

Improved chromatographic separation of modified deoxycytidine nucleosides enhances LC-MS sensitivity

Christine Isaguirre¹, Molly T. Soper-Hopper¹, Stacey L. Thomas², Rachel Shereda², Darrell Chandler², Peter A. Jones², Ryan D. Sheldon^{1§}

¹Mass Spectrometry Core, Van Andel Institute, Grand Rapids, MI, United States

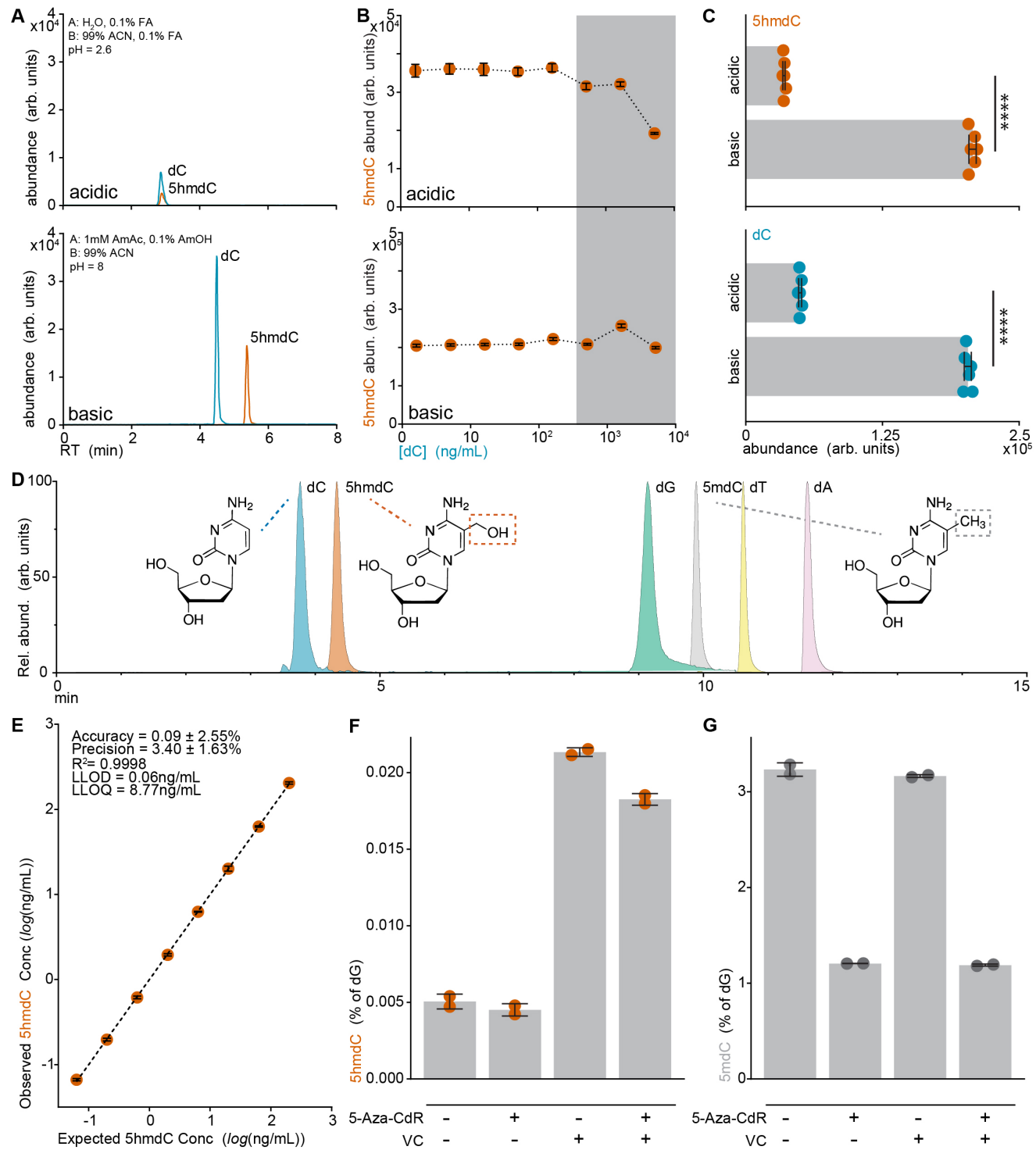
²Department of Epigenetics, Van Andel Institute, Grand Rapids, MI, United States

