



Application Note 292

Detection of ethylene oxide in medical equipment by headspace-trap with multi-step enrichment and GC-MS

Levels of residual ethylene oxide remaining on medical equipment after sterilisation are usually measured by extraction with water, but subsequent methods to analyse that water lack sensitivity. To resolve this, we present a method that involves using the focusing trap of Centri 90 to extract multiple large (5 mL) headspace volumes from a single water sample and analyse these together in one run. The method is fully automated and highly sensitive, achieving a detection limit of 0.35 µg/L from water with excellent linearity and reproducibility metrics.

Introduction

The sterilisation of medical equipment such as gauzes and bandages is critical for patient safety. A common means of sterilisation is through exposure to ethylene oxide (EtO) gas, which is highly effective at killing bacteria and other microorganisms that might otherwise cause infections. However, any residual EtO remaining after sterilisation may cause irritation and other undesirable effects when it comes into contact with patients.

To address this, methods to detect EtO and its degradation product 2-chloroethanol (2-CE) in medical equipment are used. Typically, the equipment is submerged in water, into which the EtO and 2-CE partition.¹ Analytes are then extracted

from the water, followed by separation and detection by gas chromatography-mass spectrometry (GC-MS). However, achieving sufficient extraction efficiency during this second step can be challenging.

In the technique described here, three headspace extractions, each of 5 mL, are extracted from a single closed vial and transferred to a focusing trap. The trap is electrically cooled throughout and then heated rapidly after the final extraction, so that analytes from all three extractions are desorbed to the GC column together in a narrow band of vapour. This technique, termed headspace-trap (HS-trap) with multi-step enrichment (MSE) is illustrated in Figure 1, and we have previously applied it to EtO and 2-CE on sesame seeds ([Application Note 281](#)) and other dry foods ([Application Note 284](#)).

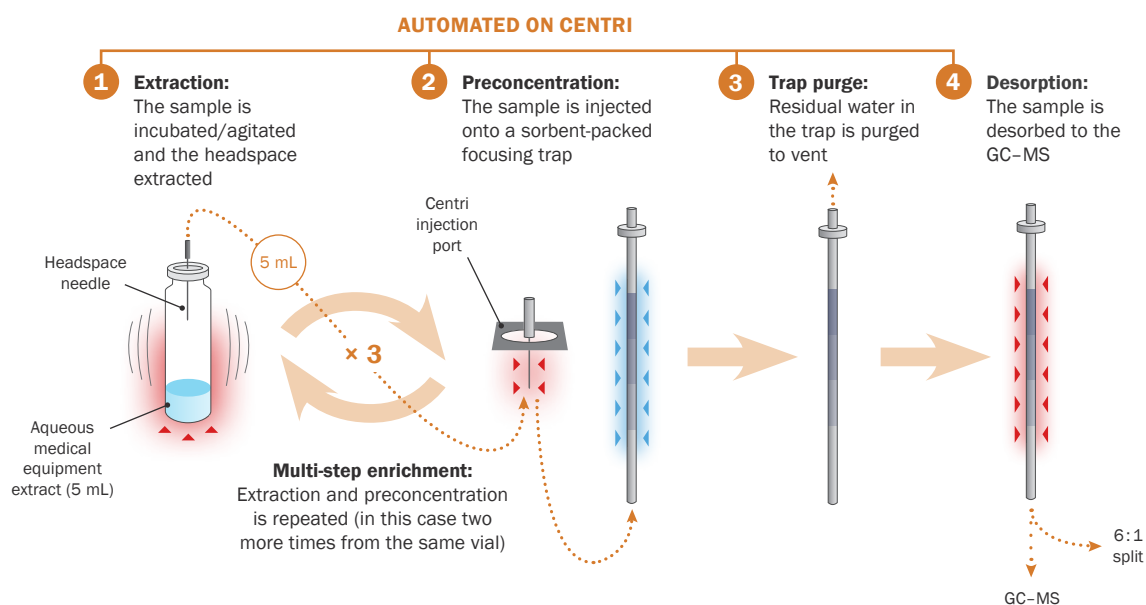


Figure 1: Headspace-trap with multi-step enrichment for the analysis of ethylene oxide in medical equipment extracts.

