Fast Analysis of Illicit Drug Residues on Currency using a Superficially Porous LC Column using LC/MS/MS

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Introduction

Illicit drugs, like cocaine, are frequently found on US currency. While a more interesting perception might be that all bills were used to inhale the drug, the truth is much more mundane. Drug trafficking is thought to be the initial source of drug residues on a small percentage of bills, and because these compounds are fine powders, they are easily transferable from one surface to another. As money is processed through counting machines and automated teller machines (ATM), small amounts of drugs are readily transferred. An Agilent application note (Agilent Publication Number 5990-4254EN) details an application kit for the screening of 25 compounds considered in forensic toxicology analyses with an Agilent 1200/6410 LC/MS/MS system. In this work, an Agilent Poroshell 120 EC-C18 column is used to analyze 25 compounds found in the Agilent LC/MS Toxicology Test Mixture (Agilent Part Number 5190-0470). This ammonium formate/ acetonitrile gradient analysis is scaled using faster flow rates to shorten analysis time and exploit the low back pressure of this superficially porous column. Calibration curves for each of the 25 compounds are generated, and as a demonstration of the method a $1 bill was extracted into methanol, analyzed and quantified.

Experimental

An Agilent 1200 Series Rapid Resolution LC (RRLC) system with an Agilent 6410 Triple Quadrupole LC/MS detector was used for this work:

•G1312B Binary Pump SL with mobile phase A: 5 mM ammonium formate with 0.01% formic acid, and B: acetonitrile with 0.01% formic acid. Gradient was 10% B at t_0, ramp to 15% B, ramp to 50% B, then ramp to 95% B and hold 95% B. Gradient times vary depending on flow rate, see Table 1.

•G1367C Automatic Liquid Sampler (ALS) SL with a 1.0 µL injection volume.

•G1316B Thermostated Column Compartment (TCC) SL, temperature set to 60 °C, with an Agilent ZORBAX RRHT Eclipse Plus C18, 2.1 mm x 100 mm, 1.8 µm column (Agilent Part Number 959764-902) or an Agilent Poroshell 120 EC-C18, 2.1 mm x 100 mm, 2.7 µm column (Agilent Part Number 695775-902).

•G6410A Triple Quadrupole LC/MS: electrospray AP-ESI, drying gas temperature and flow: 350 °C, 12 L/min, nebulizer gas pressure: 30 psi, capillary voltage: 2000 V, in dMRM mode, transitions found in Table 2.

•MassHunter versions B.02.01, B.02.00 and B.03.01 were used for data acquisition, qualitative and quantitative analyses respectively.

Experimental

Table 1. HPLC Method Parameters for Various Columns and Conditions.

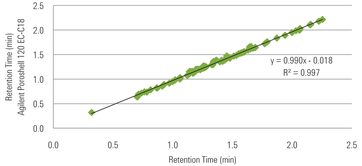
Gradient and Method Parameters	2.1 x 100 mm, 1.8-µm Agilent ZORBAX Eclipse Plus C18	2.1 x 100 mm, 2.7-µm Agilent Poroshell 120 EC-C18	2.1 x 100 mm, 2.7-µm Agilent Poroshell 120 EC-C18	2.1 x 100 mm, 2.7-µm Agilent Poroshell 120 EC-C18
Flow Rate	0.500 mL/min	0.500 mL/min	0.700 mL/min	1.000 mL/min
10% B	0.00 min	0.00 min	0.00 min	0.00 min
15% B	0.50 min	0.50 min	0.36 min	0.25 min
50% B	3.00 min	3.00 min	2.14 min	1.50 min
95% B	4.00 min	4.00 min	2.86 min	2.00 min
95% B	6.00 min	6.00 min	4.29 min	3.00 min
Stop Time	6.00 min	6.00 min	4.29 min	3.00 min
System Pressure	375 bar	280 bar	385 bar	550 bar

Table 2. MRM Transitions for 25 Compounds in Toxicology Test Mixture.

