Experimental scrutiny and project timelines: an example

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Abstract

The proper use and interpretation of 'forced degradation' experiments - performed during the early development stages of a drug project - allows analysts to identify quickly problems that may occur later. Here we report an example where forced degradation experiments allowed the rapid identification of a deleterious degradation mechanism occurring unexpectedly in a pharmaceutical formulation

Introduction

Non-receptor tyrosine kinases are cytoplasmic proteins located on membranes within the cell. The Src and Abl non-receptor tyrosine kinases are involved in cell growth and differentiation and are over-expressed in some tumor cells. AZD0424 (Fig. 1) was originally discovered by AstraZeneca. It is a dual selective inhibitor of Src and Abl non-receptor tyrosine kinases which is being developed for Phase I clinical trial through the Cancer Research UK Clinical Development Partnership initiative. Formulation, pre-formulation and development studies were performed at the Cancer Research UK Formulation Unit, University of Strathclyde. The proposed formulation was a polyethylene glycol (PEG) based melt fill capsule.

Materials and Methods

AZD0424 analysis was performed on Thermo Surveyor and Accela HPLC systems with PDA and/or Thermo LCQ Deca XP Max MS (ion trap) detection (Thermo, Hemel Hempstead, UK). HPLC analysis was performed using a Synergi Polar RP80 4µm, 150 x 4.6 mm id column (Phenomenex, Macclesfield, UK) with the gradient specified in Table 1. Flowrate was 1 ml/min, injection volume was 25µl, column temperature set to 45˚C and detection wavelength at 314 nm.

AZD0424 oxidative stress samples were by incubation of the drug in a 0.2% solution of H_2O_2 at 25˚C overnight. Formulated AZD0424 samples were generated using 1% AZD0424 in PEG heated to 55˚C.

Results

Upon closer inspection of the HPLC traces from AZD0424/PEG capsules incubated in an ICH compliant stability study [1], the retention time of the main degradant was found to coincide with the main degradant observed during the earlier oxidative stress degradation experiments (see Fig. 2).

Figure 1: Structure of AZD0424

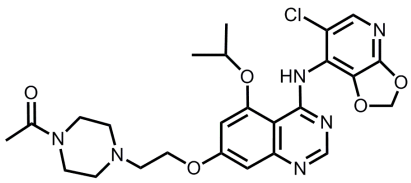


Table 1 HPLC mobile phase conditions

Time	0.3% formic acid:water	Methanol
0 min	65%	35%
30 min	15%	85%
30.1 min	65%	35%
40 min	65%	85%

Figure 2 Effect of time and temperature on main degradant concentration

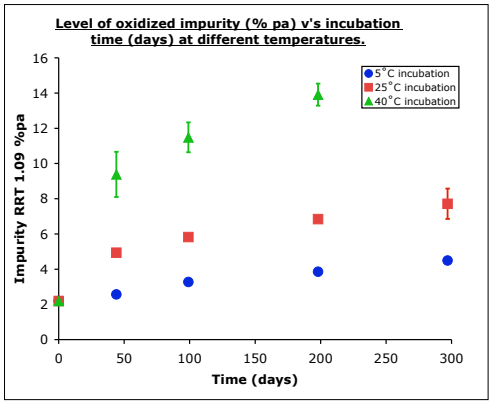
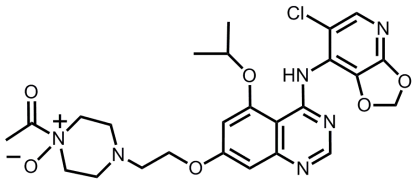


Figure 3: Structure of AZD0424 oxided degradant



Results (continued)

Armed with this information, degraded samples of AZD0424 were assayed by LCMS leading to a proposed degradant structure (Fig. 3). In addition a review of the literature affirmed the presence of peroxide contaminants in PEGs [2].

Conclusion

In the authors' lab we perform physico-chemical characterisation of putative drug compounds as part of the pre-formulation and analytical method development: working on the premise that understanding a molecule's properties is key to analysing, formulating and successfully manufacturing a drug product. This can a time consuming and labour intensive phase of the project, requiring the attention of experienced and qualified staff.

However, establishing an understanding of AZD0424 degradation early in the project was a key part of establishing the mechanism for a the deleterious degradation of AZD0424 found at the stability/pre-clinical drug development stage. A mechanism for the degradation was postulated with a few hours, confirmed by experiments within a week, and the project set back on track with the purchase of 'low-peroxide' PEGs.

The authors believe that a firm foundation of chemical and physico-chemical knowledge is key to effective progression of a clinical drug project. In the case we have presented here, the work allowed the development team to move rapidly onto other formulations that would mitigate the degradation thus allowing the project to proceed in a timeous manner.

Acknowledgements

AstraZeneca, Cancer Research UK, Nigel Westwood and Angela Patikis at Cancer Research UK's Drug Development Office, Michaela Kreiner and the Formulation Unit technical team.

References

[1] ICH Harmonised Tripartite Guideline Q1A, "Stability testing of new drug substances and products", http://www.ich.org/

[2] V. Kumar and D.S. Kalonia, "Removal of Peroxides in Polyethylene Glycols by Vacuum Drying: Implications in the Stability of Biotech and Pharmaceutical Formulations" *AAPS PharmSciTech.*, 7 (2006) Article 62

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