# A Comparison of the Performance of LC-MS/MS Analytical Methods for Workplace Drugs of Abuse Testing Using Zero-Grade Air and Nitrogen Gas

**Martin Jacques, Melissa Beals, Michael Clark, Tobin Kocour, Michael Balcha, Bengi Oguz, Zayne Williams, David Kuntz; Clinical Reference Laboratory, Lenexa, KS 66215**

## INTRODUCTION

With the continual growth and addition of Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) instruments to our laboratory, addressing the increasing consumption of nitrogen gas became a necessity. In order to support round-the-clock operation of 37 Sciex mass spectrometers nearly seven days a week, our 6,000 gallon supply of nitrogen needed to be replenished every 5 days; any unforeseen delay in delivery could result in a potential laboratory shutdown. Use of zero-grade air for source exhaust and Gases 1 and 2, which Sciex recommends, would reduce laboratory nitrogen consumption approximately 70%, extending the service of the 6,000 gallon nitrogen supply to 17 days. Prior to conversion, it was necessary to evaluate the impact of zero-grade air on analytical methods that were validated using nitrogen gas.

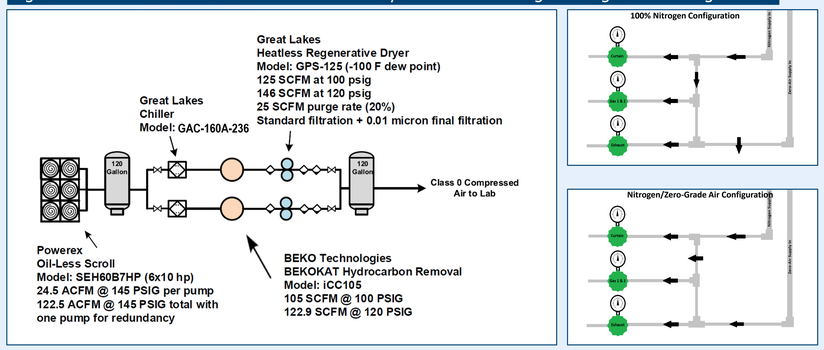
## OBJECTIVE

Determine any differences in ionization or performance for Sciex LC-MS/MS instrumentation while operating with zero-grade air compared to nitrogen for the source gas in the analysis of workplace drugs of abuse confirmation.

## METHOD

Abbreviated validation sample batches were prepared to evaluate the linearity and investigate potential interference for each assay. Linearity studies were comprised of standard replicates at concentrations equal to the lowest level of quantitation, cutoff calibrator, and upper limit of linearity for all analytes. Method interferences were examined using matrix-matched negative samples and samples formulated at 40% of cutoff concentration that were spiked with over-the-counter, prescription, and illicit drugs. Batches were initially analyzed using validated instrument methods with nitrogen gas for source exhaust and Gases 1 and 2. Gas lines were subsequently switched to zero-grade air and the samples were reanalyzed. All data was reviewed and results were compared for analyte and internal standard peak area counts and calculated concentration.

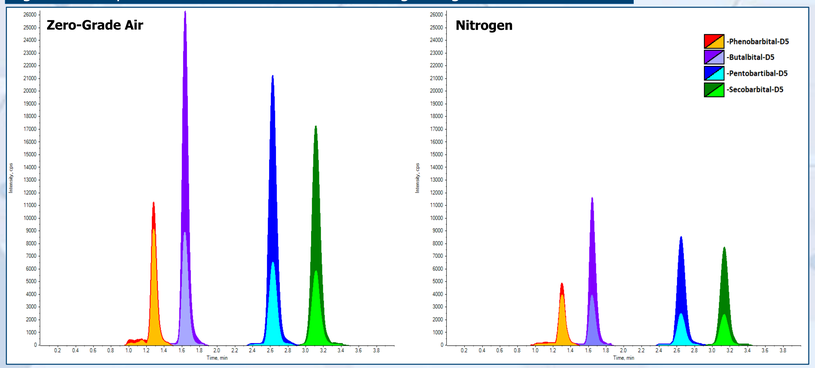
Figure A: Schematic of Zero-Grade Air Generator System and Switching Gas Regulators Configurations




## RESULTS / DISCUSSION

All methods exhibited acceptable performance operating with zero-grade air for source exhaust and Gas 1 and Gas 2. Compared to analysis conducted with the use of nitrogen gas, the only method demonstrating pronounced change was the urine barbiturate assay, which operates in negative mode using atmospheric pressure chemical ionization (APCI), and displayed a twofold increase in ionization. A few minor differences were observed with other methods. Using electrospray ionization (ESI), a 10% decrease in ionization was observed in both urine and oral fluid ethyl glucuronide analysis. The oral fluid buprenorphine assay presented a 7% decrease in buprenorphine ionization and a 10% increase in norbuprenorphine ionization. In the oral fluid benzodiazepine assay, there was a 20% increase in flurazepam ionization, and a 20% and 15% ionization decrease in alprazolam and triazolam, respectively. Out of the 83 validated methods for urine, sweat, oral fluid, and blood, only 4 assays showed any change in in ionization from nitrogen to zero-grade air.

