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Robustness testing in the determination of seven drugs in animal muscle by liquid chromatography–tandem mass spectrometry

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ABSTRACT

In this work, the robustness of the sample preparation procedure for the determination of six tranquilizers (xylazine, azaperone, propionylpromazine, chlorpromazine, haloperidol, and azaperol) and a beta-blocker (carazolol) in animal muscle by LC/MS–MS was assessed through the experimental design methodology. A 2_{III}^{7-4} fractional factorial design was performed to evaluate the influence of seven variables on the final concentration of the seven drugs in the samples, in accordance with what is laid down in Commission Decision No 2002/657/EC. The variation considered for each of those seven factors is likely to happen when preparing the samples, being the values chosen as level −1, the nominal operating conditions. The results of the experimentation were evaluated from different statistical strategies, such as hypothesis testing using an external variance previously estimated, Lenth's method, and Bayesian analysis. Both Lenth's and Bayes' approaches enabled to determine the effect of every variable even though no degrees of freedom were left to estimate the residual error. The same conclusion about the robustness of the extraction step was reached from the three methodologies, namely, none of the seven factors examined influenced on the method performance significantly, so the sample preparation procedure was considered to be robust.

1. Introduction

Assessing the potential sources of variability in one or several responses of an analytical procedure must be a key part of method development. This involves making deliberate and small changes in nominal experimental conditions and investigating their subsequent effect on performance to identify the variables with the most significant effect and ensure that they are closely controlled when using the method [1].

From this perspective, two terms referring to the evaluation of the method performance still coexist within the scientific vocabulary: robustness and ruggedness. They have often been used as synonyms [2–5], but a distinction between both has also been drawn in accordance with their information about different features of an analytical method: its practicability and stability related to experimental physicochemical variables that are internal to the method (robustness) and its interlaboratory transferability when the variables under study are external to the method (ruggedness) [6–8]. In this sense, the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) defines the robustness of an analytical procedure as "a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage" [9]. On the other hand, the United States Pharmacopeia and The National Formulary has adopted the ICH definition of robustness and defines the ruggedness of an analytical method as "the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions such as different laboratories, analysts, instruments, lots of reagents, elapsed assay times, assay temperatures, or days" [10]. However, there is still some confusion in scientific journals, guidelines, and monograph literature regarding the use of these words when applied to analytical methods [11].

Information about ruggedness/robustness should be indicated in the laboratory procedure [1]. Anyway, the strategy to be followed in a robustness and/or ruggedness test is the same. It involves performing a screening study usually by means of experimental designs after the identification of the potentially influential factors and the definition of their variation ranges and of the responses to be determined. At this point, conducting either a Plackett–Burman design [12] or a fractional

Abbreviations: ICH, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use; IUPAC, International Union of Pure and Applied Chemistry; LC/MS–MS, Liquid chromatography coupled to tandem mass spectrometry; XYL, Xylazine; AZA, Azaperone; PROP, Propionylpromazine; CHLOR, Chlorpromazine; HALO, Haloperidol; AZOL, Azaperol; CAR, Carazolol; SPE, Solid-phase extraction; MRL, Maximum residue limit; IS, Internal standard; APCI, Atmospheric pressure chemical ionization; ESI^+, Positive ion electrospray; MRM, Multiple reaction monitoring; LS, Least squares; VIF, Variance inflation factor.

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factorial design, as in the Youden test [13], is the most frequently used procedure for robustness/ruggedness evaluation. The choice of the design to be performed depends on the purpose of the test and on the number of factors to be examined [4]. Due to the minimum time and analytical effort required, Commission Decision (EC) No 2002/657 [14] encourages the application of the Youden approach to the compulsory verification of that performance characteristic both in screening and confirmatory methods for the monitoring of certain substances and residues thereof in live animals and animal products. Many examples in this field can be found in the literature [6,15–19]. The Eurachem Guide to Method Validation and Related Topics [1] recommends, whenever possible, the evaluation of the ruggedness/robustness of a method by using the Youden test. IUPAC [2] also recognizes the strategy described by Youden as adequate to study the ruggedness of an analytical method.

This work shows the evaluation of the performance of the sample preparation step prior to the simultaneous determination of seven drugs in animal muscle by liquid chromatography coupled to tandem mass spectrometry (LC/MS–MS). More precisely, the substances analyzed were five tranquilizers (xylazine (XYL), azaperone (AZA), propionylpromazine (PROP), chlorpromazine (CHLOR), and haloperidol (HALO)), one of the metabolites of azaperone (azaperol (AZOL), which is derived from the former by reduction) and a blocker agent of the β-adrenergic receptor (carazolol (CAR)). Their chemical structures are depicted in Fig. 1. As the sample preparation stage includes sampling, pretreatment, and solid-phase extraction (SPE) steps, it will be quite likely to be responsible of the highest errors in the determination. So, the effect of seven factors related to the sample preparation procedure on the final concentration of every drug in the sample was examined through an eight-experiment Youden design. These factors were deliberately changed between nominal and extreme conditions that represented the variability that may well occur when performing routine analyses. The results arising from the experimental plan were interpreted from several statistical methodologies in order to assess the robustness of the extraction step.

