MEDICINAL PLANTS

THE CHEMISTRY OF PEPTIDE ERGOT ALKALOIDS. PART 2. ANALYTICAL METHODS FOR DETERMINING ERGOT ALKALOIDS

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The analytical monitoring of ergot alkaloids is performed by various chemical, physicochemical, and physical methods or their combinations, depending on the problem to be solved.

Chemical Methods

Ergot alkaloids can be identified using general alkaloid reactions with precipitating agents such as polyiodides, heavy metal salts, heteropolyacids, etc. [1 – 3]. Some of these reagents possess certain selectivity: for example, alkaloids of the ergotoxin group are precipitated with picric and picrolonic acids and do not interact (unlike ergometrine) with antimony trichloride [4]. The TLC determination of ergot alkaloids is usually performed using Dragendorff's reagent.

Both qualitative and quantitative analyses for ergot alkaloids widely employ chromogenic reactions with concentrated acids [1, 2]. Most frequently, these are special reactions with an indole nucleus of the alkaloid molecule. The first color reaction of this type was suggested by Tarne in 1875 and involved the interaction with concentrated sulfuric acid containing iron chloride traces, leading to the characteristic (violet to blue) color development. The other well-known color reactions in concentrated sulfuric acid medium involve glyoxylic acid (blue-violet color), vanillin, Keller's reagent (purple-blue color), and Van Urk's reagent (dark-blue color) [5 – 9]. Most widely used are the condensation reactions with *p*-dimethylaminobenzaldehyde (*p*-DMB), employing Ehrlich's, Allport's, Van Urk's and some other ragents [4 – 6, 8 – 15]. This method offers high sensitivity, whereby the intensity of coloration in the reaction solution is proportional to the content of ergot alkaloids. The latter circumstance allows these reactions to be used for the quantitative spectroscopic analysis [3, 4, 6, 11 – 15]. According to the reaction mechanism proposed by Poehm in 1954, two ergot alkaloid molecules form a product of condensation at the indole nucleus with the *p*-DMB molecule (see the scheme below). The specificity of this reaction was proved in [4, 12 – 15].

Schematic diagram illustrating the formation of a product of *p*-DMB condensation (colored cation) at the indole nucleus of an ergot alkaloid molecule (for ergopeptins with R representing a tripeptide cyclole group).

Some methods proposed previously for the quantitative determination of ergot alkaloids (Keller's gravimetric technique based on the precipitation of mineral salts, Kjeldahl's micromethod, oxidimetric technique based on the ergot alkaloid oxidation by potassium bichromate), as well as the method of biological evaluation of sclerotium (employed in the 7th edition of the USSR State Pharmacopoeia), did not find use and are of not much interest now [8].

A chemical method widely used for the quantitative determination of ergot alkaloids employs the process of titration in nonaqueous media. This is a direct method based on the presence of a molecular fragment – the tertiary nitrogen atom in the ergoline nucleus – responsible for the pharmacological activity [5, 16 – 18]. The titration process is usually conducted in glacial acetic acid (either pure or mixed with acetic anhydride) using perchloric acid as a titrant. The visual titration process is performed with Crystal Violet indicator [1, 5, 17, 19 – 22]. Using this method, the ergot alkaloids can be titrated in a differential mode, since the basic properties of alkaloids decrease in the following order: ergometrine – ergotamine – ergocryptine – ergocristine.

Photocolorimetric Analysis

A method of photocolorimetric determination of ergot alkaloids based on the chemical coloration reaction with

