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Note

High-performance liquid chromatographic determination of grandaxin- (a 2,3-benzodiazepine) and its trace impurities

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Grandaxin [1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7,8-dimethoxy-5H-2,3-benzodiazepine], an anxiolytic agent marketed by Egyt (Budapest, Hungary) is synthesized as shown in Fig. 1[1]. For the simultaneous determination of grandaxin and its trace impurities (compounds III and IV), high-performance liquid chromatography (HPLC) proved to be superior to either thin-layer or gas chromatography in terms of efficiency and loadability.

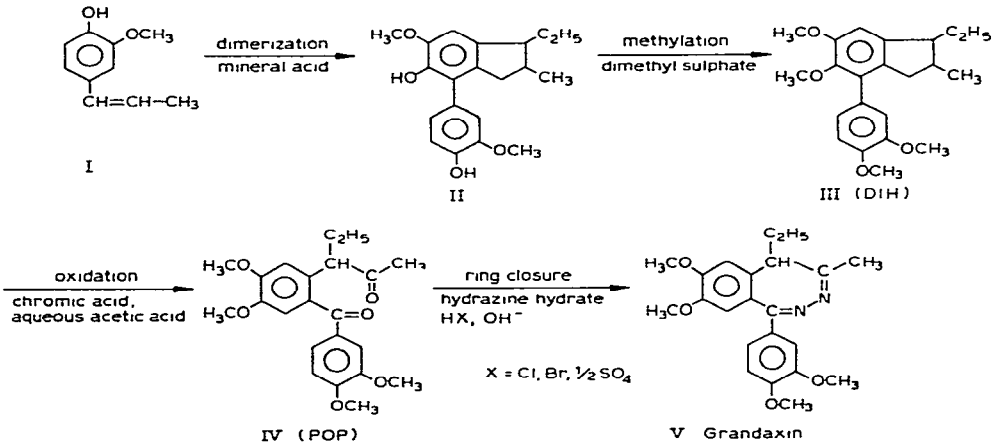

Fig. 1. Synthesis of grandaxin.

This paper reports normal- and reversed-phase HPLC methods and the sensitivity that was achieved for the trace components by optimization of column parameters. The method has also been used for the examination of grandaxin-containing tablets.

EXPERIMENTAL

Chromatographic separations were performed on a Varian 8500 liquid chromatograph equipped with a Variscan variable-wavelength UV-visible detector (231

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nm) and a Model A-25 strip-chart recorder. Two of the HPLC columns and eluents tested are specified in the legends of Figs. 2 and 3, the latter being the system of choice for the determinations. Spherisorb S10 W (10 μm) and LiChrosorb RP-8 (5 μm) packing materials were supplied by Phase Separations (Queensferry, Great Britain) and Merck (Darmstadt, G.F.R.), respectively. The columns were slurry-packed in our laboratory by an ascending technique[2]. Dioxane (Chrom AR grade) was purchased from Mallinckrodt (St. Louis, MO, U.S.A.) and acetonitrile, *n*-hexane (Uvasol) and methanol (Selectipur) were obtained from Merck. Grandaxin and tablets of grandaxin were prepared by Egyt. Other chemicals were of the highest grade available.

When tablets of grandaxin were examined, the extraction procedure was as follows. Five tablets (each consisting of 50 mg of grandaxin, 92 mg of lactose, 20.5 mg of amylum solani, 10 mg of microcrystalline cellulose, 3.5 mg of gelatine, 2 mg of stearine and 2 mg of talcum) were weighed and powdered. A portion of the powder (equivalent to 25 mg of grandaxin) and 3.3 ml of solvent [dioxane–acetonitrile–methanol (25:5:3)], the polar part of the eluent, were placed in a stoppered test-tube. The mixture was kept in a 50°C water-bath in an ultrasonic apparatus (Heat Systems Ultrasonics, Plainview, NY, U.S.A.) for 20 min, then cooled and 6.7 ml of *n*-hexane were added. The mixture was shaken and allowed to stand for a few minutes, then 40 μl of the clear supernatant liquid were injected (via a stop-flow septumless injection system) for the determination of the trace components and 2–5 μl when only the grandaxin content was to be determined. The efficiency of the extraction procedure was over 99%, so no internal standard was used.

