



BACKGROUND

Thiosulfate has been known to affect thiosulfate sulfurtransferase (TST) activity. This enzyme is related to oxidative stress and NRF2 functions in cells. In preclinical species, TST activity has been linked with the development of diabetic symptoms and obesity (1). ZD10Q is a thiosulfate compound with the organic linker and positive charges on the amino groups. This compound is the byproduct of quenching the alkylating activity of a bi-functional crosslinker. The current study was to investigate the metabolism ZD10Q in the Sprague Dawley rat, Beagle dog, Cynomolgus monkey, and human using pooled cryopreserved hepatocyte suspensions.

METHODS

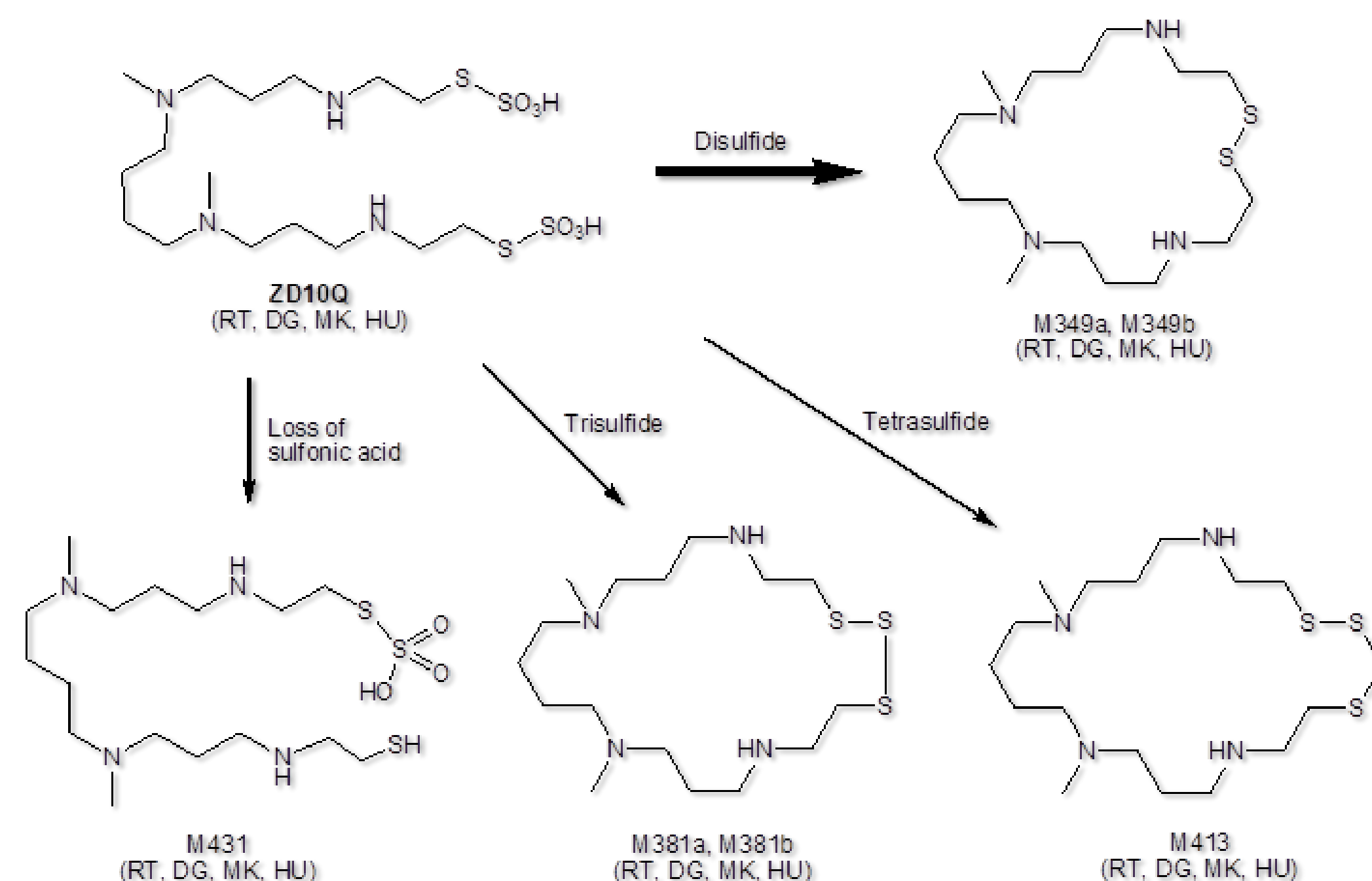
- ▶ ZD10Q was incubated in rat, monkey, dog, and human hepatocytes at 37°C. The nominal concentration was 1 µM or 10 µM for up to 2 hours. Samples were analyzed using a HPLC Q-TOF HRMS method.

