Internal Standard Correction of Results Obtained by Atmospheric Pressure Chemical Ionization Mass Spectrometry

P. R. Nelson
R.J. Reynolds Tobacco Co., Bowman Gray Technical Center, Winston-Salem, North Carolina, USA

An internal standard system has been developed for a mass spectrometer equipped with an atmospheric pressure chemical ionization source. The system has been used to overcome sensitivity drift problems that are commonly encountered when the spectrometer is used for long-term environmental monitoring. Additionally, the internal standard has been used to correct sensitivity changes induced by the matrix being analyzed. Principal components of the system are a low concentration internal standard source and a flow-delivery system for introducing the standard to the reagent gas delivery stream of the spectrometer. Following an experiment, real-time data are downloaded to a personal computer where internal standard correction and data analysis are performed. Application of the internal standard to the measurement of nicotine and pyridine is demonstrated. *(J Am Soc Mass Spectrom 1991, 2, 427–431)*

A SCIEX® (Thornhill, Ontario, Canada) TAGA® 6000 atmospheric pressure chemical ionization (APCI) mass spectrometer is used to monitor environmental tobacco smoke (ETS) generated in an 18-m^3 stainless steel controlled environment test chamber in which temperature, humidity, and ventilation can be controlled [1–4]. Real-time measurements of ETS constituents are typically obtained over a 0.5 to 7 h sampling period. One drawback of the system is that quantitative results can be adversely affected by instrument sensitivity changes that can arise from several sources. Compounds present in the sample matrix may affect the sensitivity of the instrument to selected analytes. During long sampling periods, instrument sensitivity can drift by as much as 50%. In addition, sudden changes in instrument sensitivity have been observed on occasion.

A system to overcome the problem of changes in instrument sensitivity was developed. A steady concentration of an internal standard is blended with air sampled from the environmental chamber. The response of the spectrometer to the internal standard is used to correct analyte data for both natural and electronically induced changes in instrument sensitivity. Results from tests of this system with D_5-pyridine and D_3(methyl)-nicotine as internal standards for pyridine and nicotine are presented here.

Address reprint requests to Paul Nelson, R.J. Reynolds Tobacco Co., Bowman Gray Technical Center, Winston-Salem, NC 27102.

1044-0305/91/$3.50

Received January 7, 1991
Accepted February 27, 1991

Experimental

Reagents

Pyridine (certified ACS grade) was obtained from Fisher Scientific (Pittsburgh, PA) and highest available purity (99% min.) nicotine was obtained from Eastman Kodak Company (Rochester, NY). Both chemicals were used as received. Certified pyridine (calibration) and D_5-pyridine (internal standard) permeation tubes were obtained from Kin-Tek Labs (Texas City, TX). D_3(methyl)-nicotine was synthesized, and its identity and purity (99 + %) were checked by nuclear magnetic resonance and mass spectrometry. Aldrich Chemical Company (Milwaukee, WI) glycerol (99 + %) was used to reduce the vapor pressure of D_3(methyl)-nicotine. Inlet glassware was silylated with SYLON-CT obtained from Supelco (Bellefonte, PA).

Instruments

Pyridine calibration standards were maintained in a Kin-Tek Labs model 580-3C precision gas standards generator. The nicotine calibration standard and internal standards were kept in separate Kin-Tek models 585-A and 485 precision gas standard generators. A SCIEX® TAGA® 6000 tandem mass spectrometer equipped with an APCI source was used to obtain all real-time measurements. For the experiments described here, the instrument was operated in the

single mass spectrometer mode. Internal standard correction and data analysis were performed on a COMPAQ (Houston, TX) Deskpro 386/20 personal computer.

