



PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Quality Control	SOP No.:
Title: Operation, Cleaning and Calibration of GC with Head space	Effective Date:
Supersedes: Nil	Review Date:
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1.0 OBJECTIVE:

To lay down procedure for operation, cleaning and calibration of GC with Head space

2.0 SCOPE:

This SOP is applicable for operation, cleaning and calibration of GC with Head space (Make:Agilent, Model: 6890N).

3.0 RESPONSIBILITY – Execution- Executive QC
Checking -Assistant Manager QC

4.0 ACCOUNTABILITY - Manager Quality Control

5.0 PROCEDURE:

5.1 OPERATING PROCEDURE:

5.1.1 Starting the instrument

5.1.1.1 Switch on the following

Gas chromatograph, Automatic Liquid Sampler / Headspace sampler (for HS GC), Data processing unit (Computer).

5.1.1.2 Open the N₂ (Carrier gas) from regulator valve or directly from GC key panel (if GC is controlled by EPC).

5.1.2 Column installation.

5.1.2.1 Allow the oven temperature, detector temp, injector temp. to cool bellow 50⁰C.

5.1.2.2 Replace septa and liner if required.

5.1.2.3 Switch off the oven temperature. Open the door of GC oven.

5.1.2.4 Make both the column ends flat using cutter if required.

5.1.2.5 Put the column from injector side while keeping the other end open and purge N₂ for a few seconds.

5.1.2.6 Confirm the N₂ flow from the other end by dipping it in Methanol / IPA and water mixture. Bubbles must form at the outlet.

5.1.2.7 Insert the other end of the column from detector side and ensure tightness at both ends using spanner.

5.1.2.8 Check the leakage from both ends using 1:1 mixture of Methanol / IPA and water and shut the door.

5.1.3 Column conditioning.

5.1.3.1 Open Zero-Air first then Hydrogen.

5.1.3.2 Optimum flow ratio of zero air and hydrogen shall be 400:40. Use flow meter to adjust the flow in non EPC GC.

5.1.3.3 Pressure the ignition button or ignite the flame with the help of lighter.



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5.1.3.4 Display signal by pressing from GC key panel SIG 1 for channel 1 and SIG 2 for channel 2.

5.1.3.5 Put the column on heating with the maximum temperature limit of column till the signal becomes stable.

5.1.4 Method and sequence loading from Chemstation software version A.09.01 or higher.

5.1.4.1 Press CTRL+ALT+DEL to switch over to Log in – GCLAB.

5.1.4.2 Press enter after entering password.

5.1.4.3 Click at START to choose first PROGRAMME then, choose CHEMSTATIONS.

5.1.4.4 Select instrument.

5.1.4.5 Log on by entering USER NAME and PASSWORD. Also select Chemstore database.

5.1.4.6 To create new method click in the following order METHOD/NEW METHOD and save it in the following path. C:\HPCHEM\INSTRUMENT NO.\METHODS.

5.1.4.7 To load saved method click in the following order METHOD/LOAD METHOD and select the following path to choose a method. C:\HPCHEM\INSTRUMENT NO.\METHODS.

5.1.4.8 Review the method by clicking in the following order METHOD / EDIT ENTIRE METHOD and complete the following entries. METHOD COMMENTS, INJECTION SOURCE, INSTRUMENT PARAMETERS, INTEGRATION EVENTS and SIGNAL SOURCE.

5.1.4.9 To create new sequence click in the following order SEQUENCE / NEW SEQUENCE and save it in the following path. C:\HPCHEM\INSTRUMENT NO.\SEQUENCE.

5.1.4.10 To load saved sequence click in the following order SEQUENCE / LOAD SEQUENCE and select the following path to choose a sequence. C:\HPCHEM\INSTRUMENT NO.\SEQUENCE.

5.1.4.11 Review the sequence by choosing the injector source and complete the following entries. LOCATION, SAMPLE NAME, METHOD NAME, INJ./ LOCATION and SAMPLE TYPE.

5.1.4.12 To select the data file counter and storage location click in the following order SEQUENCE / SEQUENCE PARAMETERS and choose the path. C:\HPCHEM\INSTRUMENT NO.\ DATA \YEAR\ MONTH-DATE for signal 1 and C:\HPCHEM\INSTRUMENT NO.\ DATA \YEAR\ MONTH-DATE for signal 2.

5.1.5 Enter headspace parameters (If headspace is attached).

5.1.5.1 After loading various vials for blank, standard and sample into the Headspace magazine follow the following procedure as per the required STP using the HP7694 Link software.

5.1.5.2 Click at START to choose first PROGRAMME then, HP7694 Link.

5.1.5.3 To create a new Headspace method click OPEN and save it in the following path. C:\ HP7694 \ METHODS.

5.1.5.4 To load a saved Headspace method click OPEN and select the following path. C:\ HP7694 \ METHODS.



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5.1.5.5 Review the method by checking the following entries. TEMPERATURE SETPOINTS, TIMING PARAMETERS, SAMPLE PRORERITIES, VIAL RANGE AND SAMPLING MODE.

