**Analysis of Milk Thistle (*Silybum marianum*) Extract Using High Resolution UHPLC-MS and UHPLC-MS/MS**

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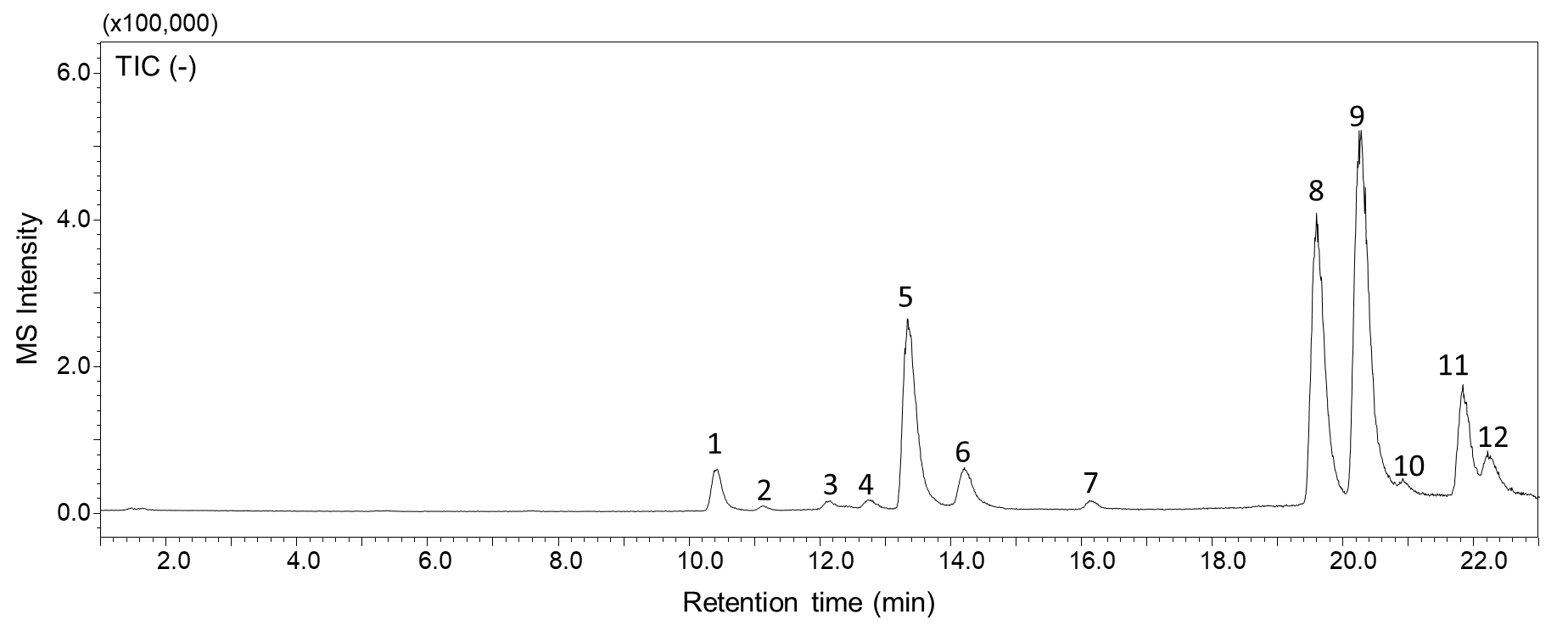
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Figure 1. Total ion chromatogram of milk thistle extract (10 µg/mL) analyzed on a Shimadzu 9030 UHPLC-Q-ToF mass spectrometer (resolving power 30,000) with negative ion electrospray. A 22-min gradient from 5% to 95% methanol containing formic acid (0.1%) in water (also containing 0.1% formic acid) was used for the UHPLC separation with a Waters Cortecs C18 (2.1 × 150 mm, 1.7 µm) column.

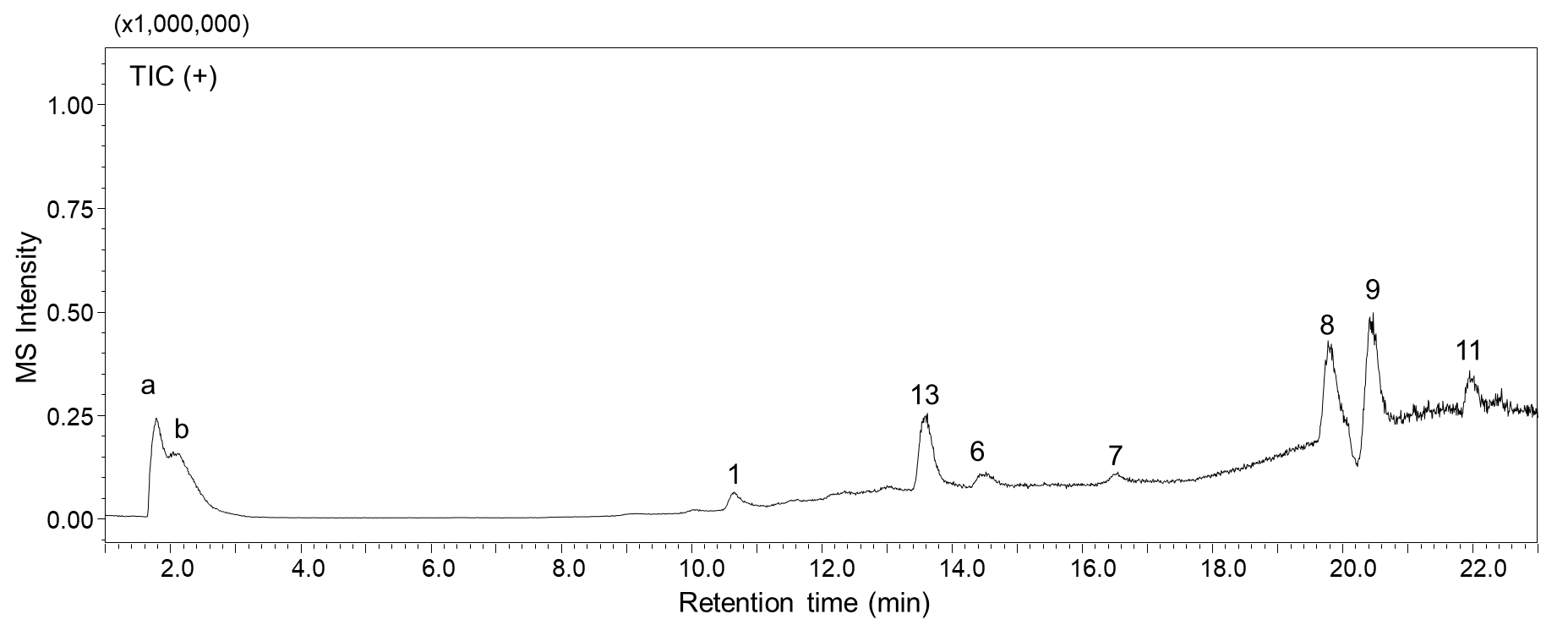
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Figure 2. Total ion chromatogram of milk thistle extract (10 µg/mL) analyzed on a Shimadzu 9030 UHPLC-Q-ToF mass spectrometer (rssolving power 30,000) with positive ion electrospray. A 22-min gradient from 5% to 95% methanol containing formic acid (0.1%) in water (also containing 0.1% formic acid) was used for the UHPLC separation with a Waters Cortecs C18 (2.1 × 150 mm, 1.7 µm) column.

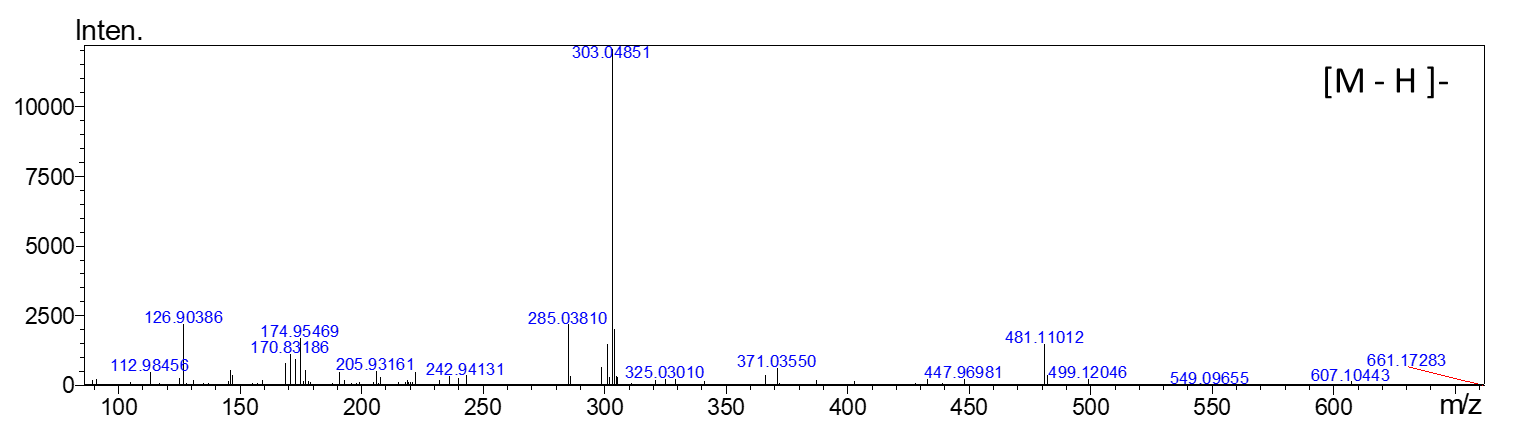
**Table 1.** Proposed and confirmed components corresponding to the peaks in the UHPLC-HRMS chromatograms in Figures 1 and 2 of milk thistle (*Silybum marianum*) extract.

