

LARGE-SCALE ASSESSMENT OF EXTRACTABLES AND LEACHABLES IN SINGLE-USE BAGS FOR BIOMANUFACTURING USING ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED TO QUADRUPOLE-ORBITRAP MASS SPECTROMETRY

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Single-use technologies (SUTs) are increasingly used in biopharmaceutical manufacturing processes. Despite their advantages, these plastic assemblies draw concern because they are a potential source of contamination due to extractable and leachable compounds (E&Ls) that result from residual polymeric fragments and different additives used in their manufacture¹. Characterizing E&Ls from such materials is a necessary step in establishing their suitability for use. There is evidence of some cytotoxic compounds that leach out of single-use bags into cell culture media in concentrations are deleterious to CHO cell growth, even at trace levels². Given the potential for these substances to adversely affect biopharmaceutical production, this discovery clearly shows an urgent need for analytical techniques to identify and quantitatively assess compounds resulting from the extraction of SUS components. This study was focused specifically in single-use bags (SUBs), one important application of the disposable technologies, used for the production of therapeutic antibodies, proteins and vaccines. 34 single-use bags from different suppliers were extracted under conditions that are relevant for the characterisation of E&Ls. Extraction with different model solvents was intended to establish a comprehensive extractables profile: water for injection, isopropanol:H₂O (1:1), 0.1 M H₃PO₄ and 50mM NH₄CH₃COO pH 9.5, while extraction with chemically defined cell culture media was used for leachable assessment. This analysis is challenging as leachables usually exist at trace levels within a very complex matrix. For this reason, a simple and fast analytical method based on a sample treatment by dispersive liquid-liquid microextraction (DLLME) was also developed and applied for analyte preconcentration and matrix elimination. Then, the extracts were analysed by LC-Orbitrap-MS, with high mass resolution performance and exceptional mass accuracy for the detection and identification of non-volatile E&Ls in SUBs. 130 E&Ls were identified, with many of these compounds being polymer additives or their degradation products. Interestingly, some leachables were not found during extractables analysis, suggesting that they might be produced by the interaction of components of the media with compounds from bags. Multivariate analysis performed on the analytical data established significant correlations between the type and concentration of compounds and bags features as brand, manufacturing date and type of polymer. New production techniques have allowed to develop new types of polymers and the advent of regulatory issues that limit/ban the use of certain raw materials and additives has produced a change in the nature of E&Ls. Consequently, it is necessary to provide practical and versatile guidelines for confident determination of these substances that would enable early identification of non-satisfactory films for control and improvement of SUBs. The analytical workflow that is presented has all the necessary features to be used as part of the quality control in bags manufacturing.

¹ Gao, Y.; Allison, N. Extractables and leachables issues with the application of single use technology in the biopharmaceutical industry. *J Chem Technol Biotechnol.* 2016, 91, 289–295.

² Hammond, M.; Nunn, H.; Rogers, G.; Lee, H.; Marghitou, A.-L.; Perez, L.; Nashed-Samuel, Y.; Anderson, C.; Vandiver, M.; Kline, S. Identification of a Leachable Compound Detrimental to Cell Growth in Single-Use Bioprocess Containers. *PDA J. Pharm. Sci. Technol.* 2013, 67, 123–134.