APPLICATION NOTE

Use of a Novel Method of Derivatization to Improve LC-MS/MS Sensitivity of 1,3-Dienes

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Key Information

- Analysis of dienes often requires increased sensitivity *via* derivatization reagents or sensitive analytical equipment.
- LC-MS/MS sensitivity of the 1,3-diene vitamin D_3 was tested using two different derivatization methods.
- The derivatization method employing the novel click chemistry-based reagent Pyr-NO provides a 10-fold improvement in sensitivity over the alternative derivatization method tested in this study.

Introduction

There is an increasing need for sensitive and accurate determination of diene compounds in samples (*e.g.*, vitamin D metabolites) due to their important biological activity.[1] However, due to their trace level and poor ionization efficiency, sensitive detection of some important diene-containing compounds is challenging when using liquid chromatography-tandem mass spectrometry (LC-MS/MS).[2] Commercial methods and reagents that react with 1,3-dienes have demonstrated some improvement in sensitivity in LC-MS/MS applications, and a novel reagent has recently shown further improvements in sensitivity.[3] Cayman has developed a new Dienes Derivatization MaxSpec® Kit (*Item No. 601510*) that employs this improved reagent (**Figure 1**). We set out to verify the increase in LC-MS/MS sensitivity by comparing the commercial derivatization methods for vitamin D_3.

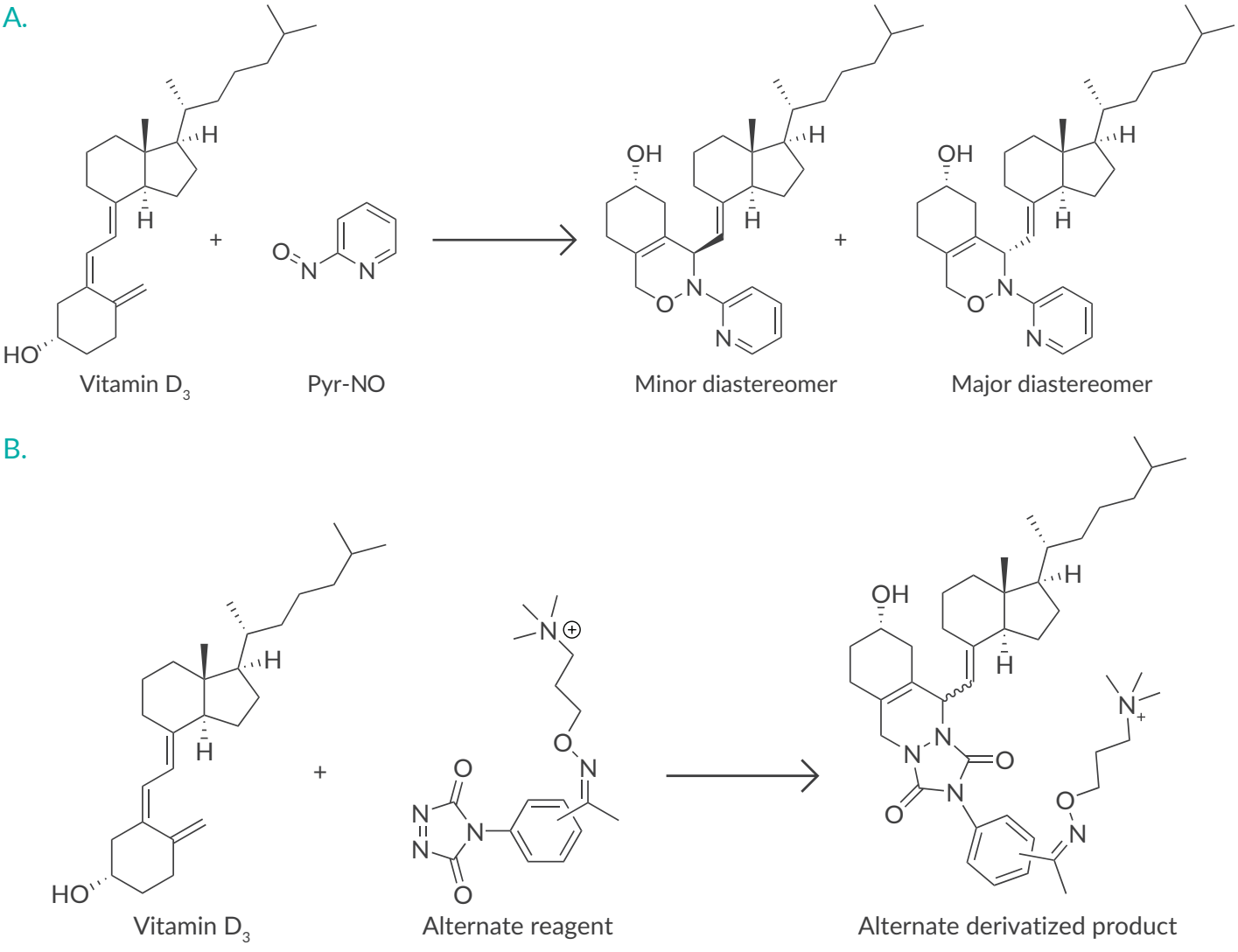

Figure 1. **A.** Example derivatization reaction of Pyr-NO (derivatization reagent) and vitamin D_3. The reaction is regioselective and produces a primary pair of diastereomers in a ratio of about 1:4. A minor pair of regioisomers may also form as minor products of the reaction. For quantitation, only the single major diastereomer is integrated and is sufficient for accurate and sensitive quantitation of vitamin D_3. The pyridine ring in the derivatized products significantly improves ionization and, as a result, overall sensitivity. **B.** Example derivatization reaction with alternate, less-stable reagent.

