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Review

Analytical methods for the determination of DEHP plasticizer alternatives present in medical devices: A review

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ABSTRACT

Until 2010, diethylhexylphthalate (DEHP) was the plasticizer most commonly used to soften PVC medical devices (MDs), because of a good efficiency/cost ratio. In flexible plasticized PVC, phthalates are not chemically bound to PVC and they are released into the environment and thus may come into contact with patients. The European Directive 2007/47/CE, classified DEHP as a product with a toxicity risk and restricted its use in MDs. MD manufacturers were therefore forced to quickly find alternatives to DEHP to maintain the elasticity of PVC nutrition tubings, infusion sets and hemodialysis lines. Several replacement plasticizers, so-called "alternative to DEHP plasticizers" were incorporated into the MDs. Nowadays, the risk of exposure to these compounds for hospitalized patients, particularly in situations classified "at risk", has not yet been evaluated, because migrations studies, providing sufficient exposure and human toxicity data have not been performed. To assess the risk to patients of DEHP plasticizer alternatives, reliable analytical methods must be first developed in order to generate data that supports clinical studies being conducted in this area. After a brief introduction of the characteristics and toxicity of the selected plasticizers used currently in MDs, this review outlines recently analytical methods available to determine and quantify these plasticizers in several matrices, allowing the evaluation of potential risk and so risk management.

Contents

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1. Introduction

Polyvinylchloride (PVC) is a plastic material which is widely used in fields as diverse as construction, automobiles, cabling, toys, luxury goods, and healthcare.

PVC possesses the largest share of the medical market. Almost 30% of all plastic-based disposable medical devices (MDs) used in hospitals are made from PVC, usually as flexible PVC [1]. The numerous benefits of PVC, which include chemical stability, biocompatibility, clarity and transparency, flexibility, durability, chemical and mechanical resistance, sterilizability, and low-cost, explain its extensive use in medical devices (e.g., tubing for infusion, dialysis, endotracheal, and feeding). To induce and maintain the flexibility and workability of these PVC medical devices, plasticizers are added to a maximum concentration of about 40% of the total weight formulation.

As plasticizers are not chemically bound to PVC, they can be released from the medical device during contact with blood, enteral or total parenteral nutrition admixtures, or lipophilic drugs, which might lead to unwanted patient exposure [2–4]. Until recently, the most commonly used plasticizers were phthalates, in particular di (2-ethylhexyl) phthalate (DEHP). However, DEHP is classed as carcinogenic, mutagenic or toxic to reproduction (CMR1B) under the CLP Regulation [5] because of its potential toxicity to fertility and reproduction. Hence, its use in medical devices has recently been challenged by the European authorities [6]. This action has forced manufacturers to quickly replace DEHP with alternative plasticizers (e.g., TOTM, DEHT, DINCH, DINP, DEHA, and ATBC), for which there is currently very little data assessing their migration from MDs, the level of exposure of the population to these alternative plasticizers and their metabolites in clinical conditions, and their toxicity [4,7].

Consequently, it is critical that the scientific community be responsible for generating reliable methods and data that support clinical studies being conducted in this area. Analytical chemists must provide the most accurate, sensitive, and robust methods possible for analyzing the alternative plasticizers found in different matrices that require these compounds to be controlled. These methods must be capable of the

- identification and quantitation of these compounds within medical devices, and the determination of the amounts of DEHP present in the mass with regards to the contamination threshold of 0.1% of the mass, as defined by the European regulation concerning the Registration, Evaluation, Authorization and Restriction of Chemical substances (REACH) [8],
- assessment of the ability of DEHP's alternatives to migrate from medical devices into fluids that are in contact with the patients (drug fluids, nutrition admixtures, patient's blood, etc.), and
- evaluation of patient exposure to these plasticizers and their metabolites by quantifying their presence in biological human fluids.

Extensive studies have been conducted on some of these alternative plasticizers, producing quantitative methods that have been used to define acceptable migration limits in the food processing industry. These methods are now the subject of an EU regulation [9].

The evaluation process for plasticized PVC MDs may be compared to the process adopted for food packaging by modifying the models to represent the MDs' clinical use, and by adjusting the migration limit calculations to take into account the exposure (biomonitoring) and toxicological data of the concerned plasticizers.

The aim of this review is to present a summary of the analytical methods developed for the quantification of the alternative plasticizers used in medical devices. Instead of reviewing all available analytical methods, this paper will focus on those suitable for the detection and quantification of the plasticizers in matrices, and which are appropriate for evaluating the patient exposure risk to the MDs' plasticized PVC (i.e., the medical device itself, clinical conditions' simulants, and human body fluids).

2. Plasticizer alternatives to DEHP

This paper focuses on the six plasticizers which have mainly substituted DEHP in all medical devices (Fig. 1).

A plasticizer is not judged on its individual qualities but rather on the qualities exhibited by the plastic into which it is integrated.

In the medical field, some properties are essential:

1) a plasticizer must have excellent plasticizer-polymer compatibility;
2) a strong and persistent plasticization efficiency is required in order to maintain the flexibility of the medical device and thus encourage patient compliance with medical care; and
3) stability to temperature, oxidation, and UV degradation to meet the life-time needs of plastified PVC in medical applications.

These criteria must be considered in parallel to the cost criterion. In the end, a plasticizer used for medical PVC must not compromise patient health.

2.1. Uses

Approximately 30% of the total PVC resin production in Europe is used for flexible PVC products. Plasticizers are by far the most common additives because they are less expensive than the others used in polymer processing and applications (e.g., flame retardants, heat stabilizers, lubricants, organic peroxydes, etc.).

The alternatives plasticizers to DEHP are used in various industrial products, both for outdoor and indoor applications, and their use is driven by the cost/performance ratios of the different raw materials used in the production of the finished goods. They are found in many PVC articles such as vinyl flooring, paint, automobiles, food wrappers, toys and childcare articles, cosmetics, and medical devices (Table 1) However, the toxic risk of DINP (one of the alternative plasticizers) has led to restrictions on its use in children's articles that can be placed in the mouth. It now must not exceed 0.1% by mass of the plasticized material [10].

In medical devices, the distribution of the alternative plasticizers is not homogeneous, as shown by Gimeno et al. [11]. They are primarily used to soften devices used in infusion and transfusion, nutrition, and hemodialysis (i.e., infusion or transfusion sets, feeding tubes for enteral and parenteral food administration, and arterio-venous lines).

Some have specific uses. For example, DINCH [12] or ATBC are mainly used, in combination with other plasticizers, in red blood

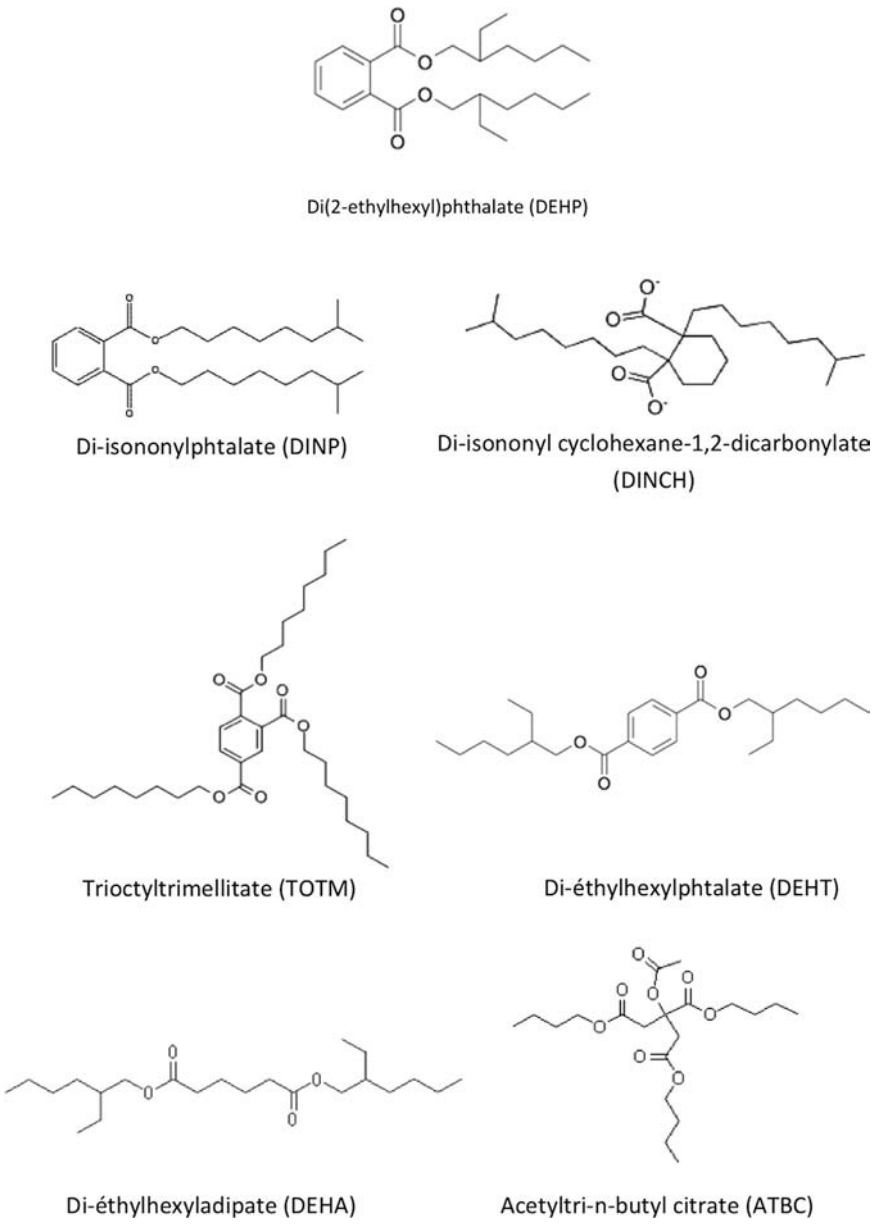

Fig. 1. Chemical structures of DEHP and plasticizers investigated as alternatives to DEHP in medical devices.

