Journal of Chromatography, 123 (1976) 193–203

CHROM. 9018

DETERMINATION OF THE CONTENT AND PURITY OF ERGOTAMINE PREPARATIONS BY MEANS OF HIGH-PRESSURE LIQUID CHROMATOGRAPHY

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(Received December 31st, 1975)

SUMMARY

A reversed-phase system of high-pressure liquid chromatography with solvent gradient is described for testing the purity of ergotamine as an active substance and for checking its concentration in pharmaceutical preparations. Because of its good resolution, this system can be used not only for the selective assay of ergotamine but also for the identification and quantitative determination, in the same chromatogram, of seven known isomerization and hydrolysis breakdown products. Simultaneous detection at two different UV wavelengths also makes it possible to measure further breakdown products formed by addition at the 9,10 double bond (lumi-compounds). The advantages of the system lie in the determination of all of the products within *ca.* 20 min, direct injection of low-dosage injection solutions and sensitive detection of polar breakdown products. The system is reproducible with regard to retention times and quantitative determination. It is suitable as a quality-control method for the routine determination of the content and purity of ergotamine preparations.

INTRODUCTION

There has been an increasing demand in pharmaceutical analysis for efficient methods which allow the determination of active substances and of their by-products and degradation products in a single operation. The accurate and reproducible, selective, quantitative determination of the active substance is one of the important requirements. In purity checks, an unambiguous and sensitive identification of breakdown products is indispensable. Verification of an increase in breakdown products, in conjunction with that of a decrease in the content of the active substance, provides additional safeguards in shelf-life analyses[1] and in checks on process technology.

The determination of small quantities of breakdown products is particularly difficult when these products are much more polar than the active substance itself and are therefore more difficult to isolate and more difficult to separate from each

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other chromatographically. Such products may also be formed as "analysis artefacts" in the process of preparing samples of active substances which are sensitive to light, air, acids or bases, thus vitiating the findings of a purity analysis. In stability investigations aimed at determining the shelf-life of an active substance by measuring the temporal rate of increase in breakdown products[2,3], the formation of artefacts may vitiate the findings completely. Amongst the various chromatographic methods for separation and determination, gas chromatography (GC) is not universally applicable because the thermal stress involved is excessive for certain compounds, particularly many types of alkaloids. Thin-layer chromatography (TLC) and high-pressure liquid chromatography (HPLC) with adsorption systems or with a distribution system on a polar stationary phase are often unsuitable for polar breakdown products.

Ergotamine (compound I in Fig. 1) is a lysergic acid derivative of the peptide type. It is extensively used in anti-migraine drugs because of its sympathicolytic action[4], and in obstetrics because it induces uterine contraction[5]. Ergotamine poses difficult analytical problems because, whilst it can be determined quantitatively quite easily by measuring[5] its UV absorbance or fluorescence or by means of the colorimetric reaction according to Van Urk[6–8], it is unstable and forms various isomerization, hydrolysis and addition breakdown products, with wide differences in polarity. Ergotamine is sensitive to acids and to bases (isomerization and hydrolysis) and to light (oxidation and addition)[5]. The most important breakdown products are shown in Fig. 1. Compounds II–IV are isomerization products, compounds V–VIII are hydrolysis products and compound IX is one of the possible addition compounds. It should be noted in this connection that the addition of water is also known with all of the other compounds (II–VIII), so that they all can form photochemical products similar to IX ("lumi-compounds")[5].

Because of the thermal instability and low vapour pressure of ergotamine and related compounds, only liquid chromatographic systems (PC, TLC, HPLC) are suitable for chromatographic separation. The separation methods of paper chromatography (PC) and TLC, described in the literature, often require very special stationary or mobile phases[9–11] and, in addition, hardly allow a quantitative determination of all of the breakdown products, either after elution[12] or by direct densitometric evaluation. In the high-pressure chromatographic separation described by Waters Assoc.[13], the peak resolution achieved is not sufficient to ensure accurate quantitative determination of all of the compounds involved.

This paper describes a HPLC system which makes it possible to solve all of the problems adumbrated above concerning the analysis of ergotamine preparations, and the determination can be carried out in *ca.* 20 min.

