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SEPARATION AND DETERMINATION OF FOUR ERGOT ALKALOIDS, DIHYDROERGOTAMINE-, DIHYDROERGOCORNINE-, DIHYDROERGOCRYPTINE- AND DIHYDROERGOCRISTINE METHANESULFONATES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A high-performance liquid chromatography (HPLC) separation of 4 ergot alkaloids, dihydroergotamine (A), dihydroergocornine (B), dihydroergocryptine (C) and dihydroergocristine (D) as their methanesulfonates is reported. The analysis is performed on two reversed-phase columns (RP 8, RP 18) with 5 different mobile phases. An optimum separation is obtained with acetonitrile–water mixtures at about pH 7 under the addition of citrate, acetate or bromide salts. α and β isomers of compound C could only be resolved with an acetonitrile–water mixture containing about 2% diethylamine at about pH 12. An ion-pair mechanism of separation is discussed. The method shows good reproducibility and high selectivity.

INTRODUCTION

High-performance liquid chromatography (HPLC) has been applied for the separation and quantitative determination of ergot alkaloids. For this purpose liquid solid chromatography on silica gel columns (Wittwer and Kluckhohn, 1973; Heacock et al., 1973; Szepesy et al., 1978), different chemically bonded reversed-phase packings (Szepesy et al., 1978; Jane and Wheals, 1973; Hartmann et al., 1978; Dolinar, 1977), micropak NH_2 columns (Würst et al., 1978) and a reversed-phase system with solvent gradient (Bethke et al., 1976) have been used. The silica packings served for group separation of some alkaloids such as analysis of ergotamine or ergocristine-rich plant extracts and contaminants (Szepesy et al., 1978), whereas the separation of active components ergotamine, ergocornine, ergocryptine, ergocristine and their dihydroderivatives was accomplished on reversed-phase systems (Szepesy et al., 1978; Jane and Wheals, 1973; Hartmann et al., 1978; Dolinar, 1977; Twitchett et al., 1978). The mobile phases used in reversed-phase systems were mostly mixtures of methanol, ethanol or acetonitrile and an aqueous solu-

tion of ammonium salts or a phosphate buffer solution. The pH values of the mobile phases varied between 8 and 13. Only the methanol–ammonium acetate mobile phase used by Hartmann et al. (1978) is reported to have shown a pH value of about 7, whereas the analysis time for the separation of components of dihydroergotoxine has increased to 30 min with a flow-rate of 3.3 ml/min. Twitchett et al. (1978) obtained only margirally slower retention with a flow rate of 1 ml/min.

The present investigation was carried out on two different reversed-phase columns, RP 8 and RP 18, with 5 different mobile phases. Except with the mobile phases IV and V, the pH value was not allowed to exceed 7.15 and was adjusted with triethanolamine. Tetradecyl trimethyl ammonium bromide was used as a paired-ion chromatographic reagent (PIC) in analogy to other quaternary ammonium PIC reagents such as tetrabutyl ammonium phosphate described in the literature (Waters, 1976). The separation of ergot alkaloids was accomplished in 10–15 min with a flow-rate of only 1 ml/min, under isocratic conditions.

MATERIALS AND METHODS

All chemicals and solvents used were of reagent grade or pure quality. The separation of ergot alkaloids was carried out simultaneously with two liquid chromatographs, a Perkin–Elmer Liquid Chromatograph 1220 and a Liquid Chromatograph series 2. In both cases a variable wavelength UV detector LC 55 and a recorder 123, both from Perkin–Elmer, were used. A Hewlett–Packard laboratory data system 3352 C was connected to both liquid chromatographs through A/D convertors. The series 2 liquid chromatograph had further a Rheodyne injection system.

Chromatographic columns

(I) A Perkin–Elmer reversed-phase column, C_{18}, 0.25 m × 4 mm i.d., packed with silica gel SI-100, 10 μm, coated with octadecylsilane chemically bonded organic phase.

(II) A Knauer reversed-phase column, Lichrosorb RP 8, 0.25 m × 4.6 mm i.d., 7 μm, coated with octylsilane chemically bonded organic phase.

Mobile-phases

(I) Acetonitrile + water + triethanolamine + citric acid (45 ml + 60 ml + 0.4 ml + 0.166 g); pH 7.1.

(II) Acetonitrile + water + triethanolamine + sodium acetate (45 ml + 60 ml + 1 ml + 0.300 g) the pH value was adjusted to 7.1 with two drops of acetic acid.

