Application Note

Food & Beverage Testing

Estimation of Ethylene Oxide and Ethylene Chlorohydrin in Foodstuffs by HS-GC/MS/MS

Agilent 8890 GC and 7010B triple quadrupole GC/MS system with an Agilent CTC PAL3 headspace sampler

Authors

Praveen Arya and
Vivek Dhyani
Agilent Technologies, Inc.

Abstract

This application note demonstrates the use of the Agilent 8890 GC system coupled with an Agilent 7010B triple quadrupole GC/MS and an Agilent CTC PAL3 Headspace sampler to detect and quantify ethylene oxide (ETO) and ethylene chlorohydrin (ECH) in sesame seed and black pepper samples.

The method provides high confidence in results for routine analysis in the food industry, whether involved in production, processing, storage, or commercial testing of food samples, or for academic purpose.

This is an automated analysis solution that does not involve any sample preparation. The sample is directly taken in a headspace vial, incubated in a headspace sampler and vapors from the headspace are injected into the GC/MS/MS system. A limit of quantitation (LOQ) of 5 parts per billion (ppb) has been demonstrated in matrix.

Introduction

ETO is used to sterilize foods to eliminate insects and bacteria such as Salmonella. ECH is a derivative produced by the reaction of ETO with chlorine ions present in foodstuffs. The use of ETO is banned in the European Union due to its carcinogenic and toxic properties.

For the analysis of ETO and ECH in foods, various methods are available that involve lengthy sample preparation and heavy matrix introduction into the GC/MS/MS system. Contamination caused by heavy matrix shortens a consumable's lifetime.

The method adopted in this application note shows the use of the headspace sampler for sample introduction into the GC/MS/MS system. The headspace sampler prevents the need for cumbersome sample preparation and can detect and quantify lower concentrations of ETO and ECH in matrices such as sesame seeds and black pepper using the 8890 GC system coupled with the 7010B triple quadrupole GC/MS and the CTC PAL3 sampler .

Experimental

Instrument parameters

The HS-GC/MS/MS parameters used in this method are outlined in Table 1.

Matrix standard calibration

Standards of ETO and ECH were combined into a stock solution (in toluene) at a concentration of 500 ng/µL. From this solution, six calibration standards were prepared in toluene between 10 and 500 ng/µL. Of the blank sample matrix, 50 mg was taken in a 20 mL headspace vial. Then, 50 µL of 10 ng/µL standard solution was added to the vial, which was then capped immediately. This produced a matrix standard concentration of 5 ng/g. Similarly, matrix standard concentrations of 10, 25, 50, 100, and 250 ng/g were prepared. All standards were stored in a refrigerator.

Sample

A 50 mg sample was taken and added to a 20 mL headspace vial. Then, 50 µL of toluene was added and the vial was capped.

Table 1. HS-GC/MS/MS parameters.

HS Parameters	
Model	Agilent CTC PAL3 Headspace Sampler 120 cm
Incubation Temperature	140 °C
Incubation Time	20 min
Syringe Temperature	150 °C
Agitation During Extraction	Yes; 250 rpm
Injection Volume	2.0 mL
Injection Flow Rate	30 mL/min
GC Parameters	
Inlet Temperature	250 °C
Inlet Liner	Agilent inlet liner, direct, 1.5 mm id (p/n 18740-80200)
Inlet Septa	Agilent inlet septa, 11 mm (p/n 8010-0239)
Column	Agilent J&W DB-VRX (p/n 122-1564)
Column Flow	1 mL/min
Split Ratio	10
Oven Program	35 °C for 4 min 15 °C/min to 150 °C, hold 1 min 40 °C/min to 240 °C, hold 7 min
Transfer Line Temperature	240 °C
Collision Gas	Argon, 0.5 mL/min
Quench Gas	Helium, 2.25 mL/min
Column Nuts	Agilent column nuts, self-tightening (p/n G3440-81011 and G3440-81013)
MS Parameters	
Ion Source Temperature	230 °C
Q1 and Q1 Temperature	150 °C
Solvent Delay	4.5 min
MRM Transitions for ETO	44 → 29 (CE:5) 44 → 28 (CE:5) 44 → 14 (CE:20)
MRM Transitions for ECH	80 → 31 (CE:5) 80 → 43 (CE:5) 82 → 31 (CE:5)
Gain Factor	20

Results and discussion

Quantifier and qualifier peaks in matrix standards

Quantifier and qualifier peaks in matrix standards are displayed in Figures 1 to 4 .Good peak shapes were observed for quantifier and qualifier peaks at LOQ level concentration of ETO and ECH in Black pepper and sesame seeds matrices.

