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Note

Picolinic acid: a mobile phase additive for improved chromatography of metal-chelating heterocyclic acids and β-diketones

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During the course of the development of high-performance liquid chromatographic (HPLC) analytical methods for some metal-chelating compounds from two distinct chemical series, one series containing a heterocyclic acid, the other a β-diketone (see Fig. 1 for general structures), severe problems were encountered due to poor chromatographic peak shapes. Thus, using reversed-phase HPLC, peaks from these compounds were characterised by excessive tailing, peak asymmetry and, in the worst cases, "chair"-shaped peaks. These problems were clearly a property of the compounds under investigation and did not reflect deficiencies in the instrumentation.
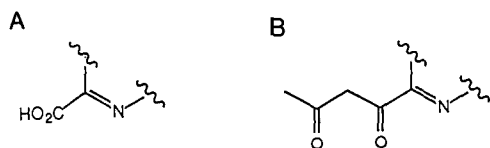

Fig. 1. Part structures of (A) the heterocyclic acid series and (B) the β-diketone series of metal-chelating compounds.

A number of strategies were therefore investigated in an attempt to eliminate this problem. However, improvements in peak shape were only obtained when the analytes themselves were added to the solvent. This resulted in a dramatic improvement and suggested that the poor peak shape was a result of a specific, saturable, interaction of the analytes with the chromatographic system.

In some ways the results obtained by us for these metal-chelating compounds are similar to those reported by a number of groups engaged in the analysis of tetracyclines[1–3]. These workers have shown that on reversed-phase systems the tetracyclines can be subject to poor chromatographic efficiencies and irreversible adsorption of trace amounts of the analytes. In order to eliminate this problem several investigators[1,4] have added related compounds to the mobile phase in order to deactivate the packing material. Another approach has been to add EDTA to the mobile phase to prevent complexation of the tetracyclines with metal ions in the chromatographic system[5–10].

Here experiments designed to further improve the chromatographic behaviour of these β-diketones and heterocyclic acids are described.

MATERIALS AND METHODS

Apparatus

The HPLC system consisted of an LC-XPD pump (Pye Unicam Cambridge, U.K.), a Rheodyne 7125 loop injector (Rheodyne, Berkley, CA, U.S.A.) fitted with a 50 μl loop, a Spectromonitor 3000 variable-wavelength UV detector (Milton Roy, Stone, U.K.) operating at 300 nm and a Model BS 273 chart recorder (Bryans Southern Instruments, Mitcham, U.K.).

Reagents. Acetonitrile and tetrahydrofuran were HPLC grade (Fisons, Loughborough, U.K.). Orthophosphoric acid (85%) and butylamine were analytical grade (Fluka, U.K.). Picolinic acid (pyridine-2-carboxylic acid), nicotinic acid (pyridine-3-carboxylic acid) and picoline (2-methylpyridine) were supplied by BDH (Poole, U.K.). Water for HPLC was produced using an Elga Spectrum ROI system (Elga, High Wycombe, U.K.).

Chromatography

Heterocyclic acid series. For the chromatography of compounds from the heterocyclic acid series stainless-steel columns (15 cm × 0.46 mm I.D.) were used packed with either 5 μm Zorbax C_8 silica (Jones Chromatography, Galmorgan, U.K.) or 5 μm Spherisorb C_1 silica (Capital HPLC Specialists, West Lothian, U.K.).

β-Diketone series. Compounds from the *β*-diketone series were chromatographed on a stainless-steel solumn (15 cm × 4.5 mm I.D.) packed with a polymer-based resin, 5 μm, 100A PLRP-S: macroporous polystyrene–divinylbenzene (Polymer Laboratories, U.K.).

Mobile phases. The mobile phase was prepared by mixing varying proportions (see figure captions) of acetonitrile with 0.1 *M* phosphoric acid–butylamine buffer. The buffer was prepared by adding *ca.* 7.0 g of orthophosphoric acid to 1 l of water followed by sufficient butylamine to give a pH of *ca.* 2 for the heterocyclic acid and between 7.5 and 10.5 for the *β*-diketone series.

Picolinic acid, nicotinic acid or picoline were added to the mobile phase to give a final concentration of 1 m*M*. Solvent was delivered at a flow-rate of 1.5–2.0 ml min^{-1}.

In the case of the *β*-diketone series the column was maintained at a temperature of 50°C. Otherwise chromatography was carried out at ambient temperatures.

Analytes were dissolved in the HPLC mobile phase for injection.

RESULTS AND DISCUSSION

Heterocyclic acids

When an otherwise unmodified mobile phase consisting only of acetonitrile–phosphoric acid–butylamine buffer was used, the peak corresponding to the analyte demonstrated excessive tailing as illustrated in Fig. 2A and B for a typical example on C_8 and C_1 columns, respectively. This phenomenon was initially assumed to be due to interaction of the analyte with residual silanols remaining on the surface of the silica-based packing materials. This type of interaction can usually be overcome by the addition of organic bases (*e.g.* butylamine or hexylamine), pH control, incorporation of ion-pair reagents into the mobile phase or the use of other packing materials *e.g.*
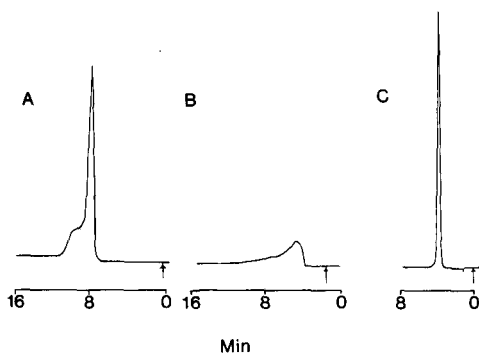
Fig. 2. (A) Typical chromatogram obtained for a member of the heterocyclic acid series on a Zorbax C_8 column using an unmodified mobile phase of acetonitrile–phosphoric acid-butylamine buffer (pH 2.5) (1:1, v/v). Flow rate 1.5 ml min^{-1} at ambient temperature. (B) The same conditions as (A) using Spherisorb C_1. Chromatographic conditions as for (A). (C) Conditions as for (A) with the addition of 1 mM picolinic acid to the mobile phase.

