SOP 412 - Quetiapine Extraction using Protein Precipitation for Quantification by Liquid Chromatography/Mass Spectrometry/ Mass Spectrometry (LC/MS/MS)

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	11.1.1.5 – Updated RT acceptance range 11.1.2.1 – Updated IRC acceptance range 11.1.3.2 – Updated Calibrator acceptance range 11.1.4.1 – Updated QC acceptance range 11.1.4.2 - Deleted	MSF – 05/07/2015
	8.4 – Corrected blank liver amount	MSF – 06/08/2015
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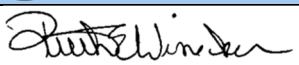
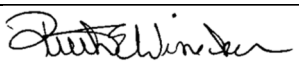
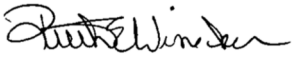
Approving Authority Name	Approving Authority Signature	Approval Date
Ruth E. Winecker, Ph.D.		04/17/2015
Ruth E. Winecker, Ph.D.		06/19/2016
Ruth E. Winecker, Ph.D.		12/07/2017

SOP 412 - Quetiapine Extraction using Protein Precipitation for Quantification by Liquid Chromatography/Mass Spectrometry/ Mass Spectrometry (LC/MS/MS)

1. **Principle of Assay**

1.1. This method is designed to confirm and quantitate quetiapine (Seroquel) in biological specimens by Liquid Chromatography Tandem Electrospray Mass Spectrometry (LC/MS/MS). Quetiapine is extracted from biological matrices by protein precipitation with methanol and identified by the retention time and ion ratio of product ions. Quetiapine is subject to matrix effects, thus a stable isotopically labeled internal standard is used (1).

1.2. Quetiapine is a second-generation, atypical antipsychotic for schizophrenia and bipolar disorder indications, as well as any number of "off-label" indications. In contrast to older, typical antipsychotics, quetiapine exhibits less extrapyramidal side-effects, such as dystonia, catalepsy, and tardive dyskinesia.

Pharmacologically, quetiapine is a serotonin 5-HT_2 and dopamine D_2 receptor antagonist, but also has affinity for other serotonin receptors. Though the complete mechanism of action is not understood, it is believed to involve the antagonism and down-regulation of 5-HT2 and partial agonism of 5-HT1A. A metabolite, norquetiapine, is a norepinephrine reuptake inhibitor, and may also play a role in the antipsychotic properties of quetiapine. (2)

In this laboratory, screening for quetiapine is typically done in central blood specimens (*e.g.* aorta, inferior vena cava) via the organic bases screen (SOP 102). Quetiapine has a high volume of distribution (Vd ~10 L/kg) and is readily distributed in perfused organs such as the liver, lung, heart, and kidneys. Along with a high volume of distribution, quetiapine is also subject to postmortem redistribution (PMR), in which drugs diffuse from areas of high drug concentration, such as organ tissue, into the blood. Because of these two features, confirmation and quantitation of quetiapine is done in peripheral blood specimens (*e.g.* femoral, iliac) and liver, to more accurately reflect quetiapine concentration at the time of death and assist in interpretation. (3)

2. **Specimens**

2.1. This procedure is applicable to urine, blood, serum, vitreous humor, properly prepared tissue specimens (typically 1:4 homogenates), bile*, and gastric contents*.

2.2. A 0.1 mL (g) sample size (in duplicate) is generally employed for urine, blood, serum, bile, and gastric contents, and a 0.1g sample size (in duplicate) for tissue homogenate (unless a dilution is required) so that the calibration curve encompasses the expected range of unknown specimens.

1.1.1. *For non-typical matrices, an additional 0.1mL aliquot shall be taken (volume permitting), spiked with appropriate QC, and analyzed to help to identify any matrix effects. (See Non-Matched Matrix Protocol section of the QA/QC manual).

