Development and validation of chiral high-performance liquid chromatographic methods for the quantitation of valsartan and of the tosylate of valinebenzyl ester

Eric Francotte*, Alexander Davatz, Paul Richert

Ciba-Geigy Limited, Pharmaceuticals Division, R&D, K-122.P.25, CH-4002 Basel, Switzerland

Abstract

A stereospecific HPLC method for the quantitation of CGP 49309 in samples of its corresponding enantiomer valsartan has been developed and validated. The enantiomeric separation was achieved on a 5 μm silica-bonded α_1-acid glycoprotein column (Chiral AGP) with a phosphate buffer, pH 7, containing 2% (v/v) 2-propanol as a mobile phase. The linearity was established in the range 0.1–4% ($r>0.999$). The limit of quantitation was 0.1% and the limit of detection was 0.04%. The accuracy of the method was found to be 96.7% (average). For the precision (repeatability), a relative standard deviation value of 2.4% was found. Similarly, a stereoselective HPLC method was also developed and validated for the quantitation of the enantiomer of the starting material used for the synthesis of valsartan, namely (R)-valinebenzyl ester tosylate. Baseline resolution of the enantiomers of valinebenzyl ester tosylate could be achieved on the chiral crown ether column Crownpak CR (Daicel) at 50°C using water–methanol–trifluoroacetic acid (850:150:1, v/v) as a mobile phase. The linearity was established in the range 0.5–5% ($r>0.999$). The accuracy of the method was found to be 100.5% (average). For the precision (repeatability), a relative standard deviation value of 3.4% was found. Both methods were found to be suitable for the analysis of the respective analytes.

Keywords: Enantiomer separation; Valsartan; Valinebenzyl ester tosylate

1. Introduction

The objective of validation of an analytical test method is to demonstrate that it is suitable for the intended analytical application. Complete documentation of the validation of an analytical test method is an integral part of any documentation submitted to regulatory agencies. For chiral drugs, enantiomeric mixtures do not escape this rule. The development of new chiral drugs implies the quantitation and the

validation of analytical assays allowing the determination of the enantiomeric composition of such drugs, the undesired enantiomer being considered as an impurity. In this context, the development of specific enantio-selective assays has become essential for the development of new chiral drugs. Some reports discussing specifically these aspects in relation to chiral separations either by HPLC [1–3] or electrophoresis [4–7] have been published. Separation of enantiomers can be achieved by indirect separation in an achiral environment after derivatization with a chiral reagent [8] but it is now preferably
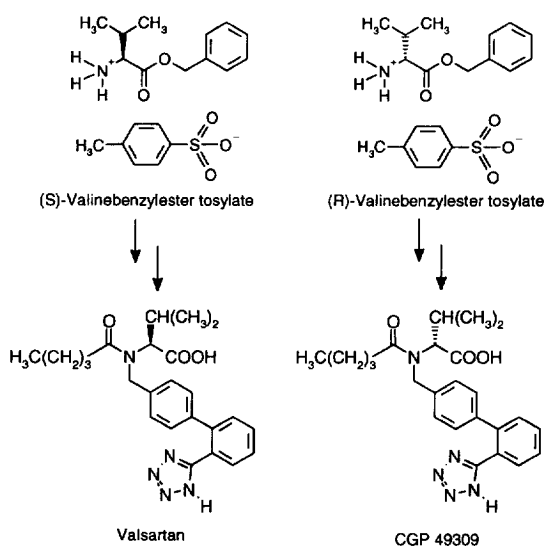
*Corresponding author.

Fig. 1. Structure of (*R*)- and (*S*)-valinebenzyl ester, and of valsartan and its enantiomer CGP 49309.

performed by direct separation using the chiral stationary phase for GC or HPLC [9–11], or employing a chiral selector in the buffer solution for capillary electrophoresis [12–14]. The purpose of this report is to assess the suitability of the method developed for the quantitative determination by chiral HPLC of the optical purity of valsartan and its valinebenzyl ester tosylate precursor in terms of the limit of detection and quantitation, linearity, repeatability, precision, accuracy, range and stability of the solutions. Valsartan is a new angiotensin II antagonist that is used for the treatment of hypertension [15]. It contains one asymmetric center and is thus chiral (Fig. 1). The active enantiomer has the *S* configuration. The starting material used for the synthesis of valsartan is the tosylate of (*S*)-valinebenzyl ester (Fig. 1).

