Journal of Chromatography, 295 (1984) 282–290
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,762

Note

Fast separation of some ergot alkaloids by high-performance liquid chromatography

J. P. CHERVET*,* and D. PLAS
Laboratoires Sandoz, Centre de Recherches Pharmaceutiques, Rueil-Malmaison, Paris (France)
(First received October 18th, 1983; revised manuscript received March 21st, 1984)

Very-high-speed liquid chromatography is a suitable technique for drug analysis. The application of this technique is only possible if the relevant instrumental factors, *e.g.*, injection volume, detector flowcell volume, detector response time, total instrumental bandwidth, sampling rate of the integrator, etc., are optimized[1–3]. In most cases, conventional systems have to be substituted by low dead volume HPLC systems having fast responses. For the user of conventional instruments it is therefore important to know under which conditions this technique may be applicable.

The present report illustrates conditions which permit fast separations with conventional instruments. As an example, the separation of ergot alkaloids in drugs was studied. In comparison with the conventional LC method, the new high-speed method has an actual chromatographic analysis time which is five times shorter, with precision, sensitivity and selectivity equal to or better than the conventional method.

EXPERIMENTAL

Instrumentation

The LC system used was a Waters liquid chromatograph equipped with a pump, Model 6000 A (maximum pressure 6000 p.s.i. $\approx$ 420 bar), an intelligent sample processor (Wisp 710) which permits variable injection volumes and automated injections, and fixed wavelength UV detector, Model 440 (280 nm). The flowcell used in this detector has a rather large total volume of 12.5 μl. The volume of the flowcell is the biggest factor contributing to extra-column band broadening. The fluorescence detector was a Jobin-Yvon, Model JY3D, with a flowcell of 20 μl and a response time of 0.5 sec.

All connecting tubing was of 0.18 mm (0.007 in.) internal diameter and a total length of *ca.* 30 cm was used throughout the system. A Hewlett-Packard laboratory data system Model 3352 was used for data handling. The sampling rate of the A/D converter was 2 RDGS/sec (readings per second).

* Present address: Sandoz Ltd., Analytical Research and Development, CH-4002 Basle, Switzerland.

0021-9673/84/$03.00

Columns

The LC columns used were Brownlee cartridges (100 × 4.6 mm I.D.) packed with 5 μm spherical C_{18} bonded phase particles (Spheri-5). These columns typically have 5000–6000 plates; see elsewhere[4] for details on column specifications. The conventional 10-μm column used for comparison was a Merck analytical column (250 × 4.6 mm I.D., LiChrosorb). An empty column (30 × 4.6 mm I.D.) filled with LiChrosorb® Si 100, 5 μm, served as a mobile phase silica presaturation column.

Reagents and chemicals

Acetonitrile and methanol, both HPLC grade, were obtained from Fisons (Loughborough, U.K.), triethylamine from Fluka (Buchs, Switzerland). The ergot alkaloid standards and the corresponding degradation products were from Sandoz (Basle, Switzerland). The ergot alkaloid drugs were obtained from Sandoz (Orléans, France).

RESULTS AND DISCUSSION

Liquid chromatographic conditions

Fig. 1 shows a conventional and a high-speed chromatogram of Co-dergocrine. The chromatographic conditions are given in Table I.

TABLE I

CONDITIONS FOR THE CONVENTIONAL AND HIGH-SPEED LC

	Conventional LC	*High-speed LC*
Column	RP-18, 10 μm, LiChrosorb® 250 × 4.6 mm I.D.	RP-18, 5 μm, Spherisorb® 100 × 4.6 mm I.D.
Mobile phase acetonitrile–water–triethylamine	200:800:25 (v/v/v)	336:645:19 (v/v/v)
Flow-rate (ml/min)	1.0	2.2
Pressure (p.s.i.)	≈ 3000 (≈ 210 bar)	≈ 3000 (≈ 210 bar)
UV detection	λ = 280 nm	λ = 280 nm
Injection	15 μl (15 μg Co-dergocrine)	5 μl (5 μg Co-dergocrine)

