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Analysis of residual Ethylene Oxide in Intraocular lenses using HS-GCMS

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1. Introduction

It is imperative to employ effective agents to disinfect and sterilize instruments and equipments, used for patient care and healthcare staff. There are many methods of disinfection and it is very important to confirm the residues of disinfectant in finished product. In this poster we discuss the quantitation of Ethylene Oxide (EtO) as disinfectant residue in intraocular lenses. Ethylene Oxide sterilization is mainly used to sterilize medical and pharmaceutical products that cannot support conventional high temperature steam sterilization, such as devices that incorporate electronic components, plastic containers and intraocular lenses[1]. Boiling point of pure EtO is 10.73°C at atmospheric pressure (The molecular structure of EtO is given in Fig. 1). It is highly volatile and can be easily removed from product. As it is highly volatile there are less chances of finding it in product as an impurity. EtO is a potential carcinogen. Since it is still used in sterilization of medical products, it is necessary to quantify EtO precisely at very low level. Occupational Safety and Health Administration (OSHA) has set the permissible exposure limit for EtO as 1.0 ppm[2]. The objective of this study is to quantify EtO at very low concentration as the intraocular lenses are permanently implant in human eye. (Picture of intraocular lens is given in Fig. 2). The analysis is carried out by using Shimadzu Headspace-Gas chromatograph - Mass Spectrometer (GCMS-QP2010 Ultra coupled with HS-20).

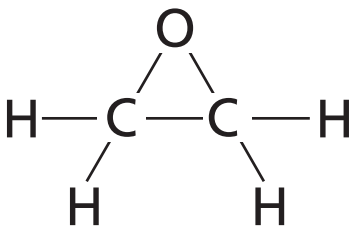

Fig. 1 Structure of Ethylene Oxide

Fig. 2 Intraocular lens

2. Method of Analysis

2-1: Extraction of Ethylene Oxide from Intraocular Lenses

Intraocular lenses were procured from local medical store. Standard stock solution of EtO (500 ppm in Dimethyl sulfoxide) was procured from Sigma Aldrich. Dimethyl sulfoxide was used as diluent for further sample and standard solution preparations. HS-GCMS technique was used for quantitation of EtO at very low concentration. Solutions were prepared as follows,

1) Blank Solution – 5 mL diluent was added to headspace vial and was crimped tightly with automated crimper.
2) Standard Solutions – 5 mL of each Std. EtO in the range of 2.5 to 3000 ppb was added to headspace vial and was crimped tightly with automated crimper.
3) Sample Solution – A piece of intraocular lens was transferred to headspace vial, 5 mL diluent was added and vial was crimped tightly with automated crimper

Partial method validation was carried out by performing Reproducibility, Linearity, LOD-LOQ determination and Recovery study. For validation, solutions of different concentrations were prepared using standard stock solution of EtO (500 ppm) as mentioned in Table 1.

Table 1 Method validation parameters

Parameter	Concentration
Reproducibility	1000 ppb
Linearity	5 levels – 100 ppb – 3000 ppb
Accuracy/Recovery	3 levels – 100 ppb – 500 ppb
Precision at LOQ level	2.5 ppb

2-2. HS-GCMS Analytical Conditions

Samples were analyzed using HS-20 coupled with GCMS-QP2010 Ultra (Fig. 3) as per the conditions given in Table 2.

Fig. 3 HS-20 coupled with GCMS-QP2010 Ultra byShimadzu

Table 2 HS-GCMS analytical parameters

Headspace parameters

Mode	: Loop
Oven Temp	: 100°C
Sample Line Temp	: 110°C
Transfer Line Temp	: 120°C
Equilibrating Time	: 30.0 min
Pressurizing Time	: 1.0 min
Pressure Equilib. Time	: 0.10 min
Load Time	: 0.50 min
Load Equilib. Time	: 0.10 min
Injection Time	: 1.0 min
Needle Flush Time	: 5.0 min
GC Cycle Time	: 26.0 min

Chromatographic parameters

Column	: Rtx-624 (60 m × 0.53 mm × 3.00 µm)		
Injection Mode	: Split		
Split Ratio	: 5.0		
Carrier Gas	: Helium		
Flow Control Mode	: Linear Velocity		
Linear Velocity	: 59.8 cm/sec		
Pressure	: 12.1 kPa		
Column Flow	: 5.50 mL/min		
Total Flow	: 33.0 mL/min		
Total Run Time	: 16.0 min		
Column Oven Temp	: Rate °C /min	Temperature °C	Hold time (min)
	30.0	40.0	5.0
		220.0	5.0

