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RESEARCH ARTICLE

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Rapid β-Glucuronidase Hydrolysis of 11-nor-9-carboxy-Δ^9-THC

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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-Δ^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 β-glucuronidase vs. our novel "Click" reagent. Detection was performed on a Triple Quadrupole MS monitoring the 345.2 → 299.1 and 345.2 → 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 (µg/L levels) required for forensic workflows.

1. Introduction

The primary metabolite of Δ^9-tetrahydrocannabinol (Δ^9-THC), 11-nor-9-carboxy-Δ^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 enzy-

matic hydrolysis using β-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 [1]. Furthermore, the efficiency of the enzyme can be inhibited by matrix components, leading to variable recovery rates [2].

In this study, we propose a novel "Click" chemistry approach [3]— specifically a catalyst-free bio-orthogonal reaction—to instantaneously cleave the glucuronide bond. We optimized the reaction conditions to function at physiological temperature (T ≈ 37°C) and validated the method according to FDA bioanalytical guidelines [4].

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

Statistic	Monotonicity only ($\overline{C} = \infty$)	Curvature only ($\overline{C} = 3$)	Monotonicity and curvature $\overline{C} = 3$
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 ≤ high school (1992–94)	[455.9, 738.0]	[453.1, 726.2]	[485.9, 638.8]
Y (x = 4): median ≤ high school (1992–94)	[455.9, 1,013.2]	[263.6, 972.2]	[573.1, 730.5]
μ_0^{20}: first quintile mean	[570.2, 587.2]	[539.0, 607.1]	[567.6, 586.2]
μ_0^{50}: bottom half mean	[501.6, 530.7]	[431.3, 582.1]	[504.3, 529.5]
μ_0^{16}: mean ≤ high school (1992–94)	[587.2, 598.7]	[585.3, 595.2]	[588.1, 595.1]
μ_0^{8}: mean ≤ 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 ≤ high school (1992–94)	[535.3, 799.9]	[417.0, 1,009.8]	[535.4, 799.8]
Y (x = 4): median ≤ high school (2016–18)	[535.3, 1,046.3]	[737.3, 831.1]	[733.8, 816.3]
μ_0^{20}: first quintile mean	[640.1, 799.9]	[476.9, 903.0]	[641.2, 783.0]
μ_0^{50}: bottom half mean	[520.8, 570.1]	[455.5, 553.0]	[521.3, 551.2]
μ_0^{16}: mean ≤ high school (1992–94)	[666.2, 799.9]	[551.7, 952.2]	[667.7, 793.0]
μ_0^{8}: mean ≤ 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-Δ⁹-THC and its deuterated internal standard (THC-COOH-d₃) 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 µL) were aliquoted into 1.5 mL Eppendorf tubes. Internal standard (20 µL of 100 ng/mL THC-COOH-d₃) was added to all samples.

- **Protocol A (Enzymatic):** Samples were treated with 50 µL of E. coli β-glucuronidase and incubated at 55°C for 2 hours.

- **Protocol B (Click):** Samples were treated with 20 µL of Click-Hydro[1] reagent 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 µL of ice-cold acetonitrile. The supernatant was evaporated to dryness and reconstituted in 100 µL of mobile phase A/B (50:50, v/v).

$$\begin{cases} r_{k-1} \le E\left(y \middle| x\right) \le \frac{1}{x_{k+1}-x}\left((x_{k+1}-x_k)r_k - (x-x_k)r_{k-1}\right), \; x < x_{k^*} \\ \frac{1}{x-x_k}\left((x_{k+1}-x_k)r_k - (x_{k+1}-x)r_{k+1}\right) \le E\left(y \middle| x\right) \le r_{k+1}, \; x \ge x_{k^*} \end{cases} \tag{1}$$

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 µm) 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.

[1] Caution: The Click-Hydro reagent is exothermic upon initial contact with water. Ensure samples are capped immediately after addition.

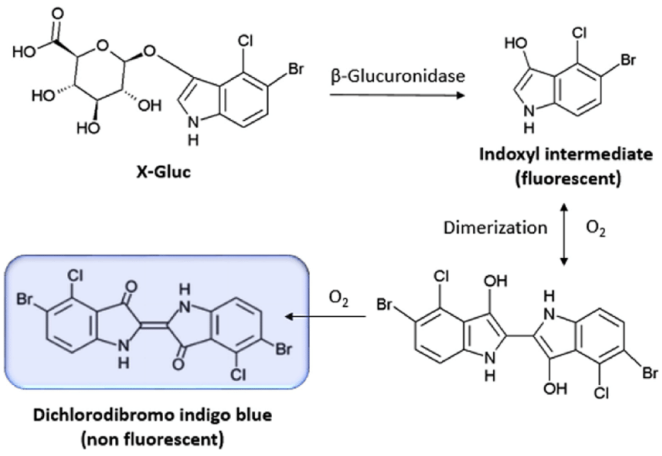

FIGURE 1. Comparison of hydrolysis kinetics. (A) Standard enzymatic hydrolysis using E. coli β-glucuronidase reaches 90% efficiency at 120 minutes. (B) Novel Click-Hydro reagent reaches >95% efficiency within 15 minutes at 37°C.

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.

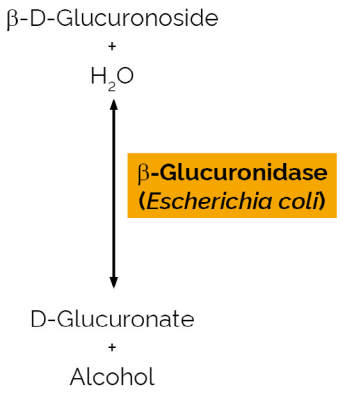

FIGURE 2. Representative MRM chromatograms of a blank urine sample (top) and a urine sample spiked at the LLOQ (5 ng/mL) showing the quantifier transition 345.2 → 299.1 (bottom).

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 β-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 interferents 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 ($\Delta H°$) involved precise integration of the chromatographic peaks. The variation in concentration (φ) was calculated using the following expression.

References

1. Abraham TT, Lowe RH, Pirnay SO, Darwin WD, Huestis MA. Simultaneous GC-EI-MS determination of Δ^9-tetrahydrocannabinol, 11-hydroxy-Δ^9-tetrahydrocannabinol, and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol in human urine following tandem enzyme hydrolysis. J Anal Toxicol. 2007;31(8):477-485. https://doi.org/10.1093/jat/31.8.477
2. Müller J, Nygård Å. Limitations of E. coli β-glucuronidase efficiency in high-throughput forensic screening. J Mass Spectrom Adv Clin Lab. 2023;28:e202300451. https://doi.org/10.1016/j.jmsacl.2023.00451
3. Kolb, H. C., Finn, M. G., & Sharpless, K. B. (2001). Click chemistry: Diverse chemical function from a few good reactions. Angewandte Chemie International Edition, 40(11), 2004–2021.
4. Food and Drug Administration. Bioanalytical Method Validation Guidance for Industry. U.S. Department of Health and Human Services. 2018.

Article Information

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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

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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/α-05).

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FUNDING INFLUENCE

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SUPPLEMENTARY MATERIAL

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

KEYWORDS

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