Development of an Advanced Toxicology Method Utilizing Turbulent Flow Technology

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Abstract

Advanced toxicology testing, specifically pain management and addiction testing, faces numerous challenges. First, many isobaric drugs (e.g. opiates) are difficult to separate and detect by liquid chromatography-tandem mass spectrometry (LC/MS/MS). Second, column life often is sacrificed when laboratories use simple “dilute and shoot” sample preparation techniques for urine. In addition, laboratories constantly are concerned about maintaining or improving lower limits of quantification (LLOQ) and detection (LLOD) as reported results depend on precise and valid quantifications at or near the LLOQ. Finally, an all-inclusive method to test for various drug classes would be of great benefit to the community. Here, we introduce a new LC/MS/MS method utilizing turbulent flow technology to test for several drug classes with only one TurboFlow HPLC column. This method yields improved separation, sensitivity and efficiency while retaining column life.

Introduction

Many labs have started to use LC/MS/MS methods to more accurately determine results. LC/MS/MS is advantageous due to a shorter amount of sample prep, thus proving cost worthy and effective. Unfortunately, dirty samples lead to decreased sensitivity over time and an increase in down time due to more necessary cleaning. With turbulent flow however, the samples are automatically cleaned up. High volume is rushed through an eluting column (1.5mL/min) flushing out all of the proteins, salts and other matrix components to reduce interferences. The drugs are retained on the column, and then transferred to an analytical column where they are separated with an appropriate LC method.

Materials and Methods

Urine samples were centrifuged, transferred to a 2mL vial, hydrolyzed with 2.5% β-Glucuronidase Type HP-2 (approx 300 units of activity), incubated at 40°C for one hour, and fortified with internal standards. Samples were then injected without further cleanup onto one turbulent flow column on a Thermo Scientific TLX^{MD} system with ammonium acetate, ammonium formate, and ammonium hydroxide in water. Next, analytes were transferred to an analytical column. Mobile phase consisted of water (pH 6.20) and methanol (pH 7.27) with ammonium formate and ammonium acetate at a gradient over three minutes to 98% organic followed by an isocratic elution for four minutes. Analytes were detected with an Applied Biosystems API QTRAP 2000 mass spectrometer utilizing positive electrospray ionization and multiple reaction monitoring. Compounds investigated include four amphetamines, 11 opiates/opioids (including propoxyphene), six benzodiazepines, 12 commonly-prescribed prescription medications, 11 tricyclic antidepressants and cocaine. Six internal standards were utilized for analyte quantification.

Results

Great separation and resolution was achieved with the advanced toxicology panel using the described method with 51 transitions in urine (Figure 1).

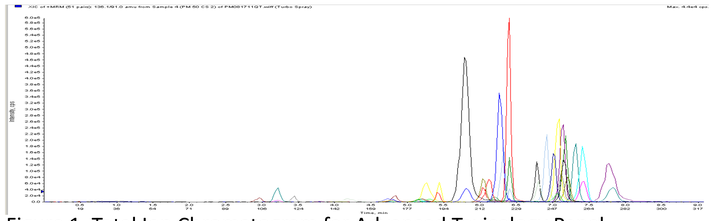

Figure 1. Total Ion Chromatogram for Advanced Toxicology Panel.

In addition to having the same parent mass ~286AMU, the isobaric compounds morphine, hydromorphone, and norhydrocodone all have the similar daughter ions. Moreover, they often tend to coelute, namely morphine and hydromorphone. In Figure 2, we see these three compounds chromatographically resolved .

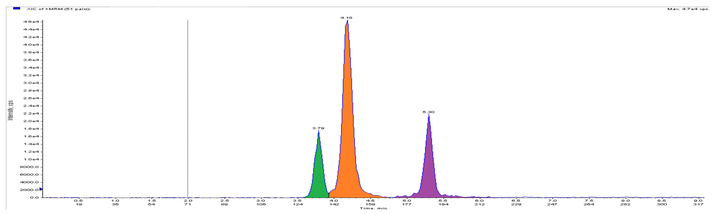

Figure 2. The complete separation of morphine, hydromorphone, and norhydrocodone

Much like the previously mentioned compounds, codeine and hydrocodone normally coelute. To make matters worse, their transitions interfere with each other, making chromatographic separation vital to correctly distinguishing the compounds. In Figure 3, complete resolution is seen.

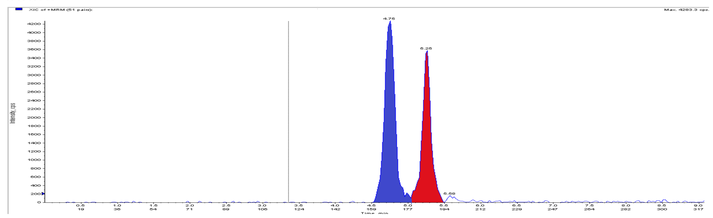

Figure 3. Chromatographic separation of codeine and hydrocodone. Additionally, the separation of oxymorphone from noroxycodone and methamphetamine from phentermine was observed (not shown).

Results Cont.

All compounds demonstrated acceptable repeatability (n = 30, <10% RSD) and linearity (Figure 4: $R^2 > 0.995$). 6 month injection repeatability yielded < 10% RSD for all compounds.

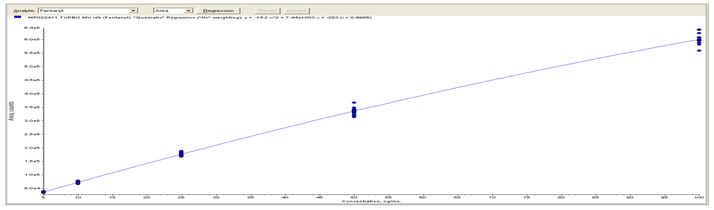

Figure 4. Repeatability is shown for five injections of five separate sets of fentanyl standards . The R^2 value is .9986.

LLOQ and LLOD were improved from our previous method (without turbulent flow) for all compounds. LLOQ ≤ 5 ng/mL were achieved for most analytes and are well below the Substance Abuse and Mental Health Services Administration (SAMHSA)’s cutoffs. One can clearly see this difference shown below in the table (Table 1).

Compound	WSL (Current)	WSL (Prior)	SAMSHA Cutoff
Amphetamine	2	5	250
Methamphetamine	2	5	250
MDMA	2	5	250
BZE	1	25	100
6MAM	5	10	10
Morphine	5	25	2000
Codeine	5	10	2000

Table 1 . Comparison of the Current WSL LLOQ, Prior WSL LLOQ, and SAMSHA cutoffs. All values are in ng/mL.

Conclusion

An advanced toxicology method utilizing turbulent flow technology was developed which tests for 51 transitions in nine minutes using a LC/MS/MS platform. The method has proven to be robust, sensitive, specific and applicable in urine. Future work will continue to improve separation and decrease method time.

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