



Drug Screen Suite – Confidently and Rapidly Screen for Unknown Drugs: Post-Mortem Casework

Abstract

The Drug Screen Suite was evaluated for its performance in forensic toxicology applications involving complex, matrix-rich post-mortem samples. Designed as an easy-to-use and highly automated solution, the system integrates retention time, exact mass, and high-resolution MS/MS spectra to enable reliable identification of toxicologically relevant

compounds. Comparative analysis with conventional screening and quantitation methods demonstrated that the Drug Screen Suite maintains excellent robustness, sensitivity, and specificity even under challenging biological matrix conditions. The following example highlights its suitability as a routine tool for efficient and accurate forensic drug screening.

Keywords:

Forensics; toxicology; drugs of abuse; screening; automated reports; spectral library search

Introduction

Growing analytical demands in forensic toxicology

Forensic analytics is facing ever-increasing demands driven by the rapid evolution of the drug landscape and the growing complexity of biological samples. The emergence of new psychoactive substances (NPS) continually expands the range of target compounds, requiring analytical methods with higher selectivity and sensitivity to ensure accurate detection while minimizing sample preparation. At the same time, laboratories are under pressure to deliver faster results to improve response speed and increase throughput in routine casework.

Performance evaluation of automated toxicological screening

In this context, the present study aimed to evaluate the analytical performance of toxicological screening methods under conditions of high biological matrix load. It reports the analysis of samples obtained from a driver fatally injured in a traffic accident, including urine, femoral blood and vitreous humor. These post-mortem casework samples were initially screened using immunochemical assays and the Toxtyper® system, followed by quantitation of relevant compounds with LC-MS/MS. These results were compared to analyses performed using

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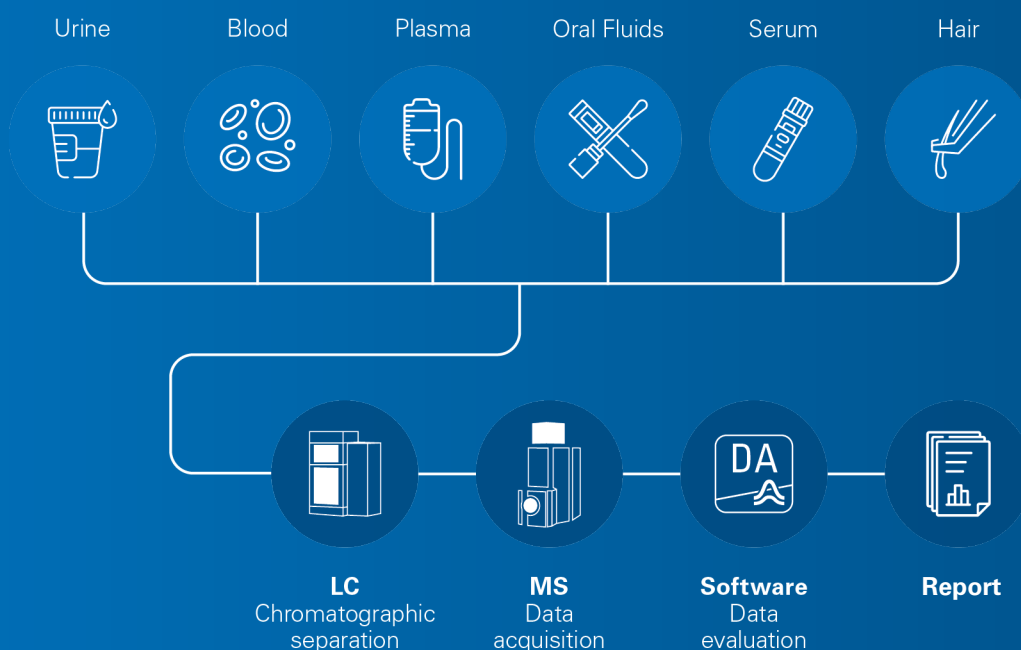
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the **Drug Screen Suite**: an easy-to-use solution offering a high degree of automation in data handling.

