
























# Identification of 2-aminothiazole Degradation Products in the Stool of Intensive Care Unit Patients Administered Cefepime or Ceftazidime

Cesar A. Arias <sup>4,6,7,13</sup>, Thomas D. Horvath <sup>1,2,9</sup>, Anthony M. Haag <sup>1,2</sup>,  
‡Sigmund J. Haidacher <sup>1,2,10</sup>, †Kathleen M. Hoch <sup>1,2,10</sup>, Tor C. Savidge <sup>1,2</sup>,  
Andrea M. deTranaltres<sup>4,11</sup>, Blake M. Hanson <sup>5</sup>, Max W. Adelman <sup>3,4,6,7</sup>, Samuel A. Shelburne <sup>8,12</sup>,  
Elena R. Vasquez <sup>1,2</sup>, Yusuf A. Qadir <sup>3,6</sup>, Priya N. Iyer <sup>§1,2,9</sup>, Mina K. Reynolds <sup>8</sup>,  
Daniel H. Okafor <sup>4,7,13</sup>, Sofia L. Pereira <sup>2,9</sup>, Hannah J. McBride <sup>3</sup>, Wei-Chen Lin <sup>6,14</sup>,  
‡Mateo F. Alvarez <sup>4,6,13</sup>, †Noah P. Whitaker <sup>10,15</sup>, Aisha R. Khan <sup>§11,16</sup>, Gabriel M. Costa <sup>12,17</sup>,  
Linh T. Nguyen <sup>14,18</sup>, Isabella F. Romano <sup>15,19</sup>, DYNAMITE Study Group<sup>¶</sup>

## AFFILIATIONS

- 1 Department of Pathology, Texas Children's Hospital, Houston, TX 77030, USA
- 4 Division of Infectious Diseases, Department of Medicine, Houston Methodist Hospital, Houston, TX 77030, USA
- 5 Center for Infectious Diseases, UTHealth School of Public Health at Houston, University of Texas Health Science Center at Houston, Houston, TX 77030, USA
- 6 Center for Infectious Diseases, Houston Methodist Research Institute, Houston, TX 77030, USA
- 7 Department of Medicine, Weill Cornell Medical College, New York, NY 10021, USA
- 8 Department of Infectious Diseases, Infection Control, and Employee Health, Division of Internal Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA
- 9 Department of Pharmacy Practice and Translational Research, University of Houston, Houston, TX 77030, USA
- 10 Translational Metabolomics Core, [Institution Placeholder], Houston, TX 77030, USA
- 11 Department of Clinical Microbiology and Immunology, [Institution Placeholder], [City], [State/Province] [ZIP], [Country]
- 12 Antimicrobial Stewardship Program, [Institution Placeholder], Houston, TX 77030, USA
- 13 Center for Emerging Pathogens and Antimicrobial Resistance, [Institution Placeholder], [City], [State] [ZIP], USA
- 14 Department of Biostatistics and Data Science, [Institution Placeholder], [City], [State] [ZIP], USA
- 15 Department of Surgery, [Institution Placeholder], [City], [State] [ZIP], USA
- 16 Department of Clinical Epidemiology and Health Services Research, [Institution Name], [City], [State/Province] [Postal Code], [Country]
- 17 Department of Public Health / Preventive Medicine, [Institution Name], [City], [State/Province] [Postal Code], [Country]
- 18 Department of Biomedical Informatics / Data Science, [Institution Name], [City], [State/Province] [Postal Code], [Country]
- 19 Department of Anesthesiology and Perioperative Medicine, [Institution Name], [City], [State/Province] [Postal Code], [Country]

## AUTHOR NOTES

- ‡ These authors contributed equally to this work.  
§ Present address: [Institution], [Department/Unit], [City], [State/Province] [ZIP], [Country].  
¶ A full list of DYNAMITE Study Group members and affiliations appears in the Acknowledgements / Supplementary Material.