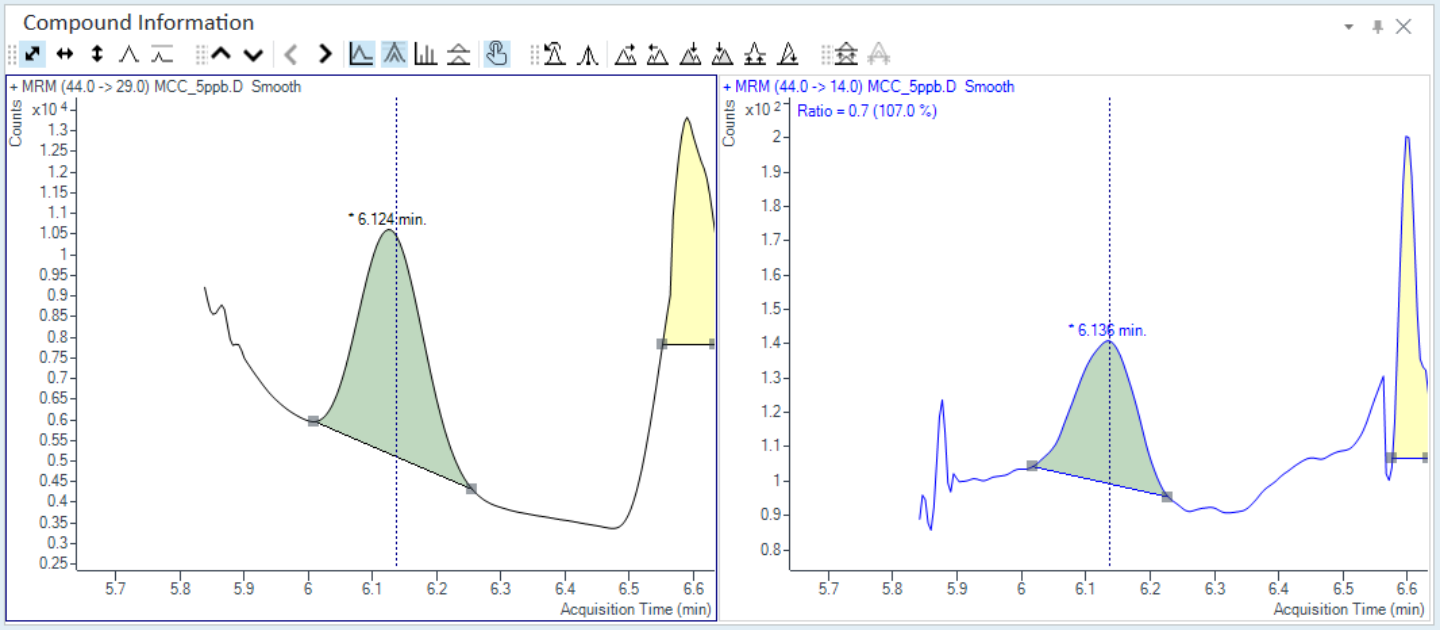

Figure 1. Quantifier and qualifier peaks of ETO at 5 ng/g matrix standard in black pepper.

