

Optimization of a GC-MS Method for the Determination of Ethylene Oxide in Heated Tobacco Product (HTP) Aerosols

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Abstract

This presentation will describe the challenges of expanding a validated method for the analysis of volatile organic compounds (VOCs) in Electronic Nicotine Delivery Systems (ENDS), to evaluate VOCs in heated tobacco product (HTP) aerosols.

In 2023, we successfully validated a method for VOCs in ENDS e-liquid and aerosol for 1,3-butadiene, vinyl chloride, ethylene oxide, isoprene, propylene oxide, acrylonitrile, benzene, and toluene. This method was based upon CRM No 70 and ISO 21330/ISO 23923, with expected modifications to the analysis (different column, ion source, and calibration range).

In 2024, we applied the new method to VOCs in HTP aerosol. While levels of VOCs in ENDS aerosol were typically near, or below, the limit of quantitation (LOQ), all analytes, except vinyl chloride, were detected in the HTP aerosol samples at or above the LOQ. The chromatograms for the HTP aerosol were more complex than for the ENDS aerosol and changes to the GC method were required to resolve some interferences from the analytes of interest. However, the biggest challenge was a large interference coeluting with ethylene oxide. Additional investigation determined the interfering compound to be acetaldehyde, which is present at a level 1000-times that of ethylene oxide in HTP aerosols. To aid in ethylene oxide selectivity, a derivatization method was adopted and incorporated into the method for HTP aerosols.

The validation of the new ethylene oxide method was conducted according to FDA/ICH Guidelines, and included selectivity, linearity and range, LOD/LOQ, accuracy, precision, stability, and robustness. The extract LOQ was 100 ng/mL, equivalent to 1 µg/collection, recoveries ranged from 91.5 to 102.8%, and method precision was 2.9%.

Pre-validation Summary

The goal of this work was to expand the scope of a validated method for VOCs in ENDS aerosol to include the analysis of VOCs in HTP aerosol. Because of the increased complexity of the HTP matrix (Figure 1), the chromatography had to be carefully evaluated. In most cases, the ENDS method was suitable for the analysis of VOCs in HTP, however, there were a few challenges.

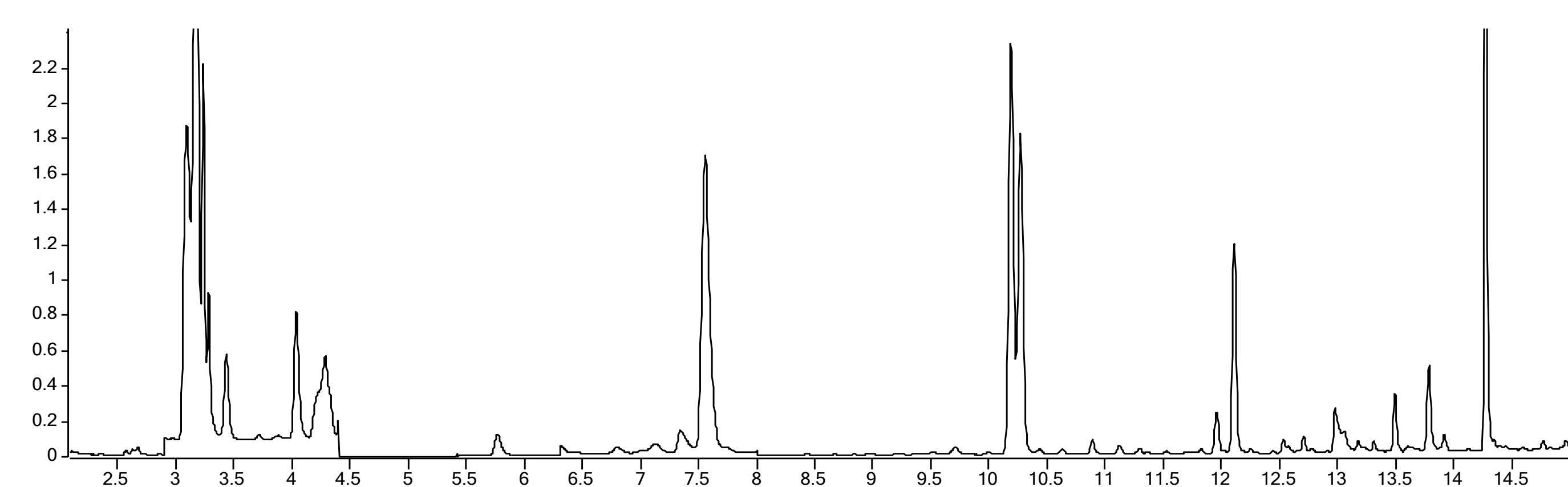


Figure 1. Total ion chromatogram for an HTP aerosol VOC extract.

