Benzene Analysis in BPO products: Strategies to Prevent False Positives in BPO Formulations

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Introduction

Benzene toxicity and carcinogenic properties have been widely studied and well-established, leading to it's strict regulatory limits in pharmaceutical products. Benzyl peroxide (BPO) is a broadly used active pharmaceutical ingredient in acne treatments. However, significant safety concerns have been recently raised, with studies showing that under certain conditions benzyl peroxide can degrade into benzene, which can lead to elevated levels of the mutagen in BPO products. This risk initiated global testing of BPO formulations for possible benzene contamination and product recalls being reported by FDA this year.[1]

While benzene formation can occur during product storage, BPO degradation may be also possible during analytical testing. This has the potential to lead to the reporting of artificially high results. Consequently, conducting false positive testing is essential to ensure accurate results are reported.

If a risk of false positive results is identified, a scavenger compound can be introduced to sample preparation. Since the formation pathway potentially involves radical species, addition of an antioxidant may be considered for prevention, with some studies demonstrating that use of antioxidants may reduce benzene formation in some BPO formulations.[2]

Two over-the-counter BPO formulations were investigated for benzene concentration with a use of GC-MS methodology. Two diluents were used for sample preparation: DMSO and an antioxidant in DMSO. Samples were analysed at multiple timepoints to investigate if benzene level increased between preparation and analysis time. Despite consistent internal standard response and low %RSD between preparations (n=10), a **3-fold increase** in benzene concentration was observed in samples prepared in the diluent with an antioxidant. No increase in benzene concentration was detected over time.

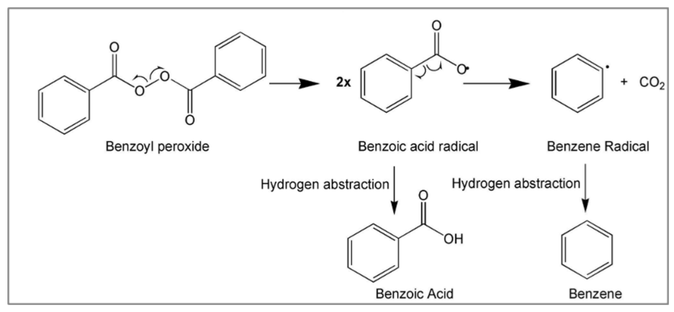

Figure 1: Benzene Formation From BPO – Possible Pathway[2]

Materials and Methods

Two widely available over-the-counter formulations, containing 5% w/w BPO were prepared by weighing a portion of each product, followed by the addition of diluent and the internal standard. Prepared samples were analysed at multiple timepoints, measured from diluent addition to the time of analysis, to investigate whether there are any changes in benzene concentration over time.

Standards and samples were prepared in duplicate using DMSO and DMSO with antioxidant. A single calibration line was plotted with both sets of standards to verify response consistency across the two diluents.

The benzene concentration was determined using an in-house Resolian methodology with stable isotopically labelled benzene as an internal standard. Incubation temperature was adjusted to minimise risk of benzene formation through thermal decomposition.

Technique	HS-GC-MS
Incubation Conditions	20 min at 30°C
Column	Restek Rxi-624 Sil MS 20 m x 0.18 mm x 1.0 µm
Acquisition mode	SIM
Monitored masses	78 (Analyte), 84 (Internal Standard)
Calibration Line Range	0.5-7.5 µg/g of product
Detection Limit	0.15 µg/g of product

Results

Linearity (R=0.9998), precision across the run (%RSD=3% in standards), specificity and sensitivity were assessed to ensure adequate method performance.

Samples in DMSO:

Benzene was detected for both formulations, at levels not exceeding concentration of 0.50 µg/g of product (quantification limit). The results were consistent between all injections in the run and no benzene increase was observed across the run.

Samples in DMSO with Antioxidant:

Samples prepared following the same process, but using a diluent containing an antioxidant exhibited significantly higher benzene levels. The benzene was detected at levels higher than 1 µg/g of product, which corresponds to approx. **3-fold increase in** detected concentration. This observation was consistent for both formulations tested.

Despite the differences in benzene concentration between the diluents, internal standard response remained consistent across the run, which indicates this is not likely caused by matrix effects interfering with the analyte detection.

Additionally, 15 hours after preparation no significant difference in benzene concentration was noted.

Product	DMSO		DMSO with antioxidant	
	Conc. (µg/g of product)	% RSD (n=10 preps)	Conc. (µg/g of product)	% RSD (n=10 preps)
Formulation A	0.326	6.69	1.09	1.64
Formulation B	0.356	11.75	1.05	3.71

For both formulations tested, elevated benzene levels were observed in the presence of an antioxidant, which was contrary to previous studies. While the reason for an increased concentration is an area of ongoing study, these results show the importance of a tailored approach when developing a method to mitigate the risk of false positive results.

