Exhaustive Extraction: Definition Of Standardized Conditions For Extractables Testing

by Michele Vasso and Simone Carrara, Eurofins BioPharma Product Testing Italy

Introduction

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.

Definitions

• **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, in which 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.

Background

Time-temperature test conditions and extraction solvents are critical parameters which affect quantitatively and qualitatively compounds released from contact materials during extractables studies. While solvents 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 behaviors 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.

Current Regulatory Guidelines on extraction conditions

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

Fixing variables

Considering the knowledge acquired in the past two decades and the current state-of-the-art of extractables testing, the number of variables can be nevertheless 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 frequent simulated in extractables testing with an alcoholic extraction solution.

• **Extraction temperature:** Extraction temperature should be set considering an appropriate aggressive condition with respect to the real use, avoiding in the same time the setting of an exaggerated level which could leads to disruption of the packaging chemical structure.

• **Extraction process:** When possible extractables testing method should represent the real interaction between drug product and packaging material. The testing of the items under orbital shaking after the filling with the extraction solutions, or the soaking when filling was not possible, was therefore our first choice for the experiment.

• **Extraction ratio:** The number of items or the surface area which has to be in contact with the extraction solutions 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 E&L study.

Study goals

Starting from the above indicated considerations and with regards of Eurofins successful expertise on E&L testing, a research study was conducted to determine the necessary contact time to achieve the asymptotic level in the extractables migration.

Methods

Instrument Parameter	GC/MS settings
Instrument	Agilent: 7890 - MSD 5975
Column	Capillary column, Agilent HP-5MSI, 30 m x 0.25 mm, 0.25 µm film thickness
Carrier gas and Flow	Helium at 1.0 mL/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, 2.1 mm x 50 mm 1.8 µm
Column temperature	65°C
Mobile phase	A) 5mM Ammonium acetate in Water B) 50:50 Acetonitrile:Methanol v/v
MS detection	ESI+ and ESI-
MS scan range	50 - 1500 amu

Results & Discussion

Seven different materials were tested by extraction with isopropanol solution at 70°C for 24, 48 and 96 hours (see Table 1 for details), maintaining the same extraction ratio in all the checkpoints. The obtained sample solutions were analyzed by GC/MS and HPLC/MS (ESI positive and negative modes).

The concentration of extractables detected above the LOQ (Signal/Noise >10) 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 achieved 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 - 0.5 µg/mL (range A); while less than a percentage increase of 20% for compounds above 0.5 µg/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 0.1 µg/mL represents for a compound at 0.4 µg/mL a percentage increase of 25%, while for a compound at 4 µg/mL represents a percentage increase of 2.5%.

Material tested	Extraction solution	Contact conditions
HDPE Bottle	Isopropanol/Water 50/50	
Polypropylene Cap	*Isopropanol/Hexane 10/90	
Chlorobutyl rubber stopper	Isopropanol/Water 50/50	24 hours 70°C
Polypropylene syringe with Bromobutyl plunger	Isopropanol/Water 50/50	48 hours 70°C
Polyurethane tubing	Isopropanol/Water 50/50	96 hours 70°C
LDPE (inner layer) and PVC (outer layer) coextruded tubing	Isopropanol/Water 50/50	
Platinum-cured Silicone tubing	Isopropanol/Water 50/50	

Table 1. Experimental design
* Considering the point of boiling of the Hexane, the Isopropanol/Hexane 10/90 solution was tested at 40°C.

GC/MS analysis

A total number of 152 peaks (concentration range: 0.04 – 804 µg/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 48h check point, the 79% of the total detected peaks resulted at the asymptotic level, showing no significant increment in the 96h checkpoint (figure 1). The 18% of the peaks which reached the plateau in 48h 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 – 117 µg/mL) were detected in the sample solutions above the LOQ by HPLC/MS analysis; 59 compounds belonging to the concentration range A while 137 compounds in the range B.

