

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY  
ASSAY ONLY TEMPLATE**

**A. 510(k) Number:** k051211

**B. Purpose for Submission:** New assay (first zonisamide assay)

**C. Measurand:** Zonisamide

**D. Type of Test:** Turbidimetric immunoassay

**E. Applicant:** Seradyn Inc.

**F. Proprietary and Established Names:** QMS® Zonisamide Assay, QMS® Zonisamide Calibrator Set, QMS® Zonisamide Control Set

**G. Regulatory Information:**

1. Regulation section:  
21 CFR §862.3350, Diphenylhydantoin test system  
21 CFR §862.3200, Clinical toxicology calibrator  
21 CFR §862.3280, Clinical toxicology control material
2. Classification: Class II
3. Product code: NWM, LAS, DLJ
4. Panel: 91

**H. Intended Use:**

1. Intended use(s):

See indications for use.

2. Indication(s) for use:

The QMS® Zonisamide Assay is intended for the quantitative determination of zonisamide in human serum or plasma samples. Zonisamide concentrations can be used as an aid in management of patients treated with zonisamide.

The QMS® Zonisamide calibrator set is intended for calibration of the QMS® Zonisamide Assay. The QMS® controls are intended for quality control of the QMS® Zonisamide Assay.

3. Special conditions for use statement(s):

The assay is to be used in conjunction with other evaluations and methods for monitoring Zonisamide concentrations.

It may be necessary to obtain multiple samples to determine expected variation of optimal (steady-state) concentrations for individual patients.

See expected range below.

4. Special instrument requirements:

The assay has currently only been validated on the Hitachi 717.

**I. Device Description:**

The device consists of ready-to-use reagents containing rabbit polyclonal zonisamide antibodies in buffer (R1), and zonisamide-coated microparticles with (R2), both with azide preservative.

**J. Substantial Equivalence Information:**

1. Predicate device name(s): Innofluor Phenytoin Assay
2. Predicate 510(k) number(s): K955562
3. Comparison with predicate: The predicate device is a fluorescence polarization immunoassay that quantitates phenytoin in serum. Both assays are for use in management of individuals treated with an anti-seizure drug. Both devices are for use on automated analyzers.

**K. Standard/Guidance Document Referenced (if applicable):**

**L. Test Principle:** The assay is a homogeneous particle-enhanced turbidimetric assay, based on antibody-binding competition between drug in the sample and microparticle-bound drug. Antibody binding causes microparticle agglutination. The rate of absorbance, measured spectrophotometrically is proportional to the rate of agglutination.

**M. Performance Characteristics (if/when applicable):** Performance was validated on a Hitachi 717 instrument.

1. Analytical performance:

a. *Precision/Reproducibility:*

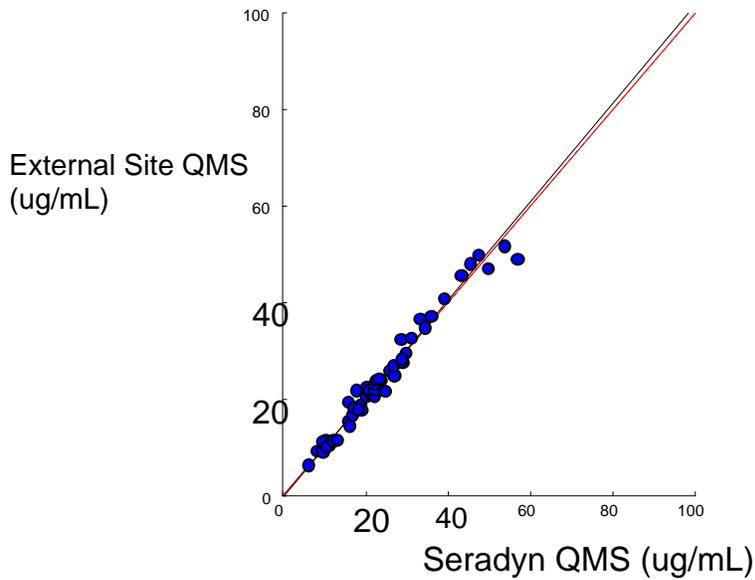
Precision studies were performed at Seradyn and at 2 external laboratories. The first study, performed at the manufacturer's site, followed CLSI EP5-A. One operator performed the evaluation, with a minimum of 2 hours between runs. Calibration was performed once. Precision was evaluated using control material as well as pooled samples from patients taking zonisamide. One reagent lot was used. Results are shown below.