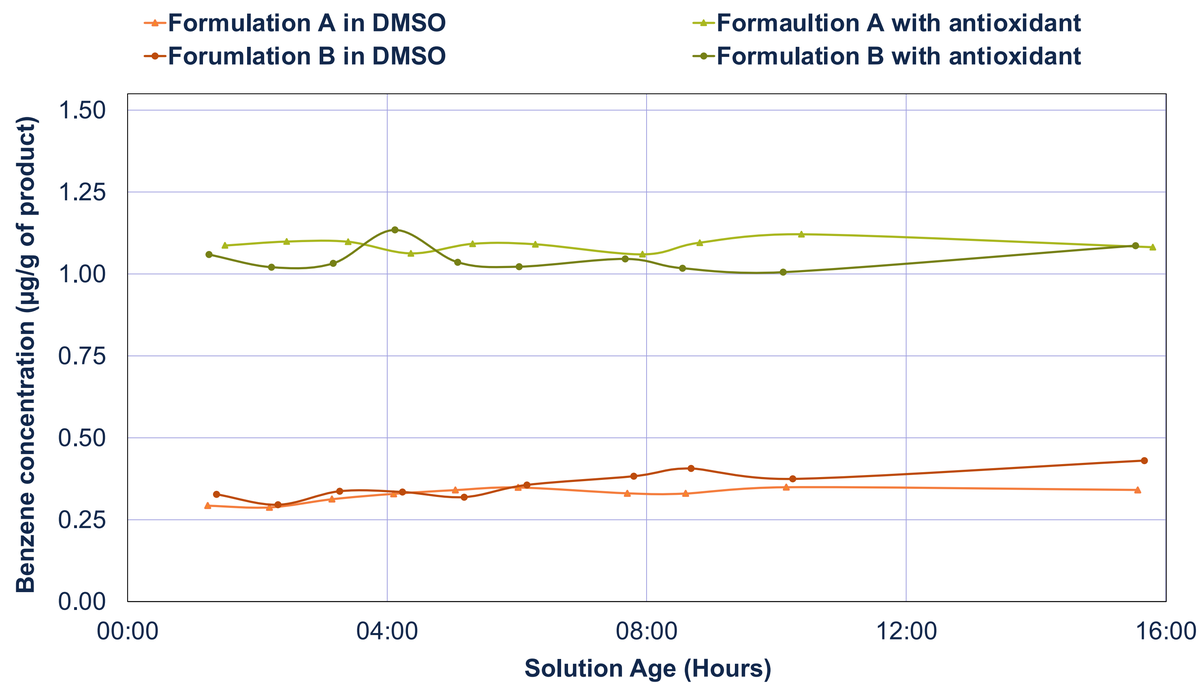

Figure 2: Short-term Stability of Benzene Concentration in BPO Product Formulations

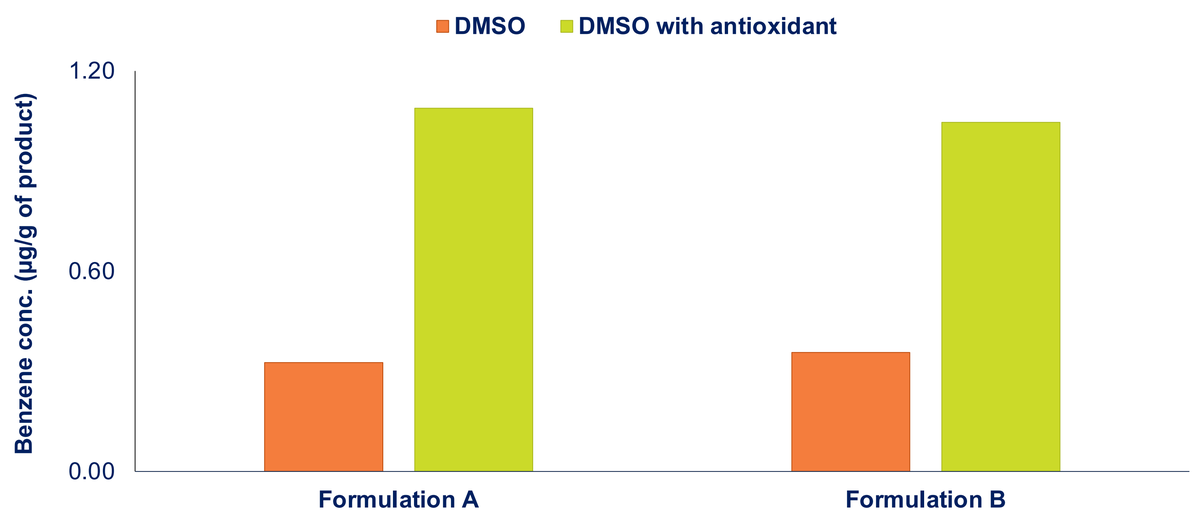

Figure 3: Mean Benzene Concentration DMSO vs DMSO with Antioxidant

Conclusions

- The addition of an antioxidant led to a significant increase in the detected benzene levels in BPO product samples.
- While the underlying cause is an ongoing area of research, the data collected highlights the importance of a tailored and formulation specific approach when developing analytical methods to prevent reporting false positive results.

References

(1) Limited number of voluntary recalls initiated after FDA testing of acne products for benzene; findings show a small number of products with elevated levels of benzene contamination. News Release. US FDA. Published March 11, 2025. Accessed April 25, 2025
(2) Valisure. 2024. "Citizen Petition on Benzene in Benzoyl Peroxide Drug Products." March 5. Division of Dockets Management, Food and Drug Administration