Journal of Pharmaceutical & Biomedical Analysis
Vol. 6, No. 1, pp. 115–119, 1988
Printed in Great Britain

0731–7085/88 $3.00 + 0.00

Short Communication

Assay of nifedipine and its by- and degradation products in the drug substance and dragees by liquid chromatography on formamide-saturated silica gel columns

H. PÖTTER and M. HÜLM

Main Department of Natural Products Research, Managerial Department of Research and Development, VEB Arzneimittelwerk Dresden, Main Factory of the VEB Pharmazeutisches Kombinat GERMED Dresden, Wilhelm-Pieck-Str. 35, DDR-8122 Radebeul

Keywords: *Nifedipine, nifedipine-related compounds, liquid chromatography, formamide-saturated columns.*

Introduction

During the synthesis of nifedipine, an anti-anginal vasodilating agent (I), three main impurities are formed as shown in Fig. 1. Impurities II and III are degradation products of I formed by a light-catalyzed intramolecular reaction and oxidation by atmospheric oxygen [1–2] and impurity IV is a by-product of the synthesis of nefidipine.

In order to determine the quality of nifedipine and Corinfar® dragees it was necessary to develop a method for the assay of nifedipine as well as the by-product IV and the impurities II and III. Gas [1, 3–11] and liquid chromatographic methods [12–14] for the assay of nifedipine in body fluids allow the simultaneous determination of the nitroso (II) and nitro (III) compounds, but not the tetrahydropyrimidine compound IV. Attempts to separate compound IV from nifedipine using published liquid chromatographic methods [12–14, 16] showed that there was no difference in the retention times of compound IV and nifedipine (Pötter and Hülm, unpublished results).

The object of the present work was to separate nifedipine and its impurities on a formamide-saturated silica gel column using the method of Pötter *et al.* [15].

Experimental

Apparatus

The liquid chromatograph comprised an Altex Pump (LC-XPO), a Pye Unicam AR 55 linear recorder, a Pye Unicam DP 88 computing integrator, a Rheodyne syringe loading

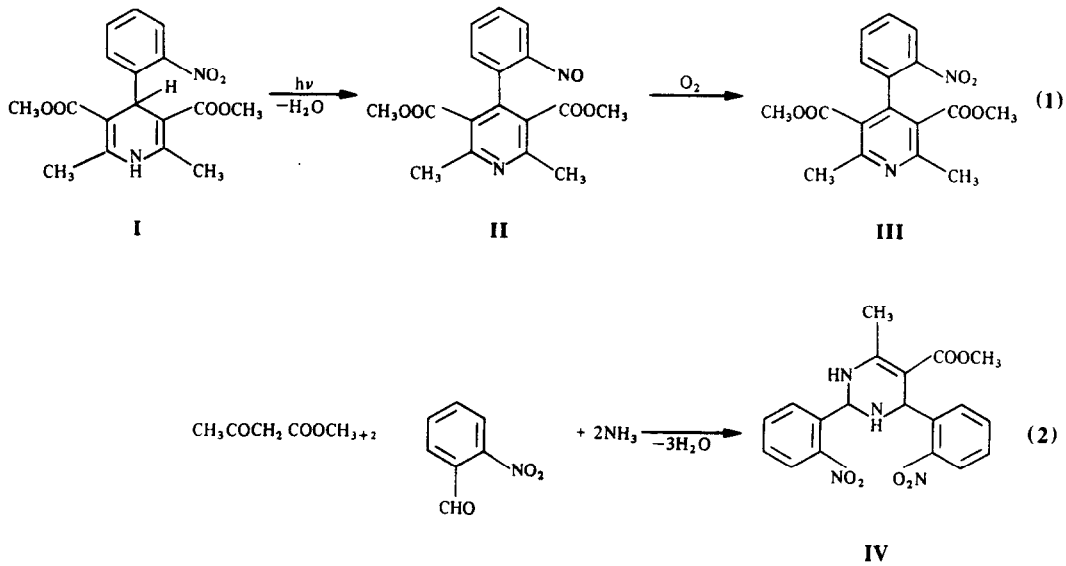

Figure 1

sample injector, Model 7120 (20 μl loop), and a Pye Unicam PU 4020 UV detector. The detector response for the assay of nifedipine was 0.32 a.u.f.s. and for the determination of small amounts of II, III and IV 0.04 a.u.f.s. Detection was carried out at 237 nm.

Separation was performed on a column containing Li-Chrosorb Si 60 (100 × 4.6 mm i.d.) and precolumn (30 × 4.6 mm i.d.) (Merck; particle size 10 μm). The formamide breakthrough was measured by means of a UV spectrophotometer (type VSU_2; VEB Carl Zeiss, Jena).

