Journal of Chromatography, 226 (1981) 423—430
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1015

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF PAPAVERINE IN WHOLE BLOOD

G. HOOGEWIJS, Y. MICHOTTE, J. LAMBRECHT and D.L. MASSART*

Farmaceutisch Instituut, Vrije Universiteit Brussel, Laarbeeklaan 103, B-1090 Brussels (Belgium)

(First received April 6th, 1981; revised manuscript received June 16th, 1981)

SUMMARY

The development and application of an assay method for papaverine in whole blood is reported. A single, simple extraction procedure at pH 10.0 using chloroform—*n*-hexane (2:3) as the solvent, results in pure extracts which can be chromatographed without further purification. Chromatography is performed on a nitrile-bonded phase, using *n*-hexane—dichloromethane—acetonitrile—propylamine (50:25:25:0.1) as mobile phase. This method is characterized by a between-day precision of 4% at the 200 ng/ml level and a detection limit of 5 ng/ml, and was successfully applied in a pharmacokinetic study.

INTRODUCTION

Papaverine is a smooth-muscle relaxant, mainly used as a peripheral vasodilator. In order to study the pharmacokinetics of a new sustained-release capsule, various published gas chromatographic (GC) and gas chromatographic—mass spectrometric (GC—MS) methods [1—4] were tested in our laboratory. At concentrations below 50 ng/ml several difficulties, mainly adsorption problems (on glassware, on the column and the GC—MS interface), were encountered. Additional attempts on various packed columns all resulted in non-zero intercepts of the calibration curves. It was therefore decided to use high-performance liquid chromatography (HPLC). When this research was started, no HPLC method for papaverine had been published, so we had to develop one. The chromatographic system used in this study was developed as one of a set of preferred HPLC systems for basic drugs, published in a previous paper from this laboratory [5]. In order to obtain statistically relevant pharmacokinetic curves, a great number of samples need to be estimated, hence necessitating a rapid and simple procedure. Since the conventional extraction

0378-4347/81/0000—0000/$02.50

procedures for papaverine are tedious and time-consuming, special attention has been paid to the extraction step.

EXPERIMENTAL

Reagents

All drugs were of pharmacopeial purity. Acetonitrile, hexane and dichloromethane were all liquid-chromatographic grade and purchased from Fluka (Buchs, Switzerland). Propylamine was synthesis grade and obtained from Merck-Schuchardt (Hohenbrunn, G.F.R.). All other reagents were analytical grade and purchased from E. Merck (Darmstadt, G.F.R.).

Apparatus

A Varian 5020 liquid chromatograph was used, equipped with a loop injector (sample loop 100 μl), a standard UV detector (254 nm), a Varian 9176 recorder and a Varian CDS 111 chromatographic data system.

Chromatography

Chromatography was performed on a Varian Micropak CN-10 column (particle size = 10 μm) (30 mm × 4 mm I.D.) at 30°C. The mobile phase consisted of *n*-hexane—dichloromethane—acetonitrile—propylamine (50:25:25:0.1). The flow-rate was 2 ml/min and the detector attenuation 0.01 or 0.02 a.u.f.s. The efficiency of the column was tested before use with a mixture of toluene and 2,6-dinitrobenzene as test samples and a mixture of hexane—ethyl acetate (99:1) as mobile phase. At a flow-rate of 1 ml/min, 2,6-dinitrobenzene eluted with a retention time of 16.1 min, giving about 10,000 theoretical plates and an asymmetry factor of 1.1. Almost daily injection of 25—40 extracts of blood samples during 4 months, caused no deterioration of the column.

Extraction procedure

To 4 ml of blood standard or sample, 100 μl of internal standard solution were added in silanized centrifuge tubes equipped with PTFE-covered screw-caps. After vortexing, 10 ml of phosphate buffer solution (μ = 0.4) were added. The mixture was homogenized by vortexing, and 5 ml of chloroform—hexane (2:3) were added. Partitioning was performed by gently shaking the tubes longitudinally in a shaking bath for 30 min. After centrifugation, 3 ml of the organic phase were transferred to a clean silanized vial with conical bottom, and evaporated to dryness under a gentle nitrogen stream at 45°C. Each extract was reconstituted just prior to chromatography with 250 μl of dichloromethane; 100 μl were injected.

