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Short communication

High-performance liquid chromatographic and megabore gas–liquid chromatographic determination of levetiracetam (ucb L059) in human serum after solid-phase extraction

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Abstract

For monitoring drug levels of the new potential anticonvulsant drug levetiracetam (ucb L059) in human serum, two assay methods were developed and compared. A solid-phase extraction procedure was followed by either reversed-phase HPLC separation and UV-detection or GLC separation using cold on-column injection on a megabore column and nitrogen-phosphorous detection. Absolute recovery of the drug exceeded 97%. Precision and accuracy values for the 16.0 μg/ml quality control sample were 2.4% and 101 ± 5% ($n = 10$), respectively, for the GLC method. Precision and accuracy values for the 12.1 μg/ml quality control sample were 1.0% and 100 ± 1% ($n = 7$), respectively, for the HPLC method. Agreement between both methods was excellent ($r = 0.993$). Both methods are suitable for pharmacokinetic studies and therapeutic drug monitoring as well. Serum level data for levetiracetam in a patient on chronic antiepileptic medication are presented.

1. Introduction

Levetiracetam (ucb L059) is a new experimental drug (UCB S.A. Pharma Sector, Brussels, Belgium) with potential anticonvulsant, anxiolytic and cognition improving properties [1]. The drug is administered as the single enantiomer *R*-levetiracetam. The in vivo stereochemical stability of the compound has been investigated in four volunteers who received a single dose of [^{14}C]-levetiracetam [2]. In plasma and urine no enantiomeric conversion was found within 48 h after drug intake. An analytical procedure for the determination of the drug in plasma and urine using capillary GLC equipped with a split/splitless injector in the split mode has already been developed [3]. The objective of this study was to develop a selective, rapid, simple and precise extraction procedure using C_{18} solid-phase extraction columns with the possibility of further automation and yielding extracts that are suitable for HPLC analysis and for GLC analysis using the on-column injection technique.

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2. Experimental

2.1. Reagents

Levetiracetam [(*S*)-α-ethyl-2-oxo-1-pyrrolidine acetamide] and the internAL standard G025 (α-methyl-5,5-dimethyl-2-oxo-1-pyrrolidine acetamide) were gifts from UCB S.A. (Brussels, Belgium). Fig. 1 shows their structural formulas.

Stock solutions of levetiracetam (2 mg/ml) and the internal standard (1 mg/ml) were prepared in methanol and stored at 4°C. The working internal standard solution was prepared by diluting the stock solution 1:50 with water. Calibration samples containing 2.5, 5.0, 10.0, 15.0, 19.9, 24.8, and 29.7 μg/ml were prepared by adding an appropriate amount of the stock levetiracetam solution to 10.0-ml portions of blank bovine serum. Bovine serum was used to avoid the risk of infection and because it is easy available. Methanol (Lichrosolv) and acetone (Uvasol) were purchased from Merck (Darmstadt, Germany), acetonitrile (HPLC reagent) was from J.T. Baker (Phillipsburg, NJ, USA). Ultra pure water was prepared using the Milli-Q-plus water purification system (Millipore, Bedford, MA, USA).

2.2. Instrumentation

HPLC analysis was carried out on a Series 4 quaternary solvent delivery system equipped with an ISS-100 automated sampling system (Perkin-Elmer, Norwalk, CT, USA), a Model 1040A diode-array detector (Hewlett-Packard, Palo Alto, CA, USA) with detection wavelength set to 205 nm and a home-made reversed-phase analytical column (15 × 0.46 cm I.D.) packed with Spherisorb 3ODS2 (Phase Separations, Deeside, UK), kept at room temperature. The mobile phase consisted of a mixture of acetonitrile (A) and water (B) and was delivered as a linear gradient with a flow-rate of 1.0 ml/min: 6% A (0 min), 20% A (5 min), 40% A (6 min), 40% A (10 min), 6% A (11 min), 6% A (15 min).