Internal Standard Selection

Proton transfer is the principal ionizing reaction in the APCI ion source, and sensitivity of the APCI ion source is strongly dependent on the gas-phase basicity and protonation kinetics of the compound being analyzed [5, 6]. In order for an internal standard to correct for the effects of other compounds present in the sample matrix, its energetics and kinetics should be as similar as possible to those of the compound being quantified. The mass-to-charge ratio of the internal standard should also fall at a mass where no interfering ions appear. In this lab, both pyridine and nicotine are frequently analytes of interest; protonated molecular ions of these compounds appear at m/z 80 and 163, respectively. Fully or partially deuterated analogs of these compounds are expected to exhibit similar ionization behavior in the mass spectrometer. Protonated D_5-pyridine and D_3(methyl)-nicotine appear at m/z 85 and 166, respectively. No interfering ions are observed at these two masses when ETS is sampled.

The presence of an internal standard must not have a significant effect on the ionization efficiency of the analytes of interest. If ionization of the internal standard requires a large fraction of available reagent ions, then the sensitivity of the instrument to other analytes may be reduced. The D_5-pyridine permeation tube used has a certified permeation rate of 1021 ng/min at 60 °C. By reducing the oven temperature to 30 °C, the permeation rate was reduced to approximately 180 ng/min. When the internal standard is fully diluted, its concentration is $\approx$ 5 ppb and it produces approximately 150,000 counts per second. The D_3(methyl)-nicotine was not readily available in permeation tubes, and its vapor pressure at 30 °C is too great to be used as an internal standard. The nicotine was mixed with glycerol in a permeation bottle until it produced approximately 30,000 counts per second in the spectrometer, which corresponds to a nicotine evolution rate of $\approx$ 80 ng/min and a fully diluted concentration of $\approx$ 0.7 ppb. At these concentrations, the internal standards provide signals large enough to be used for performing internal standard corrections, but not large enough to interfere with the sensitivity of the spectrometer to other ions of interest.

Internal Standard Introduction

A general diagram of the internal standard introduction system is shown in Figure 1. Air from the controlled environment chamber is sampled through an air-vac vacuum transducer (Air-Vac Engineering Co., Milford, CT). Dilution air, flowing at $\approx$ 8.7 L/min, is forced through an annular orifice in the transducer. This causes a reduced pressure in a vacuum passage connected with the chamber. Flow rates of 2 to 8 L/min can be induced from the chamber depending upon the size of a flow restrictor placed within the chamber. The combined flow of analyte and dilution air is then directed into the glass inlet tube shown in Figure 2.

The deuterated nicotine internal standard was shown to undergo reversible adsorption on the stainless steel "T" fitting used in a previous version of this system. The adsorption of the nicotine internal standard followed by its desorption in the presence of ETS led to unstable behavior of the internal standard. A new inlet tube was developed (see Figure 2) that minimizes the surface onto which the internal standard can adsorb. To further retard adsorption, glassware surfaces exposed to the internal standard were silylated. The threaded side-arm is used to introduce calibration gases into the ion source. The side-arm is capped during chamber sampling. A sample line from the air-vac transducer is attached to the other threaded tube. The internal standard is introduced through the tube mounted axially within the larger tube. A glass ball and a support (not shown in Figure 2) mounted at the end of the internal standard inlet tube help to provide thorough turbulent mixing of the internal standard and sample streams. At flow rates of > 1.5 L/min, good mixing occurs and stable internal standard and analyte signals are obtained. Noise in the real-time spectra is further reduced at higher internal standard flow rates. But as the flow rate is increased sample dilution is also increased with a concurrent reduction of analyte signal. The best compromise between signal and noise for this system was found at internal standard flow rates between 1.75 L/min and 2.5 L/min.

The internal standard permeation tube and bottle are housed in a Kin-Tek model 485 precision gas standards generator with oven temperature maintained at 30 °C. Nitrogen is passed through the oven at a flow of 700 mL/min, and diluted with nitrogen to obtain a total flow of 2.00 L/min.

