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Stir Bar Sorptive Extraction (SBSE) and GC/MS Determination of Polar and Non-polar Drugs and Pharmaceuticals in Post Mortem Brain Tissue

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Abstract
Stir Bar Sorptive Extraction (SBSE) is an innovative and efficient method [1] for the extraction of drugs and pharmaceuticals from blood-, urine- and tissue samples in a forensic toxicology laboratory. As shown in this application note and earlier publications [2,3,4], SBSE is an effective screening tool for drugs and pharmaceuticals in biological fluids and tissue. The SBSE technique is easy to use and the method described in this publication is performed without additional use of organic solvents, centrifugation, or the liquid transfer steps that are normally necessary when performing liquid-liquid or solid-phase extraction.

Introduction
GC/MS determination of pharmaceutical compounds and drugs in biological samples is usually done by isolating the compounds of interest and/or their metabolites from the biological matrix. The sample preparation techniques most often used in routine analysis are based on liquid-liquid extraction, protein precipitation and solid-phase extraction (SPE). The main drawback of these methods is that they are labor intensive and involve working with toxic organic solvents. The SBSE technique using the GERSTEL Twister

was developed to extract organic components from an aqueous matrix practically without any sample preparation.

The Twister is a glass-encased magnetic stir bar coated with an extraction phase of polydimethylsiloxane (PDMS). When the Twister stirs an aqueous sample or an aqueous slurry of a solid sample it extracts analytes into the PDMS phase. Just as in liquid-liquid extractions, analytes partition between the extractant phase, in this case PDMS, and the liquid sample phase. Following their extraction, analytes can be transferred to a GC system by thermally desorbing the Twister or to an LC system by re-extracting the Twister with an LC-compatible solvent. Derivatization of the analytes can alternatively be performed directly on the Twister or during the thermal desorption process by adding a derivatization agent to the carrier gas. This would be done using a derivatization pneumatic accessory installed on the thermal desorption instrument.

In this application work, the GERSTEL Twister was used to extract target analytes with different chemical properties from bovine brain and liver tissue for subsequent GC/MS determination. Basic, acidic and neutral compounds were included in a positive control mix suggested by the German Society of Toxicological and Forensic Chemistry (GTFCh, Table 1). Furthermore an extraction and screening for unknown substances in bovine brain and liver tissue was conducted during a GTFCh workshop.

In order to determine the presence of drugs of abuse in forensic samples it is mandatory that the extraction medium be absolutely free from compounds of interest. Used Twisters are cleaned and conditioned by washing them for hours in acetonitrile or methanol/dichloromethane 1/1 followed by thermal desorption overnight at 300 °C under a flow of nitrogen.

Before adding the Twister to a sample, a blank run of the Twister should by performed and the background checked to ensure that the Twister doesn't contain background levels of any compounds of interest.

Table 1. Analytes included in the positive control standard used (GTFCh mix).

Analyte	RT [min]	Target Ion [m/z]	Qual. Ions [m/z]	Conc. [ng/g]
Amphetamine	4.601	44	91/65	500
Salicylic Acid	6.160	120	92/138/64	5000
Ibuprofen	9.580	161	163/206/119	5000
Paracetamol	10.558	109	151/80/108	5000
Phenobarbital	13.646	204	117/115/103	5000
Metoprolol	14.359	72	107/223	500
Methadone	15.525	72	57/165	500
Cocaine	16.093	182	82/94/77	500
Doxepin	16.154	58	42/165	500
Codeine	17.827	299	162/214	500
Diazepam	18.329	256	258/284/257	500
THC	18.486	299	314/231/271	100
Morphine	18.246	285	162/215/115	500
THC-COOH				50

Figure 1. The GERSTEL Twister, used for stir bar sorptive extraction of drugs and pharmaceuticals from brain tissue in the work reported here.

Experimental

Materials. 0.1 M Trizma buffer solution pH 8.5 saturated with NaCl (Sigma Aldrich), PDMS Twister (2 cm lenght, 1 mm phase thickness, GERSTEL).

Instrumentation. Tissue was homogenized using an IKA® Ultra Turrax® Tube Drive. The analyses were performed using a 6890N GC equipped with a 5975 (inert XL Triple Axis) Mass Selelective Detector (Agilent Technologies), a Thermal Desorption Unit (TDU), Cooled Injection System (CIS 4) and Multi Purpose Sampler (MPS) (all GERSTEL).

