

# Interlaboratory comparison study of two gas chromatography injection techniques viz. liquid and dynamic headspace for trace level quantification of Ethylene Oxide (EtO) and 2-Chloroethanol (2-CE) in ginger powder sample by using tandem mass spectrometry.

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## 1. Purpose

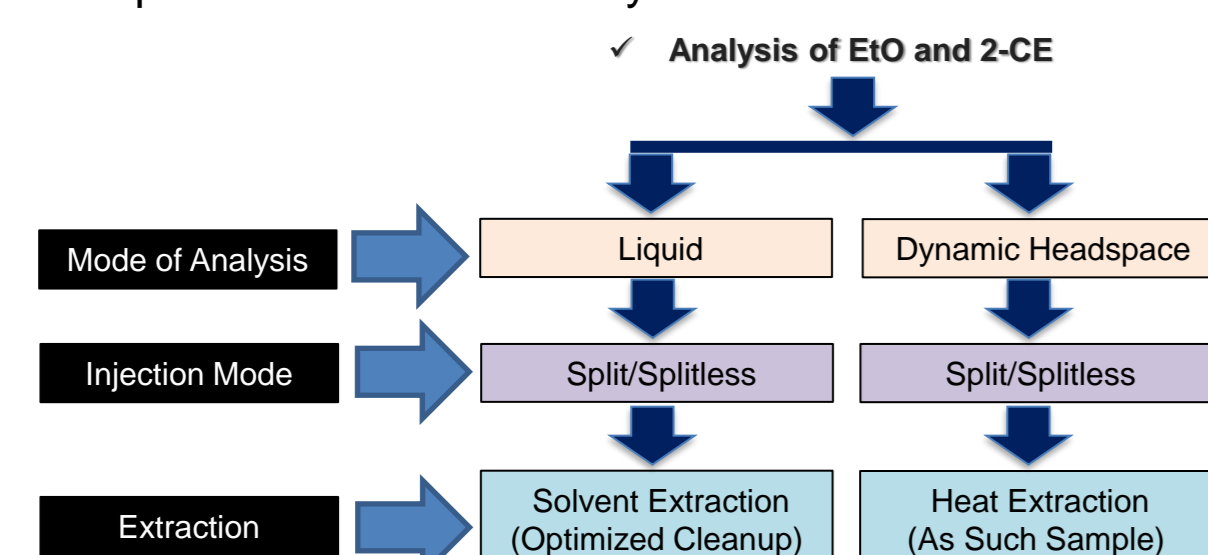
EtO is one of the most widely produced chemicals worldwide. It is colorless, odorless, flammable gaseous cyclic ether with boiling point of 10.4°C. It has a very strong antibacterial property. Due to its small size, it shows a high diffusivity and strong penetrating properties and is thus very effective in the disinfection / disinfestation of food commodities. EtO is highly carcinogenic, mutagenic and genotoxic impurity for living being. The US National Institute of Health (NIH) classified EtO as “known to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in humans, including epidemiological studies and studies on mechanisms of carcinogenesis.” Considering carcinogenicity and no acceptable threshold for exposure, no Acceptable Daily Intake (ADI) was established for EtO. 2-CE, which is one of the prominent metabolite of EtO is also considered to be genotoxic and potentially carcinogenic. Given the inconclusive toxicological picture of 2-CE, it was decided by regulatory authorities to follow the precaution approach and consider 2-CE equally toxic to EtO. Hence it is very important to quantitate EtO and 2-CE in food matrices at trace levels. EU-MRLs (Maximum Residue Levels as per European Commission) for EtO and 2-CE are summarized in Table 1.

Table 1: EU-MRLs for EO and 2-CE

No.	Products	EU-MRLs for EtO and 2-CE
1	Tea, cocoa, spices	0.10 mg/kg
2	Nuts, oil fruits, oilseeds	0.05 mg/kg
3	Fruits, fungi, pulses	0.02 mg/kg
4	Cereals	0.02 mg/kg
5	Apicultural products	0.05 mg/kg

Various methods have been presented in EURL-SRM-Analytical Observation Report for the analysis of EtO and 2-CE. The choice of the analytical approach depends on both the analytes and the matrix to be analyzed. So, it is important to have a detailed comparison study of different analytical approaches to ensure robustness, reliability and accuracy of the method for the quantification of EtO and 2-CE.

