Journal of Chromatography, 229 (1982) 417—423
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1188

QUANTITATIVE THIN-LAYER CHROMATOGRAPHIC DETERMINATION OF DIHYDROERGOT ALKALOIDS

E. RIEDEL*, G. KREUTZ and D. HERMSDORF

Institut für Biochemie der Freien Universität Berlin, Limonenstrasse 7, D-1000 Berlin 45 (G.F.R.)

(First received July 6th, 1981; revised manuscript received December 3rd, 1981)

SUMMARY

Direct, quantitative, thin-layer chromatographic methods for the determination of dihydroergot alkaloids are described, in particular the determination of dihydroergotamine with dihydroergokryptine as internal standard. The internal standard was added to plasma, which was extracted twice in dichloromethane; the organic phase was removed under nitrogen, the residue resolved in ethanol and applied on a silica gel G-60 plate. Dihydroergotamine and the internal standard can be measured directly by fluorescence, with excitation at 264 nm and with use of a Zeiss remission filter FL 39. The percentage recovery from this method is 49.17 ± 6.71% (plasma). These methods enable the determination of 10 pmol dihydroergotamine per ml of plasma (ca. 6.8 ng/ml) with a coefficient of variation of 10.3%. They have proved useful in biochemical and pharmaceutical applications.

INTRODUCTION

The majority of publications which have previously appeared concerning the determination of dihydroergot alkaloids [1—6] describe methods for quality control or special methodological aspects. Yet these methods are unsuitable for the quantitative determination of very small amounts in the nanogram range under controlled conditions. For example, in plasma or other biological fluids, these methods would be inappropriate for enzymatic [7] and biotransformation studies with these substances.

We have developed a sensitive method for the quantitative determination of dihydroergotamine with dihydroergokryptine as the internal standard and vice versa, based on the extraction of these pH basic drugs from alkaline biological solution with chloroform or dichloromethane, thin-layer chromatographic separation, and in situ fluorescence spectrometric determination on thin-layer plates. We believe that the application of unspecific spraying reagents such as

0378-4347/82/0000—0000/$02.75 © 1982 Elsevier Scientific Publishing Company

o-phthalaldehyde [6] is not to be recommended with biological extracts because endogenous substances may possibly react with these reagents. On the other hand, the fluorometric excitation wavelengths of dihydroergot alkaloids are very specific. The combination of dihydroergotamine and dihydroergokryptine as test substances and internal standards is also of pharmacological relevance because in therapy both drugs are not administered simultaneously. Our investigations on the quantitative determination of dihydroergot alkaloids by gas—liquid and high-performance liquid chromatography have shown that the thin-layer chromatography (TLC) described here is the most suitable, sensitive and exact method.

EXPERIMENTAL

Chemicals

The dihydroergot alkaloids [mesylates, dihydroergotamine (DET), dihydroergocristine (DEC), dihydroergokryptine (DEK)] were obtained as gifts from Dr. Rentschler Arzneimittel GmbH & Co., Laupheim, G.F.R. These were used as standard solutions in ethanol ($2.5 \cdot 10^{-3}$, $2.5 \cdot 10^{-4}$ and $2.5 \cdot 10^{-5}$ mol/l). Other reagents and solvents were Merck (Darmstadt, G.F.R.) p.a. products. The TLC solvents were dried and distilled before use.

Thin-layer chromatography

TLC was carried out on commercially available Merck silica gel G-60 plates (20 × 20 cm) or Merck HPTLC-Kieselgel 60 plates (10 × 10 cm) using ethanol—benzene—chloroform (1:2:4), with the addition of 1 ml of aqueous conc. NH_3 per 200 ml as a mobile phase. The plates were activated for 30 min at 100°C and then kept for 15 min before use in an incubator, which was saturated with water vapour at 60°C.

Fluorescence measurements

Fluorescence excitation spectra on the TLC plates were measured using a Zeiss fluorescence densitometer KM 3 with deuterium or mercury lamp, an emission filter FL 39 and a Siemens compensation recorder.

Extraction procedure

Ethanolic standard solutions were added to a heparinized blood sample (5 ml), mixed, and immediately centrifuged (5000 *g*). Then 2 ml of the supernatant (plasma) were combined with 1 ml of 0.1 *N* sodium hydroxide and 3 ml of dichloromethane, mixed, and centrifuged (8000 *g*, 0°C); 2 ml of the organic phase were separated, and the aqueous medium was eluted a second time with 3 ml of dichloromethane (0°C). In total 4 ml of the organic phase were evaporated under nitrogen in darkness. The residue was resolved in ethanol and applied to a TLC plate by means of a Desaga Microdoser 80 or a Camag TLC-Nanoapplicator.

