Title: Sigma-2 receptors play a role in cellular metabolism: Stimulation of glycolytic hallmarks by CM764 in human SK-N-SH neuroblastoma*

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Supplemental Figure 1:

Methods for extraction experiment:

SK-N-SH cells were plated at 60,000 cells per well in 24 well plates. Other wells were left without cells. Wells (with or without cells) were incubated in 1 ml of complete cell culture medium (with 10% FBS) containing 30 μM CM764 for 0 h (control) or for 24 h in a humidified atmosphere at 37°C and 5% CO_2. For the 0 h control, CM572 was added to the well, mixed, and media immediately removed for extraction.

The culture medium (1 ml) was removed from the wells and placed in a glass extraction tube on ice. The pH was brought from pH 7.4 to pH 8.5 using NaOH.

One ml of incubation mixture was extracted with 1 ml of ethyl acetate by vigorous vortexing. After phases separated, 0.7 ml of organic phase was removed and evaporated under nitrogen stream. The residue was reconstituted into 0.2 ml of methanol for HPLC/MS analysis.

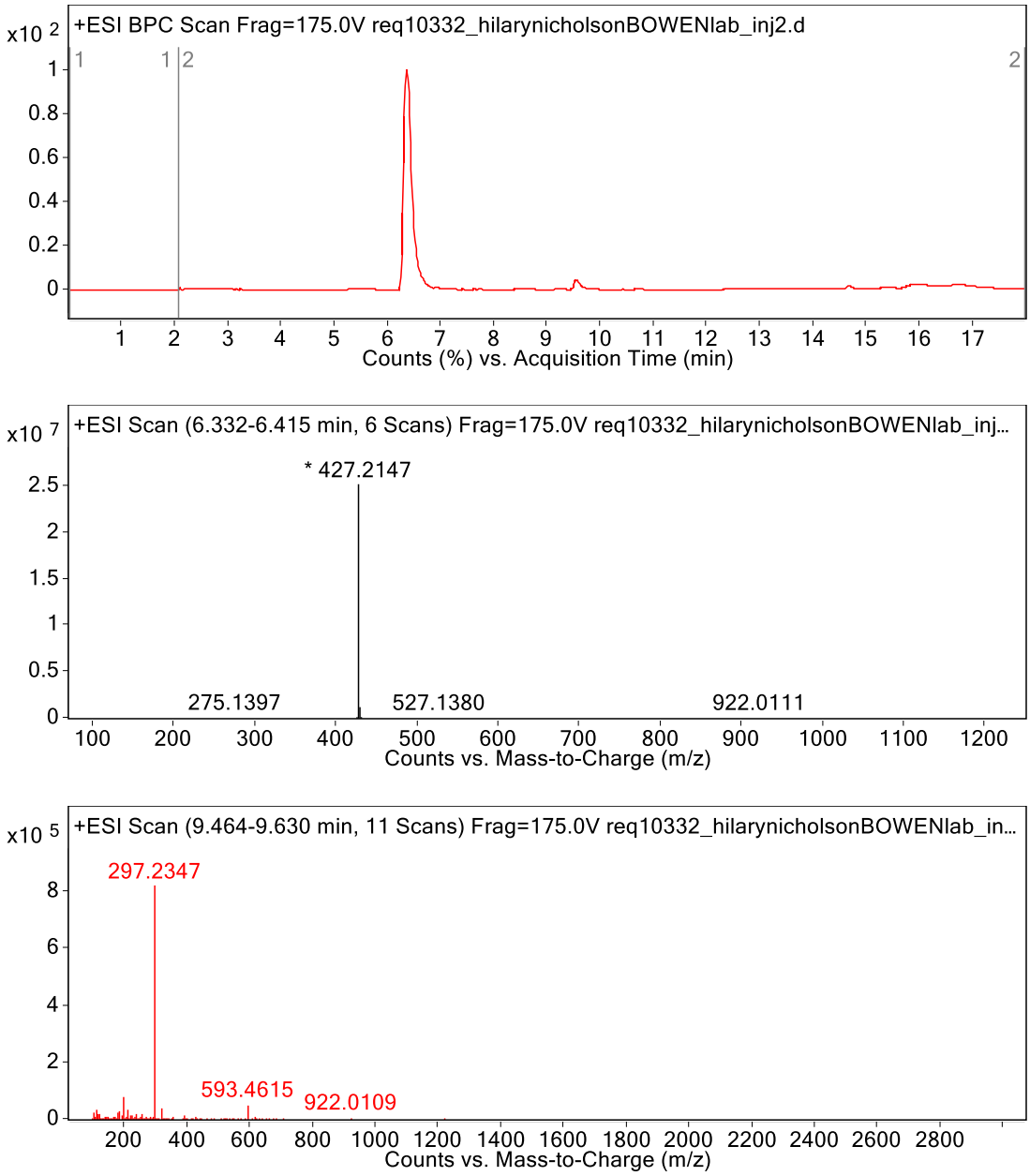
Media without cells that contained no CM764 was extracted to assess components in the ethyl acetate extract that are due to just the media.