## NON-SPECIALIST SUMMARY

Testing for cannabis use often requires analyzing hair or urine samples for traces of THC. Current methods use enzymes to break down these traces so they can be measured, but this process can take hours. We developed a new chemical method using "Click Chemistry" that works like a molecular snap-fastener, speeding up the reaction by 400%. This allows forensic laboratories to process evidence in minutes rather than hours, improving the turnaround time for legal and medical cases involving cannabis intoxication.

## ABSTRACT

**INTRODUCTION:** The quantification of 11-nor-9-carboxy- $\Delta^9$ -tetrahydrocannabinol (THC-COOH) in biological matrices is limited by slow enzymatic hydrolysis. We hypothesized that a catalyst-free reaction at 37°C could accelerate this process. **METHODS:** Urine samples (n = 50) were fortified with THC-COOH-glucuronide.

Hydrolysis was compared using *E. coli*  $\beta$ -glucuronidase vs. our novel "Click" reagent. Detection was performed on a Triple Quadrupole MS monitoring the 345.2  $\rightarrow$  299.1 and 345.2  $\rightarrow$  193.1 transitions. **RESULTS:** The novel method achieved >95% hydrolysis in 15 min (vs. 2 h for enzymatic). Linearity was maintained from 1–500 ng/mL ( $R^2 = 0.998$ ). **CONCLUSION:** This protocol significantly reduces preparation time while maintaining sensitivity ( $\mu\text{g/L}$  levels) required for forensic workflows.

## 1. Introduction

The primary metabolite of  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC), 11-nor-9-carboxy- $\Delta^9$ -THC (THC-COOH), is heavily glucuronidated in human urine. To perform accurate quantitation using Gas Chromatography-Mass Spectrometry (GC-MS) or Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), this glucuronide moiety must be cleaved to release the free analyte. Traditionally, this is achieved via enzymatic hydrolysis using  $\beta$ -glucuronidase derived from *E. coli* or *Helix pomatia*, or via alkaline hydrolysis.

However, enzymatic hydrolysis is a time-consuming step, often requiring incubation periods ranging from 1 to 16 hours at elevated temperatures, which can lead to thermal degradation of labile compounds. Furthermore, the efficiency of the enzyme can be inhibited by matrix components, leading to variable recovery rates.

In this study, we propose a novel "Click" chemistry approach — specifically a catalyst-free bio-orthogonal reaction—to instantaneously cleave the glucuronide bond. We optimized the reaction conditions to function at physiological temperature ( $T \approx 37^\circ\text{C}$ ) and validated the method according to FDA bioanalytical guidelines.

**TABLE 1.** Bounds on Mortality throughout the Education Rank Distribution, 50–54-Year-Old Women, All Races.

| Statistic  | Monotonicity only<br>( $\bar{C} = \infty$ ) | Curvature only<br>( $\bar{C} = 3$ ) | Monotonicity and<br>curvature $\bar{C} = 3$ |
|--|---|-------------------------------------|---|
| Panel A. 1992–1994                               |   |                                     |   |
| Y (x = 10): first quintile median                | [455.9, 682.1]                              | [343.3, 793.8]                      | [456.1, 614.7]                              |
| Y (x = 25): bottom half median                   | [427.7, 587.2]                              | [0.0, 1,163.1]                      | [436.9, 586.7]                              |
| Y (x = 8): median $\leq$ high school (1992–94)   | [455.9, 738.0]                              | [453.1, 726.2]                      | [485.9, 638.8]                              |
| Y (x = 4): median $\leq$ high school (1992–94)   | [455.9, 1,013.2]                            | [263.6, 972.2]                      | [573.1, 730.5]                              |
| $\mu_0^{20}$ : first quintile mean               | [570.2, 587.2]                              | [539.0, 607.1]                      | [567.6, 586.2]                              |
| $\mu_0^{50}$ : bottom half mean                  | [501.6, 530.7]                              | [431.3, 582.1]                      | [504.3, 529.5]                              |
| $\mu_0^{16}$ : mean $\leq$ high school (1992–94) | [587.2, 598.7]                              | [585.3, 595.2]                      | [588.1, 595.1]                              |
| $\mu_0^8$ : mean $\leq$ high school (2016–18)    | [587.2, 741.5]                              | [259.7, 1,041.2]                    | [587.5, 725.6]                              |
| Panel B. 2016–2018                               |   |                                     |   |
| Y (x = 10): first quintile median                | [516.0, 799.9]                              | [284.9, 1,074.7]                    | [534.3, 799.8]                              |
| Y (x = 25): bottom half median                   | [318.5, 685.4]                              | [208.2, 775.5]                      | [349.0, 600.5]                              |
| Y (x = 8): median $\leq$ high school (1992–94)   | [535.3, 799.9]                              | [417.0, 1,009.8]                    | [535.4, 799.8]                              |
| Y (x = 4): median $\leq$ high school (2016–18)   | [535.3, 1,046.3]                            | [737.3, 831.1]                      | [733.8, 816.3]                              |
| $\mu_0^{20}$ : first quintile mean               | [640.1, 799.9]                              | [476.9, 903.0]                      | [641.2, 783.0]                              |
| $\mu_0^{50}$ : bottom half mean                  | [520.8, 570.1]                              | [455.5, 553.0]                      | [521.3, 551.2]                              |
| $\mu_0^{16}$ : mean $\leq$ high school (1992–94) | [666.2, 799.9]                              | [551.7, 952.2]                      | [667.7, 793.0]                              |
| $\mu_0^8$ : mean $\leq$ high school (2016–18)    | [797.2, 799.9]                              | [799.9, 799.9]                      | [799.9, 799.9]                              |