Table 1
Fields of use of the alternative plasticizers.

Plasticizer	Main fields of use	Medical uses	Most relevant MDs	Plasticizer concentration in MDs (wt%)
TOTM	**Medical devices**, wire insulation, cable products, electric insulation	**Infusion** **Enteral and parenteral nutrition** Hemodialysis Transfusion	Infusion sets, extension sets, transfusion sets, tubes for pump administration, enteral feeding tubes, disposable infusion pumps, arteriovenous lines.	**43–57**
DEHT	**Medical devices**, toys and childcare articles, beverage closures, cables and wire.	**Infusion** **Transfusion**	Infusion, extension, and transfusion sets	**25–35**
DINP	**Medical devices**, automotive industry, cables and wires, coatings, flooring, toys, inks	**Infusion** Transfusion Parenteral nutrition	Infusion and transfusion sets, scalp vein sets (for tiny veins), feeding tubes for gravity or pump administration[a]	**30–40**
DINCH	**Medical devices**, toys, food contact applications	**Enteral nutrition** Infusion	Feeding tubes for gravity or pump administration, infusion and extension sets	**About 40**
DEHA	**Medical devices**, flooring and wall coverings, toys, clothes food packaging and films, cosmetics (nailcoating)	Hemodialysis, transfusion	Disposable infusion pumps arteriovenous lines, heating lines for blood and blood products	NA

Table 1 (*continued*)

Plasticizer	Main fields of use	Medical uses	Most relevant MDs	Plasticizer concentration in MDs (wt%)
ATBC	**Medical devices**, pharmaceutical products, toys, cosmetics (films), ink formulations, food contact applications	**Enteral and parenteral nutrition** ECMO	Feeding tubes for gravity or pump administration, enteral feeding tubes, extracorporeal tubings	**NA**

ECMO: extracorporeal membrane oxygenation; MD: medical device. NA: data not available.

[a] Blended with other alternative plasticizers.

Table 2
Overview of some physical and mechanical properties of the assessed plasticizers.

	DEHP	TOTM	DEHA	ATBC	DEHT	DINP	DINCH
Molecular weight (g/mol)	390.56	546.80	370.57	402.50	390.54	418.62	424.70
Transparency	Good			=	=	=	=
Compatibility with PVC	Good	=	−	NA	=	=	=
Viscosity (cP at 20 °C)	71	247	14	37	78	99	44–60
Water solubility (mg/L at 25 °C)	Weak (0.285)	+++	+	++	++	−	–
		100	0.78	5	NA	1×10^{-3}	0.02
Volatility (vp; at 20 °C, Pa)	8.6×10^{-4}	$< 1 \times 10^{-7}$	1×10^{-4}	6×10^{-4}	NA	$< 1 \times 10^{-4}$	1×10^{-4}
Leaching aptitude into oily media (log K_{ow})	7.6	−	−	−	−	+	+
		5.94	6.10	4.30	5.72	8.80	10.00
Low temp properties	Good	−	+++	NA	+	−	++
Flex temp		−19.2	−52.8		−27.7	−23.6	NA
Brittleness temp[a]		−31.8	−62.7		−33.4	−31.8	−43
Substitution factor[b]	**1**	1.17	0.93	NA	1.03	1.06	NA
References	[14,4,87]	[88,14,4,7,87,45]	[14,4,7]	[4,7,45]	[7,87,45]	[14,4,89,87,45]	[90,4,89]

NA: data not available.
+, - and =: Comparison symbols meaning: + better, = similar, - worse (comparison made with DEHP properties).
Brittleness temperature is the minimum temperature at which PVC can be used without becoming too brittle and is measured by ASTM D-746.
Flex temperature is the temperature at which the material is considered to have lost most of its elastomeric properties, and is measured by ASTM D 1043.
Abbreviations: DEHP (Di(2-ethylhexyl)phthalate), TOTM (Tri-octyltrimellitate), DEHA (Di-(2-ethylhexyl) adipate), ATBC (acetyltri-n-butyl citrate), DEHT (di-(2-ethylhexyl) terephthalate), DINP (Di-(isononyl) phthalate), DINCH (Di(isononyl)-cyclohexane-1,2-dicarboxilic acid)

[a] PVC at 50 phr plasticizer.
[b] Plasticizer level (phr = parts per hundred) required for 80 A durometer hardness at room temperature vs. required DEHP level (phr = 52.9).

cell (RBC) PVC bags due to their capacity to prevent excessive hemolysis during storage.

However, the trend in plasticizer use is constantly evolving as there is no referential to guide manufacturers in the choice and amount to be integrated into their products.

2.2. Physico-chemical, mechanical, and performance properties

Table 2 summarizes the most important physico-chemical, mechanical, and performance parameters of the six plasticizers used as an alternative in medical devices, compared with DEHP.

In the field of medical devices, PVC has to be flexible. The flexibility is mostly due to the non-polar moiety of the plasticizer molecule, which serves to attenuate the attractive forces between PVC chains, thus increasing the free volume of the PVC matrix in which the additives are mobile and can move [13]. The balance between the polar and non-polar moieties of the molecule is critical in controlling its solubilizing effect: if a plasticizer is too polar, it can destroy PVC crystallites, if it is too apolar, compatibility and leaching problems can arise [14].

The excellent performance of DEHP in the plasticization and processing of PVC explains its wide use in medical devices over the past few years. The strategy was to adjust the chemical nature of the lateral alkyl chains in order to reduce the leaching of the plasticizers into the surrounding medium. In general, the alternative plasticizers are strong PVC solvaters due to their sufficient polarity. This is related to their poor volatility, which is comparable to that of DEHP. Moreover, as shown in Table 2, their higher molecular weight and greater steric hindrance compared to DEHP are associated with less branching, which contributes to their chemical stability and prevents oxidative attack [14]. These are the main characteristics of trimellitates plasticizers (e.g., TOTM) where both the three alkyl chains and the ester groups contribute to higher molecular weights and give sufficient polarity to maintain PVC compatibility and improve performance in the PVC matrix. DINCH is also expected to have a low volatility due to its molecular weight and its mild polarity.

These two characteristics are of great importance in determining the leaching rates of each plasticizer, especially into an oily surrounding medium. Moreover, leaching rates are strongly related to the partition coefficient Log K_{ow}. This could explain the lesser leaching rates of TOTM compared to DEHP [15,16]. However, each molecule has a different apolar/polar ratio that results in differences in plasticizing efficiency [17], which is expressed as a substitution factor. As shown in Table 2, most of the plasticizers need to be added in higher concentrations than DEHP in order to achieve the same softness. This could explain why TOTM exhibits higher low and flex temperatures compared to the alternative phthalate counterparts, like DINP or DEHT, as plasticizing efficiency is directly related to low temperature properties (flex and brittleness temperatures). For medical devices, this highlights the critical question of the manufacturing cost.

2.3. Metabolism, toxicity and regulation in medical devices

Information on disposition and metabolism can explain the biological effects of compounds. For example, DEHP toxicity has been clearly identified as resulting mainly from its major metabolite, the monoester MEHP [18–21].

For the alternatives plasticizers, there are major concerns for patient exposure that are related to the toxicological profile of the additives, as their metabolism would likely be similar to that of DEHP.

Human biomonitoring studies allow this exposure to be assessed by measuring the levels of these chemicals, their metabolites, and/or their reaction products, in human fluids like blood (and components), urine, saliva, or expired air.

Hence, a better understanding of the human metabolism and excretion kinetics of these plasticizers is crucial for identifying metabolites that are specific to plasticizer exposure.

As shown in Table 3, literature data shows a similar metabolism process for several of the alternative plasticizers, i.e., DINP, DINCH, DEHA, and DEHT [22–28]. They are metabolized very quickly and do not bioaccumulate, leading to a negligible remaining dose after 48 h. After an initial presystemic and rapid ester hydrolysis to the corresponding monoesters, which appear in the gastrointestinal tract, they undergo further oxidation in the liver to produce secondary metabolites. These metabolites could also undergo conjugation with glucuronic acid and sulfonic acid to form the respective conjugates before being eliminated via the urine. In most cases, these secondary metabolites have been identified as specific biomarkers of plasticizer exposure.

