finding the molecular characteristics of bacterial pathogens that contribute to their pathogenicity is the first step in studying them. These characteristics may serve as therapeutic targets, and bacterial infections can be eliminated or combated by inhibiting these traits. [12] Because proteomics can give large-scale protein information involved in the etiology, infection mechanisms, and clinical symptoms of hosts, it is a valuable technique for investigating infectious diseases. From gel-based techniques to gel-free mass spectrometry (MS) methodologies known as "shotgun" proteomics, modern proteomic technologies have developed. Shotgun proteomics, which has transformed the field of proteome research by enabling large-scale protein characterization with high throughput, uses nano-high precision liquid chromatography (HPLC) equipment connected to high-resolution mass spectrometers. MS-based proteomics has several benefits for pathogen detection in the infected host or for the identification of potential biomarker candidates. [13] Compared to immunoassays, MS-based proteomics performs well in terms of detection limits, repeatability, and reproducibility. This method has the benefit of being able to be used multiplexed without the need for antibodies or similar binders. Furthermore, a lot of hospital laboratories now routinely use MS-based analysis for testing for antibiotic resistance as well as for the regular identification of bacterial infections in clinical samples. Based on target samples, proteomic research pertaining to infectious diseases can be divided into three groups: (1) pathogen in pure culture; (2) host proteome infected; and (3) pathogens within the host that is infected. [14]

3.2: LC-MS in drug metabolism studies: Before a compound can be developed into a drug, it must be studied extensively to determine its potential therapeutic value. An attractive candidate should be metabolically stable and possess a good pharmacokinetic profile.

Pharmacokinetics describes how the body affects drug absorption, distribution, metabolism, and excretion, commonly known as ADME or pharmacokinetics of the drug. Furthermore, the drug should demonstrate good bioavailability, which is defined as the quantity of administered drug that reaches the circulation and persists there for a satisfactory period during which it can produce the desired action. [15] This latter characteristic is called a half-life. An active metabolite is formed from the compound after the candidate is metabolised by the body. The metabolite(s) may be more pharmacokinetically active than the 'parent' candidate drug. Consequently, accurate main and minor metabolite characterization facilitates the discovery of medications with enhanced pharmacological activity, stability, and metabolic stability. Additionally, by improving the medicine's toxicological profile, this analysis guarantees drug safety. [16]

3.3: In Vitro drug metabolism: This branch of study is concerned with analysing the effects of a drug on organs outside the body. The liver is the organ primarily responsible for drug metabolism. For this reason, liver microsomal preparations or hepatocytes are used as the tissue for in vitro testing and provide an indication of the metabolic outcomes of a drug.3-hydroxytamoxifen is a drug derived from tamoxifen, an anti-breast cancer agent that is used to demonstrate the application of LC-MS in drug metabolism. 3- hydroxytamoxifen tested on human liver microtomes is an example of *in vitro* metabolism study.[17]

3.4: In Vivo Drug Metabolism: *In vivo* metabolism studies involve monitoring drug discovery in the body with a focus on drugs and metabolites in blood, urine, and faeces.

Samples taken from these sources contain endogenous compounds that have the potential to compromise the resolution obtained by LC-MS. It is, therefore, necessary to implement a good sample preparation technique and chromatographic separation is required to successfully apply LC-MS. This is exemplified by the *in vivo* analysis of 3-hydroxytamoxifen and its metabolites from the plasma of breast milk taken from a breast cancer patient. [18]

3.5: Identification of impurities and degradation products: To make sure that the medicine is responsible for the reported pharmacological and toxicological effects rather than impurities, it is crucial to identify impurities and degradation products. In order to stop dangerous substances from being produced while the medication is on the market, it is crucial to analyses the contaminants and degradation products.

Understanding degradation products improves comprehension of potential side effects and facilitates the creation of novel, more stable medications. The majority of drug impurities are created when pharmaceuticals are synthesized from basic ingredients. Byproducts can also develop during the synthesis process. [19]

Because the mixture produced by the synthetic process contains a variety of component concentrations, sensitive and specific detection is required to guarantee their removal. This explains why LC-MS is used so frequently in this procedure.