3. **Reagents and Materials**
   - 3.1. DI water, HPLC grade
   - 3.2. Methanol, HPLC grade
   - 3.3. Acetonitrile, HPLC grade
   - 3.4. Deuterated Quetiapine Internal Standard
   - 3.5. Quetiapine Standard
   - 3.6. Quetiapine QC Standard
   - 3.7. Drug Free Blood, Urine, Liver Homogenate
   - 3.8. Water with 0.1% formic acid
   - 3.9. Acetonitrile with 0.1% formic acid
   - 3.10. Methanol with 0.1% formic acid

4. **Standards, Controls, and Solutions**

   4.1. **Quetiapine-d8 Internal Standard (10µg/mL)**
   - 4.1.1. Into a 10mL volumetric flask, add the contents of 1 ampule (~1mL) of Quetiapine-d8 (Cerilliant – 100µg/mL).
   - 4.1.2. Fill to the line with methanol, insert stopper and invert three times to mix. Transfer to properly labeled 16x100mm screw topped test tubes and cap. Store in laboratory refrigerator (R1-2601). See SOP-010.

   4.2. **Quetiapine Calibrators and Positive Controls –** these standards are to be prepared by the QA/QC Chemist or appointee. Inform the QA/QC Chemist if calibration/control standards need to be made.

   4.3. **Water with 0.1% formic acid**
   - 4.3.1. To a 4L bottle of HPLC grade water, add 4 mL of formic acid
   - 4.3.2. Label bottle as “LC/MS” and “with 0.1% formic acid”.

   4.4. **Acetonitrile with 0.1% formic acid**
   - 4.4.1. To a 4L bottle of HPLC grade acetonitrile, add 4 mL of formic acid
   - 4.4.2. Label bottle as “LC/MS” and “with 0.1% formic acid”.

   4.5. **Methanol with 0.1% formic acid**
   - 4.5.1. To a 4L bottle of HPLC grade methanol, add 4 mL of formic acid
   - 4.5.2. Label bottle as “LC/MS” and “with 0.1% formic acid”.

5. **Equipment and Special Supplies**
   - 5.1. Test Tubes, 13 x 100 mm
   - 5.2. Centrifuge 2000 x g

5.3. Vortex mixer
5.4. GC autosampler crimp cap vials, 12 x 32 mm
5.5. GC autosampler crimp caps
5.6. Polyspring inserts, 5 mm O.D.

6. **Instrumentation and Parameters**

6.1. Windows PC with Thermo LCQuan and Xcaliber software
6.1.1. Instrument method (TSQ02): "Quetiapine"
6.1.2. Click here for instrument parameters.

6.2. Thermo Surveyor LC autosampler, or equivalent
6.3. Thermo Surveyor LC pump, or equivalent
6.4. Thermo TSQ triple quadrupole mass spectrometer

7. **Target Ions** (± 1 nominal mass)

7.1. Quetiapine-d8 (**392** 258 226)
7.2. Quetiapine (**384** 253 221)

7.2.1. Note: The precursor ion of each analyte is listed first and bolded, the first product ion- used for quantification-is second, followed by the second product ion-used for qualification/confirmation.

8. **Procedure**

8.1. Prepare a colored tape label for each standard, blank, control, and specimen to be placed on 13x100 mm test tubes.

8.1.1. **Note: follow the tube labeling and tape transfer procedure located in the Quality Assurance and Quality Control manual.**

8.2. Add the appropriate quantity (according to the Standard and Control Worksheet) of the Deuterated Quetiapine Internal Standard to all the tubes.

8.3. Add the appropriate quantity (according to the Standard and Control Worksheet) of the Quetiapine Calibration Standard and the Quetiapine QC Standard to the tubes labeled as standards and control, respectively, labeling test tubes as you go. Only internal standard should be spiked into the test tube labeled "Blank".

8.4. Add 0.1mL of blank blood to all standards, control, and blank test tubes (0.1 mL blank urine/0.1g blank liver homogenate to urine/liver blank and QC test tubes if applicable).

8.5. Add the appropriate amount of unknown specimen, labeling test tubes as you go.

8.6. Vortex all test tubes for 10 seconds.

8.7. Add 10mL methanol to each tube and vortex for 20 seconds.

8.8. Centrifuge at 2000 x g for 10 minutes.

8.9. Transfer ~1mL of supernatant to appropriately labeled autosampler vials and place in the autosampler tray of the Thermo TSQ triple-quadropole LC/MS/MS.

8.10. Build and initiate sequence as directed in SOP 053.

9. **Calculations**

9.1. Quantification

9.1.1. The method for processing the data using the Thermo LCQuan software is "Quetiapine" (SOP 055). It is used to calculate the internal standard response ratios, raw amounts, concentration, and ion ratios.