LiChrosorb RP-select, and non-silica-based materials such as the PRP1 resins. However, our attempts to overcome the peak asymmetry problems exhibited by these compounds by using such alternative approaches was unsuccesful with either poor peak shape or non-elution of the analytes from the columns. We also attempted to use EDTA as a mobile phase additive but observed no improvement in the peak shape of these analytes.

As it seemed clear that the analytes were interacting strongly with some component in the system we added the compounds themselves to the mobile phase (*ca.* 1 mM) in order to saturate the binding. This resulted in a dramatic improvement in the peak shape on subsequent injections of the analytes themselves, together with a corresponding increase in the UV background provided by the solvent. To reduce this baseline noise sufficiently to be able to employ the system for trace analysis a number of structurally related substances with maximum values for UV absorbance well below those of the analytes were studied as mobile phase additives. The first of those to be investigated was picolinic acid (pyridine-2-carboxylic acid) resulting in the chromatogram shown in Fig. 2C. Here, addition of the picolinic acid resulted in the production of excellent symmetrical peaks enabling trace analysis of the compound of interest at the ppm level. The use of mobile phase additives such as either nicotinic acid, the pyridine-3-carboxylic acid analogue of picolinic acid, or picoline (2-methyl pyridine) at similar concentrations to picolinic acid was without effect. The difference between picolinic and nicotinic acids suggest that the observed interaction was relatively specific for carboxylic acids adjacent to heterocyclic nitrogens. Injection of the analytes in the mobile phase was essential as failure to do this resulted in system peaks.

β-Diketone series

Initially we attempted to overcome the similar problems of poor peak shape associated with the β-diketone series using the approach developed for the heterocyclic acids. However, these attempts were completely unsuccessful. It was felt that the very broad tailing peaks observed with these compounds might, in addition to

problems associated with metal chelation, perhaps also be due to keto–enol tautomerism as these β-diketones were likely to be in the enol form under the conditions employed for analysis.

Such compounds can be stabilised by the use of solvents of sufficiently high pH and to enable the use of such solvents without adverse effect on the column a polymer-based packing material (PLRP-S) was adopted. This enabled the use of solvents with pH values greater than 9, and in addition also eliminated any possible silanol interactions. An example of the chromatographic behaviour of a typical β-diketone compound from this series is shown in Fig. 3A. The peak shape obtained by using such a system was still not acceptable. As seen with the heterocyclic acids the addition of EDTA to the mobile phase also proved ineffective in improving peak shape. Given that it was quite likely that these compounds were interacting with metal ions in the system, we examined the effect of adding excess ferric ions to the mobile phase. It has been shown in the case of the tetracyclines that metal ions in the mobile phase can exert a strong influence on chromatography and, for example, Reeuwiyk and Tjaden noted a large increase in the capacity ratio of tetracyclines in the presence of ferric ions[11]. A similar effect on the chromatography of β-diketones was noted by us when the effect of ferric ions in the mobile phase was studied but the chromatographic result was still unacceptable.

However, addition of picolinic acid once again resulted in a dramatic improvement in chromatography (Fig. 3B). Once again it was necessary to inject the analyte as a solution in the HPLC mobile phase to avoid system peaks.
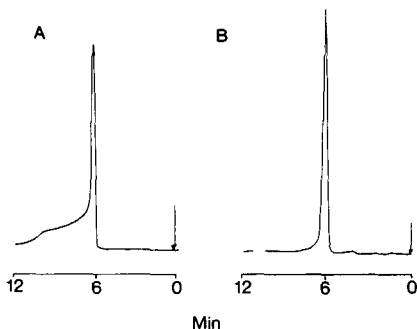

Fig. 3. (A) Typical chromatogram obtained for a member of the β-diketone series on a PLRP-S polymer column using an unmodified mobile phase of acetonitrile–phosphoric acid–butylamine buffer (pH 8.5) (30:70, v/v). Flow-rate 1.5 ml min^{-1} at 50°C. (B) Conditions as for (A) with the addition of 1 mM picolinic acid to the mobile phase.

To explain these observations for both the acid and β-diketone series some saturable interaction with a component of the chromatographic system seems likely. This interaction may be with the stationary phase, the solvent or, given the metal-chelating properties of these compounds, with the stainless-steel tubing and frits present in the system.

It is therefore probable that the mechanism by which the picolinic acid in the mobile phase causes the observed improvement in peak shape is that it saturates these active sites by effectively masking them, and thus prevents their interaction with the

analytes. It seems most unlikely that the residual silanols are responsible for the observed results as similar poor peak shape was observed on polymer-based packings from which silanols are absent. It is also unlikely that the observed dramatic improvements in peak shape are due to the action of picolinic acid as an ion-pair reagent as conventional ion-pair reagents were singularly ineffective in improving peak shape.

Whilst we are currently unable to provide complete structures for any of the compounds discussed here, we would like to emphasise that the poor chromatographic properties described here were seen in a wide range of compounds containing the part structures shown in Fig. 1 and were not significantly affected by changes elsewhere in the molecule. However, this phenomenon may be quite common for strongly metal-chelating compounds as we have subsequently been able to effect a considerable improvement in the peak shape of several structurally unrelated compounds by using a similar approach.

Further studies to understand the mechanism underlying our observations are continuing.

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