Data Treatment

Prior to each experiment, the TAGA® was calibrated by the method of Thome et al. [2]. Permeation sources

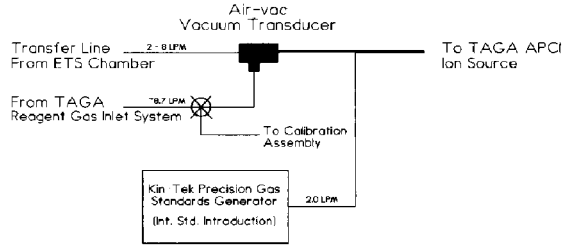

Figure 1. Block diagram of an internal standard introduction system.

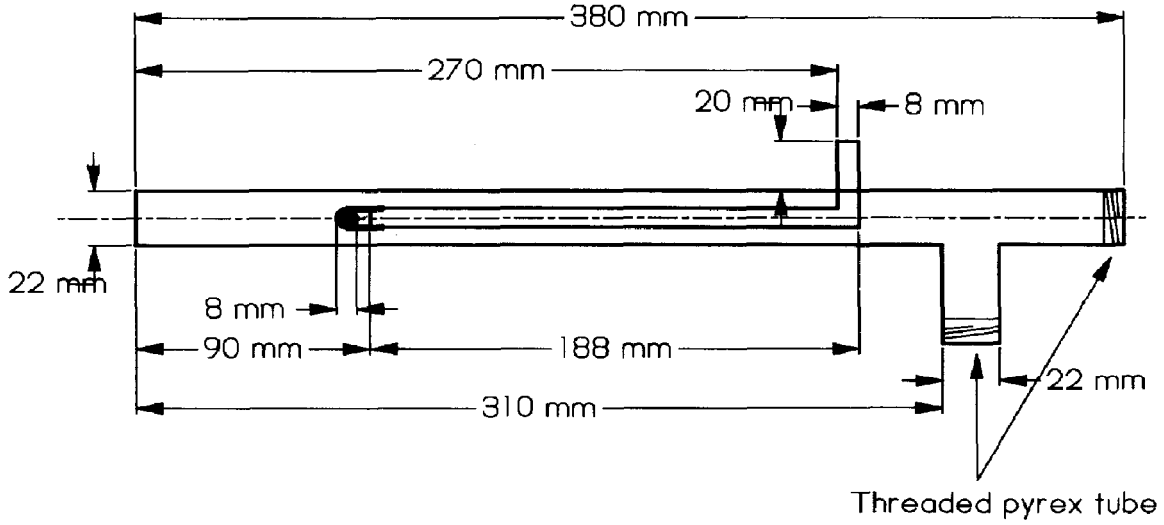

Figure 2. Glassware for blending the internal standard with the sample flow and introducing the combined flow into the ion source.

were used to generate standards of known analyte concentration. The standard was then diluted to working concentrations in a capillary dilution apparatus. A small flow of standard was blended with air (8.7 L/min) and introduced into the ion source. By changing the flow of standard through the capillary, a calibration curve was obtained which bracketed the expected sample concentration. Response factors were calculated from the calibration curve.

Data from each experiment were collected by selected ion monitoring of each analyte's protonated molecular ion: pyridine—m/z 80, D_5-pyridine—m/z 85, nicotine—m/z 163, and D_3(methyl)-nicotine—m/z 166. Following collection, the data were transferred from a PDP 11/73 minicomputer (Digital Equipment Corp., Maynard, MA) to a personal computer. Data analysis was then performed. All the data were first smoothed by using a sliding average routine. The average counts per second for each internal standard was then determined for the first 10 min (background) of the experiment. Real-time data for each internal standard were then normalized to their average background value. The normalized data were then used to correct analyte intensities for changes in sensitivity that occurred during the course of an experiment. Finally, analyte intensities were converted to concentrations by using previously determined response factors.

