

AUTOMATION OF QuEChERS EXTRACTION FOR QUANTITATION OF CBD and THC IN CANNABIS LEAVES IN 8 SECONDS PER SAMPLE

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OVERVIEW

Purpose

- Optimization of an automated extraction process and LDTD-MS/MS analysis of Tetrahydrocannabinol (THC) and Cannabidiol (CBD) in leaves.

Method

- Automated QuEChERS extraction and Derivatization.
- Samples dried and analyzed by LDTD-MS/MS.

Quantification

- Linearity: $r > 0.995$ over the calibration range.
- Between-run accuracy (%Bias), values between 0.21 to 8.83 % are obtained and precision results are lower than 18.3 %CV.
- Samples analyzed with a runtime of 8 seconds using LDTD-MS/MS technique.

INTRODUCTION

Since 2018, the Government of Canada has legalized the purchase, possession, and consumption of cannabis for recreational purposes. However, Canada is not the only country to have legalized and decriminalized cannabis. These laws make it possible to control the purchase of cannabis, to withdraw profits from organized crime and to ensure the safety of consumers. Therefore, it is important to ensure quality control on products intended for consumers. QuEChERS makes it possible to efficiently extract THC and CBD from cannabis leaves. Although this extraction method is effective, it remains time-consuming. The aim of this research is to develop automated sample extraction using the QuEChERS method to reduce preparation time. This extraction will be followed by derivatization and analysis by LDTD-MS/MS.

LUXON Ionization Source

The Luxon Ion Source (Figure 1) 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 2) 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 1 Luxon Ion Source