Compound	Precursor Ion	Fragmentor Voltage	Product Ions 1, 2	Collision Energies 1, 2
Codeine	300	158	165, 58	45, 29
Oxycodone	316	143	298, 256	17, 25
Amphetamine	136	66	119, 91	5, 17
MDA	180	61	163, 105	5, 21
Hydrocodone	300	159	199, 128	29, 65
Methamphet-amine	150	92	119, 91	5, 17
MDMA	194	97	163, 105	9, 25
Strychnine	335	195	184, 156	41, 53
Phentermine	150	66	133, 91	5, 25
MDEA	208	107	163, 105	9, 25
Heroin	370	149	268, 165	37, 61
Cocaine	304	138	182, 77	17, 61
Meperidine	248	128	220, 174	21, 17
Trazodone	372	159	176, 148	25, 37
PCP	244	86	91, 86	41, 9
Oxazepam	287	150	269, 241	12, 20
Nitrazepam	282	148	236, 180	25, 41
Verapamil	455	158	165, 150	37, 45
Lorazepam	321	102	275, 194	21, 49
Methadone	310	112	265, 105	9, 29
Alprazolam	309	179	281, 205	25, 49
Tempazepam	301	117	255, 177	29, 45
Proadifen	354	153	167, 91	29, 45
Diazepam	285	169	193, 154	45, 25
THC	315	150	193, 123	20, 30

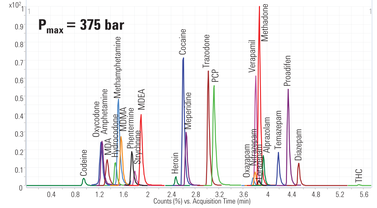
Method Transfer Results and Discussion

Previous Work Shows Highly Correlated Selectivity between Agilent Technologies' Traditional Totally Porous 1.8-µm ZORBAX Eclipse Plus C18 and their Superficially Porous 2.7-µm Poroshell 120 EC-C18 Due to Similar Bonding Chemistry (Agilent Publication 5990-6588EN).

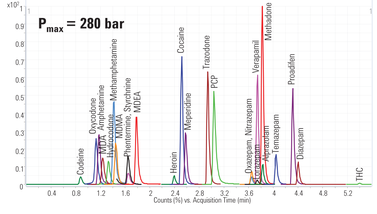


Method Parameters: Columns: 4.6 x 50 mm; A: 10 mM ammonium formate, pH 3; B: acetonitrile; Gradient: 5% B at t_0, ramp to 95% B in 2 min, hold 95% B for 1 min; Flow Rate: 2 mL/min; Sample: 1 µL of 1 mg/mL standard in water (90 compounds of various chemical classifications were used in this selectivity study).

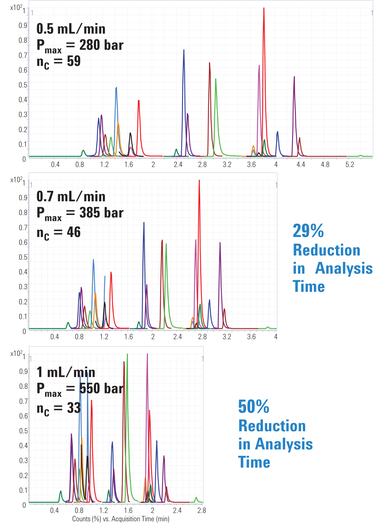
Original Analysis as Performed on a 1.8-µm Agilent ZORBAX Rapid Resolution High Throughput (RRHT) Eclipse Plus C18 Column.



A Superficially Porous 2.7-µm Agilent Poroshell 120 EC-C18 Column Offers Very Similar Selectivity, as Compared to the 1.8-µm Eclipse Plus C18 Column.



Significant Time Savings are Possible by Increasing Flow Rate with the Poroshell 120 EC-C18 Column, Exploiting the Low Back Pressure Generated by its Larger 2.7-µm Particles.



