

A Novel Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) Method for the Determination of Riluzole in Human Serum

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Overview

A robust LC-MS/MS method was developed and validated for the quantification of riluzole in human serum using LC-MS/MS with liquid-liquid extraction for sample clean up.

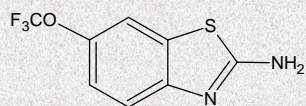
Assay Summary

- Simple sample extraction by liquid-liquid extraction
- Calibration curve range: 1 to 500 ng/mL
- Sample volume: 100 μ L
- Matrix: Human Serum
- Method: ESI(+)-HPLC-MS/MS

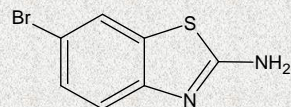
Introduction

Riluzole (Rilutek®) is the only pharmacologic agent approved by the US Food and Drug Administration for the treatment of amyotrophic lateral sclerosis (ALS), a neurodegenerative disease. Due to its speed, sensitivity, and selectivity, liquid chromatography/tandem mass spectrometry (LC-MS/MS) has become the method of choice for analyzing drug candidates in biofluids. However, there are no published LC-MS/MS methods available for the determination of riluzole in biological samples.

Figure 1. Chemical Structures of Riluzole and 2-Amino-6-Bromobenzothiazole (ABBT) as the internal standard (IS)



Riluzole



ABBT (IS)

Methods

Chromatographic Conditions

Column: Agela Venusil XBP C18 100 \AA , (50 X 2.1 mm, 5 micron).
 Mobile Phase A: 0.1 % formic acid in MeOH/H₂O (2/98)
 Mobile Phase B: 0.1 % formic acid in MeOH
 Injection Volume: 10 μ L
 Needle Wash Solvent: 0.1 % formic acid in MeOH/H₂O (50/50)
 Elution: Gradient
 Run time: 3.6 min

MS/MS Detection

Mass Spectrometer: Sciex API 4000
 Ionization: Positive Ion Electrospray
 Mode: MRM

Table 1. Monitored Transitions

| Compound | Q1 Mass m/z | Q3 Mass m/z |
|-----------|-------------|-------------|
| Riluzole | 235.1 | 138.1 |
| ABBT (IS) | 231.0 | 150.0 |

Results

Linearity

- The validated concentration range: 1 to 500 ng/mL for Riluzole
- Coefficient of Determination: $r^2 \geq 0.9985$ (Table 3)
- LLOQ: Signal/Noise at least 20
- Specificity: Six lots of blank human serum were screened and free of interference

Recovery

- The overall recovery for Riluzole was 103.2%, while IS was 96.1%. The detailed results are shown in Table 2.

Table 2. Results of Recovery Evaluation

| Sample | %Recovery | n |
|---------------|-----------|---|
| Riluzole Low | 103.0 | 3 |
| Riluzole Mid | 105.6 | 3 |
| Riluzole High | 101.0 | 3 |
| ABBT (IS) | 96.1 | 9 |

Table 3. Regression Equations and Coefficients of Determination Over Three Separate Validation Runs

| Regression Equation | R ² |
|--------------------------------------|----------------|
| $Y = -1.02E-04 + 5.451E-03 * X$ | 0.9994 |
| $Y = 1.93E-04 + 4.830E-03 * X$ | 0.9989 |
| $Y = -2.9E-05 + 2.681E-03 * X$ | 0.9985 |
| Average Coefficient of Determination | 0.9989 |

Table 4. Precision and Accuracy of QC Samples

| | Concentration (ng/mL) | 3 | 150 | 380 |
|------------|-----------------------|-------|-------|-------|
| Intrarun 1 | n | 6 | 6 | 6 |
| | Mean | 2.80 | 150 | 381 |
| | %CV | 4.0 | 1.1 | 1.0 |
| | %Nominal | 93.4 | 99.8 | 100.2 |
| Intrarun 2 | n | 6 | 6 | 6 |
| | Mean | 2.99 | 155 | 387 |
| | %CV | 1.8 | 1.9 | 1.6 |
| | %Nominal | 99.7 | 103.5 | 102.0 |
| Intrarun 3 | n | 6 | 6 | 6 |
| | Mean | 3.11 | 154 | 386 |
| | %CV | 3.0 | 3.5 | 2.4 |
| | %Nominal | 103.5 | 102.9 | 101.5 |
| Interrun | n | 18 | 18 | 18 |
| | Mean | 2.97 | 153 | 385 |
| | %CV | 5.2 | 2.8 | 1.9 |
| | %Nominal | 98.9 | 102.0 | 101.2 |

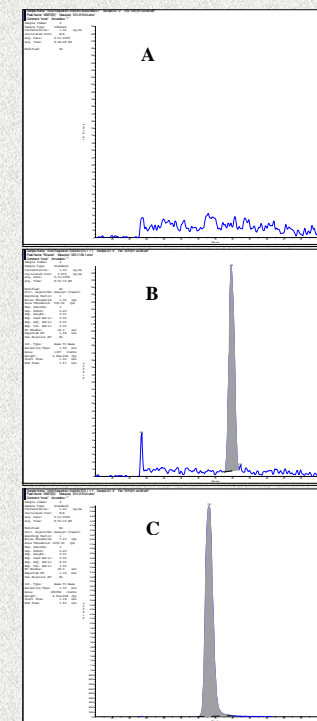
Table 5. Stability Summary

| Stability Conditions | Minimum Stability |
|-----------------------|------------------------------|
| Freeze/Thaw Stability | 3 Freeze/Thaw Cycles |
| Extraction Stability | 98 Hours at Room Temperature |
| Matrix Stability | 6 Hours at Room Temperature |

Results from Sample Assay

- Quality control samples for sample analysis: %CV < 3.7%; Accuracy: 98.8 to 102.0%
- Incurd Sample Reproducibility (ISR) has been evaluated using 20 samples from a clinical study with the results meeting the acceptance criteria

Figure 2. Chromatograms for Blank Human Serum (A), LLOQ (1 ng/mL) (B), and IS (C)



Conclusions

A simple high throughput LC-MS/MS method for determination of Riluzole in human serum has been developed and validated. The method was fast, robust, sensitive and reliable. The validated high throughput method has been successfully used in support of a clinical study sponsored by NIH.