Analysis Conditions

TDU:	
Temperature	50°C; 300°C/min; 280°C (10 min)
Pneumatics	Splitless
CIS 4:	
Temperature	-100°C; 2°C/s; 300°C (26 min)
Pneumatics	Solvent Vent @ 3 min, 50 mL/min
Liner	Quartz wool deactivated, 2 mm
GC:	
Temperature	100°C (1 min); 10°C/min; 325°C (6.5 min)
Pneumatics	He, 111.4 kPa, constant pressure Retention Time Locked (RTL) with Proadifen (SKF 525-A)
Column	Rxi-5ms 30 m d_i = 0.25 mm d_f = 0.25 µm
Detector:	
MSD	EI mode, scan, 40-570 amu

Sample Preparation

- A 2 g sample of spiked tissue was homogenized for 10 minutes using the IKA® Ultra Turrax® Tube Drive
- 1 g of the tissue homogenate was transferred to a 25 mL vial using a 5 mL Eppendorf Pipette
- 5 mL of Trizma buffer pH 8.5 saturated with NaCl was added
- A conditioned Twister was added and the vial was capped
- The sample was extracted for 3 h on a magnetic stirring plate at 1000 rpm (For the positive control mix 0.5 h extraction time is sufficient, but when screening for unknown compounds, the extraction time should be extended)
- After the extraction. the Twister was carefully removed, rinsed with deionized water, dried with a lint-free paper tissue, and placed in a thermal desorption tube which was in turn placed in the autosampler tray.

Results and Discussion

The Twister (SBSE) extraction method was chosen for screening of drugs and pharmaceuticals since the SBSE process is simpler and less labor intensive than traditional methods, and since it is solvent free. For routine analysis of large series of samples, the GERSTEL MultiPurpose Sampler (MPS) enables automated processing of up to 196 Twisters in one batch.

The evaluation of the chromatogram of the positive control standard (GTFCh mix) was done using a target database in the Agilent MSD Chemstation Software. In the chromatogram shown in figure 2, the annotated peaks represent those analytes that could be extracted from the positive control mix. Only a few highly polar (e.g. salicylic acid) or GC-incompatible compounds (THC-COOH) could not be found.

