SCIENTIFIC RESEARCH BRIEF
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ABSTRACT

Objectives

A critical step in an extended-release drug product analysis is the extraction during impurity and assay sample preparation. When both the drug substance and the impurity are unstable, this is especially challenging. This study is divided into three parts:

1. Identification of the unstable impurity by LC/MS, and its mechanism of the formation.
2. Probing the source of impurity formation during the extraction and manufacturing process.
3. Improving the processing and extraction method to eliminate the formation of the impurity.

Methods

The structure of the impurity assigned using degraded drug product and degraded API by MS and MS/MS experiments. Impurity has been identified as a dehydrated drug substance. A fishbone analysis (Ishikawa diagram) was utilized to identify the potential analytical and manufacturing steps, which could generate the dehydrated impurity.

Results

The dehydrated impurity is an unstable cyclic ester, which could convert back to drug substance after exposure to moisture and heat for a prolonged period of time. Use of pure organic solvent was found to be the source of dehydration during both the sample extraction and the manufacturing process. Addition of aqueous component minimized formation of the impurity.

Probing the Source of an Unstable Impurity and Controlling Its Formation at Release

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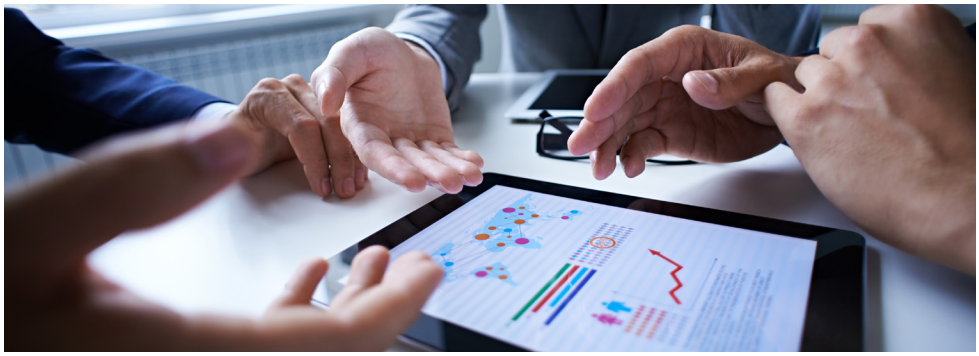


INTRODUCTION

APT-76 is an anti-neoplastic agent and currently being developed as an extended-release formulation (enteric coating) for an ongoing early Phase 1 clinical trial. The finished drug product is a capsule filled with mini tablets. Two major issues related to impurities were encountered during the formulation and analytical stages:

1. Significant growth of a known impurity (DPI, Dephosphorylated Impurity, MH+ = 286) was observed. Use of pure alcoholic solvent, sonication, and temperature for extraction facilitates the formation of DPI.
2. Formation of an unknown impurity (Unk-3), which was above the qualification threshold (0.34% at release).

STRATEGY

A systematic approach was used for impurity source analysis.

Identification of the Impurities

▼

Mechanism of the Impurities Formation

▼

Possible Source Analysis

Analytical
1. Critical Steps
2. Critical Steps Verification
3. Analytical Modifications
4. Final Analytical Method

Formulations
1. Critical Steps
2. Critical Steps Verification
3. Formulation Modifications
4. Final Formulation

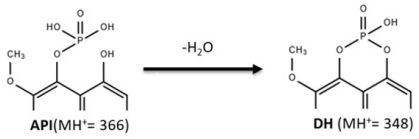
Continues >

EXPERIMENTS AND RESULTS

Identification of Impurities

Identification and Possible Mechanism of Formation of Unk-3

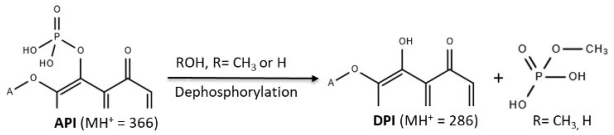
- Unk-3 (m/z = 348) was identified as dehydrate product (M-18)
- MS/MS analysis further supports the proposed structure



Anhydrous condition facilitates the formation of Unk-3

Identification and Possible Mechanism of DPI

- DPI (Dephosphorylated impurity) (m/z = 286) – DPI stucture was already established. Possible sources of formation of DPI has not been evaluated.
- Proposed Mechanism



Alcohols (methanol and ethanol) and water facilitate DPI formation

Possible Source of Impurities

Analytical Contribution

Material	Sample Method	% Unk-3	% DPI
API as a Standard	Short Method[1]	0(nd)	0.30
API as a Sample	For drug product	0.20	0.72
Impurity Introduced in Analysis[2]		0.20	0.42

