Application Note: 10014

Use of a Novel Large Volume Splitless Injection Technique and Sequential Full Scan/SIM for Simultaneous Screening and Confirmation of Toxicological Specimens

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Key Words

- DSQ Series GC/MS
- Drugs of Abuse
- Forensic
- FS/SIM
- Large Volume Injection

Overview

Purpose: The purpose of this study was two-fold. The first objective was to compare a standard splitless injection technique with a large volume splitless injection (LVSL). The second objective was to evaluate the applicability of sequential full scan/SIM EI+ GC/MS data to the analyses of forensic toxicology samples. The utility of these techniques along with directions for future research will be discussed.

Methods: Extracted, derivatized urine samples were analyzed using a standard splitless injection of 2 µL using a 15 m column. Samples at the same levels were then analyzed using a 20 µL large volume splitless injection (LVSL). A gas chromatograph/quadrupole mass spectrometer (GC/MS) was programmed to acquire sequential full scan/SIM data in EI+ mode. A custom library was created for reference for a number of derivatized and underivatized drugs to facilitate library searching.

Results: For a standard splitless injection using sequential full scan/SIM for MS acquisition, the limit of detection for BE-TMS was 30 ng/mL. This LOD was decreased 100-fold to 300 pg/mL by using a large volume splitless injection. BE-TMS in urine by LVSL was linear from 300 pg/mL to 1200 ng/mL, with a correlation coefficient of 0.9996. Automated library searching on the full scan data provided preliminary results for a number of drugs, offering additional information when evaluating unknown samples.

Introduction

Toxicology specimens present unique analytical challenges that include complex matrices, time constraints, complex sample preparation, and the need for solid scientific practices upon which to base the results. Historically, gas chromatography and mass spectrometry (GC/MS) have played a crucial role in the forensic toxicology laboratory. A novel injection technique coupled with a sequential full scan/SIM acquisition can expand that role and consequently increase the value of GC/MS as an analytical tool. By combining an injection technique that allows injection of up to 50 µL of sample with a sequential full scan/SIM acquisition, the forensic scientist can achieve several objectives with a single injection. SIM analysis enables confirmation of pre-screened presumptive positives, while the presence of full scan data allows investigation into other compounds of interest that may be present in a sample.

Extracted urine samples were analyzed for benzoylecgonine (BE) using the Thermo Scientific DSQ™ quadrupole mass spectrometer coupled to a Thermo Scientific TRACE GC Ultra™ gas chromatograph equipped with a split/splitless injector configured initially for splitless injection. For comparison, a 15 m x 0.25 mm i.d. x 025 µm column of the same phase as the LVSL analytical column was used for standard splitless injection. For LVSL, the injection port liner was changed, and a special injection port head was installed. A 5 m x 0.32 mm i.d. uncoated precolumn connected to a 15 m x 0.25 mm x 0.25 µm 5% phenyl analytical column was used for LVSL.

LVSL is accomplished on the TRACE GC Ultra with a simple modification to the standard split/splitless injector.[1] This configuration minimizes dead volume, is forward-pressure regulated and allows for an automatic pressure surge upon the injection of the large solvent volume. A process termed continuous solvent recondensation takes place as the solvent is recondensed on the precolumn, where it is retained (Figure 1). By keeping the oven temperature low through the solvent evaporation phase, volatile solutes are reconcentrated through solvent trapping. Higher boiling point components, which are spread within the uncoated precolumn, are focused by the retention gap effect.[1,2]

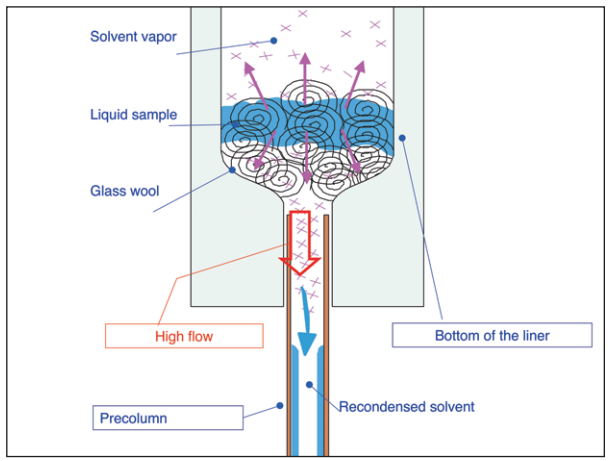

Figure 1: Basic diagram of the large volume splitless injection technique, using liner with glass wool.[1,2] Note the position of the column in the liner.

