Statistically Sound Calibration Curves for Chromatographic Methods Involving Negative Response Data

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Abstract

A statistical procedure is presented for calibrating chromatographic methods that generate negative chromatographic peaks. The technique is illustrated via data collected during the ion-chromatographic analysis of the ammonium ion in methansulfonic-acid solutions. A peak-integration protocol is explained and subsequent regression data are presented. For the concentration range of 1 to 5 ppb, a quadratic model could be fitted to the calibration data, using ordinary least squares as the fitting technique. The precision was approximately ± 0.5 ppb, at 95% confidence.

Introduction

Analysis via ion chromatography (IC) typically results in peaks that are positive (i.e., that rise above the baseline). The key exception is the so-called "water dip," which is the negative peak that often occurs when the water in the sample elutes from the column and enters the detector. This phenomenon is due to the fact that the conductivity of the sample water is less than that of the suppressed eluent, which forms the baseline. The size of the dip depends on the magnitude of this difference, as well as on the volume of the sample loop.

However, samples occasionally are prepared in some concentration of the eluent's constituent(s). In such cases, analyte peak shapes may vary from what is typical. As a result, some low concentrations of the analyte may exhibit either totally negative behavior, or may be partially negative and partially positive. The purpose of this paper is to present and illustrate a protocol for dealing with such chromatographic peaks; the technique uses net peak areas.

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The illustrative data presented involves ammonium standards in 185 mM methanesulfonic acid (MSA); analysis was effected using a 20 mM MSA eluent. (This acidic matrix is necessary when airborne ammonia must be trapped for analysis as the ammonium ion via IC.)

Experimental

Instrumentation and consumables

Unless otherwise noted, all items were from Dionex Corp. (Sunnyvale, CA). The ion chromatograph was a DX500 equipped with an isocratic pump and a conductivity detector. Separation was achieved via an IonPac CG/CS12A column set; suppression was achieved with an CSRS 300 in the external-water mode (all

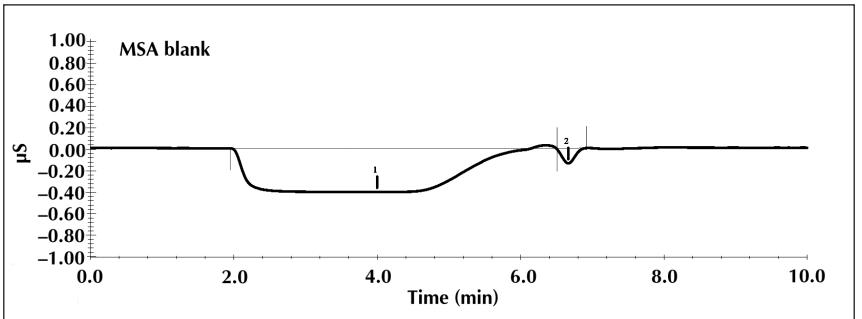

Figure 1. Chromatogram of an ammonium blank (Peak 2, which is completely negative) in 185 mM MSA.

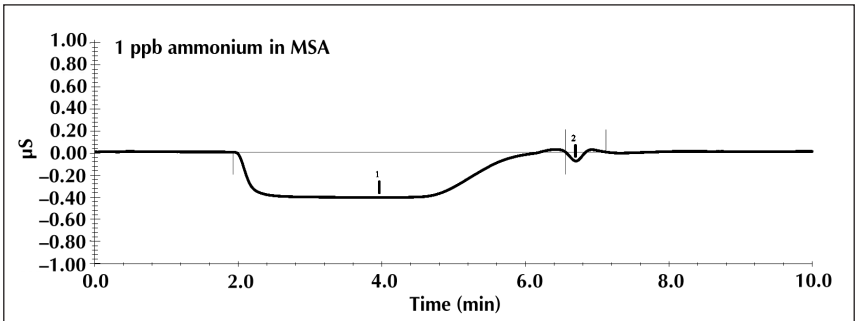

Figure 2. Chromatogram of a 1-ppb ammonium standard (Peak 2, which is partially negative and partially positive) in 185 mM MSA.