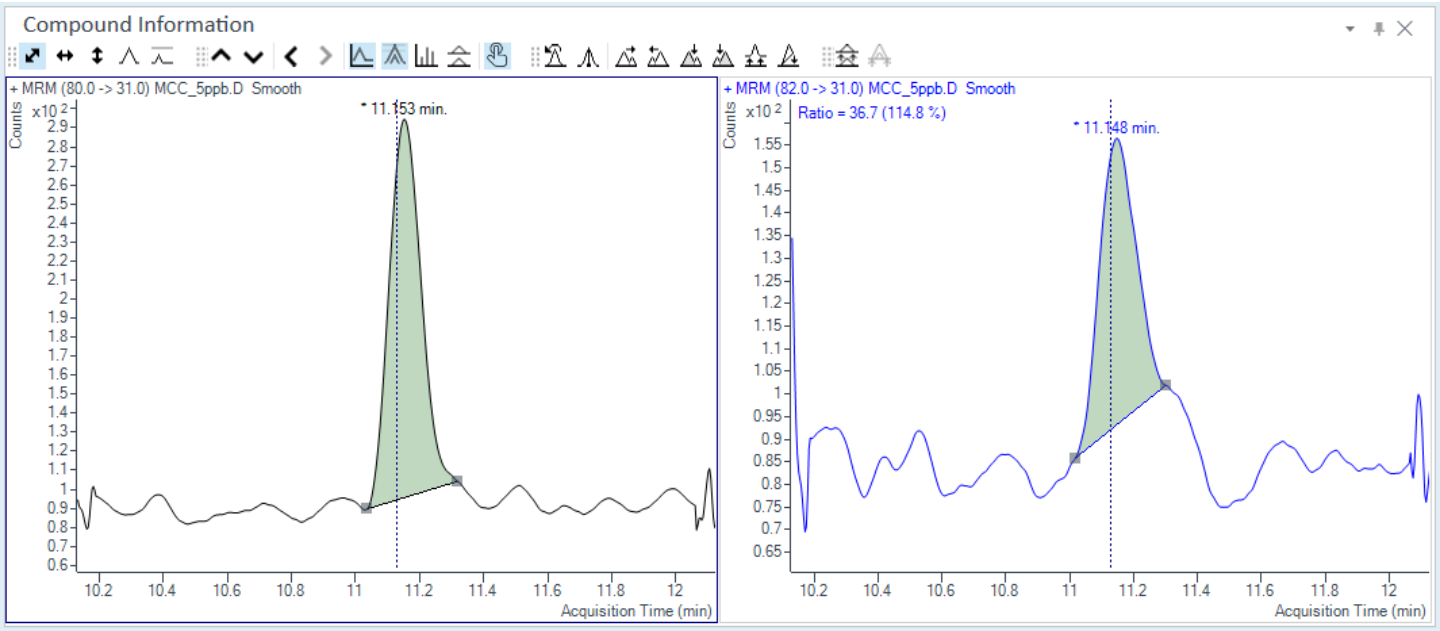

Figure 2. Quantifier and qualifier peaks of ECH at 5 ng/g matrix standard in black pepper.