(III) Acetonitrile + water + tetradecyl trimethyl ammonium bromide (45 ml + 60 ml + 0.100 g); the pH value was adjusted with two drops of triethanolamine to 7.1.

(IV) Acetonitrile + water + ammonium carbonate (45 ml + 60 ml + 0.040 g); pH value: 8.3.

(V) Acetonitrile + water + diethylamine (37.5 : 62.5 : 2.1); pH 12.3.

Standard substances

Dihydroergotamine methanesulfonate (A), dihydroergocornine methanesulfonate (B), dihydroergocryptine methansulfonate, α + β isomers (C) and dihydroergocristine

methanesulfonate (D) were obtained through the courtesy of Fa. Rentschler, Laupheim (G.F.R.). The substances were dried in vacuum over drying blue gel at room temperature and were protected from light, heat and humidity.

Of each of the substances A, B, C and D, 6.7 mg were dissolved in a water–acetonitrile mixture (60 + 45) in a 20.0 ml volumetric flask and made up to volume with the solvent. The standard solution was protected from light and air and every alternate day a new solution was prepared. Ten μl of this solution were injected directly into the liquid chromatograph. The measurement was repeated 7–8 times in order to obtain precision and accuracy of the method.

A liquid pharmaceutical product containing the substances B, C and D in the proportion 1 : 1 : 1 was also analyzed. Samples of the same product subjected to different stress conditions were also tested. Ten μl of the sample solution were injected directly into the liquid chromatograph without any pretreatment.

RESULTS AND DISCUSSION

RP 8 column

All 4 compounds A, B, C and D could be separated on the RP 8 column with the mobile phases I–IV. The α and β isomers of compound C could only be resolved with the mobile phase V in strongly alkaline medium. The chromatograms obtained with one of the mobile phases (III) on this RP 8 column is shown in Fig. 1. Capacity factors k', selectivity coefficients α and effective plates per meter, n/m are presented in Table 1.

The addition of a conventional paired-ion chromatographic reagent containing a large organic ion such as tetradecyl trimethyl ammonium bromide, did not further improve the resolution on this column in comparison to the mobile phases I and II containing citrate and acetate. As will be shown below, the counter-ion taking part in the ion-pair mechanism is not the tetradecyl trimethyl ammonium cation but the bromide anion. The pH value of three mobile phases I, II, III was adjusted between 7.0 and 7.15 using triethanolamine as a weak organic base. The mobile phase IV with 4.16×10^{-3} M ammonium carbonate showed a more alkaline pH of 8.3, whereas the mobile phase V with about 2% diethylamine was strongly alkaline with a pH of 12.3. The separation of α and β isomers of compound C could only be obtained in strongly alkaline medium with mobile phase V (Tables 1 and 2). Acetonitrile was chosen as the organic component of the eluent due to its less polar character, lower viscosity and greater selectivity. An acetonitrile content of less than 30% does not bring or retards the elution of ergot alkaloids, leading to peak broadening and bad resolution. The detection limit for all 4 alkaloids lies at about 50 μg/ml for an injected volume of 10 μl. The relative standard deviation (RSD) was found to lie between 1.6 and 2.0%. Three liquid pharmaceutical preparations subjected to different stress conditions when analyzed showed varying degrees of degradation. All degradation products appeared in the initial part of the chromatogram and were not further identified. The results with RSDs are presented in Table 3.

RP 18 column

The chromatographic parameters obtained on this column with all 5 mobile phases are presented in Table 2. RP 18 reversed-phase with a long hydrocarbon chain did not

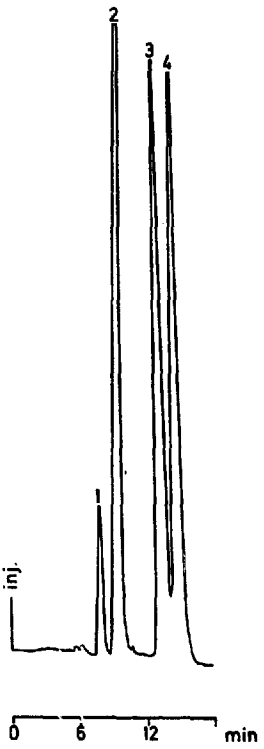

Fig. 1. Separation of four ergot alkaloids on a Knauer RP 8 column, 7 μm, with mobile phase III. Flow rate: 1 ml/min; 1, dihydroergotamine methanesulfonate (A); 2, dihydroergocornine methanesulfonate (B); 3, dihydroergocryptine methanesulfonate (C); 4, dihydroergocristine methanesulfonate (D).

separate A from the other three compounds B, C and D. The peak of A almost overlapped with the peak of compound B. There were only about 3000 effective plates per meter (Table 2). The best separation with almost no peak-broadening was obtained with the mobile phases I and III.

The major advantage of the reported method lies in the speed of analysis (about 15 min) and the low pH values of mobile phases, still rendering a very good resolution and base-line separation under isocratic conditions. Only the separation of α and β isomers of compound C needs a higher pH value of about 12.

A mobile phase containing the same amounts of acetonitrile and water, as in the case of mobile phases I, II and III, with the pH value simply adjusted to about 7 with triethanolamine did not elute the compounds A, B, C and D from the columns used. This shows clearly that the addition of citric acid, sodium acetate + acetic acid, tetradecyl trimethyl ammonium bromide or potassium bromide to the respective mobile phases was necessary for the elution and separation of all 4 compounds. The separation and resolution of all 4 compounds is governed here by some sort of ion-pair mechanism.

The principles and applications of ion-pair partition chromatography have been described elsewhere (Su et al., 1976; Huen et al., 1978; Wahlund and Sokolowski, 1978). The mode of chromatography applied in this investigation can best be termed, as sug-

TABLE 1

CAPACITY FACTORS (k'), SELECTIVITY COEFFICIENTS (α) AND EFFECTIVE PLATES PER METER (n/m) FOR ERGOT ALKALOIDS ON A REVERSED-PHASE RP 8 COLUMN, 7 μm WITH 5 DIFFERENT MOBILE PHASES

Mobile Phase		Dihydro-ergotamine methane-sulfonate	Dihydro-ergocornine methane-sulfonate	Dihydro-ergocryptine methane-sulfonate, α-isomer	Dihydro-ergocristine methane-sulfonate	Dihydro-ergocryptine methane-sulfonate, β-isomer
I	k'	2.68	3.45	4.92	5.50	
	α	1.29	1.43	1.12		
	n/m	4912	4986	9313	9195	
II	k'	3.29	4.00	5.71	6.24	
	α	1.21	1.43	1.09		
	n/m	5362	5632	10,770	10,807	
III	k'	2.08	2.65	3.84	4.22	
	α	1.27	1.45	1.10		
	n/m	3638	4624	9212	8864	
IV	k'	4.66	5.72	8.21	9.26	
	α	1.23	1.44	1.13		
	n/m	8221	7769	10,403	10,725	
V	k'		1.30	1.75	2.15	3.05
	α		1.35	1.23	1.42	
	n/m		1290	2096	3162	4259

gested by Knox (Knox and Jurand, 1975) as 'reversed phase ion-pair partition chromatography'. The species which are partitioned between the mobile and stationary phases can thus be contemplated to be ion-pairs or ion-pair complexes of weakly dissociated cations of A, B, C and D formed with acetate, citrate, bromide or sulfate anions. In the case of mobile phase III containing tetradecyl trimethyl ammonium bromide as PIC reagent, the counter-ion responsible for the separation mechanism is not the ammonium cation but the bromide anion. This was verified by replacing this reagent in mobile phase III with the same amount of potassium bromide. As Fig. 2 shows there is still a very good baseline separation of all 4 compounds. Had it been the tetradecyl trimethyl ammonium cation functioning as the counter-ion in the separation mechanism, there should not have been a good separation with sharp symmetric peaks having k' values between 1.43 and 5.57 with potassium bromide alone (Fig. 2). The same phenomenon was observed when 0.1% sodium sulfate was added to the mobile phase III instead of PIC reagent. K' values are here also increased compared to those obtained with mobile phase III (Table 1).

If the pH of the mobile phases I–III was made more acidic, between 2 and 4, with acetic acid or sulfuric acid, a bad separation with poor resolution due to earlier elution and overlapping of the peaks of A, B, C and D was obtained. In acidic medium separation is thus inadequate, probably due to an ionic-suppression mechanism. In alkaline solution with pH over 8, as in the case of mobile phase IV containing ammonium carbonate, the

TABLE 2

CAPACITY FACTORS (k'), SELECTIVITY COEFFICIENTS (α) AND EFFECTIVE PLATES PER METER (n/m) FOR ERGOT ALKALOIDS ON A REVERSED-PHASE RP 18 COLUMN, 10 μm WITH 5 DIFFERENT MOBILE PHASES

