

Identification of Dexamethasone and its Derivatives by Thin-Layer Chromatography (TLC)

According to European Pharmacopoeia by using TLC Explorer Documentation System

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Introduction

Dexamethasone (and its derivatives) is one of the most important drugs used to suppress the immune system and fight inflammation in the body. Recently, it has gained huge interest because of its use in Covid-19 infection. It reduces the body's excessive defense reaction. It is mainly prescribed to patients who are already hospitalized with severe symptoms. Studies have shown that the use of dexamethasone, together with other medical treatments such as supplemented oxygen, reduced the mortality rate by 25-35%.^{1, 2}

In this application note we show different chromatography studies described in the European Pharmacopoeia (EP).³ The identification of the substances was performed after separation by Thin-layer chromatography (TLC) method. TLC is an easy, inexpensive, and flexible method for a quick chromatographic analysis and therefore still established in compendial methods such as EP or USP. The analysis, data taking and archiving of the chromatograms were performed with the TLC Explorer device.

The first experiment (**Figure 1**) describes the identification of dexamethasone, the second (**Figure 2**) is focusing on the analysis of dexamethasone acetate, the third (**Figure 3**) on dexamethasone sodium phosphate. As requested in the method, the principal spot in the chromatogram obtained with the test solution must be similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

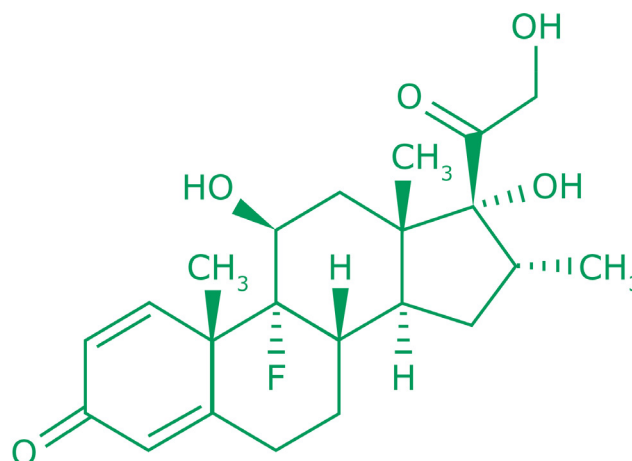


Figure 1. Structure of dexamethasone

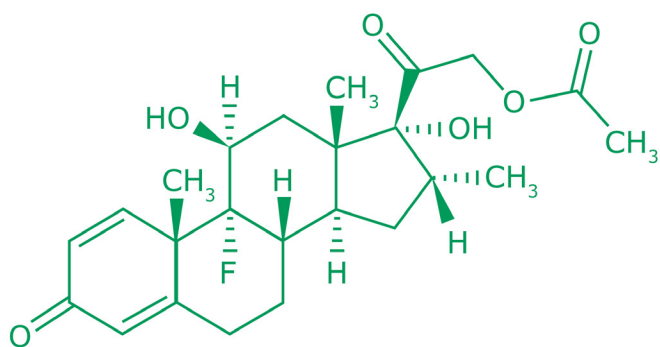


Figure 2. Structure of dexamethasone acetate

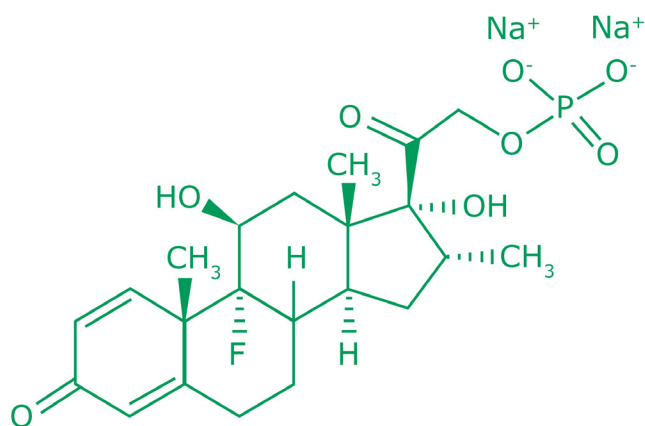


Figure 3. Structure of dexamethasone sodium phosphate

Experimental

All experimental conditions of application 1-3 are summarized in Table 1-3.