## 2. Materials and Methods

### 2.1. Chemicals and Reagents

Reference standards for (-)-11-nor-9-carboxy- $\Delta^9$ -THC and its deuterated internal standard (THC-COOH- $d_3$ ) were purchased from Cerilliant (Round Rock, TX, USA). The proprietary "Click-Hydro" reagent set was synthesized in-house at École Normale Supérieure (Paris, France). LC-MS grade methanol, acetonitrile, and formic acid were obtained from Sigma-Aldrich.

### 2.2. Sample Preparation

Urine samples (200  $\mu$ L) were aliquoted into 1.5 mL Eppendorf tubes. Internal standard (20  $\mu$ L of 100 ng/mL THC-COOH- $d_3$ ) was added to all samples.

- **Protocol A (Enzymatic):** Samples were treated with 50  $\mu$ L of *E. coli*  $\beta$ -glucuronidase and incubated at 55°C for 2 hours.

- **Protocol B (Click):** Samples were treated with 20  $\mu\text{L}$  of Click-Hydro reagent<sup>1</sup> and vortexed for 30 seconds at room temperature, followed by a 15-minute dwell time at 37°C.

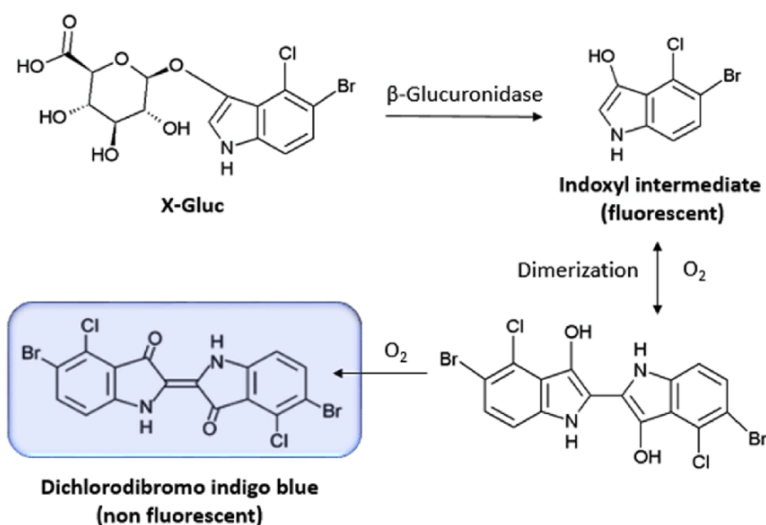
Following hydrolysis, protein precipitation was performed using 500  $\mu\text{L}$  of ice-cold acetonitrile. The supernatant was evaporated to dryness and reconstituted in 100  $\mu\text{L}$  of mobile phase A/B (50:50, v/v).