4.0: Analysis of methanolic extract of *Adenium obesum* sample with help of LC-MS technique:

Since ancient times, medicinal plants have been essential to the advancement of humankind and the treatment of a wide range of illnesses. The only secure sources of novel medications to treat both curable and incurable illnesses are medicinal plants. Approximately 25% of prescription medications on the market today come from natural resources. *Adenium obesum*, a member of the Apocynaceae family, is one of the few native medicinal plants found in Oman. The chosen plant contained various groups of chemical components, including carbohydrate, cardiac glycoside, flavonoid, crenelated flavonoids, terpenoids, prenames, etc., according to a biochemical analysis of the local Adenium species. Furthermore, the chosen Omani species' pharmacological activity demonstrated noteworthy antibacterial, antioxidant, anticancer, antiviral, and immunological modulatory properties. [20]

The native AO plant species have been the subject of very few scientific studies. Because of this, it is imperative that people, especially the younger generation, understand the toxicity, biochemistry, and pharmacological properties of the chosen Adenium plant species. This thorough review report covers phytochemicals, biological activity, taxonomy, and possible applications of the chosen medicinal plant. The study comes to the conclusion that plant extracts or isolated phytochemicals may be utilized as unique, safe medications in the near future for the treatment and prevention of various illnesses. [21]

Metabolic extract of *Addendum obesum* is packed with bioactive compounds. Among the significant compounds are beta-sitosterol, polyphenols, flavonoids, and rosemarinic acid etc.