GLC analysis was carried out on a Vega 6000 (Carlo Erba, Milan, Italy) equipped with a Model AS-550 on-column injector, a NPD-40 detector (NP-mode) and a 30 m × 0.53 mm I.D. DB-FFAP megabore column, film thickness 1 μm (J and W Scientific, Folsom, CA, USA). The following gas chromatographic parameters were used: carrier gas, helium (column head pressure 100 kPa, flow-rate 30 ml/min); flame gases, hydrogen (100 kPa, flow-rate 45 ml/min) and air (120 kPa, flow-rate 450 ml/min); make-up gas, helium (70 kPa, flow-rate 20 ml/min); detector, temperature 275°C; column temperature program, 70–190°C (40°C/min), 190–250°C (5°C/min), 250°C (2 min isothermal); precolumn, 1 m × 0.53 mm I.D. deactivated fused-silica (Interscience, Breda, Netherlands).

Extraction was performed on a Baker-10 extraction system and 100-mg C_{18} SPE-columns were obtained from J.T. Baker.

Integration of chromatographic signals and calculations (one-point calibration, internal standard method, peak-height ratios for HPLC and peak-area ratios for GLC) were performed by a Model 3390A integrator (Hewlett-Packard).

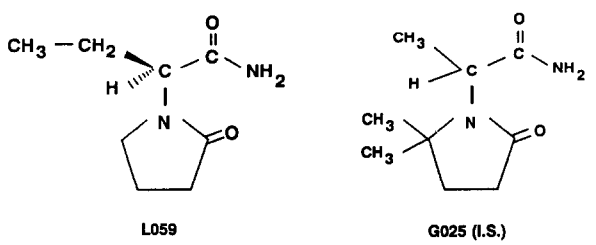

Fig. 1. Structures of levetiracetam (ucb L059) and the internal standard G025.

2.3. Sample preparation

For each sample one C_{18} SPE-column was inserted into the Baker-10 manifold which was attached to a vacuum source. For conditioning the columns, 2 ml of methanol was passed, followed by 2 ml of water. As soon as the water had passed, the vacuum was disconnected in order to prevent the columns from drying out. An aliquot of 0.2 ml of patient or calibration serum sample followed by 0.1 ml of internal standard working solution were added to each

column. Vacuum was applied to slowly draw the samples through the columns (approx. 15 s per sample). Both drug and internal standard were retained on the columns. The column with the sample matrix was flushed with 0.75 ml of water and columns were dried by applying maximum vacuum for three more minutes.

For HPLC analysis, the samples were eluted with 1.0 ml of methanol–water (1:5, v/v). The eluent was collected in 1.5-ml HPLC-vials (Chrompack, Middelburg, Netherlands) and 150 μl was injected onto the HPLC system.

For GLC analysis, the samples were eluted with 0.3 ml of methanol. After evaporating to dryness using a vortex-evaporator (Büchler Instr., Dev. of Searle Anal., Fort Lee, NJ, USA) with temperature set to 40°C, the residues were reconstituted with 0.3 ml of acetone. A 1.5-μl aliquot was injected onto the GLC system.

3. Results and discussion

3.1. Chromatography

Chromatograms of HPLC and GLC methods are presented in Figs. 2 and 3, respectively, showing calibration samples (a), patient samples before starting levetiracetam therapy (b) and patient samples during levetiracetam therapy (c).

Both the HPLC and the GLC methods are not stereospecific for levetiracetam since they are achiral and cannot differentiate between the two enantiomers of the racemic parent compound. However no enantioselective analytical method is necessary because the absence of in vivo racemization in man with *R*-levetiracetam.

