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QUANTITATIVE THIN-LAYER CHROMATOGRAPHIC ANALYSIS OF ERGOTAMINE TARTRATE AND CAFFEINE IN THE NANOGRAM RANGE

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SUMMARY

Direct, quantitative, thin-layer chromatographic methods for the determination of ergotamine tartrate and caffeine in the presence of each other in blood and in pharmaceutical preparations are described. The blood is centrifuged, the plasma decanted from the coagulum and deproteinized with acetone–methanol. After removal of the solvent mixture, the active ingredients are extracted from the remaining aqueous solution with chloroform. In the case of the pharmaceutical preparations, the active ingredients are also extracted with chloroform or methanol. Ergotamine tartrate and caffeine are separated on pre-coated silica gel 60 F_{254} plates and measured directly on the thin-layer plate, ergotamine tartrate being determined by the fluorescence method, excitation wavelength 365 nm, at $\lambda_{max.} = 450$ nm and caffeine by the reflactance method at $\lambda_{max.} = 274$ nm.

The analytical methods are suitable for the bioavailability studies of these drugs in blood as they enable the determination of 20 ng ergotamine tartrate with a coefficient of variation of 6.7% and the determination of 200 ng caffeine with a coefficient of variation of 6.1%. The methods have also proved useful in the determination of the active ingredients of drug forms such as tablets and suppositories and can be well reproduced with a maximum coefficient of variation of 4.9% for ergotamine tartrate and 3.5% for caffeine.

INTRODUCTION

In the literature numerous publications have appeared on the quantitative determination of ergotamine tartrate[1–18] and caffeine[19–49]. The methods described can be used for the quality control both of the pure substances and of the substances in pharmaceutical preparations, but they are unsuitable for stability studies of these substances in drug formulations and for the determination of very small quantities in the nanogram range, as, for example, in bioavailability studies of these substances. We therefore wanted to develop a simple, quick, specific and sensitive method for the quantitative determination of ergotamine tartrate and caffeine in the presence of each other.

EXPERIMENTAL

Pure substances examined

The drugs examined were 0.1 mg ergotamine tartrate dissolved in 100 ml chloroform and 10 mg caffeine dissolved in 100 ml chloroform.

Material examined

The material analysed was 50 ml heparinized blood with 0.5 μg ergotamine tartrate and 50 μg caffeine; tablets with 1 mg ergotamine tartrate and 100 mg caffeine (preparation A) and suppositories with 2 mg ergotamine tartrate and 100 mg caffeine (preparation B).

Preparation of the material

Pure substances. The method of quantitative determination was carried out first with pure substances. For ergotamine tartrate 100 μl, equivalent to 100 ng, and for caffeine 10 μl, equivalent to 1 μg, were applied.

Heparinized blood. In order to test the applicability and the reproducibility of the method developed for the determination of ergotamine tartrate and caffeine in the blood, 11 samples of 50 ml heparinized blood were examined. The blood was centrifuged for 10 min at 1260 *g* the plasma decanted from the coagulum and the coagulum again centrifuged for 10 min at 1260 *g* after mixing with 10 ml of a 0.9% aqueous sodium chloride solution. After decanting, the collected solutions were treated with five times the amount of acetone–methanol (1:1). The protein which flocculated in the process was filtered off and the organic solvent mixture drawn off in a vacuum at room temperature protected from light. The remaining deproteinized plasma was extracted four times by shaking with four 10-ml portions of chloroform and the combined chloroform phases were evaporated to dryness in a vacuum protected from light and at room temperature and quantitatively transferred to a 0.5-ml graduated flask with chloroform. 20 μl of this solution, equivalent to 20 ng were applied for the quantitative determination of ergotamine tartrate and 2 μl, equivalent to 200 ng, for that of caffeine.

Tablets. The active ingredients were extracted from the pulverized tablet with chloroform. The filtered solution of the active ingredients was diluted in such a way that 10 ml of solution contained 1 mg of ergotamine tartrate. This stock solution was diluted for the caffeine determination in such a way that 10 ml of solution contained 5 mg of caffeine. 1 μl of each of these solutions, equivalent to 100 ng ergotamine tartrate and 500 ng caffeine, respectively, was applied.

Suppositories. One suppository was dissolved in methanol (chloroform is unsuitable because of its good dissolving capacity with regard to the fatty mass of the suppository) with warming. The separation of the fat was accomplished by cooling the solution to −20° and filtering off the precipitated fat. The filtrates of the fatty residue treated three times in the manner described were evaporated in a vacuum at room temperature and protected from light and the residue on evaporation was dissolved in chloroform so that the solution contained about 1 mg of ergotamine tartrate in 10 ml. This stock solution was diluted in such a way that 5 mg of caffeine were contained in 10 ml of solution. 1 μl of each of these solutions, equivalent to 100 ng ergotamine tartrate and 500 ng caffeine, respectively, was applied.

