RESTEK Pure Chromatography

Consolidating LC-MS/MS Method Conditions for the Analysis of Alcohol Metabolites, Barbiturates, and Drugs of Abuse

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Abstract & Introduction

Efficiency is key in toxicology laboratories where multiple drug panels must be run daily. Consolidating multiple drug panels into large, multi-class drug assays can streamline the analytical testing process and reduce operating costs. To simplify the analysis of alcohol metabolites, barbiturates, THC metabolites, and drugs of abuse, three different LC-MS/MS methods were developed for each analyte class using the same analytical column and mobile phases. A panel of 100 drugs, novel psychoactive substances (NPS), therapeutic drugs and metabolites, a panel of biomarkers of alcohol consumption, and a panel of barbiturates and cannabinoid metabolites were all analyzed using a Force Biphenyl 50 x 3 mm, 3 µm analytical column and 0.1% formic acid in water and 0.1% formic acid in methanol as the mobile phases. The Force Biphenyl phase has unique selectivity due to the pi-pi interactions for drugs and drug metabolites when compared to a routine C18 phase allowing for improved resolution of isobars. Urinary interferences that are particularly problematic in alcohol metabolite testing were resolved without the use of buffer or additional mobile phases helping to streamline analytical testing processes.

ESI (+) Analytes and Isobars

Analyte	RT (min)	Analyte	RT (min)	Analyte	RT (min)
Morphine	1.89	Tapentadol	3.96	Buprenorphine	5.68
Hydromorphone	2.21	4’-hydroxy nitazene	3.99	Midazolam	5.75
Norhydrocodone	3.00	Dextrorphan	4.06	LSD	4.87
7-aminoclonazepam	4.49	Naltrexone	3.28	8-aminoclonazolam	5.69
Noroxymorphone	1.43	α-hydroxymidazolam	6.07	Tianeptine	5.79
Cotinine	1.67	Zolpidem phenyl-4-carboxylic acid	4.06	Sufentanil	5.86
Pregabalin	2.01	Lacosamide	4.20	Fluoxetine	5.52
Oxymorphone	1.99	O-desmethylvenlafaxine	3.46	Methadone	6.35
Noroxycodone	2.92	Tramadol	4.07	Mitragynine	5.93
Amphetamine	2.38	Nortriptyline	6.01	Amoxapine	5.86
Methcathinone	2.45	Methylphenidate	4.24	Flunitrazepam	7.31
Gabapentin	2.48	Lamotrigine	3.74	Xylazine	4.24
Olanzapine	2.54	Diphenhydramine	5.29	Eslicarbazepine	6.37
N-Desmethylclozapine	4.52	Ketamine	4.26	Sertraline	6.37
MDA	2.88	Benzoylecgonine	4.38	Zaleplon	7.21
4-hydroxy xylazine	3.10	Eutylone	3.88	Bromazepam	6.43
Methamphetamine	2.82	Pentylone	4.15	Oxazepam	6.77
Phentermine	3.00	Cocaine	4.56	Desalkylflurazepam	6.85
Oxycodone	3.22	Norbuprenorphine	4.86	Citalopram	5.28
MDMA	3.26	MDPV	4.65	α-hydroxyalprazolam	7.05
Naloxone	2.86	Cyclobenzaprine	5.97	α-hydroxytriazolam	6.89
6-Acetylmorphine	3.04	Paliperidone	4.96	Flubromazepam	6.99
Clozapine	4.56	Acetyl fentanyl	5.02	Clonazolam	7.03
Loxapine	5.93	Venlafaxine	4.68	Clobazam	7.19
Levamisole	3.36	EDDP	5.99	Temazepam	7.39
N-Desmethyltapentadol	3.78	Amitriptyline	6.05	Nordiazepam	7.09
O-Desmethyl-cis-tramadol	2.99	para-Fluorofentanyl	5.31	Flualpazolam	7.25
N-Desmethyl-cis-tramadol	4.22	Fentanyl	5.42	Promazine	5.86
Norketamine	3.90	α-PiHP	4.72	Diazepam	7.77
Norfentanyl	3.94	α-PHP	4.79	Flubromazolam	7.33
Codeine	3.01	Dextromethorphan	5.61	Alprazolam	7.48
Hydrocodone	3.32	PCP	5.62	Etizolam	7.83
Chlordiazepoxide	5.48	Mirtazapine	4.78	**Table 1.** Analyte list for positive mode DoA isobars (Isobars highlighted).	
N-Desmethylflunitrazepam	6.59	Desmethyldoxepin	5.36		

