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Application of iChemExplorer in pharmaceutical pH stress testing

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ABSTRACT

pH stress testing is an integral part of pharmaceutical stress testing and is a regulatory requirement for validating a stability indicating analytical method and elucidating degradation products and degradation pathways. This paper reports the results of an evaluation of iChemExplorer (ICE) for drug substance and drug product pH stress testing in comparison with the conventional (manual) approach. ICE is a simple and inexpensive technology, and through real case studies it was demonstrated that ICE is an efficient and "fit-for-purpose" alternative in conducting pharmaceutical pH stress testing. In addition, when using a non-isothermal ICE protocol, it is feasible to estimate the pH degradation kinetics (e.g., E_a) using the ICE software.

1. Introduction

"Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used" [1]. pH stress testing is to "evaluate the susceptibility of the drug substance to hydrolysis across a wide range of pH values [1]" and is an integral part of pharmaceutical stress testing. The conventional (manual) approach in conducting pH stress testing is placing the samples under various pH conditions in an isothermal oven or water bath until a predefined level of degradation (e.g., 5–20% assay loss) or a predefined maximum duration is reached (from hours to weeks depending on the stability of the drug and the stress temperature). This approach usually requires manual pulling of the samples periodically to check the degree of degradation; as a result, it can be quite labor intensive. Naturally, applications of commercially available or home made automated or semi-automated systems have been attractive in stress testing [2,3]. These automated or semi-automated systems can be extremely helpful in automating the repetitive operations and save the scientists' time for more value added work. However, the disadvantage is that these systems are often sophisticated and costly, and require dedicated experts to operate. Automation brings in efficiency when there are sufficient numbers of stress studies to be performed on a routine basis, and works best with a centralized laboratory dedicated to high volumes of stress testing. However, for most pharmaceutical companies, a centralized stress testing laboratory is either undesirable or unnecessary due to cost/benefit considerations. A "fit for purpose" alternative with some automated features therefore can be very attractive in a decentralized stress testing environment.

In the past few years, the authors evaluated a simple yet innovative technology iChemExplorer® (Reaction Analytics Inc., Wilmington, DE, USA) for pH stress testing of drug substances and drug products with a focus on early clinical development applications, where turnaround time and material saving can be critical. This manuscript reports the result of the evaluation.

2. Experimental

2.1. Instruments and materials

A 1290 Infinity LC system (Agilent Technologies, Santa Clara, CA, USA) with a PDA detector was used for all experiments. Empower II software (Waters Corporation, Milford, MA, USA) was used for experimental setup, data acquisition and process. The LC methods were previously developed and validated for each of the drug substance or product evaluated; however, the development or validation of these methods is out of the scope of this paper.

iChemExplorer® (ICE) is a simple add-on device to the Agilent HPLC systems. The iChemExplorer® hardware includes a specially designed sample tray and a control unit. When the ICE is installed, this sample tray replaces the original one in the HPLC autosampler. The control unit is placed directly under the autosampler,

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Fig. 1. The iChemExplorer (ICE) hardware, which includes a specially designed sample tray replacing the original sample tray of an Agilent (U)HPLC and a control unit installed directly under the Agilent (U)HPLC autosampler (courtesy of Reaction Analytics, Inc.).

as shown in Fig. 1. The temperature of the sample tray is set up using the iChemExplorer software. The ICE also allows magnetically controlled stirring if necessary.

For drug substance applications, crimp vials with a PTFE/silicone/PTFE septum (Waters P/N: PSL404231) were used.

For drug product applications, ChemGlass filter vials (part # PF-1011-150LP, which includes: 12 mm × 32 mm crimp seal vial (PF-1011-251LP), Teflon ferrule/holder for vial and filter (PF-1011-252LP), and micro coarse fritted insert (PF-1011-253LP), ChemGlass Life Sciences, Vineland, NJ, USA) and Thompson filter vials (part # 35544-500-ML, Thomson Instrument Co., Oceanside, CA, USA) were used.