Results and Discussion

Correction of Long-Term Sensitivity Drift

When measurements are carried out over a long period of time, the sensitivity of the instrument can vary considerably. For the TAGA®, factors such as contamination of the discharge needle, partial blockage of the ion extraction orifice, and accumulation of frost on cryo-cooled lens surfaces may all influence instrument sensitivity. An example of sensitivity drift is illustrated in Figure 3. A constant concentration of D_5-pyridine was introduced into the ion source and the ion signal was measured over a 4-hour period. During the first 30 min of the experiment, the sensitivity of the TAGA® to D_5-pyridine remained fairly constant. During the final $3\frac{1}{2}$ hr of the experiment, the sensitivity slowly declined. Overall, the sensitivity of the TAGA® to a constant analyte concentration decreased by 35%.

During the same experiment, pyridine vapor was generated in the chamber by volatilizing a 10% pyridine solution. Four microliters of a 10% solution of pyridine in water were volatilized in the chamber at 12 min into the experiment. The uncorrected pyridine concentration line in Figure 4 shows the real-time concentration of pyridine measured with the TAGA®. The decrease in sensitivity to pyridine is proportional to the decrease in response to D_5-pyridine illustrated in Figure 3.

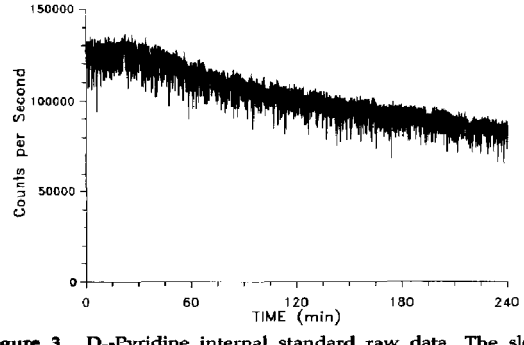

Figure 3. D_5-Pyridine internal standard raw data. The slow decline in response is due to long-term drift of instrument sensitivity.

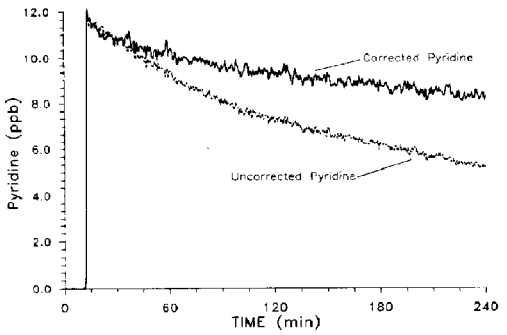

Figure 4. Correction of long-term changes in instrument sensitivity. Real-time concentration of pyridine in controlled environment test chamber that has (solid) and has not (dashed) been corrected with its internal standard is shown here.

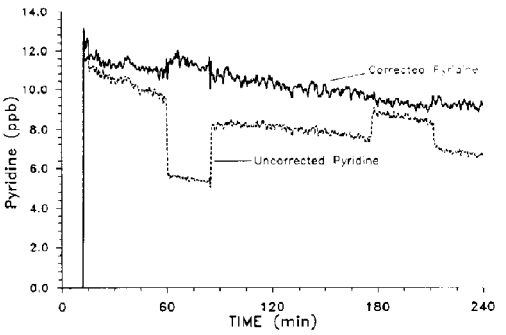

Figure 5. Correction of short-term changes in instrument sensitivity. Rapid changes in sensitivity were effected by changing the potential of a focusing lens. The Corrected Pyridine line shows the utility of the internal standard for eliminating the effect of the short-term sensitivity changes evident in the Uncorrected Pyridine line data.

The Corrected Pyridine concentration line in Figure 4 shows the results of correction. Initially, TAGA® sensitivity showed little drift, which is reflected in the similarity of corrected and uncorrected data for the first 30 min of the experiment. As the experiment progressed, sensitivity gradually declined, which caused the increasing divergence of the Corrected and Uncorrected Pyridine lines. Reliance on the uncorrected data would lead to an estimation of time-weighted-average concentration, which was biased low by 19%, and the final concentration would be low by 35%. The corrected pyridine concentration exhibits a slight decline due to the effect of air exchange within the chamber. The chamber is not perfectly sealed, and infiltration and exfiltration cause a small, real decrease in the actual concentration of compounds in the chamber.

