Fast LC/MS/MS Analytical Method with Alternate Column Regeneration for the Analysis of 125 Various Drugs and Their Metabolites in Urine in Clinical Research

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Introduction

Liquid chromatography triple quadrupole mass spectrometry (LC/MS/MS) is well suited for the rapid analysis of large numbers of analytes using a single method. A highly sensitive, specific and fast LC/MS/MS analytical method has been developed for the quantitation of 125 analytes (Table 1) of the following drug classes: antidepressants, benzodiazepines, opioids, muscle relaxants, hallucinogens, stimulants. The described method achieves high analytical sensitivity and is capable of quantitating analytes over a wide dynamic range, in addition the Alternate Column Regeneration (ACR) hardware configuration was employed to significantly increase the sample throughput. The analytical methodology was developed on an Agilent 1290 Infinity II UHPLC and 6470 TQ Mass Spectrometer with a 7.35 minute analysis time (5 minute gradient + 1.5 minute equilibration + 0.85 minute injection). ACR reduced the analysis time by 30% to 5.1 minutes, injection to injection. The ability to combine many analytes into a single run coupled with a fast analytical method and ACR could significantly improve turnaround time in a clinical research laboratory.

2-Hydroxyethylflurazepam	Fentanyl	Norcodeine
6-MAM	Flunitrazepam	Nordiazepam
7-Aminoclonazepam	Fluoxetine	Norfentanyl
7-Aminoflunitrazepam	Flurazepam	Norhydrocodone
alpha-Hydroxyalprazolam	Gabapeptin	Norketamine
alpha-Hydroxytriazolam	Heroine	Normeperidine
alpha-PVP	Hydrocodone	Normorphine
Alprazolam	Hydromorphone	Noroxycodone
Amitriptyline	Imipramine	Noroxymorphone
Amo-Pentobarbital	Ketamine	Norpropoxyphene
Amphetamine	Lorazepam	Norsertraline
Anabasine	Maprotiline	Nortriptyline
Benzoylecgonine	MDA	o-Desmethyl-cis-Tramadol
Bromazepam	MDEA	Oxazepam
Buprenorphine	MDMA	Oxycodone
Buprenorphine Glucuronide	MDPV	Oxymorphone
Butabarbital	Meperidine	Paroxetine
Butalbital	Meprobamate	PCP (Phencyclidine)
Carisoprodol	Methadone	Pentazocine
Chlordiazepoxide	Methamphetamine	Phenobarbital
Citalopram	Methylone	Phentermine
Clobazam	Methylphenidate	Pregabalin
Clomipramine	m-Hydroxybenzoylecgonine	Primidone
Clonazepam	Mianserin	Propoxyphene
Cocaethylene	Midazolam	Protriptyline
Cocaine	Mitragynine	Ritalinic Acid
Codeine	Mirtazapine	Secobarbital
Cotinine	Morphine	Sertraline
Cyclobenzaprine	Naloxone	Tapentadol
Desalkylflurazepam	Naltrexone	Temazepam
Desipramine	N-Desmethyl-cis-tramadol	THC
Desmethyldoxepin	N-Desmethylclobazam	THC-A [(-)-11-nor-9-Carboxy-Δ9-THC]
Dextromethophran	N-Desmethylclomipramine	THC-OH
Dextrophan	N-Desmethylcyclobenzaprine	Tramadol
Diazepam	N-Desmethylmirtazapine	Triazolam
Dihydrocodeine	N-Desmethyltapentadol	Trimipramine
Doxepin	N-Desmethyltrimipramine	Zaleplon
EDDP	Nicotine	Zolpidem
Estazolam	Nitrazepam	Zolpidem Phenyl-4-carboxylic Acid
Ethyl glucuronide	Norbuprenorphine	Zopiclone
Ethyl Sulfate	Norbuprenorphine Glucuronide	

Table1. 125 Compounds List

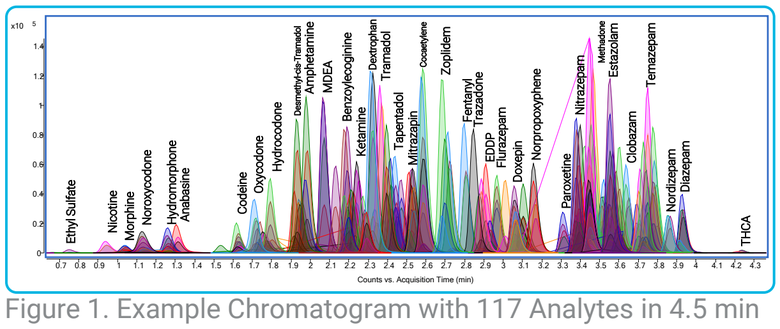

Figure 1. Example Chromatogram with 117 Analytes in 4.5 min

Experimental

Standards and Curve Preparation

Standards were spiked into drug free human urine solution (10%) and 10 µL was injected into the LC/MS system. The calibration curve was prepared by serial dilution, concentrations ranged from 1 to 1000 ng/mL. Internal standards were added at 125 ng/mL.

