# Fast Detection of Derivatized Drug Samples (Methyl Derivatives) in Spiked Horse Urine Samples Using GC-TOFMS

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Key Words: GC-TOFMS, Deconvolution, Metabolomics

## 1. Introduction

The horse racing industry in South Africa is large and spread over a considerable area, with race meetings occurring in many cities frequently two or more times per week. In an attempt to ensure that the industry is kept honest and is not subject to manipulation, numerous horse urine samples need to be analyzed for performance-enhancing substances. A centralized laboratory (the National Horse Racing Authority [NHRA] of Southern Africa Laboratory) investigates numerous samples per week.

Over the years many prohibited substances have been detected in horse urine. Among the most commonly found drugs are the non-steroidal anti-inflammatory drugs (NSAIDs): ibuprofen, diclofenac, vedaprofen, indomethacin, and eltenac; and the appetite-enhancer clanobutin which acts on the digestive system and improves the appetite. All of these drugs enhance the performance of the animal, and as such, are illegal. Analysis of these compounds can be undertaken by GC-MS provided the components are first derivatized, generally with methylation by using diazomethane.

We have analyzed these methylated drugs using GC-TOFMS, and have developed a fast GC method that permits rapid sample turnover with full-range mass spectra to provide conclusive evidence of compound identity. Fast GC times are particularly necessary as the compounds are unstable, and a rapid turnover permits more samples to be methylated at any given time.

## 2. Experimental Conditions

*Instrumentation*

LECO Pegasus® III GC-TOFMS

*GC Conditions*

Column:
10 m x 0.18 mm x 0.20 µm Rtx-5 (Restek)
Inlet:
Splitless at 200°C, purge time 60 seconds
Oven:
50°C for 1 minute, to 300°C at 50°C/minute, hold for 1.2 minutes
Transfer Line: 220°C
Injection Volume: 1 µl
Carrier Gas: Helium, 1.0 mL/minute constant flow
Total Analysis Time: 7.2 minutes

*TOFMS Conditions*

Source Temperature: 200°C
Acquisition Rate: 10 spectra/second
Stored Mass Range: 120 to 380 u

*Sample Information*

Samples were provided, as methyl derivatives, by the NHRA at four different concentration levels (high, medium, low, and very low). The drugs had been spiked into horse urine at the levels shown below, before the methylation process.

**Table 1. Concentrations (ng/ml) of the four derivatized drug samples**

| | High | Medium | Low | Very Low |
|---|---|---|---|---|
| **Ibuprofen** | 600 | 400 | 200 | 100 |
| **Diclofenac** | 300 | 150 | 75 | 37 |
| **Eltenac** | 600 | 400 | 200 | 100 |
| **Vedaprofen** | 400 | 200 | 100 | 50 |
| **Clanobutin** | 200 | 100 | 50 | 25 |
| **Indomethacin** | 200 | 100 | 50 | 25 |

## 3. Results and Discussion

TOFMS has the ability to acquire a wider spectral range (5 to 1000 u) than is shown in this application note; however the range was restricted due to the targeted nature of the analysis. This achieves two benefits. The first is that data file size is smaller. The second is that the abundant low m/z ions associated with the urine matrix are not considered in the automated peak find routine available in the ChromaTOF® software, which should lead to better deconvoluted mass spectra for the targeted compounds.

Two of the derivatized drug components were not present in the NIST Library (eltenac methyl derivative and vedaprofen methyl derivative). To be able to easily locate and identify these components, it was helpful to create a User Library. This is easily accomplished with LECO ChromaTOF software, and spectra recorded on the Pegasus can then be placed in this library, together with appropriate identification information (e.g. CAS#, molecular weight, formula, etc.), to allow searching for compounds not in the NIST library.

The advantage of this is that the recorded spectra are then obtained on the Pegasus, and not on an instrument using a different mass analyzer, which generally gives rise to better library matches. In this instance copies of spectra of the two compounds not in the NIST Library were obtained from the NHRA. The high derivatized drug sample was then run, the two compounds located in a Peak Table

generated using the Automated Peak Find and True Signal Deconvolution® capabilities of ChromaTOF, and the recorded spectra were inserted into the User Library. Subsequent identification of these compounds was achieved using the User Library.

