Reduction in Extraction Efficiency of Charged Particles from the Ion Source as the Cause of Matrix Effects in the GC–MS Analysis of Drugs*

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Abstract

Previous studies have shown that the ion response of a compound can be suppressed by the presence of a large amount of a coeluting substance in a gas chromatographic–mass spectrometric (GC–MS) system. In the present study, the change in the ion current of a constant amount of diazepam-d5 in the presence of a 100-fold amount of diazepam was used to monitor this condition in the Hewlett Packard mass selective detector (MSD). It was observed that a reduced recovery of ions occurred when the potentials of the MSD source elements were established by the autotune algorithm. Increasing the ion focus or the entrance lens potentials or both increased the recovery of the ion current of diazepam-d5 in the presence of large amounts of diazepam. The data suggested that the decreased recovery of ion current observed when the autotune source parameters were used was due to insufficient energy on the focusing lenses to extract a constant fraction of the ions from the source when a high concentration of molecules was present.

Introduction

Wu et al. (1–4) recently reported that the coelution of high concentrations of fluconazole with the trimethylsilyl (TMS) derivative of benzoylecgonine interfered with the gas chromatographic–mass spectrometric (GC–MS) detection of benzoylecgonine in urine extracts. Preliminary studies using a Hewlett Packard (Palo Alto, CA) 5890 GC and 5970B mass selective detector (MSD) indicated that the interference most likely occurred in the ion source (4). This was substantiated by observing the interference of high concentrations of fluconazole with the detection of benzoylecgonine in a direct probe MS (HP 5988B) analysis (3). Depletion of the derivatization reagent was also ruled out as the cause of false negative results by observing the negative interference after derivatizing benzoylecgonine and fluconazole separately and combining them just before GC–MS analysis (3). In other studies, it was observed that the coelution of high concentrations of a methadone metabolite caused a decrease in the ion current response of the TMS derivative of benzoylecgonine (1). Two other coeluting drug pairs, propoxyphene and propoxyphene-d7, and methaqualone and methaqualone-d4, were also studied and showed a similar concentration-dependent ion current decrease in the MSD (4). Propoxyphene and methaqualone did not require derivatization, which demonstrated that reagent depletion did not play a role in the mechanism of interference.

Matrix effects in the MS have also been reported by investigators in the analysis of environmental compounds (5–8). Previous studies by Tondeur et al. (5) and Marbury et al. (6) identified the ion source as the cause of matrix effects in these samples. The GC–MS instruments studied by these investigators were an HP 5700 GC with a VG Micromass (Altrinchem, Cheshire, U.K.) ZAB-ZFMS and a Varian (Walnut Creek, CA) model 3400 GC with an Extrel 400-1 MS, respectively. In the analysis of 2,3,7,8-tetrachlorodibenzodioxin (TCDD), a significant reduction (over 50% relative to pure standard) in the response for 100 pg of TCDD mixed with various amounts of methylstearte (400 ng to 1 mg) was observed (5). Marbury et al. (6) observed unusual nonlinearity effects (enhancement in response) at high concentrations in the analysis of TCDD.

Although this phenomenon is likely to occur in all mass spectrometers, the magnitude of the effect would likely vary depending on the ion source design of the instrument. However, characterization of the mechanism of this interference should provide a basis for the detection of this type of interference by routine drug testing laboratories independent of the type of instrumentation used. An investigation of the mechanism of this interference, using the Hewlett-Packard mass selective detector (HP 5970B) as a model, is presented in this study.

Experimental

Reagents and standards

Ethyl acetate and methanol were obtained as GC–MS-grade solvents from Burdick and Jackson (Muskegon, MI).
Perfluorotributylamine (PFTBA) was obtained from Hewlett-Packard (Kennett Square, PA).

Stock diazepam (1.0 g/L in methanol) and diazepam-d5 (0.10 g/L in methanol) standard solutions were purchased from Sigma (St. Louis, MO). Methanolic solutions containing 12.5 mg/L diazepam-d5 with 0.00, 1.25, 2.50, 5.00, 12.5, 25, 50, 125, 250, 500, 750, 1000, or 1250 mg/L diazepam were prepared from appropriate dilutions of these stock standards. For identification, each standard will be referred to as D5x, where x represents the amount (ng) of diazepam injected on the GC along with 12.5 ng of diazepam-d5 (i.e., D5125 refers to the analysis of 12.5 ng of diazepam-d5 along with 12.5 ng diazepam). Diazepam without diazepam-d5 present was prepared in methanol at 1.25, 12.5, and 1250 mg/L.