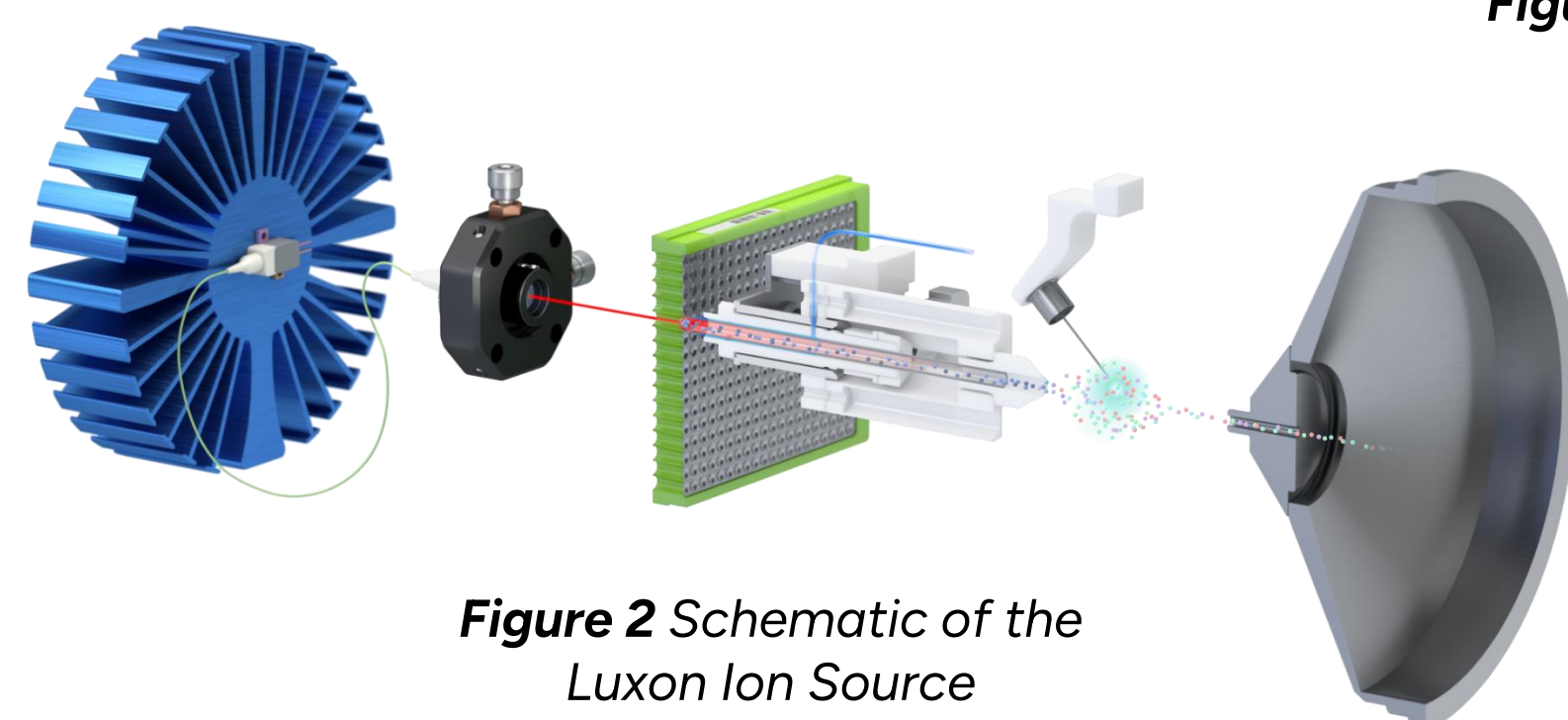


Figure 2 Schematic of the Luxon Ion Source

METHOD

Automated extraction

Dry material is homogenized with pestel and mortar to get 1 mm mesh particle. 100 mg are added in 50 mL falcon barcoded tubes, readable by the Azeo extraction system, then 10 mL water are added and let hydrate for 30 minutes. 20 mL Acetonitrile and QuEChERS salt are added, mixed and centrifuge.

Falcon tubes are transferred on Azeo carousel. Each barcoded tube is scanned by the Azeo Liquid Handler and an automatic batch file is created. Upperlayer phase is transfer and dilute with acetonitrile in glass vial. Dilute samples are mixed with internal standards solution, derivation buffer and dansyl chloride. After incubation, NH_4OH and Hexane are added and mix. 6 μL of upper layer are spotted onto LazWell™ 96 plates and evaporated to complete dryness before analysis by LDTD-MS/MS.



Figure 3 Azeo - Automated extraction system

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 55% in 3 s
 - Carrier gas flow: 3 L/min (Air)

MS Parameters

- APCI (+)
- Curtain: 10
- CAD: 7
- Time: 20 msec
- MRM mode

Table 1 MRM transitions parameters

| Compound | Q1 (m/z) | Q2 (m/z) | CE (eV) |
|--------------------|----------|----------|---------|
| THC | 538.3 | 299.2 | 45 |
| CBD | 781.3 | 533.0 | 40 |
| THC-d ₃ | 551.3 | 302.3 | 45 |
| CBD-d ₃ | 784.3 | 316.2 | 45 |

RESULTS

Derivation step

THC and CBD are isobaric molecules. To get a specific quantification, a derivatization step with dansyl chloride is performed. Figure 4 shows the reaction.

Method validation.

Calibration curves ranging from 4 to 1000 ng/mL are prepared in acetonitrile. After derivation, 6 μL are deposited onto a LazWell™ plate and dried before analysis. The peak area against the internal standard (IS) ratio is used to normalize the signal.

