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Using measurement uncertainty to assess the fitness for purpose of an HPLC analytical method in the pharmaceutical industry

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ABSTRACT

Chromatographic methods are widely used in pharmaceutical industry to assess quality, safety and efficacy of drug products. We found in literature a few number of works regarding the estimation of measurement uncertainty associated with chromatographic methods, however none of them evaluated whether the measurement uncertainty estimation values are reasonable to be applied in conformity assessment. Because of uncertainty in measurement, there is always the risk of incorrectly deciding whether or not a lot conforms to its specified requirement. In this paper, the measurement uncertainty associated with high performance liquid chromatography (HPLC) assay of amoxicillin 500 mg tablets were estimated, as well as the assessment of process capability and statistical process control of its industrial manufacturing process. In addition, consumer and producer's risks were estimated using Monte Carlo simulation. The manufacturing process was under control, but considering the measurement uncertainty, there was a considerable number of lots that may be out-of-specification. Based on Monte Carlo simulation, it was noticed that the producer's risk was significantly higher than consumer's risk. In addition, overall uncertainty and tolerance ratio was found to be higher than the recommended value. In other words, this assay cannot be deemed fit-for-purpose.

1. Introduction

Chromatographic methods are often used in pharmaceutical industry to assess qualitative and quantitative characteristics related to the quality, safety and efficacy of drug products. Chromatography is a separation technique in which the components of a sample is distributed between stationary (solid or liquid supported on a solid or a gel) and mobile phase (gaseous or liquid form). The separation may be based on adsorption, partition, ion exchange, or based on differences among the physicochemical properties of the molecules. The types of chromatography useful in pharmaceutical industry include thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), gas chromatography (GC), and high-performance liquid chromatography (HPLC). Among the several types of chromatographic methods, liquid chromatography (HPLC) is the most used [1–3].

In last decade, advances in liquid chromatography have been reported, which are mainly focus in improving the separation technique. For instance, ultra-high-pressure liquid chromatography (UHPLC) uses small particulate columns to achieve high resolution, rapid separation [4,5]. Quantitative applications of liquid chromatography depend on the type of detection used after separation. HPLC instruments are often equipped with UV or photo diode-array (PDA) detector. Moreover, fluorescence, electrochemical, light-scattering, refractive index, and mass spectroscopic detectors are also available [1–3].

Essential in the field of pharmaceutical industries and analysis, this technique is widely used to test medicines, to detect and quantify their raw ingredients, impurities, for stability studies, among other uses [6]. In this paper, we used a HPLC method to quantify the content of amoxicillin in tablets. Amoxicillin is a aminopenicillin, being a member of one of the most important groups of antibiotics available, the penicillins. As a broad-spectrum bactericide, it acts against gram-positive and gram-negative bacteria and it belongs to the Essential Medicines List of the World Health Organization, due to its relevance in public health. Amoxicillin is one of the most used antibiotics worldwide, and its monograph is available in pharmacopoeias such as those of Brazil, Britain, India and United States [7–10].

Quality, safety and efficacy of antibiotic drugs are important issues for the pharmaceutical industry, and are strictly related to the assessment of conformity of pharmaceutical products. In this context, process capability indices, including Cp and Cpk, have been widely used in the manufacturing industry to provide numerical measures on whether the process is capable of reproducing products according to the given

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specification limits. The index Cp takes into account the process variability relative to the manufacturing tolerance, reflecting product quality consistency and potential process capability. The Cpk index considers the magnitude of process variation and process diversion from the target value [11,12]. Analytical measurement is an important part of process capability and statistical process control assessment.

Analytical measurements always have intrinsic errors and therefore uncertainties. An uncertainty interval defines the range of values that could reasonably be attributed to the measurand. General rules for evaluating and reporting uncertainty in measurement have been published by the Guide to the Expression of Uncertainty in Measurement [13,14]. The procedure proposed by the Eurachem/Citac guide and the spreadsheet method are convenient for the identification of the main sources of uncertainty, in order to simplify the calculation procedure and/or reduce the final uncertainty [15,16]. As long as we found in literature, there is a few number of works regarding the estimation of measurement uncertainty associated with chromatographic methods used in pharmaceutical industry [17–19], and none of them applied to HPLC assay of amoxicillin in tablets.

The sources of uncertainty associated with chromatographic methods can include repeatability of sample and standard peak areas, nonlinearity of calibration curve, reference materials and sampling, and chromatographic equipment [20]. Estimation of measurement uncertainty based on validation results has been also reported in literature [17]. However, these papers did not evaluate whether the values of uncertainty were reasonable to be applied in conformity assessment.

Because of uncertainty in measurement, there is always the risk of incorrectly deciding whether or not a lot conforms to its specified requirement. Such errors can lead to accepting a lot that is actually nonconforming or to reject one when it complies with the specifications (consumer and producer's risks, respectively) [13,21]. Monte Carlo simulations have been used in several areas to solve mathematical problems, such as to determine the mentioned risks of incorrect decisions [22].

