Guidance Document

Calibration of Gas Chromatograph

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Introduction

Guidance is provided on general procedure applicable for calibration of Gas Chromatograph (GC) taking Agilent 7890A GC system with GC Autosampler-80 system as an example. Parameters to be considered for calibration of GC are:

1. Column Oven Temperature

- Set the column oven temperature for at least 3 points from 40º to 300º.
- After about ten minutes, record the observed temperature using a calibrated temperature probe. Ensure whether the temperature inside the oven is the same as that set. Also, the injector and detector temperatures must equate with the temperature values set for them.
- **Acceptance Criteria:** The observed temperature should be within ±2º of the set temperature.

2. Flow Rate of Gases

Check whether the flow rate of the carrier gas (Nitrogen/Helium) is within the limits of 22.5-27.5 ml/minute, hydrogen gas flow rate is within the limits of 36-44 ml/minute and air flow rate is within the limits of 360-440 ml/minute using a gas flow meter.

3. Detector Performance

a) Detector Precision and Flow Rate Consistency

- Chromatographic Parameters:

Oven temperature (1)	: 40º
Time (1)	: 0 min.
Rate (1)	: 25º/min.
Oven temperature (2)	: 90º
Time (2)	: 0 min.
Rate (2)	: 15º/min.
Oven temperature (3)	: 170°
Time (3)	: 15 min
Injector temperature	: 250º
Detector (FID) temperature	: 270º
Carrier gas (N_2/He) flow rate	: 0.5 ml/min
Split ratio	: 10:1
Hydrogen flow	: 40±4 ml/min.
Zero Air flow	: 400±40 ml/min.
Auxiliary flow (N2)	: 25±2.5 ml/min.
Injection volume	: 1.0 µl
Septum purge flow	: 5 ml/min.
Syringe used	: 10 µl
Solvent wash pre injection/	
Pre clean with solvent	: 5
Pre clean with sample	: 3
Sample pumps/ filling strokes	: 5
Post clean with solvent	: 5

- **Column:** Fused silica column 30 m long, 0.32 mm internal diameter coated with 0.25 µm film of cross linked methyl siloxane (Use HP-1 column or equivalent).

- **Standard Mixture-Preparation of stock Solution (1000 ppm):** By weighing approximately 50 mg each of n-Tetradecane, n-Pentadecane and n-Hexadecane in a 50 ml volumetric flask containing approximately 30 ml of n-hexane and finally make volume upto 50 ml with n-hexane.
- From above stock solution prepare 100 ppm, 200 ppm, 300 ppm , 400 ppm and 500 ppm respectively by dilution 1 ml , 2 ml, 3 ml, 4 ml, 5 ml in 10 ml volumetric flask of each.
- **Precision:** Inject six replicate injections of the standard mixture of 300 ppm and calculate the area ratio of Pentadecane and Hexadecane to that of the Tetradecane from the chromatograms. Determine the relative standard deviation of the ratio of the area counts of the peaks.
- Retention time of Tetradecane is about 10.6 minutes. The relative retention time of the expected peaks is given below:
- Compound name Relative retention time

n-Hexane	~ 0.41
n-Tetradecane	= 1.00
n-Pentadecane	~ 1.16
n-Hexadecane	~ 1.39

- Determine the relative standard deviation of the retention times of n-Tetradecane, n-Pentadecane and n-Hexadecane.
- **Acceptance Criteria:** %RSD of the ratio of area counts of the peaks corresponding to n-Pentadecane and n-Hexadecane to that of n-Tetradecane in the chromatograms of six replicate injections should not be more than 2.0%. %RSD of the retention times of n-Tetradecane, n-Pentadecane and n-Hexadecane in six replicate injections should not be more than 2.0% (Flow rate consistency).

b) Detector Linearity

- Inject 1.0 µl of all the five standards100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm respectively in duplicate.
- From the data obtained, plot regression curve with concentration on x-axis and mean response of standards on y-axis and calculate the value of correlation coefficient.
- **Acceptance Criteria:** Correlation Coefficient should not be less than 0.99.

Frequency of Calibration

Calibration is to be performed on half yearly basis or after any major failure or after maintenance. On completion of calibration, the GC calibration report shall be filled as per the given format.

	INDIAN PHARMACOPOEIA LABORATORY
	GC CALIBRATION REPORT

Instrument No. :
Instrument Make :
Calibration Frequency :

SOP No. :
Calibrated on :
Next Due on :

S. No.	Parameter	Acceptance Criteria		Observed Value	Complies/ Does Not Comply
1	Detector precision	%RSD of the ratio of area counts of six replicate injections of pentadecane to tetradecane should NMT 2.0%			
		%RSD of the ratio of area counts of six replicate injections of hexadecane to tetradecane should NMT 2.0%			
2	Detector linearity	n-Tetradecane	Correlation Coefficient NLT 0.99		
		n-Pentadecane			
		n-Hexadecane			
3	Flow rate consistency	%RSD of retention time of six replicate injections of hexadecane should NMT 2.0%			
		%RSD of retention time of six replicate injections of pentadecane should NMT 2.0%			
		%RSD of retention time of six replicate injections of tetradecane should NMT 2.0%			
4	Flow rate of carrier gas (Auxiliary)	25± 2.5 ml/min.			
5	Flow rate of hydrogen gas	40± 4 ml/min.			
6	Flow rate of air gas	400± 40 ml/min.			

Performed By _____ Verified By _____