# Fast Analysis of Illicit Drug Residues on Currency using Agilent Poroshell 120

## Application Note

Forensics and Toxicology


## Authors

Anne E. Mack, James R. Evans and
William J. Long
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808
USA



## Abstract

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 and toxicology analyses using an Agilent 1200 Series LC system with an Agilent 6410 Triple Quadrupole LC/MS. 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 p/n 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.


Agilent Technologies

## Introduction

The interest in superficially porous particles has led to discussions of method transfer from larger 5-µm totally porous particles, as well as from sub-2-µm totally porous particles. The high efficiency of superficially porous particles is similar to sub-2-µm totally porous particles. This is due to short mass transfer distance and substantially narrower particle size distribution.

The benefit of transferring from larger particle columns is very significant time savings, because the superficially porous particles are optimally run at faster flow rates (usually double) and are able to achieve similar resolution with a much shorter column length [1-2]. Because analysts will likely change column length and flow rate when transferring from larger totally porous particles to superficially porous columns, calculations must be performed to proportionally scale a gradient method and preserve the chromatographic selectivity (Equation 1).

**Equation 1**

$$t_2 = \frac{t_1 \bullet d_2^2 \bullet L_2 \bullet F_1}{d_1^2 \bullet L_1 \bullet F_2}$$

*Where:*

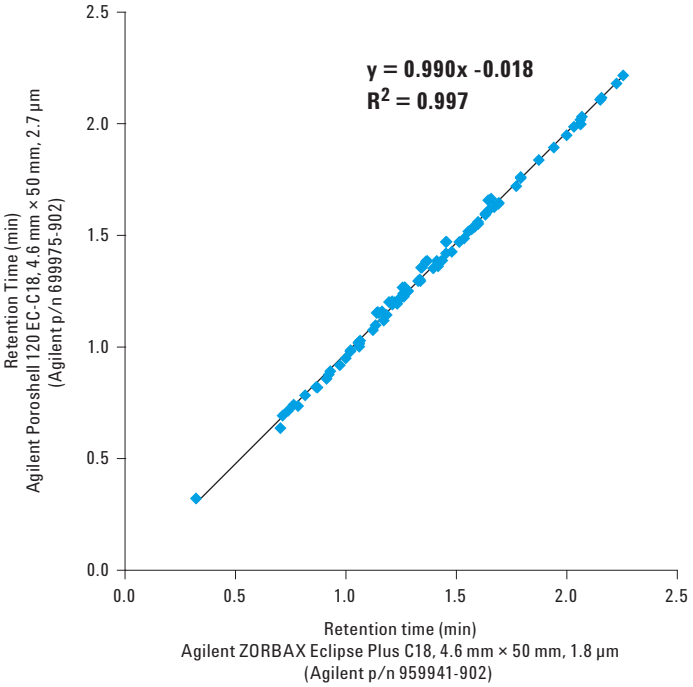
- *$t_1$ and $t_2$ are the original and new gradient times (min)*
- *$d_1$ and $d_2$ are the original and new column internal diameters (mm)*
- *$L_1$ and $L_2$ are the original and new column lengths (mm)*
- *$F_1$ and $F_2$ are the original and new flow rates (mL/min)*

In some cases, it may be useful to take advantage of the lower back pressure associated with superficially porous columns as compared to totally porous sub-2-µm columns. Depending upon operating conditions, the back pressure can be up to 50% less. This can give analysts the freedom to increase flow rates for higher throughput, or to increase column length to enhance resolution without exceeding the system pressure limits. Adjustments to flow rate and/or column length will require gradient scaling (Equation 1).

Method transfer can be especially easy, when columns like the superficially porous Agilent Poroshell 120 EC-C18 and totally porous Agilent ZORBAX Eclipse Plus C18 are manufactured to have similar bonding chemistries and use similar retention mechanisms. Figure 1 shows the similar retention of 90 compounds on Poroshell 120 EC-C18 and Eclipse Plus C18 columns using a generic gradient analysis with a variety of compounds from different chemical classifications. The high correlation coefficient ($R^2$) indicates a high degree of similarity between the interactions involved in the separation on the two C18 columns, while the slope ≈1 implies similar interaction strengths [3-4]. However, while many compounds give similar selectivity, it cannot be guaranteed that every application will transfer without adjustment.

