

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

According to European Pharmacopoeia by using TLC Explorer Documentation System

Markus Burholt, Scientist R&D Analytical Chemistry  
Monika Baeumle, Global Product Manager TLC

## 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%.<sup>1, 2</sup>

In this application note we show different chromatography studies described in the European Pharmacopoeia (EP).<sup>3</sup> 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.

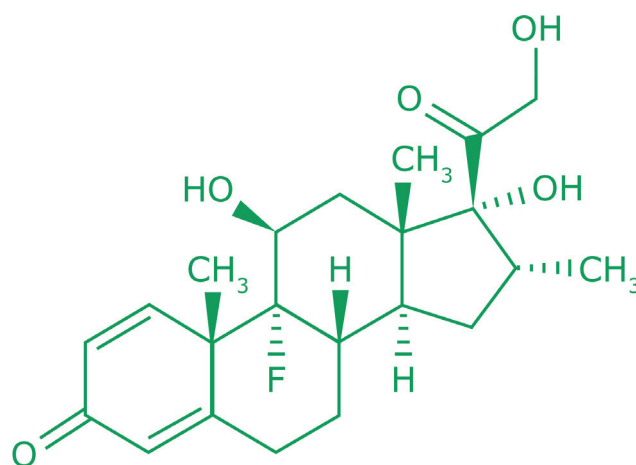


Figure 1. Structure of dexamethasone

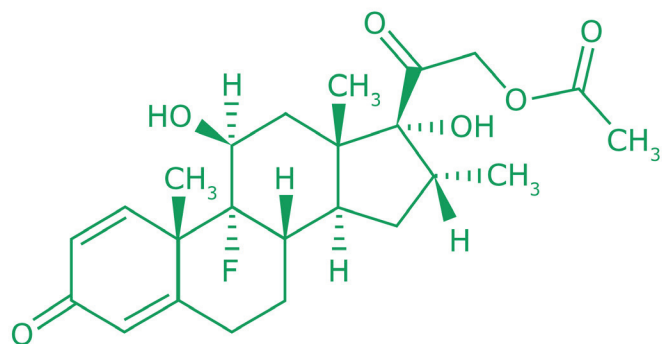


Figure 2. Structure of dexamethasone acetate

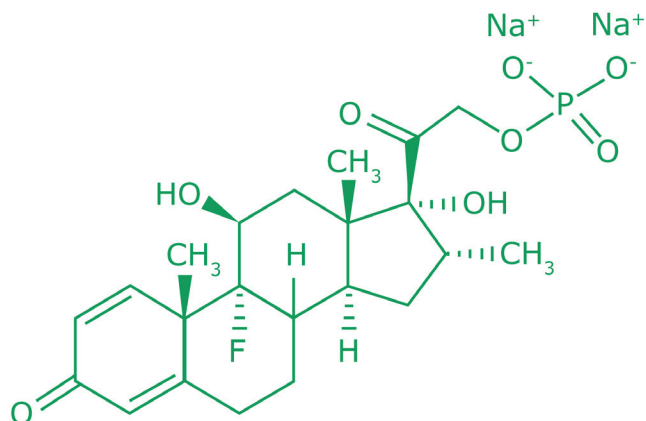


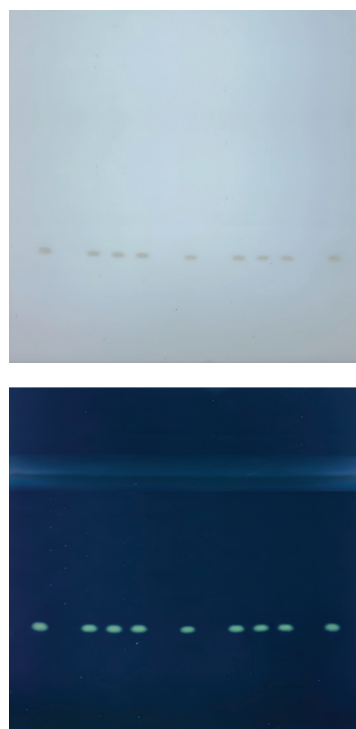
Figure 3. Structure of dexamethasone sodium phosphate

## Experimental

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

**Table 1. Chromatographic Conditions of Identification Test of Dexamethasone.**

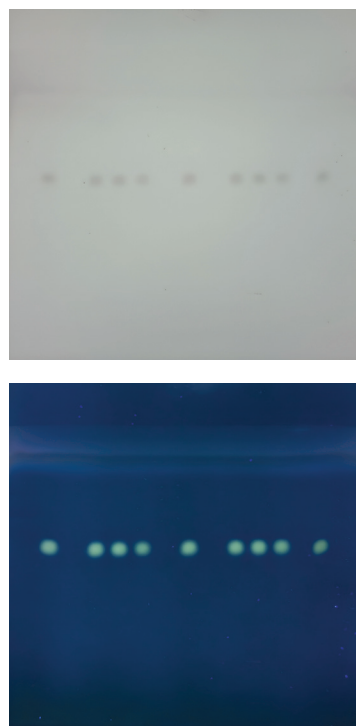
Conditions		
TLC	Plate	TLC Silica gel 60G F <sub>254</sub> , 20x20 cm, 1.00390
	Reference solution preparation	10 mg of dexamethasone CRS diluted to 10 mL of the mobile phase
	Test solution preparation	10 mg of the examined substance diluted with 10 mL of the solvent mixture
	Applied substances	1 Reference 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
Detection	Documentation	TLC Explorer
	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.**

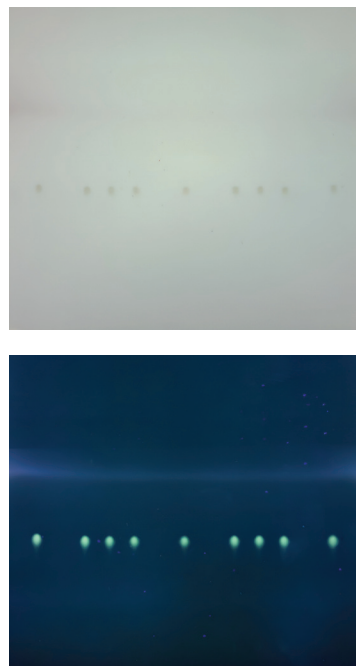
Conditions		
TLC	Plate	TLC Silica gel 60G F <sub>254</sub> , 20x20 cm, 1.00390
	Reference solution preparation	10 mg of dexamethasone acetate CRS diluted to 10 mL of the mobile phase
	Test solution preparation	10 mg of the examined substance diluted with 10 mL of the solvent mixture
	Applied substances	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
Detection	Documentation	TLC Explorer
	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		
TLC	Plate	TLC Silica gel 60G F <sub>254</sub> , 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
Detection	Documentation	TLC Explorer
	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 Rf calculation. (see **Figure 4-6**).

## Featured Products

Description	Cat.No.
TLC Explorer	1.52610
TLC-Kieselgel 60G F254 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](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"

For more information, visit [SigmaAldrich.com/tlc](https://SigmaAldrich.com/tlc)

For any assistance or technical support, please contact your local Tech Service.

## To place an order or receive technical assistance

Order/Customer Service: [SigmaAldrich.com/order](https://SigmaAldrich.com/order)

Technical Service: [SigmaAldrich.com/techservice](https://SigmaAldrich.com/techservice)

We have built a unique collection of life science brands with unrivalled experience in supporting your scientific advancements.

**Millipore® Sigma-Aldrich® Supelco® Milli-Q® SAFC® BioReliance®**

MilliporeSigma  
400 Summit Drive  
Burlington, MA 01803

