Exhaustive Extraction

With growing regulations in the pharmaceutical industry, emphasis has been placed on extractables and leachables testing to ensure patient safety and drug efficacy

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The importance of extractables and leachables testing in the pharmaceutical industry has grown significantly in the last few years, driven by a substantial growth in global regulatory requirements on patient safety and drug product interaction. A drug product container-closure system should not release chemicals that can accumulate in the drug product in quantities sufficient to present a risk of toxicity, or affect its stability or efficacy. Substances may migrate from different materials, and patients may be exposed through different routes of administration. The examination of extractable and leachable substances is extremely relevant for the protection of patients. During the drug development process and study design, it is important to evaluate the potential for various chemicals to migrate. The aim of extractables and leachables testing is to identify and quantify chemical compounds that can be released from packaging interactions with the drug product. Depending on the specific purpose of the extraction study, laboratory conditions applied may accelerate or exaggerate the normal conditions of storage and use for a packaged dosage form in order to obtain the pattern of extractables that can be released.


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Below are a list of definitions which will help with the navigation and understanding of this article:

- Extractables: chemical entities that can be released from an item (packaging system, process component, medical device) under laboratory conditions applying specific extraction solvents, temperature, and time of contact
- Asymptotic level of extraction: the situation in which the curve of release of an extractable shows a steady state, and no significant increment is noted between two subsequent checkpoints
- Exhaustive extraction: indicates the level extraction in which the most significant release, in terms of number and amount of extractables, can be considered comprehensive in the scope of the screening

Instrument parameter	GC/MS settings
Instrument	Agilent: 7890 - MSD 5975e
Column	Capillary column, agilent HP-5MSI, 30m x 0.25mm, 0.25µm film thickness
Carrier gas and flow	Helium at 1.0mL/min, constant flow
MS scan range	33-800 amu

Instrument parameter	HPLC/MS settings
Instrument	Agilent: 1260 (G1312) HPLC - 6530 Q-TOF
Column	Agilent Zorbax Eclipse XDB-C18, 2.1mm x 50mm 1.8µm
Column temperature	65°C
Mobile phase	A) 5mM Ammonium acetate in water B) 50:50 Acetonitrile: methanol v/v
MS detection	Electrospray ionisation (ESI) and ESI-
MS scan range	50 - 1,500 amu

Table 1: Methods

Background

Time-temperature test conditions and extraction solvents are critical parameters that quantitatively and qualitatively affect compounds released from contact materials during extractables studies. While solvent selection based on the drug product formulation establishes mainly extractables' chemical classes, contact time and contact temperature determine the driving force of migration in terms of quantitation. Parameter setting aims to achieve an extraction amount plateau and an asymptotic level of extraction, which is, however, difficult to determine for all extractables since different compounds show different behaviours due to their peculiar chemical-physical properties. Compounds that can be released very quickly from a packaging material could be thermally or chemically labile and may degrade before other compounds achieve the extraction plateau; in some other cases, compounds may never achieve extraction plateau before material degradation.

> **"Parameter setting aims to achieve an extraction amount plateau and an asymptotic level of extraction"**

Material tested	Extraction solution
High-density polyethylene bottle	Isopropanol/water 50/50
Polypropylene cap	Isopropanol/hexane 10/90*
Chlorobutyl rubber stopper	Isopropanol/water 50/50
Polypropylene syringe with bromobutyl plunger	Isopropanol/water 50/50
Polyurethane tubing	Isopropanol/water 50/50
LDPE (inner layer) and PVC (outer layer) coextruded tubing	Isopropanol/water 50/50
Platinum-cured silicone tubing	Isopropanol/water 50/50

Table 2: Experimental design
* Considering the point of boiling of the Hexane, the Isopropanol/Hexane 10/90 solution was tested at 40°C

Current Regulatory Guidelines on Extraction Conditions

In light of the above considerations, USP chapter (1663) ISO 10993-12 and other updated guidelines on extractables and leachables testing describe only indicative examples of these parameters as it is quite difficult to determine a unique setting that can be applied to all the packaging materials on the market and guarantee the exhaustive extraction of extractables that can potentially migrate. Moreover, if we consider the different ways of material testing (soaking, sonication, recirculation, soxhlet, reflux, etc.) and the different extraction ratios which should be applied in an extractables study, the definition of a standard set of parameters seems to be an impossible mission.

