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Fluorescence detection in packed-column supercritical fluid chromatographic separations

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Abstract

Propranolol and (9-acridinylamino)propranolol (9-AAP) have been separated by supercritical fluid chromatography with fluorescence detection by employing a simple pressure restrictor to protect the detector flow cell. The separations were carried out using a μBondapak C_{18} column with a mobile phase of 1% triethylamine in methanol-modified CO_2 at 40°C. The excitation and emission wavelengths for propranolol were 317 nm and 352 nm and for 9-AAP 245 nm and 413 nm, respectively. The sensitivity for propranolol detected by the fluorescence detector was 5.7 ng, approximately 8 times higher than by UV spectroscopic detection and was similar to the results for HPLC with fluorescence detection. A higher sensitivity (0.4 ng) was found for 9-AAP with fluorescence detection. © 1998 Elsevier Science B.V.

Keywords: Detection, SFC; Fluorescence detection; Propanolol; Acridinylaminopropranolol; Beta-blockers

1. Introduction

One of the initial attractions of supercritical fluid chromatography (SFC) was that it could be used with both GC or LC detectors. It should therefore be potentially possible to employ the high sensitivity and selectivity of fluorometric detection. Some early studies in 1982–1984 by Fjeldsted and coworkers used fluorescence detection with capillary column SFC for the determination of aromatic hydrocarbons [1–3]. However, no further work was reported for many years, until a recent capillary study for the determination of polycyclic aromatic hydrocarbons in environmental studies by Höner and coworkers [4]. Sandmann and Grayeski [5] suggested that one problem was a lack of commercially available fluorescence detectors for SFC. They developed a complex dual-restrictor interface, for the addition of a solvent, to enable a pressurised conventional fluorescence detector flow cell to be used. Using this system they examined the response from 9,10-diphenylanthracene on a packed 1-mm microbore column. Subsequently they employed a similar configuration for a chemiluminescence study [6].

The present work examined the application of fluorescence detection in packed-column SFC for the determination of propranolol. The aim was to demonstrate that by using a simple single stage restrictor as a interface it would be possible to employ a conventional unmodified HPLC fluorescence detector to enhance the sensitivity of the analysis. This work follows earlier studies in these laboratories on the potential application of conventional packed-column SFC for the separation of barbiturates [7] and benzodiazepines [8] and it was of interest to expand the application and sensitivity of the technique to other drug compounds. Propranolol is a β-blocker which has been readily determined by HPLC with

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both UV and fluorescence detection [9–11] in a wide range of biological matrixes. It has also been separated by SFC with UV spectroscopic detection on both bonded and chiral columns [12–15]. In a typical recent study Bailey and co-workers [12] reported the separation of a number of β-blockers on diol, aminopropyl, cyanopropyl, and C_1-bonded stationary phases using traces of triethylamine in methanol–carbon dioxide to improve the peak shapes.

2. Experimental

2.1. Chemicals

DL-Propranolol hydrochloride and (9-acridinylamino)propranolol triacetate salt (9-AAP), were from Sigma (Poole, UK). Methanol of HPLC grade and triethylamine (TEA), were from Fisher Scientific (Loughborough, UK). Carbon dioxide was of laboratory grade from British Oxygen Company (UK).

2.2. Chromatographic system

The SFC separations were carried out using a Jasco 880 PU pump (Toyko, Japan) with a pump head cooled to −10°C by a refrigerated bath (RB-5, Techne) to pump the carbon dioxide and a PU4015 pump (Pye Unicam, Cambridge, UK) was used to pump the modifier of 1% TEA in methanol. The eluent was mixed in a 811B Gilson dynamic mixer. Samples in methanol were injected using a Rheodyne 7125 valve fitted with a 20-μl loop (Cotati, CA, USA) onto a μBondapak C_{18} column (300×3.9 mm, Waters) held at 40°C in a Jasco 860-CO oven. The analytes were detected using a Jasco 870 UV detector equipped with a high-pressure flow cell and SFM25 fluorescence detector (Kontron, Zurich, Switzerland) fitted with a standard HPLC flow cell. The connecting tubing was manually crimped between the detectors to maintain the column inlet pressure at more than 100 bar. The pressure in the fluorescence detector was maintained at 50 bar using a 880-81 back pressure controller (Jasco). Two integrators (Model 3394 and Model 3396A, Hewlett–Packard) were used to collect the data.

