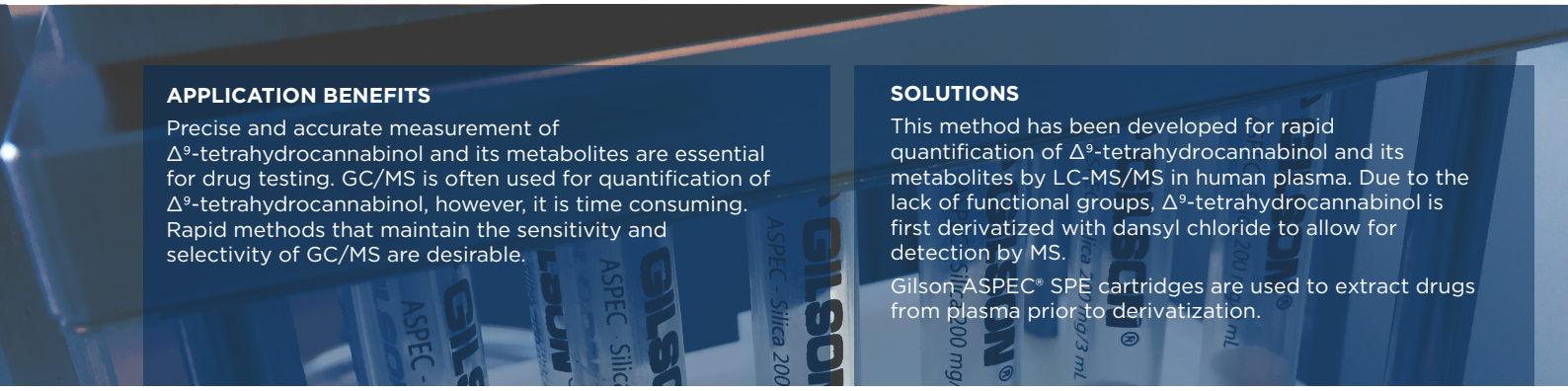
ASPEC® Positive Pressure Manifold and Cartridges: LCMS/MS detection of Δ⁹–tetrahydrocannabinol in plasma using solid phase extraction (SPE) cleanup

APPLICATION NOTE (AN0992)



APPLICATION BENEFITS

Precise and accurate measurement of Δ⁹-tetrahydrocannabinol and its metabolites are essential for drug testing. GC/MS is often used for quantification of Δ⁹-tetrahydrocannabinol, however, it is time consuming. Rapid methods that maintain the sensitivity and selectivity of GC/MS are desirable.

SOLUTIONS

This method has been developed for rapid quantification of Δ⁹-tetrahydrocannabinol and its metabolites by LC-MS/MS in human plasma. Due to the lack of functional groups, Δ⁹-tetrahydrocannabinol is first derivatized with dansyl chloride to allow for detection by MS.

Gilson ASPEC® SPE cartridges are used to extract drugs from plasma prior to derivatization.

INTRODUCTION

The analysis of Δ⁹-tetrahydrocannabinol and its metabolites is usually done by GC/MS, which has long run times[1]. In order to reduce run times without the loss of sensitivity and selectivity, Gilson has developed a method using LC-MS/MS. LC-MS/MS is a difficult method to use with this kind of compound due to the absence of functional groups, such as amines and carboxylic acid, which normally allow for good sensitivity.

The Gilson ASPEC® C_{18} stationary phase can be used to extract drugs from biological fluids. The uniform grafting on the silica surface, combined with an optimal end-capping method, provide excellent recovery and reproducibility. LC-MS/MS analysis can then be achieved by derivatization with dansyl chloride, allowing a significant increase in the sensitivity and selectivity for these drugs.[2]

This application presents the use of the Gilson ASPEC® Positive Pressure Manifold with C_{18} Cartridges for this new method for the determination of Δ⁹-tetrahydrocannabinol and its metabolites in human plasma using LC-MS/MS. The performance of Gilson ASPEC C_{18} Cartridges was comparable to SPE columns from three competitors.