Assay standards

Blood standards were prepared by spiking drug-free blood with papaverine · HCl solutions in double-distilled water, to give final concentrations of 10—600 ng/ml.

Internal standard solution

Each day a fresh solution containing 800 μg of mepyramine maleate per 100

ml of double-distilled water was prepared from a fresh stock solution.

Quantitation

The peak area ratio of papaverine to the internal standard was used for quantitation. The peak area ratios for the blood standards, which were taken through the entire assay procedure, were plotted against concentration to obtain standard calibration curves.

A new calibration curve was made with each sample set. Detector response was linear in the concentration range studied. The sequence of the samples was chosen at random and the analyses were performed blindfold. The concentration was determined from the standard curve.

RESULTS AND DISCUSSION

Extraction procedure

Most extraction procedures for papaverine are based on the method of Axelrod et al. [6]. As reported by Guttmann et al. [2], and also in our hands, the extracts yield various interfering peaks. Guttmann et al. [2] proposed an alternative procedure based upon ion-pair extraction of papaverine with di-(2-ethylhexyl)phosphoric acid as ion-pairing reagent. This ion-pairing and adduct-forming reagent has been studied systematically by us and has been found to be very useful for the extraction from aqueous solution of various basic drugs with very different structures [7]. However, when applied to biofluids a lot of endogenous compounds are coextracted, which necessitates a clean-up of the extract [2, 8]. Furthermore, since papaverine is moderately hydrophobic, the use of such a powerful extractant was considered to be unnecessary. The use of a less apolar ion-pairing reagent, sodium *n*-octylsulphate, which we also found to be applicable to the extraction of basic drugs in general, resulted in very pure extracts, but due to interactions with plasma proteins low recoveries were obtained [8]. During these preliminary experiments it was also observed that, provided an appropriate organic solvent, a pH < 11 and phosphate buffer instead of NaOH were used to alkalinize the sample, pure extracts could be obtained. Contrary to the extraction procedure of Axelrod et al. [6] and those emanating from it, interfering peaks could thus be avoided. Table I represents the overall recoveries at the 200 ng/ml of blood level obtained for papaverine and mepyramine, which was selected as internal standard (see further), using phosphate buffers ($\mu = 0.4$) of pH 5.0, pH 6.0 and pH 10.0. It can be seen that, with the chloroform—hexane (2:3) mixture as the

TABLE I

EFFICIENCY (PERCENTAGE OVERALL RECOVERY) OF THE EXTRACTION OF PAPAVERINE AND MEPYRAMINE FROM WHOLE BLOOD AT DIFFERENT pH VALUES

Solvent	pH 5.0		pH 6.0		pH 10.0	
	Papaverine	Mepyramine	Papaverine	Mepyramine	Papaverine	Mepyramine
Chloroform	44.9	84.3	27.9	85.3	21.4	80.1
Chloroform—hexane (2:3)	87.7	33.0	90.4	52.1	91.5	91.2

solvent and at pH 10.0, acceptable, reproducible (mean coefficient of variation = 2.1%) and comparable extraction efficiencies for both the analyte and the internal standard are obtained. The chloroform—hexane (2:3) mixture has furthermore the advantage over plain chloroform, that it has a lower density than blood, which makes it easy to recover after partitioning and phase separation. A simple, single extraction at pH 10.0, using the chloroform—hexane (2:3) mixture as the solvent, was hence considered as being an efficient work-up procedure.

Chromatography

A previous paper from this laboratory [5] reported on the selection of preferred HPLC systems for basic drugs in general. It was concluded that a combination of a nitrile-bonded phase with either acetonitrile—water—propylamine (90:10:0.01) or heptane (or hexane)—dichloromethane—acetonitrile—propylamine (50:50:25:0.1) as mobile phase, are very efficient for the chromatography of basic drugs in general. The latter mobile phase, being the most apolar element, was used in this study as initial investigation eluent and optimized for the determination of papaverine in whole blood. Care was taken to ensure complete resolution of papaverine and caffeine which might be present in the samples, and also to ensure minimal analysis time. The selected mobile phase was hexane—dichloromethane—acetonitrile—propylamine (50:25:25:0.1). At a flow-rate of 2 ml/min, caffeine and papaverine gave retention times of 2.9 and 3.4 min, respectively. Several drugs were tested for use as internal standard. Their retention times relative to papaverine are given in Table II. Mepyramine was chosen as internal standard since it eluted near to, but completely separated from, papaverine, and since it is reproducibly extracted from blood by the method described, with the same efficiency as papaverine. All drugs with a retention time differing by < 10% can interfere.

