Efficient LC-MS/MS Method for Determination of Δ^9 – Tetrahydrocannabinol in Human Plasma Using Solid Phase Extraction Clean-up

Application Note CL0313

Keywords

SPE (Solid Phase Extraction), Tetrahydrocannabinol, Metabolites, Human Plasma, LC-MS/MS (Liquid Chromatography Tandem Mass Spectroscopy), LC-MS (Liquid Chromatography-Mass Spectrometry), GC-MS (Gas Chromatography-Mass Spectrometry), MS (Mass Spectrometry, Solid Phase Extraction (SPE)

Introduction

The analysis of Δ^9 - Tetrahydrocannabinol and its metabolites is usually done by GC-MS, yet produces long run times.[1] In order to reduce run times without the loss of sensitivity and selectivity, Gilson, Inc. 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 amine and carboxylic acid, which normally allow for good sensitivity.

The Gilson ASPEC C18 stationary phase can be used to extract these 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[2], allowing a significant increase in the sensitivity and selectivity for these drugs.

This application presents the uses of ASPEC C18 silica and the results obtained with this new method developed for the determination of Δ^9 - Tetrahydrocannabinol and its metabolites in human plasma using LC-MS/MS analysis to compare with three other SPE column manufacturers.

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Materials & Methods

Materials

- Solid Phase Extraction Cartridges:
  - ASPEC™ C18 3 mL/500 mg
  - Gilson PN: 54350562

Sample Preparation

- Mix 250 µL of plasma with 1 mL of phosphate buffer (0.1M pH 6)

Solid Phase Extraction Steps

1. **Condition 1:** 3 mL of MeOH @ 8 mL/min
2. **Condition 2:** 3 mL of 1M HCl @ 8 mL/min
3. **Condition 3:** 3 mL of H_2O @ 8 mL/min
4. **Load:** load the full amount of prepared plasma sample (1250 µL)
5. **Wash 1:** 2 mL of H_2O @ 6 mL/min
6. **Wash 2:** 1 mL of 1M acetic acid @ 6 mL/min
7. **Wash 3:** 2 mL of (20/80) MeOH/H_2O (v/v) @ 6 mL/min
8. **Elute:** 3 mL of (50/50) CH_2Cl_2/Acetone (v/v) @ 3 mL/min

Sample Reconstitution

- Sample fractions were evaporated at 40°C for 10 minutes with nitrogen
- Samples were derivatized:
  - Mix 100 µL of carbonate buffer 0.1M with 200 µL of dansyl chloride solution under vortex for 1 minute (1 mg/mL in acetone)
- 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 vis 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 (80/20) ACN/H_2O 0.1% formic acid (v/v)

Range of Concentration and Derivatization

The range of concentration used for this application has been chosen following pharmacokinetic data evaluated from normal consumers and passive exposure to cannabis smoke.[3] In that case, a range of 2 to 200 ng/mL for the Δ^9-Tetrahydrocannabinol and the 11-nor-9-Hydroxy-Δ^9- Tetrahydrocannabinol and 10 to 200 ng/mL for the 11-nor-9-Carboxy-Δ^9- Tetrahydrocannabinol have been determined. Δ^9- Tetrahydrocannabinol and its metabolites present low sensitivity and high variability in the LC-MS/MS monitored signal as a result of unstable fragmentation.

Chromatographic Conditions

- **Mobile Phase:** 1.000 mL/min
  - **A:** 1mM ammonium formate in (10/90) H_2O/ACN, 0.1 % formic acid (v/v)
  - **B:** 1mM ammonium formate in (90/10) H_2O/ACN, 0.1 % formic acid (v/v)

GRADIENT			
Time (min)	MPA (%)	MPB (%)	Flow (mL/min)
0	10	90	1.000
1.00	10	90	1.000
1.01	0	100	1.000
3.50	0	100	1.000
3.51	10	90	1.000
5.00	10	90	1.000

- **Column:** 3.0 x 30 mm C18, 2.5 µm @ 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

Results

LC-MS/MS recovery results from this application show that the ASPEC C18 cartridges provide a high C18 loading (17%), a homogeneous layer of C18 functions, and an 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 1).

