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Application Note

Identification of Curcuminoids as per USP Monograph Using the TLC Explorer

Valanka D'Silva
R&D APAC Lab, Jigani, Bangalore, India

Abstract

This study utilizes the TLC Explorer to identify curcuminoids by TLC, following USP monograph guidelines. The system enhances TLC efficiency through automation and multiple illumination modes, accurately distinguishing curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Results confirm the suitability of the TLC Explorer for curcuminoid analysis.

Introduction

Curcuminoids are bioactive compounds present in the roots of turmeric (*Curcuma longa*), a spice widely used in Asian cuisines. These compounds include curcumin, demethoxycurcumin, and bisdemethoxycurcumin.

Known for their potential health benefits, curcuminoids possess anti-inflammatory, antioxidant, and anticancer properties.[1] They are often researched for their impact on various health issues, such as arthritis, cardiovascular diseases, and specific cancers. Typically, curcuminoids are consumed through turmeric supplements, extracts, or as part of the diet from turmeric spice.[2]

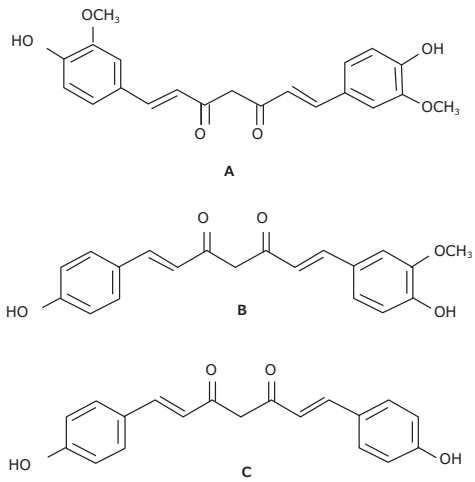

Figure 1. Chemical structures of curcumin (**A**), demethoxycurcumin (**B**), and bisdemethoxycurcumin (**C**).

The United States Pharmacopeia (USP) monograph for curcuminoids identifies thin layer chromatography (TLC) as one method for testing identification.[3] TLC is frequently referenced in pharmacopeial methods for identity testing. High-Performance Thin Layer Chromatography (HPTLC), a high-performance version of TLC and often used with automation, is a robust, reliable, rapid, and cost-effective technique used for the qualitative and quantitative analysis of pharmaceutical compounds. This method produces chromatographic fingerprints that can be visualized and stored as electronic images.[4,5] This application note presents the identification test for curcuminoids, including curcumin, demethoxycurcumin, and bisdemethoxycurcumin, as specified in the USP monograph, performed using the new TLC Explorer documentation system.

The TLC Explorer documentation system (**Figure 2**) 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 densitometric measurements, enabling quantitative analysis and reliable data interpretation.
(read more at **SigmaAldrich.com/tlc-explorer**)

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Experimental

Reagent Preparation

Mobile phase: Mix toluene and acetic acid glacial in a ratio of 4:1, v:v.

Derivatization reagent: Add 10 mL of acetic acid glacial and 5 mL of sulfuric acid to 85 mL of ice-cold methanol slowly. Mix and allow the mixture to cool to room temperature. Then add 0.5 mL of p-anisaldehyde and mix well.

Standard Preparation

Curcuminoids standard solution (1 mg/mL): Weigh and dissolve 5 mg of USP Curcuminoids RS in 5 mL of methanol.

Curcumin standard solution (1 mg/mL): Weigh and dissolve 5 mg of curcumin in 1 mL of methanol.

Demethoxycurcumin standard solution (1 mg/mL): Weigh and dissolve 5 mg of demethoxycurcumin in 5 mL of methanol.

Bisdemethoxycurcumin standard solution (1 mg/mL): Weigh and dissolve 5 mg of bisdemethoxycurcumin in 5 mL of methanol.

Sample Preparation

Test solutions I + II: Weigh 5 mg of curcuminoids in 5 mL of methanol. Sonicate for 10 minutes and centrifuge at 3000 rpm for 5 minutes. The resulting supernatant solution contains 1 mg/mL of curcuminoids.

Instrument Parameters

Table 1. TLC conditions

TLC Parameters	
Plate:	HPTLC aluminum sheets, Silica gel 60 F_{254}, 20 x 20 cm (**1.05548**), cut into 10 x 20 cm
Sample application:	2 µL of each solution
Plate conditioning:	Condition the plate at relative humidity of 33% using saturated magnesium chloride solution for 10 minutes
Mobile phase:	Toluene:acetic acid glacial (4:1 v:v)
Chamber conditions:	Twin trough chamber with chamber saturation
Migration distance:	7 cm
Drying:	Air-drying
Derivatization:	Spray the plate with derivatization reagent and heat at 100 °C for 3 minutes
Detection:	VIS and UV at 254 nm & 366 nm; evaluation under VIS and UV 366 nm

Results

The identification of curcuminoid extract performed according to USP monograph prior to derivatization under visible/white light (VIS) and 366 nm (here in addition also under 254 nm for comparison) on the TLC Explorer is demonstrated in **Figure 3**, and after derivatization under VIS and 366 nm is demonstrated in **Figure 4**. The derivatized chromatogram of the standard solutions under UV 366 nm shows three bands in the order of increasing R_f: an orange band due to bisdesmethoxycurcumin, an orange band due to desmethoxycurcumin, and a red band due to curcumin as described in the monograph. Under white light, the two lower bands appear orange, while the topmost band is reddish-pink. **Table 2** summarizes the obtained chromatographic results.

