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Technical Report

Interpretation of LC Chromatograms Facilitated by Mass Detection and the Mass-it™ Function

Katsuaki Koterasawa[1], Kazuma Maeda[1], Kazuo Mukaibatake[1], Atsuhiko Toyama[1]

Abstract:

Liquid chromatography is typically based on light absorption using an ultraviolet-visible light (UV-Vis) absorbance detector or a photodiode array (PDA) detector. However, light absorption detectors are not suited to detecting compounds that do not absorb light, compounds present in low concentrations, or compounds without adequate temporal separation (coeluted compounds). For such compounds, a mass spectrometer can serve as a supplemental detector. Because mass spectrometers are based on a different measurement principle, they can even measure compounds that do not absorb light. Furthermore, because using a mass spectrometer can provide mass information about compounds, it enables more accurate qualitative results. The LCMS-2050 mass spectrometer includes Mass-it functionality that can overlay compound mass (*m/z*) information, obtained with the mass spectrometer, onto UV chromatograms to support supplementing qualitative analysis results obtained using a UV detector.

Keywords: LCMS-2050, Mass-it, UV detector, PDA detector, i-PDeA II

1. UV Detectors and MS Detectors

Liquid chromatography (LC) is an analytical technique that uses a detector to monitor compounds separated by a column. It can provide all sorts of information about compounds contained in samples. Samples are analyzed both qualitatively and quantitatively based on a combination of qualitative information such as compound retention time and quantitative information such as detector signal intensity.

If a photodiode array (PDA) detector is used, multiple wavelengths can be analyzed at the same time to obtain UV absorption spectra for each unit of time. Because each compound has a unique UV spectrum, they improve the ability to identify compound components (qualitative analysis performance). LC analysis results obtained using a PDA detector are shown in Fig. 1-3. In Fig. 1, the UV chromatogram for absorbance at the 254 nm wavelength includes peaks for 4 compounds. The UV spectra in Fig. 2, which were obtained during the elution period between peaks 3 and 4, suggest that the corresponding compounds have completely different absorption spectra (and thus different functional groups). Fig. 3 shows a 3D plot of retention time, wavelength, and signal intensity from the information in Fig. 1-2. Though it contains all the same information, it is difficult for analysts to interpret the results at a glance.

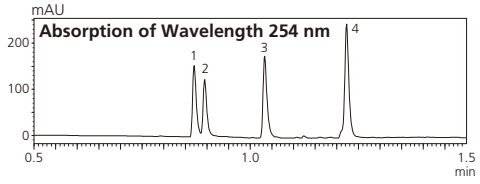


Fig. 1 UV Chromatogram of Absorbance at Wavelength 254 nm

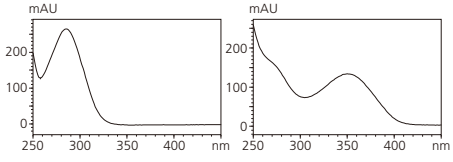


Fig. 2 UV Spectra
Left: UV spectrum of peak 3 in Fig. 1
Right: UV spectrum of peak 4 in Fig. 1

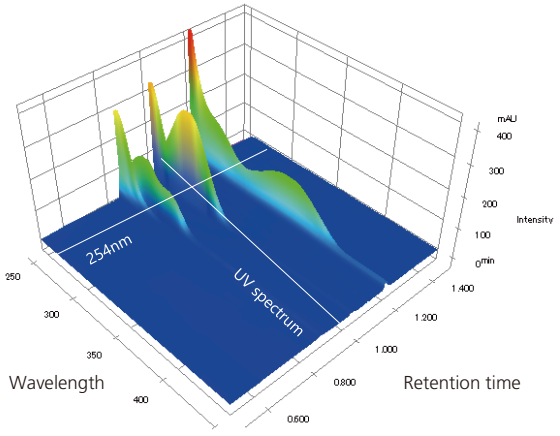


Fig. 3 3D Graph of Retention Time, Wavelength, and Signal Intensity Data Obtained from a PDA Detector

Mass spectrometers (MS) are instruments that successively ionize compounds to obtain mass information (*m/z*). The compact and simply designed LCMS-2050 model can be used as an LC detector, in the same manner as using a PDA detector, to obtain supplemental information. Whereas PDA detectors scan UV wavelengths, MS detectors scan a range of *m/z* values and display the response as a mass spectrum. Alternately, an *m/z* value can be specified and intensity plotted as a function of time to display data as a quantitative chromatogram.

