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Characterization of Tea Seed Oil for Quality Control and Authentication

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Abstract

This application note describes a streamlined UPLC System solution with PDA and single quadrupole MS (SQD) detectors for tea seed oil characterization, quality control, and authentication. This solution provides tea seed oil companies with an efficient and easy method to establish their quality standards and authenticate their products.

Introduction

Tea seed oil is an edible oil cold-pressed from the seeds of Camellia oleifera and Camellia sinensis (Figure 1). It is mainly produced in Hunan, Jiangxi, Guangxi, and other southern provinces of China. In Chinese herbal medicine, tea seed oil is considered a superior nutritional dietary supplement that benefits the digestive system, reduces bad cholesterol, lowers blood pressure, regulates the nervous system, and strengthens the immune system.[1,2] Tea seed oil is recommended by the Food and Agriculture Organization of the United Nations as a high-quality, healthy vegetable oil because of its nutritional value, which is comparable to olive oil in terms of its high oleic acid content, low saturated fat, high antioxidants, and excellent storage qualities.[3]

Figure 1. Tea seed flowers, tea seed nuts, and tea seed oil.

Tea seed trees are evergreen plants that can grow on barren land without fertilizers. They start bearing fruits eight years after initial planting, and can remain highly productive for 80 years. In an effort to create more green land, increase farmers' income, and reduce China's dependence on imported food, Chinese government agencies are setting policies to support the growth of the tea seed oil industry. At present, annual production of tea seed oil is approximately onequarter million tons and expected to reach more than three million tons by 2020 or 15–25% of the total edible oil supply in China.[3]

To satisfy legislative requirements worldwide,[4] and establish the premium quality oil brand names, tea seed oil companies in China monitor the entire production process, from tree cultivation, harvesting and cold-pressing tea seeds, to oil packaging and shipping. As a result, analytical technologies that can streamline quality control and help to differentiate their products are in increasing demand. Currently, seed oil analysis

mainly relies on GC and HPLC methods. GC methods require derivatization prior to analysis, which is timeconsuming and laborious.[5] Conventional HPLC methods require either using halogenated solvent or using non-halogenated solvent with longer runtimes to achieve adequate separation.[6–9] The use of halogenated solvents are restricted in many laboratories since they are known carcinogens and environmental hazards.

The Waters ACQUITY UPLC System is a new generation of liquid chromatographic platform. Using UPLC/UV photodiode array (PDA)/mass spectrometer detectors, fast screening and high resolution methods for seed oil characterization have been developed without using halogenated solvents.[10–13]

The ACQUITY UPLC System with PDA Detector enables the acquisition of multiple data types in a single injection to generate reproducible fingerprinting information, identify triglyceride components, and evaluate the degree of seed oil oxidation and decomposition. Compared with conventional HPLC, UPLC shortens analysis times, reduces solvent usage, and provides a higher resolution chromatogram with more information in a single injection. As a result, the UPLC method is more cost-effective.

This Application Note describes a streamlined UPLC System solution with PDA and single quadrupole MS (SQD) detectors for tea seed oil characterization, quality control, and authentication. This UPLC solution provides tea seed oil companies with an efficient andeasy method to establish their quality standards and authenticate their products. The note also compares the composition of tea seed oil with olive oil and other vegetable oils. Among the samples examined, tea seed oil exhibited the highest trioleoylglycerol content among all the oils. It contains very high omega-9 fatty acid content, but very low omega-6 and saturated fatty acid contents. This may be one of the reasons why tea seed oil has been recognized for health benefits since ancient times in Chinese medicine.

Experimental

Sample Preparation:

Commercial tea seed oil was received from China. Other edible oils were bought from local grocery stores. All oil samples were diluted with 2-propanol to make a 6 mg/mL solution for the analysis.