Guiochon[5] has shown that the maximum speed, t_R, of an LC analysis is given by

$$t_R = N \cdot \frac{1 + k'}{D_M} \cdot \frac{h}{V} \cdot d_p^2 \quad (1)$$

where N = number of theoretical plates, k' = capacity factor, D_M = diffusion coefficient in the mobile phase, h = reduced height equivalent to a theoretical plate (HETP), V = reduced flow velocity and d_p = particle diameter. The column length, L, is given by:

$$L = N h d_p \quad (2)$$

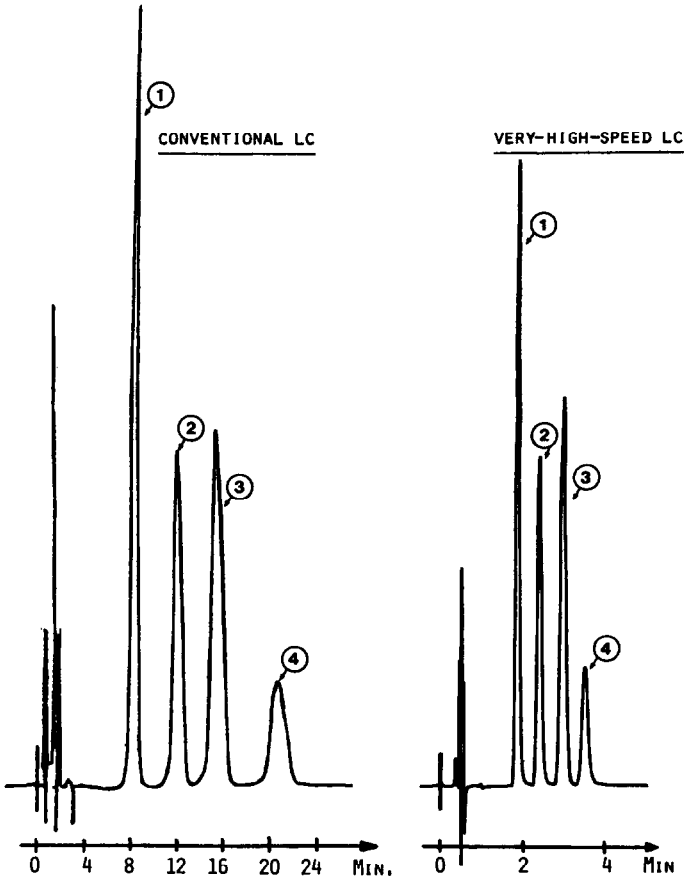

Fig. 1. Comparison of conventional *vs.* very-high-speed LC for 15 and 5 μg Co-dergocrine respectively. Peaks: 1 = dihydroergocornine; 2 = dihydro-α-ergocryptine; 3 = dihydroergocristine; 4 = dihydro-β-ergocryptine.

Combination of eqns. 1 and 2 yields:

$$t_R = L \cdot \frac{1 + k'}{D_M} \cdot \frac{d_p}{V} \tag{3}$$

By reducing the column length, *e.g.*, from 25 to 12.5 or 10 cm, and particle diameter, *e.g.*, from 10 to 5 μm, the analysis time can be reduced (up to 4–5 times for the given example) producing nearly the same plate number. In comparison with conventional 10-μm columns, the relationship between plate height and mobile phase linear velocity (flow-rate) was significantly different for the 5-μm columns used, *i.e.*, considerably smaller plate heights at substantially higher flow-rates. This indicates that it is possible to use higher flow-rates, *e.g.*, 2.2 ml/min, without loss in plate height, and makes these packings ideally suited for fast LC.

Another important factor is the optimum resolution. The resolution, R, between two peaks is a function of the selectivity, α, the plate number, N, and the capacity factor, k'. The relationship is described by the following equation:

$$R = 1/4 \cdot \frac{(\alpha - 1)}{\alpha} \cdot \sqrt{N} \cdot \frac{k'}{1 + k'} \quad (4)$$

The separations obtained often show resolutions greater than 1.5 which represents a superfluous loss of analysis time. By increasing the "strength" of the mobile phase (changing the capacity factor k') optimum resolutions for all eluted peaks can be obtained without loss of time. For the separation of Co-dergocrine, the mobile phase acetonitrile–water–triethylamine (336:645:19, v/v/v) was optimal in this respect.

Furthermore the comparison of the two chromatograms in Fig. 1 shows that the sensitivity achieved with the fast method is higher (up to three times) and with resolution equal to or better than the previous method.

Fig. 2 shows chromatograms of two different drugs containing Co-dergocrine.

