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# No Compromise! Improved Sensitivity for Negatively-Ionizing Substances

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*For forensic toxicology use only.*

This is an Application Brief and does not contain a detailed Experimental section.

## Abstract

This application brief demonstrates to assess the impact of alternate mobile phases on the sensitivity for a series of negatively ionizing substances.

### Benefits

Altered mobile phases provide improved sensitivity when using the Waters Forensic Toxicology Screening Application Solution with UNIFI in negative ionization mode.


## Introduction

In 2014, Waters released the first version of the Forensic Toxicology Screening Application Solution with UNIFI,[1] which comprised acquisition of $MS^E$ data using a time-of-flight mass spectrometer operated in electrospray positive ionization mode (ESI+). Data were subsequently compared with a comprehensive library containing more than 1,000 toxicologically-relevant substances.[2,3]

Since this time, on-going efforts have been underway to further improve the forensic solution by continuing to expand the library content, to include novel psychoactive substances and their metabolites, but also to include substances that may preferentially ionize in negative mode (ESI-), such as the barbiturates, cannabinoids, diuretics, and the non-steroidal anti-inflammatory drugs (NSAIDs).[4,5] For convenience, some screening approaches employ the same chromatographic conditions for both positive and negative ionization modes however, the impact of this approach should be evaluated, particularly with regards to the effect on sensitivity.

Consequently, the aim of this study was to compare the sensitivity obtained for a series of negatively-ionizing substances when analyzed using the mobile phases that are usually employed in ES+ mode (Method 1), with some alternative chromatographic conditions, that are based on a previously-reported method for barbiturates (Method 2).[6]

## Experimental

## Sample preparation

Individual standards were prepared at 1 mg/mL in methanol, then diluted in 10% acetonitrile in water for injection. The final concentrations ranged from 20 ng/mL to 2,500 ng/mL.

## LC conditions

| | |
|---|---|
| LC system: | ACQUITY UPLC I-Class (FTN) |
| Column: | ACQUITY UPLC HSS $C_{18}$, 2.1 x 150 mm, 1.8 µm |
| Vials: | Waters Maximum Recovery Vials |
| Column temp.: | 50 °C |
| Sample temp.: | 10 °C |
| Injection vol.: | 10 µL |
| Flow rate: | 0.4 mL/min |
| Mobile phase A method 1: | 5 mM ammonium formate pH 3.0 |
| Mobile phase A method 2: | Water containing 0.001% formic acid |
| Mobile phase B method 1: | Acetonitrile containing 0.1% formic acid |
| Mobile phase B method 2: | Acetonitrile containing 0.001% formic acid |
| Gradient: | Isocratic at 87% A for 0.5 min then to 5% A at 4.5 min, hold for 1 min before switching to 87% A |
| Run time: | 7.5 min |

$MS^E$ conditions

| | |
|---|---|
| MS system: | Xevo G2-S QTof |
| Ionization mode: | ESI- |
| Source temp.: | 150 °C |
| Desolvation temp.: | 400 °C |
| Desolvation gas: | 800 L/h |
| Reference mass: | Leucine enkephalin [M-H]- m/z = 554.2620 |
| Acquisition range: | m/z 50-1000 |
| Scan time: | 0.1 s |
| Capillary voltage: | 1.5 KV |
| Cone voltage: | 20 V |
| Collision energy: | Function 1: 6 eV<br>Function 2: Ramped 10 to 40 eV |

## Results and Discussion

Sixty-two compounds, including barbiturates, cannabinoids, diuretics, NSAIDs, and steroids, were analyzed in triplicate. The retention times for a selection of the compounds, under both sets of mobile phases evaluated, are listed in Table 1, together with the observed increase in 3-dimensional (3D) peak response and signal-to-noise ratio, with the alternative mobile phases.

Sixty of the sixty-two compounds evaluated showed an increase in 3D peak response when using the alternate mobile phases, with 75% of the compounds tested showing a greater than two-fold increase, and only two compounds showing a reduced 3D peak response. The greatest increase in 3D response was for THC, which is illustrated in Figure 1, and showed an increase of more than 50-fold alongside a dramatic increase in signal-to-noise ratio.

