



ENANTIOMERIC PURITY TESTING OF S-TIMOLOL BY NON-AQUEOUS CE USING HEPTAKIS(2,3-DI-O-METHYL-6-O-SULFO)- β -CYCLODEXTRIN AS CHIRAL ADDITIVE – VALIDATION USING THE ACCURACY PROFILE STRATEGY AND ESTIMATION OF UNCERTAINTY



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OBJECTIVE

- > To evaluate the non aqueous CE (NACE) method for the determination of the enantiomeric purity of S-timolol maleate (S-TM maleate)
- > To validate the developed NACE method for the determination of R-TM (cf. Fig.1)
- > To perform the purity testing of R-TM in S-TM maleate
- > To assess uncertainty using the validation data

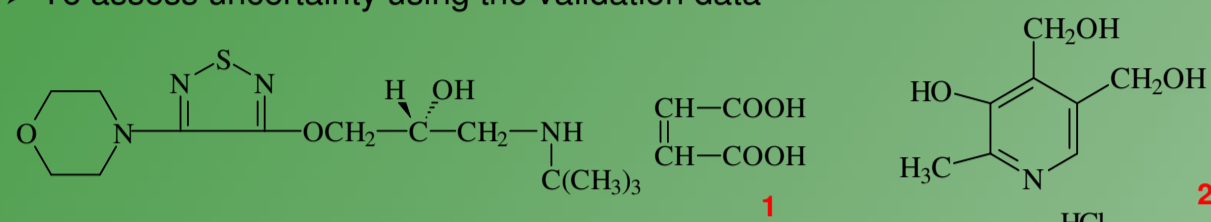


Fig. 1. Structures of R-timolol maleate (R-TM maleate) (1) and pyridoxine hydrochloride (2)

RESULTS AND DISCUSSION

The electrophoretic separations were carried with uncoated fused silica capillaries (50 μ m i.d.; 48.5 cm total length, 40 cm to the detector). The selected background electrolyte cyclodextrin (CD) was composed of potassium camphorSO₃⁻ and heptakis(2,3-di-O-methyl-6-O-sulfo)- β -CD (HDMS- β -CD) in methanol acidified with 0.75 M formic acid. The capillary was thermostated at 15 °C and UV detection was performed at 295 nm. Samples were dissolved in methanol.

> Optimisation of the CE conditions

In a previous study [1], a fast method development strategy was applied to the enantioseparation of TM maleate. Three different experimental conditions (Table 1) were evaluated.

	Concentrations (mM)		
	25	10	30
K-CamphoSO ₃ ⁻	25	10	30
HDMS- β -CD	5	30	30

Table 1. Experimental conditions

The best enantioseparation of TM was observed with the third experimental condition (Rs value = 8.5) (Fig.2.), which was therefore selected for the present study.

The use of an internal standard is required in CE for quantification. On basis of the following selection criteria (solubility, stability, absence of toxicity; achiral and basic compound presenting an absorptivity comparable to that of R-TM; proximity to R-TM and preferably migrating before TM enantiomers), pyridoxine (Fig.1) was selected.