5.1.5.6 Click at APPLY – (ACTIVATE) to send the method to active storage area.

5.1.6 Enter automatic liquid sampler parameters (IF ALS is attached).

5.1.6.1 Click instrument/edit parameters and select injector.

5.1.6.2 Select the injector source and complete the following entries. INJECTION VOLUME, SYRINGE SIZE, DELAY TIME IF ANY AND SAMPLE AND SOLVENT WASHES.

5.1.6.3 Click at APPLY to send the parameters to ALS.

5.1.7 Starting and stopping the sequence.

5.1.7.1 Click at following order CHEMSTORE/SEQUENCE SETUP and select injector source, study and custom field.

5.1.7.2 For starting a sequence on GC 5890II, 6890 and 6890N with or without ALS click the following order to RUN CONTROL/RUN SEQUENCE.

5.1.7.3 For starting a sequence on GC 6890N with Headspace sampler follow the same procedure . Then open the HP7694 link window and click at MONITOR/START.

5.1.7.4 To stop a sequence click at ABORT/START in chemstation window.

5.1.7.5 After completion of the sequence select the report style and take out the prints of the report generated .

5.1.7.6 Do the calculations as per formula given in the concerned STP

5.1.7.7 In case of any problem/ clarification contact Lab. In charge.

5.2 CALLIBRATION PROCEDURE:

Frequency: Half yearly and after each maintenance.

5.2 .1 To check the reproducibility and detect ability of the system, perform The following experiment.

G C Column : Fused silica column 30 m long, 0.53 mm internal diameter coated with 2.65 µm film of cross linked Methyl siloxane (Use HP-1 Column).

5.2.1.2 TEXT MIXTURE : Prepare sufficient quantity of solution containing 0.35 mg/ ml each of Tetradecane, Pentadecane & Hexadecane in n- Hexane.

5.2 .1.3 Injection Volume: Inject 1µL with 10µL syringe.

5.2.2 INSTRUMENT PARAMETERS :

5.2 .2.1 Initial oven temperature 40 °C

5.2 .2.2 Initial time 0 min.



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5.2 .2.3 Rate	25 °C
5.2 .2.4 Final oven temperature	90 °C
5.2 .2.5 Final time	0 min.
5.2 .2.6 Rate 'A'	15 °C/ min.
5.2 .2.7 Final oven temperature 'A'	170 °C
5.2 .2.8 Final time 'A'	15 min.
5.2 .2.9 Injector temperature	250 °C
5.2 .2.10 Detector temperature	270 °C
5.2 .2.11 Carrier flow (Helium/ N2)	10 mL/ min.
5.2 .2.12 Hydrogen flow	40+/- 4 mL/ min.
5.2 .2.13 Zero flow	40+/- 40mL/ min.
5.2 .2.14 Auxillary flow (N2)	10 +/- 2 mL/ min.

5.2.3 PROCEDURE

5.2.3.1 Inject 6 replicate injections of the test mixture and calculate the ratio of Pentadecane Hexadecane to that of Tetradecane from the Chromatogram and determine the relative standard deviation.

5.2.3.2 Retention time of Tetradecane is about 9 min. the Relative Retention time of the expected peaks is given below:

Compound Name	Relative Retention Time (RRT)
n- Hexane	~ 0.19
Tetradecane	= 1.0
Pentadecane	~1.2
Hexadecane	~ 1.5

5.2.3.3 Determine the relative Standard deviation (RSD) of the Retention time(RTs) of Tetradecane, Pentadecane & Hexadecane.

5.2.4 Acceptance criteria:

5.2.4.1 The relative standard deviation (RSD) of the ratio of Area counts of the peaks corresponding to Pentadecane and Hexadecane to that of Tetradecane should be not more than 2.0 %.

5.2.4.2 The relative standard deviation (RSD) of Retention Time(RT) Tetradecane, Pentadecane and Hexadecane in the six replicate injections should not be more than 2.0%.

5.2.5 To check the linearity of response at different concentration, performing the following



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experiment.

5.2.5.1 INSTRUMENT

Gas Chromatography equipped with flame ionization detector and GC HP Chemstation or an equivalent system.

Fused silica column HP-1 30 m x 0.53 mm x 2.65 micron or equivalent.

5.2.5.2 INSTRUMENT PARAMETERS :

Initial oven temperature	: 50 °C
Initial time	: 2 min.
Rate	: 10 °C/ min
Final oven temperature	: 200 °C
Initial time	: 1.0 min.
Injector temperature	: 250 °C
Detector temperature	: 270 °C
Carrier flow (Helium/ N2)	: 10 mL/ min.
Signal 1 range	: 8

5.2.5.3 REAGENTS:

Methylene chloride Standard

Toluene Standard

5.2.5.4 PROCEDURE

5.2.5.4.1 Prepare five different concentration of the Standard containing 0.25 % v/v

0.5 % v/v, 0.75 % v/v, 1.0% v/v and 1.25 % v/v of Methylene Chloride in Toluene.

