

DEVELOPMENT OF A PRE-SCREENING METHOD FOR bDtBPP, A CYTOTOXIC LEACHABLE FROM SINGLE-USE BIOPROCESSING CONTAINERS.

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Extractable and leachable compounds are a major obstacle in the implementation of single-use technologies. Recent studies show that a widely used trisarylphosphite-based processing stabilizer in plastic films, Irgafos 168®, can breakdown from gamma irradiation. One of the breakdown compounds, bis(2,4-di-*tert*-butylphenyl)phosphate (bDtBPP) has been found to leach out of single-use containers and suppress CHO cell growth. Due to its cytotoxic effect on bioprocessing cell lines, a targeted test for bDtBPP would be highly beneficial for pre-screening single-use bioprocessing containers and bags.

In this study, an ultra-performance liquid chromatography method was developed for the separation and detection of bDtBPP and other extractable compounds related to Irgafos 168® (such as 2,4-di-*tert*-butylphenol, 1,3-di-*tert*-butylbenzene, 2,4-di-*tert*-butylphenylphosphate and oxidised Irgafos 168®). The method was applied to an extractables study of three single-use plastic films, where bDtBPP and the oxidised form of Irgafos 168® were detected and quantified in all three films. Extraction methods using either a whole bag or a small piece of film were evaluated, and the latter shown to be more suitable for finding concentration per area of film and/or estimating the total load of extractables in the entire bag. The feasibility of monitoring bDtBPP during the bioprocess was investigated with a Waters PATROL UPLC System with a Flownamics® sampling probe as the sampling interface. bDtBPP spiked in cell culture medium was only detected in unfiltered or at-line injections, indicating that with the current sampling interface, monitoring of extractables and leachables is limited to at-line sampling.