



Evaluation of GC Inlet Liner Deactivations and GC Column Deactivations for Basic Drug Analysis Using Mass Spectrometry

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Introduction

Clinical and forensic toxicologists are required to detect low levels of abused drugs in body fluids and confirm their presence via Gas Chromatography/Mass Spectrometry (GC/MS). Typical limits of detection are less than 5ng/mL. For these nitrogen- and/or oxygen-containing compounds (benzphetamine, phenylcyclidine, ketamine, methadone, cocaine, codeine and alprazolam), selecting the proper GC column and GC inlet liner becomes important since both of these parameters can affect the MS response. Fused silica GC columns and glass inlet liners contain active silanol (Si-OH) groups (protic acids or Lewis acids) that react with compounds containing nonbonding electrons such as nitrogen or oxygen atoms. For most GC analyses, particularly for basic drug analysis, glass surfaces require "deactivation" of the -OH group in order to eliminate this acid-base reaction. The reaction will chromatographically appear as broad, tailing peaks and/or reduced response. Deactivating the surface of glass is accomplished by reacting the silanol group with a compound that yields a surface that is unreactive towards nonbonding electrons such as those found in basic drugs (nitrogen and/or oxygen atoms). By ensuring that all GC glass surfaces (GC columns and inlet liners) are inert (or deactivated), chromatographic peaks will be sharp and Gaussian making them easy to integrate and thus yielding reproducible and accurate responses.

Experimental

GC/FID responses of various basic drugs using several deactivation methods on columns & inlet liners were evaluated to maximize sensitivity. Chemical standards of basic drugs in their free base form were prepared at various concentrations. The drug mixtures included benzphetamine, ketamine, phenylcyclidine, methadone, cocaine, codeine and alprazolam. Sample preparation included extracting 1ml of the mixture with 1-chlorobutane and saturated aqueous sodium sulfate, drying the 1-chlorobutane layer (containing the drugs) down to 1ml and diluting the resulting extract into various concentrations (100, 50, 25, 10, 5 ng/mL). A testing matrix (DOE, design of experiment) comprised of 5%-diphenyl-95%-dimethylpolysiloxane stationary phase columns treated with standard and base deactivations was designed. The 4mm Single Gooseneck inlet liners included in the DOE were untreated, intermediate polar deactivated, Siltek and base deactivated. See Table 1 for testing matrix. The resulting response data for each compound was analyzed using Mini Tab statistical software to determine which combination of column and inlet liner deactivations maximized sensitivity and reproducibility. The resulting best combination was then evaluated using an Agilent 5890 GC with a 5971 MS to confirm the GC/FID data.

Table 1. Design of Experiment (DOE)

Column Deact Type	Inlet Liner Deact Type	No. of Runs
Rtx-5MS (Standard)	Undeactivated	3
Rtx-5MS (Standard)	IP (Intermediate Polar)	3
Rtx-5MS (Standard)	Siltek	3
Rtx-5MS (Standard)	Base	3
RXI-5MS (Proprietary)	Undeactivated	3
RXI-5MS (Proprietary)	IP (Intermediate Polar)	3
RXI-5MS (Proprietary)	Siltek	3
RXI-5MS (Proprietary)	Base	3
Rtx-5Amine (Base)	Undeactivated	3
Rtx-5Amine (Base)	IP (Intermediate Polar)	3
Rtx-5Amine (Base)	Siltek	3
Rtx-5Amine (Base)	Base	3

NOTE: Each column/liner combination was evaluated using standards of 5 different concentrations.

Results

MiniTab statistical software was used to analyze the DOE data. An ANOVA (analysis of variance) was performed and results (p values) showed that the inlet liner deactivation type affected drug responses more significantly than the column deactivation type. ANOVA results were used to generate box plots. Box plots display the range of data distribution. This range or variation is an indication of the reproducibility of the factors analyzed. The bigger the box, the more variation. The mean or average of the data is also displayed (line through the box) and indicates the average response for that factor. Even though data was collected on all compounds and all factors in the testing matrix, only PCP (phenylcyclidine) and cocaine plots are shown to represent the nitrogen-containing and the nitrogen/oxygen-containing drugs, respectively. The GC/FID inlet liner data obtained show that the standard IP (intermediate polar) deactivated and the undeactivated liners performed poorly for either response and/or reproducibility regardless of the column deactivation type. This is likely due to the acidic nature of the undeactivated glass surface and the small number of residual acidic sites left on the IP deactivated surface. Figure 1 shows example box plots of PCP and cocaine (5ng) for each liner deactivation. As discussed, the IP and undeactivated liners exhibit either larger variation and/or lower average response compared to the base and Siltek deactivated liners.

