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VISUAL EXPERIMENT

Chemical derivatization of pharmaceutical samples prior to Gas-Chromatography and Mass-Spectrometry analysis

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ABSTRACT

Derivatization is the process by which a compound is chemically changed, that has properties more amenable to a particular analytical method. Some samples analyzed by Gas-chromatography and Mass-spectrometry (GC-MS) require derivatization in order to make them suitable for analysis. Especially the compounds that have poor volatility, poor thermal stability, or that can be adsorbed in the injector will exhibit non-reproducible peak areas, heights, and shapes. Hence, this study focused on step-by-step visual demonstration of chemical derivatization of pharmaceutical samples prior to GC-MS analysis for their better elution and detection.

INTRODUCTION

Derivatization reactions are meant to transform an analyte for detectability in Gas-chromatography and Mass-spectrometry (GC-MS) or other instrumental analytical methods. Derivatization in GC-MS analysis can be defined as a procedural technique that primarily modifies the functionality of an analyte in order to enable chromatographic separations. A modified analyte in this case will be the product, which is known as the derivative. The derivative may have similar or closely related structure, but not the same as the original non-modified chemical compound.

Volatility of sample is a requirement for GC-MS analysis. Derivatization will render highly polar materials to be sufficiently volatile so that they can be eluted at reasonable temperatures without thermal decomposition (Knapp, 1979) or molecular re-arrangement (Kühnel et al., 2007). Understanding the chemistry of the analytes, derivatizing reagents used in sample preparation, and the detailed functionality of GC-MS are important to get reliable results.

Since GC-MS is used to separate volatile organic compounds, modification of the functional group of a molecule by derivatization enables the analysis of compounds that otherwise cannot be readily monitored by GC-MS. Derivatization process either increases or decreases the volatility of the compound of interest. It also reduces analyte adsorption in the GC system and improves detector response, peak separations and peak symmetry.It is necessary to develop and/or improve on chemical analytical methods and hence the need to familiarize with derivatization methods that are applicable to GG analysis. Generally derivatization is aimed at improving the suitability, efficiency, and detectability in Gas Chromatography.

Therefore, the objective of this study was to provide step-by-step visual demonstration of chemical derivatization of samples prior to Gas-chromatography and Mass-spectrometry (GC-MS) analysis for their better elution and sensitive detection.

MATERIALS AND EQUIPMENTS

1. Cylindrical measuring flasks

2. HPLC grade syringe filter
3. Syringe (1 mL and 5 mL)
4. HPLC grade methanol
5. Falcon tubes (15 mL and 50 mL)
6. GC glass vials
7. Liquid nitrogen gas
8. Pyridine solution
9. Methoxyamine hydrochloride
10. N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA)
11. GC operating system
12. Open cylindrical glass vial
13. Pipette (200 µL and 1000 µL)
14. Hot plate with thermal control
15. Kim wipe tissue
16. Triple distilled water

PREPARATION OF REAGENTS

Test samples: Pharmaceutical samples including standard were dissolved using suitable solvent (depending on the nature of the work) and dried under liquid nitrogen. Amount of the sample should be measured in order to define the yield of desired extract.

Derivatization chemicals: For derivatization, methoxyamine hydrochloride, pyridine and N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) were purchased from commercial chemical companies.

Organic solvents: Ready to use and purchased commercially from Sigma (USA).

Preparation of methoxyamine hydrochloride solution: Measure 50 mg of methoxyamine hydrochloride and dissolve in 1 mL of pyridine until completely dissolved.

VIDEO CLIPS

Part 1: 5 min 8 sec

Part 2: 6 min 13 sec

Part 3: 53 sec

METHOD

Gas-chromatography (GC)

Gas chromatography (GC) and mass spectrometry (MS) make an effective combination for chemical analysis. GC analysis is used for drug testing and environmental contaminant identification. GC analysis separates mixtures into individual components using a temperature-controlled capillary column and provides a representative spectral output. The technician injects the sample into the injection port of the GC device. The GC instrument vaporizes the sample and then separates and analyzes the various components. Each component ideally produces a specific spectral peak that may be recorded on a paper chart or electronically. The time elapsed between injection and elution is called the "retention time". The retention time can help to differentiate between some compounds. The size of the peaks is proportional to the quantity of the corresponding substances in the specimen analyzed. The peak is measured from the baseline to the tip of the peak.

PROTOCOL

Chemical derivatization of pharmaceutical samples

1. Pharmaceutical samples should be dissolved in a suitable solvent system (For example: for derivatization of sugar alcohol, pharmaceutical sample should be dissolved in a 1:1 ratio of water and methanol) for easy and sensitive elution of sample in GC column.
2. Samples should be filtered through HPLC grade Whatman syringe filter (size: 0.2 µm).
3. Samples should be processed only in glass tubes covered with tight caps for proper derivatization.
4. Pharmaceutical samples should be completely dried under liquid nitrogen in order to remove traces of solvent.
5. Samples should be exposed for heat at constant temperature (40°C) during drying process.
6. Once samples are dried completely, a solution of chemical derivatization agent methoxyamine hydrochloride made in pyridine (100 µL) should be mixed and left the sample at 40°C temperature for 90 minutes to occur the chemical derivatization reaction.
7. After specified time (90 min), specific GC solvent N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) (100 µL) should be mixed with samples.
8. Volume of chemical derivatization agent and GC solvent should be adjusted based on the quantity of the samples.
9. After that samples should be left at room temperature for one hour at 40°C.
10. Further, all samples should be re-filtered using a HPLC grade Whatman syringe filter (size: 0.2 µm).
11. Finally samples should be stored only in small glass vials for GC analysis.
12. Samples should be stored in cold (4°C) maximum for 24-28 hours if not analyzed instantly.
13. Based on the nature of the compounds, specific compound can be detected in GC-MS system based on its retention time and by matching with GC-MS compounds library and/or by can be analyzed quantitatively using standard compound.

