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#### 35.1 Summary

Methylphenidate and its primary metabolite, ritalinic acid, are extracted from biological samples with an acetonitrile precipitation and analyzed by high performance liquid chromatography-electrospray ionization mass spectrometry (LC-ESI-MS).

#### 35.2 Specimen Requirements

One mL blood, urine, gastric or tissue homogenate.

#### 35.3 Reagents and Standards

35.3.1 Ammonium acetate

35.3.2 Methanol

35.3.3 Acetonitrile

35.3.4 Methylphenidate, 1 mg/mL

35.3.5 Ritalinic acid ( $\alpha$ -phenyl-2-piperidineacetic acid), 1 mg/mL

35.3.6 Phenacetin, 1 mg/mL

#### 35.4 Solutions, Internal Standard, Calibrators and Controls

35.4.1 10 mM Ammonium Acetate: Weight 0.38 g ammonium acetate. Transfer to 500 mL volumetric flask and qs to volume with dH<sub>2</sub>O. Store at room temperature for up to one month.

35.4.2 Working standard solution for methylphenidate and ritalinic acid (0.01 mg/mL)

Pipet 100  $\mu$ l each of 1 mg/mL stock solutions of methylphenidate and ritalinic acid into a 10 mL volumetric flask and qs to volume with dH<sub>2</sub>O

35.4.3 Quality Control (QC) standard solution of methylphenidate and ritalinic acid (0.01 mg/mL)

Pipet 100  $\mu$ l each of separate 1 mg/mL stock solutions of methylphenidate and ritalinic acid (different manufacturer, lot number or preparation than calibrators) into a 10 mL volumetric flask and qs to volume with dH<sub>2</sub>O

35.4.4 Internal standard working solution

0.1 mg/mL phenacetin: Pipet 1 mL of 1 mg/mL phenacetin stock solution into 10 mL volumetric flask and qs to volume with dH<sub>2</sub>O

35.4.5 To prepare the calibration curve, pipet the following volumes of the 1 mg/mL and 0.01 mg/mL methylphenidate and ritalinic acid working standards into appropriately labeled 16 x 125 mm screw cap test tubes. To eliminate a solvent effect, calibrators and controls may be dried under nitrogen prior to the addition of blank blood. Add 1 mL blank blood to obtain the final concentrations listed below.

Concentration of standard (mg/mL)	Amount of Standard ( $\mu$ L)	Final concentration of methylphenidate and ritalinic acid (mg/L)
1 mg/mL	10	10

1 mg/mL	5	5
0.01 mg/mL	200	2
0.01 mg/mL	100	1
0.01 mg/mL	50	0.5
0.01 mg/mL	10	0.1

## 35.4.6 Controls

## 35.4.6.1 Methylphenidate and Ritalinic Acid Control

Pipet 100  $\mu$ L of the 0.01 mg/mL methylphenidate/ritalinic acid QC solution into an appropriately labeled tube. Add 1 mL blank blood to achieve final concentration of 1 mg/L.

35.4.6.2 Negative control. Blood bank blood or equivalent determined not to contain methylphenidate or ritalinic acid.

## 35.5 Apparatus

35.5.1 Test tubes, 16 x 125 mm, round bottom, borosilicate glass with Teflon caps

35.5.2 Test tubes, 16 x 114 mm, glass centrifuge, conical bottom

35.5.3 Centrifuge capable of 2000-3000 rpm

35.5.4 Nitrogen evaporator with heating block

35.5.5 Vortex mixer

35.5.6 GC autosampler vials with inserts

35.5.7 LCMS: Agilent Model 1100 LC-MSD

35.5.7.1 LCMS Instrument Conditions. The following instrument conditions may be modified to adjust or improve separation and sensitivity.

35.5.7.1.1 Elution conditions:

Column: C-18, 125 mm X 3 mm, 3  $\mu$ M particle size

Column thermostat: 30°C

Solvent A: 10 mM ammonium acetate in dH<sub>2</sub>O

Solvent B: methanol

Isocratic elution, stop time: 6.00 min

Time	Solv. B	Flow
0.00	48	0.5

35.5.7.1.2 Spray Chamber

Ionization Mode: Electrospray

Gas Temperature: 350°C

35 Methylphenidate and Ritalinic Acid Quantitation and Confirmation by LCMS

Drying Gas (N<sub>2</sub>): 12.0 L/min

Nebulizer pressure: 35 psig

Vcap (Positive): 3500 V

35.5.7.1.3 Selected Ion Monitoring (quantitation ions)

Polarity: Positive

Injection volume: 1 µL

Time (min)	Group Name	SIM Ion	Frag-Mentor	Gain EMV	SIM Resol.	Actual Dwell
0	Ritalinic acid	84	170	1.0	Low	352
		174	170		352	
		220	170		352	
2.3	Phenacetin	110	160	1.0	Low	529
		180	160		529	
3.5	Methylphen	84	170	1.0	Low	352
		174	170		352	
		234	170		352	

**35.6 Procedure**

- 35.6.1 Label clean 16 x 125 mm screw cap tubes appropriately with calibrators, controls and case sample IDs.
- 35.6.2 Prepare calibrators and controls.
- 35.6.3 Add 1 mL case specimens to the appropriately labeled tubes. Note: since this procedure is used for screening and confirmation, it is recommended to analyze two different aliquots (or tissues) with each case. One will serve as a screen and the second as a confirmation.
- 35.6.4 Add 50 µL 0.1 mg/mL phenacetin internal standard working solution to each tube.
- 35.6.5 Slowly, add dropwise 2 mL cold (freezer temperature) acetonitrile to each tube while vortexing. Continuous vortexing, not mere mixing, is essential.
- 35.6.6 Vortex an additional 30 seconds.
- 35.6.7 Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation.
- 35.6.8 Place tubes in freezer for at least 30 minutes to facilitate separation.
- 35.6.9 Transfer top (acetonitrile) layer to clean conical bottom tubes taking care not to transfer any lower layers.
- 35.6.10 Evaporate to dryness at approximately 50°C under nitrogen.
- 35.6.11 Reconstitute samples in 100 µL methanol. Vortex briefly. Transfer to GC microvials and inject on LCMS. Note: reconstitution volume may be adjusted as necessary to prevent saturation of detector.

**35.7 Calculation**

Drug concentrations are calculated by linear regression analysis using the ChemStation software.

**35.8 Quality Control and Reporting**

- 35.8.1 The LOQ for this procedure is defined as the lowest acceptable calibrator concentration used in the calibration curve for each analyte. The ULOL for this procedure is defined as the highest acceptable calibrator concentration used in the calibration curve for each analyte.
- 35.8.2 If the same specimen is analyzed in duplicate (for screening and confirmation) and both results are quantitative, the results should be averaged prior to reporting.
- 35.8.3 See Toxicology Quality Guidelines

**35.9 References**

- 35.9.1 Julia Pearson and Robert Steiner, in-house development.

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**36 RISPERIDONE QUANTITATION AND CONFIRMATION BY LCMS****36.1 Summary**

Risperidone is extracted from biological samples with an acetonitrile precipitation and analyzed by high performance liquid chromatography-electrospray ionization mass spectrometry (LC-ESI-MS).

**36.2 Specimen Requirements**

One mL blood, fluid or tissue homogenate.