| **Peak number** | **Retention time (min)** | ***m/z***  **(-) top**  **(+) (bottom)** | **ΔM (ppm)**  **(-) top**  **(+) bottom** | **Proposed ID**  **Molecular Formula**  **(CAS)**  **Confidence**3 | **Chemical**  **Structure** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- |
| 1 | 10.42 | 303.0485  305.0554 | 8.2  1.3 | Taxifolin  C15H12O7  24198-97-8  Reference standard |  | HRMS supports molecular formula.  MS/MS supports structure via reference standard.  Retention time matches that of reference standard |
| 2 | 11.14 | 303.0485  - | 8.2  - | Taxifolin isomer  C15H12O7  -  Tentative |  | HRMS supports molecular formula.  MS/MS supports structure through comparison of taxifolin major fragment ion of *m*/*z* 125 |
| 3 | 12.13 | 287.0538  - | 8.0  - | Taxifolin minus H2O  C15H12O6  -  Tentative |  | HRMS supports molecular formula of taxifolin minus a water molecule.  MS/MS supports structure through comparison of taxifolin major fragment ion of *m*/*z* 125 |
| 4 | 12.76 | 481.1101  - | 8.1  - | Neusilychristin  C25H22O10  -  Tentative |  | The peak elution of silybin isomers are usually close and among silychristin isomers reported in literature, neusilychristin is suggested as the peak eluting before silychristin A. The MS/MS data match that of silychristin. Neuchristin was reported in the literature [1]. |
| 5 | 13.35 | 481.1102  - | 7.9  - | Silychristin  C25H22O10  33889-69-9  Reference standard |  | HRMS supports molecular formula.  MS/MS supports structure via reference standard.  Retention time matches that of reference standard |
| 6 | 14.20 | 481.1101  - | 8.1  - | Silydianin  C25H22O10  29782-68-1  Reference standard |  | HRMS supports molecular formula.  MS/MS supports structure via reference standard.  Retention time matches that of reference standard |
| 7 | 16.15 | 257.0735  259.0899 | 8.5  1.2 | D4-Daidzein internal standard  C15H6D4O4  486-66-8  Reference standard |  | HRMS supports molecular formula.  MS/MS supports structure via reference standard.  Retention time matches that of reference standard |
| 8 | 19.60 | 481.1102  483.1283 | 7.9  0.41 | Silybin A  C25H22O10  22888-70-6  Reference standard |  | HRMS supports molecular formula.  MS/MS supports structure via reference standard.  Retention time matches that of reference standard |
| 9 | 20.27 | 481.1103  483.1284 | 7.7  0.2 | Silybin B  C25H22O10  142797-34-0  Reference standard |  | HRMS supports molecular formula.  MS/MS supports structure via reference standard.  Retention time matches that of reference standard. |
| 10 | 20.93 | 481.1102  - | 7.9  - | A silymarin isomer, likely 2,3-*cis*-silybin A  C25H22O10  --  Tentative |  | The peak elution of silybin isomers are usually close. Based on structural similarity, the suggested isomer is 2,3-cis-silybin A, which is reported in the literature [1]. The HRMS data support a silybin isomer and the MS/MS matches that of other silybins. |
| 11 | 21.85 | 481.1103  483.1254 | 7.7  6.4 | Isosilybin A  C25H22O10  142796-21-2  Reference standard |  | HRMS supports molecular formula.  MS/MS supports structure via reference standard.  Retention time matches that of reference standard |
| 12 | 22.26 | 481.1102  - | 7.9  - | Isosilybin B  C25H22O10  142796-22-3  Reference standard |  | HRMS supports molecular formula.  MS/MS supports structure via reference standard.  Retention time matches that of reference standard |
| 13 | 13.59 | -  453.1176 | -  0.88 | In source fragment of silychristin  C24H20O9  -  Tentative |  | Common fragment ion for silybins and isosilybins. The retention time suggest an insource fragment ion of silychristin. Fragmentation data of silychristin match literature data [2]. |
| 14  Shoulder of peak 6 | 14.51 | -  505.1101 | -  0.79 | Na+ adduct of silydianin  C25H22NaO10+  -  Tentative |  | Retention time and accurate mass strongly support a sodium adduct of silydianin. |

[1] Csupor D, Csorba A, Hohmann J. Recent advances in the analysis of flavonolignans of *Silybum* *marianum*. *J. Pharmaceut. Biomed. Anal.* **2016**; *130*: 301.

[2] Lee JI, Hsu BH, Wu D, Barrett JS. Separation and characterization of silybin, isosilybin, silydianin and silychristin in milk thistle extract by liquid chromatography–electrospray tandem mass spectrometry. *J. Chromatogr. A* **2006**; *1116*: 57.

Appendix



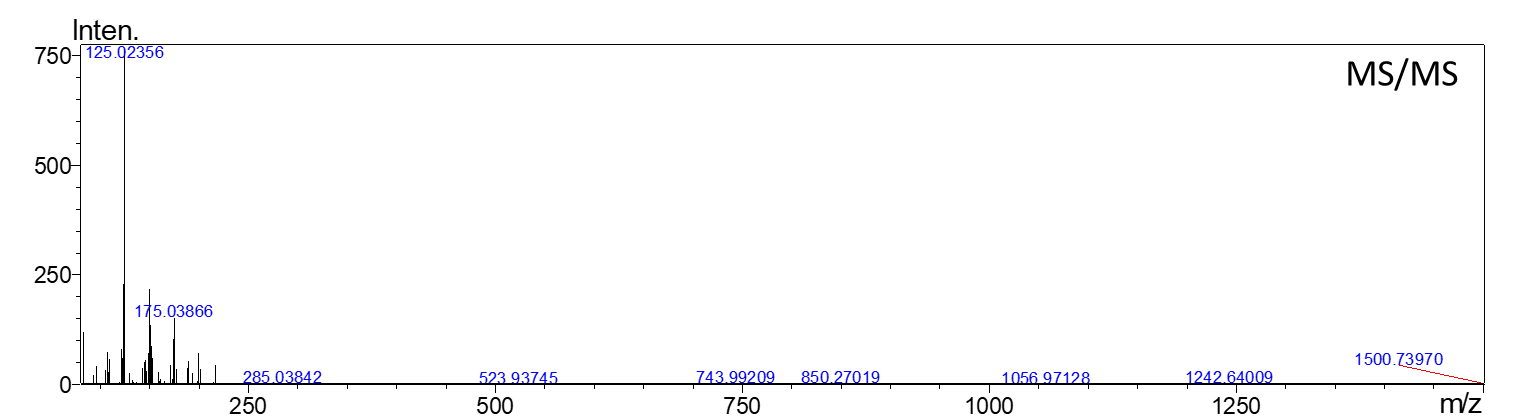
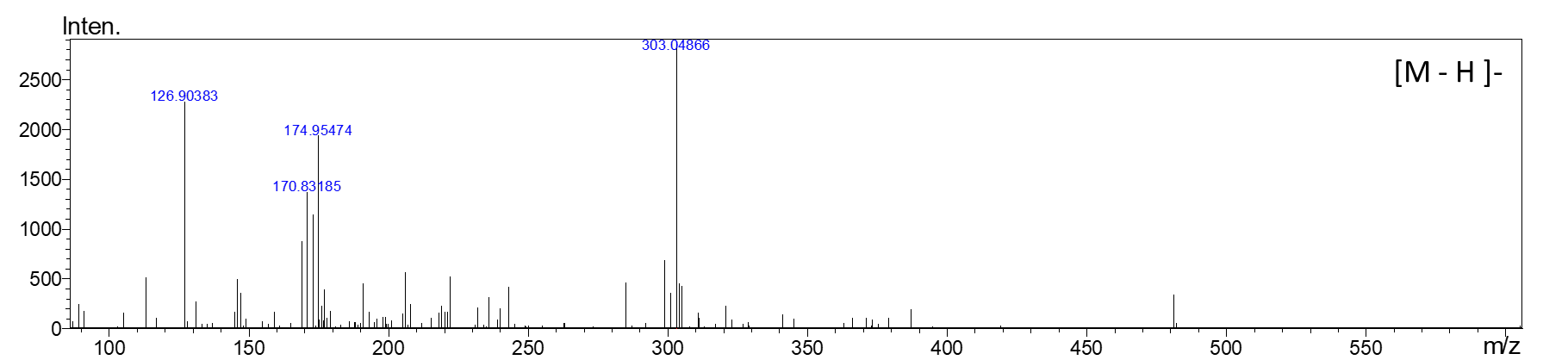


Figure 3. Negative ion electrospray HRMS (top panel) and MS/MS of peak 1 with *m*/*z* 303.04854 eluting at 10.42 min



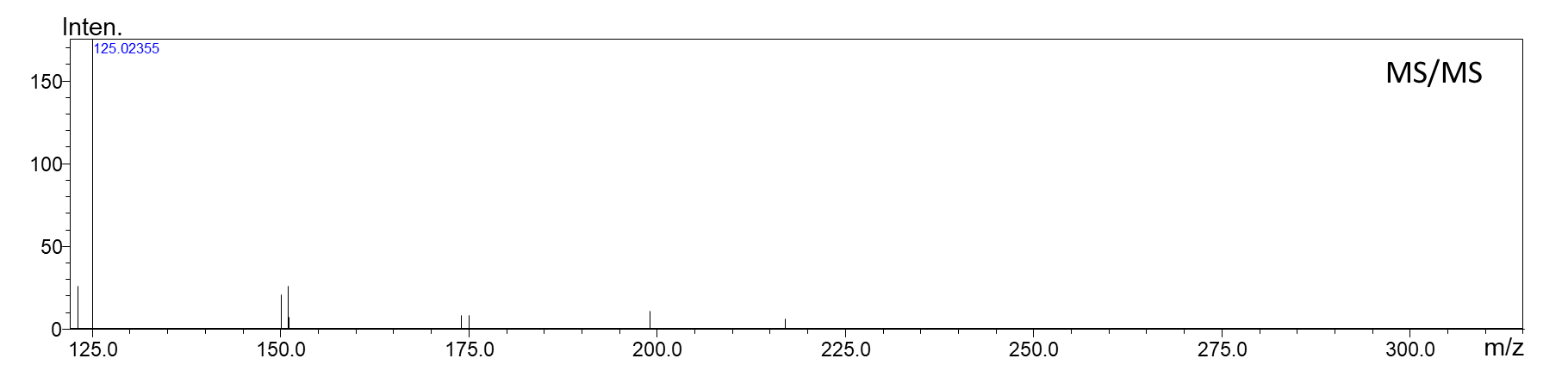


Figure 4. Negative ion electrospray HRMS (top panel) and MS/MS of peak 2 with *m*/*z* 303.04854 eluting at 11.14 min

