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TECHNICAL NOTE 21883

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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Keywords

Accucore column, drugs of abuse, isobaric separation, opioids, benzodiazepines, cannabinoids, urine, biphenyl, Vanquish Horizon UHPLC system, TSQ Quantiva MS, solid core

Application benefits

- Fast and easy separation of 23 drugs of abuse
- Excellent resolution of isobaric opioids
- High retention time and peak shape stability using a “dilute and shoot” approach

Goal

To demonstrate the fast and easy separation of 23 drugs of abuse, including the adequate and stable resolution of isobaric opioids from human urine. The new Thermo Scientific™ Accucore™ Biphenyl solid core column is used in combination with the Thermo Scientific™ Horizon™ UHPLC system and Thermo Scientific™ TSQ Quantiva™ MS/MS system.

To examine method parameters such as retention time stability and resolution robustness to increase the throughput and productivity for routine and research laboratories.

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Introduction

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.

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. The new Accucore Biphenyl 2.6 µm column shows a well-tuned balance of efficiency and selectivity and is a powerful and robust tool for the determination of drugs of abuse.

Experimental

Consumables

- Thermo Scientific Accucore Biphenyl 2.6 µm, 50 × 2.1 mm (P/N 17826-052130)
- Thermo Scientific™ Chromacol™ SureStop™ GOLD grade vial, clear 2 mL, screw thread (P/N 2-SVWGK)
- Thermo Scientific™ National™ 9 mm autosampler vial screw caps, black caps and septa, with AVCS technology (Advanced Vial Closure System) (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

Sample pretreatment

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

Sample preparation protocol

Compounds: Refer to Figure 1 and Table 3
Matrix: Human urine

Dilute and shoot protocol: 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 Pre-heater (P/N 6732.0110)

Thermo Scientific™ TSQ Quantiva™ Triple Quadrupole Mass Spectrometer

Separation conditions

Mobile phase A: 0.1% formic acid in water
Mobile phase B: 0.1% formic acid in methanol
Flow rate: 0.75 mL/min

Table 1. LC gradient conditions

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

Column temp: 45 °C, with active pre-heating and forced air
Injection volume: 2 µL
Detection: HESI-MRM

MS conditions

Table 2. MS/MS source parameters

MS Source Parameters	Setting
Source	Thermo Scientific™ Ion Max source with HESI-II probe
Polarity	Positive ionization
Spray voltage (V)	3200
Vaporizer 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 3. Compound transition details

Compound	Polarity	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)
Morphine	Positive	286.2	128.2	55
Oxymorphone	Positive	302.2	284.2	20
Hydromorphone	Positive	286.2	185.1	30
Naloxone	Positive	328.2	212.1	40
Codeine	Positive	300.2	128.1	55
Oxycodone	Positive	316.2	241.1	29
Naltrexone	Positive	342.2	270.1	27
Hydrocodone	Positive	300.2	199.1	31
Tramadol	Positive	264.2	58.2	17
Meperidine	Positive	248.2	174.2	20
Fentanyl	Positive	337.3	188.2	23
Buprenorphine	Positive	468.3	414.3	35
Methadone	Positive	310.3	265.2	15
Nitrazepam	Positive	282.2	236.1	25
Lorazepam	Positive	321.1	275.1	22
Oxazepam	Positive	287.1	241.2	23
Clonazepam	Positive	316.1	270.0	26
Flunitrazepam	Positive	314.1	268.1	26
Alprazolam	Positive	309.1	281.1	27
Temazepam	Positive	301.1	255.1	23
Diazepam	Positive	285.1	193.1	33
11-Hydroxy-THC	Positive	331.2	313.2	15
11-Carboxy-THC	Positive	345.2	327.2	17

Data processing

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

Results and discussion

Reducing analysis time is a key driver in many analytical laboratories. The use of a ballistic gradient over four minutes provides excellent peak shape for all compound 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.

