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Uncertainty evaluation for determining linezolid in injectable solution by UV spectrophotometry

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ABSTRACT

The aim of this work was to identify the main sources of uncertainty, quantify the standard deviation of each source of uncertainty, and calculated combined and expended uncertainties for the determination of linezolid in injectable dosage forms by UV spectrophotometry. The contributions of precision, linearity and weight of linezolid reference standard are the most significant, contributing with about 77% of the overall uncertainty. The uncertainties on the absorbances (sample and standard), volumetric flasks and volumetric pipettes have virtually no influence on the overall uncertainty. The estimated uncertainty of the spectrophotometric method for determination of linezolid was adequate for the scope of the method.

1. Introduction

Analytical results represent a very important part in a quality control program. Decisions of acceptance or rejection of medicine products are based on analytical results. In this context, the uncertainty estimation is a very useful tool to evaluate the quality of analytical measurement and compliance (or non-compliance) of the medicine product. The uncertainty is a parameter that, when associated with the result of a measurement, characterizes the dispersion of the values that can be fundamentally attributed to a result. The steps involved in the estimation of uncertainty measurement are: (a) specify measuring, (b) identify uncertainty sources, (c) quantify uncertainty components, and (d) calculate combined uncertainty [1–3].

Linezolid is an oxazolidinone antibiotic used for the treatment of serious infections caused by Gram-positive bacteria, including vancomycin-resistant enterococci (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA) [4,5]. The methods reported in the literature for determination of linezolid include UV spectrophotometry, HPLC using UV-detection and fluorescence detection, capillary electrophoresis, TLC followed by densitometric analysis and microbiological assay [6–12].

The uncertainty on the results may arise from many possible sources, including sampling, matrix effects and interferences, environmental conditions, uncertainties of mass and volumetric equipment, uncertainties of spectrophotometric and chromatographic equipment, uncertainties of biological and microbiological responses, purity of reagents and chemical reference substances, method validation and random variability [13–24].

The aim of this work was to identify the main sources of uncertainty, quantify the standard deviation of each source of uncertainty, and calculated combined and expended uncertainties for the determination of linezolid in injectable dosage forms by UV spectrophotometry.

2. Experimental

2.1. Instrumentation

A UV–Visible spectrophotometer (Thermo, Evolution 201) working in the range of 190–900 nm was employed to detect the absorbances of linezolid solutions. An analytical balance (Mettler Toledo, AG204) was used to

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measure the weight of linezolid reference standard. Volumetric flasks (50 mL, 100 mL and 200 mL) and pipettes (1 mL and 5 mL) (Brand or Laborglass) were used in the preparation of standard and samples.

2.2. Reagents

Linezolid reference standard (lot: 020M4707V, purity: 99%) was supplied by Sigma–Aldrich and injectable solution of linezolid (2 mg mL^{-1}) was obtained from Pfizer.

2.3. Linezolid determination by UV spectrophotometry

An accurately amount of 10.0 mg of linezolid reference standard was weighted, transferred to a 100 mL volumetric flask and diluted with ultra-purified water. An aliquot of 5 mL was transferred to a 50 mL volumetric flask and diluted with ultra-purified water. The values of absorbance from standard and sample solutions were measured in spectrophotometer adjusted at 254 nm, using ultra-purified water as blank. The assays were performed in a temperature controlled environment (between 20 and 25 °C). The amount (%) of linezolid in the injectable solution (2 mg mL^{-1}) was calculated as follows:

$$\% = \left(\frac{Au}{As}\right) \times \left(\frac{Cs}{Cu}\right) \times 100 \quad (1)$$

where *Au* is the absorbance of sample solution, *As* is the absorbance of standard solution, *Cs* is the concentration of standard solution and *Cu* is the concentration of sample solution.

2.4. Identifying the sources of uncertainty

The cause–effect figure of uncertainty source is shown in Fig. 1. The most important uncertainties sources considered were associated with the standard preparation, sample preparation, absorbances of standard and sample, and method validation [15].

2.5. Standard preparation

The uncertainty sources of the standard preparation included the weight of linezolid reference standard, the purity of linezolid reference standard, the volumetric flasks (100 mL and 50 mL) and pipettes (5 mL) used in standard preparation.

2.6. Sample preparation

The uncertainty sources of the sample preparation included the volumetric flask (200 mL) and pipette (1 mL) used in sample preparation.

2.7. Absorbances of standard and sample

The uncertainty associated to the absorbances of standard and sample solutions were estimated based on the calibration of UV–Visible spectrophotometer.

2.8. Method validation

Three kinds of uncertainty sources were considered in this study. They were linearity, precision and accuracy.

The procedure to estimate the uncertainty of the UV spectrophotometric method for determination of linezolid was validated by Monte Carlo simulation.

3. Results and discussion

3.1. Quantifying the uncertainty components

The uncertainty components of linezolid determination by UV spectrophotometry are listed in Table 1.

3.2. Standard preparation

An amount of 10.1 mg of linezolid reference standard was weight in an analytical balance. The weighting uncertainty was 0.1 mg, with a coverage factor of 2, according to the certificate of calibration of balance.

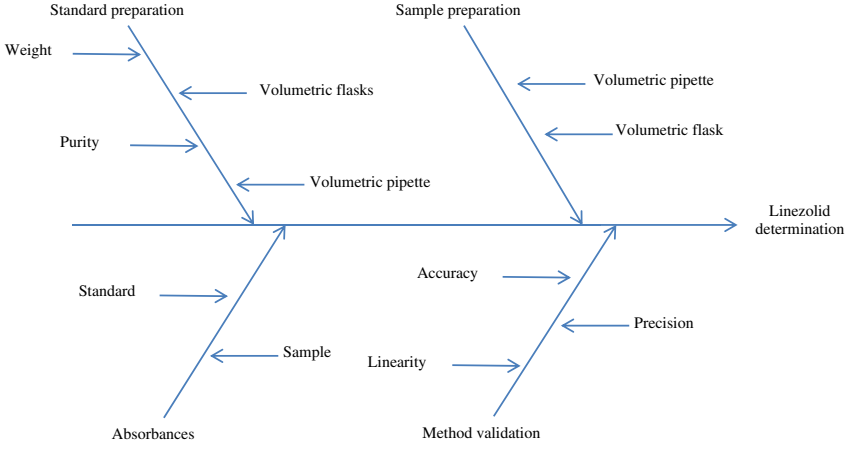

Fig. 1. The cause–effect diagram for linezolid determination by UV spectrophotometry.