Table 1. Chromatographic Conditions of Identification Test of Dexamethasone.

Conditions	
Plate	TLC Silica gel 60G F _{254r} , 20x20 cm, 1.00390
Reference solution preparation	10 mg of dexamethasone CRS diluted to 10 mL of the mobile phase
Test solution preparation	10 mg of the examined substance diluted with 10 mL of the solvent mixture
Applied substances	1 Reference solution 2 - 4 Test solution 5 Reference solution 6 - 8 Test solution 9 Reference solution
Application volume	5.0 µl of each solution
Mobile phase	Methanol / methylene chloride 1:9 (v/v)
Development time	60 Minutes
Derivatizing	Sprayed the plate with a solution of: 0.25 g dihydroxybenzaldehyde R 87.5 mL glacial acetic acid R 12.5 mL sulfuric acid R
Documentation	TLC Explorer
Detection	Wavelength VIS light, UV light 366 nm
	hRF-Value 38

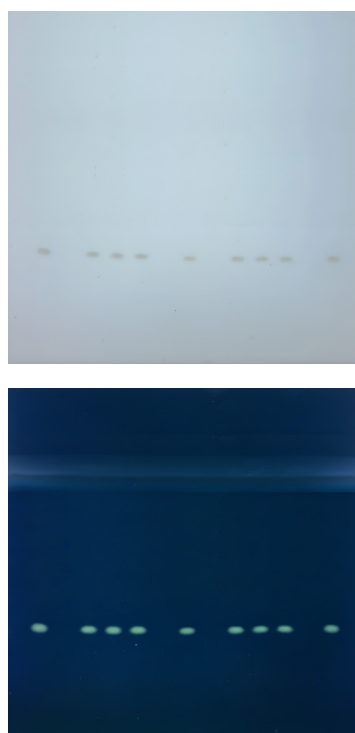


Figure 4. TLC Analysis of plate by the TLC Explorer under VIS excitation (top) and UV 366 nm excitation (bottom). The tracks 1, 5 and 9 are the references of dexamethasone and 2-4 and 6-8 are the samples. The plates were derivatized with a mixture of dihydroxybenzaldehyde, glacial acetic acid and sulfuric acid.

Table 2. Chromatographic Conditions of Identification Test of Dexamethasone Acetate.

Conditions		
Plate	TLC Silica gel 60G F _{254r} , 20x20 cm, 1.00390	
Reference solution preparation	10 mg of dexamethasone acetate CRS diluted to 10 mL of the mobile phase	
Test solution preparation	10 mg of the examined substance diluted with 10 mL of the solvent mixture	
Applied substances	1 Reference solution 2 - 4 Test solution 5 Reference solution 6 - 8 Test solution 9 Reference solution	
Application volume	5.0 µl of each solution	
Mobile phase	Methanol / methylene chloride 1:9 (v/v)	
Development time	60 Minutes	
Derivatizing	Sprayed the plate with a solution of: 0.25 g dihydroxybenzaldehyde R 87.5 mL glacial acetic acid R 12.5 mL sulfuric acid R	
Documentation	TLC Explorer	
Detection	Wavelength	VIS light, UV light 366 nm
	hRf-Value	72

