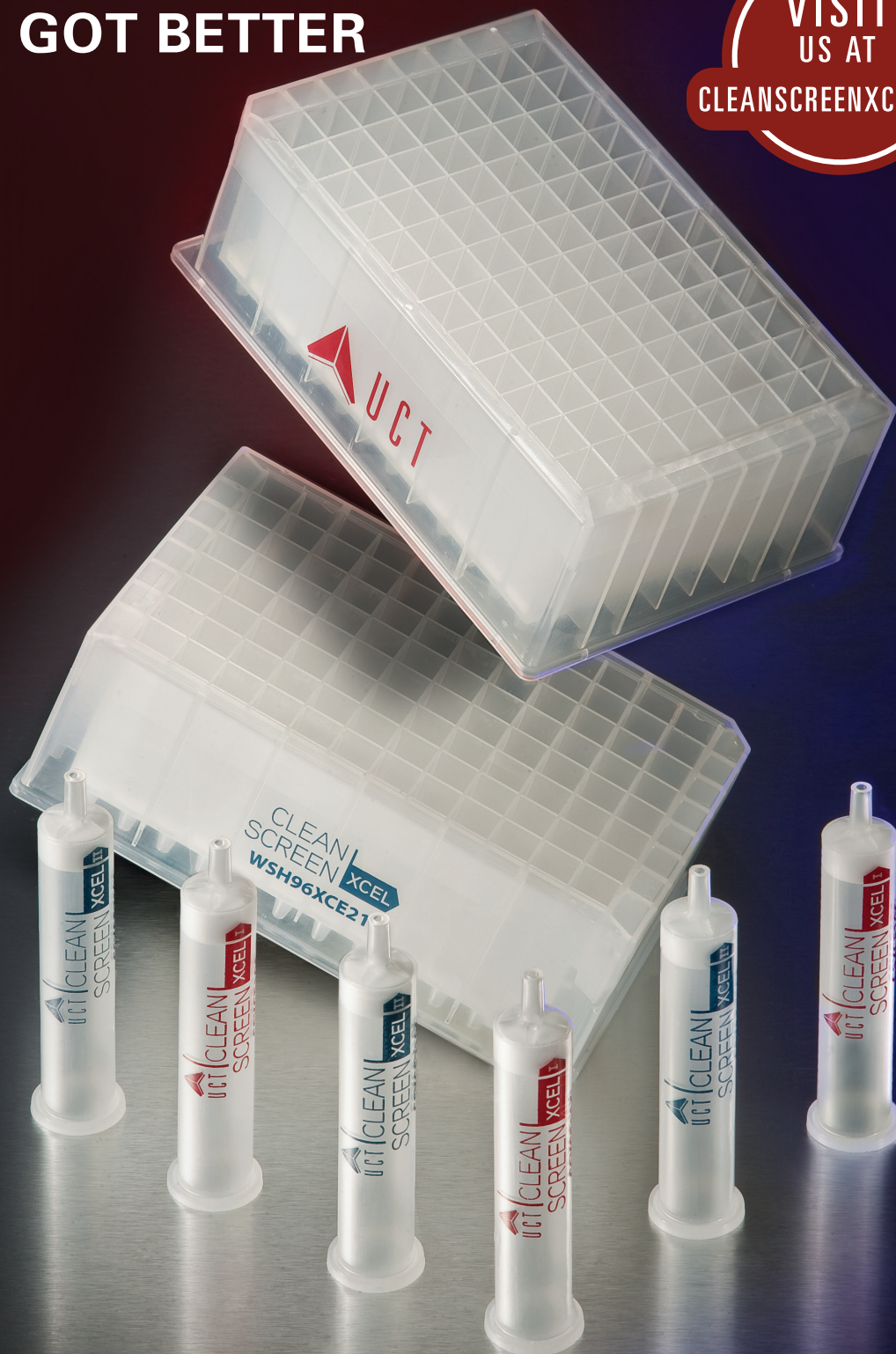


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**CLEAN SCREEN XCEL™ SPE COLUMNS**



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PHARMA

## EXTRACTION OF BASIC DRUGS AND METABOLITES FROM URINE/ BLOOD USING SPE CARTRIDGES

130mg Clean Screen Xcel I Column

Part #: CSXCE106 6 mL - 130 mg Cartridge

Part #: CSXCE103 3 mL - 130 mg Cartridge

### (Urine Hydrolysis Step)

To 1-2 mL urine sample add 500 µL of 0.1M acetate buffer (pH= 5.0) containing 5,000 units/mL β-glucuronidase. Optionally, add 500 µL of acetate buffer and 25 µL of concentrated β-glucuronidase. Add appropriate volume and concentration internal standards. Vortex and heat for 1-2 hours at 65 °C. Allow sample to cool. Do not adjust pH- sample is ready to be added to extraction column.