**36.3 Reagents and Standards**

36.3.1 Ammonium acetate

36.3.2 Acetic Acid

36.3.3 Methanol

36.3.4 Acetonitrile

36.3.5 Risperidone (Janssen Pharmaceuticals)

36.3.6 R68808 (Janssen Pharmaceuticals, internal standard)

36.3.7 Mepivacaine (alternate internal standard)

36.3.8 Phenacetin (alternate internal standard)

**36.4 Solutions, Internal Standard, Calibrators and Controls**

36.4.1 5 mM Ammonium Acetate containing acetic acid: Weight 0.19 g ammonium acetate. Transfer to 500 mL volumetric flask and qs to volume with dH<sub>2</sub>O. Add 150 µL acetic acid. Store at room temperature for up to one month.

36.4.2 Working standard solution for risperidone (0.01 mg/mL)

Pipet 100 µL of 1 mg/mL stock solution of risperidone into a 10 mL volumetric flask and qs to volume with acetonitrile

36.4.3 Quality Control (QC) standard solution of risperidone (0.01 mg/mL)

Pipet 100 µL of separate 1 mg/mL stock solution of risperidone (different preparation than calibrators) into a 10 mL volumetric flask and qs to volume with acetonitrile

36.4.4 Internal standard working solution

0.01 mg/mL R68808: Pipet 100 µL of 1 mg/mL R68808 stock solution into 10 mL volumetric flask and qs to volume with acetonitrile. If R68808 is not available from the manufacturer, alternate internal standards such as mepivacaine or phenacetin may be used.

36.4.5 To prepare the calibration curve, pipet the following volumes of the 0.01 mg/mL risperidone working solution into appropriately labeled 16 x 125 mm screw cap test tubes. To eliminate a solvent effect, calibrators and controls may be dried under nitrogen prior to the addition of blank blood. Add 1 mL blank blood to obtain the final concentrations listed below.

Amount of Standard ( $\mu\text{L}$ )	Final concentration of risperidone (mg/L)
100	1
50	0.5
20	0.2
10	0.1
5	0.05

## 36.4.6 Controls

## 36.4.6.1 Risperidone Control

Pipet 50  $\mu\text{L}$  of the 0.01 mg/mL risperidone QC solution into an appropriately labeled tube. Add 1 mL blank blood to achieve final concentration of 0.5 mg/L.

## 36.4.6.2 Negative control. Blood bank blood or equivalent determined not to contain risperidone.

## 36.5 Apparatus

36.5.1 Test tubes, 16 x 125 mm, round bottom, borosilicate glass with Teflon caps

36.5.2 Test tubes, 16 x 114 mm, glass centrifuge, conical bottom

36.5.3 Centrifuge capable of 2000-3000 rpm

36.5.4 Nitrogen evaporator with heating block

36.5.5 Vortex mixer

36.5.6 GC autosampler vials with inserts

36.5.7 LCMS: Agilent Model 1100 LC-MSD

36.5.7.1 LCMS Instrument Conditions. The following instrument conditions may be modified to adjust or improve separation and sensitivity.

## 36.5.7.1.1 Elution conditions:

Column: C-18, 125 mm X 3 mm, 3  $\mu\text{M}$  particle size

Column thermostat: 35°C

Solvent A: 5 mM ammonium acetate (500 mL) containing 150  $\mu\text{L}$  acetic acid

Solvent B: acetonitrile

Isocratic elution, stop time: 4.00 min

Time	Solv. B	Flow
0.00	80	0.6

## 36.5.7.1.2 Spray Chamber

Ionization Mode:

Electrospray

Gas Temperature: 300°C

Drying Gas (N<sub>2</sub>): 11.8 L/min

Nebulizer pressure: 30 psig

Vcap (Positive): 2500 V

36.5.7.1.3 Selected Ion Monitoring (quantitation ions)

Polarity: Positive

Injection volume: 1 µL

Time (min)	Group Name	SIM Ion	Frag-Mentor	Gain EMV	SIM Resol.	Actual Dwell
0.00	risperidone	191	115	1.0	High	114
		411				114
		412				114
	RS68808	201				114
		421				114

### 36.6 Procedure

- 36.6.1 Label clean 16 x 125 mm screw cap tubes appropriately with calibrators, controls and case sample IDs.
- 36.6.2 Prepare calibrators and controls.
- 36.6.3 Add 1 mL case specimens to the appropriately labeled tubes. Note: since this procedure is used for screening and confirmation, it is recommended to analyze two different aliquots (or tissues) with each case. One will serve as a screen and the second as a confirmation.
- 36.6.4 Add 50 µL 0.01 mg/mL R68808 internal standard working solution to each tube.
- 36.6.5 Slowly, add dropwise 2 mL cold (freezer temperature) acetonitrile to each tube while vortexing. Continuous vortexing, not mere mixing, is essential.
- 36.6.6 Vortex an additional 30 seconds.
- 36.6.7 Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation.
- 36.6.8 Place tubes in freezer for at least 30 minutes to facilitate separation.
- 36.6.9 Transfer top acetonitrile layer to clean conical bottom tubes taking care not to transfer any lower layers.
- 36.6.10 Evaporate to dryness at approximately 50°C under nitrogen.
- 36.6.11 Reconstitute samples in 100 µL acetonitrile. Vortex briefly. Transfer to GC microvials. Note: reconstitution volume may be adjusted as necessary to prevent saturation of the detector.

### 36.7 Calculation

Drug concentrations are calculated by linear regression analysis using the ChemStation software.

**36.8 Quality Control and Reporting**

- 36.8.1 The LOQ for this procedure is defined as the lowest acceptable calibrator concentration used in the calibration curve. The ULOL for this procedure is defined as the highest acceptable calibrator concentration used in the calibration curve.
- 36.8.2 If the same specimen is analyzed in duplicate (for screening and confirmation) and both results are quantitative, the results should be averaged prior to reporting.
- 36.8.3 See Toxicology Quality Guidelines

**36.9 References**

- 36.9.1 Julia Pearson, Dwight Flammia and R Steiner, in-house development.

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**37 TOPIRAMATE AND QUETIAPINE QUANTITATION AND CONFIRMATION BY LCMS****37.1 Summary**

Topiramate and quetiapine are extracted from biological samples by making the samples basic with saturated borate buffer and extracting with toluene/hexane/isoamyl alcohol (THIA). An aliquot of the extract is analyzed by high performance liquid chromatography-electrospray ionization mass spectrometry (LC-ESI-MS).

**37.2 Specimen Requirements**

2 mL blood, fluid or tissue homogenate.

**37.3 Reagents and Standards**

37.3.1 Quetiapine, 1 mg/mL

37.3.2 Topiramate, 1 mg/mL

37.3.3 Mepivacaine, 1 mg/mL

37.3.4 Sodium tetraborate decahydrate

37.3.5 Hexane

37.3.6 Isoamyl alcohol

37.3.7 Methanol

37.3.8 Toluene

37.3.9 Ammonium acetate

**37.4 Solutions, Internal Standard, Calibrators and Controls**

37.4.1 10 mM Ammonium Acetate: Weigh 0.38 g ammonium acetate. Transfer to 500 mL volumetric flask and qs to volume with dH<sub>2</sub>O. Store at room temperature for up to one month.

37.4.2 Saturated borate buffer solution. Add sodium tetraborate decahydrate to dH<sub>2</sub>O until no more dissolves after shaking vigorously. Store at room temperature for up to two years.

37.4.3 Toluene:Hexane:Isoamyl Alcohol (THIA) (78:20:2, v:v:v) Mix 78 mL toluene, 20 mL hexane and 2 mL isoamyl alcohol. Store at room temperature for up to two years.