Table I

Scheme for making reaction ratio of test sample, derivatizing reagents and standard

Sample/ Derivatizing reagent	Blank	Standard (10000, 1000, 500, 250, and 125 ppm)	Drug A (Known amount dissolved)
Drug A	-	-	1 mL of dried samples
Standard	-	1 mL of dried sample	-
Methoxyamine hydrochloride (derivatization agent)	100 µL	100 µL	100 µL
N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) (solvent)	100 µL	100 µL	100 µL

DISCUSSION

A pharmaceutical sample in its crude form contains a number of compounds. Some of the compounds of pharmaceutical importance cannot be detected during GC analysis without a reaction of chemical derivatization. Chemical derivatization enables sensitive detection of desirable compounds in GC column due to change of the chemical compounds in their different isomeric forms, thus the compounds can be detected easily by GC-MS with higher sensitivity. A general reaction of derivatization has been outlined in Figure 1. A number of research articles have been published on characterization and identification of metabolic compounds in various pharmaceutical samples, where chemical derivatization of samples prior to GC analysis has been regarded as a mandatory process for better detection profile of compounds (Farag et al., 2015; Japelt et al., 2015). Various chromatographic techniques have been used for successful detection and quantification of pharmaceutical important compounds from variety of sources. Gas-chromatography is one of the most popular and widely used separation and detection techniques to

characterize both organic and inorganic materials suggesting is potential usefulness in chemical analysis of complex extractsor crude material (Farag et al., 2015). This research visualized successful application of chemical derivatization and Gas-chromatographic techniques for the detection and characterization of biologically active compounds and/or secondary metabolites from variety of pharmaceutical samples. However, the choice of the derivatization technique for analysis of compounds will depend on the available reagent and reaction types that can produce derivatives that give desirable results in GC. The derivatives must be suitable, detectable and efficient for GC analysis. The evaluation of the functional group of the analyte, the GC detector and even the byproducts of the reaction among others considerations will guide the choice of derivatization technique.

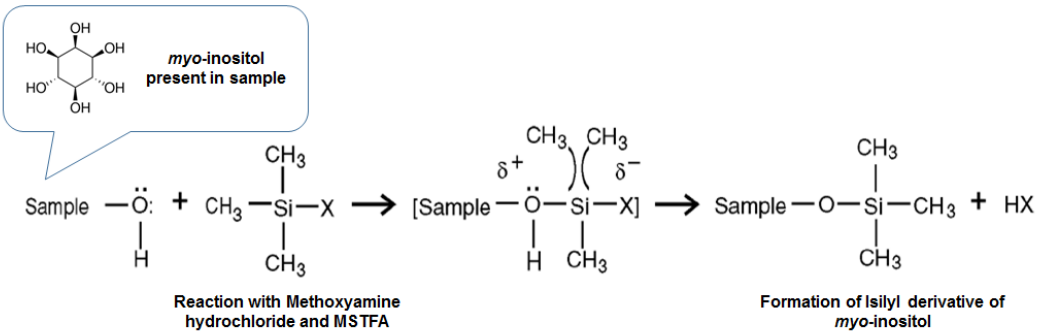

Figure 1: General reaction mechanism for the formation of silyl derivatives of *myo*-inositol

REFERENCES

Farag MA, Rasheed DM, Kamal IM. Volatiles and primary metabolites profiling in two *Hibiscus sabdariffa* (roselle) cultivars via headspace SPME-GC-MS and chemometrics. Food Res Int. 2015; 78: 327-35.

Japelt KB, Nielsen NJ, Wiese S, Christensen JH. Metabolic fingerprinting of *Lactobacillus paracasei*: A multi-criteria evaluation of methods for extraction of intracellular metabolites. Anal Bioanal Chem. 2015; 407: 6095-104.

Knapp DR. Handbook of analytical derivatization reaction. Vol. 10. New York, Wiley and Sons, 1979, p 10.

Kuhnel E, Laffan DDP, Lloyd-Jones GC, Martinez del Campo T, Shepperson IR, Slaughter JL. Mechanism of methyl esterification of carboxylic acids by trimethylsilyldiazomethane. Angewandte Chem Int. 2007; 46: 7075-78.

PRECAUTION

Precautions should be taken while drying the samples under liquid nitrogen to ensure complete drying of the samples.

Selection of solvent is very important for preparing GC samples.

During chemical derivatization, maintaining constant temperature is a critical process for better elution of desired compound in GC column.

A nasal mask and laboratory hand gloves should always put on since some of the derivatization agents have a very pungent smell and might be skin irritant to susceptible individuals.

Samples should be kept at cold (4°C) if not analyzed right after derivatization process.