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TECHNICAL NOTES

STANDARD OPERATING PROCEDURE (SOP) FOR GAS CHROMATOGRAPHY HEADSPACE SYSTEM (AGILENT- 6797A)

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ABSTRACT: Gas Chromatography-Headspace (GC-HS) is a commonly used analytical technique in research and industrial laboratories for quality control as well as identification and quantitation of volatile compounds in a mixture. A broad variety of samples can be analyzed as long as the compounds to be identified are sufficiently thermally stable and reasonably volatile. Gas chromatography can be used for the separation of gases, liquids and solids, if dissolved in appropriate solvents. Thermally stable analytes are converted to vapors, separated and detected from a mixture. Gas Chromatography- Headspace has several advantages over other analytical techniques like higher resolution, limited sample preparation compared to other Column chromatographic methods. It is one of the fastest methods of separation with results obtained within minutes, as the sample is directly changed to vapor phase within an equilibrium environment reducing the time and cost of extensive sample preparation before analysis. Sample volume can range between few µl to 1000µl. Its use can be readily understood from breadth of its applications. The range of material which can be analyzed by chromatographic methods is essentially unlimited with applications found in varied fields of Forensic Science, Food and Agriculture, Pharmaceuticals, Biological and Clinical chemistry, Environmental toxicology and many others.

KEYWORDS: Gas Chromatography- Headspace; Alcohol; Standard Operating Protocol; Internal Standard etc.

INTRODUCTION:

Static Headspace-Gas chromatography (GC) is a technique used for the concentration and analysis of volatile organic compoundswithin a matrix. Before getting injected in the inlet, analyte is changed to its volatile vapors. The volatiles injected into the instrument Inlet, enters the gas stream; Carrier gas(Helium or nitrogen) which transports the sample into a separation tube known as the "Column". Introduced volatiles get separated by means of selective interaction (Partitioning) between the stationary and mobile

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phase. The detector measures the quantity of the components that exit the column. The time taken within the sample injection and the emergence of individual peak is known as retention time, whereas the respective area/ height is proportionated to the concentration. To measure a sample with an unknown concentration, a standard sample with known concentration is injected into the instrument. The standard sample peak retention timeand area are compared to the test sample to calculate the concentration.

Its popularity has grown to gain worldwide acceptance in analyses of alcohols in blood, alcohols in other body fluids, volatiles in arson cases, and residual solvents in pharmaceutical products. Other common applications include industrial analyses of monomers in polymers and plastic, flavour compounds in beverages, and food products.Sample matrices like blood, plastic, and cosmetics contain high molecular weight, non-volatile material that can remain in the GC system and result in poor analytical performance such as column bleeding, fronting, tailing of peaks. Extensive sample preparation techniques are utilised to extract and concentrate the compounds of interest from this unwanted non- volatile material before injection into the Gas Chromatography system. These extraction and concentration techniques can become time consuming and costly requiring solvents and other extraction apparatus. Static headspace analysis reduces this time and cost by directly sampling the volatile to vapour phase and its injection into the inlet of Column.

PURPOSE:

To develop the standard Operation procedure for GC-HS; Agilent 6797A and its compliance with the provision of Good laboratory practice regulations.

SCOPE:

Describes the finest details of the steps to be followed for simplest and precise handling of the machine and analytical technique.

Responsibilities

All the scientific staff members carrying out operation of GC-HS; Agilent are responsible for strictly adhering to the procedure given in the text.

STANDARD OPERATION PROCEDURE OF GC-HS

1. Cold Start of Gas Chromatography Headspace Instrument

1. Before starting the Instrument, switch "**On**" the Air Conditioning of the room.
2. Switch "**On**" all the gases (Nitrogen, Hydrogen & Zero air) by pushing the valve up on Gas Panel.
3. Switch "**On**" the main UPS by long pressing start/stop button for 3 seconds.Before starting the GC Instrument, check the voltage stability (Voltage should be ≅233V).
4. Switch "**On**" the GC instrument by pressing the button on the lower left corner.
5. Similarly switch "**On**" the HS instrument by pressing the button on the lower left corner.
6. Switch "**On**" the CPU of computer.
7. As the system loads, from the desktop, select'GC' from Administrator and GC.

