SHIMADZU APPLICATION NEWS

LAAN-A-LM-E030

● LIQUID CHROMATOGRAPHY MASS SPECTROMETRY

No. C59

# Ultra Fast Analysis (Part 2)
# Analysis of Impurities in Curcumin

An example of ultra fast analysis using the Prominence UFLC with the LCMS-2010EV was presented in Application News No. C49. As for improvement in throughput, this combination is, of course, effective even in impurities analysis, shortening analysis time which might ordinarily take twenty or thirty minutes to several hours, greatly contributing to higher operations efficiency. Here we present an example of analysis of a curcumin standard solution using photodiode array (PDA) detection and mass spectrometry (MS).

## ■ Analysis of Curcumin Standard Solutions

Curcumin is a type of polyphenol present in turmeric, and is used as a food dye. Fig.1 shows the structrures of curcumin in addition to two similar compounds (curcuminoids). The measurement samples consisted of commercial curcumin standards of different purity, referred to as grade A and grade B. The standard solutions were prepared by dissolving the standards in methanol and adjusting the respective concentrations to 1 mg/mL. PDA detection was conducted at a wavelength of 425 nm. MS analysis was conducted using electrospray ionization, and the deprotonated molecules were detected. Grade A showed a relatively low purity of curcumin, and more than 20 minutes was required to complete the analysis using a 5 µm particle diameter column (fig.2 (a), (b)), while this analysis time was shortened by 7 minutes using the Prominence UFLC (fig.2 (c), (d)). The area percentages in the PDA chromatogram were 1.11 % for bisdemethoxycurcumin, 11.46 % for demethoxycurcumin, and 87.19 % for curcumin. Other than these compounds, minute peaks were also noticeable at $t_R$=1.34 min (λ max 338 nm, *m/z* 337), $t_R$=1.48 min (λ max 349 nm, *m/z* 367), and $t_R$=2.77 min (λ max 429 nm, *m/z* 383). As for the high-purity grade B sample, neither bisdemethoxycurcumin nor demethoxycurcumin were detected (fig.2 (e), (f)).

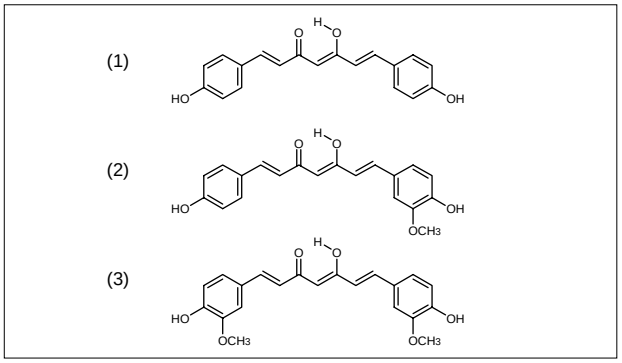


**Fig.1 Structures of Curcuminoids (1: Bisdemethoxycurcumin (MW 308), 2: Demethoxycurcumin (MW 338), 3: Curcumin (MW 368))**

**Table 1 Analytical conditions**

| | |
|---|---|
| Column | : Shim-pack XR-ODS (75 mmL. × 2.0 mmI.D., 2.2 µm, UFLC) |
| | Shim-pack VP-ODS (150 mmL. × 2.0 mmI.D., 5 µm, HPLC) |
| Mobile phase | : 0.1 % Formic acid/Acetonitrile = 60/40 |
| Flow rate | : 0.4 mL/min (UFLC), 0.2 mL/min (HPLC) |
| Column temperature | : 40 ˚C |
| Injection volume | : 1 µL |
| Detection | |
| PDA | |
| SPD-M20A | : 425 nm |
| MS | |
| Probe voltage | : -3.5 kV (ESI-Negative mode) |
| Nebulizing gas flow | : 1.5 L/min |
| Drying gas pressure | : 0.15 MPa (UFLC), 0.10 MPa (HPLC) |
| CDL temperature | : 250 ˚C |
| Block heater temperature | : 200 ˚C |
| CDL, Q-array voltages | : using Default values |
| Scan range | : *m/z* 150 - 500 |

