Development of a control strategy for peroxide in tetrahydrofuran based on a simple method to mitigate the risk of oxidation product formation for Vopimetostat (TNG462)

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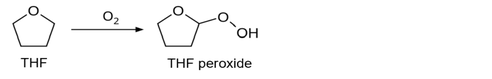
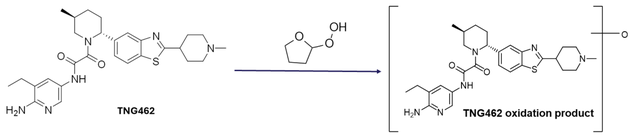
Objective

Develop a control strategy based on the simple analytical method suitable for Quality Control (QC) laboratories which can mitigate the risk of Vopimetostat (TNG462) oxidation product formation during final crystallization step.

Background

Vopimetostat is a potent synthetic lethal inhibitor of protein arginine methyl transferase 5 (PRMT5) that works selectively in cancer cells with methylthioadenosine phosphorylase (MTAP) deletion. Vopimetostat active pharmaceutical ingredient (API) process final crystallization step uses tetrahydrofuran (THF) solvent. It is well-known that THF peroxide can be formed when THF is exposed to oxygen in air, even in the presence of butylated hydroxytoluene (BHT) inhibitor. Forced degradation study has demonstrated that vopimetostat can be oxidized by peroxide to form oxidation product. Therefore, a control strategy of THF peroxide in THF needs to be established and the risk of oxidation product formation needs to be assessed.





Scheme 1: Mechanism of vopimetostat (TNG462) oxidation product by THF peroxide

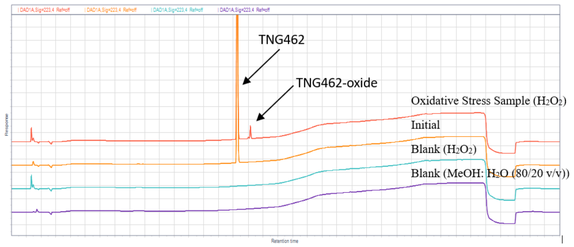
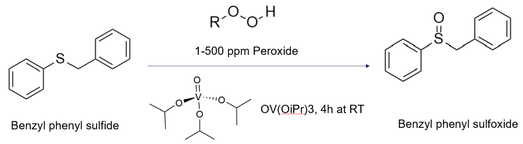


Figure 1: Overlaid LC chromatograms of vopimetostat (TNG462) peroxide forced degradation study

Methods

1. Analysis of THF peroxide through derivatization reaction

Recently, a new sensitive method for quantitation of organic peroxide through derivatization followed by liquid chromatography (LC) ultraviolet (UV) detection is published[1]. Leveraging catalytic transformation, organic peroxides are converted to sulfoxide with addition of excessive benzyl phenyl sulfide in solvents. Benzyl phenyl sulfoxide, the oxidation reaction product has a strong UV chromophore at 245 nm wavelength. Thus, commercially available benzyl phenyl sulfoxide can be prepared as standard solutions for external standard quantitation of it found in the sample solvent by modern LC techniques. Then the content of organic peroxide can be calculated by 1:1 mole ratio to benzyl phenyl sulfoxide through the indirect derivatization reaction.



Scheme 2: Mechanism of organic peroxide derivatization reaction

Pros:
Feasible method with validated range: 1-500 ppm of tert-butyl hydroperoxide (TBHP). Good recovery result: 93% on proof-of-concept experiment.
Cons:
The derivatization reaction is complex and lengthy (4 hours) and is not be suitable for QC laboratories at manufacture sites

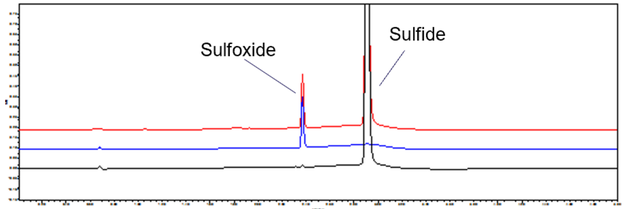


Figure 2: LC overlaid chromatograms of benzyl phenyl sulfide, benzyl phenyl sulfoxide standard, and sample solution from proof-of-concept experiment

2. Peroxide test strip

Commercially available peroxide test strips for hydrogen peroxide (H_2O_2, inorganic peroxide) are quick and economic, but semiquantitative. Our goal is to examine if a correlation between the two methods can be established, and we can implement the test strips for a quick semiquantitative determination of peroxide in THF.

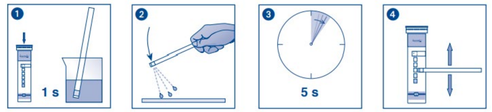
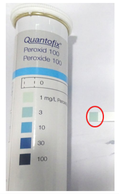


Figure 3: Peroxide test strips measurement instruction and example



Results

1. Correlation of the two methods for peroxide in the THF solvent

THF solvent solutions with and without spiking multiple levels of THF peroxide were prepared and measured by QUANTOFIX® peroxide test strips. The semiquantitative evaluation is carried out by visual comparison of the reaction color with a multi-stage color scale. As pictures demonstrated in **Figure 4**, test strips provide accurate determination for the range of 0-3 ppm, but with a degree of imprecision for levels above 3 ppm. Therefore, a range of 6-8 ppm strip reading is estimated to solution#4.