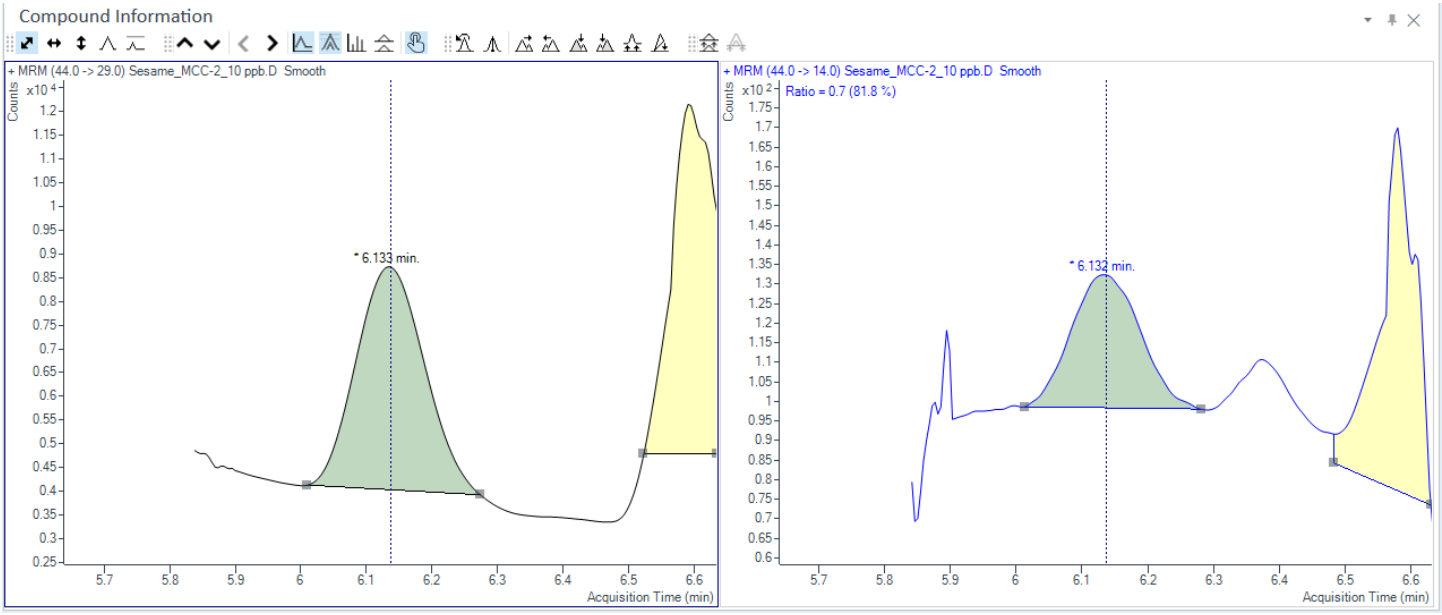

Figure 3. Quantifier and qualifier peaks of ETO at 10 ng/g matrix standard in sesame seeds.

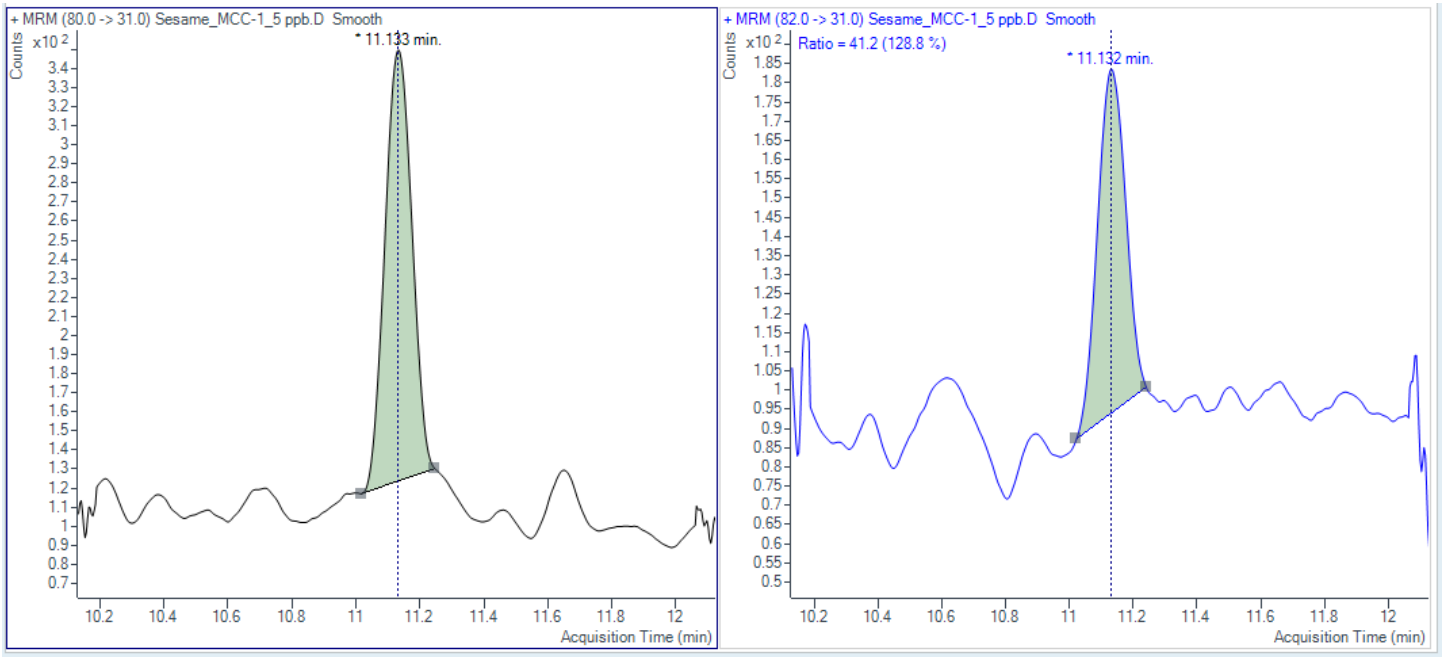

Figure 4. Quantifier and qualifier peaks of ECH at 5 ng/g matrix standard in sesame seeds.

Calibration

Matrix calibrations were plotted for ETO and ECH at concentrations of 5, 10, 25, 50, 100, and 250 ng/g for each matrix (black pepper and sesame seeds). Excellent R^2 values were obtained for six-point calibration. Figures 5 and 6 show the linearity of ETO and ECH respectively in black pepper. Figures 7 and 8 show linearity of ETO and ECH respectively in sesame seeds.