9.1.2. These calculations are computed as follows:

9.1.2.1.1. Response Ratio:

9.1.2.1.1.1. Response Ratio = response of the analyte's quantifying product ion compared to that of the internal standard

9.1.2.1.1.2. Response Ratio = QN_a / QN_{istd}

9.1.2.1.1.3. QN_a = response of the quantitative ion of the analyte

9.1.2.1.1.4. QN_{istd} = response of the quantitative ion of the internal standard Amount

9.2. Calibration

9.2.1. A quadratic regression resulting from the 6 standards is used to quantitate the analytes in the case. The area of the analyte divided by the area of the internal standard is used in the resulting formula of the calibration curve.

9.3. Dilution Factor

9.3.1. D = Total volume/Sample volume

9.4. Multiplier for homogenates, dilutions, and non-standard volumes

9.4.1. $M = (V_{curve} / V_{samp}) \times D$

9.4.1.1. M = Multiplier
9.4.1.2. D = dilution factor
9.4.1.3. V_{curve} = matrix volume of calibration curve
9.4.1.4. V_{samp} = matrix volume of specimen

9.5. Concentration

9.5.1. $C = (A / V) * M$

9.5.1.1. C = Concentration (ng/mL) of the analyte in the unknown case.
9.5.1.2. A = Amount of drug in sample
9.5.1.3. V = Volume of sample
9.5.1.4. M = Multiplier

9.6. Max/Min

9.6.1. Percent Difference = $((R_h / R_l)-1) \times 100$

9.6.1.1. R_h = high result
9.6.1.2. R_l = low result

9.7. Average

9.7.1. Average = $(R_1 + R_2) / 2$

9.7.1.1. R_1 = first result
9.7.1.2. R_2 = second result

9.8. Qualifier Ion Ratios

9.8.1.1.1. Ratio 1 = QL_1/QN

9.8.2. QL_1 = response of the quantifying product ion
9.8.3. QN = response of the qualifying product ion

10. **Validation of Method**

Parameter	Result
Bias	Blood - L: -1.5% H: -0.69% Liver - L: 2.08% H: 2.15%
Precision	Blood - L: -1.36% H: 3.68% Liver - L: -0.41% H: 1.73%
Calibration model	Quadratic: 1/x

Carryover	Insignificant carryover observed following 2X high calibrator. Carryover threshold set to 50ug/mL. (MeOH + 0.1% Formic Acid used as Wash Solution).
Interference Studies	No interfering signal from matrix, internal standard, common drugs of abuse (including metabolites), OTC drugs, and Prescription medications.
LOD (Determined experimentally)	0.05ug/mL
LOQ (Set to lowest calibrator with acceptable Bias/Precision).	0.2ug/mL
Processed Sample Stability - (re-analyze after 8 days)	Extract is stable for three days on instrument following injection and eight days re-capped - on instrument or in refrigerator.

11. **Quality Control**

11.1. Acceptance criteria

11.1.1. Chromatogram

11.1.1.1. Peaks must be Gaussian shaped (symmetrical).

11.1.1.2. Peaks sharing precursor/product ions must have baseline resolution.

11.1.1.3. The peak of interest is inspected visually for the presence of unresolved co-eluting peaks. The maximum allowable valley between adjacent peaks must not exceed ~10% by visual inspection of the analyte peak height.

11.1.1.4. The internal standard (ISTD) in each case should be inspected for evidence of signal enhancement and suppression. The area of the quantifying ion should not be less than 50% or more than 200% of the average ISTD of the calibrators.

11.1.1.5. Retention time must not deviate outside ± 3% of target, based upon the retention time of the calibrators and controls.

11.1.2. Mass spectroscopy

11.1.2.1. The ion ratio of all analytes must not be greater than ± 20% of the target ratio as determined by a mid-level calibrator (CAL 4).

11.1.2.2. Coelution of quantifying and qualifying ions must not be greater than 0.025 minutes.

11.1.3. Calibrators

11.1.3.1. Analytical curves must have a coefficient of determination (R^2) of 0.992 or greater.

11.1.3.2. Each calibrator, when calculated against the calibration curve, must not deviate outside ± 20% of the target value (± 25% at LOQ).

