



ELSEVIER

Contents lists available at ScienceDirect

Data in Brief

journal homepage: www.elsevier.com/locate/dib



Data Article

Data for the measurement of serum vitamin D metabolites in childhood acute lymphoblastic leukemia survivors

E. Delvin^{a,b,*}, M. Boisvert^a, M.-A. Lecours^a, Y. Théorêt^{a,c,d},
M. Kaufmann^e, G. Jones^e, E. Levy^{a,f}

^a CHU Ste-Justine Research Centre, University of Montréal, Montréal, Canada

^b Department of Biochemistry, University of Montréal, Montréal, Canada

^c Clinical Pharmacology Unit, Department of Clinical Biochemistry, CHU Ste-Justine, University of Montréal, Montréal, Canada

^d Department of Pharmacology, University of Montréal, Montréal, Canada

^e Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Canada

^f Department of Nutrition, University of Montréal, Montréal, Canada

ARTICLE INFO

Article history:

Received 28 December 2017

Received in revised form

24 February 2018

Accepted 10 April 2018

Available online 16 April 2018

ABSTRACT

This article describes data related to a companion research paper entitled “Vitamin D nutritional status and bone turnover biomarkers in childhood acute lymphoblastic leukemia (cALL) survivors.” (Delvin et al., submitted for publication) [1]. Various methods for the measurement of serum 25OHD₃, the accepted biomarker for assessing vitamin D nutritional status, have been described (Le Goff et al., 2015; Jensen et al., 2016) [2,3]. This article describes a novel mass spectrometry-QTOF method for the quantification of circulating 25OHD₃, 3-epi-25OHD₃ and 24,25(OH)₂D₃. It provides the description of the extraction, chromatography and mass spectrometry protocols, a sample of mass spectra obtained from standards and extracted serum, and a comparison with another HPLC-MS/MS (Jensen et al., 2016) [3] method for the measurement of serum concentrations of 25OHD₃.

© 2018 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

DOI of original article: <https://doi.org/10.1016/j.clnu.2018.02.006>

* Corresponding author at: CHU Ste-Justine Research Centre, University of Montréal, Montréal, Canada.

E-mail address: delvine@sympatico.ca (E. Delvin).

<https://doi.org/10.1016/j.dib.2018.04.030>

2352-3409/© 2018 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Specifications Table

Subject area	<i>Biology,</i>
More specific subject area	<i>Clinical Chemistry</i>
Type of data	<i>Tables, figures</i>
How data was acquired	<i>Liquid Chromatography coupled to a quadrupole time-of-flight mass spectrometer (Waters UPLC–MS system (Xevo G2 quadrupole time-of-flight))</i>
Data format	<i>Mass spectral analysis, analyzed</i>
Experimental factors	<i>Extracted serum samples, blank and standards were analyzed by Liquid Chromatography coupled to a quadrupole time-of-flight mass spectrometry.</i>
Experimental features	<i>Vitamin D metabolites were quantified after derivatization with 4-[2-(6,7-Dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxaliny)ethyl]-1,2,4-triazoline-3,5-dione (DMEQ-TAD) by isotope dilution mass spectrometry</i>
Data source location	<i>Montréal, Québec, Canada</i>
Data accessibility	<i>The data is available with this article only</i>

Value of the data

- The data describes a novel LC/MS-QTOF method for the measurement of serum vitamin D metabolites, providing the possibility of profiling.
- The details given enable other researchers to reproduce this method.
- This technology will be useful for vitamin D profiling in future clinical studies involving vitamin D supplementation.

1. Data

The data shared in this article include the description of the extraction, chromatography and mass spectrometry protocols as well sample mass spectra obtained from standards and extracted serum. The validation procedure of the method and results are also described.