This application note shows how a Poroshell 120 column can be used in a complex analysis, previously performed on a 1.8 µm column. This separation was demonstrated on Eclipse Plus in a previous Agilent application note (Publication Number 5990-4254EN) [5]. A 25-component LC/MS Toxicology Test Mixture (Agilent p/n 5190-0470) is used to illustrate the interchangeability between the two columns. Calibration curves for each of the 25 compounds on Poroshell 120 are constructed. A $1 bill is extracted in methanol to show significant presence of cocaine, as well as noticeable quantities of oxycodone, methamphetamine, PCP and THC. Trace amounts of several more illicit and prescription drugs can be detected also. Drug trafficking is assumed to be the cause for their initial presence on US currency, while ATM's and counting machines are likely the cause of their widespread presence [6]. Additionally, this gradient analysis is transferred to a Poroshell 120 SB-C18 column, which shows some selectivity differences; however it can be run at higher temperatures to allow for even faster flow rates and analysis times. Agilent Poroshell 120 columns are availabe with two different C18 phases in order to change selectivity and still have a C18 column choice. Flow rates were increased to reach 400 and 600 bar to show performance achievable on both conventional HPLC's and newer UHPLC's.

## Agilent Poroshell 120 EC-C18 has Very Similar Selectivity to Agilent ZORBAX Eclipse Plus C18




| | |
|---|---|
| Mobile phase: | A: 10 mM ammonium formate, pH 3<br>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 $H_2O$ |

| | | | | |
|---|---|---|---|---|
| Furazolidone | Biphenyl | Acetanilide | DL phenylalanine | Oxybutynin chloride 1 |
| Chloramphenicol | Acenaphthene | Fenoprofen | Doxepin hydrochloride | Diphenhydramine |
| Pyrimethamine | Methoxy naphthalene | Catechol | Ephedrine hydrochloride | Diflunisal |
| Sulfaquinoxaline | Anisole | Phenol | Loperamide | Nisoldipine |
| Sulfamonomethoxine | Dimethoxy benzene | Resorcial | Procaine hydrochloride | Diclofenac sodium salt |
| Nimopidin | Corticosterone | Hydroquinone | Fenoprofen calcium salt hydrate | Hydrocortisone |
| Sulfadimethoxine | Alpha hydroxyprogesterone | 4 nitro phenol | Erythromycin | 4 hydroxybenzoic acid |
| Sulfamethoxazole | Porgesterone | O cresol | Econazole nitrate | Procainamide hydrochloride |
| Sulfachloropyridazine | Alpha hydroxyprogesterone | P cresol | Gemfibrozil | Lidocaine |
| Sulfamethoxypyridazine | Prednisolone | 3,4 dimethyl phenol | Beta estradiol | Terfenadine |
| Sulfamethizole | Mestranol | 2,3 dimethyl phenol | Metoprolol | Terfenaine |
| Sulfamethazine | Deoxycorticosterone | 2 nitro phenol | Prednisone | Chlortetracycline hydrochloride |
| Sulfamerazine | Progesterone | 2,4 dimethyl phenol | Protriptyline | Chlorpheniramine maleate salt |
| Sulfathiazole | Chlorphenamine | 2,5 dimethyl phenol | 2-hydroxyhippuric acid | Chloramphenicol |
| Sulfadiazine | Berberine | 1 napthol | Hydroxyisophthalic acid | Buspirone hydrochloride |
| Benzaldehyde | Impramithue | Imipramine hydrochloride | Flufenamic acid | Benzocaine |
| Iodobenzene | Norethindrone | D methionine | Pramoxine hydrochloride | Antipyrine |
| Phenanthrene | Phenacetin | 3,4 dihydroxy-l-phenyl alanine | Naproxen | Acetylsalicyclic acid |

*Figure 1. Scatter plot of retention time of 90 compounds on Agilent Poroshell 120 EC-C18 versus Agilent Eclipse Plus C18.*

## Experimental

An Agilent 1200 Series Rapid Resolution LC (RRLC) system with an Agilent 6410 Triple Quadrupole LC/MS system 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 column dimensions and flow rate (Table 1).
- G1367C Automatic Liquid Sampler (ALS) SL. Injection volume was 1.0 µL.
- G1316B Thermostated Column Compartment (TCC) SL with temperature set to 60 °C or 90 °C (on Poroshell 120 SB-C18 only).
- 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.

