Journal of Chromatography, 507 (1990) 165–170
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 1770

High-performance liquid chromatography of the drug fosinopril

JOEL KIRSCHBAUM*, JOYCE NOROSKI*, ANNETRIC COSEY, DON MAYO and JOHN ADAMOVICS[a]

Squibb Institute for Medical Research, One Squibb Drive, P.O. Box 191, New Brunswick, NJ 08903-0191 (U.S.A.)

SUMMARY

High-performance liquid chromatography was used to determine the purity and impurities of fosinopril, an angiotensin-converting enzyme inhibitor used to treat hypertension. Purity values are determined using a silica column and usually are above 99%. All known possible impurities, including stereoisomeric impurities, can be resolved and quantified by injecting solutions of fosinopril onto three separate columns; silica, strong anion exchange and phenyl. Typical impurity contents total 0.5%. Validation data and a study of properties of fosinopril in solution is included.

INTRODUCTION

High-performance liquid chromatography (HPLC) methods were developed for the separation of the angiotensin-converting enzyme inhibitor[1] fosinopril, (compound I, Fig. 1), from its precursors and hypothetical impurities. Fosinopril[2] is a prodrug. The active agent is compound II[3], which is formed *in vivo*. HPLC was chosen because of its prior, successful application to the selective assays of this new class of antihypertensive agents[4,5]. Although most of the possible impurities were separated from the analyte on a silica column, a second separation on a strong anion-exchange (SAX) column was necessary to resolve two possible, stereoisomeric impurities; the *R,R,S,S* and *S,R,S,R* enantiomers. A third system was needed to both resolve a phenyl impurity (IV) and to achieve a low limit of detection of compound V (Fig. 1), another possible impurity.

In this paper, the details of the separations are described.

EXPERIMENTAL

Materials

Fosinopril sodium and related compounds were synthesized at the Squibb In-

[a] Present address: Cytogen, Princeton, NJ 08540, U.S.A.

0021-9673/90/$03.50

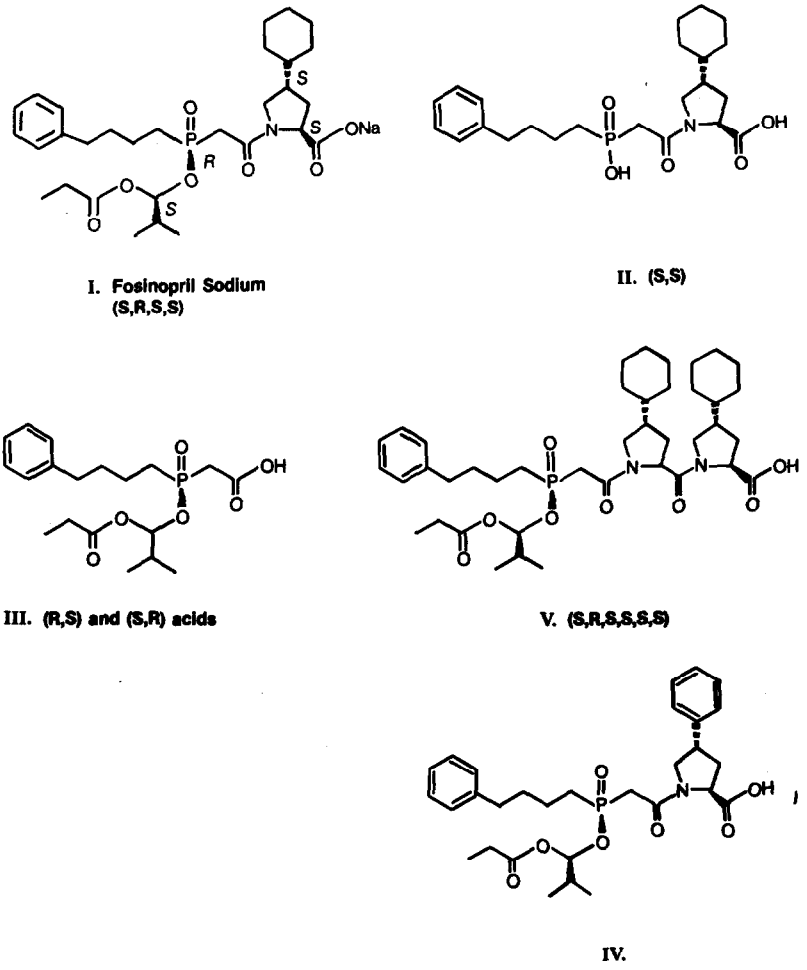

Fig. 1. Structures of fosinopril and its possible, related impurities.

stitute for Medical Research. All solvents were of HPLC grade (American Scientific Products, Edison, NJ, U.S.A.).

