Application Note: 383

Determination of LSD and Its Metabolites in Human Biological Samples by Liquid Chromatography–Tandem Mass Spectrometry

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Key Words

- TSQ Quantum
- Drugs of Abuse
- Forensic analysis
- LC-MS/MS
- LSD (Lysergic acid diethylamide
- LSD metabolites
- Toxicology

Introduction

Lysergic acid diethylamide (LSD) is a very potent hallucinogenic drug involving, particularly, behavioral disorders and is also extensively metabolized in man. Moreover, LSD and its major metabolites are present at low concentration in biological fluids, such as whole blood or urine. Identification and quantitation of such compounds for forensic use necessitate a sensitive and specific method. This study aims to describe a method using liquid chromatography/tandem mass spectrometry and permitting to quantify LSD and its metabolites at low concentrations.

Goal

The goal of this study was to identify and quantify LSD, iso-LSD, nor-LSD, nor-iso-LSD and 2-oxo-3-hydroxy-LSD in biological matrices. This report demonstrates the use of the TSQ Quantum for this application.

Experimental Conditions/Methods

Chemicals and Reagents

Lysergic acid diethylamide (LSD), d_3-LSD (internal standard), 2-oxo-3-hydroxy-LSD, iso-LSD, nor-LSD were purchased from Cerilliant (Austin, TX, USA). Ammonium formate and formic acid (>99 % pure) were purchased from Sigma. All reagents and solvents used in the extraction procedures were of analytical grade.

Sample Preparation

To 2 mL of serum, urine or whole blood content were added 100 µL of a 0.025 µg/mL aqueous solution of d_3-LSD (Internal Standard), 1 mL of a solution of pH 9.50 carbonate buffer and 8 mL of dichloromethane-isopropanol (95:5 by volume). The tubes were vortex-mixed and shaken on an oscillatory mixer. After centrifugation at 3,400 g for 5 min, the organic phase was poured in a conical glass tube and evaporated under a stream of nitrogen at 37°C. The dried extracts were reconstituted in 25 µL of acetonitrile : pH 3.0, 2 mmol/L ammonium formate (30:70 by volume) and 10 µL were injected into the chromatographic system.

Instrumentation Methods

HPLC Conditions

The chromatographic system consisted of a Shimadzu 10ADvp micro-flow rate, high-pressure gradient pumping system with a Rheodyne® Model 7725 injection valve equipped with a 5 µL internal loop. A C18, 5 µm (50×2.1 mm) column, maintained at 25°C, was used with a linear gradient of mobile phase A (pH 3.0, 2 mmol/L ammonium formate) and mobile phase B (acetonitrile:pH 3.0, 2 mmol/L ammonium formate [90:10; v/v]), flow rate of 200 µL/min, programmed as follows: 0-1.5 min, 5% B; 1.5-9 min, 5 to 50% B; 9-10 min, 50 to 90% B; 10-10.5 min, decrease from 90 to 5% B; 10.5-13 min, equilibration with 5% B.

MS Conditions

Mass Spectrometer: Thermo Scientific TSQ Quantum
Source: ESI mode
Ion Polarity: Positive
Spray Voltage: 4000 V
Sheath/Auxiliary gas: Nitrogen
Sheath gas pressure: 25 (arbitrary units)
Auxiliary gas pressure: 15 (arbitrary units)
Ion transfer tube temperature: 250°C
Scan type: SRM
Collision gas: Argon
Collision gas pressure: 1.5 mTorr

SRM Conditions

Settings were optimized by infusing at 5 µL/min a 1 µg/L solution containing the studied compound in acetonitrile: pH 3.0, 2 mmol/L ammonium formate (30:70, by volume). The structure of these compounds is shown in Figure 1.

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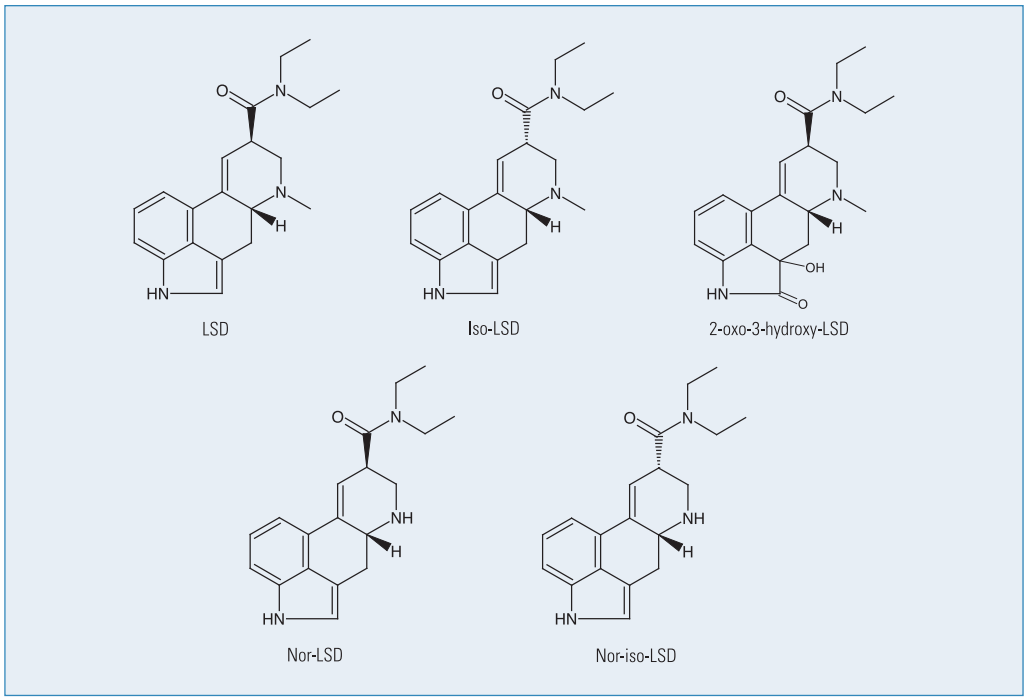