RESULTS AND DISCUSSION

For the direct in situ determination after TLC separation, it is necessary to

use optimal fluorescence excitation and emission conditions on the TLC plates. It may be seen that the region of the emission intensity for dihydroergot alkaloids ranges from 320 to 390 nm (see also ref. 1). We used the Zeiss FL 39 filter, which excludes wavelengths above 390 nm, for the thin-layer reflection measurements. Fluorescence excitation spectra were taken using a deuterium

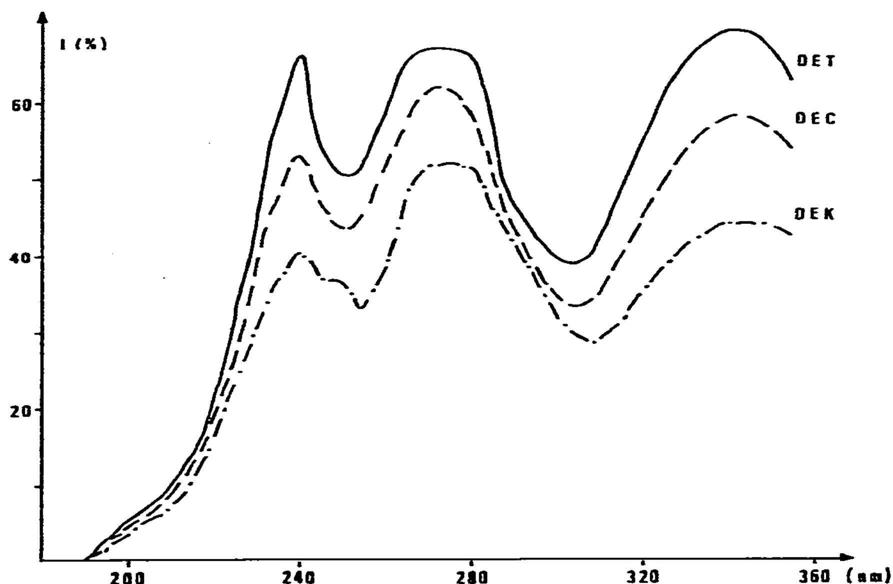

Fig. 1. Fluorescence excitation spectra of dihydroergotamine (DET), dihydroergocristine (DEC) and dihydroergokryptine (DEK) on TLC silica gel G-60 plates with deuterium lamp continuous excitation and FL 39 Zeiss remission filter. I = relative fluorescence intensity (%).

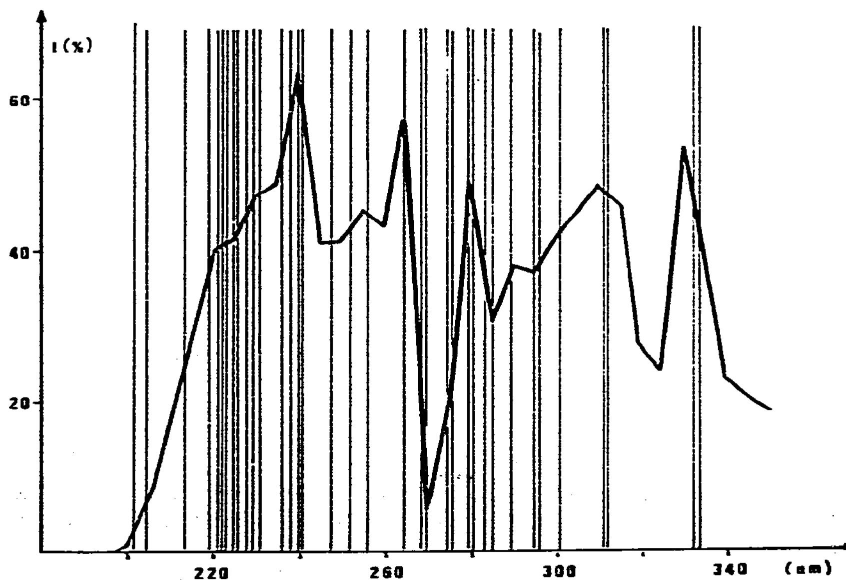

Fig. 2. Fluorescence excitation spectra of dihydroergotamine (DET) on TLC silica gel G-60 plates with mercury damp distinct line excitation and Fl 39 Zeiss remission filter. I = relative fluorescence intensity (%).

lamp (continuous spectrum) and a mercury lamp (distinct excitation lines). The two types of spectra for the dihydroergot alkaloids (DET, DEK, and DEC) on TLC silica gel G-60 plates are shown in Figs. 1 and 2, respectively. In conclusion, the optimal conditions for the fluorescence determination of the dihydroergot alkaloids on silica gel G-60 plates were found to be excitation through a mercury damp lamp of 264 nm and remission measurement with a Zeiss FL 39 filter.

