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Evaluating and Measuring Toxic Metal Impurities Assessment in Pharmaceutical

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Abstract

The evaluation and measurement of toxic metal impurities in pharmaceutical products are of paramount importance to ensure the safety and efficacy of medications. This abstract explores the significance of assessing and measuring toxic metals in pharmaceuticals and highlights the methods and regulatory frameworks involved in this critical process. Toxic metal impurities, such as arsenic, lead, cadmium, and mercury, can pose severe health risks when present in pharmaceutical formulations. Their presence can result from various sources, including raw materials, manufacturing processes, and packaging materials. Therefore, rigorous monitoring and control measures are essential to safeguard public health. Pharmaceutical regulatory agencies worldwide, including the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA), have established stringent guidelines and limits for toxic metal impurities in pharmaceuticals. These guidelines outline acceptable levels of such impurities to ensure that the risk to patients remains minimal Various analytical techniques, such as inductively coupled plasma-mass spectrometry (ICP-MS) and atomic absorption spectrometry (AAS), are employed to quantify toxic metal impurities accurately. These methods offer high sensitivity and precision, enabling pharmaceutical manufacturers to comply with regulatory requirements and meet quality standards. Additionally, risk assessment plays a pivotal role in determining the need for toxic metal testing and setting appropriate limits. Factors like the intended use of the pharmaceutical product, patient population, and potential exposure pathways are considered in this process.

Selection of plant materials for the present study

Fruits, vegetables, and medicinal plants are significant sources of bioactive substances having pharmacological and therapeutic value. Finding bioactive chemicals involves a crucial procedure called extraction. Driving on the easily accessible plants will be far more engaging and appreciated in order to encourage society to use natural goods directly instead of rare species, which will only be seen as subjects of study. A random selection process or ethnopharmacology, in which knowledge of specific medicinal characteristics passed down

from generation to generation, particularly among traditional healers, may be used to choose plant materials for biological activity screening. Chemotaxonomy, a discipline that focuses on the relationship between related plant species and the existence of comparable secondary metabolites, provides an alternative method for identifying plants for the study of their unexplored chemical contents.

Traditional medicine has long relied on plants to heal a wide range of illnesses and ailments. The present investigation was designed to evaluate an interdisciplinary study starting from phytochemistry, pharmacological activities on whole plant of Allmanianodiflora, Albizia saman (leaves, seed), Diospyros montana (bark, leaves), and Phyllanthus maderaspatensis (root). This was done using a novel approach, different from previous reports.

Research Methodology

Heavy Metal Analysis in Cholic Acid by Q-ICP-MS

Cholic acid of synthesis grade used in the study is procured from Suvidhinath laboratories. Nitric acid (65%), ethylene diamine tetra acetic acid sodium salt AR grade, and certified reference metal stock standard solutions (1000 mg/L) of V, Co, Ni, Cd, Hg, Pb, and As prepared in 2–3% HNO_3 of analytical grade were purchased from Merck (Darmstadt, Germany). Deionized water was prepared using a Milli-Q plus water purification system from Millipore (Bedford, MA, USA). Yttrium standard for ICP TraceCERT® (1000 mg/L Y in nitric acid), bismuth standard for ICP TraceCERT® (1000 mg/L Bi in nitric acid), nitric acid ≥69.0%, TraceSELECT™ for trace analysis from Honeywell were used for the study. All the autosampler vials, centrifuge tubes, and plastic bottles, were cleaned by soaking in 20% v/v HNO_3 analytical grade reagent for 4 h, followed by rinsing with deionized Milli-Q water thrice. Element impurities according to ICH Q3D, Standard 1 (containing 15 ppm of Arsenic (As), 5 ppm each of Lead (Pb) and Cadmium (Cd), 30 ppm of Mercury (Hg), 50 ppm of Cobalt (Co), 100 ppm of Vanadium (V), 200 ppm of Nickel (Ni) and three other elements i.e., 150 ppm each of Selenium (Se) and Silver (Ag) and 8 ppm of Thallium (Tl) multi-standard were procured from Sigma-Aldrich.

