COMPENDIAL AND NON-COMPENDIAL APPROACHES TO THE DEVELOPMENT OF IN-VITRO RELEASE METHODOLOGIES FOR KETOPROFEN SUPPOSITORIES

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ABSTRACT: The paper presents compendial and non-compendial approaches for the development of in-vitro release tests adapted to the composition and biopharmaceutical characteristics of suppositories containing 100 mg ketoprofen. 40 mesh and Palmieri baskets, as well as rotary dialysis adaptors were used on standard dissolution equipment. The role of the hydrodynamic conditions and design particularities was analyzed, emphasizing on the correspondence with the limiting steps of the in-vivo processes. The results confirmed that the discriminatory character is highly dependent on the experimental setup.

Keywords: ketoprofen, in-vitro release, USP apparatus, Palmieri baskets, rotary dialysis cells.

INTRODUCTION:

The suppository formulations have specific mechanisms of release, depending on the nature of the vehicle and the physical state of the active pharmaceutical ingredient. The physiological parameters of the gastro-intestinal tract and the processes controlling the drug transfer to the biological barrier have a considerable impact on both intra- and inter-individual variability (Anderson BJ et al., 1998). Nonetheless, it is rather difficult to simulate in-vitro the complexity of the in-vivo behavior, in order to develop a predictive methodology. The dissolution or drug release tests have been adopted as key quality control tests, able to signal possible underperformance or lack of reproducibility between batches of the same product. The regulatory agencies, academic media and pharmaceutical industry have focused their attention on the so called conventional dosage forms, including solid oral dosage forms such as tablets and capsules. The proven utility of the in-vitro tests in guiding the research and development and, after the adoption of biowaiver guidance, in reducing the regulatory burden (Shah VP et al., 1998), gradually increased the interest in selecting adequate procedures for the special or novel dosage forms. The latter category includes several dosage forms, such as suppositories, soft gelatin capsules, transdermal patches or drug eluting stents, characterized by particular mechanisms of release and less experience in dissolution or release testing. Despite the declared intention of the drug regulatory to limit the unnecessary proliferation of dissolution apparatus (Cohen JL et al., 1990), in many instances the accurate simulation of the in-vivo processes request for the adoption of non-compendial equipments (Azarmi S et al., 2007).

For the rectal dosage forms, the in-vitro methodologies have been categorized using different criteria. The main difference seems to be whether or not an artificial membrane is used to separate the dosage form from the release media. Dialysis bags (Roseman TJ et al., 1981) or rotary dialysis cells (Asakura S et al., 1993), either stationary, in rotation or reciprocating (Itoh O et al., 2006), allow a gradual melting, deformation and spreading of the suppository base, have been considered the most biorelevant approach. As part of regulatory submission, the advantages of a newly developed test on non-compendial apparatus have to be proved, based on the comparison with the results generated by paddle or basket method. The aim of the current paper was to comparatively assess the release of ketoprofen from industrial suppository formulations, using compendial and non-compendial approaches.

MATERIALS AND METHODS:

In-vitro dissolution testing procedure

Three rectal suppository products containing 100 mg ketoprofen and registered on the European market were included in the study (coded O, K and P, the later one being the reference listed drug). The in-vitro release tests were performed on PharmaTest PTWS 100 equipment (PharmaTest GmbH, Germany). The experimental protocol included three stages of evaluation, differing in the design of apparatus and consequent mechanism of release. In a first stage, the generic product K was tested using the USP apparatus 1 (40 mesh) and the non-compendial Palmieri baskets, at three stirring rates: 50, 75 and 100 rpm. Based on the observed relationship between the kinetics and cumulative amounts released, on one hand, and the hydrodynamic conditions on the other, the 100 rpm level was further implemented in the assessment of all three products. The rotary dialysis cells (PTWS-0, PharmaTest GmbH; RDC) were used during the last stage, on the suppository formulations displaying the highest differences during the previous protocol. 900 mL phosphate buffer pH=7.4 was selected as release media, based on the available reports regarding the solubility of the active pharmaceutical ingredient. The composition of buffer was according to the recommendations of the United States Pharmacopeia
The degassing procedure was performed by filtration under vacuum. All the evaluations were conducted on six dosage units. In case of RDC, cellulose acetate membranes (mean pore size, 0.45µm) were soaked in phosphate buffer for at least 12 hours before use. After positioning the suppository with the top facing the rotation spindle and assembling the membrane, the inner compartment was filled with media, using a 20 mL syringe. Depending on the dimensions of the suppositories, the ratio between the outer and inner volume of the assembly varied between 50 and 60. The stirring rate of the driving shafts was set at 100 rpm, with an internal reduction of 2:1.