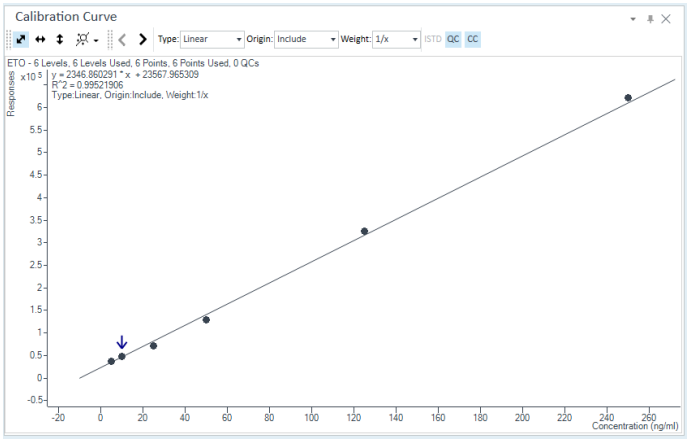

Figure 5. Calibration curve for ETO in black pepper. R^2 >0.995.

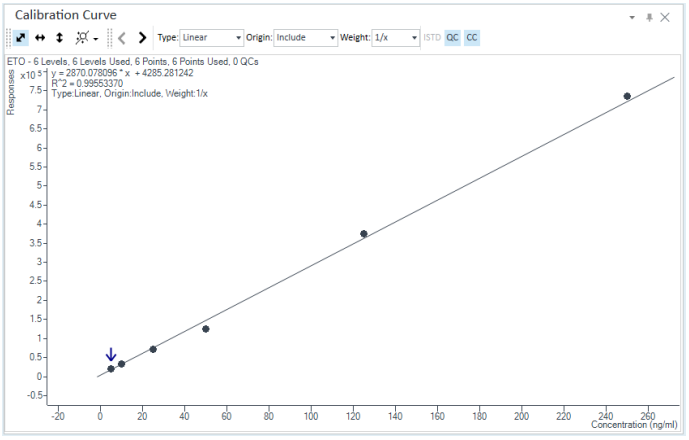

Figure 7. Calibration curve for ETO in sesame seeds. R^2 >0.995.

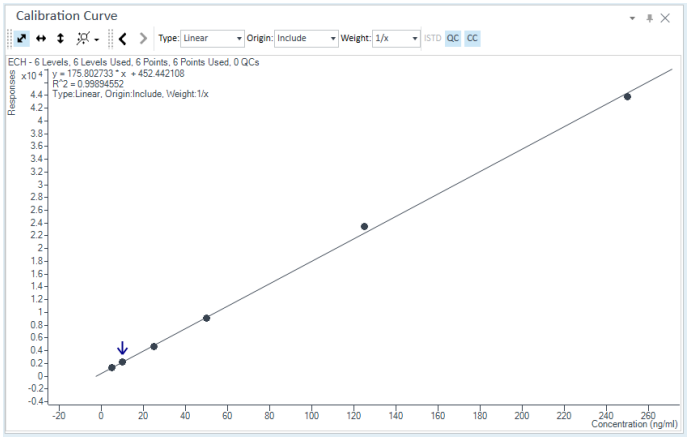

Figure 6. Calibration curve for ECH in black pepper. R^2 >0.998.

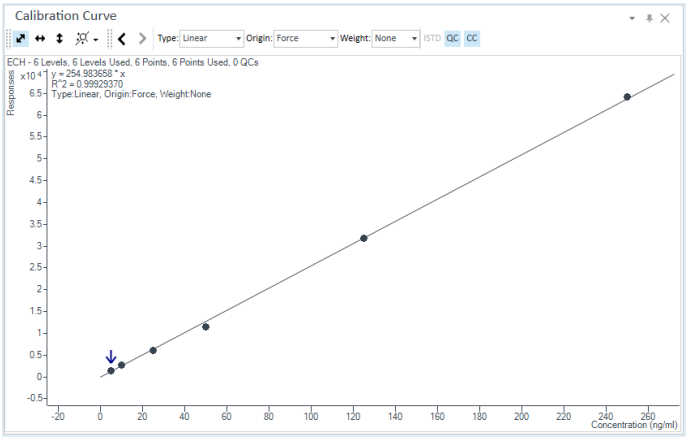

Figure 8. Calibration curve for ECH in sesame seeds. R^2 >0.999.

