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Development of a generic approach for monitoring leachable compounds in hospital pharmacy-prepared prefilled plastic packaging by ultrahigh-performance liquid chromatography coupled to high-resolution mass spectrometry with postcolumn infusion

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ABSTRACT

Prefilled plastic packaging is time- and cost-effective in hospital pharmacy because it prevents waste, preparation errors, dosage errors, microbial contamination and accidents. This packaging mostly includes prefilled syringes (PFS), intravenous (IV) bags and vials intended for long-term storage that can be used for immediate treatment. There is a rising availability in the market for prefilled drug products due to their practical approach. Leachable compounds could be evaluated in hospital pharmacy-prepared prefilled drug solutions. The Pharmacy Department at the Lausanne University Hospital has developed an innovative, highly sensitive, and generic method by postcolumn infusion based on ultrahigh-performance liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) for the analysis of plastic additives in hospital pharmacies. The postcolumn infusion solution was developed with 2% ammonium hydroxide in methanol on a representative set of 30 candidate compounds with different physical-chemical properties, such as log P and molecular structure, to represent the most important categories of additives. The LODs obtained for all compounds ranged from 0.03 to 7.91 ng/mL with linearity up to 250 ng/mL. Through this screening method, plastic additives can be rapidly identified due to the combined use of retention time, exact mass (including isotopic pattern) and MS/MS spectra. In addition, the users can screen for vast categories of plastic additives, including plasticizers, epoxy monomers, antioxidants, UV stabilizers, and others. The screening is facilitated by assessments of a complex in-house-built database for extractable and leachable trace assessment (DELTA), containing 205 compounds for unambiguous identification. Relative response factors were established for all analytes to obtain a semiquantitation of compounds. Moreover, the database also contains valuable estimative toxicology information, which was obtained through calculating their permissible dose exposure threshold; thus, estimative toxicology assessment can be performed for identified compounds in prefilled drug products. This method and the database were applied to a hospital pharmacy-prepared prefilled vancomycin syringe for paediatric use. Ultrasound-assisted dispersive liquid-liquid microextraction (UA-DLLME) was used to prepare the samples for leachable analysis. As a result, 17 plastic additives

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were formally identified, and their concentrations were estimated. A toxicology assessment was performed by comparing their concentrations with their theoretical PDE thresholds. In conclusion, the prefilled drug solution released a negligible amount of known leachables that appeared to be safe for use in neonates and children.

1. Introduction

Polymers, such as polypropylene (PP), polyethylene (PE), polyvinylchloride (PVC), cyclic olefin copolymer (COC), cyclic olefin polymer (COP) and other elastomeric materials, are currently widely used in plastic packaging in hospital care. These plastic packaging materials are easy to use and cost-effective and possess excellent mechanical, chemical and biological resistance [1,2]. These characteristics are obtained by adding plastic additives, each of which possesses different roles, such as antioxidants, plasticizers, light stabilizers, and lubricants; these properties provide specific physical and chemical attributes to assure a long shelf life. However, plastic additives from the prefilled packaging could possibly leach into the surroundings. This could be problematic for patient safety, due to the toxicity of the leached additives, including their compatibility with the drug formulation and their endocrine disrupting potential [3].

In a hospital setting, the use of optimal quality plastic material in plastic packaging is crucial when patient care is involved, such as intravenous (IV) lines, syringes, IV pouches and catheters. One of the most interesting ideas involving plastic packaging in hospital pharmacies is the use of prefilled drug products. There has been an increased use of prefilled polymer packaging in hospitals due to their practicality; these products are immediately available and considerably reduce dosage errors [4,5]. A good quality plastic used in prefilled drug products is less susceptible to leaching additives in drug solutions, as proven by industries. Before releasing the packaging in the market, pharmaceutical industries are obliged to perform extractable and leachable studies on plastic packaging to study the materials and evaluate the drug solution interaction with the material [6–8]. Guidelines and drafts are often used as guidance to perform extractable and leachable experiments. They can be found in the following entities: the United States Pharmacopoeia (USP), the International Council for Harmonization (ICH), the International Organization for Standardization (ISO) and nongovernmental organizations, such as the Product Quality Research Institute (PQRI) [9–12]. However, when prefilled drug products become unavailable in the market or are nonexistent for a certain dosage, volume, or packaging form, the hospital pharmacy could potentially produce them to fit the hospital demands. For example, hospital pharmacies would be able to make prefilled syringes (PFS) to house emergency drug solutions (atropine and fentanyl), antibiotics (vancomycin and penicillin), anaesthesia (propofol and rocuronium), chronic drugs (insulin and heparin derivative) and many more.

These leachable substances from prefilled packaging are often detected in very low concentrations in an aqueous solution and should not impact person's health. However, these substances could have an impact when a patient receives frequent administrations via the parenteral route for a long period of time. Patients, such as preterm infants, neonates and children, are at the highest risk for malnutrition, chronic illnesses, and infections when they are admitted to the hospital [13]. In industries, when a polymer additive is identified in a prefilled drug product, toxicological assessment is performed based on the dose—response relationship. The acute and subchronic systemic toxicities must be considered, such as the LD50 (lethal dose 50), NOAEL (non observed adverse effect level), LOAEL (low observed adverse effect level), etc., which are often readily available. The following key endpoints must also be evaluated: genotoxicity, irritation, sensitivity, reproduction toxicity and carcinogenicity [14]. However, some plastic additives can behave differently and possess what we call a non-monotonic dose—response curve/relationship. This is known to cause endocrine-related illnesses, such as diabetes, obesity, endometriosis and even some cancers over time because of their strong affinities for different receptors at minute concentrations. Chronic exposure of these substances in patients (i.e., neonates and children) is a major concern because the substances interact with the endocrine system [15,16]. To date, endocrine disruptors are not included in toxicological assessments because they are poorly understood; therefore, they are used only for research.

The development of a comprehensive strategy enabling sensitive, specific and reliable measurements of plastic additives in plastic packaging is essential for addressing the global health challenges putatively caused by these substances. Current analytical methodologies generally involve chromatographic techniques, such as gas chromatography (GC) with a flame ionization detector (FID) or mass spectrometry (MS) and/or ultrahigh-performance liquid chromatography (UHPLC) with a diode array detector (DAD), charged aerosol detector (CAD) or MS. GC- and UHPLC—MS are the most suited for the detection and quantification of plastic additives because of their peak capacity, sensitivity and selectivity. GC—MS is commonly used to analyse volatile and semivolatile organic compounds, whereas UHPLC—MS is the gold standard for analysing nonvolatile plastic additives. For the last few years, MS-based detection methods have been developed to analyse plastic additives in extractable and leachable studies. Plastic additive experiments by UHPLC—MS platforms generally involve hydrophobic stationary phases, such as C18. However, these studies were only conducted on small sets of compounds or on specific chemical classes of additives [17]. Complementary UHPLC methods with other stationary phase chemistry would be interesting for the scientific community to screen a wider range of plastic additives. In this context, phenyl-hexyl stationary phases are a viable strategy because they allow the retention of apolar compounds and provide alternative selectivity for aromatic compounds through π-π interactions.

Postcolumn infusion (PCI) applied to UHPLC—MS is particularly adapted for boosting the sensitivity of bisphenol derivatives. The technique involves the use of a syringe infusion installed between the LC and the MS, in which an additive could be selected to promote optimal MS sensitivity by modifying the ionization performance of the electrospray (ESI) [18].

The use of HRMS and spectral databases has dramatically improved the confidence level of compound identification, such as extractable and leachable substances. The PQRI Leachables and Extractables group as well as reviews from Christiaens and Jenke have proposed three levels of identification, i.e., confirmed, confident and tentative [19–21]. Although these three levels have been defined, the criteria used to fulfil the levels are not sufficiently precise. In other fields, such as environmental analysis or metabolomics, in which sample matrices are of high complexity, a five-level classification is generally used. The classification is established based on the information gathered by various analytical platforms, including UHPLC-HRMS. For the latter and to obtain the optimal reliability of the identification, i.e., level 1, an internal databases is needed, and properties such as retention time (RT), accurate m/z, isotopic pattern and MS/MS pattern must match compound properties to those of chemical standards measured under identical experimental conditions.

In the present work, a highly sensitive and versatile analytical method involving a UHPLC-HRMS platform was developed to screen a wide range of leachable plastic additives, i.e. plasticizers, antioxidants, UV stabilizers, bisphenol-based epoxy monomers, and lubricants in any type of prefilled drug products and also to give the user an estimative quantitation of these additives. The set-up, based on PCI using ammonium hydroxide in methanol, was built to boost signal intensity of these

compounds in both ESI positive and negative modes. Systematic evaluation on the influence of different postcolumn infusion (PCI) conditions, such as the concentration of PCI additive and its flow rate, was performed to establish generic conditions and enable the maximum sensitivity to analyse a training set of 30 representative plastic additives commonly found in products [18]. Second, a database for extractable and leachable trace assessment (DELTA), comprising a diverse range of plastic additive compounds, was implemented to monitor plastic additives in hospital-prepared drug products. Its development and purpose will be discussed. Finally, the developed generic UHPLC-PCI-HRMS method with the database was applied in a leachable-monitoring experiment on a hospital-prepared prefilled drug product, i.e., centralized intravenous additive service (CIVAS) and its risk toxicology, was evaluated.