TABLE II

RETENTION TIMES, RELATIVE TO PAPAVERINE, OF VARIOUS DRUGS

	Relative retention time*
Papaveraldine	0.62
Ethaverine	0.64
Dioxyline	0.65
Cocaine	0.77
Imipramine	0.80
Promethazine	0.87
Carbetapentane	0.89
Yohimbine	0.94
Methapyrilene	0.97
Procaine	0.98
Heroin	1.12
Mepyramine	1.17
Thonzylamine	1.18
Fluphenazine	1.36
Strychnine	2.95

*Papaverine = 1.0.

This presents no problem, however, since in the pharmacokinetic study only volunteers not undergoing drug therapy were accepted. If the method should be used for routine papaverine monitoring, however, it is essential to know the complete medication history of the patient.

Precision and detection limit

The within-day precision of the method was evaluated at the 25 ng/ml and 250 ng/ml levels by analysing replicate spiked samples (n = 6). Coefficients of variation of, respectively, 5.6% and 5.5% were found. The between-day precision was determined to be 6.4% and 4.0% at the 50 ng/ml and 200 ng/ml levels, respectively. The detection limit at a signal-to-noise ratio of 3 was estimated to be 5 ng/ml, which was considered to be sufficient for our purpose. However, it should be possible to measure much lower concentrations if the entire organic phase can be recovered, if the entire reconstituted extract is injected, and if a variable-wavelength detector, set at the maximum absorbance wavelength of papaverine, is used.

Pharmacokinetic study

The method was used to measure 400 blood samples from ten healthy volunteers, each taking four different papaverine formulations with a minimum one-week "wash-out". Fig. 1a illustrates a chromatogram of a volunteer specimen with added internal standard. The blood sample was withdrawn 30

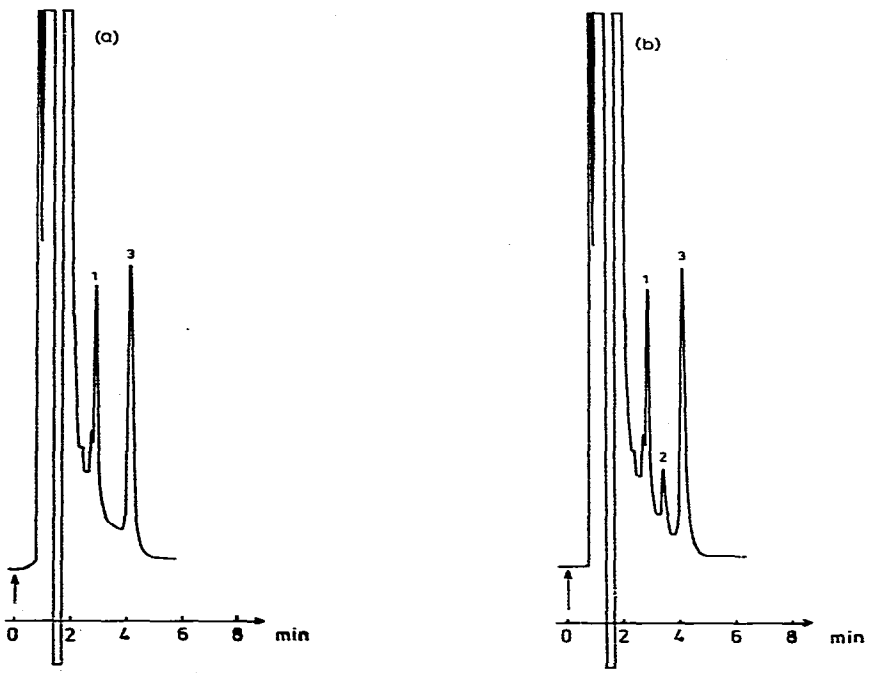

Fig. 1. (a) Chromatogram of blood from a volunteer before papaverine administration, spiked with internal standard. Peaks: 1 = caffeine, 3 = internal standard. (b) Chromatogram of blood from the same volunteer withdrawn 30 min after ingestion of a 300-mg dose of papaverine · HCl. Peaks: 1 = caffeine, 2 = papaverine (concentration found: 12 ng/ml), 3 = internal standard.

