SHIMADZU
Excellence in Science

Application News

SSI-LCMS-010

Liquid Chromatography Mass Spectrometry

Designer Cannabinoids

LCMS-8030

Summary

A rapid LC-MS-MS method for determination of designer cannabinoids in smokeable herbs was developed.

Background

Designer cannabinoids are synthetic compounds designed to mimic the effects of cannabis. Lawmakers have banned many of these substances, however drug designers can create new analogues as quickly as the old ones are banned. These analogues usually contain only minor modifications that do not affect activity yet render the substance undetectable in routine MRM-based LC-MS-MS assays.

Ultrafast precursor ion or neutral loss scanning on the Shimadzu LCMS-8030 offers a unique way of rapidly detecting and characterizing new designer cannabinoids.

Method

Authentic standards of a variety of synthetic cannabinoids were obtained. Standards were diluted for MRM optimization. A K2 Spice product marketed as "ban-compliant" was purchased from a local gas station.

A Restek Ultra Biphenyl (5 μm, 2.1 x 50 mm) column was used with a binary gradient of 0.1% formic acid (Pump A) and 0.1% formic acid in acetonitrile (Pump B). The linear gradient program started at 5% B and increased to 95% B over 10 min, followed by a 2 minute equilibration. The flow rate was 0.5 mL/min and the column oven was maintained at 40 °C.

Electrospray ionization was used in positive mode. The DL temperature was 250 °C, the Nebulizing gas was 2 L/min, the Heater Block temperature was 400 °C, and the drying gas was 15 L/min.

MS methods were used to search for both known and unknown designer cannabinoids. For known designer cannabinoids, Multiple Reaction Monitoring (MRM) of the transitions for each compound was used. MRM optimization using an automated wizard was performed to determine the highest intensity product ions as well as the optimum ion optics voltages and collision energies.

To search for unknown cannabinoids, precursor ion scanning was used. Analogues of the napthoyl-indole cannabinoids share one or more common fragment ions of *m/z* 155, 127, and 144. Precursor ion scans corresponding to these fragments were added to the MS method at a scan speed of 5,000 u/sec. In addition, data-dependent MS-MS at a scan speed of 15,000 u/sec was used to collect full product ion spectra for each precursor detected in the precursor ion scan. This information was used for library searching and to characterize unknown compounds as designer cannabinoid analogues.

Samples of K2 Spice product were divided into 100 mg portions, then mixed with 1 mL methanol, followed by vortexing and sonication. The sample was filtered and then diluted 100-fold in 50:50 water:methanol. The injection volume was 1 µL.

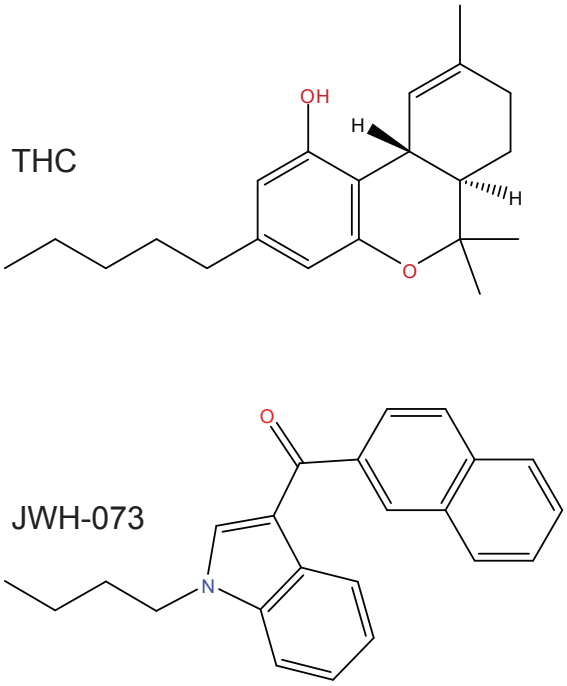


Figure 1: Structures of THC, the active component in marijuana, and JWH-073, a designer cannabinoid

Figure 2: Typical herbal incense product

Results and Discussion

Product ion scans of several representative cannabinoid standards are shown in **Figure 4**. Common product ions were observed in these and other designer cannabinoid analogues. As shown in the figure, the fragments of *m/z* 155 and 127 arise from cleavage on either side of the carbonyl linking the napt halene group from the indole group. The fragment of *m/z* 144 likely results from an intra-molecular cyclization and elimination, involving the indole group and the alkyl side chain.