Here, HS-trap with MSE is performed on the Centri® 90 sample concentration platform, which couples gold-standard robotics with Markes' unique multi-bed focusing trap design enabling simultaneous analysis of compounds across a wide volatility range.

Experimental

Calibration standards:

Solutions of EtO and 2-CE at 50 mg/L were prepared in ethanol and water respectively. Prior to each analytical run, the solutions were combined to form a working solution containing analytes at 0.5 mg/L (EtO) or 5 mg/L (2-CE) in water. To prepare a calibration standard, 1 g NaCl was placed into a 20 mL headspace vial, and water and working solution were combined to give a total liquid volume of 5 mL with concentrations as shown in Table 1. Vials were closed by crimping.

Calibration level	Volume (μL)		Final concentration ($\mu\text{g/L}$)	
	Working solution	Water	EtO	2-CE
Blank	0	5000	0	0
1	10	4990	1	10
2	20	4980	2	20
3	50	4950	5	50
4	100	4900	10	100
5	200	4800	20	200
6	500	4500	50	500

Table 1: Calibration standards for EtO and 2-CE prepared in water from a working solution of both analytes at 5 mg/L.

Medical gauze samples:

Medical gauze (Figure 2) was acquired from a local pharmacy. 1 cm² squares were cut and fortified with 1 μL ethanol containing EtO and 2-CE at 20–100 mg/L for a final concentration on the gauze of 0.1–5 $\mu\text{g}/\text{cm}^2$. Gauzes spiked only with ethanol solvent served as blanks. Spiked gauze squares were immersed in 20 mL pure water and sonicated at 35°C for 1 h, after which 5 mL water was transferred to a 20 mL headspace vial containing 1 g NaCl. Vials were closed by crimping.

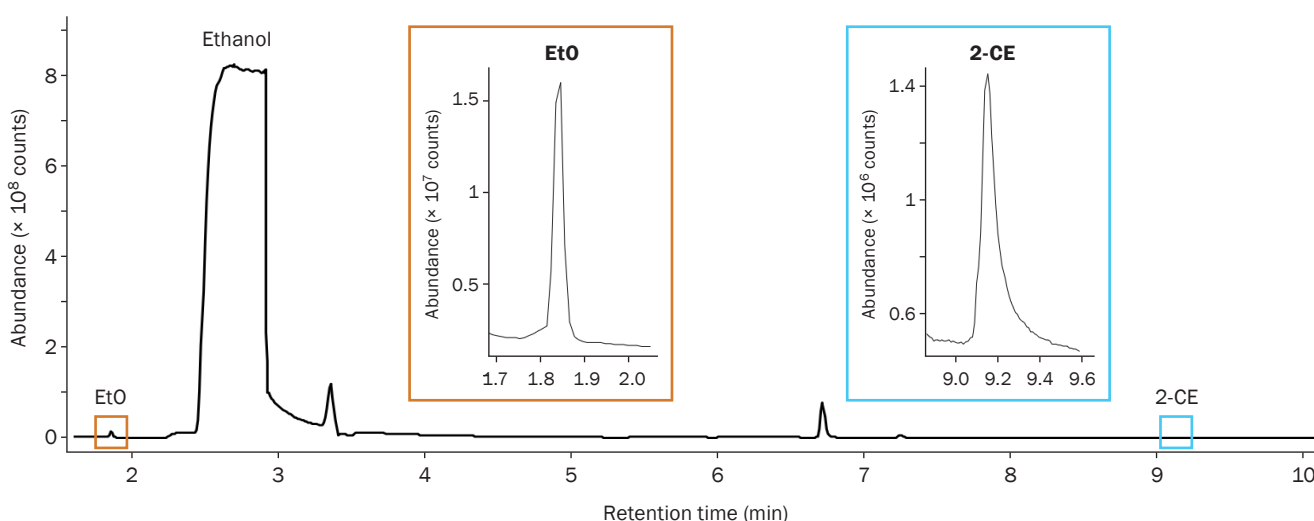


Figure 3: TIC for laboratory standard containing EtO and 2-CE at 200 $\mu\text{g/L}$. Insets are zoomed to highlight target peaks.



Figure 2: Medical gauze used in this work.