### (Blood Sample Preparation)

To 1-2 mL blood add 2 ml of 0.1M phosphate buffer (pH= 6.0). Add appropriate volume and concentration internal standards. Sample is ready to be added to extraction column.

### Applying Sample to Column

Load sample directly to column without any preconditioning. Pull sample through at a rate of 1-2 mL/ minute. Dry column thoroughly under vacuum (10 mm Hg) or positive pressure (~ 80-100 psi) for 1 minute.

### Wash (Blood Only)

Wash sample with 2 mL of 0.1M phosphate buffer (pH=6.0+0.5).

### Wash (Urine and Blood)

Wash sample with 1 mL of 2% glacial acetic acid/ 98 % methanol. Dry column thoroughly under vacuum (10 mm Hg) or positive pressure (~ 80-100 psi) for a minimum of 5 minutes.

NOTE 1: (It is important to dry the column thoroughly to achieve the highest recovery of all compounds. Any residual moisture will slow down the drying of the elution solvents prior to derivatization for GC/MS analysis. Also, any residual moisture could reduce the reactivity of the derivatization agent resulting in low GC/MS sensitivity.)

### Elution

Elute samples with ~1-2 mL dichloromethane /iso-Propanol/ ammonium hydroxide (78/20/2) Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C. (Note: For amphetamine group analysis, add 200 µL of 1% HCl/ 99% MeOH to eluate to minimize amphetamine group loss to volatilization.)

### GC/MS Analysis

Derivatize compounds with appropriate derivatizing procedure or reconstitute in 100 µL ethyl acetate and inject 1-2 µL into the GC/MS system for analysis.