For example, interferences were observed for acrylonitrile in HTP aerosol, but these were resolved by altering the oven temperature ramp. A large interference was observed eluting just before ethylene oxide (Figure 2) that could not be resolved chromatographically. Attempts to resolve the peaks by mass were unsuccessful as there was not a unique mass between them. Further investigation revealed this large interference to be acetaldehyde, which may be present at much higher levels in HTP aerosols.

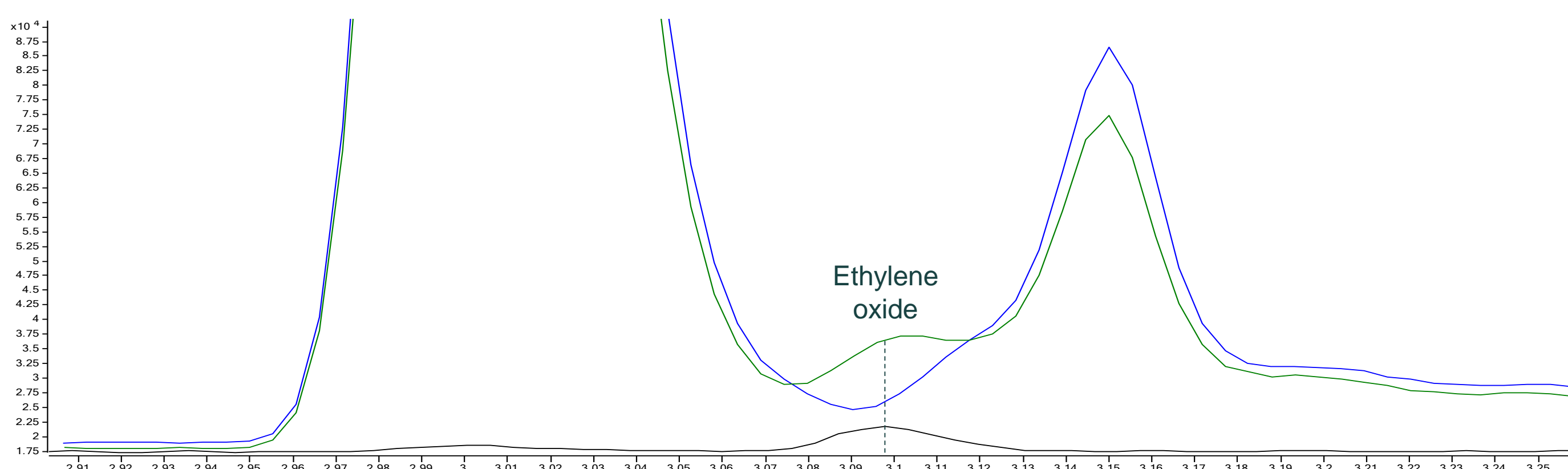


Figure 2. Overlaid ion chromatograms (m/z 44) of a calibration standard for ethylene oxide at 500 ng/mL (black), HTP aerosol extract (blue) and the same HTP aerosol extract spiked with ethylene oxide (green)

As a result of this interference, we shifted our focus from creating a single HTP aerosol method for VOCs, to validating ethylene oxide separately. The plan was to use an aliquot of the same sample collection and the same instrument (GC column) to optimize sample throughput. In order to improve selectivity, a derivatization procedure was tested for feasibility¹. Ethylene oxide was derivatized to form 2-bromoethanol using hydrobromic acid (HBr). Reaction shown in Figure 3.

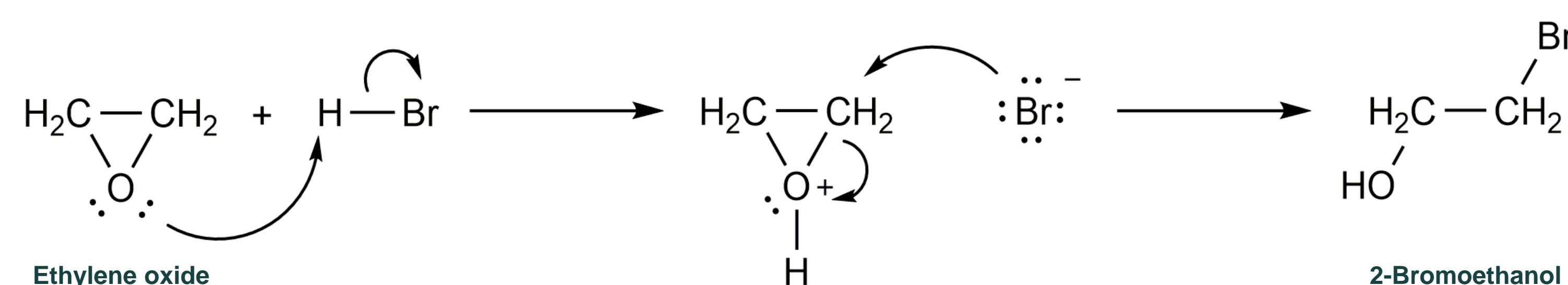


Figure 3. Reaction of ethylene oxide with hydrobromic acid to form 2-bromoethanol

Methodology

HTP aerosol was collected into a glass impinger containing methanol behind a glass fiber filter pad. An aliquot of the methanol solution was derivatized with concentrated hydrobromic acid and a separate aliquot was analyzed as is for the remaining VOCs. These extracts were each analyzed by GC-MS.