Designer cannabinoids of the napthoyl-indole type can be synthesized with one or more modifications to the napthalene group, the indole group, or to the N-alkyl chain. These modifications can include

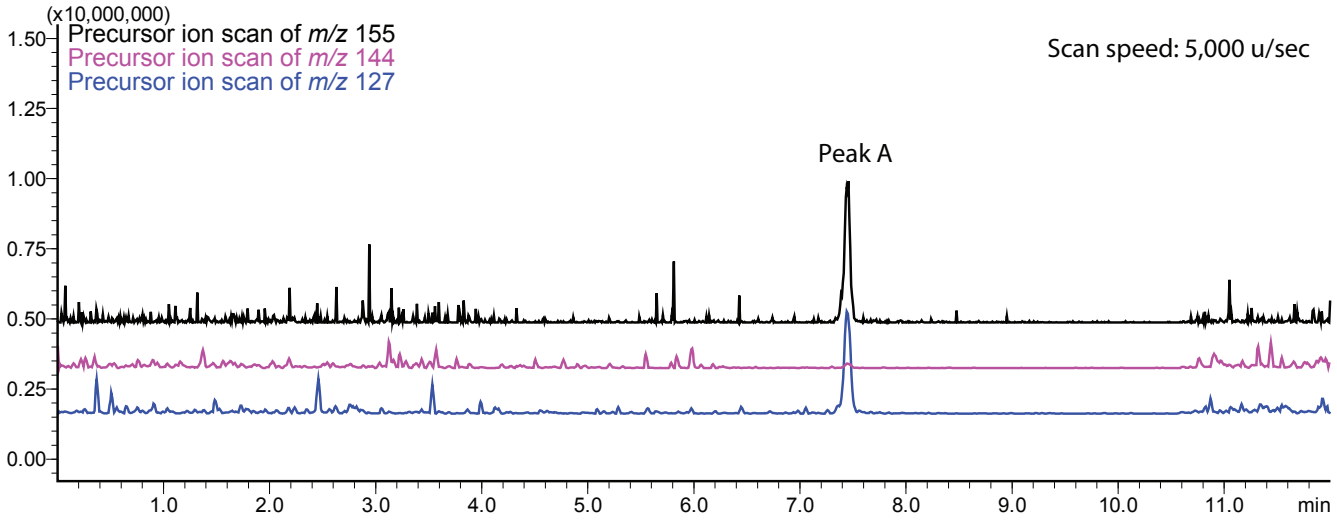


Figure 3: Chromatograms of three precursor ion scans for the K2 Spice cannabinoid product. Peak A, eluting at 7.5 min, was investigated as a designer cannabinoid.

the addition of new functional groups, and they are introduced to alter the potency of the compound or to evade detection. Precursor ion scans of *m/z* 155 and 127 can detect any analogues with modification to the N-alkyl chain or the indole group. Any analogue with modifications to the napthalene group could be detected by the fragments from the indole group using a precursor ion scan of *m/z* 144. Therefore this method has the capability to detect a wide variety of modified napthyolindole designer cannabinoids.

Because the possibility remains that some designer cannabinoids might still not be detected by these precursor ion scans, full scan MS at ultrafast scan speeds combined with ultrafast data-dependent product ion scanning can be used to further complement the semi-targeted screening approach using precursor ion scanning.

The total loop time of all events including MRMs, precursor ion scans, full scans, and data dependent scans was 520 msec. This was sufficient to collect at least 18 points per chromatographic peak.

