

THE *IN VITRO* RELEASE PROFILES OF NIMESULIDE FROM ORAL SOLID DOSAGE FORMS, IN COMPENDIAL AND MODIFIED PHYSIOLOGICAL MEDIA

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Abstract

Nimesulide is a typical Biopharmaceutical Classification System class II drug, frequently imposing the presence of high amount of tensioactive substances combined with non-physiological pH values for complete dissolution during the *in vitro* release profiling. Thus, the risk of erroneous estimation for the biopharmaceutical performance of a specific drug product is increased by these non-discriminatory conditions, with further implication on the safety and efficacy profile of the active ingredient. The current paper presents the influence of several characteristics of currently recommended compendial or physiological simulated media on the *in vitro* dissolution pattern for nimesulid, underlying their importance and biological relevance.

Rezumat

Nimesulidul este o substanță tipică pentru clasa II a sistemului de clasificare biofarmaceutică, impunând frecvent prezența unei cantități mari de tensioactiv combinată cu valori non-fiziologice ale pH-ului pentru dizolvarea completă în cursul evaluării profilului de cedare *in vitro*. Astfel, riscul unei estimări eronate a performanțelor biofarmaceutice pentru un anumit produs farmaceutic este accentuat de aceste condiții nediscriminatorii, cu implicații evidente asupra profilelor de siguranță și eficacitate ale principiului activ. Lucrarea prezintă influența caracteristicilor mai multor medii compendiale sau fiziologice simulate asupra profilului de dizolvare *in vitro* pentru nimesulid, subliniind importanța și relevanța lor biologică.

Keywords: nimesulide, dissolution tests, fasted state simulated fluids (FaSSIF), fed state simulated fluids (FeSSIF)

Introduction

For drugs with a low solubility pattern, mainly in the physiological conditions, there are frequently reported problems in proving bioequivalence, despite the fact that a given test drug product passes the pharmacopoeial tests. The low solubility – high permeability characteristics lead frequently to *in vitro* dissolution conditions without any physiological correspondence, such as high pH values or high and unjustified quantities of tensioactive substances. Although useful for batch-to-batch control or Scale Up Post Approval Changes (SUPAC) procedures, the drastic and non-discriminating dissolution tests represents the main source of *in vitro* - *in vivo* non-correlations. The simulated gastro-intestinal media proposed by Dressman JE. *et al* are currently used as valuable prediction tools for *in vivo* dissolution processes [1,2]. Nimesulide has a very low water solubility (0.01mg/mL) [3], that is a dose: solubility ratio of 10000 mL. Moreover, the n-octanol-water partition coefficient of 2.376 [4] clearly underlines the fact that sulphonamide drugs (like nimesulide) are typical Biopharmaceutical Classification System class II drugs [5,6], with a complex interplay between physico-chemical characteristics, physiological variability, as well as formulation factors, and drug product safety and efficacy profiles.

Materials and methods

The compendial, currently recommended pH values, 4.5, 6.8 and 7.2 have been used for the evaluation of the *in vitro* dissolution profiles of 100 mg nimesulide as immediate release oral dosage form (innovator formulation, Aulin® 100, CSC Pharmaceuticals Handels GmbH, batch no. 29000195). Each of the mentioned buffer systems was prepared at a concentration of 100 mM, due to high strength of the tested dosage form and to presence within the molecular structure of various functional groups, susceptible to alter the pH of the dissolution media during the test. Since the quality and sort of lecithin has been reported to strongly influence the dissolution profile, by changes induced to the micelle aggregate size, modification to the formula recommended by Dressman J.E. (Table I) have been implemented: the fasted / fed state simulated fluids (FaSSIF/FeSSIF) have been prepared without addition of lecithin. The blank media (without both lecithin and tensioactive) have been used for comparison.

Table I
Recommended composition and volume for simulated intestinal fluids [1-3,7]

Media	Component / parameter		Quantity / value
Fast State Simulated Intestinal Fluid (FaSSIF) (recommended volume: 500 mL)	Sodium taurocholate		3 mM
	Lecithin		0.75 mM
	Blank	Sodium chloride	6.186 g
		Monosodium phosphate	3.438 g
		Sodium hydroxide ad	pH=6.5
		Purified water	1 liter
	pH		6.5
	Osmolarity		270 ± 10 mOsmol/kg
	Buffer capacity		10 ± 2 mEq/l/pH
Fed State Simulated Intestinal Fluid (FeSSIF) (recommended volume: 1000 mL)	Sodium taurocholate		15 mM
	Lecithin		3 mM
	Blank	Sodium chloride	11.874 g
		Acetic acid	8.65 g
		Sodium hydroxide (pellets)	4.05 g
		Purified water ad.	1 liter
	pH		5.0
	Osmolarity		670 ± 10 mOsmol/kg
	Buffer capacity		76 ± 2 mEq/l/pH

Each dissolution test has been conducted on 6 units, using United States Pharmacopoeia Apparatus 2 (Erweka DT800 dissolution system), using 900 ml of each compendial media at 75 rpm. The simulated fluids were used in the recommended volume of 500 mL for FaSSIF and 1000 mL for FeSSIF. The media has been degassed prior to test debut by filtration under vacuum at 41°C using cellulose acetate membranes. The presence of sodium taurocholate imposed gently stirring and heating at the same temperature for 30 minutes.

