In vitro study on ciprofloxacin-food interaction

Thesis of doctoral (Ph.D.) dissertation

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Summary

During my PhD work I intended to evaluate the molecular background of the ciprofloxacin-milk/dairy products interaction using *in vitro* dissolution methods. Furthermore, I aimed to compare the *in vitro* data with earlier published *in vivo* results.

In order to determine the amount of dissolved ciprofloxacin (CPFX) in milky media – not depending on the fat content of the media - a solid phase extraction sample preparation, without precipitation of the proteins, followed by high performance liquid chromatography coupled mass spectrometry analytical method was developed, optimized and validated. The separation of CPFX and the internal standard was carried out with gradient elution; and selected ion monitoring was applied.

During the *in vitro* dissolution tests water, low- and high-fat milk, or appropriate amounts of calcium, casein or lactose were added to the dissolution media. At different pH values - simulating certain parts of the GIT – the dissolution of CPFX is pH-dependent. The low pH-values increase the dissolution of CPFX. In the presence of low- and high-fat milk the amount of dissoluted CPFX is significantly lower than in case of aqueous medium. The relatively low protein content of high-fat milk – being at about 30% less than that of low-fat milk – is in connection with the ~ 30% higher free CPFX amounts measured in high-fat milky media, in comparison to the low-fat ones.

Calcium reduced the amount of free CPFX at ~ 51-92%, while the presence of lactose caused 87-98% decrease in the dissolved amount of CPFX. According to my *in vitro* data, the presence of casein can be made responsible for the decreasing effect.

Not only the milk and dairy products, but also other foods with high protein content can have an influence on the pharmacokinetic of CPFX My results highlight the importance of correct patient information.

Introduction

The authority guidance provide recommendations to manufacturer to study the possible food-effect bioavailability studies for orally administered drug products as part of investigational new drug applications, as food can have an influence on the biopharmaceutical and pharmacokinetic properties of the products. Ciprofloxacin (CPFX), a fluoroquinolone (FQ) chemotherapeutical agent may have an interaction with milk components, inhibiting the absorption of the drug.

In vitro dissolution systems can be applied for determining the exact type and mechanism of the interaction occurring during the absorption. The biological system applied in the tests should simulate the place of the dissolution (e.g. stomach and small intestine) and the physical-chemical parameters (e.g. fed or fasted conditions), too.

In order to be able to determine drug concentrations *in vitro*, precise, reproducible, reliable, the requirements of the official specifications fulfilling sample preparation and bioanalytical methods are needed. During my research solid-phase extraction (SPE) followed by high performance liquid chromatography hyphenated with mass spectrometry (HPLC-MS) is a method of analysis to the study of ciprofloxacin-milk interaction.

The relation between *in vitro* dissolution profile and *in vivo* pharmacokinetics of a medicinal product (IVIVR), the developed dissolution test model can be used for predicting the results of costly *in vivo* clinical trials.

Objectives

The determination of the reconstituted milk components (carbohydrate, fat, protein or metal ion) – used in the *in vitro* dissolution tests – being responsible for the changes (decrease) in the free, non-complexed CPFX concentration was in the focus of my PhD research. For the determination of quantitative amounts of milk components, it is essential to analyze the reconstituted skimmed and whole milk, as well as some of the different commercial fresh milk.

In order to be able to quantify the amount of CPFX from various milky media, robust, precise, reproducible SPE and HPLC-MS methods were developed, optimized and validated.

The aim of my work was to develop an *in vitro* dissolution model – simulating the physiological conditions of the gastro-intestinal tract (GIT) parts in fed or fasted states − predicting reliable data on *in vivo* interaction studies with CPFX and milk.

Comparing my *in vitro* dissolution results and the previously, in the literature published, *in vivo* milk/yogurt-CPFX interaction data of clinical trials, my goal was to prove the relation (IVIVR) expected.