Disclosure: I have (or a member of my immediate family has) a financial relationship with a company as defined in the AACC policy on potential bias or conflict of interest

LC Column and Mobile Phases

Column	Force Biphenyl
Dimensions	50 x 3 mm
Particle Size	3 µm
Guard Column	Force Biphenyl EXP guard column, 5 x 3 mm
Pre-column Filter	UltraShield 0.2 µm
Column Temperature	30°C
Mobile Phase A	0.1% formic acid in H2O
Mobile Phase B	0.1% formic acid in MeOH

Table 2. Analytical column and mobile phases used for all methods.

ESI (+) Mode Isobar/DoA Analysis Method Conditions

Time (min)	Flow Rate (mL/min)	%A	%B
0.00	0.8	90	10
8.00	0.8	5	95
8.01	0.8	0	100
9.00	0.8	0	100
9.01	0.8	90	10
11.00	0.8	90	10

Table 3. Method conditions for positive mode isobar/DoA analysis.

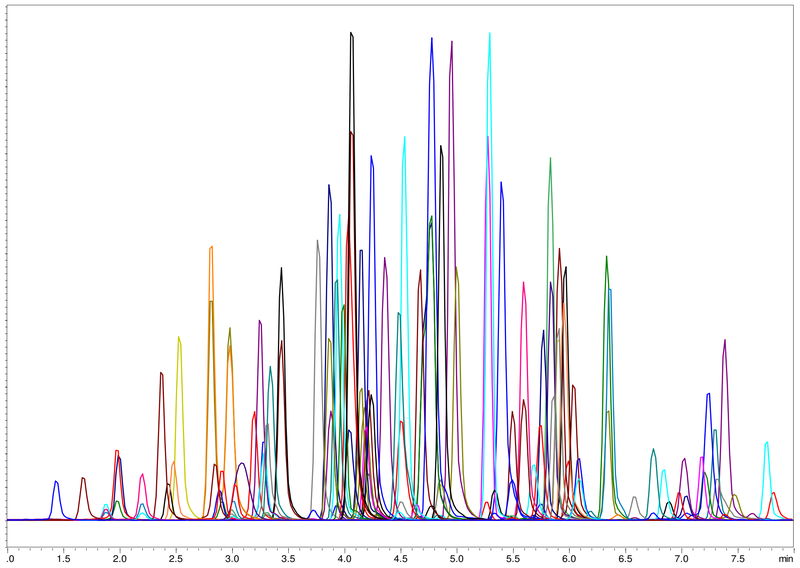

Figure 1. Compounds outlined in Table 1 were prepared at 100 ng/mL in urine and diluted with water. Column and mobile phase conditions outlined in Tables 2 & 3 were used. Method successfully differentiates between 22 sets of isobars.

