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Application Note

Identification of Fixed Oils by TLC acc. to USP General Chapter <202> (Method II) Using the TLC Explorer

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Abstract

This study presents a method for identifying fixed oils using Thin Layer Chromatography (TLC) in accordance with USP General Chapter <202>. The TLC Explorer system enabled efficient chromatographic analysis and documentation. Results revealed deviations in certain market samples from reference standards, underscoring the critical role of quality assurance in fixed oils, which can be effectively supported by High-Performance Thin Layer Chromatography (HPTLC).

Introduction

Fixed oils are lipids derived from plants that are essential in our daily diets, providing energy and supporting various biological functions. They contain numerous important components, including essential fatty acids, fat-soluble vitamins, nutrients, and sterols. In addition to their dietary applications, fixed oils play a vital role in pharmaceutical formulations, serving as carrier oils in ointments, injectables, and suppositories.[1]

The increasing demand for fixed oils has led to rising concerns over adulteration and falsification, both of which compromise product quality in efforts to maximize profit margins. To address these issues, various chromatographic techniques, including HPTLC, HPLC, LC-MS, and GC-MS, have been utilized for chemical profiling and quality assurance of these oils.[2]

The United States Pharmacopeia (USP) general chapter <202> identifies Thin Layer Chromatography (TLC) as the method for the identification of fixed oils.[3] TLC is frequently referenced in pharmacopeial methods for identity testing. High-Performance Thin Layer Chromatography (HPTLC) is an advanced version of TLC that is robust, reliable, rapid, and cost-effective for qualitative and quantitative analysis of different compounds. This technique provides chromatographic separations or fingerprints that can be visualized for identification and quantification and can also be saved as electronic images for documentation.[4,5]

In this application note, the identification test for fixed oils by TLC, as specified in the USP general chapter <202> (Method II), is conducted using the new TLC Explorer documentation system.

The TLC Explorer documentation system enables the digital and automated evaluation of TLC plates, enhancing the efficiency and accuracy of thin layer chromatography analyses. The device offers three illumination modes using LED light sources—white light (VIS), UV-A (366 nm), and UV-C (254 nm) – for the detection and fast measurement of the compounds of interest. The software offers special features like automated track recognition, simultaneous measurement of multiple plates and background signal correction. Overall, the TLC Explorer offers accurate TLC imaging for reliable video densitometric measurements, enabling quantitative analysis and reliable data interpretation (read more at **SigmaAldrich.com/tlc-explorer**).

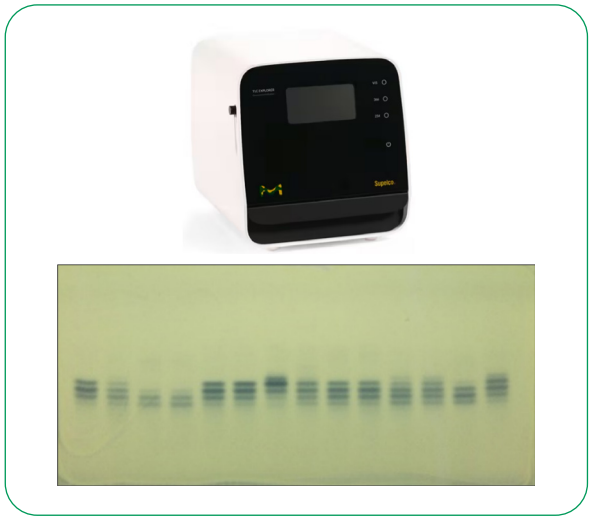

Figure 1. TLC Explorer System (**top**) and derivatized TLC plate scanned under white light by TLC Explorer (**bottom**).

Experimental

Reagent Preparation

Mobile phase: Mix methylene chloride, acetic acid glacial and acetone in a ratio of 20:40:50, v:v:v.

Spray reagent (25 mg/mL phosphomolybdic acid in 96% alcohol): Dissolve 1.26 g of phosphomolybdic acid hydrate in 50 mL of 96% ethanol.

Standard Preparation

System suitability solution 1: Dissolve 25 µL of the USP corn oil RS in 3 mL of methylene chloride.

System suitability solution 2: Dissolve 25 µL of the USP olive oil RS in 3 mL of methylene chloride.

Standard solutions I-XIV: Dissolve 25 µL of the USP reference standards of the fixed oils mentioned under **Table 1** in 3 mL of methylene chloride.

Table 1. Preparation of standard solutions for fixed oil analysis

Solution Name	Fixed oil
Standard solution I	Peanut oil
Standard solution II	Flax seed oil
Standard solution III	Borage seed oil
Standard solution IV	Cotton seed oil
Standard solution V	Palm oil
Standard solution VI	Safflower oil
Standard solution VII	Soyabean oil
Standard solution VIII	Corn oil
Standard solution IX	Olive oil
Standard solution X	Sunflower oil
Standard solution XI	Evening primrose oil
Standard solution XII	Sesame oil
Standard solution XIII	Canola oil
Standard solution XIV	Almond oil

Sample Preparation

Sample solutions I-XIV: Dissolve 25 µL of the market samples of the fixed oils mentioned under **Table 1** in 3 mL of methylene chloride.