The ions chosen to monitor as quantitation ions were based on the 2-bromoethanol mass spectrum². Early testing showed promise using m/z 95 and m/z 97 as these generated the highest responses.

Since d6-benzene was already being used as an internal standard, it was the first internal standard tested for 2-bromoethanol. However, the relative recoveries were variable. 2-Bromoethanol-d4 was evaluated and found to be a more suitable internal standard.

Validation Results

Feasibility and selectivity were determined by analysis of a 2-bromoethanol standard. A derivatized and underivatized aliquot of a stock solution containing ethylene oxide was also analyzed. The derivatized extract indicated the presence of 2-bromoethanol at the confirmed retention time. The underivatized extract confirmed the presence of ethylene oxide and its disappearance in the derivatized extract. Selectivity was further evaluated through the comparison of a derivatized and underivatized HTP aerosol extract, shown in Figure 4.

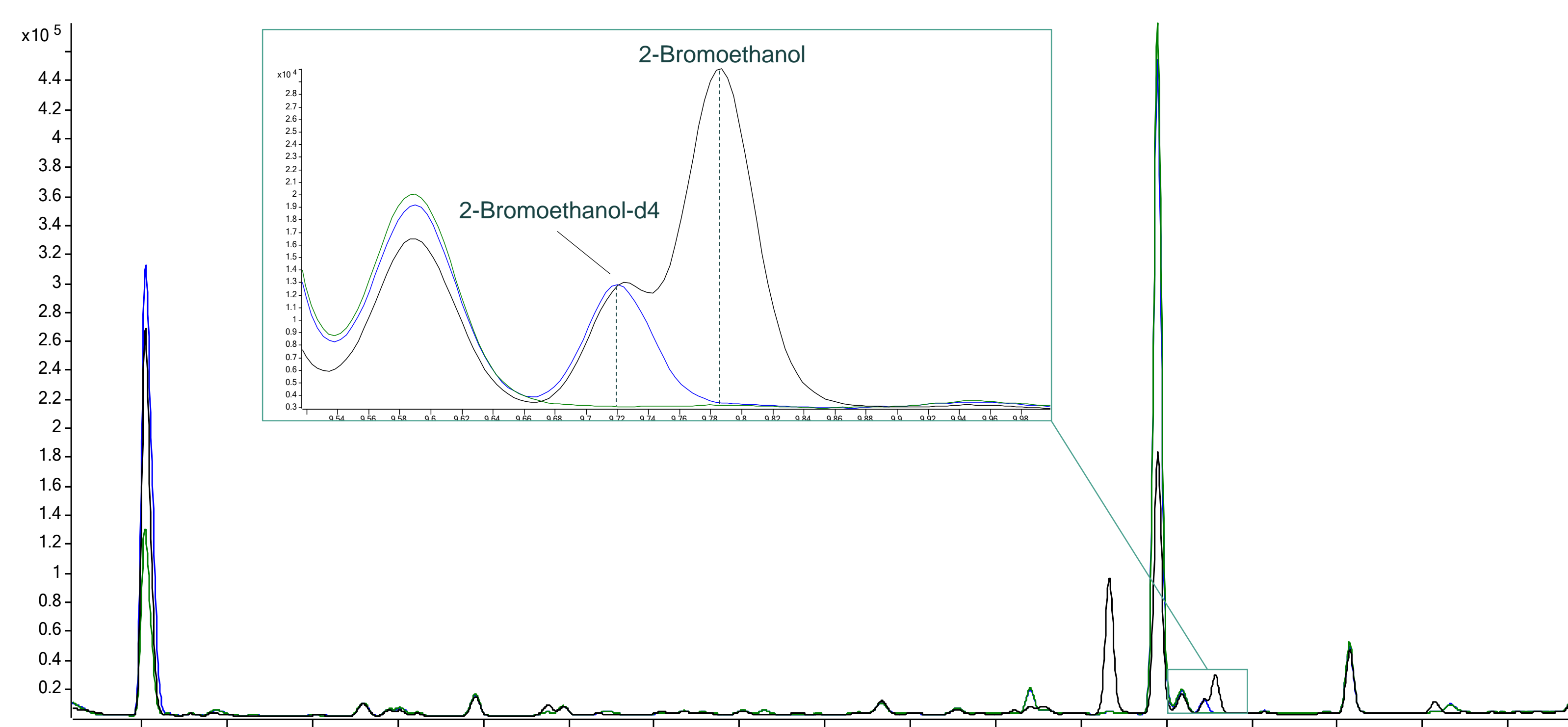


Figure 4. TIC overlay of an HTP volatiles extract injected as is (green), spiked with 2-bromoethanol-d4 and ethylene oxide (blue), and spiked and derivatized (black)