5.2.5.4.2 Inject 0.2 µL of all the five standards in duplicate and note the percent Area directly from the Chromatogram. Toluene elutes at about 4.7 min.

5.2.5.4.3 Determine the regression coefficient (R²) for the mean responses of three concentration of standard Methylene Chloride in Toluene.

5.2.5.4.4 The Relative Retention time of Eluting peaks are:

1 -Toluene : 1.0

2 -Methylene Chloride : 0.26

5.2.5.5 Acceptance criteria : R² should not be less than 0.99

5.2.6 For HEADSPACE:

5.2.6.1 PRSYSTEM CALIBRATION:



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5.2.6.1.1 Check the reproducibility of the Headspace sample injection system to ensure the reliability of analysis results.

5.2.6.1.2 TEST COLUMN : Fused silica column 50 m long, 0.32mm internal diameter coated with 0.52 micron film of cross- linked Methyl siloxane (Use ULTRA-1) .

5.2.6.1.3 TEST SOLUTION : Prepare 0.4 % v/v of Ethanol in water .

5.2.6.1.4 Prepare 10 vials each containing 2 ml test solution. Seal the vial with butyl rubber septa and place in the Headspace magazine.

5.2.6.2 GC PARAMETERS:

- 5.2.6.2 .1 Oven temperature : 60 °C
- 5.2.6.2 .2 Time : 20 min.
- 5.2.6.2 .3 Injector temperature : 120 °C
- 5.2.6.2 .4 Detector temperature : 200 °C
- 5.2.6.2 .5 Carrier gas flow (Helium/ N2): 1+/- 0.1 mL/ min.
- 5.2.6.2 .6 Carrier gas pressure : 20 psi.
- 5.2.6.2 .7 Split flow : 50+/- 1mL/ min.
- 5.2.6.2 .8 Hydrogen flow : 40 +/- 4 mL/ min
- 5.2.6.2 .9 Zero air flow : 400 +/- 40 mL/ min

5.2.6.3 HEADSPACE PARAMETERS:

- 5.2.6.3 .1 Sample temperature : 60 °C
- 5.2.6.3 .2 Needle temperature : 100 °C
- 5.2.6.3 .3 Transfer temperature : 110 °C
- 5.2.6.3 .4 Thermostat time : 20 minutes.
- 5.2.6.3 .5 GC Cycle time : 25 minutes
- 5.2.6.3 .6 Pressurizing time : 3 minutes
- 5.2.6.3 .7 Inject time : 0.04 minutes
- 5.2.6.3 .8 Withdrawal time : 0.5 minutes
- 5.2.6.3 .9 Sample shaker : ON

5.2.6.4 Acceptance criteria: The relative standard deviation (RSD) of the Area counts of the peak corresponding to ethanol should not be more than 1.5 %.(For Headspace the acceptance criteria shall be NMT 3.0 %)

5.2.7 FID CALIBRATION FOR AUTOMATIC LIQUID SAMPLER:



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5.2.7.1 FID Noise Evaluation:

5.2.7.1.1 Column: 5 m X 0.1 mm uncoated fused silica or 19091-60620 E or equivalent column.

5.2.7.1.2 Column flow : 0.2 mL/ minute

5.2.7.1.3 Inlet temperature : 250 °C

5.2.7.1.4 Detector temperature : 300 °C

5.2.7.1.5 Initial Oven temperature : 200 °C

5.2.7.1.6 Initial time : 10.5 minute

5.2.7.1.7 Rate one : 0.0 °C/ minute

5.2.7.1.8 Hydrogen flow : 30 mL/ minute

5.2.7.1.9 Air flow : 400 mL/ minute

5.2.7.1.10 Makeup flow : 25 mL/ minute

5.2.7.1.11 Specified report : Performance+ Noise

After the Test run print the report from system.

5.2.7.2 Acceptance criteria: Initial signal \leq 25 pA

ASTM noise \leq 0.10 pA

ASTM wander \leq 0.20 pA

ASTM drift \leq /2.5/ pA/hour

5.3 CLEANING PROCEDURE:

Frequency: Daily or after each use.

5.3.1 Open the front part of the instrument .

5.3.2 Wipe out any material in the sample holder assembly by means of tissue paper.

5.3.3 Clean all the sampling accessories with tissue paper after analysis and keep them in proper place.

5.3.4 Clean the outer surface of the instrument with dry cotton cloth.

6.0 SAFETY & PRECAUTIONS:

Not Applicable

7.0 REVISION HISTORY:

Revision No.	Reason for Revision	Superseded from & date



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8.0 DISTRIBUTION:

Copy No.	Issuance Record				Withdrawal Record		Destruction Record	
	Date	Dept. issued	Name / Signature of receiver	Issued By Name / Signature	By	Sign/ Date	By	Sign/ Date

9.0 REFERENCES:

Not Applicable

10.0 ABBREVIATIONS & ANNEXURES:

SOP : Standard Operating Procedure

No. : Number

QC : Quality Control

° : Degree Celsius

ANNEXURES : Not Applicable