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Fast and Easy Separation of 23 Drugs of Abuse Including High, Stable Resolution of Isobaric Opioids from Human Urine by UHPLC-MS/MS

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ABSTRACT

There is an urgent need for robust analytical methods for performing research on the measurement of drugs of abuse. This has resulted in hospital and screening laboratories needing to research new methods and tools for their screening protocols, manage the ever changing landscape of new novel psychoactive substances, and increase method throughput to meet increasing demand. Here we report an analytical method for clinical research for the quantification of 34 benzodiazepines (Table 1) in human plasma or serum.

The use of the new Thermo Scientific™ Accucore™ Biphenyl 2.6 µm column for the determination of these compounds is described. The column proved to be extremely robust with no degradation in column performance over 1000 dilute and shoot urine injections.

INTRODUCTION

The opioid class has several isobaric compounds and the adequate and stable resolution of these compounds is essential in ensuring a robust and accurate analysis. High chromatographic resolution is also important in complex mixtures such as this to minimize co-elution of both monitored and unseen matrix components to ensure optimal ionization efficiency and a reduction in matrix effects. As resolution is a function of efficiency (N), improvements in resolution can be achieved with the use of highly efficient analytical columns.

MATERIALS AND METHODS

Chromatography Consumables

Thermo Scientific Accucore Biphenyl 2.6 µm, 50 x 2.1 (P/N 17826-052130)
Thermo Scientific™ Chromacol™ GOLD-Grade Vial, clear, 2 mL, screw thread (P/N 2-SVWGK)
Thermo Scientific™ Advanced Vial Closure System (AVCS) black caps and septa (P/N C5000-54A)

Reagents

Fisher Scientific Optima™ UHPLC-MS grade water (P/N 10154604)
Fisher Scientific Optima UHPLC-MS grade methanol (P/N A458-1)
Fisher Scientific Optima UHPLC-MS grade acetonitrile (P/N A956-1)
Fisher Scientific Analytical grade formic acid (P/N F/1900/PB08)

Sample Preparation

Control human urine was spiked with 23 drugs of abuse (Figure 1 and table 3) to provide a final matrix concentration of 500 ng/mL.

100 µL of the fortified human urine sample diluted with 900 µL of 0.1% formic acid in water.

HPLC Conditions

Instrumentation:

Thermo Scientific™ Vanquish™ Horizon UHPLC system consisting of the following:

System Base Vanquish™ Horizon (P/N VH-S01-A)
Binary Pump H (P/N VH-P10-A)
Split Sampler HT (P/N VH-A10-A)
Column Compartment H (P/N VH-C10-A)
Active Preheater (P/N 6732.0110)

Thermo Scientific™ TSQ Quantiva™ Triple-Stage Quadrupole Mass Spectrometer (IQLAAEGAAXFAOUMZZZ)

Separation Conditions

Mobile phase A: 0.1% formic acid in water
Mobile phase B: 0.1% formic acid in methanol

Time (min)	%A	%B
0.0	95	5
0.15	95	5
4.0	0	100
4.0	0	100
4.6	0	100
4.6	95	5
5.5	95	5

Table 1. UHPLC Gradient Conditions

Flow rate: 0.75 mL/min
Column temperature: 45°C - active pre-heating and forced air
Injection details: 2 µL
Detection: HESI-MRM

MS/MS Conditions

	Setting
Source	Ion Max source with HESI-II probe
Polarity	Positive ionization
Spray voltage (V)	3200
Vaporiser temperature (°C)	440
Sheath gas pressure (Arb)	30
Aux gas pressure (Arb)	14
Ion Transfer tube temperature (°C)	362
CID gas pressure (mTorr)	1.5

Table 2. MS/MS Source Parameters

Compound	Polarity	Precursor (m/z)	Product (m/z)
Morphine	+ve	286.2	128.2
Oxymorphone	+ve	302.2	284.2
Hydromorphone	+ve	286.2	185.1
Naloxone	+ve	328.2	212.1
Codeine	+ve	300.2	128.1
Oxycodone	+ve	316.2	241.1
Naltrexone	+ve	342.2	270.1
Hydrocodone	+ve	300.2	199.1
Tramadol	+ve	264.2	58.2
Meperidine	+ve	248.2	174.2
Fentanyl	+ve	337.3	188.2
Buprenorphine	+ve	468.3	414.3
Methadone	+ve	310.3	265.2
Nitrazepam	+ve	282.2	236.1
Lorazepam	+ve	321.1	275.1
Oxazepam	+ve	287.1	241.2
Clonazepam	+ve	316.1	270.0
Flunitrazepam	+ve	314.1	268.1
Alprazolam	+ve	309.1	281.1
Temazepam	+ve	301.1	255.1
Diazepam	+ve	285.1	193.1
11-Hydroxy-THC	+ve	331.2	313.2
11-Carboxy-THC	+ve	345.2	327.2

Table 3. Compound Transition Details

Data Processing

The Thermo Scientific™ Dionex™ Chromeleon™ 7.2.8 Chromatography Data System was used for data acquisition and analysis.