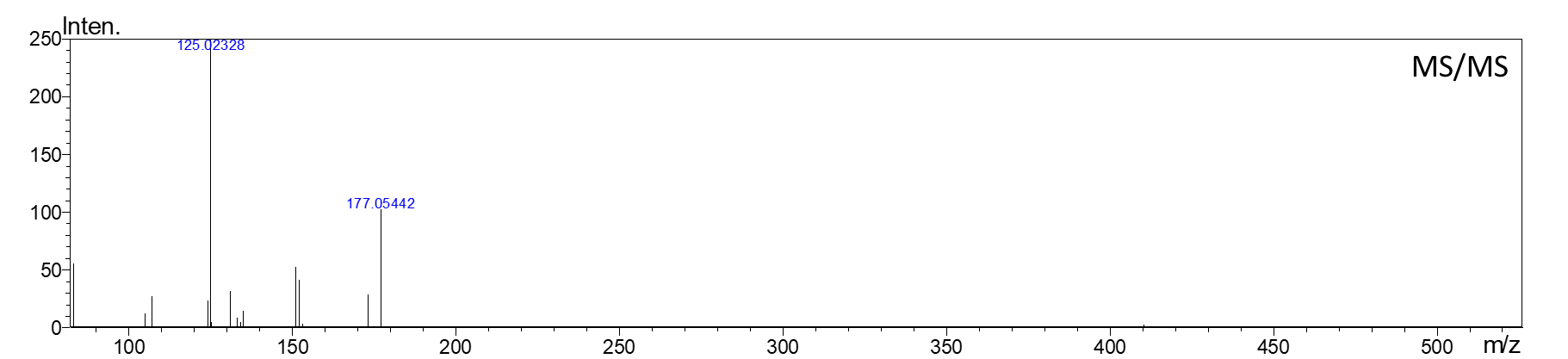
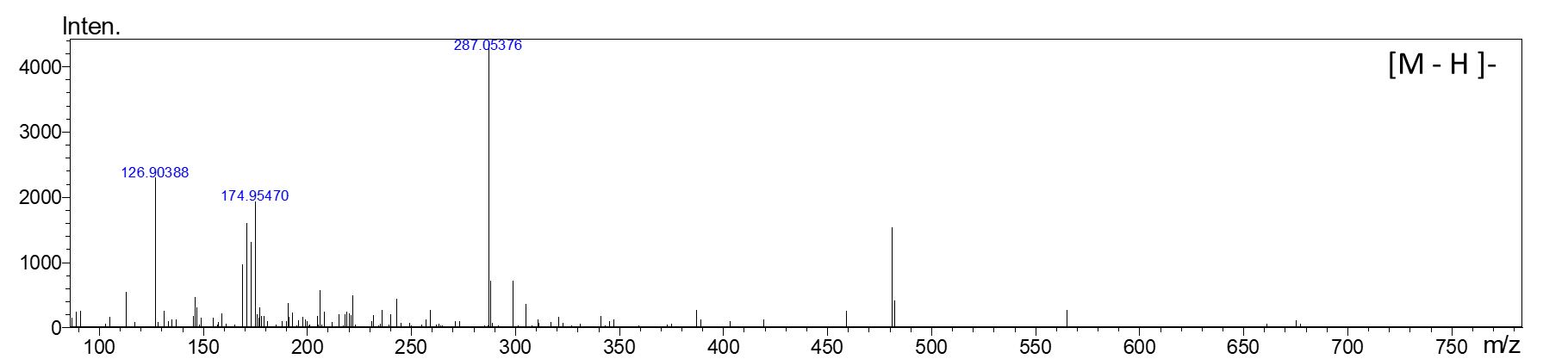
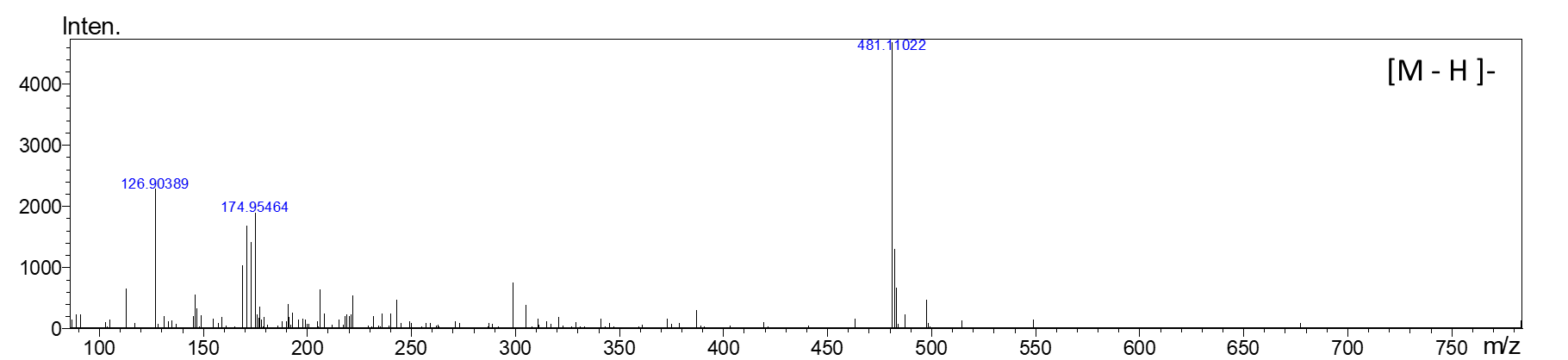


Figure 5. Negative ion electrospray HRMS (top panel) and MS/MS of peak 3 with *m*/*z* 287.0538 eluting at 12.13 min



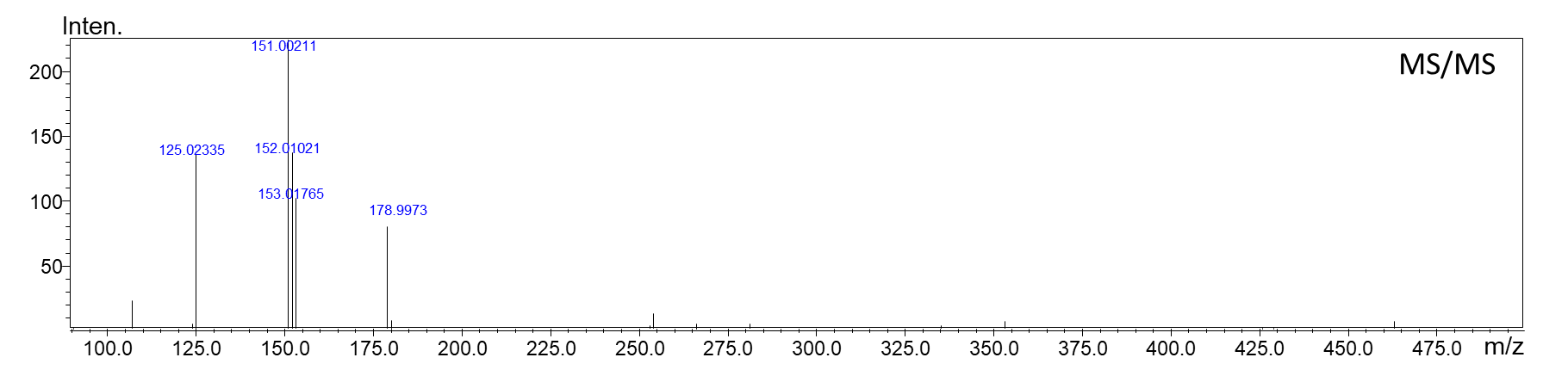


Figure 6. Negative ion electrospray HRMS (top panel) and MS/MS of peak 4 with *m*/*z* 481.1101 eluting at 12.76 min

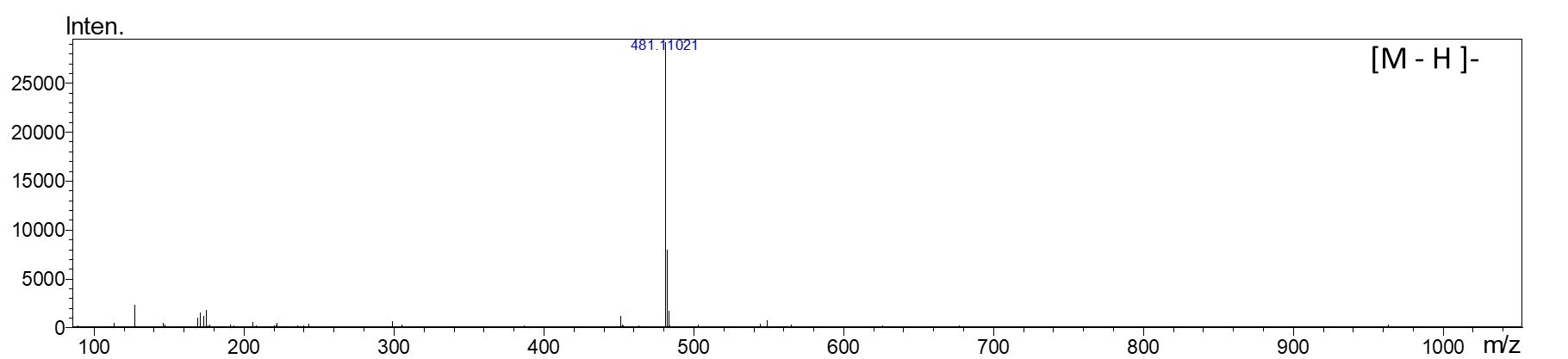
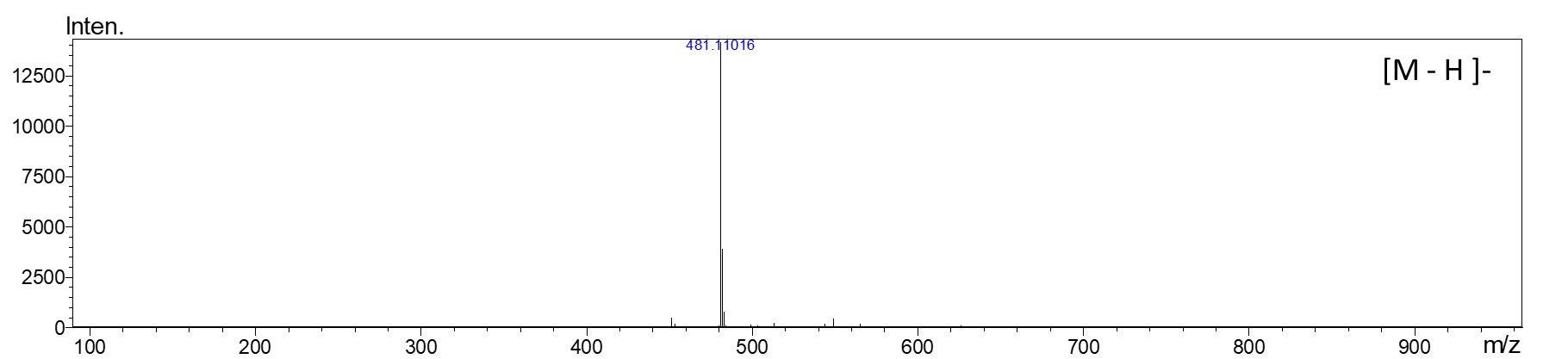