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Acquisition methods appropriate to target compounds were created. LVSL was optimized using the accompanying software tool, which facilitated development of the oven method. An autosampler was used to perform the large volume injections. Custom libraries were created containing common drugs of abuse in derivatized and underivatized form.

Methods

Sample and Standard Preparation

BE standards for calibration, along with deuterated BE-D3, were obtained from Cerilliant Corporation (Round Rock, TX). Additional BE quantitative standards for controls came from Lipomed, Inc. (Cambridge, MA). Working standards were prepared in methanol. A 1.0 mL urine sample size was used and spiked with the working standards to prepare calibrators ranging from 300 pg/mL to 1200 ng/mL. Controls were set up containing 120 and 180 ng/mL BE respectively, along with a negative control. BE-D3 internal standard was added at 75 ng/mL. Solid phase extraction using Clean Screen® DAU 200 mg 10 mL columns (United Chemical Technologies, Inc., Bristol PA) was performed according to that company's published procedure for extraction of BE from urine.[3] The extracts, eluted with 78:20:2 methylene chloride: isopropanol: ammonium hydroxide, were evaporated under N_2 at 45 °C. An unextracted standard, set up to contain the equivalent of 300 ng/mL BE, was added at this point. The dried samples were derivatized for 20 minutes at 70 °C with MSTFA with 1% TMCS (United Chemical Technologies) to form the trimethylsilyl derivative of BE (BE-TMS). For standard splitless injection, samples were diluted with 50 µL ethyl acetate; samples for LVSL were diluted with 250 µL ethyl acetate. Autosampler vials with inserts were used for all samples.

Instrument Methods

The DSQ was programmed to perform sequential full scan/SIM acquisitions. Both scan events used the same tune parameters, resulting from an optimal sensitivity Autotune. The prefilter value was increased from the Autotune value of 4.5 to 9.0 to increase sensitivity and high mass response. A detector gain of 3 x 10^5 was used for the standard splitless injection. This was decreased to a gain of 1 x 10^5 for LVSL.

The Large Volume Assistant software package was used for setting up the TRACE GC Ultra parameters for LVSL (Figure 2). The initial oven temperature was set at the maximum temperature offered by the program, 77 °C, and this temperature was held for 4.81 minutes. The oven ramp was then ramped at 30 °C/min up to 300 °C for a final hold time of 6.0 minutes. The autosampler was programmed for a 20 µL injection volume at 99 µL/sec injection speed. These instrument parameters are summarized in Table 1.

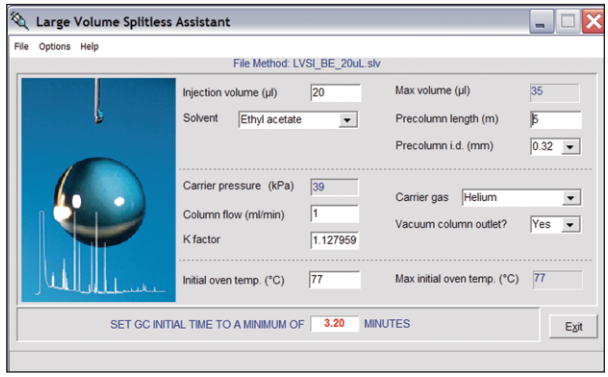


Figure 2: Large Volume Splitless Assistant method parameters for 20 µL LVSL injections.