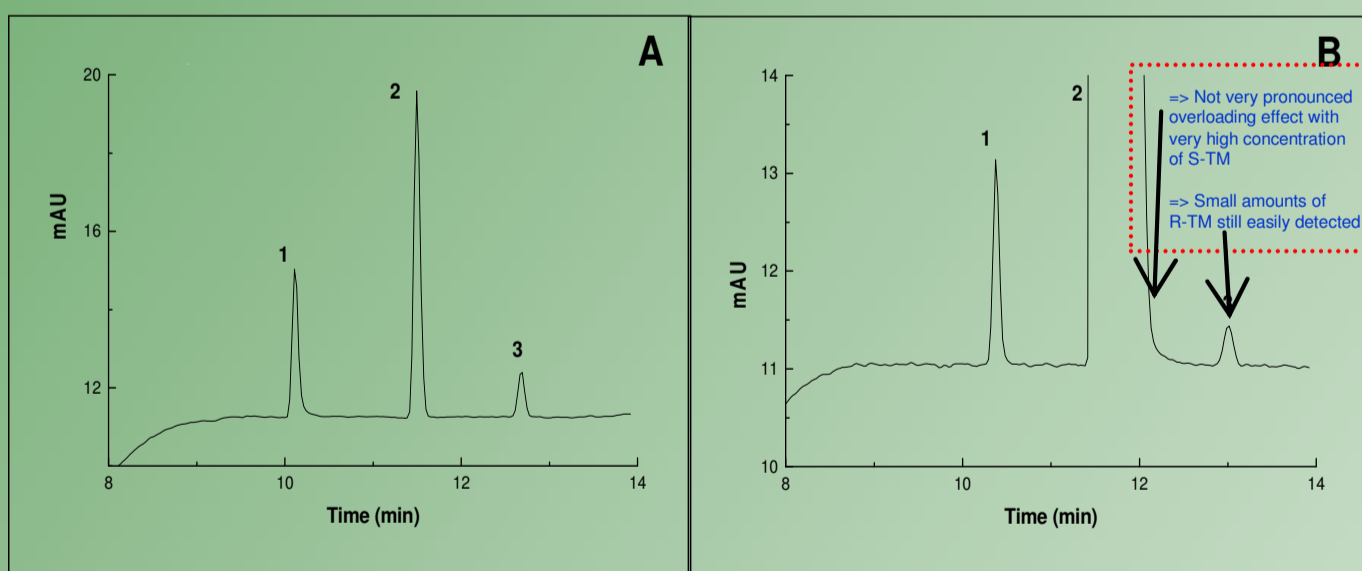


Fig. 2. Typical electropherograms of a mixture solution of pyridoxine (10 μ g/mL), S-TM (20 μ g/mL) and R-TM (5 μ g/mL) (A) and of a test sample solution of S-TM maleate (2 mg/mL) containing R-TM (2 μ g/mL or 0.1%) and pyridoxine (5 μ g/mL) (B). Peaks: 1.- pyridoxine, 2.- S-TM and 3.- R-TM; Resolutions (Rs) calculated in A : Rs_{1,2} = 9.2 and Rs_{2,3} = 8.5; Selectivity (α) calculated in A : $\alpha_{1,2}$ = 1.137 and $\alpha_{1,3}$ = 1.254

The effect on detection sensitivity of the S-TM solution concentration and the injection time was investigated from 0.5 mg/mL to 3 mg/mL and from 6 sec to 60 sec, respectively. The best results were obtained (Fig. 2B) by using a 2 mg/mL S-TM solution and an injection time of 8 sec (5 kPa). TM enantiomers as well as the internal standard and the S-TM were still separated to a large extent.

> Validation

To validate the ability of the CE method to quantify R-TM impurity in S-TM maleate samples, a novel approach using accuracy profiles was applied [2]. It is based on β -expectation tolerance intervals for the total measurement error (trueness + precision). For that purpose, two series of standard samples were prepared by dilution from independent stock solutions : the calibration samples (CS) and the validation samples (VS). Three replicates (n = 3) were prepared per concentration level (m = 4). The overall preparation step was repeated for three days (p = 3).

In order to simulating as much as possible the future routine analysis of impurities in S-TM maleate samples, the VS were independently prepared in the most pure sample of S-TM maleate. A concentration range from 2.0 to 24.0 μ g/mL of R-TM has been investigated. The response function was determined by applying different regression models and, from both responses of VS and regression line (CS) obtained, the most suitable accuracy profile was selected (Fig.3).