2. Experimental

2.1. Materials and reagents

Valsartan and its enantiomer CGP 49309 were from Ciba (Basel, Switzerland). The phosphate buffer solution (pH 7.0) was prepared by dissolution of 0.041 mol/l of disodium hydrogen phosphate and 0.028 mol/l of potassium dihydrogen phosphate.

The analytical column Chiral AGP (100×4 mm I.D.) was obtained from ChromTech AB (Hägersten, Sweden) and the Crownpak CR (150×4.6 mm I.D.) column was purchased from Daicel Chemical Industries (Tokyo, Japan).

2.2. Preparation of the solutions 1–6 of valsartan

About 75 mg of valsartan was accurately weighed and dissolved with amounts of CGP 49309 between 0.08 mg and 3 mg in 100 ml mobile phase. The solutions were diluted with mobile phase in the ratio 1:5; 10 μl were injected.

2.3. Preparation of the solutions 1–6 of valinebenzyl ester

About 20 mg of (*S*)-valinebenzyl ester tosylate was accurately weighed and dissolved with amounts of (*R*)-valinebenzyl ester tosylate between 0.1 mg and 1 mg in 50 ml mobile phase; 10 μl sample was injected.

2.4. Instrumentation

The LC system consisted of a Spectra-Physics pump SP 8800, an autosampler SP 8875, and a Focus detector (Spectra-Physics). Data acquisition and treatment was performed with an IBM computer PS/2 using the PC1000 software.

3. Results and discussion

In this section, the different steps of validation of the method developed for the determination of the enantiomeric composition of valsartan and of one of its synthetic precursors, valinebenzyl ester, are described. For the validation of a method, typical analytical parameters have to be considered. These parameters are reviewed below, and to facilitate the comprehension for the readers who are not familiar with these analytical aspects, their definition is shortly given in accordance with the definitions available from the United States Pharmacopoeia [16].

3.1. Test method

Obviously, the first step consists of elaborating an analytical procedure capable of separating the enantiomers of the considered racemate. This test method will constitute the basis for the method validation.

Separation of the enantiomers of valinebenzyl ester tosylate could be achieved on the crown ether-based chiral stationary phase Crownpak CR (Daicel) using a mixture of water–methanol–trifluoroacetic acid (850:150:1, v/v) at 50°C and at a flow-rate of 1.0 ml/min. UV detection was performed at 210 nm. (R)-Enantiomer elutes after 19.0 min while the (S)-enantiomer elutes after 22.5 min (separation factor $\alpha = 1.5$). For the separation of valsartan and its enantiomers, CGP 49309, the protein-based Chiral AGP (ChromTech) column, was used with a phosphate buffer pH 7.0 containing 2% (volume) of 2-propanol as a mobile phase at room temperature and at a flow-rate of 0.8 ml/min. UV detection was performed at 227 nm. CGP 49309 elutes after 6 min, while valsartan elutes after 10 min (separation factor $\alpha = 1.82$).

3.2. Limit of detection and limit of quantitation

The limit of detection is the lowest concentration of analyte that can be detected in a sample. It is usually expressed as the concentration of analyte in the sample. The limit of quantitation is the lowest concentration of analyte that can be determined with acceptable precision and accuracy under the stated experimental conditions. It is also expressed as the concentration of analyte in the sample [16].

For the determination of the limit of detection and of quantitation of CGP 49309 in valsartan, a series of diluted CGP 49309 samples was analyzed. From this series the peak is selected whose height h_s is about 2–10 times larger than the signal-to-noise h_n, where the following definitions apply: h_n is the largest deviation of the detector signal from the average baseline level measured in mm over a period of more or less 10 peak widths from the retention time of the analyte (Fig. 2); h_s is the peak height of the analyte measured from the average baseline level to the top of the peak measured in mm (Fig. 2). The limit of
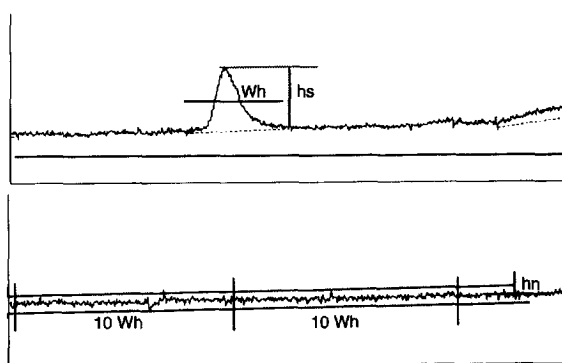