The modular HPLC apparatus consisted of a Perkin-Elmer Model 420B, LC-600 or ISS-100 autoinjector (Perkin-Elmer, Norwalk, CT, U.S.A.), a Beckman Model 110B pump (Beckman Instruments, Fullerton, CA, U.S.A.), a SYS-TEC column temperature control system (SYS-TEC, Minneapolis, MN, U.S.A.) and a Kratos-A.B.I. variable-wavelength detector Model 783 (Applied Biosystems, Ramsey Analytical Division, Ramsey, NJ, U.S.A.). Signals were processed with the aid of a Hewlett-Packard laboratory computer, Model 3357 (Hewlett-Packard, Palo Alto, CA, U.S.A.) and monitored using a Kipp & Zonen two-pen recorder, Model BD-41 (Rainen Instrument, Woburn, MA, U.S.A.).

Methods

The silica column separation utilized a Water RESOLVE column, (15 cm × 3.9 mm I.D., 5 μm, Waters, Milford, MA, U.S.A.) thermostated at 32°C, a mobile phase of acetonitrile–water–ortho-phosphoric acid (4000:15:2) with a flow-rate of 1 ml/min and detection at 205 nm. Prior to use, the column was conditioned with about 100 ml of methanol. The SAX separation utilized a Whatman PartiSphere packing (5 μm) in a 25 cm × 4.6 mm I.D. column (Whatman, Clifton, NJ, U.S.A.) thermostated at 40°C, and the same mobile phase (flow-rate, 0.8–1.0 ml/min) and wavelength as the silica assay. The third separation system consisted of an alkyl phenyl column, 5 μm (ES Industries, Marlton, NJ, U.S.A.), a mobile phase of acetonitrile–0.2% aqueous phosphoric acid (80:20) with a flow-rate of 0.5 ml/min and a detector set to 205 nm. The column temperature was 45°C. All three columns were protected by saturator columns packed with 37-μm silica located between the pump and the autoinjector. All injection volumes were 20 μl (nominal). Best separations were found using the given column temperatures.

RESULTS AND DISCUSSION

Fig. 2 is a chromatogram, obtained using a silica column, of fosinopril (0.1 mg/ml mobile phase) and added potential impurities. Because of the lack of separation between fosinopril and the possible *R,R,S,S* impurity, a second, SAX system was used to resolve these compounds, as shown in Fig. 3. (Structures of compounds III, IV and V, are depicted in Fig. 1.) Fig. 3 shows resolution of the fosinopril (0.1 mg/ml mobile phase) from added *R,R,S,S* and *S,R,S,R* possible stereoisomeric impurities using the strong anion-exchange column. The phenyl column separation of fosinopril sodium (0.1 mg/ml mobile phase) from the possible phenyl (IV) and double-proline (V) impurities is depicted in Fig. 4.

Assay validation involved repetitive injections of solutions of 0.1 mg/ml fosinopril in mobile phase. The relative standard deviations of six sets of injections, on two days using two chromatographic systems, were 0.8% and 0.5% using peak areas.

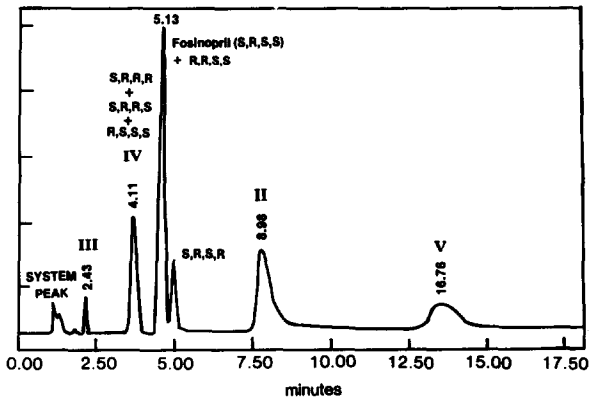

Fig. 2. Silica column chromatogram of fosinopril and added possible impurities. The mobile phase consists of acetonitrile–water–ortho-phosphoric acid (4000:15:2) with a flow-rate of 1 ml/min and detection at 205 nm. The column temperature is 32°C.