11.1.3.3. Refer to "Calibration curve point exclusion guidelines" section of the QA/QC Manual.

11.1.4. Controls

11.1.4.1. Controls must calculate within ± 20% of the target value.

11.1.5. Blanks

11.1.5.1. Matrix specific negative controls (blanks) must not contain the analyte of interest at a response greater than $1/10^{th}$ of the signal obtained from the lowest positive calibrator.

11.1.5.1.1. If an analyte of interest is detected with a response greater than $1/10^{th}$ of the signal obtained from the lowest positive calibrator, all cases specimens in which that analyte is present shall be repeated and a senior chemist notified.

11.1.6. Any deviation from the above criteria must be approved by a senior chemist.

12. **Reporting**

12.1. The percent difference of duplicate analysis for an analyte must be less than or equal to 25% (see Max/Min in Calculations section).

12.2. Reporting of duplicate analysis should be done according to the table below:

Reporting Duplicates

- Dilution factors of 1 and 1

Dil / Scenario	1	1	REPORT
A	In curve	In curve	Average
B	In curve	AQL or BQL	"In" value
C	In curve	ND *	Repeat
D	AQL/BQL	AQL/BQL	Less than/ Greater than
E	BQL	ND	ND

* ND = None Detected, due to IRC, S/N threshold, r.t., or other

12.2.1.

12.2.1.1. In Curve = Measured concentration (pre-multiplier) falls within the calibration range

12.2.1.2. AQL = Measured concentration (pre-multiplier) falls Above Quantitation Limit

12.2.1.3. BQL = Measured concentration (pre-multiplier) falls Below Quantitation Limit

12.2.1.4. ND = None Detected

12.3. Averaging reportable values

12.3.1. Results for duplicate analysis (both falling within calibration curve) shall be truncated prior to averaging.

12.3.2. Enter calculated concentration for each specimen into toxlog.

12.4. Significant figures

12.4.1. Concentrations are truncated and reported with two significant figures in mg/L (maximum of 3 decimal places eg 0.009 mg/L).

13. Reinjection:

13.1. An extract may be reinjected due to ALS failure, apparent low recovery, to check for carry-over or to meet ion ratio and/or retention time criteria. Additional solvent may be added to the ALS vial if necessary due to excessive

analyte concentration. The reinjected extract must be evaluated against the existing run. If any parameters have to be changed (e.g. thresholds due to response, smoothing due to split peaks, or windows etc.) then the control must be reinjected in addition to the patient samples under those criteria and must meet all QA/QC criteria. Finally, the entire batch may be reinjected (calibrators, controls and unknowns) in order to re-establish acceptance criteria. If ion ratio and retention time criteria for a specimen are not met, the specimen must be re-extracted. The data from the original injection and reinjection must be included in the data pack.

14. **Preparation of Load**

14.1. Enter case specimen data into LIMS in accordance with the Quality Assurance and Quality Control section of the Standard Operating Procedure manual.

14.2. The load paperwork and data is to be arranged in the following order:

14.2.1. Assignment sheet

14.2.2. Comments or note to file if applicable

14.2.3. Load summary

14.2.4. Specimen worklist

14.2.5. Chain of custody (Specimen)

14.2.6. Aliquot chain of custody

14.2.7. Standard and control worksheet

14.2.8. Sequence summaries/calibration reports – paper clipped

14.2.9. Calibrator data - paper clipped

14.2.10. Blank matrix data - paper clipped

14.2.11. Control data - paper clipped

14.2.12. Specimen data – stapled

15. **References**

15.1. Chambers, Erin, Diane M. Wagrowski-Diehl, Ziling Lu, and Jeffrey R. Mazzeo. "Systematic and Comprehensive Strategy for Reducing Matrix Effects in LC/MS/MS Analyses." Journal of Chromatography B 852.1-2 (2007): 22-34.

15.2. Sanford, M., Keating, G.M. “Quetiapine: A review of its use in the management of bipolar depression.” CNS Drugs 26.5 (2012): 435-460.

15.3. Parker, D. R., and I. M. Mcintyre. "Case Studies of Postmortem Quetiapine: Therapeutic or Toxic Concentrations?" Journal of Analytical Toxicology 29.5 (2005): 407-12.