This study covers the comparison between liquid injection technique and dynamic headspace injection technique using different extraction processes for ensuring highest extraction efficiencies. Incurred sample is used for this study.



In above study, Shimadzu's triple quadrupole GCMS-TQ8050 NX equipped with HS-20 NX dynamic headspace sampler and AOC-20i/s liquid sampler was used for analysis (Figure 1)



Figure 1: Shimadzu GCMS-TQ8050 NX with AOC-20i/s and HS-20 NX

## 2. Design of Experiment

Table 2: Methods with different experiment conditions

Exp No.	Liquid Method (Solvent Extraction)	Headspace Method (Heat Extraction)
1	Sample + Aluminium beads (Vortex for 15 min at 25°C)	Vial incubation at 65°C
2	Sample + Aluminium beads (Vortex for 45 min at 25°C)	Vial incubation at 125°C
3	Sample + Aluminium beads (Vortex for 90 min at 25°C)	Vial incubation at 65°C (Interlaboratory Study)
4	Sample + Aluminium beads (Sonicate for 90 min at 60°C)	

## 3. Methods

Instrument parameters are given in Table 3, whereas optimized MRM transitions of EtO and 2-CE are given in Table 4.

Table 3. Instrument parameters for GC-MS/MS and HS

GCMS System	: GCMS-TQ8050 NX with HS-20 NX		
Column	: SH-502.2, 60 m, 0.25 mm I.D., 1.4 µm df (S/N: 227-36341-03)		
Injection Mode	: Split (10:1) for liquid method : Split (20:1) for dynamic headspace method		
Flow Control Mode	: Column Flow		
Carrier Gas	: Helium		
Column Flow	: 2.0 mL/min		
Linear Velocity	: 36.0 cm/s		
	Ramp Rate (°C/min)	Temp. (°C)	Hold Time (min)
	-	35	5.0
	30	235	8.33
Temp. Program	: 30, 35, 235, 5.0, 8.33		
GC Run Time	: 20 min		
Ionization Mode	: Electron Ionization (EI)		
Interface Temp.	: 250 °C		
Ion Source Temp.	: 240 °C		
Headspace parameters	: 65 °C and 125 °C		
Oven Temp.	: 130 °C		
Sample Line Temp.	: 150 °C		
Transfer Line Temp.	: 150 °C		
Trap Cooling Temp.	: -10 °C		
Trap Desorption Temp.	: 280 °C		
Trap Equilibration Temp.	: -10 °C		
Shaking Level	: 5		
Equilibrating Time	: 25 min		
Multi Injection Count (MIC)	: 10		
Pressurizing Gas Pressure	: 75 kPa		
GC Cycle Time	: 35 min		

Table 4: MRM transitions for EtO and 2-CE

ID	Name	Quantifier	CE1	Qualifier	CE2	Qualifier	CE3
1	EtO	44>29	6	44>28	6	44>14	18
2	2-CE	80>31	6	80>44	5	82>31	6

## 4. Results and Discussion

Summary for linearity is given in Table 5, whereas experimental results are given in Table 6

Table 5: Summary for linearity

Details	Linearity (r <sup>2</sup> )	
	EtO	2-CE
Liquid - Matrix Matched (100,200,400,600 and 800 ppb)	0.99957	0.99641
HS - 65°C - Matrix Matched (5,10,20,40,80,160,320 and 1600 ppb)	0.99917	0.99999
HS - 125°C - Matrix Matched (5,10,20,40,80,160,320 and 1600 ppb)	0.99984	0.99971

Figure 2 and 3 depict the calibration curve, overlay of linearity standards and representative chromatograms for ginger powder sample for liquid and headspace method, respectively.

