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Basic and practical concepts of radiopharmaceutical purification methods

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The presence of radiochemical impurities in a radiopharmaceutical contributes to an unnecessary radiation burden for the patients or to an undesirable high radioactivity background, which reduces the imaging contrast or therapeutic efficacy. Therefore, if the radiolabeling process results in unsatisfactory radiochemical purity, a purification step is unavoidable. A successful purification process requires a profound knowledge about the radiopharmaceuticals of interest ranging from structural features to susceptibility to different conditions. Most radiopharmaceutical purification methods are based on solid-phase extraction (SPE), high-performance liquid chromatography (HPLC), size exclusion chromatography (SEC), ion-exchange chromatography (IEC), and liquid–liquid extraction (LLE). Here, we discuss the basic and applied concepts of these purifications methods as well as their advantages and limitations.

Introduction

Pharmaceuticals that incorporate a radionuclide are called radiopharmaceuticals. This group is divided into two main subgroups: diagnostics and therapeutics. All radioactive nuclides undergo decay with a unique half-life and decay mode; this determines the type of their application in nuclear medicine. Diagnostic radiopharmaceuticals include gamma-ray- or a positron-emitting radionuclides because their radiation has low linear energy transfer (LET) and biologic effectiveness (RBE), but is body-penetrating [1]. If a gamma-ray-emitting pharmaceutical is used, the imaging modality is single-photon emission computed tomography (SPECT), whereas, in the case of positron emitting, the modality is positron emission tomography (PET). Therapeutic radiopharmaceuticals contain an alpha particle-, beta particle- or Auger electron-emitter with high LET. For therapy, high RBE radiation of these radionuclides is preferable [1]. Nuclear medicine and biomedical imaging research advances depend on the development of diverse radiotracers. Production of radiotracers require a compatible strategy with the radionuclide half-life, structural characteristics of the final molecule or its precursor(s), and their *in vivo* application [2]. Examples of labeling strategies for radiopharmaceuticals are discussed below.

Direct labeling

In direct labeling, a radionuclide is attached to the molecule via nucleophilic substitution and isotopic exchange. The most well-known examples of substitution or isotopic exchange are 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]-FDG) and ^{131}I-metaiodobenzylguanidine ([^{131}I]-MIBG), respectively [3,4]. In addition, ^{11}C labeling through *O*- and *N*-methylation is an example of direct labeling [5]. Metallic radionuclides can also be used to label a molecule directly. For example, peptides can be labeled with ^{99m}Tc through their thiol functional group [6].

Indirect labeling

Bifunctional chelating agents (BFCAs) are typically necessary for indirect labeling with radiometals. Derivatives of BFCAs, such as diethylenetriaminepentaacetic acid (DTPA), have been used to label a variety of molecules with radionuclides, such as ^{177}Lu [7] and ^{90}Y [8]. In addition, prosthetic groups, such as tert-butyl[^{18}F]

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fluorosilyl)benzoate ([^{18}F]SiFB), can be used for indirect labeling with ^{18}F [9].

The common drawback of direct and indirect labeling methods is that neither of them guarantees a high radiochemical purity (RCP) of the final product. RCP (i.e., the percentage of radionuclide ions in the desirable chemical form) is one of the most important characteristics of a radiopharmaceutical [10]. The presence of radiochemical impurities increases the background radiation, reduces the target:nontarget ratio, and contributes to an unnecessary radiation burden for the patients without adding to the diagnostic information or improving treatment [11]. In the case of diagnostic radiopharmaceuticals, radiochemical impurities can impact the interpretation of scans and imaging accuracy [12]. Impurities in the therapeutic radiopharmaceuticals can also result in adverse effects [13]. In this review, we focus on the different methods available for the purification of radiopharmaceuticals. For each method, we highlight the basic concepts and procedure details as well as ways to improve purification of the end product.

Selection of a purification method for radiopharmaceuticals

All the data relating to the desired compound and its contaminants should be collected to enable the efficient purification of the radiopharmaceutical. A range of parameters for the method selection should be considered, including molecular weight, lipophilicity, stability in the mobile phase, the charge of the molecule [14–17], and, most importantly, the half-life of the radionuclide [18].

Molecular weight

One of the parameters that can narrow the method selection is molecular weight (M_w g/mol) (Fig. 1). If the radiotracer has a high molecular weight, such as labeled biomacromolecules, the purification method of choice is SEC. The SEC can be performed with size-exclusion HPLC (SE-HPLC) or with a SEC cartridge, such as Nap™-5, Nap™-10, or PD-10 (GE Healthcare). However, purification can also be carried out based on the features of the contaminant(s) [19,20].

Lipophilicity

For highly lipophilic radiopharmaceuticals, the purification method of choice is reverse-phase chromatography with C18 or C8 columns or cartridges. By contrast, hydrophilic radiopharmaceuticals can be purified with normal-phase chromatography. In some cases, LLE has been applied for purification of radiopharmaceuticals depending on the lipophilicity of the latter (Fig. 1).

Charge

For low-molecular-weight species, especially radionuclides or their complexes with small ligands, such as chlor or nitrate, IEC is the primary method of choice. For larger molecules, such as peptides, pH, and pI (isoelectric point), have a significant role in the purification process. The pH value of either reaction mixture or mobile phase impacts the purification [21,22].

Main parameters limiting effective purification

Factors limiting radiopharmaceutical purification include time, RCP, and radiochemical yield (RCY). These limitations originate from the fact that the radionuclide decays during the synthesis and purification of the radiopharmaceutical. Therefore, the purification process should provide maximum RCY and highest RCP in a minimum amount of time.

Time

The primary challenge to the selection of the radiopharmaceutical purification method is the purification process duration. Given radioactive decay, the selected method for purification must be as short as possible. To resolve this major limitation, different methods and efficient equipment have been introduced [23,24].