UPLC Conditions:

UPLC system:	ACQUITY UPLC with PDA and SQ detectors
Software:	Empower 2
Column:	ACQUITY UPLC BEH C_{18}, 2.1 x 150 mm
Column temp.:	30 °C
Weak wash solvent:	2-propanol (500 µL per wash)
Strong wash solvent:	2-propanol (500 µL per wash)
Seal Wash solvent:	10% CH_3CN in H_2O (every 5 min)
Mobile phase A:	CH_3CN
Mobile phase B:	2-propanol
Injection:	2 µL (full loop)

Gradient Method:

Time (min)	Flow (mL/min)	%B	Curve
0.0	0.15	10	–
22	0.15	90	6

Column Condition and Re-equilibration Method:

Time (min)	Flow (mL/min)	%B	Curve
0.0	0.13	100	–
18	0.13	10	11
21.5	0.7	10	11
24.5	0.15	10	11
25	0.15	10	11

Note: A blank injection of 2-propanol was run at the beginning of the sample set and used for PDA 3D blank subtraction.

PDA Parameters:

Detection:	195 to 300 nm
Sampling rate:	20 pts/s
Filter response:	fast

MS Conditions

Instrument:	ACQUITY SQD with IonSABRE APCI probe
Ionization mode:	APCI+
Corona (µA):	5
Cone voltage:	+30 V
Extractor:	+3 V
Source temp.:	150 °C
APCI Probe temp.:	450 °C

Desolvation gas:	700 L/hr
Cone gas:	0 L/hr
Acquisition range:	140 to 1100 *m/z*

Results and Discussion

It is difficult to separate triglycerides, the major components of seed oil, using conventional HPLC methods without halogenated solvents. The ACQUITY UPLC System includes running high-efficiency columns packed with small particles to perform faster, sensitive, well-resolved separations. The UPLC solvent delivery system can sustain back pressures up to 15,000 psi, enabling the use of high viscosity solvent such as 2-propanol for seed oil analysis. Since 2-propanol is good for dissolving seed oil,[14] low in toxicity, and allows UV detection of triglycerides due to its low limit of transparency; 2-propanol was chosen as the strong eluent. Unlike other solvents used in conventional HPLC methods, acetonitrile and 2-propanol used in UPLC methods are compatible with PDA and MS detectors for seed oil analysis. Multiple data types can be obtained in a single injection to generate reproducible fingerprinting data,[10,13] identify triglyceride components by mass spectrometry, and evaluate the degree of seed oil oxidation with multiple PDA wavelength channels.[11]

Figure 2 shows positive APCI TIC chromatograms of tea seed oil A, extra virgin olive oil, and soybean oil samples. In comparison, chromatograms of tea seed oil and olive oil show similar triglyceride peak patterns, but chromatogram patterns of tea seed oil and soybean oil are dramatically different. The triglyceride components can be identified by a well-established tandem mass spectrometry method according to observed pseudomolecular ion and relative intensity of diacylglycerol fragment ions.[15,16] Since triglycerides are well separated by UPLC, they can be identified using the ACQUITY single quadrupole mass detector (SQD). As shown in combined mass spectra of the major peaks of tea seed oil (Figure 3), using observed pseudomolecular ion and diacylglycerol fragment ions, the major components of tea seed oil are identified as follows:

- Trioleoylglycerol (OOO)
- Dioleoyl-palmitoyl-glycerol (OOP)
- Dioleoyl-linoleoyl-glycerol (OOL)

- Dioleoyl-stearoyl-glycerol (OOS)
- Palmitoyl-oleoyl-linoleoyl-glycerol(POL)
- Dilinoleoyl-oleoyl-glycerol (LLO)