Despite a similar excretion route, the toxicological profile of each plasticizer is different. The toxicological aspect of DINP seems to be similar to that of DEHP but at higher exposure levels [29], with abnormalities during embryo-fetal development occurring in rodents and significant increases in liver tumors occurring in rats and mice after oral doses. The non-observed adverse effect level (NOAEL) of 15 mg/kg bodyweight/day (against 4.8 mg/kg for DEHP) contributes to it not being considered as a CMR substance.

DINCH is neither a reproductive toxicant nor an endocrine disruptor. Although exposure to DINCH has not been found to induce mutagenicity or genotoxicity, it was found to cause renal toxicity and thyroid hyperplasia in rats, which must be taken into consideration [5]. Leaching of DEHA from food packaging is limited [9], with an established developmental and fetal toxicity that gives a NOAEL of 100 mg/kg. More recently, Ito et al. reported a possible peroxysome proliferation with DEHA that was similar to that of DEHP [30].

DEHT is DEHP's structural para-isomer. However, the structural differences have important implications for the metabolism and consequential toxicological effects. DEHT undergoes a weak conversion to its primary metabolite, MEHT, leading to a lower toxicity than DEHP. In humans, DEHT is not irritating and not sensitizing [31] and animal studies in Sprague–Dawley rats have shown no evidence of teratogenesis [32]. Moreover, no carcinogenesis was found by Deyo et al. following oral exposure of rats over a period of 2 years [33].

Lesser toxicities are also reported for TOTM and ATBC, probably due to their metabolic breakdown. TOTM shows weaker hepatotoxicity than DEHP due to its low metabolic transformation capacity; 75% of the oral dose is eliminated in the feces, primarily as unchanged parent compound [7] and to a lesser extent as the mono- and di-esters (MOTM and DOTM). This is probably due to its inability to fit into the binding sites of the PPARs receptors [34].

Table 3
Disposition and metabolism of the alternative plasticizers.

Plasticizer	Primary metabolites	Main secondary metabolites	Route of excretion	Elimination time ($t_{1/2}$ or time of elimination for the total dose)	References
DEHP	MEHP	MEOHP, MEHHP, MECCP	In urine (75%) as oxidized metabolites and, to a lesser extent, as MEHP.	$t_{1/2\ (urine)}$ = 10 h for oxidized metabolites, 5 h for MEHP	[91–93,4]
DEHT[a]	TPA, 2-EH and, to a small extent, MEHT	Oxidized metabolites	In urine (32%), as TPA (51%), 2-EH, MEHT and metabolites. In feces (56.5%) as unchanged DEHT+++, and MEHT.	Time: 24 h	[94]
DINP	MINP	OH-MINP, oxo-MINP, and cx-MINP	In urine (around 50%) as OH-MINP > cx-MINP > oxo-MINP > MINP.	$t_{1/2\ (urine)}$ = 14 h for all metabolites but faster for MINP	[4,93,95,96]
DINCH	MINCH	OH-MINCH, oxo-MINCH, and cx-MINCH CDHA	Mainly in feces as unchanged DINCH. In urine as CDHA and OH-MINP > cx-MINCH = oxo-MINCH > MINCH.	80% of the oral dose eliminated after 24 h and 90% after 48 h	[4,24,25]
TOTM[a]	MOTM, DOTM, and 2-EH	2-EH metabolites	Mainly in feces (75%) primarily as unchanged form (TOTM), MOTM, and DOTM. In urine (16%) as 2-EH metabolites.	$t_{1/2\ (urine)}$ = 30–40 h	[35,7]
DEHA	MEHA, AA, and to 2-EH	Non-specific metabolites (keto-EHA, DiEHA, 5-OH-EHA) and specific metabolites (MEHHA, MEOHA)	Mainly in urine in humans as EHA. Minimal tissue retention	$t_{1/2\ (urine)}$ = 1.5 h (humans)	[4,7,27,26,28]
ATBC[a]	Several polar metabolites		Mainly in urine, mostly as monobutylcitrate. In feces as ATBC (7%) and metabolites	99% of the oral dose is eliminated after 48 h	[4,7]

2-EH: 2-ethylhexanol; MOTM: monooctyltrimellitate; DOTM: dicotyltrimellitate; TPA: terephtalic acid; MEHT: monethylhexylterephtalate; MINP: monoisononylphtalate; OH-MINP: mono-(hydroxyisononyl)phtallate; oxo-MINP: mono-(oxo-isononyl)phtalate; cx-MINP: mono-(carboxy-isononyl)phtalate; CDHA: cyclohexane-1,2-dicarboxylic acid; MINCH: cyclohexane-1,2-dicarboxylic mono isononyl ester; OH-MINCH: cyclohexane-1,2-dicarboxylic mono hydroxyisononyl ester; oxo-MINCH: cyclohexane-1,2-dicarboxylic mono oxoisononyl ester; and cx-MINCH: cyclohexane-1,2-dicarboxylic mono carboxyisononyl ester
EHA: 2-ethylhexanoic acid; AA: adipic acid; and MEHA: mono-(2-ethylhexyl) adipate
$t_{1/2}$: half-life of elimination (when two elimination phases exist, $t_{1/2}$ corresponds to the 2nd phase of elimination)

[a] Plasticizers whose metabolism has not been studied in humans. Available data are from animal studies only.

A potential bioaccumulation after a 14-day IV administered dose [35] in rats needs further investigated. Finally, despite its low toxicity compared to DEHP, which is due mainly to its rapid absorption and excretion, ATBC is still a matter of concern because of the ease with which it leaches from PVC [4].

Major differences exist between various plasticizers used in medical devices in terms of physicochemical properties and the way they are metabolized in the body. These facts must be taken into account as they represent some of the difficulties in providing standard and reliable methods for the simultaneous analysis of multiple plasticizers and their metabolites.

3. Analysis methods for the alternative plasticizers

Due to the increasing use of alternative plasticizers in medical devices and the concerns regarding leaching, numerous studies have focused on the extraction and identification of plasticizers released into infused drug solutions and human biological fluids. However, to evaluate patient exposure to such compounds, as well as to ensure that medical devices are free from DEHP, the first step consists of identifying and quantifying the plasticizers in such devices and, consequently, those that are likely to migrate into the patient's body.

Several analytical methods are available to determine the plasticizer composition of the different matrices either directly in the medical device itself, in clinical condition simulants, or in human body fluids.

In this section, we will introduce the instrumentation used to detect and separate alternative plasticizers from the matrices mentioned above.

3.1. Analysis in the MD's matrix

The PVC MDs used during medical situations contain quantities of plasticizers ranging from 30% to 40% of the PVC mass. However, restricted phthalates like DEHP must not be present in the medical device over the contamination threshold of 0.1% of the PVC mass [8].

Thus, analytical methods must enable both the determination and the quantification of large but exact amounts of plasticizer in the PVC matrix. They must also be sensitive enough to detect trace levels of potential contaminants. Genay et al. [36] and Gimeno et al. [11] have previously shown that medical devices are not pure materials and are often contaminated with other plasticizers, especially DEHP or DEHT.

The methods can be divided into two types, direct or indirect, with the later consisting of a treatment step to extract the plasticizer from the PVC before analysis.

3.1.1. Direct analysis methods

Generally, the direct methods are not sensitive. Nevertheless, differences exist in their discrimination capabilities and in their potential use as routine techniques.

It is easy to obtain general information using very simple non-separative methods suitable for identifying the alternative plasticizers.

These methods rely on the general features of polymers, like PVC and their plasticizers, such as thermolability and spectral and electromagnetic characteristics.

Thermogravimetric analysis (TGA) has been widely used to study the thermal decomposition of polymers [37], like flexible PVC, to assess the weather aging of flexible tubing. TGA is an efficient method to evaluate and predict the desorption process of a plasticizer, which consists of two consecutive steps: diffusion from the bulk of the sample to the surface and evaporation from the surface. This evaporation is proportional to the surface area [38].

Thus, TGA could be an easy and inexpensive technique for obtaining information on the amount of plasticizer in a PVC medical device. Indeed, Perkin Elmer used TGA analysis to quantify the weight loss of PVC formulated with DINP as 50.99% [39]. Marcilla et al. also showed a correlation between the molecular structure of the plasticizers and the evaporation temperature. For example DEHA, which has a lower molecular weight, evaporates at a lower temperature than DINP [37].

Rahman et al. studied the high temperature stability of some plasticizers in 20 wt% plasticized PVC samples using TGA [40]. TOTM appears to be the most stable plasticizer in short-term and long-term thermal stability studies (Fig. 2) [40].

TGA is a useful tool to evaluate the global proportion of compounds present in a PVC matrix and therefore for the quality control of manufactured material.