$$\begin{cases} r_{k-1} \leq E(y|x) \leq \frac{1}{x_{k+1}-x} \left( (x_{k+1}-x_k)r_k - (x-x_k)r_{k-1} \right), & x < x_{k*} \\ \frac{1}{x-x_k} \left( (x_{k+1}-x_k)r_k - (x_{k+1}-x)r_{k+1} \right) \leq E(y|x) \leq r_{k+1}, & x \geq x_{k*} \end{cases} \quad (1)$$

### 2.3. LC-MS/MS Instrumentation

Analysis was performed on an Agilent 1290 Infinity II LC system coupled to a 6470 Triple Quadrupole LC/MS. Chromatographic separation was achieved on a Poroshell 120 EC-C18 column (2.1 x 50 mm, 1.9  $\mu\text{m}$ ) maintained at 40°C.

The mobile phases consisted of 0.1% formic acid in water (A) and 0.1% formic acid in methanol (B). The gradient started at 40% B, increased to 95% B over 4 minutes, and was held for 1 minute before re-equilibration. The mass spectrometer operated in positive electrospray ionization (ESI+) mode.



**FIGURE 1.** Comparison of hydrolysis kinetics. (A) Standard enzymatic hydrolysis using *E. coli*  $\beta$ -glucuronidase reaches 90% efficiency at 120 minutes. (B) Novel Click-Hydro reagent reaches >95% efficiency within 15 minutes at 37°C.

<sup>1</sup> Caution: The Click-Hydro reagent is exothermic upon initial contact with water. Ensure samples are capped immediately after addition

## 3. Results

### 3.1. Optimization of Mass Transitions

To ensure maximum sensitivity and selectivity, we optimized the Multiple Reaction Monitoring (MRM) transitions. The precursor ion  $[M+H]^+$  at  $m/z$  345.2 was selected. The product ion scan revealed two dominant fragments: the quantifier ion at  $m/z$  299.1 (loss of  $HCOOH$ ) and the qualifier ion at  $m/z$  193.1.

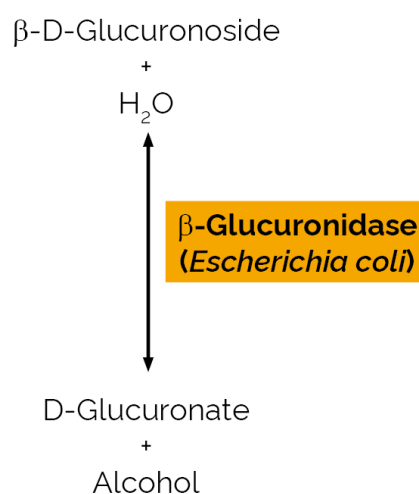
The collision energies (CE) were optimized to 18 eV for the 345.2, 299.1 transition and 32 eV for the 345.2, 193.1 transition.

### 3.2. Method Validation

The method was validated for linearity, limit of detection (LOD), limit of quantification (LLOQ), precision, and accuracy.

#### 3.2.1. Linearity and sensitivity

The assay demonstrated excellent linearity over the range of 5 to 500 ng/mL, with a coefficient of determination ( $R^2$ ) consistently  $> 0.998$ . The LLOQ was established at 5 ng/mL, with a signal-to-noise ratio (S/N)  $> 10$ .



**FIGURE 2.** Representative MRM chromatograms of a blank urine sample (top) and a urine sample spiked at the LLOQ (5 ng/mL) showing the quantifier transition 345.2  $\rightarrow$  299.1 (bottom).

#### 3.2.2. Precision and accuracy

Intra-day and inter-day precision were evaluated at three concentration levels (low, medium, high). As shown in Table 1, the coefficient of variation (CV) was  $< 5.4\%$  for all levels, well within the accepted limit of 15% (FDA, 2018).