Methods

The *in vitro dissolution* study on CPFX interaction with milk was carried out with a paddle apparatus prescribed by the United States Pharmacopeia. The drug release of Ciprinol® 500 mg film-coated tablets was analyzed at three different pH values (pH 1.2, 4.5 and 6.8) – according to the pH conditions of the gastrointestinal tract – in the presence of various food or food components (low-, high-fat milk, calcium, casein and lactose). The tests were conducted using 500 ml of dissolution medium and 250 ml of enriched water simulating the secreted gastric and intestinal juice in 120 minutes without enzymes.

A *SPE procedure* was developed and optimized to clean up the samples obtained from the dissolution medium. The sample preparation is suitable for separate drug and the disturbing matrix components and can be applied independently of the matrix.

An HPLC-MS method was developed, optimized and fully validated for qualitative determination of the dissoluted amount of CPFX with using internal standard. The separation of CPFX and the internal standard (aripiprazol) was carried out with gradient elution by mixing acetonitrile and 0.02 M ammonium acetate solution (pH 2.5 adjusted with formic acid). Mass spectrometer equipped with electrospray ionization was applied with scanning and selected ion monitoring.

Fat, protein and lactose contents of fresh and instant milk samples were analyzed with a Milko-Scan 130 flow and infrared system. Before analyses milk samples were preheated to 40°C in water bath in order to get a homogenous system.

Results - thesises

1. To eliminate interfering components of the biological matrix, a reproducible SPE method without protein precipitation was developed and optimized for quantitative analysis of CPFX in milky media. Five different methods of clean up procedures, denoted with A, B, C, D, and E, were tested and compared; in these same conditioning step but different rinsing and elution steps were used, resulting in different recovery values. The new method is independent on fat content (*Table I*).

Table I.: Comparison of different (A, B, C, D and E) SPE clean-up procedures

SPE method	Rinsing step	Elution step	Matrix	Recovery (%) mean± S.D.
A	2.0 mL 80:20 water–methanol then 2.0 mL hexane	3.0 mL acetonitrile containing 1% TFA	**instant low-fat milk**	*Not detected*
B	2.0 mL water	8.0 mL hexane		0.08 ± 0.02
C	2.0 mL methanol	8.0 mL hexane		0.05 ± 0.01
D	2.0 mL 80:20 water–methanol	8.0 mL acetonitrile		4.2 ± 0.06
E1	2.0 mL 80:20 water–methanol	6.0 mL acetonitrile containing 1% TFA		**99.06** ± 0.15
E2	2.0 mL 80:20 water–methanol	6.0 mL acetonitrile containing 1% TFA	**instant high-fat milk**	**98.57** ± 0.14

2. After extraction reliable, precise, selective and sensitive HPLC–MS method (a single-quadrupole mass spectrometer equipped with an electrospray ion source was used as detector) was validated using a quinolone derivative as internal standard, resulting 4.0 and 0.4 ng/ml as LLOQ and LOD, respectively.

3. The fat, protein, and lactose content of different reconstituted milk samples were analyzed with a Milko-Scan 130 flow and infrared system. Results were used in the dissolution tests.
4. On the basis of published literary data the bioavailability of CPFX co-administered with milk/dairy products can be decreased. To explore the molecular background of the decrease in the dissolution an *in vitro* test method was developed with dissolution media simulating the pH of certain parts of the GIT (pH 1.2, 4.5 and 6.8). Low or high-fat milk, as well as with a previously defined amount of milk component enriched water was added to the dissolution media.
5. The results of dissolution test on Ciprinol® 500 mg film-coated tablet have shown that – compared to water – in the presence of calcium, casein, lactose, low or high-fat milk – depending on the pH – the free, non-complexed amount of CPFX was reduced.

   The decrease is due to

   a) the adsorption on the surface of the precipitated protein in simulated gastric juice, and

   b) the complexation with multivalent cations (especially with calcium).
6. In a previously published *in vivo* investigation the reducing effect of calcium on the bioavailability of CPFX was assumed. The results at three different pH values give evidence, that in the molecular background of the interaction with milk/dairy products, protein has a more pronounced effect on the CPFX-dissolution and bioavailability, than the complexation with calcium being present in the milky medium *(Figure 1.*).
7. The molecular background highlights the fact that not only milk and dairy products, but also protein-rich diet may have an impact on the CPFX absorption and bioavailability, thus further clinical and pharmacokinetic studies are needed.

