Effective One/Two Step Purification of Various Materials by Solid-phase Extraction

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Simple one/two step purification procedures based on the solid-phase extraction technique were effectively exploited to clean up radiolabelled drugs represented by dihydrochloride of [6-3H]-stobadine and hydrochloride of [4-3H]-pentacaine, derivatization agents such as 4-nitrobenzoyl chloride or 3,5-dinitrobenzoyl chloride, as well as the aqueous phosphate or triethylamine acetate buffer solutions. © 1997 John Wiley & Sons, Ltd.

**INTRODUCTION**

Today solid phase extraction (SPE) is a well established and broadly exploited (pre-)separation technique (Manufacturers’ publications: Waters (1983); Analytichem International (1987); Supelco (1988); Baker (1989); Varian (1992); J. & W. Scientific Macherey-Nagel) in most laboratories devoted to monitor the levels of various compounds, e.g. therapeutic drugs, drugs of abuse, pollutants, etc. By this procedure the substances of interest are isolated from different matrices (biological fluids, animal/plant tissues, water, soil, etc.), then usually concentrated, and the level of the analytes is determined by a suitable analytical method — chromatography, spectrometry, etc. Thus on analysing such samples SPE, though inevitable, serves as a co-procedure.

In the same laboratory, however, SPE can be used also as the main step in preparing several (final, pure) materials of interest. Such applications are shown below on the examples of purification of (A) the radiolabelled drugs 3H-stobadine and 3H-pentacaine in the mass quantity of nanograms–micrograms, (B) the derivatization agents 4-nitrobenzoyl chloride and 3,5-dinitrobenzoyl chloride in quantities of milligrams–grams and (C) aqueous buffer solutions, components of the high-performance liquid chromatographic (HPLC) mobile phases, in the volume amount of millilitres–litres.

**EXPERIMENTAL**

Materials and chemicals. Standard SEP-PAK Silica and SEP-PAK C18 cartridges were purchased from Waters Associates (Milford, MA, USA). Separol SI C18 (250 mg/3 mL) and empty Separol extraction columns were the products of the Anapron Co. Ltd. (Bratislava, Slovak Republic). The latter were ‘home’ packed with approximately 0.5 g of bare wideporous silicagel of BIOSPHER SI 300 (25 μm particles; LABIO Co., Prague, Czech Republic) and subsequently cheap bovine serum albumin (BSA; A 9647; Sigma Chemical Co., St Louis, MO, USA) was in situ adsorbed into the packing pores by the procedure described in the literature (Erlandsson et al., 1986). Empty Separol syringe barrels were also packed with approximately 0.5 g of dried Na2SO4, particle fraction 63–106 μm, i.e. 150–250 mesh (Lachema, Brno, Czech Republic). CYCLOBOND I cartridges (500 mg/3 mL) used were from ‘ASTEC’ — Advanced Separation Technologies Inc. (Whippany, NJ, USA).

The dihydrochloride of [6-3H]-stobadine (I; Fig. 1) and hydrochloride of [4-3H]-pentacaine (II; Fig. 1) used were both partially decomposed as the consequence of a longer storage period. The radiolabelled drugs, supplied in the form of methanolic solutions by the Institute for Research, Production, and Uses of Radioisotopes, Prague, Czech Republic, had originally the following radiochemical parameters: I, purity >98%; activity, 40 MBq/mL; specific activity, 495 GBq/mM; II, purity >96%; activity 80 MBq/mL; specific activity 48 GBq/mM.

The stocks of 4-nitrobenzoyl chloride (4-NBC), CH2CINO (Fluka Chemie, Neu-Ulm, Germany) and 3,5-dinitrobenzoyl chloride (3,5-DNBC), C6H3CINO2 (Regis Chemical Co., Morton Grove, IL, USA) used were partially decomposed during their storage.

![Figure 1. Chemical structures of dihydrochloride of stobadine (I) and hydrochloride of pentacaine (II). (The asterisks indicate the position of the tritium atom in the radiolabelled compounds.)](image-url)
storage by the action of daylight and atmospheric humidity.

$Na_2HPO_4 \times 12H_2O$ and $KH_2PO_4$ of p.a. purity grade (Merck, Darmstadt, Germany), triethylamine (TEA; Fluka Chemie), and glacial acetic acid (Lachema) served as the HPLC mobile phase buffering components. Phosphate buffer of 'physiological composition' was prepared by mixing the aqueous $Na_2HPO_4$ and $KH_2PO_4$ solutions (each 0.067 mol/L) in the volume ratio of 80:3:19.7 (pH 7.4). Triethylamine acetate (TEAAc) buffer was prepared by titrating the 0.1% aqueous solution of TEA with glacial acetic acid up to the desired pH value (usually 4.1).

Water, CH$_3$CN, and CH$_3$OH of HPLC purity grade were purchased from J. T. Baker Chemical Co., Phillipsburg, NJ, USA. CH$_2$Cl$_2$ (Koch-Light Laboratories Ltd., Colnbrook, England) and cyclohexane p.a. (Lachema), each approximately 20 mL, were dried immediately before use by their filtration through the $Na_2SO_4$ bed held in the Separcol columns.

Procedure A$_1$. The methanolic solution (0.1–1.0 mL) of dihydrochloride of [6-3$H$]-stobadine was diluted by distilled water to the final CH$_3$OH concentration of 20% or less. This working solution was then run through the sorbent bed of the preconditioned (2 mL CH$_3$OH, 2 mL H$_2$O) Separcol SI C18. The sample components trapped by the extraction column packing were subsequently washed with 2 mL of pure acetonitrile. The trapped substance I was flushed out/displaced from the column by applying 1.0 mL of methanol. The radiochemical purity of the recovered dihydrochloride of [6-3$H$]-stobadine was determined by means of thin-layer chromatography (Soltés and Trnovec, 1987) and/or an HPLC method (Sčasnár et al., 1989).