Samples 5 mL were collected manually through resident rods, with immersed polypropylene filters (10 µm). For the mildest hydrodynamic conditions (50 rpm), the duration of tests was 120 minutes and a detailed sampling schedule was adopted. In all the other studies, the evaluations were performed for 250 minutes. The quantitative analysis of ketoprofen was performed spectrophotometrically, at λ_{\text{max}}=260 nm, using an Agilent 8453 UV-Vis Spectrophotometer (Agilent Instruments, Germany).

Analysis of the dissolution profile

Model independent, pairwise procedures were used for the assessment of the in-vitro release similarity (Costa P, 2001). The experimental data were included in the calculation of difference and similarity metrics (F1 and F2) only after the evaluation of variability, considering a threshold of 20% for the values of the coefficient of variation at early time points, respectively 10% for the subsequent part. The mean profiles were fitted with conventional kinetic models used for describing the mechanism of release.

RESULTS AND DISCUSSION:

The influence of the stirring rate on the in-vitro release profiles

The in-vitro release obtained during the initial stage in case of the generic product K displayed a pronounced dependency on the hydrodynamics. The fraction released was lower than 20% of the labeled amount and increased proportionally with the stirring rate. The mean profiles were almost parallel, proving that the mechanism of release was the same. The only explanation is that the removal of the dissolved drug from the surface of the suppository and changes in consistency of the base, with correlated decrease in diffusional resistance, were accelerated at higher rotational speeds of the basket. The meshes of the compendial apparatus were not clogged throughout the test. Unrestricted access of the phosphate media to the formulations explains the reduced impact of the basket design (Nicoara AC et al., 2014a,b). The differences in the amount of dissolved ketoprofen between the compendial and Palmieri basket were lower than 2% (Fig. 1).

![Fig. 1](image)

**Fig. 1** The influence of the stirring rate on the release of ketoprofen from generic product K using a) compendial 40 mesh baskets and b) Palmieri baskets (n=6; mean +/- standard deviation; ○ - 50 rpm; □ - 75 rpm; Δ - 100 rpm).

The dependence of the similarity on the in-vitro setup

No significant differences were concluded between the tested products and reference listed drug using the 40 mesh baskets at 100 rpm (Fig. 2a). Noteworthy, the coefficient of variation was within the compendial limits, with higher values in the first 10 to 30 minutes after initiation of the tests. Small increases of variability were observed for products P and O during the second stage of evaluation, as the released fraction almost doubled (Fig. 2b). In these two cases, faster melting and spreading were noted, together with the accumulation of suppository base in the upper part of the vessel and partial solidification around the stirring shaft. Based on the information on the qualitative composition available in the leaflet, the higher values of the released fraction can be correlated with the presence of anhydrous colloidal silicone dioxide. Its hydrophilic character generated by the presence of superficial hydroxyl groups may facilitate the access of the aqueous buffer to the suspended particles of ketoprofen. Although the information on the exact nature of the lipophilic vehicle was not available, therefore differences in the solubility of the active...
pharmaceutical ingredient in the formulation can only be speculated. Another key observation was that, despite the fact that the melted suppository base was expelled from the Palmieri baskets, no significant sedimentation occurred. The selection of an adequate media ensured sink conditions which prevented the slow dissolution of solid particles.