However, literature data also suggests that TGA is not specific enough to identify species that evolve during thermal analysis or to discriminate between plasticizers and additives in the PVC matrix. Rahman et al. observed that the early weight loss was probably due to the evaporation of moisture content, impurities, or dissolved gases [40], whereas the variations that occur in the mechanical properties of PVC geomembranes, described by Lodi et al. [41], may not be simply due to the loss of the plasticizers.

Differential Scanning Calorimetry (DSC) is also used to show polymer degradation in a medical device. It is based on the polymer's glass transition temperature (Tg), which is known to decrease with the addition of a plasticizer. DSC is a thermo-analytical technique in which the difference in the amount of heat required to increase the temperature of a polymer sample and a reference is measured as a function of temperature.

The method can be used to determine the presence of plasticizers remaining in a PVC matrix and was used by Wang et al. to compare extractions of DEHP and DEHA from PVC tubes.[42]. Similar DSC curve profiles were found in the different samples, which gave two glass transition temperatures (Tg). The comparison of the exothermic maximum of the samples allowed the authors to identify the remaining plasticizers, such as adipates, and to compare the extraction efficiencies. DSC is a rapid and simple technique that a medical device company could use to determine the effect of sterilization on their products. This approach was used by Marcella et al. in order to assess the effect of different doses of gamma irradiation on DEHP migration from PVC blood bags [43]. However, no significant difference was observed between the Tg values of the samples irradiated with different exposure doses, which could suggest that the method has poor sensitivity.

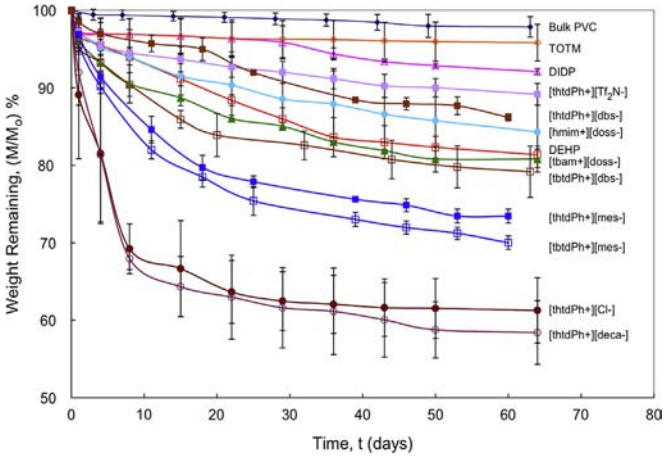

Fig. 2. Weight loss of traditional plasticizers (including TOTM) and ionic liquids during long-term stability of 20 wt% plasticized samples at 100 °C (from Rahman et al. [40]).

The techniques described above, which are based on the weight loss of the polymer, are not specific and are of little interest for the analysis of alternative plasticizers. This is also true for gravimetric analysis.

Gravimetric analysis (GA) is a technique through which the amount of an analyte, such as an alternative plasticizer, can be determined by measuring the mass after isolation by precipitation. Thus, GA may be performed in order to have a global estimation of the amount of all the additives in a PVC sample. Kastner et al. applied the American Society for Testing and Materials (ASTM) Method D1239 to a gravimetric analysis in order to measure weight loss of DINCH after extraction by de-ionized water [44]. The authors showed that the results did not reflect the total plasticizer loss due to an uptake of water that lead to an increase in the weight of DINCH in the experiment run. Repeated cycles of soaking/drying were necessary to quantify the real weight loss of the plasticizer after a long experimental time. Moreover, water absorption was different depending on the nature of the plasticizer and, though it was not apparent with DEHP, it undoubtedly existed.

Among direct analysis methods, **Nuclear magnetic resonance (NMR)** could also be appropriate, providing a good specificity due to its ability to discriminate isomers.

NMR has the advantage of efficiently separating isomers, which can be useful in the field of medical devices for discriminating DEHP from its isomer DEHT through the specific quantum mechanical magnetic properties of the atomic nuclei.

NMR is used to study microstructures and polymer-plasticizer interactions in plasticized systems. For example, a combination of a 13C solution and solid state NMR was used to reveal the crystallinity of PVC-DEHP samples that contained PVC of different tacticities [45]. NMR is, along with Mass Spectrometry, the primary analytical technique that provides both qualitative and quantitative structural informations on the analytes. Genay et al. used NMR to identify traces of DEHP in PVC medical devices presented as DEHP-free, i.e., with an amount lower than 0.1% [36]. However, Lutyten et al. identified several drawbacks, including low sensitivity, while working on the quantification of different plasticizers present in PVC tubes. The method developed by the authors can be applied to different types of plasticizers, like DINP or DEHP, within a concentration range of 10–50% by weight. The authors concluded that their technique required improvement or the development of an alternative high resolution NMR method for the detection of concentrations ranging from 0% to 10% by weight. Moreover, the NMR technique is very expensive and time consuming.

Infrared spectroscopy (IR) is widely used in plasticizer containing systems due to several advantages in terms of rapid and non-destructive measurements, good reproducibility, and accuracy. It is also more sensitive than the NMR mentioned above.

Fourier Transform-Infrared Spectroscopy (FTIR) is used extensively in the plastics industry for material characterization, i.e., for the analysis of the additives found in many plastic formulations, including plasticized tubes. The combination of high sensitivity FTIR spectrometers with ATR (attenuated total reflectance) accessories gives low noise levels, which is an advantage in the detection of trace concentrations of unexpected DEHP. Marcilla et al. used FTIR with the ATR configuration to determine plasticizer concentration in PVC resins and to estimate their diffusion coefficients [46]. Concentration profiles obtained in the study have shown that the leaching process depends on the chemical structure of the different plasticizers, allowing them to be separated into plasticizer families. According to the authors, adipates seem to have the highest leaching level. Not surprisingly, the process is also affected by the molecular weight of the compounds studied, resulting in different diffusion coefficients. However, the diffusion coefficient calculations required the construction of mathematical models and the optimization of several parameters in order to avoid biases such as the thickness of the PVC sample. Such optimization can be complex. Wang et al. used FTIR to determine the concentration of the additives in PVC that remained after an extraction process [42]. The IR technique helped the authors to select the most efficient extraction method, indicating in some cases the presence of residual solvent, and thus the difficulty in removing it after extraction, or that the plasticizers co-precipitated with the PVC or were been retained in the PVC. This was confirmed by GC analysis.

FT-Raman spectroscopy can give complementary information on the chemical composition, for example at low plasticizer levels. Based on the inelastic scattering of light, the FT-Raman technique produces an incident light with very-high energy using an UV, VIS, or NIR source. This leads to lower sensitivity due to a weaker scattering effect. The technique was used by Berg et al. in 2006 to determine the presence of adipate ester plasticizers in commercial flexible PVC products [47]. However, results showed that reliable qualitative analysis of adipates is difficult due to the spectral similarities between the molecules and that quantitative analysis of these compounds is not possible because of the limited sensitivity of the method. Only semi-quantitative estimations of additives such as adipates may be feasible. On the whole, infrared spectroscopy is useful in the analysis of the chemical types of plasticizer but is not precise enough for conclusive identification of the exact plasticizer, especially in mixtures with other extracted additives.

The near-infrared spectroscopy (NIR) method was used by Saeki et al. to discriminate different plasticizers in PVC, including TOTM, DEHA, and DINP [48]. The NIR region is usually considered to include wavelengths between 12,500 and 4000 cm^{-1}. Absorption bands originate from overtones and combinations of the fundamental (mid-IR) groups, mostly from C–H, N–H, and O–H bonds. Saeki et al. showed that the NIR spectra are insufficient for the discrimination of plasticizers belonging to the same structural group. For example, the spectra of the DINP and DEHP primary and secondary derivatives were similar, which prevented their discrimination. The solution was found by combining the NIR technique with neural network analysis. It was also found that NIR and the neural network method could predict the plasticizer content, with good correlation coefficients. The NIR technique seems to provide an interesting alternative method for separating additives, such as plasticizers, when they are blended in a complex PVC matrix. This is due, in particular, to the band combination. However, the wide and overlapping combination and the overtone spectral bands can also complicate the interpretation of the data. In addition, the non-fundamental spectra of FT-NIR, compared to FT-IR, require calibrations to be made using chemometric software, preferably over a wide range of samples.

Recently, several new spectroscopy methods have been developed, making it possible to analyze ordinary objects in their native condition, without time-consuming sample preparation steps.

The direct analysis in Real Time (DART) tandem mass spectrometry enabled Kuki et al. to screen phthalates in PVC samples [49]. It was also successfully used by Rothenbacher et al. [50] to determine if a rapid screening test for plasticizers (like DEHP, DINP, or DINCH) in PVC materials can be developed using direct analysis in real time mass spectrometry (DART-MS). An open interface would allow the direct insertion of solid specimens, such as samples of medical devices. This method may be considered as an efficient technique for the prescreening of alternative plasticizers present in a medical device, with the advantages of avoiding solvent use and of wasting valuable instrument and staff working time. It provides a rapid method for the identification of plasticizers present in the specimens prior to exact determination. Moreover, the authors hypothesized that the sensitivity of their method could be improved by using a triple-quadrupole MS

instead of a single-quadrupole MS, or with a quadrupole time-of-flight as was used by Kuki et al. [49].