Figure 1: Structures of investigated compounds

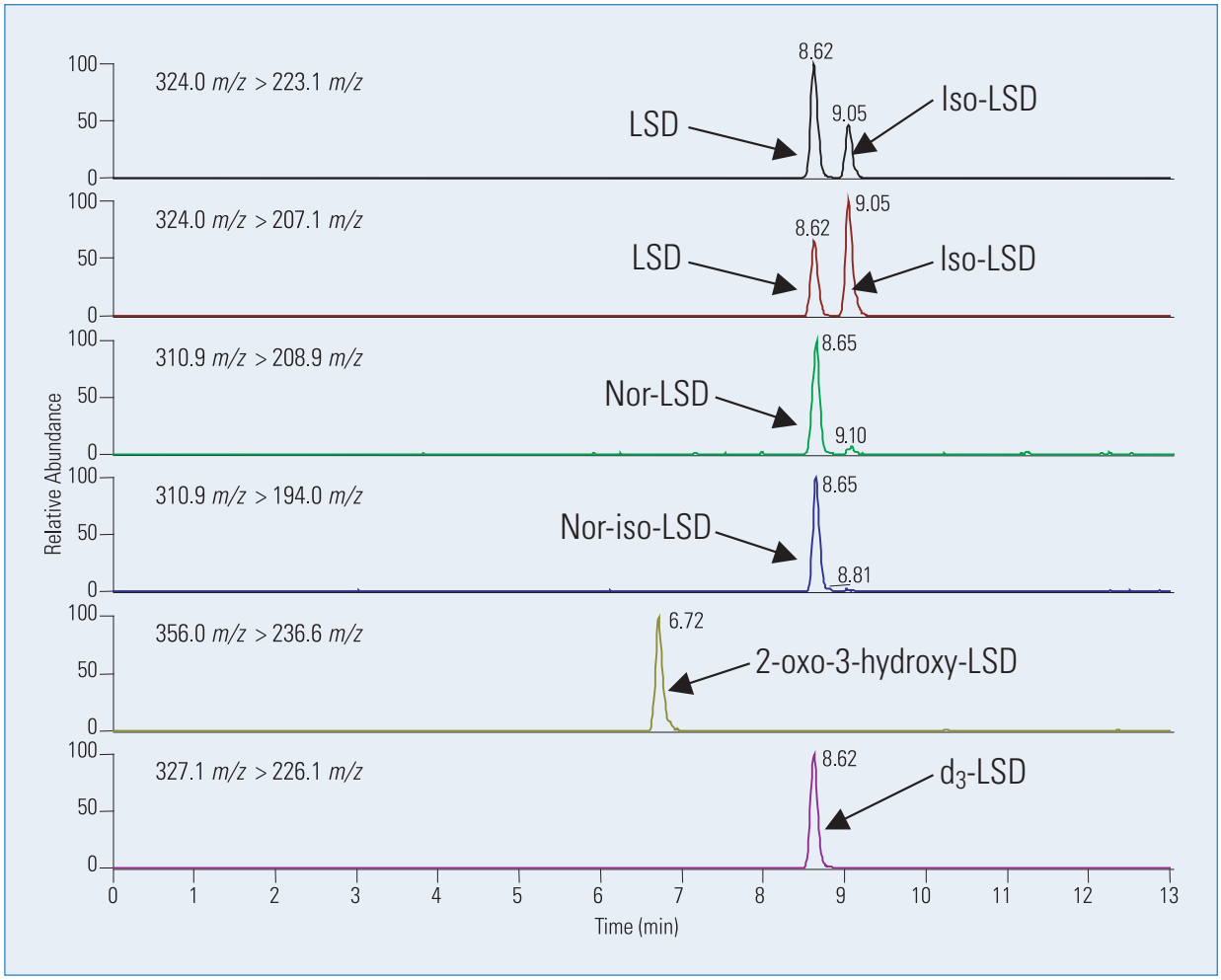

Figure 2: Chromatogram of a urine spiked at 0.5 ng/mL

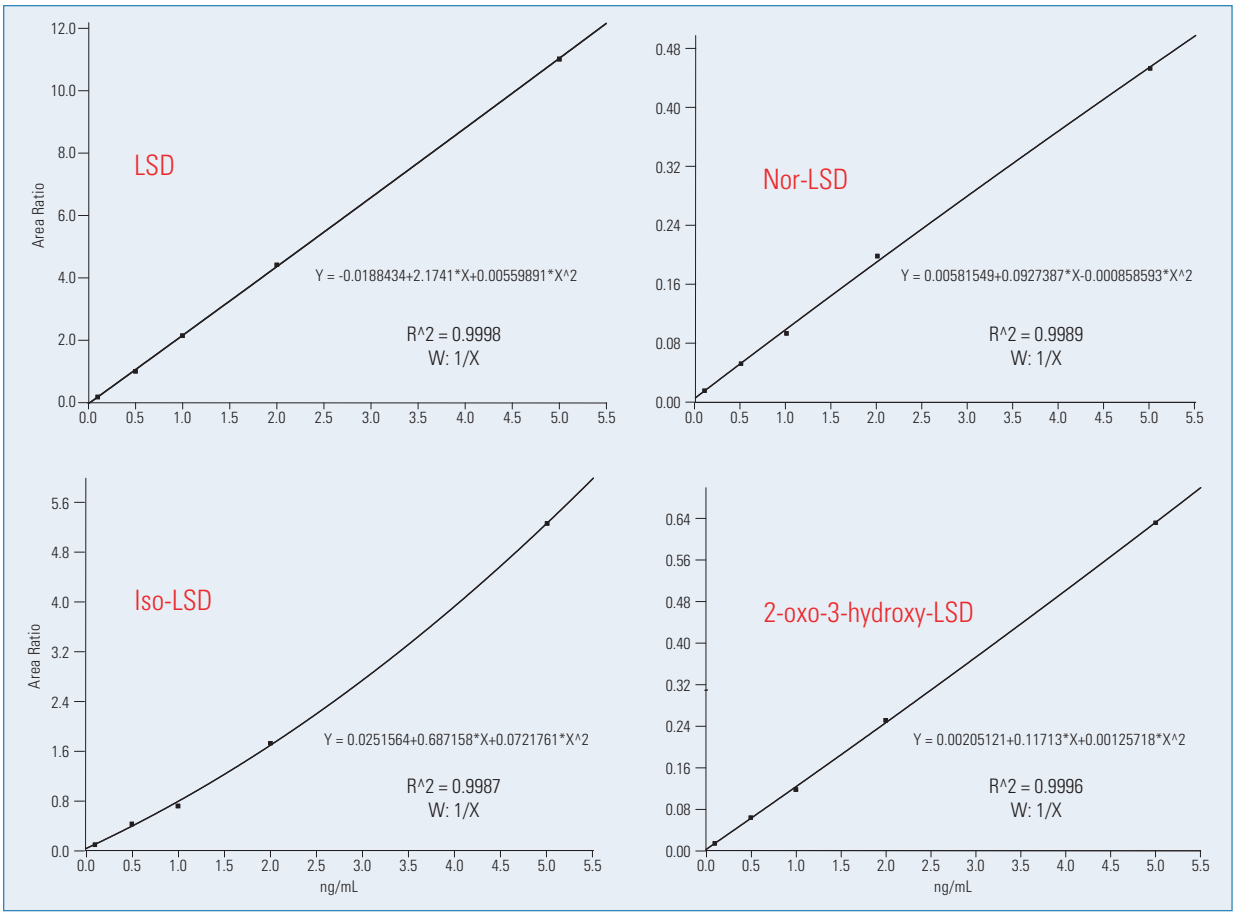

Figure 3: Representative calibration curves from standards spiked in urine

Compounds	Quantification transition	Collision energy	Confirmation transition	Tube lens voltage
LSD	324.0/223.1	30	324.01/207.1	50
Iso-LSD	324.0/223.1	30	324.01/207.1	50
Nor LSD	310.9/208.9	28	310.91/194.0	54
Nor-iso-LSD	310.9/208.9	28	310.91/194.0	54
2-oxo-3-hydroxy-LSD	356.0/236.6	30	356.01/222.0	36
d3-LSD	327.1/210.1	50	327.11/226.2	30

Results and Discussion

The LC-ESI/SRM chromatograms obtained for a blank urine spiked at 0.5 ng/mL are shown in Figure 2. As presented, LSD and iso-LSD are separated using the chromatographic conditions described previously. Identification of LSD is performed using two characteristic transitions and the retention time given by its deuterated internal standard.

Linearity

Calibration curves obtained for each compound spiked in urine samples are presented in Figure 3. Concentration ranges were comprised between 0.1 ng/mL and 5 ng/mL.

Conclusion

This application note described a sensitive, specific method developed for the quantitation of lysergide and metabolites in various biological matrices for forensic use.

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