|                   | N  | mean | Within Run    |     | Total         |     |
|-------------------|----|------|---------------|-----|---------------|-----|
|                   |    |      | SD<br>(ug/mL) | %CV | SD<br>(ug/mL) | %CV |
| Low patient pool  | 80 | 10.4 | 0.6           | 5.6 | 0.9           | 8.3 |
| Mid patient pool  | 80 | 27.7 | 1.4           | 5.0 | 2.2           | 7.9 |
| High patient pool | 80 | 40.7 | 2.5           | 6.0 | 3.1           | 7.5 |
| Low control       | 80 | 8.9  | 0.4           | 4.2 | 0.6           | 6.8 |
| Mid control       | 80 | 27.4 | 1.5           | 5.5 | 1.8           | 6.6 |
| High control      | 80 | 52.3 | 2.2           | 4.2 | 3.0           | 5.7 |

A second study was performed at 2 external sites. For control samples, the protocol followed CLSI EP-5A. For patient samples, the protocol was modified so that single measurements were taken, two times each day, over non-consecutive days. Instruments were calibrated at the beginning of the evaluation, and then as needed for quality control processes. Results obtained at one of the external sites are shown below.

|                   | N  | mean | Within Run              |     | Total         |      |
|-------------------|----|------|-------------------------|-----|---------------|------|
|                   |    |      | SD<br>(ug/mL)           | %CV | SD<br>(ug/mL) | %CV  |
| Low patient pool  | 24 | 7.3  | n/a (run in singlicate) | n/a | 0.8           | 11.5 |
| Mid patient pool  | 24 | 26.2 | n/a                     | n/a | 2.1           | 8.0  |
| High patient pool | 24 | 48.9 | n/a                     | n/a | 5.6           | 11.4 |
| Low control       | 80 | 8.1  | 0.7                     | 8.0 | 0.7           | 9.0  |
| Mid control       | 80 | 26.1 | 1.5                     | 5.7 | 1.9           | 7.2  |
| High control      | 80 | 50.5 | 3.1                     | 6.0 | 4.5           | 8.8  |

In another evaluation, results obtained at the manufacturer's site were compared to those obtained at external sites for a set of split patient samples (both serum and plasma). A representative graph is shown below, indicating good reproducibility between the two sites.



b. *Linearity/assay reportable range:*

The assay range is 3-50 ug/mL.

Linearity across this range was demonstrated by measurements of serial dilutions of a stock solution of pharmaceutical grade zonisamide in human serum (negative for zonisamide). Samples ranged between 2.5 ug/ml and the upper limit of the assay range. Solutions were assayed n=5 for each of 2 independently calibrated runs. Percents recovery were calculated as QMS® assay concentration/target concentration. Above 4 ug/mL, recovery was within +/-10%. Acceptance criteria for recovery at the assay limit of quantitation is +/- 15%. Results of a calibrator dilution study confirmed linearity within the assay range.

Samples from a zonisamide proficiency testing program were also used to evaluate recovery. Results are tabulated below:

| Target value (ug/mL) | QMS® value (ug/mL) |
|----------------------|--------------------|
| 21.9                 | 20.4               |
| 6.0                  | 6.0                |
| 10.1                 | 8.3                |
| 29.9                 | 27.9               |
| 49.9                 | 47.0               |
| 18.0                 | 17.7               |
| 34.0                 | 32.8               |

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Calibrators consist of pharmaceutical grade zonisamide gravimetrically spiked into human serum containing preservatives. Calibrator target concentrations are 0, 5, 10, 20, 40 and 80 ug/mL zonisamide. Nominal values are confirmed by a validated HPLC method. Primary calibrators are accepted if recovered by HPLC within +/- 10% of the nominal values. Working calibrators are accepted if recovered (on the Hitachi 717 calibrated with primary calibrators) within +/- 5% of the nominal value.

Ongoing real-time testing for calibrator stability is performed at 6, 12 and 18 months to ensure stability within 10% of the original HPLC value.

d. *Detection limit:*

Inter-assay precision at the detection limit was determined for serum samples spiked with zonisamide at multiple concentrations near the low end of the assay range. The evaluation was performed on 3 separate analyzers. Each sample was run in replicates of 10, for 2 runs, for a total n=60 at each zonisamide level evaluated. Results are shown below for concentrations near the lower limit of the assay range. Results support the sponsor's acceptance criteria of CV of 20% and recovery within +/- 15% at the limit of quantitation.