When examining crystalline grandaxin, the substance was dissolved in the eluent to give a 1 mg/ml solution, of which 5 μl was injected.

Calculations were based on calibration graphs prepared with the corresponding reference standards (peak height *versus* amount injected).

RESULTS AND DISCUSSION

The detection wavelength was chosen with a view to obtaining the highest sensitivity for III, the component with the weakest UV absorption and the smallest concentration (see DIH in Fig. 1 and Table I). When optimizing the column parameters from the point of view of the lowest possible detection limits, we relied mainly on the work of Karger *et al.*[3].

As the trace compounds (III and IV) exhibit a significant difference in polarity (see Figs. 1 and 2), optimization for both is impossible in reversed-phase systems

TABLE I

UV ABSORPTION DATA FOR GRANDAXIN AND ITS POTENTIAL IMPURITIES

UV absorption data	*III (DIH) (diisohomogenol)*		*IV (POP) (primary oxidation product)*			*V (grandaxin)*		
$\lambda_{max.}^{ethanol}$ (nm)	231	280	234	279	312	238	273	311
$A_1^{1\%}_{cm}$	436	209	654	322	340	686	292	450

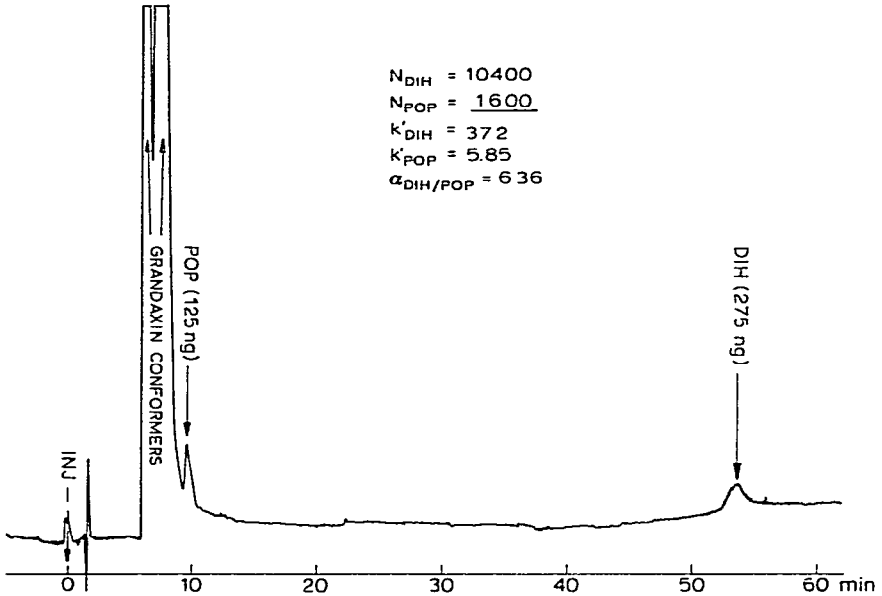

Fig. 2. Separation of grandaxin and its intermediates by reversed-phase HPLC. Sample, grandaxin containing DIH and POP as impurities; column, LiChrosorb RP-8, 200 × 4.6 mm I.D.; eluent, acetonitrile–1% (w/v) ammonium carbonate–water (40:10:50); flow-rate, 50 ml/h; chart speed, 30 cm/h; temperature, ambient.