The target derivatized drug samples were easily located and identified in all four samples. As an example, overlaid extracted ion chromatograms showing the ions at m/z 139, 155, 161, 214, and 220 for the very low level sample are shown in Figure 1.

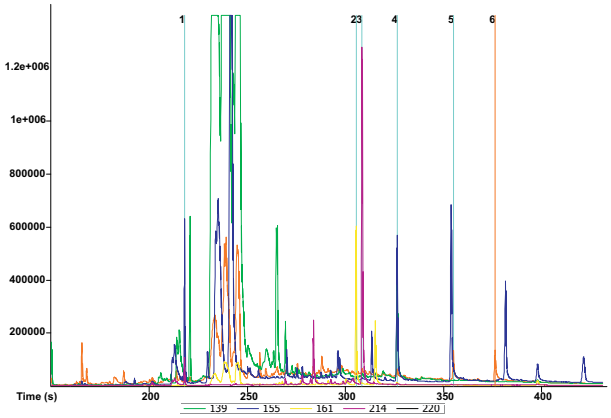


***Figure 1. Extracted ion chromatogram of the very low derivatized drug sample, expanded to permit easy visualization of the drug components.***

**Table 2. Edited peak table of the very low derivatized drug sample**

| Peak # | Name | R.T. (seconds) | Similarity |
|---|---|---|---|
| 2 | Ibuprofen methyl derivative | 218.0 | 877 |
| 189 | Diclofenac methyl derivative | 305.4 | 874 |
| 196 | Eltenac methyl derivative | 308.6 | 937 |
| 222 | Vedaprofen methyl derivative | 326.9 | 464 |
| 251 | Clanobutin methyl derivative | 356.4 | 799 |
| 269 | Indomethacin methyl derivative | 378.0 | 825 |

The edited peak table above was produced using a data processing method set up to find components with S/N ratios >100:1. Using this method, 269 components were located by the ChromaTOF software, including the six targeted drug components.

The chromatogram above shows that an adjacent compound heavily overlaps the clanobutin methyl derivative. This is more clearly demonstrated in the expanded chromatogram shown below. The clanobutin methyl derivative is a small peak lying under the tail of a much larger component. As a demonstration of spectral quality and the efficacy of the True Signal Deconvolution algorithm, the recorded spectrum after deconvolution for this component is shown below, together with the corresponding library spectrum, and the caliper spectrum, recorded at the same position as the clanobutin peak marker. The caliper spectrum has not been produced after deconvolution, but is an example of the "raw" spectrum at this point.

