Application Note: ANCCSSERVOPCP

Extraction of Phencyclidine from Urine and Subsequent Analysis by GC-MS

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Key Words

- Servo+ PCP
- TraceGOLD TG-5MS
- Phencyclidine
- Drugs of abuse
- Clinical

Abstract

This application note demonstrates a GC-MS method for identifying phencyclidine. An extraction procedure for this compound was optimized on a Thermo Scientific Servo+ PCP SPE cartridge. A urine sample was spiked with phencyclidine and analyzed by GC-MS using a Thermo Scientific TraceGOLD TG-5MS column.

Introduction

Phencyclidine (1-(1-phenylcyclohexyl)piperidine), also commonly known as PCP, was first synthesized in 1926 and later marketed in the 1950's under the trade name sernyl for use as a general anesthetic. PCP began to emerge as a recreational drug in the United States in 1967. [1] After a decline in abuse during the late 1980s and 1990s, phencyclidine (PCP) has re-emerged as a drug of abuse. PCP is now considered a "club drug" and is commonly abused by young adults. Street names include angel dust, hog, ozone, rocket fuel, shermans, wack, crystal and embalming fluid. Abuse of PCP also includes combining it with marijuana and is known by names such as killer joints, super grass, fry, lovelies, wets, and waters.

PCP can be abused by ingesting, smoking, or snorting and exhibits both hallucinogenic and neurotoxic effects.[2] The primary psychoactive effects of PCP only lasts a few hours, however the drug can persist in the body for up to eight days or longer. Typical testing for abuse of PCP is via urine, blood, or hair samples. PCP is listed as a schedule II drug by the Drug Enforcement Agency.

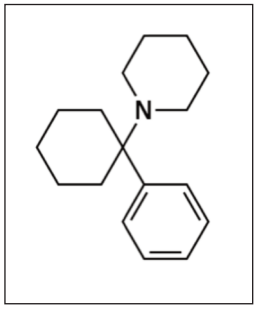

Figure 1: Phencyclidine

Experimental Details

Sample Handling Equipment	Part Number
Liquid handling hardware: eVol with 50 µL and 500 µL syringes	66002-024
SPE hardware: Universal Vacuum Manifold	60104-230
SPE cartridges / consumables:	
Servo+ PCP 30 mg/1 mL	60110-602C
Servo+ PCP 60 mg/3 mL	60110-601C
Vials and closures: 2 mL clear screw top vials	60180-599
Reacti-Vap Evaporator	TS-18826

Sample Preparation

Compounds:	Phencyclidine
Part number:	60110-602C
Phase:	HyperSep Servo+ PCP
Volume:	1 mL
Bed weight:	30 mg
Conditioning:	1 mL of MeOH followed by 1 mL of DI H_20
Application:	Load at 1 to 2 mL/min
Washing:	Wash1: 1 mL of MeOH:Formic Acid (98:2); Wash2: 1 mL of MeOH:H_20:NH_4OH (90:9.5:0.5)
Elution:	1 mL of MeOH:NH_4OH (95:5)

Separation Conditions

Instrumentation:	Thermo Scientific Trace GC Ultra
GC Column:	TG-5MS, 30 m x 0.25 mm x 0.25 µm
Oven temp:	60 °C (2 min hold) to 280 °C (2 min hold) at 20 °C /min
Carrier: Helium	(1.4 mL/min)
Injector:	250 °C split (28 mL/min, 20:1 split ratio)
Detector:	FID 300 °C (for SPE elution profiles): MS (for calibration and urine sample)
Sample injected:	1.0 µL

MS Conditions (if applicable)

Instrumentation:	Thermo Scientific Trace GC Ultra and DSQII
Transfer line temperature:	290 °C
Source temperature:	230 °C
Ionization conditions:	EI
Emission current:	100.00
Electron energy:	-70.00
Filament delay:	4 minutes
Ion range:	Scan 48 - 600
Scan time:	Scan 3 sec

Solutions

Phencyclidine (1 mg/mL in Methanol) was purchased from Alltech Associates Inc. Standards were prepared in house by making a stock solution at the highest calibration level and then diluting to achieve the various lower level standards.

