

Supplementary Materials: Electrochemical Simulation of Phase I Hepatic Metabolism of Voriconazole Using a Screen-Printed Iron(II) Phthalocyanine Electrode

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Table S1. LC-MS parameters.

| Device | Parameter | Value |
|--------|-------------------------|--|
| LC | Solvents | A – acetonitrile B – 0.1% aqueous solution of HCOOH |
| | Gradient | 5% A to 80% A |
| | Analysis time | 9 min |
| | Post-time equilibration | 2.50 min |
| | Flow rate | 0.3 mL min ⁻¹ |
| | Injection volume | 2 µL |
| | Column temperature | 35 °C |
| | Ion source | Electrospray (ESI) |
| | Mode | Positive |
| | Source temperature | 300 °C |
| MS | Drying gas flow | 10 L min ⁻¹ |
| | Nebulizer pressure | 40 psig |
| | Capillary voltage | 3500 V |
| | Fragmentor voltage | 140 V |
| | Skimmer voltage | 65 V |
| | Octopole voltage | 750 V |
| | Mass range | 90–950 m/z |
| | Acquisition rate | 1.5 spectra s ⁻¹ |

Table S2. Acute toxicity of voriconazole and its metabolites to rodents (LD₅₀ [mg/kg]).

| Compound | T.E.S.T. Rat OR | Mouse IP | Mouse OR | Mouse IV | Mouse SC | Rat IP | Rat OR |
|----------|-----------------|----------|----------|----------|----------|--------|--------|
| Vor | 779,1 | 1000 | 650 | 330 | 5000 | 490 | 740 |
| VM1 | 796,85 | 1400 | 1400 | 280 | 5600 | 520 | 800 |
| VM2 | 330,64 | 740 | 910 | 220 | 2200 | 410 | 760 |
| VM3 | 814,78 | 1200 | 730 | 390 | 6000 | 600 | 820 |
| VM4 | 732,9 | 1300 | 1000 | 450 | 13000 | 720 | 760 |

OR – oral; IP – intraperitoneal; IV – intravenous; SC – subcutaneous

Table S3. Mutagenicity (probability of positive Ames test) of voriconazole and its metabolites (red – positive, green – negative).

| Compound | T.E.S.T. Consensus ^a | Percepta ^a | Vega Consensus ^b | Vega ISS ^c | Vega KNN/Read-Across ^c |
|----------|---------------------------------|-----------------------|-----------------------------|-----------------------|-----------------------------------|
| Vor | 0,76 | 0,13 | -0,3 | 0 | 0 |
| VM1 | 0,87 | 0,28 | 0,1 | 1 | 1 |
| VM2 | 0,84 | 0,22 | -0,1 | 0 | 1 |
| VM3 | 0,75 | 0,16 | 0 | 0 | 1 |
| VM4 | 0,82 | 0,09 | 0 | 0 | 1 |

a – mutagenic > 0,5; b – mutagenic >= 0; c – 0 = non-mutagenic, 1 = mutagenic

Table S4. Developmental toxicity of voriconazole and its metabolites (red – toxic, green – non-toxic).

| Compound | T.E.S.T. Consensus ^a | Vega CAESAR ^b |
|----------|---------------------------------|--------------------------|
| Vor | 0,57 | 1 |
| VM1 | 0,63 | 0 |
| VM2 | 0,46 | 0 |
| VM3 | 0,53 | 1 |
| VM4 | 0,56 | 1 |