HS-trap with MSE:

Platform: Centri 90
 Initial incubation: 70°C for 10 min with agitation at 300 rpm
 Headspace: 3 extractions of 5 mL with 2 min incubation inbetween
 Syringe: 80°C
 Injector: 200°C
 Flow path: 180°C
 Trap low: -30°C
 Trap desorb: Max heating rate to 250°C, 3 min hold
 Split flow: 10 mL/min (split ratio 6:1)

GC:

Column type: DB 624, 60 m \times 0.32 mm \times 1.8 μm
 Column flow: 2 mL/min (constant flow)
 Oven program: 35°C (5 min), 10°C/min to 100°C (1 min), 20°C/min to 230°C (5 min)

MS:

Transfer line: 230°C
 SIM ions: EtO: m/z 29 (quant ion) and 44 (qual ion);
 2-CE: m/z 31 (quant ion) and 44 (qual ion)

Results and discussion

1. Chromatography

In the analyses of the laboratory standards, the total ion chromatogram (TIC) is dominated by the ethanol solvent that is introduced with the EtO (Figure 3). However, target analytes are apparent when zooming-in on the x-axis.

Using extracted-ion chromatograms (EICs) for quant ions, clear peaks are observed, with peak height corresponding to analyte concentration (Figure 4). Crucially, clear chromatographic separation was achieved between EtO and common contaminant methanol. Methanol shares primary ions with EtO and often co-elutes with it in GC analyses, presenting a challenge for EtO quantitation, but this was not an issue here. Hence, we conclude that our sample preparation methods and instrumental parameters are suitable for the analysis of EtO and 2-CE from water.

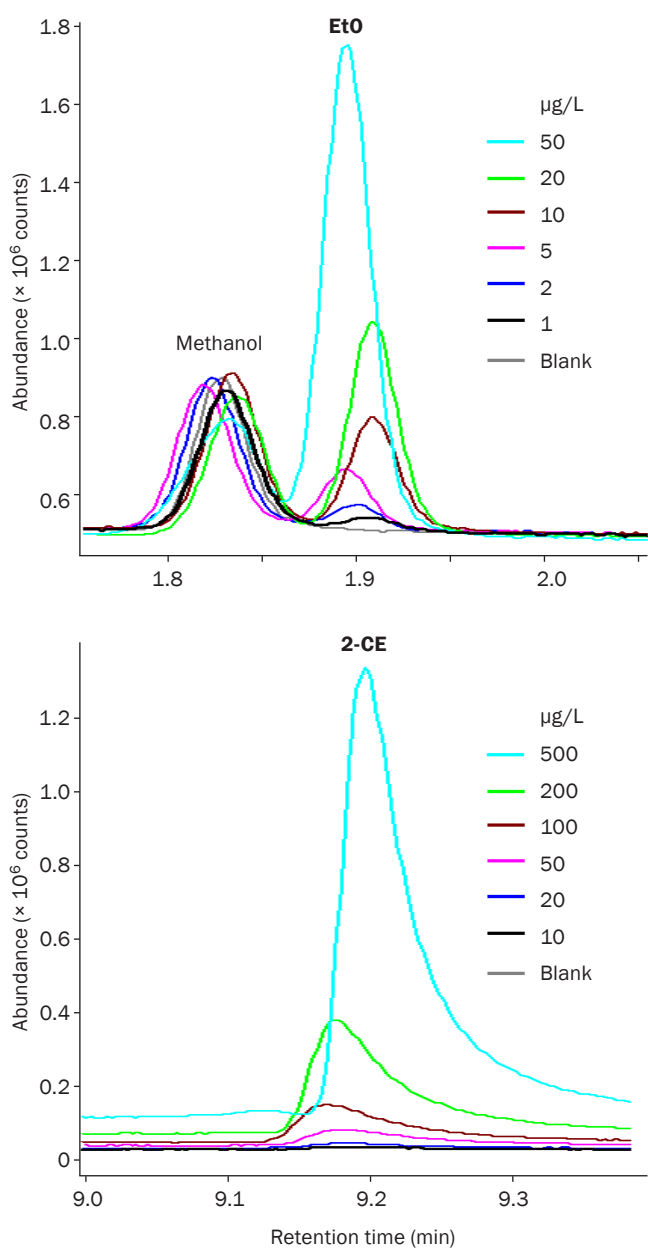


Figure 4: EICs showing target analytes at various concentrations. The peak for contaminating methanol is clearly chromatographically separated from EtO.