2.3. Separation conditions

For the separation of propranolol, the carbon dioxide was pumped at 1.4 ml min^{-1} and the modifier of 1% TEA in methanol was pumped at 0.30 ml min^{-1}. The column inlet pressure was 130 bar. The samples were detected by their absorption at 300 nm and the fluorometer was set to excitation, 317 nm and emission, 352 nm.

For the separation of 9-AAP, the modifier flow was reduced to 0.20 ml min^{-1}, which gave an inlet pressure of 120 bar and the fluorometer was set to excitation, 245 nm and emission, 413 nm.

3. Results and discussion

The principal restriction on the use of conventional HPLC UV and fluorescence detectors with supercritical fluid chromatography is the pressure limit of the spectrometer flow cell. Previous authors used specially constructed capillary flow cells [1–4] for capillary SFC or put considerable effort into the design of a dual restrictor to reduce the pressure, coupled with a curtain flow system to absorb the analyte into a liquid carrier phase before detection [5].

A much simpler system was employed in the present study. The pressure in the chromatographic column was controlled by altering the eluent flow-rate against a crimped tube post-column restrictor. The eluent was then maintained as a liquid through the detector cell by applying a low back pressure of 50 bar from an electronically controlled back-pressure regulator. This enabled a standard HPLC fluorescence flow cell rated at 50 bar to be used irrespective of the flow-rate.

3.1. Determination of propranolol

The SFC separations of propranolol were carried out on a μBondapak C_{18} column using mixtures of a 1% solution of TEA in methanol in carbon dioxide as the eluent at pump pressures of 146–108 bar and 40°C (Table 1), comparable to the conditions used previously for the SFC of propranolol with UV detection [12]. With 18% modifier in carbon dioxide, the peaks showed little tailing (Fig. 1) but as the

Table 1
The variation in the retention of propranolol with proportion of the modifier

Eluent CO_2		Modifier		Retention	
Flow-rate (ml min^{-1})	Pump pressure (bar)	Flow-rate (ml min^{-1})	% in eluent	Time(min)	*k*
1.4	146	0.50	26	2.28	0.30
1.4	125	0.30	18	3.42	1.16
1.4	118	0.20	12	5.20[a]	1.74
1.4	108	0.10	7	no peak	

Eluent 1% TEA in methanol–carbon dioxide at 40°C. Conditions as Section 2.
[a] Broad peak.

modifier concentration was reduced the peak shapes became asymmetric. The retention times also increased and by 7% modifier no peaks were eluted.

Conditions of 18% of TEA-methanol in carbon dioxide at 125 bar were selected for further study. Samples of propranolol from 0.042–21.2 μg on-column gave a linear response from the UV detector with a correlation coefficient of 0.9998 for the peak heights. With the fluorescence detector set to an excitation wavelength of 317 nm and an emission wavelength of 354 nm, a linear response for the peak heights was obtained from 0.021–0.212 μg of propranolol with a correlation coefficient of 0.9942. Previous reports using normal-phase HPLC have reported linear ranges with fluorescence detection from 1.8–26.4 ng with a correlation of 0.994 [9].