Fig. 2. Graphic representation of the parameters used for the determination of LOD and LOQ.

detection (LOD) and the limit of quantitation (LOQ) are defined as follows [17,18]:

$$LOD = \frac{C_s \times 2h_n}{h_s} \qquad (1)$$

$$LOQ = \frac{C_s \times 6h_n}{h_s} \qquad (2)$$

where C_s is the amount of analyte injected.

Fig. 3 shows the analysis of a sample of CGP 49309 used for the determination of the LOD and LOQ. By comparison of the response with the baseline noise, the limit of quantitation was estimated at 1.7 ng corresponding to 0.1% referred to valsartan. The limit of detection was 0.6 ng and corresponds to 0.04%.

For the enantiomer (R)-valinebenzyl ester tosylate, a limit of detection of 0.5% has been determined.

Fig. 4 shows a chromatogram of (S)-valinebenzyl ester tosylate spiked with 0.5% of its (R) enantiomer.

3.3. Linearity

The linearity of a test method is its ability to produce test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range [16].

Fig. 5 shows a chromatogram of valsartan spiked with 0.1% of its enantiomer CGP 49309.

It is essential that the detector response demonstrates acceptable linearity over a wide range of enantiomeric compositions for both sample concen-
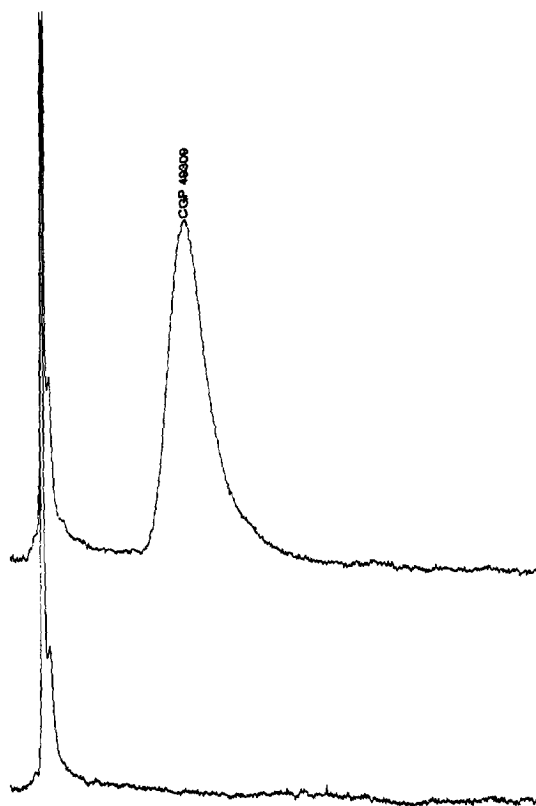
Fig. 3. Determination of LOD and LOQ for CGP 49309 (enantiomer of valsartan).

Fig. 4. Chromatogram of (S)-valinebenzyl ester tosylate spiked with 0.5% of its (R) enantiomer. Separation conditions: see Experimental.

tration and enantiomeric ratios. Linearity was assessed by analysing six mixtures of valsartan (solutions 1–6, Table 1) spiked with various amounts of its enantiomer CGP 49309 in the range 0.1–4%. The response was found to be directly proportional to the concentration in the range 6–60 ng of injected CGP 49309. The data were subjected to linear regression analysis. Linearity was assessed by the statistical data obtained and the graph is presented in Fig. 6 (correlation coefficient 0.9998; slope 52.55 ± 1.65; regression line, $y = 52.5x - 42.5$).

