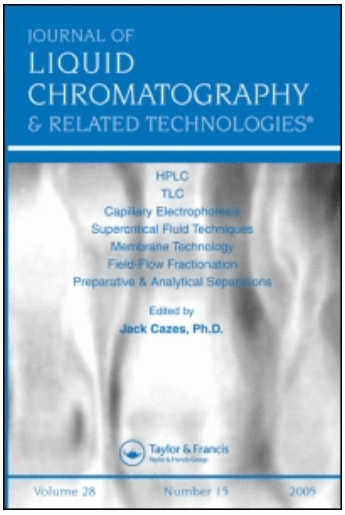

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SEP-PAK PREPARATIVE CHROMATOGRAPHY: USE IN RADIOPHARMACEUTICAL SYNTHESIS

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ABSTRACT

The use of SEP-PAK^R C_{18} cartridges for the isolation and purification of radiopharmaceuticals, labeled with the 20.4 minute half-life radionuclide carbon-11, is reported. Synthesis and SEP-PAK preparative chromatographic purification of [1-^{11}C]palmitic acid, [^{11}C-methyl]benzyl methyl ether, [1-^{11}C]butan-1-ol, and [1-^{11}C]pyruvic acid are described. The use of SEP-PAK C_{18} cartridges has allowed development of rapid and remote methods for handling of high amounts (>100 mCi) of radioactive products.

INTRODUCTION

In the synthesis of organic radiopharmaceuticals labeled with radionuclides of short half-life (for example carbon-11, $t_{1/2}$ = 20.4 minutes, or nitrogen-13, $t_{1/2}$ = 9.98 minutes) severe restrictions are placed on the methods of isolation and purification of the labeled product. Such procedures must necessarily be very rapid, yet they must be reproducible and

yield products of high radiochemical purity. Furthermore, the high radiation levels associated with handling of large amounts (up to 2 Ci) of these positron-emitting radionuclides require procedures amenable to incorporation into remotely controlled or automated apparatus, which will minimize radiation exposure of personnel involved.[1,2] Finally, procedures must be useful for the isolation of extremely small amounts of radioactive material; in no-carrier-added synthesis with these radionuclides, the actual amount of radiochemical produced is typically less than 20 μmol.

Various methods of chromatography (open column[1], flash column[3] and high pressure liquid chromatography[2,4]) are widely used in radiopharmaceutical syntheses. In many cases, however, a prior isolation and partial purification of reaction products is necessary, especially in conjunction with purification by HPLC, where resolution and column life expectancy can be severely compromised by certain impurities. We would like to describe here the application of SEP-PAK^R C_{18} preparative chromatography to the isolation and purification of several carbon-11 labeled organic radiopharmaceuticals. The use of SEP-PAK chromatography allows for the development of rapid, remotely controlled procedures for isolation of radiolabeled products.

EXPERIMENTAL

Materials and Methods

The SEP-PAK^R C_{18} cartridges were obtained from Waters Associates (Milford, MA, USA) and pre-equilibrated prior to use by

washing with 2 ml of ethanol and 5 ml of water. High pressure liquid chromatograpphy was done using a Waters Model 210 liquid chromatograph equipped with two 6000A pumps and a Waters Model 660 solvent programmer. Gas chromatographic analysis was done using a Varian Model 3700 gas chromatograph equipped with either a 5' x 1/4" stainless steel column packed with 10% FFAP on Chrom 60/80, or a 5' x 1/4" nickel column packed with Porapak Q (80/100 mesh).

The $^{11}CO_2$ was produced as previously described[7]. Syntheses of [1-^{11}C]palmitic acid, [^{11}C-methyl]benzyl methyl ether and [1-^{11}C]pyruvic acid have been previously reported (5,6,7): the modified procedures using SEP-PAK chromatography are detailed below.