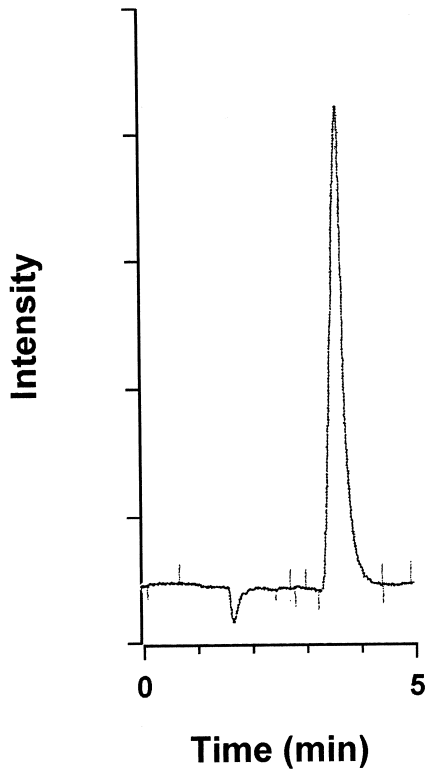

Fig. 1. Supercritical fluid chromatogram of 0.16 μg propranolol. Conditions: column, μBondapak C_{18} ; eluent, CO_2 flow-rate 1.4 ml min^{-1} and 1% TEA in methanol, flow-rate; 0.30 ml min^{-1}, temperature; 40°C; fluorescence detector, λ_{ex} 317 nm, λ_{em} 352 nm.

The limits of detection of the present method were found to be 46 ng with the UV detector and 5.7 ng with the fluorescence detector. These results were comparable to the sensitivity for HPLC coupled with fluorescence measurement reported by Jatlow and coworkers [16] who found a detection limit in blood plasma of less than 6 μg/l equivalent to 4.8 ng on-column.

3.2. Separation of 9-AAP by fluorescence detection

9-AAP is a readily available highly fluorescent derivative of propranolol first reported by Atlas and Levitzki [17]. It was therefore of interest to determine if it could also be separated and detected by SFC with fluorescence detection. A small change in the eluent to 12% modifier gave a reasonable retention time of 4.7 min (Fig. 2). Samples of 9-AAP containing between 0.2–1.0 ng gave a linear response for peak area from the fluorometer set at an excitation wavelength of 245 nm and an emission wavelength of 413 nm. Low concentrations of 9–

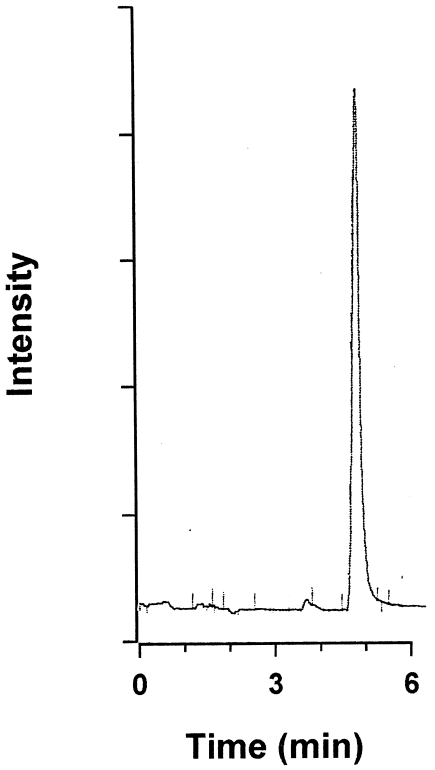

Fig. 2. Supercritical fluid chromatogram of 0.03 μg (9-acridinylamino)propranolol. Conditions: column, μBondapak C_{18}; eluent, CO_2 flow-rate 1.4 ml min^{-1} and 1% TEA in methanol flow-rate; 0.20 ml min^{-1}; temperature, 40°C; fluorescence detector, λ_{ex} 245 nm, λ_{em} 413 nm.

AAP were injected a number of times and gave a limit of detection of 0.4 ng.

4. Conclusion

With slight modifications to a standard packed-column SFC system, it is possible to readily employ a fluorometric detector with a HPLC flow cell to detect both propranolol and 9-AAP, with a similar retention and sensitivity to liquid chromatographic separations. This method should be generally applicable to a wide range of fluorescent analytes providing packed-column SFC with a highly selective and sensitive detector.

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