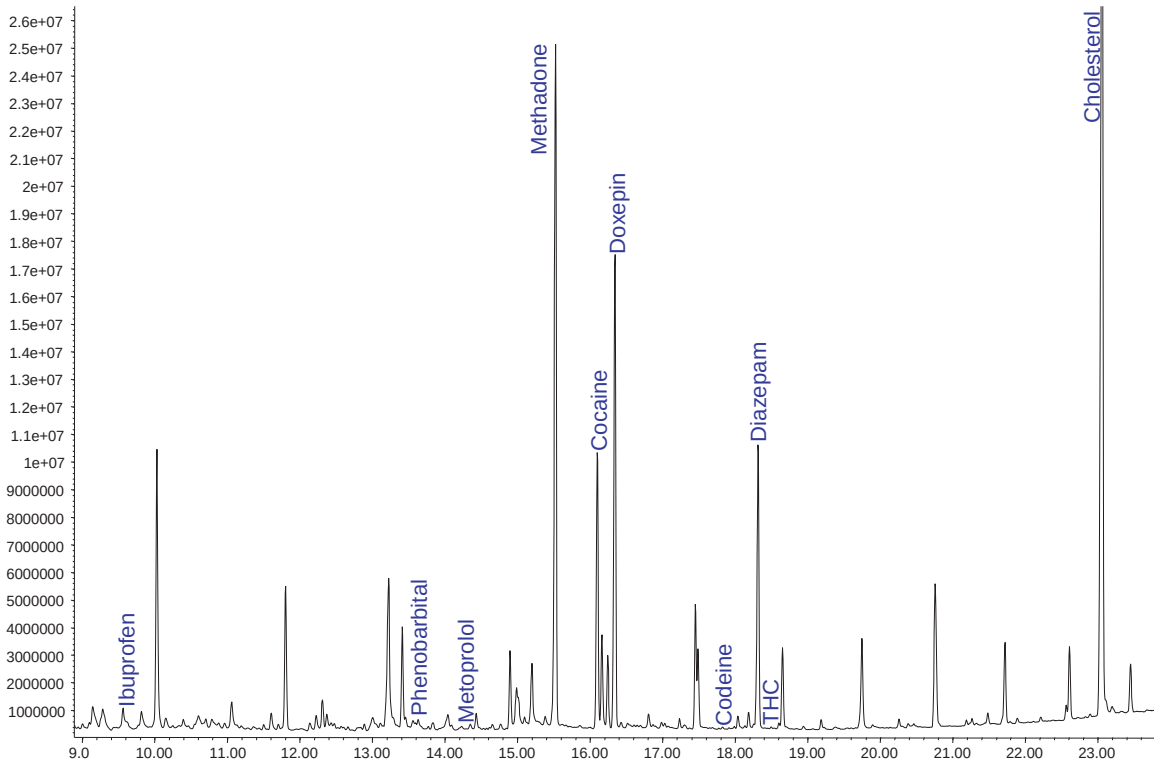

Figure 2. Chromatogram of positive control (GTFCh mix) extracted with GERSTEL twister from bovine brain tissue. Labeled analytes were identified.

When screening a large number of samples for unknown compounds, the analyst needs an efficient way to search for non-target substances in MSD-chromatograms. A useful tool for the task is the Agilent Deconvolution Reporting Software (DRS) which can be started from the Agilent MSD ChemStation.

DRS performs a deconvolution of the chromatogram based on AMDIS Software (Automated Mass Spectral Deconvolution and Identification System) combined with library searches (> 700 substances) based on the deconvoluted spectra. Not only the mass spectrum of a compound, but also the retention time is used as identification criterion (figure 3). The retention time of each analyte from the library is adjusted to the retention time stored in the library using Retention Time Locking (RTL) prior to analysis. For this purpose, one locking compound needs to be determined at different carrier gas pressures. From these results, the "correct" pressure for the actual column is determined in order to determine the exact retention times for all compounds in the library. For each spectrum found in the AMDIS deconvolution process, the matching compound is searched in the NIST08 library.

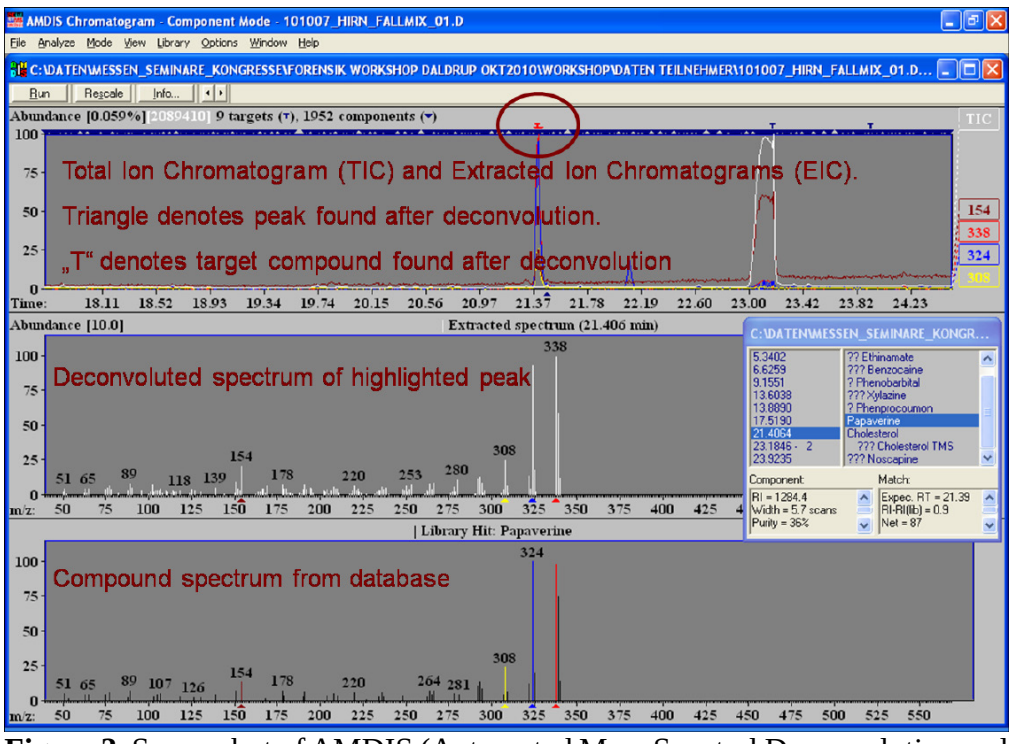

Figure 3. Screenshot of AMDIS (Automated Mass Spectral Deconvolution and Identification System) software.

