

Discussion

Comments on “Ion-exchange liquid chromatographic analysis of bisphosphonates by on-line post-column photochemical reaction and spectrophotometric detection”

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Gary W. Schieffer

Graham Development Inc., 179 River Street, Oneonta, NY 13820, USA

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Peng and Dansereau [1] recently introduced a post-column photolysis-molybdate procedure for non-UV absorbing bisphosphonates that is a simplified version of the procedure initially presented by Priebe and Howell [2,3]. Although not explicitly presented in Peng and Dansereau's introduction, the main advantage to the simplification arises from the realization that an intermediate can be photoreduced instead of chemically reduced. Priebe and Howell used ammonium peroxydisulfate-assisted photolysis to degrade eluting organophosphorus compounds to orthophosphate. The orthophosphate was then reacted with molybdate in a separate reactor to form 12-molybdophosphoric acid (12-MPA) which was then chemically reduced with ascorbic acid to a molybdenum blue complex (with added antimony) absorbing in the 800–900 nm range. In Peng and Dansereau's system, the bisphosphonate was photolyzed to orthophosphate without peroxydisulfate, reacted with molybdate to form 12-MPA, which was then photoreduced to molybdenum blue, all in the same reactor coil.

This technology was, in fact, presented in an earlier preliminary work [4] employing a laboratory-

constructed photolysis unit requiring hand crocheting of the tubing and cooling of the reactor assembly. This was later transformed into a system nearly identical to Peng and Dansereau's system for a proprietary assay of impurities in another bisphosphonate (G.W. Schieffer, unpublished results). The reference that Peng and Dansereau ascribe to the photoreduction of 12-MPA [5] actually makes no mention of UV irradiation — only chemical reduction is discussed. Thus, interested readers may wish to consult the original preliminary work for a discussion of the rationale leading to configuring this system.

Another reason for researchers interested in employing this system to read the original work pertains to the nature of the molybdate reagent added post column. Peng and Dansereau recognize that the molybdate reaction is catalyzed under acidic conditions, but control the acidity with the dilute nitric acid eluent used for the anion-exchange chromatography of the relatively strong-acid bisphosphonate. They do not add an excess concentration of nitric acid directly to the reagent. This can be a problem, especially for method transfer, when a nitric acid

gradient is used. The detector response will vary depending on the nitric acid concentration and shape of the gradient profile, especially when the nitric acid concentration is low. They also do not consider the efficacy of adding a water-soluble organic component to the molybdate reagent to facilitate the formation of 12-MPA [6].

In the preliminary work [4], the bisphosphonate ethane-1-hydroxy-1,1-diphosphonate was separated by anion-exchange chromatography with a high-pH EDTA eluent. There, the molybdate reagent used was based on the 30% methanol, 2 mM sodium molybdate, and 0.1 M nitric acid reagent of Harden and Nonidez [6] and the fact that the TLC reagent for visualizing phosphate esters photolytically with molybdate reagent employed 80% acetone [7]. It appears the use of an organic component, in addition to acidity, may be a factor in increasing the efficiency of the reaction. The final molybdate reagent in the preliminary work [4] consisting of 60% methanol, 3 mM sodium molybdate, and 0.3 M nitric

acid resulted in an apparent 10-fold lower quantitation limit [4] (G.W. Schieffer, unpublished results) than the Peng and Dansereau reagent containing just 10 mM sodium molybdate. Of course, some minor molybdate reagent optimization studies would be required for a dilute nitric acid eluent system and other organophosphorous compounds, but the reagent employed in the preliminary work [4] might be the more appropriate place to start.

References

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