TLC Conditions

The 30 solutions were applied across two HPTLC glass plates (Silica gel 60 RP-18 F_{254S}) and developed using the TLC method described in **Table 2**.

Table 2. TLC conditions for fixed oil analysis

TLC parameters	
Plate:	HPTLC glass plate, Silica gel 60 RP-18 F_{254S} 20 x 10 cm (**1.16225**)
Plate pre-treatment:	Predevelop the plate with methylene chloride to the upper edge. Dry the plate at 120 °C for 10 min.
Sample application:	2 µL as bands of 8 mm for each solution
Plate conditioning:	Condition the plate at relative humidity of 33% using saturated magnesium chloride solution for 10 minutes
Mobile phase:	Methylene chloride:acetic acid glacial:acetone (20:40:50; v:v:v)
Chamber conditions:	Twin trough chamber with chamber saturation
Migration distance:	7 cm
Drying:	Air-drying
Derivatization:	Treat the plate with spray reagent and heat at 120 °C for 3 minutes
Detection:	VIS (white light)

Results

System Suitability and Analysis

The identification of fixed oils performed according to USP general chapter <202> on the TLC Explorer under white light is demonstrated in **Figure 2**. **Table 3** summarizes the system suitability results observed for USP corn oil RS and USP olive oil RS, while **Table 4** summarizes the obtained chromatographic results for the fixed oil reference standards and their respective market samples.

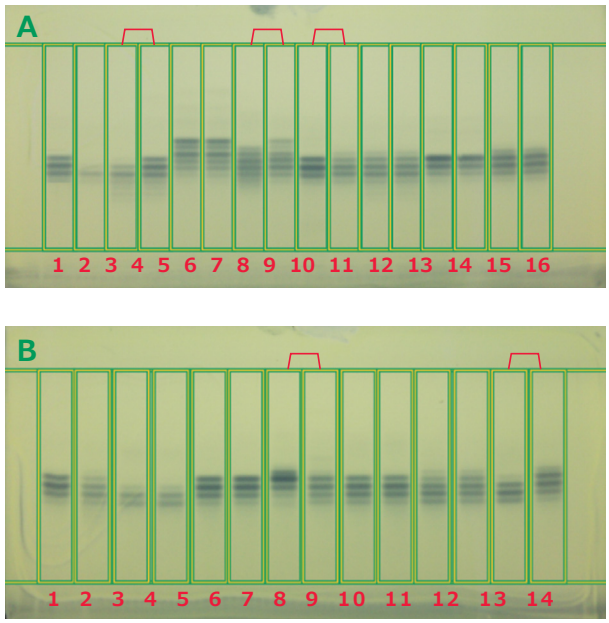

Figure 2. (**top**) TLC chromatogram of plate A demonstrating the system suitability studies using corn oil (track 1) and olive oil (track 2),as well as identification studies of fixed oil standards (odd track no.) and their market samples (even track no.) with tracks marked that showed deviations: peanut oil (tracks 3, 4), flax seed oil (tracks 5, 6), borage seed oil (tracks 7, 8), cotton seed oil (tracks 9, 10), palm oil (11, 12), safflower oil (13, 14) and soybean oil (tracks 15, 16) under white light by the TLC Explorer;

(**bottom**) TLC chromatogram of plate B demonstrating the identification studies of fixed oil standards (odd track no.) and their samples: corn oil (tracks 1, 2), olive oil (tracks 3, 4), sunflower oil (tracks 5, 6), evening primrose oil (tracks 7, 8), sesame oil (tracks 9, 10), canola oil (11, 12) and almond oil (13, 14) under white light by the TLC Explorer. (Oils where differences in the band pattern were observed between standard and market samples are marked on top of the tracks).

Table 3. System suitability data (plate A) observed for USP corn oil RS and USP olive oil RS.

Plate	Fixed oil	Solution name	Standard solution track no.	R_F of fixed oil bands
A	Corn oil	System suitability solution 1	Track 1	0.337
				0.370
				0.411
				0.450
	Olive oil	System suitability solution 2	Track 2	0.335
				0.368

As required by USP general chapter <202>, the four principal spots obtained from corn oil (plate A, track 1) are clearly identified and separated, and the two principal bands obtained from olive oil (plate A, track 2, see also plate B track 3) are clearly identified and separated.