RESULTS

Reducing analysis time is a key driver in many analytical laboratories. The use of a ballistic gradient over 4 minutes provides excellent peak shape for all compounds classes, over a wide range of polarities, while ensuring adequate resolution between the critical isobaric opioids. The consistent resolution of these isobaric compounds in drugs of abuse screening protocols helps ensure the accuracy of the analytical results and avoid false positives.

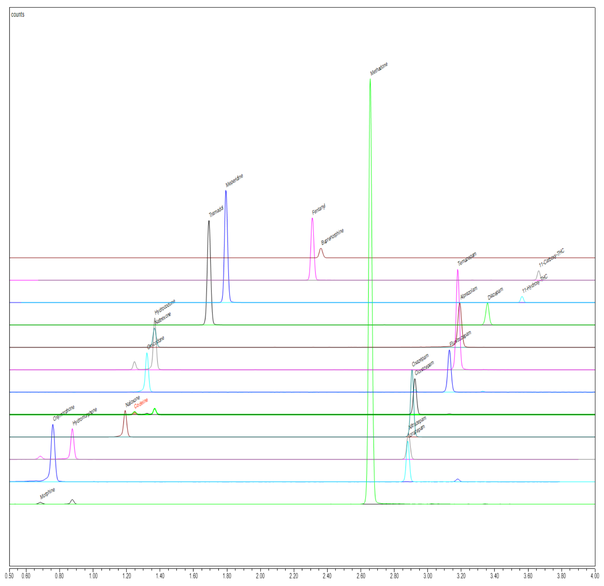

Figure 1. Separation of 23 drugs of abuse using the chromatographic conditions described

Morphine/hydromorphone (Figure 2 and Figure 3) and codeine/hydrocodone (Figure 4 and Figure 5) display excellent peak shape and resolution stability which has been tested up to 1000 replicate injections.

