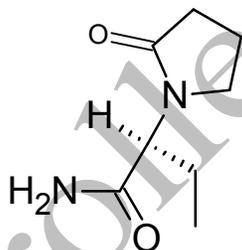


**FORENSIC TOXICOLOGY LABORATORY
OFFICE OF CHIEF MEDICAL EXAMINER
CITY OF NEW YORK**

**LEVETIRACETAM
by
SOLID PHASE EXTRACTION
and
GAS CHROMATOGRAPHY/MASS SPECTROMETRY
(Selective Ion Monitoring)**

PRINCIPLE

The chemical name of levetiracetam (Keppra[®]), a single enantiomer, is (-)-(S)- α -ethyl-2-oxo-1-pyrrolidine acetamide, its molecular formula is C₈H₁₄N₂O₂ and its molecular weight is 170.21. Levetiracetam is chemically unrelated to existing antiepileptic drugs. It has the following structural formula:



The exact mechanism(s) by which levetiracetam exerts its antiepileptic effect is unidentified. The antiepileptic activity of levetiracetam has been assessed in various animal models of epileptic seizures.

Specimens in which levetiracetam is initially detected by the gas chromatography/Mass spectrometry (GC/MS) screening procedure are verified by a selective ion monitoring (SIM) GC/MS method using levetiracetam-D₆ as the internal standard.

Levetiracetam is extracted from biological specimens by solid phase extraction. In this method the levetiracetam is temporarily bound to a sorbent in the solid phase cartridge as the prepared sample is poured through the column. The column is washed to remove interfering compounds, followed by elution of drugs from the column. The eluate is evaporated to dryness, reconstituted in an organic solvent and injected on the GCMS. Quantitative analysis is performed by SIM GCMS using a six point calibration curve.

SAFETY

The handling of all reagents, samples and equipment is performed within the guidelines which are detailed in the safety manual.

SPECIMEN PREPARATION

The procedure is applied to the following biological specimens and their aliquots unless otherwise specified:

Blood	0.5 mL of the undiluted specimen
Urine	0.5 mL for qualitative identification

REAGENTS AND MATERIALS

All chemicals should be ACS grade or better.

1. **Deionized water** (distilled can be substituted)
 2. **Methanol** (Fisher Scientific - ACS Certified or equivalent)
 3. **Certified Negative Blood.** Previously found to have no interfering peaks. Freeze validated negative blood. Discard after 3 months.
 4. **100 mM Sodium Acetate buffer (pH 4.5)**

Dissolve 2.93 g sodium acetate trihydrate in 400 mL DI H₂O; add 1.62 mL glacial acetic acid. Dilute to 500 mL using DI H₂O. Mix. Adjust pH to 4.5 ± 0.1 with sodium acetate or acetic acid.

Storage: room temperature in glass.

Stability: 6 month. Inspect daily with use for contamination.
 5. **pH 9.0 buffer**

Dissolve 20 g KHCO₃ in 800 mL DI H₂O; add 10 g K₂CO₃. Dilute to 500 mL using DI H₂O. Mix. Adjust pH to 9.0 ± 0.1 with KHCO₃ or K₂CO₃.

Store at room temperature in glass.

Stability: 6 month. Inspect each day of use contamination.
 6. **Eluting solvent:** ethyl acetate:NH₄OH (98:2). Prepare fresh each day of use.
 7. Polycrom Clin II Solid Phase Extraction Column, CEREX.
 8. System 48 Processor connected to nitrogen source.
 9. Waste Rack, SPE Rack, Collection Tube Rack.
 10. Turbopap connected to a nitrogen source.
 11. Sonicator
 12. Vortex
 13. Centrifuge
1. Levetiracetam-D₆ (internal standard), Cerilliant (L-031 1.0 mg/mL) or equivalent. Pipette 1.0 mL of 1.0 mg/mL Levetiracetam-D₆ to a 20 mL volumetric flask containing about 10 mL of methanol. QS to the mark with methanol. This yields a final concentration of 50 mg/L. Store this stock solution frozen at -10⁰ C or lower. Stable for at least one year.
 2. Levetiracetam (standard & control), Cerilliant (L-020 1.0 mg/mL) or equivalent. Pipette 4 X 1.0 mL of 1.0 mg/mL Levetiracetam to a 20 mL volumetric flask containing about 10 mL of

methanol. QS to the mark with methanol. This gives a final concentration of 200 mg/L. Store this stock solution frozen at -10⁰ C or lower. Stable for at least one year

EXTRACTION PROCEDURE

1. Aliquot 0.5 mL of validated negative matrix (in each tube labeled as calibrator or in-house control) or sample into a 16 x 125 mm disposable culture tube labeled as to the contents.