LC/MS/MS Analytical Method

The LC/MS/MS consisted of a 1290 Infinity II UHPLC system with 2 binary pumps, thermostatted multisampler, temperature controlled column compartment with 2 position-10 port valve and 6470 triple quadrupole mass spectrometer. The system was configured as shown in Figure 3.

Experimental

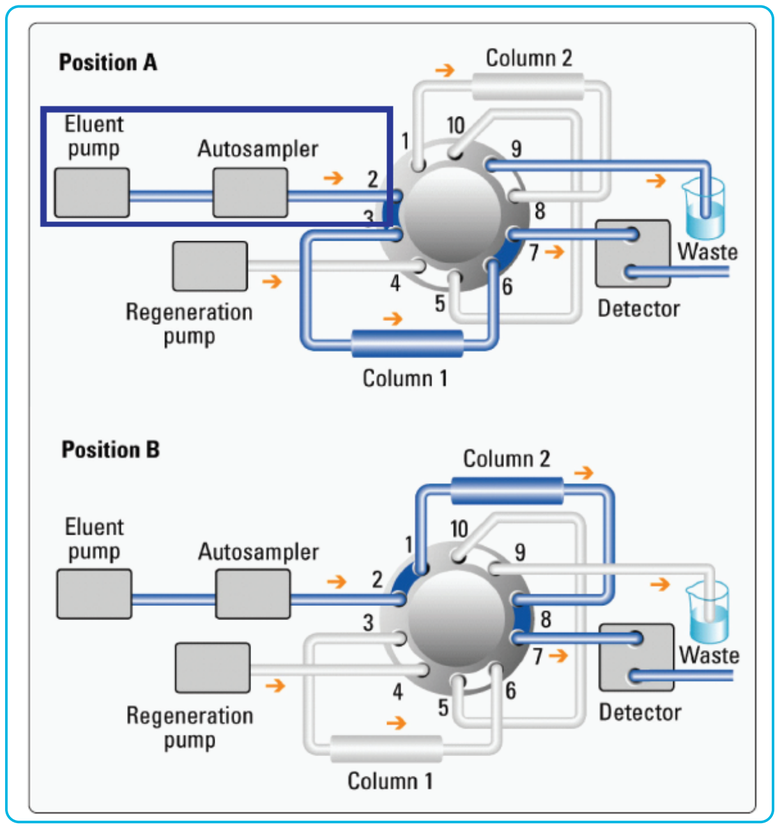

Figure 2. Alternating Column Regeneration valve configuration

Eluent pump: flowrate 0.35 mL/min Gradient: 0.00 min 12 % B 0.30 min 12 % B 1.20 min 40 % B 2.90 min 70 % B 3.30 min 98 % B 4.00 min 98 % B 4.01 min 12 % B Stop time: 4.75 min	Regeneration pump: flowrate 0.5 mL/min Gradient: 0.00 min 98% B 2.00 min 98% B 2.01 min 12% B Stop time: no limit Valve Position V1 0.00 min Current Position 4.70 min Next Position

Table 2. ACR pump gradients and switching valve timing

Columns	2 Agilent Poroshell 120 EC-C18, 2.1 x 100 mm, 2.7 µm
Injection Volume	1 µL
Mobile Phase A	H_2O + 5 mM Ammonium Formate + 0.01% Formic Acid
Mobile Phase B	Methanol + 0.01% Formic Acid
Needle Wash	50:20:20:10 IPA:MeOH:ACN:H2O
Autosampler Temp	5 °C
Column Temp	55 °C
Flow Rate	0.35 mL/min
Stop Time	5.0min

Table 3. UHPLC Conditions

	Positive Mode	Negative Mode	Units
Gas Temp	300	300	°C
Gas Flow	9	9	L/min
Nebulizer Pressure	30	30	psi
Sheath Gas Temp	380	380	°C
Sheath Gas Flow	11	11	L/min
Capillary Voltage	3750	3500	V
Nozzle Voltage	500	500	V
Delta EMV	0	100	V