Correction of Short-Term Sensitivity Changes

Occasionally, the mass spectrometer will exhibit large changes in sensitivity that take place nearly instantaneously. Causes for such variations may include electronics problems and partial blockage of the ion extraction orifice. An experiment was performed to determine the ability of the internal standard to correct for such variations. Four microliters of a 10% aqueous pyridine solution were volatilized in the chamber, and real-time pyridine concentrations were monitored for a period of 4 hr. Sharp changes in instrument sensitivity were effected by manually changing the potential applied to one of the focusing lenses. Although this particular type of change is unlikely under normal operating circumstances, the resulting change in sensitivity is similar to those that have been observed in normal operation.

Illustrated in Figure 5 are the uncorrected and corrected pyridine concentrations measured with the TAGA®. Because the internal standard experiences the same relative changes in sensitivity as the analyte, the proportional change can again be used to correct the analyte raw data. In this case, the corrected pyridine concentration showed an expected slow decay in the chamber, and the electronically induced sensitivity changes were eliminated from the data. The displacement of the uncorrected pyridine trace below the corrected pyridine trace gives evidence that some long-term sensitivity drift also took place during this experiment.

A minor drawback of the system is that use of the internal standard can increase the noise of the corrected signal. This occurs because the noise associated with the internal standard signal is combined with the noise associated with the analyte signal. For example, the signal-to-noise ratio of the corrected data is 20% lower than that of the uncorrected data in Figure 5. However, because both the analyte and internal standard signals are smoothed during processing, noise associated with the corrected signal is still much lower (3–4 ×) than that associated with unsmoothed, uncorrected data.

Correction of Matrix-induced Sensitivity Changes

When the TAGA® is used to determine concentrations of ETS constituents in the controlled environment chamber, matrix interference effects can cause difficulties. If concentrations of analyte molecules in the ion source overwhelm the available supply of reagent $[H_3O \cdot (H_2O)_n]^+$ ions in the spectrometer's ion source, competition for the reagent ions between analytes can induce changes in sensitivity. In this case, suppression of ionization is not constant for all compounds. Stronger gas-phase bases compete more effectively for the limited number of reagent ions than compounds with lower basicity. To correct for this problem properly, it is imperative that the internal standard has similar gas-phase basicity and protonation kinetics as the analyte being measured. For this reason, deuterated analogs of pyridine and nicotine were chosen as internal standards for these compounds.

To examine the effect of matrix interference on sensitivity, high ETS concentrations were generated in the controlled environment chamber. Following a

12-min background period, a smoking machine was used to smoke three pairs of cigarettes in sequence. This was done to elevate the ETS concentration in the chamber rapidly. For the remainder of the 5-hr experiment, individual cigarettes were smoked consecutively to maintain a nearly constant ETS concentration in the chamber. Throughout the experiment, the chamber was ventilated at six air changes per hour, and the temperature and relative humidity were regulated at 22 °C and 50%, respectively.

Raw, unsmoothed, D_3-nicotine internal standard data are pictured in Figure 6. During the first 12 min of the experiment the instrument response was constant. As smoke levels in the chamber increased between minutes 15 and 30, the instrument response to the internal standard decreased. This decrease was caused by competition between various components in the matrix for a limited number of reagent ions. Throughout the rest of the experiment, sensitivity slowly decreased. This is most likely due to a slight increase in ETS matrix concentration as the experiment progressed and the effect of long-term sensitivity drift. Overall, the instrument response to a constant concentration of internal standard decreased by ≈ 50%. Internal standard data obtained for D_5-pyridine displayed a similar temporal profile, but the relative decrease in sensitivity was slightly greater.