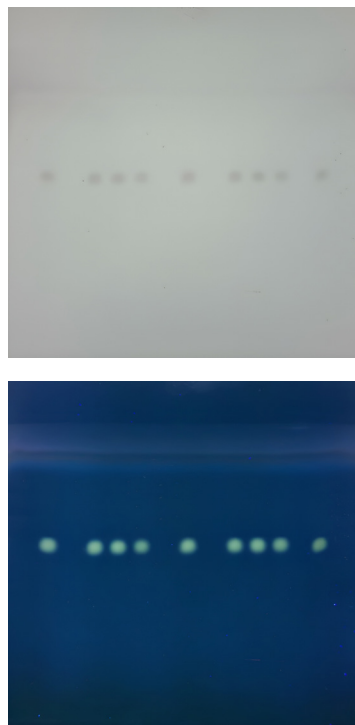


Figure 5. TLC Analysis of plate by the TLC Explorer under VIS excitation (top) and UV 366 nm excitation (bottom). The tracks 1, 5 and 9 are the references of dexamethasone acetate and 2-4 and 6-8 are the samples. The plates were derivatized with a mixture of dihydroxybenzaldehyde, glacial acetic acid and sulfuric acid.

Table 3. Chromatographic Conditions of Identification Test of Dexamethasone Sodium Phosphate.

Conditions		
Plate	TLC Silica gel 60G F _{254r} , 20x20 cm, 1.00390	
Reference solution preparation	10 mg of dexamethasone sodium phosphate CRS diluted with 10 mL of Methanol	
Test solution preparation	10 mg of the examined substance diluted with 10 mL of Methanol	
Applied substances	1 Reference solution 2 - 4 Test solution 5 Reference solution 6 - 8 Test solution 9 Reference solution	
Application volume	5.0 µL of each solution	
Mobile phase	Glacial acetic acid / Water / Butanol 20:20:60 (v/v/v)	
Development time	150 Minutes	
Derivatizing	Sprayed the plate with a solution of: 0.25 g dihydroxybenzaldehyde R 87.5 mL glacial acetic acid R 12.5 mL sulfuric acid R	
Documentation	TLC Explorer	
Detection	Wavelength	VIS light, UV light 366 nm
	hRf-Value	65



Figure 6. TLC Analysis of plate by the TLC Explorer under VIS excitation (top) and UV 366 nm excitation (bottom). The tracks 1, 5 and 9 are the dexamethasone sodium phosphate, reference and 2-4 and 6-8 are the samples. The plates were derivatized with a mixture of dihydroxybenzaldehyde, glacial acetic acid, and sulfuric acid.

Discussion

As demonstrated in experiments 1-3, all principal spots in the chromatogram obtained with the test solution are similar in position, color and size to the principal spot in the chromatogram obtained with the reference solution.

The usage of this TLC plate documentation system has confirmed reliable and convenient data collection, track identification, and R_f calculation. (see **Figure 4-6**).

Featured Products

Description	Cat.No.
TLC Explorer	1.52610
TLC-Kieselgel 60G F ₂₅₄ 25 Glass plates, 20 x 20 cm	1.00390
Dexamethasone, European Pharmacopoeia (EP) Reference Standard	D0700000
Dexamethasone acetate, European Pharmacopoeia (EP) Reference Standard	D0710000
Dexamethasone sodium phosphate, European Pharmacopoeia (EP) Reference Standard	D0720000
Methanol gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	1.06007
Butanol for liquid chromatography LiChrosolv®	1.01988
Acetic acid 100% (glacial) for HPLC LiChropur™	543808
Water suitable for HPLC	270733
Methylene chloride for liquid chromatography LiChrosolv®	1.06044
Sulfuric acid 98% for HPLC LiChropur™	5.43827
2, 4-Dihydroxybenzaldehyde 98%	168637

Reference

1. Low-cost dexamethasone reduces death by up to one third in hospitalised patients with severe respiratory complications of COVID-19. Notification from the University of Oxford, June 16, 2020.
2. https://www.bfarm.de/SharedDocs/Risikoinformationen/Pharmakovigilanz/DE/RV_STP/a-f/dexamethason.html
3. European Pharmacopoeia. 2023. Method EP 11.4 "Identification of dexamethasone and its derivatives"

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