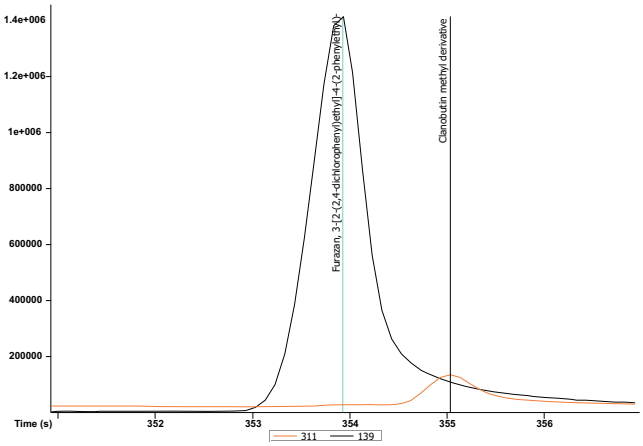


***Figure 2. Expanded chromatogram of the clanobutin methyl derivative in the very low derivatized drug sample.***

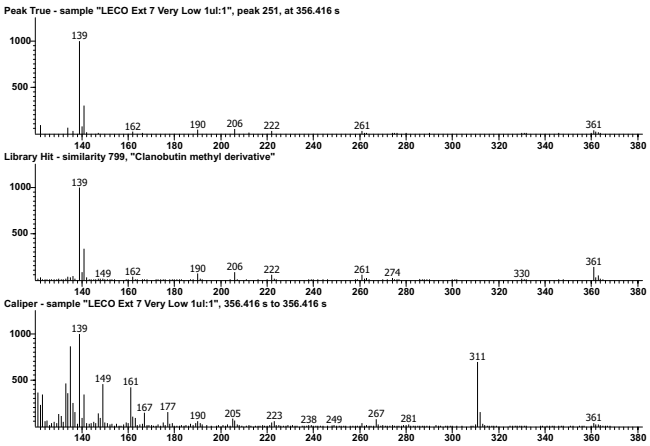


***Figure 3. Mass spectra showing the clanobutin methyl derivative spectrum after deconvolution, with the library spectrum below it, and the caliper spectrum at the bottom.***

Spectral quality is good, and a similarity of 79.9% is obtained, which along with retention time, confirms the identity of the component conclusively. Even in cases where the target compound appears as a small peak, overlapped by a much larger component, the True Signal Deconvolution algorithm produces high-quality results. It is interesting to note that if the above analysis was being undertaken using a quadrupole MS and selected ion monitoring (SIM), it is possible that the interferent might contain ions used in the SIM experiment to locate the target compound. This would disrupt the required ion ratios required for qualitative identification, and conclusive evidence of the compound identity could not then be obtained. However, the Pegasus and the True Signal Deconvolution algorithm allow unambiguous target compound identification.

*Approximate Limits of Detection*

Based on the concentrations given for the various derivatized drug samples, and using a S/N ratio of 3:1 as defining the limit of detection for the drug components, an approximate limit of detection for the derivatized drug components can be calculated. In all cases this is below 10 ng/ml (10 ppb).

*Quantification—Calibration Curves*

Although no standards of the derivatized drugs were available to assist in the preparation of calibration curves, the area results from the spiked urine samples and the concentrations of the derivatized drugs in these samples could be used to construct calibration curves. While such curves are obviously not as accurate as those using clean standards, the results obtained were nonetheless most satisfactory. Curves were constructed for the medium, low, and very low samples (the high sample was not included, as at this level with the high detector value used, some non-linearity was observed), with an example showing the calibration curve for the eltenac methyl derivative shown below.

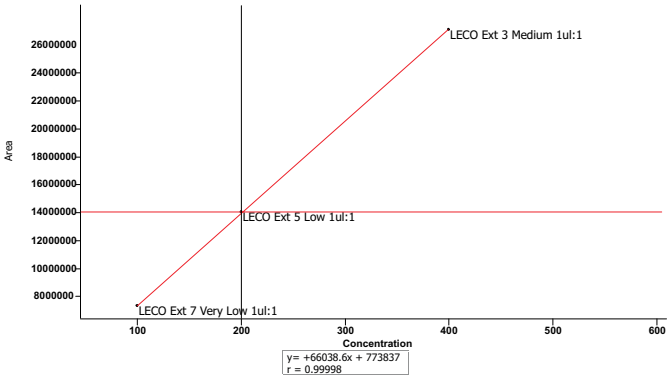


***Figure 4. Calibration curve for eltenac methyl derivative.***

As can be seen, linearity is excellent. With proper standard solutions, accurate calibration curves can easily be produced using this method, and the results used to quantify derivatized drug concentrations in horse urine samples.

## 4. Conclusion

Four horse urine samples containing different concentrations of methyl derivatives of common performance-enhancing drugs were obtained from the NHRA and analyzed by GC-TOFMS. The drug components were located in all samples, using a fast GC method with a total time of 7.2 minutes. Drugs not contained in the NIST library were entered into a User Library. A limit of detection <10 ppb was estimated for all the drug derivatives. Calibration curves were developed from the results of the spiked samples. These curves showed excellent linearity and demonstrated that the method could easily be used for the quantitation of these components in horse urine.

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Form No. 203-821-310 4/08-REV1