DSQ	
DSQ Settings	
Source Temp:	250 °C
Start Time:	6.00 minutes
Detector gain:	1E5 (1229V)
Scan Event 1	
Scan Mode:	Full Scan
Scan Rate:	2506 amu/sec
Scan Range:	40-450 amu
Scan Event 2	
Scan Mode:	SIM m/z: 240, 243, 361, 364
Width:	0.5 amu
Dwell:	30 ms

TRACE GC ULTRA	
Oven Method	
Initial Temperature (°C):	77
Initial Time (min):	4.81
Rate #1 (deg/min):	30.0
Final Temperature #1 (°C):	300
Hold Time #1 (min):	6.00
Left SSL Method	
Base Temperature (°C):	200
Mode:	Splitless
Splitless Time (min):	1.00
Constant Septum Purge:	Off
Stop Purge At: (min):	0.00
Left Carrier Mode:	Constant Flow

AUTOSAMPLER	
Sample Volume:	20.0 µL
Injection Delay:	0 sec
Pullout Delay:	0 sec
Injection Speed:	99 µL/sec
Pull-Up Speed:	10 µL/sec
Pre Injection	Washes: 5
Pre Injection	Volume: 30.0 µL
Pre Injection	Solvent: D
Post Injection Washes:	10
Post Injection Volume:	30.0 µL
Post Injection Solvent:	A

Table 1: DSQ, TRACE GC Ultra, and autosampler parameters for Large Volume Splitless Injections: The same width and dwell were used for all SIM ions. The injection speed for the autosampler ensures the delivery of a liquid droplet.

Quantitative and Qualitative Method Setup: Drug standards (Alltech-Applied Science, State College, PA) were scanned, both underivatized and derivatized with MSFTA with 1% TMCS, and custom libraries were created in the Library Browser of Xcalibur®.[4] Appropriate Xcalibur processing methods were set up for quantitative and qualitative analysis, including automatic library searching.

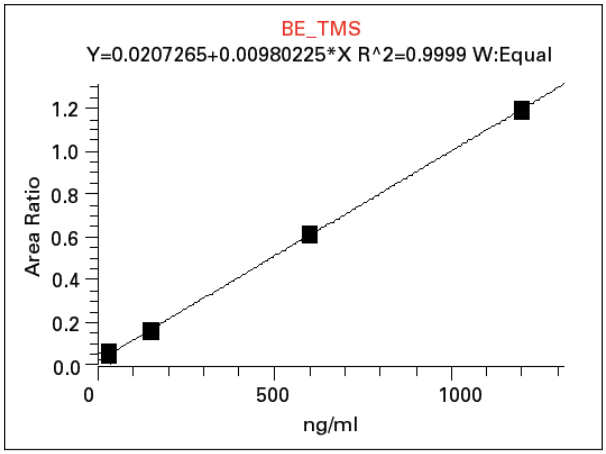


Figure 3: BE-TMS Calibration curve, from 30-1200 ng/mL, on the 15 m column using a 2 µL splitless injection and sequential full-scan/SIM (Quant Ion = m/z 240).

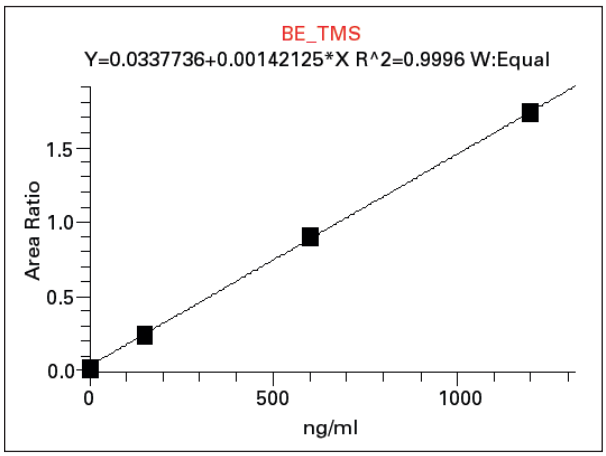


Figure 4: BE-TMS Calibration curve, from 300 pg/mL (0.3 ng/mL) to 1200 ng/ML using a 20 µL large volume splitless injection and sequential full-scan/SIM (Quant Ion = m/z 240).