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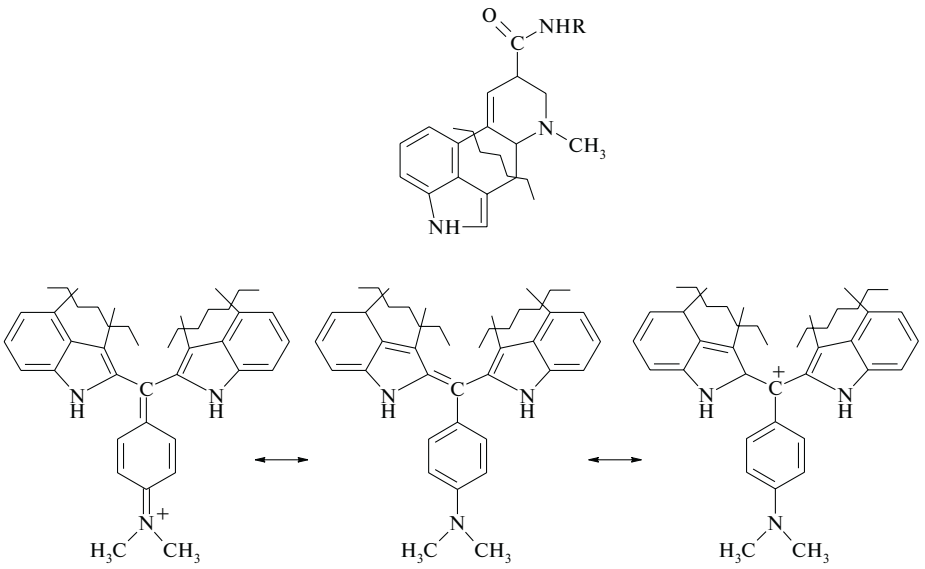

p-DMB [4, 8, 16] was originally proposed by Smith in 1930 and then continuously developed: the reaction was accelerated by adding iron chloride [4, 5, 11]. It was also suggested to catalyze the process by manganese and mercury salts or hydrogen peroxide, but these reagents possessed no significant advantage over iron chloride. The colorimetric determination of ergot alkaloids was recommended in the 8th edition of the USSR State Pharmacopoeia [8]. A related chromatophotocolorimetric method was based on the Allport reaction followed by the photocolorimetric measurements at 533 nm [4, 23 – 25].

Later, it was suggested to perform the colorimetric procedure using Van Urk's reagent. Presently, this technique is widely employed in screening analyses for determining the total content of alkaloids in ergot sclerotium [2 – 4, 26 – 28]. The colorimetric method allows up to 90% of the active

TABLE 1. Parameters of the 1 H NMR Spectra of Various Ergot Alkaloids ($CDCl_3$): Chemical Shifts, ppm (Spin–Spin Coupling Constants, Hz)

Atom	O-12′-Methylergocornine [50]	O-12′-Methyl-α-ergocryptine [50]	Ergocornine [50, 61]	Ergocornine [61]	α-Ergocryptine [50, 60]	β-Ergocryptine [60, 61]	β-Ergocryptinine [61]	Ergocristine [61]	α-Ergocryptam [60]	β,β-Ergoannam [60]	β-Ergocryptam [60]	5′-Epi-β-ergocryptine [60]
H^2			6.97 s	6.86 s	6.94/2 s	6.94/2 s	6.94 s		6.92 s	6.92 s	6.92 s	6.94 s
N^1H	8.07	8.09	7.97	8.48 s	7, 97	8.21 s	8.4 s	7.96	8.00 s	8.00 s	8.30 s	8.47 s
			8.57 s		8.37 s	8.57 s						
N^6CH_3	2.66	2.66	2.66/5 s	2.6 s	2.64/3 s	2.63/2 s	2.61 s	2.64	2.69 s	2.67 s	2.67 s	2.65 s
H^9	6.40	6.41	6.40/1 d	6.52 d	6.38 (2, 6.2)	6.35 dd	6.54 d	6.38	6.47 dd	6.46 dd	6.46 d	6.36 dd
	(2.0, 5.9)	(1.8, 5.5)	(6)	(6)	6.37 dd	(6.1, 1.4)	(6)	(2.0, 6.2)	(4.9)	(4.4, 1.5)	(4.3, 1.2)	(5.5, 1.3)
					(1.5, 6.1 – 6.4)	6.37 (6)						
(8) – CONH	9.17	9.16	9.86/3 s	10.02 s	9.86/4 s	9.88/5 s	10.05 s	9.84	9.16 s	9.26 s	9.22 s	9.84 s
									9.6	9.8	9.2	
(12′)OH	–	–	7.84 (1.6)	7.32	7.86 (1.6)	7.5	7.3 d (1.8)	7.38 (1.7)	–	–	–	–
			7.52 d (1.5)		7.38 (1.7)							
$(12')OCH_3$	3.31	3.24	–	–	–	–	–	–	–	–	–	–
$H^{3',5'}$	4.51	4.62	4.43 (3.5)	4.38 d (3.5)	4.50 (5.6,7.3)	4.49 d (2.5)	4.43 d (2.5)	4.5 t	5.08 t	4.94 d	4.96 dd	4.53 dd
	(4.1)	(3.8, 10.8)	4.49 d (3.5)		4.52 t (6.4)	4.53 d (2)		(5.6, 7.3)	(6.1)	(9.3)	(9.2)	(3.1)
$H^{2'}$	–	–	–	–	–	–	–	–	5.75 dd (8.6, 2.5)	5.94 dd (9.8, 2.4)	5.83 d (9.2, 3.1)	
$H^{11'}$	–	–	–	–	–	–	–	–		4.36 t (7.6)	4.36 t (7.8)	

