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Application

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Application Note 507†

Enhanced identification of drugs of abuse in urine using GC–TOF MS

Summary

In this Application Note, we demonstrate the benefits of BenchTOF time-of-flight (TOF) mass spectrometers and associated post-run software for the analysis of drugs of abuse in urine using fast GC.

Introduction

When screening urine for drugs of abuse (DOAs), reliability is crucial. Detection of compounds such as marijuana, cocaine, heroin and amphetamines, as well as their metabolites, is commonly carried out by GC–MS; however, the complexity of a sample such as urine can present an analytical challenge. High matrix effects and frequent co-elution can significantly compromise reliability of identification, particularly for trace-level DOAs at trace levels.

The quadrupole MS systems traditionally used for GC are significantly restricted in terms of sensitivity when used for screening (*i.e.* in scan mode). Spectra obtained are commonly affected by the phenomenon of spectral skew, resulting in false-positive or false-negative results. Increased sensitivity may be obtained with selected ion monitoring (SIM) mode, but whole-sample screening is not viable, as the number of compounds identifiable is very limited. Additionally, the identification of certain polar DOAs using GC–MS has historically necessitated derivatisation.

It is these limitations that have driven the need for a screening technique that offers high sensitivity and spectral quality for all compounds, including those at trace levels, regardless of sample complexity.

Time-of-flight technology for GC

Recently, attention has been drawn to time-of-flight (TOF) mass spectrometers as a means of screening complex mixtures. New systems can acquire data over a very wide mass range at very high scan rates. These instruments are achieving popularity due to their well-documented high levels of sensitivity.

However, a significant drawback of TOF systems has been that they generate spectra that do not resemble quadrupole-acquired spectra. This means that existing in-house or commercially available libraries (such as NIST) cannot be used for compound identification. This necessitates the creation of bespoke, TOF-specific spectral libraries, which is laborious.

Background to BenchTOF instruments

Markes' BenchTOF™ instruments are bench-top time-of-flight mass spectrometers that address the shortcomings of existing TOF systems, making them ideal for the rapid detection of compounds in complex samples such as urine.

The following three features are key to the success of BenchTOF in this particular application:

- **Sensitivity:** Highly efficient direct-extraction technology allows BenchTOF instruments to acquire full-range spectra with SIM-like sensitivity, allowing them to reliably detect trace-level analytes in a single run, which would be difficult or impossible on a quadrupole system.
- **Spectral quality:** The 'reference-quality' spectra produced by BenchTOF are a close match for those in commercial libraries such as NIST or Wiley. This enables quick and confident matching of both targets and unknowns.
- **Speed:** The ability to record full-range mass spectral information to extremely high densities (10,000 transient spectral accumulations per second) enables BenchTOF to handle the narrowest peaks encountered in well-optimised fast GC. The high stored-to-disk data rate also enables advanced spectral deconvolution and 'data-mining' algorithms to extract maximum information from weak, matrix-masked signals.

In addition, associated software incorporates baseline compensation, peak deconvolution and data-mining, which enhance the automated identification of target compounds in very complex datasets such as these.

† Formerly ALMSCO Application Note 007.

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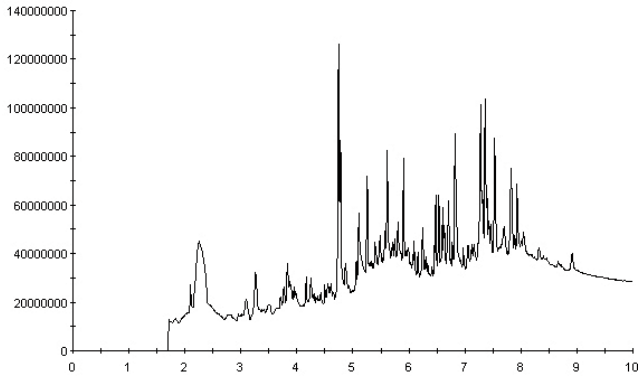

Figure 1: Unprocessed GC–MS data from the urine sample.

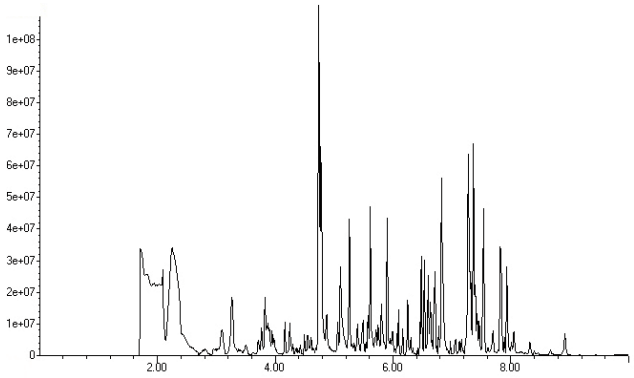

Figure 2: Baseline-compensated GC–MS data from the urine sample.