Figure 1
Gilson ASPEC® Positive Pressure Manifold

MATERIALS AND METHODS

Materials

- ASPEC® Positive Pressure Manifold: (P/N: 37012000 - P/N: 37417010)
- 3 mL Manifold (P/N: 37417012)
- ASPEC® Solid Phase Extraction Cartridges:
  - ASPEC® C_{18} 500 mg, 3 mL (P/N: 5430522NC)
- DISTRIMAN® (P/N: F164001) Distritips (P/N: F164120)

Methods

Sample Preparation

- Mix 250 µL of plasma with 1 mL of phosphate buffer (0.1 M, pH 6.0).

Solid Phase Extraction Steps

1. **Condition 1**: 3 mL of MeOH at 1 drop per second
2. **Condition 2**: 3 mL of 1 M HCl
3. **Condition 3**: 3 mL of H_2O
4. **Load**: Load 1.25 mL of diluted plasma sample at 1 drop per 3 seconds
5. **Wash 1**: 2 mL of H_2O at 1 drop per second
6. **Wash 2**: 1 mL of 1 M acetic acid
7. **Wash 3**: 2 mL of MeOH/H_2O (20/80, v/v)
8. **Elute**: 3 mL of CH_2Cl_2/Acetone (50/50, v/v) at 1 drop per 2 seconds

Sample Reconstitution

- Fractions were evaporated at 40°C for 10 minutes with nitrogen.
- Samples were derivatized:
  - 100 µL of 0.1 M carbonate buffer was mixed with 200 µL of dansyl chloride (1 mg/mL in acetone) and vortexed for 1 minute.
- Incubate for 40 minutes at 40°C.
- Samples were extracted by Liquid-Liquid Extraction (LLE):
  - Add 2 mL of 1 chlorobutane.
  - Centrifuge at 3000 rpm for 5 minutes.
- Recuperate Samples by Flash/Freeze:
  - Flash/freeze the excess water from the organic phase in a bath of dry ice/acetone for 3 minutes.
- Reconstitute with 200 µL of ACN/H_2O with 0.1% formic acid (80/20, v/v).

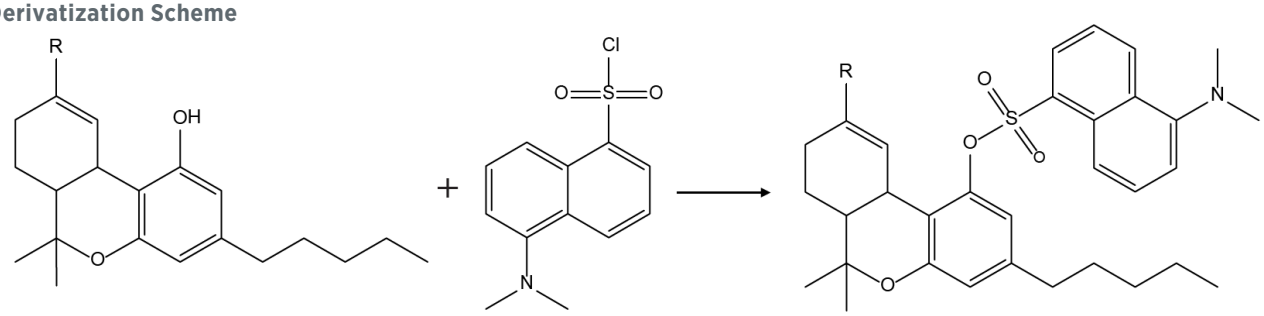
Concentration Range and Derivatization

The concentration range used for this application has been chosen based on pharmacokinetic data from normal and passive consumers of cannabis smoke[3]. A range of 2 to 200 ng/mL for Δ^9-tetrahydrocannabinol and 11-nor-9-Hydroxy-Δ^9-tetrahydrocannabinol and 10 to 200 ng/mL for 11-nor-9-Carboxy-Δ^9-tetrahydrocannabinol have been selected.