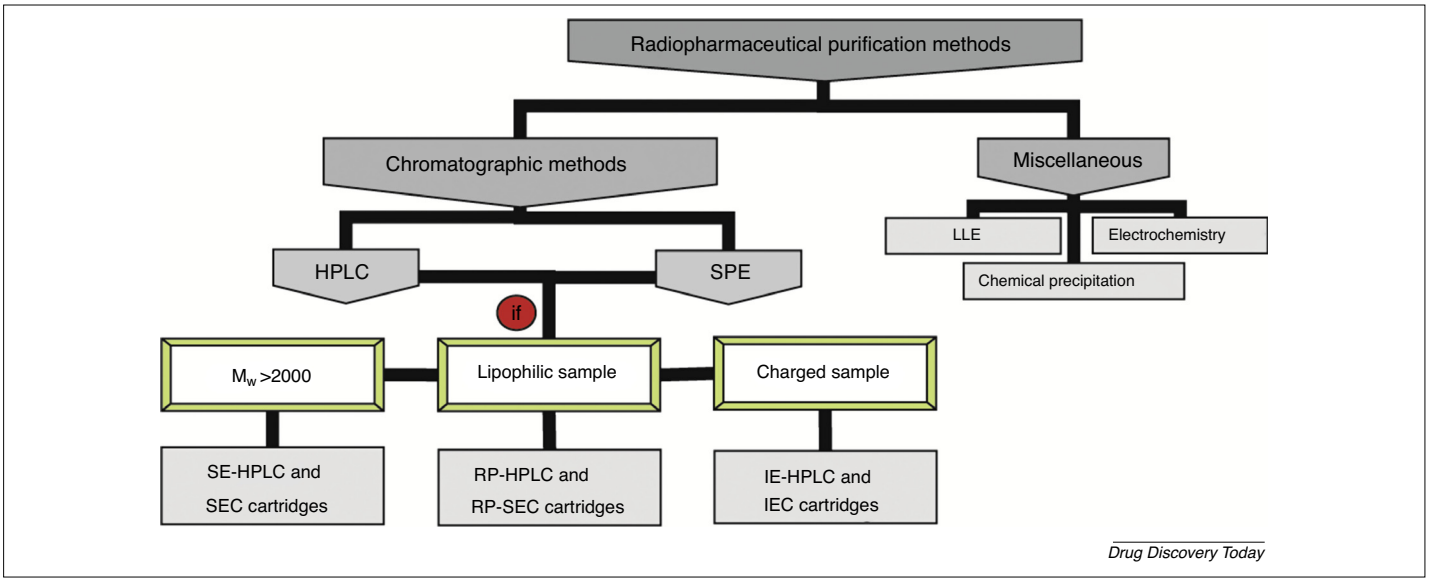

FIGURE 1

Classification of methods for the purification of radiopharmaceuticals. For definitions of abbreviations, please see the main text.

Radiochemical purity

High RCP will be achieved if the major part of the radionuclide is in the desired chemical form of the radionuclide. Typically, a RCP >95% is required, but the threshold might be reduced if the impurities are rapidly excreted [10].

Radiochemical yield

RCY is the activity ratio of the final product in the desired chemical form to the starting activity, expressed as a percentage. The RCY concept is applicable to both the labeling and purification processes. Therefore, an efficient purification process removes contaminant(s) and recovers the highest possible amount of the desired labeled compound. A purification process that provides high RCP might not necessarily provide high RCY [10].

Proper instruments

The type of the column or cartridge used affects the efficiency of the purification method. For example, different dead volumes of columns or cartridges influence the final concentration of the purified radiopharmaceutical [25]. Moreover, their capacity for the retention and recovery of the desired radiolabeled product varies. Even the back pressure caused by these columns or cartridges affects the purification process. The RCP can be unsatisfactory even if all the parameters in the purification process have been considered; thus, a combination of purification methods might be necessary, such as a combination of HPLC and a hydrophilic–lipophilic balance (HLB**)** cartridge [26].

Other parameters

Other parameters, such as sample concentration, temperature, mobile phase flow rate, and stationary phase composition and support, can impact the purification process.

Purification methods

The most frequently applied purification methods for radiopharmaceuticals are based on chromatography, such as normal and reverse SPE and HPLC, SEC, and IEC. Additionally, the use of LLE and chemical precipitation has been also reported.

HPLC

HPLC is an efficient technique for the separation, identification, and quantification of a mixture of components with a slight difference in their properties. Differences in the interaction of solutes with the column package lead to different elution rates for each component and result in the separation of compounds. The use of high pressure enables the use of smaller particles and increases the separation efficiency. Based on the solid adsorbent in the column (stationary phase), different kinds of HPLC exist (Fig. 2) [27]. A range of parameters, such as specification of mobile phase (polarity, flow rate, pH, and lipophilicity), sample matrix, type of stationary phase, and temperature, can affect the separation [28]. HPLC is a general and effective method for either the quality control or purification of radiotracers. This purification method provides high-resolution separation of a radiolabeled product from its precursor and other radiolabeled impurities or multiple radiolabeled species from a mixture. However, the use of this method can sometimes require a considerable amount of time, which is not favorable for radiopharmaceuticals labeled with short-lived nuclides. Nevertheless, this method has been applied to purify ^{11}C-labeled radiopharmaceuticals. For example, [^{11}C]β-CFT and [^{11}C]β-CIT were separated from their *N*-desmethyl precursors (nor-β-CFT and nor-β-CIT) by preparative HPLC on reverse-phase ODS (octadecylsilyl groups or C18 chemically bonded to a silica gel carrier) columns. It was observed that the separations of [^{11}C]β-CFT from nor-β-CFT and [^{11}C]β-CIT from nor-β-CIT depended on the mobile-phase pH and concentration because of the higher susceptibility of [^{11}C]β-CFT and [^{11}C]β-CIT to protonation as tertiary amines versus nor-β-CFT and nor-β-CIT as a secondary amine. The column type (non-end-capped type; YMC-Pack ODS-AL and end-capped type; YMC-Pack ODS-A) (Fig. 2) impacted the level of purification and the non-end-capped YMC-Pack ODS-AL purified the product more efficiently. Therefore, it was suggested that the silanol groups on the non-end-capped columns have an important role in separation (No. 1 in Table 1) [29]. Likewise, a semipreparative HPLC can be applied for purification of ^{11}C-labeled radiopharmaceuticals, including [^{11}C]raclopride and [^{11}C]DASB, for PET study of the brain. Given that the methylation during the labeling process increases lipophilicity and the susceptibility to protonation, the length of time of the purification process can be reduced by designing a purification process in which [^{11}C]raclopride and [^{11}C]DASB leave the column before their precursors (No. 2 and 3 in Table 1) [5]. Major disadvantages of this method are the time-consuming fraction collection and dilution of the product in a large amount of solvent because of the typically high flow rate of these columns. To resolve these drawbacks, a modified purification process for [^{11}C]raclopride and [^{11}C]DASB has been introduced that uses analytical HPLC columns instead of semipreparative columns [30].