### LC/MS Analysis

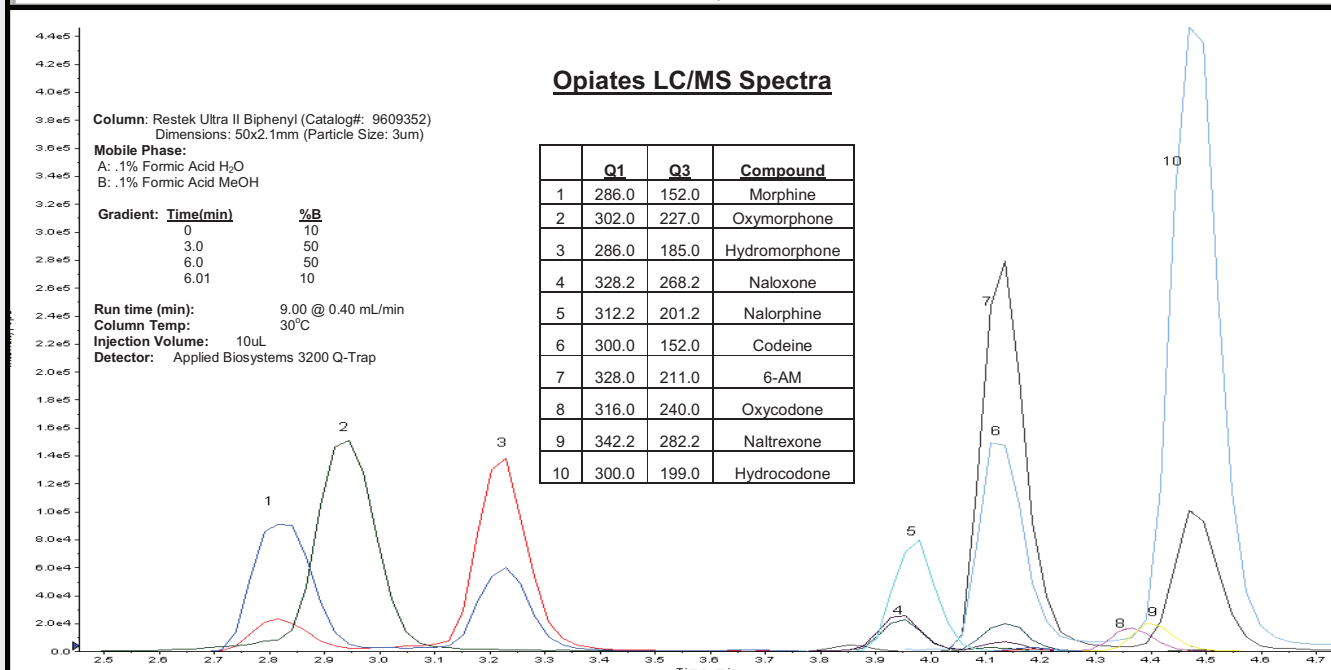
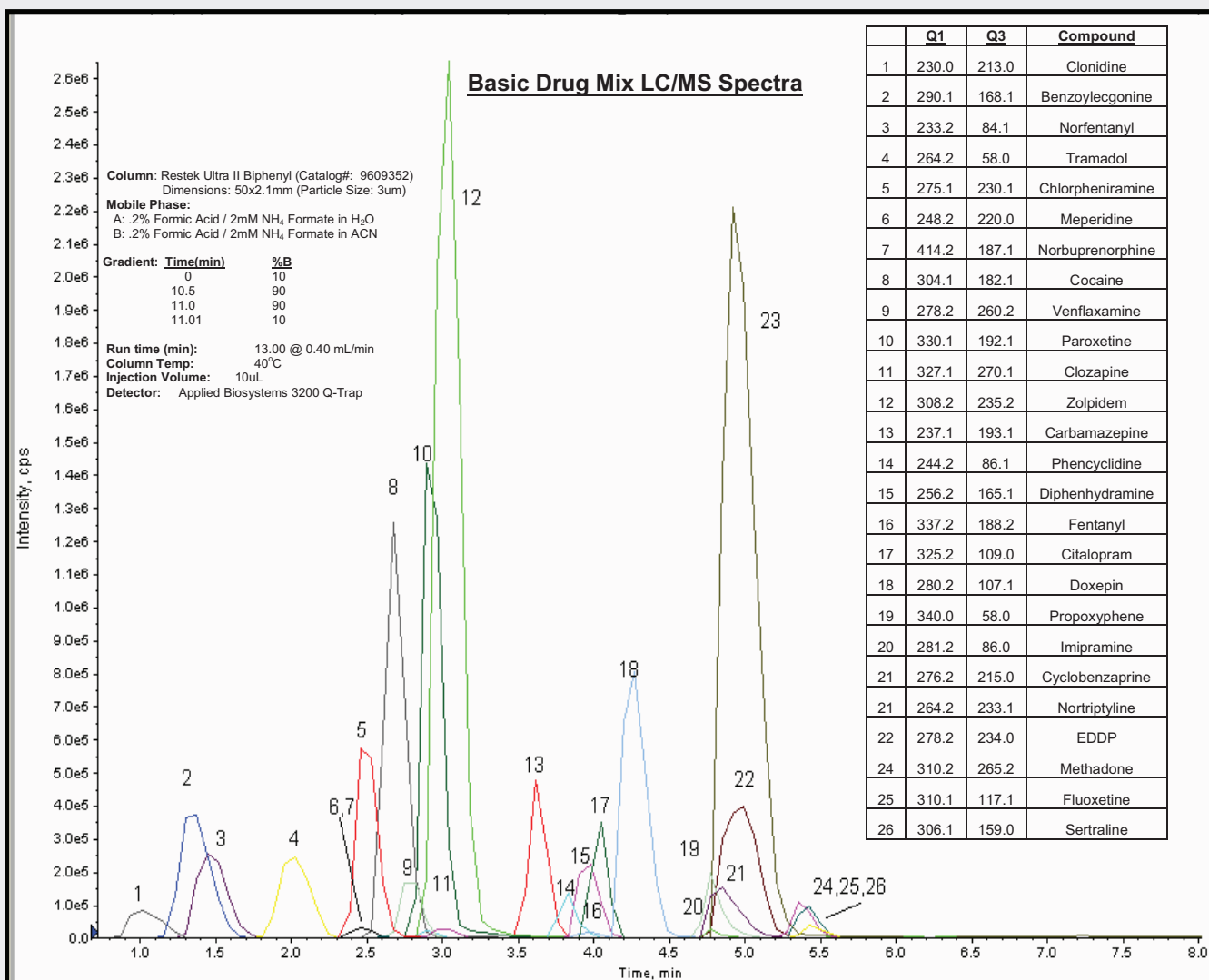
Reconstitute in methanol or appropriate mobile phase.



