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The use of heptafluorobutyric acid as a volatile ion-pair reagent in the high-performance liquid chromatographic isolation of SB-223070

John K. Roberts*, Mark J. Hughes

SmithKline Beecham Pharmaceuticals, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK

Abstract

The separation of the *E*-oxime (SB-223070) from the *Z*-oxime (SB-220582) was achieved using the ion pairing reagent sodium dodecyl sulfonate. When a pure sample of SB-223070 was required by preparative chromatography from mother liquors, modification of the chromatographic conditions was necessary. The capacity factor and resolution between the two oximes needed to be increased to isolate the *E*-oxime from the other components in the mother liquor and a method to remove the ion-pairing reagent from the isolated product was required. The volatile ion pairing reagent trifluoroacetic acid did not retain or resolve the components sufficiently but by switching to heptafluorobutyric acid, isolation of SB-223070 was achieved as the heptafluorobutyrate. This salt was liberated by hydrochloric acid to give the required hydrochloride salt and the remaining heptafluorobutyric acid was removed by extraction into dichloromethane.

Keywords: Ion-pair reagent; Heptafluorobutyric acid; SB-223070; Oximes

1. Introduction

Over the past 15 years several publications have shown that perfluorinated carboxylic acids are as effective as classical ion pairing reagents for the analysis of peptides [1,2] with the retention of a peptide increasing with chain length and concentration of the perfluorocarboxylic acid [3]. The volatility and low UV absorbance of perfluorinated carboxylic acids also makes them useful for liquid chromatography–mass spectrometry (LC–MS) studies of peptides [4]. Perfluorocarboxylic acids have also been shown to be useful for semi-preparative isolation of peptides as fractions can be freeze dried and any excess perflurocarboxylic acid removed by dissolution in an organic solvent [5,6].

The original high-performance liquid chromatography (HPLC) method for the separation of the *E*-oxime SB-223070 ((*E*)-(+/−)-α-(hydroxyimino)-1-azabicyclo[2.2.2]octane-3-acetonitrile, I) from its geometric isomer SB-220582 ((*Z*)-(+/−)-α-(hydroxyimino)-1-azabicyclo[2.2.2]octane-3-acetonitrile, II) (Fig. 1) involved the use of sodium dodecyl sulfonate as an ion paring reagent. When 5 g of I with a purity >98% were required by preparative chromatography from mother liquors, it was necessary to increase the capacity factor to separate I from other components in the mother liquor and increase the resolution between I and II without the use of classical ion-pair reagents. Although no details of the

*Corresponding author.

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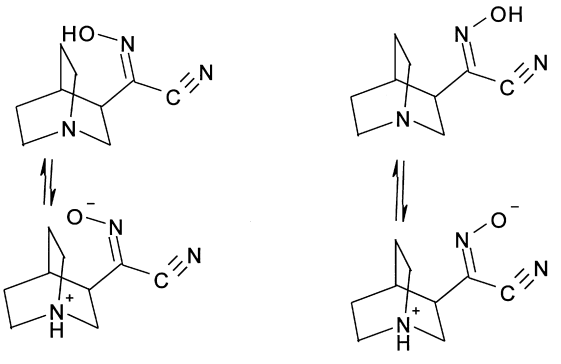

SB-223070 **I** SB-220582 **II**

Fig. 1. Structures of SB-223070, SB-220582 and their zwitterions.

use of perfluorocarboxylic acids in the chromatographic isolation of compounds such as I had been published, the properties of perfluorocarboxylic acids discussed above made them the ideal choice for this piece of work.

2. Experimental

2.1. Analytical chromatography

All chromatograms were recorded using either a Merck–Hitachi Lachrom system consisting of an L7100 quaternary pump, L7450 detector and L7200 autosampler or on a system consisting of a Merck–Hitachi L6200A ternary pump and AS-2000A autosampler with a Waters 991 detector. The column used was a Merck RP 60 select B column, 5 μ, 125×4.0 mm. Eluent A was 0.1% (v/v) heptafluorobutyric acid (HFBA) in water, eluent B was acetonitrile. The elution gradient was 100% A falling linearly to 90% A at 15 min followed by 10 min re-equilibration at a flow-rate of 1.0 ml/min, with