Three Agilent columns were used in this work:

- Agilent Poroshell 120 EC-C18, 2.1 mm × 100 mm, 2.7 µm (p/n 695775-902)
- Agilent Poroshell 120 SB-C18, 2.1 mm × 100 mm, 2.7 µm (p/n 685775-902)
- Agilent ZORBAX RRHT Eclipse Plus C18, 2.1 mm × 100 mm, 1.8 µm (p/n 959764-902)

The compounds of interest are shown in Table 2, with their respective retention times on Poroshell 120 EC-C18 at 0.5 mL/min, and their qualitative and quantitative MRM transitions. Sample is a 1 µg/mL standard in methanol purchased from Agilent Technologies (LC/MS Toxicology Test Mixture, Agilent p/n 5190-0470). Serial dilutions in methanol were prepared for the calibration standards. The $1 bill sample was extracted in 7 mL of methanol and ultrasonicated for 30 min. Additionally, acetonitrile, formic acid and ammonium formate were purchased from Sigma Aldrich (Bellefont, PA). Methanol was purchased from Honeywell, Burdick and Jackson (Muskegon, MI). Water used was 18 M-Ω Milli-Q water (Bedford, MA).

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

| Gradient and method parameters | 2.1 × 100 mm 1.8-µm Agilent ZORBAX Eclipse Plus C18 | 2.1 × 100 mm 2.7-µm Agilent Poroshell 120 EC-C18 | 2.1 × 100 mm 2.7-µm Agilent Poroshell 120 EC-C18 | 2.1 × 100 mm 2.7-µm Agilent Poroshell 120 EC-C18 | 2.1 × 100 mm 2.7-µm Agilent Poroshell 120 SB-C18 | 2.1 × 100 mm 2.7-µm Agilent Poroshell 120 SB-C18 | 2.1 × 100 mm 2.7-µm Agilent Poroshell 120 SB-C18 |
|---|---|---|---|---|---|---|---|
| Flow rate (mL/min) | 0.5 | 0.5 | 0.7 | 1.0 | 0.5 | 0.9 | 1.4 |
| 10% B (min) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 15% B (min) | 0.50 | 0.50 | 0.36 | 0.25 | 0.50 | 0.28 | 0.18 |
| 50% B (min) | 3.00 | 3.00 | 2.14 | 1.50 | 3.00 | 1.67 | 1.07 |
| 95% B (min) | 4.00 | 4.00 | 2.86 | 2.00 | 4.00 | 2.22 | 1.43 |
| 95% B (min) | 6.00 | 6.00 | 4.29 | 3.00 | 6.00 | 3.33 | 2.14 |
| Stop time (min) | 6.00 | 6.00 | 4.29 | 3.00 | 6.00 | 3.33 | 2.14 |
| Post run time (min) | 2.00 | 2.00 | 1.43 | 1.00 | 2.00 | 1.11 | 0.71 |
| Overall cycle time (min) | 8.00 | 8.00 | 5.71 | 4.00 | 8.00 | 4.44 | 2.86 |
| TCC temperature (°C) | 60 | 60 | 60 | 60 | 90 | 90 | 90 |
| Injection volume (µL) | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| System pressure (bar) | 375 | 280 | 385 | 550 | 195 | 370 | 595 |

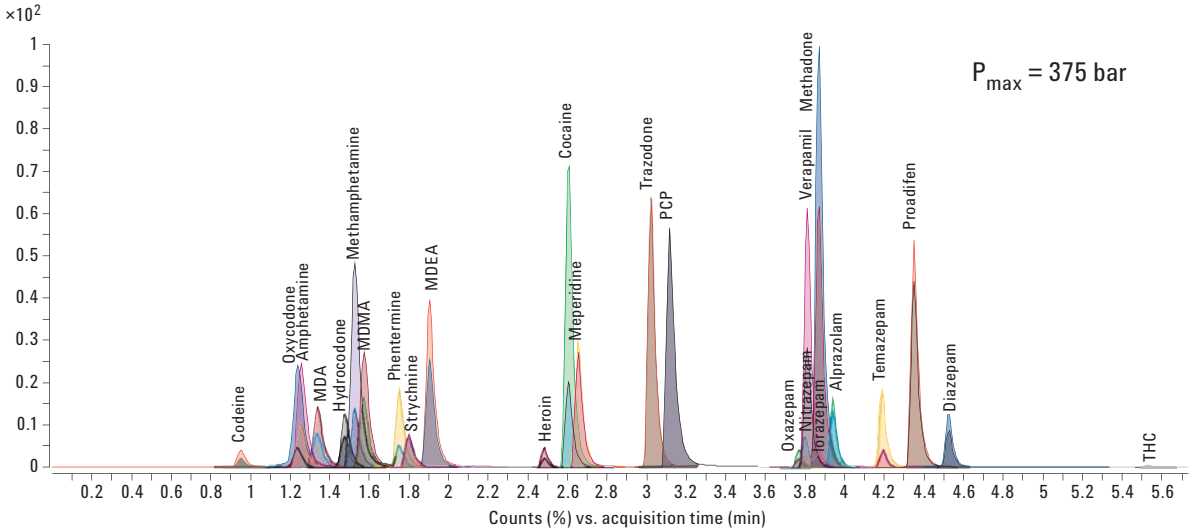
*Table 2. MRM Transitions for 25 Compounds in Toxicology Test Mixture*

