A METHOD FOR THE QUANTITATIVE DETERMINATION OF ERGOMETRINE IN RYE ERGOT

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Recently, great attention has been devoted to breeding work connected with obtaining strains of ergot-producing alkaloids and the study of their biogenesis and microbiological synthesis. These investigations require accurate and rapid methods for the quantitative determination both of the total and of the individual ergot alkaloids. Existing methods satisfy these requirements poorly [1-11].

In the present paper we give the results of investigations connected with development of a method the quantitative determination of ergometrine in rye ergot of the ergometrine strain bred in VILR (All-Union Scientific-Research Institute of Medicinal Plants), which is the main raw material for its production. The combined alkaloids of the ergometrine strain include ergometrine and ergometrinine [12]. We devoted our main attention to a study of the conditions for the extraction of the ergometrine from the raw material, its chromatography, and its removal from the sorbent. The method presupposes the defatting of the comminuted rye ergot with petroleum ether as has been described for ergotamine [13]. To extract the combined alkaloids from the raw material, we used diethyl ether in a ratio of 1:20 (Table 1); the alkaloids were extracted with continuous stirring for 2 h (Table 2).

The total alkaloids were chromatographed in chloroform on paper of type C (fast) and on plates in a thin layer of alumina [chloroform–methanol (97.5:2.5)], R_f of ergometrine 0.22 and of ergometrinine 0.41. The separation of the ergometrine by paper chromatography took rather a long time, while in thin-layer it was considerably faster.

To determine the completeness of the elution of the ergometrine from the paper and the alumina, experiments with pure ergometrine were performed (Table 3).

The accuracy of the proposed method was confirmed by the analysis of ergot with additions of pure ergometrine (Table 4). The average relative error of the method was 2.715% for the case of paper chromatography and 1.606% for thin-layer chromatography.

The total alkaloids and the ergometrine in three samples of ergot obtained from the communal farms of the "Lekrasprom" All-Union Combine were determined by the method developed. Table 5 gives the results of the analyses of one of these samples (weight of raw material 5 g).

TABLE 1

Ratio of diethyl ether to raw material taken	Ergometrine content (%) using		Total alkaloids, %
	PC	TLC	
1:10	0,042	0,048	0,064
	0,043	0,049	0,069
1:20	0,060	0,064	0,089
	0,061	0,066	0,088
1:30	0,062	0,064	0,087
	0,060	0,064	0,086

EXPERIMENTAL

Extraction of the Alkaloids from the Raw Material. The finely ground rye ergot (5.0 g, with an accuracy of 0.01 g) was defatted in a Soxhlet apparatus with petroleum ether (bp 40-60°C) for 8 h. The defatted powder was freed from solvent at a temperature not exceeding 30°C and was transferred quantitatively to a 250-ml flask with a ground-in stopper and 100 ml of peroxide-free diethyl ether, 5 ml of 10% aqueous ammonia solution was added and the mixture was shaken for 2 h. Then 5.0 g of

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TABLE 2

Sample No.	Amount (%) of ergometrine using PC, h: 0.5	1	2	3	4	Amount (%) of ergometrine using TLC, h: 0.5	1	2	3	4	Amount (%) of total alkaloids, h: 0.5	1	2	3	4
1	0,031 0,033 0,031	0,033 0,033 0,031	0,032	0,032 0,033 0,034	0,032 0,034 0,033	0,037 0,034 0,037	0,040 0,042	0,042	0,042 0,040	0,040 0,045	0,070 0,069 —	0,067 — 0,968	0,080	0,081 0,042 0,040	0,080 0,083 0,081
2		0,039 0,040	0,042 0,040	0,042 0,040	— —		0,046 0,044	0,049	0,048	—		0,060 0,059	0,060 0,061	0,059 0,054	— —

TABLE 3

Deposited, mg	Found using PC, mg	PC, %	TLC, mg	TLC, %
0,050	0,048	96,0	0,050	99,0
0,100	0,098	98,0	0,097	97,0
	0,103	102,5	0,100	100,0
0,150	0,149	99,1	0,149	99,3
	0,154	103,3	0,147	98,0
0,200	0,198	99,0	0,204	102,0
	0,206	103,0	0,200	100,0
0,250	0,258	103,2	0,253	101,2
	0,258		0,250	100,0

TABLE 4

Ergometrine, mg: amount in the	added	nominal	found	Error: absolute	relative
2,97	0,748	3,718	3,660	−0,058	1,559
2,97	1,496	4,466	4,340	−0,126	2,821
2,02	1,908	3,928	3,780	−0,148	3,767
				Mean	2,715
TLC					
3,30	0,884	4,184	4,160	−0,024	0,573
3,05	1,768	4,818	4,610	−0,208	4,317
2,34	0,668	3,008	2,990	−0,018	0,598
2,32		2,983	2,960	−0,028	0,937
				Mean	1,606

anhydrous sodium sulfate was added, and the mixture was shaken vigorously for 5 min, allowed to stand, and rapidly filtered through hygroscopic cotton.