The analyses were carried out in the Brown University Chemistry Department analytical core facility by Dr. Tun-Li Shen.

Summary of Results:

There was some concern about oxidative breakdown of CM764 over the 24 h timeline of the experiments due to the potentially sensitive 1,2-diaminophenyl ring moiety that could be oxidized to compounds analogous to an o-quinone. We incubated 30 μM CM764 in normal culture media under the conditions of cell incubation for up to 24 h, with and without the presence of SK-N-SH cells. We followed this by extraction of the media and analysis by LC/MS. The results of these experiments are shown in Supplemental Figures 2-4. There appears to be no significant degradation of the compound in 24 h compared to either compound incubated for "zero" time and extracted out of media or to pure (authentic) compound from stock stored in DMSO. The major peak under the various conditions had the correct retention time and mass of the pure compound. There were no additional peaks present that were not already present in media without CM764. The only potentially questionable peak was a very small peak of RT=6.16-6.27, m/z=429.23 that was present at 24 h, not seen at 0 h, and not seen without cells (see Supplemental Figure 4). This could either be a component from the cells or a product related to CM764. The latter is unlikely since the increased mass by 2 units could only indicate reduction, yet the compound is slightly more polar than CM764. The difference in mass by only 2 units higher shows that this is not an oxidation product of CM764, and most likely a compound coming from the cells. These results show that CM764 is apparently stable under the conditions of the experiments described.