37.4.4 Drug stock solutions:

If 1 mg/mL commercially prepared stock solutions are not available, prepare 1 mg/mL solutions from powders. Weigh 10 mg of the free drug, transfer to a 10 mL volumetric flask and qs to volume with methanol. Note: If using the salt form, determine the amount of the salt needed to equal 10 mg of the free drug, and weigh this amount. Stock solutions are stored capped in a refrigerator and are stable for 2 years.

37.4.5 Working standard solution for topiramate: 1 mg/mL stock solution of topiramate.

Working standard solution for topiramate (0.1 mg/mL): Pipet 100 µL of the 1 mg/mL stock solution of topiramate into a 1.0 mL volumetric flask and qs to volume with methanol. Prepare fresh daily.

- 37.4.6 Working standard solution for quetiapine (0.1 mg/mL): Pipet 100  $\mu$ L of the 1 mg/mL stock solution of quetiapine into a 1.0 mL volumetric flask and qs to volume with methanol. Prepare fresh daily.
- 37.4.6.1 Working standard solution for quetiapine (0.02 mg/mL): Pipet 100  $\mu$ L of the 1 mg/mL stock solution of quetiapine into a 5.0 mL volumetric flask and qs to volume with methanol. Prepare fresh daily.
- 37.4.7 Working internal standard solution (0.1 mg/mL mepivacaine): Pipet 1 mL of the 1 mg/mL stock solution of mepivacaine into a 10 mL volumetric flask and qs to volume with dH<sub>2</sub>O.
- 37.4.8 To prepare the calibration curve, pipet the following volumes of the topiramate working solution and quetiapine working solution into appropriately labeled 16 x 125 mm screw cap test tubes. To eliminate a solvent effect, calibrators and controls may be dried under nitrogen prior to the addition of blank blood. Add 2 mL with blank blood to obtain the final concentrations listed below.

Amount of 1mg/mL (0.1 mg/mL) topiramate standard ( $\mu$ L)	Final concentration of topiramate (mg/L)	Amount of 0.1 mg/mL (0.02 mg/mL) quetiapine standard ( $\mu$ L)	Final concentration of quetiapine (mg/L)
100 (1000)	50	100 (500)	5
40 (400)	20	40 (200)	2
20 (200)	10	20 (100)	1
10 (100)	5	10 (50)	0.5
4 (40)	2	4 (20)	0.2
2 (20)	1	2 (10)	0.1

#### 37.4.9 Controls

37.4.9.1 Topiramate and Quetiapine Controls. Control may be from an external source or prepared in-house using drugs from different manufacturers, lot numbers or prepared by a chemist different than the individual performing the extraction.

37.4.9.2 Negative control. Blood bank blood or equivalent determined not to contain topiramate, quetiapine or mepivacaine.

### 37.5 Apparatus

37.5.9 Test tubes, 16 x 125 mm, round bottom, borosilicate glass with Teflon caps

37.5.10 Test tubes, 16 x 114 mm, glass centrifuge, conical bottom

37.5.11 Centrifuge capable of 2000-3000 rpm

37.5.12 Nitrogen evaporator with heating block

37.5.13 Vortex mixer

37.5.14 GC autosampler vials with inserts

37.5.15 LCMS: Agilent Model 1100 LC-MSD

37.5.15.2 LCMS Instrument Conditions. The following instrument conditions may be modified to adjust or improve separation and sensitivity.

#### 37.5.15.2.1 Elution Conditions

Column: C-18, 125 mm X 3 mm, 3  $\mu$ M particle size

37 Topiramate and Quetiapine Quantitation and Confirmation by LCMS

Column thermostat: 35°C

Solvent A: 55% 10 mM ammonium acetate

Solvent B: 45% methanol

Gradient elution, stop time: 13.00 min

Time (min)	Solv. B	Flow
0.00	45	0.45
4.00	80	0.45
8.00	80	0.45
9.00	45	0.45

37.5.15.2.2 Spray Chamber

Ionization Mode: Electrospray

Gas Temperature: 350°C

Drying Gas (N<sub>2</sub>): 12.0 L/min

Nebulizer pressure: 30 psig

Vcap (Positive): 4000 V

37.5.15.2.3 Selected Ion Monitoring (quantitation ions)

Polarity: Positive

Injection volume: 2 µL

Time (min)	Group Name	SIM Ion	Fragmentor	Gain EMV	SIM Resol.	Actual Dwell
0.00	topiramate	264	150	1.5	Low	218
		282	150		218	
		340	150		218	
		<u>357</u>	150		218	
4.30	mepivacaine	98	150	0.5	Low	439
		<u>247</u>	150		439	
6.00	quetiapine	210	250	0.5	Low	218
		<u>253</u>	250		218	
		279	250		218	
		<u>384</u>	250		218	

**37.6 Procedure**

37.6.9 Label clean 16 x 125 mm screw cap tubes appropriately with calibrators, controls and case sample IDs.

37.6.10 Prepare calibrators and controls.

37.6.11 Add 2 mL case specimens to the appropriately labeled tubes. Note: since this procedure is used for screening and confirmation, it is recommended to analyze two different aliquots (or tissues) with each

case. One will serve as a screen and the second as a confirmation (unless topiramate and quetiapine have already been confirmed using the base screen procedure).

- 37.6.12 Add 40  $\mu$ L 0.1 mg/mL mepivacaine internal standard working solution to each tube for a final concentration of 2 mg/L.
- 37.6.13 Add 2 mL saturated borate buffer and 6 mL extract solvent (78:20:2 THIA) to each tube.
- 37.6.14 Cap and rotate tubes for 30 minutes.
- 37.6.15 Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation. Transfer organic upper layer (THIA) to appropriately labeled conical bottom test tubes.
- 37.6.16 Evaporate samples to dryness at approximately 50°C under nitrogen.
- 37.6.17 Reconstitute samples in 500  $\mu$ L methanol. Vortex briefly. Transfer to GC autosampler vials for analysis by LCMS. Note: reconstitution volume may be adjusted as necessary to prevent saturation of detector.

### 37.7 Calculation

Drug concentrations are calculated by linear regression analysis using the ChemStation software.

### 37.8 Quality Control and Reporting

- 37.8.9 The LOQ for this procedure is defined as the lowest acceptable calibrator concentration used in the calibration curve for each analyte.
- 37.8.10 The ULOL for this procedure is defined as the highest acceptable calibrator concentration used in the calibration curve for each analyte.
- 37.8.11 If the same specimen is analyzed in duplicate (for screening and confirmation) and both results are quantitative, the results should be averaged prior to reporting.
- 37.8.12 See Toxicology Quality Guidelines

### 37.9 References

- 37.9.9 M Contin, R Riva, F Albani and A Baruzzi. Simple and rapid liquid chromatographic-turbo ion spray mass spectrometric determination of topiramate in human plasma. *J Chrom B* 761: 133-137, 2001.
- 37.9.10 Julia Pearson, Dwight Flammia and Robert Steiner, in-house development.

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**38 SCOPOLAMINE AND ATROPINE QUANTITATION AND CONFIRMATION BY LCMS****38.1 Summary**

Scopolamine and atropine are extracted from biological samples by making the samples basic with saturated borate buffer and extracting with toluene/hexane/isoamyl alcohol. An aliquot of the extract is analyzed by high performance liquid chromatography-electrospray ionization mass spectrometry (LC-ESI-MS).

