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Collaborative study of an liquid chromatographic method for the determination of *R*-timolol and other related substances in *S*-timolol maleate

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Abstract

A collaborative study applying an enantiomeric liquid chromatographic (LC) method was carried out to determine the content of the enantiomeric impurity *R*-timolol and other related substances in three different *S*-timolol maleate samples. Eight laboratories, all located in Europe, participated in the study. The quantitative results obtained were used to estimate the uncertainty on the content of the different impurities. For that purpose, a set-up was adapted from the ISO guidelines 5725-2, which allowed the estimation of the different variances, i.e. the between-laboratories ($s^2_{\text{laboratories}}$), the between-days ($s^2_{\text{days}}$) and the between-replicates ($s^2_{\text{replicates}}$). The variances of repeatability (s^2_{r}) and reproducibility (s^2_{R}) were then calculated using the equations $s^2_{\text{r}} = s^2_{\text{replicates}}$ and $s^2_{\text{R}} = s^2_{\text{replicates}} + s^2_{\text{days}} + s^2_{\text{laboratories}}$. For the timolol impurities, it was found that the estimated uncertainty seem to be concentration-dependent. Since the LC method which combines the compendial ones for enantiomeric purity and related substances testing was applied to evaluate uncertainty in this collaborative study, it was shown how a laboratory can evaluate the uncertainty of its results when applying the method in the future.

Keywords: Collaborative study; Uncertainty estimation; Reproducibility; *R*-timolol; Degradation products; Liquid chromatography

1. Introduction

Generally, when a new analytical method is developed, it is not very common to evaluate it in an interlaboratory study, unless if the proposed method is introduced in a monograph or is used by different laboratories. Moreover, as a final aspect of an analytical method development and validation, such studies can be considered as an important step preceding a

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method transfer for a large application, not only to confirm its applicability but also to evaluate the performance of participating laboratories. At this stage of the method development, the participating laboratories can be considered as "intermediate users" whose results will be of great importance for laboratories future decision making of the method and therefore, ensure the "end users". The performance parameters, i.e. resolution values between peaks of interest, ratio of the signal-to-noise, as well as the reproducibility of the results can be evaluated either intra- or inter-laboratorycally. In the latter case, by considering the reproducibility resulting from quantitative data, one could expect that any individual measurement result, here the contents of *R*-timolol and of other related substances, issued from this study or from any future application should be encompassed in an interval of dispersion attributed to the measurand. This interval is often expressed in terms of an expanded uncertainty (U_x), which is a multiplication of the standard uncertainty (u_x) with the multiplying factor, termed the coverage factor (k) [1–3] depending on the sources of variability identified. In fact, the data of the collaborative study can be used to determine measurement uncertainty applying the "top-down" approach as defined by the Analytical Methods Committee [4]. The definition of uncertainty can be found in the literature [5]. Thus, considering the estimate uncertainty results, a given laboratory could make a statement concerning its final result uncertainty during future use of the method. Another advantage of the uncertainty evaluation [2] is the fact that the evaluation of measurement uncertainty provides starting points for method optimisation through a better understanding of the method and uncertainty contribution. The trueness which is generally also estimated in a validation process may as well be estimated by collaborative studies. Typically, both repeatability (s_r) and reproducibility (s_R) standard deviations are provided by collaborative studies. However, the estimate of trueness which is measured as bias with respect to a known reference value may also be provided [2].

Besides the approach defined by the Analytical Methods Committee (Top-down) [4], an alternative to estimate uncertainty is proposed namely by the International Organization for Standardization (ISO) also called "bottom-up" approach [3]. It considers all the contributing sources of uncertainty and combines them to assess uncertainty of measurement result. However, the latter approach is less interesting in analytical chemistry [6] since it is not evident to quantify all sources of uncertainty.

A liquid chromatographic (LC) method was recently developed in a normal-phase mode for the simultaneous determination of timolol enantiomers and three related substances [7]. Timolol maleate is a non selective β-adrenergic blocker used as a single enantiomer (*S*-timolol) against hypertension, arrhythmias and angina pectoris. It is also indicated for the secondary prevention of myocardial infarctus [8–11]. Its pharmacological effect in chronic open angle glaucoma allows it to be prescribed in the ophthalmologic fields for the topical treatment of increasing intraocular pressure [12–14]. However, the active ingredient (*S*-timolol maleate) may contain its enantiomer *R*-timolol as an impurity as well as the following related substances: isotimolol (degradation product), dimer maleate and dimorpholinothiadiazole (impurities of synthesis). Their chemical structures are given in Fig. 1. This LC method for the simultaneous determination of both

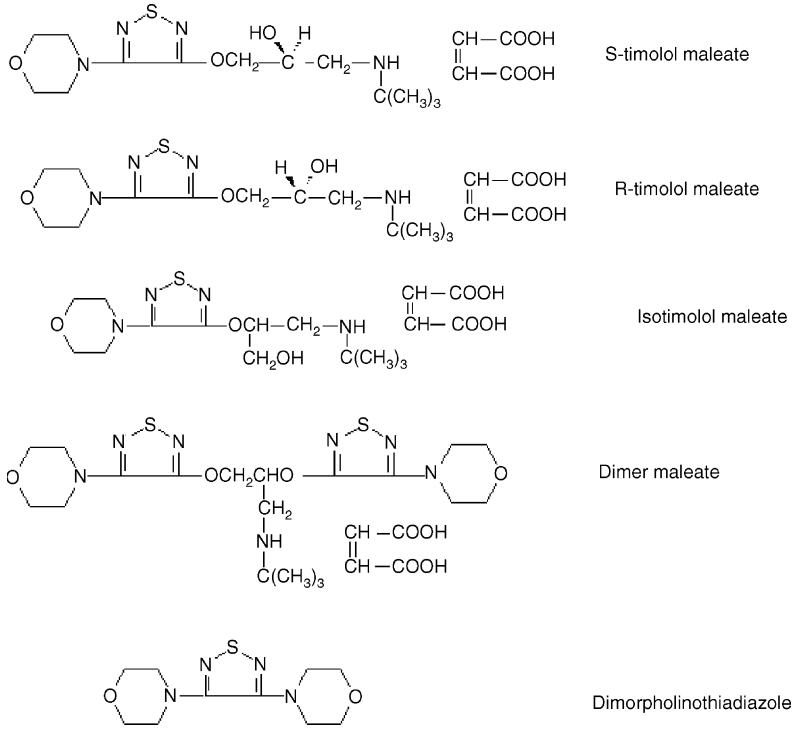

Fig. 1. Chemical structures of *S*-timolol maleate, *R*-timolol maleate, isotimolol maleate, dimer maleate and dimorpholinothiadiazole.