Journal of Chromatography A, 653 (1993) 261–265
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 435

Direct stereochemical resolution of SM-11044, a novel anti-asthmatic drug, and its stereoisomers using a chiral immobilized protein stationary phase

Masahiko Okamoto*, Yasutaka Ohgami and Hiroshi Nakazawa☆

Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., 1-98, 3-chome, Kasugade-naka, Konohana-ku, Osaka 554 (Japan)

(First received March 15th, 1993; revised manuscript received July 6th, 1993)

ABSTRACT

A high-performance liquid chromatographic separation of SM-11044, a novel anti-asthmatic drug, and its antipode and stereoisomers was achieved using a chiral protein column that permits low levels of the antipode to be measured in the SM-11044. The influence of replacing H_2O with ^2H_2O as the mobile phase and the effect of buffer ionic strength, pH, organic modifiers and temperature on the retention times and enantiomeric resolution are discussed.

INTRODUCTION

SM-11044, L-*threo*-3-(3, 4-dihydroxyphenyl)-N-[3-(4-fluorophenyl)propyl]serine pyrrolidine amide hydrogen bromide (Fig. 1), is a newly synthesized anti-asthmatic drug that has potent anti-leukotriene D_4 (LTD_4) and anti-neurokinin A (NKA) activity and an inhibitory effect on the late asthmatic response in guinea pigs [1]. Bearing in mind the current importance of the optical purity of new drugs [2,3], we have been working with several chiral HPLC columns on the chiral separation of a variety of molecules [4–10].

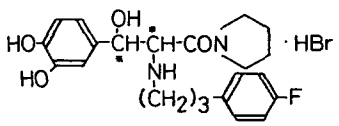

Fig. 1. Structure of SM-11044.

In this paper, we describe the direct separation of SM-11044 and its enantiomer on an ovomucoid column (Ultron ES-OVM). We also examine in detail the influence of replacing H_2O with ^2H_2O as the mobile phase, and the effect of pH, buffer strength, temperature and organic modifier on retention time and resolution of SM-11044 and its enantiomer in order to gather information about the possible mechanisms of chiral recognition [11,12].

EXPERIMENTAL

High-performance liquid chromatography

A Shimadzu LC-5A instrument equipped with an SPD-2A variable-wavelength UV monitor was used. The eluent was pumped at 1.0 ml/min. The column was an Ultron ES-OVM (Shinwakako, Kyoto, Japan; 150 × 4.6 mm). The column temperature was controlled by an Eyela Uni Cool UC-65 circulating water bath (Tokyo Rikakikai, Tokyo, Japan).

* Corresponding author.
☆ Present address: Biotechnology Laboratory, Takarazuka Research Center, Sumitomo Chemical Co., Ltd., 4-chome, Takatsukasa, Takarazuka, Hyogo 665, Japan.

0021-9673/93/$06.00

Chemicals

SM-11044, its enantiomer and its *erythro* forms were supplied by Research Laboratories, Sumotomo Pharmaceuticals (Osaka, Japan). Deuterium oxide (^2H_2O) was obtained from Wako (Osaka, Japan). Aqueous buffer solutions were prepared from potassium phosphate purchased from Wako. All other chemicals were of analytical-reagent grade.

The acidity of ^2H_2O solutions

The acidity (p^2H) of ^2H_2O solutions was measured with an ordinary glass electrode by adding 0.40 to the observed reading of the pH meter, which was calibrated with standard buffers in aqueous solution [13].

RESULTS AND DISCUSSION

Experiments with the ovomucoid chiral column (Ultron ES-OVM) were immediately successful. The chromatographic conditions for the resolution of SM-11044, its enantiomer and stereoisomers with Ultron ES-OVM were optimized by changing mobile phase conditions such as buffer strength, pH and organic modifiers, and temperature. The optimal conditions shown in Fig. 2a allowed for the complete baseline separation of SM-11044 and its enantiomer. Under these conditions four possible stereoisomers of SM-11044 could be separated, as shown in Fig. 2b. The total HPLC run time was less than 20 min. It was determined by chromatography of each individual isomer of SM-11044 that the elution order was SM-11044 first (retention time, $t_R = 7$ min), the *erythro* forms of SM-11044 second and third ($t_R = 9$ and 11 min) and the antipode of SM-11044 fourth ($t_R = 17$ min).

The precision of this method was addressed by performing a recovery test. The antipode of SM-11044 was added to SM-11044 to give a concentration of 0.5 or 1.0%. The added antipode of SM-11044 was recovered quantitatively at both concentrations using this procedure (Table I), demonstrating the chromatographic method to be precise and repeatable.