3.2. Recovery, accuracy and precision

Extraction recovery of levetiracetam was 94 ± 3% and was estimated using the HPLC method. Extracted bovine serum (16 μg/ml levetiracetam) was compared to standard solutions containing 1.6 μg/ml of levetiracetam using the internal standard method. Equal amounts of internal standard were added to equal volumes

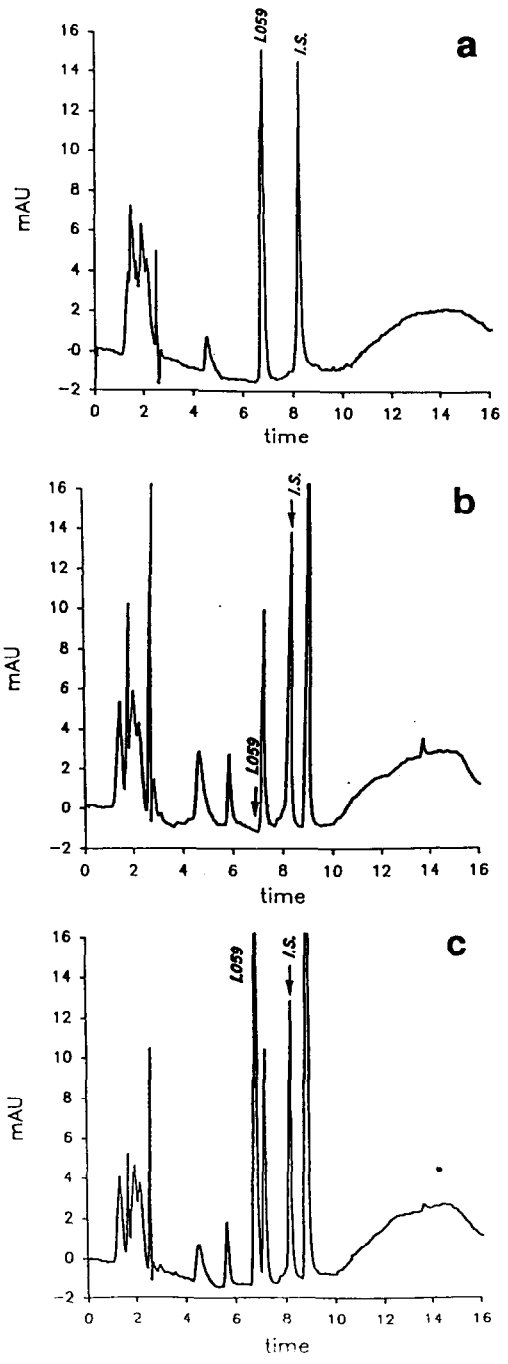

Fig. 2. HPLC chromatograms of (a) a calibration sample containing 15.0 μg/ml levetiracetam, (b) a patient sample before starting levetiracetam therapy and (c) a patient sample during levetiracetam therapy containing 16.6 μg/ml levetiracetam. Retention times are 6.4 min for levetiracetam and 7.8 min for the internal standard.