1.1. Experimental design, materials and methods

The sample preparation method was adapted from Jones et al. [4]. In brief, 300 μL of a blank, consisting of charcoal-stripped plasma (cat. # 1131-00) purchased from Biocell (Rancho Dominguez, CA, USA), sample, calibrator or control in glass tubes were spiked with 75 μL of a mixture of deuterated internal standards (IS) consisting of [25OHD₃ (26,26,26,27,27,27-d₆, IS₁), 25OHD₂ (26,26,26,27,27,27-d₆, IS₂) from Chemaphor Inc., (Ottawa, ON, Can), 3-epi-25OHD₃ (6,19,19-d₃, IS₃) from Sigma-Aldrich Canada (Oakville, ON, Can) and 24,25(OH)₂D₃ (26,26,26,27,27,27-d₆, IS₄) from Departamento de Quimica Organica, Laboratorio Ignacio Ribas, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain. 100 μL of a 0.1 M HCl was then added, mixed, and samples allowed to sit for 10 min to release vitamin D metabolites from the vitamin D binding protein. Protein precipitation was achieved by adding 450 μL 0.2 M ZnSO₄, followed by 900 μL of methanol. The solutions are mixed thoroughly and centrifuged. The supernatants were transferred to borosilicate tubes and 2.2 mL of a 1:1 hexane:methyl tert-butyl ether extraction solvent added. Solutions were vortexed for 20 s. Approximately 1.8 mL was transferred to Waters Total Recovery vials and solutions dried under a gentle nitrogen stream. The dried samples were allowed to react for 90 min with DMEQ-TAD by two successive additions of 25 μL of 0.1 mg/mL of DMEQ-TAD in ethyl acetate after which 40 μL of ethanol was added to quench the reaction and destroy excess DMEQ-TAD. Solutions

Table 1
Chromatographic retention times and extracted mass ions for each vitamin D metabolite.

Vitamin D metabolite	Extracted ion m/z	Retention time (min)
25OHD ₃	746.4740 ± 0.1	4.66 ± 0.1
[⁶ d ₂]-25OHD ₃	752.5142 ± 0.1	4.63 ± 0.1
3-epi-25OHD ₃	746.4740 ± 0.1	4.45 ± 0.1
[³ d ₂]-3-epi-25OHD ₃	749.4966 ± 0.1	4.47 ± 0.1
24,25OHD ₃	762.4680 ± 0.1	3.27 ± 0.1
[⁶ d ₂]-24,25OHD ₃	768.5040 ± 0.1	3.24 ± 0.1

The analysis was performed in the scan mode from ions 100–1000 m/z . The data acquisition time was 1 s in the continuum mode.

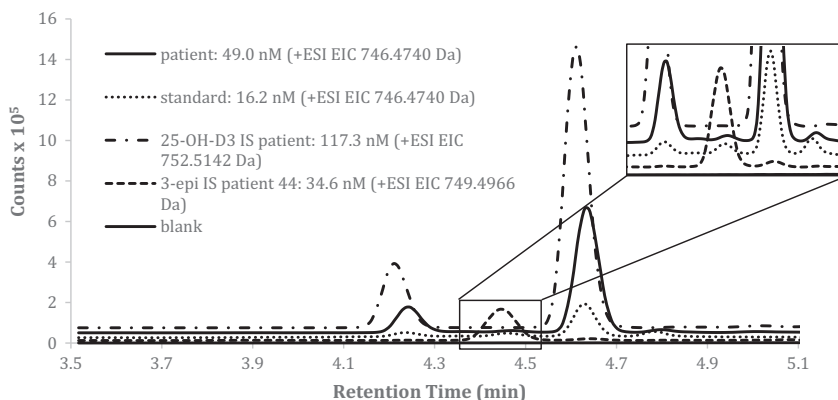


Fig. 1. Representative chromatographic profiles of 25-hydroxyvitamin D₃, 3-epi-25-hydroxyvitamin D₃ and their respective deuterated internal standards. Superimposed representative chromatographic profiles of a patient sample (—), a charcoal-stripped plasma spiked with a 25OHD₃ standard (....), a charcoal-stripped plasma spiked with the deuterated internal standard [⁶d₂]-25OHD₃ (-.-.-), and blank charcoal-stripped plasma (—) confounded with the X axis. The dotted line (—) in the inset shows profile for [³d₂]-3-epi-25OHD₃ in a patient serum extract analysed in the conditions described above.

dried under a nitrogen stream and derivatized vitamin D metabolites solubilized with 20 μ L of 40% mobile phase A and 60% mobile phase B (described below).