Data Processing

Software:	Xcalibur

Results

Both a sample extraction method and GC-MS method were developed for the identification of phencyclidine (PCP). The extraction procedure developed in this application note was the result of SPE optimization. This optimization was done in order to maximize the recovery of the analyte.

A Servo+ PCP SPE cartridge (30 mg/1mL) was conditioned with 1mL of methanol followed by 1 mL of water. A 1mL aliquot of water was spiked with 20 µg of PCP and loaded on to the Servo+ PCP SPE cartridge. The eluant from the loading was collected. Washes with increasing strengths of MeOH and H_2O/NH_4OH 95:5 (v/v) were applied, starting from 10 % MeOH/90% H_2O/NH_4OH [95:5] (v/v) and increasing in MeOH content by 10 % each. Finally four 1mL aliquots of 95 % MeOH / 5 % NH_4OH (v/v) were used to wash the SPE tube. Each eluant from the washings was collected and analyzed by GC/FID. By plotting the area/detector response for each of these eluants generates an elution profile. This data is shown in Figure 1, where L indicates the loading of the SPE cartridge, the numeric indicators are the ratios of methanol to water used in the washes, and the four elutions are marked with E1 – E4. These results show an optimum wash profile of 90 % MeOH/10 % H_2O/NH_4OH [95:5] (v/v) before phencyclidine starts to elute.

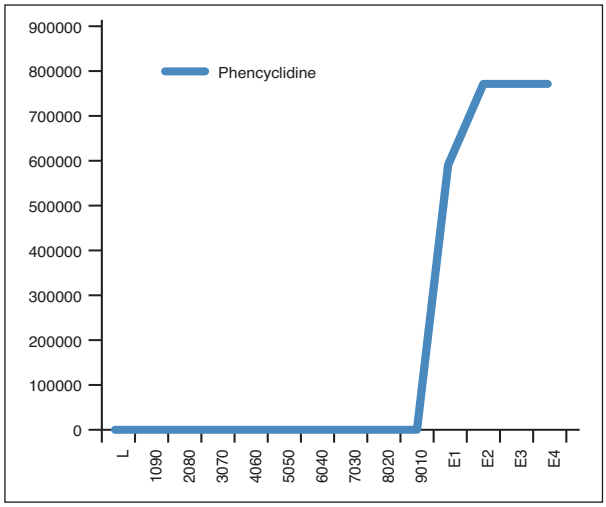

Figure 1: Elution profile for Phencyclidine

To demonstrate the robustness of the method and the Servo+ PCP SPE cartridges, percent recoveries of the phencyclidine were calculated. Using 60 mg/3 mL cartridges, 2 mL of dilute urine (1:1 dilution) spiked with 20 µg of phencyclidine, and an internal standard (naphthalene) was loaded onto the SPE devices. Nine SPE cartridges were loaded in this manner. All of the cartridges were then washed with 2 mL of 90 % MeOH/10 % H_2O/NH_4OH [95:5] (v/v) and then eluted with 1mL of 95 % MeOH/5 % NH_4OH (v/v). These samples, along with a standard prepared with 20 µg of phencyclidine and naphthalene were analyzed by GC-FID. Using the results from these analysis percent recoveries were calculated resulting, on average with a percent recovery of 98% for phencyclidine.

Using GC-MS, an instrument calibration was performed for phencyclidine. This was an external calibration curve and no internal standards were used. Using 6 different calibration levels and triple injections, percent relative standard deviation calculated from the detector response are shown in Table 1. Figure 2 shows the linear correlation for the calibration curve.