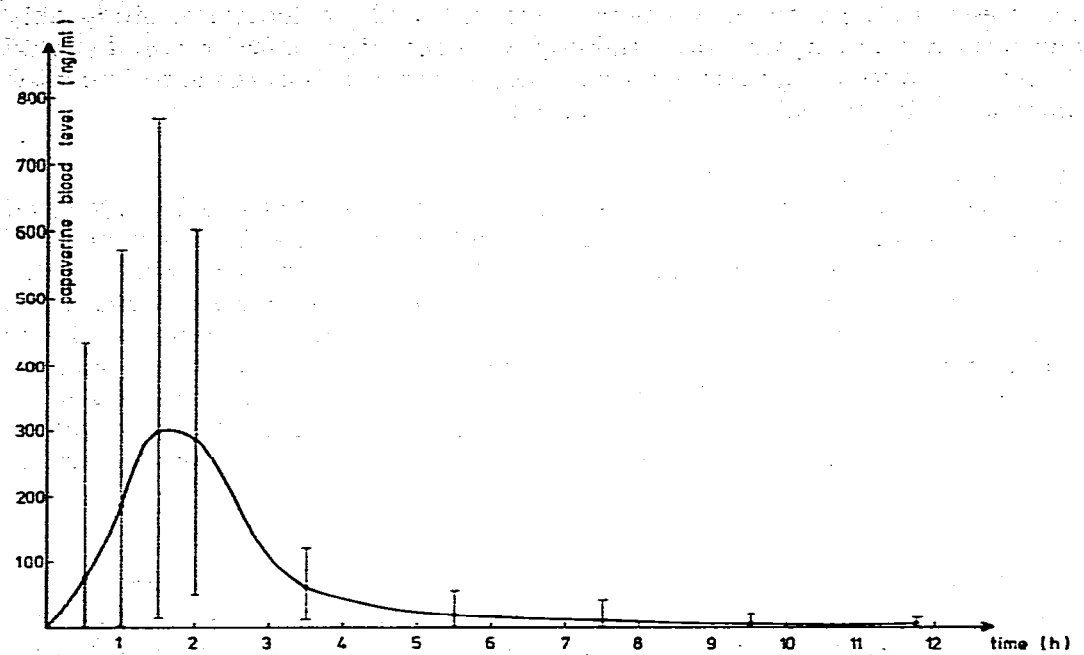

Fig. 2. Mean time course and extreme values of papaverine levels in the blood of ten volunteers each receiving a single 150-mg dose of papaverine - HCl.

