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The application of strong cation exchange high-performance liquid chromatography to drug analysis

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Abstract

The application of strong cation exchange (SCX) HPLC to the analysis of drugs in plasma, tissue and rodent feed is presented. This approach is shown to give useful separations allowing the separation of physiochemically diverse compounds in a simple isocratic system. The SCX columns are not as robust as standard reversed-phase materials and to ensure maximum column lifetimes the use of a guard column is recommended.

Keywords: HPLC; Strong cation exchange; SCX; Drug analysis; Column stability

1. Introduction

It has recently been shown that strong cation exchange (SCX) high-performance liquid chromatography is a powerful technique for the separation of basic drugs [1–3]. In contrast to typical reversed-phase systems, very high efficiencies and good peak symmetries are obtained and the approach shows broad applicability using simple organic/aqueous eluents. From a knowledge of the physicochemical properties of the analytes under study it is possible to systematically optimise separations. The ability to chromatograph simultaneously under isocratic conditions a wide variety of compounds makes the approach particularly suited to drug screening.

Much of the work to date has been carried out using simple solutions of drug compounds. We believe that this form of chromatography has broad utility in many areas particularly pharmaceutical bioanalysis. The purpose of this report is to present data showing a number of interesting applications and to highlight the some of the limitations and advantages of the technique.

2. Experimental

2.1. Equipment

For the development work, chromatography was carried out using a Hewlett Packard (HP) 1090 system which included an HP 1040 diode array detector. The application work was carried out using a variety of instrumentation.

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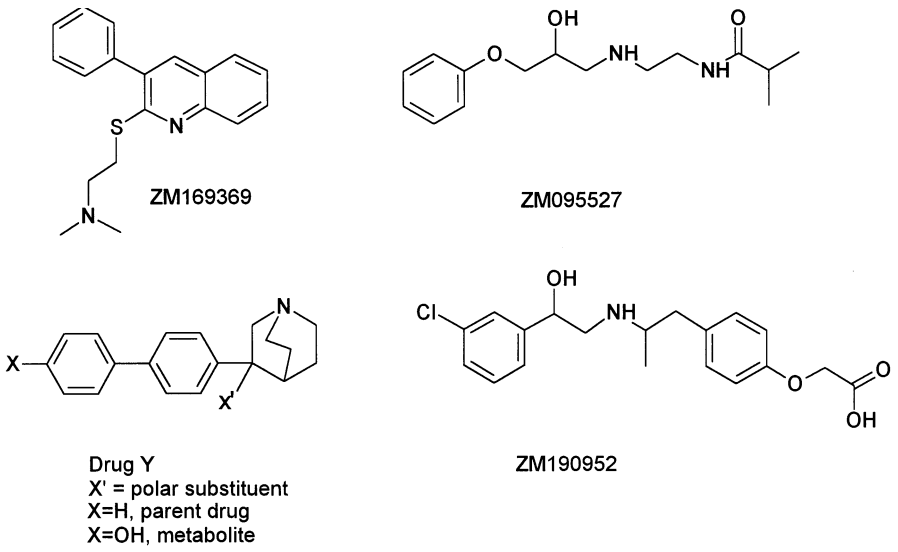

Fig. 1. The structures of the novel drug compounds studied.

Throughout, the HPLC column was stainless steel (100×4.6 mm) containing Spherisorb 5SCX (Phase Separations, Deeside, UK) packed by Hichrom, Reading, UK. The stationary phase is a bonded propyl sulphonic acid.

2.2. Materials

The following chemicals were used: ammonium formate, Analar grade (BDH, Liverpool, UK); trifluoroacetic acid (TFA) and iso-amyl alcohol (Fluka, Gillingham, UK); methanol, dichloromethane, hexane, methyl-*tert*-butyl ether (MTBE) and acetic acid were all HPLC grade (Fisons, Loughborough, UK). Solutes were a selection of drug compounds obtained from the Zeneca compound collection or from a number of commercial sources.

Blood was taken into oxalated tubes, spun to separate the plasma which was stored at 4°C before use.

2.3. Methods

The eluent consisted of methanol–water–TFA (800:200:2.3, v/v) containing ammonium formate (0.02 M) with an apparent pH of 2.45. All experiments were carried out at room temperature, the flow rate was 1 ml min^{-1} and unless stated otherwise the detection wavelength was 270 nm.