Fig. 4 SPD-M40 PDA Detector (Left) and LCMS-2050 MS Detector (Right)

[1] Analytical & Measuring Instruments Division

Fig. 5 shows the total ion current (TIC) chromatogram obtained by the LCMS-2050 detector at the same time as the PDA detector data from Fig. 1, 2, and 3. Peak A was detected by the MS unit near a retention time of 0.8 min, but not by the PDA detector. That compound was not detected by the UV detector and only detected by the MS detector because the compound has a low UV absorption level.

In addition, the mass spectra for peaks 3 and 4 are shown in Fig. 6. UV spectra from PDA detectors include spectral patterns that indicate structural information about compounds, whereas mass spectra from MS detectors include qualitative peaks that directly indicate molecular weight information about compounds with particular specificity.

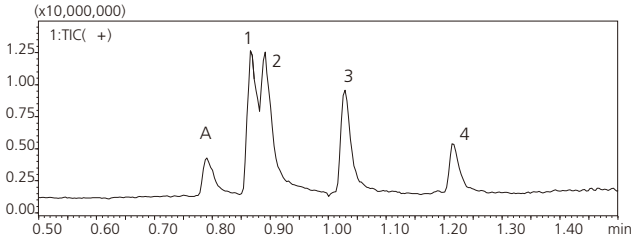


Fig. 5 TIC Chromatogram Obtained from MS Detector

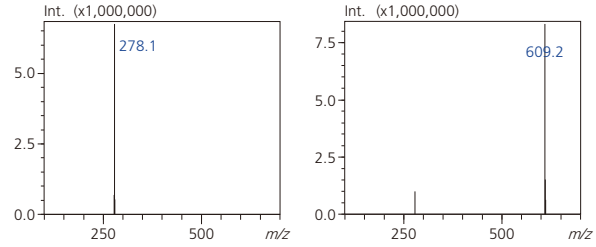
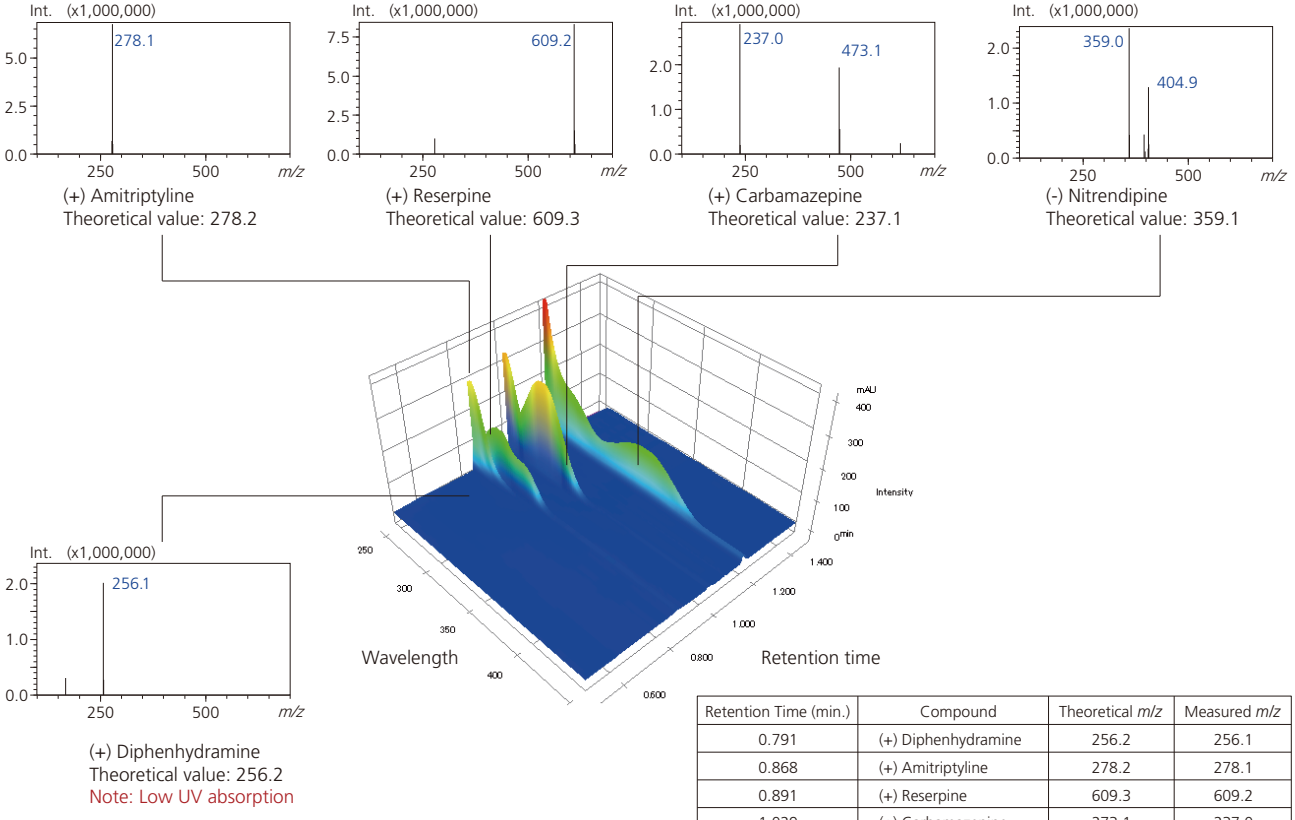