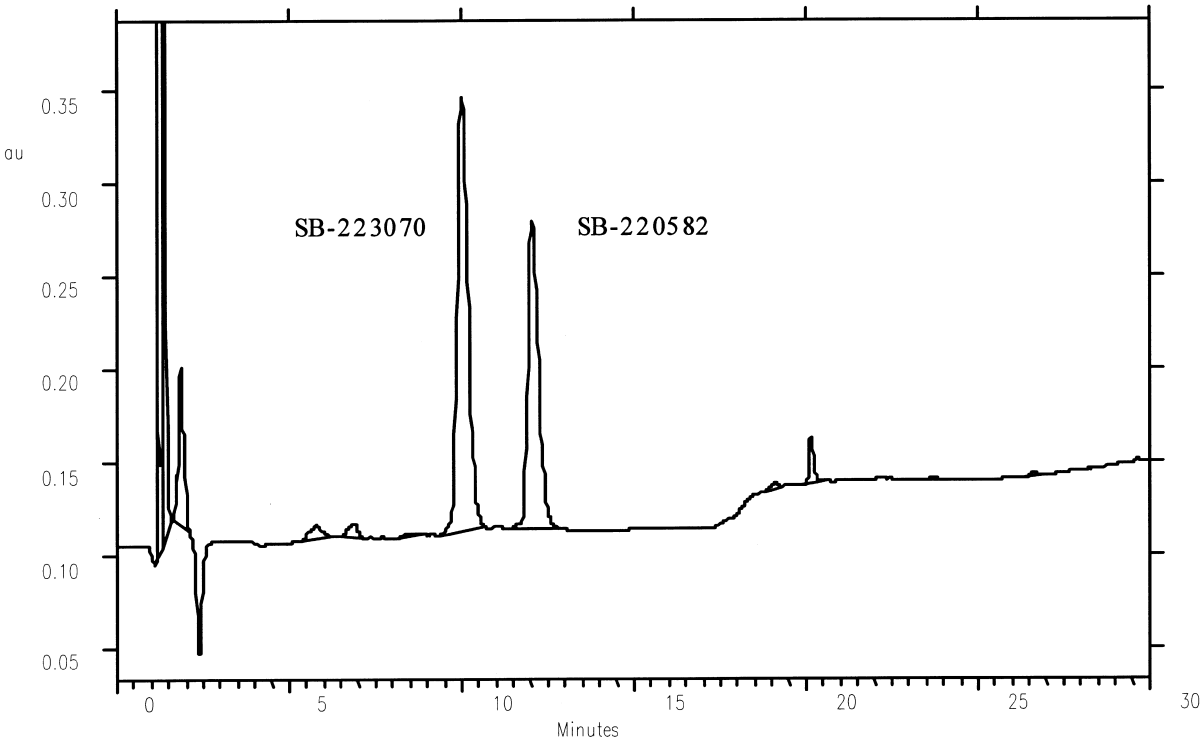

Fig. 2. Analytical chromatogram of mother liquor.

UV detection at 230 nm and a temperature of 30°C. Injection volume was 20 μl of a 0.2 mg/ml solution in A–B (80:20, v/v).

2.2. Ion chromatography

All chromatograms were recorded using a Dionex 4500I system with GPM quaternary gradient pump module, autosampler, CDM2 conductivity detector module and AMSII anion suppressor module. The column used was a Dionex Ion Pac AS11 150×4.0 mm. Eluent A was water, eluent B was 0.1 *M* sodium hydroxide in water. Elution conditions were as follows: A–B (79:21, v/v), 1.0 ml/min, temperature ambient. Injection volume was 50 μl of a 0.045 mg/ml sample in water. Detection was by chemically suppressed conductivity at 30 microSiemens FS using the AMSII anion suppresser module fed with 0.025M sulfuric acid via the Dionex autoregeneration system (% stroke=40, rate%=70).

2.3. Preparative chromatography

All preparative chromatography runs were carried out using a Merck–Septech binary pump with fraction collector and GP900A controller and a Merck–Hitachi L4000A detector fitted with preparative flow cell. Separation was carried out on a Merck RP 60 select B 12 μ stationary phase packed into a 35×10 cm column. Eluent A was 0.1% (v/v) HFBA in water, eluent B was acetonitrile. Flow-rate was 500 ml/min, detection UV at 230 nm, temperature ambient. Elution 100% A falling to 85% A at 15 min, held isocratic at 85% A for 10 min and then ramped to 30% A at 35 min. For runs 1 and 2 mother liquor was dosed onto the column head at 250 ml/min. For subsequent runs solutions of crude I at 10 mg/ml in A–B (80:20, v/v) were dosed onto the column head at 25 ml/min.