**38.2 Specimen Requirements**

2 mL blood, fluid or tissue homogenate.

**38.3 Reagents and Standards**

38.3.1 Atropine, 1 mg/mL

38.3.2 Scopolamine, 1 mg/mL

38.3.3 Pentazocine, 1 mg/mL

38.3.4 Sodium tetraborate decahydrate

38.3.5 Hexane

38.3.6 Isoamyl alcohol

38.3.7 Methanol

38.3.8 Toluene

38.3.9 Acetic Acid

38.3.10 Acetonitrile

**38.4 Solutions, Internal Standard, Calibrators and Controls**

38.4.1 Saturated borate buffer solution. Add sodium tetraborate decahydrate to dH<sub>2</sub>O until no more dissolves after shaking vigorously. Store at room temperature for up to two years.

38.4.2 Toluene:Hexane:Isoamyl Alcohol (THIA) (78:20:2, v:v:v) Mix 78 mL toluene, 20 mL hexane and 2 mL isoamyl alcohol. Store at room temperature for up to two years.

38.4.3 Drug stock solutions:

If 1 mg/mL commercially prepared stock solutions are not available, prepare 1 mg/mL solutions from powders. Weigh 10 mg of the free drug, transfer to a 10 mL volumetric flask and qs to volume with methanol. Note: If using the salt form, determine the amount of the salt needed to equal 10 mg of the free drug, and weigh this amount. Stock solutions are stored capped in a refrigerator and are stable for 2 years.

38.4.4 Working standard solution for scopolamine and atropine (SCAT, 0.001 mg/mL): Pipet 10 µL each of the 1 mg/mL stock solution of scopolamine and atropine into a 10 mL volumetric flask and qs to volume with methanol.

38.4.5 Working internal standard solution (0.001 mg/mL pentazocine): Pipet 10 µL of the 1 mg/mL stock solution of pentazocine into a 10 mL volumetric flask and qs to volume with methanol.

- 38.4.6 To prepare the calibration curve, pipet the following volumes of the 0.001 mg/mL SCAT working solution into appropriately labeled 16 x 125 mm screw cap test tubes. To eliminate a solvent effect, calibrators and controls may be dried under nitrogen prior to the addition of blank blood. Add 2 mL blank blood to obtain the final concentrations listed below.

Amount of 0.001 mg/mL SCAT working solution (µL)	Final concentration of SCAT (µg/L)
200	100
100	50
40	20
20	10
10	5
4	2
2	1

38.4.7 Controls

38.4.7.1 SCAT Controls. Control may be from an external source or prepared in-house using drugs from different manufacturers, lot numbers or prepared by a chemist different than the individual performing the extraction.

38.4.7.2 Negative control. Blood bank blood or equivalent determined not to contain scopolamine, atropine or pentazocine.

**38.5 Apparatus**

38.5.1 Test tubes, 16 x 125 mm, round bottom, borosilicate glass with Teflon caps

38.5.2 Test tubes, 16 x 114 mm, glass centrifuge, conical bottom

38.5.3 Centrifuge capable of 2000-3000 rpm

38.5.4 Nitrogen evaporator with heating block

38.5.5 Vortex mixer

38.5.6 GC autosampler vials with inserts

38.5.7 LCMS: Agilent Model 1100 LC-MSD

38.5.7.1 LCMS Instrument Conditions. The following instrument conditions may be modified to adjust or improve separation and sensitivity.

38.5.7.1.1 Elution Conditions

Column: Agilent Hypersil BDS 125 mm X 3 mm, 3 µM particle size

Column thermostat: 35°C

Solvent A: 45% water with 1% acetic acid

Solvent B: 55% methanol

Isocratic elution, stop time: 6.20 min

## 38.5.7.1.2 Spray Chamber

Ionization Mode: Electrospray

Gas Temperature: 350°C

Drying Gas (N<sub>2</sub>): 12.0 L/min

Nebulizer pressure: 30 psig

Vcap (Positive): 4000 V

## 38.5.7.1.3 Selected Ion Monitoring (quantitation ions)

Polarity: Positive

Injection volume: 8 µL

Time (min)	Group Name	SIM Ion	Frag-Mentor	Gain EMV	SIM Resol.	Actual Dwell
0.0	Scopolamine	138	220	1.0	Low	195
		156			195	
		<u>304</u>			195	
0.0	Atropine	93	220	2.0	Low	195
		124			195	
		<u>290</u>			195	
4.2	Pentazocine	173	220	1.0	Low	392
		218	250		392	
		<u>286</u>	250		392	

**38.6 Procedure**

- 38.6.1 Label clean 16 x 125 mm screw cap tubes appropriately with calibrators, controls and case sample IDs.
- 38.6.2 Prepare calibrators and controls.
- 38.6.3 Add 2 mL case specimens to the appropriately labeled tubes. Note: since this procedure is used for screening and confirmation, it is recommended to analyze two different aliquots (or tissues) with each case. One will serve as a screen and the second as a confirmation.
- 38.6.4 Add 100 µL 0.001 mg/mL pentazocine internal standard working solution to each tube for a final concentration of 2mg/L.
- 38.6.5 Add 2 mL saturated borate buffer and 6 mL extract solvent (78:20:2 THIA) to each tube.
- 38.6.6 Cap and rotate tubes for 30 minutes.
- 38.6.7 Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation. Transfer organic upper layer (THIA) to appropriately labeled conical bottom test tubes.
- 38.6.8 Evaporate samples to dryness at approximately 50°C under nitrogen.
- 38.6.9 Reconstitute samples in 500 µL acetonitrile.
- 38.6.10 Add 1 mL hexane to each tube. Vortex each sample for 30 seconds.

38.6.11 Centrifuge at approximately 2500 rpm for 10 minutes to achieve separation. Aspirate and discard upper hexane layer.

38.6.12 Evaporate samples to dryness at approximately 50°C under nitrogen.

38.6.13 Reconstitute samples in 100 µL methanol. Transfer to GC autosampler vials for analysis by LCMS.  
Note: reconstitution volume may be adjusted as necessary to prevent saturation of the detector.

### 38.7 Calculation

Drug concentrations are calculated by linear regression analysis using the ChemStation software.

### 38.8 Quality Control and Reporting

38.8.1 The LOQ for this procedure is defined as the lowest acceptable calibrator concentration used in the calibration curve for each analyte. The ULQL for this procedure is defined as the highest acceptable calibrator concentration used in the calibration curve for each analyte.

38.8.2 If the same specimen is analyzed in duplicate (for screening and confirmation) and both results are quantitative, the results should be averaged prior to reporting.

38.8.3 See Toxicology Quality Guidelines

### 38.9 References

38.9.1 J Saady and A Poklis. Determination of Atropine in Blood by GCMS. J Anal Tox 13: 296-299, 2001.

38.9.2 Julia Pearson and Robert Steiner, in-house development.

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**39 LAMOTRIGINE QUANTITATION AND CONFIRMATION BY LCMS****39.1 Summary**

Lamotrigine is extracted from biological samples with an acetonitrile precipitation and analyzed by high performance liquid chromatography-electrospray ionization mass spectrometry (LC-ESI-MS).

**39.2 Specimen Requirements**

0.2 mL blood, serum or plasma.

