



RESEARCH ARTICLE

## Development and Validation of RP-HPLC Methods for Quantifying Biotin, Vitamin D<sub>3</sub>, and Vitamin E in Bovibest-H Liquid

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First published: 12 July 2025

<https://doi.org/10.1002/sscp.70093>

**Funding:** The authors did not receive any specific funding for this work.

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## ABSTRACT

Bovibest-H Liquid is a veterinary supplement designed to support the health and recovery of animals, particularly those with nutritional deficiencies. The goal of this study is to create and verify a reliable approach for the quantification of its active constituents, mainly biotin, vitamin D<sub>3</sub>, and vitamin E, using reverse-phase high-performance liquid chromatography (RP-HPLC). The best chromatographic conditions were found using a Shimadzu LC-2050 i-series liquid chromatography system. The specifications included the use of C8 and C18 columns, isocratic elution at a flow rate of 1.0 mL/min, and detection at 254 and 200 nm. According to ICH guidelines, the methods were thoroughly validated, and the results showed that it was robust and had great recovery rates of around 99.70%, excellent specificity, linearity ( $R^2 > 0.999$ ), precision (%RSD < 2), and valid accuracy. The present study successfully created a simple, accurate, precise, and robust HPLC method for quantifying biotin, vitamin D<sub>3</sub>, and vitamin E. The developed methods demonstrated remarkable linearity, precision, and accuracy over a range of concentrations. This precise analytical technique could provide significant insights into future studies in the fields of veterinary sciences, pharmaceuticals, and nutritional supplements.

## Conflicts of Interest

The authors declare no conflicts of interest.

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**Data Availability Statement**

## References

1 A. Wal, A. Sasmal, R. Singh, et al., "Regulatory Role, Mechanism, and Metabolic Profile of Biotin in Gene Expression." *Current Pharmacogenomics and Personalized Medicine* **20**, no. 2 (2023): 73–86, <https://doi.org/10.2174/1875692120666230712160812>.

View [↗](#) | [CAS](#) [↗](#) | [Google Scholar](#) [↗](#) |

2 C. Wang, J. An, L. Bu, et al., "Effects of Biotin and Coated Cobalamin on Lactation Performance, Nutrient Digestion and Rumen Fermentation in Holstein Dairy Cows," *Journal of Animal Physiology and Animal Nutrition* **108**, no. 3 (2024): 635–645, [10.1111/jpn.13920](https://doi.org/10.1111/jpn.13920).

View [↗](#) | [CAS](#) [↗](#) | [PubMed](#) [↗](#) | [Web of Science®](#) [↗](#) | [Google Scholar](#) [↗](#) |

3 V. Sakhniuk and M. Hotsuliak, "Metabolism of Vitamin D, Calcium and Phosphorus and Their Disorders in Goats." *Naukovij visnik veterinarnoï medicini* no. 2 (2023): 159–172, [10.33245/2310-4902-2023-184-2-159-172](https://doi.org/10.33245/2310-4902-2023-184-2-159-172).

View [↗](#) | [Google Scholar](#) [↗](#) |

4 A. Falchetti, E. Rossi, R. Cosso, et al., "Vitamin D and Bone Health." *Food and Nutrition Sciences* **7**, no. 11 (2016): 1033–1051, <https://doi.org/10.4236/fns.2016.711100>.

View [↗](#) | [CAS](#) [↗](#) | [Google Scholar](#) [↗](#) |

5 F. L. Ratib, M. A. Zafendi, and M. A. Lazaldin, "The Use of Vitamin E in Ocular Health: Bridging Omics Approaches With Tocopherol and Tocotrienol in the Management of Glaucoma," *Food Chemistry: Molecular Sciences* **9** (2024): 100224, <https://doi.org/10.1016/j.fochms.2024.100224>.

[↻](#) View [↗](#) | [PubMed](#) [↗](#) | [Google Scholar](#) [↗](#) |

6 P. L. Desbene, S. Coustal, and F. Frappier, "Separation of Biotin and Its Ana Logs by High-Performance Liquid Chromatography: Convenient Labeling for Ultraviolet or Fluorimetric Detection," *Analytical Biochemistry* **128**, no. 2 (1983): 359–362, [https://doi.org/10.1016/0003-2697\(83\)90386-X](https://doi.org/10.1016/0003-2697(83)90386-X).

View [↗](#) | [CAS](#) [↗](#) | [PubMed](#) [↗](#) | [Google Scholar](#) [↗](#) |

of *Pharmaceutical Sciences* 3, no. 2 (2010): 78–86.

[CAS](#) | [Google Scholar](#)

8 L. Gamiz-Gracia, M. M. Jiménez-Carmona, and M. D. Luque de Castro, "Determination of Vitamins D 2 and D 3 in Pharmaceuticals by Supercritical-Fluid Extraction and HPLC Separation With UV Detection," *Chromatographia* 51 (2000): 428–432, <https://doi.org/10.1007/BF02490479>.

[View](#) | [CAS](#) | [Google Scholar](#)

9 S. Priya, "Development and Validation of RP-HPLC Method for the Estimation of Vitamin D3 in Liquid Formulations," *Research Gate: Pharmaceutical Sciences* 1 (2012): 5–7.

[Google Scholar](#)

10 K. Sarioglu, S. S. Celebi, and M. Mutlu, "A Rapid Method for Determination of Vitamins D2 and D3 in Pharmaceutical Preparations by HPLC," *Journal of Liquid Chromatography & Related Technologies* 24, no. 7 (2001): 973–982, <https://doi.org/10.1081/JLC-100103423>.