3. Results and discussion

The data in Table 1 show the retention times of I and II and the selectivity between them using the specified conditions. These data show clearly a significant increase in the capacity factor whilst maintaining selectivity between I and II when HFBA is added to the mobile phase. Fig. 2 shows the analytical chromatogram of the mother liquor (condition 5, Table 1) from which a pure 5-g sample of I was to be isolated. By comparison with a standard of II, the mother liquor was estimated to contain 4.3 g/l of I and 2.0 g/l of II plus other components. Due to the presence of the other components a pure sample of I was isolated in two chromatographic stages; a high compound loaded initial purification stage where the two oximes were isolated (Fig. 3) and then a lower compound loaded second stage where I only was isolated (Fig. 4). The runs carried out are shown in Tables 2 and 3. At the high loadings used in the initial purification runs the resolution between I and II is lost but the selectivity between the oximes and the other components is maintained. Attempts to extract I and II from the eluent using organic solvents failed even at pH=8 as I and II can form zwitterions (Fig. 1). All products were therefore isolated using a combination of rotary evaporation to remove the acetonitrile and freeze drying. The

Table 1
Retention time and selectivity data for I and II under the conditions described

Condition	t_R (mins) I	t_R (mins) II	Selectivity
1	3.25	3.85	1.2
2	3.6	4.6	1.4
3	5.5	6.5	1.2
4	21.9	28.6	1.3
5	10.0	12.1	1.2

Condition 1: Kromasil S5C8 column 100×3.2 mm, eluent A= 0.05 *M* sodium dihydrogenphosphate (pH=3) plus 0.015 *M* sodium dodecyl sulfonate, eluent B=acetonitrile. Elution: A–B (65:35), flow-rate 1 ml/min, detection UV 230 nm, temperature 40°C. Injection: 20 μl of 100-fold diluted mother liquor in water–acetonitrile (70:30, v/v), hold-up time, t_m=0.53 min.
Condition 2: Merck RP60 Select B cartridge 125×4.0 mm, eluent A=0.1% (v/v) trifluoroacetic acid (TFA) in water, eluent B= acetonitrile. Elution: A–B (97:3, v/v), flow-rate 1 ml/min, detection UV 230 nm, temperature 30°C. Injection: 20 μl of 100-fold diluted mother liquor in A–B (70:30, v/v), hold-up time, t_m=0.81 min.
Condition 3: as Condition 2 except elution: 100% A.
Condition 4: as Condition 2 except elution: 100% 0.1% (v/v) HFBA in water.
Condition 5: as Condition 2. Eluent A=0.1% (v/v) HFBA in water, eluent B=0.1% (v/v) HFBA in acetonitrile, elution: 100% A falling to 90% A at 15 min and to 40% A at 30 min. Injection: 30 μl of 100-fold diluted mother liquor in A–B (70:30, v/v).