Results

Sequential full scan/SIM acquisition of derivatized urine extracts using the DSQ provided linearity from 30 to 1200 ng/mL for BE using a standard 2 µL splitless injection (r^2=0.9999). SIM data was used for quantitation of BE. To evaluate the effect on sensitivity potentially offered by increasing the injection volume, 20 µL large volume splitless injections were performed on samples treated with similar extraction and derivatization techniques. The SIM data were linear using this method from 300 pg/mL to 1200 ng/mL, with a correlation coefficient of 0.9996. These curves are shown in Figures 3 and 4, respectively. Figure 5 shows the extracted ion chromatogram for *m/z* 240 for the 300 pg/mL standard.

Full scan data was used to perform automatic library searching, and reports were set up to include search results for the top 10 peaks in the total ion chromatogram. Results from one of the largest peaks in the TIC are highlighted in Figure 7. The top trace reflects the full scan data, while the lower trace is the SIM chromatogram, for all SIM ions acquired. The DSQ is able to acquire sequential full scan/SIM data for two reasons. First of all, the RF is capable of switching between the two scan ranges quickly. In addition, the curved prefilter design reduces noise and increases sensitivity. Thus offsetting any loss of sensitivity resulting from the shorter duty cycle using the sequential functions.

The injection of 20 µL was facilitated through the use of the Large Volume Assistant software program. No adjustment in the oven program was made for the effect of the derivative. The solvent delay was set to ensure solvent elution occurred before turning on the filament current, thereby increasing filament lifetime.

Setup of the TRACE GC Ultra for LVSL is not complex, although care must be taken to ensure that the injection port is leak-tight and that the press-fit connection of the pre-column to the analytical column is also leak-free. Decreased sensitivity and poor reproducibility can result from leaks. To evaluate reproducibility, the retention times for BE-D3 over 26 injections were measured (Figure 6). The %RSD for these injections was 0.027%. Additionally, 6 replicate injections of a sample at 150 ng/mL were made. The coefficient of variation for these samples was 1.5%, the average concentration was 164.3 ng/mL, and the percent difference from the nominal concentration was 9.6%.

As with any high-sensitivity technique, it is important to consider the need for pure solvents due to the amplification of any contaminants. Additional concerns stem from the potential for column overload when high levels of drugs or other compounds are present. This effect is noted in Figure 7, where trimethoprim (as the TMS derivative) and its metabolites are overloaded. Extra solvent can be added when the potential for overload exists.

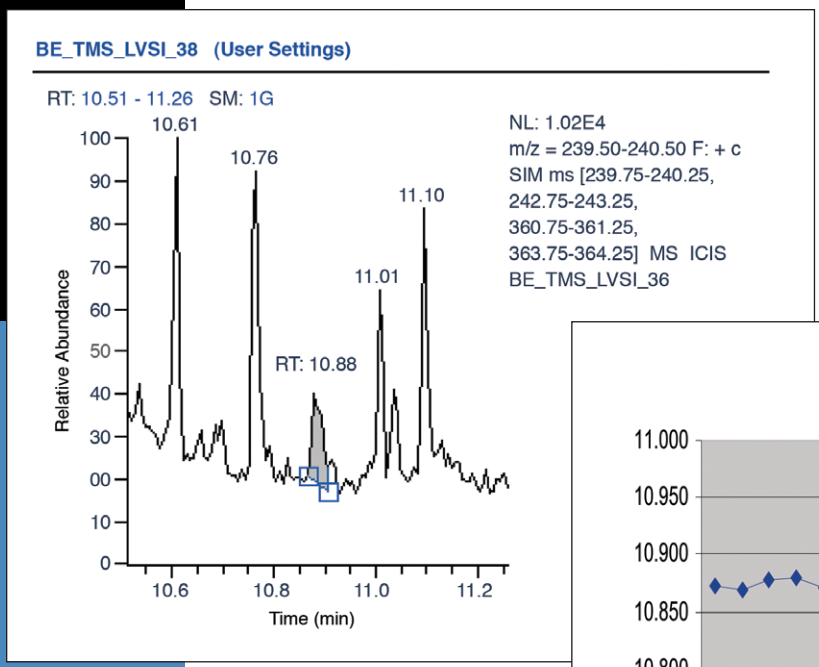


Figure 5: BE-TMS extracted ion chromatogram, m/z 240, at 300 pg/mL for LVSL, retention time at 10.88 minutes.