**39.3 Reagents and Standards**

39.3.1 Ammonium Formate

39.3.2 Methanol

39.3.3 Acetonitrile

39.3.4 Lamotrigine (Lamictal®)

39.3.5 Phenacetin

**39.4 Solutions, Internal Standards, Calibrators and Controls**

39.4.1 10 mM Ammonium formate with acetonitrile: Weigh 0.315 g ammonium formate. Transfer to 500 mL volumetric flask and qs to volume with dH<sub>2</sub>O. Then add 25 mL acetonitrile. Store at room temperature for up to one month.

39.4.2 Working standard solutions for lamotrigine

39.4.2.1 1 mg/mL lamotrigine stock solution: Weigh 10mg of lamotrigine. Transfer to 10 mL volumetric flask and qs to volume with methanol.

39.4.2.2 0.02 mg/mL lamotrigine working solution: Pipet 200 µL of 1.0 mg/mL stock solution of lamotrigine into 10.0 mL volumetric flask and qs to volume with methanol.

39.4.3 Quality Control (QC) standard solutions

0.02 mg/mL lamotrigine QC solution: Pipet 200 µL of separate 1.0 mg/mL stock solution of lamotrigine into 1.0 mL volumetric flask and qs to volume with methanol.

39.4.4 Internal standard working solution

39.4.4.1 1 mg/mL phenacetin stock solution. Weigh 10 mg phenacetin, transfer to 10 mL volumetric flask and qs to volume with methanol.

39.4.4.2 0.1 mg/mL phenacetin: Pipet 1 mL of 1 mg/mL phenacetin stock solution into 10 mL volumetric flask and qs to volume with methanol

39.4.5 Calibrators. To prepare the calibration curve, pipet the following volumes of working solution into appropriately labeled 16 x 125 mm screw cap tubes. To eliminate a solvent effect, calibrators and controls may be dried under nitrogen prior to the addition of blank blood.

Cal 1: 12 mg/L lamotrigine: 120 µL of 0.02 mg/mL lamotrigine working solution

Cal 2: 10 mg/L lamotrigine: 100 µL of 0.02 mg/mL lamotrigine working solution

Cal 3: 8 mg/L lamotrigine: 80  $\mu$ L of 0.02 mg/mL lamotrigine working solution

Cal 4: 4 mg/L lamotrigine: 40  $\mu$ L of 0.02 mg/mL lamotrigine working solution

Cal 5: 2 mg/L lamotrigine: 20  $\mu$ L of 0.02 mg/mL lamotrigine working solution

Lowest Calibrator: 1 mg/L lamotrigine: 10  $\mu$ L of 0.02 mg/mL lamotrigine working solution

39.4.5.1 For each calibrator, add .2 mL blank blood to each tube to achieve final concentration.

39.4.6 Lamotrigine Control (QC) 5 mg/L Lamotrigine: Pipet 50  $\mu$ L of 0.02 mg/mL lamotrigine QC solution into appropriately labeled 16 x 125 mm screw cap tube and add .2 mL blank blood.

39.4.7 Negative blood control: Blood bank blood (or equivalent) previously determined not to contain lamotrigine.

### 39.5 Apparatus

39.5.1 Screw cap test tubes, 16 x 125 mm

39.5.2 Centrifuge capable of 2,000-3,000 rpm

39.5.3 Vortex mixer

39.5.4 GC autosampler vials with inserts

39.5.5 LCMS: Agilent Model 1100 LC-MSD

39.5.6 LCMS Instrument Conditions. The following instrument conditions may be modified or adjusted to improve separation and sensitivity. Quantitation can be achieved by either collecting data in SIM or Scan mode.

39.5.6.1 Elution conditions:

Column: Altima HP HILIC 150 mm X 2.1 mm, 3  $\mu$ m particle size

Column thermostat: 35°C

Solvent A: 10 mM ammonium formate in dH<sub>2</sub>O containing acetonitrile

Solvent B: Acetonitrile

Solvent ramp stop time: 10 min

Time	Solv. A	Solv. B	Flow
Initial	5%	95%	.700
4.00	11%	89%	0.400
5.00	5%	95%	0.700

39.5.6.1.1 Spray Chamber

Ionization Mode: Electrospray

Gas Temperature: 350°C

Drying Gas (N<sub>2</sub>): 12.0 L/min

Nebulizer pressure: 30 psig

Vcap (Positive): 4000 V  
 Injection Volume: 1 $\mu$ L

## 39.5.6.1.2 Selected Ion Monitoring

Polarity: Positive  
 SIM parameters (quantitation ion)

Time	Group Name	SIM Ion	Frag-Mentor	Gain EMV	SIM Resol.	Actual Dwell
0.00 min	Phenacetin	180	150	1	Low	108
	Lamotrigine	256	150	1	Low	108
	Lamotrigine	257	150	1	Low	108
	Lamotrigine	258	150	1	Low	108
	Lamotrigine	259	150	1	Low	108

Scan Monitoring

Time (min)	Low Mass	High Mass	Fragmentor	Gain EMV	Threshold	Step size
0.00	55.0	300.0	150	1.0	150	0.10

**39.6 Procedure**

- 39.6.1 Label 16 x 125 mm screw cap tubes appropriately with blank, calibrators, controls and case sample IDs.
- 39.6.2 Prepare calibrators and controls.
- 39.6.3 Add .2 mL case specimens to the appropriately labeled tubes. Note: since this procedure is used for screening and confirmation, it is recommended to analyze two different aliquots (or tissues) with each case. One will serve as a screen and the second as a confirmation (unless lamotrigine has already been confirmed using the base screen procedure).
- 39.6.4 Add 50  $\mu$ L 0.1 mg/mL phenacetin internal standard working solution to each tube.
- 39.6.5 Slowly, add dropwise 6 mL acetonitrile to each tube while vortexing. Continuous vortexing, not mere mixing, is essential.
- 39.6.6 Vortex an additional 30 seconds.
- 39.6.7 Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation.
- 39.6.8 Transfer an aliquot of clear acetonitrile mixture to a GC vial containing a conical insert and transfer to autosampler tray.
- 39.6.9 Inject 1  $\mu$ L of each sample on LCMS in the API-ES/SIM or Scan Mode.

**39.7 Calculation**

Drug concentrations are calculated by linear regression analysis using the ChemStation software.

**39.8 Quality Control**

- 39.8.1 The LOQ for this procedure is defined as the lowest acceptable calibrator concentration used in the calibration curve. The ULOL for this procedure is defined as the highest acceptable calibrator concentration used in the calibration curve.

- 39.8.2 Samples greater than the ULOL should be reported as “greater than 12 mg/L” if not re-analyzed with a dilution.
- 39.8.3 Blank calibration matrix (whole blood, serum, or plasma) **must be** used as the diluent. Samples **may not be diluted** with distilled water.
- 39.8.4 Re-injection of processed samples **is not permitted**. Samples must be re-extracted for analysis.
- 39.8.5 If the same specimen is analyzed in duplicate (for screening and confirmation) and both results are quantitative, the results should be averaged prior to reporting.
- 39.8.6 See Toxicology Quality Guidelines.

### 39.9 References

- 39.9.1 Dwight Flammia and Les Edinboro, in-house development.

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## 40 OVERVIEW OF ABC ALCOHOLIC BEVERAGE ANALYSIS

### 40.1 Introduction

The Virginia Department of Alcoholic Beverage Control (ABC) regulates the sale of alcoholic beverages and enforces the alcoholic beverage control laws within the Commonwealth of Virginia. The ABC laboratory analyzes products currently available for sale or new products that are proposed for sale within the Commonwealth.