The mobile phase for the TLC (ethanol—benzene—chloroform, 1:2:4, with 1 ml of conc. NH_3 per 200 ml of solvent mixture) is similar to that used by Reichelt [3]. The R_F values are 0.41 for DET, 0.51 for DEK, and 0.54 for DEC. Plasma extracts, eluted according to the procedure described in the Experimental section, showed no spots of endogenous origin in the vicinity of these R_F values (Fig. 3). An excitation wavelength of 264 nm is highly specific for dihydroergot alkaloids. At this wavelength, endogenous substances from plasma extractions show no emission in the R_F ranges of DET and DEK. Therefore, DET and DEK can be determined quantitatively under these

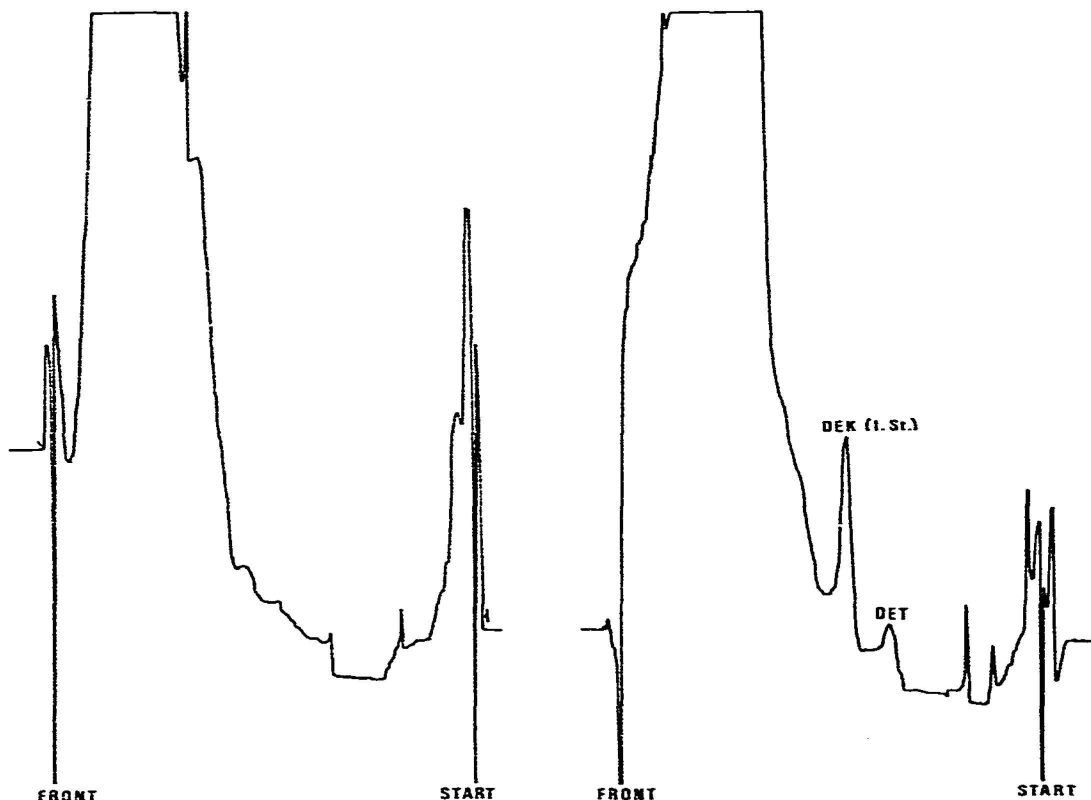

Fig. 3. TLC separation of a plasma extract (see text) without dihydroergot alkaloids. TLC plate, silica gel G-60 (Merck); mobile phase, ethanol—benzene—chloroform (1:2:4) with 1 ml of conc. NH_3 per 200 ml.

Fig. 4. TLC separation of a plasma extract with dihydroergotamine (DET) on silica gel G-60 plate (Merck). Internal standard: dihydroergokryptine (DEK). Mobile phase: ethanol—benzene—chloroform (1:2:4) with 1 ml of conc. NH_3 per 200 ml.

conditions (Fig. 4), and DET may be quantitated with DEK as the internal standard. Fig. 5 shows a plasma level determination of DET in a kinetic study with Wistar rats 2 and 3 h after oral application of 5 mg of DET (4 ml of plasma for extraction with 100 pmol of DEK as internal standard). The results here are 22 and 13 pmol/ml (13 and 8 ng/ml) DET.