Radiochemical Synthesis

[1-^{11}C]Palmitic Acid. A stream of nitrogen carrying the $^{11}CO_2$ was bubbled through 1 mL of a 0.1 M solution of pentadecylmagnesium bromide in diethyl ether. After complete transferral of the $^{11}CO_2$, 1 mL of 1 N hydrochloric acid was added, followed by 10 mL of water. The mixture was then passed through an activated SEP-PAK. The SEP-PAK was sequentially washed with 10 mL of water and 2 mL of 50% ethanol, then the [1-^{11}C]palmitic acid eluted from the SEP-PAK with 2 mL of 95% ethanol. The ethanol solution was analyzed for diethyl ether content using GC (FFAP column, 60° C, 20 cc/min helium flow, FI detector: R_T diethyl ether = 1.5 min, R_T ethanol = 2.4 min).

[^{11}C-Methyl]Benzyl Methyl Ether. A stream of $^{11}CO_2$ in nitrogen was bubbled through 0.15 mL of 1 M lithium aluminum hydride in tetrahydrofuran. After the $^{11}CO_2$ was transferred, the nitrogen flow was stopped, and 0.2 mL of water added. To the aqueous mixture was added 1.0 mL of dimethylsulfoxide, 40 µL of benzyl bromide (57 mg, 0.34 mmol), and 0.4 g of potassium hydroxide, and the mixture stirred for seven minutes. To this was added 8 mL of water and 1 mL of 1N hydrochloric acid, and the aqueous solution passed through a SEP-PAK. The SEP-PAK was rinsed with 10 mL of water, then the labeled methyl ether eluted off the SEP-PAK using 6 mL of 25% ethanol. This solution could be directly injected onto a HPLC column for purification (Waters Partisil 5 ODS-3 column, 45% ethanol, 3.0 mL/min, R_T = 3.9 min).

[1-^{11}C]Butan-1-ol. In a conical vessel were placed 0.5 mL of dry diethyl ether and 0.5 mL of a 2.2 M propyl magnesium chloride solution. Through this was bubbled helium containing $^{11}CO_2$. When the activity was transferred, the helium flow was increased and most of the ether evaporated. The helium was shut off and 0.5 mL of 1 M lithium aluminum hydride in THF added. The vessel was shaken briefly, let set 2 min, then the solution poured into 2.5 mL of cooled (ice-water bath) 1.0 M hydrochloric acid. The solution was swirled to dissolve the salts, then passed through the two SEP-PAKS. The SEP-PAKS were washed with 1 mL of water, and finally the [1-^{11}C]butan-1-ol eluted using 1.5 mL of 95% ethanol. Analysis of the ethanol solution was by HPLC (Waters Partisil 5 ODS-3 column, 30% ethanol, 3 ml/min, R_T = 5.8 min).

[1-^{11}C]Pyruvic Acid. Methyllithium (1.0 mL of a 1.6 M solution in diethyl ether) was added to 1 mL of dry tetrahydrofuran, the solution cooled (ice-water bath), and 250 µL (1.8 mmol) of 1,1,3,3-tetramethylbutylisocyanide added. After 30 min. a stream of nitrogen carrying $^{11}CO_2$ was bubbled through the solution. When transfer of the $^{11}CO_2$ was complete the nitrogen flow was stopped, 250 µL of 95% ethanol added, and the solvents evaporated. To the residue were added 3 mL of 5% hydrochloric acid, and the oily brown mixture boiled vigorously for 10 min. The solution was cooled, neutralized by dropwise addition of saturated sodium bicarbonate solution, then passed through two SEP-PAK cartridges into an empty sterile vial. The clear, colorless solution of sodium [1-^{11}C]pyruvate was analyzed by GC by withdrawal of an aliquot, acidifiction (pH 2), and injection onto a Porapak Q column. Analysis showed 95% [1-^{11}C]pyruvic acid and 5% radiochemical impurities ([1-^{11}C]acetic acid and [2-^{11}C]acetone), and no chemical impurities.

RESULTS AND DISCUSSION

The SEP-PAK C_{18} cartridges have been previously used to absorb lipophilic compounds present in low concentrations in large volumes of water (8,9). We have utilized the SEP-PAK C_{18} cartridges in a strictly analogous fashion in the isolation of [1-^{11}C]palmitic acid and [^{11}C-methyl]benzyl methyl ether. We have also used these cartridges to isolate a not-so-hydrophobic molecule, [1-^{11}C]-butanol, and to purify (but not isolate) a

hydrophilic product, [1-^{11}C]pyruvic acid. The results of these applications of SEP-PAK C_{18} chromatography are detailed below.

