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Quantitative Fluoroddensitometric Determination of Ergot Alkaloids

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Summary

The conditions for the in situ analysis of ergot alkaloids on TLC by fluorescence scanning were investigated. The excitation and emission spectra were measured and the range of linear relationship between recorded peak area and concentration established. Under the selected conditions the relative fluorescence intensities were determined.

Introduction

Rochelmeyer, in 1958 [1], was the first who separated the ergot alkaloids by TLC. Soon after, TLC was used for their quantitative analysis.

After separation ergot alkaloids can be determined either directly, without previous eluation, or indirectly, by eluating the active substance from the layer and measuring the UV-absorption [2] or photometrically by *Van Urk's* colour reaction [3–7].

The simplest direct method is the visual comparison of the coloured spots. More precise methods involve measurements in transmitted or reflected light, using densitometry of the UV absorbing or coloured spots and fluorodensitometry of the fluorescent spots [8–11].

The advantages of the direct method are the very short time of the whole analysis, small quantities of substances and more reproducible results, because there is no eluation of the active substance from the thin layer [12–15].

Method

It was found that fluorescence measurement is the most suitable method to quantify ergot alkaloids after separation by thin-layer chromatography [16–18]. The method is very sensitive and only nanogram quantities are needed. In the reflectance mode the method is nearly independent of the quality of coating; as a consequence, sample-to-sample the differences are minimal.

The following relationship exists between the intensity of fluorescence and sample concentration:

$$I_{FL} \simeq I_0 \, (1 - 10^{-w \cdot d \cdot c}) \qquad (1)$$

I_{FL} = intensity of fluorescence (λ emission)
I_0 = intensity of activation light (λ activation)
w = absorption coefficient (λ activation, mm/ng)
c = concentration (ng/mm^2)
d = layer thickness (mm)

For small quantities a linear approximation can be used:

$$I_{FL} \simeq I_0 \cdot w \cdot c \cdot d \qquad (2)$$

Determination of fluorescence was carried out in two ways:

a) D_2 lamp – monochromator – sample – photomultiplier
b) Hg lamp – sample – monochromator – photomultiplier

The respective schematic diagrams of the systems are shown in Figures 1 and 2.

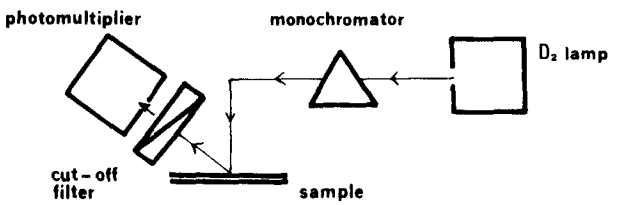

Fig. 1

● Determination of fluorescence.
D_2 lamp – monochromator – sample – photomultiplier.

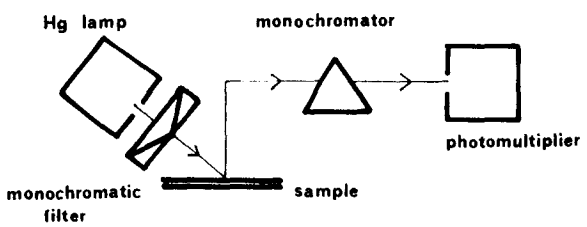

Fig. 2

● Determination of fluorescence.
Hg lamp – sample – monochromator – photomultiplier.

The first method was suitable for the determination of ergot alkaloids and therefore, it was used in this work.

The path of the excitation beam is longer, therefore it does not destroy the sample. The path of I_{FL} is shorter and the intensity losses are smaller. In the method given in Fig. 2 the alkaloid is too close to the light source and therefore, it is soon destroyed. In this case the path of I_{FL} is longer and suffers intensity losses. In order to measure the excitation and emission spectra both methods have to be used.

Experimental

Chemicals

Some ergot alkaloids used in this investigation were prepared in our own laboratories (LEK, Ljubljana). Ergobasin-Chloroformadukt and ergotaminine were supplied by Fluka AG, CH-9470 Buchs, Switzerland, while ergotamine, ergocristine and ergocryptine were purchased from Sigma Co., St. Louis, Missouri 63178, U.S.A. All samples were analysed (total assay and impurities) before being used as standards.