Mobile Phase		Dihydro-ergotamine methane-sulfonate	Dihydro-ergocornine methane-sulfonate	Dihydro-ergocryptine methane-sulfonate, α-isomer	Dihydro-ergocristine methane-sulfonate	Dihydro-ergocryptine methane-sulfonate, β-isomer
I	k'		4.08	5.92	7.17	
	α		1.45	1.21		
	n/m		1478	2040	2116	
II	k'		4.33	6.22	7.5	
	α		1.44	1.21		
	n/m		1740	2297	2390	
III	k'		2.30	3.36	4.21	
	α		1.46	1.25		
	n/m		1630	2037	2425	
IV	k'		6.66	10.12	12.72	
	α		1.52	1.26		
	n/m		1445	3363	2713	
V	k'		1.09	1.43	2.24	2.81
	α		1.31	1.57	1.25	
	n/m		544	684	1195	2007

mechanism of separation is different. Here the methanesulfonates of A, B, C and D are converted to their free bases which partition between the stationary phase of the packing and the acetonitrile–water mobile phase. In strongly alkaline medium with pH over 12, as in the case of mobile phase V containing almost 2% diethylamine, ionized free bases of ergot alkaloids are very little retarded and eluted quite early, their k' values lying between 1.3 and 3.05 (Table 1).

RP 8 column with 7 μm particle size showed a better selectivity and resolution of the 4 compounds than the RP 18 columns with 10 μm particles where the peak of the substance A overlapped with the peak of B (Table 2).

A pharmaceutical liquid sample containing the prescribed amounts of B, C and D in the proportion 1 : 1 : 1 thus analyzed for the individual content of α and β isomers of dihydroergocryptine methanesulfonate (C) showed about 19% β-isomer and 81% α-isomer.

Nevertheless it is recommended to use the mobile phases I, II and III with pH values of about 7 for the routine separation of A, B, C and D. The constant exposure of the column to highly alkaline conditions, even with organic amines, does affect the column efficiency, contrary to literature reports.

It is thus advisable to use one of the first three mobile phases, I, II or III, where α and β-isomers are eluted together and also determined as such. Pharmacological investigations

TABLE 3

RESULTS OF ANALYSIS OF ERGOT ALKALOID SAMPLES SUBJECTED TO DIFFERENT STRESS CONDITIONS

Sample X: stored at room temperature for 6 months; sample Y: stored at 61°C for 6 months; sample Z: overaged sample stored at 61°C for 1 year

	Sample	% content found	relative standard deviation %
Dihydroergocornine methanesulfonate	I	96.98	1.63 (n = 7)
	II	90.77	1.66 (n = 6)
	III	51.30	2.0 (n = 6)
Dihydroergocryptine methanesulfonate $\alpha + \beta$-isomers	I	104.00	1.44 (n = 7)
	II	97.00	1.60 (n = 6)
	III	56.80	1.85 (n = 6)
Dihydroergocristine methanesulfonate	I	104.00	1.88 (n = 7)
	II	92.80	1.62 (n = 5)
	III	54.50	1.66 (n = 6)

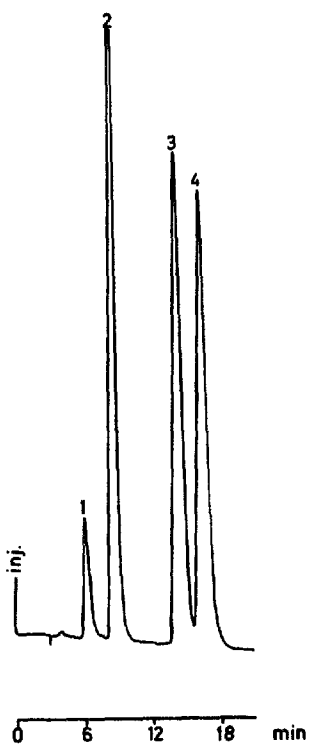

Fig. 2. Separation of ergot alkaloids on a RP 8 column, 7 μm with mobile phase III containing 0.100 g potassium bromide instead of tetradecyl trimethyl ammonium bromide; flow rate: 1 ml/min. 1, compound A; 2, compound B; 3, compound C; 4, compound D.

in the past have shown that the two isomers dihydro-α-ergocryptine and dihydro-β-ergocryptine differ only insignificantly from each other in their pharmacodynamic properties (Schlientz et al., 1968). Due to this fact there should be no immediate need for their routine separation which ultimately affects the column efficiency.

It is important that the standard solutions of all 4 compounds A, B, C and D are prepared using the mobile phase as the solvent. Highly irreproducible results were obtained when methanol was used as a solvent to dissolve the A, B, C and D standards.

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