Mass Spectrometry parameters

Ion Source Temp	: 200°C
Interface Temp	: 220°C
Ionization Mode	: EI
Event Time	: 0.30 sec
Mode	: SIM
m/z	: 29, 43 and 44
Start Time	: 2.0 min
End Time	: 4.0 min

3. Results

3-1. Fragmentation of Ethylene Oxide

According to fragmentation of Ethylene Oxide, molecular ion peak was *m/z*-44 with base peak at *m/z*-29, which was used for quantitation where as fragment ion with *m/z*-43 and *m/z*-44 were used as reference ions. Mass chromatograms of 1000 ppb EtO standard solution with *m/z* 29,43 and 44 are shown in Fig. 4, Mass spectrum of EtO is shown in Fig. 5. Validation data is summarized in Table 3. Fig. 6 and 7 shows, overlay mass chromatograms for *m/z*-29 at different concentrations and calibration curve for linearity levels, respectively.

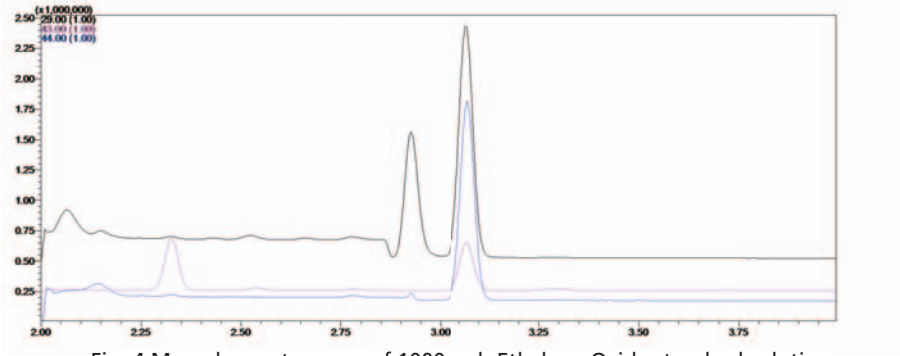

Fig. 4 Mass chromatograms of 1000 ppb Ethylene Oxide standard solution

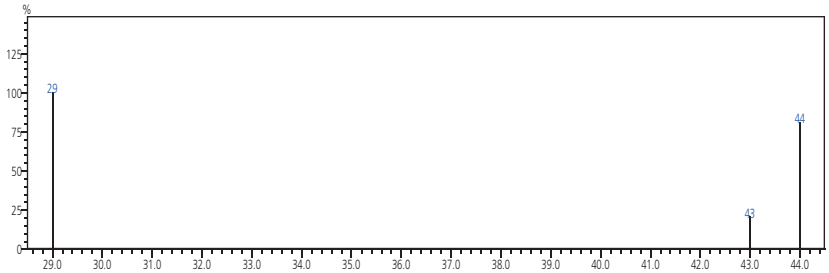

Fig. 5 Mass spectrum of Ethylene Oxide

3-2. Summary of validation results

Table 3 Summary of results for validation parameter

ID	Compound Name	Parameter	Concentration	Result
1	Ethylene Oxide	Reproducibility	1000 ppb	%RSD is 0.8 for area (n=6)
2		Linearity	100 ppb - 3000 ppb	Correlation Coefficient is 0.9999*
3		LOD	2.5 ppb - 10 ppb	0.8 ppb**
4		LOQ		2.5 ppb**
5		Precision at LOQ	2.5 ppb	Average of S/N ratio is 16 (n=6)
				%RSD is 9.6 for area (n=6)

*Linearity level– 100 ppb, 250 ppb, 500 ppb,1000 ppb and 3000 ppb. For linearity, refer Fig. 6 and Fig. 7.

