

# Fentanyl Extraction and Analysis in Soil Using Automated QuEChERS

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## OVERVIEW

### Purpose

- Comparative assay of an automated QuEChERS extraction versus manual process with MS/MS analysis.

### Method

- AOAC QuEChERS methods is used to perform fentanyl extraction in soil.
- Samples analyzed by LDTD-MS/MS.

### Quantification

- Precision results were lower than 4% CV, accuracy results were lower than 9 % from the nominal value.
- Samples analyzed with a runtime of 6 seconds using LDTD-MS/MS technique.

## INTRODUCTION

The analysis of illicit drugs, such as fentanyl, in soil is of great interest. Fentanyl analysis can be used as an indirect assessment tool of drug consumption in a specific territory (air, water, soil, sediment) or can be useful to law enforcement (presence of a drug trafficking laboratory)<sup>1</sup>. For this project, the extraction of fentanyl in soil was the selected approach. The extraction method used is QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe). However, the QuEChERS method is time-consuming. Thus, the objective of this study is to evaluate the feasibility of automating this method for the QuEChERS extraction of fentanyl from soil and proceed to the fast analysis in LDTD-MS/MS.

### HAPEX Extraction System

The HAPEX automated extraction system (Figure 1) combines an integrated centrifuge and 3 vortex mixers with a PAL RTC system to significantly reduce the time required for soil sample preparation.



Figure 1 HAPEX Extraction System

### Luxon Ionization Source

The Luxon Ion Source (Figure 2) is the second-generation sample introduction and ionization source based on the LDTD technology for mass spectrometry. The Luxon Ion Source uses a Fiber-Coupled Laser Diode (Figure 3) to obtain unmatched thermal uniformity giving more precision, accuracy and speed. The process begins with dry samples which are rapidly evaporated using indirect heat. The thermally desorbed neutral molecules are carried into a corona discharge region. High-efficiency protonation and strong resistance to ionic suppression characterize this type of ionization and is the result of the absence of solvent and mobile phase. This thermal desorption process yields high-intensity molecular ion signal in less than 1 second sample-to-sample and allows working with very small volumes.



Figure 2 Luxon Ion Source

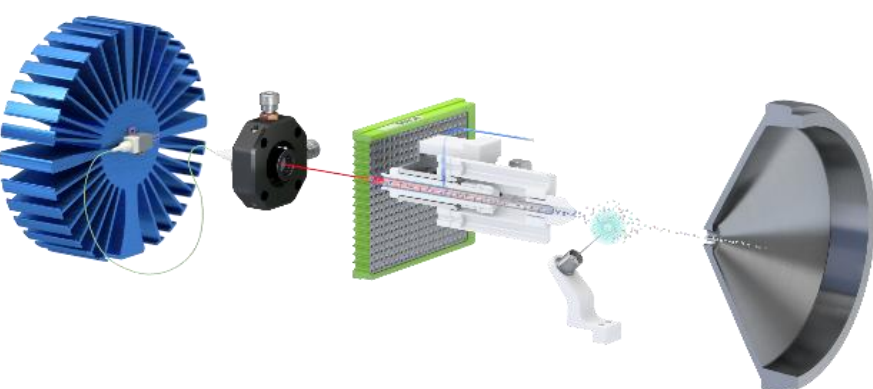


Figure 3 Schematic of the Luxon Ion Source

## METHOD

Automation of the QuEChERS extraction is performed using the HAPEX extraction system. In this method, liquid and vial handling is entirely performed by the extraction system.

Unlike the original QuEChERS AOAC method, extraction salts are added to the extraction tubes prior to extraction and the supernatant of the centrifuged sample is added on top of the salts.