EXPERIMENTAL

Reagents

Ergotamine tartrate and all of the breakdown products were obtained from Sandoz, Basle, Switzerland. The reagents and solvents used were obtained from E. Merck, Darmstadt, G.F.R., and were of analytical quality. The water was doubly distilled. The standard solutions were prepared in acetonitrile–water or in an injection solution of a placebo.

The adsorbents used were μBondapak C_{18} (particle size, 10 μm) in a 30 cm $\times$

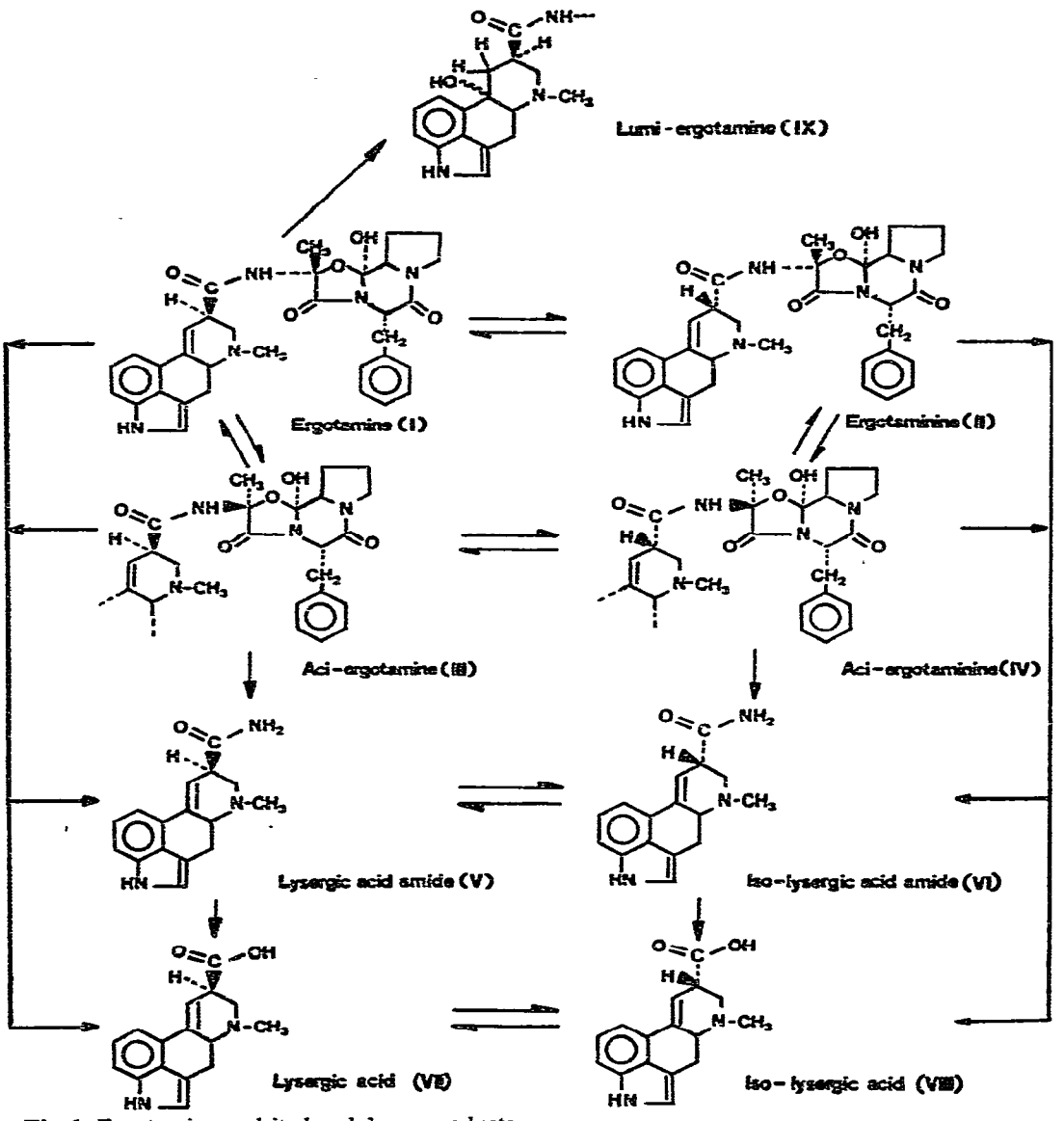

Fig. 1. Ergotamine and its breakdown products.