The sensitivity and standardisation of the quantitative determination of DET with DEK as the internal standard is shown in Table I for standard solutions in ethanol of 250—1000 pmol DET contrasted with 500 pmol DEK as internal standard, and 50—200 pmol DET contrasted with 100 pmol DEK. Fig. 6 shows a standard curve for plasma extracts where analogous amounts of the dihydroergot alkaloids were used. Serum extractions often show unsatisfactory separation of the organic and aqueous layers. We therefore consider plasma extraction a superior method. The sensitivity of the determination extends to

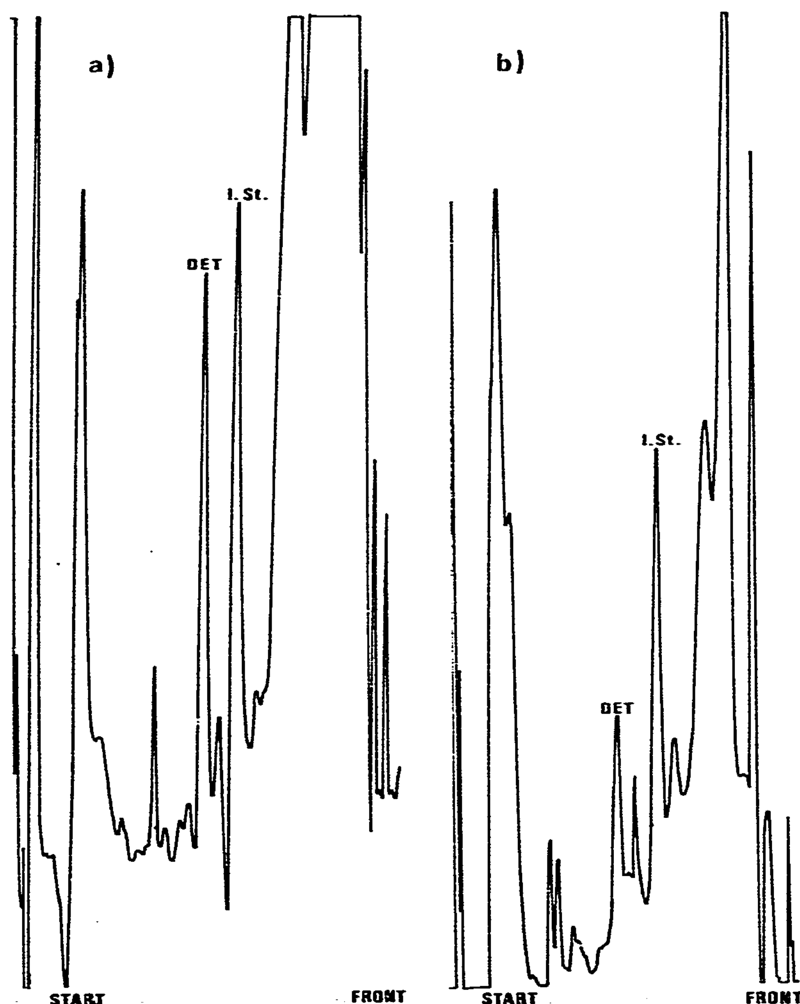

Fig. 5. Plasma level determination of dihydroergotamine (DET) in rat plasma. (a) 2 h, (b) 3 h after oral application of 5 mg of DET against 100 pmol of dihydroergokryptine as internal standard (added to the plasma before extraction).

TABLE I

STANDARD DEVIATIONS TO THE DIRECT TLC CALIBRATION AND THE STANDARD CURVES

m = (pmol dihydroergotamine)/(pmol dihydroergokryptine) applied to the TLC plates from 2 ml of plasma. Q_S = peak area quotient of the directly applied dihydroergot alkaloids. Q_E = peak area quotient of the dihydroergot alkaloids extracted from 2 ml of plasma.