[§]To whom correspondence should be addressed: ryan.sheldon@vai.org

Abstract

Cytosine methylation is an epigenetic modification that regulates transcription. DNA demethylation occurs through oxidation of methylcytosine to hydroxymethylcytosine, making hydroxymethylation an important and translationally relevant marker of DNA methylation dynamics. These epigenetic modifications can be measured in their deoxynucleoside forms from enzymatically digested DNA by liquid chromatography-mass spectrometry (LC-MS). However, 5-hydroxymethyl-2'-deoxycytidine (5hmdC) co-elutes with 2'-deoxycytidine (dC). Because 5hmdC represents <0.05% of total dC in DNA, co-elution with dC causes ion suppression that limits detection and quantitation. Here, we present a chromatographic method that separates 5hmdC from dC, eliminates ion suppression, and improves the sensitivity of 5hmdC detection.

7/13/2026 - Open Access



Compound name	Precursor (m/z)	Product (m/z)	RT (min)	RT Window (min)	Fragmentor (V)	CE (V)	Polarity
2'deoxyctidine	228	112*, 95	4.3	2	50, 50	5, 40	+
2'deoxyctidine-13C515N	231	115*, 157, 98	4.3	2	55, 140, 50	30, 15, 40	+
5-hydroxymethyl-2'deoxyctidine	258	124*, 81	5.3	2	80, 80	30, 30	+
5-hydroxymethyl-2'deoxyctidine-d3	261	127*, 145	5.3	2	50, 90	30, 5	+
2'deoxyguanosine	268	152*, 135, 110	7.7	2	25, 40, 5	20, 50, 40	+

2'deoxyguanosine-13C515N	271	155*, 117	7.7	2	30, 100	5, 15	+
5-methyl-2'deoxycytidine	242	126*, 108	9.1	2	50, 50	10, 40	+
5-methyl-2'deoxycytidine-d3	245	129*, 117	9.1	2	50, 55	10, 15	+
2'deoxythymidine	243	127*, 117	9.2	2	50, 50	10, 10	+
2'deoxyadenosine	252	136*, 119	10.1	2	50, 100	10, 50	+

Figure 1. A mobile phase containing ammonium hydroxide separates 5hmdC from dC, alleviates ion suppression, and improves LC-MS detection sensitivity:

A) Reversed-phase chromatography does not separate 5hmdC from dC if the mobile phase contains 0.1% formic acid (acidic; top), but does separate the analytes when using 0.01% ammonium hydroxide (NH₄OH) and 1 mM ammonium acetate as the mobile phase (basic, bottom).

(B) Increasing concentrations of dC suppress 5hmdC signal when the two analytes co-elute, but not when the analytes are separated with the basic mobile phase. Data are the mean $\pm$ s.d. from n=3 replicates, and were derived from a constant input of 50 ng mL⁻¹ 5hmdC in an increasing background of dC. The grey-shaded region indicates dC concentrations typically observed from DNA digests.

(C) Separation increases LC-MS signal intensity of neat 5hmdC (top) and dC (bottom) standards, likely through improved ionization efficiency. P-value between groups is <0.0001.

(D) The complete method separates all DNA nucleosides and modified deoxycytidine nucleosides with an elution order of 2'-deoxycytidine (dC), 5-hydroxymethyl-2'-deoxycytidine (5hmdC), 2'-deoxyguanosine (dG), 5-methyl-2'-deoxycytidine (5mdC), 2'-deoxythymidine (dT), and 2'-deoxyadenosine (dA).

(E) Including deuterated 5hmdC (5hmdC-d₃) as an internal standard enables absolute quantitation of 5hmdC. Data are from n=6 independent standard curves, and are the mean $\pm$ s.d. of log(5hmdC/5hmdC-d₃). The dotted line is the linear regression of the log-transformed means.

(F) Vitamin C (VC) treatment increases 5hmdC levels in HCT116 cells, but 5-aza-2'-deoxycytidine (5-Aza-CdR; decitabine) does not. Data are the mean $\pm$ s.d. from n=2 replicates per group, expressed as a percentage of dG to account for differences in DNA loading. These data demonstrate that the method is suitable for detecting an expected biological phenotype.