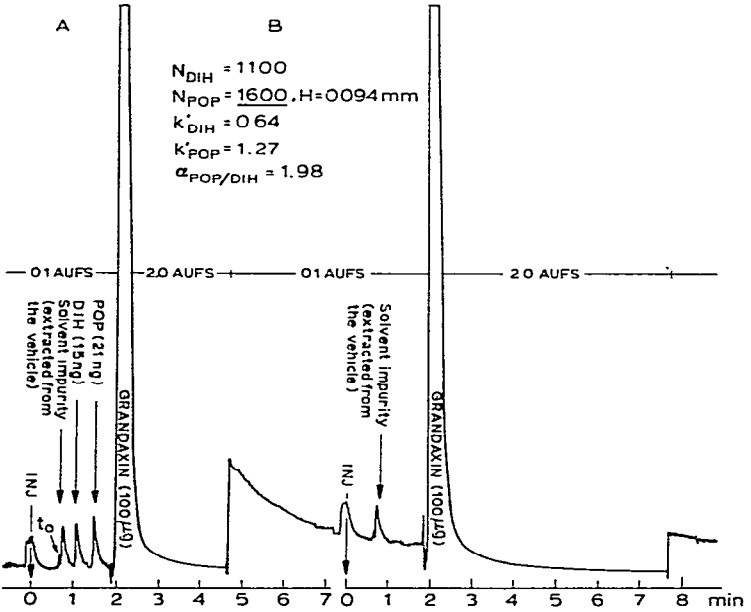

Fig. 3. Separation of grandaxin and its intermediates by normal-phase HPLC. Samples, tablet extract containing DIH and POP as impurities (A) and tablet extract with no DIH and POP (B); column, Spherisorb S10 W, 150 × 3 mm I.D.; eluent, *n*-hexane–dioxane–acetonitrile–methanol–25% (w/v) ammonia (67:25:5:3:0.02); flow-rate, 50 ml/h; chart speed, 100 cm/h; temperature, ambient.

($\alpha_{DIH/POP}$ = 6.3–8.0), and there are also problems in normal-phase systems ($\alpha_{POP/DIH}$ = 2–4).

In a satisfactorily optimized normal-phase system (see Fig. 3) capacity factors (k') of III and IV were in the optimal range (0.5–2.0), but the resolution (R_s) was still about twice as high as the optimal value (2.8 instead of 1.5). To achieve optimal resolution with selectivity factor $\alpha = 2$ and $k'_2 = 1.27$, a column with only 460 plates would be needed. With the theoretical plate height (H) in Fig. 3, this would mean an optimal column length of 4.3 cm, and a column length of about 0.5 cm or below, if efficient columns ($H \leqslant 0.01$ mm) are used. Although such short columns cause little dilution, they make high demands on minimizing the extra-column band broadening effects (dead volumes) and on the dynamic characteristics of the detection and recording units. For instance, the reduction of the column length to the theoretical optimum (4.3 cm in our case) resulted in a poorer than expected resolution, owing to the increased role of the dead volume in the lines between the injector and the column head (the adverse effect of this dead volume is demonstrated by the fact that, even with longer columns, substantially lower plate numbers were obtained for early peaks than for highly retained ones; see Figs. 2 and 3).

Thus, as control of α by gradient elution is rarely possible in sensitive trace analysis, it is reasonable to accept that combined optimization with component pairs having an α value greater than 2 is technically a greater problem than it is with components of fairly similar nature (*e.g.*, when α = 1.08–1.10).

In the system presented in Fig. 3, detection limits of DIH and POP were 2.5 and 3.0 ng, respectively. The detection limit for grandaxin, which may be of use in metabolism studies, was 2.5 ng at 238 nm. The precision was $\pm 1.5\%$ for grandaxin and $\pm 3.5\%$ for the trace components.

Grandaxin is eluted as double peaks in reversed-phase systems (see Fig. 2) and also in some normal-phase systems (with plate numbers higher than 3000). The explanation lies in the fact that, in solution, the molecule exists as a mixture of two boat conformers[4]. The ratio of conformers is a function of time, temperature and solvent.

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