Furthermore all known substances can be searched in the chromatogram in the MSD ChemStation via a target library that is based on the substances in the AMDIS database.

Following the different library searches, the results are automatically summarized in a report. After a diligent check of the report, the final analysis results can be determined.

The described method was used during a GTFCh workshop held in Düsseldorf, Germany, in 2010. Clomethiazole, phenprocoumon, papaverine and noscapine were found and identified in unknown brain and liver samples (figure 5, 7). Also xylazine was found in the brain sample, probably an artifact since it used as a sedative, for example, to help calm cattle during transport. Therefore it is probably found directly in the bovine brain sample and is not one of the spiked unknowns that should be found in the samples.

The extracts appear to be quite clean. In the chromatograms of brain samples only a very low fatty acid background is seen. (figure 4). Even the liver samples, which have high fat content, yield acceptable full scan chromatograms (figure 6).

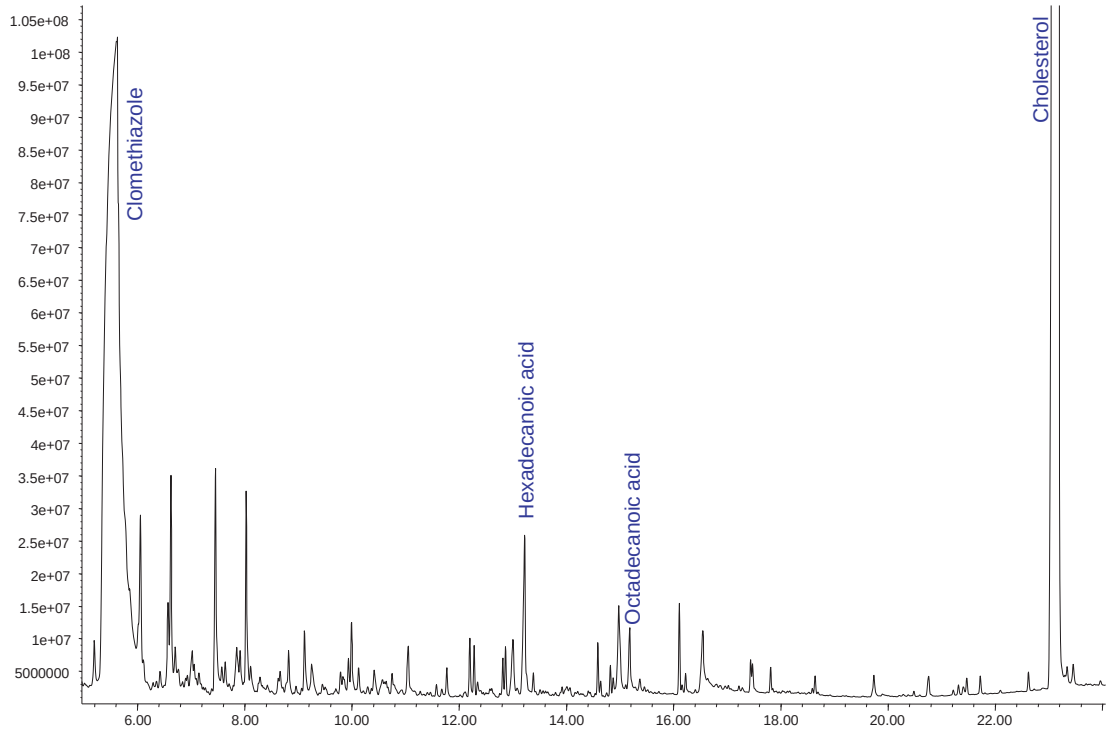

Figure 4. Chromatogram of a Twister extract of a brain sample, full scan.