**Instrumentation**

Studies were performed on a Hewlett-Packard (Palo Alto, CA) 5970B quadrupole MSD controlled by HP-UX series ChemStation software. The MSD was interfaced to a model 5890 GC that was fitted with a model 7673A automatic injector. An HP Ultra-1 capillary column (12 m x 0.2 mm; 0.33-mm film thickness) was used with a carrier gas of helium at a flow rate of 0.7–1.0 mL/min. The injector was operated in the splitless mode at 275°C. One microliter of sample was injected onto the column at 150°C. The column temperature was held at 150°C for 1 min, then increased to 280°C at 30°C/min and held at this temperature for 1 min. Electron impact ionization (EI) was performed at 70 eV. Data were acquired in the selected-ion monitoring (SIM) mode with a dwell time of 50 ms with low mass resolution. The most intense ions of diazepam (m/z 284 M+, 256, and 221) and the corresponding deuterated ions in diazepam-d5 (m/z 289 M+, 261, and 226) were monitored. Detector response was measured as peak area by baseline-to-baseline integrations of the ion chromatograms.

**Automated tuning of the MSD**

The MSD was calibrated on the m/z 69, 219, and 502 ions of PFTBA using the Hewlett-Packard MSD autotune algorithm (9) to adjust the potentials on the source elements. The accepted abundances of the m/z 219 and 502 ions relative to the m/z 69 ion in the mass spectrum of PFTBA were reported to be 50.7 and 2.6%, respectively (10). The MSD autotune algorithm adjusts the ion source and electron multiplier potentials to maximize the intensity of the m/z 502 ion while maintaining the abundances relative to the m/z 69 ion at >35% and >1% for the m/z 219 and 502 ions, respectively. The autotune calibration relative abundances obtained for the m/z 219 and 502 ions on the MSD used in the present study were frequently between 40 and 65% and 10 and 16%, respectively. The repeller potential was consistently established by the autotune algorithm at the maximum value of 10.2 V. For each experiment, the reference potentials on the source elements were determined by the MSD autotune program.

**Methods**

The study examined the effect of increasing concentrations of diazepam on the ion current response of a fixed concentration of the coeluting deuterated analogue, diazepam-d5, after ion source potentials were established by the autotune algorithm. The MSD was calibrated using the autotune algorithm. Standard methanolic solutions (1 mL) containing 12.5 mg/L diazepam-d5 and increasing concentrations of diazepam (0, 1.25, 2.50, 5.00, 12.5, 25, 50, 125, 250, 500, 750, 1000, and 1250 mg/L) were analyzed in duplicate on the GC–MSD. One microliter of 12.5, 125, and 1250 mg/L diazepam was also analyzed in duplicate. The relative peak-area ratios of the m/z 226/221 ions, the m/z 261/256 ions, and the m/z 289/284 ions were calculated and averaged from the respective ion chromatograms of diazepam. At each concentration of diazepam coanalyzed with diazepam-d5, the peak area contribution of diazepam to the m/z 226, 261, and 289 ions was calculated and subtracted from the total peak area of the respective ion chromatogram. The average corrected peak areas of the m/z 226, 261, and 289 ion chromatograms of the 12.5 ng of diazepam-d5 coanalyzed with each amount of diazepam were divided by the respective average peak areas of the 12.5 ng of diazepam-d5 analyzed without diazepam. These ratios were multiplied by 100 to give the D5x/D50 relative peak area (RPAx/o). The response factor for each concentration of diazepam (m/z 284) and the 12.5 ng of diazepam-d5 (m/z 289) was calculated for each analysis by dividing the respective ion chromatogram peak area by the mass (ng) of the injected compound.