A variety of ^{18}F radiotracers have been purified by using HPLC techniques. For the purification of 6-[^{18}F]fluoro-L-DOPA ([^{18}F]FDOPA) with HPLC, the influence of the stationary phase on the purification was investigated by using silica and polymer-based reverse-phase C18 columns. Interestingly, because of anion exchange between hydroxyl groups on the silica-based column and ^{18}F$^-$ anions in the sample, this column results in ^{18}F$^-$impurities in the purified product (No. 4 in Table 1) [31].

Facile clinical administration of ^{18}F requires automated radiosynthesis and purification procedures. A variety of conditions for the purification of [^{18}F]Fluspidine are available. Three hydroxyethyls (HE) polar end-capped silica-based C18 columns with two buffer systems (ammonium acetate and sodium dihydrogen phosphate) were evaluated. Here, the polar-nonpolar character of the columns favors purification. A slightly acidic mobile phase or addition of an organic solvent, such as acetonitrile, could reduce retention time and peak tailing (No. 5 in Table 1) [32,33].

A range of prosthetic groups have been used for site-selective labeling of biomolecules, such as small molecules, peptides, and larger molecules (including proteins) [34,35]. 4-(*p*-([^{18}F]fluorosulfonyl)phenyl)-1,2,4-triazoline-3,5-dione ([^{18}F]FS-PTAD) was introduced for the fluorination of peptides at their tyrosine (Tyr) residues. Even though the RCY of [^{18}F]FS-PTAD synthesis was high (91%), the labeling of Tyr with this prosthetic group resulted in low RCY (40–60%) for the final product ([^{18}F]FS-Tyr). HPLC was efficient in removing the unreacted prosthetic group as the main radiochemical impurity (No. 6 in Table 1) [36]. Larger molecules, such as radiolabeled peptides, also can be purified by HPLC. *N*-