2. Data correction

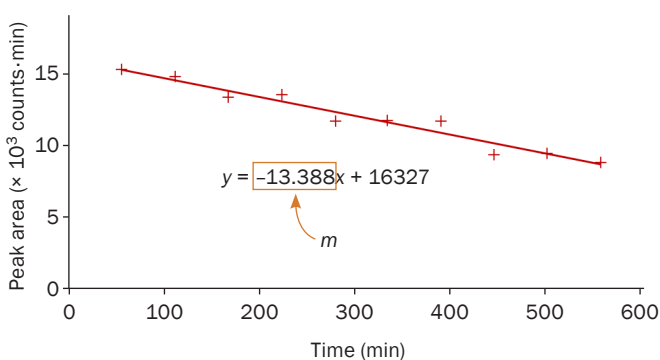
EtO is a highly volatile compound (b.p. 10.7 °C) that inevitably evaporates from vials while they wait on the sample rack to be analysed.² This leads to a reduction in signal (peak area) over time when multiple vials are prepared together and then analysed in a single batch, potentially giving rise to misleadingly low estimations of EtO concentration in samples run later. However, because evaporative loss happens at a constant rate, it can be measured and corrected for.

To perform this correction, replicates of a laboratory standard are performed, and the area of the uncorrected EtO peak (A_{uncorr}) is plotted against time (t). The slope of the resulting line (m) is determined, and the corrected area (A_{corr}) is calculated as:

$$A_{\text{corr}} = A_{\text{uncorr}} - mt$$

An example of the peak area correction is shown in Figure 5. In a series of 10 replicates with EtO at 10 µg/L, the peak area declines at a constant rate so that the relative standard deviation (RSD) across the replicates is quite high (13.34%). However, when correction is applied, the RSD is dramatically lower. Data correction was therefore used for all EtO analyses described below. 2-CE is much less volatile (b.p. 128.6 °C) and is not affected by evaporative loss, so no correction was applied.

A Uncorrected (RSD = 13.34%)



B Corrected (RSD = 3.09%)

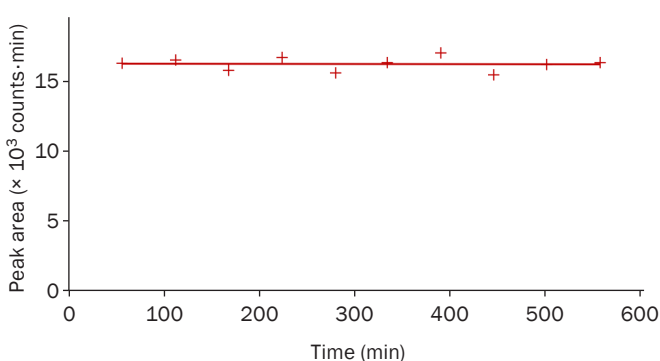
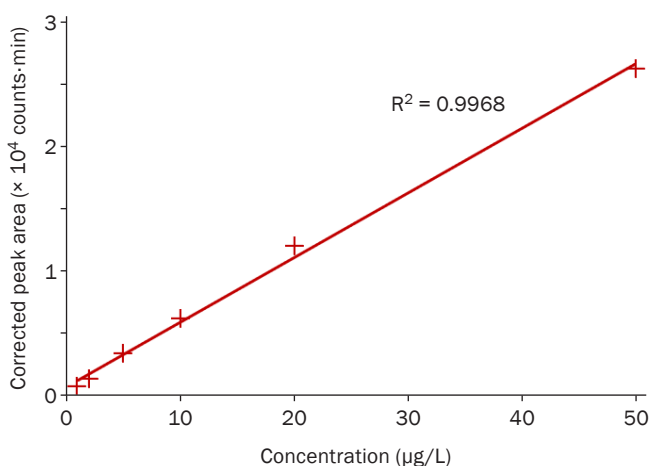


Figure 5: Replicates of EtO at 10 µg/L in water: (A) With no data correction applied, showing decline in signal over time due to evaporative loss from vial; (B) With signal correction applied.

3. Method validation (laboratory standards)

Linearity: A calibration series comprising six levels from 1–50 µg/L (EtO) or 10–500 µg/L (2-CE) was analysed (Figure 6). R^2 values were 0.9968 for EtO and 0.9964 for 2-CE, indicating excellent linearity.