Quantification at the ULOQ (200 ng/mL) with the Gilson ASPEC C18 showed excellent peak shape with no fronting or tailing for Δ^9- Tetrahydrocannabinol and its metabolites. (Figure 2)

Following the FDA acceptance guide[4], a method needs to be selective at the lowest limit of quantification (< 20% of LLOQ). Quantification samples have been prepared in the plasma matrix in order to be selective. It is well-known that a small exposition to Δ^9-Tetrahydrocannabinol is metabolized by the human body and can be detected by LC-MS/MS. In that case, matrix interferences have been observed in a few samples. By using this method, analysts can measure low concentration of this drug (LOD 200 pg/mL) which is a proof of the method's sensitivity (Figure 3).

Figure 1: Recoveries for Δ^9 - Tetrahydrocannabinol and its Metabolites

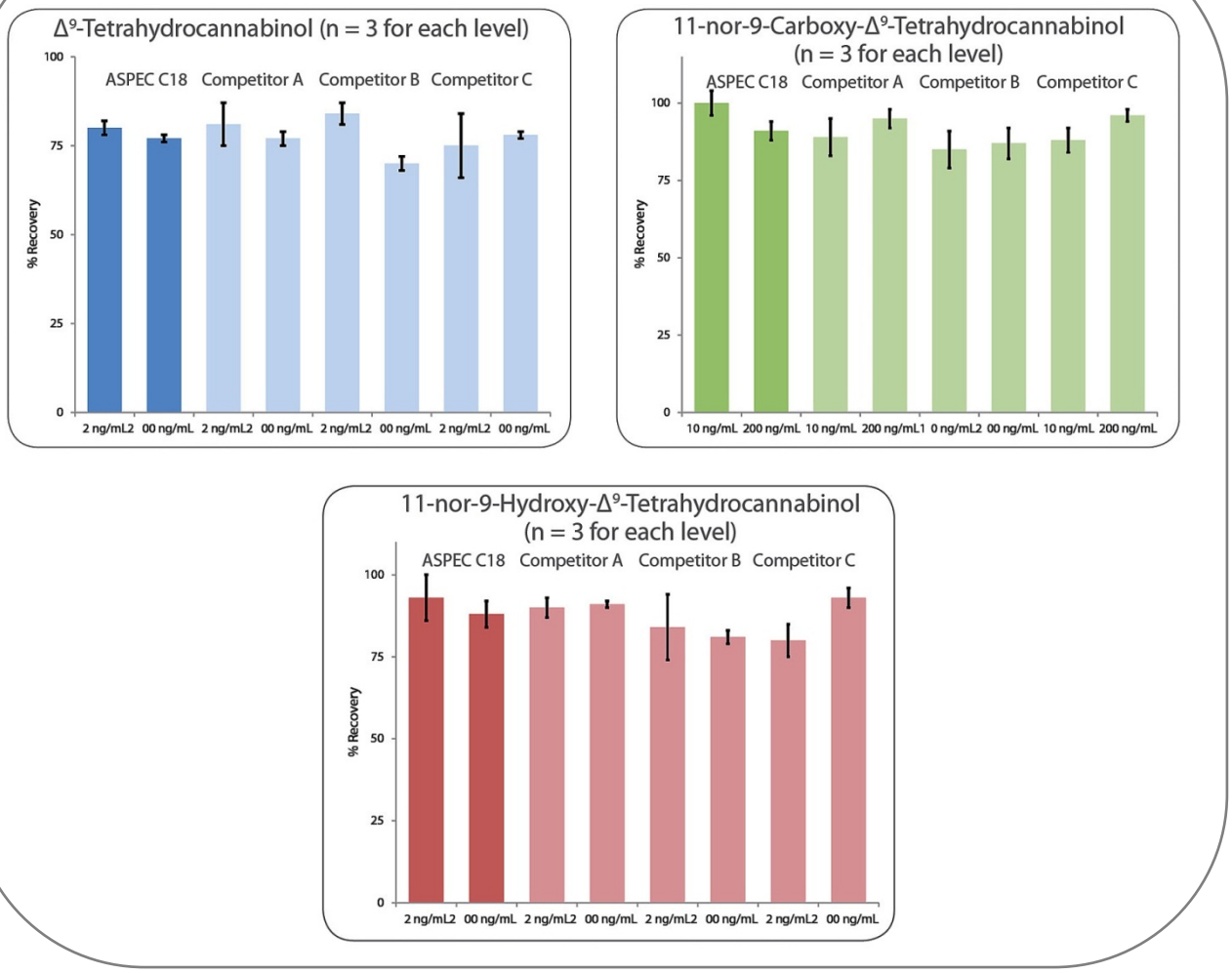


Figure 2: Quantification Chromatogram at ULOQ (200 ng/mL) for Δ^9 - Tetrahydrocannabinol and its Metabolites