Compound	Level	%RSD
Phencyclidine	5 µg/mL	4.7 %
	10 µg/mL	8.0 %
	20 µg/mL	5.1 %
	30 µg/mL	2.6 %
	40 µg/mL	6.6 %
	50 µg/mL	3.8 %

Table 1: Calibration table for phyencyclidine showing %RSD

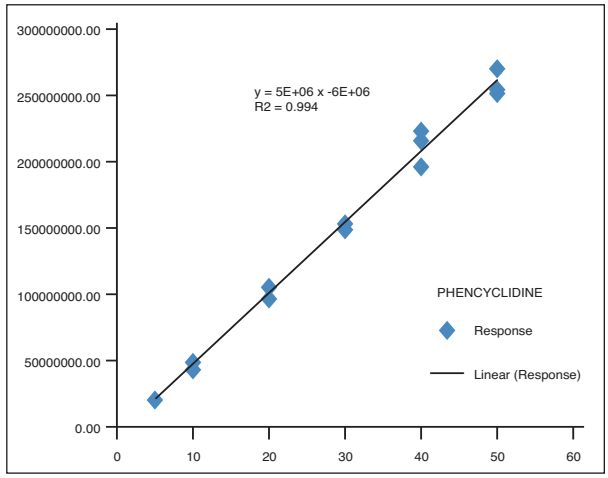

Figure 2: Calibration Curve for Phencyclidine

A 1 mL urine sample was spiked with 10 µg of phencyclidine. This sample was then diluted with 1 mL of deionized water. Following the optimized SPE method for the Servo+ PCP SPE cartridge, the urine sample was extracted and the eluant analyzed via the GC-MS method described above. The resulting chromatogram is shown in Figure 3.

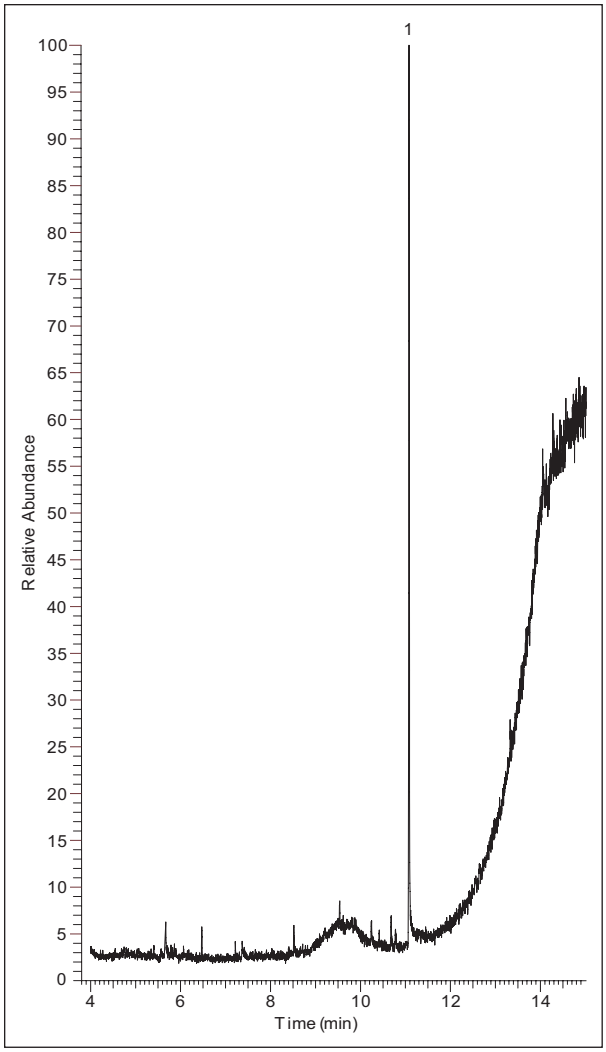

Figure 3: Analysis of spiked urine sample by GC-MS following clean-up with a 60 mg / 3 mL HyperSep Servo+ PCP cartridge.
Peak identification: 1) Phencyclidine

Conclusions

In this application note an SPE method for the extraction of phencyclidine from urine was developed. The optimized extraction for these compounds was achieved on a Servo+ PCP SPE cartridge. A GC-MS method was also developed for the identification of phencyclidine. PCP was extracted from a spiked urine sample, identified by GC-MS and quantified by a standard calibration. The TraceGOLD TG-5MS capillary columns provided the inertness and low bleed characteristics necessary for quantifying this drug of abuse.

References

[1] Inciardi, James A. (1992). The War on Drugs II. Mayfield Publishing Company, p 46.

[2] Maisto, Stephen A.; Mark Galizio, Gerard Joseph Connors (2004). Drug Use and Abuse. Thompson Wadsworth.

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