2. Experimental section

2.1. Reagents and materials

Diphenyl hydrogen phosphate, 4,4′-Sulfonyldiphenol, 4,4′-Methylenediphenol, Butylated hydroxyanisole, 4,4′-(1,1-Ethanediyl)diphenol, 4,4′-(2,2-Propanediyl)diphenol, 4,4′-(2,2-Butanediyl)diphenol, Triphenyl Phosphate, 4-(2-Phenyl-2-propanyl)phenol, 3-[4-Hydroxy-3,5-bis(2-methyl-2-propanyl)phenyl]propanoic acid, 4-Methoxy-2,6-bis(2-methyl-2-propanyl)phenol, B4-Hydroxy-3,5-bis(2-methyl-2-propanyl) benzoic acid, 4-Methyl-2,6-bis(2-methyl-2-propanyl)phenol, 4,4′-(1,3-Phenylenedi-2,2-propanediyl)diphenol, 2,4,6-Tris(2-methyl-2-propanyl)phenol, 2-(5-Chloro-2 H-benzotriazol-2-yl)− 4-methyl-6-(2-methyl-2-propanyl)phenol, 2,2′-Methylenebis[4-methyl-6-(2-methyl-2-propanyl) phenol], (9Z)− 9-Octadecenamide, [2-Hydroxy-4-(octyloxy)phenyl] (phenyl)methanone, 4,4′-Sulfanediylbis[5-methyl-2-(2-methyl-2-propanyl)phenol], 2,2′-Methylenebis[4-ethyl-6-(2-methyl-2-propanyl)phenol], 2-(2 H-Benzotriazol-2-yl)− 4,6-bis(2-methyl-2-butanyl)phenol, 3-[4-Hydroxy-3,5-bis(2-methyl-2-propanyl)phenyl]-N′-{3-[4-hydroxy-3,5-bis(2-methyl-2-propanyl)phenyl]propanoyl}propanehydrazide, Octyl 3-[4-hydroxy-3,5-bis(2-methyl-2-propanyl)phenyl]propanoate, 1,3,5-Tris[4-hydroxy-3,5-bis(2-methyl-2-propanyl)benzyl]− 1,3,5-triazinane-2,4,6-trione, 1,2-Ethanediyl bis{3,3-bis[4-hydroxy-3-(2-methyl-2-propanyl)phenyl]butanoate}, (2 R)− 2,5,7,8-Tetramethyl-2-[(4 R,8 R)− 4,8,12-trimethyltridecyl]− 6-chromanol, Didodecyl 3,3′-sulfanediyldipropanoate, 4,4',4''-[(2,4,6-Trimethylbenzene-1,3,5-triyl)tris(methylene)]tris[2,6-bis(2-methyl-2-propanyl)phenol], 3-({3-[4-Hydroxy-3,5-bis(2-methyl-2-propanyl)phenyl]propanoyl}oxy)− 2,2-bis[({3-[4-hydroxy-3,5-bis(2-methyl-2-propanyl)phenyl]propanoyl}oxy)methyl]propyl 3-[4-hydroxy-3,5-bis(2-methyl-2-propanyl)phenyl]propanoate, 2,2-Bis(4-hydroxyphenyl)propane-d16, 2,4-Di-tert-butyl-6-(5-chlorobenzotriazol-2-yl) phenol and Bis(2-ethylhexyl)phthalate-3,4,5,6-d4 were purchased from Sigma Aldrich® (Buchs, Switzerland). MS-grade water (H_2O), MS-grade methanol (MeOH) and MS-grade acetone were purchased from Biosolve® (Dieuze, France). LC-grade 1,2-dichloroethane, and ammonium hydroxide (NH_4OH) 25% were purchased from Sigma Aldrich® (Buchs, Switzerland). Since this experiment is dealing with leachable compounds, liquid solvents were obtained in glass containers to avoid plastic additive contamination.

For the development of the level 1 confidence identification list, all standards were purchased from Sigma Aldrich (Sigma Aldrich® (Buchs, Switzerland). For more information, table S2 is found in the Supplementary material.

2.2. LC-MS conditions

A Thermo Scientific™ ultra-high performance liquid chromatograph Vanquish™ Horizon™ was coupled to a Orbitrap™ Q Exactive™ mass spectrometer (Thermo Scientific™, MA, USA), equipped with a heated electrospray ionization (HESI-II) source. Samples were kept at 10 °C during analyses and a volume of 10 μL was injected.

Plastic additives were separated on a Waters™ Acquity™ BEH Phenyl (100 × 2.1 mm, 1.7 μm) (Waters™, Milford, MA, USA) and the corresponding VanGuard pre-column. Flow rate and column temperature were set at 0.2 mL/min and 60 °C respectively. Solvent A (pure water) and solvent B (pure MeOH) were used as mobile phases. The gradient profile used was as follows: a linear ascend from 70% B to 85% B in 6 min, followed by an increase to 95% B in 4 min. There is a further increase to 100% B in 2 min, holding at 100% B for 4 min, before returning back at 70% B in 0.1 min and re-equilibrating the column for 9 min.

As for the HESI-II parameters, sheath gas flow rate and auxiliary gas flow rate were set at 30 and 5 arbitrary units, respectively. Capillary temperature at 275 °C and auxiliary heater temperature at 290 °C. Analytes were analysed in both polarities, positive ion spray voltage at 3 kV and negative at 2.7 kV.

An untargeted generic approach was first used, which consisted of a data-independent acquisition (DIA). It was developed for small molecular applications to understand the behaviour pattern of small molecules and their *m/z*. It started with a full scan at 70′000 resolution, with an AGC target of 10^6 and with a maximum filling time of 50 ms. A scan range between 100 and 1200 *m/z* was used. This acquisition program is followed by two other DIA steps, both with a resolution of 17′500, with an AGC target of 10^6 and with a maximum filling time of 100 ms. For second step DIA, the loop count was placed at 4, with an isolation window of 110 *m/z* and a stepped NCE of 30 and 80 to establish a good compromise for optimum fragment generation. As for the third step DIA, it is practically the same as the second step DIA, except for the loop count which was set at 1 and the isolation window at 510 *m/z*. An inclusion list was installed for both polarities with the following *m/z*: 150, 250, 350, 450, 550 and 900. No time periods needed for the inclusion list.

The acquisition program used is a parallel-reaction monitoring (PRM), at a mass resolution of 17′500, at an AGC target of 2×10^5, using a maximum filling time of the C-trap of 50 ms. A normalized collision energy was set at 10%. All chromatograms were acquired using a *m/z* tolerance of 5 ppm. An isolation window of 1 *m/z* was set without an isolation offset and multiplexing count. A mass calibration was performed once a week in poth polarities using the Pierce™ Velos ESI Ion Calibration standard mixture (Thermo Scientific™, MA, USA). For Positive ion calibration, the mix consists of *n*-butylamine, caffeine, MRFA (peptide of Met-Arg-Ala acetate salt) and Ultramark 1621 and as for negative ion calibration, it contained sodium dodecyl sulfate, sodium taurocholate and Ultramark 1621. MS Tune 2.8 (Thermo Scientific™, MA, USA) was used to control the instrument and Chromeleon™ 7.2.7 (Thermo Scientific™, MA, USA) was used to acquire data.

2.3. Post-column infusion (PCI)

A Chemyx® Fusion 100 T syringe pump (TX, USA) was used, along with a 10 mL of glass syringe (Hamilton, Nevada, USA) containing either 0, 0.5, 2 and 5% of ammonium hydroxide in methanol infused at different flow rates, i.e. 0, 0.5, 1, 2, 4 and 6 μL per minute. The solution was pumped into the MS source via a Thermo Scientific™ Vipers™ Fingertight Fitting stainless steel capillary (Thermo Scientific™, MA, USA).

2.4. Standard solutions preparation for the optimization of the post-column infusion

A stock solution, containing 30 compounds, at 100 μg/mL was first prepared by weighing 10 mg of each compound and dissolving them in 100 mL of MeOH. The stock solution was then diluted 1000x with H_2O/MeOH (1:1) to reach a concentration of 100 ng/mL which was used as the work solution.

2.5. Prefilled plastic drug packaging used for the leachable study

The prefilled drug syringe used in the experiment is a product of the centralized intravenous additive service (CIVAS) from the Pharmacy Department of the University Hospitals of Geneva (Geneva, Switzerland) pharmacy. The material composition consists of a Becton and Dickinson and Brothers® (BD) Plastipak™ 10 mL syringe plunger and barrel that are made of polypropylene; syringe plunger head is made of rubber elastomer, using silicone oil as lubricant. The solution contains a concentration of Vancomycin at 5 mg/mL, sodium chloride and water for injection (pH 5.6).

