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A novel LC-MS compatible method for the analysis at residue levels of aminoglycoside antibiotics

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Introduction

Most LC methods for aminoglycosides require derivatisation to enable detection. Mass spectrometric detection can overcome this requirement and provide an adequate method of detection, however most HPLC methods require the use of non-volatile mobile phase reagents which are incompatible with mass spectrometry (MS). The use of volatile fluorinated carboxylic acids for aminoglycoside separation and MS detection has been reported. However, since ion-pairing reagents are known to suppress the analyte signal significantly, detection was not possible at relevant levels.

In this study, analysis of aminoglycosides without the need for ion-pair reagents, using Hydrophilic interaction chromatography (HILIC) was carried out. HILIC is a variant of normal phase chromatography, hence, gradients run from high to low percentage of organic modifier.

The aim was to investigate a chromatographic method for the analysis of gentamicin (Gent), neomycin (Neo) and kanamycin (Kan), however, the chromatographic behaviour of related compounds was also monitored.

Experimental

Separation of aminoglycosides and related compounds was carried out on HILIC Polyhydroxylethyl Aspartamide columns of different pore size (100, 500 and 1000 Å). Columns were purchased or on loan from Dr A. Alpert, Poly LC Inc., Columbia, MD, USA. Samples of 50 µl were injected.

Gradient elution was applied with various eluent compositions to study the effect of acetonitrile, buffer concentration and pH on analyte retention and peak shape.

Results and discussion

1) Effect of acetonitrile on aminoglycoside retention

The effect of acetonitrile on analyte retention was investigated. The graph in figure 1 demonstrates that, as the percentage acetonitrile in the starting gradient is increased, retention also increased between 20 and 70 % after which the rate of increase was seen to level off. Above 40 % acetonitrile, most of the analytes appear to be retained, except Salinomyin.

The effect of increasing the percentage acetonitrile at various buffer concentrations was also investigated. At lower buffer content, analyte retention was greater, the percentage acetonitrile was more influential on retention and a "levelling off" effect on retention was observed as acetonitrile was increased. The effect of acetonitrile on the ending gradient was much less influential as that in the starting gradient.

2) Effect of buffer concentration on aminoglycoside retention

Decreasing the concentration of buffer in the starting mobile phase promotes retention of the analytes, as shown in figure 2. However, as the buffer concentration was decreased below 125 mM, Neomycin peak shape deteriorates rapidly, resulting in broad, split peaks. As with acetonitrile, the percentage buffer in the ending gradient was observed to be much less influential.

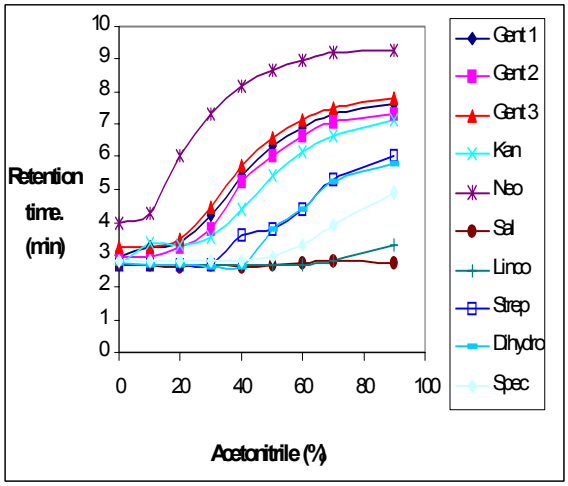

Fig 1 The effect of acetonitril in the starting mobile phase

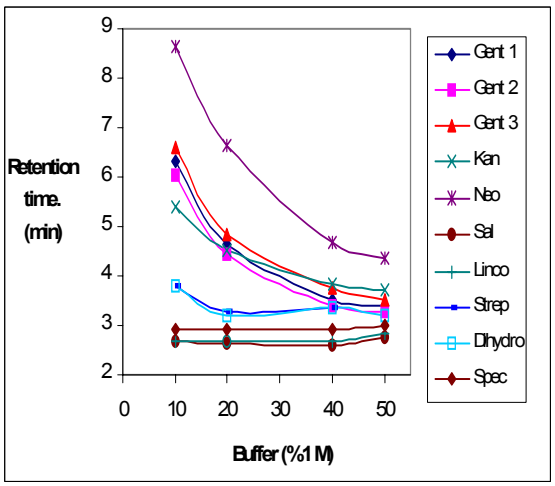

Fig2 Effect of buffer percenbtage in the starting mobile phase

3) Effect of buffer pH

In varying the pH of the mobile phase buffer, it was observed that as the pH is increased, retention is increased for most analytes. Varying the pH up to pH 6 did increase the retention of salinomycin, lincomycin, streptomycin, dihydrostreptomycin, and spectinomycin.

Conclusion

This study demonstrates the capability of Hydrophillic Interaction Chromatography for the separation of aminoglycoside residues. The method is especially suited to the Mass Spectrometric detection of these analytes, providing a highly conclusive method with superior peak shape and sensitivity than most available methods. A typical chromatogram is presented in figure 3.

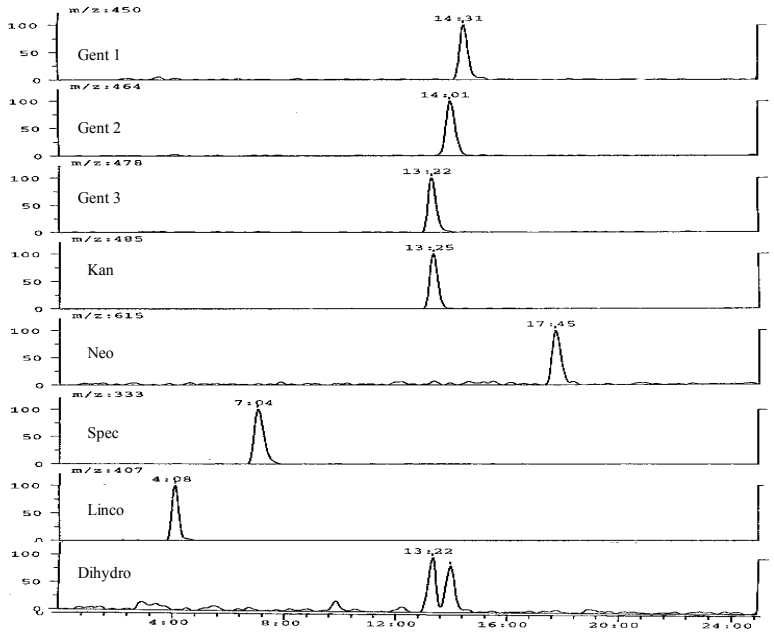

Fig 3 A typical HILIC-MS chromatogram of some aminoglycoside antibiotics,

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