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

Jamie York, Justin Steimling; Restek Corporation

Abstract & Introduction

Efficiency is key when running multiple drug panels and any way to save time or streamline a process can help reduce costs. To help simplify the analysis of alcohol metabolites, barbiturates, and drugs of abuse, three different methods were developed for each analyte class using the same analytical column and mobile phase setup. A panel of 129 drug and drug metabolite isobars in positive mode, negative mode drug and drug metabolites, and alcohol metabolites were all analyzed using a Force Biphenyl 50 x 3 mm, 2.7 µm analytical column and 0.1% formic acid in water and 0.1% formic acid in methanol 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 (+) Mode Isobars

Analyte	RT (min)	Analyte	RT (min)	Analyte	RT (min)
Methamphetamine	2.93	MDPV	4.29	N-desmethylclozapine	4.25
Phentermine	3.07	Cyclobenzaprine	5.26	Olanzapine	2.66
Norcotinine	1.35	Molindone	3.81	Flunitrazepam	6.39
Nicotine	1.39	Maprotiline	5.26	Amoxapine	5.21
Anabasine	1.51	Amitriptyline	5.27	THC	7.15
Cotinine	2.02	EDDP	5.27	Clomipramine	5.53
BZP	1.44	Venlafaxine	5.27	Clonazepam	5.97
MDA	2.98	Promazine	5.19	Oxycodone	3.19
Methylephedrine	2.68	Diazepam	6.74	Alpha-Hydroxyalprazolam	6.17
Levamisole	3.31	Psilocybin	2.04	Prazepam	7.04
Psilocin	2.42	Norcodeine	2.69	Citalopram	4.75
Methylone	3.07	7-aminoclonazepam	4.24	Escitalopram	4.75
N-Desmethyltapentadol	3.63	Hydromorphone	2.41	Midazolam	5.16
MDEA	3.54	Norhydrocodone	3.05	Norpropoxyphene	5.08
Metaxalone	5.61	Morphine	2.16	Loxapine	5.25
Tapentadol	3.76	Pentazocine	4.38	6-Acetylmorphine	3.04
Normeperidine	4.06	Asenapine	5.11	Clozapine	4.49
Methylphenidate	4.00	Noroxymorphone	1.80	JWH-073	7.54
O-Desmethyltramadol	3.02	Dihydromorphine	2.11	Naloxone	2.90
N-Desmethyltramadol	3.98	Norcocaine	4.29	Butorphanol	4.47
Methaqualone	6.10	Benzoylecgonine	4.11	Paroxetine	5.23
Lacosamide	4.03	Estazolam	6.44	XLR-11	7.02
Desmethylmirtazapine	4.27	Trimipramine	5.40	Tenoxicam	5.32
7-aminonitrazepam	3.34	Didesmethyl citalopram	4.73	Zolpidemphenylcarboxylic acid	3.82
Phenytoin	5.24	Eslicarbazepine	5.65	Topiramate	4.76
Oxcarbazepine	5.22	N-desmethylflunitrazepam	5.82	Propoxyphene	5.09
Hydroxybupropion	3.80	Chlordiazepoxide	5.05	Alpha-hydroxymidazolam	5.48
Ketorolac	5.89	Codeine	3.02	Naltrexone	3.24
Lamotrigine	3.62	Hydrocodone	3.27	JWH-018	7.64
Diphenhydramine	4.76	Clobazam	6.28	Alpha-hydroxytriazolam	6.04
Tolmetin	6.03	Temazepam	6.43	Etoricoxib	5.95
Dextrorphan	3.84	Noroxycodone	2.98	Parecoxib	6.32
Levorphanol	3.84	Oxymorphone	2.23	Thioridazine	6.03
Protriptyline	5.18	Dihydrocodeine	2.98	Haloperidol	4.80
Nortriptyline	5.29	Morphine-N-oxide	2.31	Remifentanil	4.13
Lisdexamfetamine	1.82	Ezogabine	4.84	Sufentanil	5.16
Desmethylvenlafaxine	3.38	Cocaine	4.20	Mesoridazine	5.44
Tramadol	3.86	Sertraline	5.56	9-hydroxyresiperidone	4.51
EMDP	5.68	M-hydroxy benzoylecgonine	3.59	Iloperidone	4.51
Mirtazapine	4.40	Zaleplon	6.32	Paliperidone	4.51
Desmethyldoxepin	4.82	Fluoxetine	4.91	Pimozide	5.43
Desomorphine	3.18	Methadone	5.55	Morphine-6-glucuronide	2.22
Dextromethorphan	5.01				
Normorphine	1.56				