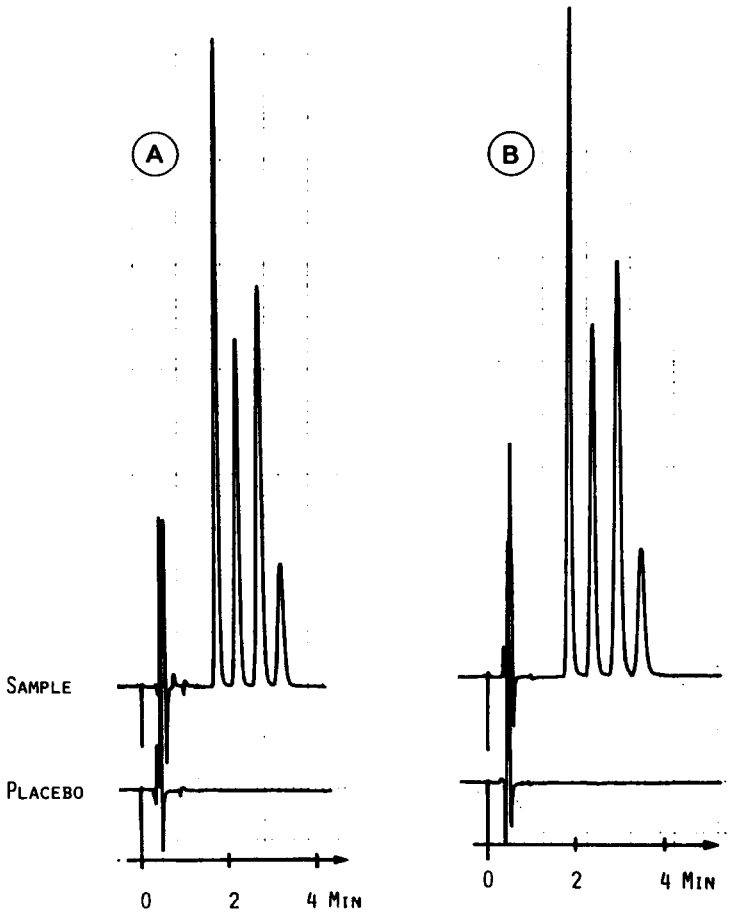

Fig. 2. High-speed LC of Co-dergocrine ampoules (A) and oral solution (B). Conditions as in Table I.

The Co-dergocrine ampoule and oral solutions were directly injected into the HPLC system without previous treatment. No interference with the placebo was observed.

Validation of the assay

Selectivity. Fig. 3 shows the selectivity of the described method. All degradation products and by-products tested are separated from the Co-dergocrine components and no interference was found. The detection limit is smaller than 0.5% ($\leqslant$ 200 ng) for each of the commonly found degradation products, except for B-*seco*-dihydroergocristine $\leqslant$1.0% ($\leqslant$400 ng).

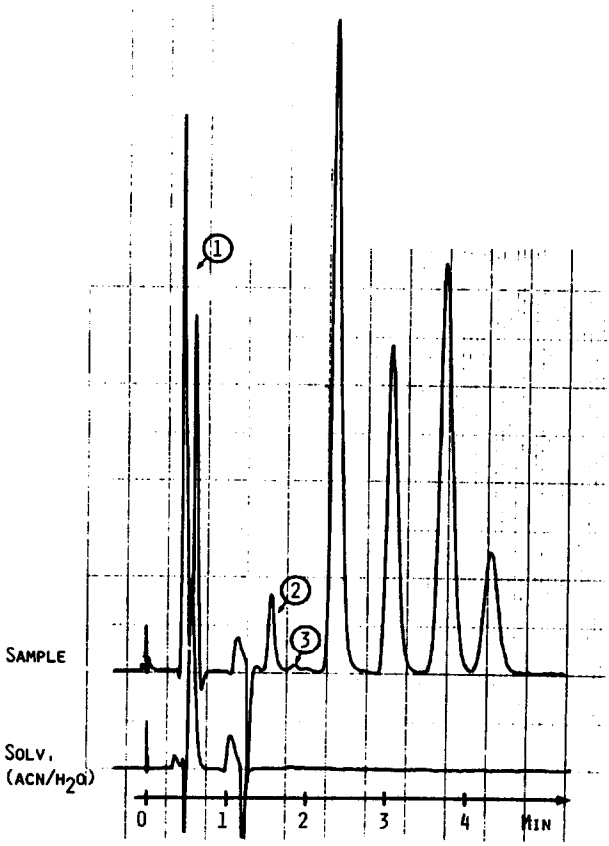

Fig. 3. Selectivity of a Co-dergocrine sample spiked with 2.0% of each degradation product. Flow-rate: 2.0 ml/min. Injection volume: 80 μl. Concentration: 4.5 mg Co-dergocrine per 100 ml solvent. Other conditions as in Table I. Peaks: 1 = aci forms + 6-methylergoline-8-carboxylic acid + 6-methylergoline-8-carboxylic acid amide; 2 = dihydroergoptine; 3 = B-*seco*-dihydroergocristine.

Linearity. Fig. 4 illustrates the linearity of the relationship between the peak areas of the four Co-dergocrine components and the concentrations. The correlation coefficients found were better than 0.999.

Relative standard deviation. The precision of the entire analytical procedure was evaluated by analysing the same sample of Co-dergocrine ten times. The relative

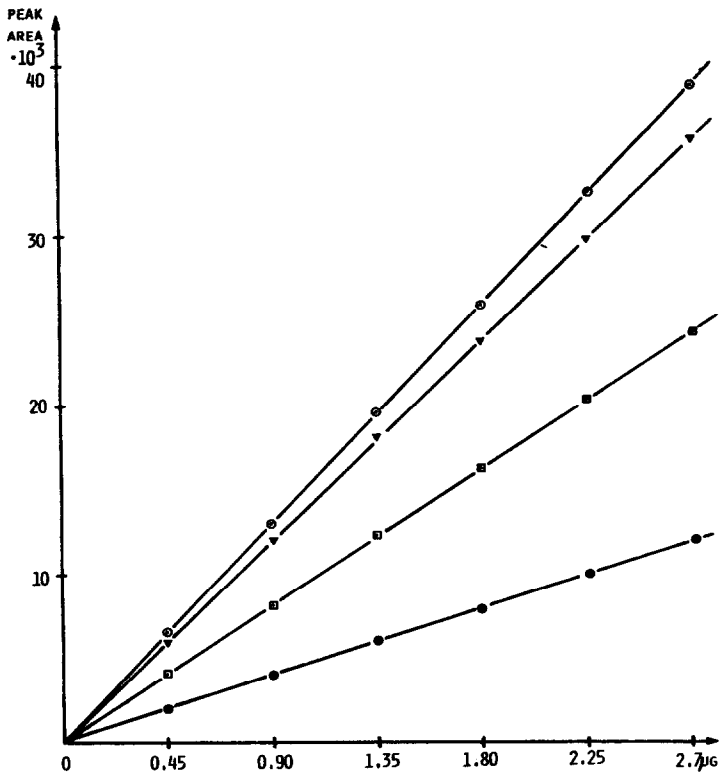

Fig. 4. Linearity of the method. Components and correlation coefficients: ⊙, dihydroergocornine, 0.9999; ▼, dihydroergocristine, 0.9999; ⊡, dihydro-α-ergocryptine, 0.9998; ●, dihydro-β-ergocryptine, 0.9998.

standard deviation for Co-dergocrine was found to be about 1%; see Table II for the individual ergot alkaloids.

Sensitivity. The following detection limits were found: dihydroergocornine, 6–9 ng; dihydroergocristine, 12–18 ng; dihydro-α-ergocryptine, 8–12 ng; dihydro-β-ergocryptine, 6–9 ng.

Column lifetime. The column life, measured by the number of injections, is

TABLE II

RELATIVE STANDARD DEVIATIONS FOR THE FOUR COMPONENTS OF CO-DERGOCRINE

Component	*Relative S.D. (n = 10) (%)*
Dihydroergocornine	0.5
Dihydroergocristine	0.5
Dihydro-α-ergocryptine	0.4
Dihydro-β-ergocryptine	1.2
Co-dergocrine	0.4

comparable with the column life of conventional LC, *e.g.*, 500 injections for the analysis of Co-dergocrine drugs, but only with presaturation of the column.

Comparison of conventional and high-speed LC. In Table III some comparative data on the use of conventional and high-speed LC are summarized. The short 5-μm column offers significant advantages in speed, sensitivity and mobile phase consumption, thus increasing the sample throughput and lowering the cost per analysis.

TABLE III

COMPARISON OF THE ANALYSIS OF ERGOT ALKALOIDS BY CONVENTIONAL AND HIGH-SPEED LC

Conditions	*Conventional LC*	*High-speed LC*
Analysis time (min per sample)	24	4
Efficiency (theoretical plates per column)	$\approx$1800*	$\approx$3000*
Detection limit (ng)	50–100*	6–9*
Mobile phase consumption (ml per sample)	24	8.8

* For dihydroergocornine.

High-speed LC with fluorescence detection

The sensitivity of UV detectors is often not sufficient to detect low concentrations of Co-dergocrine in, *e.g.*, dissolution rate tests or in testing urine or plasma samples, etc. Sometimes higher sensitivity and selectivity can be achieved by fluorescence detection.

The analysis of Co-dergocrine FAS® tablets is a good example for demonstrating the great selectivity of fluorescence detection. Fig. 5 displays a high-speed chromatogram of Co-dergocrine FAS® tablets. In A and B, the interference of diethyl phthalate (an excipient of the drug form) with the UV detection is documented. Chromatogram C displays the elution pattern of the same sample but using fluorescence detection. When comparing the two detection methods, the excellent selectivity of the fluorescence detection is revealed.