**Fig. 2** The comparative presentation of the in-vitro release profiles of ketoprofen from suppositories using a) compendial 40 mesh baskets and b) Palmeri baskets (n=6; mean +/- standard deviation; □ - reference product, P; Δ - generic product, K; ○ - generic product, O)

The conclusion on the in-vitro similarity depended on the experimental device. An equivalent release was indicated by the $f_2$ factor when the mean profiles generated by use of 40 mesh baskets. This is obviously due to the low values of released fractions and proved the lack of discriminatory character in these particular experimental conditions. The qualitative similarity (currently defined as the presence of the same excipients) induced the in-vitro similarity for products P and O, irrespective of the design of the basket (Table 1) and confirmed by both compendial metrics.

The values of difference ($f_1$) and similarity ($f_2$) factors calculated for mean in-vitro dissolution profiles

<table>
<thead>
<tr>
<th>Test product</th>
<th>O</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparatus</td>
<td>USP1 (40 mesh)</td>
<td>Palmieri</td>
</tr>
<tr>
<td>$f_1$</td>
<td>21.58*</td>
<td>9.86</td>
</tr>
<tr>
<td>$f_2$</td>
<td>77.86</td>
<td>74.88</td>
</tr>
</tbody>
</table>

* - values are outside the accepted range for in-vitro similarity

The release profiles obtained on the non-compendial rotary dialysis cells

The use of a membrane interface prospectively increased the biorelevance of the in-vitro testing procedure. The limiting role of the structural parameters after melting is directly assessed, provided that the artificial barrier serves as a mechanical, inert support of the semisolid matrix (Asakura S et al., 1993). The sedimentation of the drug in the inner compartment of the rotary cells may have contributed to a facilitate contact between the membrane and the drug particles. For this stage of evaluation, the two products displaying extreme behavior were selected, i.e. P and K. The results were consistent with the recorded differences on Palmieri baskets. The first sampling point illustrated the methodological differences. While the fraction release within 30 minutes was higher for product P on Palmieri baskets, the rank order was reversed on RDC (Fig. 3). In the first case, the direct contact between the media and the dosage form led to a faster initial release, corresponding to the dissolution of the superficial particles and dependent on the specific surface. The presence of the suppositories within the membrane-defined, liquid filled compartment of the RDC generated a solution which equilibrated with the outer media at a slower rate, compared to the melting process.

The values of both $f_1$ and $f_2$ factors underlined the in-vitro non-similarity more clearly, compared to the ones obtained in the previous setups (144.85, respectively 39.68). The overall kinetics seems to be similar, because the Korsmeyer Peppas model ($F=kt^n$, where $F$ is the fraction released, $k$ and $n$ are model-dependent constants) seems to adequately fit all the experimental profiles. The exponent values were between 0.29 and 0.39, proportional with the stirring rate. A Fickian mechanism was confirmed. Generally, the square root law has been applied (Roseman TJ et al., 1981), especially in case of membrane-based methods. Based on the current results, it can be assumed that the diffusion through the melted vehicle is not the single rate-limiting step. The sedimentation of the solid particles of drug onto the membrane and improved contact with the outer media are plausible, concurring phenomenon.
The relevance of these results is difficult to estimate. The buffer capacity and the volume of colonic fluid are lower, compared to the implemented in-vitro parameters. Considering the high permeability of ketoprofen through intestinal barriers (Shohin IE et al., 2012), any limitation in the release has to be analyzed as a potential barrier in the absorption process. None of the current in-vitro testing protocols can be used for quality control purposes, because the fraction released was below the 75-80% threshold applied in the compendial monographs. More intense shear stress is probably needed, corresponding to the colonic motility. Nevertheless, the results reflected to the difference in the qualitative composition, therefore displaying an adequate discriminatory character.

CONCLUSIONS:

Three rectal suppository products containing 100 mg ketoprofen were subject to in-vitro release evaluations. The applied methodologies included compendial and non-compendial apparatus. The results confirmed that the fraction released and the conclusion on the in-vitro similarity is highly dependent on the experimental setup. The rotary dialysis cells are feasible for development of both quality control procedures and performance tests, a considerable advantage being the concomitant simulation of melting, deformation and spreading phenomena.

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