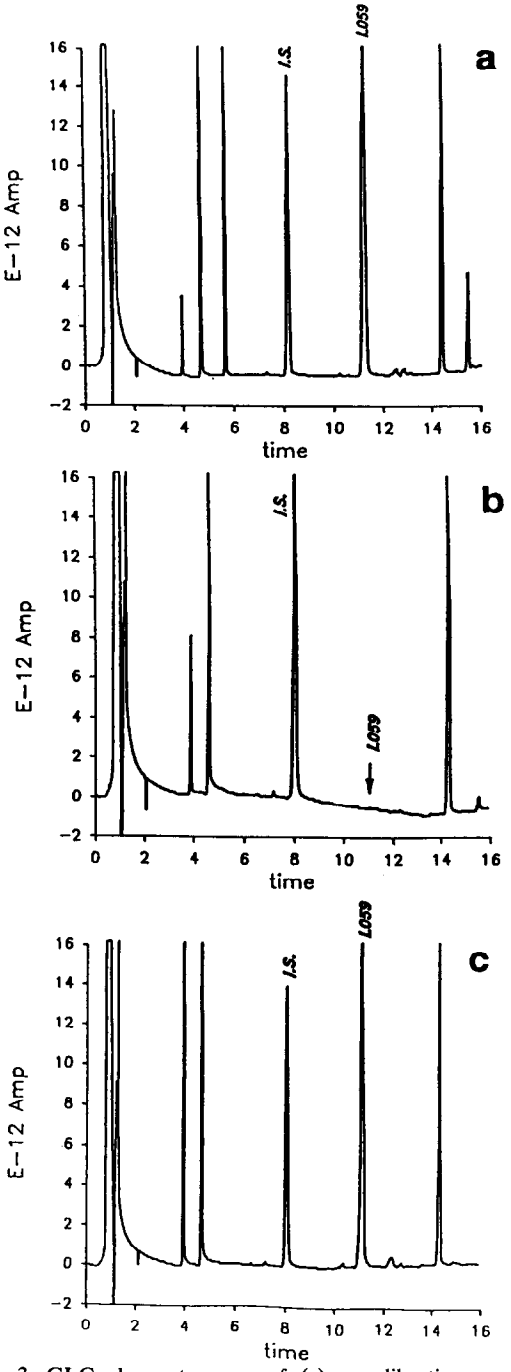

Fig. 3. GLC chromatograms of (a) a calibration sample containing 16.0 μg/ml levetiracetam, (b) a patient sample before starting levetiracetam therapy and (c) a patient sample during levetiracetam therapy containing 16.9 μg/ml levetiracetam. GLC retention times are 11.5 min for the internal standard and 14.0 min for levetiracetam.

of extract and standard solution just prior to injection.

No differences in extraction behaviour of levetiracetam and the internal standard were observed using either human serum or bovine serum.

Within-run precision and accuracy were obtained by analyzing bovine serum samples spiked with levetiracetam. The coefficient of variation (C.V.) was 2.4% for GLC analysis (16 μg/ml, $n = 10$) with an accuracy of 101 ± 5% and 1.0% for the HPLC method (12.1 μg/ml, $n = 7$) with an accuracy of 100 ± 1%.

3.3. Linearity and sensitivity

Both methods showed good linearity in the 0–30 μg/ml range. The linear regression line for GLC was $y = 0.11 + 0.989x$ ($r = 0.999$) and for HPLC was $y = 0.37 + 0.994x$ ($r = 0.999$).

We defined the limit of quantification as the concentration that can be determined with a coefficient of variation of 10%. For the GLC method this limit appeared to be 0.29 μg/ml ($n = 7$) and for the HPLC method it was 0.36 μg/ml ($n = 8$). The latter can probably be improved if a more sensitive UV-detector is available.

3.4. Interferences

The following commonly used anticonvulsant drugs and metabolites were checked for interferences in the GLC and HPLC assays: ethosuximide, primidone, phenobarbital, N-desmethylsuximide (metabolite of methsuximide), phenytoin, carbamazepine, carbamazepine-10,11-epoxide, carbamazepine-10,11-*trans*-dihydrodiol, monohydroxy-carbazepine (metabolite of oxcarbazepine), valproic acid, lamotrigine, loreclezole and vigabatrin. These drugs were either not detected or well separated using the described chromatographic conditions. For HPLC analysis, it was necessary to introduce a mobile phase gradient in order to elute the more hydrophobic anticonvulsants, preventing them from interfering in later chromatographic runs.

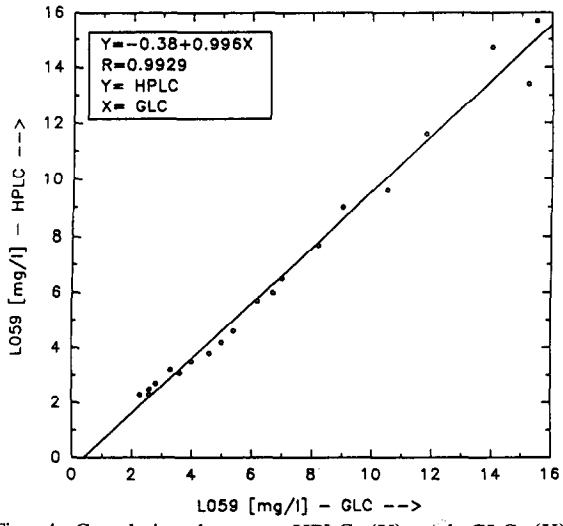

Fig. 4. Correlation between HPLC (Y) and GLC (X) methods for the determination of levetiracetam in serum ($n = 20$).