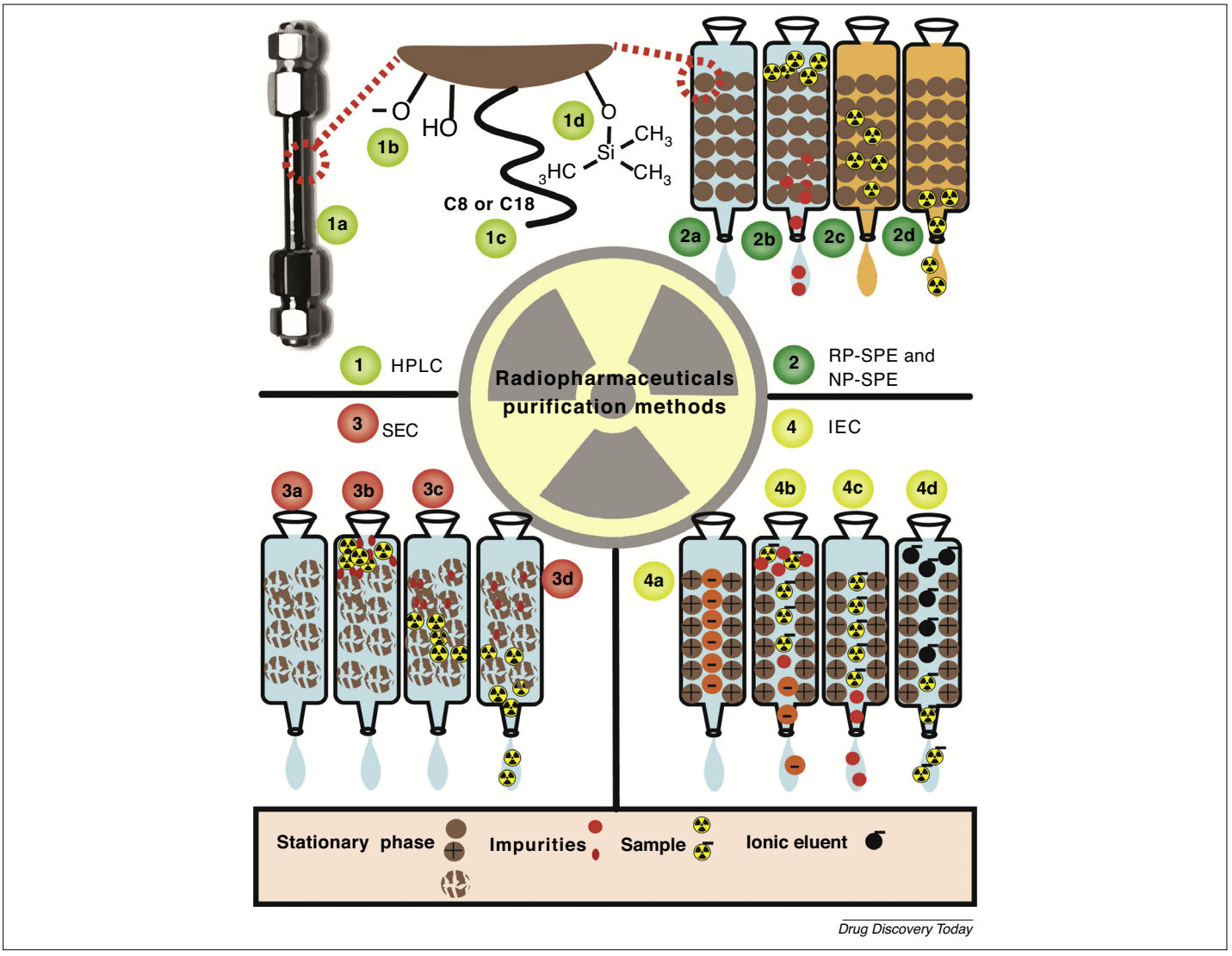

FIGURE 2

Schematic representation of radiopharmaceutical purification methods. (1) Reverse and normal phase HPLC (1a, HPLC column; 1b, silanol group in normal-phase column or cartridge; 1c, C8, C16, or C18 in reverse-phase column or cartridge; and 1d, end capping); (2) reverse and normal-phase SPE (2a, cartridge preconditioning; 2b,: loading the lipophilic/hydrophilic sample onto the cartridge and washing the polar impurities with a polar/nonpolar solvent; 2c,d,: eluting and collecting the pure sample with nonpolar and/or polar solvent); (3) SEC, mainly gel-filtration chromatography in the case of radiopharmaceuticals (3a, cartridge preconditioning with an aqueous solution; 3b, loading the sample onto the cartridge; 3c, impurities with lower molecular weights are retained; 3d, the sample has a high molecular weight and will leave the cartridge through the space between the packing particles before impurities); and (4) IEC, cationic or anionic exchange (the schematic represents anion exchange for the cationic exchange; all charges should be reversed; 4a, cartridge preconditioning with an anionic aqueous solution; 4b, loading the sample onto the cartridge; 4c, impurities with no or positive charge will leave the cartridge; 4d, the sample will be forced to leave the cartridge with an anionic solution). For definitions of abbreviations, please see the main text.

succinimidyl 4-[^{18}F]-fluorobenzoate (^{18}F-SFB) was used for labeling of Interleukin-2 (IL2). Unreacted ^{18}F-SFB and [^{18}F]fluorobenzoic acid (^{18}F-FBA), the main impurities of this reaction, were removed by RP-HPLC purification with a gradient elution (No. 7 in Table 1) [37]. For instance, direct labeling of glucose-Tyr^3-octreotate (gluc-Tyr^3-tate) and DOTA-Tyr^3-tate with ^{125}I produced gluc-^{125}I-Tyr^3-tate and DOTA-^{125}I-Tyr^3-tate, which were purified with HPLC. The purification step using an HPLC cartridge and a gradient elution improved the RCP of the final product (No. 8 in Table 1) [38].

Molecules with higher molecular weight can be purified with SE-HPLC. Radiolabeled HYNICs conjugated RGD and RGE peptides are useful for tumor imaging. During their synthesis and labeling process, purification of the cold and ^{99m}Tc-labeled RGD and RGE peptides (^{99m}Tc-RGD/E) is necessary. ^{99m}Tc-RGD/E can be purified by SE-HPLC using a phosphate buffer as the eluent (No. 9 in Table 1) [39]. In a similar case, ^{86}Y-labeled Herceptin^{TM} (^{86}Y-Herceptin^{TM}) was purified from free ^{86}Y with SE-HPLC (No. 10 in Table 1) [40].

Normal and reverse solid-phase extraction

SPE is a simple, fast, and powerful process that can separate dissolved or suspended compounds from other compounds in the mixture based on their physical and chemical properties. This technique was used for the synthesis, purification, and concentration of radiopharmaceuticals [41]. Similar to other liquid chromatography methods, it has a mobile and stationary phase. Two main types of SPE are normal and reverse-phase SPE, although other

TABLE 1

Examples of preparative HPLC for different radiopharmaceuticals

No.	Name	impurities	Column (stationary phase)	Mobile phase	Flow rate	RCY (%)	Final RCP (%)	Refs
1	[^{11}C]β-CFT and [^{11}C]β-CIT	Nor-β-CFT and nor-β-CIT	YMC-Pack ODS-AL (C18); YMC-Pack ODS-A (C18)	Acetonitrile/phosphate buffer (70:30)	–	–	>99.0	[29]
2	[^{11}C]raclopride	[^{11}C]CH_3I or [^{11}C] methyl triflate and non-radioactive precursors	Supelcosil™ Suplex pKb100 (C16)	Citrate buffer (pH 3, 0.025)/ ethanol (75:25)	1 ml/min	72.8	>99.0	[5]
3	[^{11}C]DASB		Alltima™ HP CN	Phosphate buffer (pH 7, 0.025 M)/ethanol (65:35)	1 ml/min	90.0	≥95.0	
4	[^{18}F]FDOPA	$^{18}F^-$	Silica-based reversed phase C18 column	–	–	50.0–60.0	>99.0	[31]
			Polymer-based reversed phase C18 column	–	–	–	93.0	
5	[^{18}F]Fluspidine	$^{18}F^-$	ReproSil-Pur® C18-AQ (C18 and HE)	Acetonitrile and ammonium acetate (56:44)	4 ml/min	37.0	99.3	[32,33]
6	[^{18}F]FS-Tyr	$^{18}F^-$ and [^{18}F]FS-PTAD	Nucleosil 100-5RP (C18)	MeCN/water (20:80) 0.1% TFA	1 ml/min	45.0	>99.0	[36]
7	^{18}F-FB-IL2	^{18}F-SFB and ^{18}F-FBA	Econosphere C18-column (C18)	0.1% TFA in water and 0.1% TFA in EtOH[a]	1 ml/min	20.0–35.0	>95.0	[37]
8	gluc-^{125}I-Tyr^3-tate and DOTA-^{125}I-Tyr^3-tate	^{125}I and different form of ^{125}I labeled peptide	LiChrospher® 100 RP-18 (5 mm) LiChroCART® 250-4 (C18)	NaCl 0.1 M and ethanol 95%[a]	1 ml/min	–	>99.0	[38]
9	^{99m}Tc-RGD/E	^{99m}Tc colloids and $^{99m}TcO_4^-$	–	Buffer phosphate (0.1 M, pH 7.2)	–	85.0–90.0	>95.0	[39]
10	^{86}Y- Herceptin™	–	TSK-3000 column	PBS	1 ml/min	–	100	[40]