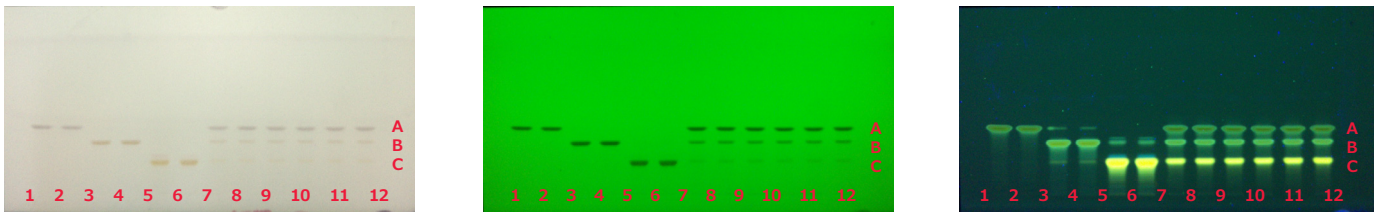

Figure 3. TLC chromatogram demonstrating the identification of curcuminoid extract prior to derivatization under VIS (left), UV 254 nm (middle) and UV 366 nm (right) by the TLC Explorer. Band IDs: curcumin(**A**), demethoxycurcumin (**B**), and bisdemethoxycurcumin (**C**). Track allocation see **Table 2**.

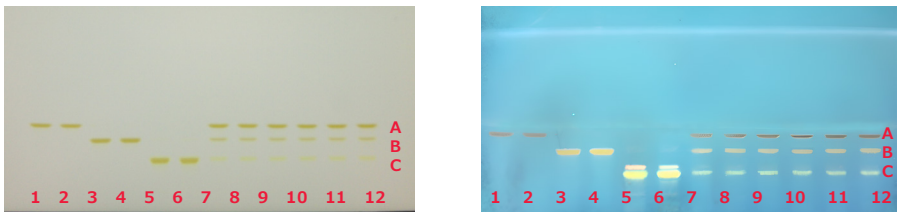

Figure 4. TLC chromatogram demonstrating the identification of curcuminoid extract after derivatization under VIS (left) and UV 366 nm (right) by the TLC Explorer. Band IDs: curcumin (**A**), demethoxycurcumin (**B**), and bisdemethoxycurcumin (**C**). Track allocation see **Table 2**.

Table 2. Chromatographic data observed for standard solutions and test solutions under UV 366 nm and determined by the TLC Explorer

Tracks no.	Solution name	Retention factor of Curcumin	Retention factor of Demethoxycurcumin	Retention factor of Bisdemethoxycurcumin
1 & 2	Curcumin standard solution	0.372	n.a.*	n.a.*
3 & 4	Demethoxycurcumin standard solution	n.a.*	0.270	n.a.*
5 & 6	Bisdemethoxycurcumin standard solution	n.a.*	n.a.*	0.139
7 & 8	Curcuminoids standard solution	0.370	0.280	0.150
9 & 10	Test solution I	0.367	0.282	0.151
11 & 12	Test solution II	0.368	0.282	0.153

*n.a.: not applicable

Conclusion

The derivatized chromatogram of the test solutions reveals two orange bands and one red band, which closely match the position and color of those found in the curcuminoid standard solution under VIS and UV 366 nm as described in the USP monograph. Under white light, the two lower orange bands and the upper darker red band correspond to bisdemethoxycurcumin, demethoxycurcumin, and curcumin in the curcuminoid standard solution, arranged in order of increasing retention factor.

This application note illustrates that utilizing the TLC Explorer documentation system for this test provides efficient data collection, 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 aluminum sheets, Silica gel 60 F_{254}, 20 x 20 cm, Pk.25	**1.05548**
Solvents, Reagents, Consumables, and Reference Materials	
Methanol, ≥99.8% (GC), HPLC grade, suitable for HPLC, LiChrosolv®	**1.06018**
Toluene, for liquid chromatography LiChrosolv®	**1.08327**
Acetic acid glacial, for chromatography	**6.18665**
Sulfuric acid, 98%, for HPLC LiChropur™	**5.43827**
p-Anisaldehyde, 98%	**A88107**
Magnesium chloride, anhydrous, ≥98%	**M8266**
Curcuminoids, United States Pharmacopeia (USP) Reference Standard	**1151866**
Curcumin, analytical standard	**08511**
Demethoxycurcumin, analytical standard	**90593**
Bisdemethoxycurcumin, analytical standard	**90594**

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More details on the TLC Explorer Documentation System is available at
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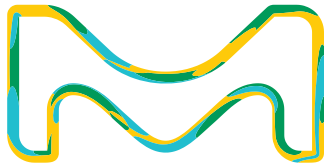

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