Standard area repeatability

A repeatable elution was obtained by injecting a 50 ppb concentration of ETO and ECH in matrix. As shown in Table 2, %RSDs of ETO and ECH were calculated from peak areas of three replicate injections of 50 ppb matrix standards in black pepper.

Table 2. Standard area repeatability for three replicates of 50 ppb matrix standard in black pepper.

Sample Name	ETO Area	ECH Area
Replicate 1	138,680	10,326
Replicate 2	138,513	10,666
Replicate 3	135,403	10,497
%RSD	1.34%	1.62%

Recovery

ETO and ECH were spiked in black pepper and sesame seed samples at a concentration level of 10 and 50 ng/g respectively. Good recoveries were obtained by quantification through matrix-based calibration. Results are highlighted in Figures 9, 10, 11, and 12.

		Sample					ETO Met...	ETO Results					Qualifier (44.0 -> 14.0) R...
!		Data File	Type	Level	Acq. Date-Time	Dil.	Exp. Conc.	RT	Resp.	Calc. Conc.	Final Conc.	Accuracy	Ratio
		Black Pepper Powder Blank.D	Sample		6/29/2022 2:54 PM	1.0		6.150	19302	0.0000	0.0000		0.5
		Matrix rep 50 ppb-1.D	Sample		7/1/2022 11:33 AM	1.0		6.159	138680	49.0494	49.0494		0.6
		Matrix rep 50 ppb-2.D	Sample		7/1/2022 12:04 PM	1.0		6.164	138513	48.9783	48.9783		0.7
		Matrix rep 50 ppb-3.D	Sample		7/1/2022 12:35 PM	1.0		6.159	135403	47.6530	47.6530		0.7
		Black pepper powder spike L1.D	Sample		6/29/2022 6:46 PM	1.0		6.155	46096	9.5991	9.5991		0.8
		Black pepper powder spike L2.D	Sample		6/29/2022 7:17 PM	1.0		6.164	133516	46.8488	46.8488		0.8
		Blank-5.D	Sample		6/30/2022 2:01 PM	1.0		6.594	0				

ETO recovery at 10 ppb spike level (L1) = 95.99%
ETO recovery at 50 ppb spike level (L2) = 93.68%

Figure 9. ETO recovery in black pepper.

		Sample					ECH Met...	ECH Results					Qualifier (82.0 -> 31.0) R...
!		Data File	Type	Level	Acq. Date-Time	Dil.	Exp. Conc.	RT	Resp.	Calc. Conc.	Final Conc.	Accuracy	Ratio
		Black Pepper Powder Blank.D	Sample		6/29/2022 2:54 PM	1.0		11.129	506	0.3023	0.3023		
		Matrix rep 50 ppb-1.D	Sample		7/1/2022 11:33 AM	1.0		11.141	10326	56.1635	56.1635		33.8
		Matrix rep 50 ppb-2.D	Sample		7/1/2022 12:04 PM	1.0		11.145	10666	58.0945	58.0945		30.6
		Matrix rep 50 ppb-3.D	Sample		7/1/2022 12:35 PM	1.0		11.141	10497	57.1366	57.1366		31.0
		Black pepper powder spike L1.D	Sample		6/29/2022 6:46 PM	1.0		11.153	2314	10.5894	10.5894		32.9
		Black pepper powder spike L2.D	Sample		6/29/2022 7:17 PM	1.0		11.145	9507	51.5020	51.5020		33.3
		Blank-5.D	Sample		6/30/2022 2:01 PM	1.0							

ECH recovery at 10 ppb spike level (L1) = 100.6%
ECH recovery at 50 ppb spike level (L2) = 103%

Figure 10. ECH recovery in black pepper.