4 mm I.D. ready-packed column (Waters Assoc., Milford, Mass., U.S.A.), and Nucleosil C_{18} (particle size, 10 μm) (Macherey, Nagel & Co., Düren, G.F.R.) in a 30 cm $\times$ 4.6 mm I.D. steel column which we packed ourselves.

Instruments

All of the chromatographic separations were carried out on a Waters ALC 202 chromatograph with two M 6000 pumps and a 660 solvent programmer. The detectors used were Hewlett-Packard Model 1030 B and Perkin-Elmer LC 55 variable-wave-

length UV detectors. The integrations were performed with a Perkin-Elmer SIP 1 integrator. 25- or 50-μl amounts were injected by means of a Hamilton syringe (50 μl, Model 705) and a Waters Model U6K universal injector. The column which we prepared was packed according to the "balanced density" technique[14–16] in which the adsorbent medium was first sedimented in octane. The height equivalent to a theoretical plate (HETP) achieved was 150 μm at a linear flow velocity of 1 cm/sec and capacity factors (k') of *ca.* 10–20. The mobile phases used were constant (isocratic) and variable (gradient) mixtures of the components, 0.01 *m* ammonium carbonate in water and acetonitrile. The compositions, flow velocities and pressures are shown on the chromatograms.

RESULTS

Chromatographic system: qualitative investigations

Of the breakdown products of ergotamine (I), shown in Fig. 1, only the C_8 epimer ergotaminine (II) is less polar than ergotamine itself. All of the other products are very polar, especially the acids VII and VIII which are formed by hydrolysis. The main problem in devising an analytical system, therefore, was to be able to detect and to determine the polar breakdown products with sufficient sensitivity and, despite the great differences in polarity, in a reasonable separation time.

On the basis of our own work on TLC of ergot alkaloids on silica gel as stationary phase, and of results obtained with HPLC of these compounds by liquid–solid chromatography on silica gel[17], it was known that it is difficult to determine very polar compounds with the help of partition chromatography on a polar stationary phase. A reversed-phase system was therefore chosen. Another advantage of the reversed-phase technique is that the solutions to be analyzed can be injected directly, *i.e.*, without any special preparation of the samples. This avoids undesirable side effects (formation of artefacts). However, preliminary experiments under isocratic conditions (see Figs. 2 and 3) showed that the range of polarity exhibited by the substances is so great that under these conditions it is impossible to separate all of the components and still determine ergotamine itself with sufficient reproducibility. A solvent gradient system was therefore worked out. Fig. 3 shows the isocratic chromatogram obtained using the final conditions of the gradient, and Fig. 2 shows the isocratic chromatogram obtained using the mean composition of the gradient.

The optimal chromatography conditions, summarized in Fig. 4, were achieved after having varied the gradient program (convex, concave, linear) and the program time as a function of the selected flow velocity. The analysis takes *ca.* 15–20 min, the reconditioning time of the column being *ca.* 2 min. These conditions make it possible to achieve the following objectives. (a) The peaks of all eight components, *i.e.*, of ergotamine (I) and of the seven by-products and breakdown products (II–VIII) are separated down to the baseline. (b) The relative capacity factors, k'_{rel}, of all of the breakdown products (i = II–VIII), referred to the factor k' for ergotamine itself ($k'_{rel} = k'_i/k'_I$), are sufficiently reproducible to allow unambiguous identification of the breakdown products. The mean values and the scatter of these factors are shown in Table I. (c) Lysergic acid (VII), which is the most polar component, is sufficiently separated from the excipients in liquid preparations (*e.g.*, water, glycerol, ethanol and tartaric acid), which give signals of varying intensity depending on the detectors used.