Note: Deionized water is used as the negative matrix for urine.

2. Add appropriate amounts of standard solutions to negative matrix specimen tubes as follows:

Concentration in mg/L	µL of 200 mg/L
40	100
30	75
25	62.5
10	25
5	12.5
2	5
QC	
2	5
35	87.5

3. Add 20 µL of internal standard solution (50 mg/L) to each tube and mix.
4. Add 2.5 mL of DI H₂O and 2mL of pH 4.5 Sodium Acetate Buffer. Vortex for 15 seconds. Centrifuge at ≈3000 rpm for 10 minutes.
5. Pour samples into the column and apply positive pressure to achieve a flow rate of 1-2 mL/min.
6. Wash each column with 1mL pH 9.0 Buffer.
7. Wash each column with 1mL DI H₂O.
8. Dry columns for 15 minutes at 40 Psig.
9. Elute each column with 2.0 mL Ethyl Acetate containing 2% NH₄OH. Into a conical 10 mL centrifuge tube.
10. Dry using the concentrator, at room temperature, to absolute dryness at 40 °C.
11. Reconstitute the dried extracts with 100 µL of Ethyl Acetate.
12. Label autosampler vials indicating aliquot and toxicology number (ex: 1-YY-xxxx), specimen type, dilution, analyst, and date.

13. Transfer extract to an insert placed in an autosampler vial. Samples are transferred into screw cap vials and capped immediately.
14. Create batch sequence as specified in Instrument Setup.
15. Enter the date completed in the Dataease database, so that the samples are not duplicated by another analyst.

INSTRUMENTATION

The primary method uses an Agilent 6890 gas chromatograph with HP5973 mass spectrometer equipped with a 7683A auto-sampler with controller and a HP 50+ 50% Phenyl Methyl Siloxane column, 30M x 0.25 mm ID x 0.25 µm DF, max temperature 310 °C.. The secondary GC method employs the use of a Restek RTX-BAC 1 (propriety Crossbond phase). 30M x 0.32 mm ID x 1.8 µm DF, max temperature 260 °C. Model # 18003

TOP LEVEL PARAMETERS

Method Information For: C:\HPCHEM\1\METHODS\LEVETIRACETAM.M

Method Sections To Run:

- Save Copy of Method With Data
- Pre-Run Cmd/Macro
- Data Acquisition
- Data Analysis
- Post-Run Cmd/Macro

Method Comments:

MSX LEVETIRACETAM METHOD

INSTRUMENT CONTROL PARAMETERS

Sample Inlet	GC
Injection Source	GC ALS
Mass Spectrometer	Enabled

HP6890 GC METHOD

OVEN

Initial temp	150 °C (On)	Maximum temp	295 °C
Initial time	2.00 min	Equilibration time	0.50 min

<i>Ramps</i>			
#	Rate	Final temp	Final time
1	20.00	280	2.0
2	0.0(Off)		
Post temp	150 °C		
Post time	5.00 min		
Run time	10.50 min		

<i>Front Inlet (Unknown)</i>		<i>Back Inlet ()</i>
Mode	Pulsed Splitless	Not used
Initial temp	260 °C (On)	
Pressure	9.32 psi (On)	
Pulse Pressure	30.0 psi	
Pulse Time	0.50 min	
Purge flow	7.5 mL/min	
Purge time	1.5 min	
Total flow	12.6 mL/min	
Gas saver	On	
Saver flow	20.0 mL/min	
Saver time	3.00 min	
Gas type	Helium	