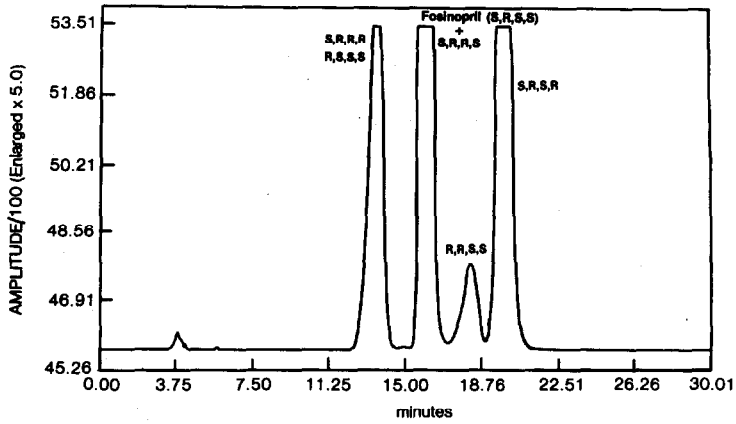

Fig. 3. Strong anion-exchange (SAX) HPLC for the separation of fosinopril from two possible stereoisomers added to the analyte. The SAX used the same mobile phase and flow-rate as the silica column. The column temperature is 40°C.

Chromatography, on different days, of three sets of solutions of fosinopril from 0.02 to 0.2 mg/ml gave linear responses (all correlation coefficients >0.9999), that, when plotted and extrapolated, showed that the lines passed through the origin.

A typical batch of the prodrug contains about 0.1% of impurity III, 0.2–0.3% of the stereoisomer triplet consisting of *S,R,R,R*, *R,S,S,S* and *S,R,R,S*, and about 0.1% of the *S,R,S,R* stereoisomer. None or trace quantities of the other possible impurities, including the active drug and the hydrolysis product (compound II), were seen. The HPLC systems have limits of detection of about 0.02 to 0.04% (three times the signal-to-noise ratio) and minimum quantifiable concentrations of about 0.1% (relative to the concentration of fosinopril) under the conditions described in the experimental section. Accuracy of the silica method was verified by chromatograph-

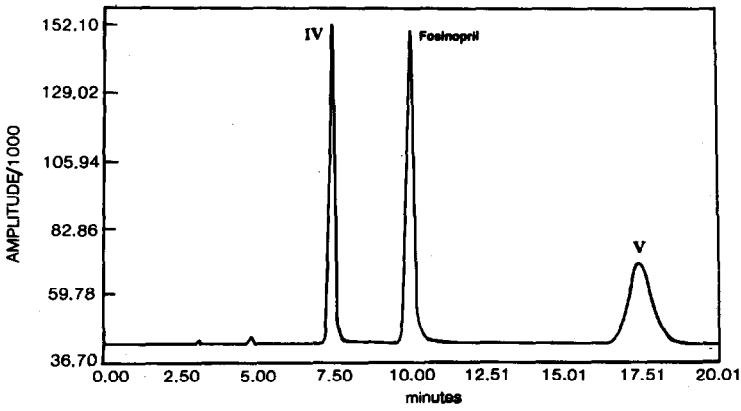

Fig. 4. Phenyl column chromatogram showing the resolution of fosinopril from added quantities of the possible phenyl (IV) and double-proline (V) impurities. The mobile phase consists of acetonitrile–0.2% aqueous phosphoric acid (80:20) with a flow-rate of 0.5 ml/min. Detection was at 205 nm and the column temperature was 45°C.