40.1.1 The ABC lab also tests beverages submitted by law enforcement agencies. Most of these cases involve the investigation of minors in possession of alcohol, open intoxicants in vehicles and illegal sale/distribution of alcohol. These types of cases require the analysis of alcohol content. Any beverage containing greater than or equal to 0.5% ethanol is defined as an alcoholic beverage (Code of Virginia § 4.1-100).

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**41 ETHANOL CONTENT OF ALCOHOLIC BEVERAGES BY HEADSPACE GC****41.1 Summary**

An aliquot of sample is diluted semi-automatically with an internal standard (IS) solution (n-propanol) into a glass vial, sealed, and placed in a heated automatic sampler. The concentration of ethanol in a dilute alcoholic beverage is directly proportional to its concentration in the gas phase or headspace. A portion of the resultant headspace vapor above the liquid is automatically injected into a gas chromatograph (GC) equipped with two flame ionization detectors (FID). Ethanol is identified by retention time and its concentration is calculated by comparison to similarly treated aqueous calibrators by using peak heights or areas.

**41.2 Specimen Requirements**

Approximately 0.5 mL liquid or 0.5 g solid material

**41.3 Reagents and Standards**

41.3.1 Absolute ethanol, 200 proof

41.3.2 N-propanol

41.3.3 Reference standard ethanol solutions (e.g. National Institute of Standards and Technology (NIST) or NIST traceable)

**41.4 Solutions, Internal Standard, Calibrators and Controls**

41.4.1 All calibrators and controls shall be stored at 2-8°C.

41.4.2 0.5% v/v n-propanol internal standard. Pipet 5 mL n-propanol into a 1 L volumetric flask and qs to volume with dH<sub>2</sub>O.

41.4.3 0.5% v/v ethanol standard. Pipet 500 µL ethanol into a 100 mL volumetric flask and qs to volume with dH<sub>2</sub>O.

41.4.4 5% v/v ethanol standard. Pipet 5 mL ethanol into a 100 mL volumetric flask and qs to volume with dH<sub>2</sub>O.

41.4.5 25% v/v ethanol standard. Pipet 25 mL ethanol into a 100 mL volumetric flask and qs to volume with dH<sub>2</sub>O.

41.4.6 50% v/v ethanol standard. Pipet 50 mL ethanol into a 100 mL volumetric flask and qs to volume with dH<sub>2</sub>O.

41.4.7 Controls

41.4.7.1 WQA Control. Burgundy wine stored in 4 ounce glass bottles (stored at 2-8°C for up to two years).

41.4.7.2 NIST ethanol solutions (7.5% and 31.25% v/v ethanol)

41.4.7.3 Negative control (dH<sub>2</sub>O)

41.4.7.4 5% v/v ethanol in-house control. Pipet 5 mL ethanol into a 100 mL volumetric flask and qs to volume with dH<sub>2</sub>O.

**41.5 Apparatus**

41.5.1 Gas chromatograph with data system, two flame ionization detectors and a headspace autosampler

- 41.5.2 Columns. Restek Rtx®-BAC1 and Rtx®-BAC2 capillary column
- 41.5.3 Glass 20 mL (23 x 75 mm) headspace vials with butyl septa and metal seals
- 41.5.4 Vial seal crimper
- 41.5.5 Hamilton Microlab 5000 Series Diluter or equivalent
- 41.5.6 Glass Test tubes (12 x 75 mm)
- 41.5.7 Headspace GC/FID parameters. Instrument conditions may be changed to permit improved performance.

## 41.5.7.1 GC parameters

Oven	50°C Isothermal
Injector	200°C
Detector (FID)	300°C
Hydrogen flow	30 mL/min
Air flow	400 mL/min
Make-up flow	20 mL/min
Make-up gas	helium
Inlet	Split
Split ratio	50:1
Split flow	674 mL/min
Total flow	690 mL/min
Pressure	8 psi constant pressure mode

## 41.5.7.2 Headspace autosampler parameters

Incubation temp	70°C
Incubation time	240 seconds
Syringe temp	80°C
Agitator speed	500 rpm
Fill speed	1000 µL/sec
Injection speed	1000 µL/sec
Syringe	2.5 mL headspace

**41.6 Procedure**

Case samples are prepared and analyzed in duplicate. Calibrators and controls are analyzed singly.

- 41.6.1 Pour approximately 0.2 mL of calibrator, control or sample into a clean 12 x 75 mm test tube.
- 41.6.2 Place the diluter delivery tip into the specimen, making sure its tip is below the surface of the sample. Activate the diluter. At this point, the diluter draws 0.05 mL of sample into its delivery tube.
- 41.6.3 Withdraw the tip and wipe it with a Kimwipe/tissue paper.
- 41.6.4 Direct the delivery tip into the appropriately labeled headspace vial and activate the diluter. The diluter will dispense the sample and 0.950 mL of the n-propanol IS solution into the vial.
- 41.6.5 Flush the diluter as necessary by activating the diluter one or more times or rinsing with dH<sub>2</sub>O, depending on the viscosity or other nature of the sample. Dispense washings into a waste beaker.

- 41.6.6 Stopper the headspace vial with the butyl septa. Vortex or manually shake for several seconds and place in the sample rack.
- 41.6.7 Repeat steps 42.6.1 through 42.6.6 for all calibrators, controls and case samples.
- 41.6.8 Seal all headspace vials by crimping the metal rings over the butyl septa.
- 41.6.9 Load headspace vials into the headspace auto sampler.

#### 41.7 Calculation

- 41.7.1 Ethanol is identified based on relative retention time compared to calibrators. Identification is performed by instrument software. Retention times for both ethanol and internal standard should be within  $\pm 2\%$  of the retention time obtained from the average of the calibrators on both columns.
- 41.7.2 Concentration is calculated automatically by the software based on linear regression of the 3 point calibration curve based on peak area or peak height measurement. The data from the Rtx-BAC1 column is utilized for quantitative results.
- 41.7.3 Solid material concentration is calculated as follows:

$$\text{Chromatogram concentration} \times \frac{0.5 \text{ g}}{\text{weighed amount}} = \text{ethanol concentration \% (w/w)}$$

#### 41.8 Quality Control and Reporting

- 41.8.1 Calibration check. The method calibration is checked with each day's batch sample analysis. Analyze the 4 calibrators, negative control (dH<sub>2</sub>O), burgundy wine QC and NIST QCs. Acceptable tolerance for calibrators and controls is 5% of the target value.
- 41.8.1.1 If the calibrators and controls satisfy quality control criteria, the method may be used as is.
- 41.8.1.2 If the calibrators and/or controls do not satisfy quality control criteria, recalibrate the method.
- 41.8.1.3 If, after recalibration, the calibrators and controls still do not fall within 5% of target concentration, then appropriate measures must be taken to rectify the problem (open or prepare new calibrators/controls, instrument maintenance etc). Document such actions and measure in the BAC instrument log.
- 41.8.1.4 Samples may not be analyzed prior to an acceptable calibration check.
- 41.8.2 Correlation of determination ( $r^2$ ). The  $r^2$  value for the linear regression curve must be 0.995 or greater. If not, the instrument must be recalibrated or other appropriate measures taken. The correlation of determination is automatically printed on the calibration table and curves (and a copy should be included with the batch data file).
- 41.8.3 Carryover. The negative control (dH<sub>2</sub>O) is used to check for carryover and is run immediately following the high 50% ethanol calibrator. An acceptable negative control must contain less than 0.1% ethanol w/v.
- 41.8.3.1 If the negative control is unacceptable, prepare a fresh negative dH<sub>2</sub>O control and reanalyze immediately after the high calibrator. If, after reanalysis, ethanol is present in the negative control greater than 0.1% w/v, perform instrument maintenance to correct the problem.
- 41.8.4 Positive controls. The acceptable tolerance for ethanol controls is 5% of target concentration. If any of the controls fall outside of the acceptable tolerance, all positive samples must be repeated. Negative results may be reported. Document actions and exceptions on the batch worksheet and the instrument log. In general,

corrective actions for failed controls may include repeating the batch, recalibrating the instrument, opening new controls or making new calibrators.