Figure 7. Negative ion electrospray HRMS (top panel) and MS/MS of peak 5 with *m*/*z* 481.1102 eluting at 13.35 min



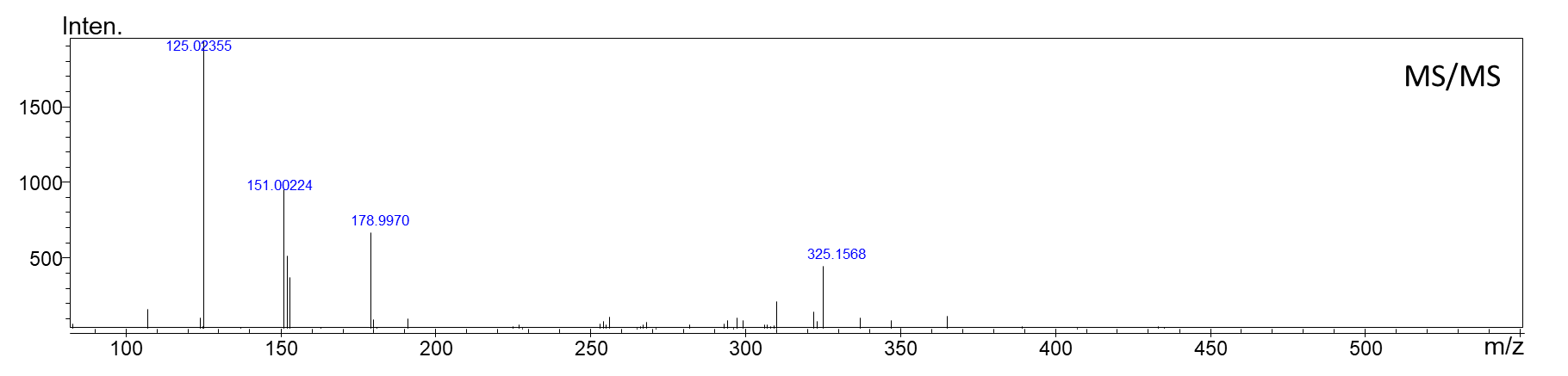
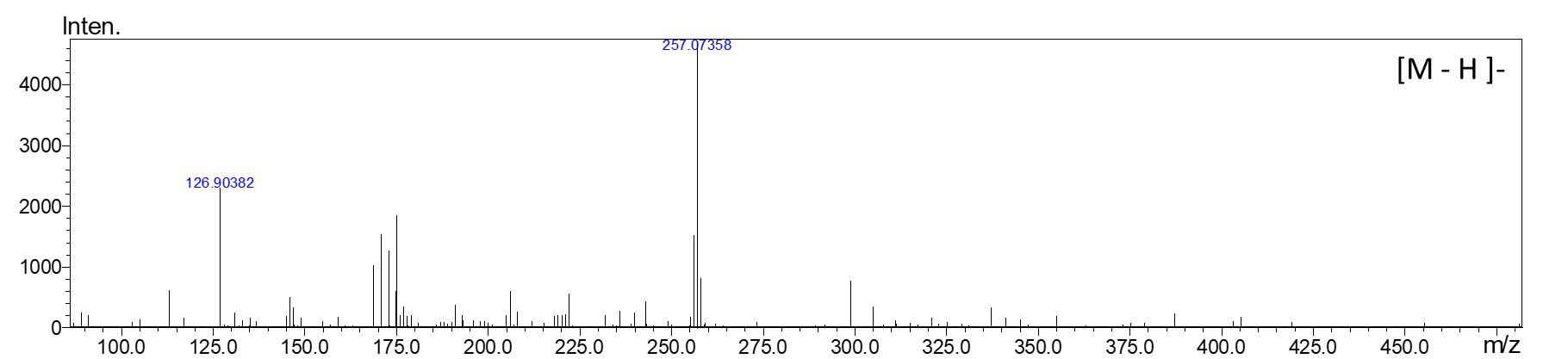


Figure 8. Negative ion electrospray HRMS (top panel) and MS/MS of peak 6 with *m*/*z* 481.1101 eluting at 14.20 min



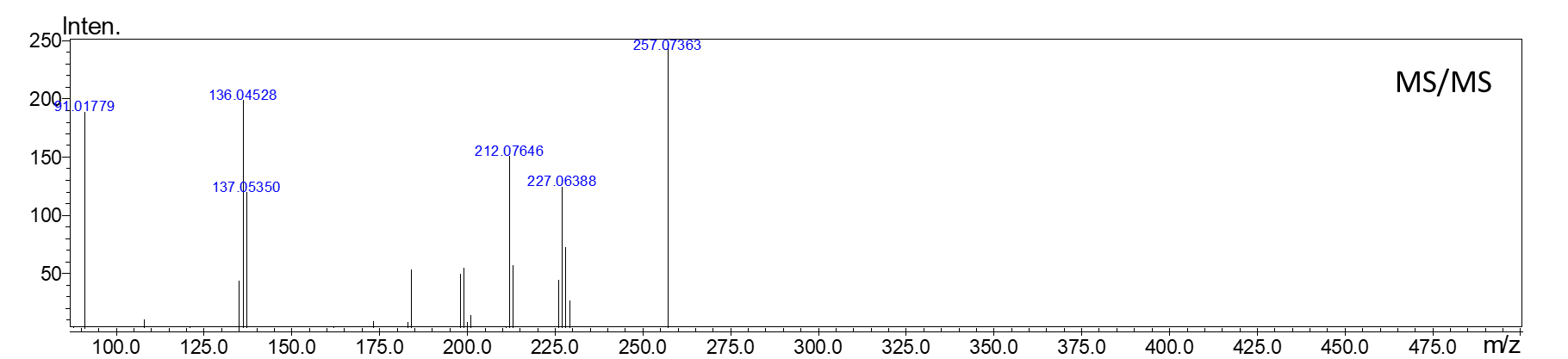
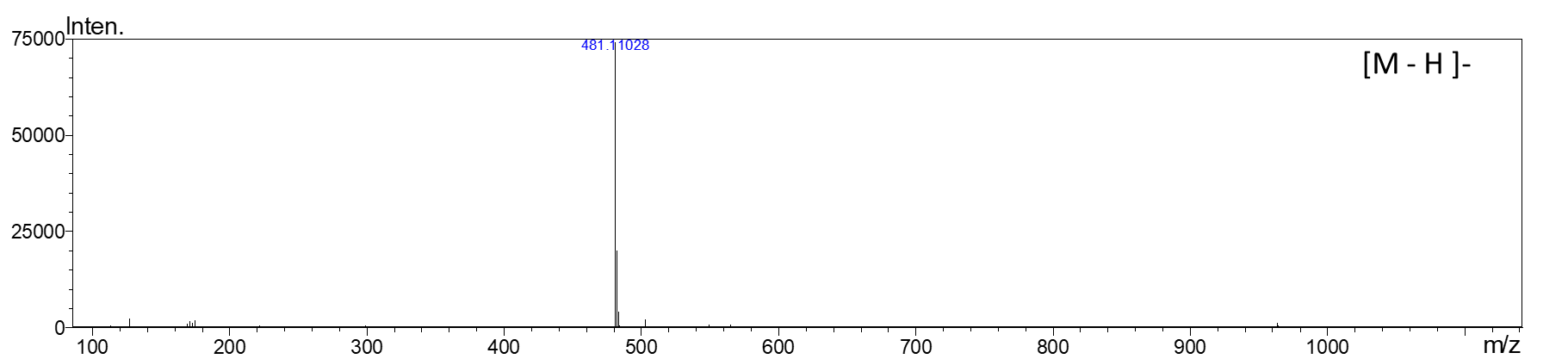


Figure 9. Negative ion electrospray HRMS (top panel) and MS/MS of peak 7 with *m*/*z* 257.0735 eluting at 16.15 min



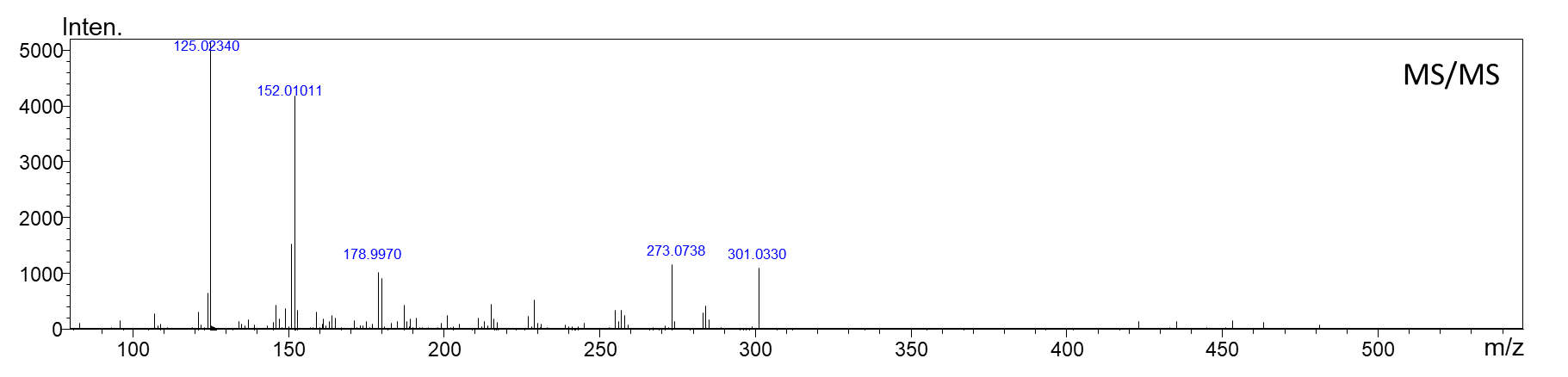
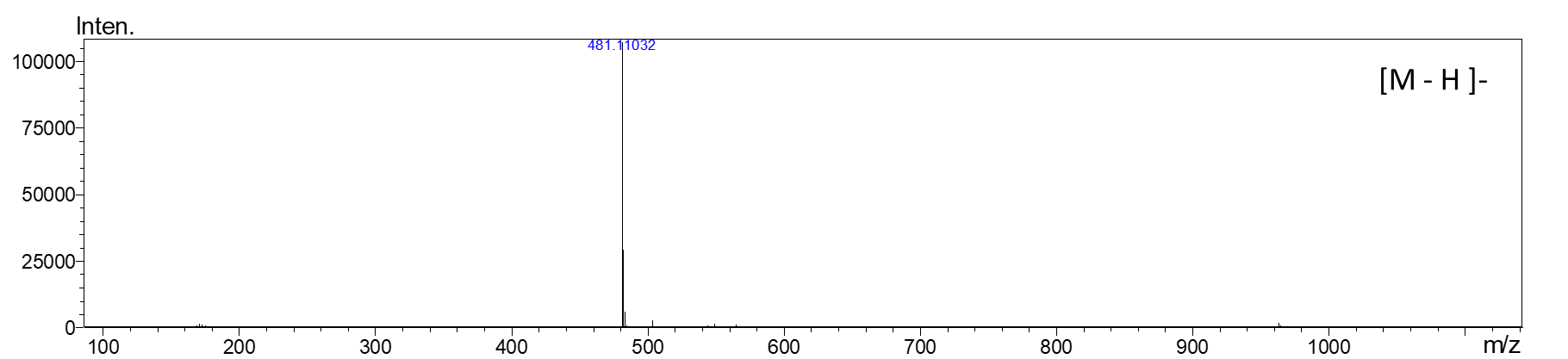