By using retention time, exact mass, and high-resolution MS/MS spectra, the Drug Screen Suite enables reliable identification of toxicologically relevant substances in

complex post-mortem matrices with ready-to-use methods. The findings of the study demonstrate that the Drug Screen Suite maintains robustness, sensitivity, and specificity even under challenging matrix-rich conditions, underscoring its suitability for routine forensic toxicology applications.

Workflow of the Drug Screen Suite



Experimental

Sample preparation

Femoral blood: Blood samples were extracted using protein precipitation and/or liquid-liquid extraction, respectively. 0.2 mL blood was precipitated with 0.5 mL ice-cold acetonitrile. For liquid-liquid extraction, 1 mL blood was adjusted to pH 9 using 0.5 mL borate buffer and extracted with 1.5 mL chlorobutane.

Vitreous humor: Samples (1 mL) were extracted using a two-step solid-phase extraction (Biotage Isolute HXC 100 mg/3 mL). Analytes were eluted after washing steps with 3 mL hexane/ethyl acetate (75/25) and 3 mL ethyl acetate/ammonia (98/2), respectively, and both eluates were combined.

Urine: 100 μ L of urine was extracted using 500 μ L cold acetonitrile.

For all extraction methods, the organic phase was transferred to an LC vial, evaporated to dryness under nitrogen and the residue reconstituted in 25 μ L mobile phase.

Instrumentation and software

The Drug Screen Suite was used for the analysis of the samples. It consists of an Elute⁺ UHPLC system, a compact QTOF instrument and DataAnalysis as software (all Bruker). Method parameters are summarized in Table 1. Samples were analyzed in data-dependent acquisition (DDA) mode using auto MS/MS. For data evaluation also the Maurer, Meyer, Helfer, Weber: LC-HR-MS/MS Library of Drugs, Poisons, and Their Metabolites was used.

Spectral verification and identification criteria

During post-processing, automated spectral matching is performed. The MS/MS precursor masses and fragment ions are verified requiring a mass accuracy of ± 5 mDa. A purity score is calculated, taking mass accuracy into account. In addition, retention time matching is assessed. The combined evaluation of all these criteria results in an overall Effective Purity Score, which must exceed 600 for a valid identification. The workflow is shown in Figure 1.

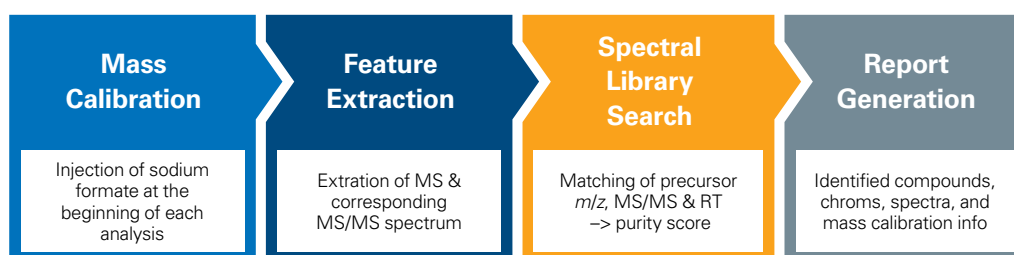


Figure 1. Automatic data processing workflow of the Drug Screen Suite.

Table 1. Method parameters

Elute ⁺ SL UHPLC	
Column	Bruker Intensity Solo 1.8 C18-2 2.1x100 mm
Column oven temperature	40°C
Injection volume	2 μ L
Mobile phase	TargetScreener LC method
Gradient	TargetScreener LC gradient
compact QTOF Mass Spectrometer	
Mass range	m/z 20 - 1300
Polarity	Positive
Scan modes	Classification: MS full scan Identification: Auto MS/MS