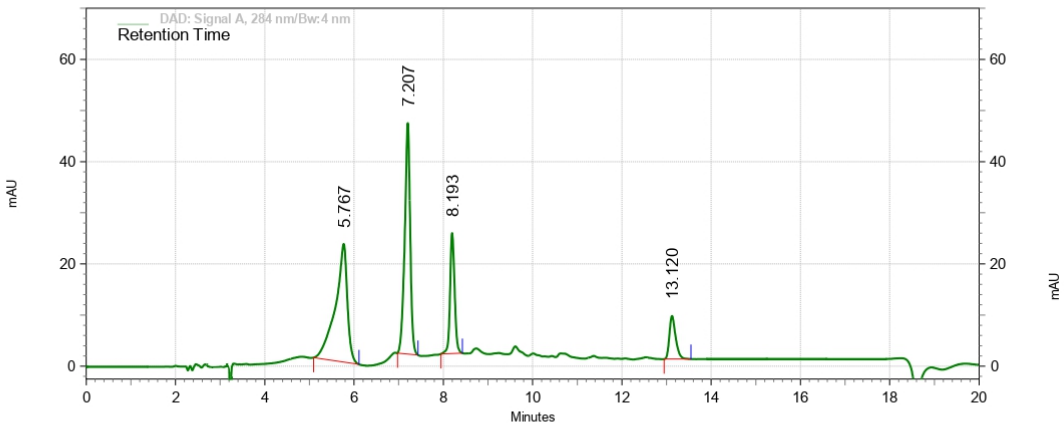
Quantitative Analysis Sample Report

hydroxyphenylacetic acids	6.454	185413	480.0000	mcg/kg
3,4-dihydroxyphenylacetic acid	6.518	35178	442.0000	mcg/kg
2-hydroxy-2-phenylacetic acid	6.689	2078	166.0000	mcg/kg
hydroxyphenylpentanoic acids	6.704	183473	175.0000	mcg/kg
3-hydroxy-3-(3-hydroxyphenyl)propionic acid	7.588	344193	271.0000	mcg/kg
(+)-catechin 3-*O*-gallate	7.609	140339	274.0000	mcg/kg
(+)-gallocatechin 3-*O*-gallate	7.736	154358	129.0000	mcg/kg
(−)-epigallocatechin	7.815	105280	227.0000	mcg/kg
procyanidin trimer C1	7.961	57531	474.0000	mcg/kg
cinnamtannin A2	8.082	11012	107.0000	mcg/kg
(−)-epicatechin	8.263	261452	104.0000	mcg/kg
4″-*O*-methylepigallocatechin 3-*O*-gallate	8.264	231580	481.0000	mcg/kg
procyanidin dimer B1	8.473	112349	540.0000	mcg/kg
Neritaloside	8.597	38440	78.0000	mcg/kg
naringin	8.722	199063	346.0000	mcg/kg
Honghelin	8.854	194299	91.0000	mcg/kg
hesperidin	8.895	116122	275.0000	mcg/kg
Rosemarinic acid	9.081	295808	543.0000	mcg/kg
apigenin 7-O-apiosyl-glucoside	9.123	276639	442.0000	mcg/kg
apigenin 7-O-glucuronide	9.287	26576	150.0000	mcg/kg
apigenin 6,8-di-C-glucoside	9.377	264250	440.0000	mcg/kg
Oleanolic acid	9.514	436876	519.0000	mcg/kg
apigenin 6-C-glucoside	9.676	104039	80.0000	mcg/kg
neodiosmin	9.716	127475	393.0000	mcg/kg
patuletin	9.919	64119	392.0000	mcg/kg
3-O-glucosyl-(1->6)-[apiosyl(1->2)]-glucoside				mcg/kg
quercetin 3-O-xylosyl-rutinoside	10.371	855408	199.0000	mcg/kg
myricetin 3-O-rutinoside	10.479	20422	76.0000	mcg/kg
quercetin 3-O-glucosyl-xyloside	10.507	452348	408.0000	mcg/kg
kaempferol 3,7-O-diglucoside	10.556	3406	216.0000	mcg/kg
myricetin 3-O-glucoside	10.739	29613	145.0000	mcg/kg
kaempferol 3-O-glucosyl-rhamnosyl-galactoside	10.833	36162	242.0000	mcg/kg
kaempferol 3-O-(2″-rhamnosyl-galactoside) 7-O-rhamnoside	10.945	4300	108.0000	mcg/kg
				mcg/kg
quercetin 3′-O-glucuronide	11.093	567900	175.0000	mcg/kg
myricetin 3-O-rhamnoside	11.272	189474	170.0000	mcg/kg
quercetin 3-O-arabinoside	11.322	326216	363.0000	mcg/kg
isorhamnetin	11.416	305651	332.0000	mcg/kg
dihydrochalcones	11.522	13954	459.0000	mcg/kg
3-hydroxyphloretin 2′-O-xylosyl-glucoside	11.635	56793	187.0000	mcg/kg
3-hydroxyphloretin 2′-O-glucoside	11.706	279595	344.0000	mcg/kg
phloridzin	11.805	278976	261.0000	mcg/kg
anthocyanins	12.012	165071	239.0000	mcg/kg
peonidin 3-O-diglucoside-5-O-glucoside	12.131	825636	145.0000	mcg/kg
cyanidin 3-O-(6″-p-coumaroyl-glucoside)	12.274	593935	409.0000	mcg/kg
delphinidin 3-O-glucoside	12.361	158245	177.0000	mcg/kg

Quantitative Analysis Sample Report

delphinidin 3-O-glucosyl-glucoside	12.485	81014	65.0000	mcg/kg
isopeonidin 3-O-arabinoside	12.582	112968	86.0000	mcg/kg
cyanidin 3,5-O-diglucoside	12.632	88400	398.0000	mcg/kg
pelargonidin 3-O-rutinoside	12.766	91061	70.0000	mcg/kg
Beta-sitosterol	12.888	84742	532.0000	mcg/kg
5,6,7,3′,4′-pentahydroxyisoflavone	13.035	239694	317.0000	mcg/kg
6″-O-acetyldaidzin	13.240	64871	446.0000	mcg/kg
3,5,7,3,4,5-Hydroxy flavonone	13.387	492839	320.0000	mcg/kg
3′-hydroxydaidzein	13.619	228757	358.0000	mcg/kg
Ursolic acid	13.788	226902	85.0000	mcg/kg
3′-hydroxygenistein	13.910	45909	364.0000	mcg/kg
dihydrobiochanin A	14.024	194915	443.0000	mcg/kg
5,7,3,4-Tetrahydroxy flavone	14.101	197210	388.0000	mcg/kg
3-Acetoxy ursolic acid	14.353	125408	88.0000	mcg/kg
coumarin	14.438	560	171.0000	mcg/kg
esculin	14.638	5603	292.0000	mcg/kg
Stigmasterol	14.779	61866	241.0000	mcg/kg
scopoletin	14.818	142959	505.0000	mcg/kg
alkylmethoxyphenols	15.127	533112	537.0000	mcg/kg
4-vinylsyringol	15.234	91460	130.0000	mcg/kg
hydroxybenzoketones	15.418	138903	215.0000	mcg/kg
2,3-dihydroxy-1-guaiacylpropanone	15.875	458347	455.0000	mcg/kg
2-hydroxy-4-methoxyacetophenone 5-sulfate	16.480	3595	372.0000	mcg/kg
hydroxytyrosol 4-O-glucoside	8.264	124797	487.0000	mcg/kg
demethyloleuropein	8.729	206757	492.0000	mcg/kg
3,4-DHPEA-AC	8.895	63118	516.0000	mcg/kg
todolactol A	9.083	267752	496.0000	mcg/kg
7-hydroxymatairesinol	9.142	9091	241.0000	mcg/kg
Stilbenes	9.151	141612	253.0000	mcg/kg
matairesinol	9.181	119015	258.0000	mcg/kg