The LC-MS-MS chromatogram of the precursor ion scans of the K2 Spice product is shown in **Figure 3**. One major peak, **Peak A**, is observed at a retention time of 7.5 min. The precursor ion scans corresponding to this peak are shown in **Figure 5**. The peak at *m/z* 342 is observed as the base peak in each spectrum, indicating the presence of a compound at this mass that fragments to the three products at *m/z* 155, 127, and 144.

The data-dependent MS-MS of Peak A is shown in Figure 7. The spectra were searched in a library containing tandem mass spectra of commercially available designer cannabiniod standards. The top hit from the search was the synthetic cannabinoid JWH-018. Other hits in the search were metabolites of synthetic cannabinoids that, while having some product ion similarities with JWH-018, could be easily rejected because they either contained different product ions or had different precursor masses.

If no hits had been found in the database search, the data dependent tandem mass spectra could be used to further characterize the unknown compound by comparing the product ions to those of known cannabinoids.

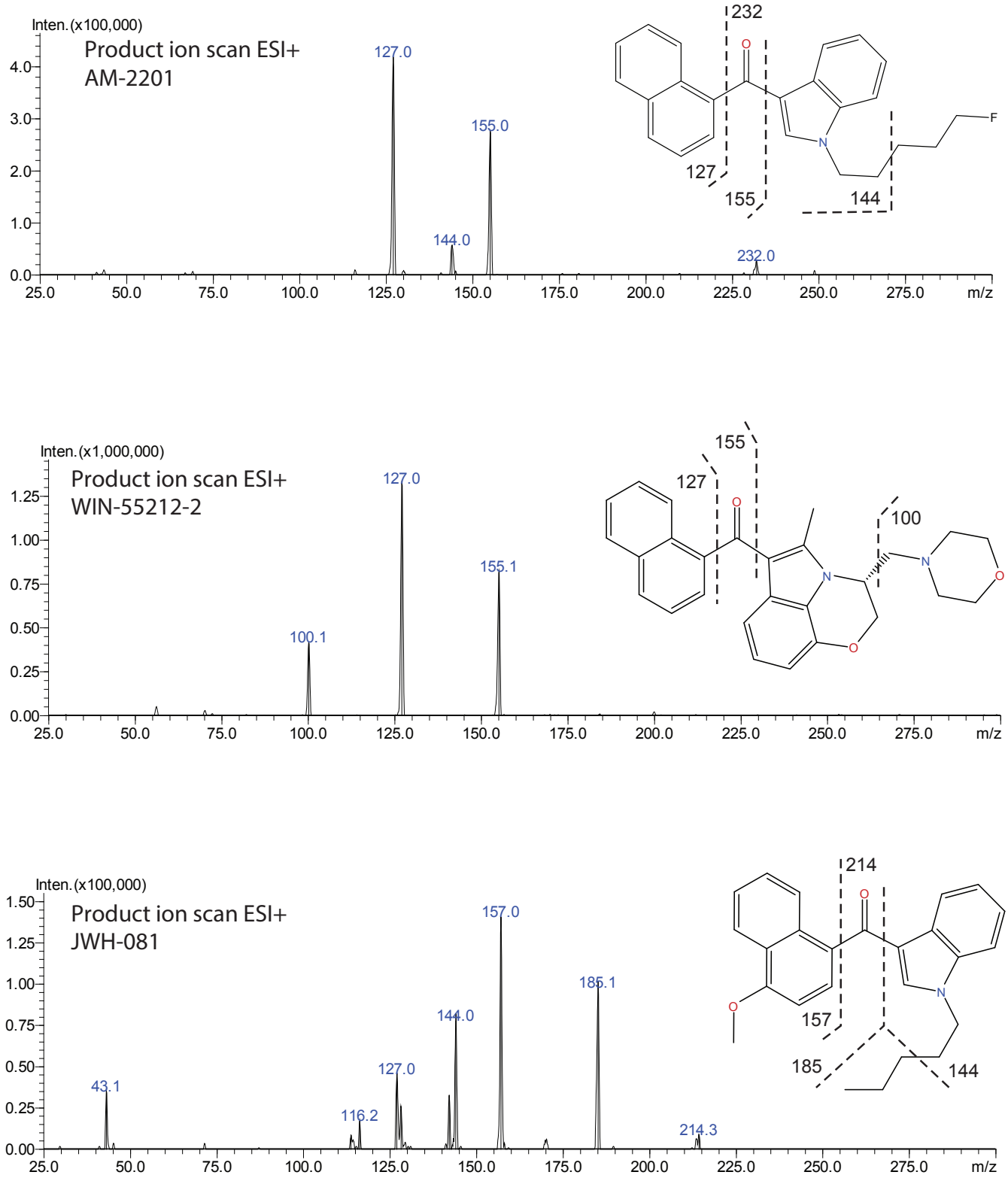


Figure 4: Tandem mass spectra of three representative designer cannabinoids showing common product ions and neutral losses