To gather information on the mechanism of

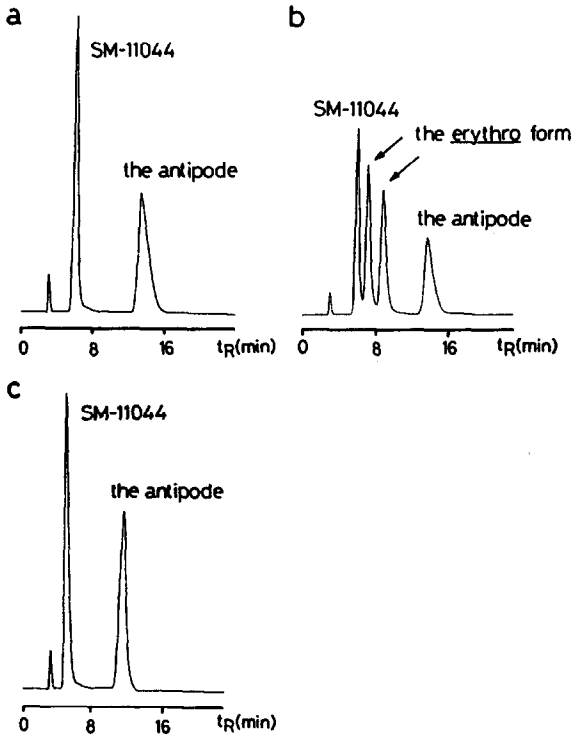

Fig. 2. (a) Resolution of SM-11044 and its enantiomer on an Ultron ES-OVM column. Mobile phase, 20 m*M* phosphate buffer (pH 5.2)–acetonitrile (94:6); flow-rate, 1.0 ml min^{-1}; temperature, 25°C; UV detection at 280 nm; injection volume, 5 μl (1 μg each). (b) Resolution of SM-11044 and its stereoisomers. Other conditions as in (a). (c) Chromatogram of separation of SM-11044 and its enantiomer at p^2H around 5.2. Other conditions as in (a).

interaction between ovomucoid and SM-11044 and its enantiomer, we examined the influence on the enantiomeric separation of replacing water (H_2O) with deuterium oxide (^2H_2O) as

TABLE I

RESULTS OF TESTS ON THE RECOVERY OF THE ANTIPODE OF SM-11044 FROM SM-11044

Calculated (%)	Found (%)	Recovery (%)	Standard deviation
1.00	1.00	100.0	$8.9 \cdot 10^{-3}$ ($n = 5$)
0.50	0.49	98.0	$7.5 \cdot 10^{-3}$ ($n = 5$)

the mobile phase. The viscosity, dipole moment, dielectric constant and ionization of ^2H_2O are significantly different from those of H_2O [14,15]. We could not observe any apparent effect on the resolution, although the retention of the solutes was slightly decreased (Fig. 2c). Camilleri and Dyke [16] have reported that for atenolol there is no significant increase in resolution of the enantiomer on an α_1-acid glycoprotein (α_1-AGP) column using ^2H_2O as the mobile phase, whereas the effect of ^2H_2O on the enantiomeric separation of ibuprofen is drastic. We suppose that such an effect is not universal with all compounds.

The retention and resolution of SM-11044 can be regulated in four ways: by varying the buffer ionic strength or the pH of the mobile phase, by addition of an organic modifier to the mobile phase or by changing the column temperature.

The effects of buffer ionic strength were examined at pH 5.2 using three phosphate buffer concentrations ranging from 5 to 50 m*M*. All the mobile phases contained 6% acetonitrile as the organic modifier. The data are shown in Table II. At higher buffer strength, the capacity factors (k') were increased, the separation factors (α) were slightly decreased and the peaks were broadened. This indicates that hydrophobic interactions are involved in the retention of SM-11044.

The effect of mobile phase pH was studied in the range between 4.5 and 7.3 while maintaining a 20 m*M* phosphate concentration and buffer–acetonitrile (9:1, v/v) composition. The results, as shown in Table III indicate increased resolution with decreasing pH. Retention of SM-11044 became stronger with increasing pH. Ovomucoid has an isoelectric point of 4.1 and has a net negative charge at higher pH. Our preliminary experiment shows that SM-11044 has a pK_a of 8.6. At pH 5.2 the protein has a negative charge, and the solute is positively charged. Hence Coulombic interaction is important for the chiral recognition of SM-11044 as well as for its retention.

The retention and selectivity were greatly influenced by the content and type of organic modifier in the mobile phase. Increasing the concentration of the organic modifier reduces the

TABLE II

INFLUENCE OF BUFFER IONIC STRENGTH (m*M*) ON THE CAPACITY FACTOR (k_1'), SEPARATION FACTORS (α), RESOLUTION (R_s), THEORETICAL PLATE NUMBER (N) AND BAND ASYMMETRY FACTOR (*asf*)

Mobile phase: phosphate buffer (pH 5.2)–acetonitrile (94:6); column temperature: 25°C.