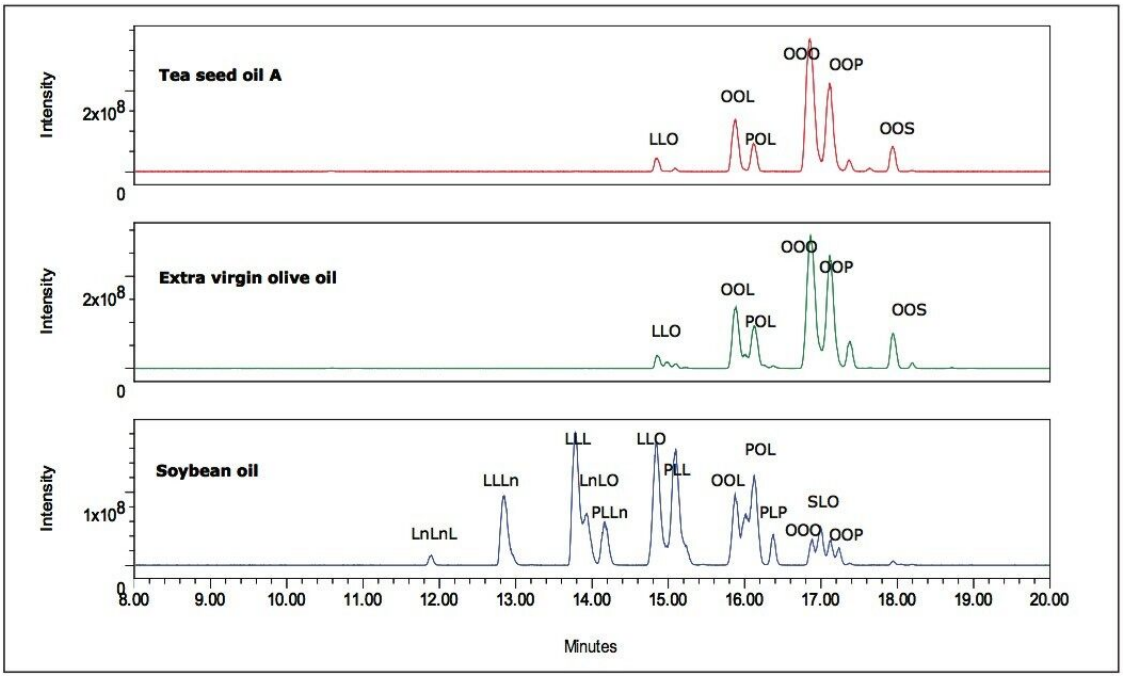

Figure 2. Positive APCI TIC chromatograms of tea seed oil A, extra virgin olive oil, and soybean oil samples.