Sample preparation

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Weighed accurately about 100 mg of cholic acid commercial sample into a 15 mL calibrated plastic tube. Transferred 90 mL of (65%) HNO_3 into 3000 mL volumetric flask containing 1000 mL of deionized water mixed well and diluted up to the mark with water, and shaken well. Added 3 mL of concentrated nitric acid to the sample in the sample tube and allowed the sample to digest with intermittent shaking. After sample digestion, when the sample became clear and no more fumes of nitric acid were evolved from the sample tube, the content is made up to 10 mL mark with water.

Microwave Digestion

There are open and closed-vessel approaches to microwave-assisted digestion. A closed vessel method is appropriate for a majority of pharmaceutical applications. Digestion was performed using Mth 2018–001, STD 75 manufactured by PerkinElmer 16 position unit size microwave digestion system. Weighed accurately 0.2 g sample into 10 mL volumetric flask and mixed it with 7.0 mL conc. HNO_3. Transferred into the digester vessel and selected the digestion method as above and digested the sample. Cooled to the room temperature and transferred into 10 mL volumetric flask and made up with purified water. Pipetted out 5.0 mL into 10 mL volumetric flask and dilute up to the mark with deionized water.

Results and Discussion

Bioassay-Directed Segregation and Construction Clarification of Dynamic Mixtures Preparative.

Normal phase preparative HPLC was used to fractionate and purify the cold-preserved petroleum ether extract. The following are the prerequisites: Mobile phase: Chloroform: MeOH 95:5; flow rate: 15 ml/min; injection volume: 2.0 ml; run time: 20 minutes; detection at (max): 280 nm; column: silica column, 250 x 21.5 mm, 10 um (Unisphere). A sample of the main peak from the chromatogram in was taken, dried, and sent to HTS for analysis of its activity. According to HTS measurements, the peak has maintained its level of activity.

Excitingly, the HRMS and NMR analyses of this noteworthy peak revealed that it was composed of three different chemicals. There were three peaks seen in the HRMS, each corresponding to a different molecular ion: m/z 293 (M+H), m/z 291 (M+H), and a little peak with a probable mass peak at m/z 295 (M+H). The inhomogeneity of the preparative HPLC sample meant that further analytical reverse-phase HPLC separation was required to identify the pure active chemicals (responsible for the individual mass peaks seen in the HRMS data).

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Once again, the procedure prerequisites are as follows: Mobile phase: gradient combination of 0.1% formic acid and acetonitrile; column: reverse phase, 150 x 4.6 mm, 2.7 micron (Ascentis); flow rate: 1.0 ml/min; injection volume: 5.0 l; run time: 10 min; detection at (max): 295 nm.

Separately isolating the chemicals at peak A and peak B allowed for the creation of dry, solid samples of both compounds via lyophilization. Using LC-MS/MS, we found that the mass of the compound at peak A was m/z 291 (M+H), while the mass of the compound at peak B was m/z 293. Compound B with molecular weight m/z: 292 (peak B) showed good activity in the glucose-uptake HTS experiment at 30 M concentration, so it was chosen for more studies to elucidate its structure and biological function.

Structure elucidation of compound B

The substance was obtained in the form of a solid that ranged in colour from white to off-white and had a purity level of 99.7 percent. The FT-IR data that was acquired in the KBr pellet suggested that the structure may contain carbonyl, methyl, and methylene moieties. (High-resolution mass) ESI-HRMS (-ve ion) measurements obtained for the chemical revealed a peak at m/z 291.1955 [M-H].The calculated mass was 292 in 1966.