The effect of varying the potentials of the ion source elements (Figure 1) on the ion response recovery of 12.5 ng diazepam-d5 in the presence of 1250 ng diazepam was studied. Methanolic solutions (1 mL) containing 12.5 mg/L of diazepam-d5 and either 12.5 or 1250 mg/L were analyzed in duplicate on the GC–MSD system following an MSD autotune calibration of the mass spectrometer. The standard solutions were then reanalyzed in duplicate on the same system with each source element, in turn, set at selected potentials while leaving all other elements at their autotune settings. Ion current recoveries were evaluated at repeller potentials of 0.0, 2.0, 4.0, 6.0, 8.0, and 10.2 V; ion focus potentials of 0.0, –50, –100, –150, and –200 V; and entrance lens potentials of –25, –48, –75, –100,
-125, and -255 V. The average peak area (m/z 289) of the 12.5 ng of diazepam-ds that was coanalyzed with 1250 ng of diazepam was divided by the average peak area (m/z 289) of the 12.5 ng of diazepam-ds that was coanalyzed with 12.5 ng of diazepam multiplied by 100 to give the D51250/D5125 relative peak area (RPA1250/125).

Results

Figure 2 shows the RPAx/0 of the m/z 221, 261, and 289 ions of 12.5 ng of diazepam-ds in the presence of increasing amounts of coeluting diazepam (0 to 1250 ng) following tuning of the mass spectrometer to PFTBA using the MSD autotune algorithm. A decrease in the RPAx/0 of diazepam-ds response was observed for each of the ions when greater than 25 ng of diazepam was present. Less than 10% of the initial diazepam-ds m/z 256 and m/z 289 ion response was observed when 1250 ng of diazepam was present. The m/z 284 ion response factor of diazepam (0 to 1250 ng) and the m/z 289 ion response of diazepam-ds (12.5 ng) are plotted against the total amount of injected solutes in Figure 3. The response factor for both compounds decreased when greater than 37.5 ng of total solutes were analyzed.

The effect of the repeller potential on the RPA1250/125 of diazepam-ds is shown in Figure 4. The RPA1250/125 increased from 36% at the autotune repeller potential (10.2 V) to 106% at a repeller potential of 4.0 V. The RPA1250/125 was less than that of the autotune value at repeller potentials of 6, 8, and 10 V. Figure 5 shows the ion chromatogram peak area response of D5125 and D51250 observed at increasing repeller potentials. The greatest response for D5125 and D51250 was observed at repeller potentials of 6.0 and 8.0 V.

A linear increase in RPA1250/125 was observed with an increase in ion focus potential (Figure 6). The maximum peak area response of D5125 and D51250 occurred at an ion focus potential of -100 V (Figure 7) which corresponded to an RPA1250/125 of approximately 60% (Figure 6).

As the entrance lens potential was increased, RPA1250/125 initially decreased and then increased to a value greater than 100% (Figure 8). Figure 9 shows that the ion chromatogram peak areas of D5125 and D51250 did not respond in the same manner to the changes in entrance lens potentials. The D5125 response initially increased dramatically from -25 to -50 V potentials, and then slowly decreased with increasing entrance lens potentials to a response lower than that of D51250 at a -150 and -250 V. The D51250 response increased with increasing entrance lens potential from -25 to -100 V and then decreased slightly at -125 and -250 V.

Discussion

Previous work with benzoylecgonine, propoxyphene, and methaqualone showed a decrease in mass spectrometric ion current response of moderate amounts of these compounds in the presence of high amounts of coeluting substances (1-5). In the present study, diazepam and diazepam-ds were evaluated as probes to monitor this effect. These compounds were selected because they do not require derivatization and have low adsorptive properties in the injection port, column, and mass spectrometer. When using ion source potentials set by the MSD autotune algorithm, these compounds exhibited the same decrease in ion response (Figure 2) that was previously observed. It was also observed that as the total amount of

![Figure 2](https://example.com/figure2.png)

Figure 2. RPAx/0 of the m/z 221, 261, and 289 ions of 12.5 ng of diazepam-ds in the presence of increasing amounts of diazepam. Detector settings established by autotune algorithm: electron multiplier, -1800 V; repeller, 10.2 V; ion focus, 0.0 V; entrance lens, -58 V.

