Miriam Ehrnhöfer-Reßler / Wolfgang Platzer / Bernhard Wagner / Erwin Zinser

Development of a multi-dimensional screening model to investigate the metabolic effects of extractables and leachables from packaging materials

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Abstract

Risk assessment of migrating packaging components into final products entails significant challenges for regulators and manufacturers. Apart from the analytical requirements associated with the identification and quantification of extractables and leachables, adverse effects on the human metabolism with regard to versatile impacts on human health are of particular concern.
This work evaluates different extraction approaches requiring individual analytical techniques for the detection of very volatile, volatile and semi-volatile compounds. A robust screening method was established considering the impact of polarity, boiling point and vapour pressure of target compounds. Using this multi-dimensional model, residual solvents, extractables and leachables such as acetaldehyde, phthalates, methacrylates and siloxanes were detected in different packaging materials including syringes, glass vials with stoppers, and PET-bottles. Further studies including an *in vitro* cell culture model will be conducted to gain information about the molecular mechanisms underlying potential oxidative and immune modulatory effects.

Keywords:

GC-MS, headspace analysis, extractables, leachables, packaging materials

1. Introduction

The risk assessment and mitigation of extractables and leachables from food packaging materials, medical containers and drug products poses significant challenges to regulators and manufacturers. Apart from the analytical requirements associated with the identification and quantification of migrating packaging components, adverse biological effects of the individual chemical structures and metabolites thereof must be considered (Li et al., 2015; Jenke, 2007). Compounds such as for example, bisphenol A or phthalates used as plasticizers in the production process were identified as

endocrine disrupting chemicals suspected of influencing the immune system in its regulatory and functional levels (Hansen et al., 2014).

To study the effectiveness and limitations associated with methodologies used to detect very volatile, volatile, semi-volatile and non-volatile compounds, a holistic screening model was developed involving robust extraction studies combined with selective and quantitative detection techniques suitable for sensitive and unstable substances. Following a concrete and specific approach, firstly, the final product and the packaging material were subjected to a defined analytical process with careful consideration of solvation effects, pH, ionic strength, and temperature.
In a second step, the metabolic effects of identified extractables and leachables were investigated by means of an *in vitro* cell culture system focusing on immune modulatory properties and markers related to oxidative stress. Herein, the focus was on the specific requirements related to analyses of volatile and semi-volatile compounds present in aqueous product matrices.

2. Materials and Methods

The analytical screening of migrating packaging components was divided into two major parts comprised of sample preparation together with targeted and untargeted data analyses. For determination of volatile and semivolatile extractables, packaging materials and product containers were directly exposed to solvents with different polarities, e.g. hexane, methylene chloride, tert-methyl butyl ether, isopropanol and water over time (2 weeks to 6 months) at room temperature and/or thermal treatment with 60°C. Migration studies focusing on volatile and semi-volatile leachables in aqueous products thermally stressed at 60°C for two weeks were conducted. Meanwhile, impurities were isolated from the product via liquid-liquid-extraction using organic solvents. The individual solvent extracts were concentrated by centrifugation under lower pressure using a speed vac (Labconco) and the final volume of the organic extract was adjusted to 1.5 mL. The concentrated solvent extracts were injected into a 6890 gas chromatograph (GC, Agilent Technologies) coupled with a single quadruple mass spectrometer (MS, Agilent Technologies) operated at 70eV using a split/splitless injector.
Analysis of volatile and very volatile compounds was done via direct static-headspace sampling of the aqueous extracts or parts of the packaging materials using a 7697 headspace-sampler (Agilent Technologies) also connected to GC-MS. Headspace equilibration was carried out at 80°C for 20 min. The injection duration was set for 0.5 min with loop and transferline operated at 95°C and 110°C, respectively. Direct analysis of packaging materials was additionally carried out using a harsher temperature program, with 121°C oven temperature held for 15 mins.
For data analyses, the individual GC-chromatograms (total ion current, TIC) and area counts of respective peaks, either assigned to extractables or leachables, were extracted and compared among individual samples.
Identification of all peaks exceeding an area count of 2500 was carried out, by matching the retention time and the corresponding mass spectra with an internal screening method built up using the Masshunter Quantitative Data Analysis Software (Agilent Technologies). This

screening method contained about 210 reference substances including volatile and semivolatile compounds e.g. phthalates, polycyclic aromatic hydrocarbons (PAH), linear hydrocarbons (C8-C40), solvents and individual reference compounds related to plastic materials.

Substances that had not been confirmed via reference standards were registered in the Masshunter data analyses method after matching the MS-spectra with reference spectra from the Wiley 2011 database. The area counts of all extractables and leachables found in the samples were extracted and checked for quality controls, especially quantifier/qualifier-ratio and retention time shifts.

3. Results

Considering the chemical properties of the final product and the packaging material, different extraction approaches were evaluated while focusing on very volatile, volatile and semi-volatile compounds (see **Figure 1**).