3.1.2. Indirect analysis methods

Gas chromatography (GC) and liquid chromatography (LC) are the preferred indirect methods for the identification of plasticizers. GC and LC, preceded by different extraction procedures, have been the usual techniques for determining the presence of phthalates in the routine analysis of a wide variety of samples ranging from indoor air, natural water and sewage sludge, to food-packaging materials, toys, and medical products.

In gas chromatography analysis, different injection modes, columns, and detectors are available. Most GC methods involve the use of 5% dimethylpolysiloxane and/or phenyl-methyl-polysiloxane non-polar columns, with bonded and cross-linked stationary phases. In general, mass spectroscopy (MS) was the detection mode used in the studies involving phthalates. GC is often mentioned in the analysis of phthalates in various solid matrices ranging from food packaging, child toys, to cosmetic products [51–54]. Recently, gas chromatography was used for the separation and quantification of 12 phthalates, including 8 phthalates regulated in the cosmetics field [55]. The chromatographic method developed was also proposed as a working document for the possible elaboration of a new standard for a phthalate assay of cosmetic samples at the CEN (European Committee for Standardization).

For alternative plasticizers, gas chromatography with both mass spectroscopy detection and a flame ionization detector (FID) has been employed to quantify additives in PVC tubes.

Mass spectroscopy is the most suitable method for the simultaneous detection and quantification of mixtures of low concentrations (or traces) of plasticizers because it provides selectivity and sensitivity in the analysis of complex samples. Wang et al. found DEHA in two out of three tubes using GC/MS analysis [42]. The technique was also employed in the field of food and food packaging in order to detect amounts of plasticizers, especially phthalates, in different types of matrices like olive oil fat or “fat-free” foods [56–60]. A very recent study used GC–MS to identify and quantify 14 phthalates and 5 non-phthalate plasticizers, including alternative plasticizers used in medical devices (TOTM, DINCH, DINP, DEHA, DEHT, and ATBC) [11]. Using a traditional crosslinked poly (5% diphenyl/95% dimethylsiloxane) capillary column, a split mode, and an acquisition on SIM mode, the resulting method provided an acceptable separation of most compounds and the possibility to specifically detect each analyte using specific m/z ions.

Flame ionization detection (FID) is also commonplace because it is versatile, simple, and is readily accessible to most analytical laboratories and gas chromatographs. FID is also suitable for the detection of various alternative plasticizers (Sautou, poster Matbim), including TOTM, despite its steric hindrance. However, FID needs an efficient upstream separation method to quantify these additives, unlike MS detection which provides structural information Consequently, GC/FID could be considered as a routine technique to quantify known analytes in a PVC matrix.

Liquid chromatography (LC) has not yet been employed to determine the presence of alternative plasticizers in PVC medical device matrices. Indeed, LC is a valuable analytical technique that is conventional to most laboratories conducting trace analysis in liquid and non-volatile samples.

LC–UV is a technique originally developed to quantify phthalates in environmental matrices or intravenous pharmaceutical solutions [61]. It has also been used extensively in the field of cosmetics for the separation, identification, and quantification of phthalates, with several phthalates being determined simultaneously [55]. However, UV detection is not suitable for non UV-absorbent plasticizers such as ATBC, DEHA, and DINCH. Moreover, UV detectors have some limitations in separating compounds, like plasticizers, whose chemical structures are similar (e.g., the structural isomers DEHP and DEHT). Nowadays, it is not uncommon for manufacturers to provide marketed medical devices containing a mixture of two or three plasticizers whose separation could be difficult with HPLC–UV for the reason mentioned above.

The use of a diode array detector (DAD) may provide an interesting addition for the discrimination of alternative plasticizers, since it is capable of the simultaneous and accurate detection and quantification of DEHP and its major metabolite MEHP in seminal plasma [62]. Currently, there is no published data on the use of DAD to detect alternative plasticizers.

Finally, **LC–MS/MS** provides numerous advantages, especially in terms of sensitivity and specificity and will be discussed later.

Supercritical fluid chromatography (SFC) is a hybrid of gas and liquid chromatography. This technique uses a supercritical fluid, mostly carbon dioxide, as a mobile phase and sometimes enables, in a short analysis time, the separation and identification of a group of compounds that are not conveniently handled by either gas or liquid chromatography. SFC has been recently used to analyze 4 of the 6 plasticizers mainly found in medical devices [63]. The authors demonstrated that SFC could be an interesting alternative for the detection and quantification of alternative plasticizers, after condition optimization [63]. A chemometric strategy was used in order to optimize the hyphenation of the SFC detector. The use of an evaporative light scattering detector (ELSD) offers the advantage of a more uniform sensitivity for the detection of the analytes, regardless of their physical and chemical properties. In this study, ATBC, which cannot be detected by even low wavelength HPLC–UV, has been successfully analyzed. Due to the fact that liquid carbon dioxide has a higher density than the gas, the mobile phase has a greater chance of interacting with the plasticizer, thus giving more flexibility in optimizing the separation. However, this is also a disadvantage of the technique. A sensible selection of chromatographic parameters is required in order to have a good efficiency with the selected detector. Moreover, the need of specific equipment, such as a restrictor to maintain a high pressure in the column, and continuous assessment of the temperature and the pressure of the mobile phase to keep it as a supercritical fluid, could prevent SFC from being considered for routine use.

3.1.3. Extraction procedure

Analytical laboratories aim to develop sample preparation techniques that are accurate, reproducible, robust, simple, cost-effective, time-efficient, and safe (non-toxic and less organic solvents). Common examples of isolation techniques which could be applied to extract plasticizers from PVC medical devices can be divided into two types: solid–liquid extractions and head-space methods.

Table 4 is a comparison of potentially suitable extraction techniques for the alternative plasticizers in medical devices.

According to some literature articles, the traditional extraction techniques are occasionally used because of a combination of criteria that are suitable for routine procedures. The separation methods include the traditional Soxhlet technique, solvent extraction, or a method that firstly dissolves the whole polymer and then separates the plasticizers from the PVC by precipitation.

The Soxhlet method was the most commonly used semi-continuous method for the extraction of polymeric additives [64] and remains the standard against which the performance of newer techniques are compared. It has been the subject of numerous

Table 4
Comparison of possible extraction methods for analysis of alternative plasticizers to DEHP in PVC medical devices (adapted from Moller et al., 2008).

Method	*Polymer dissolution*	*Solvent extraction*	*Soxhlet*	*ASE (=PSE)*	*MAE*	*UAE*	*SFE*	*HS*	HS-SPME
Main solvent	– Toluene, THF, – EtOH, MeOH,	Hexane, Et_2O $CHCl_3$, dichloromethane	Ethylacetate, Et_2O	2-propanol, acetone, ACN, cyclohexane	Solvents with microwave absorbing component: 2-propanol, hexane, isooctane, MeOH, EtOH	Ethyl acetate	Mainly CO_2	No	No
Solvent volume	> 10 mL	50–100 mL	> 100 mL	< 50 mL	10–50 mL	5–50 mL	Low (analytes collected)	No	No
Sample size	1 g	1–5 g	1–5 g	< 1 g	0.5–1 g	0.5–5 g	< 1 g	< 1 g	< 1 g
Analysis time	> 60 min	30 min–24 h	6–24 h	10–15 min	5–30 min	15–60 min	< 60 min		5–60 min
Advantages	Easy, inexpensive	Possibility to dissolve small molecular weight compounds	High temperature during procedure, inexpensive equipment	Short extraction time, low solvent consumption, high reproducibility	Fast and effective, low solvent use, several samples in one extraction (10–14), controlled pressure and Temp °C	Simple, less expensive, several samples in one extraction	Low solvent use, fast, low critical temperature and pressure, high purity and low toxicity, low critical temperature and pressure, high purity and low toxicity	Inexpensive, no complex equipment	Allows selective extraction, low cost, fast
Disadvantages	Uses volatile solvents, cleanup steps, time	Choice of solvent (solubility) which provides specific extraction efficiency	Time consuming extraction, large amount of solvent, environmental risk	Expensive, time consuming sample preparation, difficult choice of solvent	Expensive equipment, reproducibility problems	Not always effective	Difficult to optimize, expensive equipment	Only for volatile compounds	Only for volatile compounds
Extraction yield	High	High	High	Very high	High		Medium-yield	Medium	?
Optimization	Repeated extractions	–	High pressure, automated or microwave-assisted Soxhlet extraction		+CPEExtraction conditions	Use of a mechanical shaker	Adding a modifier which increases the extraction of polar compounds		

EtOH: ethanol; MeOH: methanol; THF: tetrahydrofuran; EtO2: diethylether; CHCl3: chloroform.
ASE: accelerated solvent extraction; PSE: pressurized solvent extraction; MAE: microwaves extraction; UAE: ultrasonic-assisted extraction; SFE: supercritical fluid extraction; HS: head-space; and HS-SPME: head-space-solid phase micro extraction.

papers [65]. Acting as a continuous-discrete technique, analytes from the polymer are extracted by repeated washing with an organic solvent under reflux in special glassware. To this end, the system remains at high temperature over long extraction periods, which may result in decomposition of thermolabile target species. This was the case for the DEHA and DEHP additives when extracted with diethylether by Wang et al. [42].