When the internal standard response was used to correct the real-time nicotine concentration measured in the chamber, the results shown in Figure 7 were obtained. Although there is some noise associated with the corrected nicotine data in Figure 7, it is possible to pick out the increase in nicotine signal that occurred with the smoking of each individual cigarette. Each peak that appears at 10-min intervals is due to changes in nicotine concentrations induced by the burning of each cigarette. Without internal standard correction, estimates of the nicotine concentration in the chamber would have been biased significantly. From Figures 6 and 7, it can be seen clearly that the matrix can interact strongly with the analyte of interest in the APCI ion source, and that interaction can lead to significant errors in quantitation. However, an internal standard with similar ionization properties to the analyte of interest can be used to correct matrix-induced changes in sensitivity.

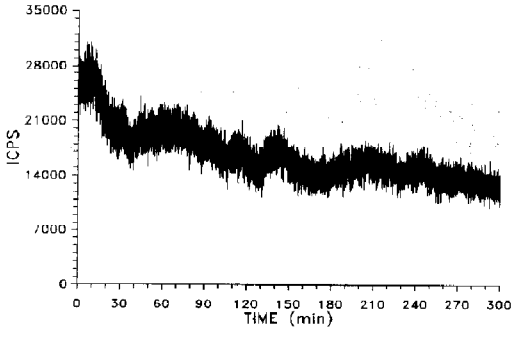

Figure 6. D_3(methyl)-nicotine internal standard raw data. The sharp drop in sensitivity occurring between 15 and 30 min is caused by matrix interferences.

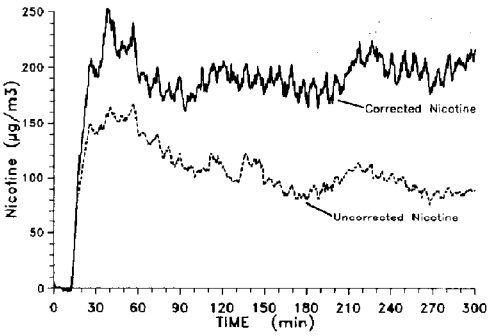

Figure 7. Correction of matrix-induced sensitivity changes. Real-time concentration of nicotine in a controlled environment test chamber that has (solid) and has not (dashed) been corrected with its internal standard is shown here.

Conclusions

The utility of an internal standard system for correction of sensitivity changes in a mass spectrometer equipped with a corona discharge APCI ion source has been demonstrated. Introduction of a constant, low concentration of an internal standard with gas-phase basicity and protonation kinetics similar to those of an analyte of interest can be used to overcome both long- and short-term changes in sensitivity, as well as correct for matrix-induced changes in sensitivity. The system is simple and could probably be adapted to work with other spectrometers that can perform atmospheric sampling in real time.

Acknowledgments

The help given by Barbara Collie and David Heavner with the daily operation of the TAGA® and controlled environment test chamber is gratefully acknowledged. Thanks are also due to Bill Davidson of SCIEX®, who provided a data conversion program.

References

1. Heavner, D. L.; Thome, F. A.; Eudy, L. W.; Ingebrethsen, B. J.; Green C. R. *Proceedings of the 79th Annual Meeting of the Air Pollution Control Association*, 1986, 86-37.9.
2. Thome, F. A.; Heavner D. L.; Ingebrethsen B. J.; Eudy L. W.; Green C. R. *Proceedings of the 79th Annual Meeting of the Air Pollution Control Association*, 1986, 86-37.6.
3. Nelson P. R.; Heavner D. L.; Collie B. B. In *Present and Future of Indoor Air Quality*; Bieva, C. J. et al., Eds.; Excerpta Medica, Int. Cong. Ser. 1989, *860*, 277.
4. Nelson, P. R.; Heavner, D. L.; Oldaker G. B. III. In *Proceedings of the 1990 EPA/A&WMA International Symposium on Measurement of Toxic and Related Air Pollutants*; Air & Waste Management Association: Pittsburgh, 1990, p. 550.
5. Reid, N. M.; French, J. B.; Buckley, J. A.; et al. *4th Joint Conference on Sensing of Environmental Pollutants*; ACS: Washington, 1977; p. 594.
6. Sunner, J.; Nicol, G.; Kebarle, P. *Anal. Chem.* 1988, *60*, 1300.