Table 1: Analyte list for positive mode DOA isobars. (Isobars highlighted)

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 mm x 3 mm ID
Particle Size:	3 µm
Guard Column:	Force Biphenyl EXP guard column 5 mm, 3 mm ID
Pre Column Filter:	Ultra Shield 0.2 µm frit
Temp.:	30 °C
Mobile Phase	
A:	Water, 0.1% formic acid
B:	Methanol, 0.1% formic acid

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

ESI (+) Mode Isobar Analysis

Time (min)	Flow (mL/min)	%A	%B
0.00	0.8	96	4
7.00	0.8	0	100
8.00	0.8	0	100
8.01	0.8	96	4
10.00	0.8	96	4

Table 3. Mobile phase conditions outlined for positive mode drug panel isobars.

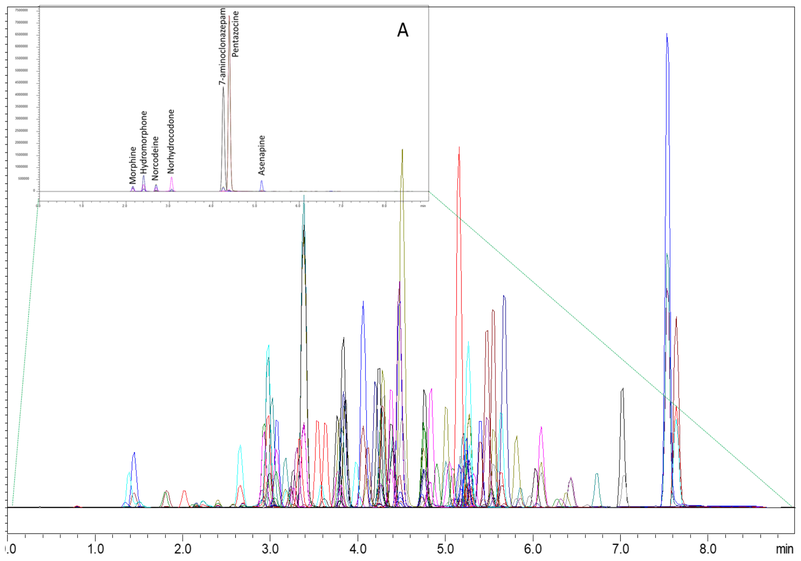

Figure 1. Compounds outlined in Table 1 were prepared at 80 ng/mL in water and 1 µL injected. Column and mobile phase conditions outlined in Tables 2 & 3 were used. A. Separation achieved for seven isobaric compounds that share the *m/z* of 286.

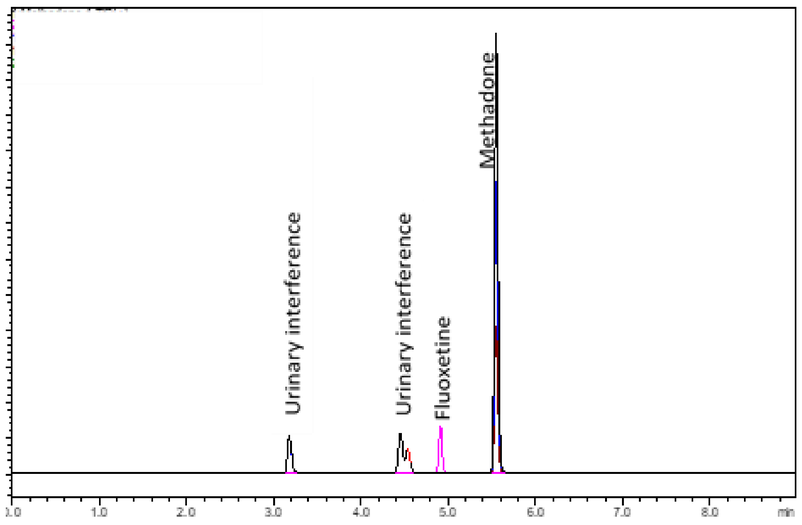

Figure 2. Separation of methadone and fluoxetine from urinary matrix interferences using the outlined conditions in Tables 2 & 3 without the use of additional buffered mobile phases.

ESI (-) Mode Barbiturates, THCA-A, and THC-COOH

Time (min)	Flow (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. Mobile phase conditions outlined for negative mode compounds- barbiturates, THCA-A, and THC-COOH.

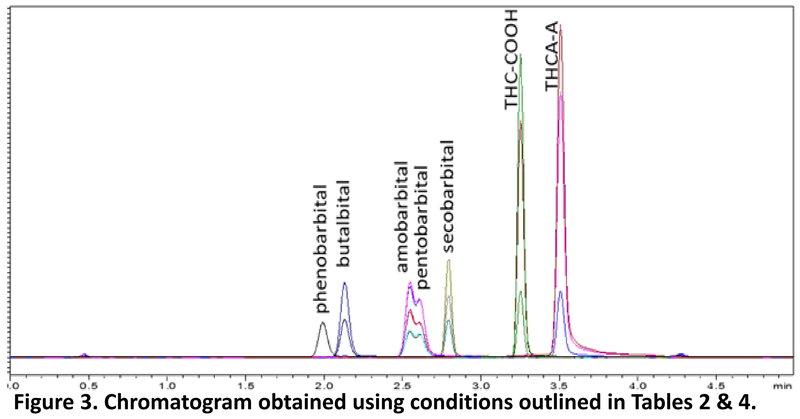

Figure 3. Chromatogram obtained using conditions outlined in Tables 2 & 4. Partial resolution of amobarbital and pentobarbital allows labs to identify which barbiturate is present in a sample. Sample concentration is 500 ng/mL for barbs and 5 ng/mL for THCA and THC-COOH. 1:10 dilution in urine and 1 µL injection performed.

Alcohol Metabolites- EtG & EtS

Time (min)	Flow (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. Mobile phase conditions outlined for the analysis of EtG & EtS

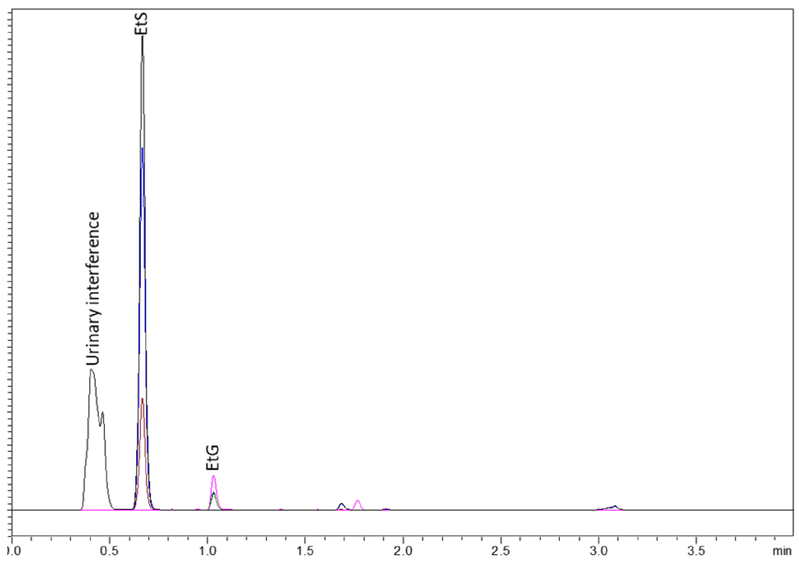

Figure 4. EtG and EtS prepared in urine with a 1/10 dilution in water and a 10 µL injection performed.

Conclusions

A panel of 129 drug and drug metabolite isobars in positive mode, negative mode drug and drug metabolites, and alcohol metabolites were all analyzed using the same column and mobile phases, without the use of buffer or additional mobile phases. The use of an UltraShield PreColumn filter helps prevent buildup on the guard and analytical column, improving their lifetime.