| Compound name | Precursor ion | Fragmentor voltage | Product ion 1 | Collision energy 1 | Product ion 2 | Collision energy 2 | Retention time (min) | Delta retention time |
|---|---|---|---|---|---|---|---|---|
| Codeine | 300.2 | 158 | 165.1 | 45 | 58.1 | 29 | 0.89 | 0.4 |
| Oxycodone | 316.2 | 143 | 298.1 | 17 | 256.1 | 25 | 1.14 | 0.4 |
| Amphetamine | 136.1 | 66 | 119.1 | 5 | 91 | 17 | 1.19 | 0.4 |
| MDA | 180.1 | 61 | 163 | 5 | 105 | 21 | 1.25 | 0.4 |
| Hydrocodone | 300.2 | 159 | 199 | 29 | 128 | 65 | 1.34 | 0.4 |
| Methamphetamine | 150.1 | 92 | 119 | 5 | 91 | 17 | 1.43 | 0.4 |
| MDMA | 194.1 | 97 | 163 | 9 | 105 | 25 | 1.46 | 0.4 |
| Strychnine | 335.2 | 195 | 184 | 41 | 156 | 53 | 1.66 | 0.4 |
| Phentermine | 150 | 66 | 133 | 5 | 91 | 25 | 1.66 | 0.4 |
| MDEA | 208.1 | 107 | 163 | 9 | 105 | 25 | 1.8 | 0.4 |
| Heroin | 370.2 | 149 | 268.1 | 37 | 165 | 61 | 2.4 | 0.4 |
| Cocaine | 304.2 | 138 | 182.1 | 17 | 77 | 61 | 2.52 | 0.4 |
| Meperidine | 248.2 | 128 | 220.1 | 21 | 174.1 | 17 | 2.59 | 0.4 |
| Trazodone | 372.2 | 159 | 176 | 25 | 148 | 37 | 2.95 | 0.4 |
| PCP | 244.2 | 86 | 91 | 41 | 86.1 | 9 | 3.05 | 0.4 |
| Oxazepam | 287 | 150 | 269 | 12 | 241 | 20 | 3.66 | 0.4 |
| Nitrazepam | 282.1 | 148 | 236.1 | 25 | 180 | 41 | 3.66 | 0.4 |
| Verapamil | 455.3 | 158 | 165 | 37 | 150 | 45 | 3.75 | 0.4 |
| Lorazepam | 321 | 102 | 275 | 21 | 194 | 49 | 3.75 | 0.4 |
| Methadone | 310.2 | 112 | 265.1 | 9 | 105 | 29 | 3.83 | 0.4 |
| Alprazolam | 309.1 | 179 | 281 | 25 | 205 | 49 | 3.84 | 0.4 |
| Temazepam | 301.1 | 117 | 255.1 | 29 | 177 | 45 | 4.05 | 0.4 |
| Proadifen | 354.2 | 153 | 167 | 29 | 91.1 | 45 | 4.33 | 0.4 |
| Diazepam | 285.1 | 169 | 193 | 45 | 154 | 25 | 4.41 | 0.4 |
| THC | 315.2 | 150 | 193.2 | 20 | 123.3 | 30 | 5.4 | 0.4 |

## Results and Discussion

Figure 2 shows the original method developed by P. Stone on an Agilent ZORBAX Eclipse Plus C18 2.1 mm × 100 mm, 1.8 µm column. This analysis is accomplished in 6 min with a 2-min post run time at 375 bar. Figure 3 shows the same method with an Agilent Poroshell 120 EC-C18 2.1 mm × 100 mm, 2.7 µm column. Analysis and post run time are identical to the Eclipse Plus method, while the system back pressure is reduced to 280 bar. While there are slight variations between elution patterns in Figures 2 and 3, overall selectivity is very similar, as would be predicted by Figure 1.

