Journal of Chromatography, 382 (1986) 412—414
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3291

Letter to the Editor

Chromatographic selectivity and maintenance of column efficiency during the high-performance liquid chromatographic analysis of progabide and its acid metabolite

Sir,

Recently, a high-performance liquid chromatographic (HPLC) method for the determination of a new γ-aminobutyric acid receptor agonist, progabide (PG), and its active acid metabolite (PGA) was published in this journal.

The method, developed in our laboratory, involved a single extraction of PG and PGA from blood and plasma and chromatography of the extracts on a silica column with UV detection. In the routine application of this method, we had to deal with two important points: (i) selectivity, in order to avoid chromatographic interference due to other co-administered anticonvulsant drugs; (ii) maintenance of column efficiency, to ensure good performance during the analysis of the large number of samples arising from clinical studies and extensive drug monitoring.

Concerning the selectivity, we have investigated some drugs that could interfere with the determination of PG and PGA, such as carbamazepine (CBZ), phenytoin (PHEN) and theophylline (THEO). The HPLC operating conditions were those described previously [1], but the eluent mixture was slightly different, having the composition methanol—water—acetic acid—dichloromethane (2.3:0.1:0.3:97.3).

The results depicted in Fig. 1A can be summarized as follows: CBZ elutes before PGA and is well separated from this compound; PHEN elutes close to CBZ but has a very low absorbance at 340 nm; THEO is eluted after PG and also absorbs poorly at this wavelength. Hence, none of the compounds investigated interfered with PG and PGA during the chromatographic analysis.

Concerning the problem of column efficiency, we observed that after injecting about 500 biological extracts, a column (10-μm Porasil, Millipore-Waters, Milford, MA, U.S.A.) loses its efficiency. It was possible to restore the original efficiency of the column, for PGA and PG, by washing it with about 80 ml of methanol—25% ammonia solution—dichloromethane (4:0.2:95.8). After a further washing step with 80 ml of neutral methanol—water—dichloromethane (4:0.1:95.9), the column was re-equilibrated with the

0378-4347/86/$03.50

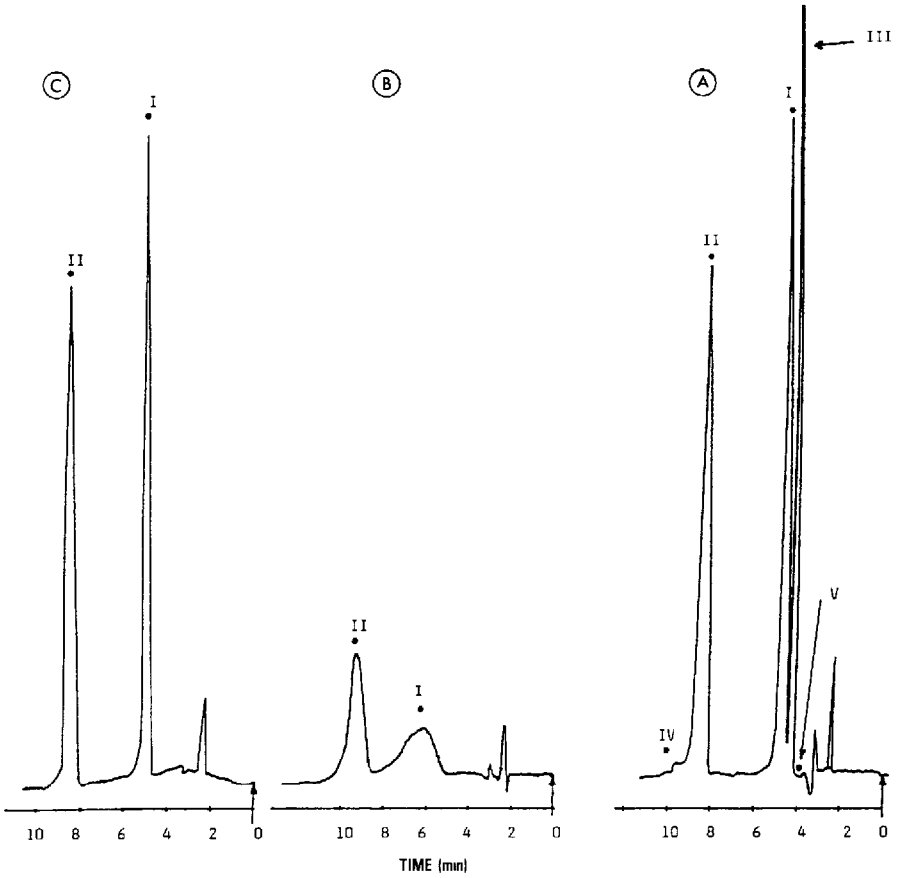

Fig. 1. (A) Chromatogram of an extract of human plasma spiked with the standards. Peaks: I = acid metabolite of progabide (0.5 μg injected); II = progabide (0.5 μg injected); III = carbamazepine (2.5 μg injected); IV = theophylline (2.5 μg injected); V = phenytoin (2.5 μg injected). (B) Chromatogram of a mixture containing 0.25 μg of both progabide (II) and its acid metabolite (I) on a degraded column. (C) Chromatogram of a mixture containing 0.5 μg of both progabide (II) and its acid metabolite (I) injected on to a regenerated column.

original eluent mixture. The problem and its solution are illustrated in Fig. 1B and C; Fig. 1B shows a chromatogram obtained after injection of a mixture of PG and PGA on a deteriorated column and Fig. 1C shows a chromatogram of the same sample on the same column after it had been restored to the original efficiency by the above procedure using the ammoniacal eluent mixture.

We are as yet unable to explain why the column loses its efficiency. One hypothesis that can be formulated concerns the instability of Schiff bases, such as PG and PGA, in acidic media; the acidity of the column should increase as a consequence of the numerous injections of acidic biological extracts.

Following several hundred determinations, the resolution between CBZ and PGA may be lost. The original performance of the column can be restored with the described regeneration procedure. Therefore, we suggest that the efficiency of the chromatographic column should be checked periodically by

analysing a standard test mixture containing PG, PGA and CBZ and comparing the results with those reported in Fig. 1A. We recommend, however, carrying out the regeneration treatment (with 30 ml of ammoniacal mixture) periodically, after 150—200 injections of biological extracts, or at the beginning of the week, without waiting for a serious decrease in column performance.

Pharmacokinetic Laboratory,
Clinical Research Unit, L.E.R.S.-Synthélabo,
C. so Matteotti 7, 20121 Milan (Italy)

V. ASCALONE*
L. DAL BO'

1 V. Ascalone, P. Catalani and L. Dal Bo', J. Chromatogr., 344 (1985) 231—239.

(Received June 12th, 1986)