Supplemental Figure 2: **Pure CM764 from DMSO stock**

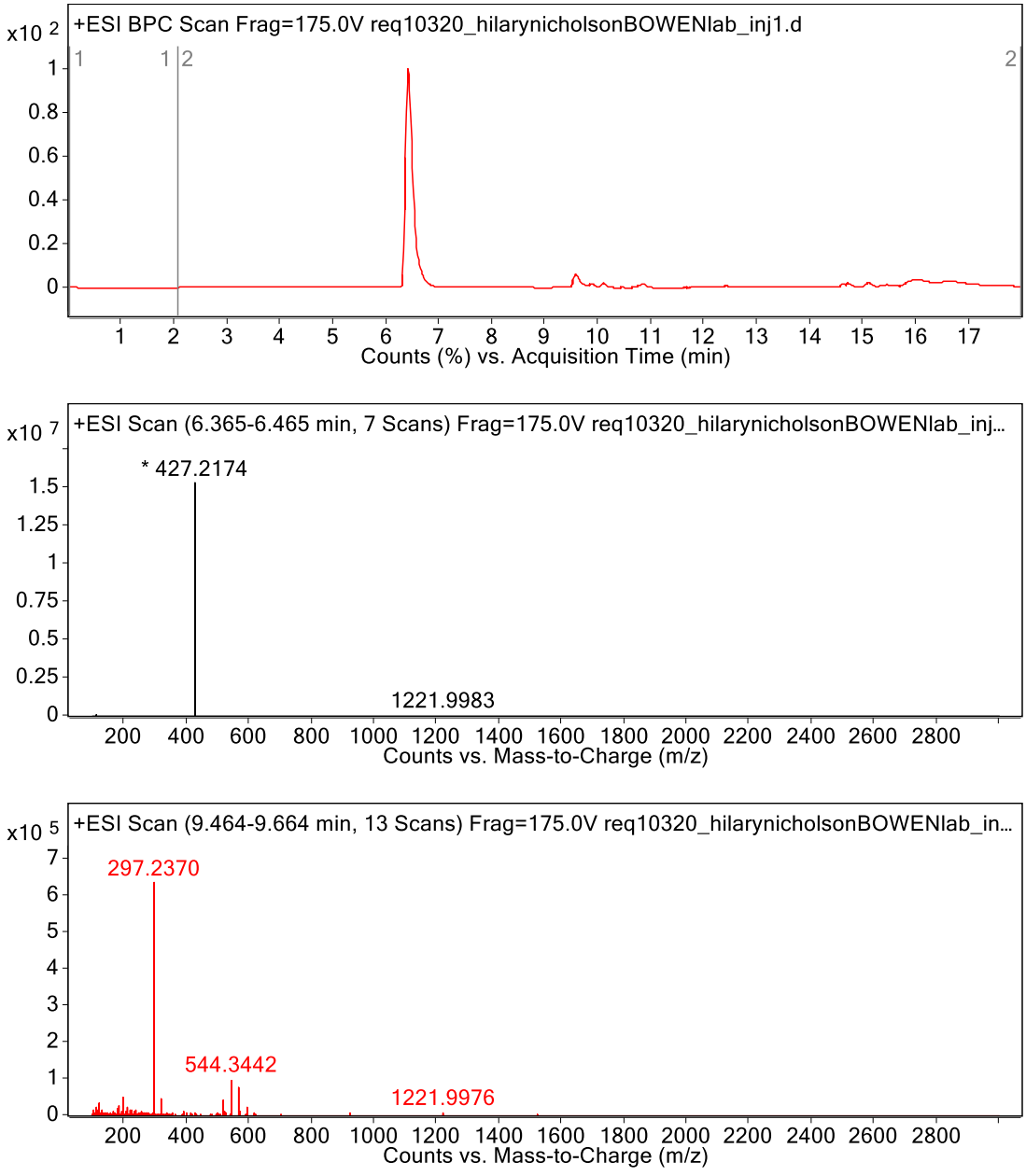


CM764 m/z = 427.21, RT = 6.33-6.41 min

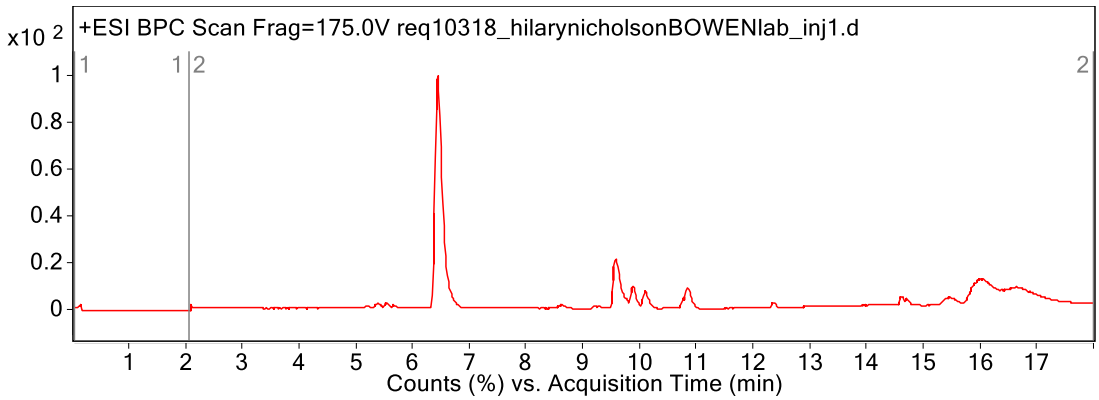
Small peak at RT=9.46-9.63, m/z = 297.23 is not an