Solution#1 (unspiked): 0 ppm	Solution#2: 1 ppm	Solution#3: 2 ppm	Solution#4: 6-8 ppm

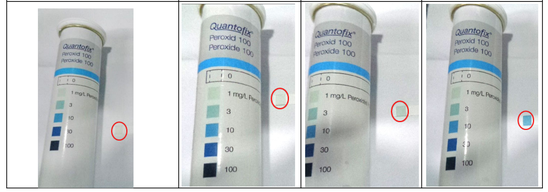


Figure 4: Peroxide test strips data for THF solutions spiked with multiple levels of THF peroxide

Then, derivatization reaction experiments are conducted immediately on those freshly prepared THF solvent solutions. Linear regression analysis are performed on test strip variable determinations (6, 7, and 8 ppm) for THF solvent solution#4 due to the relatively wider range (3-10 ppm) of the test strip color scale. Results presented in **Table 1** and **Figure 5** have demonstrated that peroxide test strips measurement (0-8 ppm range) has a 10.2-13.6 fold reduction in sensitivity, but a linear regression correlation (correlation coefficient, R^2>0.997) to THF peroxide results measured by LC analysis from derivatization reaction. This data aligns with results measured by differential scanning calorimetry (DSC) method[2], which indicates 7-13.5 fold reduction in sensitivity. Therefore, the control strategy of THF peroxide in THF measured by peroxide test strips can be implemented if a correlation of peroxide test strips measurement (≤8 ppm levels) to levels of vopimetostat oxidation product is established.

Solution#	Test strip measurement (ppm)	LC measurement (ppm)	Linear regression slope/correlation coefficient (R^2)
1 (unspiked)	0	Measurement#1: 1	N/A
	0	Measurement#2: 1	
	0	Measurement#3: 2	
2	1	12	
3	2	25	
4	6	83	13.6/0.9971
	7		11.7/0.9997
	8		10.2/0.9982

Table 1: Correlation of peroxide test strips measurement vs. LC measurement through derivatization reaction on THF solvent solutions with and without spiking THF peroxide

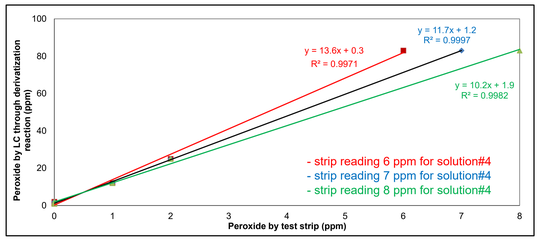


Figure 5: Linear regression plots for correlation of peroxide test strips measurement vs. LC measurement through derivatization reaction on THF solvent solutions with and without spiking THF peroxide

2. Correlation of the level of vopimetostat oxidation product with peroxide level in THF

Finally, selected THF solvent solutions (with and without spiking THF peroxide) with test strip readings at 0, 2, and 8 ppm are used for vopimetostat crystallization step to examine if a correlation between the test strip reading and vopimetostat oxidation product can be established to mitigate the risk of oxidation products formation in the process. When process experiments are completed, vopimetostat API samples are collected and analyzed by LC for vopimetostat oxidation product. Results presented in **Table 2** and **Figure 6** have demonstrated a linear regression correlation (correlation coefficient, R^2=1.000) between the test strip reading on THF peroxide in THF solvent and vopimetostat oxidation product. Considering the quantitative range (≤1 ppm) on peroxide test strips and the LC detection limit for the oxidation product (0.02%), a specification on peroxide test strip (≤1 ppm) to control THF peroxide in THF solvent is established to guide crystallization process of vopimetostat, as shown in **Table 3**. This approach has been successfully implemented vopimetostat manufacturing at 1 Kg scale and will be executed on future registration batches manufacturing.

Experiment#	Test strip measurement (ppm)	Vopimetostat oxidation product by LC (%)	Linear regression correlation coefficient (R^2)
1	0	0.00	0.9996
2	2	0.03	
3	8	0.13	

Table 2: Correlation of peroxide test strips measurement vs. vopimetostat oxidation product through crystallization process step

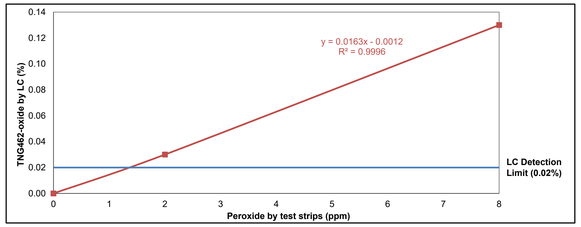


Figure 6: Linear regression plot for correlation of peroxide test strips measurement vs. vopimetostat (TNG462) oxidation product through crystallization process step

Specification of peroxide test strip	Vopimetostat oxidation product LC detection limit
≤1 ppm	0.02%

Table 3: Specification of peroxide test strip for peroxide in THF and vopimetostat oxidation product detection limit

Conclusion

In conclusion, a control strategy based on the simple peroxide test strip method with quantitative measurement (≤1 ppm) is established which can mitigate the risk of vopimetostat oxidation product formation during the final crystallization step. The method is accurate, precise, and suitable for QC laboratories at manufacturing sites.

Reference

[1] Beutner, G.; Ayers, S.; Chen, T.; Leung, S.W.; Tai, H.C.; Wang, Q. "General Method for Quantitation of Organic Hydroperoxides" *Org. Process Res. Dev.* 2020, 24, 1321-1327
[2] Jiang, Y.; Song, X.; Hou, N.; Pan, C. "Can Dip Strips Really Test for Peroxide in Organic Solvents Semiquantitatively?" *Org. Process Res. Dev.* 2019, 23, 2540