Rapid Screening Method for Illicit Drugs, Using an Advanced Solid Core UHPLC Column and UHPLC System with MS/MS Detection

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Key Words
Vanquish, Accucore, opiates, benzodiazepines, amphetamines, drugs of abuse, forensic, toxicology

Abstract
This application shows the advantages of using the Thermo Scientific™ Accucore™ Vanquish™ C18 UHPLC, 1.5 µm column and Vanquish UHPLC system for the analysis of 47 illicit drugs.

Advanced capabilities of the Vanquish UHPLC system allow the Accucore Vanquish UHPLC columns to be operated at high flow rates that enable development of rapid analytical methods while maintaining performance. The need for chromatographic separation of isobaric compounds prior to MS detection is also highlighted.

Introduction
There is increasing demand to provide rapid and selective screening techniques for an expanding range of illicit drugs for forensic methodology and workplace drug screening. Creating screening methods for multiple analytes is more cost-effective than dedicated methods for fewer analytes. Reduced analysis times provide for quicker release of data, reduced costs per assay, and greater sample throughput overall.

Other methodologies exploit the high mass resolution capabilities of specialty mass spectrometers[1]. Where these are not readily available, and where common precursor and product ions are present, there is a requirement for good chromatographic resolution, prior to MS detection.

Accucore Vanquish UHPLC columns use Core Enhanced Technology™ to facilitate fast and highly efficient separations. This next-generation column features 1.5 µm solid core particles that are not totally porous, but instead have a solid core and a porous outer layer. The optimized phase bonding creates a high coverage, robust phase. This coverage results in a significant reduction in secondary interactions and delivers highly efficient peaks. The tightly controlled 1.5 µm diameter of Accucore Vanquish particles, in combination with controlled manufacturing processes, results in a column that delivers the increased chromatographic performance required for rapid screening methods commonly used in forensic toxicology.

The Accucore Vanquish UHPLC column and Vanquish UHPLC system were designed in combination to achieve the best possible chromatographic performance. The system is optimized to reduce extra column band dispersion and allow users to significantly improve the separation power in their analytical assays. By exploiting the 1500 bar high-pressure capability of the Vanquish UHPLC system, the flow rates can be increased while maintaining peak capacity, resulting in shorter method times and increased assay throughput.

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Experimental Details

Consumables	Part Number
Accucore Vanquish C18, 1.5 µm UHPLC column, 100 × 2.1 mm	17101-102130
LC-MS grade 18 MΩ water from Thermo Scientific™ Smart2Pure™ system	50129845
Fisher Chemical™ LC-MS grade methanol	10653963
Fisher Chemical analytical grade formic acid	10559570
Fisher Chemical ammonium acetate	10598410
Thermo Scientific™ Virtuoso™ 9 mm wide opening, 2 mL screw thread vial and cap kit	60180-VT400

Sample Preparation

Solutions of the 47 compounds shown in Table 3 were prepared by dissolving 1 mg amounts in 1 mL of water/methanol (1:1 v/v) to produce 1 mg/mL primary solutions. Where required, the methanol proportion was increased to ensure sample solubility. Serial dilutions were made with water/methanol (90:10 v/v) to produce 100 ng/mL working solutions.
Vial labeling is supported by the Virtuoso Vial Identification System.

Instrumentation	Part Number
Vanquish UHPLC system consisting of:	
Binary pump H	VH-P10-A
Split sampler HT	VH-A10-A
Column compartment H	VH-C10-A
Thermo Scientific™ TSQ Vantage™ triple quadrupole mass spectrometer	
Virtuoso Vial Identification System	60180-VT-100

LC/MS Conditions

UHPLC column:	Accucore Vanquish C18, 1.5 µm, 100 × 2.1 mm
Mobile phase A:	10 mM ammonium acetate in water
Mobile phase B:	0.1% formic acid in methanol
Flow rate:	500 µL/min
Column temperature:	(Forced air mode) with active eluent preheating set to 50 °C
Injection details:	2 µL
Gradient:	Refer to Table 1

Time (min)	% B
0.00	10
0.16	10
2.88	90
3.20	90
3.28	10
5.60	10

Table 1: LC gradient conditions

Compound detection was achieved by selected-reaction monitoring (SRM) experiments on a TSQ Vantage triple quadrupole mass spectrometer.
Source and tuning conditions are set out in Table 2 and compound specific parameters for the 47 different SRM transitions are shown in Table 3, together with the analyte retention time.