Chromatographic separation was achieved using a Waters Acquity UPLC HSS T3 C18, 3.5 µm 4.6x100 mm column on a high-speed Triple TOF 6600 LC-MS/HRMS mass spectrometer.

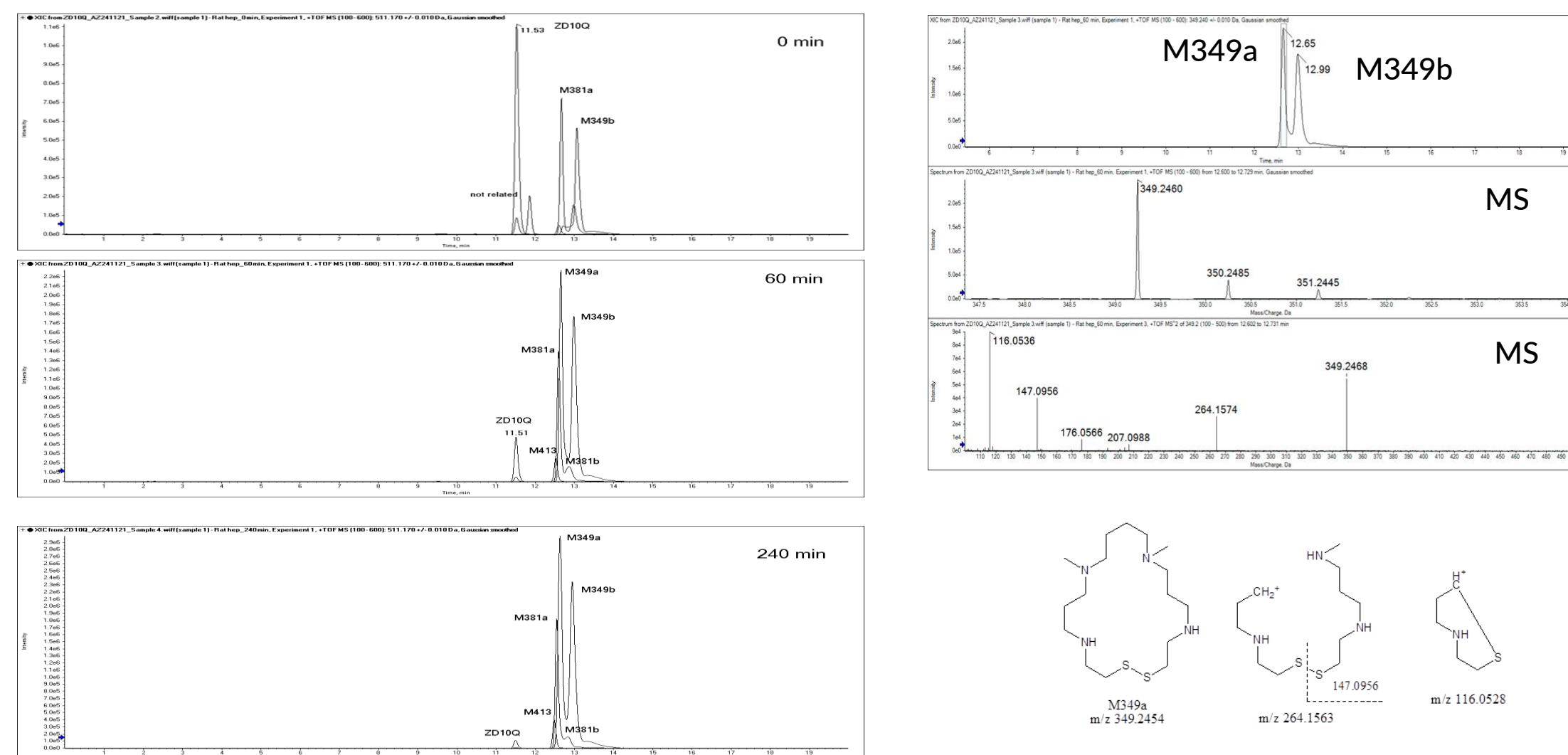
RESULTS and CONCLUSIONS

- ▶ The metabolites of ZD10Q in rat, dog, monkey, and human hepatocytes were profiled. Based on the mass spectrometry responses, the following components