The LC conditions were the same as in Table I, with the following modifications: flow-rate 1.5 ml/min; detection, fluorescence, excitation at 290 nm, emission at 360 nm; injected amount 1.8 μg. Due to the large cell volume (20 μl), the flow-rate had to be decreased to 1.5 ml/min in order to obtain a satisfactory resolution.

Comment on the developed method

For conventional HPLC systems, the time reduction gained by very-high-speed LC is limited by the detector flowcell. The largest contribution to extra-column band broadening results from the relatively large flowcell volume which decreases the resolution drastically. The maximum analysis speed is achieved if the resolution is just sufficient and/or the detector response time is just fast enough to follow the peak signal. In all cases the suitability has to be proved by validation of the method.

From the 2000 routine analyses performed in the course of our investigations, it appears that fast LC can be realized with conventional LC systems without practical problems, compared to conventional HPLC.

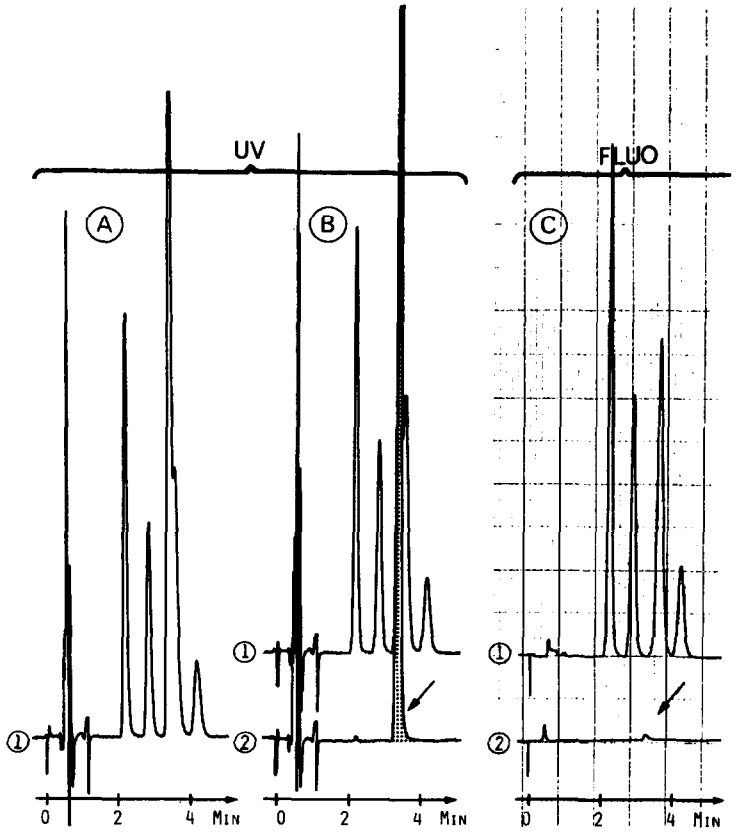

Fig. 5. Comparison UV and fluorescence detection for co-dergocrine FAS® tablets (1) and a placebo (2).

CONCLUSION

Fast LC is a powerful technique for drug analysis and can be performed with conventional instrumentation. For super-speed LC, modified and adapted instrumentation is absolutely necessary. The most important benefit of the described fast LC over conventional LC is the reduction in analysis time (up to a factor of 5) without changing the instrumentation. The sample throughput is increased by the same factor, but not the number of analyses because the sample preparation is often the most time-consuming step of the analysis.

ACKNOWLEDGEMENTS

The authors thank Mrs. R. Hobl (Sandoz, Basle), Mr. F. Erni (Sandoz, Basle) and Mr. J. R. Kiechel (Sandoz, France) for their advice and suggestions.

REFERENCES

1 J. L. DiCesare, M. W. Dong and J. G. Atwood, *J. Chromatogr.*, 217 (1981) 369–386.
2 M. W. Dong and J. L. Di Cesare, *J. Chromatogr. Sci.*, 20 (1982) 49–54.
3 W. Th. Kok, U. A. Brinkman, R. W. Frei, H. B. Hanekamp, F. Nooitgedacht and H. Poppe, *J. Chromatogr.*, 237 (1982) 357–369.
4 *Technical notes reversed phases and product bulletin*, Brownlee Labs., Santa Clara, CA, U.S.A, 1980.
5 G. Guiochon, *Anal. Chem.*, 52 (1980) 2002–2008.