In fact, Δ^9-Tetrahydrocannabinol and its metabolites present low sensitivity and high variability in the LC-MS/MS monitored signal caused by the unstable fragmentation.

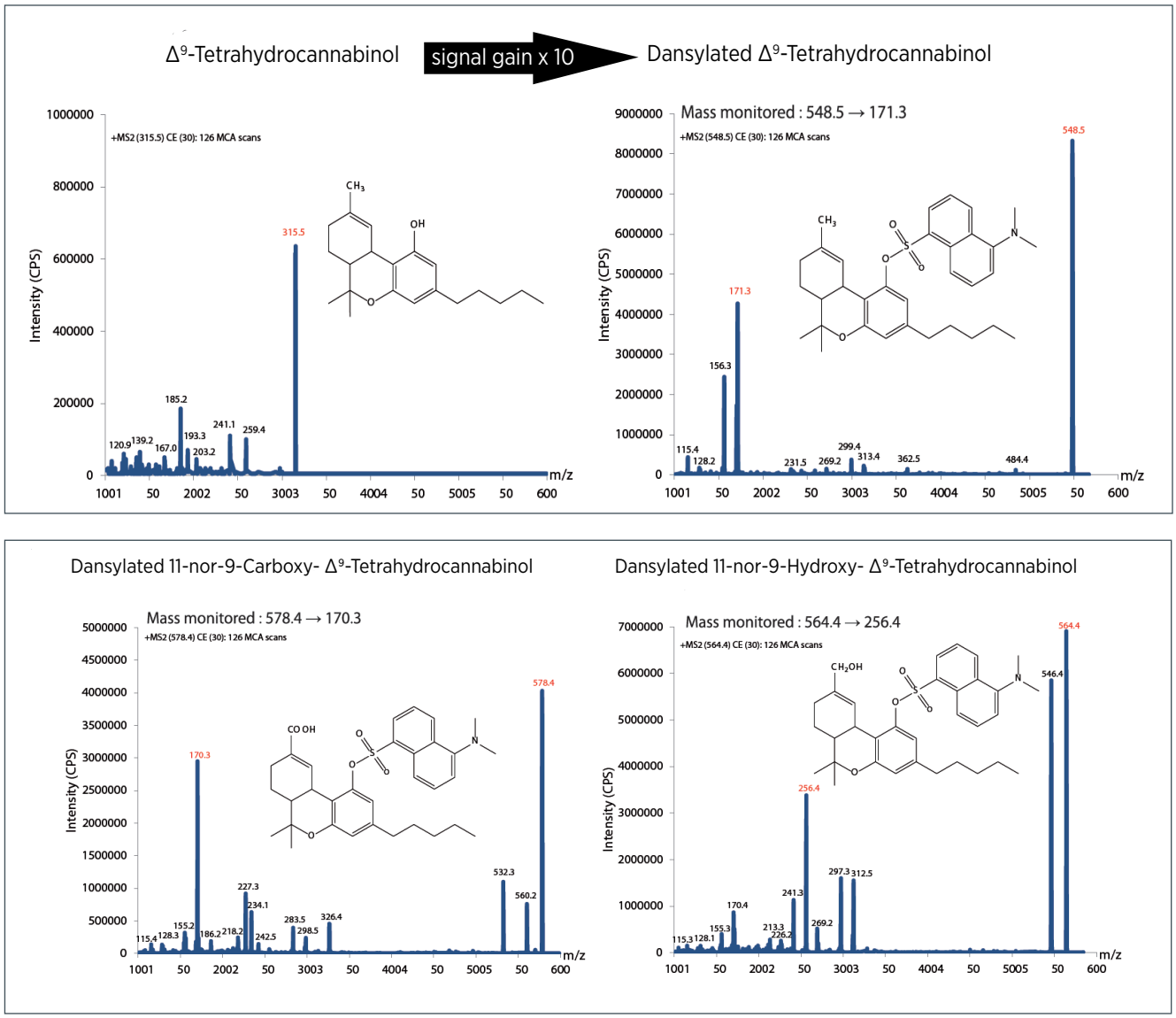
Derivatization using dansyl chloride is a well-known reaction used in different LC-MS/MS applications. This reaction is selective for phenol functions present in Δ^9-Tetrahydrocannabinol and its metabolites.