a – mutagenic > 0,5; b – 0 = non-mutagenic, 1 = mutagenic

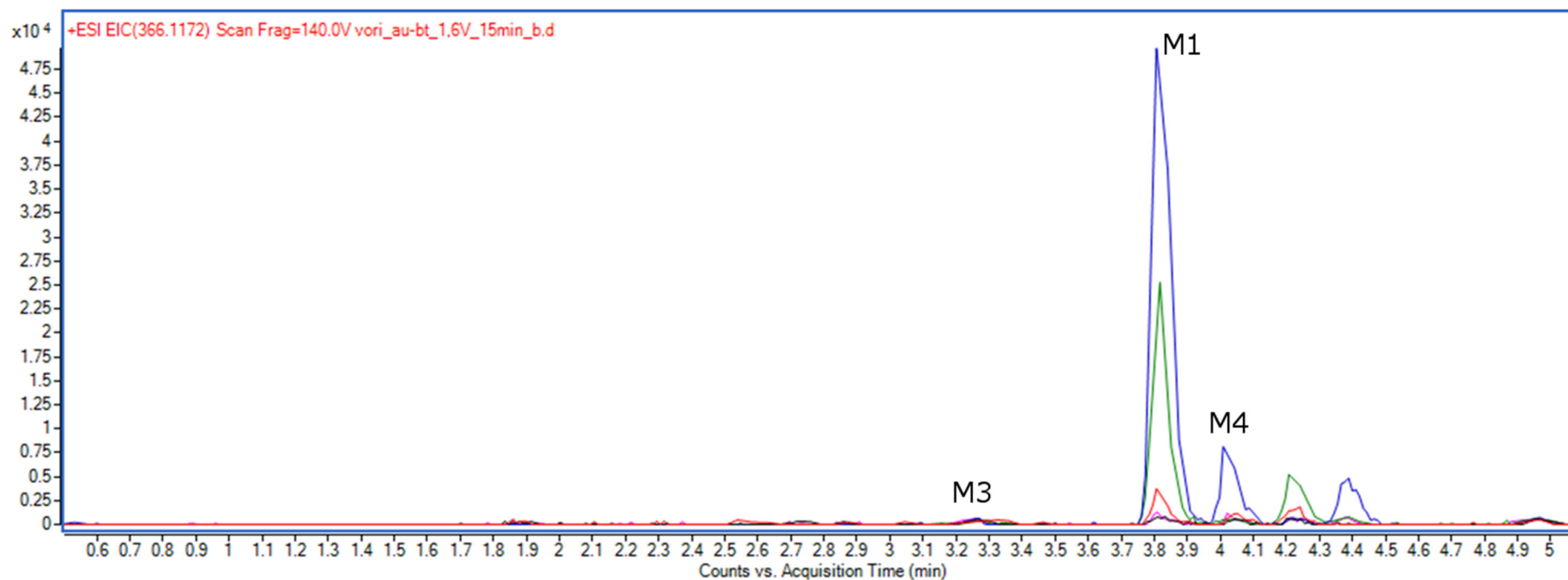


Figure S1. Overlaid EIC chromatograms of m/z 366.1172 obtained from the examined electrodes. The color-coding is as follows: blue corresponds to Fe(PH), pink to Cu(PH), green to Pt, red to Au, and black to GC SPEs. The optimal conditions for obtaining metabolites with a mass of m/z 366.1172 for the given electrodes were as follows: 1.0 V for the Fe(PH) electrode, 1.4 V for Cu(PH), 1.2 V for Pt, 1.6 V for Au, 1.4 V for GC. The chromatogram shows all the products resulting from monooxygenation, with M1 appearing at a retention time of 3.80, M3 at 4.03, and M4 at 3.25. The peak at a retention time of 4.97 aligns with the retention time of the parent compound (in source clustering). Additionally, two products of electrochemical transformation are observable on the chromatogram at retention times of 4.23 and 4.40.

