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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-^{3}H]-stobadine and hydrochloride of [4-^{3}H]-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.

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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 ^{3}H-stobadine and ^{3}H-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 C_{18} cartridges were purchased from Waters Associates (Milford, MA, USA). Separcol SI C18 (250 mg/3 mL) and empty Separcol 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 Separcol syringe barrels were also packed with approximately 0.5 g of dried Na_2SO_4, 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-^{3}H]-stobadine (**I**; Fig. 1) and hydrochloride of [4-^{3}H]-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), $C_7H_4ClNO_3$ (Fluka Chemie, Neu-Ulm, Germany) and 3,5-dinitrobenzoyl chloride (3,5-DNBC), $C_7H_3ClN_2O_5$ (Regis Chemical Co., Morton Grove, IL, USA) used were partially decomposed during their

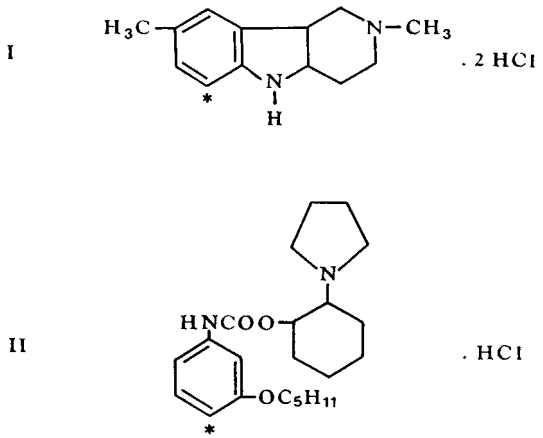

Figure 1. Chemical structures of dihydrochloride of stobadine (**I**) and hydrochloride of pentacaine (**II**). (The asterisks indicate the position of the tritium atom in the radiolabelled compounds.)

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storage by the action of daylight and atmospheric humidity.

$Na_2HPO_4 \times 12\ H_2O$ 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_3CN, and CH_3OH of HPLC purity grade were purchased from J. T. Baker Chemical Co., Phillipsburg, NJ, USA. CH_2Cl_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_3OH concentration of 20% or less. This working solution was then run through the sorbent bed of the preconditioned (2 mL CH_3OH, 2 mL H_2O) 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 (Šoltés and Trnovec, 1987) and/or an HPLC method (Ščasnár *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 (Šoltés *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 Ščasnár *et al.* (1984).

Procedure B_1. 4-NBC, 0.2 g (light-brownish powder) was dissolved in 2 mL of dry CH_2Cl_2. Next 8 mL of dry cyclohexane was added into this solution to weaken the solvent polarity. The final sample solution was then pressed through the SEP-PAK Silica cartridge (flow rate approximately 1–2 mL/min). By such a treatment the dark-brownish sample component(s) remained trapped at the top of the sorbent bed, while a light-yellow solution passed unretained through the cartridge. Subsequent crystallization — CH_2Cl_2 evaporation — yielded clear yellow needles (67%). The melting point (m.p.) was 74–75°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 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, 36.41:1.31:12.15; found, 36.20:1.38:12.20. (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 (Šoltés *et al.*, 1994; Šoltés, 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 minocolumn.

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.