### Automated QuEChERS AOAC Method

- 5 g of soil are weighed and placed in a **40 mL glass vial** (off-line).
- Add 10 mL of Water.
  - Vortex (1000 rpm / 1 minute).
- Add 15 mL of acidified Acetonitrile (1 % acetic acid) fortified.
- Add 2 mL of internal standard solution in acetonitrile.
  - Vortex (1000 rpm / 1 minute) and centrifuge (3700 rpm / 4 minutes).
- Transfer 10 mL upper layer to **40 mL screw cap glass tube containing QuEChERS AOAC salt mixture**.
  - Vortex (1000 rpm / 1 minute) and centrifuge (3700 rpm / 4 minutes).
- Transfer 1 mL of upper layer into a **2 mL vial containing dSPE**.
  - Vortex (1000 rpm / 30 seconds) and centrifuge (3700 rpm / 4 minutes).
- Transfer 100 µL of upper layer into a 2 mL glass vial and add 100 µL of acetonitrile/water (1:1).
  - Vortex (1000 rpm / 1 minute).
- Spot 5 µL of upper layer on a LazWell™96 plate.
  - Dry at 40°C for 4 minutes.
- Analyze by LDTD-MS/MS.

**For 1 sample**  
**AOAC Salts:** 6 g MgSO4 and 1.5 g NaOAc  
**dSPE:** 25 mg PSA, 150 mg MgSO4 and 25 mg C18

### Instrumentation

- Ion source: Phytronix Luxon Ion Source S-960
- Mass spectrometer: Sciex, Q-Trap System 5500

### Luxon Parameters

- Laser power pattern:
- Increase laser power to 65% in 5s.
- Hold 1 s. at 65%.
- Carrier gas flow: 6 L/min (Air)

### MS Parameters

- APCI (+)
- Curtain (CUR): 20
- CAD: 8
- Time: 20 msec

Table 1 MRM transitions parameters

Drugs	Transition	CE	DP
Fentanyl	337.2 → 188.1	26	60
Fentanyl-d <sub>5</sub>	342.2 → 188.1	35	80

## RESULTS

### Validation

The following acceptance criteria are used:

- Linearity with R ≥ 0.995
- Each standard concentration must not exceed 15% CV.
- Each standard concentration must be ±15% of the nominal value (%Bias).

### Linearity

Five-point calibration curves (ranging between 10 ng/g and 100 ng/g) are used to validate the method.