Procedure A$_2$. A procedure analogous to that of A$_1$ was applied on purifying hydrochloride of [4-3$H$]-pentacaine. In this case, instead of the Separcol SI C18 column the SEP-PAK C$_18$ cartridge was applied advantageously (Soltés et al., 1983). The purified drug — II — was collected as the fraction eluted between 0.5–2.5 mL (Fig. 2). The radiochemical purity of hydrochloride of [4-3$H$]-pentacaine was determined by the method of Sčasnár et al. (1984).

Procedure B$_1$. A treating procedure analogous to that of B$_1$ was successfully used also to purify the sample of 3,5-DNBC. The yield of the dark yellow crystalline material was 88% with a m.p. of 69–70°C. The elemental C:H:N analysis was: theoretical, 45.31:2.17:7.55; found, 45.10:2.29:7.60.

Procedure B$_2$. A procedure analogous to that of B$_1$ was applied on purifying hydrochloride of [4-3$H$]-pentacaine. In this case, instead of the Separcol SI C18 column the SEP-PAK C$_18$ cartridge was applied advantageously (Soltés et al., 1983). The purified drug — II — was collected as the fraction eluted between 0.5–2.5 mL (Fig. 2). The radiochemical purity of hydrochloride of [4-3$H$]-pentacaine was determined by the method of Sčasnár et al. (1984). The recovered materials had identical physico-chemical parameters — IR (KBr disc); MS; and H-/C-NMR spectra — with those reported for the chemically pure 4-nitrobenzoyl chloride and 3,5-dinitrobenzoyl chloride.

Procedure C$_1$. The freshly prepared aqueous phosphate buffer (0.2–1.0 L), used at the chiral HPLC analysis on working with the BSA-bond column (Soltés et al., 1994; Soltés, L., and Sébille, B. unpublished data), was clarified by its fast running (approximately 20 mL/min) through the barrel of the BSA modified Separcol silica minicolumn.

Procedure C$_2$. The TEAAc buffer (up to 1.0 L), preferably used for chiral HPLC at working with the cyclodextrin-bond columns in aqueous media, was cleansed by a procedure analogous to that of C$_1$. To trap the impurities from the aqueous TEAAc buffer solution, the cartridge of CYCLOBOND I was here exploited effectively.

Figure 2. Cumulative elution of the hydrochloride of [4-3$H$]-pentacaine from the SEP-PAK C$_18$ cartridge by the weak solvents aqueous methanol — H$_2$O:CH$_3$OH, 80:20, v/v (1) and acetonitrile (2), and by strong methanol (3).
RESULTS AND DISCUSSION

Procedures A

Radiolabelled substances are broadly used in biomedical research. In determining the experimental pharmacokinetics of a new drug, the required tracer purity is ≥ 95%. If decomposition occurring during sample storage reduces the content of the radiolabelled drug, the tracer has to be cleansed. Since labelled substances are usually characterized by high specific activity, their cleansing should yield only a minimal volume of waste. The use of extraction in the system of two liquids (liquid–liquid extraction — LLE) appears to be a priori questionable as LLE operates mostly with large volumes (tens to hundreds of millilitres) of aqueous/non-aqueous solutions. On the other hand, exploitation of SPE cartridges for the purification of isotopically labelled substances can be characterized as a particularly appropriate alternative. Compared to LLE, SPE provides the advantage (i) that the volumes of liquids which are eventually wasted are small, and (ii) that the small corpus of the cartridge allows its safe long-term storage.

In our case, fractional cleaning of tracers I/II by procedures A/I, A/II resulted essentially in the following four low-volume fractions: (1) the ‘original’ aqueous–methanolic (volume ≈ 1.0 mL); (2) the acetonitrile wash (Soltés, 1992) (volume 2 mL); (3) the recovered methanolic eluate (1.0 mL) containing the purified substance I/II, and (4) the ‘contaminated’ SPE cartridge. Should subsequent checking of the radiochemical purity of dihydrochloride of [6-3H]-stobadine or of hydrochloride of [4-3H]-pentacaine still detect an inadequate purity grade, the solid and liquid waste obtained by using procedures A/I, A/II presents an acceptable material of a volume below 10 mL, which can easily be stored over a long period of time.

Procedures B

The derivatization agents 4-nitrobenzoyl chloride and 3,5-dinitrobenzoyl chloride are highly reactive substances which react very readily with alcohols, phenols, as well as primary and secondary amines (Manufacturer’s publication: Regis Chemical Co). Thus due to the action of air humidity, gradual decomposition of the substance sets in at each opening of the storage jar. The reaction products can be characterized by high specific activity, their cleansing should yield only a minimal volume of waste. The use of extraction in the system of two liquids (liquid–liquid extraction — LLE) appears to be a priori questionable as LLE operates mostly with large volumes (tens to hundreds of millilitres) of aqueous/non-aqueous solutions. On the other hand, exploitation of SPE cartridges for the purification of isotopically labelled substances can be characterized as a particularly appropriate alternative. Compared to LLE, SPE provides the advantage (i) that the volumes of liquids which are eventually wasted are small, and (ii) that the small corpus of the cartridge allows its safe long-term storage.

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CONCLUSION

As demonstrated on the examples given, the SPE purification technique presented can be assessed as both quality- and cost-effective.

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