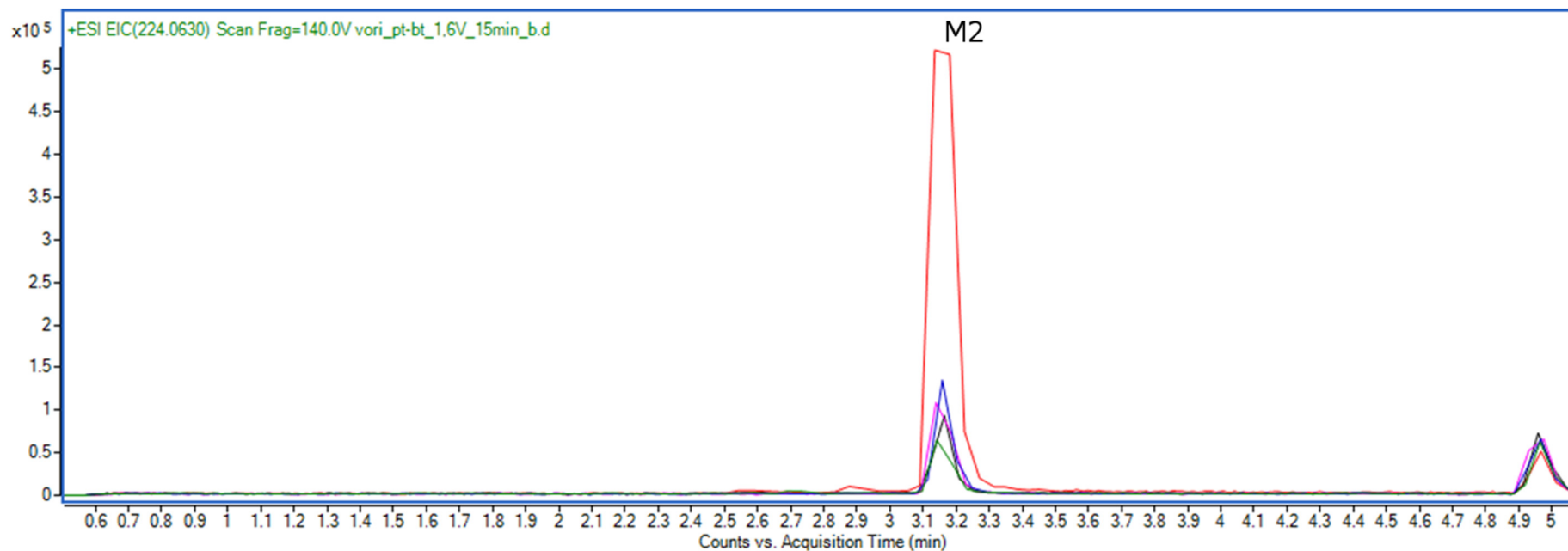


Figure S2. Overlaid EIC chromatograms of m/z 244.0630 obtained from the examined electrodes. The color-coding is as follows: blue corresponds to Fe(PH), pink to Cu(PH), green to Pt, red to Au, and black to GC SPEs. The optimal conditions for obtaining M2 for the given electrodes were as follows: 1.4 V for the Fe(PH) electrode, 1.4 V for Cu(PH), 1.6 V for Pt, 1.8 V for Au, 1.4V for GC. The peak belonging to M2 is visible in the chromatogram at a retention time of 3.15 min. The peak with a retention time of 4.97 aligns with the retention time of the parent compound undergoing decomposition in the ion source of mass spectrometry.