Data Processing

Software:	Thermo Scientific™ Xcalibur™ 2.1 (MS control)
	Thermo Scientific™ Dionex™ Chromeleon™ 7.2 Chromatography Data System

Parameter	Setting
Ionization conditions	HESI
Polarity	Positive
Spray voltage (V)	4500
Vaporizer temperature (°C)	500
Sheath gas pressure (Arb)	75
Aux gas pressure (Arb)	20
Capillary temperature (°C)	380
Collision pressure (mTorr)	1.0
Scan time (s)	0.005
Q1 resolution (FWHM)	0.7
Q3 resolution (FWHM)	0.7

Table 2: TSQ Vantage MS/MS experiment conditions

Standard	Precursor Ion	S-Lens (V)	Product Ion	Collision Energy (V)	Retention Time (min)
Ecgonine methyl ester	200	73	182	15	0.47
Normorphine	272	110	165	36	0.63
Dihydromorphine	288	120	185	31	1.06
Anhydroecgonine methyl ester (AEME)	182	75	91	27	1.21
Oxymorphone	302	97	161	33	1.29
Norcodeine	286	96	193	21	1.34
Morphine	286	112	201	24	1.35
Noroxycodone	302	86	187	23	1.42
Hydromorphone	286	120	185	28	1.45
Norhydrocodone	286	102	241	21	1.49
Amphetamine	136	41	91	16	1.54
MDA	180	49	163	6	1.55
Methamphetamine	150	53	91	17	1.61
O-desmethyl(-cis-) tramadol	250	72	58	16	1.62
Dihydrocodeine	302	120	201	29	1.62
N,O-didesmethyl tramadol	236	68	44	13	1.65
MDEA	208	63	163	11	1.73
Benzoylecgonine	290	95	168	17	1.75
Oxycodone	316	100	298	16	1.76
6-acetylmorphine	328	120	165	36	1.83
Codeine	300	105	215	29	1.85
Norfentanyl	233	70	177	13	1.92
Hydrocodone	300	120	199	29	1.94
N-desmethyl tapentadol	208	75	107	23	2.05
N-desmethyl tramadol	250	59	44	14	2.06
cis-tramadol	264	75	58	16	2.06
Naltrexone	342	118	270	24	2.06
Tapentadol	222	80	107	25	2.09
Normeperidine	234	83	160	14	2.16
Meperidine	248	100	174	18	2.31
Nortilidine	260	75	77	42	2.33
Naloxone	328	91	253	25	2.36
6-acetylcodeine	342	138	165	43	2.37

Table 3: Compound SRM transition details and retention time

Standard	Precursor Ion	S-Lens (V)	Product Ion	Collision Energy (V)	Retention Time (min)
Norbuprenorphine	414	160	187	36	2.39
PCP (phencyclidine)	244	53	91	34	2.43
1,5-dimethyl-3,3-diphenylpyrrolodone (EDDP)	278	120	234	29	2.46
Clonazepam	316	95	270	23	2.66
Tilidine	274	76	155	18	2.77
Oxazepam	287	87	241	20	2.81
Methadone	310	84	265	13	2.81
Lorazepam	321	100	275	20	2.82
Alprazolam	309	101	281	24	2.84
Flurazepam	388	100	315	21	2.95
Fentanyl	337	106	188	22	2.97
Nordiazepam	271	100	140	27	2.99
11-nor-Δ-9-carboxy-THC	345	104	193	24	3.49
Buprenorphine	468	138	187	39	3.75

Table 3 (continued): Compound SRM transition details and retention time

Results

By exploiting the high pressure capabilities of the Vanquish UHPLC system, in conjunction with the Accucore Vanquish UHPLC column and a simple binary gradient, it was demonstrated that a screening method for 47 compounds within a 4 minute detection window (and a full method cycle time of less than 6 minutes) can be achieved.