Derivatization Scheme



R = CH_3, CH_2OH or COOH

Structure Determination by Ion Mass Spectra



Chromatographic Conditions

- Mobile Phase (MP): 1.0 mL/min
  - A: 1 mM ammonium formate in H_2O/ACN (10/90, v/v) with 0.1% formic acid
  - B: 1 mM ammonium formate in H_2O/ACN (90/10, v/v) with 0.1% formic acid
- **Column**: 3.0 x 30 mm C_{18}, 2.5 µm at 23°C
- **Detector**: Sciex API 3000
  - **Turbo Ion Spray Heater Gas Flow**: 8,000 cc/min
  - **Turbo Ion Spray Heater Temperature**: 325°C, ESI+, MRM SCAN
- **Injection Volume**: 5 µL

Table 1

Gradient and Rate Parameters

Time (min)	Mobile Phase A (%)	Mobile Phase A (%)	Flow (mL/min)
0	10	90	1
1.00	10	90	1
1.01	0	100	1
3.50	0	100	1
3.51	10	90	1
5.00	10	90	1

RESULTS AND DISCUSSION

The ASPEC® C_{18} cartridges have high C_{18} loading, a homogeneous layer of C_{18} functional groups and efficient end-capping that result in high recoveries and excellent reproducibility for Δ^9-tetrahydrocannabinol and its metabolites. Comparable results were obtained for the three competitor cartridges (Figure 2).

Quantification at the upper limit of quantification (ULOQ) (200 ng/mL) prepared with the Gilson ASPEC C_{18} column showed excellent peak shape with no fronting or tailing for derivatized Δ^9-tetrahydrocannabinol and its metabolites (Figure 3).

Following the FDA guide[4], a method needs to be selective at the lowest limit of quantification (< 20% of LLOQ). Careful sample preparation is critical for sensitive methods. Gilson's ASPEC C_{18} cartridges remove interfering substances from human plasma allowing for accurate measurements. The interference in the matrix blank was calculated and is reported as a percentage of LLOQ response in Table 2. It is well-known that a small exposure to Δ^9-tetrahydrocannabinol is metabolized by the human body and can be detected by LC-MS/MS. By using this method, analysts can measure low concentrations of this drug (LLOQ 200 pg/mL), proof of the method's sensitivity (Figure 4).