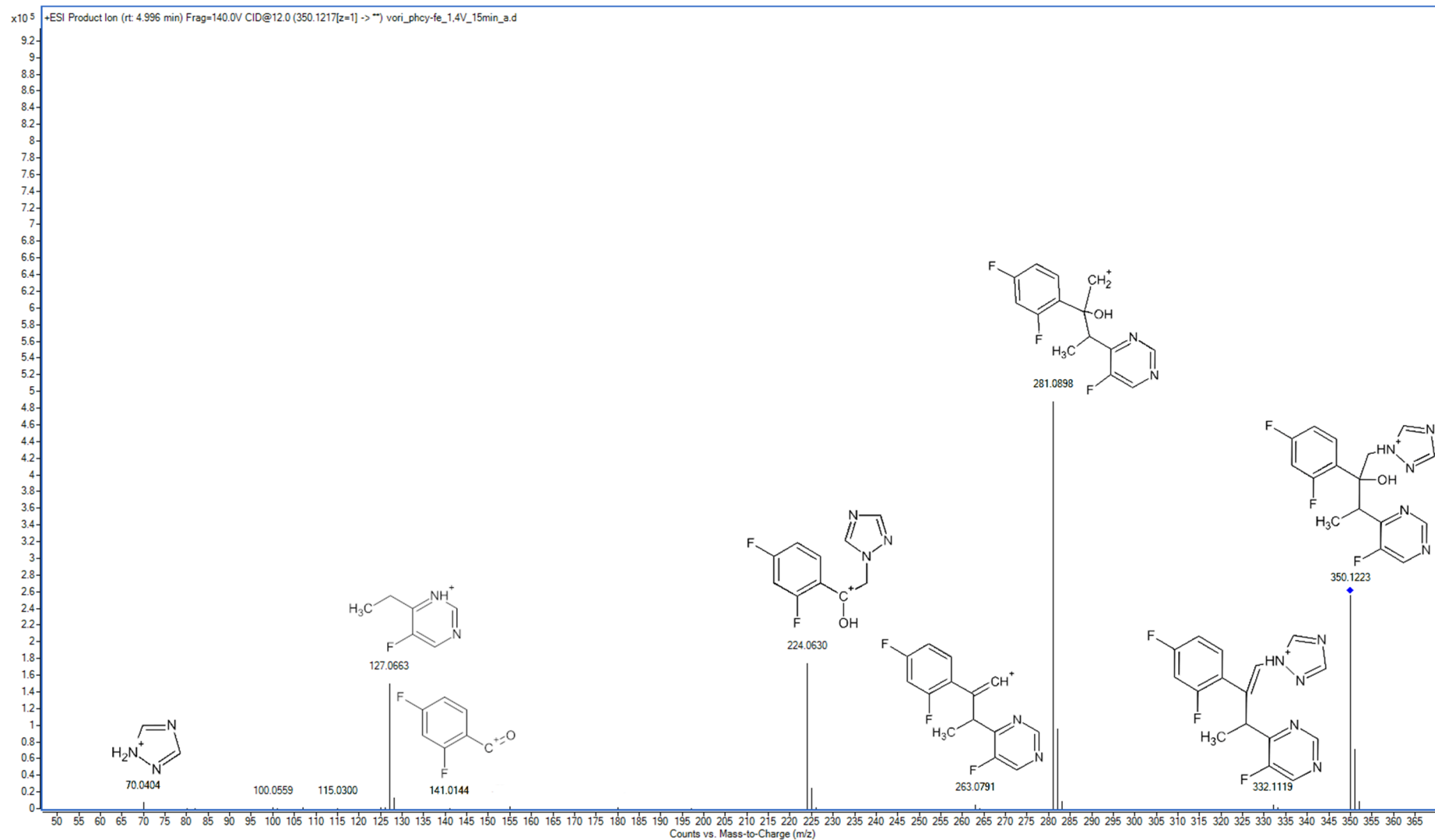


Figure S3. MS/MS spectrum and fragmentation pattern of voriconazole.