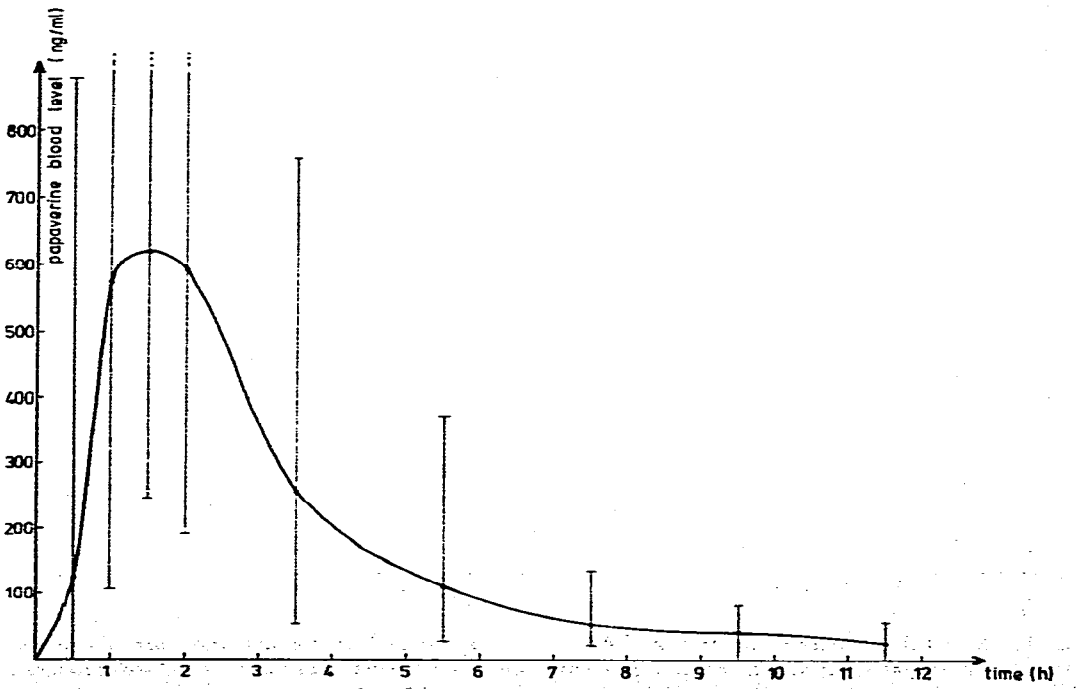

Fig. 3. Mean time course and extreme values of papaverine levels in the blood of ten volunteers each receiving a single 300-mg dose of papaverine - HCl.