Table 4. Chromatographic data (R_f) observed for standard solutions and sample solutions under white light by the TLC Explorer

Plate	Fixed oil	Standard solution	Standard solution track no.	R_F of bands	Sample solution	Sample solution track no.	R_F of bands
A	Peanut oil	Standard solution I	3	0.334	Sample solution I	4	0.332
				0.367			0.366
				0.406			0.406
				n.d*			0.440
	Flax seed oil	Standard solution II	5	0.399	Sample solution II	6	0.399
				0.428			0.428
				0.465			0.465
				0.500			0.501
				0.532			0.530
	Borage seed oil	Standard solution III	7	0.333	Sample solution III	8	0.334
				0.368			0.366
				0.401			0.401
				0.432			0.435
				0.466			0.468
				0.495			0.494
				n.d*			0.529
	Cotton seed oil	Standard solution IV	9	0.330	Sample solution IV	10	0.330
				0.363			0.365
				0.400			0.401
				0.439			0.438
				n.d*			0.468
	Palm oil	Standard solution V	11	0.330	Sample solution V	12	0.330
				0.367			0.366
				0.405			0.406
				0.444			0.445
	Safflower oil	Standard solution VI	13	0.338	Sample solution VI	14	0.337
				0.372			0.373
				0.406			0.407
				0.446			0.447
	Soyabean oil	Standard solution VII	15	0.345	Sample solution VII	16	0.349
				0.374			0.378
				0.412			0.415
				0.450			0.454
B	Corn oil	Standard solution VIII	1	0.376	Sample solution VIII	2	0.376
				0.414			0.414
				0.454			0.452
				0.497			0.494
	Olive oil	Standard solution IX	3	0.369	Sample solution IX	4	0.369
				0.410			0.410
	Sunflower oil	Standard solution X	5	0.371	Sample solution X	6	0.373
				0.407			0.407
				0.447			0.447
				0.488			0.489
	Evening primrose oil	Standard solution XI	7	0.373	Sample solution XI	8	0.375
				0.408			0.409
				0.447			0.449
				0.487			0.488
				0.516			n.d*
	Sesame oil	Standard solution XII	9	0.377	Sample solution XII	10	0.377
				0.413			0.412
				0.452			0.452
				0.492			0.493
	Canola oil	Standard solution XIII	11	0.379	Sample solution XIII	12	0.381
				0.418			0.418
				0.454			0.456
				0.488			0.488
	Almond oil	Standard solution XIV	13	0.385	Sample solution XIV	14	0.388
				0.422			0.426
				0.464			0.466
				0.499			0.505
				n.d*			0.536

* not detected

Conclusion

A method was utilized for the identification of fixed oils using TLC in accordance with USP general chapter <202>. The assessment and the documentation of the chromatographic results were done with the TLC Explorer documentation system. The R_F values of the principal bands in the chromatogram obtained from most fixed oil sample solutions correspond to those obtained from the respective standard solution. However, the market samples for five oils, namely peanut oil (plate A, track 4) borage seed oil (plate A, track 8), cotton seed oil (plate A, track 10), evening primrose oil (plate B, track 8), and almond oil (plate B, track 14), exhibited differences in the band pattern from their respective USP RS standards (marked track sets in **Figure 2**).

This application demonstrates that for this test the TLC Explorer documentation system has proven to be an efficient and practical approach for data collection and documentation, track identification, and R_f calculation.

References

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Product List

Description	Cat. No.
Digital TLC analysis and documentation device	
TLC Explorer, digital TLC analysis and documentation device	**1.52610**
TLC Plate	
HPTLC Silica gel 60 RP-18 F_{254S} Glass plates 20 x 10 cm, PK.25	**1.16225**
Solvents and Reagents	
Methylene chloride, for liquid chromatography LiChrosolv®	**1.06044**
Acetic acid glacial, for chromatography	**6.18665**
Acetone, HPLC Plus, for HPLC, GC and residue analysis, ≥99.9%	**650501**
Magnesium chloride, anhydrous, ≥98%	**M8266**
Phosphomolybdic acid hydrate, ACS reagent	**221856**
Ethyl alcohol, pure, ≥99.5% (GC), EMPARTA® ACS reagent	**1.07017**
Water, suitable for HPLC	**270733**
Reference Materials	
Corn oil, United States Pharmacopeia (USP) Reference Standard	**1148806**
Olive oil, United States Pharmacopeia (USP) Reference Standard	**1478254**
Sunflower oil, United States Pharmacopeia (USP) Reference Standard	**1642347**
Evening primrose oil, United States Pharmacopeia (USP) Reference Standard	**1269017**
Sesame oil, United States Pharmacopeia (USP) Reference Standard	**1612404**
Canola oil, United States Pharmacopeia (USP) Reference Standard	**1090150**
Almond oil, United States Pharmacopeia (USP) Reference Standard	**1013206**
Peanut oil, United States Pharmacopeia (USP) Reference Standard	**1500557**
Flax seed oil, United States Pharmacopeia (USP) Reference Standard	**1270774**
Borage seed oil, United States Pharmacopeia (USP) Reference Standard	**1076057**
Cotton seed oil, United States Pharmacopeia (USP) Reference Standard	**1150207**
Palm oil, United States Pharmacopeia (USP) Reference Standard	**1492040**
Safflower oil, United States Pharmacopeia (USP) Reference Standard	**1607200**
Soybean oil, United States Pharmacopeia (USP) Reference Standard	**1617500**

More details on the TLC Explorer Documentation System are available at **SigmaAldrich.com/tlc-explorer**

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