Samples of 1.0 mL were collected at 5, 10, 20, 30, 45 and 60 minutes after the simultaneous introduction of the tablets. A supplementary sample (90 minutes) was collected for the simulated fluids, in either blank or modified composition, due to provisioned higher amount and longer dissolution process. The media was not replaced, since the total sampled volume remained below the 1% threshold of the initial dissolution volume. The samples were filtrated immediately through 0.45 µm regenerated cellulose filters, 15 mm diameter, PhenexTM, Phenomenex.

For the preparation of dissolution media, analytical grade anhydrous monobasic and dibasic sodium phosphate, acid acetic glacial and anhydrous sodium acetate, sodium hydroxide, sodium taurocholate were used. Purified water was obtained using a reverse osmosis SG Ultra Clear UV plus TM system. For the chromatographic determinations, HPLC grade acetonitrile (Sigma-Aldrich) and analytical grade trifluoroacetic acid and trichloroacetic acid were also used.

Chromatographic separations have been carried out in isocratic conditions, using an Agilent Instruments 1200 system, equipped with G1311A quaternary pump, G1316A column thermostat, G1329A thermostatted autosampler and G1315D diode array detector. Two monitoring wavelength were used, 300 and 230 nm, corresponding to the absorption maxima of the analyte, nimesulide, and of the internal standard, glyburide, added after samples filtration. The stationary phase consisted of an ODS Hypersil Column, 4 x 150mm, 5 μ m, 150 mm. Samples of 10 μ L were injected into the column and run time was set at 5 min for compendial media, and 10 minutes for physiological simulated media, respectively, with a mobile phase composed by acetonitrile:trifluoroacetic acid 0.1% = 60:40, delivered at 1.0 mL/minute flow rate.

Results and discussion

The simple chromatographic method led to accurate separation for both analyte and the internal standard, the retention times being 2.8 and respectively 3.3 minutes, without any significant interference of the dissolution media components (figures 1, 2).

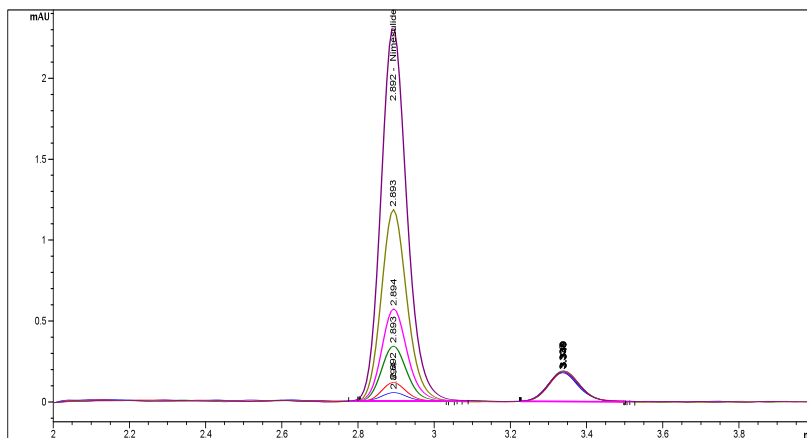


Figure 1

Overlaid chromatograms of calibration samples for nimesulide (0.04 – 40 μ g/mL) in the presence of the internal standard, glyburide.