For the column longevity studies, biological extracts were prepared from dog plasma containing two test compounds; propranolol and atenolol, (100 ng ml^{-1} each) using a solid phase extration procedure. Plasma (either 0.25 or 1 ml) was applied to a Bond Elut C2 cartridge which had previously been conditioned with methanol and water (1 ml of each). The cartridge was washed with water (2×1 ml) and then eluted with methanol (0.8 ml) containing ammonium acetate (0.005 M). A 0.2 ml aliquot of the extract (equivalent to either 0.0625 or 0.25 ml of plasma) was injected onto HPLC. The chromatographic efficiency was determined using the HP system suitability software. This involved injecting a methanolic solution of five test compounds (ZM190952 (Fig. 1), atenolol, propranolol, metoclopramide and clomipramine), the k' for which varied from 2.49 to 10.9 and efficiency from 36000 to 66500 plates m^{-1}. The mean efficiency of the five test compounds was used as an overall index of efficiency.

3. Results and discussion

3.1. Column longevity

Initial attempts at the analysis of plasma extracts using the SCX system gave disappointing results since the chromatographic efficiency fell away rapidly. The number of theoretical plates dropped to around half the starting value after injecting extracts equivalent to 4–5 ml of plasma. Repacking the top 1–3 mm of the column re-established the original efficiency, although this dropped off in a similar manner. These results indicated that the column was not undergoing irreversible damage or deactivation. Further experiments were carried out involving the same total volume of plasma extract, injected either as a small number of large volume injections or a large number of small volume injections. These experiments clearly showed that the decrease in performance was associated with the volume of plasma injected rather than the number of injections per se.

It was clear that the Sperisorb SCX phase was not as robust as the more commonly used reversed-phases materials so attempts were made to improve the situation through the use of a guard column. However, Spherisorb SCX is not available in large particle sizes suitable for use as a guard column. We therefore evaluated four comparable and readily available materials viz. Bond-Elut SCX (40 μm, Analytichem), silica (60 μm particle size, Phase Separations) and Whatman and Alltech pellicular SCX materials (both 40 μm). These were all packed into a direct connect guard column (30 × 2 mm) from Alltech, which were then tested for retentivity and peak shape using a set of basic compounds. The Bond-Elut material appeared to be highly retentive and failed to allow elution of any of the test compounds. The silica material was found to be the best in terms of having minimum retention for the test compounds and hence giving narrow peaks. When tested in conjunction with the analytical column, it failed to afford any protection and the loss in efficiency was the same as that seen previously.

The two pellicular materials gave similar results, with peaks marginally wider than those seen with silica. A number of experiments were then carried out using both these materials which were found to afford good protection to the analytical column. After injecting the equivalent of approximately 11 ml of plasma, efficiency was usually around 90% of that seen originally (Fig. 2). In one experiment using the Whatman material we were still able to retain 80% of the original efficiency after the injection of extracts containing the equivalent of 42.5 ml of plasma.

As well as monitoring column efficiency, retention reproducibility was also examined in the same experiments. It was found that even without a guard column in place, retention times were highly reproducible. After the injection of the equivalent of 11.25 ml plasma (at least 45 injections) the relative standard deviation of the retention times for the five compounds was $< 0.15\%$.

3.2. Bioanalytical applications

The SCX system has been used in our laboratory for the analysis for a range of drug candidates in biological materials, particularly plasma. This approach has been found to work well with both solid-phase and liquid–liquid extraction procedures. For the latter we have employed solvents with a range of polarities such as hexane/iso-amyl alcohol, *tert*-butyl methyl and dichloromethane.

A typical application involving the analysis of a drug development candidate (Drug Y, Fig. 1) in

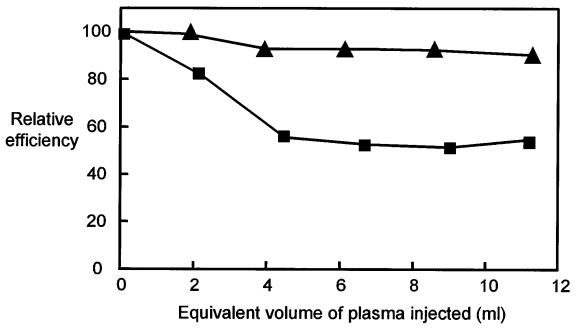

Fig. 2. The variation in the efficiency shown by a Spherisorb 5SCX column as a function of the volume of extracted plasma injected. Data is shown for a standard column (100 × 4.6 mm) (■) and a column protected with a guard column (30 × 2 mm) packed with Alltech pellicular SCX (▲).