- 41.8.5 Batch sample analysis. Headspace ethanol analysis is performed as a batch analysis. Analyze one control after every 10 injections. All controls within a batch must fall within acceptance tolerance in order to report any positive cases from that batch.
- 41.8.6 Vial Verification. After the completion of a batch, the identity of each vial in the headspace sampler is verified with the sequence table and the alcohol worksheet. Vial verification is performed by an analyst other than the operator and is documented by initials and date on the batch alcohol worksheet.
- 41.8.7 Case samples are analyzed in duplicate. Duplicate results must agree within 10%. Otherwise, reanalyze samples in duplicate.
- 41.8.8 The LOQ for this procedure is defined as the lowest acceptable calibrator concentration used in the calibration curve. The ULOL for this procedure is defined as the highest acceptable calibrator concentration used in the calibration curve.
- 41.8.9 Report the average of the duplicates truncated to 1 decimal place (e.g. 5.4% v/v)

#### 41.9 References

- 41.9.1 B. Kolb, "Head Space Analysis by Means of the Automated Gas Chromatograph F-40 Multifract", Bodenseewerk Perkin-Elmer and Co., Technical Manual #15E.
- 41.9.2 K. M. Dubowski, "Manual for Analysis of Ethanol in Biological Liquids," Department of Transportation Report No. DOT TSC NHTSA-76-4, Jan 1977.

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**Appendix A - COMMONLY USED ABBREVIATIONS**

The following is a list of abbreviations commonly used by examiners and toxicologists in the Section. This list has been generated to assist in the interpretation of case file notes and is not a standardized list of required abbreviations. While as comprehensive as possible, the list may not be complete. The abbreviations are appropriate written in either lower or upper case, with and without punctuation such as periods. Empirical formulas, chemical, mathematical and shorthand abbreviations are equally acceptable and are not listed here. In all instances, abbreviations used in note taking should be readily interpretable within the context of the subject material and in conjunction with the associated Certificate of Analysis.

**A.1 General Abbreviations**

<b>Abbreviations</b>	<b>Definitions</b>
/nor	desmethyl metabolite
?	unidentified/unknown
@	at
6AM, 6MAM, MAM	6-monoacetylmorphine
A/N	acidic/neutral drugs
Aa	automobile accident
ABC	Alcoholic Beverage Commission
Abs	absorbance
Accd	accepted
Ace	acetone
ACI	acute coronary insufficiency
Acids	acidic drugs
ADM	administrative
Alc	alcohols/alcohol screen
alp, alpraz	alprazolam
ami, amit, amitrip	amitriptyline
Amp	amphetamine
Amps	amphetamine type drugs
Anal	analysis
APAP	acetaminophen
Approx	approximately
ASA	salicylate
ASCVD	Arteriosclerotic cardiovascular disease
Ass	assault
Ave	average
Axsym	fluorescence polarization immunoassay
BAC	blood alcohol content
barb, barbs	barbiturates
Bases	basic drugs
BE	benzoylecgonine
BE-d3	benzoylecgonine d3
Benz, benzos, BZ	benzodiazepines
Bld	blood
Bldy	bloody
BR	barbituates
Brn	brain
Buprop	Bupropion
BUP	buprenorphine

butal	butalbital
C, cont	container
caff	caffeine
cal	calibrator
cannabs	cannabinoids
carbam	carbamazepine
cariso	carisoprodol
cav fl	cavity fluid
CE	cocaethylene
Cer	Cerilliant
CHLORDIAZ	chlordiazepoxide
CHROMAT	chromatograph
CIT, citalo	citalopram
cntl, ctl, cont, con	control
c/o	complained of
CO	carbon monoxide
COC	cocaine
coc metab	cocaine metabolite (benzoylecgonine)
COD	cause of death
Cod	codeine
conc	concentrated/concentration
conf, conf'd	confirmed
cot	cotinine
cpds	compounds
CSF	cerebral spinal fluid
CYCLOBENZ	cyclobenzaprine
d	driver
d(followed by a number)	deuterated with specified number
dec fl, decomp fl	decomposition fluid
decomp	decomposed
DEX, DXM	dextromethorphan
DFSA	drug-facilitated sexual assault
dH2O	deionized water
dia, DIAZ	diazepam
dil	dilution/diluted
DIP, DPH	diphenhydramine
DM	diabetes mellitus (diabetic)
DNE	did not extract
DOX	doxepin
Dx	diagnosis
DZ	disease
ECD	electron capture detector
EG, ETH GLY	ethylene glycol
ELISA	Enzyme Linked Immunosorbant Assay
ETOH	ethanol
ext	extraction/extracted
f	femoral
fent	fentanyl
FID	flame ionization detector

FLUNITRAZ	flunitrazepam
FLUOX	fluoxetine
form	formaldehyde
FPIA	fluorescence polarization immunoassay
FRAGS	fragments
fs	full scan
FS#	Department of Forensic Science unique case number
Fx	fracture
Gastric	gastric contents
GBL	gamma butyrolactone
GC	gas chromatograph/gas chromatography
GC	gastric contents
GCMS	gas chromatograph/mass spectrometer
GHB	gamma hydroxybutyrate
GSW	gunshot wound
GT	greater than
H	homicide
h/o	history of
Hb	hemoglobin
HB, Hbl	heart blood
HbCO	carboxyhemoglobin
HBLD, Hbl	hospital blood
HCD, HYC, hydrocod	hydrocodone
hdz	heart disease
HPLC	high pressure liquid chromatography
hrnd	heart related natural death
HSPB	heat sealed plastic bag
Ht	heart
HTN	hypertension
Hx	history
HYM, hydromorph	hydromorphone
ID	identification
Immunal	Immunoanalysis
imp	impurities
info	information
Int Std, ISTD, IS	internal standard
IPA, iso, isoprop, ISOP	isopropyl alcohol or isopropanol
IT, ITM	item
IVC	inferior vena cava
L	left
LCMS	liquid chromatography/mass spectrometry
LCMSMS	liquid chromatography/ tandem mass spectrometry
lid, lido	lidocaine
liq	liquid
Liv	liver
LKA	last known alive
LL	liquid/liquid
LLOQ	lower limit of quantitation
LOD	limit of detection

