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Discussion

Rapid and highly sensitive high-performance liquid chromatographic method for the determination of histamine and 3-methylhistamine in biological samples using fluorescamine as the derivatizing agent

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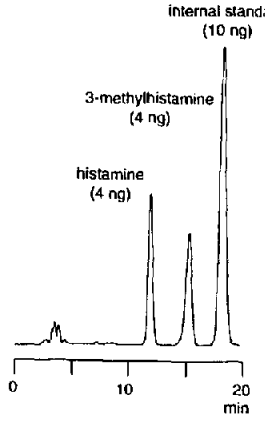
We recently have described a high-performance liquid chromatographic method for the determination of histamine and 3-methylhistamine in biological samples with 1-methylhistamine as the internal standard. The 'classical' reversed-phase octadecyl column (250 mm × 4 mm I.D., 5 μm particles, Chrompack, Bergen op Zoom, Netherlands), as used in this study, was not optimal, since it requires a relatively long elution time (ca. 30 min) for an adequate separation of the compounds. Moreover, chromatograms show relatively broad and tailing peaks probably due to the presence of small amounts of unesterified silanol groups on the surface of the silica, which will delay the elution of nitrogen-containing compounds such as fluorescamine-derivatized histamines.

Here we wish to report that major improvements are obtained by using an Inertsil ODS-2 column (200 mm × 3 mm I.D., 5 μm particles, Chrompack), which is characterized by a high carbon load and complete end-capping of the residual silanol groups by silylation. A representative chromatogram, as shown below, demonstrates that the elution time, as well as peak tailing and peak broadening were significantly



reduced by using this column. The mobile phase was water–acetonitrile–methanol–H_3PO_4 (750:150:100:2, v/v), brought to pH 6.87 with NH_4OH. The injection volume was 20 μl and the flow-rate was 0.4 ml/min. Other conditions were similar to those described earlier [1].

Reference

[1] C.M.C.J. van Haaster, W. Engels, P.J.M.R. Lemmens, G. Hornstra and G.J. van der Vusse, *J. Chromatogr.*, 617 (1993) 233–240.

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