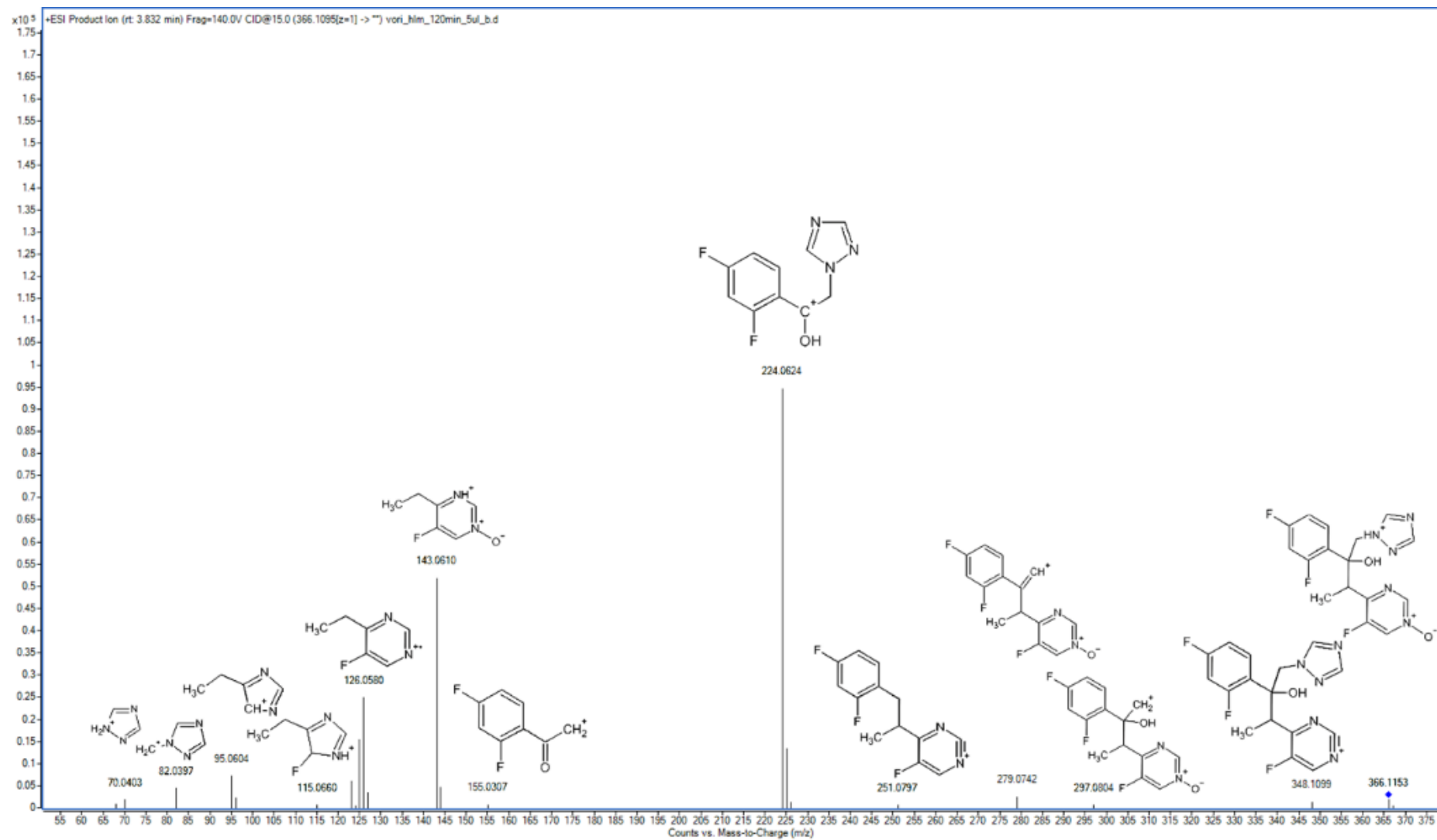


Figure S4. MS/MS spectrum and fragmentation pattern of M1.