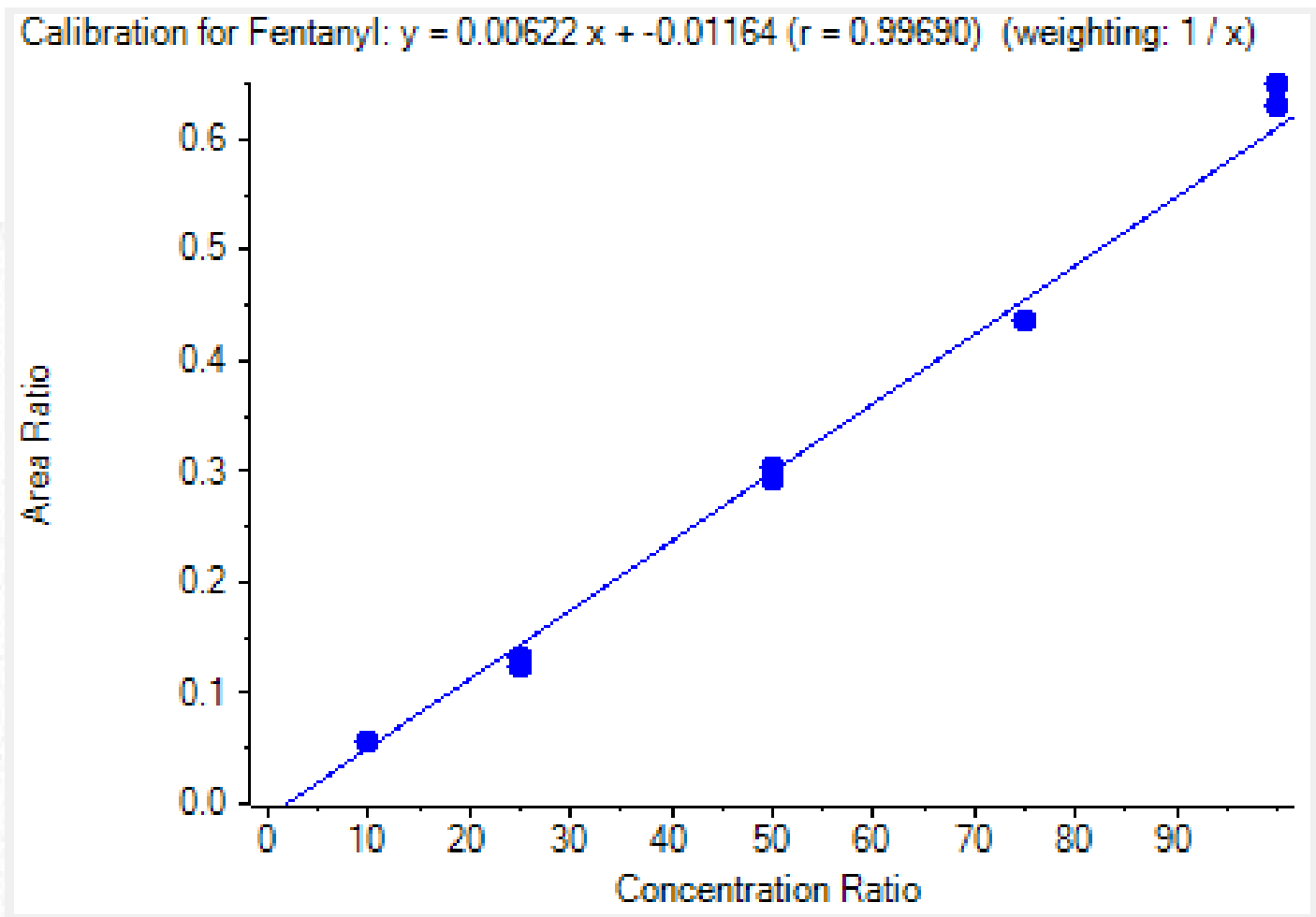


Figure 4 Calibration curve for automated QuEChERS AOAC

Table 2 Linearity results

	Correlation coefficient (r)
Manual	0.99679
Automated	0.99690

## CONCLUSION

- Efficient Automated QuEChERS Method using HAPEX System.
- Difference of 6 % in extraction yields between manual and automated QuEChERS AOAC Method.
- Linearity, accuracy, precision and stability within the acceptance criteria.
- Sample-to-sample analysis of **12 seconds using LDTD-MS/MS**.

#### REFERENCES

1) Carlos A. Valdez, José A. Rosales, Roald N. Leif (2023). Determination of fentanyl and acetylfentanyl in soil in their intact form and orthogonal corroboration of their presence by EI-GC-MS using chloroformate chemistry, Forensic Chemistry, Volume 34, 100504, <https://doi.org/10.1016/j.forc.2023.100504>.

### Precision

QCs samples were extracted in sextuplicate. %CV and %Bias were evaluated.

Table 3 Precision and accuracy results

	QCL	QCM	QCH
Conc (ng/g)	30	60	80
N	6	6	6
Mean (ng/g)	31.4	64.8	82.9
%CV	2.3	3.3	3.1
%Bias	4.8	8.1	3.6

### Recovery

Samples spiked at the highest calibration level, from both the AOAC manual extraction method and the automated extraction method, are extracted in triplicate and analyzed in triplicate to assess the recovery percentage of fentanyl in both methods.

Table 4 Extraction yield for the manual QuEChERS AOAC method and the automated.

	Recovery (%)	±	N
Manual	83	19	9
Automated	78	6	9

Table 5 Extraction difference between the manual QuEChERS AOAC method and the automated.

Difference (%)	±
6	2