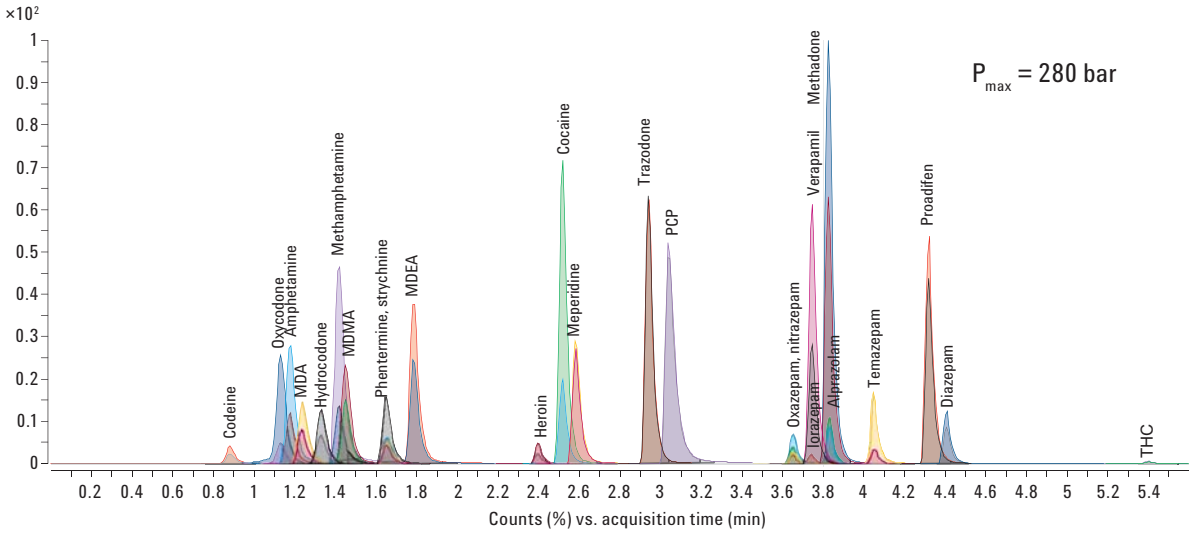
**Original Toxicology Method on Agilent ZORBAX Eclipse Plus C18 2.1 mm × 100 mm, 1.8 µm (Agilent p/n 959764-902)**




A: 5 mM ammonium formate w/ 0.01% formic acid (1 L water + 0.3153 g ammonium formate + 0.1 mL formic acid),
B: acetonitrile w/ 0.01% formic acid (1 L acetonitrile + 0.1 mL formic acid); 0.5 mL/min; 10% B at $t_0$, ramp to 15% B in 0.5 min,
ramp to 50% B in 2.5 min, ramp to 95% B in 1 min, hold 95% B for 2 min; stop time 6 min, post run 2 min;
Sample: injector program: draw 5 µL water, draw 1 µL LC/MS Toxicology Test Mixture (p/n 5190-0470), inject; TCC = 60 °C
MS Source: electrospray AP-ESI, drying gas temperature and flow: 350 °C, 12 L/min, nebulizer gas pressure: 30 psi, capillary voltage: 2000V;
MS Acquisition: dynamic MRM (see Table 2 for MRM transitions), positive ionization polarity

*Figure 2. Agilent LC/MS Toxicology Test Mixture (Agilent p/n 5190-0470) analyzed on Agilent ZORBAX Eclipse Plus C18 via an Agilent 1200 Series LC system with detection by an Agilent 6410 Triple Quadrupole LC/MS.*

**Original Toxicology Method on Agilent Poroshell 120 EC-C18 2.1 mm × 100 mm, 2.7 µm (Agilent p/n 695775-902)**




A: 5 mM ammonium formate w/ 0.01% formic acid (1 L water + 0.3153 g ammonium formate + 0.1 mL formic acid),
B: acetonitrile w/ 0.01% formic acid (1 L acetonitrile + 0.1 mL formic acid); 0.5 mL/min; 10% B at $t_0$, ramp to 15% B in 0.5 min,
ramp to 50% B in 2.5 min, ramp to 95% B in 1 min, hold 95% B for 2 min; stop time 6 min, post run 2 min;
Sample: injector program: draw 5 µL water, draw 1 µL LC/MS Toxicology Test Mixture (p/n 5190-0470), inject; TCC = 60 °C
MS Source: electrospray AP-ESI, drying gas temperature and flow: 350 °C, 12 L/min, nebulizer gas pressure: 30 psi, capillary voltage: 2000V;
MS Acquisition: dynamic MRM (see Table 2 for MRM transitions), positive ionization polarity