Results and Discussion

Analysis of different matrices

In this study, a case sample from a driver who was fatally injured in a traffic accident was analyzed. Routine testing was performed using immunochemical assays and the Toxtyper system, and the results were compared with those obtained from the Drug Screen Suite. All substances detected in femoral blood, vitreous humor, and urine were identified by both the routine methods and the Drug Screen Suite. The only compounds not detected by the Drug Screen Suite were

ethyl glucuronide (EtG) and ethyl sulfate (EtS). This is expected, as these analytes are normally measured in negative ionization mode. Results are shown in Figure 2. The analysis of a post-mortem case without toxicological findings did not lead to any findings. Despite the high matrix load of post-mortem samples, no false positive results were obtained in any of the three matrices examined, demonstrating the high analytical selectivity of this approach.

Femoral Blood (A)



Substance	Routine Analysis	Drug Screen Suite
Amlodipine	60 µg/L	✓
Fentanyl	< 1.0 µg/L	✓
Ketamin	220 µg/L	✓
Metoprolol	230 µg/L	✓
Midazolam	16 µg/L	✓
Paroxetine	200 µg/L	✓
Ramipril	1,1 µg/L	✓
Ephedrine	350 µg/L	✓
Norephedrine	21 µg/L	✓
Tilidine	110 µg/L	✓
Nortilidine	160 µg/L	✓
Naloxone	detected	✓
4-Acetylaminoantipyrine	detected	✓
4-Formylaminoantipyrine	detected	✓
4-Methylaminoantipyrine	detected	✓
Aminoantipyrine	detected	✓
Ethyl glucuronide*	detected	✗
Ethyl sulfate*	detected	✗
Ondansetron	detected	✓
Torasemide	detected	✓

Vitreous Humor (A)



Substance	Drug Screen Suite
4-Acetylaminoantipyrine	✓
4-Formylaminoantipyrine	✓
4-Methylaminoantipyrine	✓
Aminoantipyrine	✓
Amlodipine	✓
Clopidogrel	✓
Ephedrine	✓
Ketamine	✓
Metoprolol	✓
Midazolam	✓
Naloxone	✓
Nortilidine	✓
Ondansetron	✓
Paroxetine	✓
Tilidine	✓

Urine (A)



Substance	Drug Screen Suite
4-Acetylaminoantipyrine	✓
4-Formylaminoantipyrine	✓
4-Methylaminoantipyrine	✓
Aminoantipyrine	✓
Amlodipine	✓
Clopidogrel	✓
Metoprolol	✓
Naloxone glucuronide	✓
Nortilidine	✓
Paroxetine	✓
Ramipril	✓
Tilidine	✓
Torasemide	✓

Figure 2. Comparison of the analysis of femoral blood, vitreous humor and urine with routine analysis vs. Drug Screen Suite.

*Typically observed in negative ionization mode and not detected in positive ionization mode used in these analyses.

Automated reporting

Figure 3 shows an example of a report of the Drug Screen Suite. The report includes a comprehensive list of identified compounds. With the DataAnalysis software, users can review library search results with a single click, enabling fast and interactive evaluation

through chromatogram and MSⁿ spectra views. Identified compounds can be easily sorted by parameters such as signal intensity, retention time, or alphabetical order. Reports can then be saved as PDFs for convenient sharing via email.