Thin-layer chromatography (TLC)

Pre-coated silica gel 60 F_{254} plates, 20 × 20 cm with a layer thickness of 0.25 mm (E. Merck, Darmstadt, G.F.R.) divided into 1.5-cm bands, were used. The solutions were applied with micro-pipettes [automatic spotting pipettes (1, 2 and 5 μl) according to Dr. Barrolier] in the form of spots. To set up the calibration curves amounts of ergotamine tartrate between 20 and 80 ng per spot and amounts of caffeine between 200 and 800 ng per spot were applied. Since the emission values were not suitable for accurate analysis in samples with less than 20 ng ergotamine tartrate per spot, the addition of 50 ng of ergotamine tartrate per sample spot proved useful.

The evaluation took place in this case on the basis of the resulting differential calibration line.

After saturation of the chambers the following mobile phase systems were used. (1) Chloroform–benzene (1:1) as preliminary mobile phase for separation of the plasma components and the excipients of the suppositories and tablets. Length of run, 2 × 15 cm; elution time, approx. 60 min per run. (2) Chloroform–ethanol (9:1) as mobile phase for ergotamine tartrate. Length of run, 1 × 20 cm; elution time, approx. 45 min. (3) Acetone–cyclohexane–methanol (49:49:2) as mobile phase for caffeine. Length of run, 1 × 20 cm; elution time, approx. 60 min.

Attention is drawn to the fact that the development of the plates must take place with exclusion of light.

Measurement and evaluation

The ergotamine tartrate and caffeine spots were measured directly on the TLC plate with the thin-layer chromatogram spectrophotometer PMQII. Ergotamine tartrate was determined by the fluorescence method, excitation wavelength 365 nm and measuring wavelength 450 nm, and caffeine by the reflectance method at 274 nm.

The quantitative evaluation in the blood took place on the basis of the calibration lines set up on the same TLC plate and in the pharmaceutical preparations with reference to the degree of remission (caffeine) of a reference spot of known concentration[50] or to the emission (ergotamine tartrate) of a reference spot of known concentration according to the following formulae:

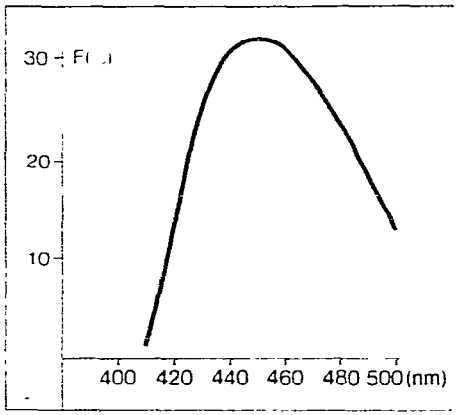
$$x = \frac{C_v \cdot Em_p}{C_p \cdot Em_v} \cdot 100 \text{ or } x = \frac{C_v \cdot F_p}{C_p \cdot F_v} \cdot 100$$

where x is the substance content of the sample (%); C_v is the amount of reference substance applied (μg); C_p is the amount of sample substance applied (μg) (calculated from the declaration of the preparation); Em_p is the emission or differential emission of the sample spot; Em_v is the emission or differential emission of the reference spot; F_p is the emission area or differential emission area of the sample spot (cm^2) and F_v is the emission area or differential emission area of the reference spot (cm^2) (emission area = height × width, measured mid-way).

Figs. 6 and 7 were recorded with Camag Reprostar at 366 nm and 244 nm, respectively.

RESULTS

Fig. 1 shows the emission spectrum of ergotamine tartrate and Fig. 2 the re-



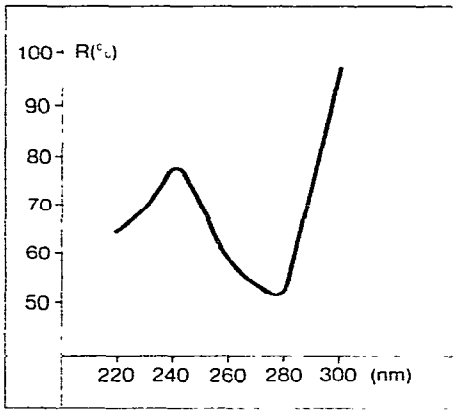


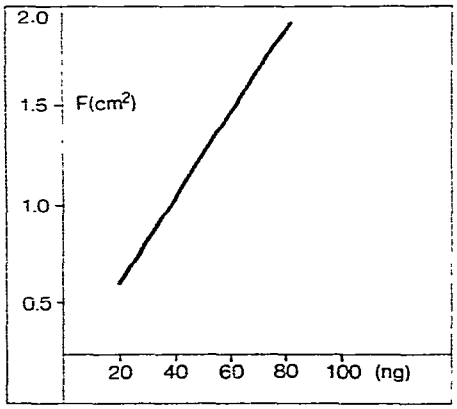
Fig. 1. Emission spectrum of 100 ng ergotamine tartrate measured on the silica gel plate. Excitation wavelength, 365 nm; $\lambda_{max.} = 450$ nm.

