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LC method for the determination of R-timolol in S-timolol maleate: Validation of its ability to quantify and uncertainty assessment

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Abstract

This article presents the validation results of a chiral liquid chromatographic (LC) method previously developed for the quantitative determination of R-timolol in S-timolol maleate samples. A novel validation strategy based on the accuracy profiles was used to select the most appropriate regression model, to assess the method accuracy within well defined acceptance limits and to determine the limits of quantitation as well as the concentration range.

The validation phase was completed by the investigation of the risk profiles of various acceptable regression models in order to ensure the risk of obtaining the future measurements outside the acceptance limits fixed a priori.

On the other hand, the present paper also shows how data used in this validation approach can be used to estimate the measurement uncertainty. The uncertainty derived from β-expectation tolerance interval (σ^2_{Tol}), which is equal to the uncertainty of measurements as well as the expanded uncertainty (U_x) using a coverage factor $k = 2$ was estimated. The uncertainty estimates obtained from validation data were finally compared with those obtained from interlaboratory and robustness studies.

Keywords: Timolol maleate; R-timolol impurity; Liquid chromatography; Validation; Accuracy profile; Risk; Uncertainty assessment

1. Introduction

During the development of analytical methods, it becomes more and more obvious and essential that after the optimisation step the analysts have to demonstrate that the obtained results are reliable for the intended use of the method. In this way, many procedures are available, such as those established by ICH and SFSTP commissions [1–4]. However, in a statistical point of view and by considering the decision making according to the defined acceptance limits and the risk related to the future use of the method, some drawbacks were noticed. Recently, a novel validation strategy based on the use of accuracy profiles has been introduced [5,6]. The notion of including the use of accuracy profiles 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 that the laboratory will have to determine. In fact, what is expected from all analysts when using an analytical method is that the difference observed between the measured result (x_i) and the "true value" (μ_{T}) of the sample (which will always remain unknown) is inferior to an acceptance limit (λ), as can be expressed in the following Eq. (1):

$$|x_i - \mu_{\mathrm{T}}| < \lambda \quad (1)$$

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The acceptance limits are different and depend on the requirement of the analyst or the objective of the analytical method [5,6]. At each concentration level, the accuracy profile is obtained by computing the β-expectation tolerance interval that allows to predict where β% of the future measurements are expected to lie. Therefore, this new strategy clearly shows an advantage over the commonly used procedures by allowing the control of the risks associated to the use of the method. In fact, this notion of risk is linked to the notion of guarantee concerning the future analysis of unknown samples using the validated method [5,6]. As suggested in our previous paper [7], a procedure can be qualified as acceptable if it is very likely, i.e. with a "guarantee", that the difference between every measurement (x_i) of a sample and its "true value" (μ_T) is inside the acceptance limits predefined by the analyst. From this, one can refer to the risk expressing the proportion of measurements that are expected to fall out of acceptance limits ($\pm\lambda$) during the routine analysis. That risk can be evaluated by means of a profile by level of investigated concentration and can be translated by the following Eq. (2):

$$Pr[|x_i - \mu_T| > \lambda] \leq \beta \quad (2)$$

where Pr is the probability that a measurement will fall outside the acceptance limits and β the maximum risk that the analyst is able to take during routine use [6–8].

On the other hand, by considering this new validation strategy, Feinberg et al. [9] introduced the possibility to estimate the uncertainty using the validation data. The definition of uncertainty can be found in the Eurachem guide [10]. From an analytical perspective, this can be considered as straight forward for many analysts. Indeed, even though few approaches have been described for the estimation of uncertainty from validation process [11–16], there is still a need to clarify the relationship between validation and uncertainty for many analysts and particularly to show how the validation data can be practically used to estimate the uncertainty measurement. A recent draft of guide ISO/DTS [17] suggests that experimental data obtained from repeatability, reproducibility and trueness studies could be used to determine uncertainty measurement [9]. Other approaches, such as those proposed by the International Organization for Standardization (ISO) [18] and the Analytical Methods Committee [19] can be applied to estimate the uncertainty.

The first objective of this paper is to fully validate the liquid chromatographic (LC) method for the determination of R-timolol in S-timolol samples, applying this novel validation strategy based on the accuracy profiles. Indeed, the method was previously developed for the simultaneous determination of R-timolol and other related substances in S-timolol maleate bulk material but was not validated [20].

The second objective is to estimate the measurement uncertainty from validation data for the determination of R-timolol content. For this purpose, the approach described in [9] is applied.

Finally, the third objective is the comparison of different studies to evaluate the uncertainty, namely the present validation, the interlaboratory [21] and the robustness [22] studies. In these three studies, the present LC method was concerned to analyze S-timolol maleate samples containing R-timolol impurity at similar concentrations.

2. Experimental

2.1. Chemical and reagents

Samples of S-timolol maleate, R-timolol maleate, isotimolol, dimer maleate and dimorpholinothiadiazole were obtained from the European Pharmacopoeia Secretariat (Strasbourg, France).

N-Hexane of LC grade was purchased from Hipersolv (Poole, England), 2-propanol for analysis from Merck (Darmstadt, Germany) and diethylamine (DEA) for analysis from Sigma (St. Louis, MO, USA).

2.2. Apparatus

The chromatographic system from Shimadzu (Shimadzu Corporation, Kyoto, Japan) was composed of a model LC-10 AT pump, a model SIL-10 AVL automatic injector, a model CTO-10 AC oven and a model SDP-M10 AVP diode array detector. To control the LC system, a Class LC-10 software from Shimadzu was loaded on a Pentium 166 MHz computer. A model CBM-10 Shimadzu interface was used to send the signals from the detector to the computer.

A Chiralcel OD-H column (250 mm × 4.6 mm, i.d.) packed with cellulose tris(3,5-dimethylphenylcarbamate) coated on silica particules (5 μm) from Daicel Limited Industries (Tokyo, Japan) was used. A guard column (4 mm × 4 mm, i.d.) packed with LiChrospher 100 Diol (5 μm) (Merck) and maintained with a holder was used.

The accuracy profiles as well as the statistical calculations including the validation results and the different uncertainty estimates were obtained using the e-noval® software (Arlenda, Belgium). JMP® software Version 5.1 for Windows (SAS Institute, Cary, NC, USA) was also used for further statistical calculations.

2.3. Analytical conditions

The chromatographic separation was carried out using a mobile phase consisting of a mixture of hexane, 2-propanol and DEA pumped at a constant flow rate of 1.0 mL min^{-1}. UV detection was set at 297 nm. Prior to use, the mobile phase was degassed for 15 min in an ultrasonic bath. The injection volume was 10 μL.

2.4. Preparation of standard solutions

The dissolution of analytes and dilution of sample solutions were realized in 2-propanol containing 1% (v/v) of