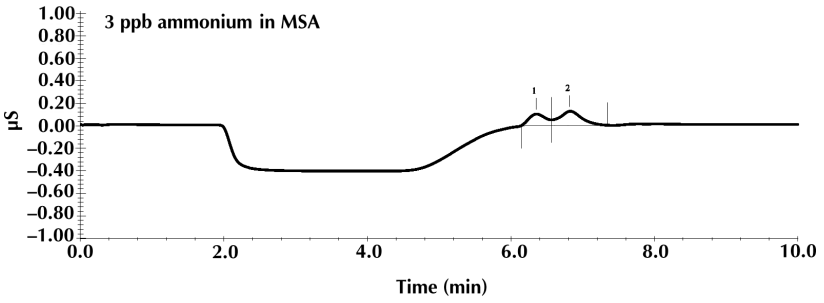

Figure 3. Chromatogram of a 3-ppb ammonium standard (Peak 2, which is totally positive) in 185 mM MSA.

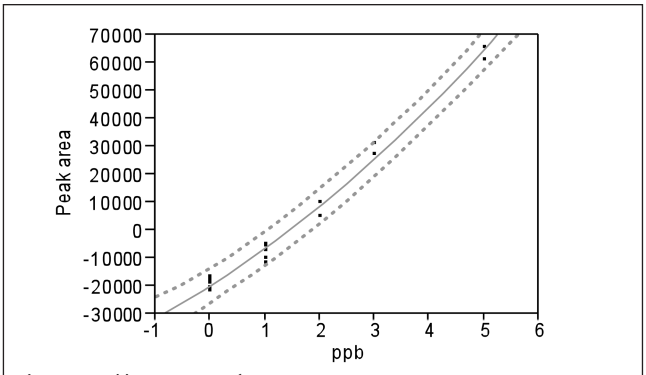

Figure 4. Calibration curve for ammonium in 185 mM MSA.

formats were 2-mm i.d.). Samples were introduced into a 1000-µL loop via a pressurized vessel (1), following a 5-min helium purge (to remove any ammonium-containing air from the vessel).

Instrument control and data collection were effected via PeakNet 5.1 software (Dionex). Statistical analyses were performed using JMP 8.0 software (SAS Institute, Cary, NC).

Chemicals and standards

The 20 mM and 185 mM solutions were prepared from 99% methanesulfonic acid (Aldrich, Milwaukee, WI) for use as eluent and standard-preparation solvent, respectively. Deionized water was obtained from an in-house purification system.

Beginning with a 1000-ppm (parts per million, w/w) ammonium standard (Spex, Metuchen, NJ), intermediate concentrations were prepared in deionized water until a 100-ppb (w/w) solution was obtained. From this concentration, working standards of 1, 2, 3, and 5 ppb (as well as a standard-preparation blank) were made in 185 mM MSA. All solutions were prepared in 125-mL wide-mouth HDPE bottles (Nalgene, Rochester, NY). Transfer pipets were from Fisher Scientific (Pittsburgh, PA).

Results and Discussion

Integration protocol

A typical blank chromatogram is shown in Figure 1 (Peak 2 is the ammonium response; integration of the water dip was allowed to ensure consistent drawing of the baseline). The baseline was very well-behaved throughout the run, starting and ending at virtually the same conductivity. Thus, a line could be drawn from the beginning of the water dip to the post-ammonium return to baseline; the negative and positive (if any) components of the analyte could then be defined for integration purposes. Such a process led to consistent integration across all chromatograms.

At the 1-ppb level, a slight positive peak was seen in addition to the dip (Figure 2); Peak 2 yields the net integration of the negative and positive components. By 3 ppb, the ammonium peak had become completely positive (Figure 3); Peak 2 is due to ammonium.

Calibration study

A small calibration study was conducted to determine if a "net-area" approach to integration could generate a usable curve. The design consisted of the following concentrations (all in ppb) a blank, 1, 2, 3, and 5; the number of replicates were 6, 7, 2, 2, and 2, respectively. More replicates were collected at the lowest two concentrations because tighter precision was desired in that range. Data were collected over the course of four consecutive days. Net peak areas were calculated.

Calibration diagnostics were the same as reported earlier (2,3). Analysis showed that a quadratic model with ordinary-least-squares fitting was appropriate. At 95% confidence, the half-width of the prediction interval was ~0.5 ppb. This level of uncertainty was deemed acceptable. The curve and its interval are shown in Figure 4.

Conclusion

This work shows that statistically sound calibration curves can be generated even when analytes exhibit negative or partially negative peaks. The key to the process is obtaining a stable baseline and being consistent in integrating the peaks. When peaks exhibit both negative and positive components, care must be taken in integrating each portion; the two signed areas are then combined to obtain the net result for use in the calibration curve.

References

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