impurity in CM764, as it has appeared in all chromatographs, even those without CM764 present.

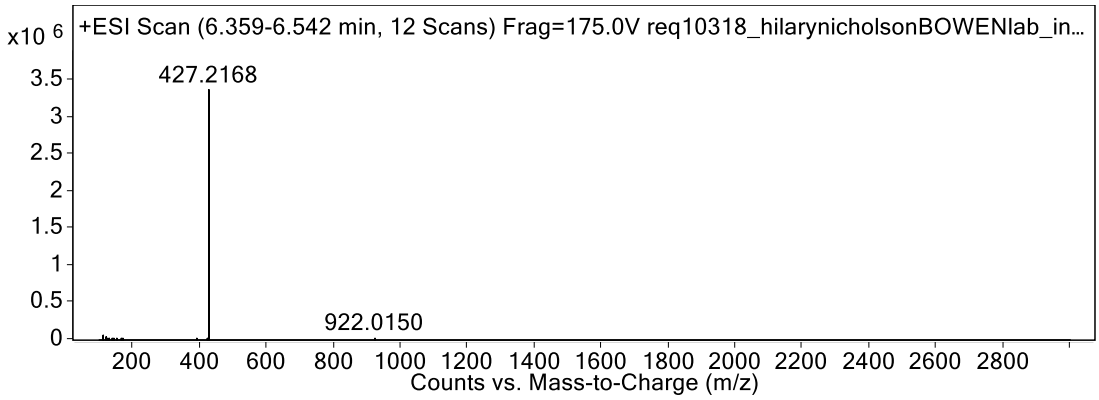
Supplemental Figure 3: **CM764 (30 µM) incubated in culture media without cells for 0 h or 24 h**

