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Short Communication

Comparison of Different Approaches for Calculating LOD and LOQ in an HPLC-Based Analysis Method

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Abstract

Background: Sensitivity in the determination of the drug concentration is critical in pharmaceutical analysis. This research investigates several approaches for determining two sensitivity parameters, the Limit of Detection (LOD) and the Limit of Quantification (LOQ), in the analysis of the drug concentration using High-Performance Liquid Chromatography (HPLC).

Methods: The study evaluates the FDA's Lower Limit of Quantification (LLOQ) parameter, following global standards and quantitatively comparing sensitivity parameters for an established HPLC-UV method for the analysis of carbamazepine and phenytoin.

Results: The study found that the LOD and LOQ values obtained by different methods varied significantly. The signal-to-noise ratio (S/N) method provided the lowest LOD and LOQ values for both drugs, while the standard deviation of the response and slope (SDR) method resulted in the highest values. This highlights the variability in sensitivity depending on the method used.

Conclusion: The results show significant differences among calculated sensitivity values, emphasizing the influence of methodological variations on sensitivity values. It recommends following FDA criteria in chromatographic-based pharmaceutical analysis to improve the accuracy of drug concentration determination.

Introduction

Achieving precise and sensitive determination of drug concentrations for both quality control and biological fluid monitoring purposes requires the use of a highly accurate analytical approach.[1-3] Consequently, the development and validation of analytical methods play a crucial role in pharmaceutical discovery and assessment.[4] Method validation seeks to guarantee that the chosen approach not only satisfies but exceeds the minimum requirements established by regulatory authorities in terms of sensitivity, accuracy, precision, and other relevant factors.[5,6]

In the field of chemistry, a characteristic aspect of any analytical procedure is the lowest amount of the analyte that is able to be identified or measured with a certain level of reliability.[7,8]

For this purpose, the two fundamental indicators widely used to evaluate the sensitivity of an analytical technique are the Limit of Detection (LOD) and the Limit of Quantification (LOQ).[9] LOD and LOQ are described by various terminology, but broadly, LOD refers to the minimum amount of an analyte present in a sample that could be identified but, under the given testing circumstances, is not accurately measured. Conversely, the LOQ is defined as the minimum concentration of an analyte within a sample that can be precisely and accurately measured according to the required standards under the specified conditions of the test.[10]

Various methodologies determine these detection limits based on the recommendations of different regulatory agencies.[11,12] For instance, the International Conference on Harmonization (ICH) has proposed three distinct strategies, which are explained as follows:[13,14]

1. Visual evaluation: This involves preparing samples with specified concentrations of analyte and subsequently evaluating the concentration at which the analyte can be consistently detected or quantified for LOD and Limits of Quantification LOQ.

2. Signal-to-noise ratio: A commonly favored approach, particularly for instrumental methods and chromatographic techniques with constant background noise. When determining the LOD, the signal-to-noise ratio should be 2 or 3, while the LOQ should have a ratio of 10. Notably, even though a minimum of 10 separate determinations is required to establish the LOD, increasing the number of determinations is preferable when the LOD is specified as 2 × blank to prevent incorrect results.

3. The ratio of the standard deviation of the response and the slope of the calibration curve: In this approach, the

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LOD is calculated by taking 3 times the standard deviation (SD) of the response divided by the slope of the calibration curve. Similarly, the LOQ was determined by multiplying the ratio of SD to the calibration curve slope by 10.

There are several ways to determine the SD of the response, including calculating the standard deviation of the blank response, computing the regression line's residual standard deviation, measuring the standard deviation of the line's y-intercept, or assessing the standard error associated with the estimate.[2,15]

Furthermore, the regulations established by the Association of Official Analytical Chemists (AOAC) regard the LOD as greater than 20 blank values, and they do not provide any suggestions for the LOQ.[16]

The USP (United States Pharmacopeia) proposes that LOD be defined as measuring a sample with a known concentration of analyte and defining the minimal level at which the analyte is able to be reliably identified. Additionally, the USP recommends using the same method as the International Council for Harmonization (ICH) for determining the Limit of Quantification (LOQ).

It is important to mention that the FDA has yet to publish any guidelines for LOD measurement. Instead of using LOQ, they have suggested an alternative metric termed LLOQ (lower limit of quantification). In order for the LLOQ to gain approval, it must satisfy particular requirements. The criteria for measurement are as follows: (A) The signal at the lowest possible concentration of the analyte, known as the LLOQ, must be at least five times greater than the signal produced by the blank sample; (B) Precision must be in the range of 20%; and (C) Accuracy must be within the range of 80% to 120%.

In our study, we determined the LOD, LOQ, and LLOQ of a previously published research for the quantification of two anti-epileptic drugs i.e., Carbamazepine and Phenytoin, employing different approaches.[17] Subsequently, we conducted a numerical comparison of these values. The calculations were performed with careful consideration of various strategies suggested by the regulatory bodies. This thorough approach ensures the accuracy and reliability of the results of the analysis, following the rigorous criteria established by regulatory authorities.