A EtO



B 2-CE

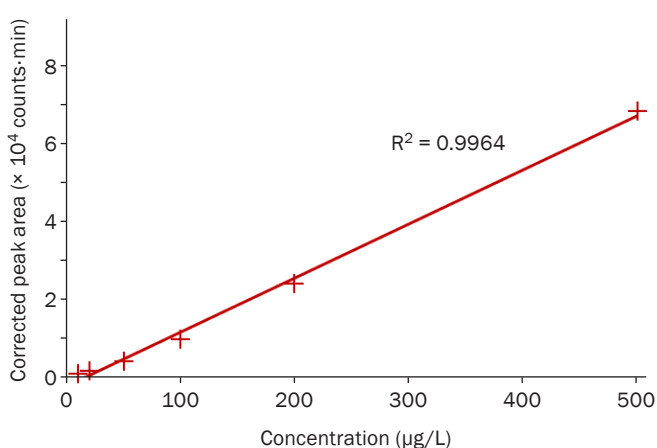


Figure 6: Calibration series for EtO and 2-CE, extracted from water by HS-trap with MSE.

Reproducibility: 10 replicates of target analytes at 10 µg/L (EtO) or 25 µg/L (2-CE) were analysed, and RSDs were calculated at 3.09% and 2.73% for EtO and 2-CE respectively. Reproducibility was therefore determined to be excellent.

Limits of detection and quantitation: 10 replicates at 1 µg/L (EtO) or 10 µg/L (2-CE) were analysed, and the standard deviation of the peak areas was calculated for each compound. The deviation was compared against a calibration curve to determine an equivalent concentration. The LOD was taken as this equivalent concentration multiplied by 3, while for the limit of quantitation (LOQ), it was multiplied by 10.

By this method, we calculated an LOD and LOQ for EtO of 0.35 µg/L and 1.17 µg/L respectively, while for 2-CE these values were 2.29 µg/L and 7.62 µg/L. We therefore determine that HS-trap with MSE is a highly sensitive technique able to detect EtO in water at sub-ppb levels.

4. Analysis of medical gauze extracts

Medical equipment spiked with EtO and 2-CE was immersed in water, and this water was subsequently analysed as for the laboratory standards (Figure 7). Trace methanol was still detected, but this was well separated from EtO. A calibration series was performed with six analyte concentrations from 0.1–5 µg/cm², and R^2 values were 0.9996 and 0.9997 for EtO and 2-CE respectively (Figure 8). This demonstrates that the concentration of EtO and 2-CE in water following extraction is proportional to that originally on the sample, as previously observed.¹

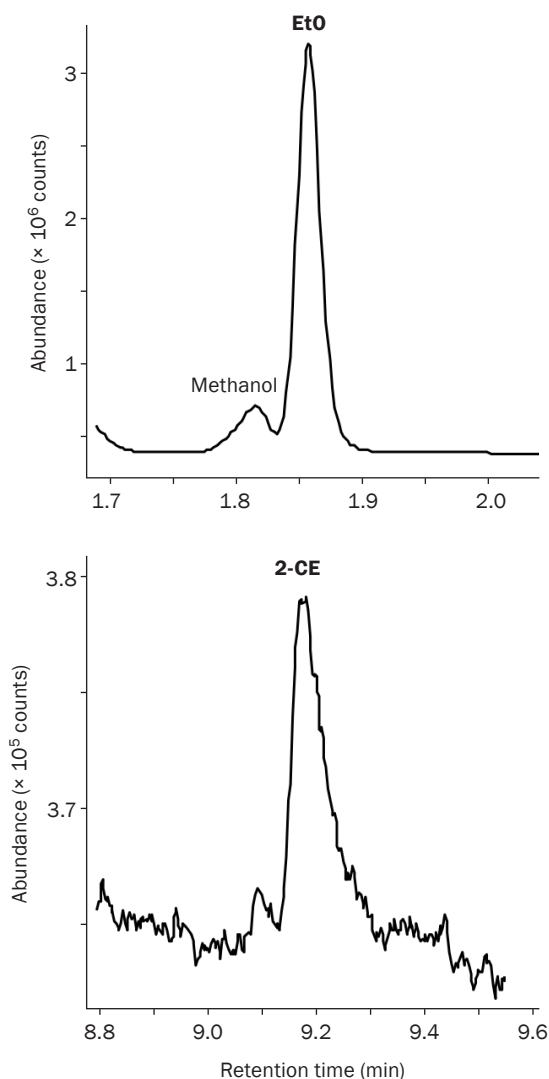


Figure 7: EICs for EtO and 2-CE, extracted from medical gauze spiked at 5 µg/cm².