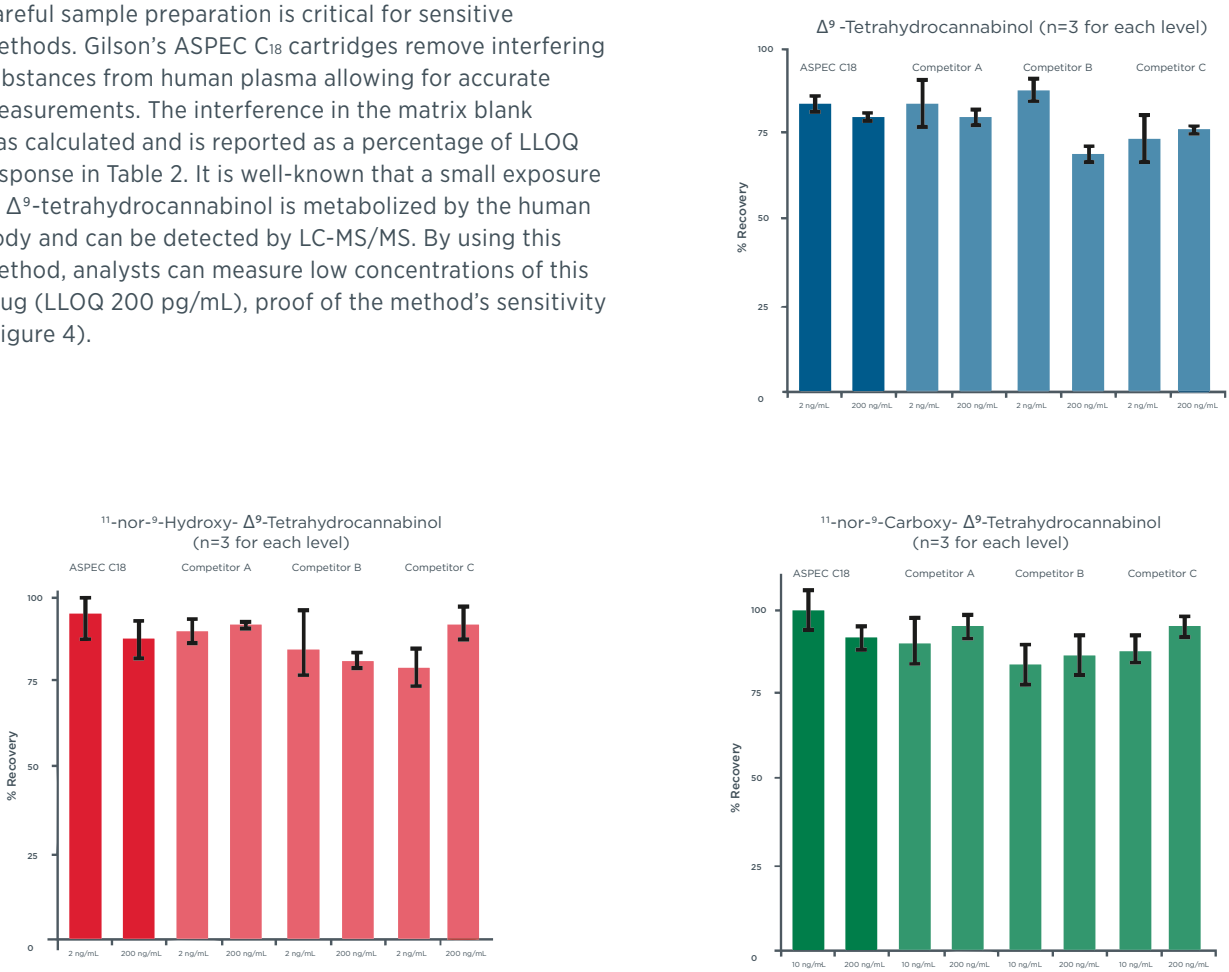


Figure 2
Recovery for Δ^9-tetrahydrocannabinol and its Metabolites

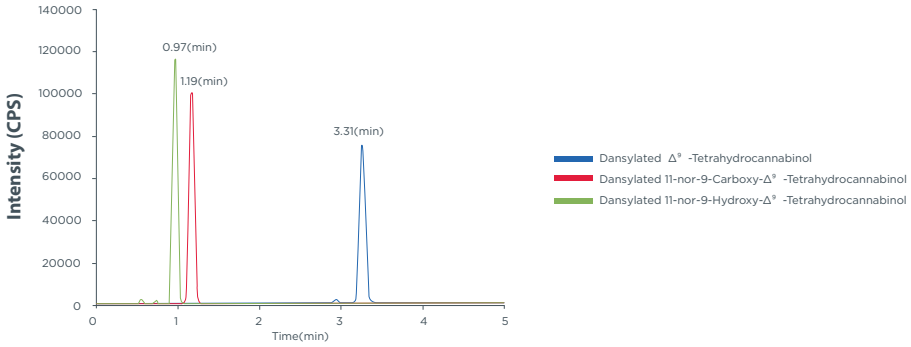


Figure 3
Quantification Chromatogram at ULOQ (200 ng/mL) for Δ^9-tetrahydrocannabinol and its metabolites