Fixing Variables

Considering the knowledge acquired in the past two decades and the current state-of-the-art extractables testing, the number of variables can nevertheless be reduced by the following considerations:

- Extraction solution: most drug products are designed to be delivered by oral, intravenous, intramuscular, or inhalation administration, and for this reason the components of their formulation are very frequently simulated in extractables testing with an alcoholic extraction solution
- Extraction temperature: this should be set considering an appropriate aggressive condition with respect to the real use, simultaneously avoiding in the same time the setting of an exaggerated level that could lead to disruption of the packaging chemical structure
- Extraction process: when possible, extractables testing methods should represent the real interaction between drug product and packaging material
- Extraction ratio: the number of items or the surface area, which has to be in contact with the extraction solutions

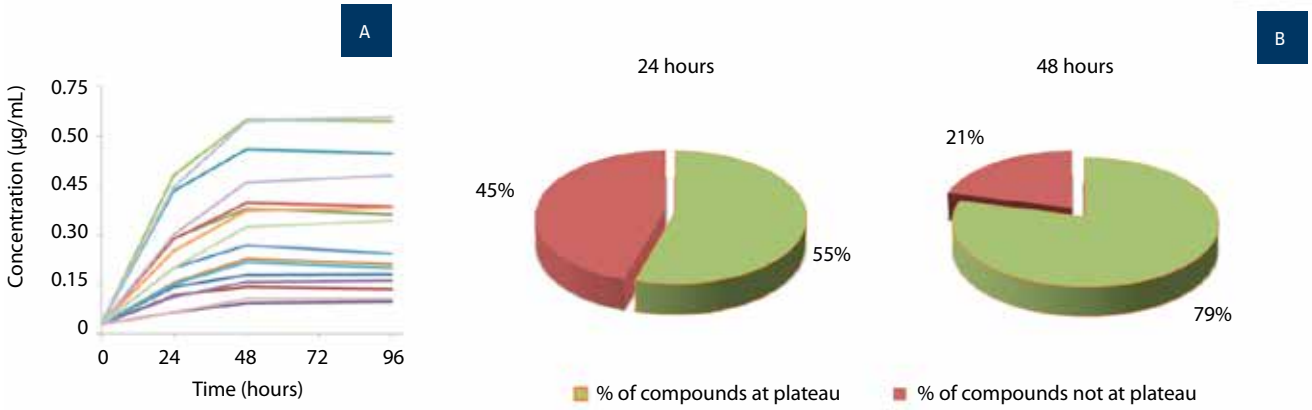

Figure 1: GC/MS analysis. Examples of the compounds concentration trend is shown in Panel A, while the percentage of compounds that reached the asymptotic level at 24 and 48 hours is reported in Panel B

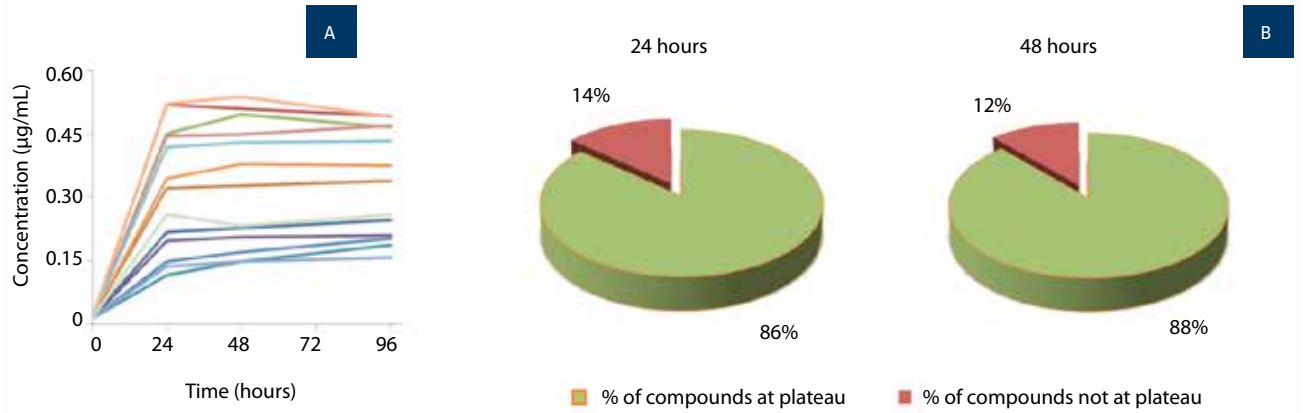

Figure 2: HPLC/MS analysis. Examples of the compounds concentration trend is shown in Panel A, while the percentage of compounds that reached the asymptotic level at 24 and 48 hours is reported in Panel B

during the extractables testing, is chosen to permit that the limit of detection of the analytical techniques covers the reporting threshold set (AET). For this reason, it has to be set case by case in each extractables and leachables study

Study Goals

Starting from the above, a research study was conducted to determine the necessary contact time to achieve the asymptotic level in the extractables migration.