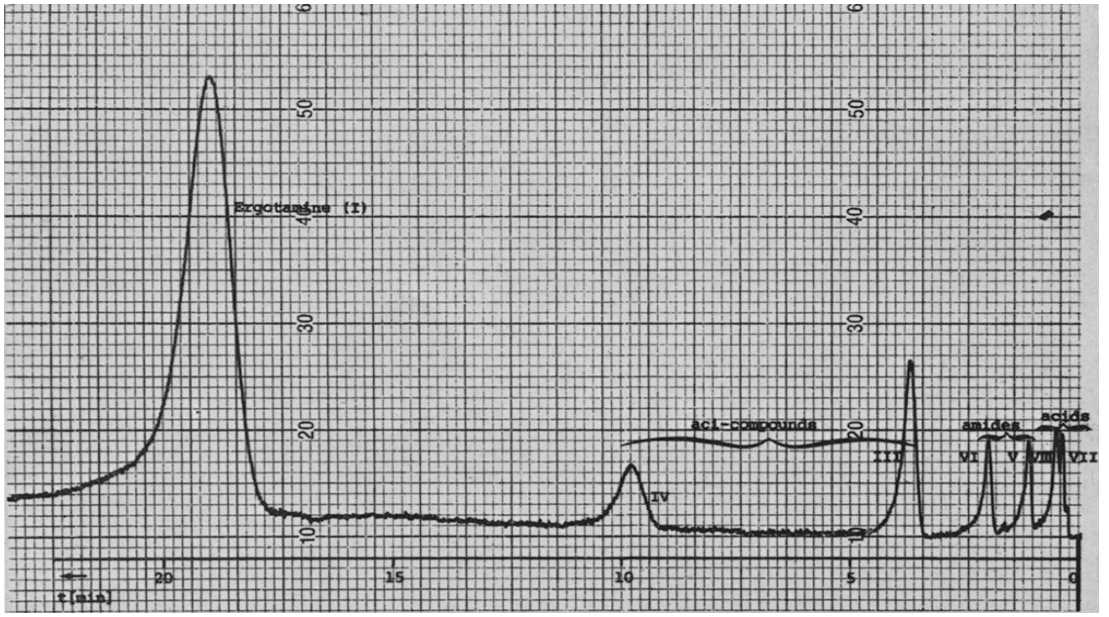

Fig. 2. Isocratic chromatogram. Waters Assoc. ready-packed steel column (30 cm × 4 mm I.D.). Stationary phase, μBondapak C_{18} (particle size, 10 μm). Mobile phase, 0.01 *m* ammonium carbonate in water–acetonitrile (2:1). Flow velocity, 8.0 ml/min. Pressure, 3000 p.s.i. UV detection: 320 nm, 0.02 *A*. Injection: 50 μl of a solution containing 9 μg of I, 7 μg of II and 1–2 μg of the breakdown products III–VIII.

(d) Because of the gradient system, all of the signals have comparable half-peak widths, and are thus amenable without difficulty to electronic integration for quantitative evaluation.

By contrast, the isocratic conditions indicated in Figs. 2 and 3 make it possible to solve problems only partially, *i.e.*, they allow the quantitative determination either of the main components ergotamine (I) and ergotaminine (II) (see Fig. 3) or of the other components III–VIII (see Fig. 2). In principle, therefore, it is possible to determine all of the components within a reasonable time by using a step gradient instead of a steady gradient. This, however, would result in widely varying half-peak widths of the signals, *i.e.*, in poorer detection limits and reproducibility of the integration than those achievable with the system with a linear gradient.

Quantitative analyses

The quantitative analyses were based on the use of two distinct evaluation methods. Ergotamine itself (I) was determined with the aid of external calibration, whereas the by-products and breakdown products II–VIII were determined using ergotamine as an internal standard. The correlation of the linear calibration graphs was highly significant for all of the compounds (Table II).