September 9, 2009

Analyte Peak Name	RRT (min)	% Recovery (Urine)	% Recovery (Blood)
6-MAM	4.14	97%	100%
Codeine	4.13	96%	99%
Hydrocodone	4.48	98%	94%
Hydromorphone	3.22	91%	91%
Morphine	2.83	92%	85%
Nalorphine	3.97	99%	97%
Naloxone	3.94	99%	94%
Naltrexone	4.40	99%	90%
Oxycodone	4.37	98%	94%
Oxymorphone	2.94	94%	88%

Analyte Peak Name	RRT (min)	% Recovery (Urine)	% Recovery (Blood)
Amphetamine	4.35	112%	104%
Benzoylcegonine	3.19	54%	58%
Buprenorphine	6.21	92%	92%
Chlorpheniramine	3.72	85%	94%
Citalopram	6.05	99%	95%
Clonidine	2.35	85%	98%
Clozapine	5.34	98%	94%
Cocaine	4.85	103%	96%
Cyclobenzaprine	7.48	102%	92%
Diphenhydramine	6.29	87%	88%
Doxepin	6.39	97%	93%
EDDP	7.52	85%	88%
Ephedrine	3.86	75%	57%
Fentanyl	6.31	88%	89%
Fluoxetine	7.95	97%	92%
Imipramine	7.18	97%	97%
MDA	5.08	95%	84%
MDEA	6.14	100%	93%
MDMA	5.62	99%	90%
Meperidine	4.59	82%	95%
Methadone	7.93	92%	93%
Methamphetamine	4.93	99%	104%
Norfentanyl	3.41	84%	85%
Nortriptyline	7.24	79%	89%
Paroxetine	7.09	90%	92%
Phencyclidine	5.72	90%	93%
Propoxyphene	7.22	90%	95%
Pseudoephedrine	3.86	79%	56%
Sertraline	8.08	75%	70%
Tramadol	4.01	89%	81%
Venlafaxine	4.94	102%	93%
Zolpidem	5.23	92%	95%





## EXTRACTION OF BENZODIAZEPINES FROM URINE/ BLOOD USING SPE CARTRIDGES

130mg Clean Screen Xcel I Column

Part #: CSXCE106 6 mL - 130 mg Cartridge

Part #: CSXCE103 3 mL - 130 mg Cartridge

### (Urine Hydrolysis Step)

To 1-2 mL urine sample add 500 µL of 0.1M acetate buffer (pH= 5.0) containing 5,000 units/mL

β-glucuronidase. **Optionally**, add 500 µL of acetate buffer and 25 µL of concentrated β-glucuronidase.

Add appropriate volume and concentration internal standards.

Vortex and heat for 1-2 hours at 65 °C.

Allow sample to cool.

Do not adjust pH- sample is ready to be added to extraction column.

### (Blood Sample Preparation)

To 1-2 mL blood add 2 mL of 0.1M phosphate buffer (pH= 6.0±0.5).

Add appropriate volume and concentration internal standards.

Sample is ready to be added to extraction column.

### Applying Sample to Column

Load sample directly to column without any preconditioning.

Pull sample through at a rate of 1-2 mL/ minute.

Dry column thoroughly under vacuum (10 mm Hg) or positive pressure (~80-100 psi) for 1 minute.

### Wash (Blood Only)

Wash sample with 2 mL of 0.1M phosphate buffer (pH=6.0±0.5).

### Wash (Urine and Blood)

Wash sample with 1 mL of methylene chloride.

Dry column thoroughly under vacuum (10 mm Hg) or positive pressure (~80-100 psi) for a minimum of 5 minutes.

NOTE 1: (It is important to dry the column thoroughly to achieve the highest recovery of all compounds. Any residual moisture will slow down the drying of the elution solvents prior to derivatization for GC/MS analysis. Also, any residual moisture could reduce the reactivity of the derivatization agent resulting in low GC/MS sensitivity.)

### Elution

Elute samples with 1 mL ethyl acetate/ ammonium hydroxide (98/2)

Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C.

### GC/MS Analysis

It is recommended to add 50 µL of Ethyl Acetate to 50 µL of derivatization agent and react at ~70 °C for 15 minutes. Inject 1-2 µL of cooled (50:50) solution in the GC/MS system for analysis.

### LC/MS Analysis

Reconstitute in methanol or appropriate mobile phase.

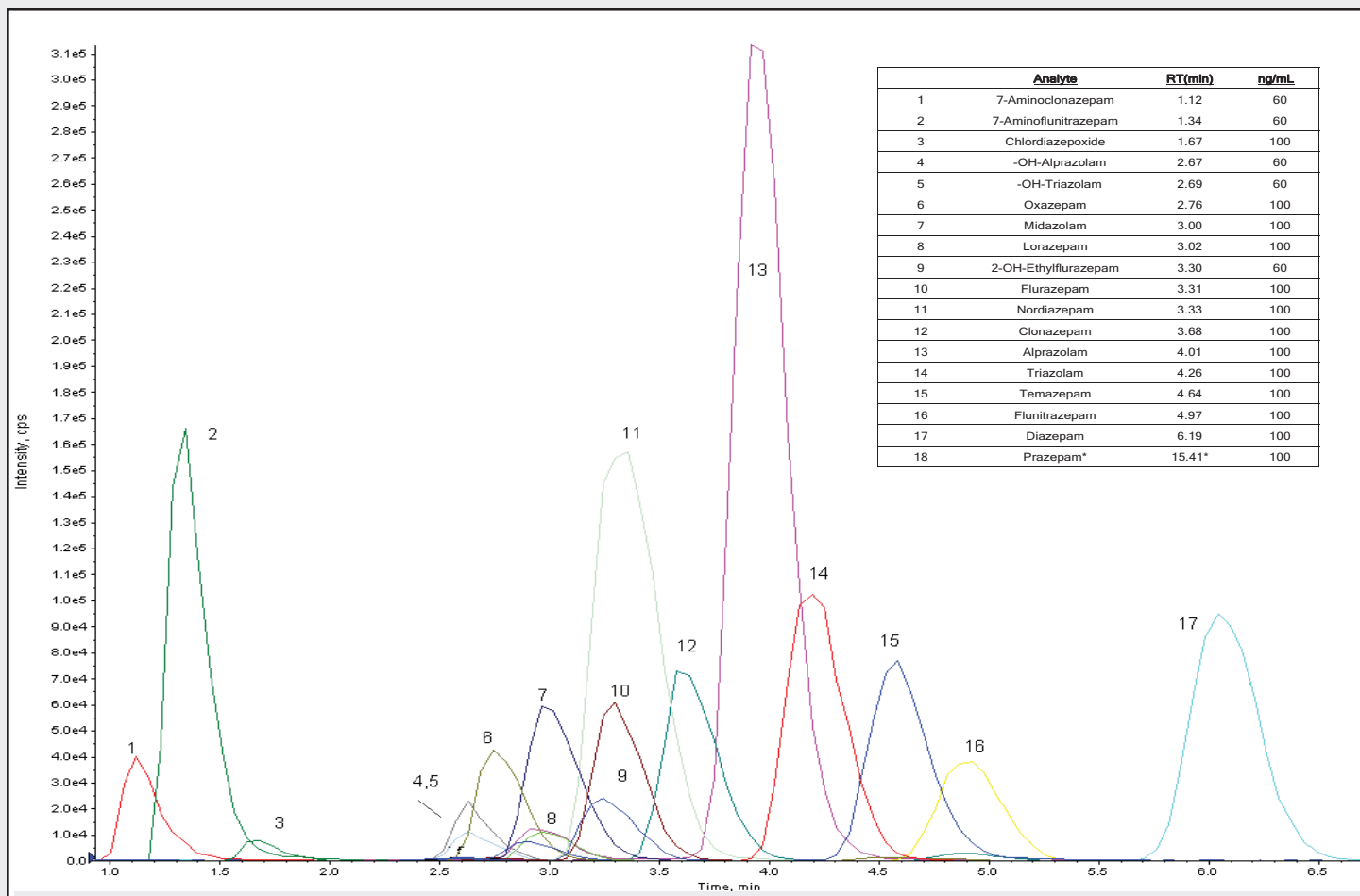
### Benzodiazepine Analytes Extracted

Diazepam Clonazepam Alprazolam Midazolam



September 9, 2009

Analyte Peak Name	Analyte Peak Area (counts)	RRT (min)	% Recovery (Urine)	% Recovery (Blood)
2-Hydroxyethylflurazepam	1.98E+05	3.25	97%	89%
7-Aminoclonazepam	1.27E+05	1.12	93%	95%
7-Aminoflunitrazepam	9.50E+05	1.32	80%	83%
alpha-Hydroxyalprazolam	1.16E+05	2.64	103%	82%
alpha-Hydroxytriazolam	6.59E+04	2.64	97%	82%
Alprazolam	2.33E+06	3.93	90%	89%
Chlordiazepoxide	2.33E+04	1.67	75%	96%
Clonazepam	4.97E+05	3.60	78%	78%
Diazepam	4.64E+05	6.19	100%	90%
Flunitrazepam	3.39E+05	4.87	85%	85%
Flurazepam	9.75E+05	3.31	73%	78%
Lorazepam	7.17E+04	3.02	93%	76%
Midazolam	2.35E+05	3.00	89%	89%
Nordiazepam	4.01E+05	3.29	101%	80%
Oxazepam	1.93E+05	2.75	78%	96%
Prazepam	2.97E+06	15.1	89%	98%
Temazepam	2.64E+05	4.64	79%	68%
Triazolam	8.24E+05	4.18	95%	82%