**As per software calculations.

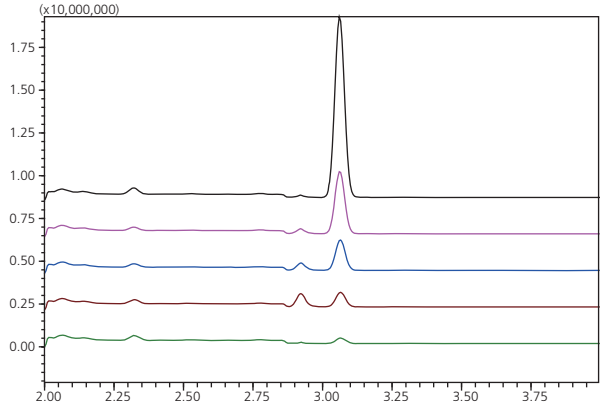

Fig. 6 Linearity levels overlay mass chromatogram

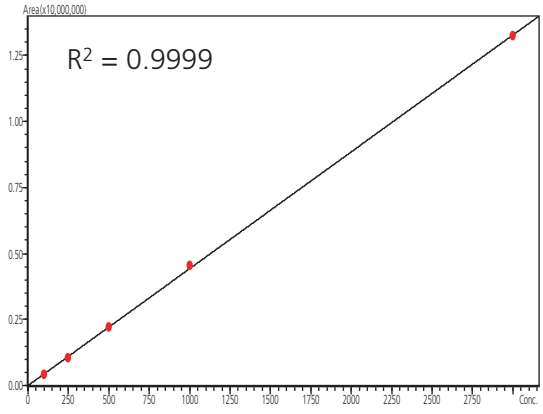

Fig. 7 Linearity levels calibration curve

3-3. Quantification of Ethylene Oxide in intraocular lens sample

Analysis of intraocular lens samples was done as per the given method. Recovery studies were carried out by spiking 100 ppb, 250 ppb and 500 ppb of standard solutions in sample of intraocular lenses. Fig. 8 gives overlay mass chromatogram of spiked and unspiked samples. Table 4, gives the summary of results.

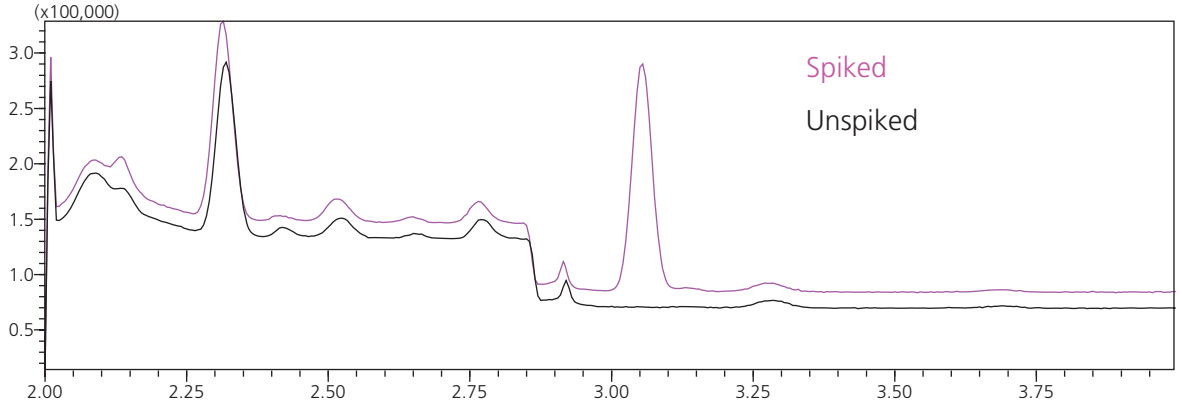

Fig. 8 Spiked and unspiked sample solution overlay mass chromatograms

Table 4 Summary of results for sample analysis

ID	Sample Name	Parameter	Concentration	Result
1	Unspiked Sample	Precision	NA	Below LOQ level
2	Intraocular lens samples spiked with different linearity levels standards	Recovery	100 ppb spiked	Recovery - 87%
			250 ppb spiked	Recovery - 92%
			500 ppb spiked	Recovery - 97%

4. Conclusion

- HS-GCMS method was developed for quantitation of residual EtO present in intraocular lenses sample. Part method validation was performed successfully. Results obtained for Reproducibility, Linearity, LOQ and Recovery studies were well within limit, as per ICH guidelines[3].
- With "Low Carryover" – The characteristics feature of HS-20 headspace, reproducibility even at very low concentration level could be achieved easily.
- High speed scan rate 20,000 u/sec is the characteristic feature of GCMS-QP2010 Ultra mass spectrometer, useful for quantitation of residual EtO at very low level (ppb level) with high sensitivity.

5. References

[1] "Biological Evaluation of Medical Devices-Part 7: Ethylene Oxide Sterilization Residuals," ANSI/AAMI/ISO 10993-7, Arlington, VA, Association for the Advancement of Medical Instrumentation, 1995.
[2] Potter, Wayne "OSHA Method No. 30, Ethylene Oxide ", OSHA Analytical Laboratory, Salt Lake City, Utah 84115, August 1981.
[3] ICH guidelines Q2(R1), Validation of Analytical Procedure Text And Methodology, 2005

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