At a concentration of 0.5 mg of ergotamine tartrate per ml of injection solution, and an injection volume of 25 μl of this solution (corresponding to a total content of ergotamine tartrate of 12.5 μg), the detection limit for all of the hydrolysis

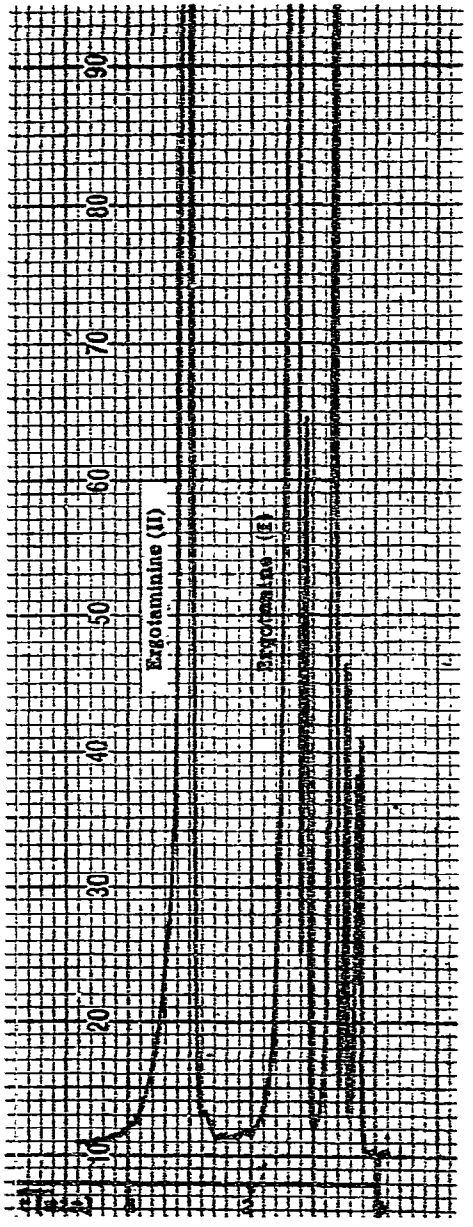

Fig. 3. Isocratic chromatogram. Mobile phase, 0.01 *m* ammonium carbonate in water–acetonitrile (1:1). Pressure, 3300 p.s.i. Other conditions as in Fig. 2.