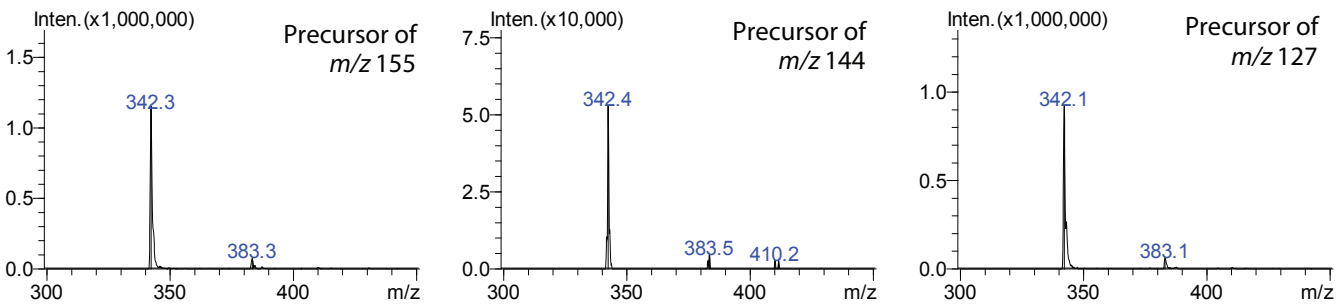


Figure 5: Precursor ion spectra for Peak A

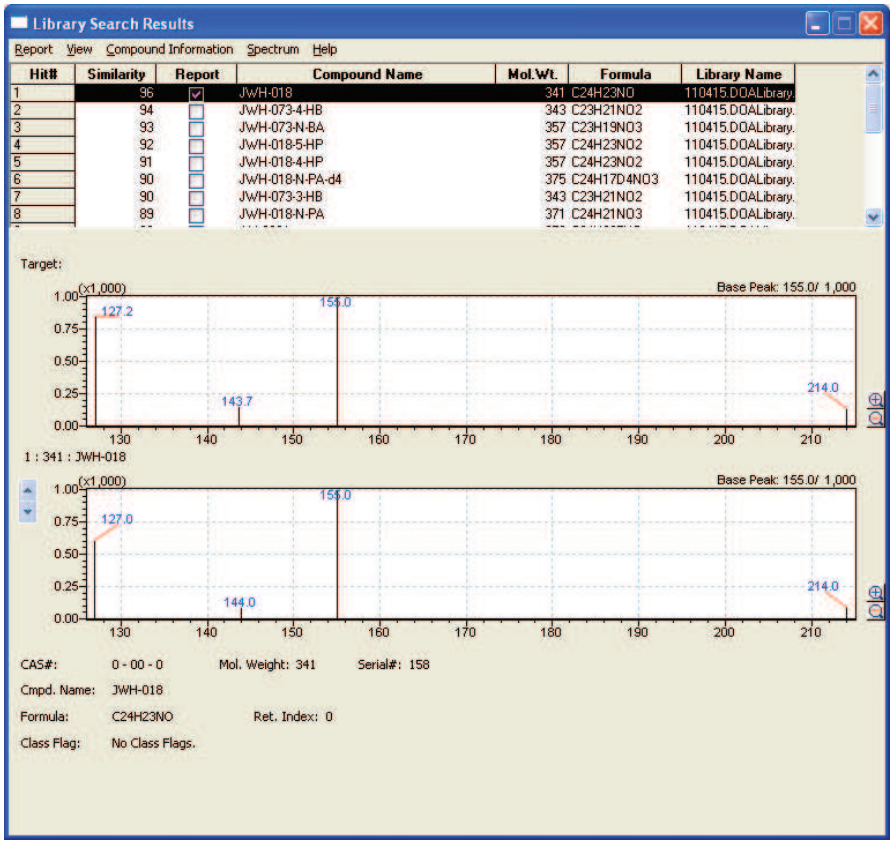


Figure 6: Library search results for the tandem mass spectrum of Peak A. The top hit is the designer cannabinoid**JWH-018**. The other hits found are metabolites of synthetic cannabinoids which have different precursor masses and therefore can be distinguished from JWH-018.

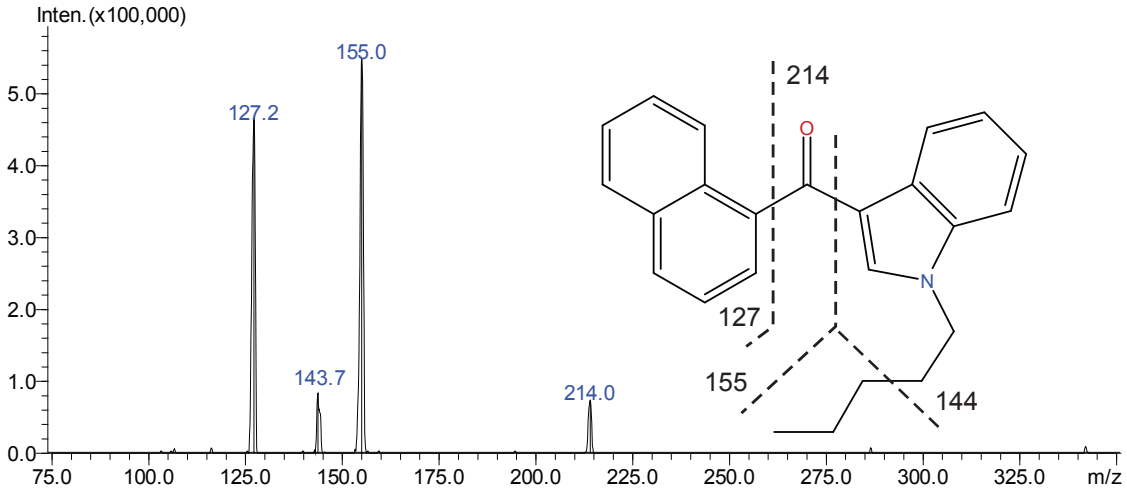


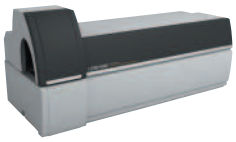
Figure 7: Tandem mass spectrum of Peak A, identified as JWH-018, and fragment assignment

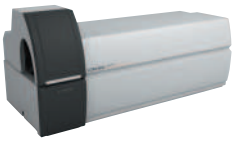
It should be noted that even at the ultrafast precursor ion scan speeds of 5,000 u/sec and data dependent product ion scan speeds of 15,000 u/sec that no significant shift in precursor or product ion masses were observed and no sensitivity was lost.

Conclusion

The fast scan capabilities of the LCMS-8030 enabled MRM, precursor ion scanning and full scanning with data dependent MS-MS for detection of known and unknown designer cannabinoids in commercially available herbal incense products.

ULTRA FAST MASS SPECTROMETRY


LCMS-8040


LCMS-8030


LCMS-8080


LCMS-2020


LCMS-IT-TOF

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