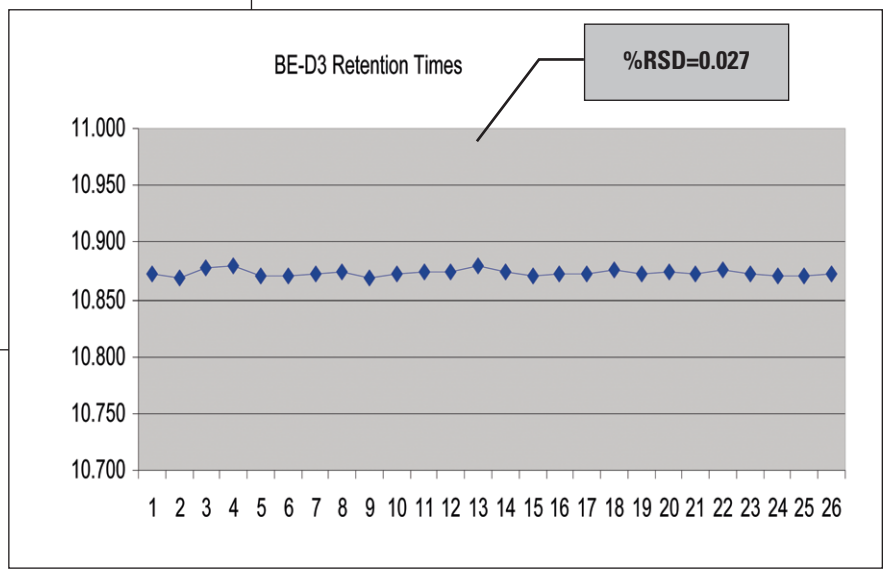
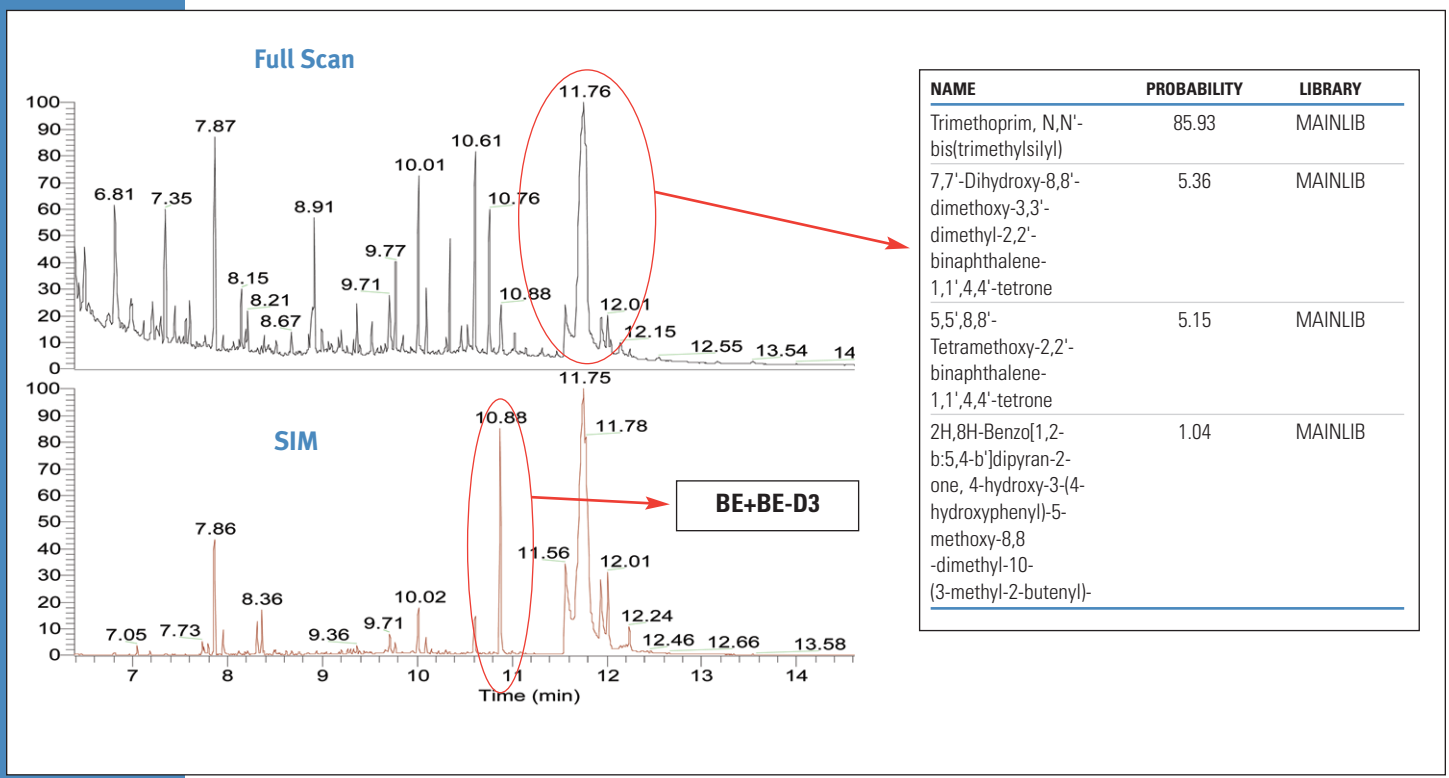