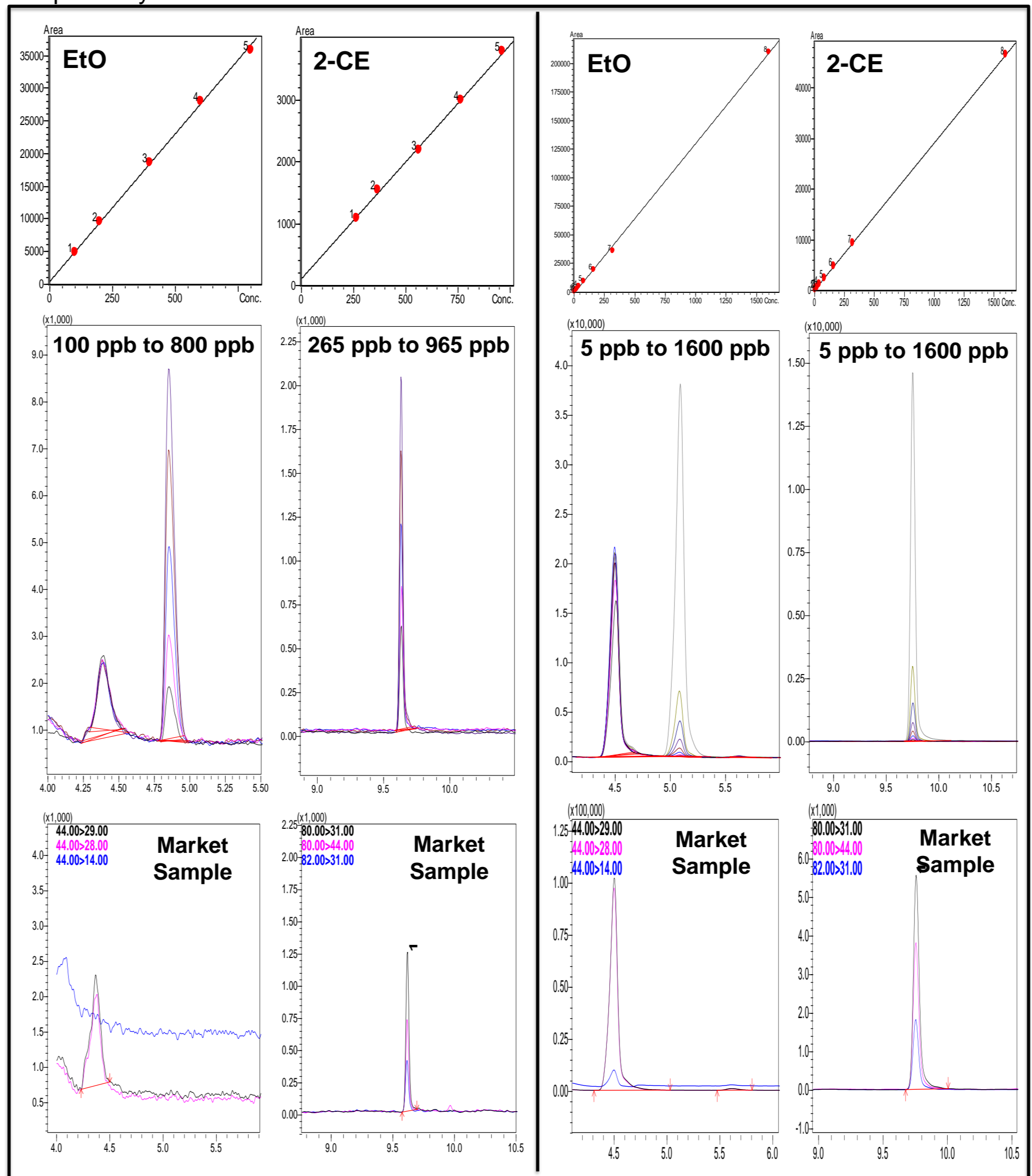