(G) Same as panel F, except quantifying 5mdC. 5-Aza-CdR treatment decreases 5mdC concentration in HCT116 cells, as expected. N=2 replicates per condition.

Table: Transition list for LC-MS acquisition of DNA nucleosides and corresponding internal standards. * denotes transition used for quantitation. Each fragment ion is separated by a comma, with corresponding fragmentor and collision energy (CE) voltages listed in the same order as their corresponding fragment ions.

Description

Naturally modified DNA bases and their associated DNA:protein interactions are fundamental to normal biology (Gommers-Ampt & Borst, 1995; Raiber et al., 2017). 5-Methylcytosine, in particular, is a key transcriptional regulator (Jones & Takai, 2001) with genome-wide DNA methylation patterns established in early embryonic development (Li, 2002). These patterns are maintained across cell divisions and are considered relatively stable across the lifespan (Bird, 2002). However, the discovery that TET enzymes can actively oxidize 5-methylcytosine to 5-hydroxymethylcytosine (Tahiliani et al., 2009) revealed that DNA methylation is more dynamic than originally thought (Wu & Zhang, 2010). Importantly, 5-hydroxymethylcytosine levels in tissues or circulating cell-free DNA are diagnostic hallmarks of various cancers and other diseases (Buscarlet et al., 2015; Chen et al., 2016; Li & Liu, 2011; Miao et al., 2015; Song et al., 2017; Song et al., 2025; Xu & Gao, 2020; Yu et al., 2021), with the distribution and ratio of 5-methylcytosine to 5-hydroxymethylcytosine providing important clues about the tissue-of-origin (Li et al., 2024; Li et al., 2017; Song et al., 2017), disease stage (Li et al., 2024; Liao et al., 2016), treatment response (Chen et al., 2021; Lian et al., 2012; Shao et al., 2024), and patient outcomes (Chen et al., 2017; Chiu et al., 2019; Dong et al., 2015; Liao et al., 2016; Orr et al., 2012). Accurate and sensitive quantitation of 5-hydroxymethylcytosine is therefore important for detecting epigenetic dysregulation and supporting biomarker development.

LC-MS is frequently used to quantify the deoxynucleosides of modified cytosine bases, including 5-hydroxymethyl-2'-deoxycytidine (5hmdC), 5-methyl-2'-deoxycytidine (5mdC), and unmodified 2'-deoxycytidine (dC) from enzymatically digested DNA. Most commonly, reversed phase separations are performed using mobile phases containing formic acid. In such methods, however, 5hmdC co-elutes with the far more abundant analyte dC, which limits sensitivity and quantitative accuracy of 5hmdC measurements (Figure 1A, top). To address this, we evaluated several chromatography parameters,

including stationary phase (BEH Amide, phenylhexyl), flow rate and gradient, and mobile phase salt addition (10 mM ammonium formate), and discovered that adding 0.01% ammonium hydroxide (NH_4OH) with 1 mM ammonium acetate (basic) to the mobile phase, instead of 0.1% formic acid (acidic), resulted in baseline chromatographic separation of dC and 5hmdC (Figure 1A, bottom).

We next compared the amount of ion suppression observed with each solvent system by titrating 50 ng mL^{-1} 5hmdC into half-log dilutions of dC (range = $5 \text{ } \mu\text{g mL}^{-1}$ to 1.58 ng mL^{-1}). Acidic chromatography decreased 5hmdC signal at increasing dC concentrations (Figure 1B, top), indicating ion suppression of 5hmdC by dC. With the basic mobile phase, however, there was no detectable ion suppression (Figure 1B, bottom). We also saw a marked increase in signal intensity for dC and 5hmdC with the basic method (Figure 1C), presumably from enhanced ionization efficiency and/or less in-source adduct/fragment formation. Thus, using 0.01% NH_4OH with 1mM ammonium acetate in the mobile phase alleviates ion suppression and improves signal intensity.