component to be determined, while the side products of oxidation are not detected [29].

Spectrophotometric Analysis

The spectrophotometric technique used for the quantitative determination of ergot alkaloids offers a high sensitivity (2 – 10 ng/ml). However, the method is insufficiently selective: most of the lysergic acid derivatives, including some degradation and polymerization products, also possess UV absorption spectra with absorption maximum at 312 – 313 nm [5, 8, 10, 16, 30, 31].

The spectrophotometric analysis can be used either for directly determining argot alkaloids or for detecting the products of color reactions with *p*-DMB (using Van Urk's, Allport's, and other reagents). The condensation products exhibit maximum absorption in the spectral region of 570 nm [32, 33].

Increase in selectivity can be achieved by combining the spectrophotometric and chromatographic techniques. Such a chromatospectrophotometric method [18, 34] was successfully used in the study of selected ergot strains.

Spectrofluorimetric Analysis

Being capable of detecting about 1 ng of lysergic acid derivatives, the method of fluorescence spectroscopy (or spectrofluorimetry) possesses a high sensitivity [5, 10, 16,

TABLE 2. Parameters of the ^{13}C NMR Spectra of Various Ergot Alkaloids ($CDCl_3$): Chemical Shifts (ppm)

Atom	O-12′-Methylergocornine [50]	O-12′-Methyl-α-ergocryptine [50]	Ergocornine [50]	α-Ergocryptine [50, 60]	β-Ergocryptine [60]	Ergometrine [16]	Ergotamine [16]	5′-Epi-β-ergocryptine [60]	α-Ergocryptam [60]	β,β-Ergoannam	β-Ergocryptam [60]
C(2)	119.0	119.0	119.2	119.2/1	119.1	119.1	119.4	119.2	119.8	118.8	118.9
C(3)	110.8	110.3	110.6	110.6/7	110.7	108.9	118.8	110.5	111.0	111.1	110.9
C(4)	26.6	26.4	26.6	26.5	26.6	26.6		26.7	29.1	29.8	29.6
C(5)	59.4	59.5	59.3	59.2/64.5	64.0	62.6	62.4	64.1	62.5	63.3	63.2
N(6)-CH_3	44.1	44.6	44.4	44.3	44.3	43.4	43.4	44.3	44.3	44.4	44.3
C(7)	49.2	48.4	48.2	48.1	48.0	55.5	55.1	48.2	50.8	50.4	50.6
C(8)	41.3	41.3	40.9	40.9	40.9	42.8	42.5	40.9	41.8	41.7	41.7
C(9)	119.2	119.2	118.8	118.8	119.2	120.1	118.3	118.9	118.8	119.8	119.2
C(10)	138.8	138.8	139.1	139.1/2	139.2	135.0	136.0	139.0	137.6	137, 9	137.9
C(11)	129.8	129.8	129.6	129.6	129.7	127.4	127.1	129.6	129.4	129.7	129.4
C(12)	112.0	112.1	111.9	111.9	112.0	111.0	111.0	111.9	111.9	111.8	111.8
C(13)	123.4	123.5	123.3	123.3	123.3	122.4	122.2	123.2	123.5	123.3	123.2