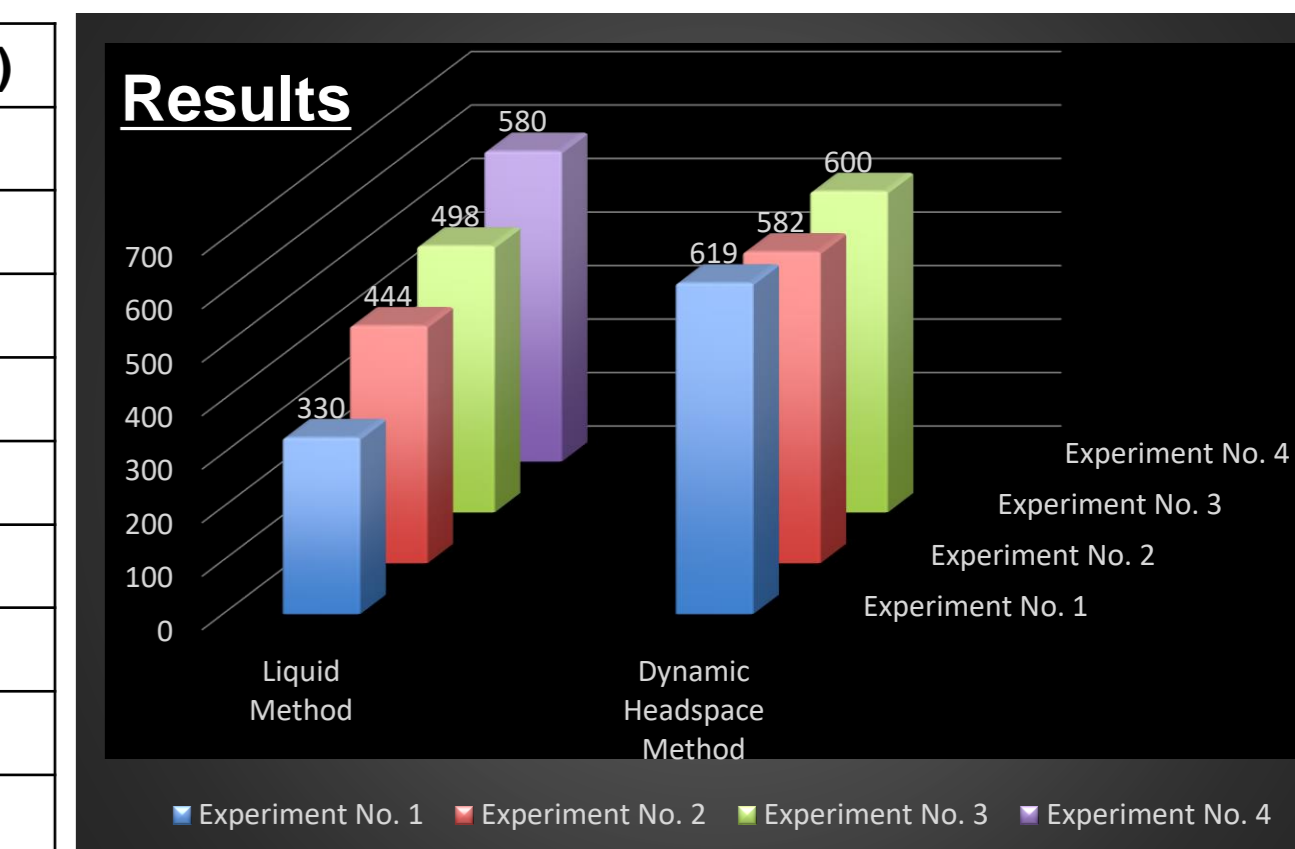