[1-^{11}C]Palmitic Acid

This labeled fatty acid is prepared by a classical Grignard reaction. The separation of the labeled palmitic acid from diethyl ether and excess hydrochloric acid is crucial; at the end of the synthesis the [1-^{11}C]palmitic acid is prepared for in vivo injection by addition of a saline solution of human serum albumin, and traces of diethyl ether or hydrochloric acid can cause denaturation of the albumin and render the preparation useless[5]. Previous methods for ^{11}C-palmitic acid purification have involved extraction of the product into diethyl ether, repeated washings of the ether with saline to remove HCl, then evaporation of the ether. The use of a SEP-PAK C_{18} cartridge, as shown in Table 1, can substitute for these isolation and purification steps. The ethanol solution of [1-^{11}C]palmitic acid obtained via SEP-PAK isolation is at neutral pH and contains less than 0.5% diethyl ether impurity.

TABLE 1
Isolation and Purification of [1-^{11}C]Palmitic Acid

	Solution through C-18 SEP-PAK	Activity, mCi SEP-PAK	Eluant
(1)	Crude reaction mixture	32	2
(2)	10 ml H_2O	25	7
(3)	2 ml 50% ethanol	23	2
(4)	2 ml 95% ethanol	5	18

[^{11}C-Methyl]Benzyl Methyl Ether

This labeled ether is prepared in two steps, the reduction of $^{11}CO_2$ to $^{11}CH_3OH$ using lithium aluminum hydride, and the reaction of this labeled methanol with benzyl bromide using potassium hydroxide in water-dimethylsulfoxide solution. Although a reverse-phase HPLC separation of methanol, benzyl bromide, and benzyl methyl ether is easily achieved, the presence of dimethylsulfoxide and potassium hydroxide in the crude reaction mixture (both deleterious to HPLC columns) makes a preliminary isolation of the product absolutely necessary. In this regard the SEP-PAK works exceptionally well; the lipophilic benzyl methyl ether and benzyl bromide are quantitatively retained on the SEP-PAK, and washing with water removes the KOH, DMSO, and 90% of the ^{11}C-methanol (see Table 2). Washing of the SEP-PAK with 25% ethanol is effective in eluting off 84% of the ^{11}C-benzyl methyl ether, along with only a small amount of the benzyl bromide. Using higher concentration of ethanol (up to 50%) gives more complete elution of the labeled benzyl methyl ether, but also elution of proportionately more benzyl bromide. Finally, a reverse-phase HPLC

TABLE 2
Isolation and Partial Purification of [^{11}C]Benzyl methyl ether

	Solution through C-18 SEP-PAK	Activity, mCi SEP-PAK	Eluant
(1)	Crude reaction mixture + 10 ml H_2O	125	62
(2)	6 ml 25% ethanol	19	105

separation affords [^{11}C-methyl]benzyl methyl ether with a radiochemical purity of 99% and free of chemical impurities.

[1-^{11}C]Butan-1-ol

This low molecular weight alcohol is obtained by a two step synthesis, a Grignard synthesis of [1-^{11}C]butanoic acid followed by lithium aluminum hydride reduction to the alcohol. The ^{11}C-butanol is separated from chemical impurities (diethyl ether, tetrahydrofuran, and hydrochloric acid) using two SEP-PAK C_{18} cartridges in series; typical results are shown in Table 3. As butanol is somewhat water soluble, a procedure using a minimum of water and a slower flow rate is necessary: however, 20% of the ^{11}C-butanol is lost in the water wash step. HPLC analysis of the final product solution shows [1-^{11}C]butanol in 100% radiochemical purity with small amounts of diethyl ether and methanol as chemical impurities. Attempts to entirely remove these chemical impurities by selective washings of the SEP-PAK with various ethanol:water mixtures proved unsuccessful. At present, these impurities do not interfere with the use of [1-^{11}C]butan-1-ol for in vivo animal studies. In those instances where a product of