Despite recent improvements in the Soxhlet technique, including the use of high-pressure, automated or micro-assisted Soxhlet extractors, it is considered as obsolete in many laboratories mainly due to the equipment, the large volume of solvent needed, and the long procedure times.

Solvent extraction at room temperature may be an efficient and simple alternative with good recovery rates. Classical solvent extraction is a phase transfer of solutes from the solid phase to solution. The mechanism of this extraction procedure is based on a transport process which depends on a variety of physical properties such as diffusion, viscosity, partitioning, solubility, and surface tension. Thus, the choice of the solvent is the most important factor determining extraction efficiency. The technique is easy and reproducible, very suitable for routine application, and is appropriate for the plasticized PVC of medical devices. In the study by Wang et al., the simple room temperature extraction using chloroform was found to be the most efficient method for extracting alternative plasticizers like DEHA [42].

Polymer dissolution is also a solid–liquid extraction procedure that may be used to extract alternative plasticizers from medical device samples. The whole polymer, i.e., PVC, is first dissolved in a solvent like tetrahydrofuran (THF) or dimethylacetamide. The dissolution is then followed by alcohol re-precipitation of the polymer (e.g., using ethanol or methanol), which enables the separation of the plasticizers [42,36]. This technique is easy and inexpensive, providing good extraction yields. However, it is considered too time-consuming. Indeed, exhaustive extractions may require several dissolution/precipitation steps, extended extraction times, or changes in solvents and temperatures to obtain quantitative results. Moreover, the large excess of solvent used for dissolution might interfere with the interpretation of the chromatogram, obscuring some of the peaks of interest.

Besides these traditional extraction methods, recent techniques using high pressure during extraction have been developed. In ASE (accelerated solvent extraction), MAE (microwaves extraction), UAE (ultrasonic-assisted extraction), and SFE (Supercritical Fluid Extraction), high pressure accelerates solvent extraction by forcing the solvent into the matrix pores. These techniques may theatrically be applied to the extraction of alternative plasticizers from PVC medical devices. They may provide good recovery values, but only if used under optimized conditions. While conventional methods of polymer extraction use large quantities of chlorinated solvents, these recent high pressure techniques are more appealing because of low solvent consumption. Also, the extraction may be performed either off-line or on-line with the analytes being sent directly to a chromatograph or a spectrometer, especially in SFE.

An on-line SFE could be suitable for trace analysis of PVC plasticizers where analytes are transferred directly to assay instruments. Cano et al. demonstrated the successful and highly reproducible extraction of DEHA and DINP from PVC plastisols [66]. Moreover, the difficulties encountered with the HPLC separation of plasticizers with similar structure and similar retention times (e.g., DINP and DINCH) may be overcome by combining SFE with SFC. Despite the generally lower precision of this technique, the on-line SFE-SFC method for the quantitation of polymer additives appears to be sufficiently reliable and robust for application in routine quality control analysis, as demonstrated by Zhou et al. [67].

Microwaves extraction (MAE), which consists of heating the extraction solvent or sample with electromagnetic radiation, can be applied to the extraction of plasticizers in medical devices. The advantages of this method are numerous. MAE is fast and effective, requires low-solvent use, and can handle several samples in one extraction. The technique has been used to extract DEHA and DINP from plastisols [66]. According to the authors, after condition optimization in terms of the choice of the extraction solvent, microwave power, temperature, and number of vessels, the whole DEHA content was extracted in only 10 min using MeOH at 120 °C. MAE belongs to the group of techniques where the extraction solvent is kept under high pressure during extraction.

The main disadvantages of the technique include the need of specific and expensive equipment and the long length of time for the optimization of the extraction conditions in order to have sufficient recovery. The limiting factor is the diffusion of the plasticizers to the surface of the medical device. This diffusion-limited extraction process has been described by the “hot ball” model of Bartle et al. [68]. The choice of the extraction solvent is essential, and can partly predict the diffusion of the plasticizers due to its solubility parameters.

Head-space (HS) extraction methods enable the identification and quantification of analytes from solid samples like PVC medical devices. HS methods are often coupled online with gas chromatography (HS–GC). HS–GC is an automated, reproducible, and inexpensive method. The efficiency of the technique may be improved by coupling HS with solid phase microextraction (SPME), which greatly increases sensitivity and provides highly selective extraction.

In Frankhauser-Noti's study, an injector-internal thermal desorption was performed in order to extract a range of plasticizers, including DINP, DEHA, and ATBC, from edible oils [69] The technique, which can be considered as a dynamic headspace analysis, required high temperatures in order to achieve complete desorption and the transfer of the analytes into the GC column. The authors demonstrated that using this extraction technique made the GC–MS detection of the plasticizers easier, allowing a detection limit below 0.1 mg/kg for plasticizers forming single peaks and 1 mg/kg for mixtures of isomers, like DINP. Also, the analysis presupposes a higher injector temperature than usual. This may cause evaporation of the bulk material, whose other components may enter into the column. Frankhauser-Noti overcame this problem by back flushing a coated pre-column toward the end of each run.

However, the extraction efficiency is affected by the volatility of the analytes. The vapor pressure of most of the alternative plasticizers is low, particularly for TOTM (see Table 2). This is a barrier for analysis by HS–GC

3.2. Analysis in human body fluids: methods for the assessment of human exposure

In order to assess patient exposure to the alternative plasticizers and to characterize the risk they induce, human biomonitoring studies are essential.

Human biomonitoring represents an unambiguous assessment and allows actual individual exposure to be quantified for each subject, independent of the various possible routes of external exposure [70].

In some cases, a useful and convenient alternative solvent to the biological fluid is more suitable for use in a screening test to evaluate the release of the plasticizers from the medical devices that have contact with such fluids.

3.2.1. Parent molecule analysis

Human biological monitoring includes the measurement of a parent chemical of interest in the human body or other tissues. For alternative plasticizers, such studies mean the direct contact between the medical device and a biological fluid, e.g. the contact between the patient's blood and a device used in a cardiopulmonary bypass procedure or for hemodialysis. It is also the case for blood transfusions or plateletpheresis procedures, where blood may extract plasticizers from the PVC of the transfusion set. A sample preparation is required for the preconcentration of the plasticizers due to the complex sample matrix.

As shown in Table 5, very few studies have measured the levels of parent plasticizers in biological matrices. TOTM concentrations have been determined in blood from chronic renal failure patients undergoing chronic hemodialysis treatment, which has been shown to be a medical procedure with a large risk of exposure [4]. Two different chromatography methods have been employed, both of which enable TOTM quantification at low levels (in the ng/mL range) [71,16]. In another study by Fromme et al., DEHA and DINP were detected in breast milk [72]. In these three studies ([71,16,72]), a traditional liquid–liquid extraction procedure using an organic solvent was required. Moreover, due to the complex nature of the breast milk matrix, Fromme et al. performed a subsequent clean-up of the extracts using selective pressurized liquid extraction (sPLE). High oven temperatures for GC analysis were also needed for plasticizer determination.

In general, plasticizer parent molecules are extracted in large amounts from the PVC matrix since the PVC MDs used during at-risk medical situations contain high quantities of plasticizers ranging from 30 to 40% of the PVC mass (see Table 1). GC is widely used for the detection of these molecules because it is simple, rapid and senstitive. HPLC is also useful for the analyses of organic compounds and may be superior to GC when it is used for the determination of compounds with high boiling points, i.e alternative plasticizers to DEHP.

3.2.2. Simulants

Studies conducted on the types of medical devices that have contact with body fluids usually need to recruit volunteers to collect blood or milk from patients, which could be very difficult or impractical when a certain type of exposure assessment is needed. Moreover, leaching measurements are complicated because the additional media that are in contact with the PVC have complex and variable compositions. It is usually not practical to study real materials.

Consequently, an alternative contact medium is often preferred and simulants have been developed to resemble a group of products. Leaching tests are used to evaluate the leaching capacity of the different plasticizers present in PVC MDs into the contacting solutions or biological fluids under close to real clinical conditions. To reach this aim, optimization of the test conditions (i.e., choice of simulants, temperature, contact time, mechanical aggressions, etc.) and the analytical method used are crucial, whether they include a previous extraction step or not.

According to the literature, gas and liquid chromatographic methods with MS detection are the most widely employed techniques. These methods are suitable for the detection and quantification of plasticizer amounts over a broad range of values, depending on the media and the plasticizer [73]. In the work by Luo et al., an ethanol/water mixture was used as an extraction screening vehicle in order to evaluate DEHP released from medical devices that come into contact with human blood or blood components [74]. Without any details on either the preparation of the simulant or the conditions used for simulating the clinical use of the devices, Luo et al. showed that GC–MS could be an appropriate technique for the determination of the amount of plasticizer in an alternative extraction solution. However, there were some difficulties in determining the detection and quantification limits of the method due, in particular, to an important DEHP background that resulted from various contamination sources.

