A Fast LC/MS/MS Method for the Simultaneous Analysis of Barbiturates and 11-nor-9-Carboxy-Δ9-Tetrahydrocannabinol (THC-A) in Urine Using Negative ESI Ionization Mode and Alternating Column Regeneration

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Introduction

The vast majority of the compounds in large toxicology drug panels are analyzed using positive mode; however, barbiturates and 11-nor-9-carboxy-Δ⁹-THC (THC-A) perform better in negative mode with the mobile phase pH favorable for the negative ionization. Included in the analysis were 8 Barbiturates and 11-nor-9-carboxy-Δ⁹-THC (Figure 1). The main goal of this work was to develop a fast method that combines Barbiturates and THC-A in a single analysis and to utilize Alternating Column Regeneration (ACR) to increase sample throughput. This analytical method employed the ability of LC/MS/MS to detect compounds over a wide range of concentrations simultaneously; the calibration concentrations ranged from 0.1 ng/mL to 5000 ng/mL. The standard curve preparation was done by spiking standards into drug free urine, diluting 1 to 10 and injecting into the LC/MS system. The methodology was developed on an Agilent 1290 Infinity II UHPLC and 6470 QQQ Mass Spectrometer with a 5 minute analytical gradient. ACR reduced the analysis time by 26% to 3.7 minutes injection to injection.

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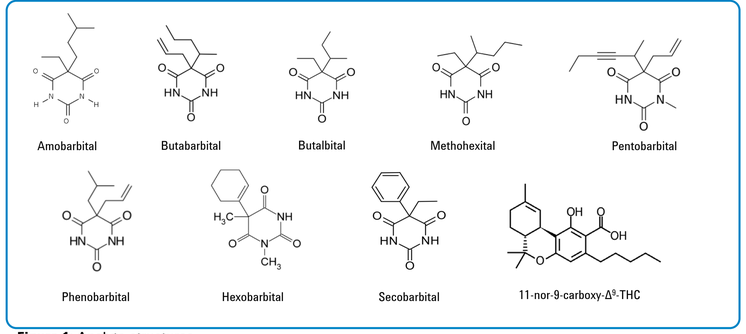

Figure 1: Analyte structures

Experimental

Standards and Curve Prep

Standards were spiked into drug free human urine solution (10%) and 10 µL was injected into the LC/MS system. The calibration curve was done by serial dilution following a pattern of 1:2:2:2.5 and concentrations ranged from 0.5 to 5000 ng/mL. Internal standards were added to final concentration of 200 ng/mL.

LC/MS/MS Analytical Method

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

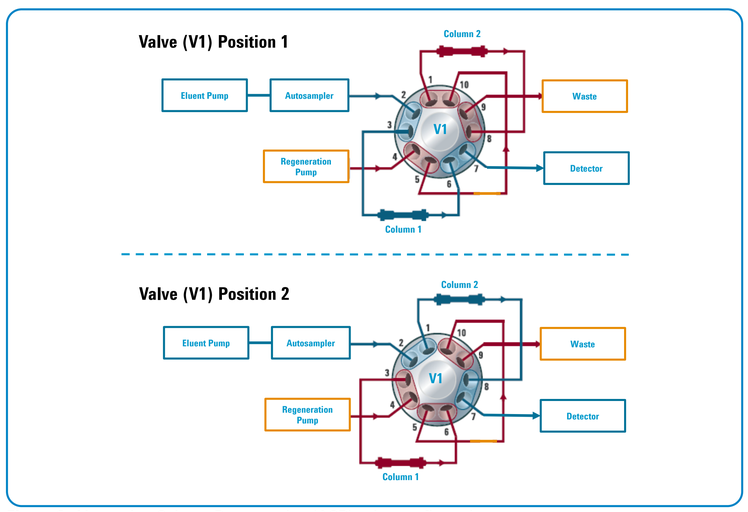

Figure 2: Alternating Column Regeneration (ACR) valve configuration

Eluent pump	**Regeneration pump**
Gradient: 0.00 min 35 % B	**Gradient: 0.0 min 98% B**
1.60 min 45 % B	**1.4 min 98% B8% B**
1.61 min 98 % B	**1.5 min 35% B**
3.00 min 98 % B	**Stop time: no limit**
3.01 min 45 % B	**Valve Position V1**
3.59 min 45 % B	**0.00 min Current Position**
Stop time: 3.65 min	**3.59 min Next Position**

