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Research paper

Evaluation of the light scattering and the turbidity microtiter plate-based methods for the detection of the excipient-mediated drug precipitation inhibition

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ABSTRACT

The excipient-mediated precipitation inhibition is classically determined by the quantification of the dissolved compound in the solution. In this study, two alternative approaches were evaluated, one is the light scattering (nephelometer) and other is the turbidity (plate reader) microtiter plate-based methods which are based on the quantification of the compound precipitate.

Following the optimization of the nephelometer settings (beam focus, laser gain) and the experimental conditions, the screening of 23 excipients on the precipitation inhibition of poorly soluble fenofibrate and dipyridamole was performed.

The light scattering method resulted in excellent correlation ($r > 0.91$) between the calculated precipitation inhibitor parameters (PIPs) and the precipitation inhibition index ($PI_{classical}$) obtained by the classical approach for fenofibrate and dipyridamole. Among the evaluated PIPs AUC_{100} (nephelometer) resulted in only four false positives and lack of false negatives. In the case of the turbidity-based method a good correlation of the $PI_{classical}$ was obtained for the PIP maximal optical density (OD_{max}, $r = 0.91$), however, only for fenofibrate. In the case of the OD_{max} (plate reader) five false positives and two false negatives were identified. In conclusion, the light scattering-based method outperformed the turbidity-based one and could be reliably used for identification of novel precipitation inhibitors.

1. Introduction

A sufficient drug oral bioavailability followed by the desired clinical efficacy is of critical importance for the positive outcome of the drug discovery and the formulation development projects [1]. The improvement of the oral bioavailability by the proper formulation design has gained importance and is now recognized as a powerful tool, not only just in the early drug development formulation design, but also in the later stages of the drug discovery. In the past several years, research groups and drug discovery units have developed high-throughput experimental (HTE) techniques to assist in the search for effective formulations. In addition, HTE techniques have also been recognized by the generic drug development particularly in the field of the improvement of available therapies by reformulation [2–4]. For example, HTE techniques have been applied not only for the estimation of thermodynamic and kinetic compound solubility [5–7], but also for the *in vitro* detection of drug precipitation out of parenteral solutions [8–10] and for the rapid identification of solubility-enhancing formulations based on precipitation inhibitors [2]. The latter approach is related to the excipient-mediated intestinal drug supersaturation that was recognized as a valuable approach toward the bioavailability improvement of some BCS class II compounds [11–14]. In the state of supersaturation, the compound in the solution is present at a concentration higher than its thermodynamic solubility [15]. Hence, the higher fraction of the dissolved compound in the GIT lumen results in an increased flux across the intestinal epithelia. However, supersaturated solutions are thermodynamically unstable and are prone to precipitation that could result in the failure to achieve a satisfactory flux across the intestinal epithelia [15]. The latter issue greatly depends upon the extent and the duration

Abbreviations: AUC, area under curve; Fa/FeSSIF, fasted/fed state simulated intestinal fluid; GIT, gastrointestinal tract; HTE, high throughput experimentation; OD, optical density; OD_{max}, maximum optical density; PI, precipitation inhibition index; $PI_{classical}$, precipitation inhibition index determined by the classical approach; PIPs, precipitation inhibition parameters; RNU, relative nephelometric unit; RNU_{max}, maximum relative nephelometric unit; S_{PI}/S_B, precipitation inhibitor to McIlvain buffer pH 6.8 signal ratio; UPLC, ultrapressure liquid chromatography; UV, Ultraviolet.

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of the supersaturated state which can be prolonged by certain excipients also known as precipitation inhibitors [12,15]. Even though extensively studied [11,12,14–19], the mechanism of the action of the precipitation inhibitors still remains unclear. Moreover, these processes are dissolution medium and drug–excipient combination dependent and therefore cannot be straightforwardly predicted. In combination with the material and the time savings, the screening of the plethora excipients in order to develop/optimize a formulation with precipitation inhibitor(s) is of great importance in the pharmaceutical industry. *In vitro* kinetic methods for the detection of the precipitation of poorly soluble compounds for oral and intravenous administration have been described for rank-ordering of precipitation inhibitors [9,10,20]. In these methods the effect of the precipitation inhibitors is measured by the quantification of the dissolved compound in a time-dependent manner using chromatography or UV spectrophotometry [12,19,21–23]. Although this classical approach provides a valuable estimation of the precipitation inhibitor efficacy, some drawbacks of the described methods cannot be ruled out. Initially, sample preparation steps could represent a source of variability. In particular, chromatographic methods are time-consuming due to the time required for each sample to be analyzed. Finally, a continuous time-dependent monitoring of the drug precipitation by the described approaches is not possible [12,19,21–23]. In contrast to the detection of the dissolved compound, a recent study has emphasized the application of the nephelometer for the detection of the drug precipitate in the screening of the excipient-mediated precipitation inhibition of danazol [24]. However, the method evaluation steps as well as the correlation of the classical approach, i.e. determination of the concentration of the dissolved compound, were not reported [24]. This makes the validity of the applied method specifically designed to detect the precipitation inhibition effect questionable. In order to ensure the quality of the information arising from the screening experiment, the method also has to be evaluated for its accuracy, precision and needs to outline its potential drawbacks.