Two different methods, one stringent and one a simulated method, have been developed to estimate DINP migration into child saliva [75]. Both methods correspond closely to the mean oral contact time of the toys with young children. Although selected ion monitoring (SIM) provides higher sensitivity, all analyses were performed in a fullscan mode in order to discriminate between unexpected additives. This study highlights the importance of the reliability of the analytical method in order to compare results obtained from the various participating laboratories.

Gas chromatography was also used to quantify DINCH release from PVC into an aqueous simulant matrix following liquid–liquid extraction (LLE) performed with chloroform [44]. Among the different “green” plasticizers tested, DINCH was found to be the least likely compound to migrate from the PVC into water, compared to the DEHP reference.

The capacity of plasticizers to migrate into IV drug solution simulants has been investigated in three different studies [76,77,15]. However, the data from these studies are not comparable due to the plasticizers studied (DINCH, TOTM, or DEHT), the simulant nature (fatty or aqueous), and the infusion conditions used (contact time and dynamic or static process). Although both Welle and Wirnitzer failed to describe the analytical procedure used, both found that TOTM or DEHT were released to a lesser extent than DEHP. In both cases, larger amounts of plasticizers were released into lipophilic intravenous preparations, probably due to the nature of the surfactant added to the pharmaceutical product as a solubilizer [15]. In the agri-food sector, such migration tests should be performed using vegetal oils or the allowed substituents (95% ethanol or isooctane) as the referent simulant for fatty foods [9].

Table 6 summarizes the little data available in the literature on the analytical method employed to detect and quantify alternative plasticizers in different simulants.

3.2.3. Analysis of metabolites

Human biomonitoring also consists of the identification and quantification of the metabolites of the molecules of interest that are present in body fluids. This avoids significant errors caused by

Table 5
Methods for concentration determination of alternative plasticizers from human fluids matrix

Separation technique	Matrix	Stationary phase	Detection	Sample preparation technique	Compound of interest of medical applications	Limit of detection (μg/L)	Limit of quantification (μg/L)	Reference
LC–UV	Blood	C18 (4.6 × 150 mm; 5 μm)	UV	LLE	TOTM	–	25	[16,9]
GC–MS?	Blood	?	MS		TOTM			[71,7]
GC–MS	Breast milk	Capillary column (30 m × 0.25 mm, 0.5 μm)	EI-quadrupole	LLE + sPLE	DINP and DEHA	0.1 ng/g		[72,8]

Table 6
Methods for concentration determination of alternative plasticizers from simulant matrix.

Separation technique	Matrix	Stationary phase	Detection	Sample preparation technique	Compound of interest for medical applications	Limit of detection (μg/mL)	Limit of quantication (μg/mL)	References
GC–MS	Saliva simulant solution	50% dimethyl–50% diphenylpolysiloxane column (30 m × 0.25 mm; 0.15 μm)	EI	LLE	DINP	2.5–3.5	–	[75,4]
Gravimetricanalysis	De-ionized water				DINCH	–		[44,7]
GC–MS		NA	NA	LLE		NA		
LC–MS/MS	IV pharmaceutical solutions	C8 (2.1 × 50 mm; 5 μm)	ESI-quadrupoleQ_3	SPE on-line on Capcell PAK 5u C18-MG-II	TOTM	0.0005	0.001	[15,8]
GC–MS	Middle-chain-triglycerideemulsion Balanced cristalloid solution Balanced crystalloid solution with 10% ethanol				DEHT			[76,3]

contamination by ubiquitous parent substances, which can occur during sample collection, transportation, storage, and throughout the analytical process [70]. Moreover, the assessment of these metabolites corresponds to the measurement of the biologically active species of the parent compounds. It has previously been shown that DEHP (and other phthalates) metabolites, in particular the monoesters, are associated with many of the toxic endpoints produced by exposure to DEHP.

For such studies, the techniques require sophisticated and reliable analytical instruments and methods in order to detect very small amounts of plasticizers or their metabolites in human biological samples. A high separation capability is required as well as the possibility of detecting extremely small amounts of plasticizers.

Traditionally, concentrations of non-persistent pollutants, like the alternative plasticizers, are measured in urine samples because urine collection procedures are very simple, noninvasive, straightforward, and enable the collection of large volumes of urine with very little discomfort to the study participants [78].

Plasticizer metabolites (as opposed to parent compounds) are the specific biomarkers of exposure. In general, the concentrations of plasticizer secondary metabolites are higher than the monoester metabolites, which are not considered as a sensitive exposure biomarker. There is no current biomonitoring data concerning exposure to a medical device. The leachability and the toxic risk related to MD-induced exposure cannot be estimated. Moreover, the availability of biomonitoring data on alternative plasticizers is very limited. Data from studies conducted to measure these metabolite concentrations in biological fluids are summarized in Table 7.

LC is the preferred method to analyze metabolites of alternative plasticizers, mainly due to the high separation capability and because it is a conventional technique in most laboratories conducting trace analysis. As shown in Table 7, the combination of LC and mass spectrometry (LC–MS) is frequently used in biomonitoring studies due to the sensitivity, specificity, and speed offered by MS. MS is widely considered to be the most sensitive and discerning detector for LC analysis.

Studies on the identification of plasticizer metabolites in urine samples have been especially performed on two alternatives plasticizers, DINP and DINCH. Most have been carried out after oral doses, occupational or environmental exposure, on different specific population groups or on the general population, and with various sample sizes. However, the metabolite concentrations reported in these studies are very similar and have been detected at low levels. As expected, the concentrations of the monoester MINP were often under the detection limit (see Table 7). For DINCH, the primary metabolite MINCH also proved to be a very weak biomarker of DINCH exposure with only one urine sample above the LOQ, according to the work by Schütze [24].

The accuracy and reliability of the method employed are of greater importance as both plasticizers are available as a mixture of isomers, which cannot be differentiated. Koch and Angerer [79] chose not to chromatographically resolve all of the different oxidized DINP isomers but rather to integrate each m/z signal over the time range of metabolite elution. Similarly, the cis and trans isomers of the DINCH metabolites could not been distinguish by Schütze because of the same retention characteristics and the same fragmentation patterns and responses. Therefore, the chromatographic results represented the sum of the cis and the trans isomers [24]. Although Silva et al. [28] successfully differentiated the specific secondary metabolites MEHHA (mono-2-ethylhydroxyhexyl adipate) and MEOHA (mono-2-ethyloxohexyl adipate) as two separate peaks., the co-eluted isomers of each metabolite were not separated and were analyzed together to facilitate their detection. In contrast to the phthalate plasticizer DEHP, the

Table 7
LC methods for determination of trace levels of metabolites of alternative plasticizers from urine samples.