2. Preparation of Sample and Standards for Analysis

2.1. Preparation of Standard

1. From the standard GC grade analyte (99.95% GC grade), pipette 1ml and dilute the analyte in 100ml Ultra-pure water.
2. Pipette out serial dilutions of 2000µl, 1000µl, 500µl, 250µl, 100µl, from stock solution of 1ml/100ml and dilute in 10ml of Ultra-pure water.

2.2. Preparation of Internal Standard

1. From the standard GC grade analyte (n-propanol), pipette 300µl and dilute the analyte in 100ml distilled water.

2. Take 90µl of internal standard in the vial along with Standard and Sample if required.

2.3. Sample Preparation

1. In a clean, dry 20 ml HS vial take 1000µl/1ml of liquid analyte.
2. If required as per analytical conditions, add 90µl of Internal Standard (n-propanol).
3. Seal the mouth of vial using fresh PTFE septa and metallic crimp.
4. Shake the vial slowly to mix sample and standard.

3. Creating New Method for analysis

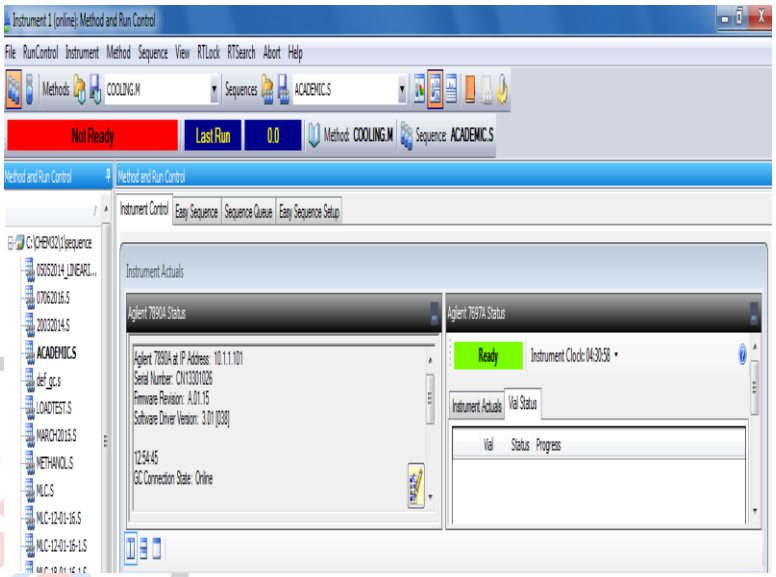
3.1. Creating New Method

1. On the Desktop, Double click on "**Instrument1 online**" icon; Method and Run Control screen will appear.
2. From the Menu bar click on "**Method**", from the drop-down list select "**New Method**".

3.2. Creating Calibration of a Method

1. Successively run samples of serial dilutions; 2000µl, 1000µl, 500µl, 250µl, 100µl in a sequence.
2. Double click on "**Instrument1 offline**" icon on desktop; Data analysis screen will appear.
3. From the Menu bar click on "**Method**", from the drop-down list select "**Load Method**". From the list, select the method on which calibration is to be drawn.
4. Select the graph of maximum concentration and load.
5. On the "**Menu bar**" click on "**Calibration**", from the drop-down list select "**New Calibration Table**".
6. "**Calibrate: Instrument1, New Calibration Table**" window will appear, click on "**OK**".
7. All the peaks appear highlighted and table appears with RT and area.
8. For every peak specify "**Name**" and "**Amount**".
9. For next dilution, from the "**Menu bar**" click on "**Calibration**", from the drop-down list select "**Add Level**".
10. Specify only amount. After all concentrations have been labelled, click on "**OK**".
11. From the Menu bar click on "**Method**", from the drop-down list select "**Save Method**".