## BENZODIAZEPINE LC/MS PARAMETERS

### PEAK LIST:

Q1	Q3	Dwell Time (MS)	Compound	DP (volts)	EP (volts)	Cerilliant Part #
333.10	211.20	150	2-Hydroxyethylflurazepam	60	10	F-901
286.10	121.10	150	7-Aminoclonazepam	60	10	A-915
284.10	135.10	150	7-Aminoflunitrazepam	60	10	A-911
325.10	205.10	150	Alpha-Hydroxyalprazolam	60	10	A-905
341.00	297.00	150	Alpha-Hydroxymidazolam	60	10	H-902
359.00	239.10	150	alpha-Hydroxytriazolam	60	10	T-911
309.10	281.10	150	Alprazolam	60	10	A-903
300.00	227.00	150	Chlordiazepoxide	60	10	C-022
316.10	270.10	150	Clonazepam	60	10	C-907
289.00	140.10	150	Desalkylflurazepam	60	10	D-915
300.10	245.20	150	Desmethylflunitrazepam	60	10	D-918
285.10	193.10	150	Diazepam	60	10	D-907
314.10	268.10	150	Flunitrazepam	60	10	F-907
388.10	315.10	150	Flurazepam	60	10	F-003
321.00	229.10	150	Lorazepam	60	10	L-901
326.10	291.10	150	Midazolam	60	10	M-908
271.10	140.10	150	Nordiazepam	60	10	N-905
287.10	241.10	150	Oxazepam	60	10	O-902
325.10	271.10	250	Prazepam	60	10	P-906
301.10	255.10	150	Temazepam	60	10	T-907
343.00	239.00	150	Triazolam	60	10	T-910

### COLUMN:

Restek Ultra II Biphenyl

Catalog#: 9609352

Dimensions: 50 x 2.1mm

Particle Size: 3 µm

### CONDITIONS:

**Mobile Phase:** A: .2% Formic Acid / 2mM NH<sub>4</sub> Formate in H<sub>2</sub>O  
B: .2% Formic Acid / 2mM NH<sub>4</sub> Formate in ACN

**Isocratic Flow:** .2 mL/min A : .1 mL/min B

**Run time:** 20 minutes

**Injection Volume:** 10 µL

**Column Temp:** 40°C

**Detector:** Applied Biosystems 3200 Q-Trap LC/MS/MS

## SPE EXTRACTION OF THC-DELTA-9-CARBOXY METABOLITE FROM URINE/ BLOOD

130mg Clean Screen Xcel II Column

PART #: CSXCE2106 6 ML - 130 MG CARTRIDGE

### (Urine Sample Preparation - Hydrolysis / Neutralization Step)

To 2 mL urine sample add appropriate volume and concentration internal standards.

Add 50 µL of 10N NaOH and heat for 15 minutes at 70 °C.

Add 50 µL of 1:1 (glacial acetic acid: DI water). pH should be ~ 6.5.

Add 100 µL 0.1M pH=7.0 phosphate buffer

Sample is ready to be added to extraction column.

### (Blood Sample Preparation)

To 1-2 mL blood add appropriate volume and concentration internal standards

Add 2 mL of 0.1M phosphate buffer (pH= 7.0).

Sample is ready to be added to extraction column.

### (Alternate Blood Preparation)

To 1-2 mL blood add appropriate volume and concentration internal standards

Add dropwise 1-2 mL of cold acetonitrile. Centrifuge and decant acetonitrile.

Reduce acetonitrile by evaporating at ≤ 40 °C to ~ 100-200 µL.

Add 2 mL of 0.1M phosphate buffer (pH= 7.0).

Sample is ready to be added to extraction column.

### Applying Sample to Column

Load sample directly to column without any preconditioning.

Pull sample through at a rate of 1-2 mL/ minute.

Dry column thoroughly under vacuum (10 mm Hg) or positive pressure (~ 80-100 psi) for ~1 minute.

### Wash (Blood Only)

Wash sample with 2 mL of 0.1M phosphate buffer (pH= 7.0).

Wash (Urine and Blood)

Wash sample with 1-2 mL of hexane.

Dry column thoroughly under vacuum (10 mm Hg) or positive pressure (~ 80-100 psi)

for a minimum of 5-10 minutes.

NOTE 1: (It is important to dry the column thoroughly to achieve the highest recovery of all compounds. Any residual moisture will slow down the drying of the elution solvents prior to derivatization for GC/MS analysis. Also, any residual moisture could reduce the reactivity of the derivatization agent resulting in low GC/MS sensitivity.)

### Elution

Elute samples with ~1-2 mL hexane/ ethyl acetate/ glacial acetic acid (49/49/2)

Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C.

### GC/MS Analysis

Derivatize compounds with appropriate derivatizing procedure inject 1-2 µL into the GC/MS system.

