

# Analysis of hallucinogenic mushrooms by CE-ESI-MS

## Application Note

Drug Testing

### Authors

Dat T. Phan  
Peter B. Harrsch  
Agilent Technologies,  
Waldbronn, Germany

### Abstract

Extracts from hallucinogenic mushrooms were analyzed by capillary electrophoresis coupled with electrospray ionization mass spectrometry (CE-ESI-MS). The data shown here demonstrate feasibility. Additional method development and/or validation may be required for routine use.

### Experimental

CE-ESI-MS analysis was performed using the Agilent Capillary Electrophoresis system with CE-MS capillary cassette coupled to the Agilent MSD which was equipped with electrospray source and orthogonal sprayer for CE-MS. Sheath liquid was delivered by an Agilent binary pump equipped with a 1:100 flow splitter. The Agilent ChemStation software was used for instrument control.

The fused silica capillary was prepared by flushing for 30 minutes with 1 N NaOH followed by a 10 minute wash with de-ionized water prior to insertion into the sprayer needle. Once installed, the capillary was flushed with running buffer for 30 minutes.

The sample was provided by a law enforcement agency specifically for this study. The hallucinogenic mushroom sample was prepared by methanolic extraction with no further dilution.



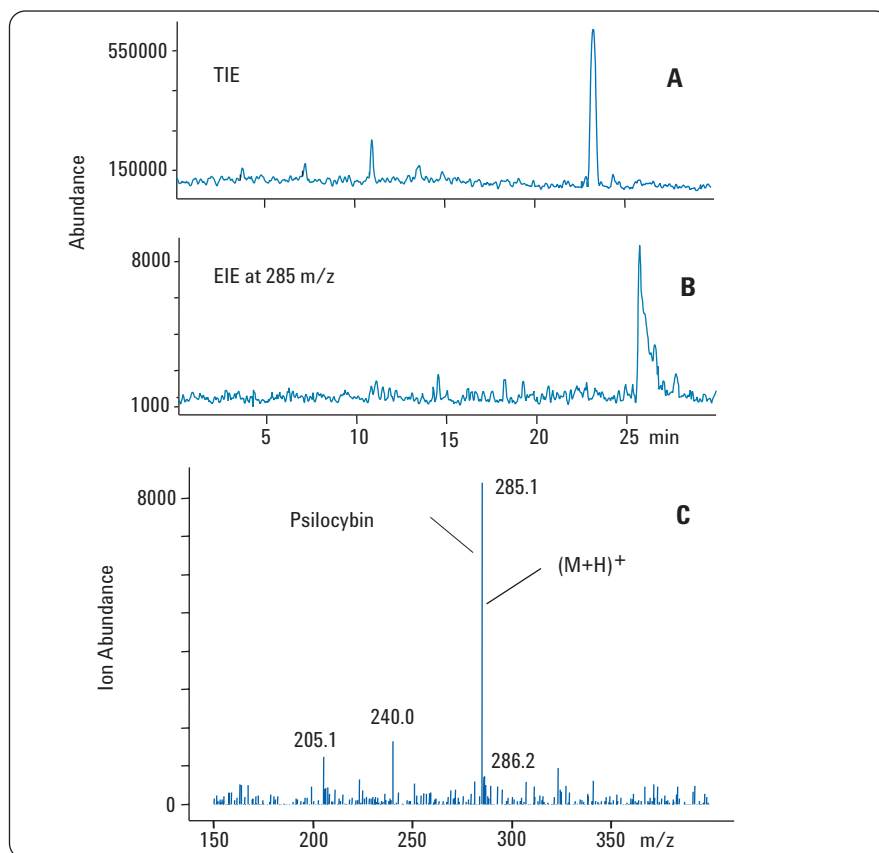
**Agilent Technologies**

## Results

Psilocybin is the major of two psychoactive compounds found in hallucinogenic mushrooms, the other component being psilocin. Analysis of a methanolic extract of hallucinogenic mushrooms produced a total ion electropherogram (TIE) with two peaks (figure 1A). The spectra from both peaks were examined and did not appear to contain the expected molecular ion of 285 m/z characteristic for psilocybin. However, an extracted ion electropherogram (EIE) at 285 m/z gives a single peak at 26 minutes (figure 1B). Evaluation of the spectrum (figure 1C) from this peak clearly shows that the major ion has a mass of 285 and is most likely the protonated molecular ion of psilocybin. An ion at 205 m/z is also present which would be consistent with a fragment ion resulting from the loss of a phosphate from the psilocybin yielding the dephospho-analog psilocin. The data shown here demonstrate that CE-ESI-MS can be used to identify these drugs of abuse. Confirmation of psilocybin was accomplished by the presence of molecular ions specific for the drug substance.

## Equipment

- Agilent Capillary Electrophoresis system
- Agilent CE-MS Adapter Kit
- Agilent LC/MSD module with API Electrospray Source
- Agilent CE-ESI-MS Sprayer Kit
- Agilent ChemStation and CE-MS software



**Figure 1**  
Analysis of a methanolic extract from hallucinogenic mushrooms.

### Chromatographic conditions

Sample:	methanolic extract from hallucinogenic mushrooms
Injection:	3 sec @ 50 mbar
Capillary:	bare fused silica, total length 100 cm, 50 $\mu$ m id
Buffer:	50 mM ammonium acetate, pH 4.5
Voltage:	25 kV
Temperature:	25°C
Preconditioning:	3 min flush with buffer at 1 bar
Sheath liquid:	0.5 % acetic acid in 50 % acetonitrile, 5 $\mu$ L/min
Nebulizing gas:	nitrogen, 20 psi
Drying gas:	nitrogen, 7 L/min, 200 °C
Acquisition:	positive mode, Vcap -3.5 kV, fragmentor, 100 V, step size 0.1, PW 0.25 min, time filter on
Scan range:	80–500 m/z

[www.agilent.com/chem/ce](http://www.agilent.com/chem/ce)

© Agilent Technologies Inc., 2000-2009

Published March 1, 2009  
Publication Number 5990-3393EN



**Agilent Technologies**