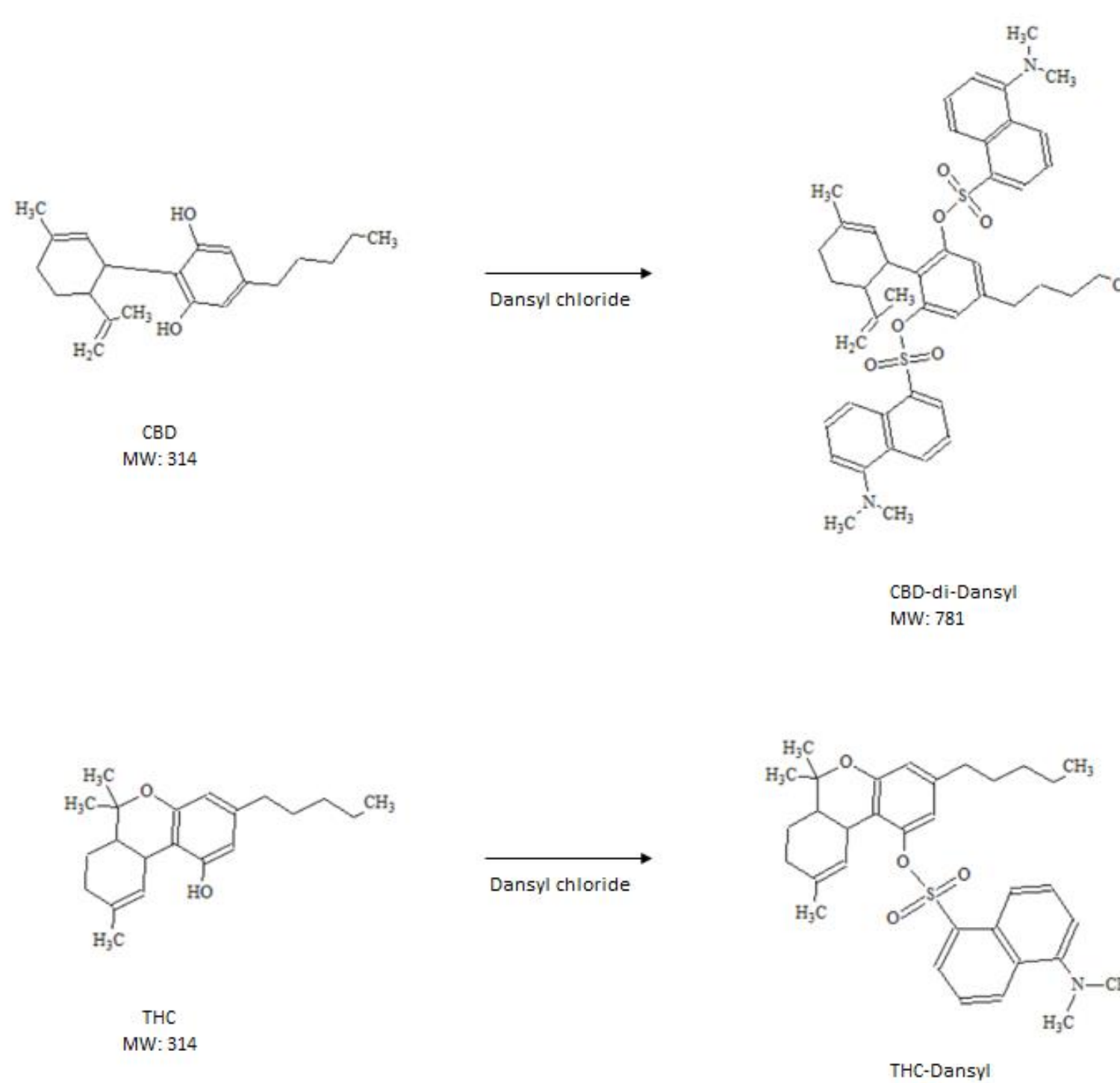


Figure 4 Derivatization reaction

RESULTS

Linearity

The calibration curves are plotted using the peak area ratio and the nominal concentration of standards. For the linearity test, the following acceptance criterion is used:

- Linear regression (r) must be ≥ 0.995

Table 2 shows the inter-day correlation coefficients for THC and CBD. Values greater than 0.995 are obtained. Figure 5 and 6 shows a typical calibration curve result for THC and CBD.

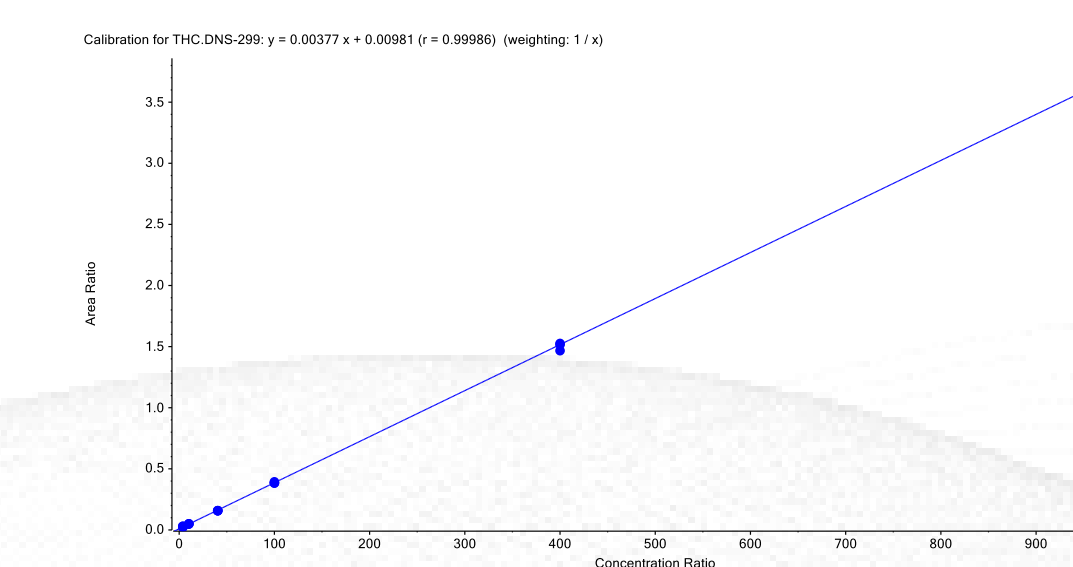


Figure 5 THC calibration curve

Table 2 Inter-day calibration curve correlation coefficients (r)

| | THC | CBD |
|---------|---------|---------|
| Curve 1 | 0.99966 | 0.99937 |
| Curve 2 | 0.99972 | 0.99970 |
| Curve 3 | 0.99986 | 0.99985 |
| Curve 4 | 0.99978 | 0.99983 |
| Curve 5 | 0.99986 | 0.99959 |