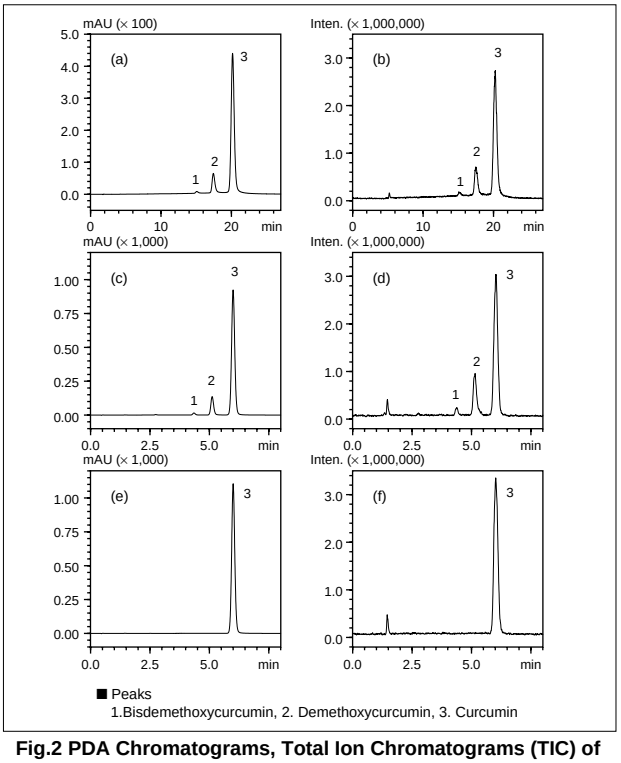


**Fig.2 PDA Chromatograms, Total Ion Chromatograms (TIC) of Curcumin Standard Solutions**
**(a): PDA chromatogram of curcumin (grade A) by HPLC,**
**(b): TIC of curcumin (grade A) by HPLC-MS**
**(c): PDA chromatogram of curcumin (grade A) by UFLC**
**(d): TIC of curcumin (grade A) by UFLC-MS**
**(e): PDA chromatogram of curcumin (grade B) by UFLC**
**(f): TIC of curcumin (grade B) by UFLC-MS**

## ■ PDA Data

Fig.3 shows the UV spectra of curcuminoids. All of them clearly possess a maximum absorption wavelength near 420 nm. When the peak purity is calculated from the grade A data, an impurity was detected at 5.35 minutes (fig.4 (2)).