*Figure 3. Agilent LC/MS Toxicology Test Mixture (Agilent p/n 5190-0470) analyzed on Agilent Poroshell 120 EC-C18 via an Agilent 1200 Series LC system with detection by an Agilent 6410 Triple Quadrupole LC/MS.*

Table 3 shows calibration data for all 25 compounds found in the Agilent LC/MS Toxicology Test Mixture on Poroshell 120. All compounds exhibit strong linear correlations, with $R^2$ > 0.9979. Calibration data was used to quantify a methanol-extracted US $1 bill sample; chromatographic and quantitative results are shown in Figure 4. A significant amount of cocaine was found on the dollar bill. Oxycodone, methamphetamine, PCP and THC were also detected. Smaller quantities of amphetamine, hydrocodone, MDMA, heroin, methadone and diazepam were also found. Quantities of these substances on US currency are consistent with previous findings [6-8].

*Table 3. Calibration Data for 25 Toxicology Compounds on Poroshell 120*

| Compound name | Linear calibration curve | Correlation coefficient, $R^2$ |
|---|---|---|
| Codeine | y = 25.4023 × + 3.1628 | 0.99990276 |
| Oxycodone | y = 138.9535 × – 0.6269 | 0.99938632 |
| Amphetamine | y = 196.3425 × + 50.1606 | 0.99987385 |
| MDA | y = 121.2945 × + 180.2165 | 0.99945701 |
| Hydrocodone | y = 72.1351 × – 8.1010 | 0.99964622 |
| Methamphetamine | y = 286.7936 × + 429.4970 | 0.99789141 |
| MDMA | y = 121.4217 × – 55.0435 | 0.99874569 |
| Phentermine | y = 110.8083 × – 65.1028 | 0.99914972 |
| Strychnine | y = 39.3465 × – 9.5339 | 0.99964358 |
| MDEA | y = 200.4804 × – 14.2886 | 0.99980092 |
| Heroin | y = 18.2969 × + 0.4442 | 0.99987634 |
| Cocaine | y = 295.8654 × – 5.6261 | 0.99963342 |
| Meperidine | y = 145.0367 × + 17.2273 | 0.99986118 |
| Trazodone | y = 286.1986 × – 12.4408 | 0.99969366 |
| PCP | y = 287.4395 × – 24.8090 | 0.99989199 |
| Oxazepam | y = 14.7883 × – 0.4919 | 0.99900677 |
| Nitrazepam | y = 49.1750 × + 69.2747 | 0.99876656 |
| Verapamil | y = 273.3001 × + 17.3890 | 0.99986678 |
| Lorazepam | y = 11.2911 × + 6.0687 | 0.99896851 |
| Methadone | y = 439.7238 × – 6.7890 | 0.9997511 |
| Alprazolam | y = 80.2721 × + 18.5435 | 0.99969734 |
| Temazepam | y = 70.9899 × + 15.5246 | 0.99976598 |
| Proadifen | y = 243.9474 × – 13.0696 | 0.99990655 |
| Diazepam | y = 68.9622 × + 26.0608 | 0.99948978 |
| THC | y = 3.1838 × – 2.7072 | 0.99801611 |