For the enantiomers of valinebenzyl ester tosylate a good linearity was also observed with a correlation coefficient of 0.99902 (Fig. 7). This linearity was assessed by analysing six mixtures of (S)-valinebenzyl ester (solutions 1–6, Table 2) spiked with various amounts of (R)-valinebenzyl ester (slope
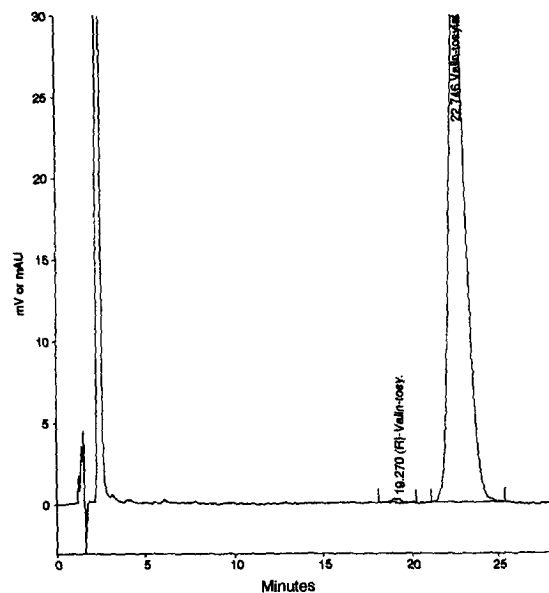
110723 ± 2454; regression line, $y = 110723.3x - 882.6$).

3.4. Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy is usually expressed as percent recovery by the assay of known, added amounts of analyte [16].

The accuracy was determined in terms of recovery using six mixtures of valsartan (solutions 1–6, Table 1) spiked with various amounts of its enantiomer CGP 49309 in the range 0.1–4%. It was obtained by comparing the average peak height of the six solutions with the average peak height of a standard solution. The average recovery was determined as 96.7% with a relative standard deviation (R.S.D.) of 2.8%, which is quite satisfactory. The linearity plot shown in Fig. 6 confirms recovery, as each point was obtained by spiking a solution of valsartan with a known amount of its enantiomer CGP 49309.

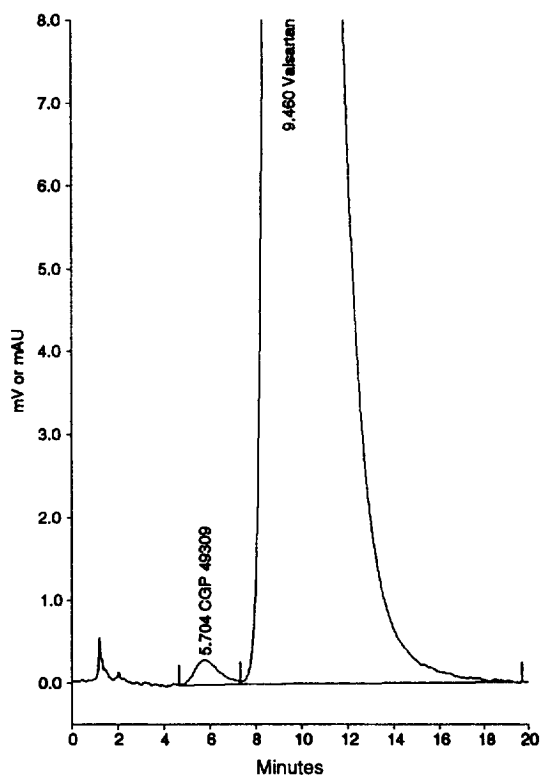For the method developed to analyze valinebenzyl

Fig. 5. Chromatogram of valsartan spiked with 0.1% of its enantiomer CGP 49309. Separation conditions: see Experimental.
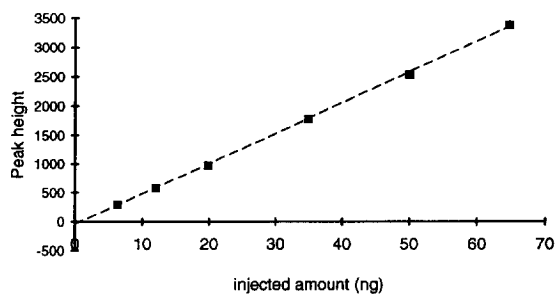