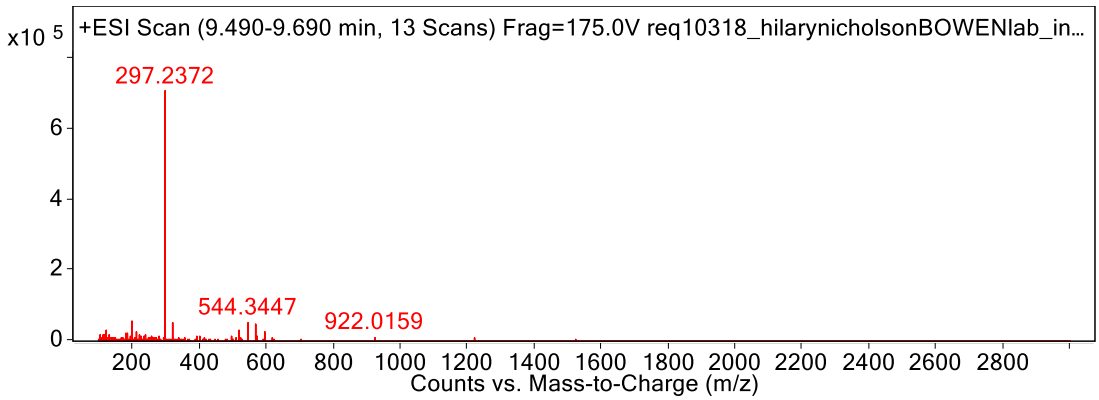
A) Incubation for 0 h (compound added to media and immediately extracted), without cells

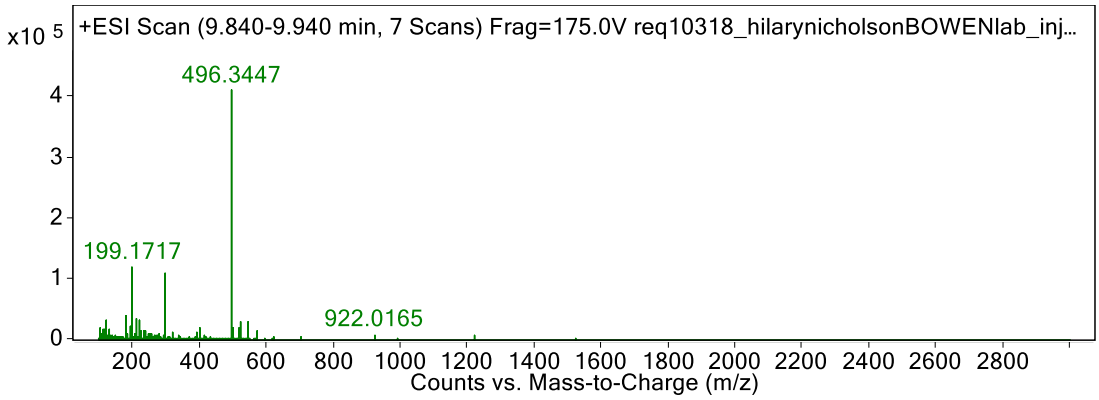


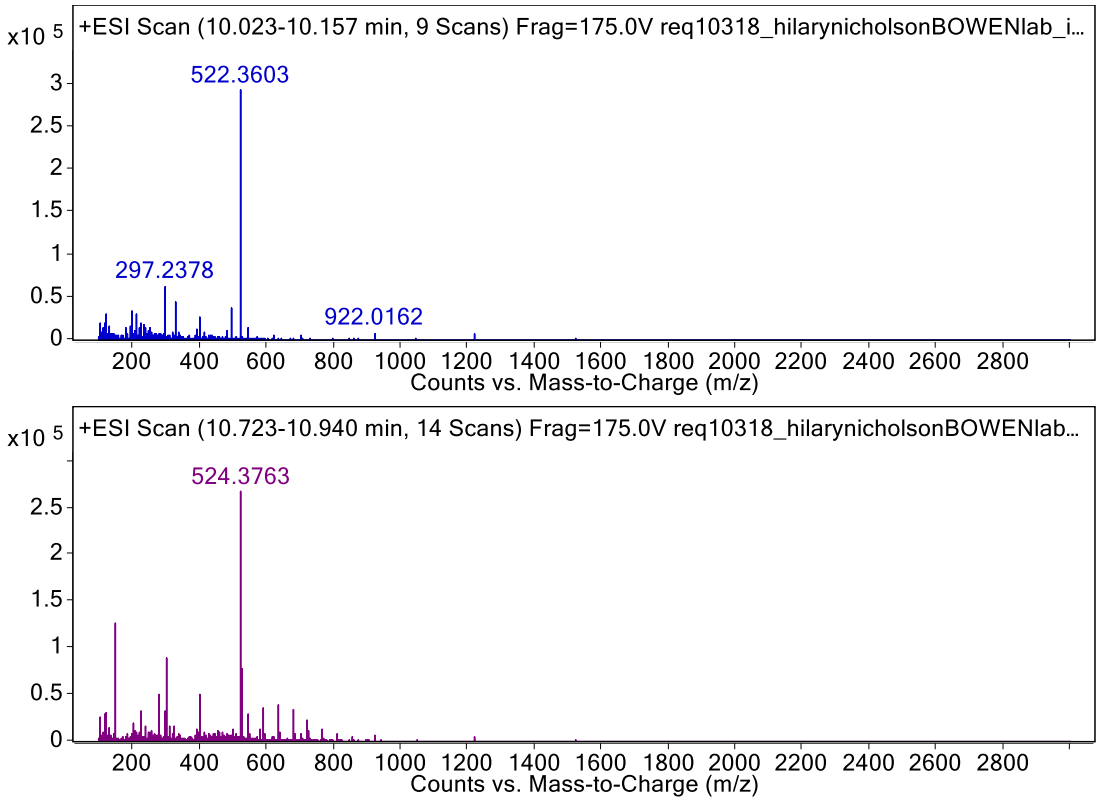
B) Incubation for 24 h in media at 37°C in 5% CO_2 humidified incubator, with no cells present