C(14)	110.0	110.0	110.1	110.1	110.1	109.2	110.2	110.2	109.8	109.8	109.9
C(15)	133.8	133.8	133.9	133.8/9	133.8	133.7	133.8	133.9	133.9	133, 9	133.9
C(16)	126.2	126.2	126.2	126.2/3	126.3	125.8	125.9	126.3	126.4	126, 3	126.3
-CONH (17)	173.9	174.0	176.2	176.2/3	176.3	171.2	174.3	176.1	174.5	174.7	174.4
C(2′)	89.2	89.2	89.6	89.7	89.5	–	–	89.5	58.2	56.5	58.1
C(3′)	164.7	165.7	164.7	165.8	164.6	–	–	165.0	173.7	173.6	173.6
C(5′)	60.7	53.1	60.7	53.3	59.7	–	–	60.4	59.0	60.0	60.0
C(6′)	168.7	167.4	166.8	166.2	166.6	–	–	167.1	165.6	165.6	165.6
C(8′)	45.8	46.1	45.8	46.0	45.9	–	–	46.0	45.5	45.5	45.5
C(9′)	22.1	21.9	21.7	21.6	21.3	–	–	21.7	22.9	22.9	22.9
C(10′)	22.2	22.0	22.1	22.1/2	22.2	–	–	22.1	23.3	23.1	23.3
C(11′)	61.4	61.9	64.1	64.5/59.3	59.2	–	–	59.3	59.5	60.3	60.4
C(12′)	106.8	106.4	103.6	103.5/4	103.6	–	–	103.7	169.9	170.0	170.0
(12′)-OCH_3	49.2	48.8	–	–	–	–	–	–	–	–	–
C(13′)	35.7	35.9	34.3	34.3	34.3	–	–	34.3	30.3	36.8	30.3
C(14′)	16.0	16.0	15.3	15.4/3	15.3	–	–	15.3	16.0	27.4	16.1
C(15′)	16.7	16.8	16.9	16.9	17.0	–	–	16.9	20.1	11.7	20.1
C(16′)	32.3	42.2	32.8	43.5	39.4	–	–	39.8	40.3	13.6	37.2
C(17′)	–	25.0	–	25.1	27.9	–	–	26.3	25.1	37.3	25.7
C(18′)	19.5	22.1	19.0	22.2/1	12.6	–	–	12.5	21.6	25.7	10.9
C(19′)	19.2	21.4	20.5	22.6	15.2	–	–	16.6	23.2	10.9	15.5
C(20′)	–	–	–	–	–	–	–	–	–	15.5	–

20, 35, 36]. Although the spectra of various alkaloids are identical, the individual detection is possible by using different excitation wavelengths [16, 30]. The fluorescence spectra of ergot alkaloids depend on the solvent and the pH value. In particular, ethanol solutions exhibit maximum intensity at an excitation energy of 320 – 340 nm and show emission peaks at 400 – 425 nm.

This method is widely employed in studying the products of metabolism of ergot alkaloids and other lysergic acid derivatives in the human and animal organisms. As a rule, the spectrofluorimetry is used in combination with chromatographic techniques [37].

Infrared Spectroscopy

The IR spectra of ergot alkaloids are mostly used for identification and characterization of the individual (natural and synthetic) compounds [5, 6, 13 – 15, 38 – 45].

The bands in the IR spectrum observed in the region of 3460, 3160, and 3300 cm^{-1} correspond to the OH and NH groups of alkaloids; the bands at 1721 – 1723, 1663, 1644 – 1648, and 1540 – 1550 cm^{-1} are signals characteristic of the CO groups [38, 39, 45 – 48].

