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LC method for therapeutic drug monitoring of levetiracetam: Evaluation of the assay performance and validation of its application in the routine area

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Abstract

Objectives: An accurate and precise high-performance liquid chromatographic method using diode array detection for the determination of levetiracetam in human plasma has been developed and validated for use in pharmacokinetic studies.

Methods: A harmonized validation strategy based on the accuracy profiles was used to select the most appropriate regression model and to determine the limits of quantitation as well as the concentration range of the developed analytical procedure. On the other hand, the present paper also shows this validation approach as a suitable tool to guaranty the quality of the results obtained by the use of the analytical validated methodology for plasma levetiracetam determination in a routine setting and to ensure the risk of obtaining the future measurements outside the previously fixed acceptance limits.

Results: As pointed recently, the FDA, a weighted $1/x^2$ quadratic regression model ranging from 0.53 to 107.00 mg/L was selected as the simplest calibration model that maximized the accuracy all over the range. Relative bias was <5%, assay imprecision was always <6% and mean extraction recovery from plasma was >90%. So, accuracy did not exceed the acceptance limits settled at ±20% according to the FDA or Washington conference regulatory requirements for bioanalytical methods. Internal quality control has been assessed over a 2 year time period. All controls were essentially found to provide levetiracetam concentrations within the target range according to the FDA.

Conclusions: The validated analytical procedure complies with strongest regulatory standards. The validated method has a sufficiently rapid turnaround time and their results are good enough to enable the laboratory to routinely provide useful and accurate pharmacokinetic data in time to adjust patient regimens.

Keywords: HPLC; Therapeutic Drug monitoring; Accuracy profile; Levetiracetam; Validation

Introduction

Levetiracetam, *S*-α-ethyl-2-oxo-1-pyrrolidine acetamide (Keppra, UCB Pharma, Braine-l'Alleud, Belgium) is a new antiepileptic drug, unrelated chemically to other anticonvulsants in current use [1,2]. Levetiracetam is indicated as adjunctive therapy in the treatment of partial seizures, with or without secondary generalization [3].

Levetiracetam exhibits good bioavailability, linear pharmacokinetic profile, minimal plasma protein-binding, insignificant hepatic metabolism and consequently lack of drugs interactions and inactive, renal excretable metabolites [4–6]. Nevertheless, it is recommended to monitor the plasma concentrations of levetiracetam, its recent introduction in therapy means that its pharmaco-toxicological profile is not fully understood. So, TDM of patients undergoing therapy with levetiracetam, especially in different physiopathological states such as renal impairment or in specific clinical characteristics or age groups (elderly patients with decreasing renal function, pediatric patients with higher apparent clearance of levetiracetam than adults) is worthwhile and advisable [7,8]. Moreover, drug plasma concentration monitoring is helpful to determine if optimal drug concentration is achieved, to assess the treatment compliance when facing a therapeutic failure or to evaluate an overdose. Therapeutic drug monitoring is routinely performed

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in patients treated with antiepileptics and it is particularly useful for new drugs, such as levetiracetam.

Several chromatographic assays have been reported for the determination of levetiracetam in biological fluids including gas chromatographic [9,10], high performance liquid chromatographic [9,11–18] methods or the more recent specific and sensitive but expensive liquid chromatography-tandem mass spectrometry (LC-MS-MS) technologies [19–21] with equipment cost not available for most clinical laboratories. Most of the HPLC reported methods lack sensitivity for levetiracetam routine monitoring in clinical practice according to the steady-state trough levetiracetam concentrations reported by May et al. [22] and to the large interindividual concentration variability observed in patients receiving a similar levetiracetam dosage. Moreover, most of them encounter tedious and time-consuming sample preparation procedures. Only the HPLC of Lancelin et al. [17] has a limit of quantification similar to the reported in the LC-MS methodologies.

Therefore, our first objective was to develop a reliable HPLC-UV method for the quantification of levetiracetam in plasma/serum samples suitable for therapeutic drug monitoring. Our second objective was to validate the developed methodology in order to check its applicability in the routine area and the future analysis of unknown samples using the validated method. Recently, a novel validation strategy based on the use of accuracy profiles has been introduced [23–28]. The notion of including the use of accuracy profiles as a decision tool to select the most appropriate response function, to estimate the limit of quantitation or to evaluate the concentration range, is in accordance with the objective of an analytical method that can be summarized as its ability to quantify as accurately as possible each of the unknown quantities in human samples that the laboratory will have to determine.

So, in the present work, a simple, rapid and sensitive HPLC method for determination of levetiracetam in plasma/serum has been developed and validated in our laboratory according to the mentioned validation strategy. Moreover, to the best of our knowledge, this is the first account of a levetiracetam HPLC assay for which a specific evaluation of the risk of the procedure related to the future use of the method during its daily use for the therapeutic drug monitoring of levetiracetam in the clinical setting is reported. Recently, we have recently published a paper reporting a lamotrigine bioanalysis method validated according with this strategy [29].

Material and methods

Chemical and reagents

Levetiracetam reference standard and UCB 17025 (α,2,2-trimethyl-5-oxo-1-pyrrolidine acetamide) used as internal standard were kindly supplied by UCB Pharma (Brain-l`Alleud, Belgium).

99.9% purity HPLC grade solvents methanol and acetonitrile were obtained from Merck (Barcelona, Spain). Potassium dihydrogen phosphate and triethylamine, analytical grade, were purchased from Panreac (Barcelona, Spain).

For method validation, human plasma (University hospital blood bank) to prepare calibration, validation and quality controls standards and to study the specificity of the method was obtained from pooled drug-free samples collected from healthy volunteers.

Drug solutions

Stock standard solutions of levetiracetam and the internal standard were prepared by dissolving appropriate amounts of compounds in a known volume of ultrapure water and methanol respectively and stored at −30 °C.

Working standard solutions for the preparation of calibration, validation and quality controls standards were prepared by appropriate dilutions of the stock standard solutions in blank human plasma obtained from the University hospital blood bank.

Levetiracetam serum lyophilized controls purchased from Chromsystems (Münchem, Germany) were reconstituted with HPLC water according to manufacturer instructions, aliquoted and frozen at −20 °C until analysis. All frozen samples were slowly defrosted and vortex-mixed prior to analysis.

Calibration and validation standards

In order to validate the analytical method, we prepared two kinds of samples for calibration and validation in an independent way.

The calibration standards consist of plasma/serum samples, containing known concentrations of the analyte of interest. The samples are only used for calibration and they are prepared according to the protocol that will be applied routinely. Two calibration standard series of five concentration levels replicated on three different days were performed. Spiked plasma samples used as calibration standards (0.53, 3.42, 26.75, 75.00 and 107.00 mg/L) were prepared by addition of different volumes of the corresponding standard solution of levetiracetam in blank human plasma obtained from the University hospital blood bank. The most appropriate response function was selected according to the accuracy profile approach in order to guaranty a reliable quantification.

The validation standards are also matrix samples containing known concentrations of the analyte of interest. They were independently prepared in the matrix simulating as much as possible the future routine analysis of levetiracetam samples. In the validation phase, the validation standards represent the future samples that the analytical procedure will have to quantify. The concentration levels selected for the validation standards were the same as the levels of the calibration standards. Six replicates were prepared at each concentration level for three days.

Quality control samples were prepared in human plasma at the concentrations of 1.5, 15.0 and 75.0 mg/L, as described above for the calibration and validation standards. Chromsystems plasma/serum controls with mean levetiracetam concentrations of 9.61 and 53.20 were also employed for internal quality assurance. Calibration and validation standards and quality control samples were analyzed in the same way as patient plasma samples.