As the proposed design was saturated, an independent estimation of the experimental error as standard deviation at a previous stage of the method development was used to evaluate the significance of the factors. In addition, Lenth's and Bayes' approaches [20] have also been

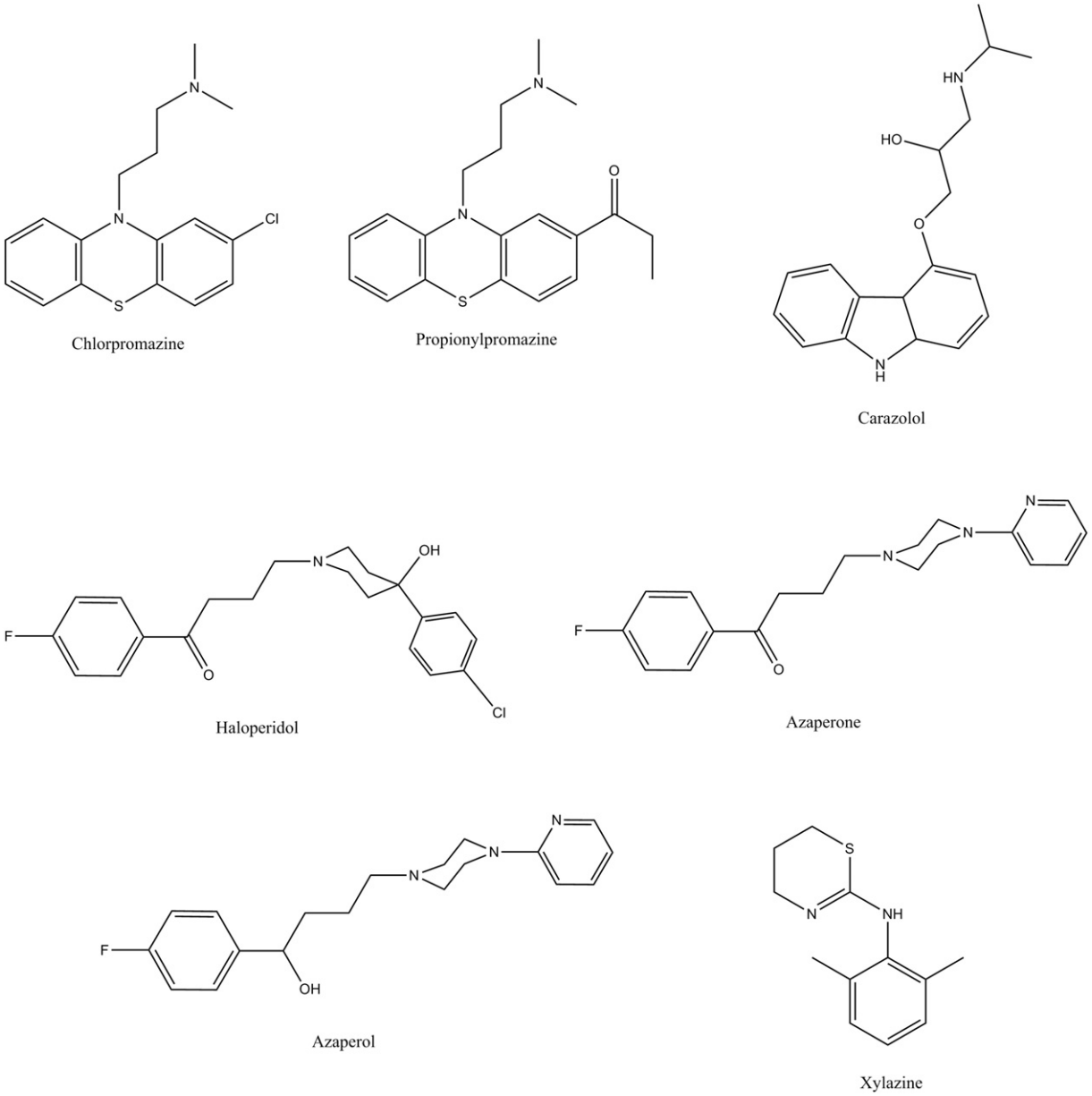

Fig. 1. Chemical structures of the seven drugs analyzed.