were observed in each species: M349a, M349b (cyclized disulfide), M381a, M381b (cyclized trisulfide), M413 (cyclized tetrasulfide), and M431 (loss of sulfonic acid). There were no human specific metabolites observed under the in vitro conditions evaluated.

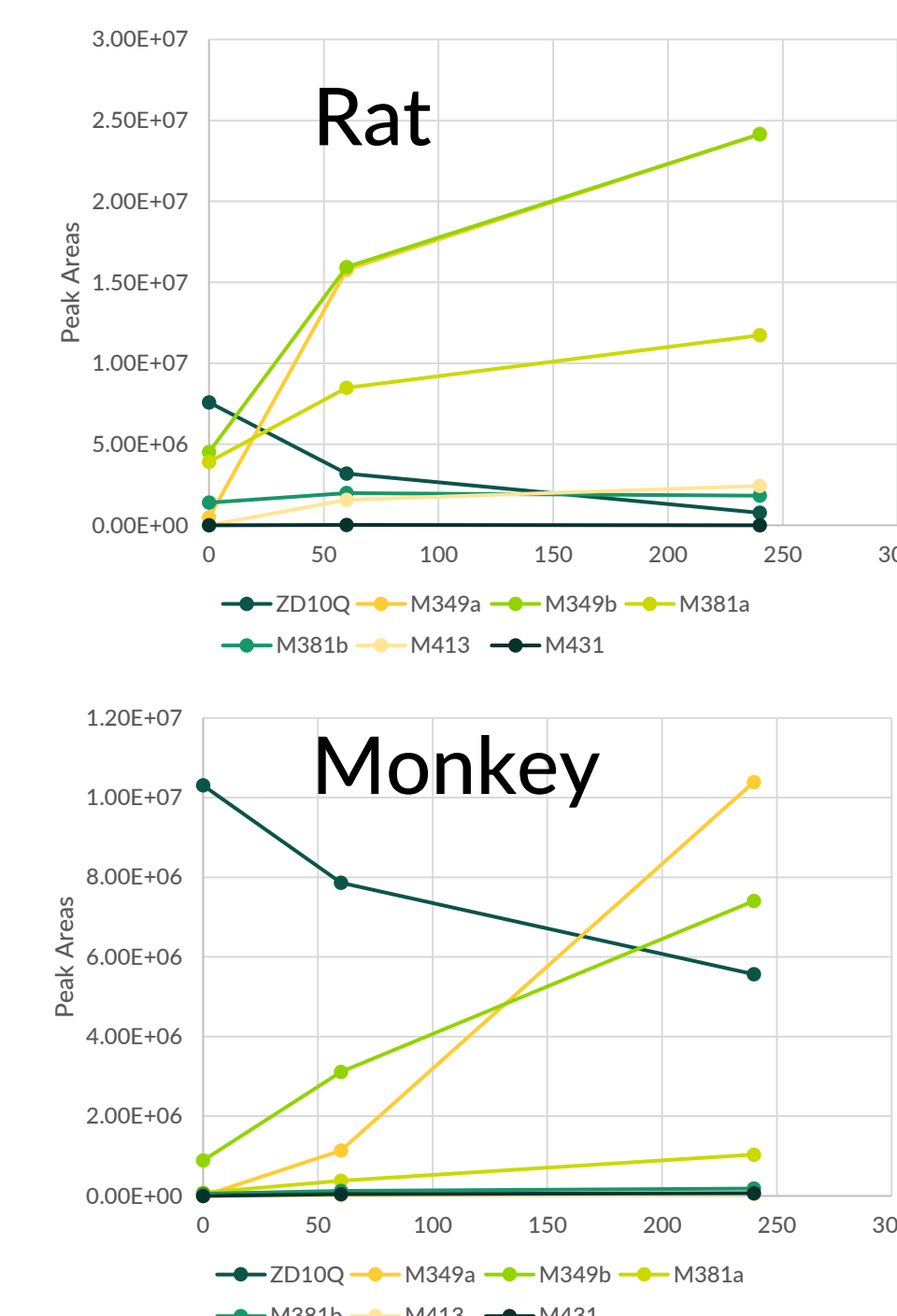
Proposed Biotransformation Products of ZD10Q in Rat, Dog, Monkey, and Human Hepatocytes