We then expanded the method to include 5-methyl-2'-deoxycytidine (5mdC) and the other DNA nucleosides, which cleanly separate over a 19-minute analytical gradient and are easily detected by MS (Figure 1D). Because dC, 5mdC, and 5hmdC are typically reported as a percentage of dG to account for DNA loading differences, we extended this method to include isotopically labeled internal standards of these four deoxynucleosides. Transitions for these internal standards are included in Table 1. For absolute quantitation, we prepared a stock mixture for each nucleoside at concentrations expected in 500 ng DNA, serially diluted the mix, and then separated the analytes with the basic method to generate a calibration curve. This approach enabled absolute quantitation of 5hmdC with $0.09 \pm 2.6\%$ accuracy and $3.4 \pm 1.6\%$ precision across six independently prepared standard curves (Figure 1E; transitions for all compounds and internal standards are included in Table 1; * denotes quantifier transitions). Lower limit of detection (LLOD) and lower limit of quantitation (LLOQ) were determined to be 0.06 ng mL^{-1} and 8.77 ng mL^{-1} , respectively.

Finally, as a proof of principle, we evaluated whether the method was sufficient to detect expected biological effects. HCT116 cells were treated with 5-aza-2'-deoxycytidine (5-Aza-CdR, decitabine) and/or vitamin C to decrease 5mdC and increase 5hmdC, respectively (Liu et al., 2016). DNA was isolated, digested to nucleosides, and used to determine absolute concentrations of 5mdC, 5hmdC, dC, and dG. As expected, vitamin C increased 5hmdC content independently of 5-Aza-CdR, whereas 5-Aza-CdR decreased 5mdC independently of vitamin C. These data demonstrate the utility of this method to detect biologically relevant epigenetic cytosine modifications.

Methods

Cell culture. HCT116 cells were seeded on Day -1 at 300,000 cells per 100 mm dish in McCoy's 5A medium (16600-082, Gibco) supplemented with 1% Pen Strep (15140-122, Gibco) and 10% FBS (F0926, Sigma-Aldrich). On Day 0, cells were treated with 300 nM 5-aza-2'-deoxycytidine (5-Aza-CdR) as a single dose. Culture medium was replaced on Day 2. Vitamin C was administered at 57 μM once daily for 5 consecutive days (Days 0-4). Cells were harvested on Day 5 for downstream analysis.

DNA preparation. DNA was purified and digested using an AllPrep DNA/RNA Mini Kit (80204, Qiagen). After following the kit protocol, DNA was eluted with 50 μL of LC/MS grade water (W6, Fisher) and further purified using the Genomic DNA Clean & Concentrator-25 (D4065, Zymo Research). DNA was again eluted with 50 μL of LC/MS grade water, and the DNA concentration of each sample was measured using a Qubit Fluorometer. The DNA was then degraded to single nucleosides using Nucleoside Digestion Mix (M0649S, NEB) by incubating 50 μL of sample containing 500-800 ng of DNA with 5 μL of enzyme mix for 4 hr at 37°C . The completeness of digestion was evaluated by running 10 μL of each sample on a 1.5% agarose gel, looking for the absence of ethidium bromide/SYBR Safe fluorescent signal. 100 ng of DNA was used as a positive control. When complete digestion was confirmed, the remaining DNA digest mixture was stored at -80°C until LC/MS sample preparation and analysis.

Nucleoside preparation for LC-MS. 35 μL of DNA digests and standard curve samples were extracted with 315 μL of 80% methanol (v/v) (Fisher, A456) containing [$^{13}\text{C}_5$ ^{15}N]2'-deoxycytidine (100 ng/mL) (D239552, Toronto Research Chemicals), [D_3]5-methyl-2'-deoxycytidine (200 ng/mL) (M295902, Toronto Research Chemicals), [D_3]5-hydroxy-methyl-2'-deoxycytidine (200 ng/mL) (H946632, Toronto Research Chemicals), and [$^{13}\text{C}_5$] 2'-deoxyguanosine (200 ng/mL) (D232617, Toronto Research Chemicals) as internal standards. After adding extraction solvent, samples were pulse vortexed, sonicated in a water bath for 5 min, and incubated on wet ice for one hr. Samples were centrifuged at $17,000 \times g$ at 4°C for 10 min, and 280 μL of the supernatant was collected and dried overnight in a rotary vacuum evaporator. Samples were resuspended in LC/MS grade water, pulse vortexed, and sonicated in a water bath for 5 min. Samples were then transferred into autosampler vials (Fisher, 6PSV9-03FIVP) for LC-MS analysis.