Methods

Material

Carbamazepine was purchased from Sobhan Pharmaceutical Company (Iran). Phenytoin was purchased from Alhavi Pharmaceutical Company (Iran). Methanol, acetonitrile, potassium dihydrogen phosphate and orthophosphoric acid were obtained from Merck Company (Germany). Deionized water was purchased from Shahid Ghazi Pharmaceutical Company (Iran).

Apparatus

HPLC analysis was performed using a WellChrom Maxi-Star K-1000 pressure pump, a WellChrom K-2500 spectrophotometer, a Knauer K-5003 four-channel degasser, and EuroChrom 2000 data processor software (Berlin, Germany). A Nova-Pak® C18 analytical column (Milford, Ireland; 250 × 4.6 mm, 4 µm) was used for the separation process. A Grace Vydac space column heater (Worms, Germany) was used to set the column temperature at 25 °C.

HPLC method

The calibration curves for carbamazepine and phenytoin were plotted using the previously established analytical approach developed using HPLC for the simultaneous determination of antiepileptic medications.[17]

The mobile phase was composed of a mixture of phosphate buffer, 2-propanol and acetonitrile in a ratio of 63:15:22 (v/v/v) and the pH of the mobile phase was set to 6.0 with the help of orthophosphoric acid. 0.89 g of potassium dihydrogen phosphate (KH_2PO_4) was dissolved in 100 mL of distilled water to form the phosphate buffer. Freshly prepared mobile phase, filtered using a 0.45 µm membrane filter, then degassed for 15 minutes. The chromatograms were recorded at 220 nm.

The obtained data from the calibration curve was used to calculate important sensitivity parameters such as the LOD, LOQ, and LLOQ using the distinct strategies aforementioned suggested by the regulatory bodies.

Results and Discussion

Table 1 provides the linear range, the calibration curve equation and its SD. The results of the experiments, including LOD, LOQ, and LLOQ are shown in Table 2. It has been shown that distinct LOD and LOQ values (sensitivity parameters) were obtained by using various methods. These methods were based on the peak of the blank and its associated standard deviation, the equation of the calibration curve, the linear range, and the standard deviation of the calibration curve (Table 1). It was found that these findings were considerably different from the sensitivity criteria that were given in the FDA regulation (LLOQ).

According to FDA guidelines, the sensitivity of the developed method (LLOQ) for the quantification of carbamazepine and phenytoin was 1 and 2.5 mg/L, respectively. At the LLOQ concentration, the back-calculated error was in the range of ±20%. These results support the validity of the established analytical approach

Table 1. Details of calibration curve for determination of carbamazepine and phenytoin by HPLC method.

Analyte	Linear range, mg/L	Calibration curve equation	SD of the blank	SD of the calibration curve
Carbamazepine	1.0-30	y = 11205x + 10515	5354.18	10945.12
Phenytoin	2.5-30	y = 3278.8x + 2203.1	1338.69	2631.21

Table 2. Sensitivity parameters, LOD, LOQ, and LLOQ calculated using three distinct approaches in the HPLC analytical methods for the evaluation of carbamazepine and phenytoin.

Analyte	Visual Evaluation		Signal-to-Noise Ratio		Response's SD-to-Calibration curve's slope Ratio		Blank's SD-to-Calibration curve's slope Ratio		LLOQ (mg/L)
	LOD (mg/L)	LOQ (mg/L)	LOD (mg/L)	LOQ (mg/L)	LOD (mg/L)	LOQ (mg/L)	LOD (mg/L)	LOQ (mg/L)	
Carbamazepine	0.20	0.50	0.15	0.50	2.93	9.77	1.43	4.73	1.0
Phenytoin	0.30	2.50	0.25	2.50	2.41	8.02	1.22	4.04	2.5

with the aforementioned sensitivity levels. However, estimated LOQ values from various methodologies provide unreliable and varying sensitivity values ranging from 0.5 to 9.77 mg/L for carbamazepine and 2.5 to 8.02 mg/L for phenytoin, as shown in Table 2. Various approaches provide significant variations in the computed parameters of LOD and LOQ, with the LLOQ where this variance is determined by the numerical standard deviation (SD) values of the blank samples. The SD values of blank samples have the potential to substantially impact both LODs and LOQs.

Conclusion

The validation of pharmaceutical analysis methods has revealed that the LOD and LOQ values are prone to fluctuation and lack of consistency, as indicated here by the various approaches used to determine these values. Therefore, the criteria suggested by the FDA are more suitable for the sensitivity assessment of chromatographic methods in pharmaceutical and biomedical analysis. Implementing this approach would significantly help to reduce the variations and differences observed when employing diverse methods. By following the FDA criteria, researchers can ensure the sensitivity of their chromatographic methods is accurately evaluated and compared. This would contribute to greater reliability and reproducibility in biomedical research.

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Author Contributions

Raha Kaviani: Conceptualization, Methodology, Investigation, Formal Analysis, Writing - Original Draft.

Conflict of Interest

The author declares no conflict of interest.

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