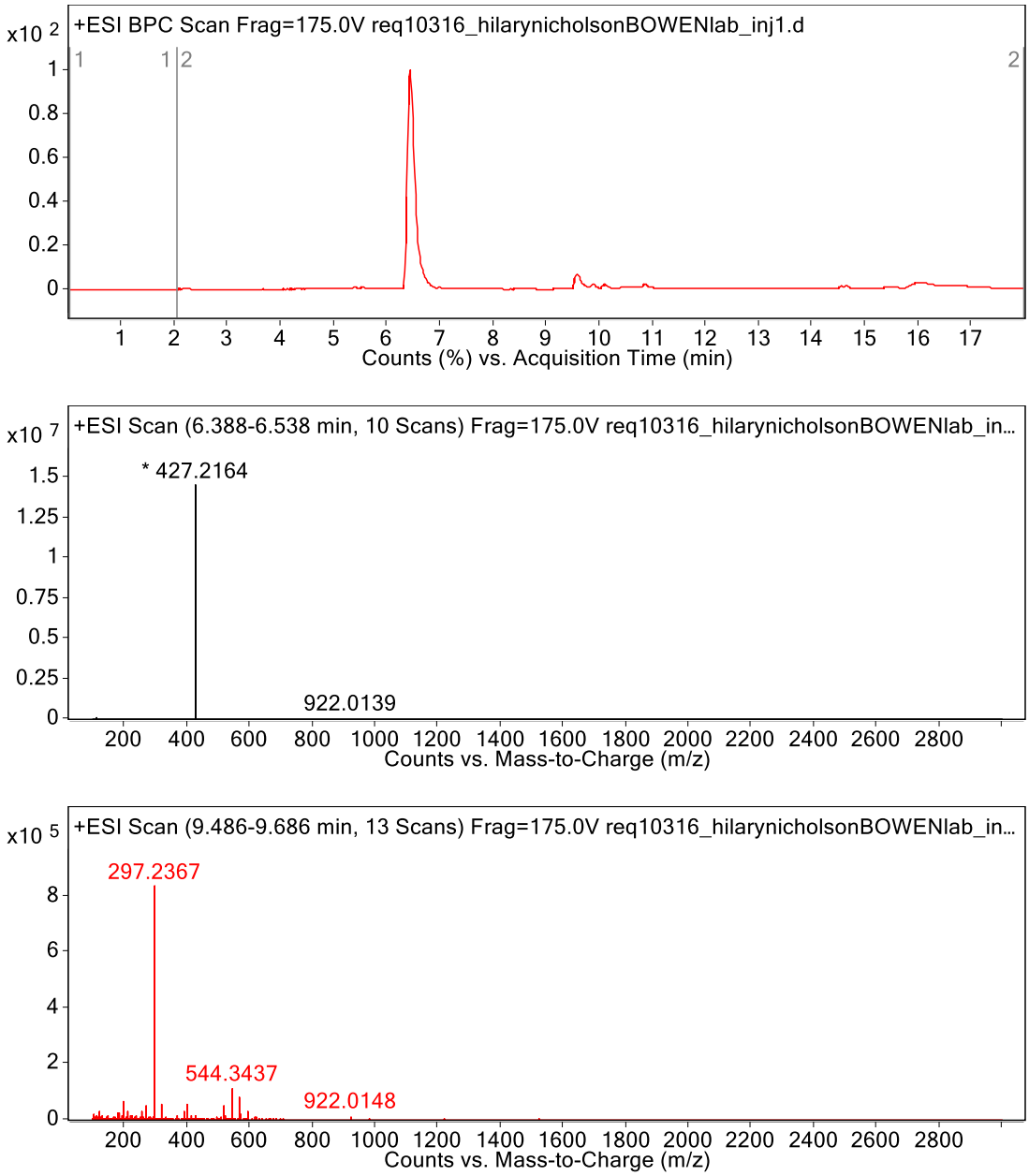


CM764 m/z = 427.21, RT = 6.36-6.46 min

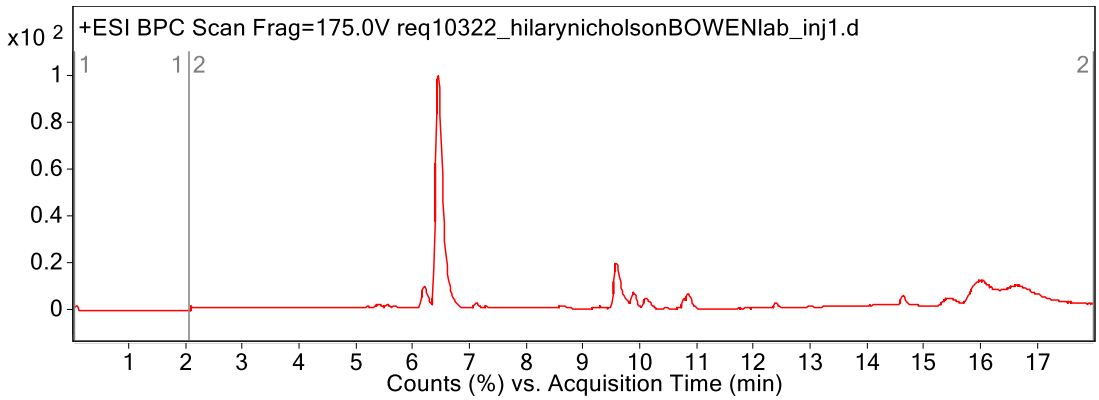
The small peaks in the RT range 9.49 – 10.94 are not related to CM764 as they appear in extracted media that has not been exposed to CM764.

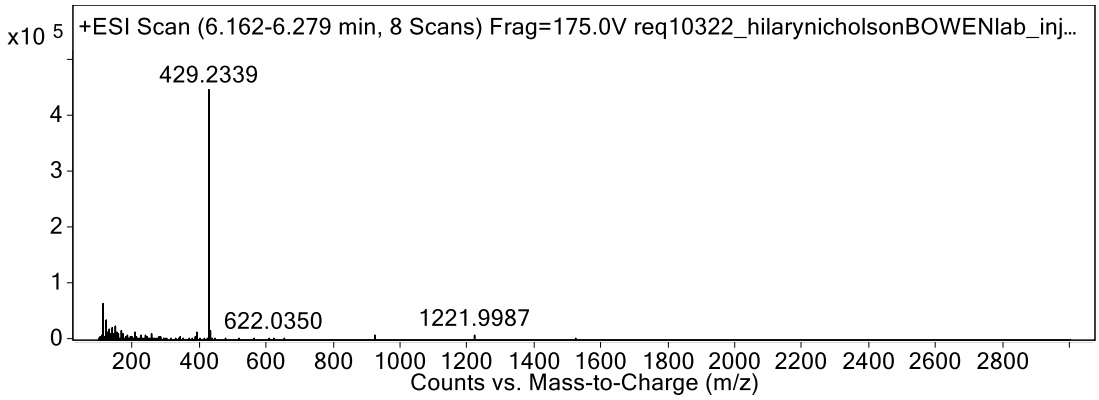
Supplemental Figure 4: **CM764 (30 µM) incubated in media with cells for 0 h or 24 h**

