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Short Communication

The detection of [^{14}C]propranolol following supercritical fluid chromatography using in-line radioactivity detection*

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Introduction

Whilst many technical problems remain, it is clear that supercritical fluid chromatography (SFC) has matured to the stage where it can be seen to complement the "traditional" methods of high-performance liquid (HPLC) and gas liquid chromatography (GLC). In particular SFC has advantages for compounds which are difficult to analyse by HPLC and unsuited to GLC because of thermal instability or the need for derivatization in order to increase volatility. The possibility of using both HPLC and GLC detectors and facile coupling to mass spectrometers is another attractive feature of SFC (see for example Shoenmakers [1]).

For polar or ionizable molecules it is often necessary to add an organic modifier, such as methanol, to the liquid carbon dioxide used as the mobile phase and such substances are generally best analysed using packed column SFC. Our preliminary studies to explore the potential applications of SFC in drug metabolism studies, whilst promising, were hampered by the lack of a suitable radioactivity monitor to detect radiolabelled compounds and their metabolites [2]. Here we describe our initial experiences with packed column SFC and an in-line radioactivity detector exemplified using [^{14}C]-labelled propranolol.

Materials and Methods

[^{14}C]propranolol was synthesized in the radiochemical laboratories at ICI Pharmaceuticals and had a specific activity of 38.4 μCi mg^{-1} and a radiochemical purity of >99%. The radiolabelled propranolol was dissolved in HPLC grade methanol or acetonitrile (Fisons, Loughborough, UK) at approximately 1 mg ml^{-1}.

Chromatography was performed on an SFC system constructed from two Pye Unicam LC3-XP HPLC pumps, one of which was used to deliver methanol. The other pump was fitted with a clamp-on heat exchanger cooled by a modified refrigerator unit (Ash Instruments, Macclesfield, UK) in order to enable it to pump supercritical carbon dioxide. The supercritical carbon dioxide was delivered from a cylinder fitted with a syphon via an alumina-filled column to the pump. Supercritical carbon dioxide and methanol were mixed in a magnetically stirred chamber. The proportions of methanol used as modifier were controlled by a Beckman 421A gradient controller. Samples were introduced into the system via a Rheodyne 7120 valve, fitted with a 100-μl injection loop, mounted inside a modified Pye 104 GC oven which was used to control the column temperature (further details of this system are given in ref. 2).

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Chromatography was performed on a 15 cm by 0.46 cm (i.d.) aminopropyl-bonded silica cartridge HPLC column (Capital HPLC, Edinburgh, UK).

Radiolabelled compounds eluting from the column were detected using a Berthold LB507A radioactivity monitor fitted with a high pressure flow cell (Berthold UK Ltd, St Albans, UK) and recorded using a Phillips PM 8252 chart recorder.

The chromatographic conditions employed were as follows. The mobile phase consisted of carbon dioxide containing 10% (by volume) methanol and 0.1% triethylamine. The triethylamine used was of sequencer grade (Rathburn Chemicals, Scotland). The flow rate used was 5 ml min^{-1} at an indicated back pressure of between 3200–3400 psi. The back pressure was regulated by using a Tescom Model 26 back pressure regulator. Chromatography was performed with an oven temperature of 50°C.

The amount of radioactivity injected onto the system was determined using liquid scintillation counting of aliquots of the [^{14}C]propranolol solution.

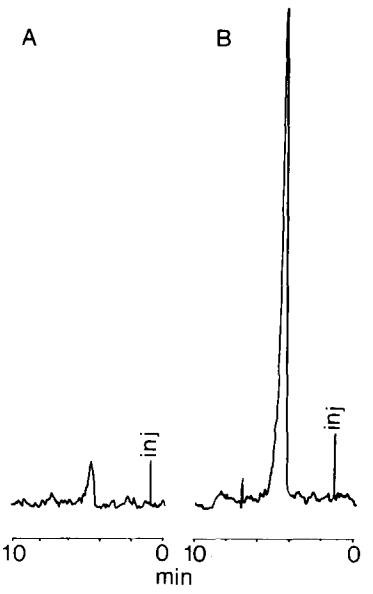

Figure 1
Supercritical fluid chromatography of [^{14}C]propranolol, with in-line radioactivity detection on an amino-bonded column with supercritical carbon dioxide and methanol (90:10) containing 0.1% triethylamine at 50°C and 5 ml min^{-1}; (A) 10,000 dpm and (B) 100,000 dpm on-column.

Results and Discussion

The chromatograms shown in Fig. 1 illustrate the results obtained for SFC with an in-line radio-flow cell following the injection of 10,000 and 100,000 dpm of [^{14}C]propranolol. Under the chromatographic conditions used the radiolabelled compound eluted with good peak shape approximately 3 min after injection. Under these conditions as little as 2000 dpm of radioactivity on column were detectable, although a practical limit of detection probably lies in the region of 3000–4000 dpm. This is somewhat higher than the equivalent result for HPLC with this type of flow cell where a detection limit of approximately 500 dpm might be expected. However, the flow rate of 1–2 ml min^{-1} used for HPLC is much lower than the 5 ml min^{-1} used here for SFC. The lower sensitivity obtained for the radio-flow cell with SFC probably simply reflects the difference in residence time in the detector. The response of the detector was linear over the range tested (0 to 100,000 dpm).

Initially the authors were concerned that radiolabelled material would be adsorbed onto solid scintillant contained within the flow cell (yttrium silicate) under SFC conditions. This would have lead to an increased background and severely limited the use of the radio-flow cell. However, no evidence for any such absorbtion in this series of experiments was observed.

A practical problem encountered, which was related to the chromatographic system rather than the radio-flow cell, concerned the solvent used for injection. The [^{14}C]propranolol was injected into the SFC system in quantities of between 5–100 μl of methanol via the loop injector but it was found that with volumes in excess of approximately 30 μl peak splitting occurred. This was no doubt due to some radiolabelled material eluting with the poorly retained "solvent front" of highly eluotropic methanol. When acetonitrile was substituted for methanol as injection solvent this problem was much less apparent. This was probably due to the fact that acetonitrile is a very poor eluent for this type of compound under SFC conditions. It has not been possible to elute β-blockers from amino-bonded columns by SFC with acetonitrile as the only organic modifier (Roberts and Wilson, unpublished observations). Clearly careful selection of the solvent used for sample introduction is required when the injection of large volumes is contemplated (for example for preparative isolation of metabolites).

On the basis of this brief study it is clear that a high pressure radio-flow cell capable of withstanding the back pressures associated with SFC has been developed. The detection of [^{14}C]-labelled material in line with SFC is therefore a practical possibility and this will allow the full investigation of the use of SFC in metabolism studies on radiolabelled compounds.

References

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