Methods

A series of vitamin D_3 standard solutions (5 pM, 10 pM, 25 pM, 50 pM, 100 pM, 250 pM, 500 pM, and 1 nM) were prepared in methanol. All prepared solutions (50 µl), as well as pure methanol, were transferred to their individual Eppendorf tubes and dried under speed vacuum. Afterward, 50 µl of the dienes derivatization reagent was added into each tube. The tubes were vortexed vigorously for 30 seconds to reconstitute vitamin D_3. Each solution was transferred into HPLC autosampler vials with glass inserts and incubated at 70°C for 1 hour to derivatize vitamin D_3 using Cayman's dienes derivatization reagent. The derivatized vitamin D_3 samples were directly analyzed by LC-MS/MS after cooling down to room temperature. The detailed LC-MS/MS approach is described in the Dienes Derivatization MaxSpec® Kit booklet (*Item No. 601510*).

For comparison, the above procedure was repeated using a similar, commercially available kit. After reconstitution, the vitamin D_3 standards were derivatized following the protocol provided by the manufacturer. After derivatization, vitamin D_3 was analyzed by LC-MS/MS using a method specifically optimized for the derivatized analyte.

Results

Comparison of lower limit of quantitation between the two diene derivatization methods

A series of concentrations (5 pM-1 nM) of vitamin D_3 solutions were derivatized by both the Cayman and competitor kits. The derivatized vitamin D_3 solutions were measured by their respective optimized LC-MS/MS methods. Lower limit of quantitation (LLOQ) was estimated by the concentration of the derivatized vitamin D_3 solutions observed to produce 10-times the blank response. Comparing the blank response with the derivatized vitamin D_3, the LLOQ of the derivatized vitamin D_3 is 25 pM (peak area 91) for the Cayman kit and 250 pM (peak area 3,012) for the competitor kit (**Figure 2**).

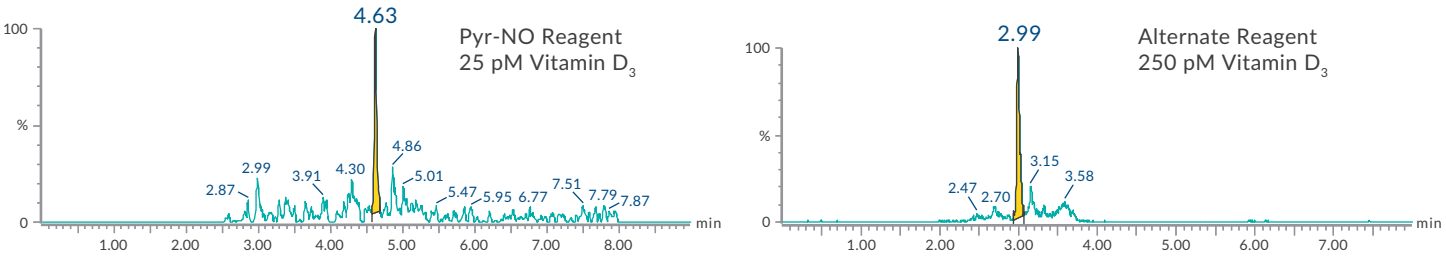

Figure 2. Extracted mass chromatogram of LLOQ for derivatized vitamin D_3 by the Cayman (left) and competitor (right) diene kit.

Conclusions

This study demonstrates that Cayman's Dienes Derivatization MaxSpec® Kit, which uses a one-step, click chemistry method, offers superior sensitivity over a similar commercially available kit for the detection and quantitation of vitamin D_3 using LC-MS/MS. The increase in sensitivity is primarily attributed to improvements in ionization efficiency of Cayman's reagent. In addition, preliminary data shows increased stability of derivatized products compared to other methods, allowing for storage of derivatized samples prior to analysis.

Cayman products used in this application

Item No.	Product Name
601510	Dienes Derivatization MaxSpec® Kit
11792	Vitamin D_3

Please send questions and comments regarding this application to techserv@caymanchem.com
Visit www.caymanchem.com to view all products and research services available through Cayman Chemical.

References

1. Pauwels, S., Jans, I., Billen, J., *et al. J. Steroid Biochem. Mol. Biol.* **173**, 341-348 (2017).
2. Fraser, W.D. and Milan, A.M. *Calcif. Tissue Int.* **92(2)**, 118-127 (2013).
3. Hammock, B.D., *et al. J. Lipid Res.* **58(4)**, 798-808 (2017).