Chemicals and reagents

Nifedipine, the nitroso compound (II), the nitro compound (III), the tetrahydropyrimidine compound (IV) and hexobarbitone were prepared by VEB Pharmazeutisches Kombinat GERMED Dresden.

The mobile phase comprised *n*-hexane (spectroscopically pure for UV purposes, VEB Petrolchemisches Kombinat Schwedt): tetrahydrofuran (reagent grade, Merck; purified on an alumina column before use): dichloromethane (reagent grade, Merck): formamide (reagent grade, Merck): ammonia (reagent grade, 24%, Merck) in the proportions by volume 400 : 100 : 20 : 1 : 1. The mobile phase was mixed and vigorously shaken for 2 min in a separating funnel. The formamide that separates on standing was removed. For the preparation of solutions for the calibration series, nifedipine, compounds II, III and IV and hexobarbitone (the internal standard) were dissolved in tetrahydrofuran : *n*-hexane (1 : 1) and diluted to give a series of concentrations of the analytes ranging from 40 to 200 μg ml^{-1} and 800 μg ml^{-1} of internal standard. For the determination of nifedipine, 10 mg of nifedipine and 30 mg of hexobarbitone, accurately weighed, were dissolved in 50.0 ml of tetrahydrofuran : *n*-hexane (1 : 1).

For the determination of the by- and degradation products, 250 mg of nifedipine and 2 mg of hexobarbitone, accurately weighed, were dissolved in 50.0 ml of tetrahydrofuran : *n*-hexane (1 : 1). For the determination of nifedipine content of dragees (labelled

content 10 mg), an amount corresponding to the average weight of a dragee, finely powdered, was weighed accurately. A solution containing 30 mg of hexobarbitone in 10.0 ml of dichloromethane was added and the mixture was extracted in an ultrasonic bath for 5 min and then filtered by means of a G4 suction filter. The filtrate was then diluted to 50 ml with tetrahydrofuran : *n*-hexane (1 : 1). For the determination of the by-products, a quantity of powder equivalent to two dragees was extracted with 10.0 ml of a solution containing 10 mg of hexobarbitone in 250.0 ml of dichloromethane.

Impregnation of the column

The silica gel column was impregnated with formamide by pumping the formamide-saturated mobile phase through the column for 180 min at a flow rate of 2 ml min^{-1}. The saturation time was determined by measuring the concentration of formamide in the eluate by means of a UV spectrophotometer at 250 nm. The work was carried out at room temperature (20 ± 2°C) and in the complete absence of light.

Results and Discussion

At the beginning of the impregnation, most of the formamide was adsorbed by the silica gel, but after 3 h, an equilibrium between the mobile and stationary phases was achieved, provided the mobile phase was saturated with formamide (Fig. 2). Nifedipine and the related compounds were separated with satisfactory efficiency (Fig. 3). The calibration curves for each of the substances separated (I to IV) were linear in the range 0.8–4.0 μg 20 μl with correlation coefficients of +0.99(I), +0.98(II), +0.99(III) and 0.97(IV).

The limits of detection were found to be 8 ng/20 μl for the nitroso compound II and the nitro compound III and 17 ng/20 μl for the tetrahydropyrimidine compound IV. Thus, approximately 0.01% of the degradation products II and III and 0.02% of the by-product IV can be detected in a loading of nifedipine of 100 μg/20 μl. The column was found to give reproducible retention times for the four compounds for 30 days (Table 1). To assess the method, the assay of nifedipine was carried out nine times in a sample of the drug substance and eight times in a sample of Corinfar® dragees. The results in Table 2 show that the nifedipine was assayed with satisfactory accuracy and precision. The

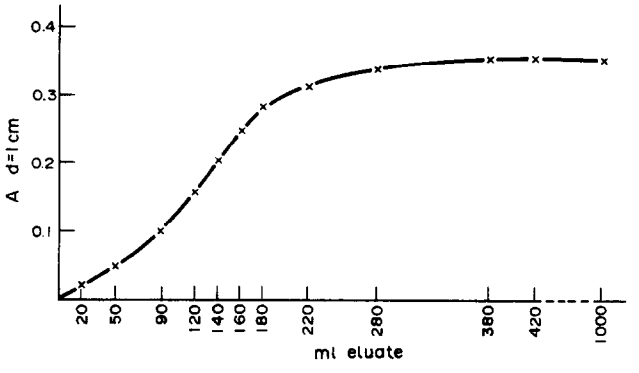

Figure 2
Saturation with formamide of a Li-Chrosorb-Si 60 column as measured in the eluate fractions (10 ml each) at 250 nm.