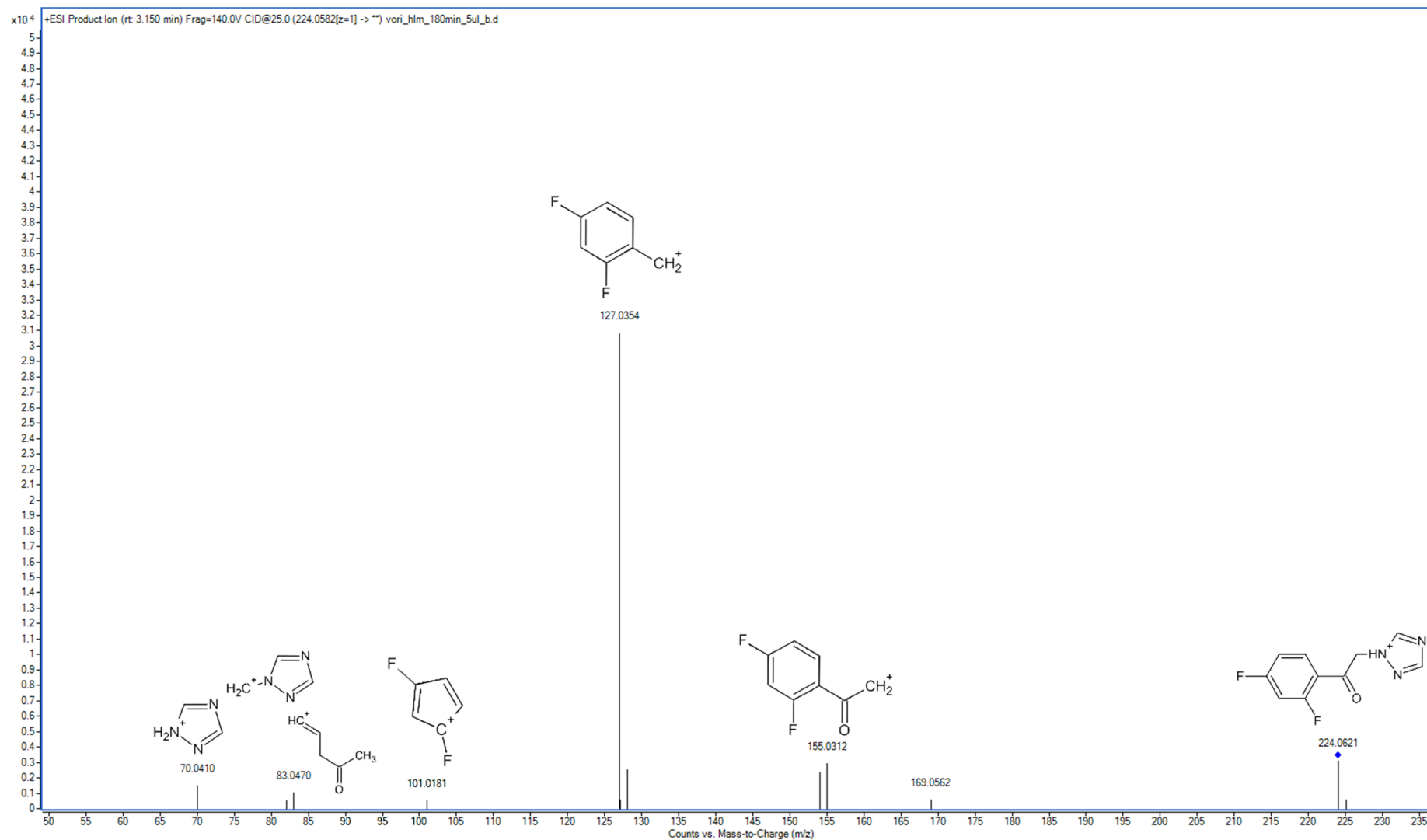


Figure S5. MS/MS spectrum and fragmentation pattern of M2.