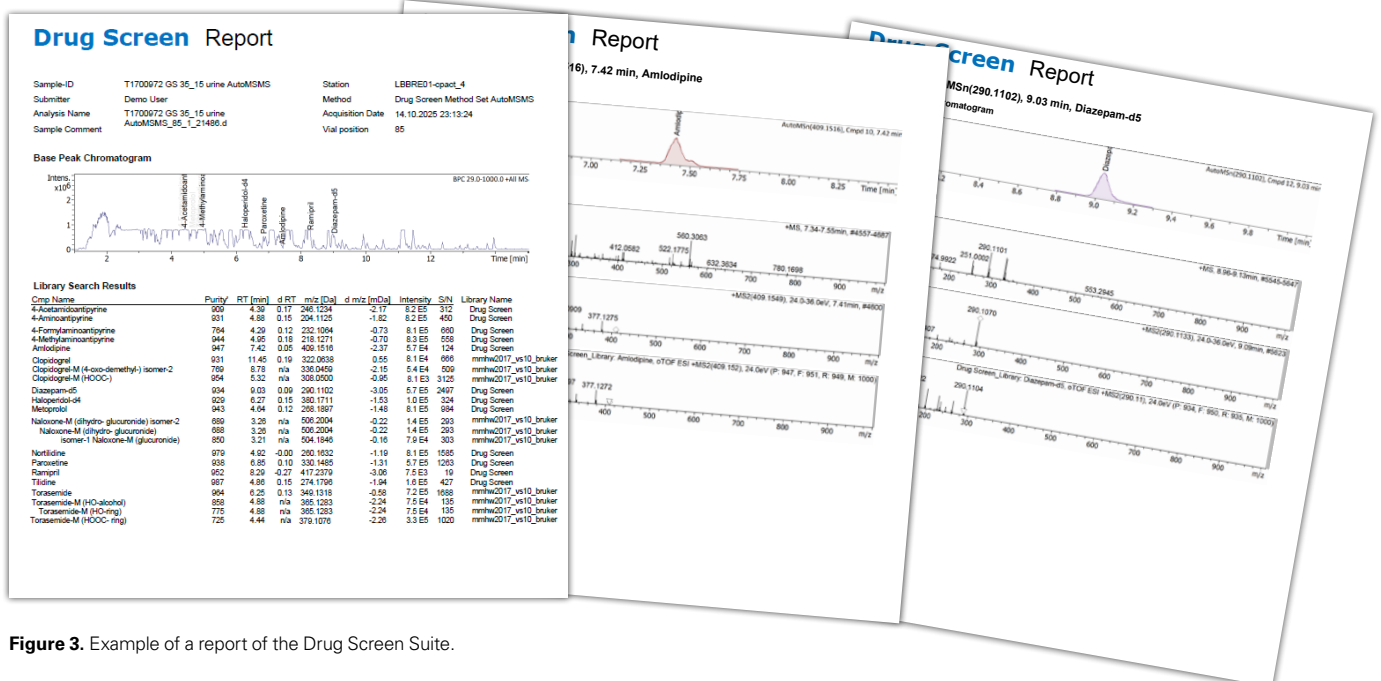


Figure 3. Example of a report of the Drug Screen Suite.

Open library concept

A key feature of the Drug Screen Suite is its open library concept, which enables users to quickly and easily add unknown compounds to their database. This allows for efficient storage and comparison of future samples, helping forensic analysts stay current with emerging new psychoactive substances (NPS). New substances can be added by

extending the spectral libraries, without the need for additional method optimization as the Drug Screen Suite already includes a specialized method for the addition of new compounds (Figure 4). A compound standard should be measured to obtain a pure spectrum and to ensure the identity of the substance being added.

Only a few steps are necessary to add a new compound to the library:

1. Selection of MS and MS/MS spectra
2. Add to library
3. Add compound name and additional information
4. Library ready for use