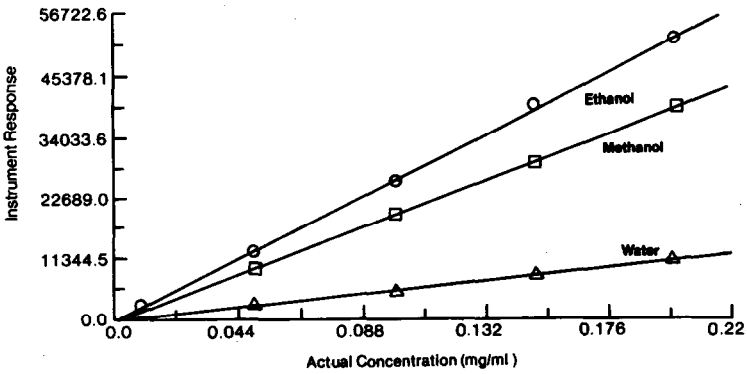

Fig. 5. Dependence of instrument response (peak area) on injection solvent. Linearity plots of various concentrations of fosinopril dissolved in either methanol, ethanol or water and injected into an identical HPLC system using the silica column. All injection volumes were 20 μl (nominal) and the chromatographic conditions were identical to those described in the text and for Fig. 2. The only variation was the solvent used to dissolve the fosinopril.

ing a solution of 0.00500 mg/ml of compound II and 0.09500 mg/ml fosinopril. After assay, 0.00503 mg/ml of compound II was found (n = 7, relative standard deviation = 2.7%) and 0.09504 mg/ml of fosinopril (n = 7, relative standard deviation = 0.4%). This also indicates no hydrolysis product to be present.

The same weight of fosinopril sodium dissolved in various quantities of either water, methanol or ethanol, and injected into an identical LC system with respect to autoinjector, pump, mobile phase, flow-rate, detector, wavelength and data reduction system, gave different peak responses using the silica column. Fig. 5 shows linearity plots for fosinopril in water, methanol and ethanol of concentration *vs.* instrument response (peak area). The correlation coefficients were all <0.9999. The hydrolysis product, compound II, also exhibited this property of peak area being dependent on injection solvent. Void volume effects were not responsible since the retention time of fosinopril was 5 min and that of its hydrolysis product was about 9 min. Injection of the solvents gave a straight baseline, with no negative or positive peaks visible. This phenomenon of the peak response being dependent on the injection solvent has been discussed previously for captopril[6,7] vancomycin[8], vinblastine[8], aztreonam[7] and several other drugs[7]. Since the solvents and fosinopril are over 99.5% pure, the dependence of peak area on injection solvent used to dissolve the fosinopril appears to be a chromatographic phenomenon.

In summary, HPLC systems are presented for quantifying the angiotensin-converting enzyme inhibitor, fosinopril, and its possible isomeric and non-isomeric impurities.

ACKNOWLEDGEMENTS

The authors thank Drs. Berry Kline and Glenn Brewer for their helpful comments and thoughtful criticism. We are grateful to Drs. John Grosso and William Winter for supplying many of the compounds used in these studies, to Mrs. Maria Berrios and Ms. Kim Shields who assisted in this work, and to Mrs. Nancy Garside

Thompson who obtained independent evidence for the varying peak responses of the analyte in various solvents.

REFERENCES

1 Z. P. Horovitz (Editor), *Angiotensin Converting Enzyme Inhibitors*, Urban & Schwarzenberg, Baltimore-Munich, 1981.
2 J. Krapcho, C. Turk, D. W. Cushman, J. R. Powell, J. M. DeForrest, E. R. Spitzmiller, D. S. Karanewsky, M. Duggan, G. Rovnyak, J. Schwartz, S. Natarajan, J. D. Godfrey, D. E. Ryono, R. Neubeck, K. S. Atwa and E. W. Petrillo, Jr., *J. Med. Chem.*, 31 (1988) 1148.
3 M. A. Ondetti, *Circulation*, 77 (1988) 174.
4 S. Perlman and J. Kirschbaum, *J. Chromatogr.*, 206 (1981) 311.
5 J. Kirschbaum and S. Perlman, *J. Pharm. Sci.*, 73 (1984) 686.
6 S. Perlman and J. Kirschbaum, *J. Chromatogr.*, 357 (1986) 39.
7 J. Kirschbaum, *J. Pharm. Biomed. Anal.*, 7 (1989) 813.
8 E. L. Inman, A. M. Maloney and E. C. Rickard, *J. Chromatogr.*, 465 (1989) 201.