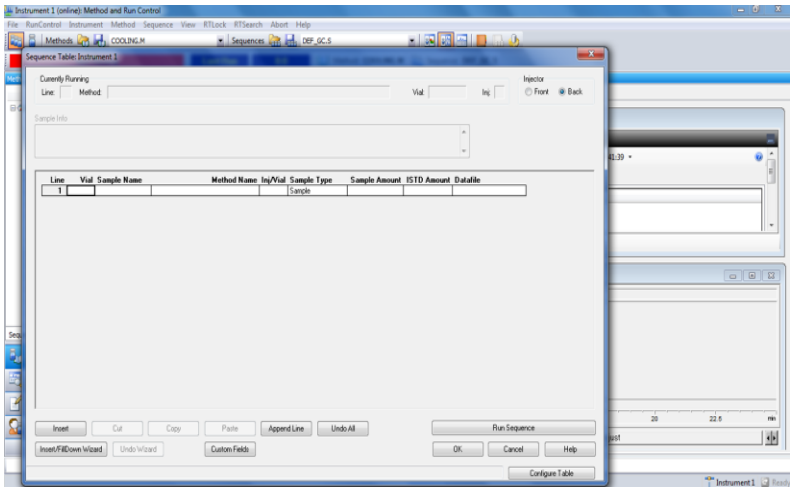
4. Operation of Gas-Chromatography Headspace System



1. Double click on"**Instrument1 online**" icon on desktop; Method and Run Control screen will appear.
2. From the Menu bar click on "**Method**", from the drop-down list select "**Load Method**".
3. Run "**CONDITIONING.M**" method for at least 30 minutes before analyzing any sample.

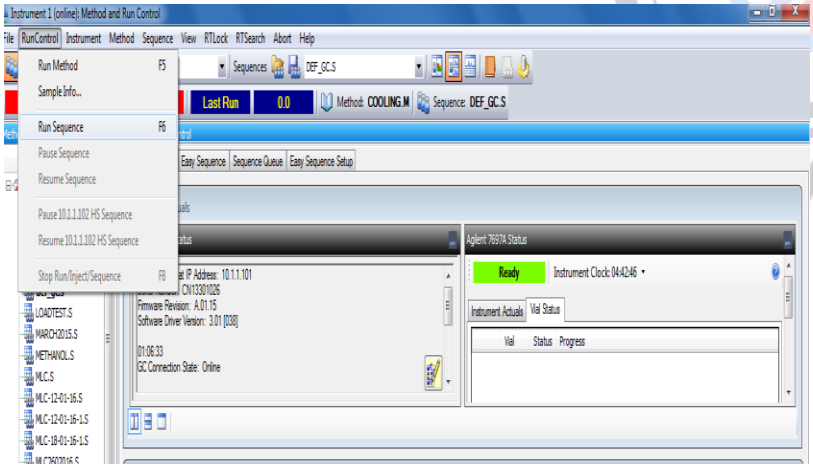
5. Analyzing Sample on GC-HS

1. From the Menu bar click on "**Method**", from the drop-down list select "**Load Method**". From the list, select the method to be run (recent calibration method), and click '**OK**'.
2. From the Menu bar click on "**Sequence**", from the drop-down list select "**Sequence Table**".
3. On the Sequence Table: Instrument1 window,fill the following data of



- Vial position where vial is kept
- Sample Name ID of the sample to be analyzed
- Method name Method on which sample is to be analyzed
- Inj/vial Always "1"
- Sample type Sample/ Standard

4. For every next line, press down key.
5. For every vial in the carousal, information should be filled up.
6. Start sequence by clicking on "**Run Sequence**" or by pressing F6.



6. Viewing Report of Sample Run

1. On the Desktop, Double click on "**Instrument1 offline**" icon on desktop; Data analysis screen will appear.
2. From the Menu bar click on "**Method**", from the drop-down list select "**Load Method**". From the list, select the method to be loaded.
3. From "**Data Analysis**" window, on the left side of screen, select the sequence run.
4. Load the graph.
5. From the Menu bar click on "**Report**", click on "**Print Report**".
6. View report and print if required.