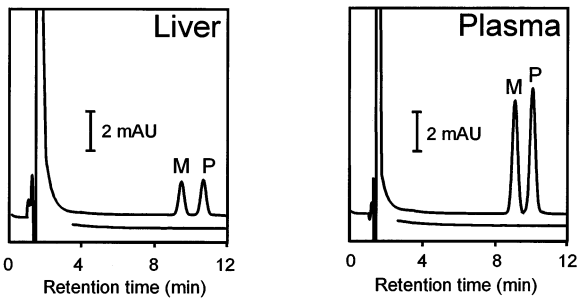

Fig. 3. Chromatograms showing the results following the analysis of rat plasma (1 ml) and liver (1 g), from animals dosed with a drug candidate. Comparable blank samples are also shown (in part). The liver samples were first homogenised with water and both plasma and homogenised liver were basified and extracted with methyl-*tert*-butyl ether. The concentrations of the parent compound (P) and metabolite (M) were around 15 ng g^{-1} for the liver and 200 ng ml^{-1} for the plasma. Column: Spherisorb 5SCX (100 × 4.6 mm), eluent: methanol–water–TFA (800:200:2.3, v/v) containing ammonium formate (0.02 M), with an apparent pH of 2.45, detection was as 253 nm.

plasma and liver is shown in Fig. 3. Despite the relatively simple and generic approach to the analysis, the chromatographic performance is good and the chromatograms are relatively free from interfering peaks. As well as parent drug (peak P), the chromatograms also show a peak corresponding to a ring hydroxylated metabolite (peak M, Fig. 1), which elutes just prior to the parent compound peak. The fact that the metabolite and parent have similar elution characteristics, but are well resolved, is a useful feature of this system. This is in contrast to the situation in reversed-phase HPLC where polar hydroxy metabolites are frequently eluted early and lost in the solvent front whilst the parent compound chromatographs with a k' of approximately five [4].

3.3. Analysis of medicated rodent diet

One research programme in our laboratory involved an investigation of supercritical fluid extraction for the isolation of drugs from medicated rodent diet. To minimise extract-to-extract variability and to maximise sample throughput it was desirable to extract and quantify several drugs simultaneously. Because the analytes (propranolol, ZM95527, tamoxifen and ZM169369 (Fig. 1)) exhibited widely differing physicochemical properties (log P 1.07–6.63) it was not possible to use a simple isocratic reversed-phase HPLC. However, since the separation on SCX is controlled in the main by the basicity of the solutes it was possible to obtain excellent separation of the compounds using this mode of chromatography. Fig. 4 shows the separation of four test compounds extracted from rodent feed using a Hewlett Packard 7680T extractor with supercritical CO_2–methanol (85:15, v/v). Baseline separation was easily achieved for this diverse set of compounds (see Figure legend) and the quality of the chromatography in each case was very good.

4. Conclusions

Whilst not as robust as the more widely used reversed-phase materials Spherisorb SCX would appear to be suited to bioanalysis when protected by a suitable guard column. Retention times are highly reproducible and following the analysis of extracts equivalent to 42.5 ml of plasma, 80% of the original efficiency was still retained.

This approach has been shown to be useful in a wide range of situations and the fact that compound lipophilicity is only a minor factor in the

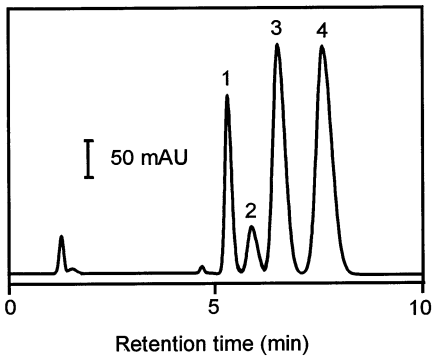

Fig. 4. Chromatogram showing the results of the analysis of four compounds extracted from rodent diet. The compounds are: 1 = propranolol (log P 3.56, pK_a 9.42), 2 = ZM95527 (log P 1.07, pK_a 7.93), tamoxifen (log P 6.63, pK_a 8.57) and ZM169369 (log P 4.50, pK_a 8.23). Each compound was present at a concentration of around 5 mg g^{-1} and 0.3 g of diet was extracted. Column: Spherisorb 5SCX (100 × 4.6 mm), eluent: methanol–water–TFA (800:200:2.3, v/v) containing ammonium formate (0.02 M), with an apparent pH of 2.45, detection was at 270 nm.

control of retention makes it suitable for the simultaneous analysis of compounds having wide ranging physicochemical properties.

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