Consumer and producer's risk assessment and management are important issues in improving quality, safety and efficacy of pharmaceutical products. Thus, the aim of this paper was to estimate the measurement uncertainty associated with HPLC assay of amoxicillin 500 mg tablets, as well as to assess the process capability and statistical process control of an industrial manufacturing process of amoxicillin 500 mg tablets. In addition, consumer and producer's risks were estimated using Monte Carlo simulations.

2. Material and methods

2.1. Amoxicillin 500 mg tablets assay by HPLC

Forty-five lots of amoxicillin 500 mg tablets were analyzed for assay. Amoxicillin reference standard (USP RS) was weighed (about 12 mg) and transferred to a 100 mL volumetric flask (Brand). An aliquot of 2 mL was pipetted (Brand) to a 200 mL volumetric flask (Brand), to final concentration of 1.2 µg/mL (standard solution). Twenty tablets of each lot were weighed and powdered. About 72 mg of the powdered tablet was weighed and transferred to a 500 mL volumetric flask (Brand). An aliquot of 2 mL was pipetted (Brand) to a 200 mL volumetric flask (Brand), to final concentration of 1.2 µg/mL (sample solution).

The liquid chromatograph (Thermo Scientific Accela or Agilent Technologies 1200 Series) was equipped with a 230 nm detector and a 4 mm × 25 cm column (octadecylsilane, C18), with mobile phase (mixture of phosphate buffer pH 5 and acetonitrile, 96:4) at a flow rate about 1 mL per minute. Volumes of 10 µL of standard and sample solutions were injected (3 replicas each) and the areas of peaks were measured. System suitability was assessed by the capacity factor (between 1.1 and 2.8), theoretical plates (not less than 1700), tailing factor (not more than 2.5), and standard deviation for replicate injections (not more than 2%). Amoxicillin content was calculated using Eq. (1).

$$Amox\% = \frac{A_{sp}}{A_{st}} \times \left(\frac{m_{sp} \times 50}{AW \times 500vf} \times \frac{2vpa}{200vfa} \right) \Big/ \left(\frac{m_{st} \times purity}{100vf \times 500} \times \frac{2vpb}{200vfb} \right) \times 100 \quad (1)$$

where, A_{sp} and A_{st} are the areas of peaks for sample and standard solutions, respectively, m_{sp} and m_{st} are the weighed mass of sample and standard, AW is the average weight of amoxicillin tablets, $purity$ is the purity of amoxicillin reference standard (USP RS), $500vf$, $100vf$, $200vfa$ and $200vfb$ are the volume of volumetric flasks, and $2vpa$ and $2vpb$ are the volume of volumetric pipettes.

2.2. Process capability and statistical process control

Manufacturing process of amoxicillin 500 mg tablets was assessed by calculating the process capability index (Cpk). Cpk is an index which measures how close a process is running to its specification limits, relative to the natural variability of the process. The larger the Cpk, the less likely it is that any lot of amoxicillin tablet will be out-of-specification. Regulatory specification from 95 to 105% of the label content was adopted to calculate Cpk.

In addition, a Statistical Process Control (SPC) chart was used to monitor and control the manufacturing process of amoxicillin 500 mg tablets over time. Lower and upper limits (LL and UL, respectively) were calculated as function of manufacturing process variability. By comparing HPLC assay results (in time order) to UL and LL, we could draw conclusions about whether the manufacturing process variation was consistent (in control) or was unpredictable (out of control).

Process capability and Statistical Process Control was assessed using Minitab 17™.

2.3. Measurement uncertainty estimation

Eurachem/Citac guideline was used to estimate the measurement uncertainty associated with HPLC assay of amoxicillin 500 mg tablets. The main sources of uncertainty were identified based on Equation (1). Individual sources of uncertainty were quantified from calibration certificate analysis (Type I) or calculated as the standard deviation of replicates (Type II). A list of these uncertainties is presented in Table 1.

Combined uncertainty was estimated using equation (2). Alternatively, measurement uncertainty was also estimated using spreadsheet method.

Table 1
Individual sources of uncertainty associated with HPLC assay of amoxicillin 500-mg tablets.

Sources of uncertainty	Symbol	Value	U	Distribution	u
Areas of sample solution	A_{sp}	185,624	–	Normal	1018
Areas of standard solution	A_{st}	185,971	–	Normal	1020
Mass of sample	m_{sp}	72	0.1 mg	Normal	0.05
Average weight of tablets	AW	600.5	–	Normal	2.9
500 mL volumetric flask	$500vf$	500	0.15	Triangular	0.09
2 mL volumetric pipette	$2vpa$	2	0.006	Triangular	0.003
200 mL volumetric flask	$200vfa$	200	0.10	Triangular	0.06
Mass of standard	m_{st}	12	0.1 mg	Normal	0.05
Purity of reference standard	$purity$	0.995	0.005	Rectangular	0.002
100 mL volumetric flask	$100vf$	100	0.08	Triangular	0.05
2 mL volumetric pipette	$2vpb$	2	0.006	Triangular	0.003
200 mL volumetric flask	$200vfb$	200	0.10	Triangular	0.06