7. Shut Down of Gas Chromatography Headspace Instrument

1. On the Desktop, Double click on "**Instrument1 offline**" icon. From the Menu bar click on "**Method**", from the drop-down list select "**Load Method**". From the list, select "**COOLING.M**" method.
2. As soon as Detector temperature of GC system and Oven temperature of Headspace system acquire temperature below 50^0C, close the "**ChemStation Online**" or "**Offline**" windows.
3. Switch "**Off**" the Instruments by pressing the button at the left down corner. Push the valve down for gases (Nitrogen, Hydrogen & Zero Air) from as panel.
4. Press the "**Start/Stop**" button on the UPS, wait till the supply becomes zero.
5. Switch "**Off**" the Air conditioners of the room.

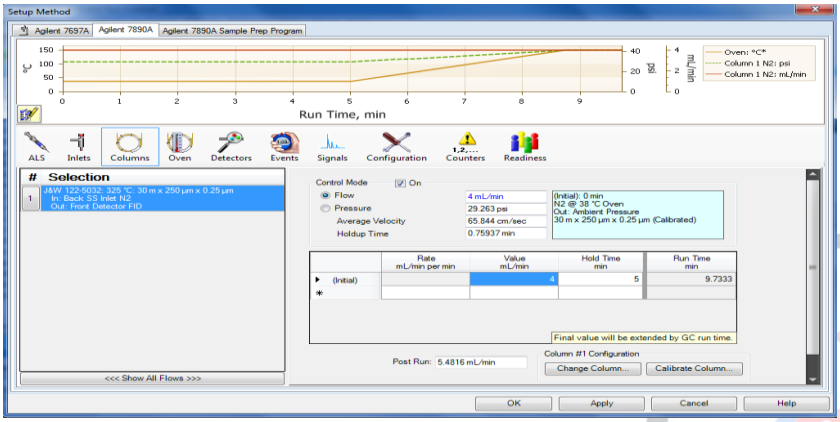
FITTING OF NEW COLUMN FOR ANALYSIS

1. Fitting New Column in Oven Compartment

1. Open the Oven compartment, ensuring that system is cooled and not in working condition.
2. Open the nut of Inlet and Detector with a spanner.
3. Insert the new column within the hanger in oven. Take one end of column to be fixed in Inlet end and other at Detector end.
4. To fix the Inlet end, cut the capillary column at about a length of 1mm with the help of cutter. Insert a septa and ferrule leaving a length of 4mm-6mm from tip.

5. Insert the column tip in the Inlet end, leaving a distance of 1mm from top. Seal the attachment by screwing the nut using a spanner.
6. Similarly, seal the detector end by cutting the capillary column at about a length of 1mm with the help of cutter. Insert a septa and ferrule leaving a length of 4mm-6mm from tip.
7. Insert the end in detector, leaving a tip of 1mm and seal using spanner.

2. Configuring New Column



1. From the Menu bar click on "**Method**", from the drop-down list select "**Load Method**". From the list, select the method on which new column is to be configured.
2. On the Menu bar click on "**Edit Instrument Parameters**", Setup Method window will appear.
3. Select 7690A (GC Sampler), different conditions of Auto sampler; Column; Oven; Inlet; Detector etc. will appear.
4. Click on "**Column**", column conditions will appear.
5. Click on "**Show All Columns**", list of columns appears. Select your column specifying the Identification and its length etc. click on "**Install**", specify Inlet and Outlet.
6. Click on "**Calibrate Column**".
7. Click on "**OK**" and save method changes.
8. Run Conditioning method for 1 hour before running any sample on a new column.