Table 1: ACR pump gradients and switching valve timing

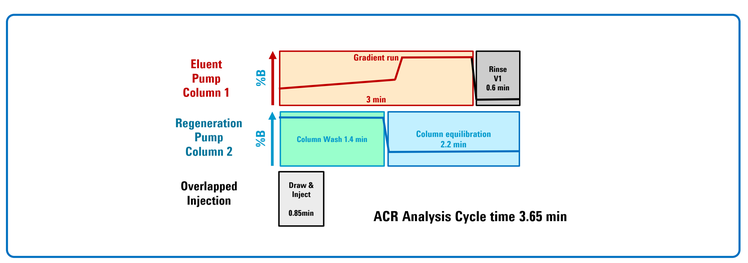

Figure 3: Graphical timeline for ACR analysis

Experimental

UHPLC and MS Method Conditions

Columns	2 Agilent Poroshell 120 EC-C18, 2.1 x 100 mm, 1.9 µm
Injection Volume	10 µL
Mobile Phase A	H_2O + 5 mM ammonium acetate
Mobile Phase B	Acetonitrile
Needle Wash	50:20:20:10 IPA:MeOH:ACN:H2O
Autosampler Temp	10 °C
Column Temp	55 °C
Flow Rate	0.35 mL/min
Stop Time	3.65 min

Table 2: UHPLC parameters

	Negative Mode	Units
Gas Temp	150	°C
Gas Flow	11	L/min
Nebulizer Pressure	30	psi
Sheath Gas Temp	350	°C
Sheath Gas Flow	11	L/min
Capillary Voltage	6000	V
Nozzle Voltage	2000	V
Delta EMV	800	V

Table 3: 6470 QQQ AJS source parameters

Compound Name	Ret Time (min)	Prec Ion	Prod Ion	CE (V)	Polarity
Amo/Pentobarbital	1.78	225.1	182	12	Negative
Amo/Pentobarbital	1.78	225.1	42	24	Negative
AmoPentobarbital-D5	1.78	230.1	42	24	Negative
Butabarbital	1.32	211.1	168	12	Negative
Butabarbital	1.32	211.1	42	40	Negative
Butalbital	1.45	223.1	180	8	Negative
Butalbital	1.45	223.1	42	36	Negative
Butalbital-D5	1.45	228.1	42	36	Negative
Hexobarbital	1.85	235.1	42	20	Negative
Methohexital	2.66	261.1	42	20	Negative
Phenobarbital	1.17	231.1	188	8	Negative
Phenobarbital	1.17	231.1	42	36	Negative
Phenobarbital-D5	1.17	236.1	42	36	Negative
Secobarbital	2.02	237.1	194	12	Negative
Secobarbital	2.02	237.1	42	36	Negative
Secobarbital-D5	2.02	242.1	42	36	Negative
THC-A	2.88	343.2	299.2	24	Negative
THC-A	2.88	343.2	245	36	Negative
THCA-D9	2.88	352.2	254.1	32	Negative

Table 4: MS transitions and dMRM acquisition details

Results and Discussion

Chromatography

The main emphasis of this work was on increased throughput. Therefore, under these chromatographic conditions, the isobars Amobarbital and Pentobarbital do not separate and are reported as a single peak. However, if there is a need for separation between Amobarbital and Pentobarbital, the gradient and the runtime could be adjusted to achieve baseline separation between those isobars, as shown in Figure 5.

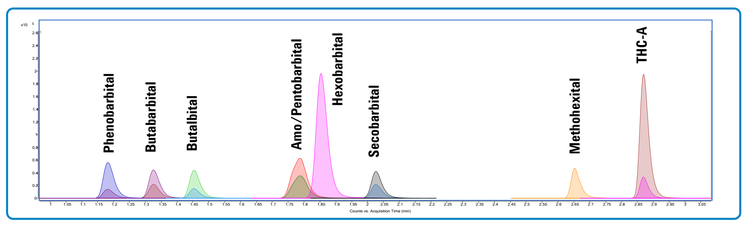

Figure 3: Example dMRM chromatogram showing elution of the 9 compounds at 500 ng/mL.