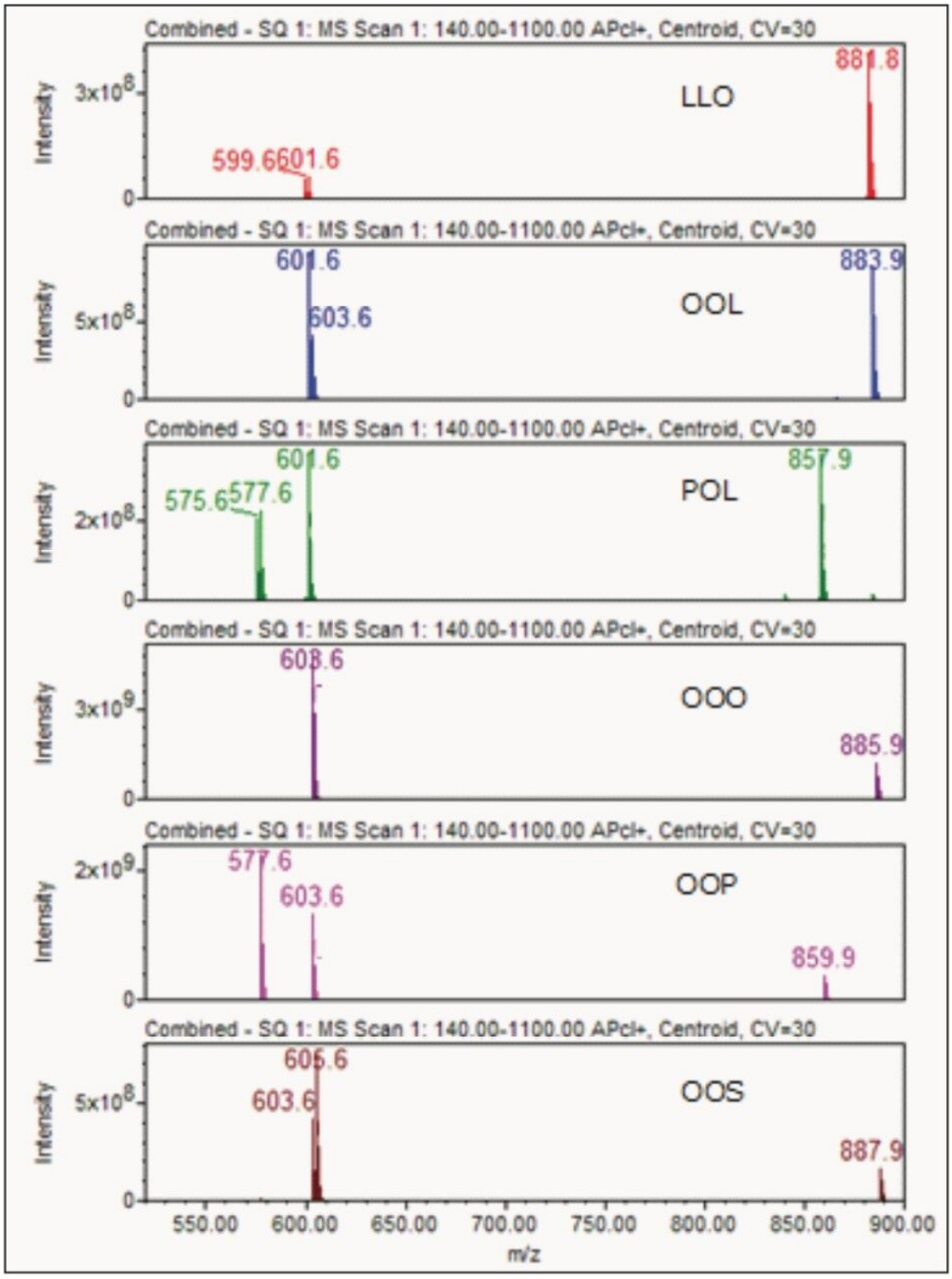

Figure 3. Combined positive-ion mass spectra of major peaks of tea seed oil A; Dilinoleoyl-oleoyl-glycerol (LLO) is confirmed with m/z 881.8: $[M+H]^+$, m/z 599.6: $[LL]^+$,and m/z 601.6: $[LO]^+$. Dioleoyl-linoleoyl-glycerol (OOL) is confirmed with m/z 883.9: $[M+H]^+$, m/z 601.6: $[LO]^+$, and

m/z 603.6: $[OO]^+$. Palmitoyl-oleoyl-linoleoyl-glycerol (POL) is confirmed with m/z 857.9: $[M+H]^+$, m/z 575.6: $[PL]^+$, m/z 577.6: $[PO]^+$ and m/z 601.6: $[LO]^+$. Trioleoylglycerol (OOO) is confirmed with m/z 885.9: $[M+H]^+$, and m/z 603.6: $[OO]^+$. Dioleoylpalmitoyl- glycerol (OOP) is confirmed with m/z 859.9: $[M+H]^+$, m/z 577.6: $[PO]^+$ and m/z 603.6: $[OO]^+$. Dioleoyl-stearoyl-glycerol (OOS) is confirmed with m/z 887.9: $[M+H]^+$, m/z 603.6: $[OO]^+$, and m/z 605.6: $[OS]^+$. Note: The regioisomer ratio of triglycerides can be further quantified using ion ratio calibration curves of diacylglycerol fragment ions with isomeric pure standards.[16]