DO'S & DON'T'S REGARDNG OPERATION OF GC-HS

DO'S

1. Before starting the instrument, verify the volume of gas in cylinder.If the primary gas pressure is less than 4 psi, replace it.
2. Purity of carrier gas should be maintained to prevent degradation of chromatographic hardware.
3. Purity of gases should be 99.999% and cylinder should be supplied with a purity certificate.
4. Gases should be passed through a gas panel containing Hydrocarbon and Oxygen traps to ensure no impurity or moisture enters the Inlet or Column.
5. Immediately change the expired oxygen and moisture traps (when blue colour changes to white) to avoid gas contamination.
6. During cartridge replacement, check valves and close off the system to the atmosphere, further minimizing the entry of contaminants.
7. Start the Instrument (GC-HS) first then Load the software from the system.
8. Once the Instrument is "On", always start the machine by running 'Conditioning' method for half hour.
9. Samples and Standards should be clearly marked and labelled, to avoid error.
10. Always use fresh, clean and sterile vials for sample preparation and storage.
11. Septa with a PTFE face down, should be used to seal the headspace vial to eliminate bleed from the septa.
12. Shaking or vibrating the vial containing high viscosity sample matrices during heating can assist in achieving equilibrium faster.
13. Adjust the temperature of the sample to change the solubility of the analyte as well as to drive the equilibrium in towards the gaseous phase.

14. To minimize matrix problems and prevent water condensation from aqueous samples, use a higher transfer line temperature, usually 10^0 higher than sample heating temperature.
15. Heat the syringe to a temperature comparable to the sample vial temperature to minimise pressure differences, when using gas tight syringes.
16. Ensure the septum of the GC injection port is well maintained to decrease the possibility of a leak.
17. In Headspace, septa can be changed after 500 injections.
18. Use injection port liners of small internal diameters and lower buffer volumes to maintain a narrow bandwidth.
19. Use an on-column syringe when injecting into an on-column inlet so that the injector, syringe and column are not damaged.
20. Inject standards and samples in order from low to high concentrations to help minimize carryover.
21. For high concentration samples in a sequence of samples, run a blank after the suspected samples to reduce carryover contamination.
22. Increase the oven temperature after the samples are completely transferred to the column to increase the movement of compounds inside the column.
23. Built sufficient time into the sample cycle to achieve constant state of equilibrium.
24. GC instrument maintenance should include checking fittings and connections with a gas leak detector.
25. To prevent stationary phase decomposition, the oven and inlet should be at room temperature when not in use and when changing the septum.
26. After running the samples, condition the system at least 30min before shut down.
27. Always ensure Detector temperature reaches 50^0C and lower, before switching "Off" the GC Instrument.

DON'TS

1. Constant exposure of capillary columns to oxygen and moisture should be avoided especially at high temperatures as it may produce rapid and severe column damage.
2. Improper handling or installation of gas lines should be avoided as moisture introduced by this can be a common cause of column stationary phase degradation.
3. Do not use sample matrices containing high molecular weight compounds to avoid incomplete or inefficient transfer into the GC injection port.
4. Transfer line temperature should not be kept low than sampler oven as water from the sample matrix can cause problems by condensing in the transfer line.
5. Avoid using high-concentrated samples lest they produce ghost peaks in subsequent analyses due to carryover of sample from previous injections.
6. Do not use reuse unclean or unpacked vials.
7. Septa with punctured PTFE face should not be reused.
8. Do not use transfer line having smaller internal diameter than the injection port liner to avoid broader peaks, tailing peaks, lower sensitivity, and loss of resolution.
9. Use company recommended regulator materials and tubing. These should only be changed by an company engineer.
10. Non-metallic types of tubing such as polyethylene and Teflon are not recommended for GC applications due to their gas permeability and difficulty in cleaning.
11. Unclean or improperly cleaned tubing can lead to contamination of the system with disastrous results.

12. Never open the GC door if oven temperature is more than 100^0C or during running conditions.
13. Do not inject air into the vials to prevent the vacuum. This often damages the cap seal.
14. Avoid cleaning agents that are alkaline, contain phosphates, or are strongly acidic for syringes or glassware.

DISCUSSION AND CONCLUSION:

In the developed Standard Operative Procedure (SOP), all the information regarding how to operate the system, how to resolve problems encountered during analysis and what should be the precautions taken during running the system were gathered in one platform bit by bit. It will be immensely helpful to the laboratory personals to accomplish routine complex analysis and also play an important part to achieve effectiveness, great output and consistency in performance.

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