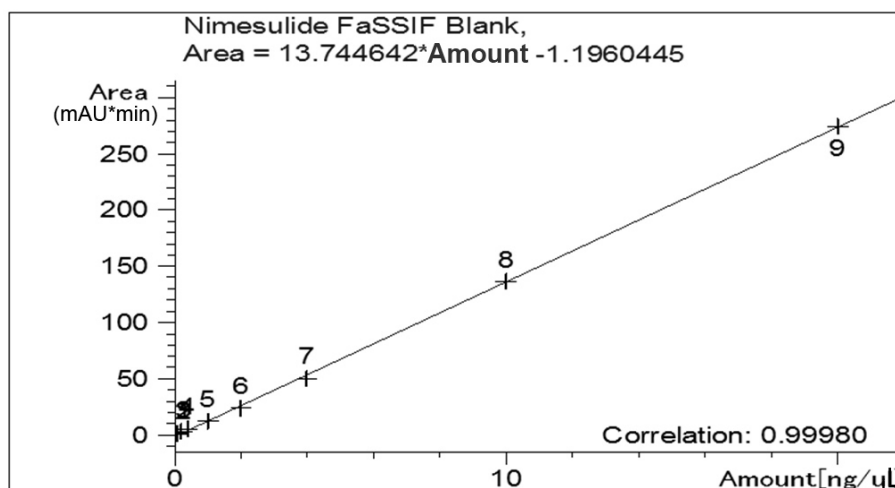


Figure 2

Calibration curve for nimesulide in FaSSIF blank

The presence of bile acid salt in the FaSSIF/FeSSIF collected samples determined, by the lack of composition uniformity (various impurities and/or degradation products), the necessity of supplementary processing procedure. Adding trichloroacetic acid and centrifugation led to precipitation of acidic components susceptible to interfere with the quantification of the analyte. The acquisition time was doubled, in order to achieve complete elution of all these components.

It concerns the *in vitro* release profiles, none of the implemented tests could be used as a quality control procedure for oral solid dosage forms containing nimesulide. The percentage dissolved after 60 minutes in case of compendial media, and after 90 minutes in case of simulated fluids did not exceed 25 % from the claimed content (figure 3 a,b).

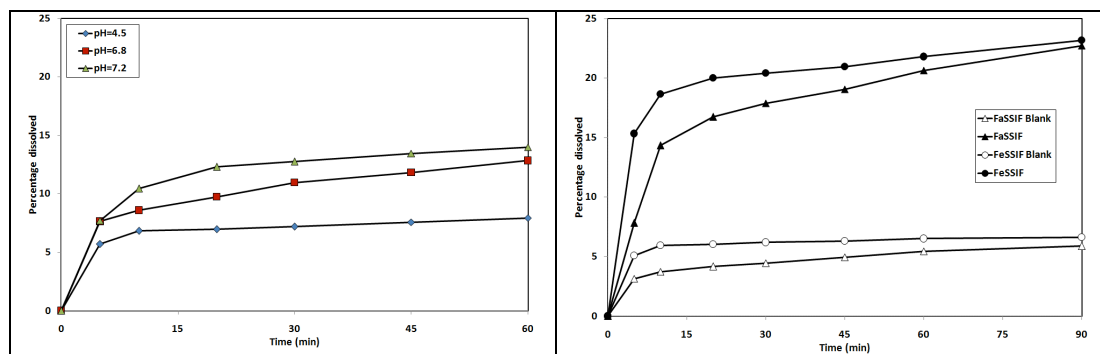


Figure 3

Mean *in vitro* release profile (N=6) for nimesulide from immediate release oral solid dosage forms in compendial and simulated physiological media.

The comparative evaluation of the entire *in vitro* release profile reveals at least three groups. The first group is characterized by final dissolution percentage within 5 and 10% of the claimed content, induced by blank physiological relevant media and the pH=4.5 buffer system. The reason of excluding from the experiments design the gastric conditions was based on the very low solubility of the active ingredient at low pH values. In this context, the results obtained for the blank FaSSIF media (pH=6.5) may appear unexpected, but the influence of ionic strength for the corresponding system should be considered.

For the next group of profiles, generated in 100 mM phosphate buffer system with pH values of 6.8 and 7.2, respectively, only a reduced, 1% difference in the dissolved percentage is induced.

The presence of sodium taurocholate as tensioactive agent determines a net increase of dissolution rate for nimesulide, probably by facilitating the wetting process of the solid particles. The percentage dissolved was increased 3.8 fold for a concentration of 3 mM, in case of FaSSIF and 3.35 fold for 15 mM, in case of FeSSIF, compared to the blank media.

Conclusions

The release rate seems to be critically influenced not by pH value or the concentration of endogenous surfactant, but by the combination of the two characteristics of the *in vitro* dissolution media. Moreover, the lecithin may play a major role in the development of *in vitro* - *in vivo* relations or correlation for hydrophobic active ingredients, offering a more physiological relevant composition.

Currently, there are various dissolution methodologies implemented by drug manufacturers, in the context created by the particular "regulatory fate" of nimesulide (concerns related to the safety profile) leading to the lack of official monographs. The solution developed for overcoming the particular solubility profile consisted of non-relevant pH values, such as 8.0, combined with high quantities of anionic or non-ionic surfactants (1-2%). While high amounts of active ingredient are released in these drastic conditions, a lack of discriminatory character can also be assumed. Although not applicable as quality control tests, the physiological simulated intestinal fluids, even in the modified composition described in this paper, can be implemented for more *in vivo* relevant comparison of dissolution profiles.

Acknowledgements

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