Primary Column

<i>Column 1</i>		<i>Column 2</i>
Capillary Column	HP 50+ 50% Phenyl Methyl Siloxane	(not installed)
Max temperature	310 °C	
Nominal length	30 m	
Nominal diameter	250.00 µm	
Nominal film thickness	0.25 µm	
Mode	Ramped flow	
Initial flow	1.9 mL/min	
Initial time	4.50 min	
Rate	Final flow	Final time
0.10	1.5	0.0
0.0(Off)		
Post flow	2.5 mL/min	
Nominal init pressure	23.93 psi	
Average velocity	52 cm/sec	
Inlet	Front Inlet	
Outlet	MSD	
Outlet pressure	vacuum	

<i>FRONT DETECTOR (NO DET)</i>	<i>BACK DETECTOR (NO DET)</i>
COLUMN COMP 1	COLUMN COMP 2
(No Detectors Installed)	(No Detectors Installed)

<i>Thermal Aux 2</i>	
Use: MSD Transfer Line Heater	
Description	
Initial temp	150 °C (On)
Initial time	0.00 min
# Rate Final temp Final time	
1 0.0(Off)	

<i>Post Run</i>	
Post Time	1.00 min
Oven Temperature	150 °C
Column 1 Flow	2.5 mL/min

<i>Time Table</i>		
Time	Specifier	Parameter & Setpoint
7673 Injector		
<i>Front Injector:</i>		
Sample Washes	1	
Sample Pumps	2	
Injection Volume	1.0 microliters	
Syringe Size	10.0 microliters	
PreInj Solvent A Washes	1	
PreInj Solvent A Washes	1	
PostInj Solvent A Washes	3	
PostInj Solvent B Washes	3	
Viscosity Delay	0 seconds	
Plunger Speed	Fast	
PreInjection Dwell	0.00 minutes	
PostInjection Dwell	0.00 minutes	
<i>Back Injector:</i>		
No parameters specified		

MS ACQUISITION PARAMETERS

<i>General Information</i>	
Tune File	ATUNE.U
Acquisition Mode	SIM
<i>MS Information</i>	
Solvent Delay	3.00 min
EM Absolute	False
EM Offset	0
Resulting EM Voltage	varies

[SIM Parameters]

<i>Group 1</i>			
Group ID	1		

Resolution	High		
Plot 1 Ion	58.0		
Ions/Dwell In Group	(Mass, Dwell)	(Mass, Dwell)	(Mass, Dwell)
	(58.0, 30)	(64.0, 30)	(98.0, 30)
	(104.0, 30)	(126.0, 30)	(132.0, 30)
	(170.0, 30)	(176.0, 30)	

[MS Zones]

MS Quad: 150 °C maximum 200 °C

MS Source: 230 °C maximum 250 °C

Timed Events

[Timed MS Detector Entries]

Time (min) State (MS on/off)

END OF MS ACQUISITION PARAMETERS

Alternate Column

<i>Column 1</i>		<i>Column 2</i>
Capillary Column	Restek RTX18003 BAC 1	(not installed)
Max temperature	240 °C	
Nominal length	30 m	
Nominal diameter	320.00 um	
Nominal film thickness	1.80 um	
Mode	Ramped flow	
Initial flow	1.9 mL/min	
Initial time	4.50 min	
Rate	Final flow	Final time
0.10	1.5	0.0
0.0(Off)		
Post flow	2.5 mL/min	
Nominal init pressure	9.33 psi	
Average velocity	52 cm/sec	
Inlet	Front Inlet	
Outlet	MSD	
Outlet pressure	vacuum	

<i>FRONT DETECTOR (NO DET)</i>	<i>BACK DETECTOR (NO DET)</i>
COLUMN COMP 1	COLUMN COMP 2
(No Detectors Installed)	(No Detectors Installed)

<i>Thermal Aux 2</i>	
Use: MSD Transfer Line Heater	
Description	
Initial temp	280 °C (On)
Initial time	0.00 min
# Rate Final temp Final time	
1 0.0(Off)	

<i>Post Run</i>	
Post Time	5.00 min
Oven Temperature	150 °C
Column 1 Flow	2.5 mL/min

<i>Time Table</i>		
Time	Specifier	Parameter & Setpoint
7673 Injector		
<i>Front Injector:</i>		
Sample Washes	1	
Sample Pumps	2	
Injection Volume	1.0 microliters	
Syringe Size	10.0 microliters	
PreInj Solvent A Washes	1	
PreInj Solvent A Washes	1	
PostInj Solvent A Washes	3	
PostInj Solvent B Washes	3	
Viscosity Delay	0 seconds	
Plunger Speed	Fast	
PreInjection Dwell	0.00 minutes	
PostInjection Dwell	0.00 minutes	
<i>Back Injector:</i>		
No parameters specified		