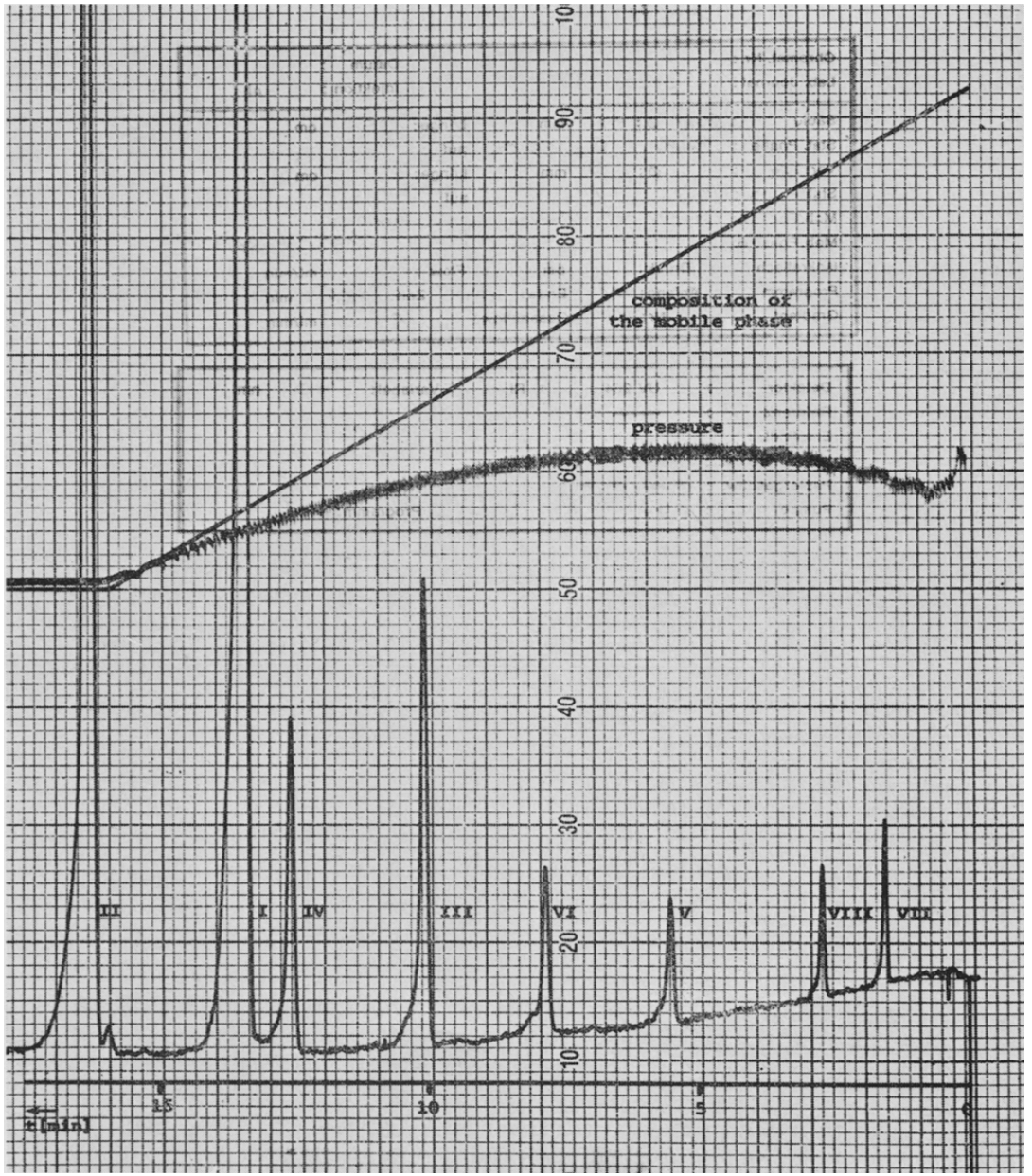

Fig. 4. Gradient chromatogram. Waters Assoc. ready-packed steel column (30 cm × 4 mm I.D.). Stationary phase, μBondapak C_{18} (particle size, 10 μm). Initial conditions, 0.01 *m* ammonium carbonate in water–acetonitrile (3.7:0.3). Final conditions, 0.01 *m* $(NH_4)_2CO_3$ in water–acetonitrile (1:1). Gradient shape, linear in 15 min. Flow velocity, 8.0 ml/min. Pressure, 3000–3600 p.s.i. UV detection: 320 nm, 0.02 *A*. Injection: 50 μl of a solution containing 9 μg of I, 7 μg of II and 1–2 μg of the breakdown products III–VIII.

TABLE I

RELATIVE CAPACITY FACTORS, $k'_{rel} = k'_i/k'_I$, OF THE BREAKDOWN PRODUCTS *i* (II–VIII) AND THEIR REPRODUCIBILITY

Eleven measurements were made in each case.

Compound i	*Mean values of* k'_{rel}	*Rel. standard deviation (%)*
Ergotaminine (II)	1.18	0.3
Aci-ergotamine (III)	0.77	0.4
Aci-ergotaminine (IV)	0.98	0.3
Lysergic acid amide (V)	0.45	1.0
Isolysergic acid amide (VI)	0.61	0.8
Lysergic acid (VII)	0.08	1.2
Isolysergic acid (VIII)	0.18	1.5

TABLE II

CALIBRATION RANGE, EVALUATED MEASURED SIGNAL AND CORRELATION CO EFFICIENTS OF THE STRAIGHT-LINE CALIBRATION GRAPHS

Compound	*Concentration range of the calibration per 50-µl injection volume*	*Content referred to I (%)*	*Measured value*	*Correlation coefficient, r (number of measurements)*
Direct calibration				
Ergotamine (I)	5–11.25 µg	40–90	area	0.9996 (6)
Indirect calibration referred to I				
Ergotaminine (II)	1– 6 µg	10–60	area	0.9999 (6)
Aci-ergotamine (III)	50–800 ng	0.5–16	area	0.9997 (5)
Aci-ergotaminine (IV)	50–800 ng	0.5–16	area	0.9997 (5)
Lysergic acid amide (V)	25–400 ng	0.5–16	height	0.9995 (5)
Isolysergic acid amide (VI)	25–400 ng	0.5–16	height	0.9999 (5)
Lysergic acid (VII)	25–400 ng	0.5–16	height	0.9976 (5)
Isolysergic acid (VIII)	25–400 ng	0.5–16	height	0.9991 (5)

products (signal to noise ratio of *ca.* 3:1) is *ca.* 25 ng, corresponding to *ca.* 0.5%. By injecting 50 µl of the solution, the detection limit can be lowered to *ca.* 10 ng, corresponding to *ca.* 0.2%, without disturbance of the chromatography by the excipients.