Figure 10. Negative ion electrospray HRMS (top panel) and MS/MS of peak 8 with *m*/*z* 481.1102 eluting at 19.60 min



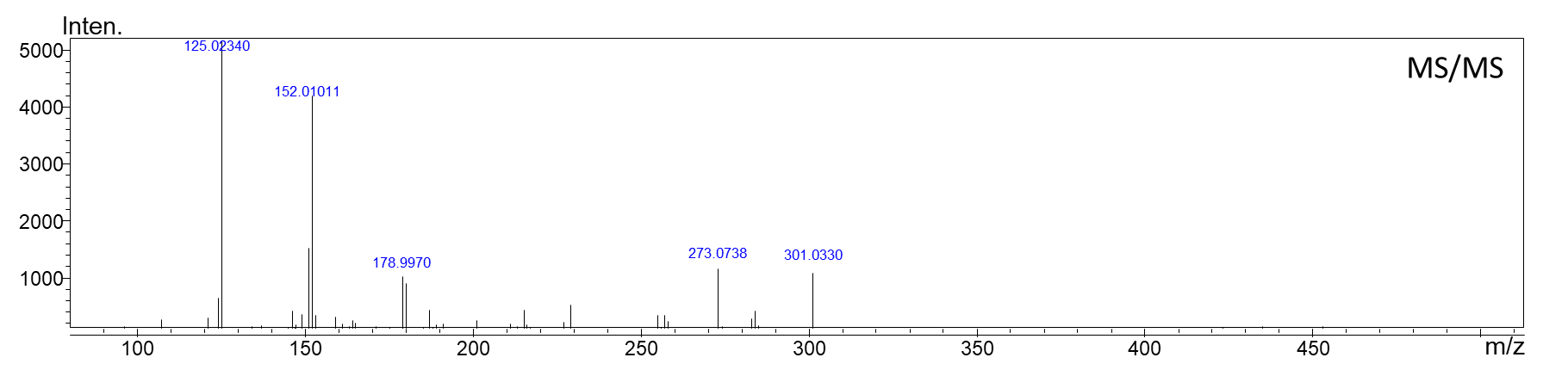
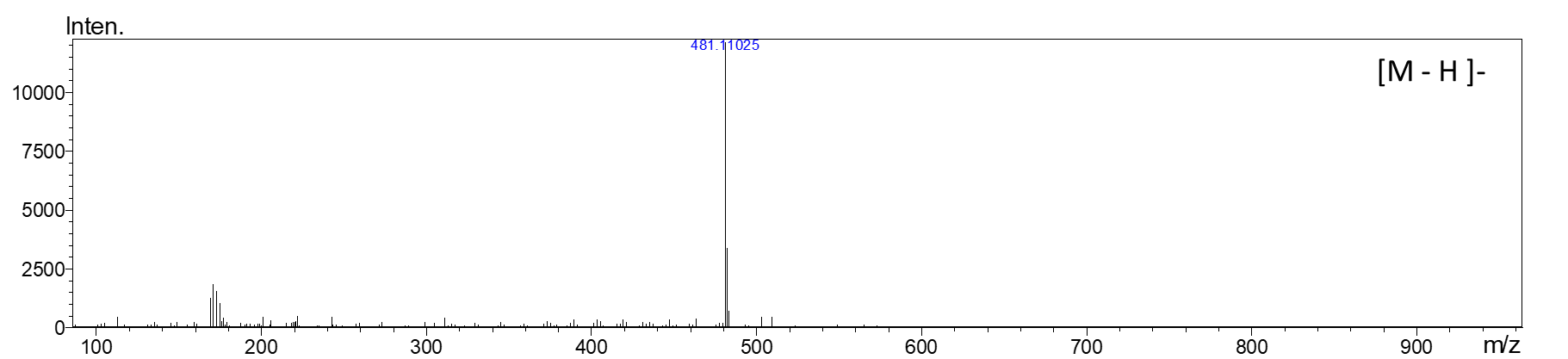


Figure 11. Negative ion electrospray HRMS (top panel) and MS/MS of peak 9 with *m*/*z* 481.1103 eluting at 20.27 min



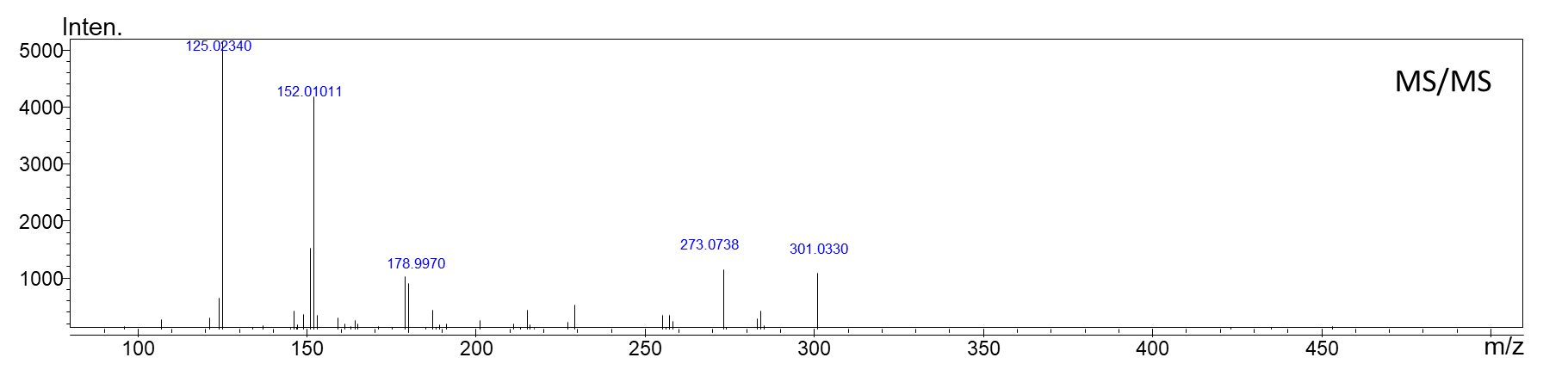
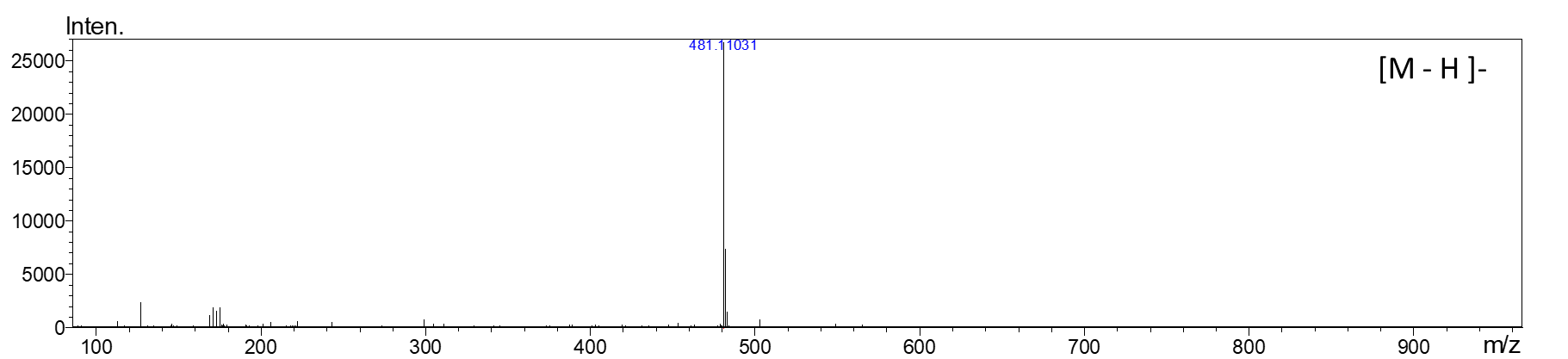


Figure 12. Negative ion electrospray HRMS (top panel) and MS/MS of peak 10 with *m*/*z* 481.1102 eluting at 20.93 min