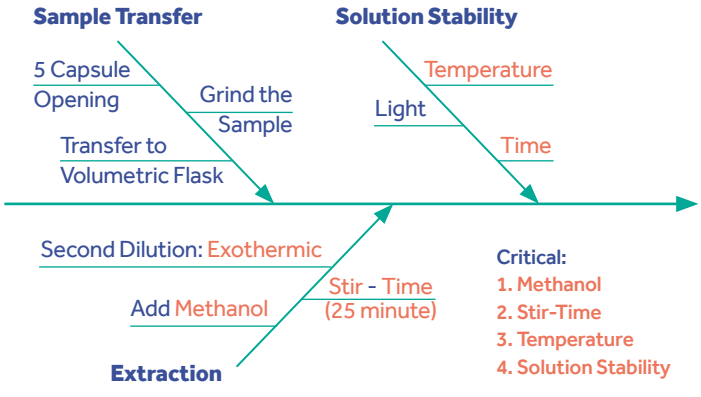
1. API directly dissolved in diluent.
2. A rough estimation to determine **impurity produced during analysis**.
Impurity introduced during analysis = impurities in "API as a sample" - Impurity in "API as a standard"

Formulation Contribution

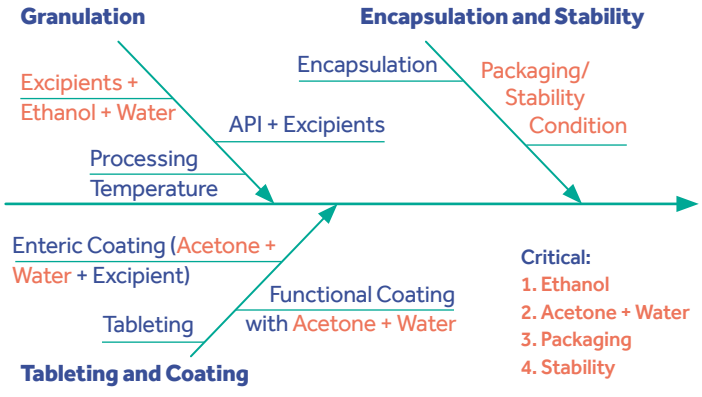
Material	Sample Method	% Unk-3	% DPI
Finished Product	For drug product	0.31	1.54
API as a Sample	For drug product	0.20	0.72
Impurity Introduced in Formulation[1]		0.11	0.82

1. A rough estimation to determine **impurity produced during formulation**.

Critical Analytical Steps



Critical Formulation Steps



EXPERIMENTS AND RESULTS

Analytical Results

- Stirring the API as a sample in Methanol for 25 minutes generates 0.20% of Unk-3 and 0.72% of DPI.
- DMSO completely dissolve the sample, but will generate significant amount of Unk-3.
- A pre-chilled (10-15 deg) Methanol/Buffer (60/40) was used as an extracting solvent.
- 1 minute vortex was found to be very effective and provided the result equivalent to 25 minute stirring.
- At 2.8°C, sample solution is stable for 24h.

Formulation Results

- API was treated with Acetone, Methanol and Isopropanol. In Acetone, no Unk-3 was detected after 24h.
- DPI (Specification NMT 5.0%) exceeded the limit in 6 months at ±2°C/60% ± 5% RH.
- Stability study at 5°C ± 3°C, significantly slowed the formation of DPI.

Final Analytical Method

1. Mixed five capsules well.
2. Transfer content equivalent to one capsule to Mortar.
3. Grind and transfer the content quantitatively to volumetric flask.
4. Added Pre-chilled extracting solvent (60/40, Methanol/ pH 10 Buffer), and vortex 1 minute.
5. Dilute to volume with pre diluent (60/40, Methanol/ pH 10 Buffer), filtered and analyzed.

Final Formulation

1. Acetone was proved to be the solvent, but it was not able to dissolve all the excipients. A mixture of Acetone-IPA was used in granulation process.
2. Finished Product was packaged in a sealed bottle with desiccant.
3. A low temperature (5°C) condition growth of DPI is significantly slower.

CONCLUSION

Analytical Contribution

Critical Analytical Steps		Critical Formulation Steps	
Before	After	Before	After
Pure Ethanol	Methanol + pH 10 Buffer	Ethanol-Water (For Granulation)	iso-propanal+ Acetone+Water
25 minute Stir	1.0 minute Vortex	Stability Study Only 25°C/60% RH 40°C/75% RH	Stability Study 5°C 25°C/60% RH 40°C/75% RH
Addition of Diluent	Pre-mixed, Pre-Chilled Solvent		
Solution at Ambient	Solution at 2-8°C		

Formulation Contribution

Combined Results	Unk-3 (%)	DPI (%)
Results before Implementation	0.20	0.72
Results after Implementation	nd	0.48

Acknowledgements

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