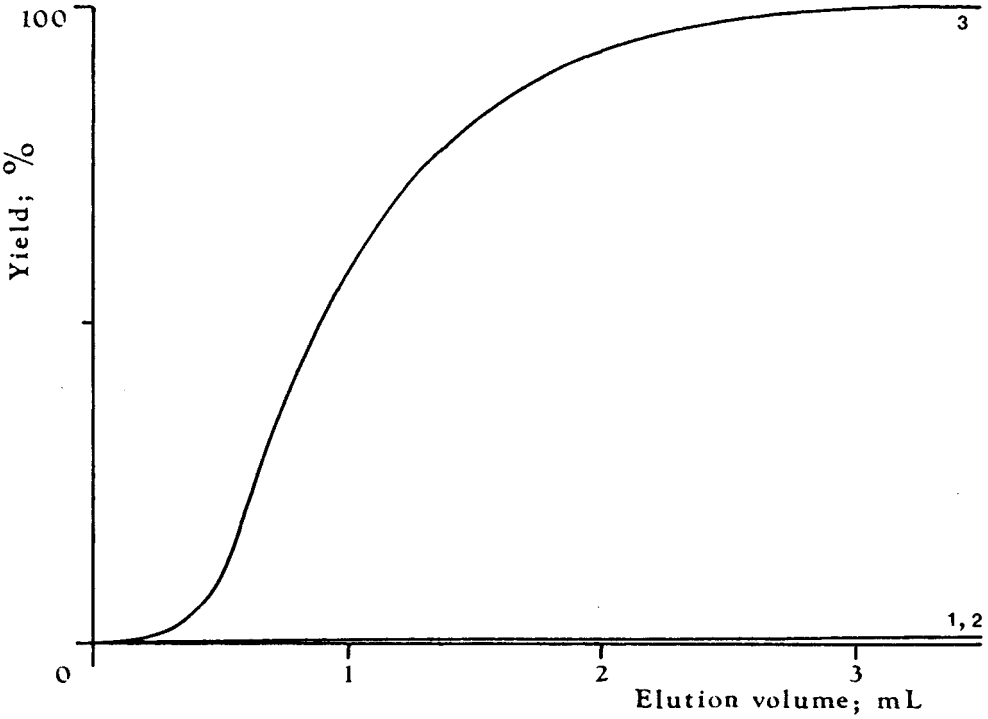

Figure 2. Cumulative elution of the hydrochloride of [4-^{3}H]-pentacaine from the SEP-PAK C_{18} cartridge by the weak solvents aqueous methanol — H_2O:CH_3OH, 80:20, v/v (1) and acetonitrile (2), and by strong methanol (3).

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 $\geqslant 95\%$. 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_1/A_2 resulted essentially in the following four low-volume fractions: (1) the 'original' aqueous–methanolic (volume $\leqslant 1.0$ mL); (2) the acetonitrilic wash (Šoltés, 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-^{3}H]-stobadine or of hydrochloride of [4-^{3}H]-pentacaine still detect an inadequate purity grade, the solid and liquid waste obtained by using procedures A_1/A_2 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 subsequently undergo further decomposition effected by heat, light, and also as a consequence of their reaction with the parent 4-NBC and 3,5-DNBC. Effective derivatization, however, requires a reagent of the purest quality to warrant one single resulting derivative with defined properties.

The SPE technique of purification of the given derivatization substances developed is based on the precondition that in the course of their reaction with H_2O vapors both 4-NBC and 3,5-DNBC are most probably contaminated by substances of higher polarity. Owing to the higher affinity of the latter to silica gel, these products can be separated from the molecules of the parent derivatization agent by exploiting, for example, the commercial SEP-PAK silica cartridge. The two-component liquid phase used (cyclohexane:CH_2Cl_2 80:20, v/v) proved to be particularly appropriate, though only for the elution of the purified substance (4-NBC, 3,5-DNBC), while its decomposition products remained trapped on the cartridge top. By the subsequent evaporation of the substantially more evaporative CH_2Cl_2 (Manufacturer's publication, Spectra-Physics) (boiling point (b.p.) 39.8°C), the purified 4-NBC or 3,5-DNBC (both substances are relatively poorly soluble in cyclohexane — b.p. 80.7°C) crystallize in the form of clear yellow needles.

Procedures C

On using aqueous chiral HPLC, both the ionic strength and the pH value of the mobile phase are set by adding inorganic or polar organic buffers, which should be substances of highest purity. Yet, as mentioned also in the literature (Manufacturer's Publication: Advanced Separation Technologies Inc.; Šoltés *et al.*, 1994, 1996; Šoltés, L. and Sébille, B., unpublished data), even some substances of declared p.a. quality contain impurities strongly adsorbing to the chiral stationary phase and thus gradually diminishing the resolution of the method. The suggested use of a guard precolumn with a chiral sorbent identical with that contained in the analytical column does present a certain solution, nevertheless pre-purification of the mobile phase is of advantage in any case.

Our approach, i.e. the application of SPE cartridges, removed most impurities both from the aqueous phosphate and the TEAAc buffer, which would have gradually blocked the chiral precolumn/column used. Although the purification of the mobile phase is time-consuming, the cost of the required disposable cartridges is low, so that the approach proves to be ultimately cost-effective, considering the relatively expensive chiral BSA-bond/CYCLOBOND I guard and/or analytical column(s).

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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