Table 4 6470 AJS Source Conditions

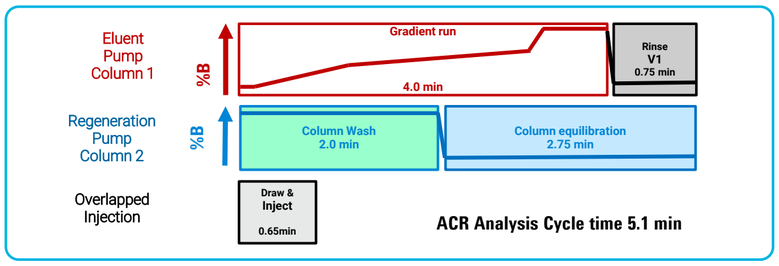

Figure 3. Graphical Timeline for ACR Analysis

Results and Discussion

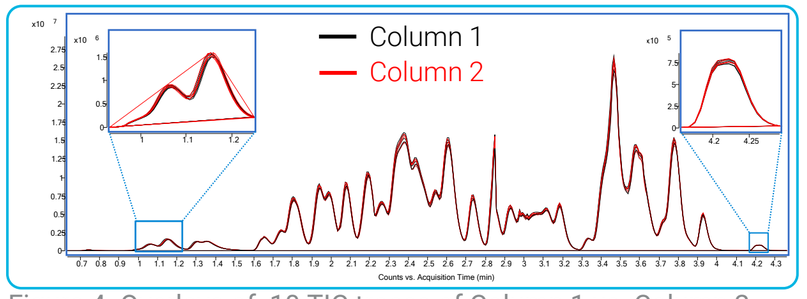

Figure 4. Overlays of 10 TIC traces of Column 1 vs. Column 2

Columns Comparison Test

Two columns used in this method were tested for peak retention time reproducibility by making 5 injection for each column. Both columns were virtually identical as shown in Figure 4.

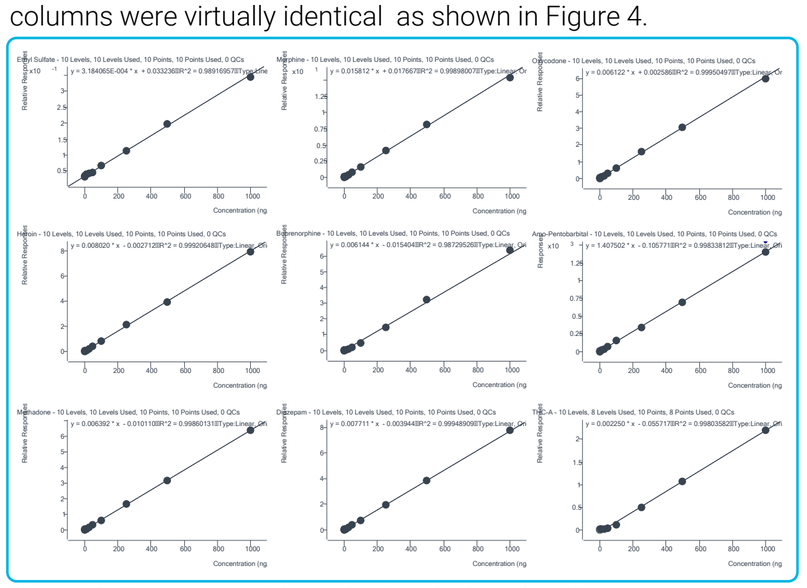

Figure 5. Examples of Quantitative Calibration Curves

Calibration Curves

Majority of calibration curves were linear from 1 to 1000 ng/mL for few of analytes quartic fit was used. Examples of calibration curves are shown in Figure 5.

Exp. Conc.	Nicotine		Morphine		Fentanyl		Buprenorphine		Hydrocodone	
(ng/mL)	Resp.	Final Conc.	Resp.	Final Conc.	Resp.	Final Conc.	Resp.	Final Conc.	Resp.	Final Conc.
1	200	1.55	570	1.14	870	1.44	27	0.97	217	1.10
2.5	368	2.31	1042	2.98	2465	2.31	64	2.62	568	2.24
5	830	4.56	1171	3.71	6029	4.30	117	4.94	1402	4.98
10	1686	8.56	2514	8.77	14477	8.87	251	10.41	2803	9.52
25	4578	22.43	6312	24.58	38876	22.64	631	26.78	7697	25.54
50	8992	44.09	12293	50.69	83930	48.17	1229	51.33	14557	49.65
100	19647	94.60	24286	98.99	170035	94.79	2428	99.98	28752	100.11
250	53201	257.86	59423	260.40	451903	261.34	5942	251.24	70769	264.18
500	104254	497.40	115458	514.02	865763	515.45	11545	501.76	125968	498.01
1000	205825	1010.14	192631	998.16	1556435	984.16	19263	996.85	209324	999.18

Table 5. Examples of Quantitative Results

Quantitation Results

Examples of quantitation results are shown in Table 5. This was a 10 point calibration curve ranging from 1 ng/mL to 1000 ng/mL for all compounds. All compounds were analyzed down to 1 ng/mL (1pg on column injection) and 104 showed a signal to noise better then 10 at 1 ng/mL level and 112 curves were linear from 1 to 1000 ng/mL analysis range, for others, quadratic fit was used. Excellent reproducibility was observed for majority of analytes (CV < 15%) for all techniques and configurations.

Conclusions

This fast, sensitive, simple, specific and accurate analytical LC/MS/MS method was developed and verified for the simultaneous measurement of 125 various drugs and their metabolites in urine. The use of ACR reduced the analysis turnaround time by 30%. Future work will include testing multiple sources of human urine for interferences that may impact the quantitation of any of the compounds in the analytical method.

References

Simultaneous Determination of Multiple Drugs of Abuse and Relevant Metabolites in Urine by LC-MS-MS, June Feng et al, J of AT, Vol. 31, September 2007

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