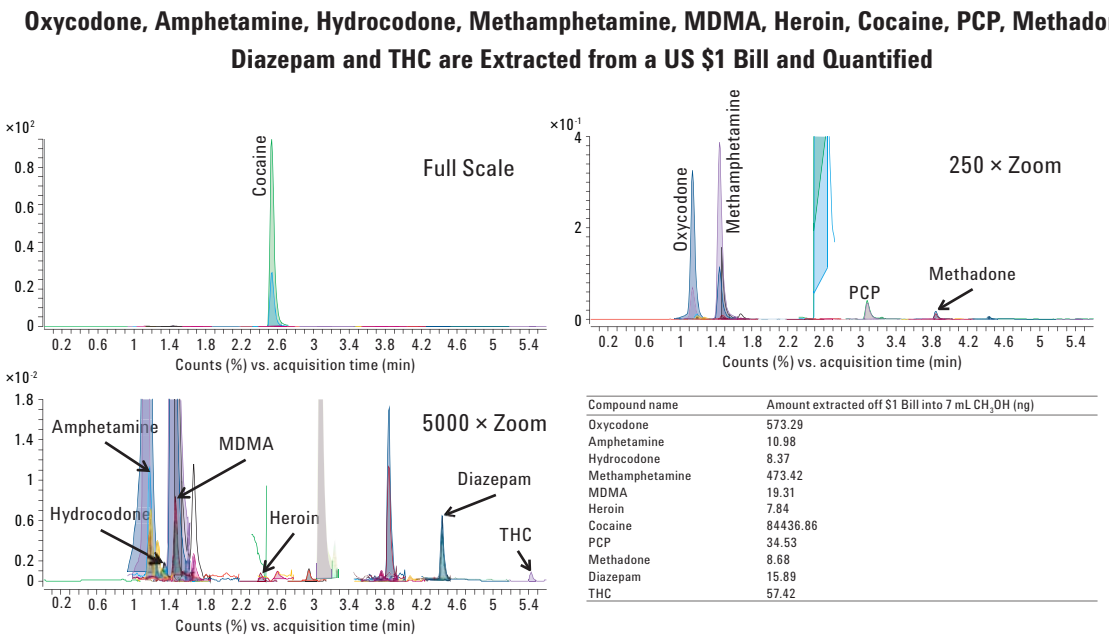



*Figure 4. Chromatographic and quantitative results from a random US $1 bill sample extracted with 7 mL of methanol and ultrasonicated for 30 minutes.*

Due to the low system back pressure generated with the Poroshell 120 column, the flow rate can be increased from 0.5 mL/min to 0.7 mL/min without exceeding 400 bar for use on a standard HPLC, or it can be increased to 1 mL/min without exceeding 600 bar for use on a UHPLC, as shown in Figure 5. The increased flow rate may be desirable when high throughput is important and when a UHPLC is available for use. Overall cycle time can be decreased by 2.3 minutes while keeping pressure below 400 bar, or by 4 minutes while keeping pressure below 600 bar (a 50% reduction in cycle time). Increasing the flow rate to this degree does cause some loss in resolution, but with MS detection this is not critical.

**Significant Time Savings are Possible by Increasing Flow Rate with Agilent Poroshell 120 EC-C18 to LC System Pressure Limits, whether 400 or 600 bar**

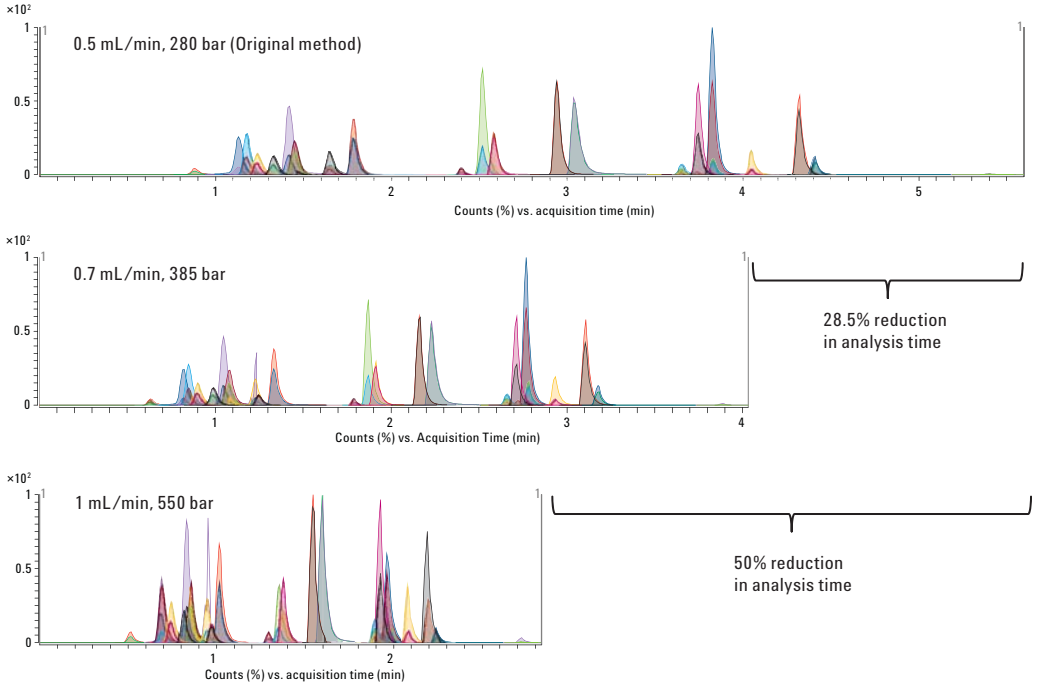



*Figure 5. Overlay of Agilent Poroshell 120 EC-C18 toxicology analysis showing time savings by increasing flow rate to reach a 400 or 600 bar system limit.*