Figure 6: Retention time stability for the BE-D3 internal standard using 20 µL injections, n=26. The % RSD for 26 injections was 0.027%, looking at all sample types injected.



NAME	PROBABILITY	LIBRARY
Trimethoprim, N,N'-bis(trimethylsilyl)	85.93	MAINLIB
7,7'-Dihydroxy-8,8'-dimethoxy-3,3'-dimethyl-2,2'-binaphthalene-1,1',4,4'-tetrone	5.36	MAINLIB
5,5',8,8'-Tetramethoxy-2,2'-binaphthalene-1,1',4,4'-tetrone	5.15	MAINLIB
2H,8H-Benzo[1,2-b:5,4-b']dipyran-2-one, 4-hydroxy-3-(4-hydroxyphenyl)-5-methoxy-8,8 -dimethyl-10-(3-methyl-2-butenyl)-	1.04	MAINLIB

Figure 7: TIC and Extracted ion chromatogram for 1200 ng/mL LVSL standard, showing library search result table with tentative identification of the compound at 11.75 minutes as trimethoprim, N,N'-bis(trimethylsilyl), an antibiotic.

Conclusions

LVSL is a useful tool for forensic toxicology that is easily configured and does not require sophisticated software or hardware. Injections are reproducible, and the resulting lower detection limits indicate that this method could be suitable for alternate matrices, i.e. hair, sweat, blood/body fluids, or oral fluid, where lower concentrations are present. However, due to the sensitivity to contaminants and the presence of many organics co-extracted with solid phase extraction (primarily urea), additional sample cleanup or cleaner extraction methods should be pursued.

Sequential full scan/SIM acquisitions give the availability of full-scan data to evaluate unknown peaks, without significantly compromising SIM data for quantitation. Automated library searching gave a top hit for trimethoprim, as the di-TMS derivative, which corresponded to medication being taken by the matrix donor during this study. This further reflects the suitability of this type of analysis to toxicology applications. Alternate matrices could benefit from the larger injection volume and therefore utilize EI+ as the analytical technique.

References

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Acknowledgement

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