2.6. Sample preparation by UA-DLLME for the prefilled plastic drug packaging

Sample preparation was performed via an ultrasound-assisted dispersive liquid-liquid micro-extraction (UA-DLLME) method. Sample volume of 10 mL from the prefilled plastic packaging was transferred into 15 mL centrifugal glass tube. A mixture of 2 mL of acetone and 0.35 mL of 1,2-dichloroethane was then rapidly injected into the sample via a 2.5 mL Hamilton® glass syringe. A dispersion of organic solvents in the aqueous solution was obtained. Samples were then sonicated in an ultrasonic bath (Branson Ultrasonics, CT, USA) for 5 min and then centrifuged (Hettich AG, Bäch, Switzerland) for 5 min at 3′500 g. After centrifugation, the sedimented phase was collected into a small glass vial by using a 1 mL Hamilton glass syringe. A second extraction was performed by injecting this time 0.35 mL of 1,2-dichloroethane into the samples. After that, they underwent ultra-sonication followed by centrifugation before extracting again the sedimented phase, which was then transferred into the same glass vial. A third and final extraction was done again with 0.35 mL of 1,2-dichloroethane. Yet again, the sedimented phase was extracted and transferred into the same glass vial. The collected sedimented phases were evaporated by nitrogen gas and reconstituted with 0.2 mL of H_2O:MeOH (1:1) and vortexed before injecting for analysis.

2.7. Internal Standard solution preparation

A stock solution of five internal standards (4,4′-Sulfanediylbis[5-methyl-2-(2-methyl-2-propanyl)phenol], 4,4′-(1,3-Phenylenedi-2,2-propanediyl)diphenol, Bis(2-ethylhexyl)phthalate-3,4,5,6-d4, 2,4-Di-tert-butyl-6-(5-chlorobenzotriazol-2-yl) phenol and Bisphenol A-d16) at 100 μg/mL was first prepared by weighing 10 mg of each compound and dissolving them in 100 mL of MeOH. The stock solution was then diluted 1000x with H_2O/MeOH (1:1) to reach a concentration of 100 ng/mL which was used as the work solution. A blank solution was prepared by spiking a solution of 10 mL containing 0.9% of sodium chloride with 250 μL of the work solution. The sample is also spiked the same way as the blank, before undergoing sample preparation by UA-DLLME.

3. Results and discussion

3.1. Development of the postcolumn infusion (PCI) parameters

The 30 substances used for the PCI method development were chosen based on their physical-chemical properties, including log P and pKa, as well as their ability of different functional groups (esters, amides, carboxylic acids, thiols, phenyls and hydroxyls) to form adducts (H^-, H^+, $NH4^+$). They have also been selected as representatives of their chemical class based on numerous scientific articles and reviews on leachable and extractable analysis. Moreover, they were also chosen for their molecular size, ranging from low (diphenyl phosphate) to high molecular weight, such as over 1000 daltons (Irganox® 1010). Some analytes were considered for their relative stability, i.e., alpha-tocopherol, which tends to be very vulnerable to volatility and oxidative and thermal stress, to test the extent of the method [22]. All 30 compounds were combined in a mixture at 100 ng/mL to develop the optimal parameters of post-column infusion as well as the chromatographic and MS settings. The MS/MS analysis was performed first by data-independent acquisition (DIA) to establish the observed adducts of each standard before proceeding to a parallel reaction monitoring acquisition mode (PRM). This acquisition program was built using an inclusion and exclusion list based on the DIA results. Twenty-five substances were detected in negative mode as molecular ion $[M-H]^-$ and 5 substances in positive mode $[M+H]^+$ as molecular ion and/or with their ammonium adducts $[M+NH_4]^+$. All technical information of the 30 candidates are found in Table S1 as Supplementary material.

Some compounds (small antioxidants and bisphenols) do not produce an optimal MS response with current LC parameters, such as the use of mobile phase additives; thus, the sensitivity of detection is low, as shown in the literature for BHT and BHA as well as bisphenols [17,22,23]. To solve issues of detection by LC—MS, PCI was considered. In the field of extractable and leachable substances, this is an innovative setup that boosts signals of trace plastic additive molecules that usually show low sensitivity with traditional LC—MS methods. Ammonium fluoride as a PCI additive was used in other domains, such as to perform steroid profiling [24]. However, one major issue is the risk of fluoride contamination, which is the formation of fluoric acid in the source house and in the waste. The use of postcolumn infusion with ammonium hydroxide (NH_4OH) results in better ionization of small phenolic molecular structures due to a high pH content [18]. Moreover, it was more interesting to investigate the extent of PCI effects in the negative mode on similarly structured compounds, such as antioxidants and UV stabilizers, and in the positive polarity mode on ester and amide compounds, as this has never been attempted before. Hence, the idea was adopted and applied to a set of different molecular plastic additive structures with diverse functional groups in both ESI polarities. By choosing NH_4OH as a PCI additive, different concentrations (no PCI, 0.5%, 2% and 5%) as well as different flow rates (no PCI, 0.5, 1, 2, 4 and 6 μL/min) were assessed. All experiments were conducted with 6 replicates to obtain the signal average, standard deviation and relative standard deviation calculations.

Each flow rate remained constant while the concentrations of NH_4OH were varied. According to Fig. 1 and Fig. 2, the results show that the optimal NH_4OH concentration happens to be at 2% at a flow rate of 2 μL/min for most analytes in positive and negative ESI modes. In negative mode, antioxidants and bisphenols possess a very high pKa since they are weak phenolic acid structures. At 2% NH_4OH, the environment in the ion source possesses a high pH value; thus, these substances remain in their ionized state, enabling much better performant ionization [18]. At 0.5% NH_4OH, the performance on almost all standards is reduced. At 5% ammonium hydroxide, the signal intensity of most of the analytes starts to plummet. A lack of ionization efficiency is also noticed when a low flow rate, i.e., 0.5 μL/min, is applied. Moreover, flow rates below 2 μL/min showed relatively high fluctuating RSD (%) results as well as lower signal responses due to the lack of ions in the source house. Due to a dilution effect in the source, the signal response started to drop when working at high flow rates, i.e., 4 or 6 μL/min. Finally, the optimal conditions, i.e., a concentration of 2% NH_4OH at a flow rate of 2 μL/min, enabled a 2- to 100-fold enhancement in signal intensity for the tested compounds in comparison to that without PCI. For further information, two chromatograms with all 30 compounds are shown in Fig. S1 and Fig. S2 in the Supplementary materials.

3.2. Negative mode

As seen in Fig. 1. and Fig. 2., with NH_4OH as a PCI additive, the signal intensity significantly increased for phenolic-like structures, such as bisphenols and antioxidants, which exhibited poor sensitivity with traditional LC—MS parameters. This was clearly observed with butylhydroxyanisole, butylhydroxytoluene, and other bisphenol analogues, and this is explained by their poor ionisable capacity because they do not

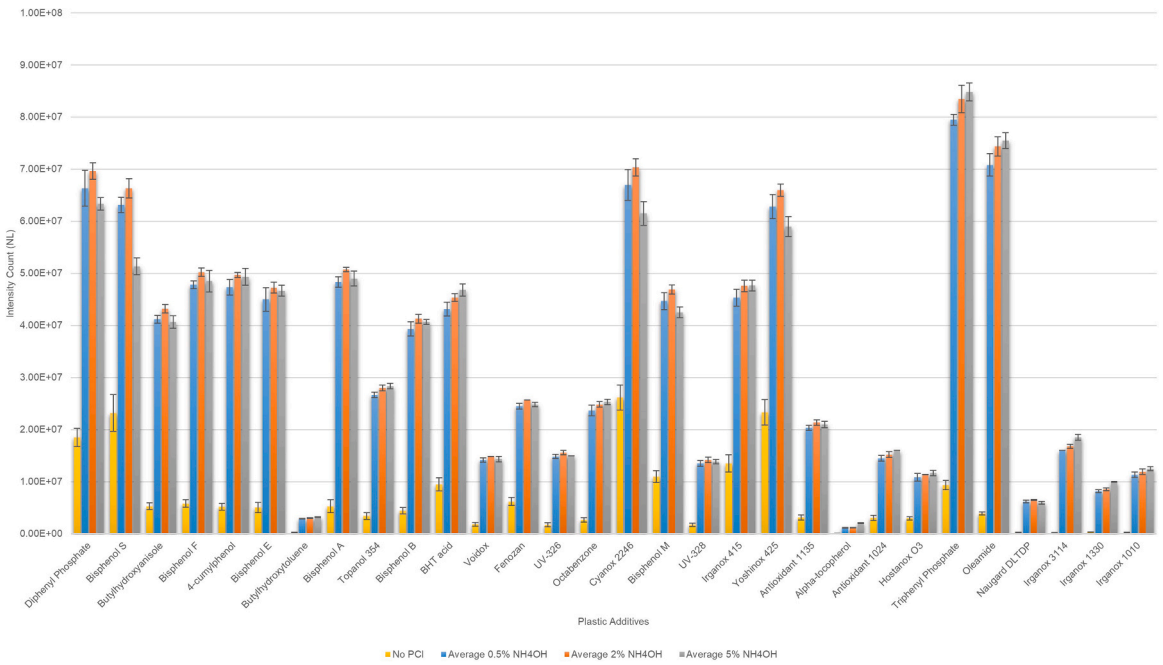

Fig. 1. Histogram describing the response in intensity counts of all 30 candidates at 100 ng/mL under different concentrations of NH4OH (no PCI, 0.5%, 2.0% and 5.0%) at a fixed flow rate of 2 μL/min.

