



ELSEVIER

Journal of Chromatography A, 868 (2000) 141–142

JOURNAL OF  
CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Letter to the Editor

## Chromatographic analysis of bisphosphonates

Rolf W. Sparidans\*, Jan den Hartigh, Serge Cremers, Pieter Vermeij

*Leiden University Medical Center, Department of Clinical Pharmacy and Toxicology, P.O. Box 9600, 2300 RC Leiden, The Netherlands*

Received 22 September 1999; received in revised form 16 November 1999; accepted 19 November 1999

*Keywords:* Bisphosphonates

In the paper of Lovdahl and Pietrzyk [1], published recently in your journal, the development of a post-column reaction for bisphosphonates and related analytes in ion-exchange chromatography (IEC) has been described. The use of the aluminium(III)morin reagent with indirect fluorescence detection resulted in a sensitive and selective analytical method; however, the separation efficiency seems to be rather poor and the applicability to biological samples was not (yet) shown.

In this letter, we would like to make a few remarks on the Introduction Section of this article. The extensive literature review in this section suggests a more or less complete review of the chromatographic analysis of bisphosphonates. However, from all relevant research articles published after 1994 (ca. 15) only one has been reported and this lack of topicality resulted in missing a few new strategies for bisphosphonate analysis; we recently published a more complete review on this subject [2].

While Lovdahl and Pietrzyk [1] used an anion-exchange separation and post-column indirect fluo-

rescence detection methodology for etidronate {[ (1-hydroxyethylidene)bisphosphonate], clodronate [(dichloromethylene)bisphosphonate], (difluoromethylene)bisphosphonate and alendronate [(4-amino-1-hydroxybutylidene)bisphosphonate]} several more complex bisphosphonate structures can be readily detected by selective detection of native properties. Previously published examples are the bisphosphonate tiludronate {[ (4-chlorothiophenyl)methylene]bisphosphonate}, containing an aromatic ring, that has been determined in urine and plasma using ion-pair liquid chromatography with UV detection [3], {1-hydroxy-2-(imidazo[1,2-*a*]-pyridyl)ethylidene}bisphosphonate (YM-529) using fluorescence detection of the imidazopyridyl moiety [4] and incadronate [(cycloheptylamino)methylene]bisphosphonate using electrochemical detection of the cycloheptylamino group [5]. Further, (2-thioethylidene)bisphosphonate and probably other bisphosphonates can be determined rather sensitively with capillary electrophoresis in a glycerol-coated column using electrokinetic injection under stacking conditions and non-selective UV detection at 185 nm [6].

Finally, from the IEC methods for bisphosphonate determination reported in the Introduction Section of this paper, which can be related most directly to the new presented method, we believe a very competitive IEC assay is missing. We previously reported a

\*Corresponding author. Utrecht University, Faculty of Pharmacy, Department of Pharmaceutical Analysis, Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands. Tel.: +31-30-2536-918; fax: +31-30-2535-180.

*E-mail address:* r.w.sparidans@pharm.uu.nl (R.W. Sparidans)

chromatographic method for bisphosphonates using IEC with in-line copper(II) complexation and UV detection [7]. This assay is approximately equally selective and sensitive {8 ng pamidronate [(3-amino-1-hydroxypropylidene)bisphosphonate]} compared to 10 ng alendronate for the new presented method. However, the detection method is much more simple, the assay has already been validated {for the pharmaceutical analysis of pamidronate and olpadronate [(3-dimethylamino-1-hydroxypropylidene)bisphosphonate] preparations} and the assay has been used routinely in our laboratory for several years now for the quality control of all raw bisphosphonate material and their pharmaceutical preparations. The assay is superior compared to the previously applied method using IEC with conductivity detection [8] and should, in our opinion, also be preferred above the new method using post-column indirect fluorescence detection.

## References

- [1] M.J. Lovdahl, D.J. Pietrzyk, J. Chromatogr. A 850 (1999) 143.
- [2] R.W. Sparidans, J. den Hartigh, Pharm. World Sci. 21 (1999) 1.
- [3] J.-P. Fels, J. Guyonnet, Y. Berger, W. Cautreels, J. Chromatogr. 430 (1988) 73.
- [4] T. Usui, R. Kawakami, T. Watanabe, S. Higuchi, J. Chromatogr. B 652 (1994) 67.
- [5] T. Usui, T. Watanabe, S. Higuchi, J. Chromatogr. 584 (1992) 213.
- [6] S.X. Peng, R. Takigiku, D.E. Burton, L.L. Powell, J. Chromatogr. 709 (1998) 157.
- [7] R.W. Sparidans, J. den Hartigh, P. Vermeij, J. Pharm. Biomed. Anal. 13 (1995) 1545.
- [8] J. den Hartigh, R. Langebroek, P. Vermeij, J. Pharm. Biomed. Anal. 11 (1993) 977.