Sample							ETO Met...	ETO Results					Qualifier (44.0 -> 14.0) R...
		Data File	Type	Level	Acq. Date-Time	Dil.	Exp. Conc.	RT	Resp.	Calc. Conc.	Final Conc.	Accuracy	Ratio
		Sesame Blank.D	Sample		7/1/2022 6:30 PM	1.0		6.137	9652	1.8699	1.8699		0.7
		Sesame_MCC-1_5 ppb.D	Cal	1	7/1/2022 8:20 PM	1.0	5.0000	6.133	20620	5.6913	5.6913	113.8	0.9
		Sesame_MCC-2_10 ppb.D	Cal	2	7/1/2022 9:03 PM	1.0	10.0000	6.133	33932	10.3295	10.3295	103.3	0.7
		Sesame_MCC-3_25 ppb.D	Cal	3	7/1/2022 9:46 PM	1.0	25.0000	6.137	71518	23.4253	23.4253	93.7	1.0
		Sesame_MCC-4_50 ppb.D	Cal	4	7/1/2022 10:18 PM	1.0	50.0000	6.137	125171	42.1194	42.1194	84.2	0.7
		Sesame_MCC-5_125 ppb.D	Cal	5	7/1/2022 10:49 PM	1.0	125.0000	6.137	374278	128.9139	128.9139	103.1	0.7
		Sesame_MCC-6_250 ppb.D	Cal	6	7/1/2022 11:20 PM	1.0	250.0000	6.137	734779	254.5206	254.5206	101.8	0.7
		Sesame spike_L1.D	Sample		7/2/2022 12:23 AM	1.0		6.137	32260	9.7470	9.7470		0.8
		Sesame spike_L2.D	Sample		7/2/2022 12:54 AM	1.0		6.137	120763	40.5833	40.5833		0.7

ETO recovery at 10 ppb spike level (L1) = 97.47%
ETO recovery at 50 ppb spike level (L2) = 81.17%

Figure 11. ETO recovery in sesame seeds.

Sample							ECH Met...	ECH Results					Qualifier (82.0 -> 31.0) R...
		Data File	Type	Level	Acq. Date-Time	Dil.	Exp. Conc.	RT	Resp.	Calc. Conc.	Final Conc.	Accuracy	Ratio
		Sesame Blank.D	Sample		7/1/2022 6:30 PM	1.0							
		Sesame_MCC-1_5 ppb.D	Cal	1	7/1/2022 8:20 PM	1.0	5.0000	11.133	1325	5.1968	5.1968	103.9	41.2
		Sesame_MCC-2_10 ppb.D	Cal	2	7/1/2022 9:03 PM	1.0	10.0000	11.141	2783	10.9139	10.9139	109.1	30.4
		Sesame_MCC-3_25 ppb.D	Cal	3	7/1/2022 9:46 PM	1.0	25.0000	11.141	6053	23.7394	23.7394	95.0	34.7
		Sesame_MCC-4_50 ppb.D	Cal	4	7/1/2022 10:18 PM	1.0	50.0000	11.145	11413	44.7597	44.7597	89.5	31.0
		Sesame_MCC-5_125 ppb.D	Cal	5	7/1/2022 10:49 PM	1.0	125.0000	11.145	31701	124.3245	124.3245	99.5	33.2
		Sesame_MCC-6_250 ppb.D	Cal	6	7/1/2022 11:20 PM	1.0	250.0000	11.141	64121	251.4714	251.4714	100.6	31.4
		Sesame spike_L1.D	Sample		7/2/2022 12:23 AM	1.0		11.149	2656	10.4150	10.4150		31.4
		Sesame spike_L2.D	Sample		7/2/2022 12:54 AM	1.0		11.149	10957	42.9728	42.9728		30.4

ECH recovery at 10 ppb spike level (L1) = 104.1%
ECH recovery at 50 ppb spike level (L2) = 85.94%

Figure 12. ECH recovery in sesame seeds.

Conclusion

An accurate and robust method was developed for the analysis of ETO and ECH in black pepper and sesame seed samples. This approach avoids the need for sample preparation, as the sample is processed directly by the PAL3 headspace sampler. Heavy matrix in sample is not introduced in to the triple quadrupole GC/MS system, which greatly enhances the consumable's lifetime. The LOQ of the method was demonstrated at 5 ng/g for sesame seed and black pepper samples. Repeatable results were found for three successive replicates of black pepper samples at a 50 ng/g spiked concentration of ETO and ECH. Excellent recoveries were obtained for black pepper and sesame seed matrices at 10 and 50 ng/g spiked concentration levels. Thus, this application note demonstrates the usefulness of the developed method for routine analysis of food samples for ETO and ECH analysis at trace levels.

References

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2. Fully Automated Cryogen-Free Analysis of Ethylene Oxide and 2-Chloroethanol in Contaminated Sesame Seeds Using Headspace–Trap with Multi-Step Enrichment (MSE) GC–MS. *Markes International application note 281*.
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