Using a 500 µL/min flow rate, the system pressure at the start of the gradient was 890 bar, rising to a maximum of 1233 bar during the gradient cycle. The Vanquish UHPLC system is able to routinely operate at these pressure conditions.

Although the data set is complex, the low dispersion of the Vanquish UHPLC system coupled with the Accucore Vanquish UHPLC column, in combination with the selected-reaction monitoring experiment, provides sufficient performance to resolve the individual compounds (Figure 1).

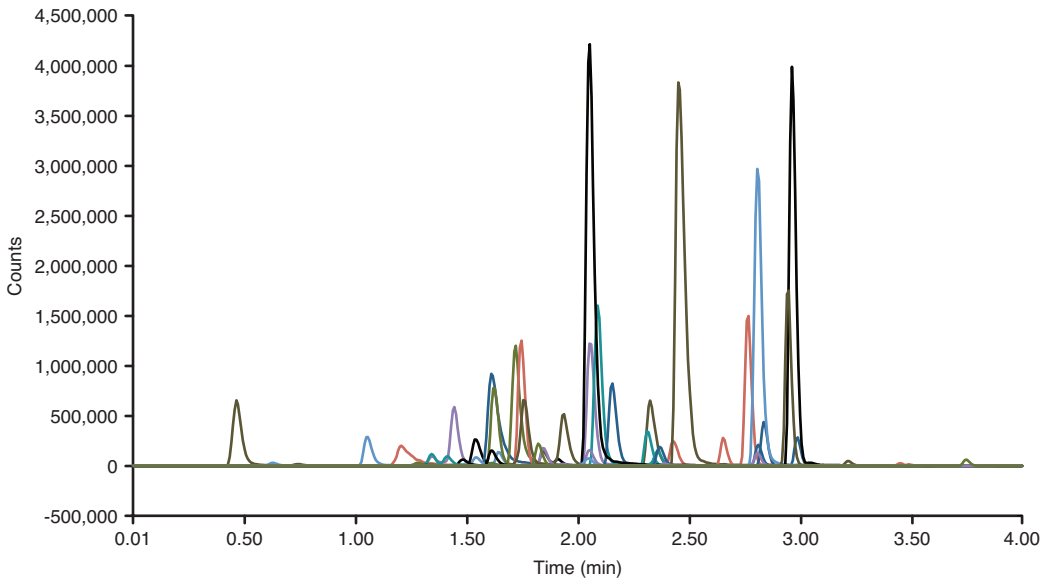

Figure 1: Overlaid selected-reaction monitoring chromatograms showing detection of 47 compounds within a 4 minute detection window

When isobaric compounds are present in the screening portfolio it is not possible to rely solely on the mass resolution capability of the mass spectrometer, particularly as structurally similar compounds can have common precursor and product ions. In these cases the separation of the components using chromatography provides a clear advantage.

Figures 2 to 4 show the SRM chromatograms of compounds that demonstrate this capability. Each figure represents data from two isobaric compounds.

Figure 2 relates to compounds with molecular weight of 208 u and without common product ions, the chromatograms have a single peak.

Figures 3 and 4 relate to compounds with a molecular weight of 300 u and 342 u, respectively. In this situation the compound pairs have common precursor and product ions so multiple peaks are observed but are able to be individually resolved in time.

Without this extra chromatographic resolution the identification of these compounds in a screening set would be compromised as the signals would appear at the same place.