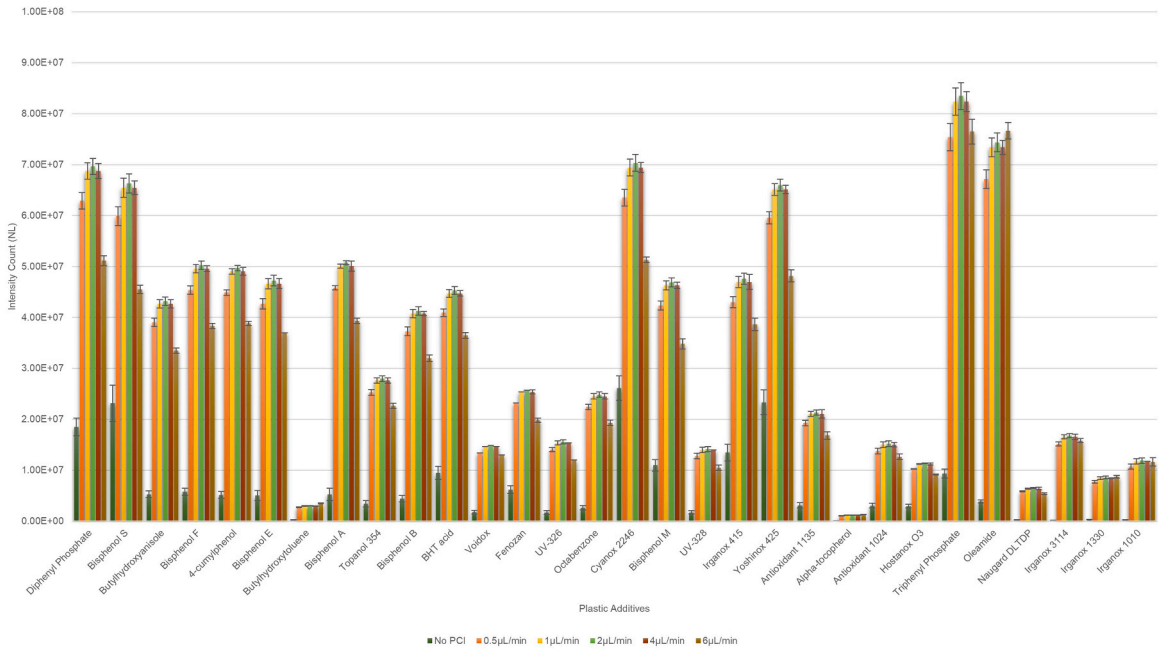

Fig. 2. Histogram describing the response in intensity counts of all 30 candidates at 100 ng/mL under different flow rates of NH4OH (no PCI, 0.5, 1, 2, 4 and 6 μL/min) at a fixed concentration of 2% NH4OH.

enter the MS as gas phase ions as per the ESI theory [18].

3.3. Positive mode

Positive ionization was also performed. According to the results, it has been shown that using high-pH modifiers in mobile phases or in postcolumns enables at least a twofold enhancement in the signal-to-noise ratio. Molecules bearing ester, amide, thiol and phosphate functional groups were detectable with this PCI additive. This was observed with oleamide, triphenyl phosphate, Naugard® DLTDP, Irganox® 3114, 1330 and 1010. By using NH_4OH, analytes could be detected with proton and/or ammonium adducts, which is favourable to their MS2 fragmentation. This could be explained by the dissociation of the ammonium ion (NH_4^+) into ammonia gas (NH_3) and protons (H^+) in positive mode. Small molecules possessing a single ester group tend to form $[M+H]^+$ adducts more easily than ammonium adducts $[M+NH_4]^+$. However, in some cases, the opposite is observed when molecules possess many ester bonds, such as Irganox® 1010, produce a higher signal response for adduct $[M+NH_4]^+$. Phosphate and amide groups form proton adducts and never ammonium adducts because the latter causes steric hindrance with the oxygen of each respective group. The major advantage of using PCI is the absence of a sodium adduct, which

could negatively affect the MS2 fragmentation of many molecules. With the help of PCI, the lower limit of detection (LLOD) was either comparable or even more sensitive in most cases than that of traditional LC—MS methods.

3.4. Method selectivity

To obtain a clear profile for any plastic additive residuals, blanks of the diluent as well as that of the sample preparation solvents were considered. If any contamination is present, these blanks could help by indicating the source of contamination. The signal response that was observed in the blank was that of 2,4-di-tert-butylphenol, which is a monomer of Irgafos® 168, but it was very low in signal intensity (near-baseline) and therefore not considered. However, it could become a serious issue depending on the batch of solvents used. Indeed, in industries, organic solvents are purified via a series of distillation steps and undergo ultrafiltration through a 0.1–0.2 μm polypropylene filter. The solvent in contact with the filter could cause the breakdown of Irgafos® 168 into 2,4-di-tert-butylphenols [17,25]. Therefore, it is recommended to perform solvent profiling before starting extractable and leachable experiments. Moreover, oleamide could sometimes be a primary source of contamination. The polypropylene filters used in industries contain oleamide on their surface, which acts as a lubricant to ease the transition flow, but it could also cause oleamide to enter the organic solvent [25]. However, in our case, the oleamide signal response caused little to no interference. These assays were performed with clean glassware rather than plastic materials due to the possible presence of plastic additive contaminations, such as 2,4-di-tert-butylphenol, oleamide and antioxidant 425, which could be detected in plasticware. The only plasticware used was micropipette tips, and the impact on contamination was determined to be negligible.

Molecules that cause difficulties in extractable and leachable experimental analysis, namely, Irgafos® 168, Irganox® 1076, erucamide, and myristic acid, were observed as products of the LC system or from the mobile phase solvent inlet tubes, silicone tubing in the pump module and PEEK capillaries. The latter were changed to stainless steel capillaries to prevent contamination, and after replacement of the tubing, less oleamide, Irganox® 1076 and erucamide contamination was observed, leading to a cleaner baseline.

3.5. Choice of column

The choice of column was based on experiments that were conducted on a series of reversed-based stationary phase chemistry (C18, C8 and phenyl-hexyl). Estimating the presence of very lipophilic compounds such as oleamide, Naugard® DLTDP and Irganox® 1330, 3114 and 1010, the molecular affinity between the C18 column and these compounds is much stronger than the affinity between the mobile phase and the compounds. These could affect the chromatographic performance with peak tailing, ghost peaks and cross contaminations. Most researchers in the extractable and leachable analytical field have achieved analysis with C18 stationary phases [17]. Through C8 chemistry, less interaction occurred with the compounds. However, it did not solve the peak specificity issues and was not entirely resolute. Most extractable and leachable compounds are aromatic by nature, and the phenyl-hexyl column exhibited optimal results with an increased resolution for phenolic molecular structures via π-π interactions, allowing important isomers to be baseline resolved. Therefore, the phenyl-hexyl stationary phase was selected.

3.6. Relative standard deviation, limit of detection, domain linearity and matrix effect

The limit of detection (LOD) as well as the response function towards the analytical range were tested by dividing the maximum concentration by half each time, from a concentration of 250 ng/mL to a concentration of 0.03 ng/mL, to obtain a generic LOD. The LOD for all 30 analytes ranged from 0.03 to 7.81 ng/mL with good linearities between 0.9991 and 0.9999. All compounds showed a variable and wide concentration (limit of quantification – LOQ) range between 0.09 and 250 ng/mL. The compound with the lowest LOD is triphenyl phosphate with a concentration of 0.03 ng/mL, and the compound with the highest LOD is alpha-tocopherol at 7.81 ng/mL, probably due to its fragile structure. After these results were observed, it was confirmed that PCI drastically increased the sensitivity of these compounds, with a signal enhancement between 2- to 100-fold. All 30 candidate compounds with different molecular sizes, logP, pKa and functional groups were detected in under 16 min of LC run time, with a good peak resolution. The variability of the method was assessed by the relative standard deviation (RSD) of all 30 analytes. After performing 6 injections on 6 different preparations, RSD values lower than 10% were obtained.