Separation technique	Stationary phase	Detection	Sample preparation technique	Compound of interest for medical applications (dosage of its metabolites)	Limit of detection (μg/L)	Limit of quantification (μg/L)	Reference
LC/LC–MS/MS	RP C18 (dC18 2.1 × 150 mm; 3 μm)	ESI-ion trap	SPE on-line on Capcell PAK 5u C18-MG-II	DINCH	–	MINCH and CHDA 0,1; OH-, oxo- and cx-MINCH 0.05	[24]
LC/LC–MS/MS	RP C18 (dC18 2.1 × 150 mm; 3 μm)	ESI-ion trap	SPE on-line on Capcell PAK 5u C18-MG-II	DINCH	MINCH 0.05; OH-, oxo- and cx-MINCH 0.025	MINCH 0.1; OH-, oxo- and cx-MINCH 0.05	[25]
LC/LC–MS/MS	Fusion-RP (3 × 250 mm; 4 μm)	ESI-quadrupole Q_2	SPE on-line on LiChrospher RP-8 ADS, 25 μm, 25 mm × 4 mm	DINP	0.25	0.5	[95]
LC/LC–MS/MS	RP C18 (dC18 2.1 × 150 mm; 3 μm)	ESI-quadrupole Q_2	SPE on-line on Capcell PAK 5 u C18-MG-II	DINP	–	0.25	[97]
LC/LC–MS/MS	RP C18 (dC18 2.1 × 150 mm; 3 μm)	ESI-quadrupole Q_2	SPE on-line on LiChrospher RP-8 ADS, 25 μm, 25 mm × 4 mm	DINP	0,1	0.2	[98]
LC–MS/MS	NA	NA	SPE on LiChrospher RP-8	DINP	-	MINP 4; OH-, oxo- and cx-MINP 1	[99]
LC/LC–MS/MS	Fusion-RP (3 × 250 mm; 4 μm)	ESI-quadrupole Q_2	SPE on-line on LiChrospher RP-8 ADS, 25 μm, 25 mm × 4 mm	DINP		0,25	[100]
LC/LC–MS/MS	Fusion-RP (3 × 250 mm; 4 μm)	ESI-quadrupole Q_2	SPE on-line on LiChrospher RP-8 ADS, 25 μm, 25 mm × 4 mm	DINP	0.25	0.5	[101]
LC–MS/MS	Betasilphenyl (2.1 × 100 mm; 3 μm)	ESI-quadrupole Q_3	SPE	DINP	MINP 0.36; OH-, oxo- and cx-MINP 0.25	–	[93]
LC/LC–MS/MS	Fusion-RP (3 × 250 mm; 4 μm)	ESI-quadrupole Q_2	SPE on-line on LiChrospher RP-8 ADS, 25 μm, 25 mm × 4 mm	DINP	0.25	–	[102]
LC/LC–MS/MS	Fusion-RP (3 × 250 mm; 4 μm)	ESI-quadrupole Q_2	SPE on-line on LiChrospher RP-8 ADS, 25 μm, 25 mm × 4 mm	DINP	1	–	[79]
LC–MS/MS	Fusion-RP (2 × 75 mm; 4 μm)	ESI-quadrupole Q_3	Automated SPE	DINP	MINP 0.61; OH-MINP 0.26, oxo-MINP 0.25 and cx-MINP 0,11	-	[103]
LC–MS/MS	Phenyl-hexyl (3 × 150 mm; 3 μm)	ESI-quadrupole Q_3	Protein precipitation	DINP	MINP 1.5; OH-MINP 1, oxo-MINP 0.5 and cx-MINP 1.3	-	[104]
LC/LC–MS/MS	Fusion-RP (2 × 75 mm; 4 μm)	ESI-quadrupole Q_2	SPE on-line on LiChrospher RP-8 ADS, 25 μm, 25 mm × 4 mm	DINP		0.5	[105]
LC–MS/MS	Fusion-RP (3 × 250 mm; 4 μm)	ESI-quadrupole Q_2	SPE on-line on C18 PhenomenexPrimesphere 30 × 4.6 mm; 5 μm	DINP		0.25	[96]
LC–MS/MS	Fusion-RP (2 × 75 mm; 4 μm)	ESI-quadrupole Q_2	Automated SPE	DINP	MINP 0.62; OH-MINP 0.31, oxo-MINP 0.16 and cx-MINP 0.10	–	[106]

Table 7 (*continued*)

Separation technique	Stationary phase	Detection	Sample preparation technique	Compound of interest for medical applications (dosage of its metabolites)	Limit of detection (μg/L)	Limit of quantification (μg/L)	Reference
LC/LC–MS/MS	Betasil phenyl (2.1 × 150 mm; 3 μm)	ESI-quadrupole Q_3	SPE on-line on Chromolith Flash RP-18 (2 μm, 25 mm × 4.6 mm)	DINP	MINP 0.8; cx-MINP 0.7	–	[107]
LC/LC–MS/MS	Phenyl-hexyl 150 × 4.6 mm, 3 μm	ESI-quadrupole Q_2	SPE on-line on LiChrospher RP-8 ADS, 25 μm, 25 mm × 4 mm	DINP	–	0.2	[108]
LC–MS/MS	Fusion-RP (2 × 75 mm; 4 μm)	ESI-quadrupole Q_2	Automated SPE	DINP	MINP 0.15; OH-MINP 0.12, oxo-MINP 0.11, and cx-MINP 0.05	-	[109]
GC–MS	ChrompackCPSil 52CB (25 m × 0.32 mm, 0.5 μm)	EI	LLE (NaOH+HCl+diethylether)	DEHA	-	-	[26]
GC–MS	BP1 column (25 m × 0.32 mm × 0.5 μm)	EI	LLE (NaOH+HCl+diethylether)	DEHA	-	-	[27]
LC–MS/MS	Betasil phenyl (2.1 × 150 mm; 3 μm)	ESI-quadrupole Q_2	SPE on-line on Chromolith Flash RP-18 (2 μm, 25 mm × 4.6 mm)	DEHA	0.5		[28]

"specific background exposure" of the alternative plasticizers is not the main metabolite but other sensitive compounds and their isomers that are present at very low concentrations.

Electronspray ionization (ESI), in combination with single ion monitoring (SIM), was used in all studies presented in Table 7 to determine the presence and quantity of DINCH or DiNP metabolites in urine. This approach offers versatility, sensitivity, and selectivity for trace analysis, which are all criteria followed in biomonitoring studies. Ion-trap detection was used for DINCH metabolite analysis, although it is less selective than a quadrupole analyzer. The later was, however, a better solution for the detection of DINP and its discrimination from other phtalates because of higher signal-to-noise ratios and selectivity. More effective analyzers, like time-of-flight analyzers, may improve resolution and mass accuracy of the detected analytes and might increase selectivity. However, in the case of exposure to alternative plasticizers from MDs, especially in high risk clinical situations and considering only the 6 alternative plasticizers, such advanced analysis technologies may not be absolutely necessary. In any case, detection and quantification within a range of 0.05–0.5 μg/L requires separation and concentration steps before analysis. Common examples of isolation techniques for the separation of phthalates from biological matrices, and which can be found in the literature, include liquid–liquid extraction (LLE), solid phase extraction (SPE), cloud point extraction (CPE), solid-phase microextraction (SPME), and stir bar sorptive extraction (SBSE) [80]. For DINP and DINCH determination from urine samples, automated SPE has been frequently used because of the high levels of recovery, high volumes of sample throughput, and good reproducibility. C18 sorbent, which is the most commonly used sorbent may be optimized as required by the addition of nanomaterial.

Loftus et al. used GC–MS to determine and quantify DEHA metabolites in urine after oral dose [26,27], while more recently, Silva et al. were the first to characterize the specific biomarkers of DEHA exposure using LC–MS to a sensitivity of 0.5 ng/mL (LOD) [28].

3.2.4. *Other techniques for the assessment of exposure*

Other techniques have recently been developed to assess the human exposure to plasticizers, but only for DEHP or other phthalates. A new direct competitive ELISA method for detecting MEHP has been developed and applied to real assay samples where it demonstrated a high detection rate in human urine [81]. The recovery rates from a MEHP-spiked matrix ranged from 87.4% to 91.78% with CVs less than 5%. The technique seems to be specific, sensitive, and effective. However, by comparing it with a traditional HPLC method, a little discrepancy was detected that highlighted a lack of specificity of the Elisa technique, thus leading to an approximation of the MEHP concentration in the human urine. Moreover, this immunoassay needs specific equipment and antibody preparation, which may not be suitable for routine use.

An alternative to chromatographic methods is capillary electrophoresis (CE) or capillary zone electrophoresis (CZE), which enables the separation and quantification of various analytes in a single run based on molecular size, charge/mass ratio, and isoelectric points (the differences in electrical field-induced migration properties of the analytes and the run buffer) [61]. Use of CE to determine trace amounts of plasticizers or their metabolites in, for example, urine samples is limited because of low sensitivity, i.e., low concentrations in low sample volumes. There is no data on the use of CE in detecting alternative plasticizers. A few studies have used the technique to separate phthalate esters from aqueous media [82–85,9]. Huang et al. developed a novel technique based on non-aqueous capillary electrophoresis which was able to separate organic compounds, like phthalates, using the MEKC

(miscellaneous electrokinetic chromatography) separation mechanism [86]. However, their study required the optimization of several factors. It is also difficult to know if such a process can be applied to the 6 alternative plasticizers incorporated in MDs.

4. Conclusion

Much emphasis has been placed on the alternative plasticizers to DEHP, especially those integrated into medical devices. This is due to their widespread use and the direct exposure of hospitalized patients. The main risk is their ability to leach out of the PVC matrix of the MD, which is related to their overall characteristics and their compatibility with PVC.

This review suggests that several techniques are available to analyze the alternative plasticizers within different matrices. The identification and quantification of the plasticizers within the MDs can be directly performed by rapid and destructive, or non-destructive, techniques that are currently in routine use. However, other methods could be preferentially applied to identify and quantify these chemical substances within the various solutions that they are in contact with, i.e., infused medical solutions, nutrition admixtures, or alternative solvents, which are also known as simulants. Finally, advanced and sensitive analytical techniques are employed to detect and to quantify the plasticizers in complex matrices such as biological fluids.

These techniques seem to meet different objectives:

- firstly, to determine the exact composition of the medical devices, particularly the amount of the plasticizer used to soften the PVC matrix,
- secondly, to assess the leaching rate of the plasticizers from these devices, and consequently the doses to which patients are exposed to on a daily basis,
- and finally, to assess the real multiple exposures of patients in at risk medical situations by measuring trace levels of their specific metabolites in 24-hour urine samples.

These required objectives correspond to the ARMED (Assessment and Risk Management of Medical Devices in plasticized polyvinylchloride) study group's scientific commitment to the interests of the community, which is supported by the French Medicine Agency (ANSM: Agence Nationale de Sécurité des Médicaments et des Produits de Santé) within the framework of the 2012 call for proposals on health commodity security.

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