These features make BenchTOF instruments ideal for the routine screening of the chemical profiles of complex samples. In this application, a urine sample was analysed for the presence of a number of DOAs using fast GC, simultaneously demonstrating the high sensitivity and spectral quality of BenchTOF instruments, as well as their ability to handle narrow peaks produced by the GC method.

Experimental

A urine sample was collected from a participant in a methadone substitution programme. Glucuronide separation was followed by sorptive extraction using SPEC DAU cation exchangers to extract the organic compounds from the urine. Sorptive extraction is a highly selective and sensitive technique for sampling volatile compounds such as DOAs from aqueous matrices.

The underivatised sample was evaporated and concentrated in MeOH (50 µL). A 1 µL aliquot was used for GC–TOF MS analysis under the following conditions:

GC:

Column:	Optima 5 Accent, 10 m × 0.2 mm × 0.35 µm
Transfer line:	50 °C
Run time:	10 min

MS:

Mass range:	m/z 35–635
Scan rate:	2 Hz with 5000 spectra per data point
Ion source:	260 °C

A target library of the compounds of interest was created in the BenchTOF software package. The GC–MS data for the urine sample was then imported, and processed to automatically identify targets.

Results and discussion

Processing using TargetView

The total ion chromatogram (TIC) obtained for the urine sample is shown in Figure 1. As expected from the nature of the sample, this is affected by high levels of background interference. To deal with this, a baseline-compensation algorithm was applied to remove static or slowly-changing ion signals (such as solvents or column bleed), flattening the baseline and giving 'cleaner' spectra (Figure 2).

To address analyte co-elution, the peak spectra were then deconvolved. The speed of data acquisition by BenchTOF instruments allows many single scans to be summed in a single scanset. This provided high signal-to-noise ratios, and spectra unaffected by spectral skew, aiding effective deconvolution.

The next step was to compare the mass spectra chemometrically, again within the BenchTOF software package. The ability of BenchTOF instruments to produce 'classical' spectra allowed commercial spectra to be used to create the target library, increasing confidence in any matches detected. A derived match coefficient indicates the quality of the match, and the results are output in a report (Table 1).

Compound name	Retention time (min)	Match factor	Peak sum
Amphetamine	2.257	0.664	625 697 019
Methylecgonine (a cocaine metabolite)	3.869	0.890	11 188 961
Codeine	7.064	0.511	306 946
Diazepam	7.191	0.772	2 162 616
Methadone	6.249	0.825	37 260 860
2-Ethylidene-1,5-dimethyl-3,3-diphenyl-pyrrolidine (EDDP, a methadone metabolite)	5.905	0.899	78 941 200
Morphine	7.255	0.677	7 900 944
Nicotine	3.261	0.870	81 285 680
Cotinine (a nicotine metabolite)	4.742	0.624	115 129 070
Nordiazepam	7.429	0.895	24 555 590
Oxycodone	7.400	0.602	6 286 203
Temazepam	7.466	0.697	2 499 606

Table 1: Report listing the drugs of abuse found in the urine sample.