At the 48h check point the 88% of total detected peaks resulted at the asymptotic level (figure 2). As different with respect to the behavior noted in the GC/MS technique, no compounds showed a significant decrement in the 96h checkpoint with respect to the 48h.
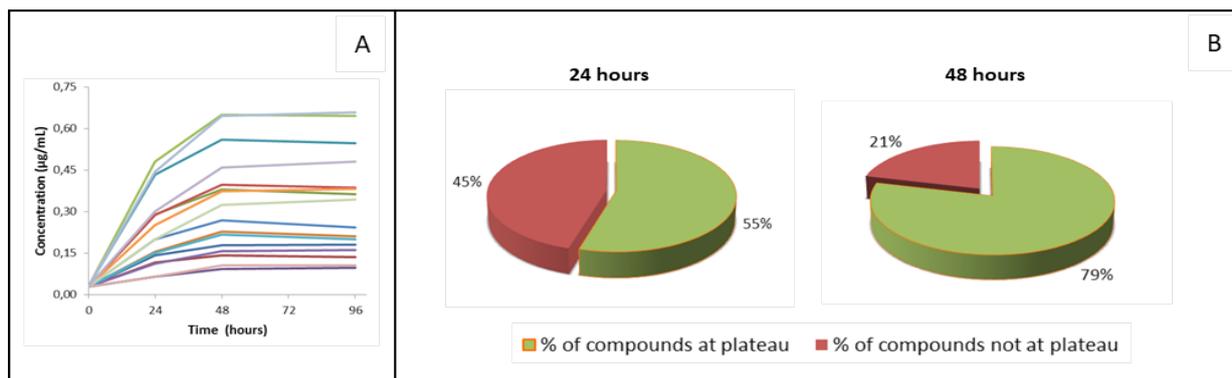

Figure 1. GC/MS Analysis. Example of the compounds concentration trend is showed in panel A while the percentage of compounds that reached the asymptotic level at 24 and 48 hours is reported in panel B.
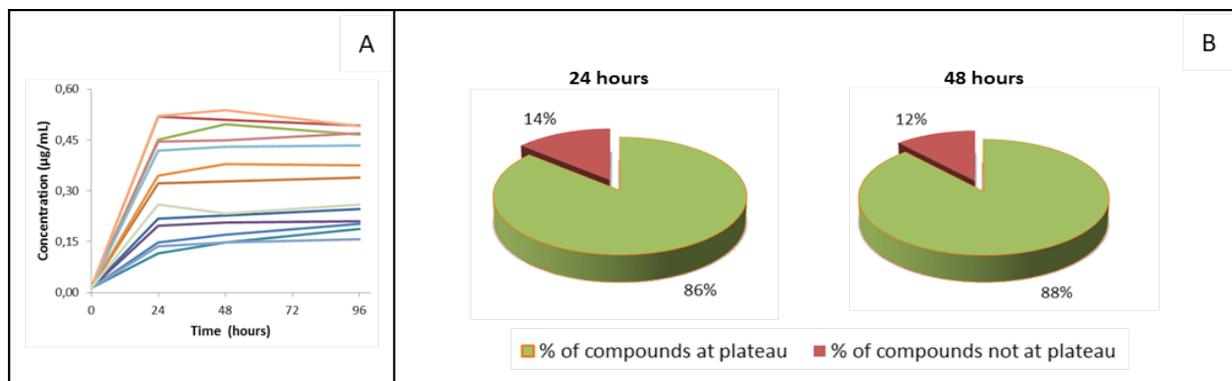

Figure 2. HPLC/MS Analysis. Example of the compounds concentration trend is showed in panel A while the percentage of compounds that reached the asymptotic level at 24 and 48 hours is reported in panel B.

Conclusion

In the present study, in order 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 different items tested 24 hours contact time was demonstrated as not the perfect timeframe to allow the achievement of the extraction plateau for the desired extractables quantity. As expected, the highest compound release was achieved at 96 hours checkpoint but during this timeframe a partial degradation of several compounds was detected. 48 hours contact time resulted instead 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 to consider the obtained data statistically relevant, it cannot be representative for all the materials present on the market which can manifest different behaviors 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 standardized and exhaustive approach.