Fig. 6 Mass Spectra for Respective Peaks in Fig. 5
(Left: Mass spectrum for peak 1, Right: Mass spectrum for peak 2)

Fig. 7 combines the 3D graph data in Fig. 3 with the compound information (compound name and theoretical *m/z* value) and the mass spectrum obtained for each component. Thus, using a combination of both PDA and MS detectors can provide extensive qualitative information that cannot be adequately shown by 3D graphing, which is useful for determining all components contained in samples.



Retention Time (min.)	Compound	Theoretical *m/z*	Measured *m/z*
0.791	(+) Diphenhydramine	256.2	256.1
0.868	(+) Amitriptyline	278.2	278.1
0.891	(+) Reserpine	609.3	609.2
1.029	(+) Carbamazepine	273.1	237.0
1.216	(-) Nitrendipine	359.1	359.0

Fig. 7 3D Data Obtained from a PDA Detector and Mass Spectral Data Obtained from an MS Detector

2. Mass-it Function

The Mass-it function is used to automatically overlay *m/z* information obtained from a mass spectrum over the corresponding UV chromatogram (Fig. 8). That can improve the reliability and overall visibility of compound identification in UV chromatograms.

2-1 Using the Mass-it Function to Detect Components with No UV Absorption

Due to the low light absorption of the diphenhydramine eluted near a retention time of 0.8 min, the peak was not detected by the PDA detector but was detected by the MS detector. Consequently, using the Mass-it function to add *m/z* information to a UV chromatogram can prevent overlooking compounds not detectable based on UV absorption.

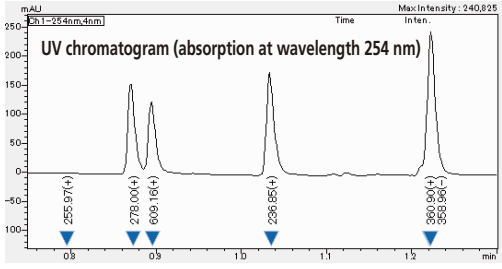


Fig. 8 UV Chromatogram with *m/z* Information Added Using the Mass-it Function

2-2 Using the Mass-it Function to Check the Molecular Weights of Synthesized Substances

When UV detectors are used to quantitate substances for drug discovery or chemical synthesis applications, mass information is sometimes obtained to check the synthesis process. Mass-it is especially useful for such data analysis performed for analytical purposes.

Fig. 9 shows the results of measuring a 1,000 ng/µL atorvastatin sample that contains impurities. The principal component peak was detected at about 10.6 min in the UV chromatogram. At that point, the Mass-it function automatically adds the information obtained from the mass spectrum (*m/z* 559.3) to the UV chromatogram, making it easy to see at a glance that atorvastatin is the main peak (Fig. 10).