Oxidation of triglycerides, known as rancidification, occurs upon exposing triglycerides to air. Most seed oil companies make great efforts to prevent seed oil oxidation during production processes, packaging, and storage. The degree of oxidation of seed oil samples can be monitored using the ACQUITY UPLC System with PDA Detector. While unspoiled triglycerides have UV absorption at about 210 nm, oxidized triglycerides containing conjugated diene, aldehyde, ketone, or carboxylic acid functional groups have UV adsorption at higher wavelength. Figures 4a to 4f show UV PDA extracted chromatograms at 210, 240, and 280 nm wavelengths of two different brands of tea seed oil samples from different provinces in China. The 210 nm chromatograms of tea seed oil A and B have similar triglyceride peaks but different relative intensities, as shown in Figures 4a and 4b. The difference in triglyceride component ratios between the two brands of tea seed oil samples may due to the variation of tea trees, geography, or production conditions. The 240 nm chromatogram of tea seed oil B has more high intensity peaks than that of tea seed oil A, as shown in Figures 4c and 4d, which indicate more oxidized triglycerides with conjugated dienes in tea seed oil B relative to tea seed oil A.18 Interestingly, the 280 nm chromatogram of tea seed oil B has much lower intensity peaks than tea seed oil A (Figure 4e and 4f), indicating that tea seed oil A contains more oxidized triglycerides with conjugated aldehyde or ketone functional groups. Clearly, the two tea seed oil samples are in different stages of oxidation.

