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Return of the lysergamides. Part III: Analytical characterization of N6-ethyl-6-norlysergic acid diethylamide (ETH-LAD) and 1-propionyl ETH-LAD (1P-ETH-LAD)

http://researchonline.ljmu.ac.uk/6073/

Citation (please note it is advisable to refer to the publisher's version if you intend to cite from this work)

Brandt, SD, Kavanagh, PV, Westphal, F, Elliott, SP, Wallach, J, Stratford, A, Nichols, DE and Halberstadt, AL (2017) Return of the lysergamides. Part III: Analytical characterization of N6-ethyl-6-norlysergic acid diethylamide (ETH-LAD) and 1-propionyl ETH-LAD (1P-ETH-LAD). Drug Testing and
Return of the lysergamides. Part III: Analytical characterization of $N^6$-ethyl-6-norlysergic acid diethylamide (ETH-LAD) and 1-propionyl ETH-LAD (1P-ETH-LAD)

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Drug Testing and Analysis – Brandt et al. – Supporting Information

![Graph showing infrared spectra and GC-EI-MS analysis of ETH-LAD isomers.](Image)

**ETH-LAD Isomer I base (GC-sIR)**
**ETH-LAD Isomer II (IR not detectable) (GC-sIR)**
**ETH-LAD Isomer III base (GC-sIR)**
**ETH-LAD Isomer IV base (GC-sIR)**
Liquid chromatography diode array detection

A Dionex 3000 Ultimate liquid chromatography system coupled to a UV diode array detector (Thermo Fisher, St. Albans, UK) was used for analysis using a Phenomenex Synergi Fusion column (150 mm × 2 mm, 4 μm) that was protected by a 4 mm × 3 mm Phenomenex Synergi Fusion guard column (Phenomenex, Cheshire, UK). The Mobile phases were 70% acetonitrile with 25 mM of triethylammonium phosphate buffer (TEAP) and aqueous TEAP (25 mM) buffer. The gradient elution commenced with 4% acetonitrile and ramped to 70% acetonitrile in 15 min and held for 3 min, resulting in a total acquisition time of 18 min at a flow rate of 0.6 mL/min. The diode array detection window was set at 200 nm–595 nm (collection rate 2 Hz).
1P-ETH-LAD incubation (10 μg/mL) in human serum at 37°C.

Fifty μL and 950 μL (acetonitrile/water, 1/1 + 0.1 % formic acid); centrifuged at 18,000 rpm for 3 min, then passed through a Nylon spin filter (0.2 μm). Samples were further diluted for LC-MS: 50 μL of the above and 950 μL (acetonitrile/water, 1/1 + 0.1 % formic acid).

Serum (blank)

Serum Spiked with 1P-ETH-LAD 10 μg/mL at 0 h

Serum Spiked with 1P-ETH-LAD 10 μg/mL at 1 h
Serum spiked with 1P-ETH-LAD 10 μg/mL at 2 h

Serum spiked with 1P-ETH-LAD 10 μg/mL at 3 h

Serum spiked with 1P-ETH-LAD 10 μg/mL at 4 h
Serum Spiked with 1P-ETH-LAD 10 μg/mL at 5 h

Serum Spiked with 1P-ETH-LAD 10 μg/mL at 5 h

Serum Spiked with 1P-ETH-LAD 10 μg/mL at 6 h

Serum Spiked with 1P-ETH-LAD 10 μg/mL at 6 h

Serum Spiked with 1P-ETH-LAD 10 μg/mL at 24 h

Serum Spiked with 1P-ETH-LAD 10 μg/mL at 24 h

LC-Q-MS SIM m/z 338 (ETH-LAD)
Fragmentor voltage: 50 V

LC-Q-MS SIM m/z 394 (1P-ETH-LAD)
Fragmentor voltage: 50 V
ETH-LAD hemitartrate
$^1$H-NMR (400 MHz)
d$_6$-DMSO
ETHALAD hemitartrate
HMQC
$d_6$-DMSO

TA = Tartaric acid
Drug Testing and Analysis – Brandt et al. – Supporting Information

ETH-LAD hemitartrate
HMBC
$\delta_{f}$-DMSO

TA = Tartaric acid
^{1}P-ETH-LAD hemitartrate

^{1}H-NMR (400 MHz)
d_{6}-DMSO
1P-ETH-LAD hemitartrate

13C-NMR (150 MHz)

$\delta$-DMSO

TA = Tartaric acid
1H-ETH-LAD hemitartrate
HSQC
d$_6$-DMSO
Drug Testing and Analysis – Brandt et al. – Supporting Information

1H-NMR of 1P-ETH-LAD hemitartrate
HMBC
d$_6$-DMSO

$\text{TA} = \text{Tartaric acid}$