LC parameters. 2 μL of each sample was injected and separated using a 21-minute gradient on a Cortecs T3 column (1.6 μm , 2.1 mm $\times$ 150 mm, 186008500, Waters, Eschborn, Germany) fitted with a Cortecs T3 VanGuard pre-column (1.6 μm , 2.1 mm $\times$ 5 mm, 186008508, Waters). Mobile phases for the acidic solvent system were A: LC/MS grade water and B:

99% acetonitrile (A955, Fisher), both with 0.1% (v/v) formic acid (A117, Fisher). For the basic solvent system, mobile phase A consisted of LC/MS grade water with 1 mM ammonium acetate (73594, Sigma) and 0.01% ammonium hydroxide. Mobile phase B consisted of 99% acetonitrile (A955-4, Fisher) and 1% LC/MS grade water. The column temperature was kept at 30°C, the flow rate was held at 0.3 mL min⁻¹, and the chromatography gradient was as follows: 0-6 min held at 0% B; 6-9 min ramp from 0 to 10% B; 9-14 min ramp from 10% to 50% B; and 14-18.9 min ramp from 50% to 99% B. At 19 min, the mobile-phase composition was changed to 0% B and the flow rate was changed to 0.4 mL min⁻¹ and held until 20.5 min, then decreased to 0.3 mL min⁻¹ by 21 min.

MS parameters. Samples and standards were separated on an Agilent1290 Infinity II ultra-high performance liquid chromatography system analyzed with an Agilent 6470 triple quadrupole mass spectrometer. Mass spectrometer parameters were: gas flow at 13 L min⁻¹ at 80°C; sheath gas flow at 11 L min⁻¹ at 275 °C; and the nebulizer was set to 30 psi. Capillary voltage was +2500 V and nozzle voltage was +500 V. Data were acquired using dynamic multiple reaction monitoring (dMRM) with at least two transitions per compound. The transition list was developed and optimized using neat analytical standards, with parameters provided in Table 1. The dMRM parameters were determined based on running LC/MS grade analytical standards for each target compound. Peak picking and integration was performed using Skyline software (v 24.1).

Absolute concentration. A stock standard curve mix was prepared with individual analyte concentrations selected to account for their endogenous levels. The stock mix contained 2'-deoxycytidine (dC; 250 µg mL⁻¹; Caymen Chemical #30125), 5-methyl-2'-deoxycytidine (5mdC; 10 µg mL⁻¹; Caymen Chemical #16166), 5-hydroxy-methyl-2'-deoxycytidine (5hmdC; 0.5 µg mL⁻¹; Caymen Chemical #18162), and 2'-deoxyguanosine (dG; 250 µg mL⁻¹; Caymen Chemical #9002864). This stock was diluted 2.5X to generate the initial dilution, then serially diluted in half-log steps. Each dilution was then extracted as described above.

For each analyte, peak area was expressed as the ratio of analyte signal to the corresponding internal standard signal, and the response ratio was log-transformed. Expected analyte concentrations were also log-transformed, and linear regression of log-transformed response ratio versus log-transformed concentration was used to generate the calibration curve. For biological samples, analyte concentrations were interpolated from this calibration curve. The resulting concentrations (ng/mL) were then used to calculate the ratios 5hmdC/dG and 5mdC/dG, which were expressed as percentages to account for variation in DNA loading.

Statistical analysis. Standard curve concentrations and unknown response ratios (i.e. signal normalized to the cognate internal standard) were log-transformed, and linear regression was used to interpolate concentrations. Back-transformed concentrations were used to calculate accuracy as $(\text{observed-expected})/\text{expected} \times 100$ and precision as $(\text{standard deviation})/\text{mean} \times 100$ of observed versus expected concentrations.

The lower limit of detection and lower limit of quantitation were estimated from blank responses on the log scale. Blank responses were expressed as log₁₀(signal/internal standard signal), and the mean and standard deviation of the blank responses were calculated. The detection and quantitation thresholds on the response scale were defined as $\bar{Y}_{\text{blank}} + 3.3s_{\text{blank}}$ and $\bar{Y}_{\text{blank}} + 10s_{\text{blank}}$, respectively. A calibration curve was generated by linear regression of log₁₀(response ratio) versus log₁₀(known concentration) according to $Y = 0.9867X - 2.280$. The corresponding log-transformed concentration was calculated as $X = (Y + 2.280)/0.9867$, and the final concentration was obtained by back-transformation as 10^X. All data are represented as means ± s.d.