types, such as ion exchange, are available (see below). Based on the desired compound characteristics (e.g., lipophilicity) and the type of SEP, the compound can be retained during the stationary phase or pass through it (Fig. 2).

Despite the simplicity of this method, its efficiency can be comparall even with HPLC [42]. This tool has been frequently utilized for pre-and postlabeling purification [43]. For reverse-phase cartridges, such as C18 Sep-Pak, the cartridge should be activated based on the manufacturer's instructions, usually by passing an organic solvent (ethanol) and water through it. The next steps are loading the reaction mixture on to the column, applying water to remove the impurities, and then eluting the product with an appropriate lipophilic eluent. Purification with SPE cartridges occurs when the cartridge retains most of the product while impurities freely leave the column or vice versa (Fig. 2). If the cartridge retains the product, a high percentage desorption should be achievable. Parameters, such as type of cartridge, cartridge conditioning, sample concentration, eluent flow rate, and lipophilicity, influence the efficiency of the purification step [44]. Different purification procedures lead to different results. For example, ^{68}Ga-NOTA-ubiquicidin fragments (^{68}Ga-NOTA-UBI29-41 and ^{68}Ga-NOTA-UBI30-41) and ^{68}Ga-DOTA-TATE were purified by using Strata™-X, C18-E light and Sep-Pak C18, which resulted in slightly variable RCPs (No. 1, 2, and 3 in Table 2) [45].

For a DOTA-like chelator-modified peptide labeled with $^{68}Ga^{3+}$, the main radiochemical impurity is free $^{68}Ga^{3+}$, which can be removed by Sep-Pak cartridges because the hydrophilic species of $^{68}Ga^{3+}$ can be eluted before the labeled compound of interest (No. 4 in Table 2) [14]. This method has been used to remove free $^{68}Ga^{3+}$ from ^{68}Ga-DOTATOC labeled peptides, ^{66}Ga-DOTA-Tyr^3-octreotide, and ^{66}Ga-DOTA-biotin (No. 5, 6 and 7 in Table 2) [46,47]. A similar purification procedure was applied for ^{68}Ga,^{111}In,^{177}Lu, and ^{90}Y-radiolabeled somatostatin analogs [48].

Given the simplicity and speed of this method, it is apprpriate for the purification of radiopharmaceuticals labeled with short-lived radionuclides. For instance, [^{18}F]-2-(5-fluoro-pentyl)-2-methyl malonic acid ([^{18}F]-FPMA) as an apoptosis PET imaging agent needs to be purified to remove free $^{18}F^-$as the main impurity. By loading the sample onto a C18 Sep-Pak column, free $^{18}F^-$can be washed away with water and then the product can be eluted from the column with ethanol (No. 8 in Table 2) [49]. The production of other ^{18}F-labeled radiotracers also requires certain purification steps. Purification procedures of ^{18}F-labeled radiotracers are not always straightforward and more complicated purification steps can be necessary. For example, to perform an automated synthesis for 2-[^{18}F]fluoroethylcholine ([^{18}F]-FECH) in one step, a combination of Sep-Pak C18 and Sep-Pak QMA (anion exchanger) is required for purification. In addition to free $^{18}F^-$as the main impurity, this anion acts as a counterion for the [^{18}F]-FECH cation, which should be replaced with a nonradioactive anion. Therefore, the reaction mixture should be passed through a Sep-Pak C18 cartridge to remove free $^{18}F^-$and then through two Sep-Pak QMA cartridges to remove $^{18}F^-$counterions (No. 9 in Table 2) [50].

After the application of an innovative microfluidic reactor chip technique for radiosynthesis of PET radiotracers [51], this method was used as an on-chip SPE technique for the purification of 2-[18F] fluoro-2-deoxy-D-glucose ([^{18}F]FDG). Two different approaches have been reported for on-chip SPE purification: single chip and train chips. To that end, four kinds of SPE particle [PS-H+ (to