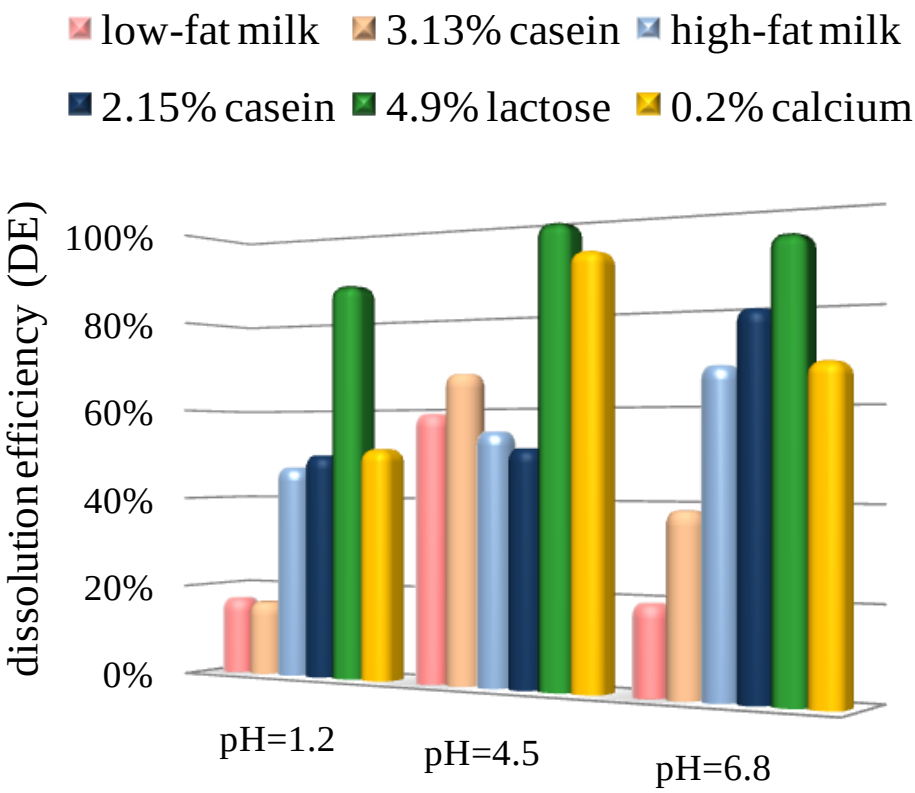

Figure 1: Effect of milk components on the dissolution of Ciprinol® 500 mg film-coated tablet

8. The new *in vitro* dissolution method is considered to be a model test for studying milk interaction with other active substance(s). The *in vitro* method is simpler, faster and cheaper than *in vivo* clinical testing, and due to the IVIVR it is suitable for the prediction of the *in vivo* data.

Conclusions

The HPLC-ESI-MS bioanalytical technique characterized with low LOD and LLOQ values can be applied in pharmaceutical researches, in particular for pharmacokinetic and *in vitro* food/milk and CPFX/FQ interaction studies.

On the basis of the test results of casein/milk – CPFX interaction, the pronounced alterations in the dissoluted amount of CPFX can lead to inefficient clinical therapy and as a consequence of it to bacterial resistance.

My results highlight the importance of correct patient information. In case of patients suffering from bacterial infection there is a great emphasis on possible interactions, and should be drawn to patients' attention, that the medicinal product containing CPFX cannot be applied with milk or food with high protein content. On the basis of my results the standard operating procedure (SOP) and patient information of the CPFX products should contain not only that the medical product can not be taken with food containing calcium, antacid or dairy supplement, but also should notify of the prominent role of protein. According to my experiments it is contraindicated to administer CPFX containing products in the vicinity of a food containing protein. The food intake should take place at least 1 hour before or 2 hours after the medicinal CPFX application.

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