TABLE 3
Isolation and Purification of [1-^{11}C]butan-1-ol

	Solution through C-18 SEP-PAK	Activity, mCi SEP-PAK	Eluant
(1)	Crude reaction mixture	117	12
(2)	1.5 ml H_2O	94	23
(3)	2.0 ml 95% ethanol	85	9

higher chemical purity is desired, the solution of ^{11}C-butanol in ethanol isolated using SEP-PAKS in this manner can be diluted with water and safely injected onto a reversed-phase HPLC column for preparative chromatographic purification. The use of the SEP-PAKS has been particularly rewarding in this synthesis: it should be noted that the alternative method of isolation by liquid-liquid extraction and evaporation cannot be utilized, due to the volatility of the radiolabeled product.

[1-^{11}C]Pyruvic acid

This α-keto acid is prepared in two steps: the addition of $^{11}CO_2$ to a lithium aldimine followed by acid hydrolysis of the intermediate α-imino acid[7]. The crude product solution contains two major impurities, 1,1,3,3-tetramethylbutylamine and [1-^{11}C]-2-(1,1,3,3-tetramethylbutyl)-iminopropionic acid. By using two SEP-PAK C_{18} cartridges in series, 100% of these impurities can be easily and quickly removed. As a typical example, the passage of a solution of 50 mCi of crude products through two SEP-PAKS gave 17 mCi of [1-^{11}C]pyruvic acid, with 11 mCi retained on the SEP-PAK cartridges (93% of which could be eluted with 2 mL of 95% ethanol). The eluant from the SEP-PAK chromatography then contains the desired [1-^{11}C] pyruvic acid in greater than 95% radiochemical purity (by GC analysis: remainder is [1-^{11}C]acetic acid and [2-^{11}C]acetone). The absence of chemical impurities was confirmed by both GC (FI detector) and thin layer chromatography. In this example, the SEP-PAKS are used to purify (but not isolate) the desired radiolabeled product by selective absorption of the

hydrophobic side products, and is thus in a sense an application "opposite" to the three previous examples of isolation of hydrophobic products.

The use of SEP-PAK C_{18} cartridges in the syntheses of carbon-11 labeled palmitic acid, butanol, benzyl methyl ether, and pyruvic acid are only four examples of our many applications of SEP-PAK C_{18} chromatography to radiochemical syntheses. The syntheses of these compounds were chosen to illustrate the versatility of this isolation and purification technique. We have also used SEP-PAK C_{18} chromatography in syntheses of carbon-11 labeled ethers[6] and long-chain alcohols[10], in the syntheses of several other carbon-11 labeled α-keto acids, and in the synthesis of a carbon-11 labeled α-amino acid, [1-^{11}C]norvaline. In general, incorporation of a SEP-PAK chromatography step has allowed for shorter preparation times and safe handling of larger amounts of radioactive products. Finally, in the synthesis of fluorine-18 labeled spiroperidol[11] (fluorine-18 is a positron-emitting radionuclide with $t_{1/2}$ = 110 minutes), we have recently used SEP-PAK C_{18} chromatography to concentrate the organic products prior to HPLC separation. For fluorine-18 and other longer-lived radionuclides the speed of synthesis is not as crucial as with carbon-11, but methods amenable to incorporation in remotely controlled apparatus are still needed.

We feel that SEP-PAK chromatography, and related methods of C_{18}-silica gel bonded phase chromatography[12], will be a valuable

addition to the chromatographic options of the radiopharmaceutical chemist. The SEP-PAK cartridges have proven very versatile; they can be used for product isolation, for isolation and purification, or product purification alone. Finally, use of a SEP-PAK C_{18} cartridge should allow for concentration of a radiolabeled product obtained in an eluant from an HPLC column; this is important in situations where the product is obtained in a solvent or volume incompatible with in vivo animal or human studies.

SEP-PAK chromatography has thus taken its place alongside column and high-pressure liquid chromatography as a purification technique used daily in our laboratories.

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