[View](#) | [CAS](#) | [Web of Science®](#) | [Google Scholar](#)

11 M. Topkoska, M. Miloshevska, M. Piponski, et al., "Greenness Assessment and Validation of HPLC Method for Simultaneous Determination of Resveratrol and Vitamin E in Dietary Supplements," *Journal of AOAC International* 107, no. 2 (2024): 248–253, <https://doi.org/10.1093/jaoacint/qsad131>.

[View](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

12 Ž Temova and R. Roškar, "Stability-Indicating HPLC–UV Method for Vitamin D3 Determination in Solutions, Nutritional Supplements and Pharmaceuticals," *Journal of Chromatographic Science* 54, no. 7 (2016): 1180–1186, <https://doi.org/10.1093/chromsci/bmw048>.

[View](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

13 S. Kucukkolbasi, O. Bilber, H. F. Ayyildiz, and H. Kara, "Simultaneous and Accurate Determination of Water-and Fat-Soluble Vitamins in Multivitamin Tablets by Using an RP-HPLC Method," *Química Nova* 36 (2013): 1044–1051, <https://doi.org/10.1590/S0100-40422013000700020>.

[View](#) | [CAS](#) | [Web of Science®](#) | [Google Scholar](#)

1-7085(95)01644-9.

View [View](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

---

15 G. A. Klaczkow, E. Czyz, and E. L. Anuszevska, "Elaboration of HPLC Method for Biotin Determination in Multiple Vitamin Drugs and Comparison With Microbiological Method," *Acta Poloniae Pharmaceutica* **58**, no. 2 (2001): 93–96.

[CAS](#) | [Google Scholar](#)

---

16 L. B. Thompson, D. J. Schmitz, and S. J. Pan, "Determination of Biotin by High-Performance Liquid Chromatography in Infant Formula, Medical Nutritional Products, and Vitamin Premixes," *Journal of AOAC International* **89**, no. 6 (2006): 1515–1518, <https://doi.org/10.1093/jaoac/89.6.1515>.

View [View](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

---

17 Q. Lin, Y. Ding, F. Poh, C. Zhang, S. J. Pan, and K. J. Schimpf, "Determination of Biotin in Infant, Pediatric, and Adult Nutritionals by High-Performance Liquid Chromatography and Fluorescence Detection: Single-Laboratory Validation, First Action 2016.11," *Journal of AOAC International* **100**, no. 1 (2017): 145–151, <https://doi.org/10.5740/jaoacint.16-0257>.

View [View](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

---

18 A. E. Ekpe and C. Hazen, "Liquid Chromatographic Determination of Biotin in Multivitamin-Multimineral Tablets," *Journal of Pharmaceutical and Biomedical Analysis* **16**, no. 8 (1998): 1311–1315, [https://doi.org/10.1016/S0731-7085\(97\)00143-X](https://doi.org/10.1016/S0731-7085(97)00143-X).

View [View](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

---

19 Q. S. Qu, D. Mangelings, F. Shen, et al., "Pressurized Capillary Electrochromatographic Assay of Trimethoprim Impurities Using 1 µm Particle-Based Columns," *Journal of Chromatography A* **1169**, no. 1–2 (2007): 228–234, <https://doi.org/10.1016/j.chroma.2007.08.068>.

View [View](#) | [CAS](#) | [PubMed](#) | [Google Scholar](#)

---

20 S. Kumar, D. Chawla, and A. K. Tripathi, "An Improved and Sensitive Method for Vitamin D3 Estimation by RPHPLC," *Pharmaceutica Analytica Acta* **6**, no. 8 (2015): 1–6, <https://doi.org/10.4172/2153-2435.1000410>.

View [View](#) | [Google Scholar](#)

Dosage Form," *Pharmaceuticals* 17, no. 4 (2024): 505, <https://doi.org/10.3390/ph17040505>.

View [View](#) | [CAS](#) | [Web of Science®](#) | [Google Scholar](#) |

22 H. M. Lee, B. M. Kwak, J. H. Ahn, et al., "Simultaneous Determination of Vitamin A and E in Infant Formula by HPLC With Photodiode Array Detection," *Food Science of Animal Resources* 31, no. 2 (2011): 191–199, <https://doi.org/10.5851/kosfa.2011.31.2.191>.

View [View](#) | [Google Scholar](#) |

23 International Council for Harmonisation (ICH), Validation of *Analytical Procedures: Text and Methodology*, Q2 (R1) (2005): 04.

[Google Scholar](#) |

24 U. M. Patel, A. B. Chokshi, and P. R. Desai, "Development and Validation of RP-HPLC Method for Determination of Hydrochlorothiazide, Olmesartan Medoxomil and Their Related Substances in Combined Tablet Dosage Form," *International Journal of Pharmacy and Pharmaceutical Sciences* 6, no. 9 (2014): 318–323.

[CAS](#) | [Google Scholar](#) |

25 D. García-Ferrer, J. Peris-Vicente, D. Bose, A. Durgbanshi, and S. Carda-Broch, "An Assay to Quantify Methylphenidate and Atomoxetine in Pharmaceutical Preparations by Micellar Liquid Chromatography," *Separation Science Plus* 8, no. 1 (2025): e202400302.

View | [CAS](#) | [Web of Science®](#) | [Google Scholar](#) |

26 A. A. Habib, S. F. Hammad, M. M. Amer, and A. H. Kamal, "Stability Indicating RP-HPLC Method for Determination of Dimethyl Fumarate in Presence of Its Main Degradation Products: Application to Degradation Kinetics," *Journal of Separation Science* 44, no. 3 (2021): 726–734.

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