Figure B: Comparison of Barbiturates Ionization using Nitrogen and Zero-Grade Air




CRL completed a thorough evaluation of the impact of using zero-grade air in place of nitrogen gas for LC-MS/MS source exhaust and Gases 1 and 2 on all workplace drug testing confirmation methods. The comparison of validation data generated while operating with zero-grade air and nitrogen gas revealed few significant differences; however, the potential cost savings and increased business autonomy created by a 70% reduction in nitrogen consumption was compelling.

Figure C: Comparison of Ethyl-β-D-glucuronide (EtG) Ionization via Nitrogen and Zero-Grade Air

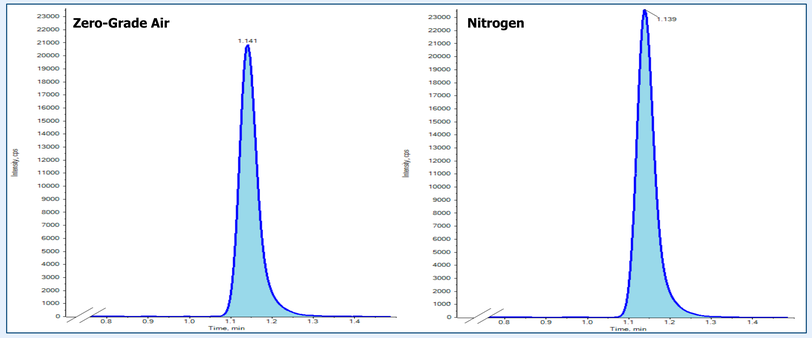



Figure D: Comparison of Benzodiazepines Ionization using Nitrogen and Zero-Grade Air

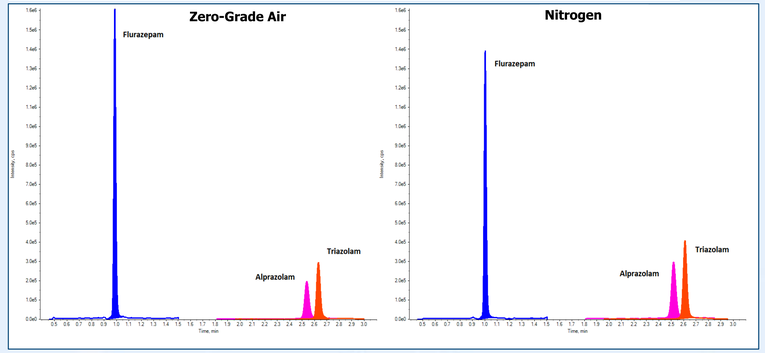
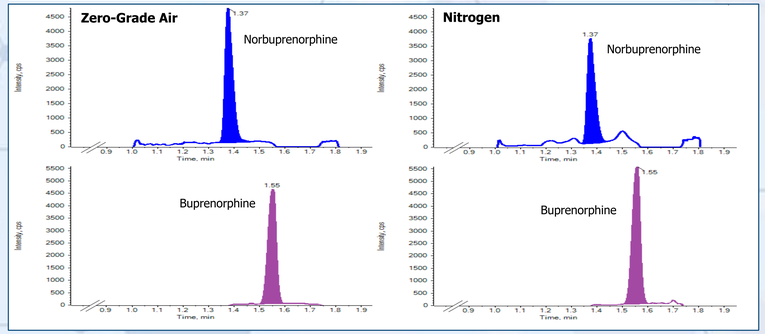



Figure E: Buprenorphine and Norbuprenorphine Ionization: Nitrogen vs. Zero-Grade Air




## CONCLUSION

This study revealed that the vast majority of compounds tested did not exhibit noticeable difference in ionization when using zero-grade air as compared to using nitrogen for source exhaust and for Gases 1 and 2. The compounds that displayed a loss in ionization all have labeled internal standards, which helped to ensure there was no change in quantitation. Additionally, the compounds that experienced reduced signal still had adequate sensitivity for the testing range of the laboratory. Ultimately, the minor loss in sensitivity is a disadvantage that is drastically outweighed by the financial savings and aversion of interruption in laboratory production.

## REFERENCES

Sciex. (2021, June) SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready Site Planning Guide
Sciex. (2019, August) 6500+ Series of Instruments Site Planning Guide

## DISCLOSURE

No relevant financial or nonfinancial relationships to disclose.