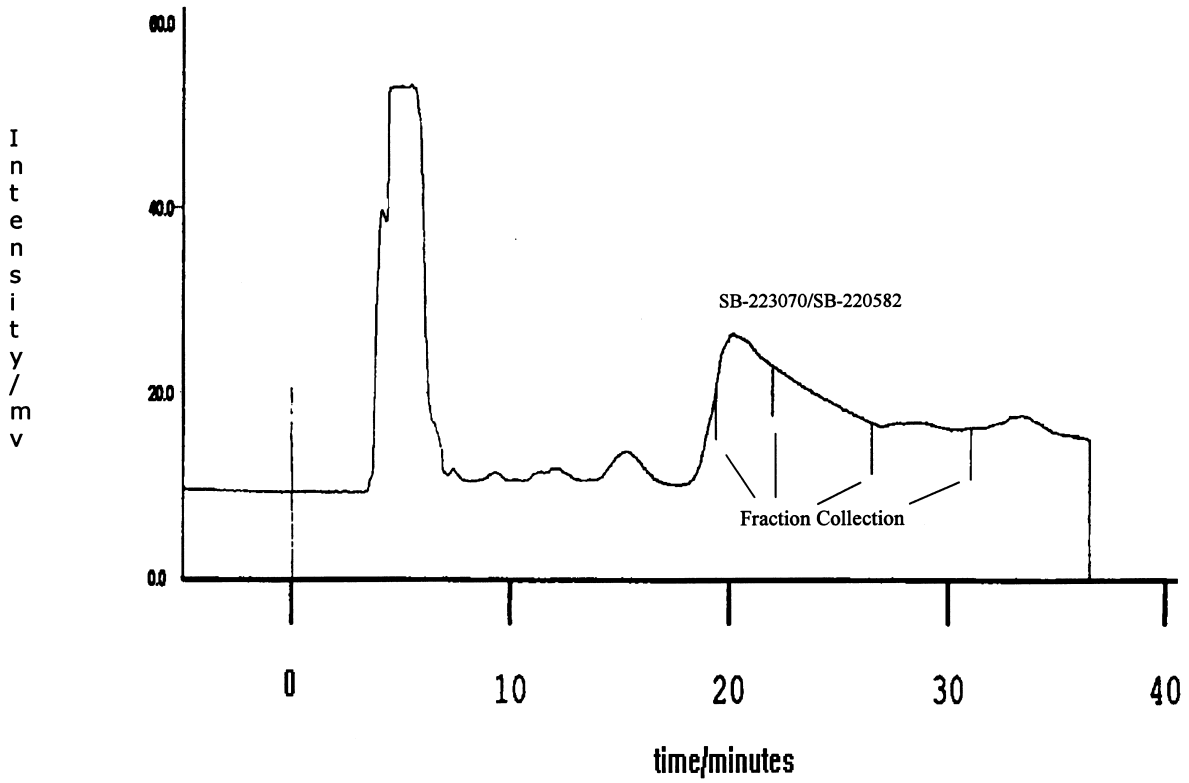

Fig. 3. High compound loaded initial purification, data from run 2.

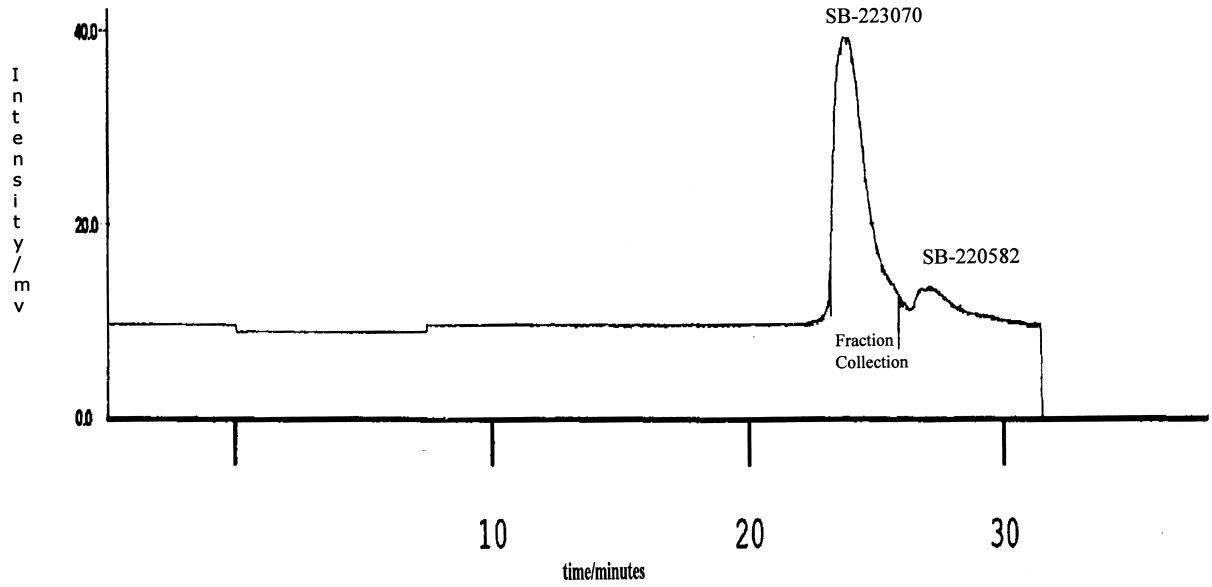

Fig. 4. Low compound loaded SB-223070 purification, data from run 7.

Table 2
Initial purification runs

Run	Volume of mother liquor /l	Fraction 1 % by area I, mfd[a]	2 % by area I, mfd[a]	3 % by area I, mfd[a]
1	0.5	88.9, 4.84	56.7, 4.36	16.5, not freeze dried
2	1.0	75.8, 3.89	68.9, 4.03	67.7, not freeze dried

[a] mfd is the mass of Crude I (in grams) after freeze drying.

isolated crude product from the initial purification runs was a mixture of free base and heptafluorobutyrate salt as there is insufficient HFBA present to form a stoichiometric salt. A loading of 1.5 g of crude product per final purification run gave I as the HFBA salt with acceptable purity.