A Waters UPLC system with a Waters BEH phenyl, 2.1 \times 50 mm column with 1.7 μ m particle preceded by a guard column was used for the chromatographic separations. Mobile phase A and B consisted of 2 mM ammonium acetate/0.1% formic acid in water and methanol, respectively. Initial conditions were 35% phase A and 65% phase B with a 5 min-gradient to reach 90% phase B, followed by a one-minute equilibration time. The flow rate was 300 μ L/min, the column temperature was set at 40 $^{\circ}$ C, and the auto-sampler at 4 $^{\circ}$ C. A Waters Xevo G2 QTOF was used to detect and quantify the vitamin D metabolites. The instrument was operated in positive mode using the sensitivity mode. Capillary voltage was set at 1.0 kV, cone voltage at 35 V, with a source temperature of 150 $^{\circ}$ C, a desolvation gas temperature of 650 $^{\circ}$ C, with a flow rate of 900 L/h. Mass spectra were acquired in the target-enhanced mode with an acquisition time of 1 s. The chromatographic retention times and ionic transition masses are listed in Table 1.

Fig. 1 illustrates superimposed representative chromatographic profiles of a blank charcoal-stripped plasma, a charcoal-stripped plasma spiked with the deuterated internal standards [⁶d₂]-25OHD₃, a charcoal-stripped plasma spiked with a standard and a patient sample. The inset in Fig. 1 shows the profile for [³d₂]-3-epi-25OHD₃ in a patient serum extract analysed in the conditions described above. Note that the derivatization of the different vitamin D metabolites yielded, for each,

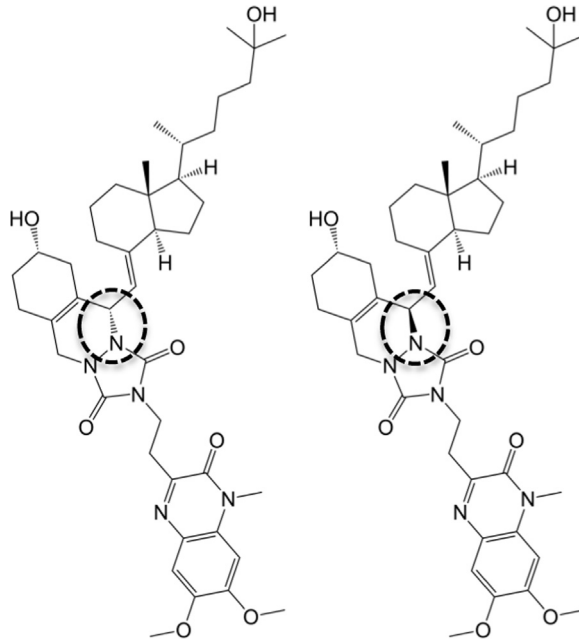


Fig. 2. 25-hydroxyvitamin D₃-DMEQ-TAD enantiomers. 25OHD₃ - DMEQ-TAD: 25-hydroxyvitamin D₃-4-[2-(6,7-Dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyloxy)ethyl]-1,2,4-triazoline-3,5-dione. The dotted circles indicate the chirality centre.

Table 2A

Method performance characteristics.