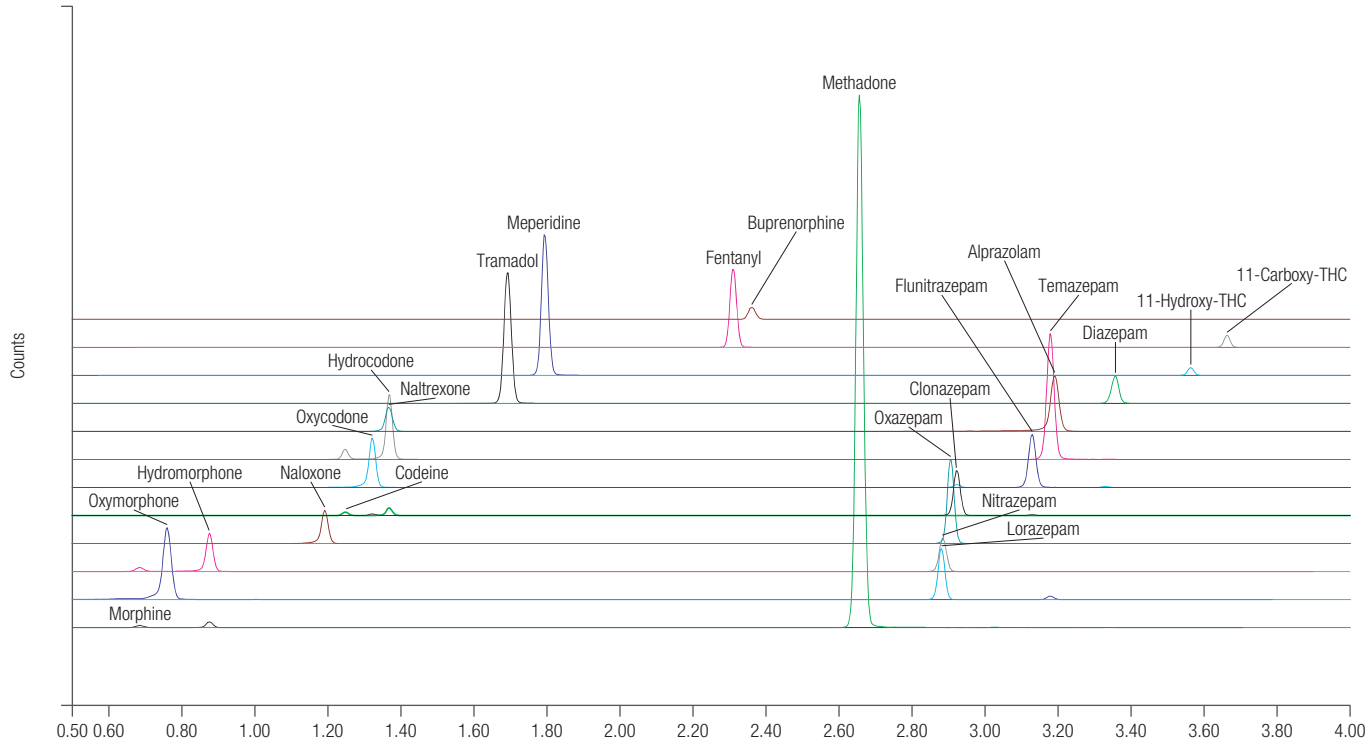

Figure 1. Separation of 23 drugs of abuse under 4 minutes on the Accucore Biphenyl column

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.