Thin-Layer-Chromatography

The chromatographic conditions and solvent concentrations suggested in other publications were modified [19, 20]. Thin-layer chromatography was carried out on commercially available cellulose plates (Merck TLC plates Cellulose – without fluorescent indicator – precoated 20 cm × 20 cm, layer thickness 0.10 mm). The plates were activated for 30 minutes at 105 °C, and impregnated in 15 % (v/v) solution of formamide in acetone. Subsequently, the plates were dried and then heated for 2.5 min at 105 °C. The solvent system used consisted of ethylacetate-n-heptane-diethylamine (5 : 6 : 0.005).

The samples (0.02 % – 0.05 % chloroform solutions of the alkaloids) were applied with 1 – mm^3 Desaga microcaps. The chromatograms were developed in ascending mode in nonsaturated chromatographic chambers at 4–6 °C for about 60 minutes until the solvent front rose 17 cm from the origin line. Subsequently, they were dried in a stream of cool air. The plates were stored in dark place for 30 minutes and then scanned. A typical scan is shown in Fig. 3.

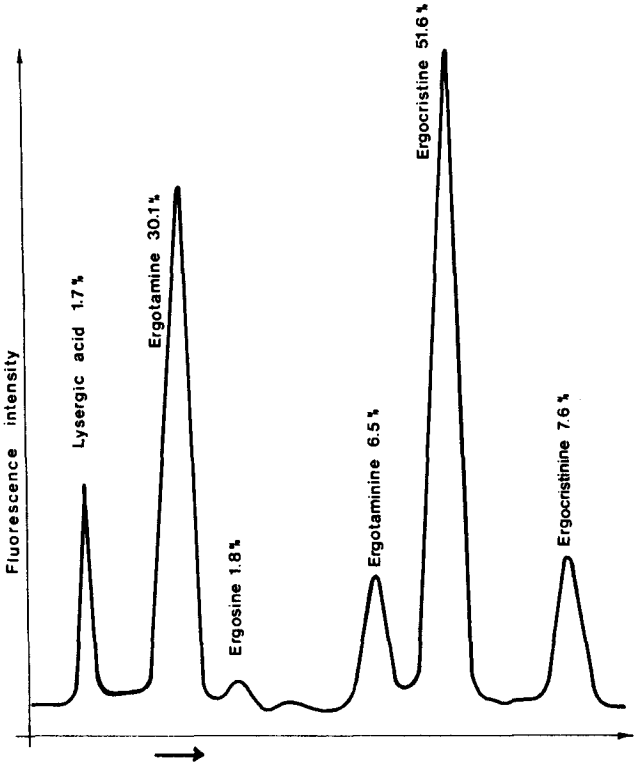

Fig. 3

● Typical scan of the ergocristine-type sample.

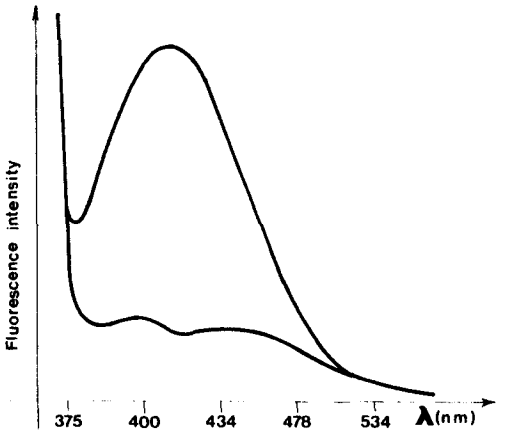

Fig. 4

● Emission spectrum of ergocristine.

Quantification of the Thin-Layer Chromatograms

Measurements were carried out with the Opton Model PMQ II Chromatogram Spectrophotometer (Opton Company, Oberkochen, FRG) which was combined with a Photovolt TLC 530 densitometer (Photovolt Corp., 1115 Broadway, New York, N.Y. 10010, U.S.A.) because the suitable parts for the Opton instrument were not yet available.

The excitation spectra were obtained with the original Opton densitometer, but using the Photovolt monochromatic filter (445 nm). Fig. 5 shows a typical excitation spectrum.