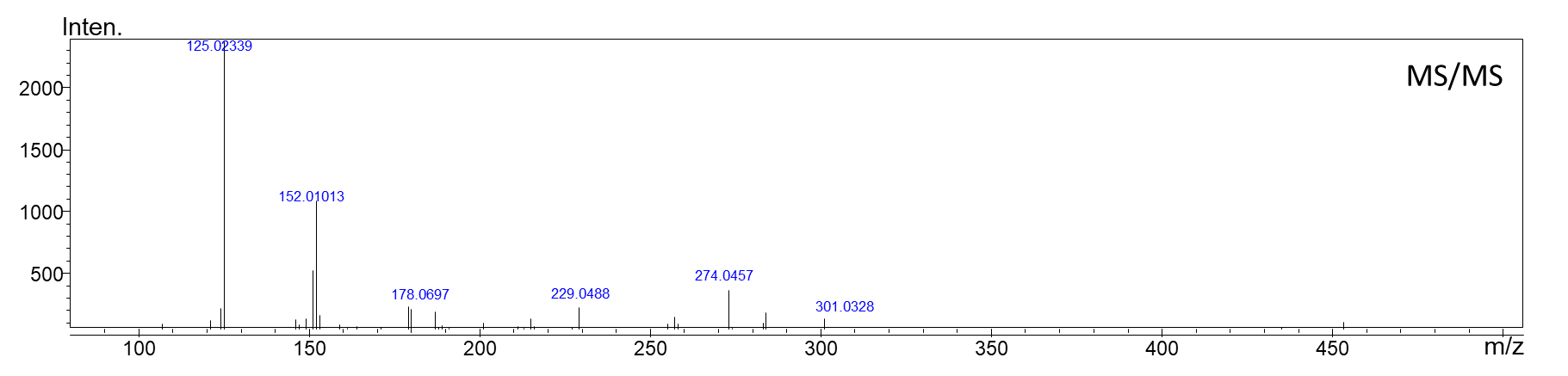
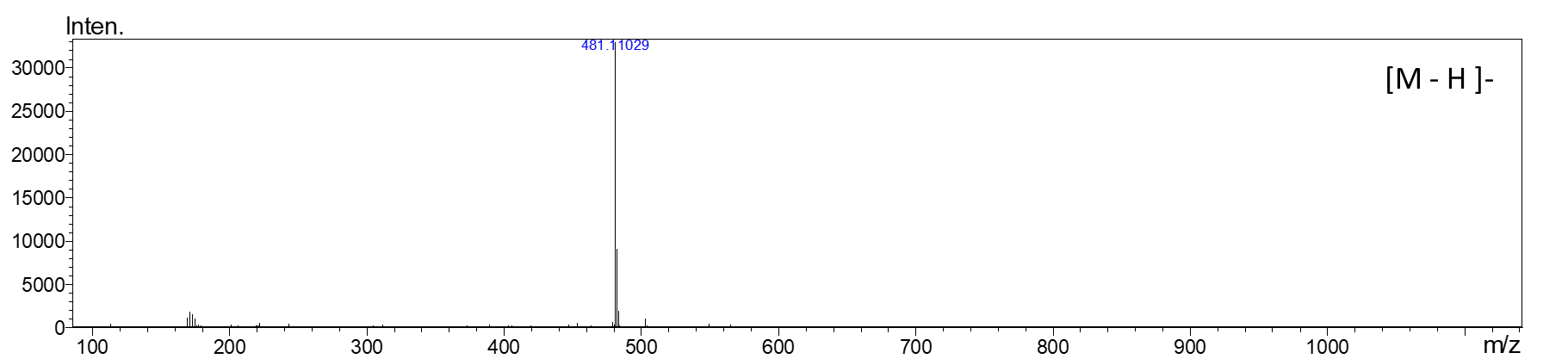


Figure 13. Negative ion electrospray HRMS (top panel) and MS/MS of peak 11 with *m*/*z* 481.1103 eluting at 21.85 min



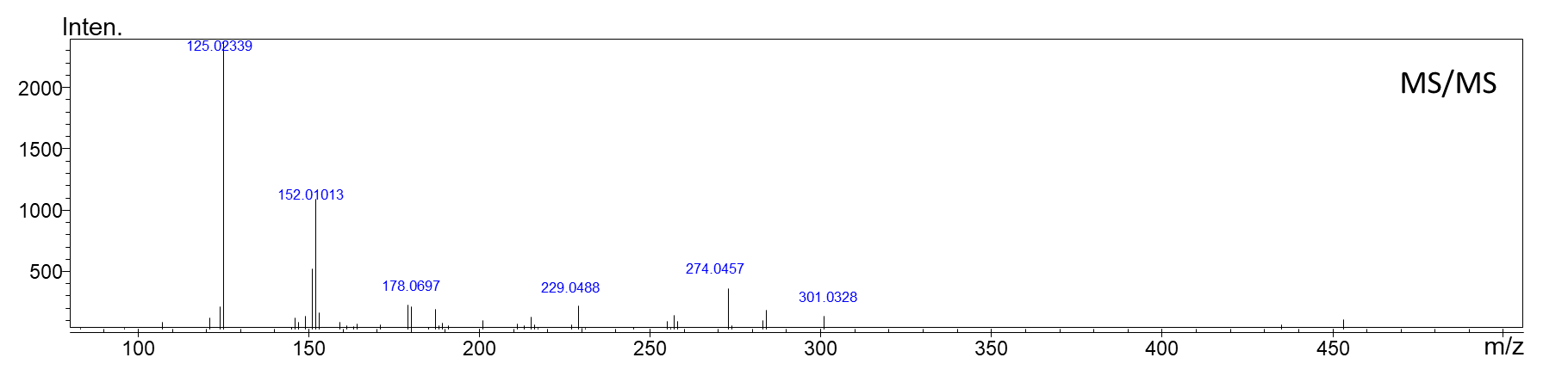
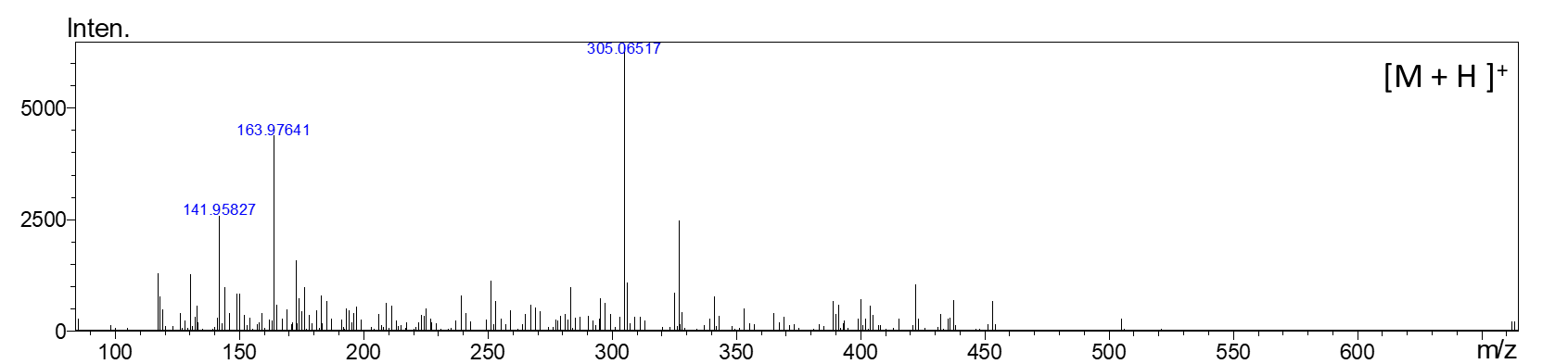


Figure 14. Negative ion electrospray HRMS (top panel) and MS/MS of peak 12 with *m*/*z* 481.1102 eluting at 22.26 min



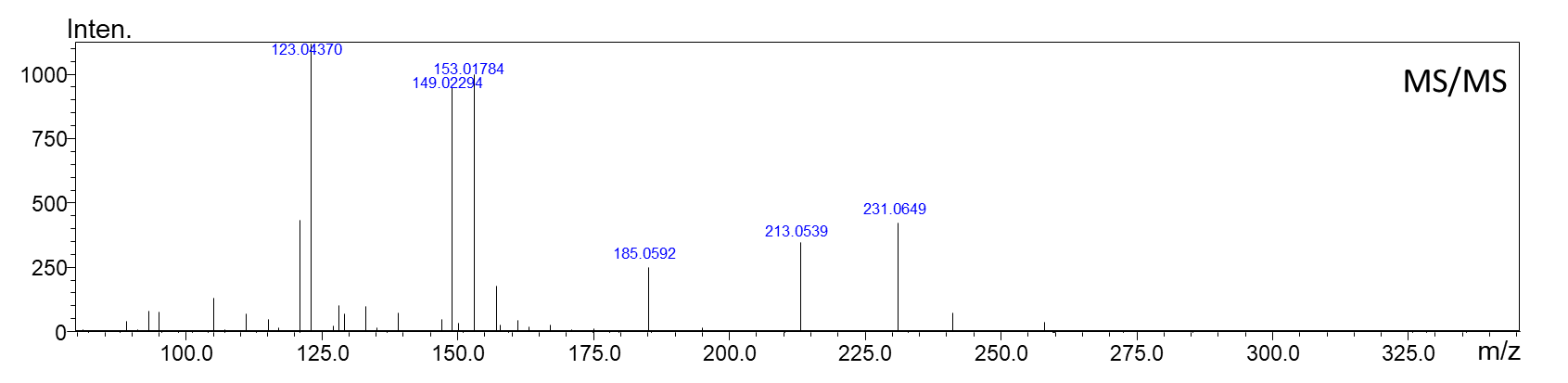
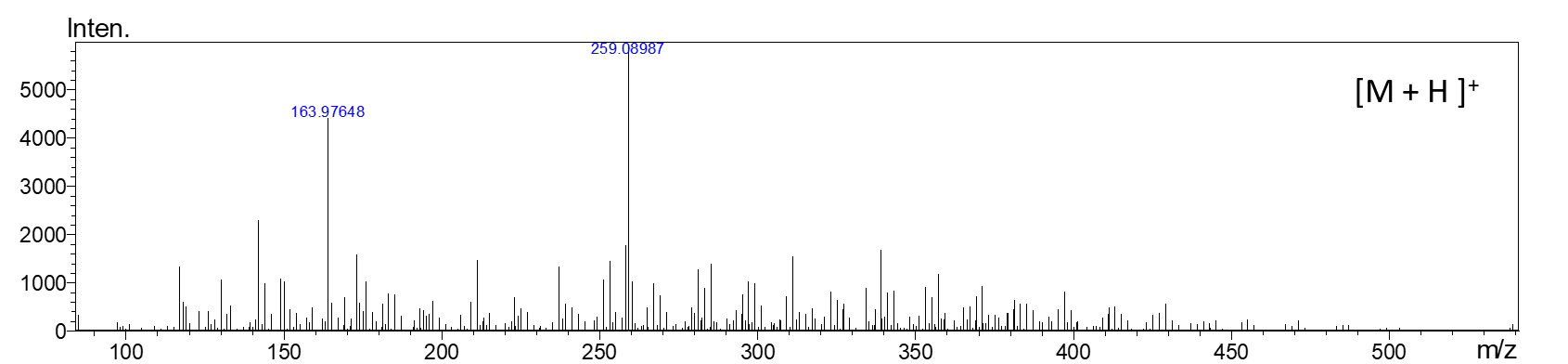


Figure 15. Positive ion electrospray HRMS (top panel) and MS/MS of peak 1 with *m*/*z* 305.0651 eluting at 10.64 min