The 1H NMR spectra that was acquired for the chemical (500 MHz, CDCl3) revealed peaks that appeared in the aliphatic portion of the spectrum. These peaks are suggestive of a scaffold that is abundant in methylene groups. In the spectrum, there were clearly distinguishable peaks that corresponded to approximately 28 protons. The 13C-NMR spectrum revealed the presence of two carbonyl carbons, normally responsible for carbonyl carbons (C 178.28 and C 199.63 ppm), as assessed by these two values.

Through the utilisation of 2D and 1H13C HSQC techniques, an unambiguous HSQC spectrum was achieved, which demonstrated the presence of eleven methylene, two (ene) CH, and one methyl system inside the structure. In order to determine further the structure's connectedness, an analysis of the 2D-COSY spectral data as well as an analysis of the 2DHMBC spectral data were both carried out. An intriguing COSY correlation was seen between a doublet of protons in the ene system (with a H value of 6.67 and a C value of 124.07 ppm) and a doublet of CH protons (with a H value of 6.46 and a C value of 136.51 ppm). A strong HMBC association may be seen between carbonyls measured at ppm C 199.63 and the ene system. A HMBC association was found between the CH group and another CH2 group (H 2.36, C 19.83 ppm).

Last but not least, it demonstrated HMBC association with quaternary carbons, which sheds light on the triple bond. These correlations between CH2 groups in the 2D COSY spectra were quite comparable to those reported in a succession of CH2 groups in a linear aliphatic chain. These correlations were caused by two triplet protons. There was a COSY correlation between the CH2 protons that appeared at H 2.36 ppm and those that appeared at H 1.56 ppm (C 28.02 ppm), and this correlation was further connected with the CH2 proton H 1.34 ppm and H 1.34 ppm (C 31.04 and C 22.15, respectively). At the very end of the carbon chain, a CH3 molecule, also known as a methyl group, exhibits a triplet with a H value of 0.92 ppm and a C value of 13.94. On the opposite side, a CH2 triplet with a H value of 2.53 and a C value of 40.81 exhibited correlations between COSY and its surrounding CH2 protons.

It was inferred, on the basis of these correlations, that the CH2 groups were arranged in a seven-carbon long chain of the aliphatic skeleton, with the CH2 group H 2.36 (C 34.01 ppm) at one end of the chain. This chain began at the other end with the CH2 group. Based on the typical chemical shift values of the CH2 group, which was observed to have a H value of 2.53, it was hypothesised that it was situated directly next to the carbonyl group, which had a C value of 199.63 ppm. In the same manner, the other end CH2 H 2.36 ppm was discovered next to carbonyl group groups that had a concentration of 178.24 ppm of carbonyl.These two CH2 units were located between two carbonyl groups, creating a sandwich structure.

Additional research was used to assist in doing an in-depth investigation into the relationships between COSY, HSQC, and HMBC. A triple bond structure was postulated for the compound, and this was done on the basis of the typical chemical shift values of each structural fragment and compound. Therefore, after examining all of the available data, (E)-9-octadec-10-en-12-ynoic acid was determined to be the most accurate representation of the compound's overall structure. For brevity's sake, we'll refer to compound B from now on as (E)-9-oxooctadec-10-en-12-ynoic acid (compound-1).

IR, Elemental analysis (CHN), 1H, 13C, H H-COSY, HSQC and HMBC correlation data of (E)-9-octadec-10-en-12-ynoic acid provided in Tables. UV Spectrum: (E)-9-octadec-10-en-12-ynoic acid in methanol displays max at 270 nm.