LOQ	limit of quantitation
lor, loraz	lorazepam
LRL	lower reporting limit
LT	less than
lvr	liver
mc	motorcycle
MCC	motorcycle collision
MDA	3,4-methylenedioxyamphetamine
MDMA	3,4-methylenedioxymethamphetamine
MDO, Mdon, MTD	methadone
ME	medical examiner
MeOH	methanol
mepro	meprobamate
metab	metabolite
meth, methamp	methamphetamine
methapyr, mp	methapyrilene
min	minutes
MIRTAZ	mirtazepine
MJ	marijuana
mL	milliliter
MOD	manner of death
mor, morph	morphine
MS	mass spectrometer
MSD	mass spectrometer detector
MVA	motor vehicle accident
MVC	motor vehicle collision
N, neg	negative
NA	not analyzed
nal	nalorphine
nat	natural
NCI	negative chemical ionization
ND	not detected or none detected
NDD	no drugs detected
neutrals	neutral drugs
nic	nicotine
NIST	National Institute of Standards and Technology
NOA	no other acidic or neutral drugs
NOB	no other alkali extractable drugs
nor, nordiaz	nordiazepam
norprop, norpropox	norpropoxyphene
nortrip	nortriptyline
NPD	nitrogen phosphorous detector
n-prop	n-propanol
O:, op	opened
OCME	Office of Chief Medical Examiner
OD	overdose
OLANZ	olanzapine
OP, opi	opiate(s)
OP'D, OPN'D	opened

ORG, orig	original
OXAZ	oxazepam
OXC, oxy, oxymor	oxycodone
OXM	oxymorphone
oxymor	oxymorphone
P, pass	passenger
PAROX	paroxetine
PCP	phencyclidine
PdCl <sub>2</sub>	palladium chloride
PE	pulmonary embolism
ped	pedestrian
perf	perforated
pf	purge fluid
PGT, PRGT	present greater than
PHENOBARB, pheno	phenobarbital
pk	peak
Pl fl	pleural fluid
PLB	plastic bag
PLT, PRLT	present less than
PM	preventive maintenance
pos	positive
pr	present
prob	probably
PROMETH	promethazine
PROP GLY, PG	propylene glycol
propox	propoxyphene
pur	purge
q	quantity
QA	quality assurance
QAS	Quality Assurance Service Corp
QC	quality control
QNS	quantity not sufficient for analysis
QNS	quantity insufficient for further analysis
QNSFFA	quantity not sufficient for further analysis
QQQ	liquid chromatography/mass spectrometry triple quadrupole
qs	add quantity sufficient to bring up to volume
quant	quantitation
QUET	quetiapine
R	right
r/o	rule out
R:, rec'd, revd	received
RRT	relative retention time
RT	retention time
Rx	prescription
S, suic	suicide
SBX	sealed box
SC	subclavian
SCHIZ	Schizophrenia
scr, scrn	screen

SD	standard deviation
SD	sudden death
SDH	subdural hematoma
SENV	sealed envelope
SERT	Sertraline
sgsw	single gunshot wound
SIM	selected ion monitoring
sl	slightly
SPB	sealed plastic bag
SPE	solid phase extraction
SPLB	sealed plastic bag
Std	standard
Subclav	subclavian
SUD	sudden unexpected death
SUID	sudden unexpected infant death
Supraclav	supraclavian or supraclavicular
surg	surgery/surgical
sx	sexual
syr	syringe
sys	systemic/system
sys imp	systemic impurities
SZ	seizure
T1	Tray 1
T2	Tray 2
TDx	fluorescence polarization immunoassay
TEMAZ	temazepam
TGT	target
THA, THCA	THC carboxylic acid
THC	tetrahydrocannabinol
THIA	toluene/hexane/isoamyl alcohol
Tox, TX	toxicology
tr	trace
TRAM	tramadol
TRAZ	trazodone
UFA, unsuit, Uns	unsuitable for analysis
ULOL	upper limit of linearity
ULOQ	upper limit of quantitation
unid	unidentified
UR	urine
UV	ultraviolet spectrophotometer
UV/VIS	ultraviolet/visible spectrophotometer
V	vehicle/vehicular
V	vial
VA, valp	valproic acid
VC	vena cava
VENLA	venlafaxine
VH, vit	vitreous humor
Vic	victim
vol, vols	volatile(s)

vols	volatiles
w/in	within
WQA	Wine Quality Assurance
zolp	zolpidem
ZPLB/T	Ziploc plastic bag sealed with tape

## A.2 DUI/DUID Evidence Codes – DUID Worksheet

### Abbreviations

### Definitions

CBW	Certificate of Blood Withdrawal
OA	Name order different on RFLE.
QN	Quantity insufficient for further analysis.
01	Received broken vial. No analysis possible. Blood and tube discarded.
02	Container not sealed. Vial sealed.
03	Last name on Certificate of Blood Withdrawal not present.
04	Blood vial was not provided by the Department.
05	Blood was coagulated when received. No analysis was possible.
06	Spelling of last name on CBW is questionable.
07	Spelling of first name on CBW is questionable.
08	Name order on CBW is questionable.
09	First name on CBW is not legible.
10	Last name on CBW is not legible.
11	The quantity of blood received was insufficient for analysis.
12	First and last names on CBW are not legible.
13	There is no information on the CBW.
14	Spelling of first and last names on the CBW is questionable.
24	Container sealed. Vial not sealed.
27	Neither vial nor container was sealed.
30	There was no CBW submitted.
31	CBW was detached from the vial at the perforation.
33	The vial number on the vial does not match the vial number on the CBW.
41	Blood vial cracked and leaking when received.
44	Vial number is not complete.
53	CBW not fully recoverable from vial.
55	Vial cap loose. No blood available for analysis.
58	CBW not recoverable from vial.
65	The attached is a photocopy of the vial label.
66	Vial leaking.
67	No analysis performed.
68	Sample may be analyzed if resubmitted with identifying information.
69	No name on CBW.
70	CBW was not attached to the vial.













**Appendix C - DUI/DUID TESTING PROTOCOLS AND REPORTING LIMITS**

Level I Alcohol Testing (Limits are in units of % by weight by volume)

	REPORTING LIMIT	STOP ANALYSIS LIMIT***
Ethanol	0.01	0.10

\*\*\* - Do not proceed further in the analytical scheme when results at or above this concentration are obtained.

Level II Drug Screening and Identification/Quantitation (Limits are in units of mg/L)

Screening	Identification/Quantitation		
DRUG CLASS	DRUG	SCREENING CUTOFF	STOP ANALYSIS LIMIT ***
Barbiturate	Amobarbital	-	2
	Butobarbital	-	2
	Butalbital	1	10
	Pentobarbital	-	2
	Phenobarbital	-	30
	Secobarbital	-	2
	Cocaine/ Benzoyllecgonine	Cocaine	-
Benzoyllecgonine		0.05	-
Cocaethylene		-	-
Benzodiazepine	Alprazolam	0.04	0.10
	Chlordiazepoxide	-	4
	Clonazepam	0.04	0.10
	Diazepam	-	1
	Lorazepam	0.04	0.3
	n-Desalkylflurazepam	-	-
	Nordiazepam	-	1
	Oxazepam	-	2
	Temazepam	0.04	1
Carisoprodol	Carisoprodol	-	10
	Meprobamate	4	10
Fentanyl	Fentanyl	0.002	0.002
Methadone	Methadone	0.05	review
Methamphetamine	Methamphetamine	0.05	0.1
	3,4-MDMA	0.05	0.1
	MDA	0.05	0.1
Opiate	Codeine	-	0.5
	Morphine	0.04	0.1
	Hydrocodone	-	0.10
	6-Acetylmorphine	-	present
	Hydromorphone	-	0.1
	Oxymorphone	-	0.1
Oxycodone	Oxycodone	0.04	0.10
	Hydrocodone	0.04	0.10
Phencyclidine	Phencyclidine	0.01	0.01
THCA	THC	-	0.005
	THCA	0.01	-
Zolpidem	Zolpidem	0.05	0.1

\*\*\* - Do not proceed further in the analytical scheme when results at or above this concentration are obtained.