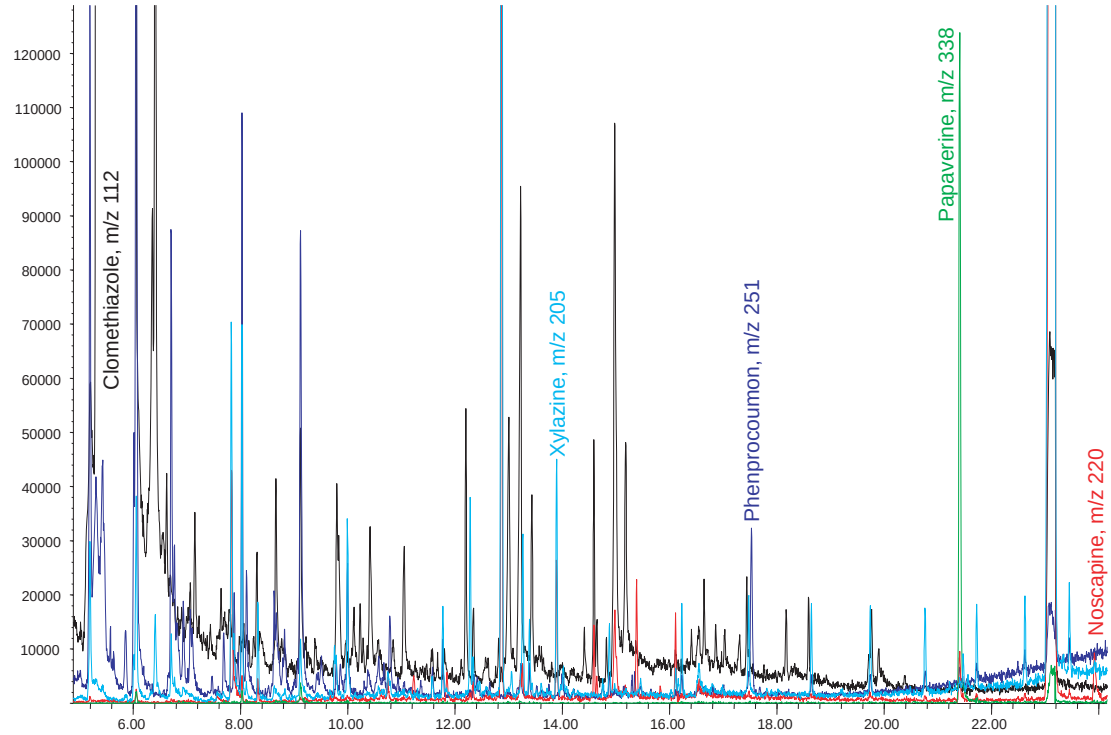

Figure 5. Chromatogram of a Twister extract of a brain sample, extracted ion chromatograms.