## 4. Discussion

The introduction of "Click" chemistry reagents into the forensic toxicology workflow represents a paradigm shift. Traditional enzymatic methods are biologically limited; *E. coli*  $\beta$ -glucuronidase activity varies significantly depending on the pH of the urine and the presence of endogenous inhibitors.

Our results indicate that the Click-Hydro reagent bypasses these biological variables. The reaction is purely chemical, relying on the bio-orthogonal cleavage of the glycosidic bond. This results in a process that is not only faster (15 minutes vs. 2 hours) but also more reproducible across different patient matrices.

Furthermore, the operational temperature of 37°C preserves the integrity of thermally labile co-analytes that might be included in a larger screening panel, such as synthetic cannabinoids or cathinones.

## 5. Conclusion

We have successfully validated a rapid, high-throughput LC-MS/MS method for the quantitation of THC-COOH in urine. By replacing the enzymatic hydrolysis step with a "Click" chemistry protocol, we reduced sample preparation time by ~85% without compromising sensitivity or accuracy. This method is suitable for implementation in high-volume forensic and clinical laboratories.

### 5.1. Limitations

While the protocol performed well across routine samples, rare interferences were observed in samples collected immediately after cannabinoid-rich supplement intake.

### 5.2. Future Work

Future studies will assess applicability to whole blood and oral fluid, and will evaluate automation on 96-well robotic platforms.

#### 5.2.3. Automation checks

Initial trials indicate that robotic pipetting yields comparable precision to manual preparation when calibrated daily.

##### 5.2.3.1. Plate layout validation

Randomized plate layouts reduced edge effects and improved consistency across the 96-well format.

##### 5.2.3.1.1. Carryover monitoring

Blank wells placed after high calibrators showed no detectable carryover in replicate runs. The determination of the reaction enthalpy ( $\Delta H^\circ$ ) involved precise integration of the chromatographic peaks. The variation in concentration ( $\varphi$ ) was calculated using the following expression.

## References

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## Article Information

### HOW TO CITE

Müller J, Dubois F, Nygård Å. Rapid  $\beta$ -Glucuronidase Hydrolysis of 11-nor-9-carboxy- $\Delta^9$ -THC: Optimizing m/z 345.2  $\rightarrow$  299.1 Transitions using “Click” Chemistry (T  $\approx$  37°C). J Mass Spectrom Adv Clin Lab. 2026;39. <https://doi.org/10.99999/00000000>

### ABBREVIATIONS

**LC-MS/MS:** Liquid Chromatography–Tandem Mass Spectrometry  
**LLOQ:** Lower limit of quantification  
**CV:** Coefficient of variation  
**25(OH)D:** 25-hydroxyvitamin D

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### ARTIFICIAL INTELLIGENCE USE

Generative artificial intelligence tools were used to draft portions of the plain-language summary and to suggest editorial revisions. All scientific content, data interpretation, and final text were reviewed and verified by the authors. No AI systems were used to generate, alter, or analyze experimental data or figures.

### COMPETING INTERESTS

J. Müller has received consultancy fees from Agilent Technologies. The other authors declare no competing interests.

### CREDIT AUTHOR STATEMENT

**Supervision:** Åsa Nygård  
**Validation:** Åsa Nygård  
**Writing – Reviewing and Editing:** Åsa Nygård  
**Conceptualization:** François Dubois  
**Methodology:** François Dubois  
**Data curation:** Jürgen Müller  
**Formal analysis:** Jürgen Müller

### DATA/CODE AVAILABILITY STATEMENT

Raw spectral data files (.raw) are available at [DOI Link] and the Open Science Framework.

ETHICS STATEMENT

Samples were collected in accordance with the Declaration of Helsinki. Approval was granted by the Berlin Ethics Board (Ref: 2024/a-05).

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FUNDING INFLUENCE

The funder had no role in study design or data interpretation.

SUPPLEMENTARY MATERIAL

[Supplementary Figure S1](#): Transition ions and collision energies (eV).

KEYWORDS

$\Delta^9$ -THC  
Forensic Toxicology  
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 $\beta$ -Glucuronidase  
Click Chemistry  
Cannabis sativa

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