MS ACQUISITION PARAMETERS

<i>General Information</i>	
Tune File	ATUNE.U
Acquisition Mode	SIM
<i>MS Information</i>	
Solvent Delay	6.00 min
EM Absolute	False
EM Offset	0
Resulting EM Voltage	varies

[SIM Parameters]

<i>Group 1</i>			
Group ID	1		

Resolution	High		
Plot 1 Ion	58.0		
Ions/Dwell In Group	(Mass, Dwell)	(Mass, Dwell)	(Mass, Dwell)
	(58.0, 30)	(64.0, 30)	(98.0, 30)
	(104.0, 30)	(126.0, 30)	(132.0, 30)
	(170.0, 30)	(176.0, 30)	

[MS Zones]

MS Quad: 150 °C maximum 200 °C

MS Source: 230 °C maximum 250 °C

Timed Events

[Timed MS Detector Entries]

Time (min) State (MS on/off)

END OF MS ACQUISITION PARAMETERS

INSTRUMENT SETUP

An acceptable autotune must be obtained prior to batch analysis.

All autosampler syringe wash vials are filled with ethyl acetate.

Prepare a sequence using the following steps.

When Chemstation is opened, the **Openlab ECM Login** screen appears, Enter the instrument name (ms3, ms4, etc.) as appropriate for username and the current password. Verify that Account field says production and Domain field says Built-In. if Chemstation is already running, it may be necessary to log out and relog in. Using the Chemstation software, at the top Method and Run toolbar under **ECM**, select **Logon to ECM**. Follow the instructions above to log on.

14. On the Method and Run toolbar, under **Sequence**, select **Load Sequence**. Select **default.s**. Click on Select.
15. In the Method and Run toolbar, under **Sequence**, select **Edit Sequence**. At the top of the screen under Data Path, click on Browse. Under **Select Data Path**, click on the **msdchem** folder. The click **1** and then click on the **Data** folder to highlight it. In lower left of dialog box, select **Make New Folder**. A folder with the name New Folder is created under DATA. Right click on **New Folder** and **Rename** or double click to highlight the folder name and change the entry. Rename the file using the format MSNMMDDYYx, where N is instrument number, MM = month, DD = day, YY= year and x = a letter indicating the batch being run, e.g. MS1041013a. Click **OK** which will take you back to the sample log table.

16. In the Sample column verify the correctness of pre-loaded entries. Starting at the first empty field, enter sample or QC information. For samples this would include aliquot number, laboratory number, specimen source, dilution if any (i.e., 2-13-2432 fem). If the sample has been diluted, enter the appropriate dilution factor in the Multiplier column. For QC samples use an appropriate designator. Enter the next sample in the batch in the next open field down the column.
17. In the Type column, select the corresponding sample type for each vial: Sample, Blank, Calibration or QC.
18. In the Vial column, click in the cell with the number 1, hold down the left mouse button and drag to the last vial number in the sequence (cells will be highlighted). Right click and select Fill Column and Increment. Verify that the vial numbers are correct.
19. In the Method column, verify that the correct method is loaded in the first cell. Then click on the first cell containing the method name, hold down the left mouse and drag to the last vial number in the sequence (cells will be highlighted). Right click and select Fill Column, No Increment. Verify that the method for each vial is correct.
20. In the Data File column, in the cell corresponding to the first vial, enter the data file name in the format MSNMMDDx001, where N = instrument number, MM = month, DD = day, and x = a letter indicating the batch being run, i.e. MS10410a001a. Click on this cell, hold down the left mouse and drag to the last vial number of the sequence (the cells will be highlighted). Right click and select Fill Column and Increment. Verify that the data file information for each vial is correct.
21. In the Comment column, enter any additional information for the vials.
22. In the Multiplier column, enter any sample dilutions.
23. In the **Level** column, verify that the correct level numbers are entered for calibrators in this batch.
24. Verify **No Update** is selected for all vials under **Update Rf** and **Update Rt**.
25. Review the information typed for the sequence. Correct any information as needed. Verify that the Data Path is C:\MSDCHEM\1\Data\current sequence name. Verify that the Method Path is C:\MSDCHEM\1\METHODS. Then Click **ok**.