### LC/MS Analysis

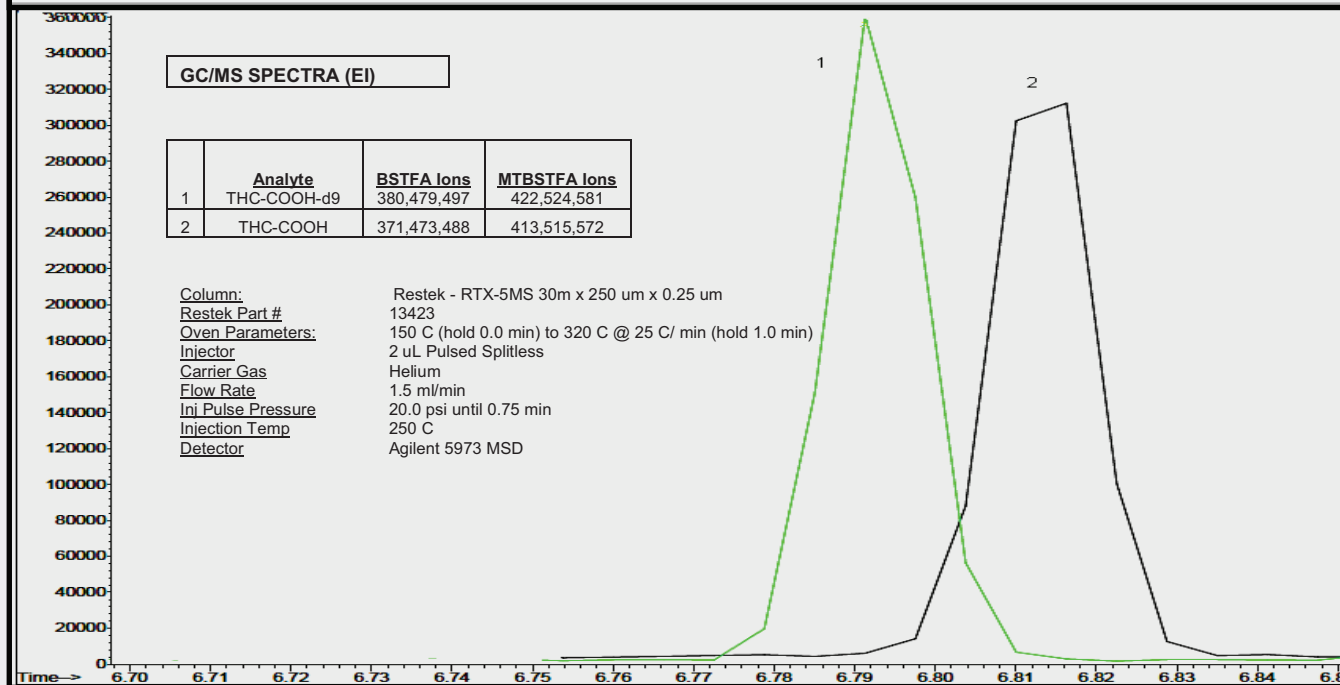
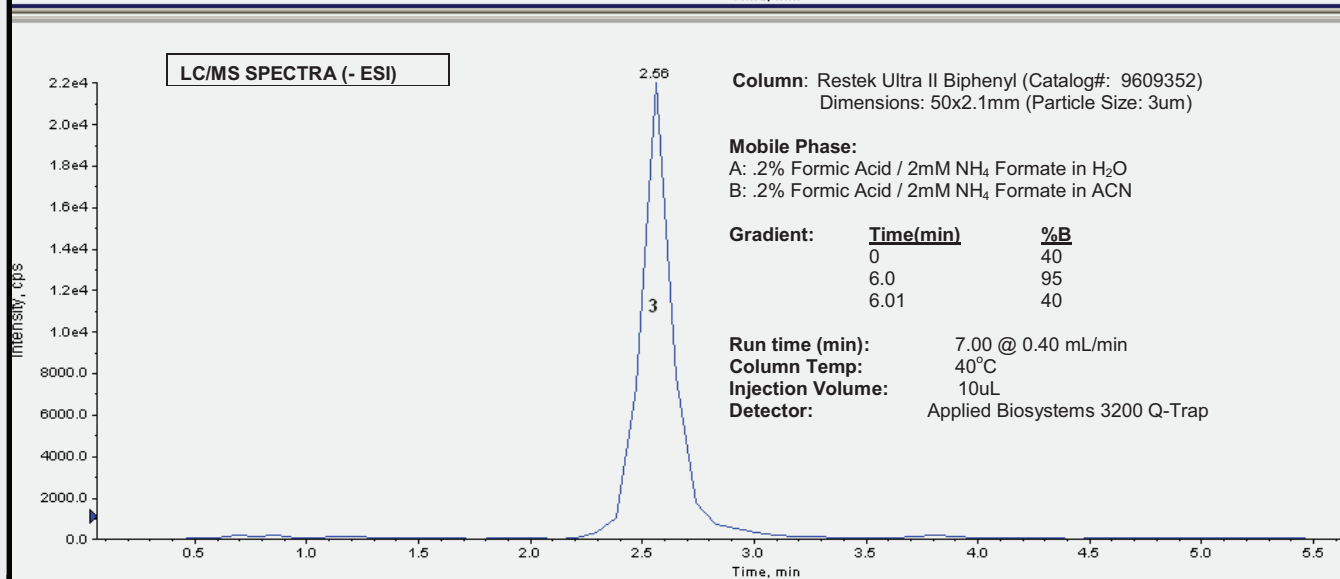
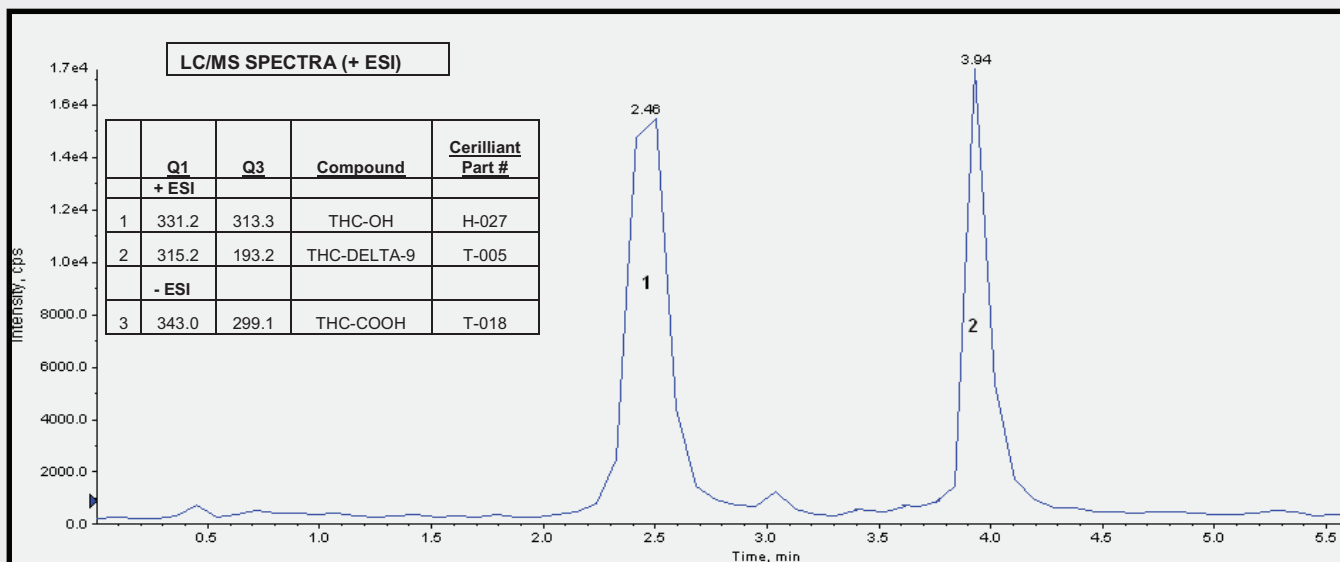
Reconstitute in methanol or appropriate mobile phase.



September 9, 2009

## THC-delta-9-COOH Comparison of Extraction Methods Xcel II versus Liquid/ Liquid

SAMPLE ID #	Liquid/ Liquid Result (ng/ml)	2 Step Xcel II SPE Result (ng/ml)	Liquid/ Liquid Area Response	2 Step Xcel II SPE Area Response
60 control	60.8	63.2	193689	521993
300 control	226	299	663859	1793767
9141-1	157	171	366285	1210294
9141-2	26	30.1	74689	283042
9141-4	57	73	125662	497268
9141-5	13.8	14	50238	168674
9141-6	57.9	56.2	148767	469350
30 control	26.5	27	95809	215516
9140-2	602	617	1475613	1874264
9140-6	158	183	315248	421658
9140-8	24.3	36	92673	187997
9140-9	23.7	29	89165	203170
9148-3	15	12	48302	65258
9152-08	259	218	929062	1271537
9154-13	257	238	945812	1536918
9154-10	31	38	83919	198698
9153-6	△500	476	2119187	2516835
9153-3	68	48	56757	53761
9152-8	259	219	929062	1271537
Control-70	72	65	218237	415581



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