Nuclear Magnetic Resonance Spectroscopy

NMR spectroscopy is one of the main methods used for identifying ergot alkaloids, studying the stereoconfiguration, structure, and lability of molecules, and solving problems related to changes in the configuration and structure of these compounds [38, 39, 41, 44, 45, 48 – 60]. Using this method, it is possible to determine the ratio of alkaloids in a mixture while simultaneously confirming the component structures, which facilitates the solution of many technological problems [57 – 59].

Tables 1 and 2 present a summary of data on the ^{1}H and ^{13}C NMR spectra, describing signals characteristic of some atoms, functional groups, and derivatives of ergot alkaloids.

Mass Spectrometry

The method of mass spectrometry (MS) is used for identifying ergot alkaloids, determining their structures, and characterizing the peptide fragments of molecules [16, 50, 60 – 62]. Under the same ionization conditions, the fragmentation of alkaloid molecules in the peptide part proceeds by a general scheme in which the ion masses depend only on the peptide fragment and, hence, are characteristic of the given compound. This general scheme of the fragmentation of ergot alkaloids was presented in [50, 61].

Using the MS method, it is possible to determine quite rapidly the possible pattern of changes in the peptide part of an ergot alkaloid molecule. For example, this technique was successfully employed in the study of ergometrine and ergine rotational isomers [56].

Paper Chromatography

The first successful application of the paper chromatography to the analysis of ergot alkaloids dates back to 1949, when Foster used this method to separate ergometrine isomers [5]. Then Stoll and Ruegger [63] adapted paper chromatography to separate ergot alkaloids of various groups using formamide – water mobile phases with various pH values. This method [23, 64] was successfully employed to separate and study ergot peptide alkaloids, ergotoxins, and ergotamine tartrate [2, 3, 26, 65]. This was achieved using various mobile phases such as benzene – formamide, chloroform – methanol, and acetone or ether with concentrated ammonia additives.

Thin-Layer Chromatography

At present, TLC techniques have practically displaced paper chromatography [5, 12, 16, 66] and are widely used for separating and identifying ergot alkaloids, characterizing the alkaloid composition of ergot sclerotium, studying the stability and degradation pathways of related drugs during storage, determining the content of impurities in parent substances, etc. [18, 67 – 72]. In addition to the instrumental analytical techniques, TLC is employed for determining the amino acid composition of the peptide part of ergot alkaloid molecules [50, 73].

TLC can be used both separately and in combination with spectrophotometric, colorimetric, spectrofluorimetric, or densitometric quantitative analysis [28, 73]. Ergot alkaloids are detected by treating the TLC plates with chromogenic reagents (Dragendorff's, Van Urk's, etc.) or by measuring the intrinsic fluorescence of ergot alkaloids in the UV range at 254 or 360 nm [12, 16, 25, 28, 50, 66, 69, 70, 72 – 79].

Conditions for the TLC separation and identification of ergot alkaloids have been extensively studied. The components of complex mixtures were separated using sorbents of various types, including cellulose [78], aluminum oxide [16, 27, 37, 80], and (most frequently) silica gel [28, 75 – 77, 81]. The best results were obtained using specially pretreated sorbents, whereby the TLC plates are activated or impregnated (usually, with formamide or its mixtures with ethanol and ammonia). It should be noted that both the TLC conditions and the quality of reagents have to be thoroughly monitored [20, 21].

The best results in separating alkaloids of the ergotoxin group, some representatives of the ergoxine and ergotamine groups, and dihydro derivatives of ergot alkaloids were obtained using mobile phases based on diisopropyl ester with ethyl or methyl alcohols, toluene, tetrahydrofuran (THF), *p*-heptane, dichloromethane, benzene, or diethylamine [35, 69, 70, 81]. Also widely used are mobile phases based on chloroform and dichloromethane with additives of ethyl, methyl, or butyl alcohols, acetone, dioxane, heptane, diethylamine, ammonia, etc. [28, 34, 50, 82 – 84]. Usually,

these are two- to four-component mixtures of the types dichloromethane – methanol (9 : 1), chloroform – ethanol (8 : 2), chloroform – ethanol – acetone – ammonia (4 : 1 : 1 : 0.1), dichlorometane – dioxane – ethanol – ammonia (36 : 3:1 : 0.1), etc.