3.5. GLC versus HPLC

A correlation study between the two methods using 20 patient samples (concentrations ranged from 2 to 16 μg/ml) showed excellent agreement. The linear regression line was $y = -0.38 + 0.996x$ (where y stands for the HPLC method) with $r = 0.993$ (Fig. 4).

3.6. Application

Fig. 5 shows an example of a serum concentration vs. time profile for levetiracetam in a patient receiving 750 mg levetiracetam in combination with 800 mg carbamazepine per day. Curves for carbamazepine (CBZ) and its metabolites carbamazepine-10,11-epoxide (CBZE) and carbamazepine-10,11-diol (CBZD) are also plotted.

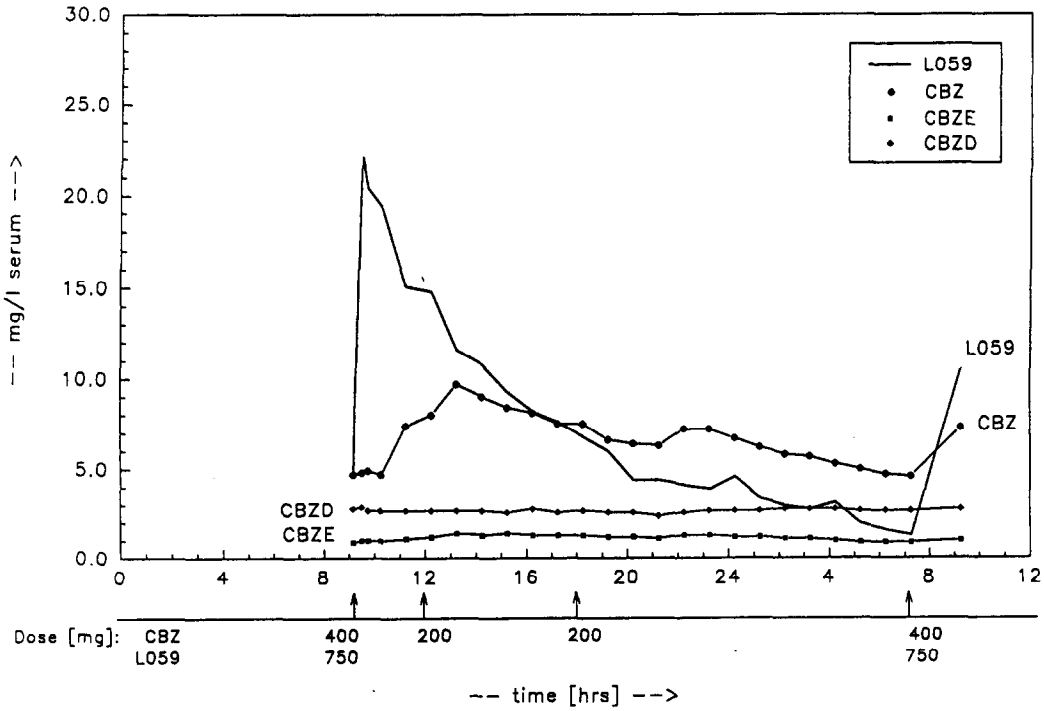

Fig. 5. Serum concentration vs. time profiles of levetiracetam, carbamazepine (CBZ), carbamazepine-10,11-epoxide (CBZE) and carbamazepine-10,11-diol (CBZD) of a patient receiving 750 mg levetiracetam and 800 mg carbamazepine.

4. Conclusions

The GLC and HPLC methods are both suitable for therapeutic drug monitoring as well as for pharmacokinetic studies. Taking into account the shorter extraction procedure and the possibility of further automation using an on-line extraction system, we prefer the HPLC method for routine therapeutic drug monitoring of levetiracetam.

References

[1] A.J. Gower, M. Noyer, R. Verloes, J. Gobert and E. Wülfert, *Eur. J. Pharm.*, 222 (1992) 193.
[2] UCB Report RR LE 942301.
[3] UCB Report LE 90A092 (Appendix 2): Detailed analytical procedure for the determination of ucb L059 in plasma.