Title: Dual Design Spaces for Micro-Extraction Together with the Core–Shell Chromatographic Determination of Dorzolamide and Timolol in Rabbit Plasma: An Example of Quality by Design Method Development

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An innovative strategy dedicated to quality-by-design (QbD) principles has been comprehensively applied in the set-up of a vortex aided salting-out-assisted liquid–liquid microextraction (VA-SALLME) combined with a core–shell chromatographic method for the simultaneous analysis of two anti-glaucoma drugs, dorzolamide hydrochloride (DOR) and timolol maleate (TIM), in rabbit plasma. Each step of the QbD workflow has been implemented, starting from using the design of experiments with a Plackett–Burman design for screening and a Box–Behnken design for response surface methodology up to using Monte Carlo simulations for error propagation. The optimal chromatographic conditions were: a core–shell Kinetex XB C\textsubscript{18} column with ACN: 45.56 mmol L\textsuperscript{-1} phosphate buffer at pH 3.76 (18.17 : 81.83%, v/v) as the mobile phase at a 1 mL min\textsuperscript{-1} flow rate,
using hydrochlorothiazide as an internal standard and detection at 254 and 295 nm for DOR and TIM, respectively. Incorporating QbD principles in the method development allowed the visualization of two definite design spaces for the VA-SALLME and the HPLC methods, providing assurance of the quality in accordance with ICH guideline Q8(R2). Linearity was found over the concentration ranges of 0.9–50 and 1.5–50 ng mL\(^{-1}\) with quantitation limits of 0.87 and 1.40 ng mL\(^{-1}\) for DOR and TIM, respectively. As a consequence of the obtained unprecedented chromatographic separation and sensitivity, it was possible to carry out simultaneous pharmacokinetic studies of the two anti-glaucoma drugs after their single instillation.