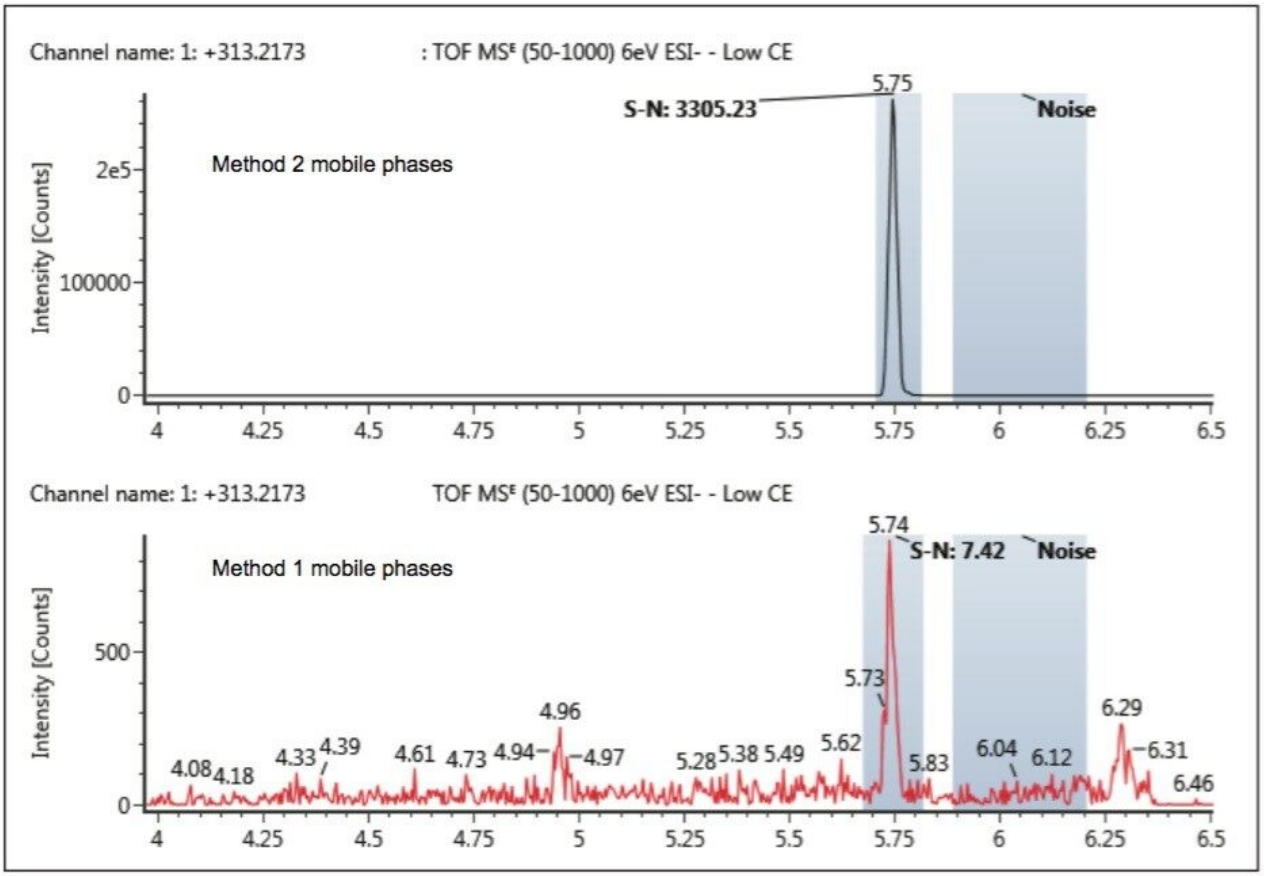


*Figure 1. Improvement in both 3D peak response and signal-to-noise ratio for 2500 ng/mL injection of THC standard when using the altered mobile phases (upper chromatogram) in comparison with the original mobile phases (lower chromatogram).*

A small number of compounds were only identified, at the concentrations investigated, when using the altered mobile phases. The signal-to-noise ratio comparison between the two methods, for a selection of compounds, is highlighted in Table 1, and complements the increase in 3D response. Only small differences in retention time were observed when switching between the two mobile phases.

| Analyte | Drug class | Injection conc. | Degree of improvement (method 2/1) | | Retention time (min) | |
|---|---|---|---|---|---|---|
| | | ng/mL | 3D peak response | Signal-to-noise ratio | Method 1 | Method 2 |
| Phenobarbital | barbiturate | 100 | 15 | 17 | 2.9 | 2.9 |
| Secobarbital | barbiturate | 100 | 11 | 11 | 3.5 | 3.5 |
| Carboxy-THC | cannabinoid | 20 | 3 | 3 | 5.0 | 5.0 |
| THC | cannabinoid | 2500 | 67 | 445 | 5.7 | 5.8 |
| Amiloride | diuretic | 250 | 3 | 3 | 1.1 | 1.0 |
| Furosemide | diuretic | 250 | 7 | 3 | 3.1 | 3.1 |
| Naproxen | NSAID | 400 | 23 | 20 | 3.8 | 3.8 |
| Ibuprofen | NSAID | 1000 | 52 | 73 | 4.4 | 4.5 |
| Hydrocortisone | steroid | 400 | 6 | 5 | 3.0 | 3.0 |
| Triamcinolone | steroid | 400 | 5 | 3 | 2.6 | 2.6 |

*Table 1. Improvement in 3D peak response and sensitivity for a selection of analytes, using the two sets of mobile phases along with their retention times.*

## Conclusion

While it is certainly possible to use the same mobile phases for screening analysis in both positive and negative mode, this simple study clearly demonstrates that these compromises in chromatographic conditions can influence analytical performance and sensitivity, and in some cases, this can be significant. In a toxicological screening this can yield false negative results, particularly for the cannabinoids which are the most-commonly encountered illicit drug substances. For this reason the expanded Waters Forensic Toxicology Screening Application Solution with UNIFI includes a fully-optimized chromatographic method for more efficient, more accurate toxicological screening of negatively-ionizing substances.

## References

1. Forensic Toxicology Screening Application Solution with UNIFI. Waters Brochure (P/N 720004830EN).

2. Wood M. The Utility of $MS^E$ for Toxicological Screening. Waters Tech Brief 2014 (P/N 720005198EN).

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4. Lee R and Wood M. Using the Waters Forensic Toxicology Screening Application Solution with UNIFI to determine diuretics in urine. Waters Application Note 2015 (P/N 720005391EN).

5. Lee R and Wood M. Screening for Cannabinoids using the Waters Forensic Toxicology Application Solution with UNIFI. Waters Application Note 2015 (P/N 720005413EN).

6. Lee R, et al. Quantitative Analysis of Barbiturates in Urine Using UPLC/MS/MS. Waters Application Note 2014 (P/N 720004466EN).

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ACQUITY UPLC I-Class PLUS System <https://www.waters.com/134613317>

Forensic Toxicology Screening Application Solution with UNIFI <https://www.waters.com/134779723>

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