Column deactivation data shows that the standard deactivated column (Rtx-5MS) produced the largest variation. This is thought to be due to the slightly acidic nature of the column deactivation. Figure 2 displays the PCP and cocaine box plots on all column deactivation types.

Figure 1. Even though the IP deactivated liner has a higher mean response for PCP and Cocaine, it has a larger variation indicating that it is not reproducible. The undeactivated liner shows less variation, but has the lowest mean response.

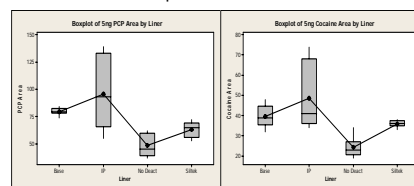
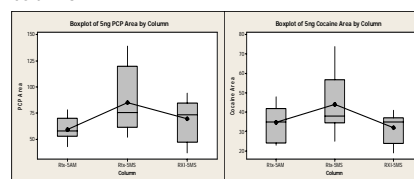


Figure 2. The mean responses for all columns are very similar. However, the variation on the Rtx-5MS is relatively larger than the Rtx-5Amine and RXI-5MS columns.



Since the IP deactivated liner, undeactivated liner and the Rtx-5MS column deactivation type proved to exhibit high variation (not reproducible) and/or the lowest response (lowest mean), the testing matrix data was reanalyzed excluding these factors. By excluding these factors, we were able to compare the remaining data for response and reproducibility. As shown in Figures 3 and 4, this reanalyzed data indicates that the base deactivated column (Rtx-5Amine) coupled with the base deactivated inlet liner yields the best response and reproducibility overall. Because these drugs are regularly analyzed using GC/MS, the base deactivated column and inlet liner were evaluated on an Agilent 5890 coupled to an Agilent 5971 MS. See Figure 5.

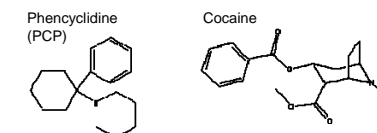


Figure 3. The mean PCP response and variation are similar for both columns. For cocaine, not only is the mean response significantly higher, but the variation is smaller.

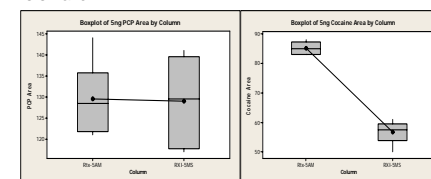


Figure 4. For PCP, the mean response is much higher on the base deactivated inlet liner and the variation is similar to the Siltek inlet liner. The mean cocaine response and variation are similar for both inlet liner deactivation types.

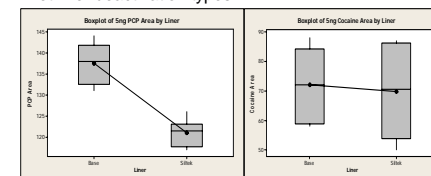
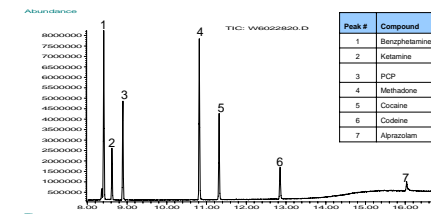


Figure 5. GC/MS drug analysis using base deactivated column and inlet liner.



Conclusion

Because nitrogen- and oxygen-containing drugs have nonbonding electrons that react with protic or Lewis acids such as those on glass surfaces (silanol groups), it is important to have properly deactivated GC glass inlet liners and columns. The work presented in this poster demonstrates that basic drugs analyzed on a base deactivated GC column (Rtx-5Amine) coupled with a base deactivated inlet liner produces reproducible responses and the highest responses overall.