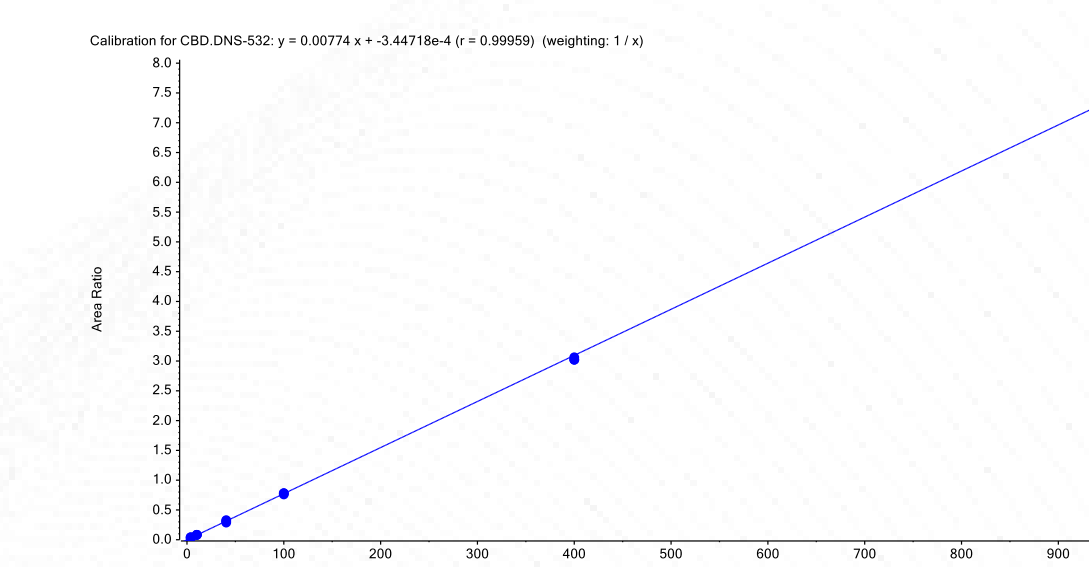


Figure 6 CBD calibration curve

Precision and Accuracy

For the accuracy and precision evaluation, the following acceptance criteria are used:

- Each concentration must not exceed 15 %CV.
- Each concentration must be within $100 \pm 15\%$ of the nominal concentration.

For the inter-run precision and accuracy experiment, each standards are analyzed in triplicate, on five different runs. Table 3 and 4 show the inter-run precision and accuracy results for THC and CBD. The obtained %CV is below 15% and the accuracy is within 15% of the nominal value.

Table 3 Inter-run precision and accuracy for THC

| THC | S1 | S2 | S3 | S4 | S5 | S6 |
|--------------|------|------|------|-------|-------|-------|
| Conc (ng/mL) | 4 | 10 | 40 | 100 | 400 | 1000 |
| N | 15 | 15 | 15 | 15 | 15 | 15 |
| Mean (ng/mL) | 3.98 | 10.3 | 39.4 | 100.4 | 400.4 | 999.6 |
| %CV | 14.8 | 5.4 | 2.5 | 1.9 | 2.4 | 1.8 |
| %Bias | -0.4 | 3.2 | -1.5 | 0.4 | 0.1 | -0.1 |

Table 4 Inter-run precision and accuracy for CBD

| CBD | S1 | S2 | S3 | S4 | S5 | S6 |
|--------------|------|------|------|-------|-------|-------|
| Conc (ng/mL) | 4 | 10 | 40 | 100 | 400 | 1000 |
| N | 15 | 15 | 15 | 15 | 15 | 15 |
| Mean (ng/mL) | 3.98 | 10.4 | 40.2 | 102.1 | 404.8 | 992.5 |
| %CV | 6.4 | 5.4 | 2.9 | 1.8 | 2.8 | 2.0 |
| %Bias | -0.6 | 3.9 | 0.6 | 2.1 | 1.2 | -0.8 |

Cannabis product evaluation

Dry Cannabis product (Super Lemon Haze Biologique) from Société Québécoise du Cannabis (SQDC) is used to evaluate THC and CBD. After extraction, THC and CBD concentration are evaluated using Luxon-MS/MS method and LC-MS/MS as a reference method. Replicate extracts are reported in Table 5. For THC and CBD, the %CV and %Bias is below 20%.

Table 5 Cross Validation results

| | THC (mg/g) | CBD (mg/g) |
|-----------|------------|------------|
| Ref value | 30.1 | 24.5 |
| Replica 1 | 34.5 | 19.8 |
| Replica 2 | 33.3 | 22.8 |
| Replica 3 | 33.0 | 24.5 |
| Mean | 33.6 | 22.3 |
| %CV | 2.4 | 10.8 |
| %Bias | 11.6 | -8.8 |

CONCLUSION

- Automated QuEChERS process.
- Efficient derivatization to get specific analysis of THC and CBD
- High-throughput analysis using LDTD-MS/MS.
- Linearity, accuracy and precision within the acceptance criteria.
- Sample-to-sample analysis of 8 seconds.