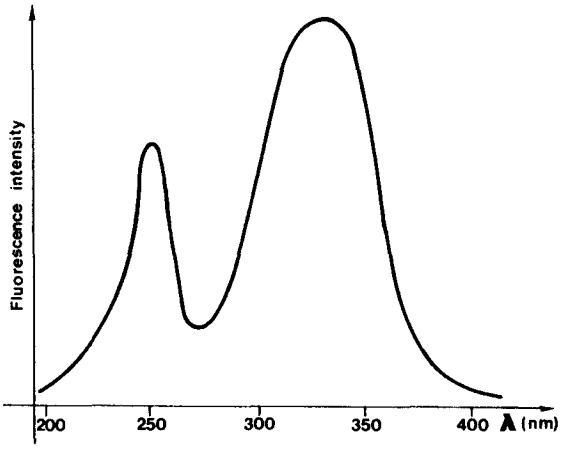

Fig. 5

● Excitation spectrum of ergocristine.

For emission spectra, the Opton densitometer was combined with some parts of the Photovolt densitometer. A General Electric Germicidal lamp (254 nm) was used as the light source while the UV filters were Photovolt Cat. No. 5265 and Cat. No. 5267.

The most suitable conditions for fluorescence scanning were determined from the spectra (see e.g. [4 and 5]).

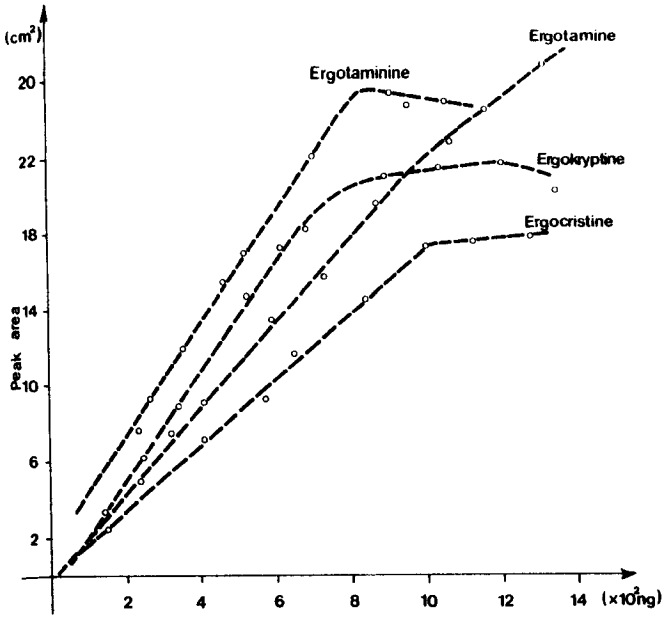

Fig. 6
• Calibration curves for some ergot alkaloids.

The wavelength 325 nm was chosen at the monochromator for the excitation beam, while the monochromatic filter at 445 nm was used in the emission side. The best reproducibility and the minimal dependance on the quality of coating were obtained under these conditions.

The scans were recorded on a Varicord 42 B recorder with a Photovolt integrator and a Varian A-25 recorder (Varian Aerograph Product, Palo Alto, California 94303, U.S.A.).

Results and Discussion

The peak areas were integrated by multiplying peak height by the peak width at half heigt. The linear response range (peak area vs. sample amount) for alkaloids was found up to 800 ng and for isomers of alkaloids up to 600 ng (see Fig. 6). The following relationship exists for the concentrations in these ranges:

$$A = K_1 m + K_2 \qquad (3)$$

A = peak area
K_1 = constant (relative fluorescence per ng)
m = amount of sample, ng
K_2 = background constant

The value of constant K_1 is different for the different alkaloids. This means that the relative fluorescence per ng is not the same for all ergot alkaloids. Under well selected conditions (slit, gain, etc.) it was possible to hold the background constant, K_2, at zero.