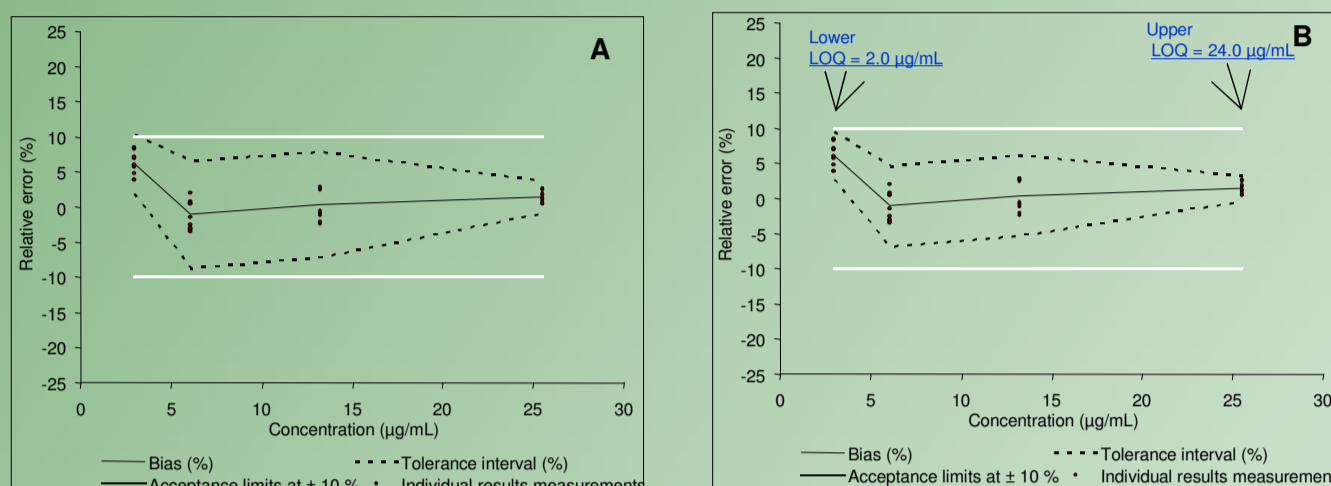


Fig. 3. Accuracy profiles obtained by using one concentration level (24.0 μ g/mL) for calibration and considering a risk of 5% (A) and of 10% (B).

As currently practiced in the Pharmacopoeia's monographs, only one calibration level was investigated (Fig. 3). By using 24 μ g/mL as calibration level (external standard procedure) the accuracy profile obtained with a risk of 10% was inside the acceptance limit (Fig. 3A). On the other hand, it can be noticed that at the concentration levels above the maximum concentration tolerated for R-TM impurity (≥ 20 μ g/mL) the acceptance limits could even be reduced to 5%. Consequently, this simple regression model was used to evaluate the different validation criteria. The LOQ (see Fig. 3B), the trueness and precision were evaluated (cf. Table 2). The observed bias was below 6.07%. The RSD values were between 0.7% and 1.8% for repeatability and less than 2.4% for the intermediate precision. By considering a 5% risk (Fig 3A) the model was not suitable even by applying simple linear and weighted linear regression models. Indeed, most of the β -expectation tolerance intervals were not within the acceptance limits, whatever the risk considered.

Table 2. Trueness and precision of the method, and uncertainty assessment

Concentrat. level (μ g/mL)	Trueness		Precision (R.S.D.)		Uncertainty of bias (10^2 μ g/mL)	Uncertainty (10^2 μ g/mL)
	Relative bias (%)	Recovery (%)	Repeatability (%)	Intermediate precision (%)		
2.0	6.07	106.1	1.8	1.8	1.74	5.50
5.0	- 1.12	98.88	1.4	2.3	6.96	15.47
12.0	0.37	100.4	1.7	2.4	15.39	35.54
24.0	1.37	101.4	0.7	0.8	9.30	23.27

> Purity testing

To confirm the suitability of the validated NACE method, several samples of S-TM maleate from different sources were analyzed. The content of the chiral impurity in those samples was determined. As can be seen in Table 3, the content of R-TM never exceeded 1.0% which is the maximum content tolerated for this impurity as mentioned in the monograph. The quantification results were compared to those obtained by LC and were found equivalent.

Table 3. Content (in %) of R-TM in six S-TM maleate samples (n = 3)

Analytical method		S-TM maleate samples					
		Batch N° 11351	Batch N° 11486	Batch N° A5799	Batch N° 11483	Batch N° 11484	Batch N° A5800
CE	Mean	0.10	0.11	0.26	0.29	0.35	0.52
	(RSD, %)	(11.4)	(6.3)	(6.8)	(2.3)	(3.4)	(7.4)
LC	Mean	0.11	0.11	0.24	0.25	0.31	0.49
	(RSD, %)	(2.1)	(4.7)	(3.7)	(0.6)	(3.4)	(1.2)

> Uncertainty assessment

As a parameter of performance, the uncertainty of measurement was evaluated at each concentration level of R-TM as defined in validation. Taking into account the demonstration of Feinberg et al. [3], the uncertainty can be determined using the data of validation that is derived from the variance used to construct the β -expectation tolerance limits. As shown in Table 2, the values are comprised between 0.0174 – 0.154 μ g/mL and 0.0550 – 0.355 μ g/mL for the bias uncertainty and the measurement uncertainty, respectively. It was remarked that the uncertainty seems to increase with the concentration.

[1] A.-C. Servais, M. Fillet, P. Chiap, W. Dewé, P. Hubert, J. Crommen, *Electrophoresis* 2004, 25, 2701
 [2] P. Hubert et al, *STP Pharma Pratiques* 2003, 13, 101.
 [3] M. Feinberg, B. Boulanger, W. Dewé, Ph. Hubert, *Anal. Bioanal. Chem.* 2004, 380, 502.