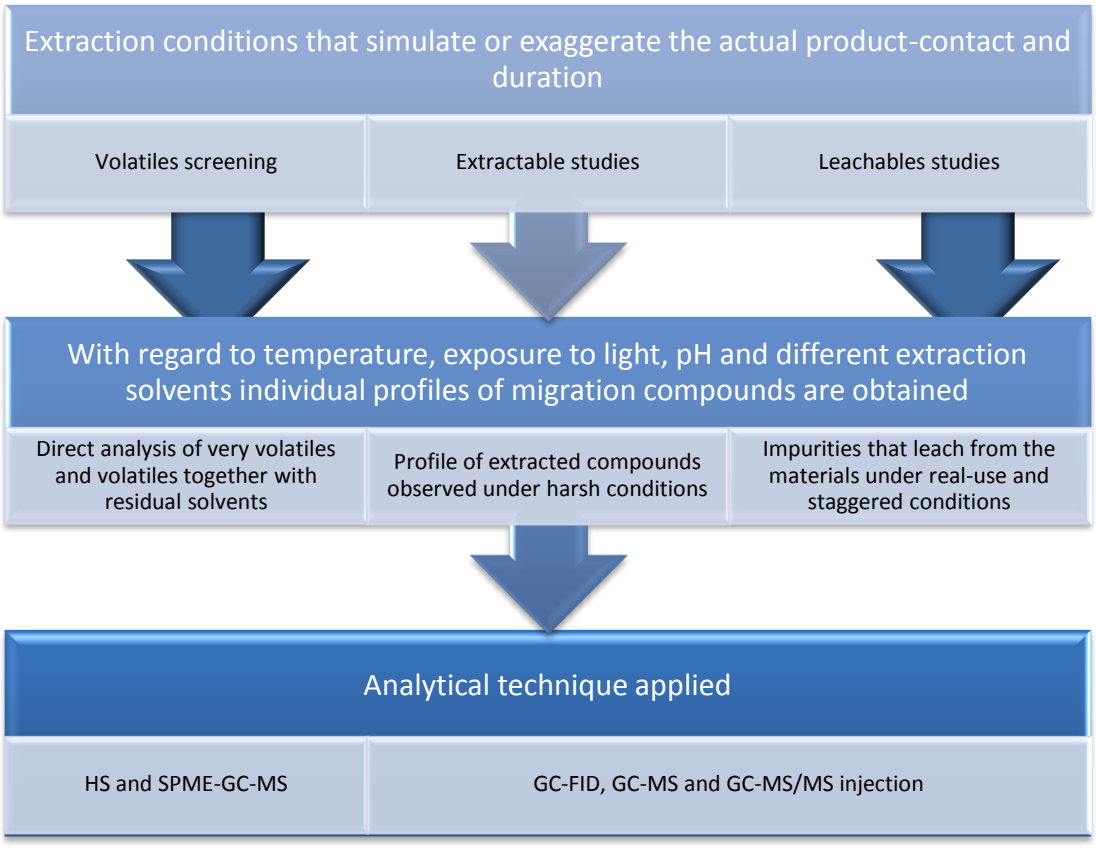

Figure 1: Holistic analytical approach for extractables and leachables screening.

The extractables and leachables screening involved syringes, glass vials with stoppers, and PET-bottles made from different polymer-based materials e.g. polyethylene (PE), polyethylene terephthalate (PET), polycyclohexylenedimethylene terephthalate (PCT), polypropylene (PP), and

polystyrene (PS). Preliminary tests including direct compound analyses via headspace (HS)-sampling, as well as liquid-liquid-extraction (LLE) with different organic solvents were conducted using spiked aqueous solutions with different pH (pH 1, pH 7 or pH 11.5) to consider polarity and vapour pressure of potential analytes.

For detection of very volatile and volatile compounds with boiling points up to 150°C and high vapour pressure, direct analyses using headspace (HS) coupled with gaschromatography-mass spectrometry (GC-MS) were proven to be the most suitable technique. Compounds such as acetaldehyde, N,N-dimethylacetamide, acetic acid, methyl acetate or ethyl acetate were identified to be pH sensitive due to dissociation processes or formation of salts. Thus, the pH of aqueous samples was determined before the HS-GC-MS analyses were carried out.

HS-GC-MS analysis of the aqueous extract from packaging materials did not reveal specific volatile material components. However, a characteristic profile was obtained for direct material extraction at 80°C and 121°C equilibration temperature (**Figure 2, A**). Apart from the compounds that could be easily identified as e.g. 2-methyl-pentane, 3-pentene-2-one and 2-octane, unknown but structural related hydrocarbons with m/z 43, m/z 45, m/z 57, m/z 71, m/z 85, m/z 98, m/z 112 and m/z 127 were also obtained. Using the internal data analyses method, these compounds were also identified in the final product (**Figure 2, B**).

Depending on solvent polarity, different patterns of volatile and semi-volatile compounds were extracted from packaging materials. Comparable results including material specific components were obtained for the hexane and the ether extracts, whereas substances derived from the production process or the material composition such as BHT, styrene, methylacrylates, diethylphthalate, diisobutylphthalate and 9-octadecanamide were determined. Extraction with methylene chloride did not yield a significant quantity of components at all. Using isopropanol, more polar compounds such as siloxanes were extracted.