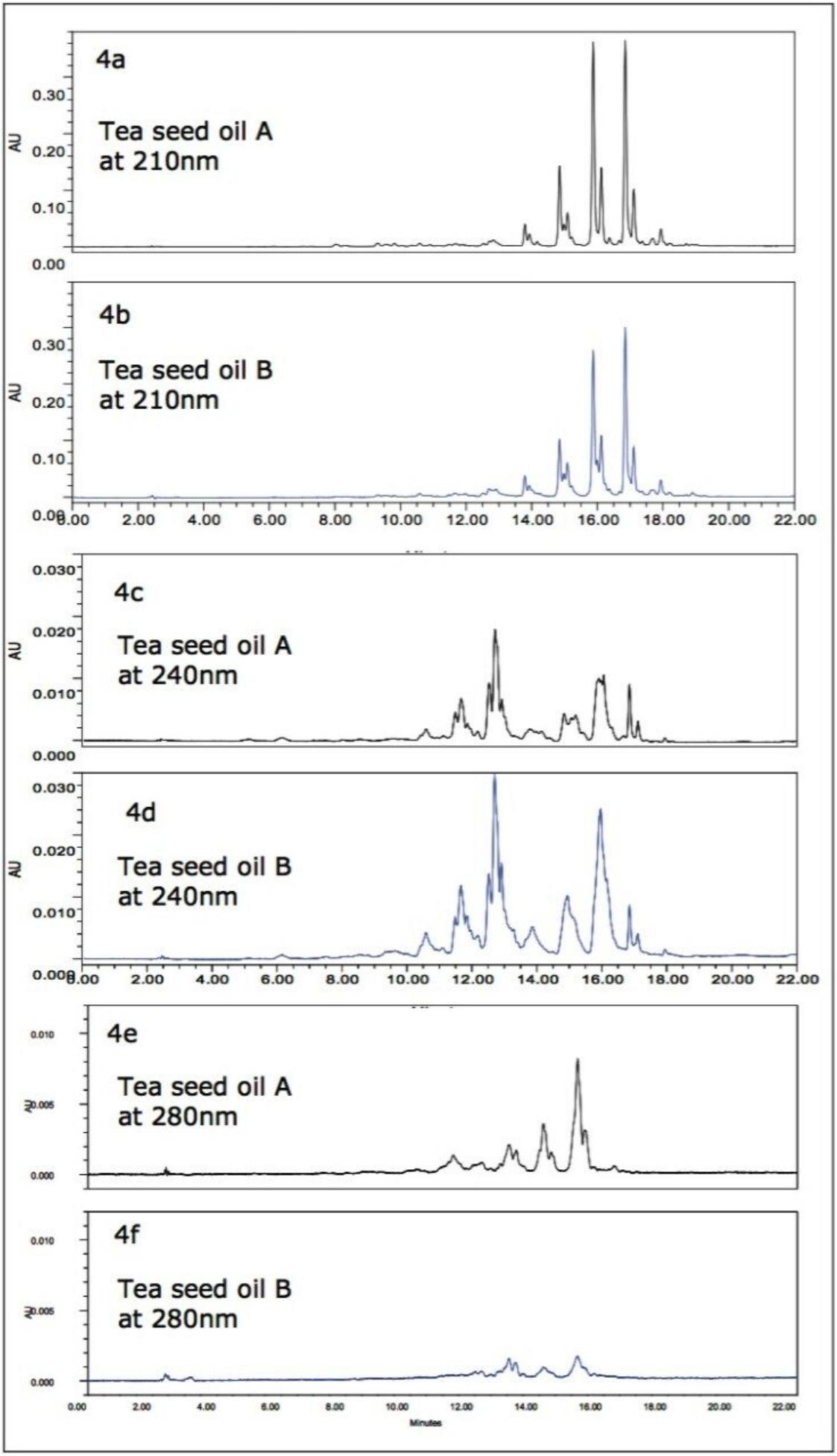

Figure 4. PDA extracted chromatograms at 210 nm, 240 nm, and 280 nm wavelengths of two diff

2. http://seed.aweb.com.cn/2009/0106/142425500.shtml

3. http://finance.aweb.com.cn/2008/9/12/2252008091208142370.html

4. http://www.fediol.org/5/pdf/legislation.pdf <http://www.fediol.be/>

5. V G Dourtoglou *et al. JAOCS*, 80,.3: 203-208, 2003.

6. LCGC, The Application Notebook, Sept 1, p 51, 2006.

7. A J Aubin, C B Mazza, D A Trinite, P. McConvile, Analysis of Vegetable Oils by High Performance Liquid Chromatography Using Evaporative Light Scattering Detection and Normal Phase Eluents, Waters Corporation Application Note no. 720002879en < https://www.waters.com/nextgen/us/en/library/application-notes/2008/analysis-vegetable-oils-by-high-performance-liquid-chromatography-using-evaporative-light-scattering-detection-normal-phase-eluents.html> , 2008.

8. P. Sandra *et al. J. Chromatogr. A*. 974, 231–241, 2002.

9. International Olive Oil Council standard method COI/T.20/Doc.no20 2001.

10. P J Lee, C H Phoebe, A J Di Gioia, "ACQUITY UPLC Analysis of Seed Oil (Part 1): Olive Oil Quality & Adultration," Waters Corporation Application Note no. 720002025EN < https://www.waters.com/webassets/cms/library/docs/720002025en.pdf> , 2007.

11. P J Lee, C H Phoebe, A J Di Gioia, "ACQUITY UPLC Analysis of Seed Oil (Part 2) Olive Oil Quality & Adultration," Waters Corporation Application Note no. 720002026EN < https://www.waters.com/webassets/cms/library/docs/720002026en.pdf> , 2007.

12. P J Lee, and A. J. Di Gioia, "ACQUITY UPLC/ELS/UV: One Methodology for FFA, FAME and TAG Analysis of Biodiesel," Waters Corporation Application Note, no.720002155EN < https://www.waters.com/webassets/cms/library/docs/720002155en.pdf> 2007:.

13. P J Lee, Y Ichikawa, R R Menard, and A J Di Gioia, "Automating Calculations for Rapid Seed Oil Quality Control and Authentication," Waters Corporation, Technical note 2009 no. 720002973en < https://www.waters.com/webassets/cms/library/docs/720002973en.pdf> . In press.

14. F O Oyedeji *et al. J. Applied Sci.,* 6, 2510–2513, 2006.

15. http://www.byrdwell.com/Triacylglycerols/ <http://www.byrdwell.com/Triacylglycerols/>

16. A Jakab et al. Rapid Commun. *Mass Spectrom.* 17, 2295–2302, 2003.

17. S Terés et al. *Proc Natl Acad Sci* U S A. 105, no.37, 13811–6, 2008

18. A Lezerovich, *JAOCS*, 63, no.3, 883–889, 1986.

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