A drawback to using 2-bromoethanol-d4 as the internal standard is that common masses in both spectra led to less than ideal resolution of the internal standard peak from the analyte peak (Figure 5). In order to combat this, m/z 95 was chosen as the quantifier ion to increase the overall analyte response and the internal standard concentration was decreased from 2 µg/mL to 1 µg/mL.

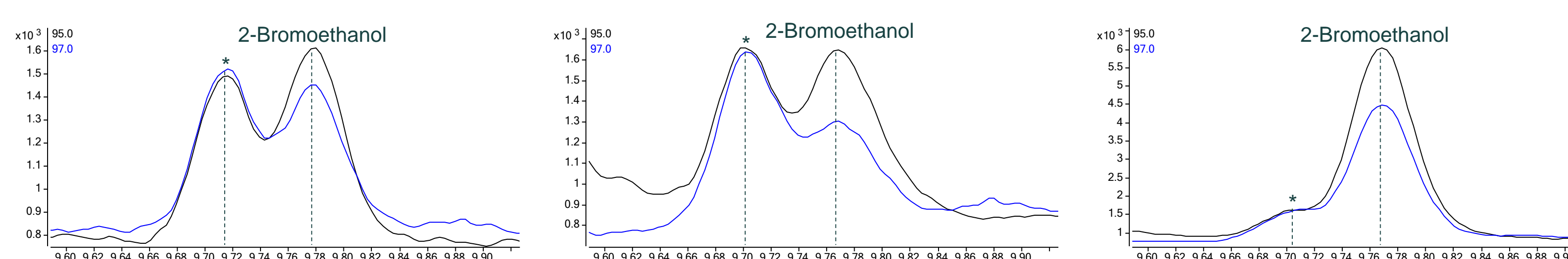


Figure 5. Overlaid quantifier (m/z 95) and qualifier (m/z 97) ion chromatograms of a calibration standard (left), HTP sample (middle), and a mid-level spiked HTP sample. Peaks marked with an asterisk indicate the common fragment ions shared between 2-bromoethanol and 2-bromoethanol-d4.

Validation Results Continued

Quantitation was evaluated using both area and height. While all validation parameters were acceptable for both, quantitation by area required significantly more manual integrations to 2-bromoethanol peaks in both standards and samples than quantitation by height. Additionally, quantitation by height resulted in improved method accuracy and precision.

Since the method used as the starting point for this scope expansion was for the determination of ethylene oxide in mainstream cigarette smoke, the amount of HBr used for derivatization was also evaluated. A composite HTP aerosol sample was spiked at the upper end of the calibration range and aliquots were taken for derivatization using different amounts of HBr in order to determine the appropriate amount of derivatizing reagent required.

Table 1. Validation Summary

Parameter	Value
Calibration Range	0.1-3.0 µg/mL
LOD	0.12 µg/collection
LOQ	1 µg/collection
Accuracy	91.5-102.8%
Instrument Precision	1.0-3.3%
Intermediate Accuracy and Precision _(3-day) (by area)	97.9 ± 6.7%
Intermediate Accuracy and Precision _(3-day) (by height)	98.3 ± 2.8%
Sample Stability	Derivatized extracts are stable for 4 days when stored under ambient conditions

Conclusions

A method was successfully validated for the determination of ethylene oxide in HTP aerosols by utilizing HBr derivatization to form 2-bromoethanol. We were able to use a single GC configuration and aerosol collection method for ENDS and HTP for 8 VOCs, with minor alterations to the sample preparation procedure and GC method parameters.

Despite HTP aerosols having more complex matrices than ENDS aerosols, we were able to validate this method with comparable linearity, range, LOD/LOQ, accuracy, and precision to the ENDS aerosol method.

Limitations and Future Work

- Limited number of HTP products were evaluated
- Although the objective of this work was to use an existing aerosol collection method and GC configuration, it may be possible to achieve lower LOQs and improve peak resolution using different collection methods and a different column.
- We may be able to apply this derivatization procedure to other analytes (e.g., propylene oxide) as well as mainstream cigarette smoke. Other GC columns have been shown to provide acceptable resolution of ethylene oxide from acetaldehyde, however, this typically results in higher LOQs and coelution with other large peaks.

References

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