Determination of the Total Alkaloids. A 25-ml portion of the filtrate (1.25 g of ergot) was placed in a separating funnel, and the alkaloids were extracted with 1% aqueous sulfuric acid (4×10 ml). The flask with the combined sulfuric acid extracts was placed in the water bath and heated to 40-50°C to eliminate traces of ether. The cooled solution was passed through absorbent cotton into a 50-ml measuring flask; the flask and the funnel with the cotton were washed with 1% sulfuric acid, and the solution was made up to the mark with the same solvent (solution A).

To 2 ml of solution A, 4 ml of the Van Urk reagent was added, the mixture was stirred, and after 30 min the optical density was measured in a photocolorimeter with a green filter (500-560 nm) in a cell with a layer thickness of 5 nm. The amount of ergot alkaloids in 1 ml of solution A was found from a calibration graph.

The percentage of total alkaloids calculated as ergometrine (X) was calculated from the formula

$$X = \frac{a \cdot 50 \cdot 100}{1,25},$$

where a is the amount of alkaloids calculated as ergometrine in 1 ml of the solution A under investigation found from the calibration graph, g.

Determination of the Ergometrine Content. A 50-ml portion of the filtrate (2.5 g of ergot) was evaporated to dryness on the water bath. The solution was dissolved in 2 ml of methanol (solution B).

A. Paper Chromatography. With a micropipette, 0.2 ml of solution B was deposited in a narrow band on the starting line of a sheet (55×17.5 cm) of type C (medium) chromatographic paper. The paper was impregnated with a 50% ethanolic solution of formamide with a pH of 7.2-7.5, leaving the starting line dry to a width of about 2 cm; the excess of formamide was carefully squeezed out between sheets of filter paper, and the starting line was sprayed with the same solution of formamide from an atomizer. The

TABLE 5

Ergometrine content (%) using					
PC			TLC		
found	$(x-\bar{x})\cdot 10^{-4}$	$(x-\bar{x})^2\cdot 10^{-8}$	found	$(x-\bar{x})\cdot 10^{-3}$	$(x-\bar{x})^2\cdot 10^{-6}$
0,035	+2	4	0,037	0	0
			0,036	−1	1
			0,035	−2	4
0,033	−18	324	0,040	+3	9
0,035	+2	4	0,037	0	0
			0,038	+1	
0,036	+12	144	0,036	−1	1
0,035	+2	4	0,038	+1	
0,034	−8	64	0,037	0	0
$\bar{X}=0,0348$		Σ 560	$\bar{X}=0,037$	Σ 0,000021	

Statistical characteristics	PC	TLC
S	0,00079	0,00153
$S_{\bar{x}}$	0,00025	0,00048
α	0,95	0,95
t_α	2,23	2,23
E_α	0,000558	0,00108
E_{rel}	1,6%	2,92%

ethanol was allowed to evaporate from the sheet of paper in the air in a dark place for 10 min. Then chromatography was performed for 5 h in a darkened chamber by the descending method in the chloroform system, after which the paper was freed from the solvent by drying and was examined in UV light, the luminescing ergometrine band being marked. Over the whole width of the paper, this band was cut out from one end with serrations, and the other end was placed in a cell containing a 1% solution of tartaric acid present in a chamber saturated with water. The ergometrine was eluted with the 1% solution of tartaric acid, and the eluate was collected in a 10-ml cylinder. Overnight, 6-8 ml of eluate was obtained, and this was made up to 10 ml and mixed (solution C). To 2 ml of solution C was added 4 ml of the Van Urk reagent, the mixture was stirred, and after 30 min its optical density was measured in a photoelectric colorimeter with a green filter (500-560 nm) in a cell with a layer thickness of 5 mm. The amount of ergometrine in 1 ml of solution C was found from a calibration graph. The percentage of ergometrine (X) was calculated from the formula

$$X = \frac{b\cdot 10\cdot 100}{0,25},$$

where b is the amount of ergometrine in 1 ml of solution C found from the calibration graph, g.

B. Thin-Layer Chromatography. From a micropipette, 0.2 ml of solution B was deposited in the form of a narrow band at the starting line of a plate (18×12 cm) of alumina (activity grade IV). Chromatography was performed in a darkened chamber in the chloroform–methanol (97.5 : 2.5) system until the front had traveled 12-15 cm, after which the plate was dried in a dark place and was inspected in UV light, the luminescent band of the ergometrine being marked. The alumina with the adsorbed ergometrine was transferred to a test tube, 10 ml of a 1% solution of sulfuric acid was added and the mixture was shaken for 50 min. It was then allowed to stand and was centrifuged at 1200 rpm for 5 min (solution D).

To 2 ml of solution D, 4 ml of the Van Urk reagent was added, and after mixing, the procedure was the same as in the determination of ergometrine using paper chromatography.

SUMMARY

A chromatophotometric method for the quantitative determination of ergometrine in rye ergots is proposed.

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