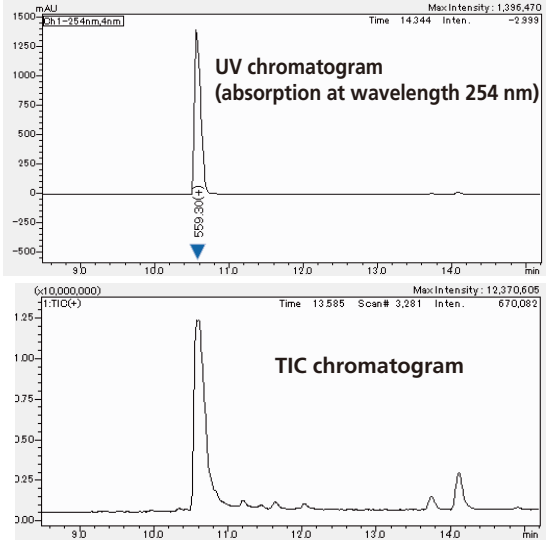


Fig. 9 UV Chromatogram with *m/z* Information Added to a Principal Component Peak Using the Mass-it Function

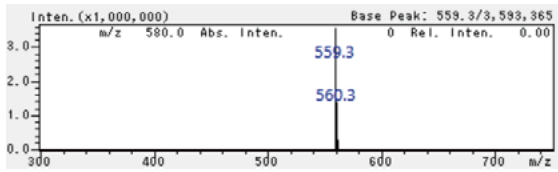


Fig. 10 Mass Spectrum with Peaks for Principal Component Atorvastatin

In addition to checking principal components, Mass-it functionality can also be useful for analyzing impurities. Fig. 11 is an enlargement of an area near the baseline of the chromatogram in Fig. 9. *m/z* information can even be added to peaks with an area value that is 0.1 % or less of the main peak, which is useful for predicting impurities.

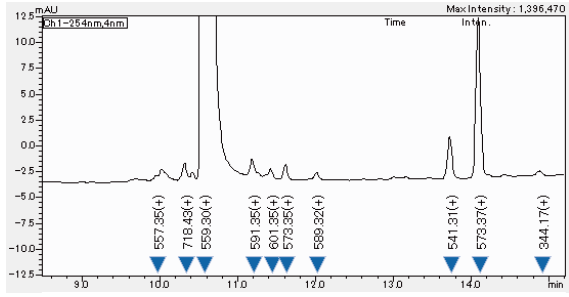


Fig. 11 UV Chromatogram with *m/z* Information for Impurity Components Added Using the Mass-it Function

2-3 Using the Mass-it Function to Detect Coeluted Peaks

If multiple compounds are eluted simultaneously (coeluted) without being separated in the column, they appear as one peak in UV chromatograms. If the retention times are particularly close, it can be difficult to discover a substance was coeluted. However, coelution can be determined from the mass spectrum obtained by MS. Furthermore, the Mass-it function provides support for discovering coeluted compounds by displaying *m/z* information obtained from mass spectra on UV chromatograms.

A UV chromatogram from simultaneous analysis of 7 drug components is shown in Fig. 12. It shows the coelution of both lorazepam and oxazepam near a retention time of 4.9 minutes. Given that lorazepam and oxazepam were detected at *m/z* 320.8 and 286.8, respectively, the Mass-it function adds that *m/z* information to the UV chromatogram in the same location. That information can be used to determine that the substances eluted at the same time.

Shimadzu PDA detectors include an i-PDeA II function that can derive UV chromatograms from UV spectral information. Using both i-PDeA II and Mass-it functions can provide more reliable analytical results.