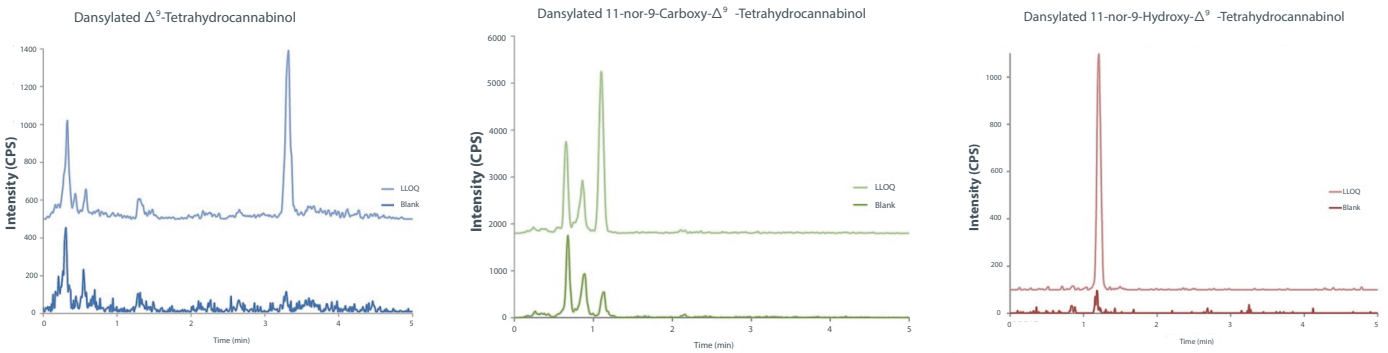


Figure 4

Selectivity and Sensitivity for dansylated Δ⁹-tetrahydrocannabinol and its metabolites

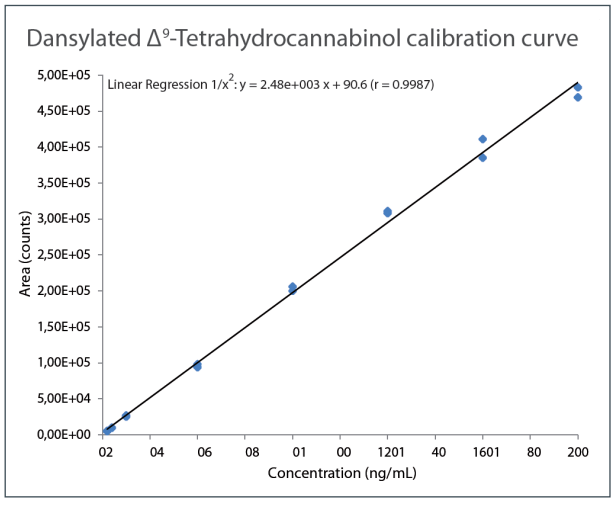
Table 2

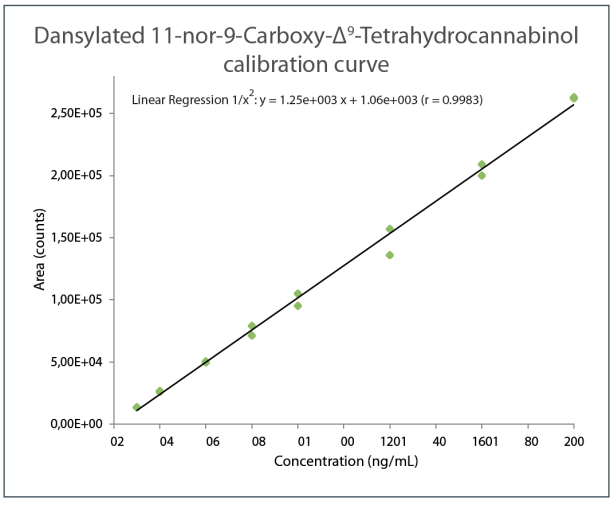
Interference in the Matrix Blank

Drug	% of LLOQ Response
Δ⁹-tetrahydrocannabinol	5
11-nor-9-Hydroxy- Δ⁹-Tetrahydrocannabinol	5
11-nor-9-Carboxy- Δ⁹-Tetrahydrocannabinol	15

Linearity

For each analyzed compound, the calibration curve was linear for all ranges of concentrations.