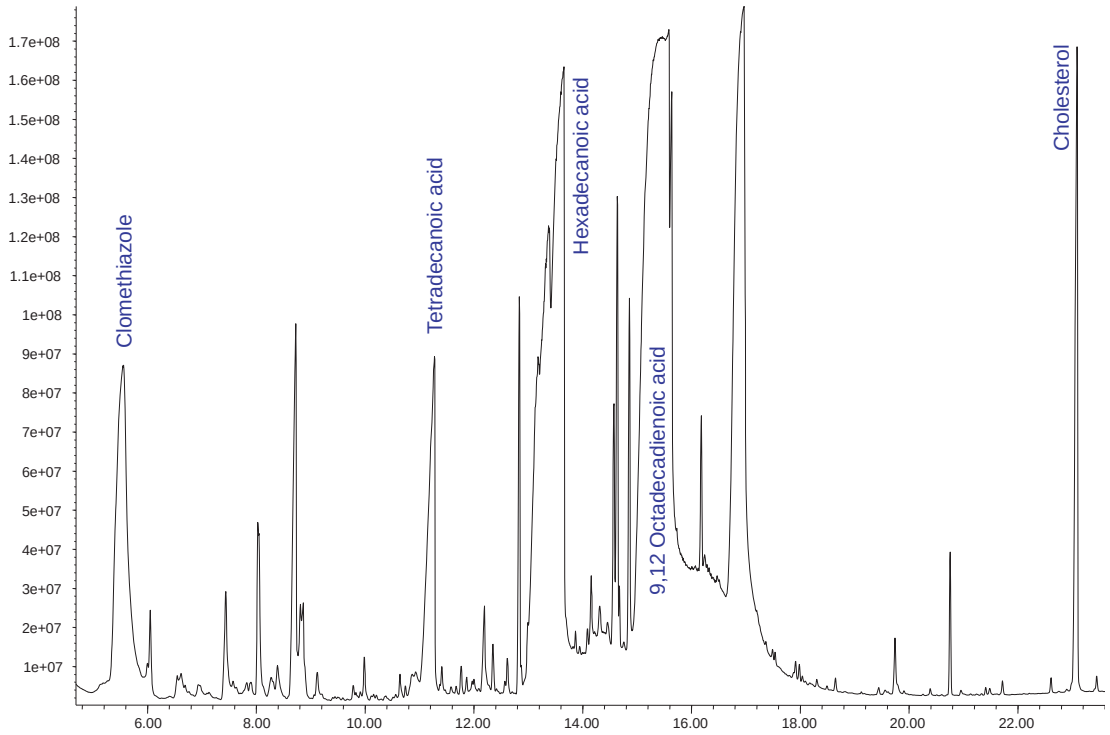

Figure 6. Chromatogram of a Twister extract of a liver sample, full scan.

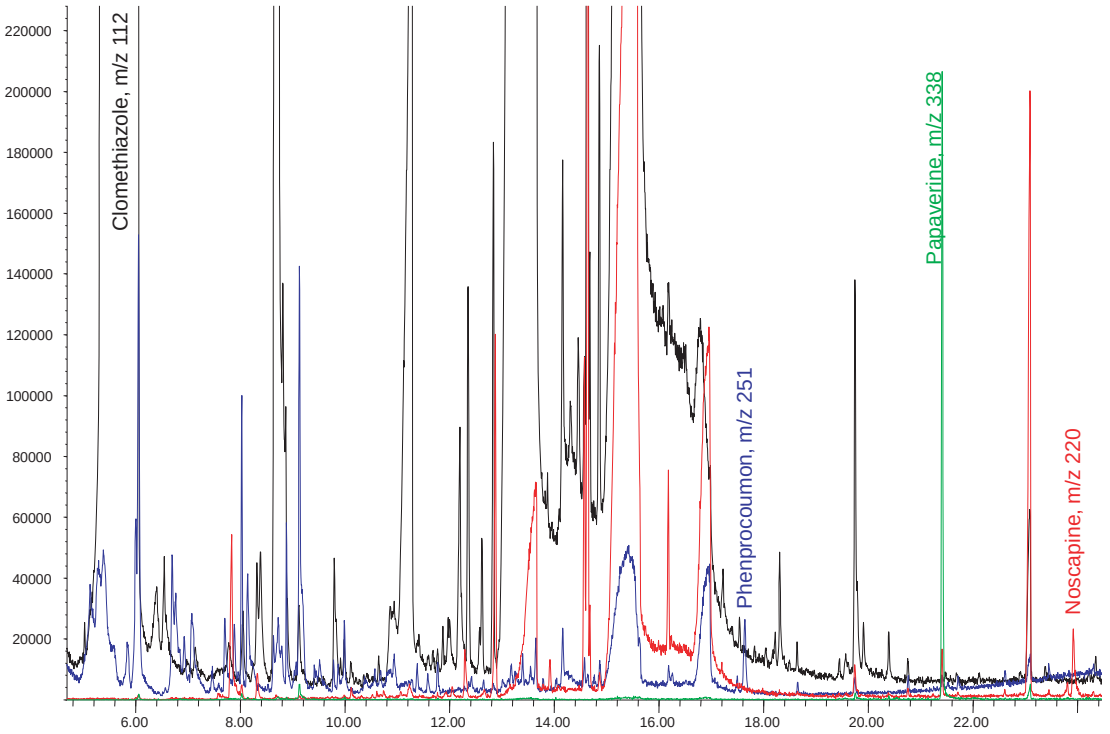

Figure 7. Chromatogram of a Twister extract of a liver sample, extracted ion chromatograms.

The analysis provides good repeatability as can be seen by comparing the two repeat analyses of spiked brain tissue shown in figure 8.