TABLE 2

Examples of RP-SPE and NP-SPE for different radiopharmaceuticals

No.	Name	Radio impurity	Stationary phase (cartridge)	Mobile phase	RCY (%)	RCP (%)	Refs
1	^{68}Ga-NOTA-UBI29-41	$^{68}Ga^{3+}$	Strata™-X 100 mg polymer or C18-E light 100 mg or Sep-Pak C18 online light or Sep-Pak C18 500 mg 3 cc	Water; ethanol: saline; or acetonitrile/water	62.0	86.2–96.4	[45]
2	^{68}Ga-NOTA-UBI30-41	$^{68}Ga^{3+}$			67.0	90.7–98.0	
3	^{68}Ga-DOTA-TATE	$^{68}Ga^{3+}$			71.0	82.7–96.4	
4	^{68}Ga-labeled peptide	$^{68}Ga^{3+}$	C18 Sep-Pak	Water; ethanol: saline	–	>95.0	[14]
5	^{68}Ga-DOTATOC	$^{68}Ga^{3+}$			59.3	>98.0	[46]
6	^{66}Ga-DOTA-Tyr^3-octreotide	$^{66}Ga^{3+}$	C18 Sep-Pak	Ammonium acetate, (pH 5.0); ethanol	–	93.2	[47]
7	^{66}Ga-DOTA-biotin	$^{66}Ga^{3+}$			–	98.6	
8	[^{18}F]-FPMA	$^{18}F^-$	C18 Sep-Pak	Water; ethanol	≤79.0	≥98.0	[49]
9	[^{18}F]-FECH	$^{18}F^-$	Sep-Pak C18 Plus and Sep-Pak plus QMA	Water; ethanol	48.0	>99.0	[50]
10	[^{18}F]FDG	Acetylated-[18F]FDG and $^{18}F^-$	PS-H$^+$, PS-HCO$_3$, ALOX N and HR-P	Water	47.0	90.0–91.0	[52]
11	[^{18}F]FAZA	$^{18}F^-$	Chromabond® Set V	Ethanol 80%; ethanol 15%	20.56–24.22	>95.0	[53]
12	[^{18}F]FMISO	Multiple impurities	Sep-Pak C18 and HLB cartridge	Water; ethanol 5%	34.5	>98.0	[54]

eliminate cationic impurities), PS-HCO3 (to eliminate anionic impurities), ALOX N (to eliminate polar impurities), and HR-P (to eliminate hydrophobic impurities)] were used for the purification of [^{18}F]FDG. In single-chip SPE purification, a glass microfluidic device with two separate chambers was used, whereas, for the train chips, a train of four chips connected end-on-end to a glass microfluidic device was used. Each chamber of the microfluidic device in the single-chip SPE purification was filled with two types of resin, whereas, in the train mode, each chamber was filled with one type of resin. The RCP of [^{18}F]FDG in both methods was ~90%, although the train mode was supplied with twice the amount of resin compared with the single mode (No. 10 in Table 2) [52].

PET radiotracers for hypoxia imaging, such as 1-(5-[^{18}F]fluoro-50-deoxy-a-D-arabinofuranosyl)-2-nitroimidazole ([^{18}F]FAZA), also require purification before administration. [^{18}F]FAZA has been purified by SPE using Chromabond® Set V, which comprises PS-H +, PS-HCO3, ALOX N, and HR-P columns. The reaction mixture was loaded on to the preconditioned columns, rinsed with 80% ethanol and the product eluted with 15% ethanol (No. 11 in Table 2) [53].

[^{18}F]fluoromisonizadole ([^{18}F]FMISO) is another well-known radiopharmaceutical for hypoxia imaging. Different conditions were evaluated for the purification of [^{18}F]FMISO, including: (i) a Sep-Pak C18 cartridge; (ii) a single HLB cartridge; and (iii) two serial HLB cartridges. Column specification and research design both significantly impact the purification process. To that end, two serial HLB cartridges trapped almost 98% of [^{18}F]FMISO, whereas for one HLB cartridge, this rate was ~68%. By contrast, the Sep-Pak C18 cartridge retained only 15.8% [^{18}F]FMISO. In this case, a mixed-mode cation exchange (MCX) cartridge trapped ~17% of the protonated form of [^{18}F]FMISO (No. 12 in Table 2) [54].

Size-exclusion chromatography

SEC separates a mixture of substances with different molar masses and provides a way to purify macromolecules, such as antibodies, proteins, peptides, nucleic acids, and industrial polymers with high molecular weight [55,56], and even nanoparticles [20]. The SEC chromatography column is packed with fine, porous polymeric beads. The pore size of the packing material determines the dimensions of macromolecules that can be retained. If the molecules are smaller than the pore sizes, they enter the pores and are retained. If they are larger than the size of the pores, they pass through the spaces between the packing material. Thus, the first fraction obtained has a higher molecular weight than ones that follow (Fig. 2) [57].

The labeling of macromolecules, such as monoclonal antibodies, with metallic radionuclides requires conjugation of a proper chelator, which can be associated with problems. In such an approach, the chelator has a higher concentration than the macromolecule; thus, free chelator can compete to react with the radionuclide, which results in radiochemical impurities during the labeling step [58,59]. Based on this strategy, *p*-isothiocyanatobenzyldesferrioxamine as a BFCA was conjugated to monoclonal antibodies (mAbs) for labeling with zirconium-89 (^{89}Zr–mAb). The labeling step resulted in attachment of >85% of ^{89}Zr to the antibody, and SEC increased the RCP to >95% (No. 1 in Table 3) [59]. Another labeled macromolecule that has been purified with SEC is [^{213}Bi]CHX-A-DTPA-HuM195. This radiolabeled humanized mAb is active against CD33 and emits α and β particles. The initial RCP of the labeling reaction reached 81%, but SEC improved this to 99% (No. 2 in Table 3) [60]. In a similar study, a single-chain variable fragment of prostate-specific membrane antigen (PSMA)-binding antibody was labeled with ^{99m}Tc tricarbonyl (^{99m}Tc-J591) for prostate cancer imaging. The labeling yield in the presence of different NaCl concentrations was in the range of 74–90%, which was unsatisfactory for imaging purposes. To improve the RCP, SEC was performed and increased the RCP to >99% (No. 3 in Table 3) [15].