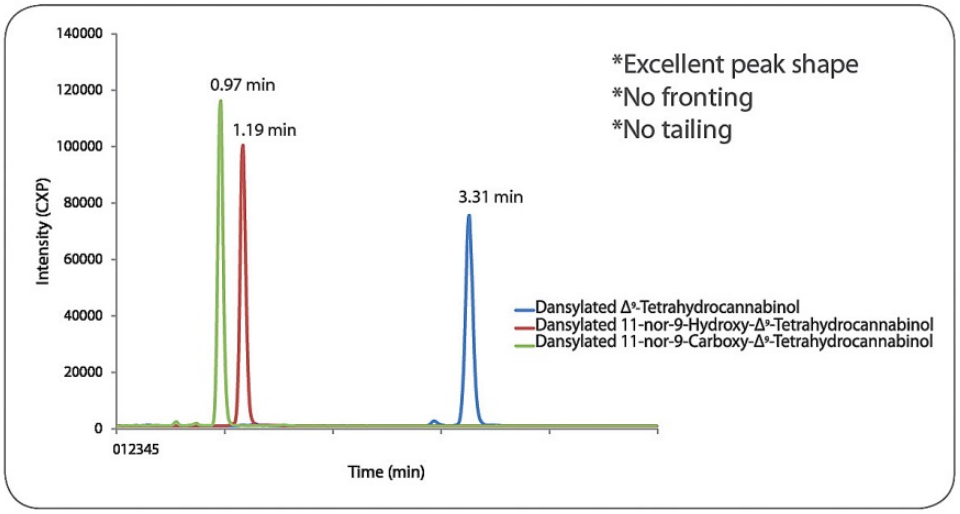
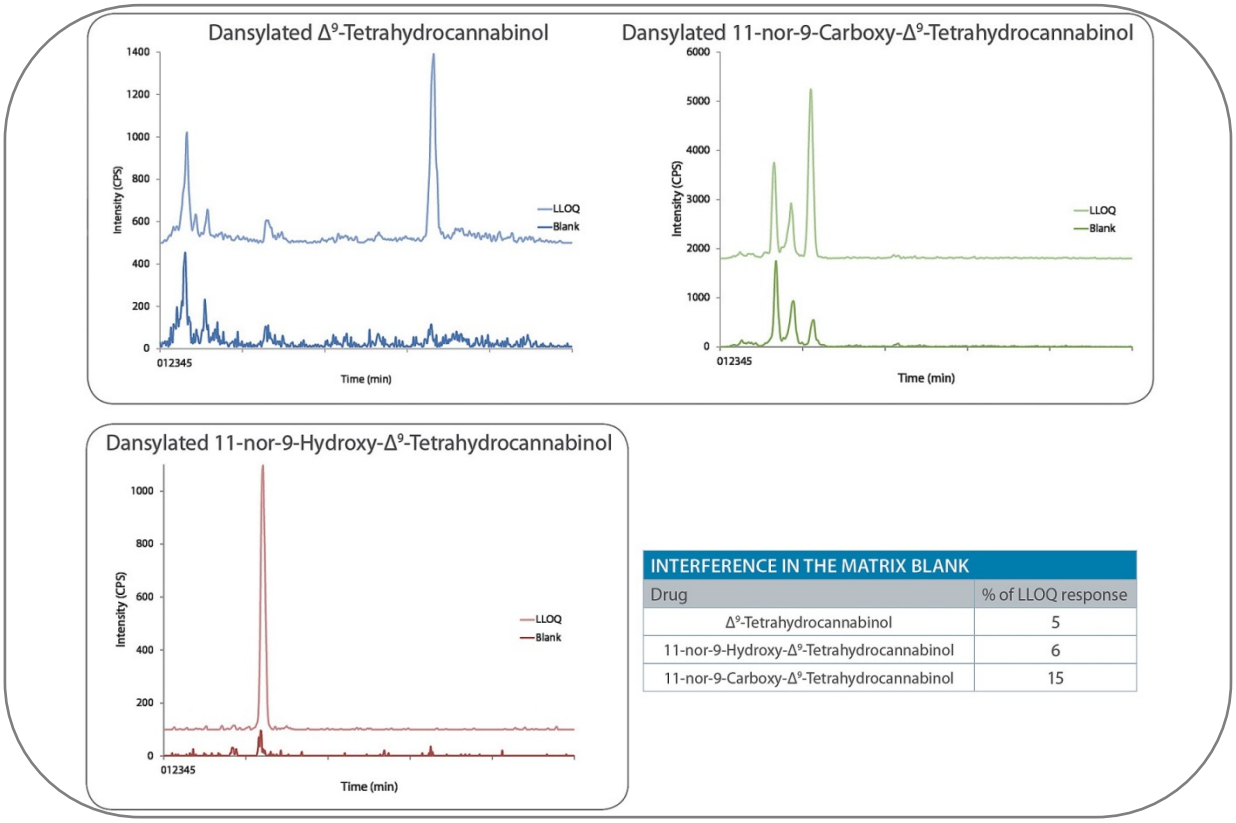