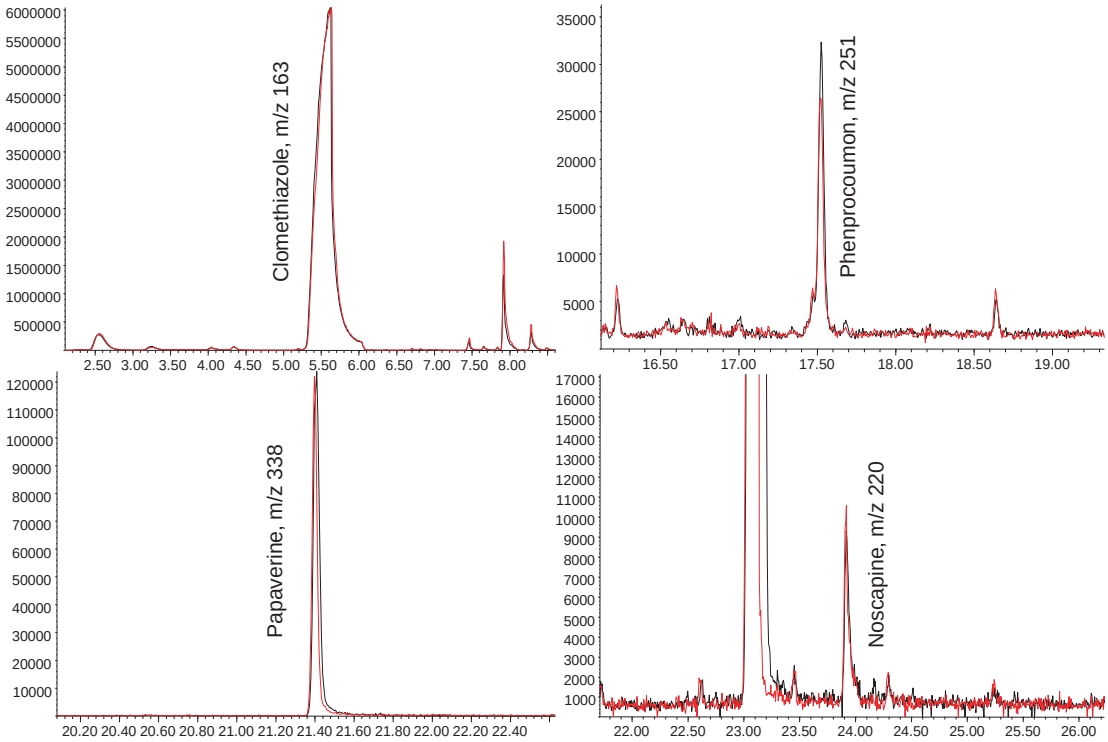

Figure 8. Two separate extractions of drugs from brain tissue using the GERSTEL-Twister® showing good repeatability.

Conclusions

Stir bar sorptive extraction (SBSE) using the GERSTEL Twister is a powerful tool for screening tissue for drugs of abuse and pharmaceuticals. Almost no sample preparation is needed and thermal desorption of the Twisters is performed automatically by the MPS autosampler. Screening for unknowns can be facilitated using Agilent Retention Time Locking (RTL) combined with the Deconvolution Reporting Software (DRS) and the AMDIS software.

The chromatograms are surprisingly clean considering the very complex sample matrix. The method shows good repeatability which means that quantification should be possible.

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References

[1] P. Sandra, E. Baltussen, F. David, A. Hoffmann: ”A Novel Extraction Technique for Aqueous Samples: Stir Bar Sorptive Extraction”, GERSTEL AppNote 1/2000

[2] J. Crifasi, M. Bruder, C. Long, K.J. Janssen: “Performance Evaluation of Thermal Desorption System (TDS) for Detection of Basic Drugs in Forensic Samples by GC–MS”, J. Anal. Tox. 30 (2006), 1-12

[3] A. Vendenin, V. Sovorkin, E. Kachur: ”Stir Bar Sorptive Extraction-Thermal Desorption-Capillary GC-MS applied for Analysis of Amphetamine Derivatives in Biological Fluids”, GERSTEL AppNote 7/2004

[4] E. Pfannkoch, J. Whitecavage, R. Bramlett: “Feasibility of Extraction and Quantitation of Δ9-Tetrahydrocannabinol in Body Fluids by Stir Bar Sorptive Extraction (SBSE) and GC/MS“, GERSTEL AppNote 6/2005

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