# ASPEC<sup>®</sup> Positive Pressure Manifold and Cartridges: LCMS/MS detection of $\Delta^9$ -tetrahydrocannabinol in plasma using solid phase extraction (SPE) cleanup



# **APPLICATION NOTE (AN0992)**

#### APPLICATION BENEFITS

Precise and accurate measurement of  $\Delta^{9}$ -tetrahydrocannabinol and its metabolites are essential for drug testing. GC/MS is often used for quantification of  $\Delta^{9}$ -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  $\Delta^9$ -tetrahydrocannabinol and its metabolites by LC-MS/MS in human plasma. Due to the lack of functional groups,  $\Delta^9$ -tetrahydrocannabinol is first derivatized with dansyl chloride to allow for detection by MS.

Gilson ASPEC<sup>®</sup> SPE cartridges are used to extract drugs from plasma prior to derivatization.

# INTRODUCTION

The analysis of  $\Delta^{9}$ -tetrahydrocannabinol and its metabolites is usually done by GC/MS, which has long run times<sup>1</sup>. 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<sup>®</sup> C<sub>18</sub> 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.<sup>2</sup>

This application presents the use of the Gilson ASPEC® Positive Pressure Manifold with C<sub>18</sub> Cartridges for this new method for the determination of  $\Delta^9$ -tetrahydrocannabinol and its metabolites in human plasma using LC-MS/MS. The performance of Gilson ASPEC C<sub>18</sub> Cartridges was comparable to SPE columns from three competitors.

# MATERIALS AND METHODS

#### **Materials**

- ASPEC<sup>®</sup> Positive Pressure Manifold: (P/N: 37012000 - P/N: 37417010)
- 3 mL Manifold (P/N: 37417012)
- ASPEC<sup>®</sup> Solid Phase Extraction Cartridges: o ASPEC<sup>®</sup> C<sup>10</sup> 500 mg, 3 mL (P/N: 5430522NC)
- DISTRIMAN<sup>®</sup> (P/N: F164001) Distritips (P/N: F164120)



Figure 1 Gilson ASPEC<sup>®</sup> Positive Pressure Manifold

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# Methods

# **Sample Preparation**

- Mix 250  $\mu L$  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<sub>2</sub>O
- 4. Load: Load 1.25 mL of diluted plasma sample at 1 drop per 3 seconds
- 5. Wash 1: 2 mL of H<sub>2</sub>O at 1 drop per second
- 6. Wash 2: 1 mL of 1 M acetic acid
- 7. **Wash 3**: 2 mL of MeOH/H<sub>2</sub>O (20/80, v/v)
- 8. Elute: 3 mL of CH<sub>2</sub>Cl<sub>2</sub>/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:
  - $_{0}$  100  $\mu L$  of 0.1 M carbonate buffer was mixed with 200  $\mu L$  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):
  - o Add 2 mL of 1 chlorobutane.
  - o 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  $\mu$ L of ACN/H<sub>2</sub>O 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<sup>3</sup>. A range of 2 to 200 ng/mL for  $\Delta^9$ -tetrahydrocannabinol and 11-nor-9-Hydroxy- $\Delta^9$ -tetrahydrocannabinol and 10 to 200 ng/mL for 11-nor-9-Carboxy- $\Delta^9$ -tetrahydrocannabinol have been selected.

In fact,  $\Delta^{9}$ -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  $\Delta^9$ -Tetrahydrocannabinol and its metabolites.

# **Derivatization Scheme**





 $R = CH_3$ ,  $CH_2OH$  or COOH





Dansylated 11-nor-9-Carboxy- Δ9-Tetrahydrocannabinol

Dansylated 11-nor-9-Hydroxy-  $\Delta$ <sup>9</sup>-Tetrahydrocannabinol



#### **Chromatographic Conditions**

- Mobile Phase (MP): 1.0 mL/min
  - A: 1 mM ammonium formate in H<sub>2</sub>O/ACN (10/90, v/v) with 0.1% formic acid
  - B: 1 mM ammonium formate in H<sub>2</sub>O/ACN (90/10, v/v) with 0.1% formic acid
- Column: 3.0 x 30 mm C<sub>18</sub>, 2.5 μm at 23°C
- Detector: Sciex API 3000
  - o 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<sup>®</sup> C<sub>18</sub> cartridges have high C<sub>18</sub> loading, a homogeneous layer of C<sub>18</sub> functional groups and efficient endcapping that result in high recoveries and excellent reproducibility for  $\Delta^9$ -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<sub>18</sub> column showed excellent peak shape with no fronting or tailing for derivatized  $\Delta^9$ -tetrahydrocannabinol and its metabolites (Figure 3).

Following the FDA guide<sup>4</sup>, 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<sub>18</sub> 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  $\Delta^9$ -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 $\Delta^{9}$ -tetrahydrocannabinol and its Metabolites



#### Figure 3

Quantification Chromatogram at ULOQ (200 ng/mL) for ∆<sup>9</sup>-tetrahydrocannabinol and its metabolites

<sup>11</sup>-nor-<sup>9</sup>-Carboxy-  $\Delta^9$ -Tetrahydrocannabinol (n=3 for each level)





#### Figure 4

Selectivity and Sensitivity for dansylated  $\Delta^9$ -tetrahydrocannabinol and its metabolites

#### Table 2

Interference in the Matrix Blank

Drug	% of LLOQ Response
$\Delta^9$ -tetrahydrocannabinol	5
11-nor-9-Hydroxy- $\Delta^9$ -Tetrahydrocannabinol	5
11-nor-9-Carboxy- $\Delta^9$ -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 (%)	
Δ <sup>9</sup> -Tetrahydrocannabinol	0.2	103 ± 5	103 ± 7	102 ± 6	100 ± 2	97 ± 3	
11-nor-9-Hydroxy-Δ°-Tetrahydrocannabinol	0.2	100 ± 4	103 ± 6	102 ± 3	106 ± 4	101 ± 3	
11-nor-9-Carboxy-∆ <sup>9</sup> -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 $\Delta^9$ -Tetrahydrocannabinol	9.0	6.7	5.3	6.4	4.8		
11-nor-9-Hydroxy-∆°-Tetrahydrocannabinol	8.1	4.8	2.4	5.9	2.3		
11-nor-9-Carboxy-Δ <sup>9</sup> -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  $\Delta^9$ -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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### ACKNOWLEDGMENTS

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