Vitamin D metabolites	25OHD ₃	2425OHD ₃	3-epi-25OHD ₃	25OHD ₂
LLOD (S/N ≤ 3)	0.3 nM	0.3 nM	0.1 nM	0.3 nM
LLOQ (CV ≤ 20%)	2.5 nM	2.0 nM	2.0 nM	2.5 nM
Intra-assay CV	4.2%	5.1%	4.5%	7.0%
	(70 nM)	(15 nM)	(20 nM)	(40 nM)
Inter-assay CV	7.0%	10.0%	8.9%	10.2%
	(70 nM)	(15 nM)	(20 nM)	(40 nM)
Bias (UTAK vitamin D control)	5%			6%
Linearity r ² > 0.99	2.5–462 nM	2.0–149 nM	2.0–148 nM	2.5–158 nM
Spiking % recovery	+ 37.5 nM (118%)	+ 36 nM (92%)	+ 37.5 nM (117%)	+ 37.8 nM (105%)
Carry-over	< LLOD	< LLOD	< LLOD	< LLOD

2 DMEQ-TAD enantiomers (R & S) due to an asymmetric carbon (Fig. 2). The major peak was used for quantification.

The method validation was performed with modified CLSI Guidelines [5,6]. Briefly, the Lower Limit of quantification (LLOQ) was estimated by the serial dilution of the standard solution ($n = 5$ per dilution) and was defined as the concentration at which precision was $\leq 20\%$. Linearity was evaluated by serially diluting a pool of high 25OHD₃ concentration samples with charcoal-stripped serum to generate 8 samples of intermediate concentrations that were measured in duplicate. Within assay imprecision was characterized by 5 measurements of a plasma sample pool spiked with 3-epi-25OHD₃, 25OHD₂, and 24,25(OH)₂D₃. Between-assay imprecision was assessed by analyzing 1 reference sample at 2 different concentrations in each batch over 14 months. Bias was determined by using UTAK vitamin D controls and a DEQAS test sample set.

Table 2A summarizes the performance characteristics of the method. Linear responses were observed up to 462 nM for 25OHD₃, 158 nM for 25OHD₂, 148 nM for 3-epi-25OHD₃ and 149 nM for

Table 2B
Method bias.

DEQAS controls vitamin D metabolites		25OHD ₃	25OHD ₂	Total (25OHD ₃ + 25OHD ₂)
461	Measured value	56.5 nM	2.7 nM	59.2 nM
	Reference value	55.0 nM	2.1 nM	57.1 nM
	bias	2.7%	28.8	3.7%
462	Measured value	77.5 nM	1.2 nM	78.7 nM
	Reference value	80.0 nM	1.2 nM	81.2 nM
	bias	- 3.1%	0%	- 3.1%
463	Measured value	90.8 nM	1.0 nM	91.8 nM
	Reference value	85.7 nM	0.7 nM	86.4 nM
	bias	6.0%	43%	6.3%
464	Measured value	60.4 nM	1.9 nM	62.3 nM
	Reference value	57.7 nM	2.0 nM	59.7 nM
	bias	4.7%	- 5%	4.4%
465	Measured value	60.0 nM	2.0 nM	62 nM
	Reference value	57.9 nM	2.0 nM	59.9 nM
	bias	3.6%	0%	3.5%

LLoD: Lower Limit of Detection; LLoQ : Lower Limit of Quantification; S/N: Signal/Noise ratio; UTAK : UTAK Laboratories.

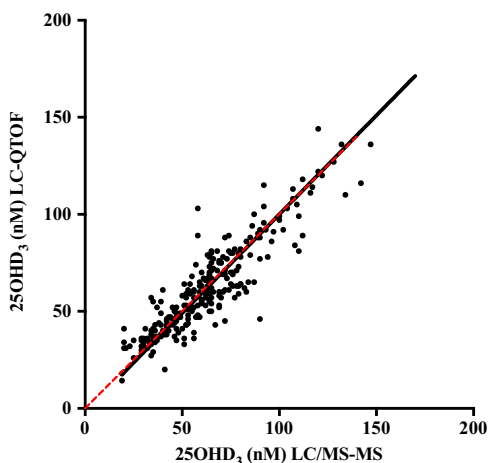


Fig. 3. Comparison between the HPLC-MS/QTOF and LC-MS/MS methods for measuring serum 25OHD₃ concentrations. The Deming linear regression analysis was used for comparing the 2 methods. Dashed line: Line of identity; $n = 248$. Equation: $Y = 1.02 \cdot X - 1.91$; Slope 95% Confidence Interval (C.I.): 0.957–1.08; $P = 0.0001$.