|                                    |      |      |      |
|------------------------------------|------|------|------|
| Avg concentration (ug/mL)          | 4.25 | 3.2  | 2.0  |
| N                                  | 60   | 59   | 60   |
| SD (ug/mL)                         | 0.37 | 0.54 | 0.42 |
| %CV                                | 9    | 17   | 21   |
| % recovery relative to known value | 106  | 107  | 100  |

e. *Analytical specificity:*

The assay was evaluated for potential interference from the zonisamide metabolites NAZ (N-acetyl zonisamide) and SMAP (2-sulfamoyl acetyl phenol); commonly co-administered and OTC drugs and other compounds; and endogenous interferents. Potential interferents were spiked into 3 serum pools, each containing 0, 12, or 36 ug/mL zonisamide. Samples were evaluated in triplicate and interference was evaluated by comparison to control samples without interferent. The concentrations of metabolites and endogenous compounds tested are shown below. The commonly co-administered drugs tested are listed in the package insert.

| <b>Metabolite</b>  | <b>Concentration of metabolite (ug/mL)</b>          | <b>Percent cross-reactivity relative to control without metabolite</b>        |
|--|---|---|
| NAZ  | 500   | 0.2   |
|  | 100   | Not detected  |
| SMAP   | 500   | 0-2.6   |
|  | 100   | 1.3 (at 12 ug/mL zonisamide)<br><br>11.3 (at 0 ug/mL zonisamide)              |
| <b>Endogenous compound</b>   | <b>Concentration of endogenous compound (ug/mL)</b> | <b>Percent recovery relative to control without added endogenous compound</b> |
| Albumin  | 12 g/dL   | Within 90 to 110 %  |
| Bilirubin  | 20 mg/dL  | Within 90 to 110 %  |
| Cholesterol  | 500 mg/dL   | Within 90 to 110 %  |
| Hemoglobin   | 1150 mg/dL  | Within 90 to 110 %  |
| IgG  | 12 g/dL   | Within 90 to 110 %  |
| RF*  | 500 IU/mL   | Within 90 to 110 %  |
| Triglycerides*   | 1500 mg/dL  | Within 90 to 110 %  |
| Uric Acid  | 20 mg/dL  | Within 90 to 110 %  |
|  |   |   |
| * prepared by dilution of patient samples containing high values of these compounds. |   |   |

f. *Assay cut-off:*

See Limit of quantitation.

2. Comparison studies:

a. *Method comparison with predicate device:*

Results obtained with the QMS® assay were compared to results of validated HPLC methods performed at 3 separate clinical laboratory reference sites. Samples were serum and plasma samples, previously received for evaluation at the reference laboratories. No specific selection criteria (in terms of patient demographics) were applied. Most samples were banked, frozen samples. Minimally, 40% of the samples represent individual patients. The table below lists results summary provided by the sponsor, using Passing Bablok analysis.

| <u>study</u> | <u>Site performing QMS</u> | <u>QMS range</u><br>µg/mL | <u>N</u>                   | <u>Slope</u>              | <u>intercept</u>           | <u>R</u>                                | <u>Standard error of estimate</u>     |
|--------------|----------------------------|---------------------------|----------------------------|---------------------------|----------------------------|---|---------------------------------------|
| 1            | External                   | 2.71-43.60                | 83                         | 0.918<br>(0.814 to 0.988) | 1.672<br>(0.326 to 2.718)  | 0.921                                   | 4.17                                  |
| 2            | Manufacturer               | 5.39-49.63                | 145<br>(148 with outliers) | 0.933<br>(0.884 to 0.984) | 1.285<br>(0.3432 to 2.235) | 0.933<br>(0.914 with outliers included) | 3.49<br>(4.70 with outliers included) |
| 3            | Manufacturer               | 2.59-49.49<br>µg/mL       | 97                         | 1.063<br>(1.019 to 1.120) | 0.184<br>(-0.368 to 0.735) | 0.976                                   | 2.67                                  |

*b. Matrix comparison:*

Linearity, precision, sensitivity and method comparison studies used both serum and plasma samples. Similar performance was observed for both.

3. Clinical studies:

*a. Clinical Sensitivity:* NA. Not typically submitted for this type of assay.

*b. Clinical specificity:* NA. Not typical for this type of assay.

*c. Other clinical supportive data (when a. and b. are not applicable):* The sponsor provided balanced and representative literature discussing measurement of zonisamide for clinical use.

4. Clinical cut-off: see expected values.

5. Expected values/Reference range:

A therapeutic range for zonisamide has not been well-established and there is no clear relationship between zonisamide serum concentrations and clinical response. Considerable overlap in zonisamide concentrations has been observed between serum responders and non-responders as well as between serum levels associated with seizure control and adverse effects. According to some reports (see package insert for references), adverse effects are more commonly associated with patients with zonisamide concentrations above 30 µg/mL.

**N. Proposed Labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**O. Conclusion:**

The submitted information in this premarket notification is complete and supports substantial equivalence decision.