Fig. 2. Reflection spectrum of 5 μg caffeine measured on the silica gel plate. $\lambda_{max.} = 274$ nm.

flection spectrum of caffeine. The linear relation between concentration and the emission area or the differential emission area is shown in Figs. 3 and 4 for ergotamine tartrate. Fig. 5 shows the linearity of the relation between the concentration and the remission area in caffeine. Figs. 6 and 7 show the separation of ergotamine tartrate and caffeine from the blood.

The reproducibility of the quantitative analyses for ergotamine tartrate by the fluorescence method and for caffeine by the reflectance was examined in the pure substances and the results are summarized in Table I.

The 11 examinations of 0.5 μg ergotamine tartrate and 50 μg caffeine per 50 ml blood showed that only 64.4 $\pm$ 6.7% of the amounts of active ingredient used were recovered (Table II). It can be assumed that this lost portion of active ingredient was



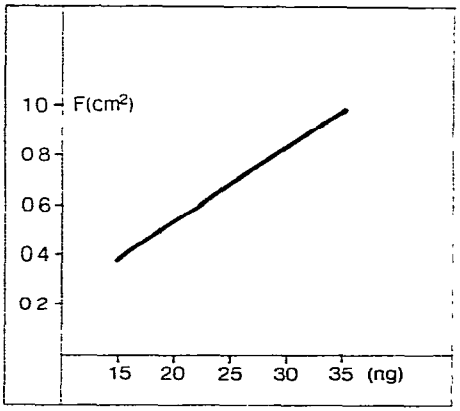


Fig. 3. Emission calibration line of ergotamine tartrate measured on the silica gel plate. Excitation wavelength, 365 nm; $\lambda_{max.} = 450$ nm.

Fig. 4. Differential emission calibration line of ergotamine tartrate measured on the silica gel plate. Excitation wavelength, 365 nm; $\lambda_{max.} = 450$ nm.

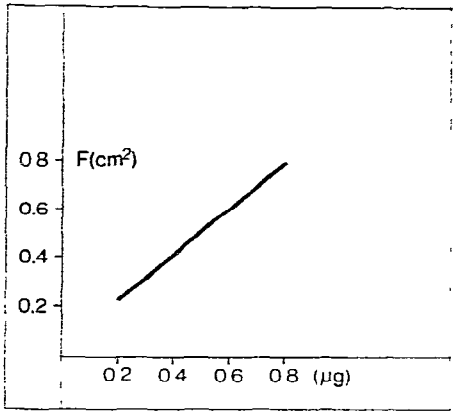


Fig. 5. Calibration line in reflected light of caffeine measured on the silica gel plate. $\lambda_{max.} = 274$ nm

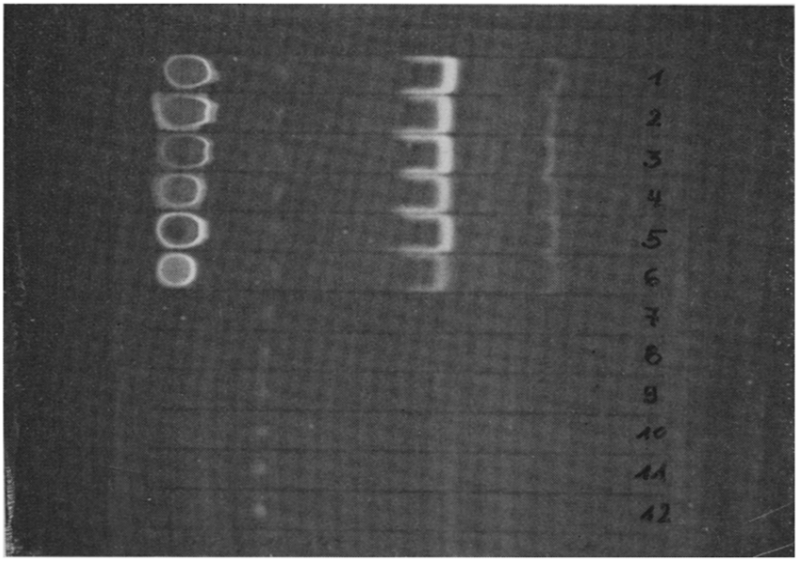


Fig. 6. Thin-layer chromatogram of ergotamine tartrate from the blood. Bands 1–6, ergotamine tartrate from blood; bands 7–12, ergotamine tartrate from the reference solution. Preliminary mobile phase, chloroform–benzene (1:1); main mobile phase, chloroform–ethanol (9:1).