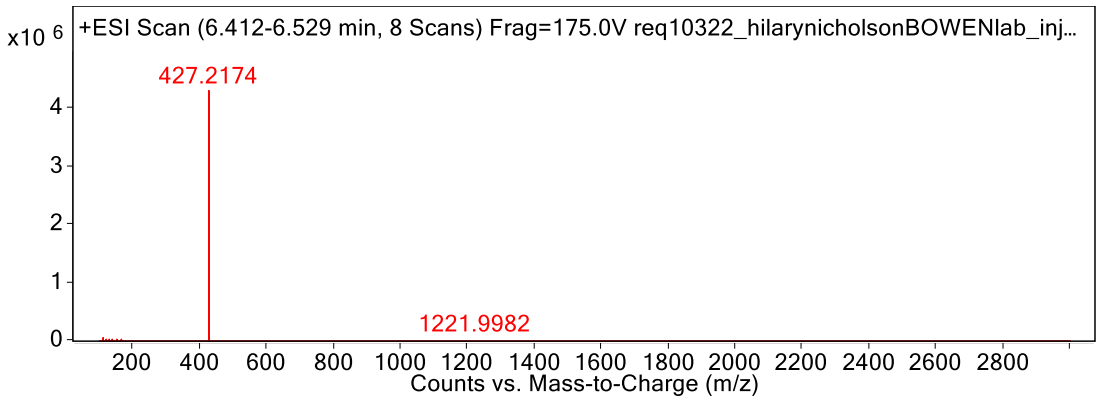
A) Incubation for 0 h with cells present (compound added, mixed, and immediately extracted)

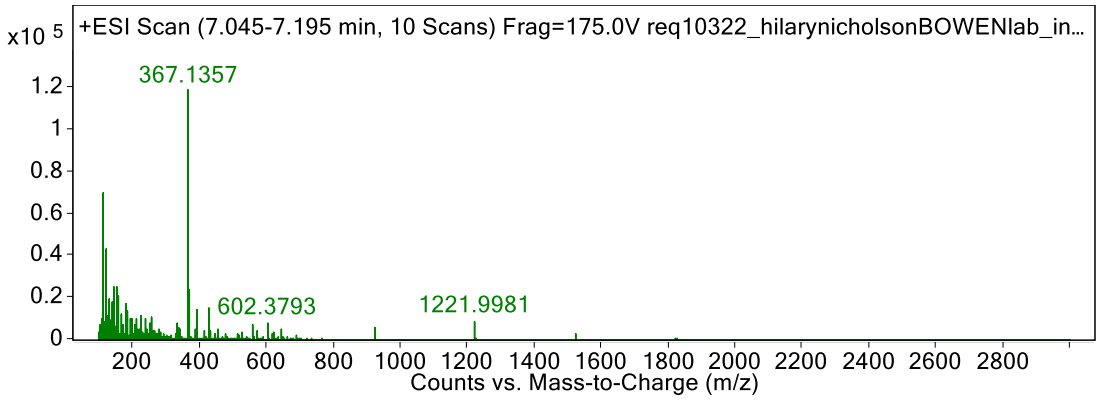


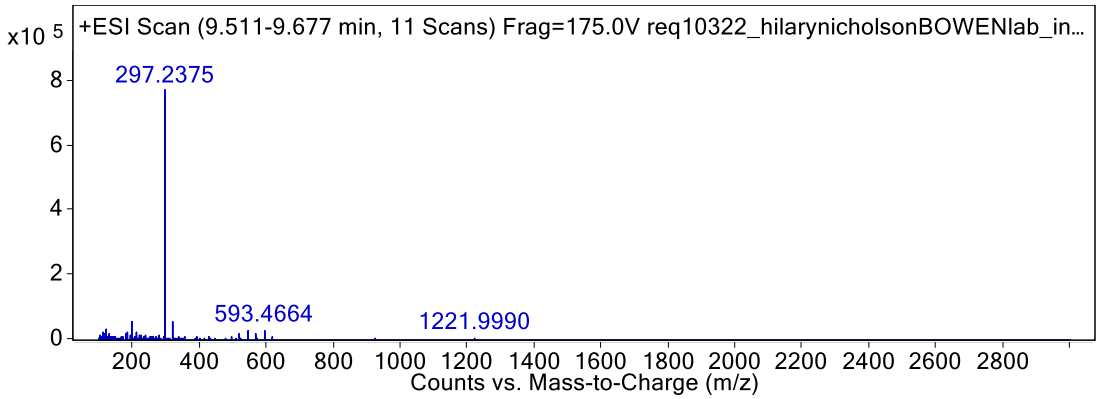
B) Incubation for 24 h in media at 37°C in 5% CO_2 humidified incubator, with cells present