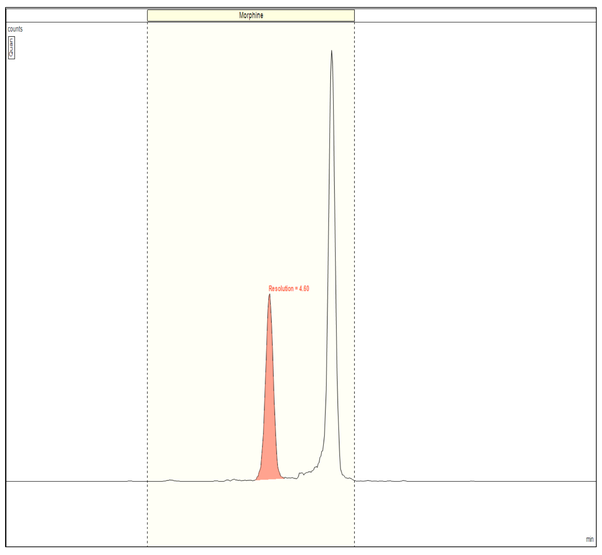

Figure 2. Resolution of morphine/hydromorphone injection 10

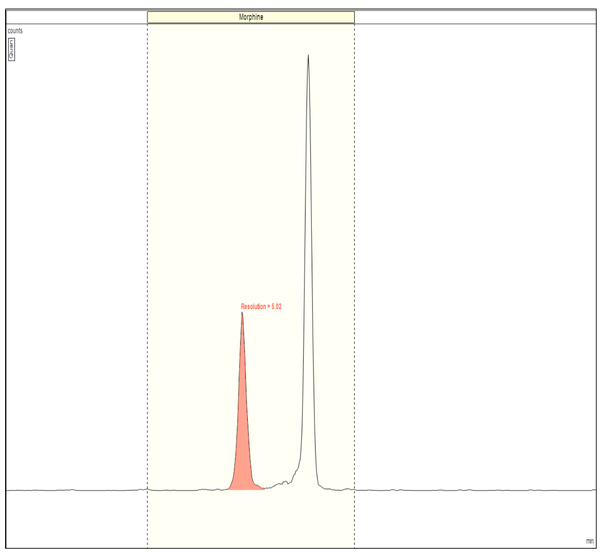

Figure 3. Resolution of morphine/hydromorphone injection 1000

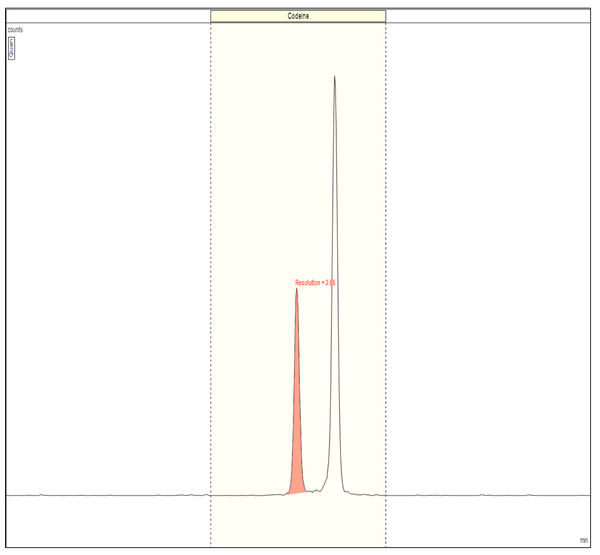

Figure 4. Resolution of codeine/hydrocodone injection 10

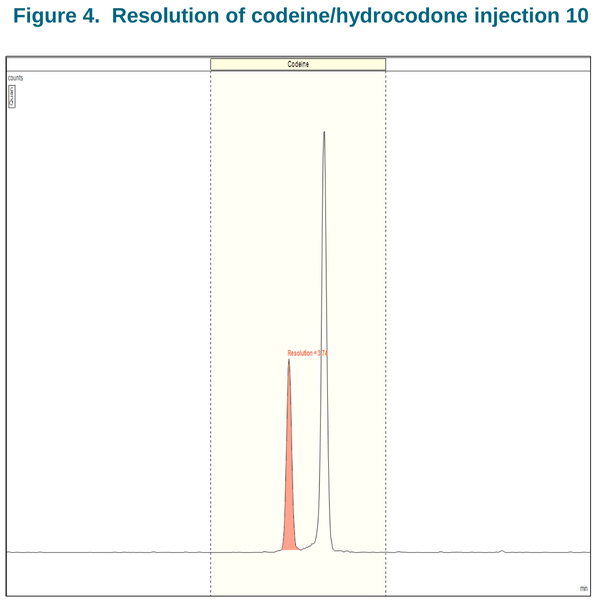

Figure 5. Resolution of codeine/hydrocodone injection 1000

In order to meet the demands of rapid screening assays for drugs of abuse, it is necessary to have highly robust columns.

Rapid degradation of column performance mid-run is highly undesirable. This may be a loss of resolution, shifting of retention time, degradation of peak shape or even a blockage. The Accucore Biphenyl 2.6 µm has been designed with robustness in mind. Over 1000 injections of dilute and shoot urine were performed on the column and key chromatographic parameters monitored.

The Accucore Biphenyl showed no degradation in key chromatographic parameters over these 1000 injections, with no filter and no switching valve used. The drugs of abuse panel is divided into three distinct classes, namely opiates, benzodiazepines and cannabinoid metabolites. Representative examples from each compound class are presented.

For morphine, alprazolam and 11-hydroxy-THC the %CV for the retention variability was 2.49%, 0.07% and 0.14% respectively (Figure 6).

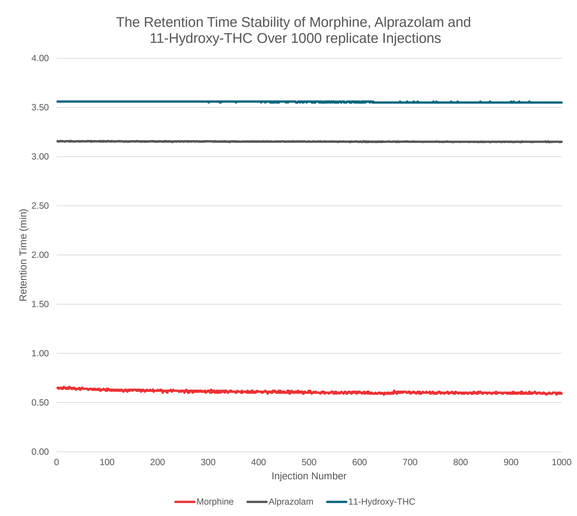

Figure 6. Retention stability of a representative opiate, benzodiazepine and cannabinoid

Furthermore, the peak widths at half height (FWHH) and peak asymmetry are consistent over this large number of injections giving confidence that the column will not fail even when exposed to the most demanding of applications.

CONCLUSIONS

- Fast and easy separation of 23 drugs of abuse from diluted human urine within 5.5 minutes cycle time for clinical research. The sample preparation is simple and potentially easy to automate.
- Excellent and stable resolution of isobaric steroids up to 1000 replicate injections.
- High retention time stability observed for all compounds ensuring data processing will be as easy as possible.
- Excellent consistency of key chromatographic parameters over 1000 injections of diluted urine showing the robustness of the new Accucore Biphenyl 2.6 µm.
- Streamlined integration of a robust and powerful analytical solution combining the Accucore Biphenyl 2.6 µm, Vanquish Horizon UHPLC system, Quantiva MS and Chromeleon CDS with advanced MS data processing.

REFERENCES

Find out more at www.thermofisher.com/appslab

Find out more at www.thermofisher.com/biphenyl

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