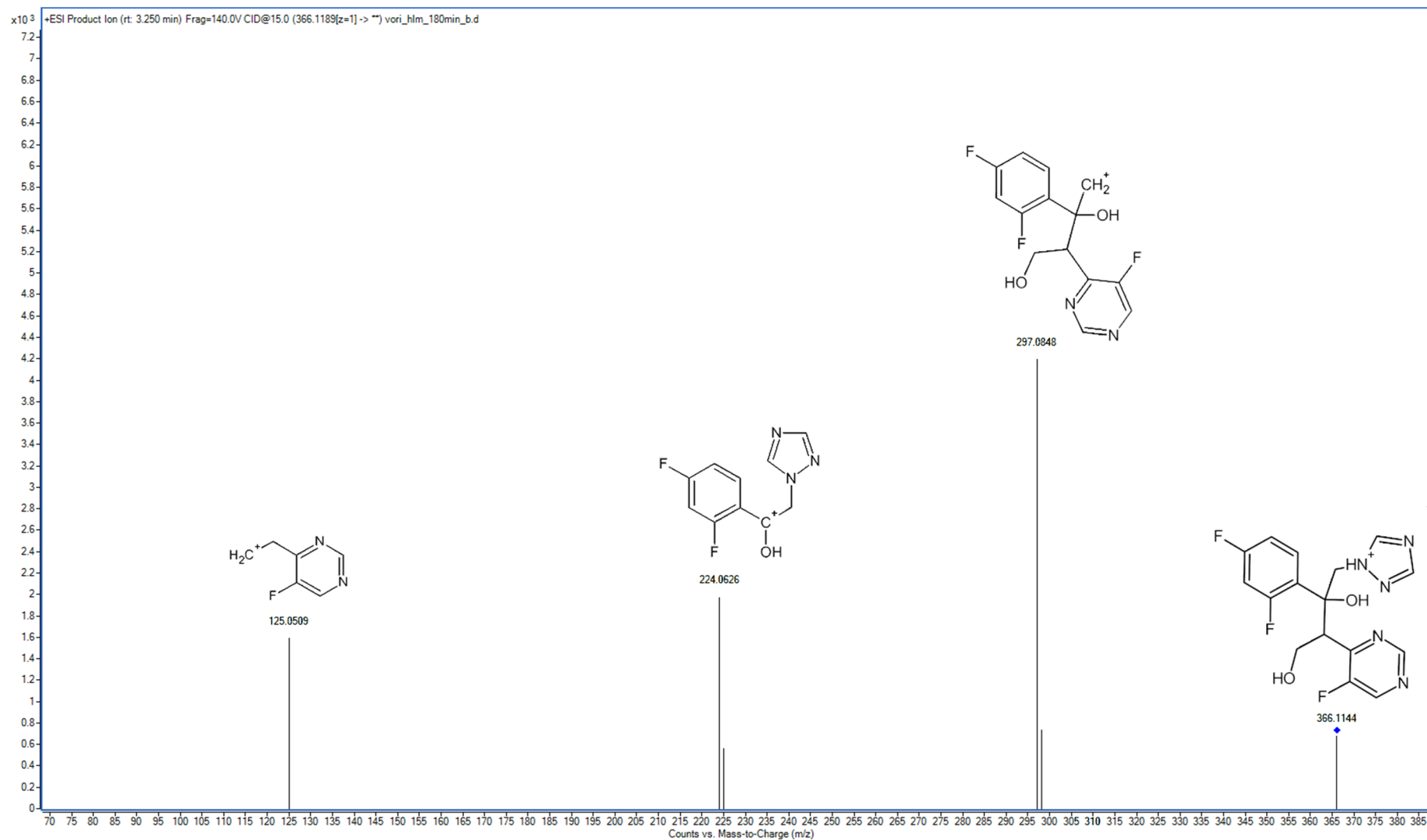


Figure S6. MS/MS spectrum and fragmentation pattern of M3.

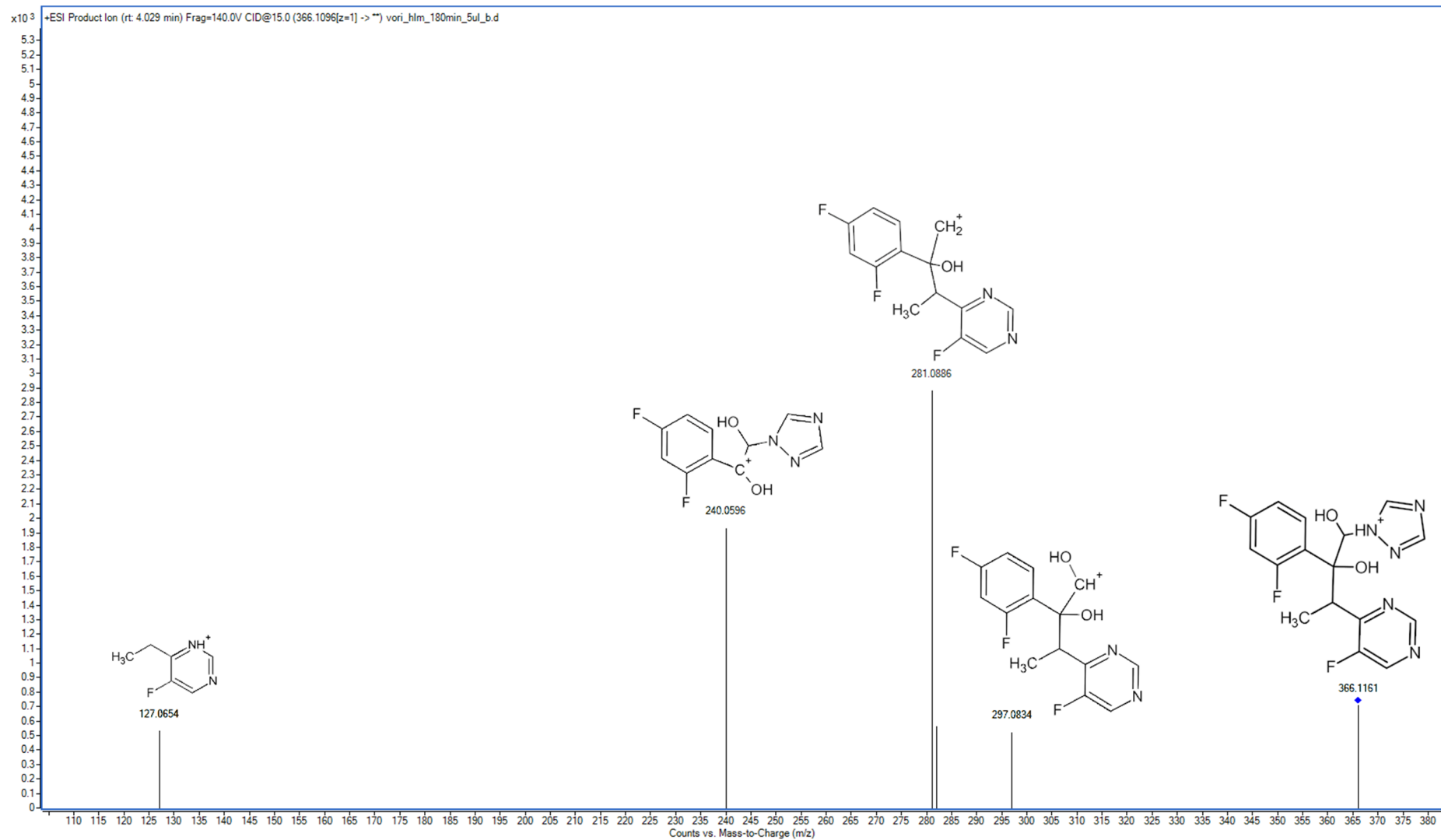


Figure S7. MS/MS spectrum and fragmentation pattern of M4.