![Figure 3](https://example.com/figure3.png)

Figure 3. Ion current response factor (peak area/ng) of 12.5 ng of diazepam-ds (m/z 289 ion) and of diazepam (m/z 289 ion) with increasing amounts of diazepam injected on the column. Source element settings established by autotune algorithm: electron multiplier, -1800 V; repeller, 10.2 V; ion focus, 0.0 V; entrance lens, -58 V.
solute in the source increased, the response per mass of injected compound decreased for both the compound that was increasing in concentration (diazepam) and the compound that was maintained at a constant concentration (diazepam-d5). This indicated that all of the compounds present in the source were affected by this condition, not just compounds at low concentration.

Decreased sensitivity attributed to the ion source may be caused by one of the following factors; a reduction in the extraction efficiency of charged particles from the ion source because of space-charge effects or poor ion focusing; a low fraction of total emission current traversing the ionization chamber; a build-up of ions on the source elements which reduces the electrical field strength; or a high source pressure which decreases ionization because of a reduction in the mean free path (MFP) of the ionizing electrons (5,11–15). The detector may be a source of low sensitivity if the electron multiplier is contaminated, has a low gain, or becomes saturated (15,16).

Based on previous reports (5,11–15), it was postulated that the recovery of ions from the ion source (Figure 1) could be enhanced by increasing the repeller potential, by increasing the size of electron entrance apertures and ion exit apertures, by changing the volume of the source, or by adjusting the potentials of the focusing lenses (ion focus and entrance lenses). On the MSD, the autotune calibration function routinely sets the repeller potential to the maximum setting of 10.2 V. Higher potentials could not be evaluated. Changing the size of the apertures or source volume on the HP MSD was not feasible and was not evaluated. The focusing lens potentials could be adjusted on the MSD and potentials different from those set at autotune calibration were studied.

Although the RPA1250/12.5 was approximately 100% at a repeller potential of 4 V, the peak area response of D512.5 was 8 to 11 times less than that observed at repeller potentials of 6 and 8 V. Even though the D512.5 and D51250 responses were similar at the 4 V potential, the sensitivity of the D512.5 response was compromised at this potential. Interestingly, the D512.5 response at the autotune setting of 10.2 V was 3.5 to 4.8 times less than that at the 6 and 8 V repeller potentials, although the RPA1250/12.5 at these three potentials were similar. It would appear that the optimum repeller potential for these ions (m/z 284 and m/z 289) was 6 to 8 V.

The autotune algorithm routinely sets the ion focus potential to 0.0 V. In evaluation of the effect of repeller potential on RPA1250/12.5, the ion focus was set at this potential. It was observed that, with the repeller potential set at 10.2 V, RPA1250/12.5 increased with increasing ion focus potential (Figure 6). At an ion focus potential of -200 V, RPA1250/12.5 was almost 100%. As the ion focus potential was increased from

![Figure 4](https://academic.oup.com/jat/article-abstract/21/1/17/812975)

**Figure 4.** RPA1250/12.5 (m/z 289 ion) of 12.5 ng of diazepam-d5 with increasing repeller potential. Other detector elements held constant at: electron multiplier, -2400 V; ion focus, 0.0 V; entrance lens, -56 V.

![Figure 5](https://academic.oup.com/jat/article-abstract/21/1/17/812975)

**Figure 5.** Ion current response (m/z 289 ion) of 12.5 ng of diazepam-d5 in the presence of 12.5 ng and 1250 ng diazepam with increasing repeller potential. Electron multiplier, -2400 V; ion focus, 0.0 V; entrance lens, -56 V.

![Figure 6](https://academic.oup.com/jat/article-abstract/21/1/17/812975)

**Figure 6.** RPA1250/12.5 (m/z 289 ion) of 12.5 ng of diazepam-d5 with increasing ion focus potential. Other detector elements held constant as follows: electron multiplier, -2000 V; repeller, 10.2 V; entrance lens, -48 V.

