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# Quality by design approach for development and validation of a RP-HPLC method for simultaneous determination of co-administered levetiracetam and pyridoxine HCl in prepared tablets

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ABSTRACT

A new simple, rapid and sensitive reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed by employing Quality by Design (QbD) approach for determination of levetiracetam and pyridoxine HCl in prepared tablets. Levetiracetam and pyridoxine HCl are co-administered because treatment with the antiepileptic levetiracetam leads to a deficiency in pyridoxine HCl. Fractional factorial design (FFD) was applied for screening of the four independent factors: pH of the aqueous part of the mobile phase, flow rate, injection volume and % of the organic modifier. Analysis of variance (ANOVA) confirmed that the four factors were significant. Optimization of the chromatographic conditions was performed using central composite design (CCD). The Analysis was achieved on BDS Hypersil $C_8$ (250 × 4.6 mm, 5 µm) column applying an isocratic mobile phase containing MeOH and 25 mM $KH_2PO_4$ buffer pH 3 (38.4:61.6, v/v) at 0.8 mL/min flow rate with UV detection at 214 nm and 5 µL injection volume. The proposed method was validated according to ICH guidelines. Under optimized conditions, linear ranges of 1.56–100 µg/mL and 0.39–100 µg/mL were obtained for levetiracetam and pyridoxine HCl, respectively, with correlation coefficients ($R^2$) of 0.999. The average recoveries ranged from 95.46 to 101.14%. The inter- and intra-day precisions were lower than 1%. The proposed method showed good predictability and robustness.

## 1. Introduction

Levetiracetam is a new non-inducing antiepileptic drug. It is used as adjuvant medication as well as monotherapy in refractory partial seizures with or without generalization [1]. Its mechanism of action is unknown. However, it is believed that it binds to a synaptic vesicle protein and consequently slowing down nerve conduction across synapses. Chemically, levetiracetam is (*S*)-ethyl-2-oxo-1-pyrrolidineacetamide (Fig. 1a) [2]. It is official in BP [3] and USP [4]. Various analytical methods were reported for the determination of levetiracetam in pure and pharmaceutical dosage forms including spectrophotometry [2,5–9], spectrofluorimetry [10], HPLC [11–15], HPTLC [16], capillary electrophoresis [17] and LC-MS [18,19].

Treatment with enzyme-inducing or non-inducing antiepileptic drugs commonly causes pyridoxine deficiency with similar incidence [20], and some reports indicated that pyridoxine HCl can alleviate behavioral side effects related to the use of levetiracetam [21–23].

Pyridoxine HCl is a naturally occurring form of vitamin B6. Its chemical name is 3, 4-pyridine-diacetonitrile, 5-hydroxy-6-methyl, hydrochloride (Fig. 1b). Pyridoxine HCl is involved in the formation of hemoglobin and metabolism of amino acids, carbohydrates, and fats [24]. It is used for the prophylaxis or treatment of depression, nausea, and vomiting of pregnancy and as vitamin B6 dietary supplement [25]. Pyridoxine HCl was assayed by potentiometric titration and HPLC in BP [3] and USP [4], respectively. The other previously reported analytical methods described the quantitation of pyridoxine HCl alone [25–27] and in a mixture with other vitamins [28–30] or drugs [24,31,32].

The traditional optimization of HPLC methods requires studying one factor at a time (OFAT) while keeping the others fixed. OFAT results in a large number of experiments with a lack of understanding of critical parameters. Recently, QbD is used as a powerful tool for chromatographic method development [33–37]. The advantages of this approach include the better understanding of the factors influencing chromatographic separation by determination of critical ones, their positive or negative effect on the selected responses, and the multidimensional interaction between them. In addition, QbD facilitates the purposeful

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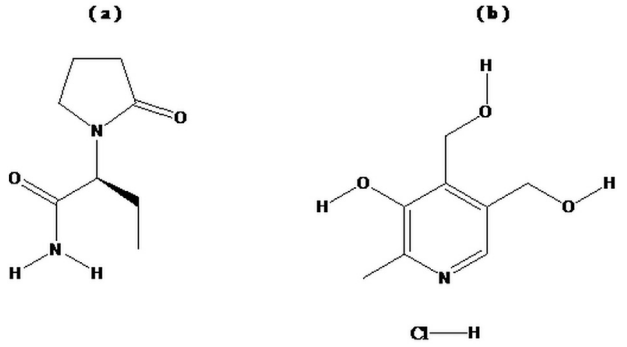


**Fig. 1.** Chemical structure of (a) levetiracetam and (b) pyridoxine HCl.

**Table 1**
ANOVA results of the fractional factorial design (insignificant interaction effects were excluded).

| Item | *p*-Value (N) | | *p*-Value (R) | |
|---|---|---|---|---|
| | F | *p*-Value | F | *p*-Value |
| A-pH | – | – | 302.93 | **0.0033** |
| B-F R | 7.59 | **0.0401** | 10.66 | 0.0824 |
| C-I V | 51.74 | **0.0008** | 16.71 | 0.0550 |
| D-% MeOH | – | – | 28.87 | **0.0329** |
| AD | – | – | 183.56 | **0.0054** |
| Adjusted $R^2$ | 0.8912 | | 0.9871 | |

Bold values indicate that the independent factor has a significant effect on the selected response.

variables change to obtain the desired response and suggests the optimal solution with variables value that best gives the maximum, minimum or target response, while at the same time it finds the spot with the minimum error transmitted to the responses. So, this should represent process conditions that are robust to slight variations in factor settings. It also suggests a mathematical model that relates the response and the experimental variables, thus allowing response prediction with minimum error transmitted to the response (propagation of error, POE) [35,38].