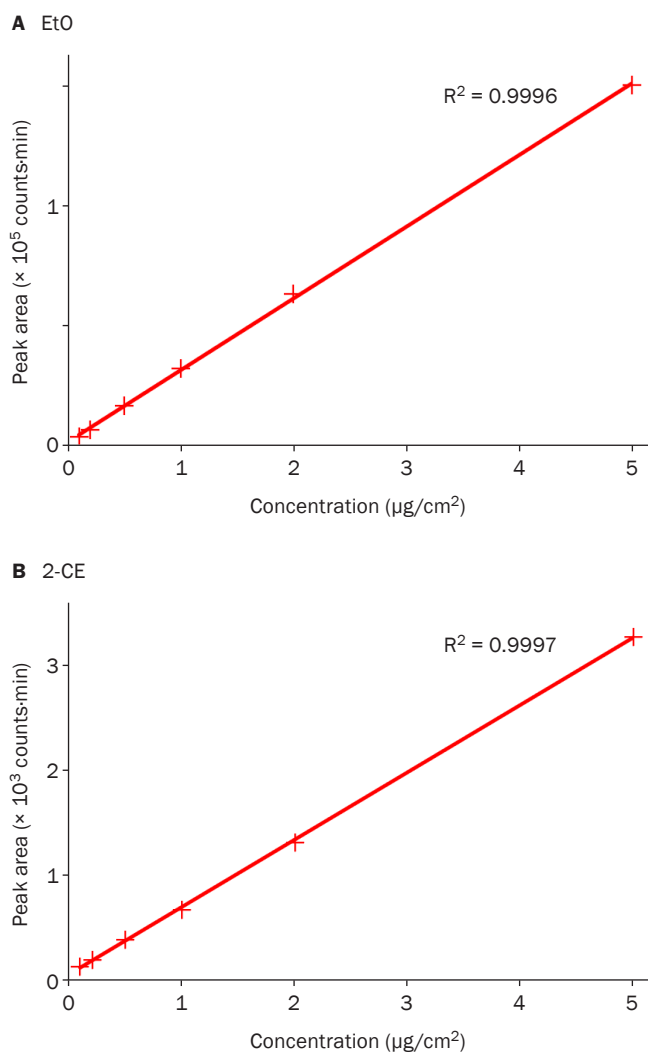


Figure 8: Calibration series for EtO and 2-CE, extracted from medical gauze extracts by HS-trap with MSE.

Conclusion

This application note shows how HS-trap with MSE was successfully implemented for the fully automated analysis of ethylene oxide and 2-chloroethanol from water. Using the focusing trap of Centri 90, multiple large (5 mL) headspace volumes were extracted from a single sample and analysed together in one run.

The method is highly sensitive, achieving a limit of detection at 0.35 and 2.29 $\mu\text{g}/\text{L}$ (= parts per billion) for EtO and 2-CE respectively with excellent linearity and reproducibility metrics. The method was applied to water extracts of medical gauze, which, like other medical equipment, is commonly sterilised with EtO. The high sensitivity and full automation intrinsic to this approach make it particularly well-suited to routine, regulatory-driven workflows for medical equipment.

References

1. A.D. Lucas and M.E. Stratmeyer, Extraction and stability of ethylene oxide residue in medical devices, *Biomedical Instrumentation & Technology*, 2008, 42: 76–79, [https://array.aami.org/doi/full/10.2345/0899-8205\(2008\)42\[76:EASOEO\]2.0.CO;2](https://array.aami.org/doi/full/10.2345/0899-8205(2008)42[76:EASOEO]2.0.CO;2).
2. Analysis of ethylene oxide and its metabolite 2-chloroethanol by the QuOil or the QuEChERS method and GC-MS/MS, EURL-SRM, 2020, https://www.eurl-pesticides.eu/library/docs/srm/EurlSrm_Observation_EO_V1.pdf.

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