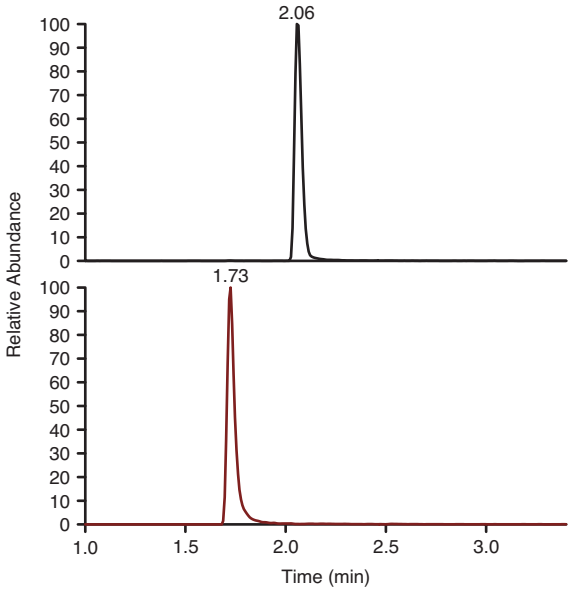

Figure 2: Chromatographic resolution of N-desmethyltapentadol and MDEA with common precursor ion (208 u) and different product ions

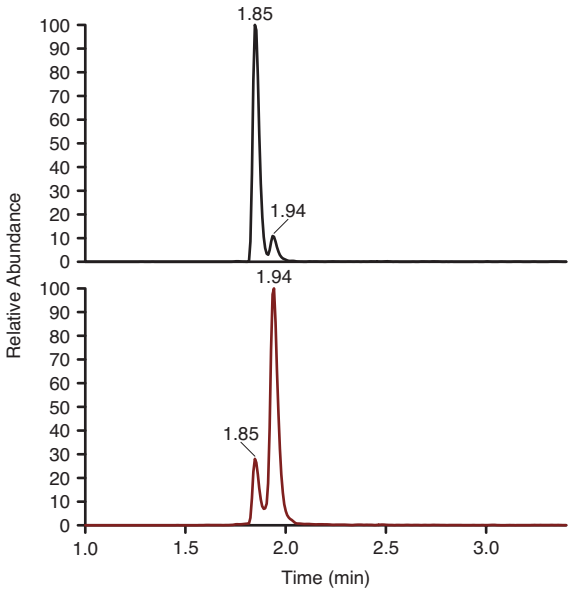

Figure 3: Chromatographic resolution of codeine and hydrocodone with common precursor (300 u) and product ions

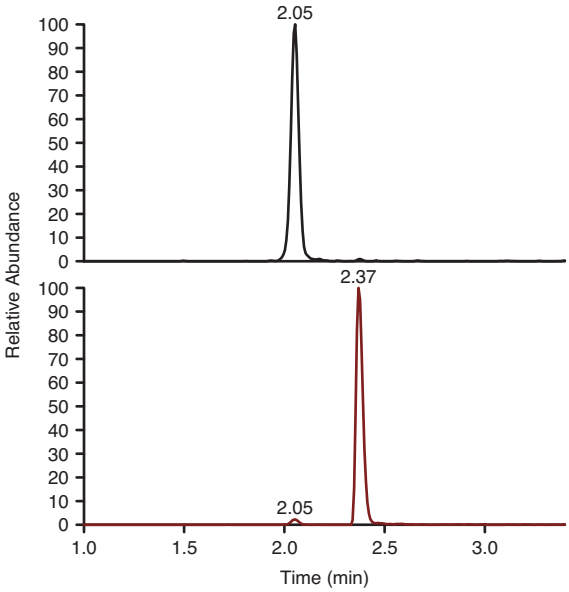

Figure 4: Chromatographic resolution of naltrexone and 6-acetylcodeine with common precursor (342 u) and product ions

Conclusion

This application note demonstrates the advantages of using the Accucore Vanquish C18 1.5 µm UHPLC column and Vanquish UHPLC system. This solution:

- Delivers a rapid screening method for 47 drugs of abuse
- Provides method time of less than 6 minutes
- Exploits the high pressure capabilities of the Vanquish UHPLC system
- Supports separation of isobaric compounds prior to MS detection

References:

[1] McHale, K., Thermo Scientific, Quantitative LC-MS Screening for Illicit Drugs Using Ultrahigh Resolution Mass Analysis and Accurate Mass Confirmation, Thermo Scientific Application Note (2010) AN63166b_E08/10S.

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