As little was known about the stability of HFBA salts, a procedure was required to convert this salt to the hydrochloride. An aqueous suspension of material from runs 6/7 was treated with hydrochloric acid to liberate the HFBA salt and the HFBA was removed by extraction with dichloromethane. Concentration of the resulting solution, addition of isopropyl alcohol and cooling gave rise to crystals of I as the hydrochloride salt with a yield of 78%. Analysis of this material showed no interconversion between I and II and ion chromatography confirmed that a stoichiometric hydrochloride salt had been formed (Fig. 5). It was decided to see if crude material from the initial purification runs could be treated in this way and yield acceptably pure I as the hydrochloride salt in good yield. Crude material from run 2 fraction 1 (Table 2) was treated in a similar way and two crops of crystals of the stoichiometric hydrochloride salt were produced. Crop 1 was 98.5% by area I and crop 2 was 96.7% by area I with an overall yield based on an initial stoichiometric HFBA salt of 68%. Further work would be necessary to see if a crude chromatographic purification followed by work up to form the hydrochloride salt would be a more efficient way to produce further supplies of I. All the material from runs 3 to 10 and the material previously converted to the hydrochloride salt were combined to yield 6.68 grams of stoichiometric I as the hydrochloride salt with a purity of 99.8%.

4. Conclusions

HFBA was shown to be a useful volatile ion pairing reagent which gives significantly increased retention of amine containing compounds over its shorter chain homologue trifluoroacetic acid. The heptafluorobutyric acid salt of I was readily liberated by hydrochloric acid and the resulting heptafluorobutyric acid easily removed by extraction into dichloromethane. The conversion of heptafluorobutyric

Table 3
Final purification runs

Run	Mass of crude I (g)	Source of crude I	% by area I	Mass of HFBA salt (g)
3	1.00	fr.2 R1[a]	100	0.74
4	1.00	fr.2 R1	100	Combined with R5
5	1.25	fr.2 R1	100	2.78 (R4+R5)
6	1.50	0.6g fr.2 R1 0.9g fr.1 R1	100	Combined with R7
7	1.50	fr.1 R1	100	3.37 (R6+R7)
8	1.50	fr.1 R1	100	Combined with R9
9	1.50	0.4g fr.1 R1, 1.1g fr.2 R2	100	3.49 (R8+R9)
10	1.50	fr. 2 R2	98.4	1.45

[a] fr.2 R1 stands for fraction 2 run 1 the details of which are shown in Table 2.

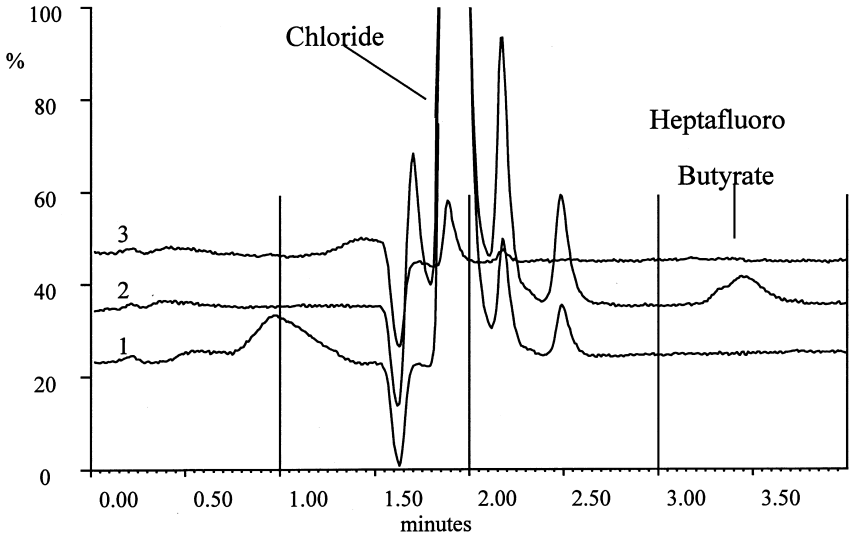

Fig. 5. Ion chromatography following extraction procedure. Trace 1: SB-223070 hydrochloride, trace 2: sample from trace 1 spiked with 1% (w/w) heptafluorobutyrate, trace 3: blank.

acid anion to the chloride anion was monitored readily by the use of standard ion chromatography. These properties make HFBA a particularly suitable choice for preparative chromatography of amines and for analytical chromatographic method development in general where ion-pairing is necessary.

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