Using GC-MS for natural product analysis in Nigeria: Problems and prospects

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Abstract

The absence or idle state of analytical equipment such as nuclear magnetic resonance spectroscopy, mass spectrometers and chromatographic techniques has constrained natural products research in Nigeria. Presently, gas chromatography-mass spectrometry has become the more easily available method in Nigeria for the identification of natural products in extracts. This method is sometimes fraught with non-reproducible results and the identification of non-natural or biosynthesized compounds. Several of the extracts analyzed are not volatile, some of the analysts are not competent and certified reference standards are hardly used. These coupled with poor library or database matches and data processing among others lead to the identification of compounds not recognizable from the extract or plants materials under study. This review discusses some of the problems involved in the use of this method for dereplication studies of plant extracts in Nigeria and makes some suggestions for improvement.

Key words: Dereplication, natural products, medicinal plants, extracts, GC-MS, spectroscopy

Introduction

The relevance of natural products and medicinal plants in drug discovery and identification of active molecules has been well reviewed [1-6]. The problem today is the slow pace of discovery of new active compounds from reportedly active medicinal plant extracts [7]. The identification of novel drug candidate molecules from forests, soils, oceans, fungi, bacteria or other microbial sources requires considerable investments in techniques, equipment, time and human resources [8]. While several reports of active extracts abound, they are not backed up by the isolation and characterization of their true active constituents. Rather efforts to determine the active constituents have ended up in the isolation or identification of well-known or previously isolated compounds [18,19]. One process of avoiding the waste of time and resources only to isolate previously known constituents is dereplication studies which enables the identification of known substances present in a mixture and enable focus on the discovery of new ones [8]. A range of dereplication methods are currently available to meet this challenge of improving natural product screening programs and thus distinguish novel entities from known

natural compounds at an early stage [8,20]. The advent of hyphenated techniques such as GC-MS, HPLC-MS, HPLC-NMR and HPLC-NMR-MS has eased and enabled rapid compound separation and identification. They have also made possible metabolomics and dereplication studies and the identification of new metabolites and drug molecules. However, basic chromatographic and separation techniques, NMR, MS and HRMS are absent in Nigeria hence the most common practice in Nigeria now is GC-MS analysis of crude extracts. This method, GC-MS analysis [23,24] ideally separates the constituents of the extracts (which must be volatile or semi-volatile) and simultaneously identifies them, facilitating dereplication [25], such that the need to tediously isolate and characterize the components, especially known compounds, is removed [37]. However, the method, as applied presently in Nigeria, is fraught with errors and a lot of unusual and non-natural or non-biosynthesized compounds are identified. This paper examines some of the challenges in the use of GC-MS, problems of its application in Nigeria and makes suggestions for improvement to obtain better and credible results.

Challenges in the use of GC-MS for analysis

GC/MS database

Compared to other types of chromatography and mass spectrometry hyphenated techniques, GC-MS has a large number of databases, such as national institute of standards and technology (NIST), dictionary of natural products (DNP) and MZmine. However, many GC-MS peaks still cannot be resolved by the available commercial MS databases [19]. The structures of metabolites in plants are complex, and so a metabolomics-targeted database will be more appropriate. Many databases, such as human metabolomics database, plant metabolomics database, and species-specific metabolomics database, are being continuously established and improved [28-31].

Thermal Degradation

Multi-peak phenomena result from metabolites producing multiple peaks, and this can be caused by sample degradation, by-product formation and the introduction of exogenous contaminants. The multi-origination phenomenon means one peak has multiple origins or precursors. Thermal non-stable compounds are prone to be thermally degraded, resulting in multi-peak and multi-origination phenomena [32].

Deconvolution

The aim of deconvolution is to analyze overlapping or co-eluted peaks and obtain the MS peak of a single pure substance. Currently, there are only a few good deconvolution software such as AMDIS, and commercial software such as ChromaTOF and AnalyzerPro [33,34].

Poor Baseline

Baseline position could change suddenly during the run. This usually results from a range or attenuation change. It can also be as a result of valve operations, if valves are switched during the run, septum leak, wander and drift may occur when a flow or temperature setting is changed, or a damaged column is used. If the system is not stabilized at the new conditions

before starting a run, some baseline changes are to be expected. An erratic or wandering baseline or one that moves up and down could be due to contamination in carrier gas, injector or a leak in the system. If there is a leak at the detector end of the system, then retention times should be stable and only sensitivity may be reduced. A leak at the inlet end of the column will result in decreased sensitivity and delayed retention times. Hence baseline problems could arise from several sources, some of which are electronic or mechanical failure, contamination in critical areas, such as detectors, incorrect or inappropriate setpoints; leaks, column or septum bleed, and other chromatographic difficulties [32,35,36,38].