adsorbed by the coagulum or by substances of high molecular weight in the blood. It is therefore necessary in bioavailability studies of these substances in the blood to multiply the readings obtained by this method by a correction factor of 1.56. The correction factor found has been confirmed in determinations of 0.25, 1.0 and 2.0 μg ergotamine tartrate and 25,100 and 200 μg caffeine per 50 ml blood. No significant deviations of the correction factors were found in blood samples of various origins (dog and man). The results of the studies of the active ingredients from the pharmaceutical preparations are summarised in Table III. The values demonstrate the good reproducibility of the methods used.

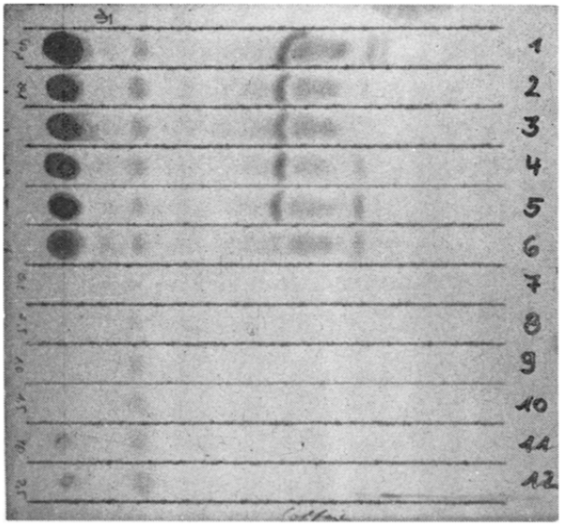


Fig. 7. Thin-layer chromatogram of caffeine from the blood. Bands 1–6, caffeine from blood; bands 7–12, caffeine from the reference solution. Preliminary mobile phase, chloroform–benzene (1:1); main mobile phase, acetone–cyclohexane–methanol (49:49:2).

TABLE I

REPRODUCIBILITY OF THE DIRECT QUANTITATIVE ANALYSIS CARRIED OUT WITH PURE SUBSTANCES

Values from 11 measurements	*Ergotamine tartrate (fluorescence method)*	*Caffeine (reflectance method)*
Amount applied per spot (ng)	100	1000
Arithmetic mean value of the peak area (cm^2)	1.22	1.00
Standard deviation of the individual values (cm^2)	0.0553	0.035
Coefficient of variation (%)	4.51	3.5

TABLE II

RESULTS OF THE BLOOD EXAMINATIONS WITH 0.5 μg ERGOTAMINE TARTRATE AND 50 μg CAFFEINE PER 50 ml BLOOD

Blood analysis No.	*Ergotamine tartrate*		*Caffeine*	
	Recovery (%)	*Calculated correction factor*	*Recovery (%)*	*Calculated correction factor*
1	68	1.47	72	1.39
2	70	1.43	63	1.59
3	59	1.69	62	1.61
4	67	1.50	59	1.70
5	64	1.56	66	1.57
6	67	1.50	65	1.54
7	68	1.44	70	1.43
8	60	1.67	62	1.61
9	58	1.72	60	1.67
10	67	1.50	66	1.52
11	60	1.67	64	1.56
Mean value	64.36	1.56	64.45	1.56
Standard deviation	4.32	0.108	3.96	0.09
Coefficient of variation	6.71	6.93	6.14	6.03

TABLE III

RESULTS OF THE STUDIES OF THE ACTIVE INGREDIENTS IN PHARMACEUTICAL PREPARATIONS

Values from 11 measurements	*Ergotamine tartrate (fluorescence method)*		*Caffeine (reflectance method)*	
	Preparation A (theoretical amount 1 mg)	*Preparation B (theoretical amount 2 mg)*	*Preparation A (theoretical amount 100 mg)*	*Preparation B (theoretical amount 100 mg)*
Amount applied per spots (ng)	100	100	500	500
Mean value (mg)	1.04	1.952	99.45	101.6
Standard deviation (mg)	0.0513	0.0882	3.14	3.48
Coefficient of variation (%)	4.93	4.52	3.16	3.45

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