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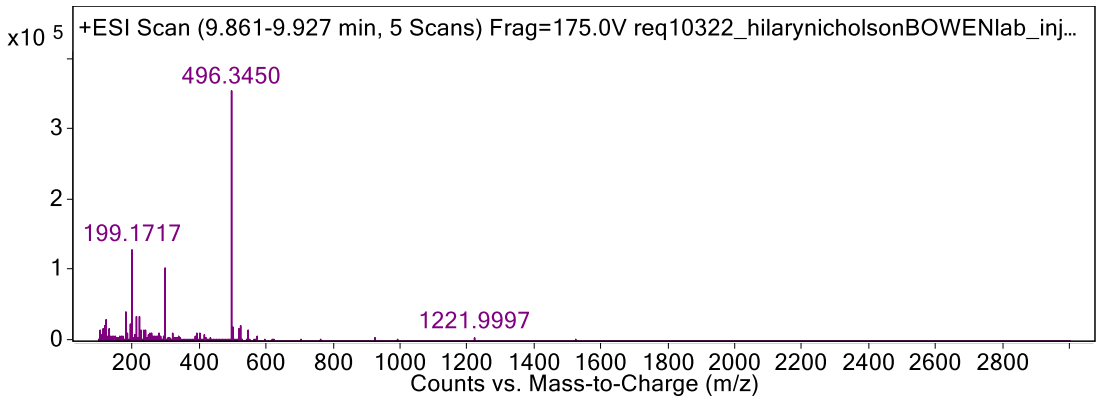
Counts vs. Mass-to-Charge (m/z)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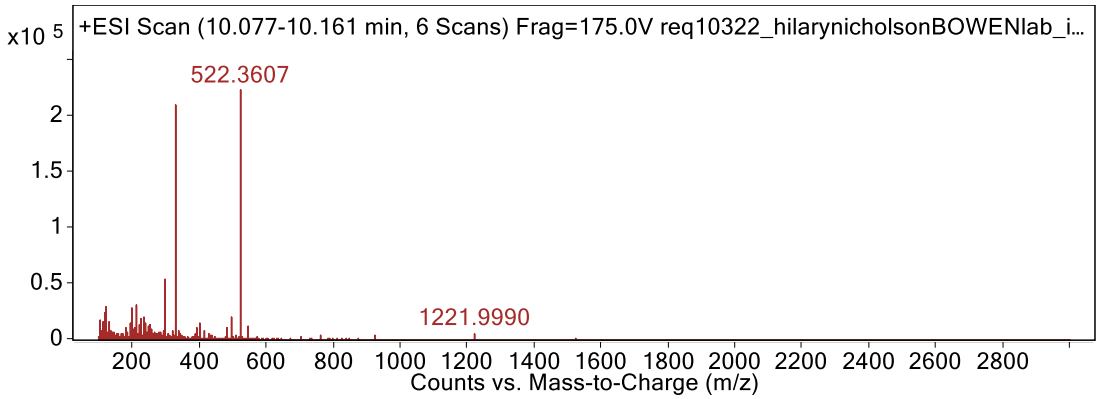
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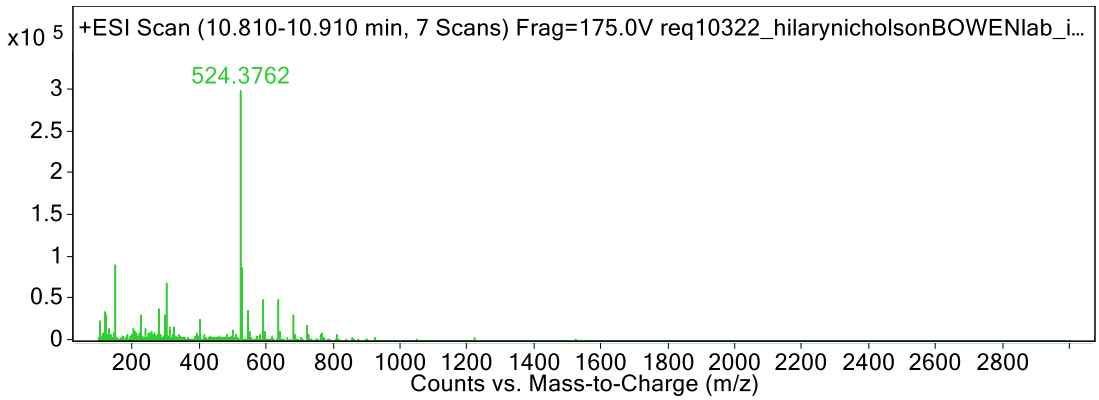
Counts vs. Mass-to-Charge (m/z)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x10 5
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Counts vs. Mass-to-Charge (m/z)

CM764 m/z = 427.21, RT = 6.41-6.53 min

The small peaks in the RT range 9.51 – 10.91 are not related to CM764 as they appear in extracted media that has not been exposed to CM764.

The small peak at RT=6.16-6.27, m/z=429.23 was not seen without cells (Supplemental Figure 2) and could be either be a component from the cells or a product related to CM764. The latter is unlikely since the increased mass by 2 units could only indicate reduction, yet the compound is slightly more polar than CM764.