Interestingly, each of the identified extractables was hardly detected in the final product. Depending on the individual composition, it may be possible that extractables were overlaid by formulation specific substances like the API, or by high amounts of other volatile product constituents. In accordance with this, high amounts of product specific hydrocarbons with similar and specific product ions were also detected.

Further studies regarding the biological activity will be conducted, including a validated quantification of identified migration compounds together with an investigation of the dose-dependent biological effects by means of an *in vitro* cell culture model. In a second step, molecular mechanisms underlying potential oxidative and immune modulatory effects will be explored.

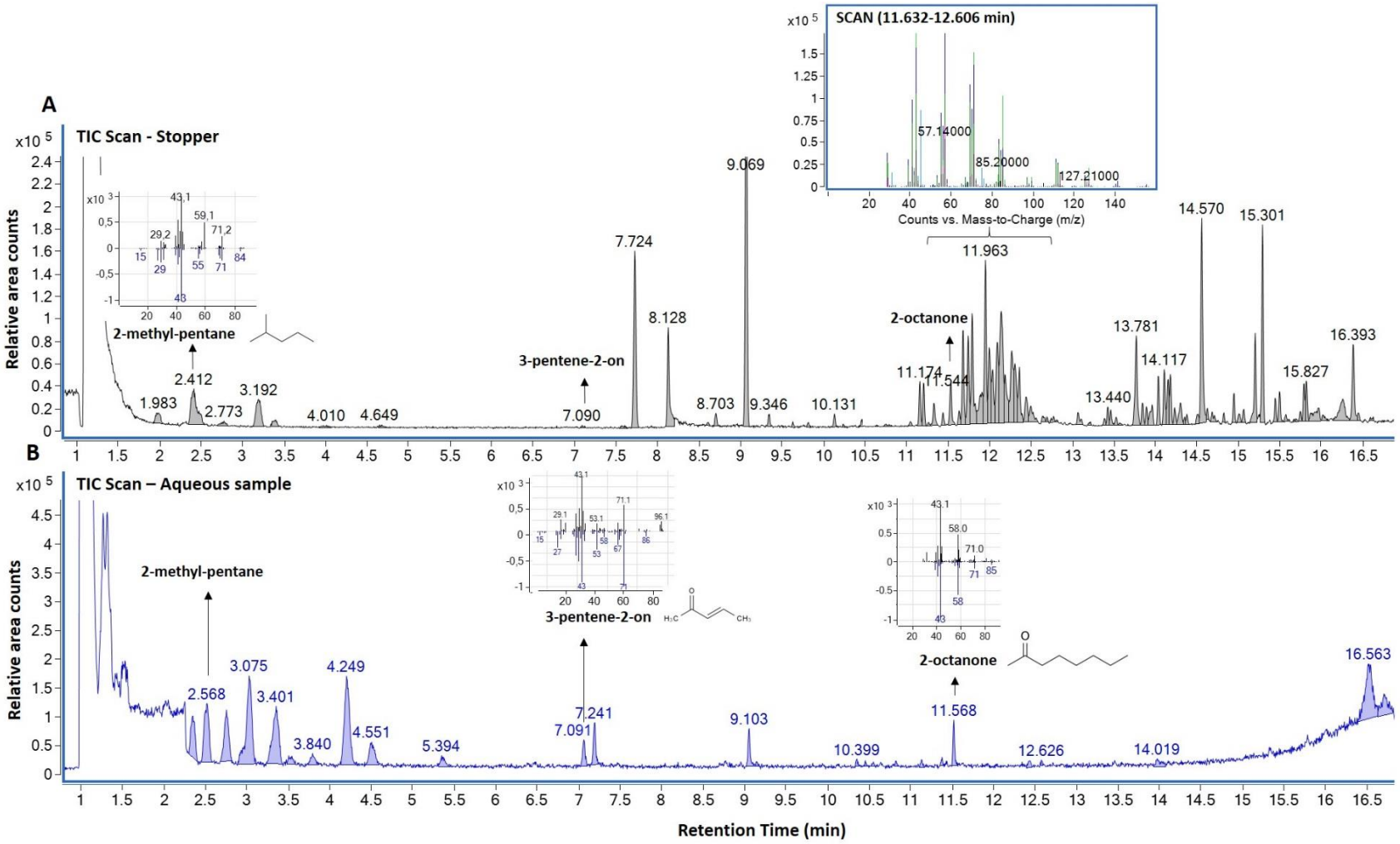

Figure 2: Chromatogram of total ion current generated via GC-MS-headspace analysis from packaging materials (A) and the final

4. Conclusion

These experiments relating to very volatile, volatile and semi-volatile migration compounds serve as part of a holistic approach to effectively evaluate and minimize the risk of leachables present in final products with regard to improvements in product quality, patient safety and consumer acceptance covering the production process and lifecycle management. Targeted and untargeted screenings of potentially harmful substances using tailored analytical techniques are ultimately impaired by concentration issues and by the complexity of sample composition.

References:

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