QbD was considered as a systematic approach to design a quality product which is the main aim of pharmaceutical development. The principles of QbD were well explained by International Conference on

**Table 2**
Regression coefficients of polynomial equation along with p-value of ANOVA of central composite design (bold *p*-values indicate a significant effect).

| Factors[a] | Theoretical plates[b] | | Resolution | |
|---|---|---|---|---|
| | Coefficient | *p*-Value | Coefficient | *p*-Value |
| Intercept | 8.97974E−007 | | 2.91733 | |
| A | −1.73217E−008 | 0.2556 | −0.602917 | < **0.0001** |
| B | 7.98437E−008 | < **0.0001** | −0.08125 | 0.4011 |
| C | 2.05059E−007 | < **0.0001** | −0.182083 | 0.0694 |
| D | −2.70278E−008 | 0.0850 | −0.287083 | **0.0068** |
| AB | 6.49821E−009 | 0.7224 | 0.011875 | 0.9194 |
| AC | −4.63134E−009 | 0.7999 | 0.086875 | 0.4625 |
| AD | −7.54371E−009 | 0.6802 | 0.599375 | < **0.0001** |
| BC | −2.98599E−009 | 0.8701 | 0.060625 | 0.8190 |
| BD | 2.09645E−008 | 0.2610 | −0.026875 | 0.8190 |
| CD | −3.35486E−008 | 0.0813 | 0.020625 | 0.8606 |
| A^2 | −5.09168E−009 | 0.7155 | | |
| B^2 | 5.7412E−009 | 0.6813 | | |
| C^2 | 4.29198E−008 | **0.0069** | | |
| D^2 | −3.88775E−008 | **0.0125** | | |
| Model | Quadratic | | 2FI | |

[a] A: pH, B: flow rate, C: injection volume, D: % MeOH.
[b] Transformation is power (Lambda = −1.6).

Harmonization (ICH) harmonized triplicate guidelines Q8(R2) that outlined the important concepts and tools required to build in the quality by design. These outlined concepts include critical process parameters (CPPs) which refer to the higher ranked variables. These CPPs may have a significant effect on a desired characteristic (critical quality attribute, CQA) that should be within a certain range to ensure the quality of product. The design space was also referred to as a multidimensional interaction of CPPs that represents a satisfactory response. It is considered as a robustness zone as working within a design space is not considered as a change [39].

Literature survey revealed that there is no previously reported analytical method for simultaneous determination of levetiracetam and pyridoxine HCl. Thus, the main goal of this work was to establish a precise, accurate and sensitive HPLC method for simultaneous determination of levetiracetam and pyridoxine HCl using a QbD-based approach. Screening of the critical factors was achieved using FFD, while CCD was applied in optimization step. The proposed method was tested for linearity, precision, accuracy, and robustness. Levetiracetam and pyridoxine HCl were simultaneously determined in prepared

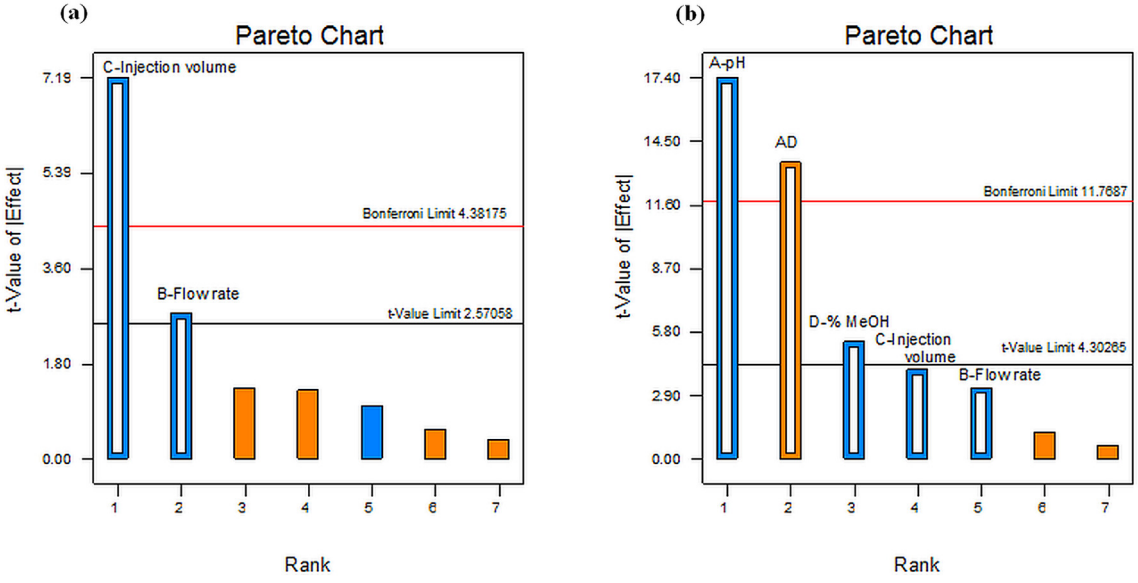


**Fig. 2.** Pareto chart showing effect of factors on (a) theoretical plates (levetiracetam) and (b) resolution.