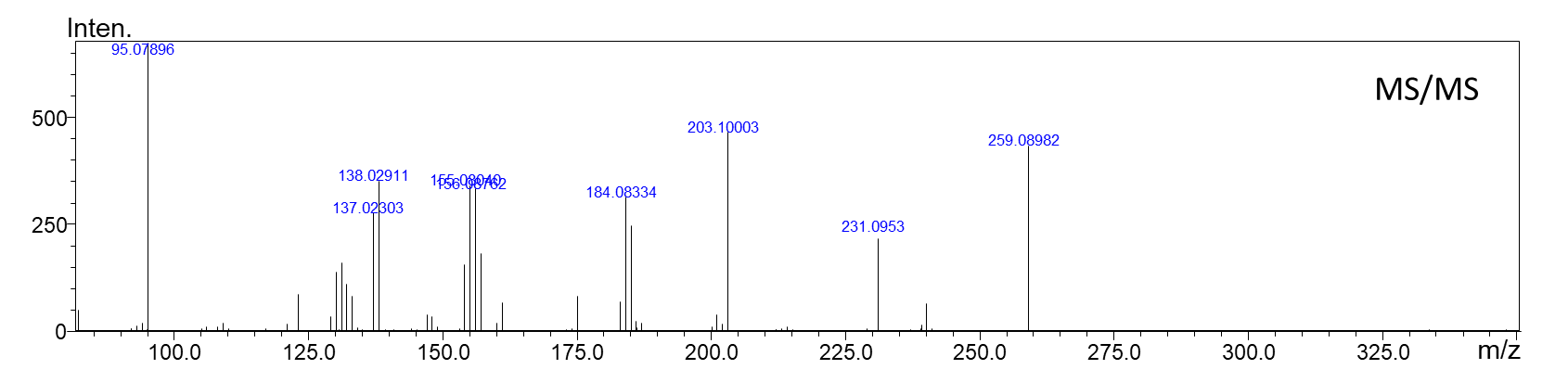
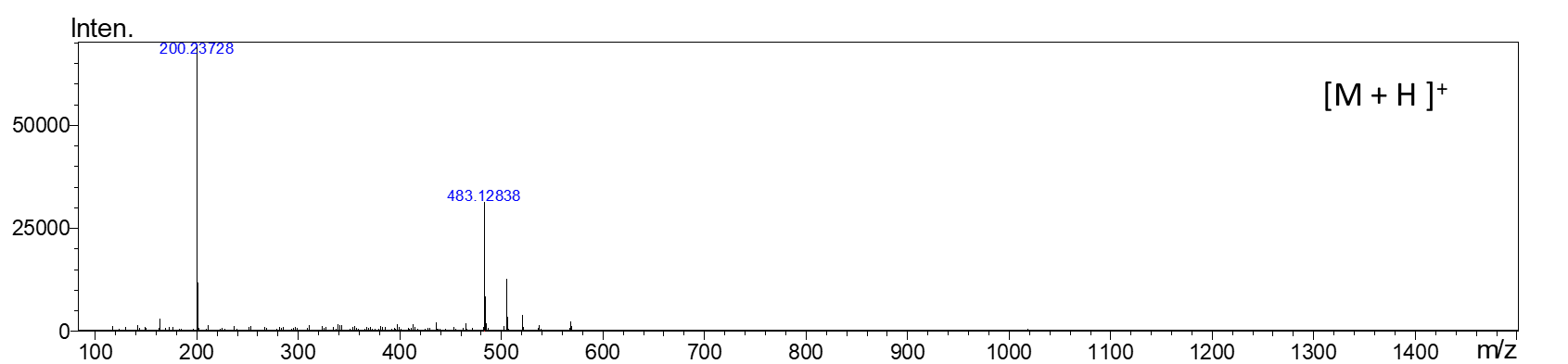


Figure 16. Positive ion electrospray HRMS (top panel) and MS/MS of peak 7 with *m*/*z* 259.0899 eluting at 16.51 min



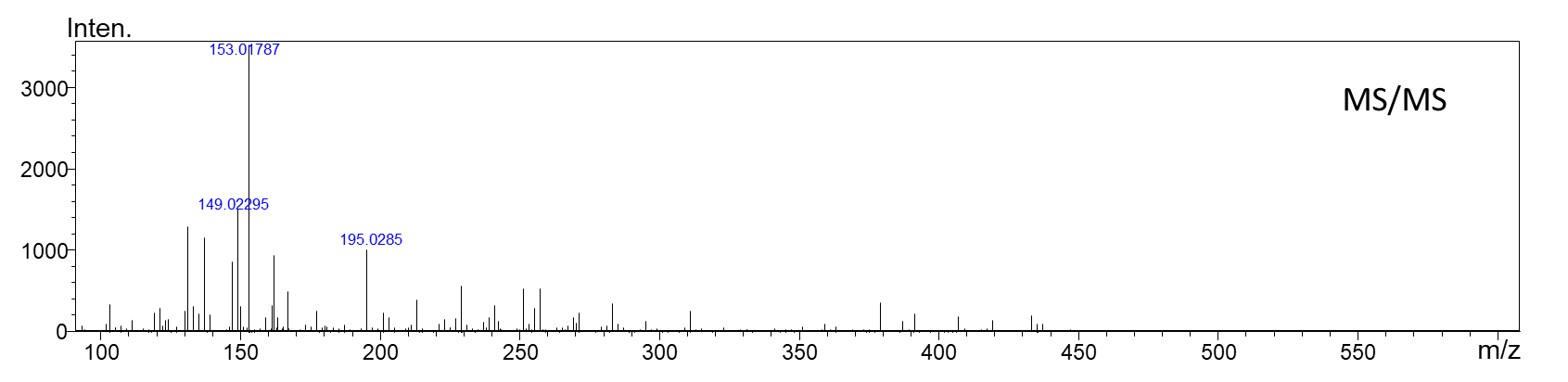
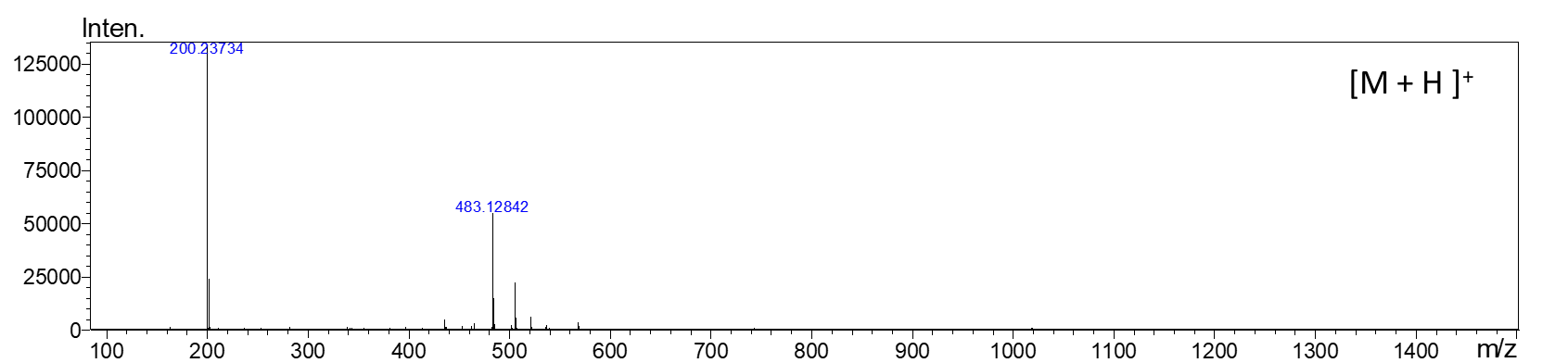


Figure 17. Positive ion electrospray HRMS (top panel) and MS/MS of peak 8 with *m*/*z* 483.1283 eluting at 19.80 min



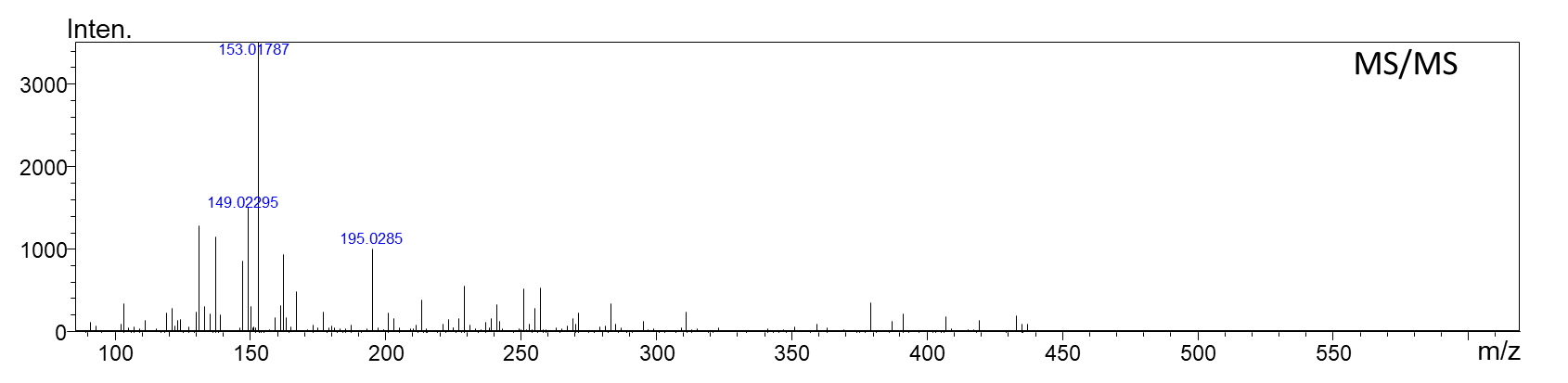
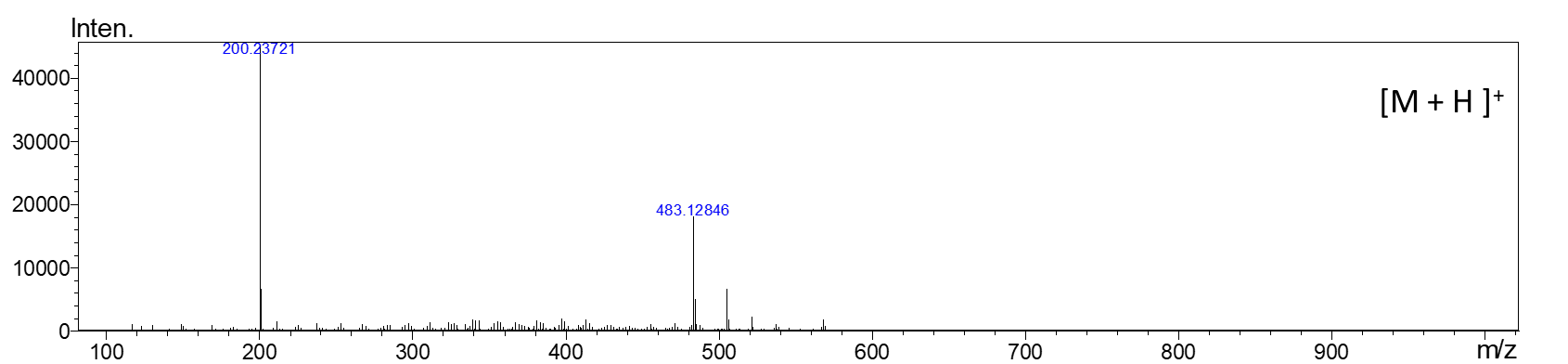