In order to determine the concentrations of ergot alkaloids in samples it was neccessary to know relative values of constant K_1. Therefore, standard alkaloid mixtures with known concentrations were prepared and applied on the plates. Only results from one plate were used for each determination of K_1. A number of determinations and calibration curves were made before the constants were established. The results of these studies are listed in Table I. The quantity of each ergot alkaloid in the sample was calculated as:

Table I. Relative values of the constant K_1

Substance	K_1	Substance	K_1
Ergotamine*	1.00	Ergocryptine	1.10
Ergotaminine	1.10	Ergocryptinine	1.15
Ergosine	1.13	Ergocornine	1.25
Ergosinine	1.18	Ergocorninine	1.30
Ergocristine	0.78	Ergometrine	3.60
Ergocristinine	0.88	Lysergic acid	2.00

* used as the reference substance

$$m_x = \frac{A_x}{K_1, x} \qquad (4)$$

m_x= amount of substance x
K_1 = constant
A_x = peak area

We also studied the possibility of change in the fluorescence intensity with time. Practically no difference in fluorescence was found during 24 hours if the chromatograms were hold in dark place at room temperature. It was impossible to find any correlation between time and fluorescence; slight changes in the fluorescence were most probably the effect of UV light destroying part of the alkaloids.

Table II. Results of reproducibility studies

Method	Concentration %	No. of analyses	Range of relative standard deviation [a]
Indirect	〉25	17[b]	± 5 %
	10–25		±10 %
	〈10		±30 %
Direct	〉25	19[c]	± 3 %
	10–25		± 8 %
	〈10		±12 %

[a] Relative standard deviation (%) = $(6 / \bar{x}) \cdot 100$
Relative standard deviation of the instrument [21] for normaly detected spots is better than 0.3 %

[b] For the indirect method 17 plates were selected among the 20 prepared. Three plates were not taken in account while the separation was unsatisfactory.

[c] For the direct method 19 samples on two plates were used for the determination while 20 were spotted.

The reproducibility studies (see Table II) indicated that direct analysis could be carried out with a somewhat smaller relative standard deviation than the indirect analysis. For practical work the differences between the results obtained

Table III. Relationship between the results obtained by direct (D) and indirect (P) determination of a number of samples
(A) ergocristine-type samples
(B) ergocornine-type samples

A	lysergic acid	ergom-etrine	ergot-amine	ergo-sine	ergot-aminine	ergo-cristine	ergo-cristinine
1. D	0.6	–	29.7	1.5	8.8	49.7	9.7
P	1.0	–	26.3	1.3	8.8	49.6	13.0
2. D	1.3	–	35.5	2.4	6.1	50.4	4.2
P	1.9	–	38.6	0.6	7.4	47.7	4.0
3. D	1.3	–	77.7	5.7	2.7	12.7	–
P	2.9	–	77.4	5.5	3.8	10.1	0.3
4. D	5.0	2.5	79.8	5.6	1.4	5.5	–
P	5.6	–	77.9	6.9	3.8	4.7	1.1

B	lysergic acid	ergom-etrine	ergot-amine	ergo-sine	ergot-aminine	ergo-sinine	ergo-cornine	ergo-cryptine	ergo-corninine	ergo-cryptinine
5. D	10.0	–	3.6	18.3	–	1.6	33.5	25.2	3.0	2.5
P	11.3	–	0.6	15.5	–	5.3	32.9	24.3	9.8*	
6. D	10.3	2.2	–	17.2	–	3.8	26.8	24.1	9.0	4.7
P	8.8	–	2.1	13.7	2.1	5.6	28.4	24.5	13.9*	
7. D	–	–	–	1.3	3.0	5.2	11.0	15.7	32.9	33.8
P	1.9	–	–	1.9	–	5.0	12.2	14.0	65.0*	
8. D	44.3	–	8.3	37.4	–	–	6.8	3.0	–	–
P	45.8	–	5.7	36.7	1.8	1.4	6.9	1.2	0.5*	
9. D	1.0	–	–	13.6	2.2	–	42.9	31.3	5.8	3.3
P	0.3	–	0.5	11.1	2.2	2.8	41.8	32.9	8.4*	

* by the indirect method only the sum of ergocorninine and ergokryptinine was determinde.

by these two methods can be neglected while the direct method is more rapid. For our work all samples were spotted at least twice on each plate and two plates were made for each analysis. If differences in the concentrations were smaller than ± 2 %, the results were accepted; otherwise the analysis was repeated. The difference between direct fluorodensitometric and indirect photometric results was smaller than 5 % (see Table III).

Acknowledgements

The authors wish to express their appreciation to Opton Company specially to Dr. *Hezel* and Ing. *Ottelinger* for technical help.

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Received: Oct. 8, 1975
Accepted: Jan. 20, 1976