Results and Discussion

Seven different materials were tested by extraction with an isopropanol solution at 70°C for 24, 48, and 96 hours, maintaining the same extraction ratio in all the checkpoints (see Table 1, page 82). The obtained sample solutions were analysed by GC/MS and HPLC/MS (ESI positive and negative modes). The concentration of extractables detected above the LOQ (Signal/Noise less than10), and the percentage variation between two subsequent checkpoints was calculated. Considering the screening nature of analytical methods employed in extractables studies, the asymptotic level of extraction was considered achievable if the percentage increase of a compound concentration in the subsequent checkpoint was less than 50% of the concentration level detected in the previous checkpoint within the extractables range LOQ-0.5µg/mL (range A); while less than a percentage increase of 20% for compounds above 0.5µg/mL (range B). The application of this percentage range is due to the different impact on variation that the same amount can have at low and high concentration levels. For example, concentration increase of 0.1µg/mL represents, for a compound at 0.4µg/mL, a percentage increase of 25%, while a compound at 4µg/mL represents a percentage increase of 2.5%.

> **Storage conditions for 48 hours at 70°C establish a satisfactory basis for further testing**

GC/MS Analysis

A total number of 152 peaks (concentration range: 0.04 – 804µg/mL), 96 compounds belonging to the concentration range A, while 56 compounds in the range B were detected above the LOQ in the sample solutions by the GC/MS analysis.

At the 48 hour check point, the 79% of the total detected peaks resulted at the asymptotic level, showing no significant increment in the 96 hour checkpoint (see Figure 1, page 84). 18% of the peaks that reached the plateau in 48 hours showed instead an important decrease at 96 hours, underlying a degradation due to the prolonged contact.

HPLC/MS Analysis

A total number of 196 peaks (117 by ESI positive mode and 79 by ESI negative mode; concentration range: 0.05 –117µg/mL) were detected in the sample solutions above the LOQ by HPLC/MS analysis; 59 compounds belonged to the concentration range A, while 137 compounds in the range B. At the 48 hour checkpoint, the 88% of total detected peaks resulted at the asymptotic level (see Figure 2, page 84). With respect to the behaviour noted in the GC/MS technique, no compounds showed a significant decrement in the 96 hour checkpoint compared to the 48 hour mark.

Full Analysis

In the present study, to evaluate the impact of contact time necessary to achieve the asymptotic level in extractables release, the following parameters were taken into account:

- Extraction solution: alcoholic solution
- Extraction process: filling or soaking
- Extraction temperature: 70°C

For all seven items tested, 24 hours contact time was demonstrated as an imperfect timeframe to allow the achievement of the extraction plateau for the desired extractables quantity.

As expected, the highest compound release was achieved at the 96 hour checkpoint, however, during this timeframe a partial degradation of several compounds was detected. The 48 hours contact time resulted as the best compromise between the achievement of the asymptotic level of extraction, the necessity to gain reliable extractables data in a short period, and the necessity to avoid the loss of compounds due to exaggerated extraction conditions. Although the high number of peaks evaluated in the present study allows for the consideration of the obtained data that are statistically relevant, it cannot be representative for all the materials present on the market that can manifest different behaviours to those detected in this study. Storage conditions for 48 hours at 70°C establish a satisfactory basis for further testing, with the aim to reach, in the near future, a more standardised and exhaustive approach.

About the authors

Simone Carrara currently serves as Extractables and Leachables and Impurity Characterisation Laboratory Manager at Eurofins BioPharma Product Testing in Italy. He holds a Biotechnology degree from the University of Milan, Italy, and has a vast experience as a bio-analytical researcher through LC/MS method development for Pharmacokinetics analysis and ADMET profiling. In his previous role as Project Leader he successfully managed different projects in a wide range of areas, including analytical method development and validation with LC/MS-GC/MS, and drug product impurities characterisation. For the last five years he has been focusing on extractables and leachables studies to support drug-manufacturing companies to fulfil regulatory requirements.

Michele Vasso, PhD, has served as Project Leader of Extractables & Leachables and Impurity-Characterization Laboratory at Eurofins BioPharma Product Testing in Italy since 2016. Michele holds a Biotechnology degree from the University of Naples, Italy. He received the PhD in Molecular Medicine from the University of Milan in 2009. During this period he consolidated his expertise as bio-analytical researcher in mass spectrometry. He joined the Italian National Research Council (CNR) as a researcher where he was involved in relevant projects on method development by mass spectrometry (LC/MS and MALDI/MS) for the characterisation of biological molecules and low-weight compounds. After joining Eurofins BioPharma Product Testing Italy, his main expertise lies in extractables and leachables studies.