Chromatofluorimetric Analysis

Using the TLC procedure followed by scanning the chromatograms, it is possible to perform a rapid analysis of the content of ergot alkaloids. This is important for the screening analyses in the course of ergot strain selection, the comparative study of various strains, the monitoring of technological processes, etc. This combination of methods offers a high sensitivity (detecting 15 ng of alkaloid per spot). Moreover, using a specially developed procedure, whereby the spots of ergot alkaloids in the TLC patterns are treated with sulfuric acid containing *o*-phthalic aldehyde, it is possible to detect as small as 1 ng of lysergic acid derivatives [16, 87].

Pollack [88 – 90] theoretically studied the principles of TLC with fluorescent scanning, including the effects of sorbent type and thickness, the amount of a sample, and the size and shape of spots. The role of the sorption properties and pH of sorbate and the conditions of fluorescence measurements were studied by Prosek et al. [78, 91, 92]. The fluorescence spectra of ergotamine, ergometrine, ergotoxin alkaloids, and their isomers usually exhibit identical spectra with maxima at 440, 520, 540, 560, and 590 nm [35, 36, 93, 94]. The fluorescence intensity of ergot alkaloids was determined using excitation at 313 nm and optical filters for 520 nm [35, 95] or 445 nm [96]; in [78, 79], the spectra excited at 325, 313, and 230 nm were measured at 445, 390, and 250 – 320 nm. In order to reduce the measurement error, it was suggested to perform the calculations with special corrections for the properties of particular alkaloids, the amount of sample, and the spot size. The coefficients of sorption for individual alkaloids were determined.

The development of new optical scaners for TLC, making the fluorescence measurements quite rapid, markedly improved the prospects for this method.

Gas Chromatography

The GC method was used for the separation and identification of ergot alkaloids. The analyses were performed in glass columns because metal parts catalyzed decomposition of the samples in the course of measurements [30, 86, 97, 98]. Szepesy et al. [86, 98] used GC in a temperature-programmed mode to analyze “dihydroergotoxin,” representing a mixture of dihydro derivatives of alkaloids of the ergotoxin group.

This method did not find wide application, since ergot alkaloids readily decompose on heating. The GC procedure

TABLE 3. Sorbents and Mobile Phases for the HPLC Analysis of Ergot Alkaloids

Sorbent (particle size, μm)	Mobile phase	Ref.
Lichrosorb Si-60 (10)	$CHCl_3$ – MeOH, 95 : 5	[86]
Silica Si-60 (10)	$CHCl_3$ – hexane – EtOH, 40 : 40 : 10;	
Lichrosorb RP-2 (5)	MeCN – 0.01 M $(NH_4)_2CO_3$, 2 : 3	
Lichrosorb RP-18, 12		
Silica RP-18, 12		
Silica RP-18 (10)		
Lichrosorb RP-18 (10)	THF – 0.01 M $AcONH_4$, 4 : 6	[103, 112]
Lichrosorb RP-18 (5)	MeCN – $(NH_4)_2CO_3$ solution, 30 : 70, 50 : 50	[104 – 105]
Lichrosorb RP-8		
Lichrosorb RP-2		
Spherisorb ODS (5)		
MicroPak NH 10	Diethyl ether – EtOH, 9 : 1	[56]
Zorbax ODS C-8	MeCN – 0.005 M $AcONH_4$, 44 : 56 to 65 : 35	[106 – 107]
	MeOH – 0.005/0.01 M $AcONH_4$, 70 : 30, 65 : 35	
	EtOH – 0.05 M $AcONH_4$, 70 : 30, 65 : 35	
	THF – 0.01 M $AcONH_4$, 32 : 68 to 65 : 35	
Lichrosorb Si-60 (5)	Hexane – $CHCl_3$ – MeCN, 56 : 22 : 22, 55 : 20 : 25, 55 : 20 : 23	[108]
Lichrosorb Si-60 (10)	56 : 22 : 22 (5)5 : 20 : 25 (5)5 : 20 : 23	
ODS-Hypersil (5)	MeOH – phosphate buffer (pH 8.1), 60 : 40	[109]
Bondapak C18	MeCN – 0.01 M $(NH_4)_2CO_3$, gradient 3.7 : 0.3 to 1 : 1	[110]
Nucleosil C18		
Lichrosorb 2P (10)	Water – MeCN – triethylamine, 80 : 20 : 2	[29]
Corasil	$CHCl_3$–MeOH–AcOEt–MeCOOH, 60 : 20 : 50 : 3	[111]
MicroPak Si-18	$CHCl_3$–EtOH, 95 : 5	[83]