In order to avoid problems with peak measurements at low signal to noise ratios and electronic integration, the hydrolysis products V–VIII were evaluated by measuring the signal peak heights instead of the areas. This is permissible in this instance because the half-peak widths are approximately constant, so that the peak heights provide an adequate measure of the peak areas. As far as the isomerization products (II–IV) are concerned, the detection limit is not a critical factor because these isomers may be present in all pharmaceutical solutions of ergotamine, due to chemical equilibria, at concentrations ranging from 1% for the aci-forms III and IV to *ca.* 40% for ergotaminine (II).

The reproducibility of the quantitative determination is good. Thus, in eight

repetitions, the relative standard deviations for ergotamine and for ergotaminine were found to be 1.2% and 1.0% respectively.

Applications

The HPLC system described in this paper was used, for example, to study the establishment of the isomerization equilibrium between I, II, III and IV and the formation of the hydrolysis products V–VIII in ergotamine tartrate solutions in relation to the temperature and duration of the heat treatment. One ampoule per experiment was sufficient. All of the products could be determined using an injection volume of 25 μl. In Fig. 5 (ref. 18) concentration has been plotted against time during heat treatment at a particular temperature (121°), to which the pharmacopoeia attaches considerable importance.

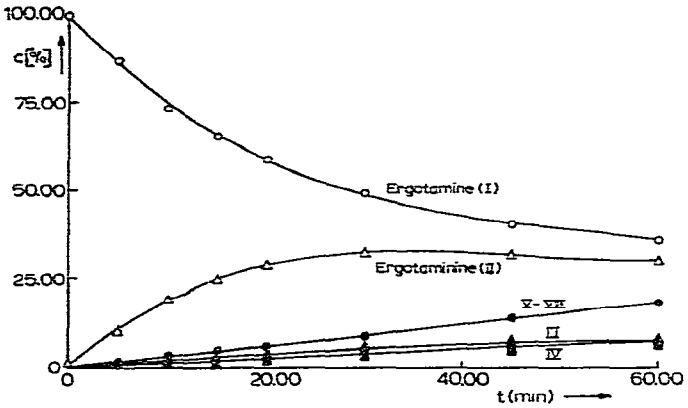

Fig. 5. Results of the effect of heat treatment (121°) on ergotamine injection.

Another series of experiments was concerned with the investigation of improperly stored (high temperature, exposure to light, long storage time) ampoule batches in which lumi-compounds (see Fig. 1) could be expected to occur. Comparison of simultaneous detection findings at 320 nm (absorbance maximum of lysergic acid derivatives with a 9,10 double bond) and at 280 nm (absorbance maximum for saturated C-9 and C-10 positions) showed that: (a) with detection at 320 nm the determination of the compounds I–VIII is not disturbed by the presence of lumi-compounds; (b) with detection at 280 nm the sensitivity for lumi-compounds is increased by a factor of over 1000 compared to detection at 320 nm so that, even with insufficient chromatographic selectivity (see Fig. 6), these breakdown products can be estimated quantitatively with good accuracy. At 280 nm the sensitivity for compounds I–VIII is reduced by a factor of *ca.* 2.5.

CONCLUSIONS

The HPLC system with reversed-phase chromatography and the use of a solvent gradient, described in this paper, is suitable for routine analyses of ergotamine tartrate and of its pharmaceutical preparations because: (a) it yields reproducible