Note: Occasionally, it will be desirable to run several subsequences in one batch. Use the instructions below to accomplish this.

Setting-Up a Subsequence

On the Sample Log Table:

1. Select "**Keyword**" for "Type"
2. Select "**DataPath**" for "Method/Keyword"
3. Under "Comment/KeywordString" type in the *new data path* for your subsequence i.e.: **C:\MSDCHEM\1\DATA\MSNMMDDYYx** where "N" is the instrument's number and "x" is the letter designated to the subsequence (**it must be different than that of the original sequence**).

4. The suffix of the data files must be different from that of the original; i.e.: MSNMMDDy001; **the subsequence data files must start with 1 again.**
5. After typing in the entire sequence, save sequence accordingly.
6. Go to Sequence → Simulate Sequence → Run Sequence.
7. A dialog box will pop-up: DataPath **C:\MSDCHEM\1\DATA\MSNMMDDYYy** does not exist. Edit Sample Log Table? → Click **No** if the sequence was set up correctly.
8. A 2nd dialog box will pop-up: Create **C:\MSDCHEM\1\DATA\MSNMMDDYYy**? → Click **Yes**.
9. A 3rd dialog box will pop-up: Sequence Verification Done! View it? → Click **Yes** or **No**.

Setting-Up a Subsequence with a Different Method

On the Sample Log Table:

1. After inserting the DataPath keywords and Commands, Insert a Row.
2. Select "**Keyword**" for "Type"
3. Select "**MethodPath**" for "Method/Keyword"
4. Check that the method for each sample is changed to the new method.
5. Follow Steps 5-9 in the "**Setting-Up a Subsequence**" Section (see previous section).
6. On the Method and Run toolbar, under **Sequence**, select **Run Sequence**. In the dialog box under **Sequence Comment** enter the initials of the individual who has entered the sequence in the **Operator Field** (e.g., MPM). Under **Data File Directory** verify that the data file path is C:\MSDCHEM\1\DATA\batch name.
7. Under **Sequence**, select **Save Sequence As**. Under File Name, type in the name of the folder that the batch will be saved to under DATA, MSNMMDDYYx (e.g. MS1041013a) Select **Save**. The extension ".s" will automatically be added.
8. Under **Sequence** select **Print Sequence**. Verify that **Brief Format** is selected and click on **OK**. The sequence will be printed. Apply the preprinted labels for documenting verification of process steps to the printed sequence list.
9. Use the printed sequence list to load vials into the appropriate autosampler positions as indicated by the order on the printed sequence list. Check vial information against the sequence list and ensure that the vial is inserted in the correct numeric position in the autosampler as indicated on the sequence list. Document correct positioning by dating and initialing the appropriate line on the sequence list.

10. Under Sequence select **Run Sequence**. Verify that the Sequence comments and Data Field information are correct (i.e. verify that the proper sequence is loaded. If not, load the proper sequence). Click on **Run Sequence**.
11. After the batch is finished, unload the vials. Compare the vial information to the sequence list as they are removed, to verify that the correct vial was in the correct position. Date and initial the sequence list when this is completed. Annotate discrepancies if necessary

DATA TRANSFER AND PROCESSING - EXPORTING RAW DATA FOR PROCESSING

All processing and review of the processed data should be performed on a processing computer.

SAVE METHOD TO ECM

1. After the run finishes, the data files will be in the data subdirectory on the local chemstation and also automatically transferred to ECM. From the acquiring computer, make sure the proper method, the one used to acquire the data, is loaded. On the top toolbar under **ECM** click on **Save Method to ECM**.
2. Click on the GCMS, the correct instrument name folder, the appropriate month and batch to which the method will be saved.