Large molecules, such as [^{99m}Tc(CO)$_3$]–DTPA–rituximab, can be purified by using aPD-10 column. ^{99m}Tc $(H_2O)_3(CO)_3^+$ synthon is used for DTPA–rituximab labeling, and its unreacted portion,

TABLE 3

Examples of SEC for different radiopharmaceuticals

No.	Name	Column (Stationary phase)	Mobile phase	pH	RCP (%)		Refs
					Initial	Final	
1	^{89}Zr–mAb	PD-10 (Sephadex G-25)	Gentisic acid in 0.25 M sodium acetate	5.4–5.6	>85.0	>95.0	[59]
2	(^{213}Bi)CHX-A-DTPA-HuM195	TSK 3000SWXL	0.15 mol/L NaCl/0.02 mol/L sodium acetate	6.5	81.0	99.0	[60]
3	^{99m}Tc-J591	(Sephadex G-25)	PBS	7.0	7.4.0–90.0	>99.0	[15]
4	[^{99m}Tc(CO)$_3$]–DTPA–Rituximab	PD-10 (Sephadex G-25)	PBS (0.05 M)	7.4	<90.0	>95.0	[61]
5	^{99m}Tc-AuNP and ^{99m}Tc-AuNP-mannose	PD-10 (Sephadex G-25)	Water	–	30.0	95.0	[62]
6	^{64}Cu-SLN	G-50 Sephadex	Saline (0.9%)	–	66.5	>95.0	[17]

^{99m}Tc $(H_2O)_3(CO)_3^+$, can be removed by a PD-10 column because of its low molecular weight, resulting in a final RCP of 95%. [^{99m}Tc $(CO)_3$]–DTPA–rituximab tends to aggregate; therefore, RCP after purification cannot exceed 95% (No. 4 in Table 3) [61].

SEC even can purify nanoparticles. For instance, ^{99m}Tc-labeled gold nanoparticles conjugated to the HYNIC-Gly-Gly-Cys-NH2 peptide (^{99m}Tc-AuNP) and thiol-mannose (^{99m}Tc-AuNP-mannose) for sentinel lymph node identification were purified with this method. The initial RCP of both ^{99m}Tc-AuNP and ^{99m}Tc-AuNP-mannose was ~30%. However, the purification process increased RCP to 95%. In this method, after collecting the void volume, the first nanoparticles leave the PD-10 column, followed by labeled co-ligands, whereas free $^{99m}TcO_4^-$ and ^{99m}Tc-colloid are unable to leave the column (No. 5 in Table 3) [62]. ^{64}Cu-labeled solid lipid nanoparticles (^{64}Cu-SLN) have also been purified using SEC. The labeling was carried out with a Cu-specific chelator, 6-[p-(bromoacetamido)-benzyl]-1,4,8,11-tetraazacyclotetradecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid (BAT). This chelator is a surfactant that can place itself in the SLN monolayer to form SLN-BAT. During the labeling of SLN-BAT, the unreacted ^{64}Cu complexed with EDTA and the resulting ^{64}Cu-EDTA as the radiochemical impurity was separated using SEC (No. 6 in Table 3) [17].

Ion-exchange chromatography

ICE is a chromatography technique that separates ions and polar molecules. The two main types of ICE are anion exchange and cation exchange. The stationary phase in cation-exchange chromatography is negatively charged; hence, it can separate cations from other ions. By contrast, the stationary phase in anion-exchange chromatography is positively charged and, therefore, it can separate anions from other ions. Before performing an IEC, the stationary phase should achieve electroneutrality. Two main types of stationary phase in IEC exist: strong or weak ion-exchangers. Depending on the type of stationary phase, after separation, the bound ions can be eluted and collected using a proper eluent, which acts either by using a higher concentration of a competing ion or through changing the eluent pH (Fig. 2) [63,64].

Simple ions and more complex charged molecules can be purified with IEC. For example, this purification technique was used in the purification of 1-^{11}C-acetoacetic acid. Briefly, the reaction mixture was passed through a Dowex 50WX8-100 (cation-exchange resin) and an AG 1X-8 (anion-exchange resin). Given the anionic nature of 1-^{11}C-acetoacete, it remained on the AG 1X-8 column and was eluted by citrate buffer (No. 1 in Table 4) [65]. Based on the rapidity and simplicity of purification with SPE cartridges, this technique is favored for the purification of ^{11}C-acetate (^{11}C-AC) because of the short half-life of the ^{11}C radionuclide. Therefore, the automated method for production of ^{11}C-AC uses IEC as a purification method. Briefly, the reaction mixture of ^{11}C-AC is hydrolyzed with HCl and passed through an SCX cartridge to replace all the Mg^{2+} ions with H^+ and turn ^{11}C-$CH_3COOMgBr$ into ^{11}C-CH_3COOH. ^{11}C-CH_3COOH is then passed through a Sep-Pak plus C18 cartridge and a Sep-Pak AG11A8 cartridge to remove organic impurities and Br^-, respectively (No. 2 in Table 4) [66].

Radio-iodinated mAb and other materials are usually contaminated with free iodine. For example, ^{131}I-labeled MOv18 (^{131}I-c-MOv18), a candidate for radioimmunotherapy of ovarian cancer, is contaminated with free ^{131}I. The free ^{131}I can be removed by Dowex AG1-X8 anion-exchange resin in PBS (No. 3 in Table 4) [67].

A simplified kit has been introduced for ^{99m}Tc protein radiolabeling using an N_3S triamide mercaptide. This kit has been applied for labeling NR-LU-10 Fab with ^{99m}Tc (^{99m}Tc-N_3SNR-LU-10 Fab). The ingredients of this kit including gluconate (transfer ligand), the hydrolyzed form of a N_3S bifunctional chelating agent, and free pertechnetate, can jeopardize the RCP. However, these radiochemical impurities are negatively charged and can be retained with QAE Sephadex anion exchange resin (No. 4 in Table 4) [68]. In some cases, to purify a sample with IEC, it should be converted into an ionic form. For example, the reaction mixture of [^{18}F]FMISO was acidified with HCl and then the protonated form of [^{18}F]FMISO was purified with a mixed-mode cation exchange (MCX) cartridge (No. 5 in Table 4) [54].