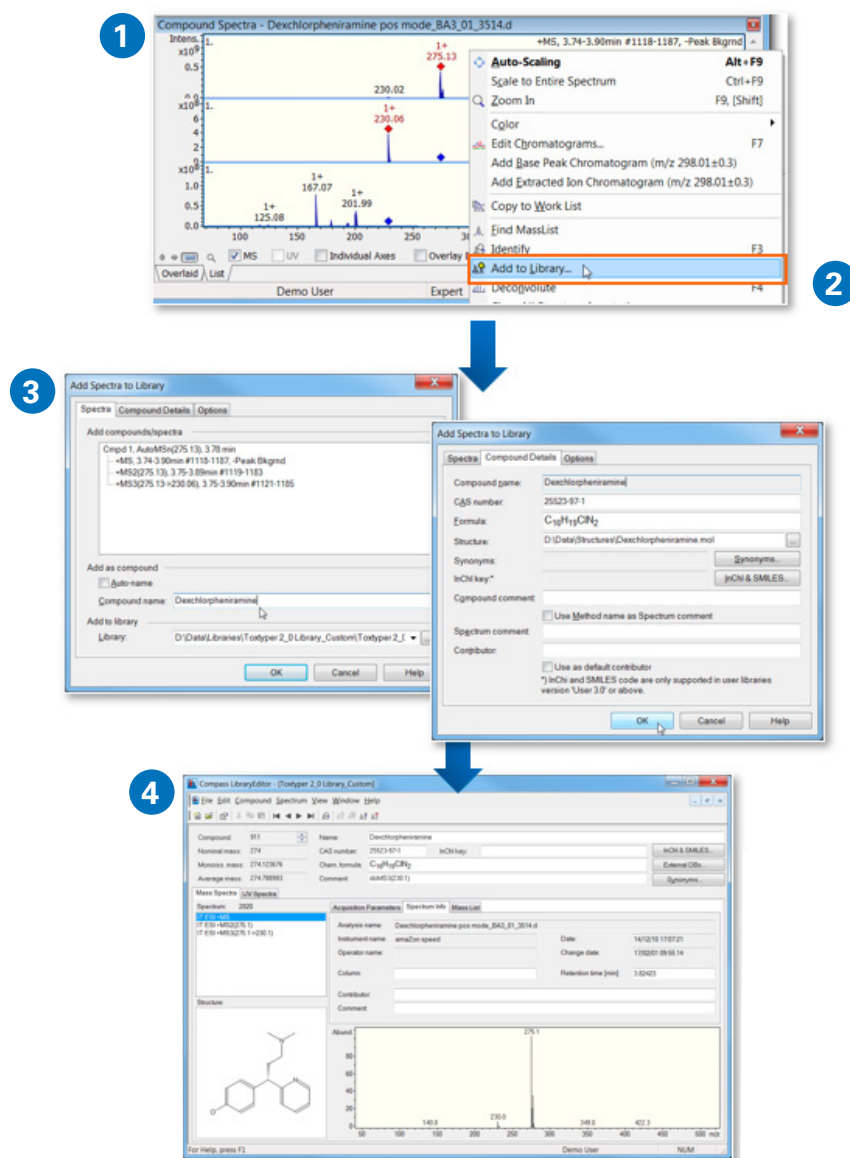


Figure 4. Addition of a new compound to the library.

Compatibility with national and international forensic libraries

The Drug Screen Suite enables seamless comparison of samples against a network of national and international forensic databases to confirm the presence of drugs and other medications. It integrates with leading forensic libraries, including:

- **Bruker Drug Screen Library**, featuring retention times for reliable compound identification, containing 390 substances
- **Maurer/Meyer/Helfer/Weber (MMHW) LC-HR-MS/MS Library**, containing 2,000 substances and 3,000 metabolites
- **NIST/EPA/NIH Mass Spectral Library 2023**, offering approximately 4,900 drug and forensic compounds and 9,700 metabolites

Access to these comprehensive libraries allows forensic, clinical research, and routine laboratories to rapidly and accurately identify drugs, poisons, and their metabolites. This enhances metabolite-based mass spectrometry screening and reduces the risk of false negatives. The public libraries are continually updated through contributions from customers and collaboration partners.

Conclusion

The Drug Screen Suite provides a ready-to-use solution for the rapid detection and identification of compounds in forensic toxicology. Its high sensitivity enables the identification of a wide range of active substances, even in challenging forensic work case samples.

Even in complex matrices, the use of high mass accuracy (± 5 mDa) ensures an

exceptionally low rate of false positives. The open library concept supports continuous and timely method updates, helping laboratories stay current with emerging substances.

Additionally, automated data processing and reporting significantly reduce turnaround times and simplify the integration of HRMS into routine workflows.

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