Chromatogram of ZD10Q and the Detected Metabolites (Rat Hepatocytes, 0, 60, 240 min incubation)

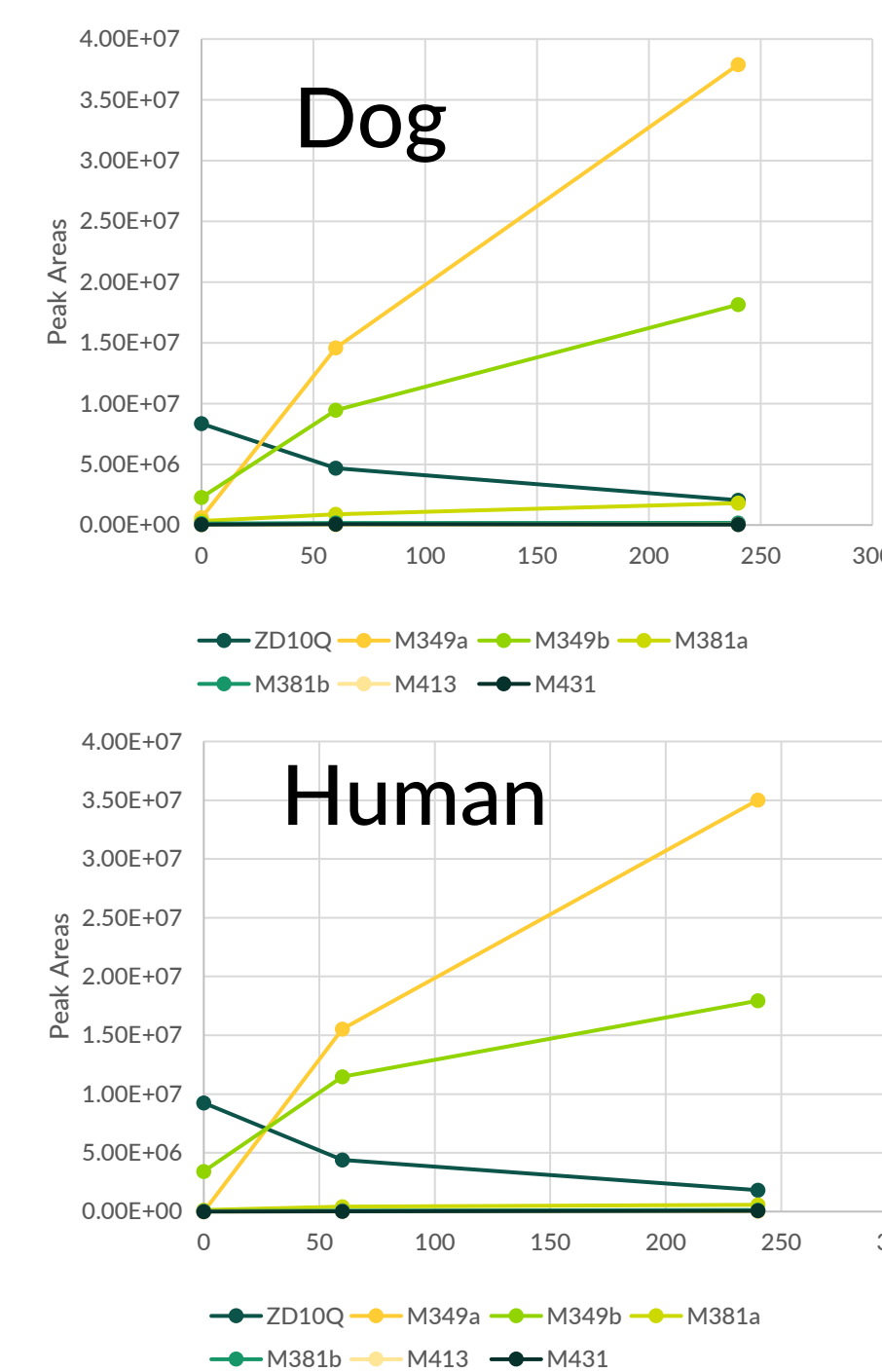


ZD10Q Metabolites Formation in Rat, Dog, Monkey, Human Hepatocytes (0-240 min incubations)



Metabolic Stability of ZD10Q in Rat, Dog, Monkey, and Human Hepatocytes

| Species | Incubation (min) | ZD10Q Mean (Peak Area Ratio) | Stdev | % Remaining of ZD10Qa |
|---------|------------------|------------------------------|-------|-----------------------|
| Rat | 0 | 5.65 | 0.311 | 100 |
| | 5 | 6.36 | 0.720 | 113 |
| | 15 | 6.01 | 0.255 | 106 |
| | 30 | 6.31 | 0.137 | 112 |
| | 45 | 4.76 | 0.385 | 84.3 |
| | 60 | 3.71 | 0.483 | 65.7 |
| Dog | 0 | 6.77 | 0.864 | 100 |
| | 5 | 6.98 | 0.346 | 103 |
| | 15 | 7.33 | 0.726 | 108 |
| | 30 | 5.84 | 0.406 | 86.3 |
| | 45 | 4.90 | 0.384 | 72.3 |
| | 60 | 4.14 | 0.137 | 61.1 |
| Monkey | 0 | 7.62 | 0.333 | 100 |
| | 5 | 7.03 | 2.04 | 92.3 |
| | 15 | 7.20 | 0.425 | 94.5 |
| | 30 | 8.06 | 0.608 | 106 |
| | 45 | 6.81 | 0.084 | 89.4 |
| | 60 | 6.39 | 0.279 | 83.8 |