Flow rate can be further increased by elevating temperature, thereby reducing mobile phase viscosity. The original method however was run at 60 °C, which is the maximum operating temperature for both Eclipse Plus C18 and Poroshell 120 EC-C18. In order to perform this analysis at a higher temperature, the column must be replaced with a Poroshell 120 SB-C18, which has a maximum operating temperature of 90 °C. Figure 6 shows the fast chromatography possible with Poroshell 120 SB-C18. With a 600 bar system pressure limit, it is possible to reduce run time by 64.3%, however this comes at the cost of reduced resolution. For an analysis as complex as this toxicology method, this loss of resolution and significant coelution will cost the analysts a reduction in data points across all peaks, therefore reducing the quality of the results. A simple solution may be to increae column length. A slight increase in column length from 100 mm to 150 mm will increase the resolution of all compounds. While the longer column cannot be run at quite as fast flow rates the analyst can still glean significant time savings by running it at its respective highest flow rate without exceeding system limitations.

**Very Significant Time Savings are Possible by Increasing Temperature and Flow Rate with Agilent Poroshell 120 SB-C18 to LC System Pressure Limits, whether 400 or 600 bar**

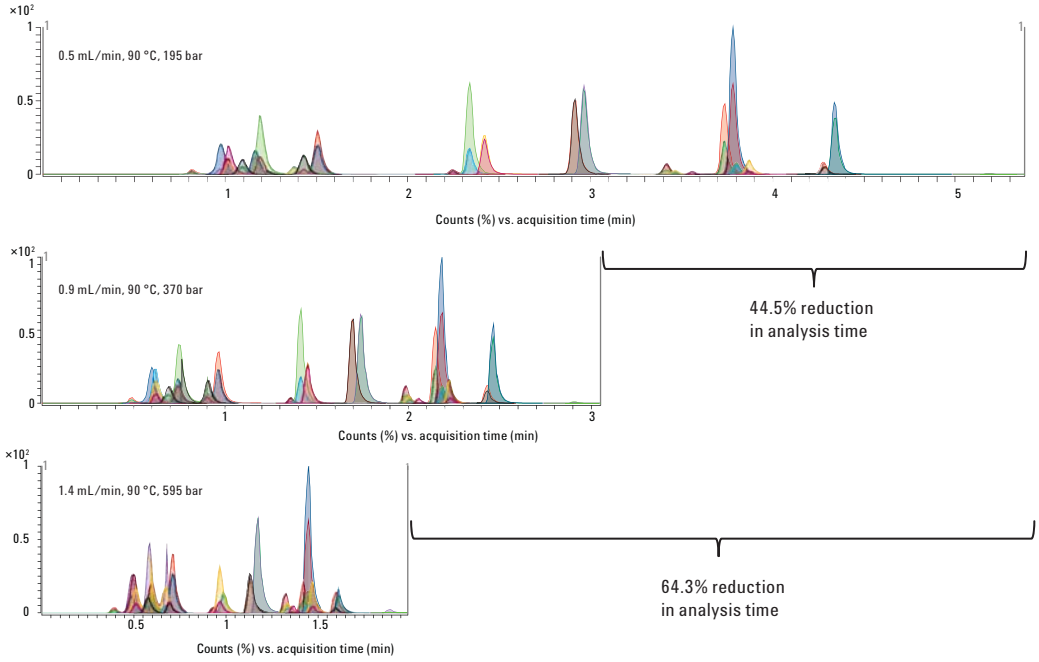



*Figure 6. Overlay of Agilent Poroshell 120 SB-C18 toxicology analysis showing time savings by increasing temperature and flow rate to reach a 400 or 600 bar system limit.*

## Conclusion

A complex analysis of 25 toxicology compounds, that was originally performed on an Agilent ZORBAX 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 C18 to Poroshell 120 EC-C18 of the same dimensions 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 of abuse found on a $1 bill, including: cocaine, oxycodone, methamphetamine, PCP and THC.

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