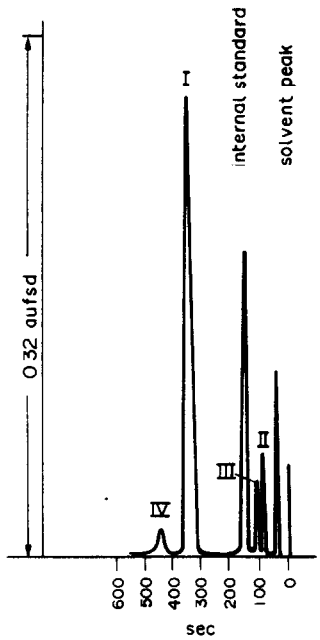

Figure 3
Chromatogram of I = nifedipine, II = nitroso compound, III = nitro compound and IV tetrahydropyrimidine compound. Internal standard: hexobarbitone, Detection: 237 nm.

Table 1
Reproducibility of the retention times of nifedipine and related compounds

	Retention time(s) Day 1	Day 5	Day 10	Day 15	Day 20	Day 30
Nitroso compound	96	94	97	95	96	98
Nitro compound	120	118	122	121	119	123
Hexobarbitone	168	167	170	168	167	172
Nifedipine	360	360	363	361	362	364
Tetrahydropyrimidine Compound	460	458	463	460	461	465

Table 2
Precision of the assay of nifedipine in the drug substance and in Corinfar® dragees

	Nifedipine substance %	Corinfar® dragees (mg of nifedipine/dragee)
	101	9,5
	99,7	9,6
	99,7	9,7
	99,9	9,9
	99,5	10,0
	99,5	9,7
	98,8	9,6
	98,4	9,5
	98,0	
n	9	8
$\bar{x}$	99,39	9,7
s	0,9	0,18
s %	0,9	1,9

mean value of 9.7 mg per dragee determined by the HPLC procedure was in good agreement with the value of 9.8 mg per dragee determined by a UV spectrophotometric procedure. No interference from excipients was observed.

The method is thus suitable for the assay of nifedipine in the pure substance and in dragees and for the detection of small amounts of by- and degradation products.

References

[1] R. Testa, E. Dolfind, C. Reschiotto, G. Secchi and P. A. Biondi, *SL Farmaco* **34**, 463–473 (1979).
[2] K. Thoma and R. Klimek, *Pharm. Ind.* **47**, 207–215 (1985).
[3] M. Eggstein and F. H. Kreutz, *Klin. Wochenschr.* **33**, 879–884 (1955).
[4] P. J. Geiger and S. P. Bessman, *Anal. Biochem.* **49**, 467–473 (1972).
[5] S. Higuchi and Y. Shiobara, *Biomed. Mass. Spectrom.* **5**, 220–223 (1978).
[6] P. Jakobsen, O. Lederballe Pedersen and E. Mikkelsen, *J. Chromatogr.* **162**, 81–87 (1979).
[7] S. Düpre and M. Aureli, *Anal. Biochem.* **105**, 97–100 (1980).
[8] S. Kondo, A. Kuchiki, K. Yamamoto, K. Akimoto, K. Takanashi, N. Awata and J. Sugimoto, *Chem. Pharm. Bull.* **28**, 1–7 (1980).
[9] M. T. Rosseel and M. G. Bogaert, *J. Chromatogr.* **279**, 675–680 (1983).
[10] S. R. Hamann and R. G. McAllister Jr., *Clin. Chem.* **29**, 158–160 (1983).
[11] G. Pabst, D. Lütz, K. H. Molz, W. Dahmen and H. Jaeger, Arzneim.-Forschung, *Drug Res.* **36**, 256–260 (1986).
[12] J. Dokladalova, J. A. Tykal, S. J. Coco, P. E. Durkee, G. T. Quercia and J. J. Korst, *J. Chromatogr.* **231**, 451–458 (1982).
[13] P. Pietta, A. Rava and P. Biondi, *J. Chromatogr.* **210**, 516–521 (1980).
[14] Katsumi Miyazaki, Naonori Kohri and Takaichi Arita, *J. Chromatogr.* **310**, 219–222 (1984).
[15] H. Pötter, M. Hülm and S. Schumann, *J. Chromatogr.* **319**, 440–445 (1985).
[16] United States Pharmacopoea XXI, 4. Suppl.

[Received for review 2 February 1987; revised manuscript received 26 June 1987]