3.7. Development of the plastic additive internal database

To obtain the highest confidence level for identification, an innovative analytic- and toxicology-based database containing 205 additives was created as a generic means to screen diverse categories of plastic additives, such as plasticizers, antioxidants, UV stabilizers, bisphenols, bisphenol-based epoxy derivatives, lubricants and even non-intentionally added substances (NIASs), such as oligomers and degradation of additives. All standard additives were analysed, and retention properties, exact mass and isotopic pattern, their MS/MS fragmentation spectra and their domain of linearity were acquired for the database. This database should facilitate plastic additive screening in both ESI polarities, i.e., positive and negative for prefilled drug products compounded in hospital pharmacies. It was also built to help in the semi-quantitation of additives in extractable and leachable research in diverse public establishments and was designed using sources of information [19–21]. The list of additives was built using multiple extractable and leachable (E/L) reports, several external E/L and toxicology databases, diverse articles and reviews and different sources of toxicology background from authorities. Some nonofficial endocrine disrupting compounds (EDCs) were also integrated due to their sound experimental evidence [26]. The database is organized through the diverse characteristics of compounds, which are used to identify compounds internally and in samples (laboratory product code, IUPAC, compound name, CAS N°, European N°, SMILES, InChI and InChIKey), physico-chemical properties (logP, pKa, solubility in H_2O (mg/mL)), plastic additive role and application (polymer category), linear toxicology (NOAEL, LOAEL, LD_{50}, Uncertainty Factor T_{1-4}, carcinogenicity alerts In-silico A-B-*In-Vitro*, permissible dose exposure, Cramer), standard compound provider reference (catalogue number, batch N°, provider name), nonlinear toxicology (EDC name, category, system-level perturbations, European EDC list category, EDC reference) and last but not least, analytical data of compound properties. Fig. 3 describes the database organization as well as the suborganisation for potential endocrine disruptors. All 205 compounds are found in table S2, containing analytical information such as chromatographic and mass spectrometric data, that is found as supplementary information.

In the database, chemical classes are organized into the following different plastic additive categories: plasticizers, bisphenols, antioxidants, UV stabilizers and miscellaneous (containing some monomers and oligomers, antimicrobials, epoxy residues, rubber cross-linkers and slip agents/lubricants). This list is comprised of additives, monomers, oligomers, their degradation compounds and some complex molecules. It was built to ease the transition into the screening for these compounds in hospital pharmacies. For example, it was observed that 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propanoic acid or fenozan acid appeared in an aqueous solution contained in coextruded polypropylene IV bags (CEPP-IV) after heat sterilization [27]. It can be assumed that Irganox® 1010 was present in CEPP-IV bags because fenozan acid is among four monomers of Irganox® 1010. There are other complex antioxidants that

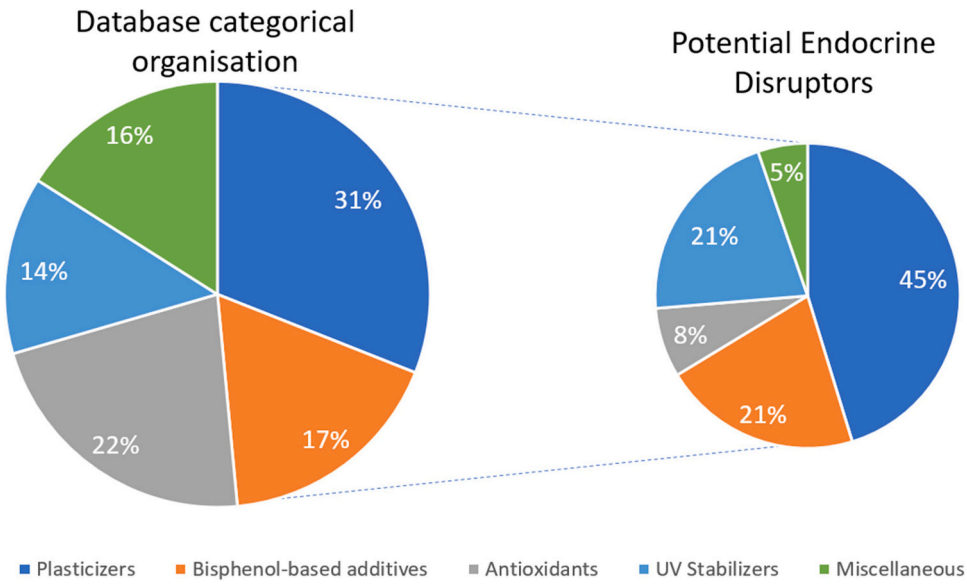

Fig. 3. Pie chart describing the categorical organisation in the database as well as a detailed pie chart on potential endocrine disruptors.

contain fenozan acid, such as Irganox® 259, Irganox® 1076, Antioxidant 1135, Irganox® 3125 and many more. In another example is the identification of 2,4-di-tert-butylphenol, which is a small phenolic molecule found in most plastic packaging. If such a molecule is identified, this could provide information that a bigger and more complex antioxidant could be present in the plastic packaging, such as in a polypropylene syringe. This compound is part of a complex phosphite antioxidant, which could be Irgafos® 168 in this case. Degradation of complex structures could lead to many byproducts. Thanks to this database, how these byproducts were generated could be clarified. Statistics were also performed on many parts of the database, linking toxicology (linear and nonlinear) to the additives, retention time to mass (m/z), and log P to toxicology. One disadvantage is that identification of compounds with a level 1 confidence is limited to 200 compounds with this list. Medical polymers are known to possess top quality with minimal release of additives. The identification of molecules was obtained due to the matching of properties with analytical information (e.g., chromatographic retention time, exact mass and isotopic pattern recognition, MS/MS spectra). The MS2 spectrum of a molecule often exhibited a specific signature, and often one or more fragment(s) that is unique to any given functional group was obtained. For example, adipates provides a signature fragment of 129.054 m/z, citrate 157.013 m/z, phosphate 98.098 m/z, azelate 153.091 m/z, phthalate 149.023 m/z, and bisphenol 93.034 m/z. This approach solves issues for isobaric structural distinction by searching for the unique fragment ion to identify the functional group of that molecule. However, dealing with isomers is another issue. As the list of molecules increases, so does the number of isomers. Therefore, one of the best approaches would be to look for that unique signature fragment ion and check for its abundance.

For example, all the phthalate isomers (phthalate, isophthalate and terephthalate) have the same retention time, but for phthalates, the 149.023 m/z ion is near 100%; isophthalates have an abundance that is approximately 50%; and terephthalates are always low in abundance at approximately 10%. Another example is the differentiation of the isomers of bisphenol F (2,4′-bisphenol F, 2,2′-bisphenol F and 4,4′-bisphenol F), which all of them possess similar retention times. However, the compounds are still distinguishable by MS. As mentioned previously, the best approach would be to look for a unique signature fragment; however, just looking for the signature fragment ion (93.033 m/z) can sometimes be insufficient, and it would be best to search for the abundance in precursor ions (199.075 m/z). 2,4′-bisphenol F has an 80% abundance of 93.033 m/z and a 100% abundance of precursor ions at 199.075 m/z, 2,2′-bisphenol F has 100% abundance of the first fragment and 10% abundance of the second fragment, and 4,4′-bisphenol F has 50% abundance for the first fragment and 100% abundance for the second fragment. The last example is with butylhydroxyanisole, which consists of 2-tert-butyl-4-methoxyphenol and 3-tert-butyl-4-methoxyphenol in equal proportions. The differentiation is obtained due to the signature fragment 164.083 m/z. The 2-tert-butyl-4-methoxyphenol possesses the fragment, and its counterpart does not. Hence, it is important to approach isomeric distinction carefully by looking into the fragment pattern elucidation thoroughly and by comparing the unique fragment and precursor ion patterns with the database. Nevertheless, chromatographic separation remains most recommended in most cases when MS2 spectral identification becomes too complex.

An estimative and semiquantitative approach was elaborated due to a relative response factor (RRF) bank for all 200 additives. The RRF was established via five internal standards (IS), one for each main category (Bis(2-ethylhexyl)phthalate-3,4,5,6-d4 for the plasticizer group, 4,4′-(1,3-Phenylenedi-2,2-propanediyl)diphenol for the bisphenol group, 4,4′-Sulfanediylbis[5-methyl-2-(2-methyl-2-propanyl)phenol] for the antioxidant group, 2,4-Di-tert-butyl-6-(5-chlorobenzotriazol-2-yl) phenol for the UV absorber and stabilizer group and Bisphenol A-d16 for the miscellaneous group). They were selected for their structural similarity to their corresponding groups. A RRF dynamic value range criteria was set up; all values that are between 0.5 and 2 are viable for semiquantitation, and those that are lower than 0.5 and higher than 2 would result in a suboptimal semiquantitation [20].