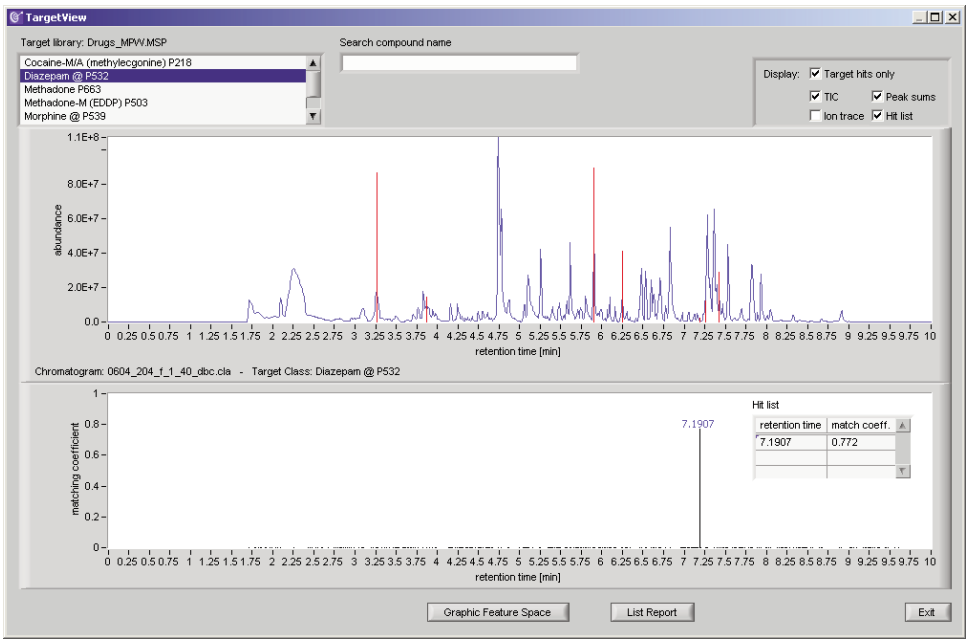

Figure 3: Software interface for analysis of the urine sample. The upper window shows the baseline-compensated TIC, with target drugs marked by the red bars. The lower window shows the target match for diazepam, with a match coefficient of 0.772.

The baseline-compensated chromatogram was then interrogated for compounds in the DOA library. In Figure 3, detected target compounds are indicated by red bars, with their heights being proportional to the peak sum.

High sensitivity and spectral quality

A number of compounds that would have been difficult or impossible to identify from quadrupole data or by using traditional compound identification software were identified in the sample. Of particular note are the benzodiazepines, which have been historically difficult to detect by GC–MS due to their polarity. However, the high sensitivity and non-skewed spectra provided by BenchTOF instruments, in combination with post-run software, has ensured the confident identification of this class of compounds in their underivatised form.

Identification of co-eluting compounds

To further illustrate the performance of the system, Figure 4 shows a saturated amisulpride peak which completely overlaps a small concentration of alprazolam. By limiting the mass range to m/z 100–400, alprazolam is positively identified, and shows a very good match to the library spectrum (Figure 5), with a correspondingly high match coefficient of 0.88. This is possible despite the large matrix interference.

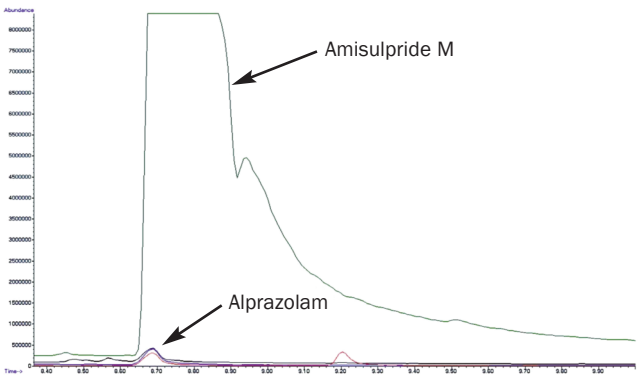

Figure 4: Overlaid chromatograms showing co-elution of amisulpride M and alprazolam.

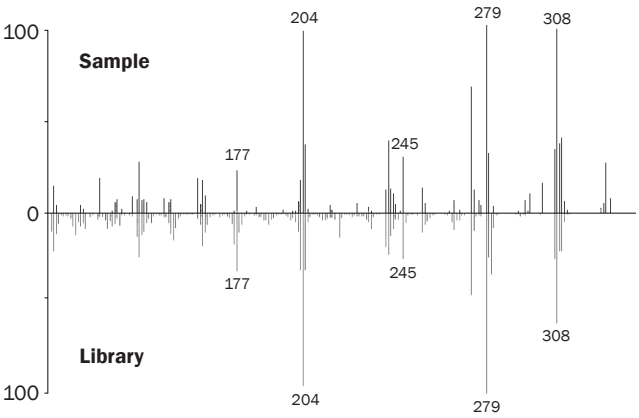

Figure 5: Comparison of sample and library spectra for alprazolam.

Conclusion

Sample complexity may pose a problem for many analytical detection systems, but this is not an issue for BenchTOF instruments, which can acquire high-quality mass spectra over a wide concentration range.

Even in the presence of a high matrix background, the performance of BenchTOF, in conjunction with post-run processing, allowed trace-level DOAs to be identified with a high degree of confidence.

In particular, the ability of BenchTOF instruments to deliver NIST-searchable spectra means that existing spectral libraries can be employed without modification.

Trademarks

Applications were performed under the stated analytical conditions. Operation under different conditions, or with incompatible sample matrices, may impact the performance shown.

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