Acknowledgements: LC-MS/MS analysis was conducted in the Van Andel Institute Mass Spectrometry Core (RRID:SCR_024903) and supported by the MeNu (Metabolism and Nutrition) program (RRID: SCR_027494) and a grant to P.A.J from the National Institutes of Health (R35CA209859).

References

- Bird A. 2002. DNA methylation patterns and epigenetic memory. *Genes Dev.* 16: 6-21. 5. PubMed ID: [11782440](#)
- Buscarlet M, Tessier A, Provost S, Busque L. 2015. Human blood cell level of 5-hydroxymethylcytosine (5hmC) declines steadily during aging and is multifactorial. *Blood.* 126: 4113-4113. 13. DOI: [10.1182/blood.V126.23.4113.4113](#)
- Chen HY, Zhang WL, Zhang L, Yang P, Li F, Yang ZR, et al., Jing HM. 2021. 5-Hydroxymethylcytosine profiles of cfDNA are highly predictive of R-CHOP treatment response in diffuse large B cell lymphoma patients. *Clin. Epigenetics.* 13: 33. 21. PubMed ID: [33573703](#)
- Chen K, Zhang J, Guo Z, Ma Q, Xu Z, Zhou Y, et al., Ci W. 2016. Loss of 5-hydroxymethylcytosine is linked to gene body hypermethylation in kidney cancer. *Cell Res.* 26: 103-18. 9. PubMed ID: [26680004](#)
- Chen Z, Shi X, Guo L, Li Y, Luo M, He J. 2017. Decreased 5-hydroxymethylcytosine levels correlate with cancer progression and poor survival: a systematic review and meta-analysis. *Oncotarget.* 8: 1944-1952. 23. PubMed ID:

[27911867](#)

Chiu BC, Zhang Z, You Q, Zeng C, Stepniak E, Bracci PM, et al., Zhang W. 2019. Prognostic implications of 5-hydroxymethylcytosines from circulating cell-free DNA in diffuse large B-cell lymphoma. *Blood Adv.* 3: 2790-2799. 25. PubMed ID: [31570490](#)

Dong ZR, Zhang C, Cai JB, Zhang PF, Shi GM, Gao DM, et al., Fan J. 2015. Role of 5-hydroxymethylcytosine level in diagnosis and prognosis prediction of intrahepatic cholangiocarcinoma. *Tumour Biol.* 36: 2763-71. 22. PubMed ID: [25480415](#)

Gommers Ampt JH, Borst P. 1995. Hypermodified bases in DNA. *FASEB J.* 9: 1034-42. 1. PubMed ID: [7649402](#)

Jones PA, Takai D. 2001. The role of DNA methylation in mammalian epigenetics. *Science.* 293: 1068-1070. 3. DOI: [10.1126/science.1063852](#)

Li E. 2002. Chromatin modification and epigenetic reprogramming in mammalian development. *Nat. Rev. Genet.* 3: 662-73. 4. PubMed ID: [12209141](#)

Li JJN, Liu G, Lok BH. 2024. Cell-free DNA hydroxymethylation in cancer: Current and emerging detection methods and clinical applications. *Genes.* 15 17. PubMed ID: [39336751](#)

Li W, Liu M. 2011. Distribution of 5-hydroxymethylcytosine in different human tissues. *J. Nucleic Acids.* 2011: 870726. 8. PubMed ID: [21772996](#)

Li W, Zhang X, Lu X, You L, Song Y, Luo Z, et al., Liu J. 2017. 5-hydroxymethylcytosine signatures in circulating cell-free DNA as diagnostic biomarkers for human cancers. *Cell Res.* 27: 1243-1257. 16. PubMed ID: [28925386](#)

Lian CG, Xu Y, Ceol C, Wu F, Larson A, Dresser K, et al., Shi YG. 2012. Loss of 5-hydroxymethylcytosine is an epigenetic hallmark of melanoma. *Cell.* 150: 1135-46. 19. PubMed ID: [22980977](#)

Liao Y, Gu J, Wu Y, Long X, Ge DI, Xu J, Ding J. 2016. Low level of 5-hydroxymethylcytosine predicts poor prognosis in non-small cell lung cancer. *Oncol. Lett.* 11: 3753-3760. 18. PubMed ID: [27313688](#)