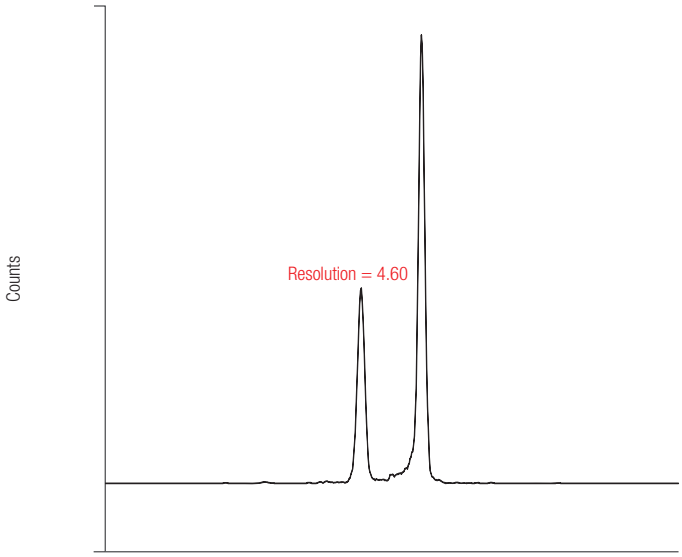

Figure 2. Resolution of morphine/hydromorphone injection 10

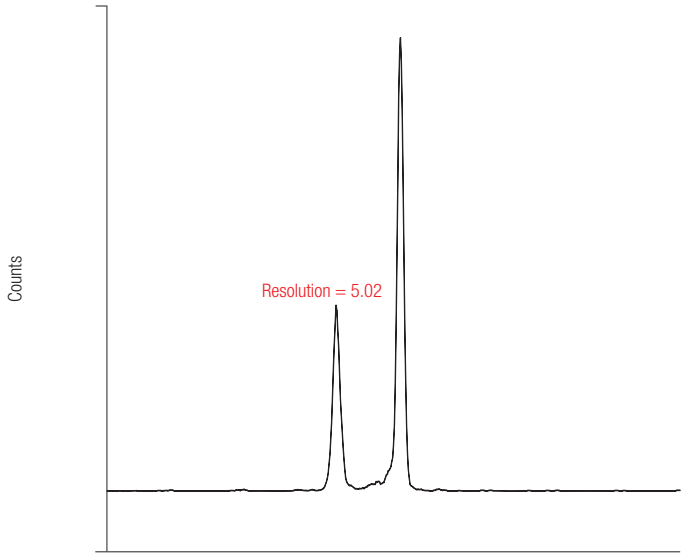

Figure 3. Resolution of morphine/hydromorphone injection 1000

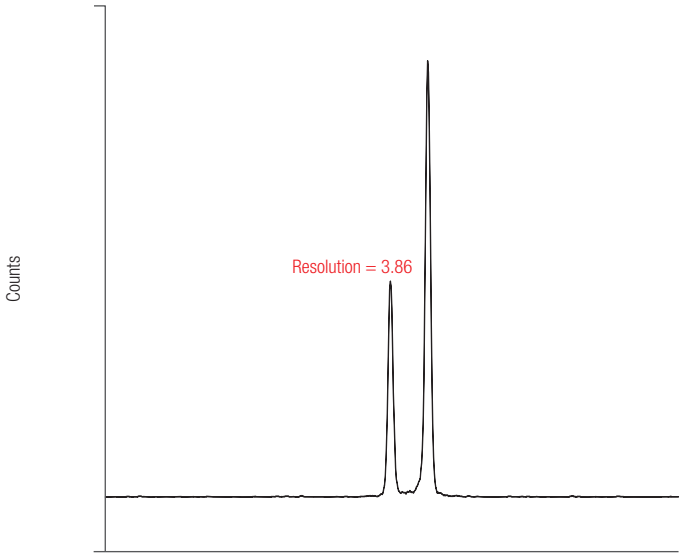

Figure 4. Resolution of codeine/hydrocodone injection 10

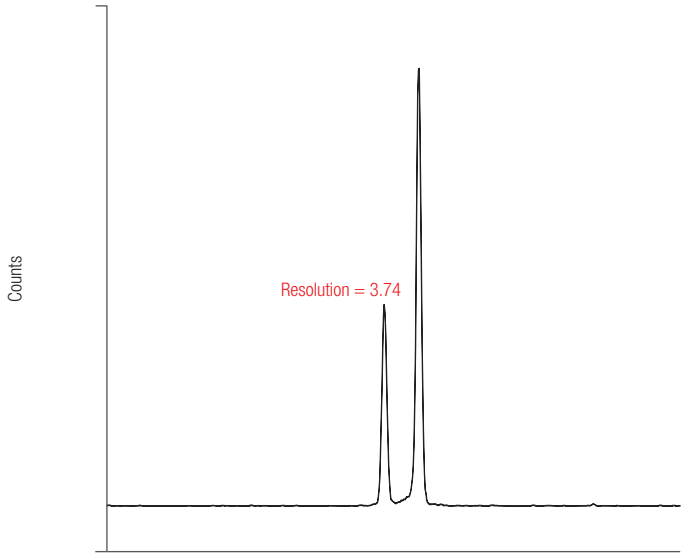

Figure 5. Resolution of codeine/hydrocodone injection 1000

To meet the demands of rapid screening methods 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 column 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 column 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).