A novel maleimide derivative of [^{90}Y]-DOTA ([^{90}Y]DOTA-butanoyl-maleimide) has been introduced as a prosthetic group for the labeling of peptides in mild conditions. The purification of such prelabeled agents is necessary because of the susceptibility of maleimide to hydrolysis. The byproduct and precursor of [^{90}Y]DOTA-butanoyl-maleimide are negatively charged; hence, purification of [^{90}Y]DOTA-butanoyl-maleimide can be carried out with anion exchange SPE. In this case, the mobile phase should contain a low concentration of phosphate ions to avoid further hydrolysis of the product. To that end, the purification step is performed by using a Sep-Pak QMA Acell Plus cartridge and water (No. 6 in Table 4) [8].

Liquid–liquid extraction

Other techniques, such as LLE, can be established for the purification of radiopharmaceuticals. LLE is a simple, fast, and effective

TABLE 4

Examples of IEC for different radiopharmaceuticals

No.	Name	Column (Stationary phase)	Mobile phase	Final RCP (%)	Refs
1	1-^{11}C-acetoacetic acid	AG 1X-8	Water, citric acid, citrate buffer	98.0	[65]
2	^{11}C-AC	Sep-Pak AG11A8 and SCX cartridge	Water	>99.0	[66]
3	^{131}I-c-MOv18	Dowex AG1-X8	PBS	99.5	[67]
4	^{99m}Tc-N_3SNR-LU-10 Fab	QAE Sephadex	Normal saline	90.0–96.0	[68]
5	[^{18}F]FMISO	MCX cartridge	Water, ethanol 5%	>98.0	[54]
6	[^{90}Y]DOTA-butanoyl-maleimide	Sep-Pak QMA Acell Plus cartridge	Water, NaCl 0.5 M and CH3CN (95:5 v/v)	>98.0	[8]

method in which impure radiopharmaceuticals are dispensed between two immiscible phases (an organic solvent and an aqueous phase). Based on the partition coefficient of the radiochemical impurities and radiopharmaceuticals, each will be concentrated in either the organic or aqueous phase. For example, ^{99m}Tc-labeled AS1411 aptamer (^{99m}Tc-AS1411) as a potential prostate tumor imaging was purified by LLE. In this method, ^{99m}Tc-AS1411was dispersed between *n*-butanol/water to remove radiochemical impurities such as ^{99m}Tc-co-ligand and $^{99m}TcO_4^-$ [69]. Similarly, ^{99m}Tc-HMPAO was extracted with chloroform from the aqueous phase, which led to an RCP >90% [70].

Miscellaneous purification methods

Essentially, radiolabeling and purification are two separated procedures, but in some cases combining them minimizes the duration of the procedure. To that end, a SPE cartridge with two types of stationary phase was developed for simultaneous radioiodination and purification. The first part of this cartridge was filled with ordinary fluorous silica, whereas the second part was filled with a mixture of fluorous arylstannanes coated on fluorous silica, [^{125}I] NaI, and an oxidant, such a chloramine-T. Passing an arylstannanes precursor through this cartridge resulted in a radio-iodinated product with RCP >98% in one step [71]. Other types of SPE stationary phase, such as Zeolite 13X cartridges, have been reported for [^{11}C]palmitate purification [72].

In some cases, chemical precipitation can be used for purification. For example, it was reported that barium (the decay progeny) can be removed from ^{137}Cs as a source of brachytherapy through barium precipitation with carbonate [73]. In addition, this method is applicable to more complicated radiotracers, such as the Pt(IV)(IT-[^{131}I] carnosine) complex. To provide this radiotracer, radio-iodinated carnosine (^{131}I-carnosine) was synthesized and then combined with Pt(IV) through its free sulfhydryl group to obtain a Pt(IV)(IT-[^{131}I] Carnosine) complex. The unsatisfactory RCP of the product was improved (to 95%) by reducing the pH of the reaction mixture, which resulted in precipitation of the final pure product [74].

Concluding remarks

After labeling, purification is often required to isolate the radioactive compound from either radioactive or cold contaminant(s). Given the short half-life of radionuclides, the purification method should be as short as possible and effective enough to ensure a high RCP. Diverse purification methods for radiopharmaceuticals have been introduced. Most radiopharmaceutical purification methods are chromatographic methods, including normal and reverse SPE and HPLC, SEC, and IEC, while other methods based on LLE and chemical precipitation also have been reported. To select the most suitable purification method for a desirable radioactive compound, the intrinsic characteristics of the compound and separation equipment should be evaluated. These include molecular weight, lipophilicity, and stability in the mobile phase, the charge of the molecule, and, most importantly, the half-life of the radionuclide. For lipophilic low-molecular-weight radiopharmaceuticals, such as [^{18}F]FDOPA, RP-HPLC is a method of choice, whereas NP-HPLC is useful for hydrophilic compounds. RP-SEP and NP-SEP are similar to HPLC, except HPLC removes most cold organic contaminants. The purification of charged radiopharmaceuticals (usually with a low molecular weight) can be carried out with IEC, such as 1-^{11}C-acetoacetic acid. Some reports have suggested the purification of large molecules with IEC, such as ^{131}I-c-MOv18. SEC is an efficient method for the purification of high-molecular-weight molecules, such as ^{89}Zr–mAb. Therefore, selecting an appropriate purification method and equipment will lead to radiopharmaceuticals with a high purity. Finally, for all the purification methods, there should be a balance between the duration of the purification process and final RCP and RCY. In other words, an efficient purification method should provide high RCP and RCY in the shortest possible time.

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