Fig. 6. Linearity response for peak area versus spiking levels of CGP 49309 into a solution of the valsartan. Separation conditions: see Experimental.
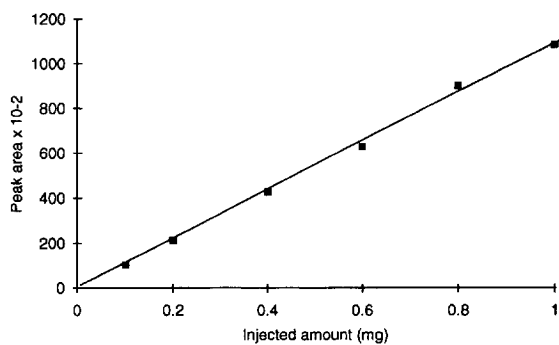

Fig. 7. Linearity response for peak area versus spiking levels of (R)-valinebenzyl ester tosylate into a solution of (S)-valinebenzyl ester tosylate. Separation conditions: see Experimental.

Table 1
Solutions and measured response for each individual concentration of CGP 49309

Solution no.	Weight[a] (mg)	Injected amount (ng)	Average peak height	Found (mg)	Recovery (%)
Used for	LAP	LAP	LAP	A	A
1	0.310	6.20	302	0.290	93.5
2	0.597	11.94	587	0.563	94.4
3	0.986	19.72	981	0.942	95.5
4	1.739	34.78	1785	1.713	98.5
5	2.497	49.94	2539	2.437	97.6
6	3.232	64.64	3389	3.253	100.7
7[b]	1.507	30.14	1570		

[a] Sum of added CGP 49309 and CGP 49309 contained in valsartan (0.3%).
[b] Standard solution of CGP 49309.
L: data used for the determination of linearity.
A: data used for the determination of accuracy.
P: data used for the determination of precision.

Table 2
Solutions and measured response for each individual concentration of (R)-valinebenzyl ester tosylate

Solution no	Weight (mg)	Average peak area	Peak area relative to 0.668 mg	Found (mg)	Recovery (%)
Used for	LAP	LAP	P	A	A
1	0.0998	10508	70288	0.1029	103.08
2	0.1995	21573	72187	0.2028	101.66
3	0.3990	42918	71806	0.3956	99.14
4	0.5985	63038	70312	0.5773	96.46
5	0.7980	90306	75545	0.8236	103.20
6	0.9975	108751	72780	0.9902	99.26

L: data used for the determination of linearity.
A: data used for the determination of accuracy.
P: data used for the determination of precision.

ester, the R.S.D. obtained by testing solutions of (S)-valinebenzyl ester tosylate spiked with various amounts of its (R) enantiomer was 2.63% and a recovery of 100.5% was determined.

3.5. Precision (repeatability)

The precision (repeatability) of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample or to artificially prepared samples (same laboratory, same analyst, same instruments, short intervals of time). The precision is usually expressed as the standard deviation or relative standard deviation [16].

Precision (repeatability) was determined by using six mixtures of valsartan (solutions 1–6, Table 1) spiked with various amounts of its enantiomer CGP 49309 in the range 0.1–4%. It was determined by calculating the relative standard deviation (R.S.D.) of the average heights obtained for the six solutions after conversion of these heights into average height per ng injected (Table 1). The R.S.D. value was found to be 2.4%.

For valinebenzyl ester tosylate the R.S.D. value determined from the analysis of six solutions of (S)-valinebenzyl ester tosylate spiked with various amounts of its (R)-enantiomer was 3.4%.

3.6. Precision (reproducibility)

The precision (reproducibility) of an analytical method is the degree of agreement of test results obtained by the analysis of the same sample under various test conditions (different laboratories, different analysts, different instruments). The precision is usually expressed as the lack of influence on test results of operational and environmental variables or as standard deviation or relative standard deviation [16].

For valsartan, precision (reproducibility) was determined by analysing samples of five different batches of the drug for the content of its enantiomer CGP 49309 in two laboratories. The data show very good agreement.

3.7. Range

The range of a test method is the interval between the upper and lower levels of analyte that have been demonstrated to be determinable with acceptable precision, accuracy and linearity using the method as described [16].