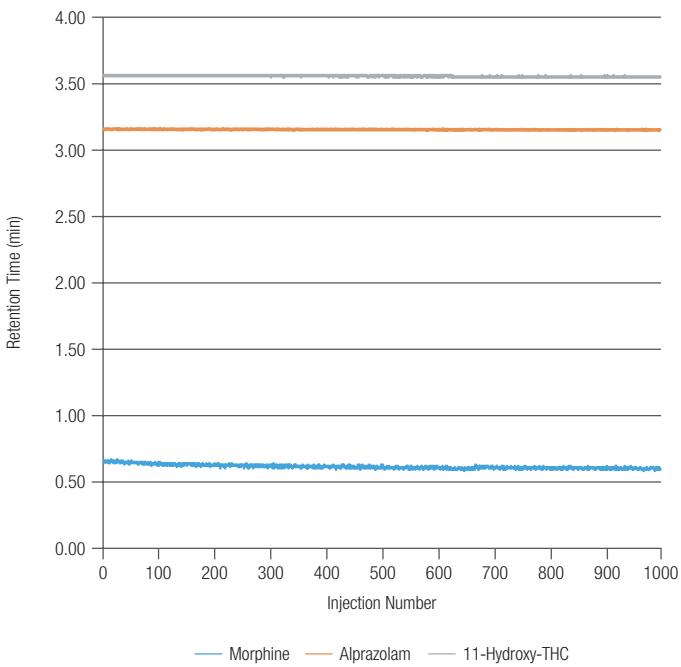

Figure 6. Retention stability of a representative opiate, benzodiazepine, and cannabinoid (morphine, alprazolam, and 11-hydroxy-THC) over 1000 replicate injections

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 (Tables 4 and 5).

Table 4. Summary of peak width at half height and peak asymmetry stability from injection 10

	Peak Name	Width (50%) (min)	Asymmetry
Injection 10	Morphine	0.025	1.10
	Oxymorphone	0.025	0.93
	Hydromorphone	0.024	0.88
	Naloxone	0.025	0.72
	Codeine	0.021	0.85
	Oxycodone	0.022	0.86
	Hydrocodone	0.023	0.89
	Naltrexone	0.024	0.87
	Tramadol	0.025	0.96
	Meperidine	0.023	1.04
	Fentanyl	0.022	0.97
	Buprenorphine	0.024	1.05
	Methadone	0.024	0.97
	Nitrazepam	0.023	0.99
	Lorazepam	0.025	1.28
	Oxazepam	0.026	1.13
	Clonazepam	0.025	1.03
	Flunitrazepam	0.024	0.90
	Temazepam	0.025	1.11
	Alprazolam	0.027	0.84
	Diazepam	0.026	0.88
	11-Hydroxy-THC	0.021	1.07
	11-Carboxy-THC	0.021	1.24

Table 5. Summary of peak width at half height and peak asymmetry stability from injection 1000

	Peak Name	Width (50%) (min)	Asymmetry
Injection 1000	Morphine	0.023	0.83
	Oxymorphone	0.025	0.88
	Hydromorphone	0.023	0.91
	Naloxone	0.024	0.83
	Codeine	0.022	0.89
	Oxycodone	0.023	0.93
	Hydrocodone	0.021	0.90
	Naltrexone	0.024	0.87
	Tramadol	0.023	1.04
	Meperidine	0.022	0.97
	Fentanyl	0.023	1.05
	Buprenorphine	0.023	1.12
	Methadone	0.023	1.17
	Nitrazepam	0.024	0.97
	Lorazepam	0.024	1.01
	Oxazepam	0.024	1.02
	Clonazepam	0.023	0.99
	Flunitrazepam	0.026	0.95
	Temazepam	0.024	1.07
	Alprazolam	0.024	0.92
	Diazepam	0.024	0.91
	11-Hydroxy-THC	0.023	1.20
	11-Carboxy-THC	0.022	0.99

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 column
- Streamlined integration of a robust and powerful analytical solution combining the Accucore Biphenyl 2.6 µm column, Vanquish Horizon UHPLC system, TSQ Quantiva MS, and Chromeleon CDS with advanced MS data processing

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