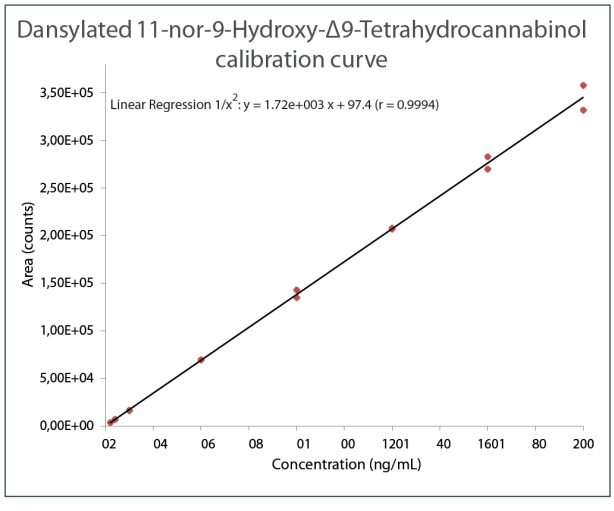


Table 3

METHOD ACCURACY AND PRECISION RESULTS FOR THE SAME RUN (N=6)						
Drug	**LOD (ng/mL)**	**Accuracy LLOQ (%)**	**Accuracy 3 x LLOQ (%)**	**Accuracy 35% LLOQ (%)**	**Accuracy 75% LLOQ (%)**	**Accuracy ULOQ (%)**
Δ⁹-Tetrahydrocannabinol	0.2	103 ± 5	103 ± 7	102 ± 6	100 ± 2	97 ± 3
11-nor-9-Hydroxy-Δ⁹-Tetrahydrocannabinol	0.2	100 ± 4	103 ± 6	102 ± 3	106 ± 4	101 ± 3
11-nor-9-Carboxy-Δ⁹-Tetrahydrocannabinol	0.2	102 ± 5	95 ± 5	97 ± 3	104 ± 2	98 ± 4

Table 4

METHOD REPRODUCIBILITY RESULTS FOR 3 SUBSEQUENT DAYS					
Drug	**Intra-assay LLOQ (%)**	**Intra-assay 3 x LLOQ (%)**	**Intra-assay 35% LLOQ (%)**	**Intra-assay 75% LLOQ (%)**	**Intra-assay ULOQ (%)**
Dansylated Δ⁹-Tetrahydrocannabinol	9.0	6.7	5.3	6.4	4.8
11-nor-9-Hydroxy-Δ⁹-Tetrahydrocannabinol	8.1	4.8	2.4	5.9	2.3
11-nor-9-Carboxy-Δ⁹-Tetrahydrocannabinol	8.0	8.8	6.4	6.3	7.4

For each analyzed compound, the calibration curve was linear for all range of concentrations. The accuracy and the precision of this method was measured using 5 points on each calibration curve and the reproducibility was measured for 3 subsequent days. The results show that the method is accurate (Table 3) and reproducible (Table 4) even if no internal standard was used. For a future validation, addition of an internal standard is highly recommended to avoid matrix effects.

CONCLUSIONS

This application presents data representing the usefulness of this new method with supporting data for peak shape, recovery, accuracy and precision.

Selectivity and sensitivity of the LC-MS/MS method for the determination of Δ⁹-tetrahydrocannabinol and its metabolites in plasma, which is usually measured by GC-MS are maintained with this protocol.

With this method, run times are reduced and high selectivity maintained compared to the GC-MS method.

REFERENCES

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2. T. Santa, O.Y. Al-Dirbashi, T. Fukushima, Derivatization reagents in liquid chromatography/ electrospray ionization tandem mass spectrometry for biomedical analysis. Drug Discov Ther.1, 108-118 (2007).
3. J.P. Goullé, E. Saussereau, C. Lacroix. Delta-9-tetrahydrocannabinol pharmacokinetics. Ann Pharm Fr. 66, 232-244 (2008).
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ACKNOWLEDGMENTS