Ionic strength (m*M*)	k_1'[a]	α[b]	R_s[c]	N[d]	*asf*[e]
5	1.43	4.20	2.81	1350	1.20
20	1.80	4.70	3.56	1300	1.20
50	2.09	3.33	5.01	1150	1.21

[a] The capacity factors, k_1' (of the first-eluted enantiomer) and k_2' (of the second-eluted enantiomer), were calculated as follows: $k_1' = (t_{R1} - t_0)/t_0$. $k_2' = (t_{R2} - t_0)/t_0$.

[b] The separation factor $\alpha = k_2'/k_1'$.

[c] R_s (resolution factor) = 2 × (distance of the two enantiomer peak positions/sum of the band widths of the two peaks at their bases); $R_s = 2\ (t_{R2} - t_{R1})/(w_1 + w_2)$.

[d] N (theoretical plate number) = 5.54 × (t_{R1}/the peak width at half-height)^2. The peak of SM-11044 was used for calculation.

[e] ASF (band asymmetry factor) = $(a + b)/2a$ where a and b are the peak width of SM-11044, as shown below.

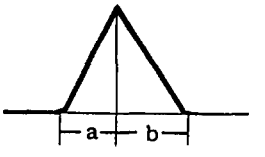

TABLE III

INFLUENCE OF pH ON THE CAPACITY FACTORS (k_1'), SEPARATION FACTORS (α), RESOLUTION (R_s), THEORETICAL PLATE NUMBER (N) AND BAND ASYMMETRY FACTOR (*asf*)

Mobile phase: 20 m*M* phosphate buffer–acetonitrile (90:10); column temperature: 25°C. Footnotes as in Table II.

pH	k_1'[a]	α[b]	R_s[c]	N[d]	*asf*[e]
4.5	0.76	1.55	3.55	980	1.18
5.2	1.80	3.56	4.70	1300	1.20
6.5	4.79	1.38	3.72	550	1.43
7.3	9.87	1.32	2.75	270	1.72

TABLE IV

INFLUENCE OF MONOVALENT ALCOHOLS AND ACETONITRILE ON THE RESOLUTION OF SM-11044 AND ITS ANTIPODE

Mobile phase: modifier + 20 m*M* phosphate buffer (pH 5.2); column temperature: 25°C. Footnotes as in Table II.

Modifier	k'^a	α^b	R_s^c	N^d	asf^e	Elution order
Acetonitrile (6%)	1.80	3.56	4.70	1300	1.20	L/D
Methanol (15%)	1.96	4.86	3.58	700	1.29	L/D
Ethanol (10%)	1.10	3.18	2.79	1030	1.25	L/D
1-Propanol (2%)	2.13	6.29	4.37	700	1.29	L/D
2-Propanol (3%)	1.97	7.70	4.58	700	1.25	L/D

capacity factor. Table IV gives some results obtained using monovalent alcohol and acetonitrile as an organic modifier. Acetonitrile was found to be the best modifier for SM-11044. Although 1- and 2-propanol gave good separation factors for SM-11044 (6.29 and 7.70 respectively), the peaks were broadened in both cases. The elution order was not changed by changing the organic modifier used. These results show that the organic modifiers are involved in the hydrophobic interactions required for the chiral recognition process.

The chromatography was evaluated at temperatures from 5 to 45°C. These data are shown in Table V. As expected, the resolution increased at the expense of longer retention times as well as broadened peak shape when the column temperature was decreased. No significant column degradation was observed upon operation at higher temperatures. Around 25°C chromatography was not affected by slight changes in temperature.

In conclusion, excellent resolution can be obtained for SM-11044, its antipode and stereoisomers by using ovomucoid as the chiral stationary phase. The enantiomeric purity of SM-11044 can be also evaluated directly, rapidly and accurately by this HPLC method.

TABLE V

EFFECT OF TEMPERATURE ON THE CAPACITY FACTORS (k'_1), SEPARATION FACTORS (α), RESOLUTION (R_s), THEORETICAL PLATE NUMBER (N) AND BAND ASYMMETRY FACTOR (asf)

Mobile phase: 20 m*M* phosphate buffer (pH 5.2)–acetonitrile (94:6). Footnotes as in Table II.

Temperature (°C)	k'^a_1	α^b	R_s^c	N^d	asf^e
5	3.15	4.16	5.86	1820	1.20
15	2.35	3.80	5.04	1390	1.23
25	1.80	3.56	4.70	1300	1.20
35	1.36	3.20	3.15	1410	1.21
45	1.06	2.81	1.41	780	1.22

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