This semiquantitative approach was selected to simplify the screening procedure for leachable studies in the prefilled drug products prepared in hospital pharmacy. Since these drug products are not meant for the open market but for hospitals with specific medical demands, they are not subjected to legal requirements. That is why a semiquantitative system was built to monitor directly leachable compounds in hospital pharmacy-prepared prefilled drugs based on the 200 most common leachable compounds. This approach was adopted from the screening semiquantitative system done during Extractable and Leachable (E&L) studies. In current E&L semiquantitative screening procedure, E&L compounds are given toxicological meaning via analytical evaluation threshold (AET). Compounds that are found above this value are selected for further toxicology assessment. The safety concern threshold (SCT) is also needed in the equation, which is the threshold below which a leachable compound would present such a low dose that all safety concerns are negligible from carcinogenic and non-

carcinogenic toxic effects. If an AET is needed to be calculated for Vancomycin PFS, some technical information is required on the PFS such as the volume per unit, the number of doses administered daily or maximum daily dose (MDD) and the SCT (a constant determined via the means of administration). This PFS has a total volume of 10 mL per unit, perfused around four times a day which results in a MDD total of 40 mL per day. The SCT constant selected is at 1.5 μg per day because of its parenteral use over a considerable period of time. Two formulas could be used to express the AET, one in μg per unit and the other in μg per mL. When the AET is finally obtained, it requires an analytical correction called uncertainty factor which is a 50% reduction of the current AET value. This is applied to account for the RF variation in the screening process. The present formula below are based on Singh et al. as follows [28]:

$$\mathbf{AET}\ \left[\frac{\mu g}{unit}\right] = \frac{\mathbf{SCT}\ \left[\frac{\mu g}{day}\right] \times \frac{Doses}{Unit}}{\mathbf{Maximum\ Daily\ Dose}\ \left[\frac{Doses}{day}\right]}\ \mathbf{AET}\ \left[\frac{\mu g}{mL}\right] = \frac{\mathbf{SCT}\ \left[\frac{\mu g}{day}\right]}{\mathbf{Maximum\ Daily\ Volume}\ \left[\frac{mL}{day}\right]}$$

$$\mathbf{AET}\ \left[\frac{\mu g}{unit}\right] = \frac{1.5\ \frac{\mu g}{day} \times 10\ \frac{mL}{unit}}{40\ \frac{mL}{day}} = 0.375\ \frac{\mu g}{unit}\ \mathbf{AET}\ \left[\frac{\mu g}{mL}\right] = \frac{1.5\ \frac{\mu g}{day}}{40\ \frac{mL}{day}} = 0.0375\ \frac{\mu g}{mL}$$

$$\mathbf{Final\ AET} = \mathbf{AET} \times \mathrm{UF}\ (50\%) = \frac{0.375\ \frac{\mu g}{unit}}{2} = 0.188\ \frac{\mu g}{unit}\ \left(\mathbf{or}\ 0.01875\ \frac{\mu g}{mL}\right)$$

It is important that the AET or the final AET is not found below the LOD/LOQ of the current analytical method (LOD = 0.03–7.81 ng/mL). An AET below the LOD/LOQ would not enable the user to identify compounds and even evaluate them for further toxicology assessment.

To evaluate the risk toxicology of all compounds in the database, the permissible daily exposure (PDE), risk index (RI) or acceptable dose intake (ADI), which is human weight and compound specific, can be estimated. All non observed adverse effect levels (NOAELs) were obtained by using a non in vitro and in vivo-based approach, such as Layton's approach. The lethal dose 50 (LD50) was obtained from *in silico* calculations performed on the EPA T.E.S.T platform via consensus mode, which is the average of many *in silico* results [29]. Other approaches, such as those by Hall, Conine, LeBlanc, and Kramer, were also assessed [30]. However, Layton's approach seemed to be easier and straightforward to use to estimate the PDE when compounds have little to no experimental toxicological data available, which is the case for many of them in the database. One of the pitfalls to the nonanimal approach is that compared to in vitro and in vivo-based approaches, NOAEL and LD50 are over- or underestimated. The Layton's approach was selected nonetheless as the toxicology part of the database, despite being oversimplified. The NOAEL is estimated by dividing the LD_{50} (oral-rat) by an empirical constant of 2000 [30]. The result often gives a lower NOAEL value than the experimental value as well as the PDE value. The calculation of the PDE is adapted from Jenke et al. as follows [8]:

$$\mathbf{PDE} = \frac{\mathbf{Estimated\ NOAEL}}{\mathbf{T1\ X\ T2\ X\ T3\ X\ T4}}\ \mathbf{X\ Body\ Weight}$$

PDE is obtained by using the estimated NOAEL, multiplying it by the weight of the relevant human (for example, 2 kg was selected for neonates, 12 kg for children and 70 kg for adults) and dividing the final result by the uncertainty factors T_{1-4}, which after performing a serial multiplication of the factors give us a value of 10′000 (10×10×10 ×10) (accounting for interspecies variation, interindividual variation, from acute to chronic exposure, and from oral to IV administration).

For the nonlinear toxicology part of the database, it was simplified to provide answers on whether a compound is a potential EDC and its source of explanation [26]. The main drawback is the absence of harmonization with these databases. This database can tell the user whether a compound is a potential endocrine disruptor but does not give a 100% assurance that it is a disruptor.

For all information concerning database organization, *m/z* distribution in the database as well as chromatogram compilation of all diverse additives and physical-chemical and toxicology information, Figs. S3-S7 and Table S2 are available as Supplementary materials.

3.8. Leachable experiment on prefilled syringes

Prefilled syringes (PFS) could be important in any hospital setting, although not all hospitals compound PFS. They are prepared in batches to be stored in stable conditions for a defined period. They improve patient safety by preventing drug and concentration errors [4,5]. PFS often contains a drug concentration in normal saline (NaCl). Since it is produced in advance and usually stored for a considerable time (i.e., 3–12 months), there is always a potential for plastic syringes to leach additives over time into the solution. The ready-to-use injectable vancomycin IV was selected as a candidate with the following rationale: paediatric use, antibiotic drug and frequent drug administration for a considerable amount of time (depending on the medical situation) [31, 32]. Once manufactured, this PFS is stored for 6 months in refrigerated areas between 2 and 8 °C. It is administered to preterm infants, neonates and children intravenously. This drug targets gram-positive bacteria, such as coagulase-negative Staphylococci (CoNS), methicillin-resistant Staphylococcus aureus (MRSA), and Enterococci species. According to SwissPedDose, for neonates (<44 weeks), it is generalized twice daily, and for children (>44 weeks to 18 years old), it is generalized four times daily. The duration of treatment can depend on the severity of the bacterial infection, sometimes ranging from a week to a month [32]. It is important to focus on preterm, neonate- and child-oriented medication because they are in a rapidly growing phase, which makes their metabolism ultrasensitive to any molecules, including plastic additives and even endocrine disruptors [33]. Syringes, such as Plastipak from BD®, have already undergone thorough analysis of extraction and time-based leachable studies to determine the potential release of plastic additives. However, since these prefilled drug products have been prepared in a hospital pharmacy setting, it would be interesting to perform a leachable experiment for traces of polymer-related compounds and further evaluate their toxicology. With this analytical method at ultrahigh sensitivity and the developed plastic additive database, screening and estimation and semiquantitation of additives could be emphasized. The experiment was performed on three vancomycin PFS at 5 mg/mL (3 months after production, stored in a refrigerated area between 2 and 8 °C). By using UA-DLLME, the sample was preconcentrated approximately 50-fold. As a result, a total of 17 plastic additives were identified in the screening of vancomycin 5 mg/mL PFS (Table S3).

All 17 compounds were identified with the following tolerance: ΔRT % (criteria <2%), criteria mass error < 5 ppm and MS/MS spectra comparison. All 17 compounds were identified with level 1 confidence. Myristic acid was identified in the chromatogram of BPA. Its presence is due to LC system contamination, deriving from the silicone and peek tubing in the instrument. It is observed in the chromatogram of BPA because both molecules possess nearly the same *m/z* (227.20165 for myristic acid vs. 227.10775 for BPA), and the isolation window of the Orbitrap quadrupole was set at 1 *m/z*.

To illustrate the identification process, di-ethylhexyl adipate (DEHA) was taken as an example. The retention time of the analyte found in the sample was obtained to the reference standard, with a difference in percentage of 0.58% (<2%). Their exact masses were also compared and interpreted with a mass error of 0.84 ppm (<5 ppm). The spectra of both analytes were also cross-referenced, and their MS2 fragment patterns are identical. Its signature fragment is 129.054 *m/z*, which is unique for adipates. This concludes the level 1 confidence identification, which

confirms that the analyte found in the vancomycin PFS is DEHA. To calculate the concentration of DEHA via semiquantitation, the RRF of DEHA is used (0.741), along with the area under the curve (AUC) of DEHA, as well as the response factor of the internal standard, which, for the plasticizer category, is DEHP-d_4 [20]. The sample preparation by UA-DLLME gave out a recovery extraction for DEHA at 85.1%. However, since the aim of semiquantitative approach is to estimate the concentration of plastic additives, all potentially identified compounds would feature a 100% recovery extraction yield. The average concentration via semiquantitation was 15.1 ± 0.3 ng/mL (RSD: 2.01%). Chromatogram results as well as MS2 spectra are found in Figs. S8-S11 as Supplementary materials.