According to this definition, we demonstrated that the method developed for valsartan can be considered as suitable for the determination of CGP 49309 in the concentration range 0.1–4.0%.

3.8. Specificity

The specificity of an analytical method is its ability to measure accurately and specifically the presence of components that may be expected to be in the sample or the presence of ingredients other than the analyte, whether pharmaceutically active or inert. Specificity can be assessed by providing data which demonstrate the ability of a test method to

differentiate between the analyte and the other components [16].

In the case of valsartan and of valinebenzyl ester, as the expected contaminant is the respective enantiomer, the method must be stereospecific. The specificity was determined by comparing test results from the analysis of solutions containing the active substance valsartan and its enantiomer CGP 49309 with those obtained from the analysis of a solution containing CGP 49309 only. Fig. 5 shows the chromatograms of a solution of unspiked valsartan and of valsartan spiked with 0.1% of CGP 49309. The method allows the two enantiomeric compounds to be separated and thus establishes that it is suitable or specific for the desired separation.

Analogously, it has been established that the method demonstrates specificity for the enantiomers of valinebenzyl ester tosylate which are baseline separated on the chiral column Crownpak CR (Fig. 4).

3.9. Stability of the solution

The stability of the solution has been demonstrated by analysing solutions of CGP 49309 after 16 h storage. The solutions did not undergo any decomposition within 16 h if stored at room temperature; the method is thus suitable for automation.

4. Conclusion

Efficient and reliable HPLC methods for the stereoselective analysis and quantitation of valsartan and its enantiomer CGP 49309, and for the enantiomers of valinebenzyl ester, were developed. The methods were validated to meet the pharmaceutical industry requirements in terms of limit of detection and quantitation, linearity, repeatability, precision, accuracy and range.

References

[1] W. Naidong and J.W. Lee, J. Pharm. Biochem. Anal., 11 (1993) 785.
[2] W. Naidong, J.W. Lee and J.D. Hulse, J. Liq. Chromatogr., 17 (1994) 3747.
[3] W. Naidong and J.W. Lee, J. Pharm. Biochem. Anal., 12 (1994) 551.
[4] K.D. Altria, R.C. Harden, M. Hart, J. Hevizi, P.A. Hailey, J.V. Makwana and M.J. Portsmouth, J. Chromatogr., 641 (1993) 147.
[5] K.D. Altria, A.R. Walsh and N.W. Smith, J. Chromatogr., 645 (1993) 193.
[6] K.D. Altria, D.M. Goodall and M.M. Rogan, Electrophoresis, 15 (1994) 824.
[7] J.E. Noroski, D.J. Mayo and M. Moran, J. Pharm. Biochem. Anal., 13 (1995) 45.
[8] J. Gal, in I.W. Wainer (Editor), Drug Stereochemistry, Analytical Methods and Pharmacology, Marcel Dekker, New York, Basel, 2nd ed., 1993, Ch. 4.
[9] A.M. Krstulovic (Editor), Chiral separations by HPLC, Application to pharmaceutical compounds, Ellis Horwood, Chichester, 1989.
[10] S. Ahuja (Editor), Chiral Separations by Liquid Chromatography, ACS Symposium Series, Vol. 471, Washington, 1991.
[11] G. Subramanian (Editor), A Practical Approach to Chiral Separations by Liquid Chromatography, VCH Publishers, New York, 1994.
[12] S. Terabe, K. Otsuka and H. Nishi, J. Chromatogr. A, 666 (1994) 295.
[13] H. Nishi and S. Terabe, J. Chromatogr. A, 694 (1994) 245.
[14] E. Francotte, S. Cherkaoui and M. Faupel, Chirality, 5 (1993) 516.
[15] P. Buehlmayer, P. Furet, L. Criscione, M. de Gasparo, S. Whitebread, T. Schmidlin and R. Lattmann, J. Wood, Bioorg. Med. Chem. Lett., 4 (1994) 29.
[16] United States Pharmacopeia XXIII, p. 1982.
[17] J.P. Foley and J.G. Dorsey, Chromatographia, 18 (1984) 503.
[18] J.E. Knoll, J. Chromatogr. Sci., 23 (1985) 422.