Figure 18. Positive ion electrospray HRMS (top panel) and MS/MS of peak 9 with *m*/*z* 483.1284 eluting at 20.43 min



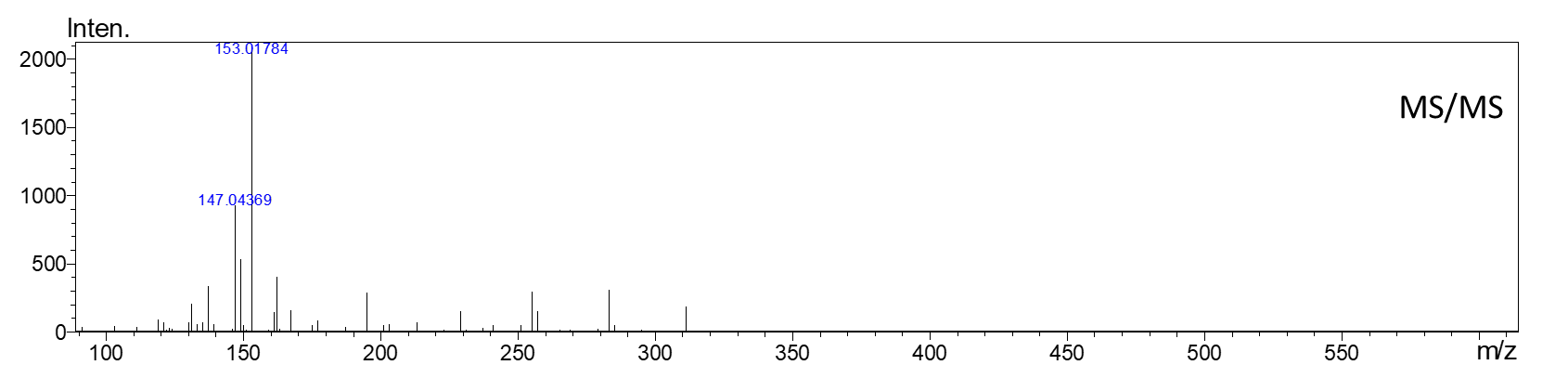
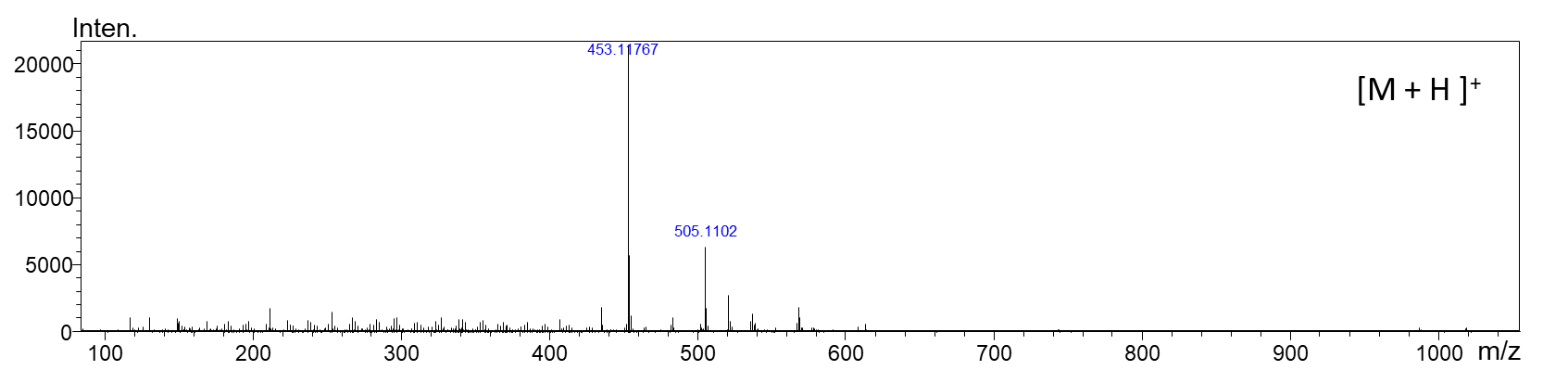


Figure 19. Positive ion electrospray HRMS (top panel) and MS/MS of peak 11 with *m*/*z* 483.1254 eluting at 21.99 min



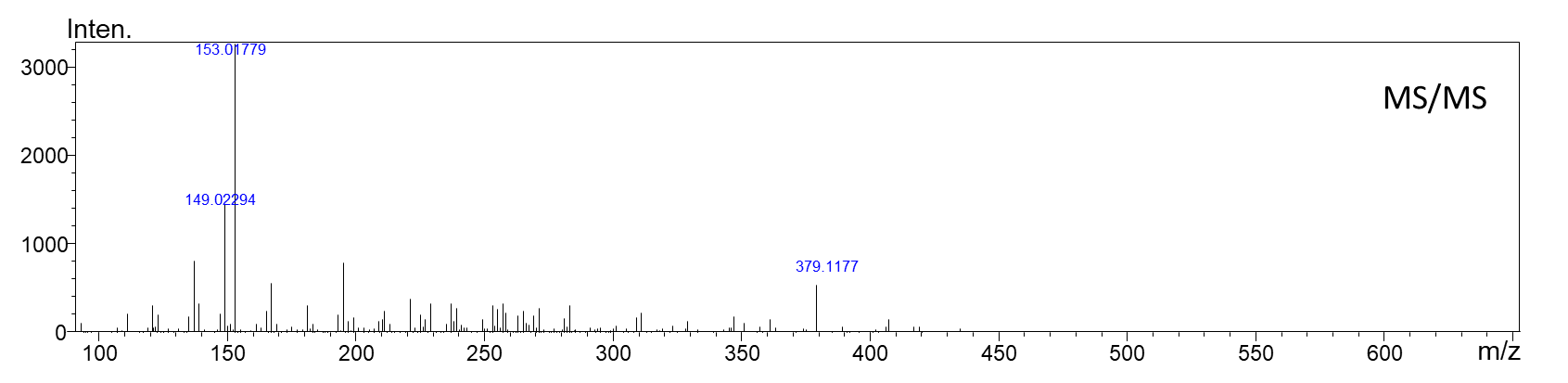
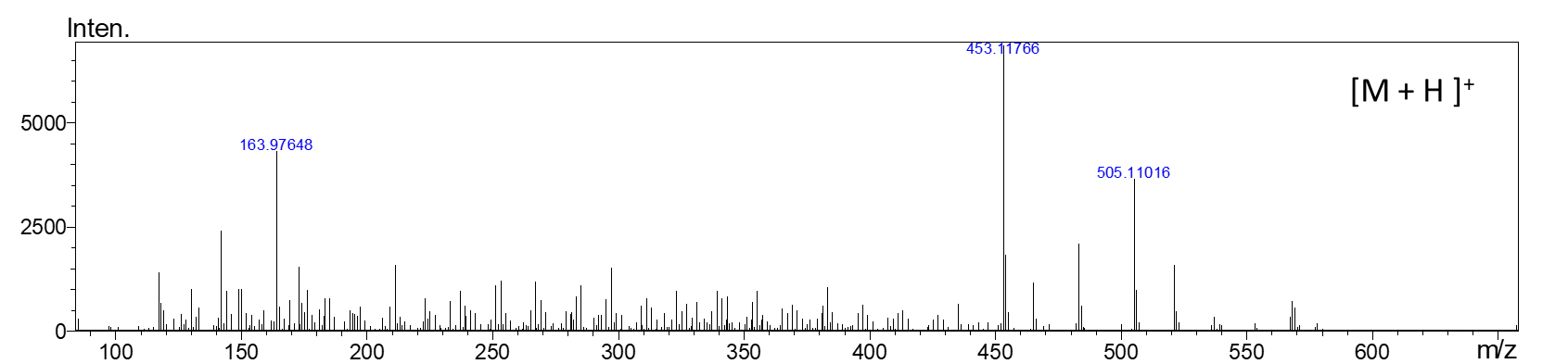


Figure 20. Positive ion electrospray HRMS (top panel) and MS/MS of peak 13 with *m*/*z* 453.1176 eluting at 13.59 min



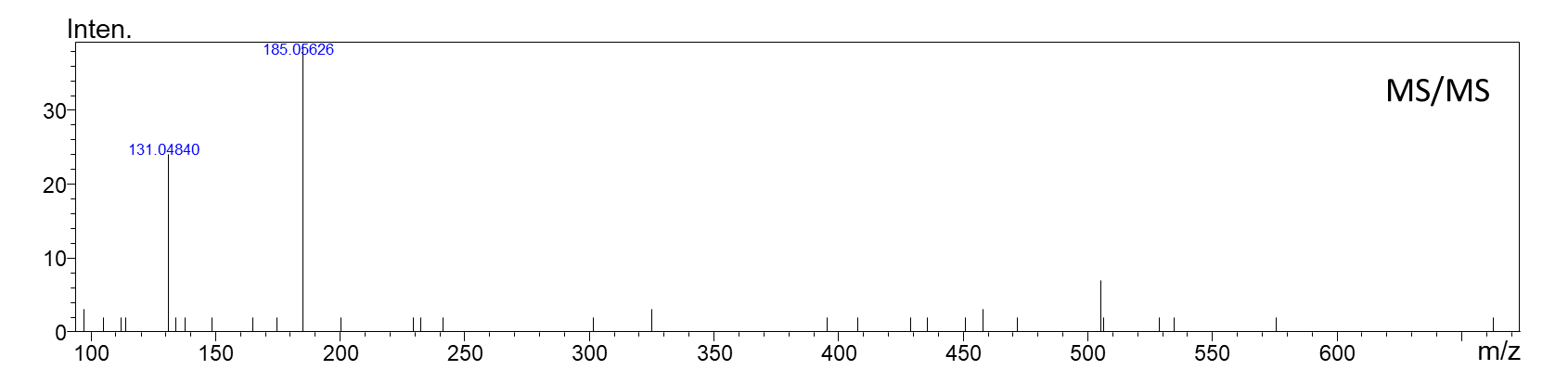


Figure 21. Positive ion electrospray HRMS (top panel) and MS/MS of peak 14 with *m*/*z* 505.1101 eluting at 14.51 min