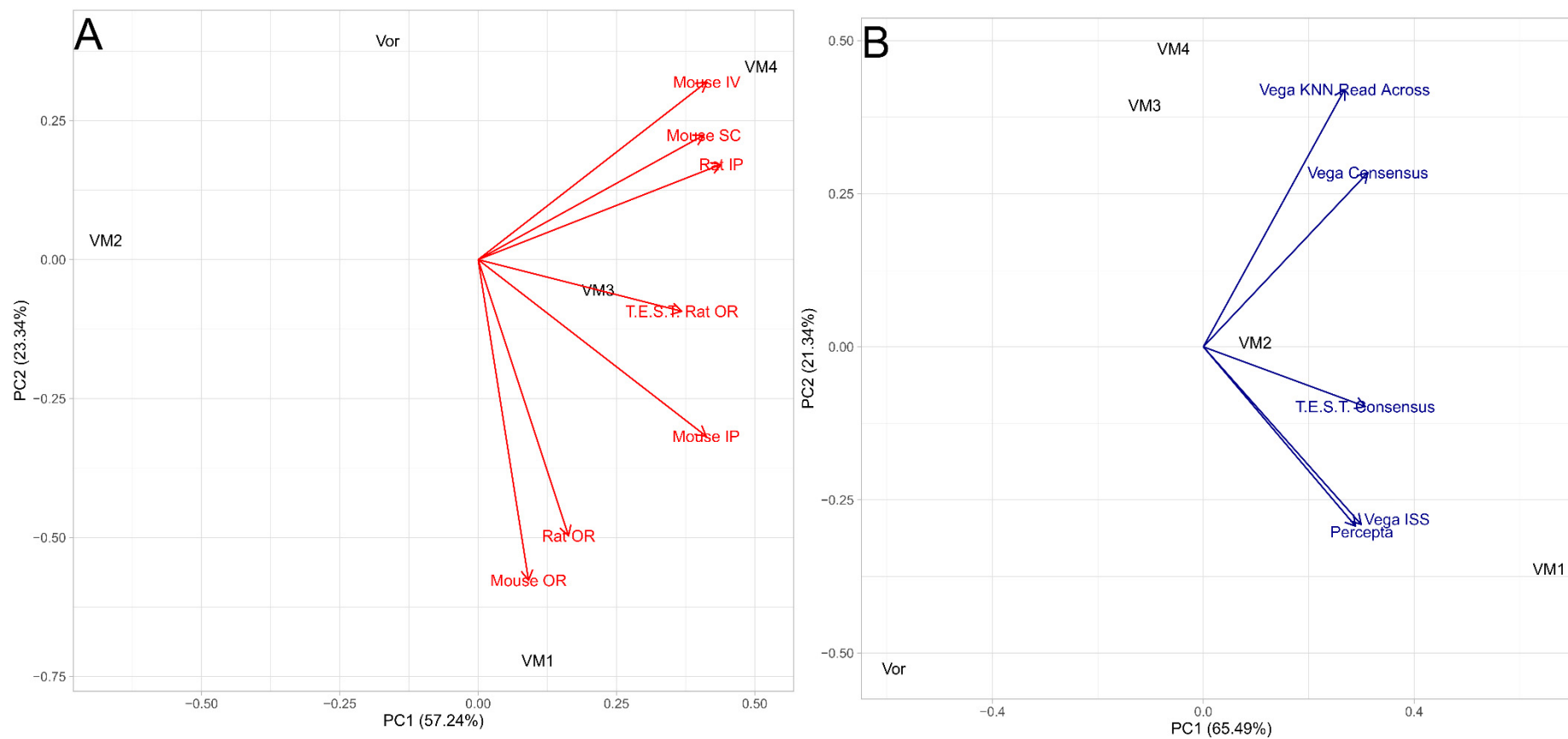


Figure S8. Comparison of toxicity of metabolites of voriconazole by PCA; IP – Intraperitoneal, IV – Intravenous, OR – Oral, SC – Subcutaneous.