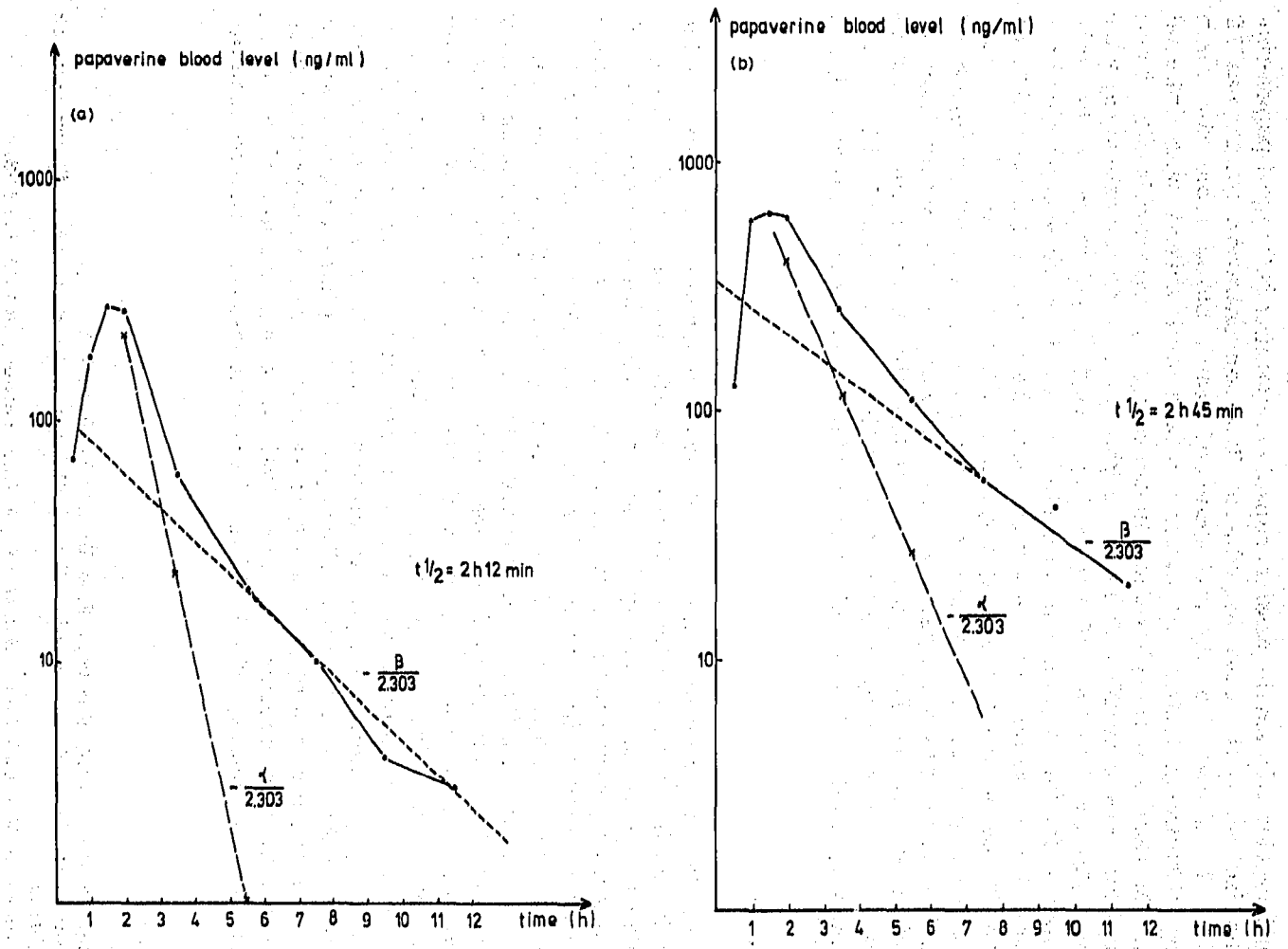

Fig. 4. (a) Mean time course (logarithmic coordinates) of papaverine levels in the blood of ten volunteers each receiving a single 150-mg dose of papaverine · HCl. (b) Mean time course (logarithmic coordinates) of papaverine levels in the blood of ten volunteers each receiving a single 300-mg dose of papaverine · HCl.

min after ingestion of a single classical capsule containing 300 mg of papaverine · HCl and lactose as the only ingredients. The concentration found was 12 ng/ml. Fig. 1b shows the chromatogram of blank blood from the same volunteer, withdrawn before papaverine intake and spiked with internal standard. The results of the pharmacokinetic study are reported in Figs. 2 and 3 as mean blood—time profiles. Only the results obtained with classical capsules containing 150 or 300 mg of papaverine · HCl and lactose as the only ingredients are shown. As could be expected, a similar curve was obtained for both doses. The extreme values obtained show the inter-subject variability of the papaverine blood levels to be very important. From Figs. 2 and 3 it can also be seen that the peak level is reached between 1.5 h and 2 h after ingestion of the drug. The results of the pharmacokinetic study are plotted on semi-logarithmic coordinates (Fig. 4). This figure suggests that the change in the concentration of the drug in the body as a function of time can be described by a two-compartmental model. The elimination half-life, determined graphically, is about 2.5 h.

CONCLUSION

A simple, fast and reproducible method for the determination of papaverine in whole blood has been developed. During the editorial review of this paper, we became aware of an article by Gautam et al. [9], dealing with the HPLC determination of papaverine in plasma and urine. These authors, claiming their method to be the first HPLC method for that purpose, used a single extraction with chloroform—isopropanol (95:5) followed by reversed-phase chromatography on a C_8 column. While their method is more or less similar in simplicity and reproducibility, our method is more sensitive. Furthermore, we obtained larger extraction recoveries and, although Gautam et al. [9] used a faster flow-rate (which also means that larger eluent volumes are needed), the time needed for the chromatographic step is longer than in our method. While Gautam et al. [9] presented no pharmacokinetic data, the procedure developed by us has been shown to be successfully applicable to a pharmacokinetic study. The results of the pharmacokinetic study show that there is an important inter-subject variability in papaverine blood levels (and elimination of the drug, proceeding in two phases). The half-life of the drug was estimated to be about 2.5 h.

REFERENCES

1 E. Mussini and A. Marzo, Biochem. Exp. Biol., 10 (1973) 331.
2 D.E. Guttmann, H.B. Kostenbaunder, G.R. Wilkinson and P.H. Dubé, J. Pharm. Sci., 63 (1974) 1625.
3 J. De Graeve, J. Van Cantfort and J. Gielen, J. Chromatogr., 133 (1977) 153.
4 V. Bellia, J. Jacob and H.T. Smith, J. Chromatogr., 161 (1978) 231.
5 D.L. Massart and M.R. Detaevernier, J. Chromatogr. Sci., 18 (1980) 139.
6 J. Axelrod, R. Shofer, J.K. Iuscoe, W.M. King and A. Sjoerdsma, J. Pharmacol. Exp. Ther., 124 (1958) 9.
7 G. Hoogewijs and D.L. Massart, Anal. Chim. Acta, 106 (1979) 271.
8 G. Hoogewijs and D.L. Massart, J. Chromatogr., submitted for publication.
9 S.R. Gautam, A. Nahum, J. Baechler and D.W.A. Bourne, J. Chromatogr., 182 (1980) 482.