29% Reduction in Analysis Time

50% Reduction in Analysis Time

Gradient Scaling Equation:

$$k^* = (t_g F)/(d/2)^2 L(\Delta\%B)$$

where:
t_g = gradient time
F = flow rate
d = column internal diameter
L = column length
Δ%B = change in organic content across gradient segment

Conditional Peak Capacity Equation:

$$n_c = (t_{Rn} - t_{R1})/W$$

where:
t_{Rn} = retention time of the last peak
t_{R1} = retention time of the first peak
W = average 4σ peak width = $(W_{1/2}/2.35)x4$
$W_{1/2}$ = average peak width at half height

Calibration Data Results and Discussion

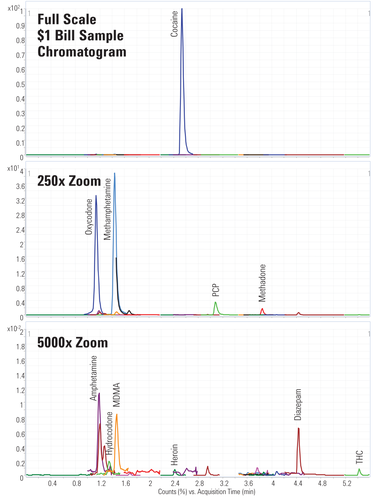
Table 3. Calibration Data for 25 Compounds on Poroshell 120 EC-C18

Compound	Linear Calibration Curve	Correlation Coefficient, R^2
Codeine	y = 25.4023 x + 3.1628	0.9999
Oxycodone	y = 138.9535 x - 0.6269	0.9994
Amphetamine	y = 196.3425 x + 50.1606	0.9999
MDA	y = 121.2945 x + 180.2165	0.9995
Hydrocodone	y = 72.1351 x - 8.1010	0.9996
Methamphetamine	y = 286.7936 x + 429.4970	0.9979
MDMA	y = 121.4217 x - 55.0435	0.9987
Strychnine	y = 39.3465 x - 9.5339	0.9996
Phentermine	y = 110.8083 x – 65.1028	0.9991
MDEA	y = 200.4804 x - 14.2886	0.9998
Heroin	y = 18.2969 x + 0.4442	0.9999
Cocaine	y = 295.8654 x - 5.6261	0.9996
Meperidine	y = 145.0367 x + 17.2273	0.9999
Trazodone	y = 286.1986 x - 12.4408	0.9997
PCP	y = 287.4395 x - 24.8090	0.9999
Oxazepam	y = 14.7883 x - 0.4919	0.9990
Nitrazepam	y = 49.1750 x + 69.2747	0.9988
Verapamil	y = 273.3001 x + 17.3890	0.9999
Lorazepam	y = 11.2911 x + 6.0687	0.9990
Methadone	y = 439.7238 x - 6.7890	0.9998
Alprazolam	y = 80.2721 x + 18.5435	0.9997
Tempazepam	y = 70.9899 x + 15.5246	0.9998
Proadifen	y = 243.9474 x - 13.0696	0.9999
Diazepam	y = 68.9622 x + 26.0608	0.9995
THC	y = 3.1838 x - 2.7072	0.9980

Table 4. Quantitative Results from a Random $1 Bill Sample Extracted with 7 mL of Methanol and Ultrasonicated for 30 Minutes.

Compound	Amount Extracted off $1 Bill into 7 mL of Methanol
Oxycodone	573.29 ng
Amphetamine	10.98 ng
Hydrocodone	8.37 ng
Methamphetamine	473.32 ng
MDMA	19.31 ng
Heroin	7.48 ng
Cocaine	84436.86 ng
PCP	34.53 ng
Methadone	8.68 ng
Diazepam	15.89 ng
THC	57.42 ng

Chromatographic Results from a Random $1 Bill Sample Extracted with 7 mL of Methanol and Ultrasonicated for 30 Minutes.



Conclusions

A complex forensic analysis of 25 compounds, that was originally performed on an Agilent ZORBAX RRHT Eclipse Plus C18 column, was easily carried out on a superficially porous Agilent Poroshell 120 EC-C18 column with high quality results and substantial time savings. Other complex analyses can likely be transferred from 1.8-µm Eclipse Plus to Poroshell 120 EC-C18 of the same dimension without method modification, due to very similar selectivity and efficiency. The lower back pressure of Poroshell 120's 2.7-µm particles can be exploited for productivity gains; faster flow rates may be used to shorten analysis time without exceeding system pressure limits for 400 bar HPLC's or higher pressure UHPLC's. This method was used to detect and quantify several drugs found on a $1 bill, including: cocaine, oxycodone, methamphetamine, PCP and THC.