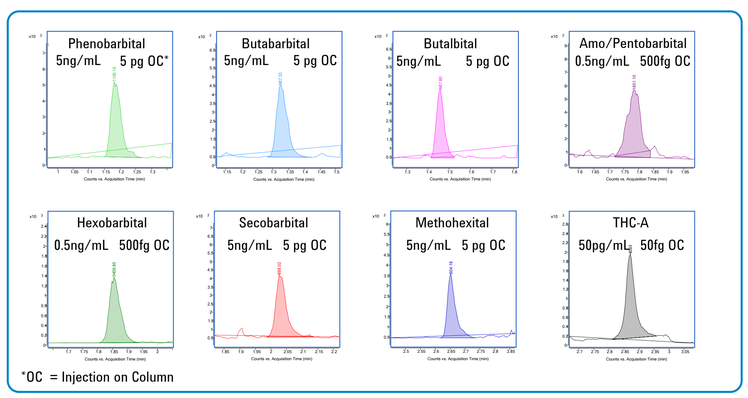

Figure 4: Chromatograms at LLOQ

Results and Discussion

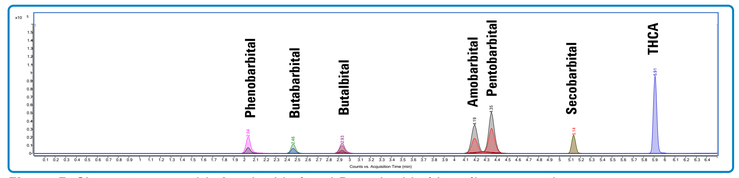

Figure 5: Chromatogram with Amobarbital and Pentobarbital baseline separation

Calibration Curves

All calibration curves were linear and a 1/x weighting factor was used. Examples of calibration curves are shown in Figure 6 and curve fit correlations (R^2) are listed in Table 5. For better visual presentation, Amo/Pentobarbital and THC-A are shown with logarithmic scales so all calibration points can be displayed.

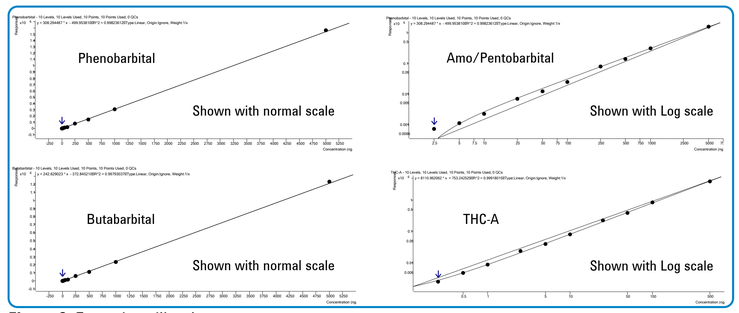

Figure 6: Example calibration curves

Name	Transition	Correlation R^2
Amo/Pentobarbital	225.1 -> 42.0	0.9963
Butabarbital	211.1 -> 42.0	0.9980
Butalbital	223.1 -> 42.0	0.9982
Hexobarbital	235.1 -> 42.0	0.9928
Methohexital	261.1 -> 42.0	0.9916
Phenobarbital	231.1 -> 42.0	0.9968
Secobarbital	237.1 -> 42.0	0.9974
THC-A	343.2 -> 299.2	0.9992

Table 5: Linear curve correlation coefficients

Quantities Results

All quantitation results are shown in Table 6. This was a 10 point calibration curve ranging from 5 ng/mL to 5000 ng/mL for all compounds except Amo/Pentobarbital and THC-A, which ranged from 0.5 ng/mL to 500 ng/mL. All compounds were analyzed down to 1 ng/mL (0.1 ng/mL for Amo/Pentobarbital and THC-A) and showed a signal/noise of 5 or better.