Table 3

25OHD₃, 3-epi-25OHD₃ and 24,25(OH)₂D₃ serum concentrations in childhood acute lymphoblastic leukemia survivors.

Analyte	LC-MS/MS nM		MS-QTOF nM		P value
	Median	2.5–97.5 th iles	Median	2.5–97.5 th iles	
25OHD ₃ ^b	60	22–131	57	29–121	= 0.3262
3-epi-25OHD ₃ ^a	N/A	N/A	3.1	2.0–12.6	-
24,25(OH) ₂ D ₃ ^b	N/A	N/A	6.3	2.8–20	-

^a 3-epi-25OHD₃ concentration was \geq LLoQ in 55 samples.

^b $N = 247$.

24,25(OH)₂D₃. The LLOD spanned from 0.1 to 0.3 nM and the LLOQ from 2.0 to 2.5 nM for the 4 vitamin D metabolites. The intra-assay imprecision ranged from respectively 4.2% to 7.0% and the inter-assay imprecision from 8.9% to 10.2% depending on the metabolite measured. The recovery of spiked samples ranged from 92% for 24,25(OH)₂D₃ to 118% for 25OHD₃. As shown in Table 2B, using DEQAS samples, the mean bias for 25OHD₃ ranged between 6.0 and – 3.1% at 85.7 and 80.0 nM respectively, within the limits set by the Vitamin D Standardization Program [7].

Fig. 3 is the Deming regression plot comparing serum 25OHD₃ concentration observed in the 248 serum samples of cALL survivors (1) by the present QTOF method and by HPLC-MS/MS (3). Table 3 lists the 25OHD₃, 3-epi-25OHD₃ and 24,25(OH)₂D₃ serum concentrations for the same samples.

Acknowledgements

This work was funded by the J.A.DeSève Research Chair in Nutrition (EL).

Transparency document. Supplementary material

Transparency document associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.dib.2018.04.030>.

References

- [1] E. Delvin, N. Alos, F. Rauch, V. Marcil, S. Morel, M. Boisvert, M.A. Lecours, C. Laverdière, D. Sinnett, M. Krajinovic, J. Dubois, S. Drouin, G. Lefebvre, M. Samoilenko, C. Nyalendo, E. Cavalier and E. Levy. Vitamin D nutritional status and bone turnover markers in acute lymphoblastic leukemia survivors, *Clin. Nutr.* 2018, <http://dx.doi.org/10.1016/j.clnu.2018.02.006>. pii: S0261-5614(18)30069-4, (Epub ahead of print) Feb 21.
- [2] C. Le Goff, E. Cavalier, J.-C. Souberbielle, Gonzalez-Antunia, E. Delvin, Measurement of circulating 25-hydroxyvitamin D: a Historical review, *Pract. Lab. Med.* 2 (2015) 1–14.
- [3] M.E. Jensen, F.M. Ducharme, Y. Théorêt, A.-S. Bélanger, E. Delvin, Data in support for the measurement of serum 25-hydroxyvitamin D (25OHD) by tandem mass spectrometry, *Data Brief* 8 (2016) 925–929.
- [4] G. Jones, M. Kaufmann, Vitamin D metabolite profiling using liquid chromatography-tandem mass spectrometry (LC-MS/MS), *J. Steroid Biochem. Mol. Biol.* 164 (2016) 110–114.
- [5] CLSI, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline. CLSI Document EP17-A2, second ed., Clinical Laboratory Standards Institute, Wayne, PA, 2012.
- [6] CLSI, Evaluation of the Linearity of Quantitative Measurement Procedures. A Statistical Approach; Approved Guideline. CLSI Document EP6-A, Clinical Laboratory Standards Institute, Wayne, PA, 2003.
- [7] N. Binkley, C.T. Sempos, for the Vitamin D Standardization Program (VDSP), Standardizing vitamin D assays: the way forward, *J. Bone Miner. Res.* 29 (2014) 1709–1714.