In this PFS, a variety of additives from different categories are found. There are plasticizers, which are to be expected since polymers, such as polypropylene, possess a series of different plasticizers to obtain optimal material flexibility to serve their purpose. Although phthalates are lipophilic in nature, they form a weak interaction within the polymer strand, which causes them to be released in an aqueous environment. This process tends to work best when different plasticizers, such as di-ethylhexyl adipate, acetyl tributyl citrate, and di-ethylhexyl sebacate, are combined to assure optimal purposes. A lack of plasticizers cause the material to rigidify and break easily as a result whereas an excess of plasticizers could lead to over flexibility, which could lead to deformity of the material, causing it to lose its purpose. Furthermore, the presence of antioxidants and UV stabilizers is also highlighted. The role of antioxidants and UV stabilizers is to protect the material against the formation of peroxide and free radicals that are caused by oxidation and/or photooxidation, which could destroy the strand of the polymer, leading to breakdown of the structural integrity [14]. These additives are needed in synergy to protect the polymer material from photooxidative breakdown. Most of the time, they protect the polymer by undergoing oxidation. Examples of leachable antioxidants, UV stabilizers and their degradants in packaging are 2,6-di-tert-butyl-p-cresol (BHT), 3, 5-di-tert-butyl-4-hydroxybenzaldehyde (BHT-COH), 2,6-di-tert-butyl-4-hydroxy-4-methylcyclohexa-2,5-dien-1-one (BHT-OH), 3-(3, 5-di-tert-butyl-4-hydroxyphenyl)propanoic acid (Fenozan), 3-(3–5-di-tert-butyl-1-hydroxy-4-oxo-2,5-cyclohexadiene-1-yl)propanoic acid, Irganox® 1010 and benzotriazole. Fenozan is a common antioxidant degradant found in most polymer materials, especially in polypropylene materials, such as syringes and coextruded IV bags after heat sterilization [27]. This prefilled drug product is not heat sterilized, which explains its low concentration of fenozan. Moreover, N-butylformamide, a rubber cross-linker degradant, was found to be a leachable compound, probably coming from the rubber plunger that was in contact with the aqueous drug solution. Rubber cross-linkers are molecules that give rubber elastic properties through a process called vulcanization, hence their importance in a syringe plunger head. Moreover, N-vinyl caprolactam was identified in very low concentrations in aqueous solution. This molecule could be derived from ink, adhesive, paper coating of the label or from industrial in-process contamination [14].

Most of the compounds found above are non-intentionally added substances (NIAS). In other words, these compounds are not meant to be added to the plastic material. These include degraded forms of plastic additives and industrial contamination. Other NIAS-like flame retardants were identified (tris(2-chloro-1-methylethyl) phosphate, tributyl phosphate and triphenyl phosphate) and traces of epoxide materials, such bisphenol A diglycidyl ether (BADGE) derivatives and bisphenol A. Flame retardants are normally found in plastic materials that are used for electrical devices and cables to prevent the start of a fire or slow its growth. BADGE is an epoxide used as a starting material for coatings of diverse packaging (food contact and pharmaceutical) [14]. Bisphenol A (2,3-dihydroxypropyl) glycidyl ether is issued when one of the ether cycles opens up. As observed, NIAS contamination could be due to industrial in-process contamination, which may appear with intra- and interbatch variations. This is observed for BPA, which is present in vancomycin PFS at varying concentrations, causing its RSD to skyrocket at 17.8%, according to Table S3 found in the Supplementary materials.

To perform a toxicological risk assessment of all 17 additives, the PDE can be calculated for each observed plastic additive. As a reminder, PDE is compound- and bodyweight specific, and with Layton's approach, three weight categories were selected. Regarding regular toxicology values, none of the observed plastic additives has far surpassed their respective PDE, which makes this PFS safe to use. Moreover, the possibility of a cocktail effect caused by the combination of additives is difficult to evaluate. From an EDC point of view, there are approximately 0.22 ± 0.01 ng/mL BPA in the vancomycin CIVAS in comparison to the safety threshold limit for BPA that the European Commission has fixed at 0.4–12 ng/kg bw/day for long-term exposure (>1 month) [34]. The same is true with plasticizers, phthalates in general. According to the European Commission Scientific Committee on Health, Environmental and Emerging Risk Guidelines on Phthalates, medical devices containing potential endocrine-disruptor phthalates or potentially of CMR nature above 0.1% (w/w) should be justified [35]. In other words, phthalates identified in this packaging have not surpassed this threshold. To summarize, our results suggested that the vancomycin solution, which was packaged in a polypropylene syringe, was viable for long-term storage as a prefilled drug product intended by the hospital pharmacy, meaning that it is safe for neonates and children. However, more studies are needed to complete the profile of plastic additives as well as their toxicology in prefilled drug syringes, including volatile and semivolatile compounds such as silicone-based oligomers and other rubber-related compounds, by GC—MS as well as trace analysis by ICP—MS.

4. Conclusion and perspective

An original UHPLC-HRMS method with postcolumn infusion (PCI) was developed with 30 representative candidate compounds. These substances were selected to represent different categories of plastic additives due to the presence of specific functional groups such as phenol, ester, amide, thiol and amine. The optimal PCI was established with 2% NH_4OH in methanol at a flow rate of 2 μL/min, enabling us to obtain LODs between 0.03 and 7.81 ng/mL for all 30 analytes in both negative and positive modes. To screen plastic additives within plastic packaging in hospital pharmacies, an internal database was considered. The latter was built using extractable and leachable reports, articles and reviews, as well as toxicology information sourced from external databases. All toxicology data, including LD50, NOAEL and PDE, were obtained via calculations, and the concentrations obtained from the analyses were compared to evaluate their toxicology. Hence, this approach could be considered a generic and near-universal method to screen different categories of plastic additives, including plasticizers, bisphenol derivatives, antioxidants, UV stabilizers, antimicrobials, rubber cross-linkers, nonsilicone-based lubricants, acrylate adhesives and oligomers.

Furthermore, leachable compounds in a hospital pharmacy-prepared prefilled drug syringe containing a solution of vancomycin were monitored. As a result, 17 plastic additives were identified by using different identification criteria, such as retention time, exact mass, and MS2 spectra. These compounds are plasticizers, antioxidants and UV stabilizers. Some NIAS were also detected. A risk assessment was then performed by comparing the concentration of the identified compounds to their PDE threshold. According to the results, the concentrations of the 17 compounds were at safe levels since none surpassed their PDE threshold. Despite being at low concentrations, some are potential endocrine disruptors, which could be consequential at very low concentrations, especially when administered frequently to neonates and children. According to the EDC guidelines, the concentration of BPA was below its threshold limit.

In general, prefilled drug products generated in batches by hospital pharmacies are increasingly being used because the approach is

practical and decreases dosage errors. The use of plastic packaging to house frequently administered drug products, such as antibiotics, is a practical approach. To determine drug product safety in the hospital pharmacy, LC—MS-based analysis together with an in-house database could help to estimate their content trace and potential human toxicity.

CRediT authorship contribution statement

William Bello: Conceptualization, Investigation, Methodology, Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing. **Julian Pezzatti**: Conceptualization, Methodology, Supervision, Writing – review & editing. **Serge Rudaz**: Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration. **Farshid Sadeghipour**: Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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None.

Novelty statements

An innovative and highly sensitive postcolumn infusion UHPLC-HRMS method was developed to screen plastic additives in hospital-prepared prefilled drug products. This method was used to generate an in-house database, containing 205 compounds, to enable the unambiguous identification and semiquantitation of plastic additives in leachable studies in hospital pharmaceutical domain.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jpba.2023.115640.

