

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).

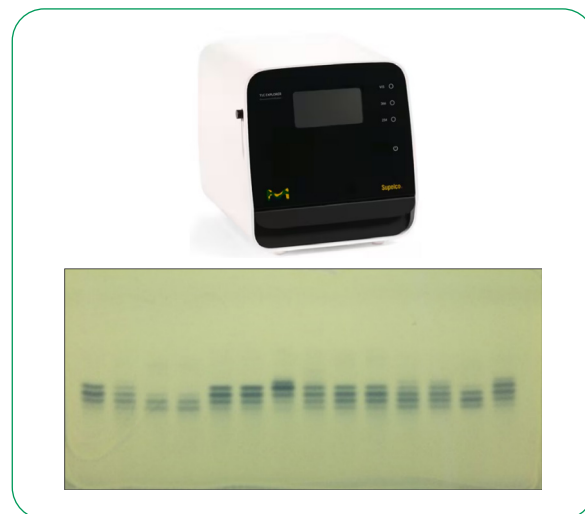


Figure 1. TLC Explorer System (top) and derivatized TLC plate scanned under white light by TLC Explorer (bottom).

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.

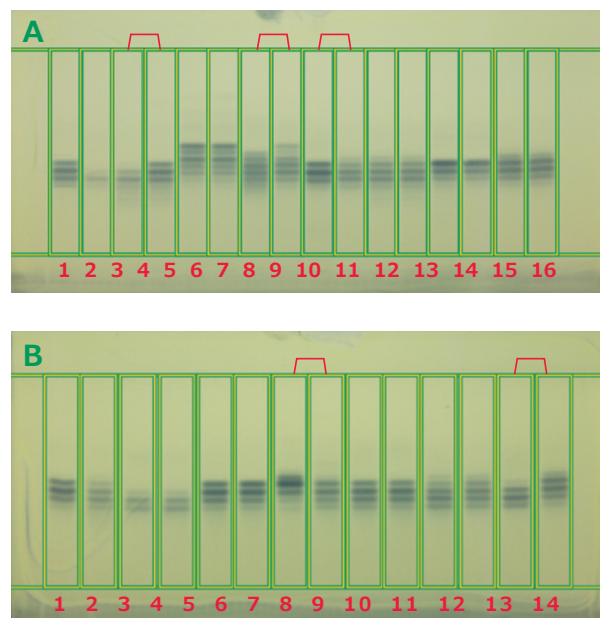


Figure 2. (top) TLC chromatogram of plate A demonstrating the system suitability studies using corn oil (track 1) and olive oil (track 2), as well as identification studies of fixed oil standards (odd track no.) and their market samples (even track no.) with tracks marked that showed deviations: peanut 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
	Borage seed oil	Standard solution III	7	0.532	Sample solution III	8	0.530
				0.333			0.334
				0.368			0.366
				0.401			0.401
	Cotton seed oil	Standard solution IV	9	0.432	Sample solution IV	10	0.435
				0.466			0.468
				0.495			0.494
				n.d*			0.529
	Palm oil	Standard solution V	11	0.330	Sample solution V	12	0.330
				0.363			0.365
				0.400			0.401
				0.439			0.438
	Safflower oil	Standard solution VI	13	n.d*	Sample solution VI	14	0.468
				0.330			0.330
				0.367			0.366
				0.405			0.406
	Soyabean oil	Standard solution VII	15	0.444	Sample solution VII	16	0.445
				0.338			0.337
				0.372			0.373
				0.406			0.407
B	Corn oil	Standard solution VIII	1	0.446	Sample solution VIII	2	0.447
				0.345			0.349
				0.374			0.378
				0.412			0.415
	Olive oil	Standard solution IX	3	0.450	Sample solution IX	4	0.454
				0.376			0.376
				0.414			0.414
				0.454			0.452
	Sunflower oil	Standard solution X	5	0.497	Sample solution X	6	0.494
				0.369			0.369
				0.410			0.410
				0.371			0.373
	Evening primrose oil	Standard solution XI	7	0.407	Sample solution XI	8	0.407
				0.447			0.447
				0.488			0.489
				0.373			0.375
	Sesame oil	Standard solution XII	9	0.408	Sample solution XII	10	0.409
				0.447			0.449
				0.487			0.488
				0.516			n.d*
	Canola oil	Standard solution XIII	11	0.377	Sample solution XIII	12	0.377
				0.413			0.412
				0.452			0.452
				0.492			0.493
	Almond oil	Standard solution XIV	13	0.379	Sample solution XIV	14	0.381
				0.418			0.418
				0.454			0.456
				0.488			0.488
				0.385			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.

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 ₂₅₄ S 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

Find more information on our TLC portfolio at SigmaAldrich.com/tlc

To place an order or receive technical assistance

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References

1. Bhattacharya S. Prophylactic Role of Dietary Fixed Oils and Fats: A Review. Journal of Advanced Pharmacy Education & Research. 2012 [accessed January 30, 2025];2(4):177-184. <https://japer.in/prophylactic-role-of-dietary-fixed-oils-and-fats-a-review.pdf>
2. Ali S, Ekbbal R, Salar S, Yasheshwar, Ali SA, Jaiswal AK, Singh M, Yadav DK, Kumar S, Gaurav. Quality Standards and Pharmacological Interventions of Natural Oils: Current Scenario and Future Perspectives. ACS Omega. 2023;8(43):39945-39963. <https://doi.org/10.1021/acsomega.3c05241>
3. USP general chapter <202>: Identification of Fixed Oils by Thin-Layer Chromatography. https://doi.usp.org/USPNF/USPNF_M6338_01_01
4. Attimarad M, Ahmed KK, Aldhubaib BE, Harsha S. High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery. Pharm Methods. 2011;2(2):71-5. <https://doi.org/10.4103/2229-4708.84436>
5. Srivastav M. An Overview of HPTLC: A Modern Analytical Technique with Excellent Potential for Automation, Optimization, Hyphenation, and Multidimensional Applications. Berlin, Heidelberg: Springer; 2011. https://doi.org/10.1007/978-3-642-14025-9_1

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