Noise

Noise occurs as rapid vertical baseline fluctuations, broadening of the baseline and giving it a hairy appearance. Noise is quite distinct from the random, large spiking peaks. Small amounts of noise are present in most detectors. At high attenuation, noise may be invisible but appears as attenuation is decreased. If noise appears suddenly on a previously clean baseline then attenuation should be reduced but if the noise decreases when the inlet temperature is lowered, then this is the likely cause of the noise. Noise could also appear from contaminated carrier gases. Similarly, air currents from a fan or air conditioner blowing across the top of the instrument may interfere with gas exiting from the detector and a contaminated detector results in noise. When noise increases gradually to an unacceptable level, it indicates a gradual buildup of the noise source, rather than an abrupt change [39,40].

Common problems in using GC-MS for natural products dereplication studies in Nigeria

The problems associated with using GC-MS in Nigeria for natural products analysis include poor quality of extracts and in many cases the same column is used for all analysis. An overused column with residual components or fixated impurities could result in a high or poor baseline and a lot of noise from impurities in the

spectrum. Some of the compound libraries for matching and comparison are not well extended to cover all classes of compounds or may not be regularly updated. Since most of the assays are on the “active” extracts, these extracts are usually methanol or aqueous extracts which are non-volatile. These extracts contain polymeric (polysaccharides or starch) and polar substances which may not be volatile under run temperatures (80-300 °C). Some low melting point compounds or compounds with poor stability may decompose or undergo thermal degradation and rearrangements under the conditions. Plasticizers from solvents such as diisobutylphthalates are usually detected as they come from poorly purified solvents or industrial grade solvents transferred into Winchester bottles as analytical grade solvents. Cases of petrol being transferred into Winchester bottles as petroleum ether have been found. The lack of references and standards in their GC-MS runs (spiking- addition of internal standards for quantification) makes the spectra to be absolute rather than referenced spectra thus jeopardizing correlation of identified compounds to expected or previously isolated compounds.

Skilled personnel to handle the instrument are lacking as most organizations in Nigeria who acquire this equipment never put in place adequate training and retraining plan for the technicians. Also, many of the schools do not educate the students enough on the basics of GC-MS hence, many of the postgraduate and undergraduate students who are in most instances asked by their supervisors to use the method have little or no idea about it. And they in turn submit poorly prepared samples for GC-MS analysis. Similarity index is usually low in most cases as other compounds or derivatives compete with the components of interests, this can be overcome when proper extraction is done using standard protocols.

Suggestions for improving the use of GC-MS for natural product studies in Nigeria

Sample analysis using GC-MS technique has been a most promising research aim in Nigeria though the results hardly meet expectations or are of any significant scientific value.

Improvements in this aspect of research can be brought about by

- (a) Sample preparation: Extracts should be cleaned or pre-fractionated using silica gel or alumina so as to have a free-flowing extract. If column chromatography is not desired, a simple small column cleaning should be used and all eluates collected in a single vial, evaporated free of the solvent and reconstituted for analysis.
- (b) Recognition of trace compounds or signal molecules: It is often desirable to add a trace of a known compound or standard for each run. This will serve as a signal compound among the other compounds or metabolites. The correct identification and quantification of such a compound gives integrity to the spectrum. As much as possible, similarly or previously isolated or reported compounds from the plant should be identified and a private library created for the spectrum. The spectrum should be reproducible qualitatively and quantitatively. This can be checked by running a known mixture of reference compounds. This known mixture can be used to spike the extract samples and by comparison to the reference compounds, the quantities of the sample constituents can be rationalized.
- (c) Methods validation should be conducted, when new methods or modified methods are used. Laboratories should participate in Inter –Laboratory Comparison Tests, this should be part of the Analytical lab policy.
- (d) Where novel compounds or constituents are inferred, efforts should be made to isolate such constituents and the GC-MS repeated and coupled with high resolution mass spectrometry and other spectroscopic techniques, the novel compound(s) can only then be identified.
- (e) Several blanks and controls should be run at the initial stage. Blanks could be run with the extraction solvents or solvent mixtures. Controls could be run

with standards or known compounds and can be used as references to establish the integrity of the spectrum.

- (f) A proper and detailed literature survey to identify all previously identified compounds either by GC-MS or any spectroscopic method should be carried out and such compounds should be confirmed to be in the database. Compounds that are not natural or biosynthesized should be discarded and removed from the results. Similarly, for impurities and compounds arising from the column such as silicon esters should be discarded and removed from the results, unless the compounds were derivatives in the first instance.
- (g) Improved data processing using statistical approaches and chemometrics [24,25] or deconvolution methods [26].

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