imposes very high requirements on the quality of materials and is highly sensitive to the conditions of analysis.

High-Performance Liquid Chromatography

HPLC is now widely used for both qualitative and quantitative analysis of ergot alkaloids, for the characterization of raw materials (ergot sclerotium and saprophytic cultures), and for the investigation of biological fluids, technological products, and medicinal preparations containing ergot alkaloids [16, 99 – 113].

There are many papers devoted to determining the optimum conditions for the HPLC analysis of ergot alkaloids, including selection of the sorbents and eluents (solvent system, polarity, pH, etc.) [12, 86, 99]. In view of the high lability of ergot alkaloids, the conditions of HPLC analysis have to be thoroughly specified. The best results in separating ergot alkaloids were obtained using a reverse-phase HPLC column eluted in an acetonitrile-based system. Wehrli et al. [102] showed that the quality of separation of ergot alkaloids depends on the eluent pH and alkalizing additive. Some data on the conditions of HPLC analyses of ergot alkaloids are summarized in Table 3.

Isolation of Ergot Alkaloids from Raw Materials

Methods used for the isolation of ergot alkaloids depend primarily on the physicochemical properties of an individual target product (group of alkaloids) and on the particular analytical problem to be solved.

Organic solvents most frequently used to isolate alkaloids in the form of bases include ether, chloroform, dichloromethane, and some other. In order to obtain salts, the extraction is performed with acidified aqueous solutions of acetone, methanol, ethanol, etc. Sometimes, the raw materials are preliminarily purified before extraction, whereby the material is degreased with petroleum ether or some other solvent [16, 24].

The ergot peptides exhibit solvation, with the solvate depending both on the solvate and the ergot alkaloid structure. For example, β-ergocryptine does not crystallize from methyl alcohol, while ergocornine forms a crystal solvate containing one methanol molecule [9, 41]. Crystallized from benzene, α- and β-ergocryptines are characterized by the formation of crystal solvates containing two benzene molecules, while ergostine forms a solvate with a single benzene molecule [39, 65]. Ergocryptine mesylate is virtually insoluble in water but, upon forming a solvate with ethanol, can be mixed with water in any proportion. The formation of solvates is a necessary stage in the technology of isolation of ergot alkaloids.

The first methods proposed for the isolation of ergot alkaloids were based on their extraction with ether in the presence of ammonia [6, 24, 26, 35, 64, 93]. A more complete and rapid extraction is provided by chloroform and acetone. However, attempts at the subsequent purification of such products showed that the passage of alkaloids from chloroform extracts into tartrate solutions is complicated by certain factors and may lead to losses of the target products [24]. Ergot alkaloids can be extracted with acidified (pH 2) aqueous solutions [24]. Ergot alkaloids can be extracted from the raw material with benzene [42]. Extraction with methylene chloride [50], followed by chromatographic purification, was used for the rapid analysis of individual ergot sclerotium.

In all cases, the highly labile ergot alkaloid samples should be handled with great care. Most of the procedures are usually performed under controlled temperature conditions in the dark (or under red light), and if necessary, in an inert gas atmosphere. The quality of solvents and other reagents should be thoroughly checked.

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