References

[1] E.J. North, R.U. Halden, Plastics and environmental health: the road ahead, Rev. Environ. Health 28 (1) (2013) 1–8, https://doi.org/10.1515/reveh-2012-0030.
[2] L. Fishbein, Additives in synthetic polymers: an overview, Prog. Clin. Biol. Res 141 (1984) 19–42.
[3] L. Czuba, Application of plastics in medical devices and equipment, Handb. Polym. Appl. Med. Med. Devices (2014) 9–19, https://doi.org/10.1016/B978-0-323-22805-3.00002-5.
[4] S. Makwana, B. Basu, Y. Makasana, A. Dharamsi, Prefilled syringes: an innovation in parenteral packaging, Int J. Pharm. Invest. 1 (4) (2011) 200–206, https://doi.org/10.4103/2230-973X.93004.
[5] G. Sacha, J.A. Rogers, R.L. Miller, Pre-filled syringes: a review of the history, manufacturing and challenges, Pharm. Dev. Technol. 20 (1) (2015) 1–11, https://doi.org/10.3109/10837450.2014.982825.
[6] D. Jenke, Extractable/leachable substances from plastic materials used as pharmaceutical product containers/devices, PDA J. Pharm. Sci. Technol. 56 (6) (2002) 332–371.
[7] T.H. Broschard, S. Glowienke, U.S. Bruen, L.M. Nagao, A. Teasdale, C.L. Stults, K. L. Li, L.A. Iciek, G. Erexson, E.A. Martin, D.J. Ball, Assessing safety of extractables from materials and leachables in pharmaceuticals and biologics - current challenges and approaches, Regul. Toxicol. Pharm. 81 (2016) 201–211, https://doi.org/10.1016/j.yrtph.2016.08.011.
[8] D. Jenke, T. Carlson, A compilation of safety impact information for extractables associated with materials used in pharmaceutical packaging, delivery, administration, and manufacturing systems, PDA J. Pharm. Sci. Technol. 68 (5) (2014) 407–455, https://doi.org/10.5731/pdajpst.2014.00995.
[9] United States Pharmacopeia (2022). General Chapter, ⟨1663⟩ Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems. USP-NF. Rockville, MD: United States Pharmacopeia.
[10] United States Pharmacopeia (2022). General Chapter, ⟨1664⟩ Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems. USP-NF. Rockville, MD: United States Pharmacopeia.
[11] ISO ISO 10993–18:2020 Biological evaluation of medical devices - Part 18: Chemical characterization of medical device materials within a risk management process; International Organization for Standardization (ISO): Geneva, Switzerland, 2020.
[12] D. Paskiet, D. Jenke, D. Ball, C. Houston, D.L. Norwood, I. Markovic, The product quality research institute (PQRI) leachables and extractables working group initiatives for parenteral and ophthalmic drug product (PODP), PDA J. Pharm. Sci. Technol. 67 (5) (2013) 430–447, https://doi.org/10.5731/pdajpst.2013.00936.
[13] L.M. Iribarne-Durán, F. Artacho-Cordón, M. Peña-Caballero, J.M. Molina-Molina, I. Jiménez-Díaz, F. Vela-Soria, L. Serrano, J.A. Hurtado, M.F. Fernández, C. Freire, N. Olea, Presence of Bisphenol A and Parabens in a neonatal intensive care unit: an exploratory study of potential sources of exposure, Environ. Health Perspect. 127 (11) (2019), 117004, https://doi.org/10.1289/EHP5564.
[14] J.N. Hahladakis, C.A. Velis, R. Weber, E. Iacovidou, P. Purnell, An overview of chemical additives present in plastics: migration, release, fate and environmental impact during their use, disposal and recycling, J. Hazard Mater. 344 (2018) 179–199, https://doi.org/10.1016/j.jhazmat.2017.10.014.
[15] F. Lagarde, C. Beausoleil, S.M. Belcher, L.P. Belzunces, C. Emond, M. Guerbet, C. Rousselle, Non-monotonic dose-response relationships and endocrine disruptors: a qualitative method of assessment, Environ. Health 14 (2015) 13, https://doi.org/10.1186/1476-069X-14-13.
[16] EFSA Scientific Committee, S. More, D. Benford, S. Hougaard Bennekou, V. Bampidis, C. Bragard, T. Halldorsson, A. Hernandez-Jerez, K. Koutsoumanis, C. Lambré, K. Machera, E. Mullins, S.S. Nielsen, J. Schlatter, D. Schrenk, D. Turck, J. Tarazona, M. Younes, Opinion on the impact of non-monotonic dose responses on EFSA's human health risk assessments, EFSA J. 19 (10) (2021), e06877, https://doi.org/10.2903/j.efsa.2021.6877.
[17] C. Moreta, M.T. Tena, Determination of plastic additives in packaging by liquid chromatography coupled to high resolution mass spectrometry, J. Chromatogr. A 1414 (2015) 77–87, https://doi.org/10.1016/j.chroma.2015.08.030.
[18] D. Tan, J. Jin, L. Wang, X. Zhao, C. Guo, X. Sun, Dhanjai, X. Lu, J. Chen, Ammonium hydroxide enhancing electrospray response and boosting sensitivity of bisphenol A and its analogs, Talanta 182 (2018) 590–594, https://doi.org/10.1016/j.talanta.2018.02.033.
[19] P. Christiaens, J.M. Beusen, P. Verlinde, J. Baeten, D. Jenke, Identifying and mitigating errors in screening for organic extractables and leachables: Part 1-introduction to errors in chromatographic screening for organic extractables and leachables and discussion of the errors of omission, PDA J. Pharm. Sci. Technol. 74 (1) (2020) 90–107, https://doi.org/10.5731/pdajpst.2018.009761.
[20] P. Christiaens, J.M. Beusen, P. Verlinde, J. Baeten, D. Jenke, Identifying and mitigating errors in screening for organic extractables and leachables: Part 2-errors of inexact identification and inaccurate quantitation, PDA J. Pharm. Sci. Technol. 74 (1) (2020) 108–133, https://doi.org/10.5731/pdajpst.2018.009779.
[21] D. Jenke, Identifying and mitigating errors in screening for organic extractables and leachables: Part 3-considering errors of implementation and the use of a database to judge and promote good science and efficient practices, PDA J. Pharm. Sci. Technol. 74 (1) (2020) 134–146, https://doi.org/10.5731/pdajpst.2018.009787.
[22] C.M. Sabliov, C. Fronczek, C.E. Astete, M. Khachaturyan, L. Khachatryan, C. Leonardi, Effects of temperature and UV light on degradation of α-Tocopherol in free and dissolved form, J. Am. Oil Chem. Soc. 86 (2009) 895, https://doi.org/10.1007/s11746-009-1411-6.
[23] P.P. Hao, Determination of bisphenol A in barreled drinking water by a SPE-LC-MS method, J. Environ. Sci. Health A Tox Hazard Subst. Environ. Eng. 55 (6) (2020) 697–703, https://doi.org/10.1080/10934529.2020.1732764.
[24] L. Schiffer, F. Shaheen, L.C. Gilligan, K.H. Storbeck, J.M. Hawley, B.G. Keevil, W. Arlt, A.E. Taylor, Multi-steroid profiling by UHPLC-MS/MS with post-column infusion of ammonium fluoride, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1209 (2022), 123413, https://doi.org/10.1016/j.jchromb.2022.123413.
[25] C. Pouech, F. Lafay, L. Wiest, R. Baudot, D. Léonard, C. Cren-Olivé, Monitoring the extraction of additives and additive degradation products from polymer packaging into solutions by multi-residue method including solid phase extraction and ultra-high performance liquid chromatography-tandem mass spectrometry analysis, Anal. Bioanal. Chem. 406 (5) (2014) 1493–1507, https://doi.org/10.1007/s00216-013-7551-4.
[26] B.S. Karthikeyan, J. Ravichandran, S.R. Aparna, A. Samal, DEDuCT 2.0: an updated knowledgebase and an exploration of the current regulations and guidelines from the perspective of endocrine disrupting chemicals, Chemosphere 267 (2021), 128898, https://doi.org/10.1016/j.chemosphere.2020.128898.
[27] G. Singh, R. Gollapalli, A. Blinder, M. Patel, Identification of leachable impurities in an ophthalmic drug product originating from a polymer additive Irganox 1010 using mass spectroscopy, J. Pharm. Biomed. Anal. 152 (2018) 197–203, https://doi.org/10.1016/j.jpba.2018.01.053.
[28] G. Singh, D. Lu, C. Liu, D. Hower, Analytical challenges and recent advances in the identification and quantitation of extractables and leachables in pharmaceutical and medical products, TrAC Trends Anal. Chem. 141 (2021), 116286, https://doi.org/10.1016/j.trac.2021.116286.
[29] Toxicity Estimation Softwarte Tool (T.E.S.T). ⟨https://www.epa.gov/chemical-research/toxicity-estimation-software-tool-test⟩, 2022 (Accessed 22 December 2022).
[30] A. Walsh, M. Ovais, T. Altman, E. Sargent, Cleaning validation for the 21st century: acceptance limits for cleaning agents, Pharm. Eng. 33 (6) (2013) 1–11.

[31] H.L. Girand, Continuous infusion vancomycin in pediatric patients: a critical review of the evidence, J. Pedia Pharm. Ther. 25 (3) (2020) 198–214, https://doi.org/10.5863/1551-6776-25.3.198.
[32] Drug Administration guide in paediatrics and neonatology [Internet]. ⟨https://pharmacie.hug.ch/infomedic/utilismedic/ped_admin_medic_inj.pdf⟩, 2022 (Accessed 11 December 2022).
[33] R. Scheuplein, G. Charnley, M. Dourson, Differential sensitivity of children and adults to chemical toxicity. I. Biological basis, Regul. Toxicol. Pharm. 35 (3) (2002) 429–447, https://doi.org/10.1006/rtph.2002.1558.
[34] SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), Safety of the use of bisphenol A in medical devices, 18 February 2015
[35] SCHEER (Scientific Committee on Health, Environmental and Emerging Risks), Guidelines on the benefit-risk assessment of the presence of phthalates in certain medical devices covering phthalates which are carcinogenic, mutagenic, toxic to reproduction (CMR) or have endocrine-disrupting (ED) properties, final version adopted at SCHEER plenary on 18 June 2019.