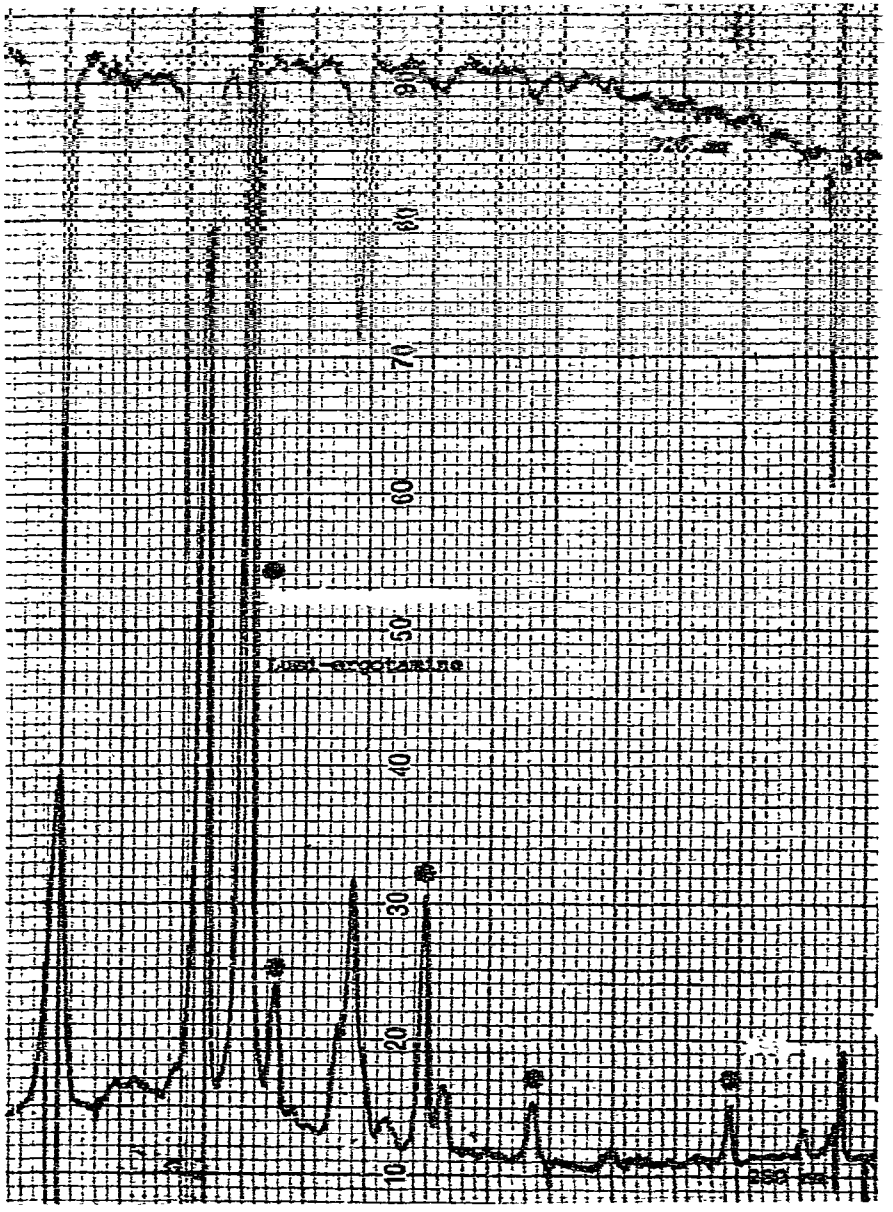

Fig. 6. Gradient chromatogram. Simultaneous detection at 280 and 320 nm for selective identification of lumi-compounds. Peaks indicated by an asterisk have selective absorbance at 280 nm. Steel column (30 cm × 4.6 mm I.D.). Stationary phase, Nucleosil C_{18} (particle size, 10 μm). UV detection: 320 nm, 0.02 *A*; 280 nm, 0.02 *A*. Injection: 50 μl of solution, containing 6 μg of I,4 μg of II, *ca.* 0.25 μg of each of the breakdown products III–VIII and *ca.* 4 μg of IX. Other conditions as in Fig. 4.

separations and relative capacity factors, k'_{rel}, so that it permits the unambiguous identification of components; (b) it yields reproducible concentration data by means of integration and/or peak-height evaluation, thus making possible a quantitative determination of all of the compounds, including breakdown products with extreme differences in polarity; (c) it is a simple method of analysis of liquid preparations, which does not require any special preparation of the sample with the attendant risk of formation of artefacts; (d) it requires only *ca.* 20 min per analysis.

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