Table 1: 5 IR spectrum (E)-9-octadec-10-en-12-ynoic acid; KBr Pellet

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No.	Wave no. in cm-1	Intensity	Assignment
1	2931	Strong	CH3, CH2 and CH Stretching
2	2213	Strong	C≡C stretch
3	1696, 1598	Strong	Aldehyde C=O Stretch
4	1120, 1267, 1312, 1467	Strong	CH2 bending
5	980, 1075, 1115	Strong	CH2 bending

Table 2: Elemental Analysis of (E)-9-octadec-10-en-12-ynoic acid

Elements	Calculated	Found
C	73.95	74.08
H	9.67	10.10
N	0	0

Table 3: 1H NMR Spectrum of (E)-9-octadec-10-en-12-ynoic acid; 500 MHz; Solvent: DMSO-d6

Sr. No.	Chemical shift (δ ppm)	Multiplicity and no. of protons	J (Hz)	Assignment
1	11.97	1H	-	-OH
2	6.72	d, 1H	18	H on C-11
3	6.48	d, 1H	18	H on C-10
4	2.60	t, 2H	9	CH2 on C-8
5	2.52	solvent	-	DMSO-d6
6	2.44	t, 2H	14	CH2 on C-14
7	2.20	t, 2H	9	CH2 on C-2

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8	1.53	m, 4H	-	CH2 on C-15
9	1.51	m, 6H	-	CH2 on C-3, C-7
10	1.37	s, 4H	-	CH2 on C-16
11	1.34	s, 4H	-	CH2 on C-17
12	1.26	s, 6H	-	CH2 on C-5,C-4, C-6
13	0.89	t, 3H	9	CH3 on C-18

Table 4: 1H13C-HSQC data of (E)-9-octadec-10-en-12-ynoic acid; 500 MHz; Solvent: DMSO-d6

Sr. No.	The chemical shift of carbon (δ ppm)	Shows cross-peak with proton signal at δ ppm	Assignment of Carbon	Assignment of Proton
1	137.67	6.48, d, 1H	C-10	H on C-12
2	123.82	6.72 d, 1H	C-13	H on C-13
3	40.14	2.60, t, 2H	C-10	CH2 on C-10
4	34.11	2.20, t, 2H	C-4	CH2 on C-4
5	30.96	1.37,m,2H	C-18	CH2 on C-8
6	29.03	1.26, s, 2H	C-8	CH2 on C-16
7	28.90	1.26, s, 2H	C-6, C-7	CH2 on C-6 and C-8
8	27.99	1.53, m, 2H	C-17	CH2 on C-18
9	24.93	1.51, m, 2H	C-5	CH2 on C-3
10	23.93	1.51, m, 2H	C-9	CH2 on C-17
11	22.10	1.34, m, 2H	C-19	CH2 on C-5
12	19.49	2.44, m, 2H	C-16	CH2 on C-14
13	14.32	0.89,t,3H C	C-20	CH2 on C-20

Conclusion

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The evaluation and measurement of toxic metal impurities in pharmaceuticals are indispensable practices that underscore the commitment of the pharmaceutical industry to patient safety and product quality. This critical assessment process, guided by stringent regulatory frameworks, ensures that pharmaceutical products are free from harmful levels of toxic metals, such as arsenic, lead, cadmium, and mercury. The presence of toxic metal impurities in pharmaceuticals can have serious health consequences, making their assessment a non-negotiable aspect of drug development and manufacturing. Regulatory agencies like the FDA and EMA have set clear guidelines and limits for these impurities, serving as a vital safeguard for public health. The application of advanced analytical techniques, including ICP-MS and AAS, empowers pharmaceutical manufacturers to accurately quantify and control toxic metal impurities, maintaining the integrity of their products. These methods offer both precision and sensitivity, ensuring that impurity levels remain well below established limits. Furthermore, risk assessment is a crucial component of this process, allowing pharmaceutical companies to tailor their evaluation efforts to specific product characteristics, patient populations, and potential exposure pathways. This risk-based approach enhances the effectiveness and efficiency of toxic metal impurity assessments. In the pursuit of safer and more effective pharmaceuticals, the ongoing commitment to evaluating and measuring toxic metal impurities is unwavering. By adhering to robust regulatory standards, leveraging advanced analytical tools, and considering risk factors, the pharmaceutical industry continues to prioritize patient well-being, providing assurance that medications are not only therapeutic but also devoid of potential harm from toxic metal impurities.

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