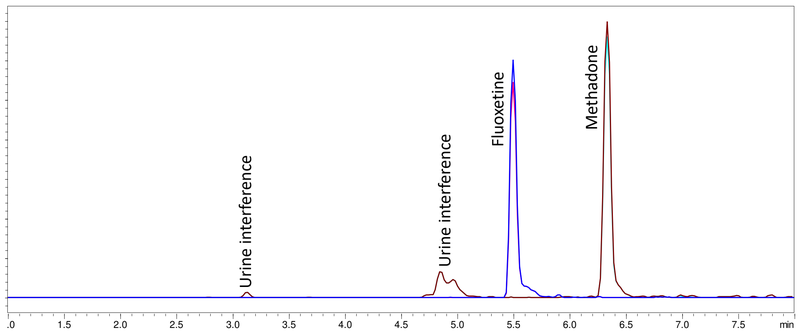

Figure 2. Separation of methadone and fluoxetine (*m/z* 310) from urine matrix interferences using outlined conditions in Tables 2 & 3 without the use of additional buffered mobile phases.

ESI (-) Mode Method Conditions

Time (min)	Flow Rate (mL/min)	%A	%B
0.00	0.8	55	45
2.00	0.8	40	60
2.50	0.8	0	100
4.00	0.8	0	100
4.01	0.8	55	45
5.00	0.8	55	45

Table 4. Method conditions for negative mode (barbiturates/cannabinoid) analysis.

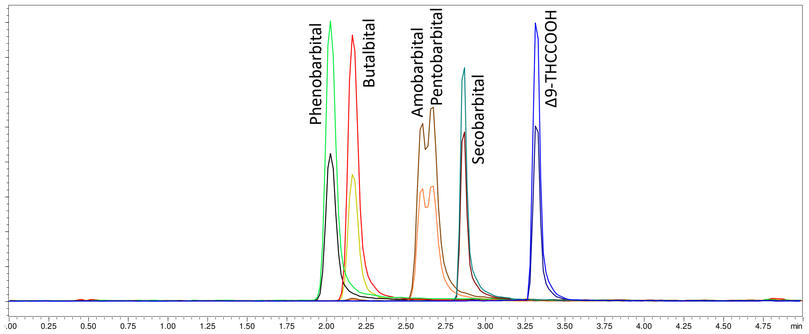

Figure 3. Chromatogram obtained using conditions outlined in Tables 2 & 4. Partial resolution of amobarbital and pentobarbital allows labs to identify which isomer is present in a sample. Sample was prepared at 500 ng/mL for barbiturates and 5 ng/mL for Δ9-THCCOOH in urine and diluted with water.

Alcohol Metabolites Method Conditions

Time (min)	Flow Rate (mL/min)	%A	%B
0.00	0.8	100	0
3.00	0.8	5	95
3.01	0.8	0	100
3.50	0.8	0	100
3.51	0.8	100	0
5.00	0.8	100	0

Table 5. Method conditions for EtG and EtS analysis.

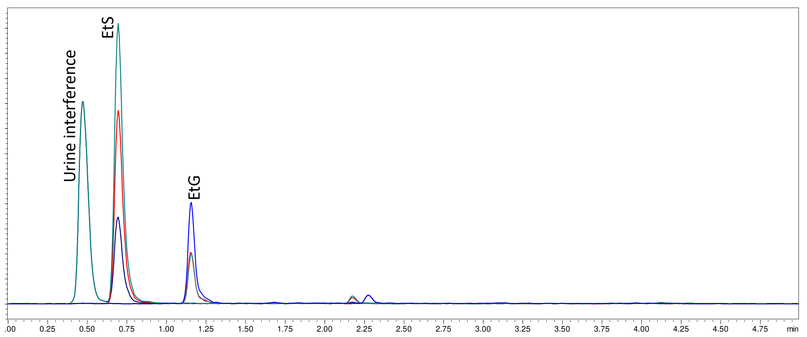

Figure 4. Chromatogram obtained using conditions outlined in Tables 2 & 5. EtG and EtS were prepared at 100 ng/mL in urine and diluted with water.

Conclusions

A panel of 100 drugs of abuse/NPS/therapeutic drugs in positive mode, a panel of 5 barbiturates and a cannabinoid metabolite in negative mode, and alcohol metabolites were all analyzed using the same column and mobile phases without the use of buffer or additional mobile phases. This work highlights the high selectivity and robustness of the biphenyl phase.