![Figure 7](https://academic.oup.com/jat/article-abstract/21/1/17/812975)

**Figure 7.** Ion current response (m/z 289 ion) of 12.5 ng of diazepam-d5 in the presence of 12.5 ng and 1250 ng diazepam with increasing ion focus potential. Electron multiplier, -2000 V; repeller, 10.2 V; entrance lens, -48 V.
The entrance lens potential setting was the potential established at the entrance lens when m/z 502 was being monitored. The actual entrance lens potential varied relative to this value as each mass-to-charge ratio was being detected. The entrance lens value was established to regulate the velocity of each ion entering the mass analyzer, the value being directly proportional to the mass-to-charge ratio of the ion being monitored. With the ion focus potential set to 0 V, increasing the entrance lens potential resulted in an initial increase in the response of D512.5 and D51250 (Figure 9). However, as the entrance lens potential was increased further, the peak-area response of D512.5 and D51250 decreased. Initially, the increased energy was extracting a greater fraction of the total ions from the source; however, because the entrance lens potential also controls the velocity of the ions entering the mass analyzer, as the potential was increased over -50 V, the efficiency of detecting the m/z 284 or 289 ion decreased because the velocity through the mass analyzer was too high. Although the RPA1250/12.5 was near 100% (Figure 8) at an entrance lens potential of -100 V, this was not the optimum setting for maximum efficiency of detecting these ions.

The data suggested that the mechanism of concentration-dependent ion current reduction involved a decrease in ion extraction and ion focusing efficiency of charged particles from the ion source into the mass analyzer. The source potentials set during an autotune calibration were not sufficient to extract a constant fraction of the 289 ions generated by 12.5 ng of diazepam-d5 when the amount of coeluting diazepam exceeded 25 ng (Figure 2).

This matrix phenomenon has serious effects on quantitative and qualitative analysis and therefore compromises the validity of data obtained for detection and confirmation of drugs in urine. The ability to detect matrix effects was affected by the mode of analysis (full scan or SIM). Unknown substances that coelute and interfere with the target compound can be readily detected in the full scan mode by the presence of additional ions contaminating the mass spectrum of the target drug. In an SIM analysis, where at least three ions are monitored, interference can be recognized by unacceptable ion ratios when the interfering compound has ions in common with the compound of interest. If a coeluting deuterated analogue of the compound of interest, present at the threshold concentration for the assay, is used as an internal standard, interference can further be detected by a reduction in or complete absence of ions monitored for the internal standard. This means of identifying an interfering compound is particularly important in cases in which the ion ratios are not altered by the coeluting compound.

In an SIM analysis in which the internal standard does not coelute with the compound of interest, detection of an interfering compound with either the compound of interest or the internal standard may be impossible. Suppression of the ion response of the analyte below the threshold concentration or the presence of altered ion ratios most likely would, under the standard operating procedures used by many drug testing laboratories, result in a negative report without further investigation.

Stringent sample cleanup procedures are needed to remove...
matrix interferences to improve the specificity of the analysis. Because of the complex nature of a urine sample, it is almost impossible to design a cleanup procedure that would account for all of the possible exogenous substances that may be present. To prevent lowered or elevated results from occurring, analysts must be aware of this type of interference and modify their procedures to incorporate strategies to detect it. This may involve performing the analysis in the full scan mode or using a deuterated coeluting internal standard at the threshold concentration and monitoring at least three ions when performing the analysis in the SIM mode. If a coeluting substance is detected, the problem can be eliminated by identifying the substance and processing the sample to remove the interferant.

Conclusion

Concentration-dependent decreases in ion current in the mass selective detector appeared to be due to insufficient energy for extraction of the ions from the source at the source element potentials established by the auto tune algorithm. Increasing the potential of the source elements to aid in the extraction of ions at high concentrations caused inefficient focusing of ions into the mass analyzer. The data indicated that there was a limited dynamic range of total solute concentration in the ion source at which efficient detection of compounds could be obtained. To prevent false negatives results for the detection of target compounds, one must be aware of the presence of coeluting substances. The most effective way to determine the presence of interferants is to perform the analysis in the full scan mode. Alternatively, when performing the analysis in the SIM mode, the recovery of a coeluting deuterated analogue should be critically evaluated of a coeluting deuterated analogue should be critically evaluated to detect possible detrimental effects of a coeluting substance.

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References


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