| Human | 0 | 7.51 | 0.105 | 100 |
| | 5 | 7.18 | 0.392 | 95.6 |
| | 15 | 7.28 | 0.269 | 97.0 |
| | 30 | 6.68 | 0.956 | 88.9 |
| | 45 | 5.36 | 1.09 | 71.4 |
| | 60 | 3.82 | 0.338 | 50.9 |



Summary of ZD10Q Metabolites Observed in Hepatocyte Incubations

| Component | Description | Theoretical Mass [M+H] ⁺ (m/z) | Detected Mass [M+H] ⁺ (m/z) (ppm) | RT | Proposed Structure |
|----------------|--|---|--|------|--------------------|
| ZD10Q | Parent C ₁₈ H ₃₈ N ₄ O ₃ S ₄ | 511.1747 | 511.1733 (-2.7) | 24.0 | |
| M349a | Cyclized Disulfide C ₁₈ H ₃₈ N ₄ S ₂ | 349.2454 | 349.2460 (1.7) | 12.7 | |
| M349b (isomer) | Cyclized Disulfide C ₁₈ H ₃₈ N ₄ S ₂ | 349.2454 | 349.2460 (1.7) | 13.0 | |
| M381a | Cyclized Trisulfide C ₁₈ H ₃₈ N ₄ S ₃ | 381.2175 | 381.2178 (0.8) | 12.6 | |
| M381b (isomer) | Cyclized Trisulfide C ₁₈ H ₃₈ N ₄ S ₃ | 381.2175 | 381.2172 (-0.8) | 12.9 | |
| M413 | Cyclized Tetrasulfide C ₁₈ H ₃₈ N ₄ S ₄ | 413.1896 | 413.1889 (-1.7) | 12.5 | |
| M431 | Loss of sulfonic acid C ₁₈ H ₃₈ N ₄ O ₃ S ₃ | 431.2179 | 431.2171 (-1.9) | 14.1 | |