RETRIEVE BATCH FROM ECM - RAW DATA FOR PROCESSING

1. At the processing computer, click on Processing Data Analysis. Log on using your OCME network username and password.
2. On Enhanced Data Analysis screen, click on ECM at the top toolbar and select Retrieve entire sequence from ECM.
3. This will open up Openlab ECM screen. Select GCMS, then the appropriate instrument, the month, and, finally the batch to be retrieved. On the status line at the bottom of the screen that the batch is being retrieved. The batch will be downloaded to the following location: C:\msdchem\1\ECM\Retrieve\batch name"

DATA REVIEW

There are three levels of review; the first level of review is the transference and processing of the raw data, this may be performed by any trained analyst; the second level of review is performed by an experienced analyst who is trained and signed off in data review, he / she will review the processed data; the third level of review is considered the final level of review, this can only be performed by the Laboratory Managers. He/she will review the data for the entire case ensuring that screening, confirmatory and quantitative analysis on the case have been completed and reported accurately. As needed, he/she will also schedule additional analysis

and contact the Medical Examiner on the case to discuss any findings and / or review case history.

LOAD METHOD AND BATCH – FIRST LEVEL REVIEW

1. On the left side of the screen under the C drive, open C:\msdchem\1\ecm\retrieve
2. Under retrieve, click on the batch that was retrieved. When all files have been downloaded to the processing computer, verify that the appropriate method is present in the batch.
3. To load the method, right click on the method under the batch being processed and select load. This will bring up “Be sure changes are saved. Load now?” Click yes. If the method is not present, load the method by retrieving the method from ECM.
4. Click on any file in the batch to load it.

PERFORM BATCH CALIBRATION – PROCESSING – FIRST LEVEL REVIEW

Under enhanced data analysis:

1. Process the calibrator. Select Tools from the toolbar, DoLIST, and Quant, No Report (QT 1). Press Add, and OK. Select the files for this action to be performed on, in this case, a a single point calibrator only. Verify that the selected files are located in the correct subdirectory. Change the path if necessary. Click the → Arrow and Process..
2. Review the integrations of the targeted compounds for the calibrator, checking that the ion peaks are present and integrated correctly (i.e. the baseline is the most scientifically accurate one that can be drawn). Select View from the toolbar, QEDIT. Answer appropriately when prompted to save changes made to quantitation results when moving from file to file. Return to Data Analysis by selecting View from the toolbar, return to Data Analysis.
3. Update the existing calibration table (level 1). Select Calibrate, Update One Level. When asked to requant files before update, select NO. Next update Calibration.
4. Select Update One Level, select replace responses and replace retention time and Replace Qualifier Ion Relative Responses, and choose the existing level ID (#1). Click Do Update.
5. Review the Compound database. Double click on the internal standard listed on the left to reveal the compounds quantitated with it. Select the calibration tab to reveal compound responses. Click OK or Cancel when review is complete
6. Save Method before proceeding. Select Method from the toolbar, Save method, make sure that the path is correct. Save to OpenLab ECM at this time. On Update Calibration screen, select Update Level. Then click on Responses and Replace and on Retention Times and Replace. Then under Existing level ID, select the cal level to update and click on Do Update.

7. Requantitate the calibrator with the updated calibration curve. Select Tools from the toolbar, DoLIST, Requant, no report (QT 2), Add, and OK. Remove any existing commands. Select files to process. Click the → Arrow and Process. Review with QEDIT. Check the responses, retention times and ion ratios.
8. Process controls and cases. Select Tools from the toolbar, DoLIST, Quant, No Report (QT 1), Add, and OK. Select appropriate files. Click the → Arrow and Process. Review with QEDIT. The blank must not contain detectable amounts of target analytes or significant interfering peaks.
9. When review is complete, return to Data Analysis. Select report format by choosing Quantitate from the toolbar, Report Options. Check SIM style report and uncheck Internal Standards. Press OK.
10. To print reports, select Tools from the toolbar, DoLIST, Profile Quant w/o Calculations (QT 0,1,'P'), Add, and OK. Select files to print, click the → Arrow and Process.
11. Print the calibration table for the current batch by clicking **Calibrate** on the command line. Select **List, Calibrate Report** and click OK. The Calibration report will print to the screen. Review the r^2 values, then right click on the screen report to print it.
12. Save files to ECM. Select ECM from the toolbar, select "Save multiple data files to ECM". Select all files.
13. Save method to ECM. Select ECM from the toolbar, Save Method to ECM. Make sure data path is correct.