Data File: D:\Sample_Extract.dat
Method: D:\HPLC DATA FOOD\METHOD\Antioxidant.met
Acquired: 03-12-2022 20:00:00 (GMT +05:30)
Printed : 05-12-2022 16:44:06 (GMT +05:30)
SampleID: Sample Extract_1
Injection Vol: 20



DAD: Signal A, 284 nm/Bw:4 nm Results

Name	Retention Time	Area	Conc.(mg/kg)
BHT	13.120	172718	42.56
Totals			

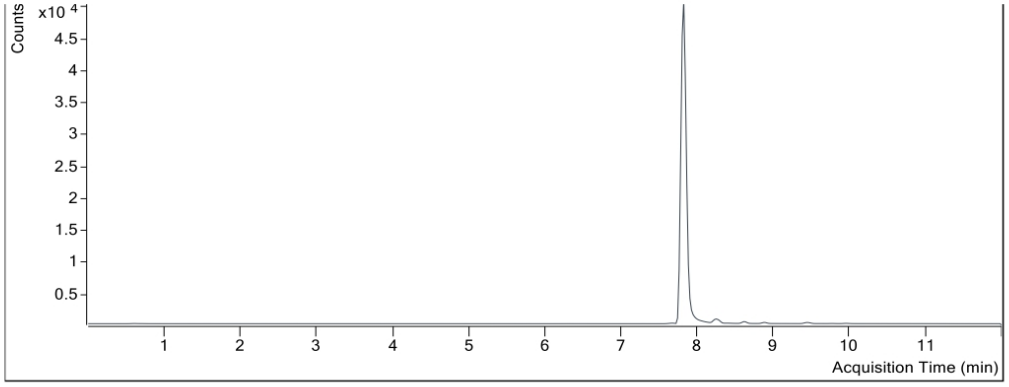
Quantitative Analysis Sample Report

Batch Data Path	D:\Data\2022.batch.bin		
Analysis Time	12-5-2022 9:33 PM	**Analyst Name**	DESKTOP-1F4BJ25\Agilent
Report Time	12-5-2022 9:33 PM	**Reporter Name**	DESKTOP-1F4BJ25\Agilent
Last Calib Update	12-5-2022 9:33 PM	**Batch State**	Processed

Analysis Info

Acq Time	03-12-2022 15:22	**Data File**	Sample_Extract.d
Position	P1-A2	**Sample Name**	Sample_Extract.d
Dilution	1	**Sample Info**	
Inj Vol	20.00	**Acq Method File**	Ascorbic Acid.M
Sample Type	Sample	**Comment**	

Sample Chromatogram



Quantitation Results

Compound	RT	Response	Conc	Unit
Ascorbic Acid	7.827	91383	53.7800	mg/kg

5.0: Result & discussion:

Here, we describe a sensitive and quick LC-MS method for determining the chemical composition of the *Adenium obesum* extract quantitatively. The present article suggested method works well for precisely measuring the bio actives found in the Adenium extract. The effectiveness of plant extracts has been demonstrated by the analysis of numerous chemical components from the methanolic extract of Adenium obesum. Target substances, also known as analyses, are physically separated in LC-MS analysis and then their mass is detected. Despite being relatively new, its sensitivity, selectivity, and accuracy have made it the method of choice for identifying levels of micrograms or even monograms of a wide range of analyses, including natural product extracts, pesticides, drug metabolites, and food adulterants.

Conflict of interest:

There is no any conflict of interest in this work, according to the authors.

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