Figure 2: For Liquid Method

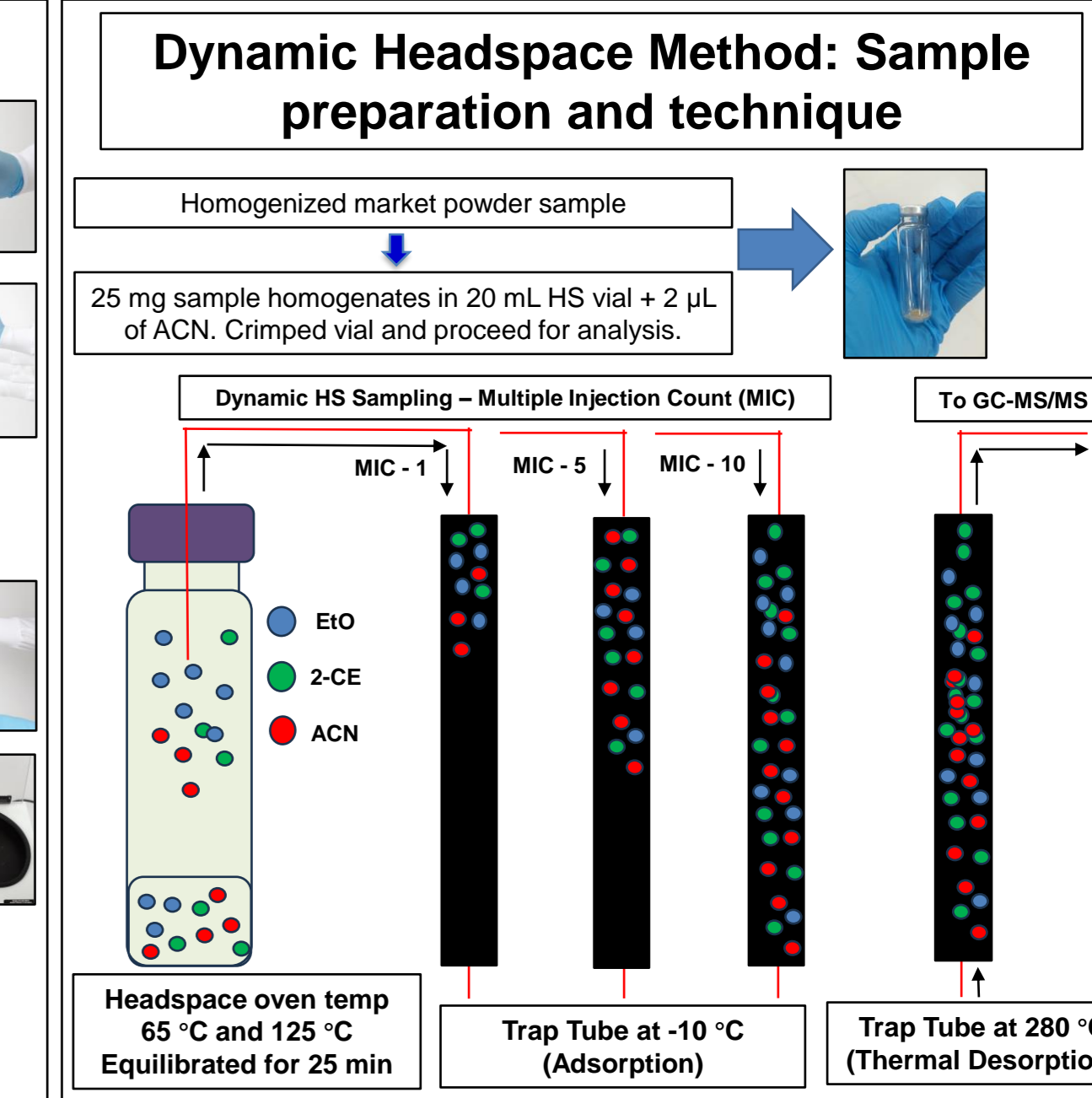
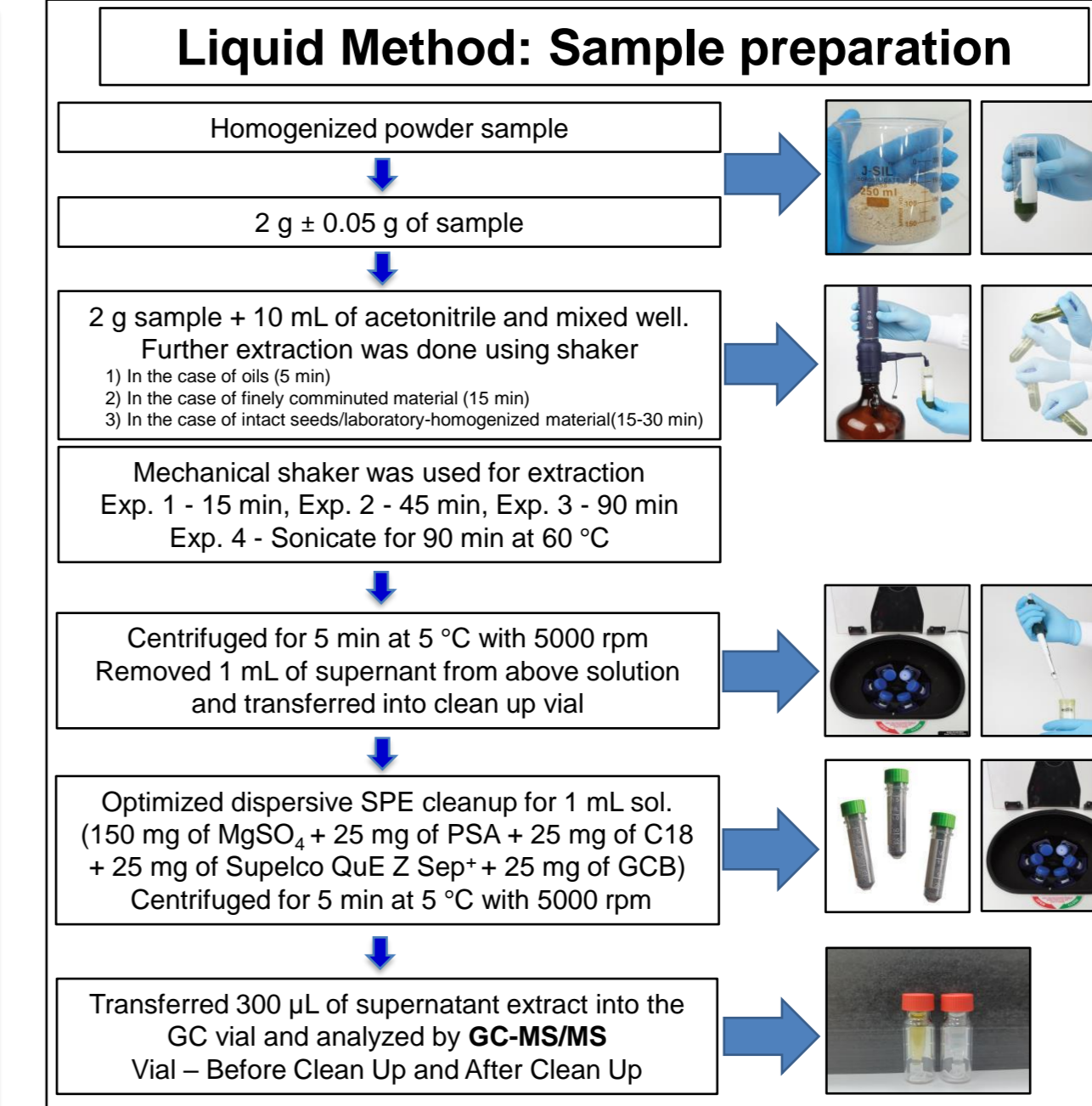
Figure 3: For Headspace Method

Table 6: Comparison of results

Experiment Details	Avg Conc. ppb (n=3)	
	EtO	2-CE
1 Liquid Method (Exp No.1)	-	330
2 Liquid Method (Exp No.2)	-	444
3 Liquid Method (Exp No.3)	-	498
4 Liquid Method (Exp No.4)	-	580
1 HS Method (Exp. No.1)	-	619
2 HS Method (Exp. No.2)	-	582
3 Interlaboratory study	-	600



## Detailed sample preparation for liquid method and HS method



## 5. Conclusion

- In comparison between liquid and HS-Dynamic mode, it is evident that HS Dynamic has advantage in method optimization for accurate recoveries. Liquid extraction needs multiple trials to ensure complete extraction of analytes, majorly depending on matrix. Similarly, temperature and extraction time also plays a critical role
- Quantification of EtO and 2-CE impurities in ginger powder sample was successfully performed by using Shimadzu GCMS-TQ8050 NX with AOC-20i/s and HS-20 NX dynamic headspace samplers
- HS Dynamic requires less sample preparation, demonstrates high recovery due to closed system, high accuracy and easy method optimization

## 6. References

[1] EURL-SRM – Analytical Observation Report, Version 1.1 (December 2020)