Sample			Phenobarbital Results			Butabarbital Results			Butalbital Results			Amo/Pentobarbital		
Name	Type	Level	RT	Final Conc.	Resp.	RT	Final Conc.	Resp.	RT	Final Conc.	Resp.	RT	Final Conc.	Resp.
002-DB	Blank		1.17	1.73	34	1.34	1.60	15	1.45	1.99	39	1.77	0.09	50
004-2p5	Cal	2.5/0.25	1.19	2.68	326	1.33	3.84	558	1.46	3.44	381	1.78	0.24	1155
005-5	Cal	5/0.5	1.18	5.21	1107	1.32	5.60	987	1.46	6.00	988	1.79	0.59	1971
006-10	Cal	10/1	1.19	8.86	2230	1.33	8.96	1802	1.46	8.99	1696	1.78	0.96	3911
007-25	Cal	25/2.5	1.18	23.92	6875	1.33	23.70	5376	1.46	23.48	5129	1.79	2.18	11737
008-50	Cal	50/5	1.18	40.24	11906	1.33	40.86	9540	1.46	39.96	9035	1.79	3.71	21176
009-100	Cal	100/10	1.18	80.44	24298	1.33	74.78	17770	1.46	78.94	18270	1.79	9.20	62732
010-250	Cal	250/25	1.19	255.89	78389	1.33	254.05	61268	1.46	256.42	60317	1.79	23.49	140534
010-500	Cal	500/50	1.18	454.96	139761	1.33	461.05	111491	1.45	464.14	109530	1.79	42.42	257751
011-1000	Cal	1000/100	1.18	994.75	306175	1.33	979.64	237317	1.46	1041.56	246330	1.79	88.96	545678
013-5000	Cal	5000/500	1.18	5074.33	1563889	1.33	5090.02	1234614	1.46	5019.58	1188795	1.79	523.99	3240808

Sample			Hexobarbital Results			Secobarbital Results			Methohexital Results			THC-A Results		
Name	Type	Level	RT	Final Conc.	Resp.	RT	Final Conc.	Resp.	RT	Final Conc.	Resp.	RT	Final Conc.	Resp.
002-DB	Blank		1.87	0.20	30	2.07	2.36	53	2.59	0.49	5	2.87	0.10	1573
004-2p5	Cal	2.5/0.25	1.86	3.81	1777	2.03	3.73	365	2.65	2.51	391	2.87	0.21	2471
005-5	Cal	5/0.5	1.85	6.11	3410	2.03	6.02	885	2.65	5.85	604	2.87	0.50	4773
006-10	Cal	10/1	1.85	9.35	7489	2.03	8.98	1559	2.65	9.60	1554	2.87	0.98	8687
007-25	Cal	25/2.5	1.86	19.74	20576	2.04	22.80	4702	2.65	21.59	4594	2.87	2.81	23554
008-50	Cal	50/5	1.86	34.93	39720	2.03	36.86	7898	2.65	33.59	7637	2.87	4.80	39706
009-100	Cal	100/10	1.86	71.48	85748	2.03	79.09	17502	2.65	69.44	16727	2.87	9.87	80784
010-250	Cal	250/25	1.86	230.17	285638	2.03	239.81	54046	2.65	223.89	55890	2.87	27.89	226961
010-500	Cal	500/50	1.85	409.39	511383	2.03	464.13	105055	2.65	399.36	100383	2.87	48.75	396146
011-1000	Cal	1000/100	1.85	931.90	1169527	2.03	1076.06	244202	2.65	883.23	223071	2.87	103.10	836973
013-5000	Cal	5000/500	1.86	5224.62	6576592	2.03	5005.01	1137604	2.65	5290.95	1340690	2.87	495.35	4018515

Table 6: Quantitation results

Conclusions

The main goal of this work was to combine Barbiturates and THC-A into a single method. Additional emphasis was on increasing throughput by the use of Alternating Column Regeneration (ACR). In order to have a fast method, there was no separation between the isobars Amobarbital and Pentobarbital and the initial analysis time for all compounds was 5.0 min. The addition of ACR reduced the analysis runtime to 3.7 min injection to injection, which translates to a 26% improvement of the analysis throughput. Calibration curves for all compounds were linear with correlations of 0.99 or better. LLOQs for urine-spiked Phenobarbital, Butalbital, Butabarbital, Secobarbital and Methohexital were at least 5.0 ng/mL or better and for Amo/Pentobarbital and THC-A the LLOQs were 0.5 ng/mL or better.

References

A Combined Method for the Analysis of Barbiturates and 11-nor-9-carboxy Δ9THC in Urine by LC/MS/MS; Heather Workman, Erica A. Guice, Matthew Montgomery, and Brad Holden; SOFT 2011, Poster

LC/MS/MS Analysis of Barbiturates in Urine, Oral Fluid and Blood; Julie Cichelli and Rory M. Doyle; MSACL 2015, Poster

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