m (pmol/pmol)	Q_S (DET/DEK)	s	C.V. (%)	n	Q_E (DET/DEK)	s	C.V. (%)	n
$\frac{25}{100} = 0.25$	0.12	0.01	8.3	6	0.29	0.03	10.3	6
$\frac{50}{100} = 0.5$	0.47	0.06	12.8	18	0.73	0.08	10.9	11
$\frac{100}{100} = 1.0$	1.00	0.11	11.0	21	1.20	0.13	10.8	11
$\frac{200}{100} = 2.0$	1.90	0.10	5.3	8	2.84	0.10	3.5	5
$\frac{100}{500} = 0.2$	0.19	0.01	5.3	6	0.19	0.02	10.5	6
$\frac{200}{500} = 0.4$	0.40	0.03	7.5	8	0.45	0.04	8.9	6
$\frac{500}{500} = 1.0$	0.98	0.03	3.1	8	1.00	0.06	6.0	6
$\frac{1000}{500} = 2.0$	1.93	0.14	7.2	10				

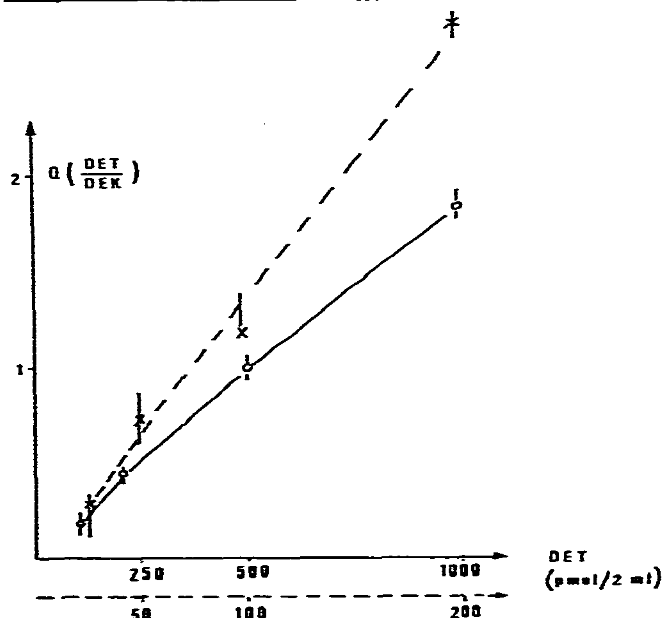

Fig. 6. Standard curves for the quantitative determination of dihydroergotamine (DET) in plasma extracts with dihydroergokryptine (DEK) as internal standard. Q = peak area quotient a_{DET}/a_{DEK}. Standard curves: o——o, with 500 pmol internal standard in 2 ml of plasma; x — — — x, with 100 pmol internal standard in 2 ml of plasma. For standard deviations see Table I.

10 pmol of DET per ml of plasma or test solution. The recovery of DET for extraction between 25 and 500 pmol per ml of plasma is 49.17 ± 6.71% (n = 15).

The standard deviations (Table I) can be minimized if the molar proportions of DET and the internal standard (DEK) in the plasma sample remain between 0.2 and 2.0. Therefore, within the range of 25—1000 pmol of DET, two standard curves are needed — one from 25 to 200 and another from 100 to 1000 pmol. The direct quantitative analysis carried out with pure substances in ethanol solution shows definite regression lines with correlation coefficients $r = 0.9972$ (range 1000 pmol) and $r = 0.9939$ (range 200 pmol). In the extraction procedure a slight difference in the partition coefficients of DET and DEK created the convex slopes in the standard curves (Fig. 6).

The quantitative determination of DEK with DET as the internal standard can be done in a manner analogous to the study given here. As a further example, these methods have been used in the determination of the specific radioactivity of di-[9,10-^{3}H]hydroergot alkaloids as synthesized from native ergot alkaloids catalytically hydrogenated with tritium [8].

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Rentschler, Arzneimittel GmbH & Co., for financial assistance and the gift of the ergot alkaloids. We are indebted to H. Schröder and M. Nündel for helpful technical assistance.

REFERENCES

1 M. Prošek, E. Kucan, M. Katić and M. Bano, Chromatographia, 9 (1976) 325.
2 V. Hartmann, M. Rödiger, W. Ableidinger and H. Bethke, J. Pharm. Sci., 67 (1978) 98.
3 J. Reichelt, Ceskoslov. Farm., 25 (1976) 213.
4 J. Reichelt and S. Kudrnac, Ceskoslov. Farm., 23 (1974) 13.
5 B. Berde and H.O. Schild, Ergotalkaloids and Related Compounds, Springer Verlag, Berlin, 1978.
6 A. Szabó and E.M. Karácsony, J. Chromatogr., 193 (1980) 500.
7 E. Riedel, I. Kyriakopoulos and M. Nündel, Arzneim.-Forsch., 31 (1981) 1387.
8 E. Riedel, R. Roetz and M. Nündel, Arzneim.-Forsch., 28 (1978) 1319.