Liu M, Ohtani H, Zhou W, Orskov AD, Charlet J, Zhang YW, et al., Jones PA. 2016. Vitamin C increases viral mimicry induced by 5-aza-2'-deoxycytidine. *Proc. Natl. Acad. Sci. USA.* 113: 10238-44. 26. PubMed ID: [27573823](#)

Miao Z, He Y, Xin N, Sun M, Chen L, Lin L, et al., Xu X. 2015. Altering 5-hydroxymethylcytosine modification impacts ischemic brain injury. *Hum. Mol. Genet.* 24: 5855-66. 14. PubMed ID: [26231219](#)

Orr BA, Haffner MC, Nelson WG, Yegnasubramanian S, Eberhart CG. 2012. Decreased 5-hydroxymethylcytosine is associated with neural progenitor phenotype in normal brain and shorter survival in malignant glioma. *PLoS ONE.* 7: e41036. 24. DOI: [10.1371/journal.pone.0041036](#)

Raiber EA, Hardisty R, Van Delft P, Balasubramanian S. 2017. Mapping and elucidating the function of modified bases in DNA. *Nat. Rev. Chem.* 1: 0069. 2. DOI: [10.1038/s41570-017-0069](#)

Shao J, Xu Y, Olsen RJ, Kasparian S, Sun K, Mathur S, et al., Li Z. 2024. 5-hydroxymethylcytosine in cell-free DNA predicts immunotherapy response in lung cancer. *Cells.* 13 20. PubMed ID: [38667328](#)

Song CX, Yin S, Ma L, Wheeler A, Chen Y, Zhang Y, et al., Quake SR. 2017. 5-hydroxymethylcytosine signatures in cell-free DNA provide information about tumor types and stages. *Cell Res.* 27: 1231-1242. 10. PubMed ID: [28820176](#)

Song D, Zhang Z, Zheng J, Zhang W, Cai J. 2025. 5-hydroxymethylcytosine modifications in circulating cell-free DNA: frontiers of cancer detection, monitoring, and prognostic evaluation. *Biomark. Res.* 13: 39. 11. PubMed ID: [40055844](#)

Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, et al., Rao A. 2009. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science.* 324: 930-935. 6. PubMed ID: [19372391](#)

Wu SC, Zhang Y. 2010. Active DNA demethylation: many roads lead to Rome. *Nat. Rev. Mol. Cell Biol.* 11: 607-20. 7. PubMed ID: [20683471](#)

Xu T, Gao H. 2020. Hydroxymethylation and tumors: can 5-hydroxymethylation be used as a marker for tumor diagnosis and treatment?. *Hum. Genomics.* 14: 15. 15. PubMed ID: [32375881](#)

Yu Z, Tong Y, Liang Y, Li Y, Yang H, Liu SY, et al., Zou X. 2021. Highly sensitive fluorescence detection of global 5-hydroxymethylcytosine from nanogram input with strongly emitting copper nanotags. *Anal. Chem.* 93: 14031-14035. 12. PubMed ID: [34637276](#)

Funding:

Supported by National Institutes of Health (United States) R35CA209859 to Peter Jones.

Conflicts of Interest: The authors declare that there are no conflicts of interest present.

Author Contributions: Christine Isaguirre: conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, writing - original draft. Molly T. Soper-Hopper: data curation, investigation, visualization, writing - original draft, writing - review editing. Stacey L. Thomas: investigation, writing - review editing, methodology. Rachel Shereda: investigation, writing - review editing. Darrell Chandler: writing - original draft, writing - review editing. Peter A. Jones: supervision, writing - review editing, conceptualization, funding acquisition. Ryan D. Sheldon: conceptualization, data curation, formal analysis, investigation, methodology, project administration, supervision, visualization, writing - original draft, writing - review editing.

Reviewed By: Anonymous

History: **Received** May 13, 2026 **Revision Received** June 18, 2026 **Accepted** July 9, 2026 **Published Online** July 13, 2026 **Indexed** July 27, 2026

Copyright: © 2026 by the authors. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Citation: Isaguirre C, Soper-Hopper MT, Thomas SL, Shereda R, Chandler D, Jones PA, Sheldon RD. 2026. Improved chromatographic separation of modified deoxycytidine nucleosides enhances LC-MS sensitivity. microPublication Biology. [10.17912/micropub.biology.002202](https://doi.org/10.17912/micropub.biology.002202)