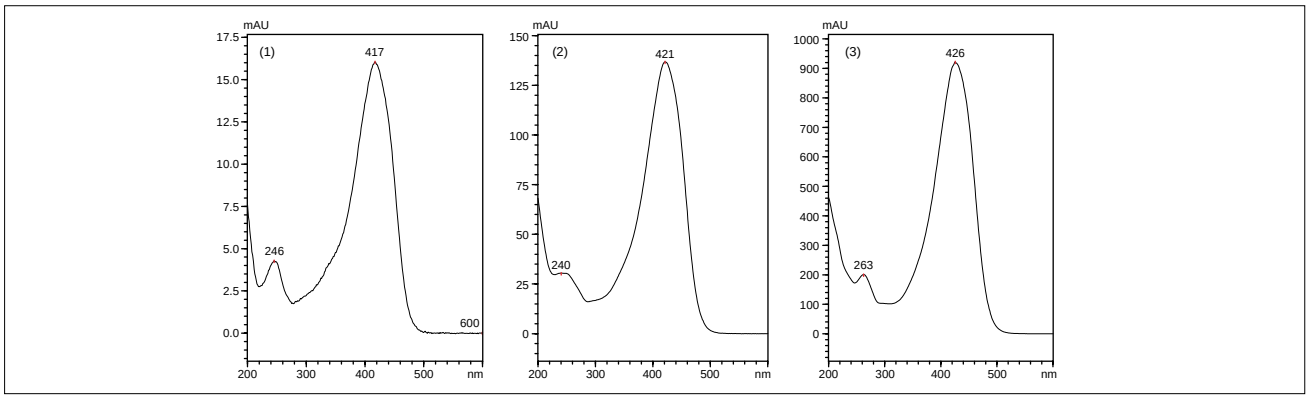


**Fig.3 UV Spectra of Curcuminoids (1: Bisdemethoxycurcumin, 2: Demethoxycurcumin, 3: Curcumin)**

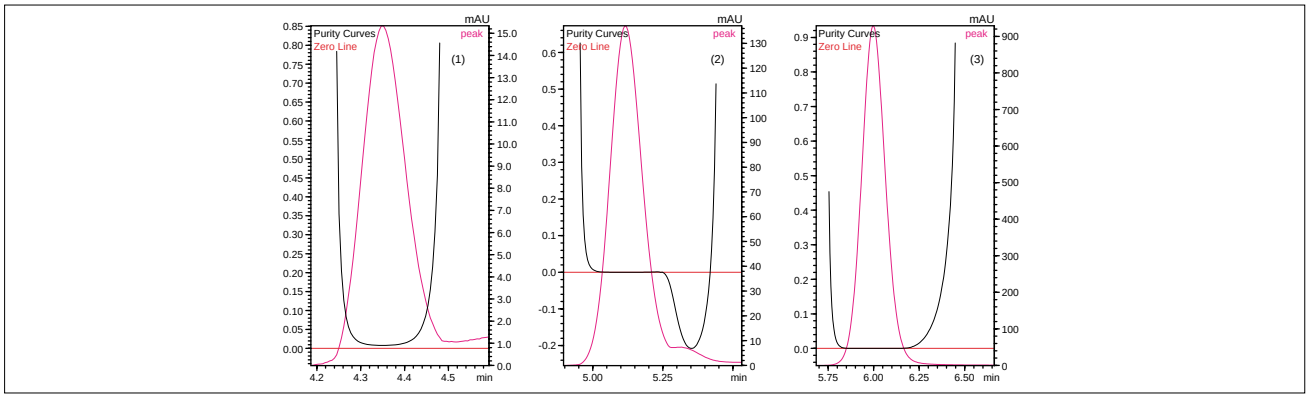


**Fig.4 Purity Curves of Curcuminoids (1: Bisdemethoxycurcumin, 2: Demethoxycurcumin, 3: Curcumin)**

## ■ Another Impurity

Focusing on the peak (compound X) at 5.35 minutes in the mass chromatograms of fig.4 (2), the UV spectrum and mass spectrum were obtained for this peak, as shown in the fig.5. The maximum absorption wavelength is in the vicinity of 370 nm, and the *m/z* 369 negative ion was detected. Therefore, the molecular weight of compound X is presumed to 370. Thus, by using both PDA and MS in UFLC detection, greater efficiency is believed to be possible.

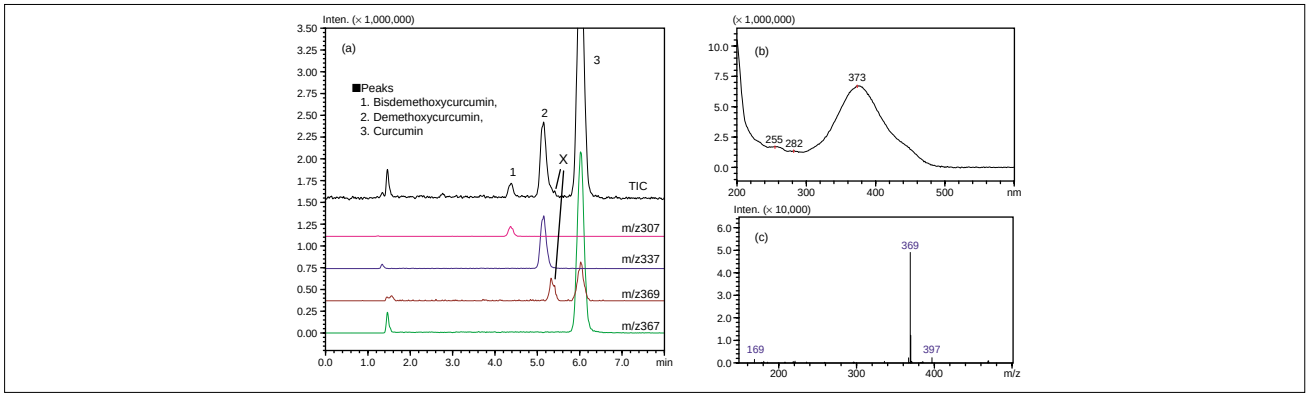


**Fig.5 Mass chromatograms of curcumin standard solution (grade A, (a)) and UV spectrum (b), mass spectrum (c) of compound X**

**NOTES:**

✻This Application News has been produced and edited using information that was available when the data was acquired for each article. This Application News is subject to revision without prior notice.

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