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Validation parameters cannot be obtained without using pure substance

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Oswald et al. [1] describe the validation of a method of analysis for simultaneous determination of clarithromycin (CLA), rifampicin (RIF) and their main metabolites in horse plasma, epithelial lining fluid and broncho-alveolar cells (BAL) by LC-MS/MS.

A recent report by de Velde et al. [2] described the simultaneous determination of all four compounds in human plasma. Oswald et al. state this method is not sufficiently sensitive to investigate the influence of RIF on the plasma concentration of CLA and the active metabolite 14-hydroxyclarithromycin (OH-CLA). However, concentrations are measured after multiple dosing. It is unlikely to find plasma concentrations below the lower limit of quantification of 0.1 μg/ml at steady state, since peak concentrations of 0.9 μg/ml are found in foals after multiple administrations (7.5 mg/kg) [3]. Moreover, the influence of RIF on the concentration of CLA was investigated by Alffenaar et al. [4]. Peak plasma concentrations of 0.5–2.1 μg/ml CLA and OH-CLA were found at steady state. The method of de Velde et al. [2] was most likely sufficiently sensitive for the research of Oswald et al. [1].

Although the authors further state their method was validated according to FDA guidelines, we have to make an important remark about the quantification of OH-CLA. The authors describe that OH-CLA was commercially not available and they therefore used a quantification method based on the parent compound. The mean ratio of the slopes of the signal to concentration curves between CLA and OH-CLA was calculated. Consequently, all estimated concentration values of OH-CLA were divided by this factor to conclude the concentration of the metabolite. As OH-CLA has an extra OH-group, one cannot assume that CLA and OH-CLA ionize equally and therefore have a similar signal to concentration response when analysing with LC-MS/MS. By not using an OH-CLA standard, results and conclusions are only based on assumptions. Recovery rates and other validation parameters (e.g. matrix effect and stability) cannot be obtained without using the pure substance. de Velde et al. received the pure substance of OH-CLA from Abbott, USA and used it for calibration purposes [2].

Last, the authors assume only small differences between plasma, epithelial lining fluid and BAL. Potential interferences arising from the diversity of the matrices have not been studied and the validation procedure is therefore not complete [5]. These tests should be performed before using this method in clinical practice.

Response

First of all we would like to thank van der Elst et al. for their critical comments. Unfortunately, the authors have not carefully read our manuscript because most of the criticized points are clearly stated or discussed in the article.

We agree with the authors that their very recently published article (de Velde et al., 2009) describes an elegant and very easy quantification method for clarithromycin, rifampicin and their main metabolites in human plasma. Nevertheless, this assay was not useful for our study due to the high LLOQ (100 ng/ml for clarithromycin and 200 ng/ml for rifampicin) because our study investigated the influence of the enzyme inducer rifampicin on the disposition of clarithromycin in foals (not in human as described by Alffenaar et al., 2010). Consequently, in the presence of rifampicin, most mean values of the plasma concentration-time curves of clarithromycin (10 of 16) were below 100 ng/ml (range: 4.2–156 ng/ml), which represents about 42% of the respective AUC. Additionally, nearly all mean plasma concentrations of OH-CLA (3.5–118 ng/ml) and all mean plasma values of 25-O-desacetylrifampicin (7–89 ng/ml) of our study on foals were below the LLOQ of 100 or 200 ng/ml. Finally, measuring the drug levels in the broncho-alveolar lavage (BAL) and in broncho-alveolar cells (BAC) also required a substantially lower LOQ and a different sample preparation than presented by de Velde et al. This clearly demonstrates that a modified method (extraction vs. protein precipitation) with a considerably lower LOQ (2.5 ng/ml for all analytes) was needed and had to be validated.

Referring the second major critics, we totally agree with the authors that for an analytical method validation pure reference compound is required. Thus, we clearly stated in the article that "*quantitative determination of OH-CLA could not be validated*". Because OH-CLA was commercially not available and was also not provided by Abbott despite several requests, we decided to use our indirect approach based on the quantification of the parent compound. We agree that OH-CLA and CLA are expected to show different ionization behaviour. For this purpose we calculated a correction factor from the ratio of the slopes of the signal-to-concentration function between CLA to OH-CLA. Consequently, all estimated concentration values of OH-CLA generated by this approach (based on the measured peak area ratio of OH-CLA over the internal standard) were divided by this correction factor to avoid over or underestimation of OH-CLA. In conclusion, this approach is of cause no state-of-the-art procedure but may be an interesting experimental option in cases if no pure reference compound is available, e.g. for drug metabolites, which is underlined

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by very similar pharmacokinetic results compared to previously published studies.

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References

[1] S. Oswald, J. Peters, A. Venner, W. Siegmund, LC-MS/MS method for the simultaneous determination of clarithromycin, rifampicin and their main metabolites in horse plasma, epithelial lining fluid and broncho-alveolar cells, J. Pharm. Biomed. Anal. 55 (2011) 194–201.
[2] F. de Velde, J.W. Alffenaar, A.M. Wessels, B. Greijdanus, D.R. Uges, Simultaneous determination of clarithromycin, rifampicin and their main metabolites in human plasma by liquid chromatography-tandem mass spectrometry, J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci. 877 (2009) 1771–1777.
[3] A.Y. Womble, S. Giguère, E.A. Lee, T.W. Vickroy, Pharmacokinetics of clarithromycin and concentrations in body fluids and bronchoalveolar cells of foals, Am. J. Vet. Res. 67 (2006) 1681–1686.
[4] J.W. Alffenaar, W.A. Nienhuis, F. de Velde, A.T. Zuur, A.M. Wessels, D. Almeida, J. Grosset, O. Adjei, D.R. Uges, T.S. van der Werf, Pharmacokinetics of rifampin and clarithromycin in patients treated for mycobacterium ulcerans infection, Antimicrob. Agents. Chemother. 54 (2010) 3878–3883.
[5] E. Rozet, R.D. Marini, E. Ziemons, B. Boulanger, Ph. Hubert, Advances in validation risk and uncertainty assessment of bioanalytical methods, J. Pharm. Biomed. (2011), doi:10.1016/j.jpba.2010.12.018.

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