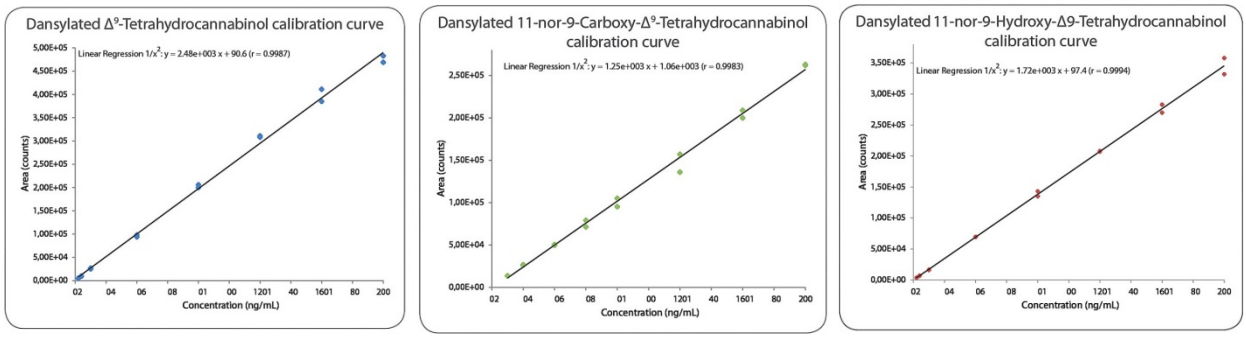
Figure 3: Selectivity and Sensitivity for Δ^9 - Tetrahydrocannabinol and its Metabolites



INTERFERENCE IN THE MATRIX BLANK	
Drug	% of LLOQ response
Δ^9-Tetrahydrocannabinol	5
11-nor-9-Hydroxy-Δ^9-Tetrahydrocannabinol	6
11-nor-9-Carboxy-Δ^9-Tetrahydrocannabinol	15

For each analyzed compound, the calibration curve was linear for the full 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 (Figure 4). The recovery results show that the method is accurate and reproducible even if no internal standard was used. For a future validation, addition of an internal standard is highly recommended to avoid matrix effects.

Figure 4: Linearity and Recovery Accuracy) for Δ^9 - Tetrahydrocannabinol and its Metabolites



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

METHOD ACCURACY AND PRECISION RESULTS FOR THE SAME RUN (N=6)					
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

Conclusion

This application presents data representing the usefulness of this new method with supporting data for peak shape, recovery, accuracy and precision, as well as selectivity and sensitivity of the LC-MS/MS method for the determination of Δ^9 - Tetrahydrocannabinol and its metabolites in plasma, which is usually measured by GC-MS. Moreover, with this method, it has been demonstrated that it is possible to reduce run time and maintain high selectivities compared to the GC-MS method by using the ASPEC C18 cartridges.

References

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