BATCH CLEAN UP

1. Select "my computer". Find the batch on the C drive at C:\msdchem\1\ecm\retrieve\batch. Right click on the batch to be deleted and select delete. Do not delete a batch that has not been successfully uploaded to ECM.

ACCEPTANCE CRITERIA

1. Review the entire batch, checking that the ion peaks are present and integrated correctly (e.g., that the baseline is the most scientifically accurate one that can be drawn), that the ion ratios are $\pm 20\%$ of the average of a calibrator, that the peaks are $\pm 2\%$ of the calibrator retention times, and the peaks meet chromatography criteria. It is acknowledged that with some compounds the ion ratios will vary with concentration. As the target for ion ratio's is set at the mid calibrator, for compounds at the lower or upper range of the calibration curve the acceptable ion ratios can be either $\pm 30\%$ of the target value or $\pm 20\%$ of the nearest calibrator provided that the peaks meet all other chromatographic criteria. The blank must not contain detectable amounts of target analytes or significant interfering peaks. The blood controls must be within $\pm 20\%$ of the target value. For tissues, the controls are acceptable up to $\pm 30\%$.

Note: In some instances ratios will be off in exceptionally low or high concentrations. The operator must evaluate this and schedule proper dilutions or other methodologies, as

needed. See REPORTING section.

2. The analyst shall choose from the standard levels a level at their discretion. All internal standard areas/peak heights for that sequence shall be compared to the internal standard(s) in that level. The acceptance criteria for the internal standard recovery/response shall be $\pm 30\%$ of the selected internal standard. If the internal standard recovery/response is outside these limits the specimen shall be repeated unless the analyte in question is not related to the internal standard that is outside the acceptable limits. If the internal standard is still unacceptable in the repeat analysis in the same way (i.e. both times too high or both times too low), then the sample shall be repeated a third time using a "blank and spike" method. The "blank and spike" run shall be viewed as acceptable if the following conditions are met: All standards and QC materials are within acceptable ranges, the "blank and spike" samples internal standards are within $\pm 20\%$ of their average responses, the calculated amount of the spiked sample is $\pm 20\%$ of the theoretical amount. If any of these conditions are not met than consult with a toxicology manager for further direction.
3. Make copies of all controls, the r^2 report and the sequence list, enough to attach a set to each case in the batch.

SECOND LEVEL REVIEW

The Second Level Reviewer will review the processed data in its entirety according to the acceptance criteria section of this SOP.

REPORTING

After the batch has undergone second level review and has been printed, either the first level reviewer or the second level reviewer may report the data in the appropriate case file.

Report using the following guidelines:

1. Each case printout must have a copy of the sequence and all controls appended.
2. Concentrations greater than or equal to 2 mg/L are reported in mg/L.
3. Concentrations lower than 2 mg/L but meeting all other criteria are reported less than 2mg/L. ". If the drugs are not detected or the reporting criteria are not met, the drug is reported as "not detected".
4. Sample concentrations greater than the highest acceptable calibrator must be re-extracted with appropriate dilution(s) to bring it within quantitative curve limits.
5. Review other findings in the case, especially GCMS and GC results to see if they are consistent with these GCMS findings. If there are discrepancies, schedule additional testing to resolve them. If in doubt, consult with a supervisor.

6. If the positive matrix controls are greater than $\pm 30\%$ of target for non-blood matrices, the samples may be reported qualitatively, as “detected” or “not detected”. If quantitative results are needed, the sample must be repeated.
7. Submit the case file with all reports for Third Level Review.

THIRD LEVEL REVIEW (FINAL REVIEW)

The third and final level review can only be performed by the Laboratory Managers. He/she will review the data for the entire case according to all established criteria. They will ensure that screening, confirmatory and quantitative analysis on the case have been completed and reported accurately. As needed, they will also schedule additional analysis and contact the Medical Examiner on the case to discuss any findings and / or review case history.

REFERENCES

Clarke's Identification and Isolation of Drugs in Pharmaceuticals, Body Fluids and Post-Mortem Materials, Ed. A.C. Moffat, The Pharmaceutical Press, London, Second Edition, 1986.

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REVISION HISTORY

Ver 01.12.2015

Ver 08.31.2015

1. New method implemented.
 1. Added internal standard acceptance criteria.
 2. Defined the level of review(s).
 3. Revised reporting limits.

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