Drug substance and drug product samples were all small molecule investigational drug candidates from internal sources. Drug substance or drug product stock solutions were prepared at concentrations according to the procedure for that drug substance or drug product using the diluents specified in the procedure. For acid and base stress testing, typically, a 1 N HCl and 1 N NaOH aqueous solutions were prepared, respectively. The final stress solution was prepared by mixing appropriate aliquots of the drug substance or drug product stock solution and the 1 N HCl or 1 N NaOH solution in a volumetric flask to make a 0.1 N HCl or 0.1 N NaOH stress solution with a concentration of the drug ranging from 0.1 to 1 mg/ml in accordance with the analytical procedure. Likewise, a stress solution at pH 7.4 was prepared using phosphate buffer. For ICE studies, the pH stress solution was then transferred to a regular crimp vial or a filter vial in the case of drug product.

A more comprehensive pH stability study was performed with 8 pH values: pH 1.3, 2, 3, 4.5, 6, 6.8, 7.4, and 8. In this case all samples were prepared using phosphate buffer except pH 4.5 which was prepared using citrate buffer.

For pH stress testing using the conventional (manual) approach, stress solutions were prepared in volumetric flasks, which then were placed in an oven preheated to 40 °C.

All solvents (e.g., water, methanol, acetonitrile) were HPLC grade. All chemicals (e.g., NaOH, HCl and phosphate buffer) were ACS grade.

2.2. pH stress testing protocols

pH stress studies were carried out using both the conventional and ICE approaches for comparison. The experimental designs of both approaches are summarized in Table 1. For optimization of the ICE approach, two ICE protocols were initially evaluated.

For ICE studies, the temperature program was set up using the ICE software. Empower II software was used for HPLC instrument control and data processing. If applicable, the data were also processed with the iChemExplorer software for additional information (e.g., kinetic estimation).

3. Results and discussion

3.1. Design and application of the ICE protocol

ICH Q1A recommends stress testing including pH stress testing [1], however, the specifics on the conditions and durations of the stress testing are left to the individual pharmaceutical company. Internally, we adopted a general pH stress testing protocol (which henceforward is referred to as the conventional protocol) as shown in Table 1. pH stress testing is carried out at 40 °C for a maximum of 2 weeks or 5–20% degradation, whichever is achieved first. This protocol is in line with the industry's best practice in performing pH stress testing [4–6] although a quicker turnaround time is always desirable. To take full advantage of the flexibility of the ICE in temperature programming and evaluate the feasibility of its use in pH stress testing, the following were considered when designing an ICE protocol:

(1) Maintain the same level or slightly excessive stress with that of the conventional approach so that the extent of the pH stress would not be significantly altered due to the use of ICE.
(2) A higher temperature than 40 °C would be evaluated to shorten the duration of the ICE stress studies; however, the temperature would be increased gradually from room temperature to a higher temperature as needed for achieving the target level of degradation. In this way, degradation products only formed at high temperature can be monitored and thus differentiated from the more relevant degradation products (those that are readily formed at ambient or moderate temperature).
(3) The highest temperature would be 70 °C to limit the potential for formation of irrelevant degradation products or secondary degradation products, as 70 °C has been generally accepted as an appropriate temperature for stress testing in the pharmaceutical industry [5,6].

The equivalency of the ICE approach to the conventional approach was estimated by applying the principle of Arrhenius equation. It has been reported that the activation energies for pharmaceutical degradations are mostly in the range of 12–30 kcal/mol or higher [6,7]. Without knowing the actual activation energy of a compound, an activation energy of 12 kcal/mol can be considered an extremely conservative estimation. At this activation energy, the rate increase roughly follows the "2 for 10" rule-the rate approximately doubles with every 10 °C temperature increase.

Two ICE temperature protocols were initially evaluated. The first protocol used a staged isothermal approach:

(1) Keep the temperature at 40 °C for up to 48 h,
(2) If the target level of degradation is not reached after 48 h at 40 °C, increase the temperature rapidly (in 10 min) to 55 °C,
(3) If the target level of degradation is still not reached after 24 h at 55 °C, increase the temperature rapidly (in 10 min) to 70 °C and keep it for up to 24 h,
(4) Stop the stress testing after 24 h at 70 °C regardless the level of degradation.

This 4-day ICE protocol is roughly equivalent to a 13-day stress at 40 °C estimated according to the "2-for-10" rule. The second ICE protocol is consisted of the following steps: