APPLICATION NOTE

# In-process control of ergoline psychedelics during chemical synthesis by HPTLC

A-99.1


## Keywords

LSD, LSP, LSM, LSZ, mass detection, in-process control, quality control, HPTLC-MS, scanning densitometry


## Introduction

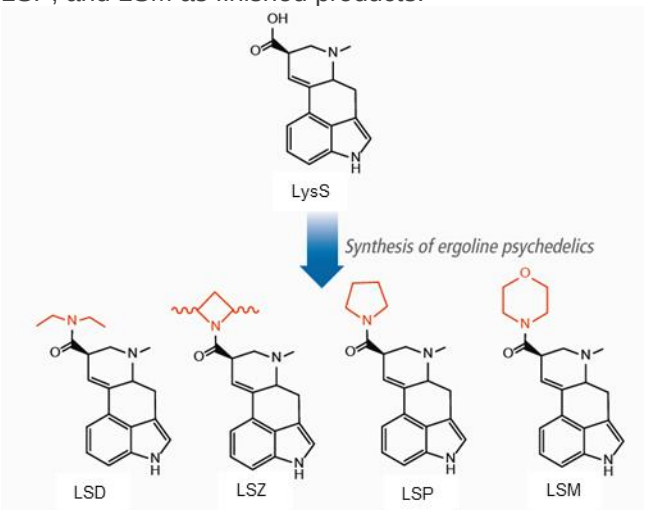
TLC is a simple and convenient tool for monitoring classical organic syntheses. Standardized HPTLC may additionally provide reliable analytical endpoints and significantly improved separation. Structure confirmation can be achieved with HPTLC-MS hyphenation. In addition, HPTLC can be used as a technique complementary to existing HPLC methods for ensuring a product of high purity.

## Scope

This method is suitable for identification of synthesis products. The CAMAG TLC-MS Interface 2 is used to directly elute target zones from the HPTLC plate into the Waters ACQUITY QDa® for mass detection. A second confirmation can be achieved by recording the UV spectra with the TLC scanner. A fast way for in-process and quality control during synthesis of ergoline psychedelics is shown below.

### Synthesis of ergoline psychedelics

In a first step lysergic acid (LysS) is produced from ergotamine as starting material. Then the formation of different amides takes place, with LSD, LSZ, LSP, and LSM as finished products.




*LysS is the starting material for the synthesis of LSD, LSZ, LSP, and LSM; different side chains are highlighted in red*

## Required or recommended devices

Automatic TLC Sampler 4 or Linomat 5, Automatic Developing Chamber ADC 2, Immersion Device 3, TLC Visualizer, visionCATS software, TLC Scanner, TLC-MS Interface 2, Waters ACQUITY QDa Detector (Performance), Empower® or MassLynx® software, optional: UV Cabinet

## Sample

Samples from different synthesis steps are applied directly onto HPTLC plates without further preparation. Depending on the process step the applied volume can vary as well as the solvent (in most cases dichloromethane and/or methanol). The purified synthesis products are dissolved in methanol (1 mg/mL). Samples and purified synthesis products were provided by Lipomed AG (Arlesheim, Switzerland).

## Standards

Methanolic solution of LSD (0.1 mg/mL). Standard was provided by Lipomed AG (Arlesheim, Switzerland).

## Chromatography

| | |
|---|---|
| Stationary phase: | HPTLC Si 60 $F_{254}$, 20 x 10 cm (Merck). |
| Sample application: | Bandwise application with ATS 4 or Linomat 5, 15 tracks, band length 8 mm, track distance 11.4 mm, distance from left edge 20 mm, distance from lower edge 8 mm, application volume between 0.5 und 15 µL for sample solutions, 1 µL for dissolved purified synthesis products and 8 µL for the standard solution. |
| Developing solvent: | Dichloromethane – methanol – trimethylamine 9:1:0.002 (v/v/v)<br>In the case of LSZ: dichloromethane – isopropanol – triethylamine 9:1:0.002 (v/v/v) |
| Development: | In the ADC 2 with chamber saturation (with filter paper) 20 min and after conditioning at 33% relative humidity for 10 min using a saturated solution of magnesium chloride. |
| Developing distance: | 70 mm (from the lower edge) |
| Plate drying: | Drying 5 min in the ADC 2 |
| Documentation: | With the TLC Visualizer under UV 254 and UV 366 nm |
| Densitometry: | Densitometric analyses are performed at 254 nm (absorbance) and 366/>400 nm (fluorescence), slit dimension 5.0 x 0.2 mm, scanning speed 20 mm/s, spectra recording 200 to 500 nm |
| MS confirmation: | The zones to be eluted are marked with a soft pencil under UV 254 nm and/or UV 366 using the UV cabinet or TLC Visualizer.<br>Target zones are directly eluted using the TLC-MS Interface 2 with oval elution head into the ACQUITY QDa Detector at a flow rate of 0.5 mL/min with methanol (with 0.1% formic acid) – water 90:10 (v/v). For a full scan spectrum it is recommended to first elute a blank, which can be subtracted from the spectra of the target zones. For confirmation of substances between 50 and 500 ng per zone are required. |

NOTE: The presented results are to be regarded as examples only!

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MS parameter: The ACQUITY QDa Detector is operated in ESI$^+$ mode using default parameters. The ESI capillary is set to 0.8 kV, cone voltage to 15 V, and desolvation temperature at 600 °C. A full scan mass spectrum between m/z 50 and 650 is acquired at a sampling rate of 10.0 points/sec (continuum). Data processing and evaluation of mass spectra are performed with Empower. For routine use in quality control Single Ion Recording (SIR) can be performed.

## Results

System Suitability Test (SST):
LSD shows a quenching zone (under UV 254 nm) and a blue fluorescent zone (under UV 366 nm) at $R_F$ ~ 0.43

a) In-process control during synthesis of LSP:

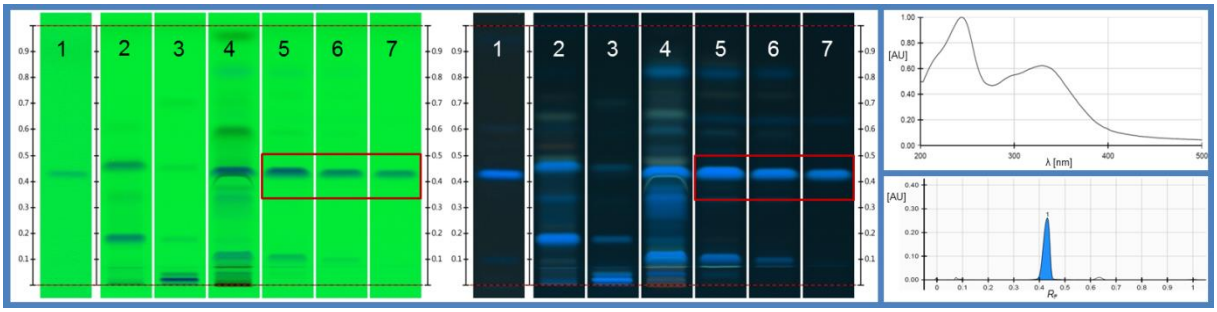


*Fig. 1 Chromatograms under UV 254 nm und 366 nm that show the different synthesis steps, including the purification, 1: LSD as reference, 2: Hydrolysis of lysergic acid from ergotamin, 3: purified lysergic acid (starting material for the chemical synthesis), 4: crude synthesis product (LSP and side products), 5: Column purification, 6: second column purification, 7: purified LSP; UV spectrum and densitogram of LSP 366/>400 nm (fluorescence)*

b) Confirmation of the purified synthesis products:

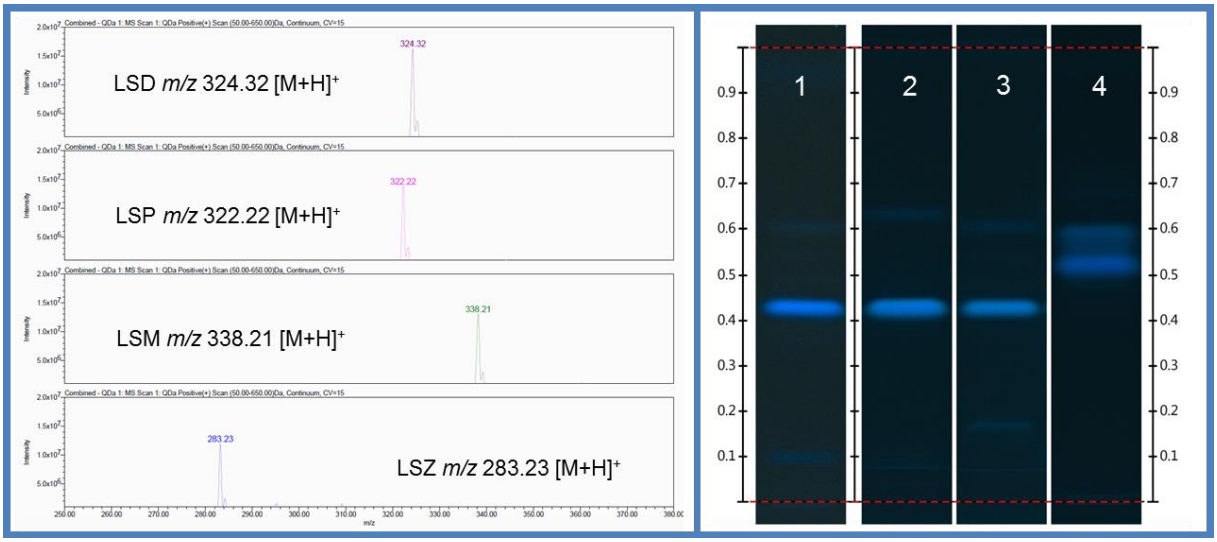


*Fig. 2 HPTLC-MS mass spectra of LSD, LSP, LSM, and LSD, displayed range m/z 250 to 380 and HPTLC Image Comparison of the purified synthesis products under UV 366 nm (left; tracks 1: LSD as reference; 2: purified LSP, 3: purified LSM, 4: purified LSZ(two product zones due to the formation of the isomers [1]))*

## Acknowledgment

This work was done at Lipomed AG (Arlesheim, Switzerland). We are grateful to Dr. Matthias Grill for the excellent collaboration.

## Literature

[1] David E. Nichols *et al.*, J Med Chem 45 (2002) 4344
[2] Alexander Shulgin *et al.*, TIHKAL: The Continuation, 1997
[3] Matthias Grill and Melanie Broszat, CAMAG Bibliogr Service CBS 115 (2015) 11-12