In this report, the authors evaluated the light scattering and the turbidity microtiter plate-based methods as alternatives to the classical approach for the detection of the excipient-mediated precipitation inhibition. The validity of the methods was evaluated by the calculation of five precipitation inhibitor parameters (PIPs) which were correlated with the precipitation inhibition index ($PI_{classical}$) determined by the classical approach. The AUC_{100} for the laser scattering microtiter plate-based method and the OD_{max} for the turbidity-based one resulted in an excellent correlation of the $PI_{classical.}$ However, due to the lack of false negatives in the screening experiments, the laser scattering PIP AUC_{100} was chosen as the most reliable one for determination of the excipient-mediated precipitation inhibition of the poorly soluble fenofibrate and dipyridamole. The results outline the suitability of the laser scattering microtiter plate-based method to be used as the "first" screening line with complete confidence, followed by a proof-of-concept classical methodology.

2. Materials and methods

2.1. Reagents

The tested model compounds and their physicochemical properties are given in Table 1. The microtiter plate layout of the tested excipients and their suppliers are given in Table 2. All the chemicals were purchased from Merck (Germany). The composition of the dissolution media (i.e. McIlvaine buffer) was 21.1 g/L citric acid monohydrate (0.1 M) and 35.6 g/L disodium hydrogen phosphate dihydrate (0.2 M) adjusted to pH 6.8. Ultra-pure ion exchanged water was used for the chromatographic analysis.

2.2. Excipient-mediated drug precipitation inhibition assay

2.2.1. Preparation of the incubation plate

The screening of the 23 precipitation inhibitors (Table 2) was performed in a simple aqueous buffer i.e. McIlvaine buffer pH 6.8 simulating the pH of the intestinal fluids. More biorelevant media such as FaSSIF and FeSSIF yielded a high background signal due to the presence of micelles that interfered with the sensitivity of the nephelometer/UV plate reader and therefore could not be used. All liquid dispensing steps, besides the addition of the compound DMSO stock solution, were performed on a Freedom EVO™ workstation (Tecan, Switzerland) equipped with a robotic manipulator arm (RoMa) and eight Teflon-coated stainless washable tips (LiHa). Briefly, the 23 different excipients i.e. precipitation inhibitors (Table 2) were diluted with McIlvaine buffer pH 6.8 at 0.1% (w/v) followed by LiHa transfer (247.5 μL) into each well of the 96-well microtiter plate (Cliniplate, Fisher Scientific, Germany or Corning®, Germany). These plates were chosen on the basis of our previous experience since they yielded the lowest background and the smallest standard deviation between wells. A 2.5 μL DMSO was dispensed very rapidly into the solutions containing excipients using the MultidropCombi™ (ThermoScientific, Switzerland) equipped with a small tube dispensing cassette. The plate was immediately shaken for five seconds to ensure mixing of the solutions and the absence of bubbles. The 96-well plate was then placed either on the nephelometer or on the UV plate reader to check whether the precipitation inhibitor background signal could interfere with the measurement of the excipient-mediated precipitation inhibition. Shaking was omitted since both instruments provide the possibility for shaking only at the end of each cycle which would not allow a uniform experimental condition in the adjacent wells of the microtiter plate. The accuracy and the precision of the liquid handling steps and its application in the screening of the excipient-mediated precipitation inhibition have been described in the authors' previous study [23].

2.2.2. Investigation of liquid evaporation during measurement

Since the drug precipitation kinetics experiments were performed in uncovered microtiter plates, we investigated how much of the liquid evaporates during the measurement inside the plate reader. A 200 μL Orange G solution (dissolved in a McIlvaine buffer pH 6.8) was added to each well of the 96-well plate followed by the incubation of covered and uncovered microtiter plate at 26 °C and 37 °C. The solution evaporation was followed by weighing the plate at 0, 0.5, 2 and 24 h. The "edge" effect and well-to-well variability were investigated by measuring the time-dependent (0, 0.5, 2 and 24 h) changes in the Orange G optical density (OD) at 380 nm. The absorbance was measured on an Infinite M1000 plate reader (Tecan, Switzerland). Due to data simplification results at 26 °C where 8% liquid evaporation after 24 h was observed are omitted from the manuscript.

2.2.3. Optimization of the instrumental and the experimental conditions

The nephelometer Nephelostar from BMG LabTechnologies (Germany) was used with a polarized helium–neon laser that lases at 632.8 nm. The nephelometer settings i.e. beam focus (1.5, 2.5, and 3.5 mm) and laser gain (50, 60 and 70) were varied in order to optimize the measurement parameters. The number of the measurement cycles (120), the measurement time per well (0.1 s) and the positioning delay (0.3 s) were fixed instrument settings. The optimization of the nephelometer instrument settings was performed when only the precipitation inhibitors (Table 2) were