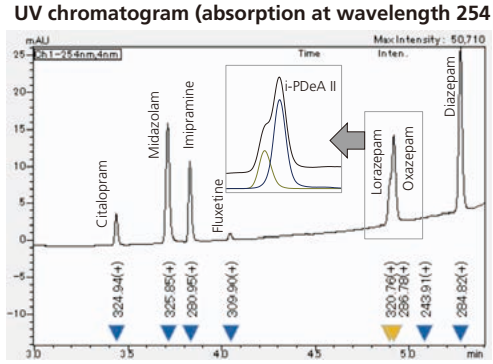
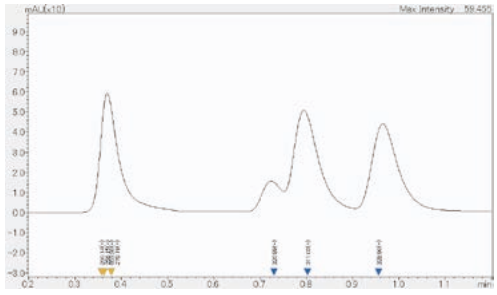


Fig. 12 Measurement of 7 Drug Components (with Separation of Unseparated Peaks by i-PDeA II and Mass-it Functions)

Analytical results with even more coeluting components are shown in Fig. 13. Even if 4 components are eluted simultaneously within a single peak, MS can determine the corresponding *m/z* information for each compound, with the Mass-it function overlaying that information on the UV chromatogram.

UV chromatogram (absorption at wavelength 254 nm)



Mass chromatogram

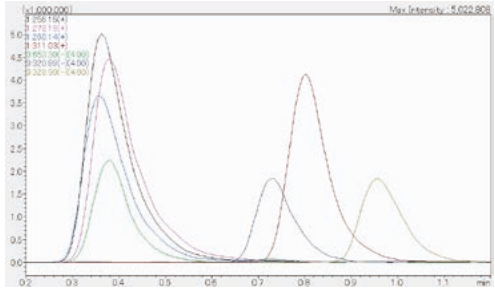


Fig. 13 Chromatograms with 4 Components Coeluted near a Retention Time of 0.3 to 0.5 Minutes

3. Algorithm of Mass-it Function

Mass-it extracts *m/z* information from TIC chromatograms based on the following algorithm.

(1) Detect peaks with a TIC chromatogram.
(2) Calculate mass spectra within that TIC chromatogram.
(3) Plot mass chromatograms for detected *m/z* values.
Mass chromatograms without a peak are excluded.
Peaks with the same retention time are considered identical components.
If peaks do not have matching retention times, they are considered peaks for separate components.
(4) Add the *m/z* information to the UV chromatogram.

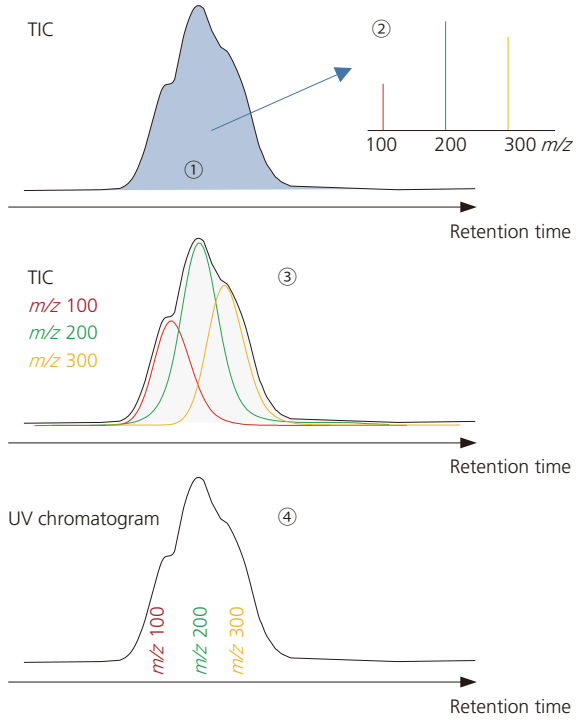


Fig. 14 Algorithm of Mass-it

4. Summary

- The Mass-it function helps identify compounds by displaying *m/z* information obtained by MS on UV chromatograms. It is especially useful for checking molecular weights or impurities in synthesized chemicals.
- Even if a compound with no UV absorption is detected, mass information is displayed on UV chromatograms, which helps prevent overlooking compounds.
- If a column coelutes inadequately separated peaks, then mass information is displayed in the same location. That can result in discovering the coelution.

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