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Note

Use of Sep-Pak cartridges for the recovery of compounds from scintillation fluids

PETER L. DEVINE and B. V. MILBORROW*
University of New South Wales, P.O. Box 1, Kensington, N.S.W. 2033 (Australia)
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Compounds obtained in biosynthetic experiments may contain so little radioactive label that often the whole sample has to be counted. It is then extremely difficult to recover the compound so that it can be subjected to further analysis or re-used for further feeding experiments[1]. The very small quantities of labelled compound are contaminated with a many-fold excess of scintillant and solvents so that purification is complicated and causes considerable losses of the material under investigation.

We have used Millipore Sep-Pak cartridges[2] to overcome many of the difficulties encountered and while, for example, the recovery of trace amounts of labelled compounds from Triton-based scintillation mixtures is difficult, this problem can often be avoided if a scintillation mixture without a detergent is selected for experiments in which the need for a material to be recovered can be anticipated. We describe a method for the removal of solvents and common scintillants using either silica or alumina Sep-Pak cartridges.

MATERIALS

Millipore Silica or alumina B Sep-Pak cartridges (Millipore, Bedford, MA, U.S.A.) were used in this study. Wheaton Scientific supplied low potassium glass scintillation vials and polypropylene caps containing a metal foil liner. Toluene, acetone and 2-methoxyethanol of analytical-reagent grade were purchased from BDH, 2,5-bis-(5′-*tert.*-butyl-benzoxazolyl-2′-thiophene) (BBOT) from Ciba (Duxford, Cambridge, U.K.), naphthalene and 2,5-diphenyloxazole (PPO) from Sigma (Port Fairy, Australia). Radiochemicals used in this study were [1,2-$^3H_2$(N)]androst-4-ene-3,17-dione (45 Ci/mmol) (New England Nuclear, North Ryde, Australia) and β-[U-^{14}C]carotene (81 Ci/mol) (The Radiochemical Centre, Amersham, U.K.). These will be referred to as [^{3}H]androstenedione and [^{14}C]-β-carotene. Millipore HPLC-grade hexane was dried over molecular sieves type 13X (Union Carbide, Danbury, CT, U.S.A.) for at least an hour prior to use.

Two scintillation mixtures were used: scintillation mixture I, PPO in toluene (5 g/l); scintillation mixture II, toluene–2-methoxyethanol (3:2, v/v) containing naphthalene (80 g/l) and BBOT (6 g/l).

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METHODS

Removal of solvents

After liquid scintillation counting, the toluene and/or 2-methoxyethanol are removed by evaporation under a very gentle stream of high-purity nitrogen, while maintaining the temperature at 30°C in a water bath. The resulting powder is taken up in 10 ml dry hexane and this is again evaporated under nitrogen to ensure the complete removal of the original solvents.

BBOT, in particular, when pure, is deposited from solution as a friable powder which is prone to blow away under strong agitation, as from a jet of gas. Care has to be taken to avoid this occurrence or 2,6-di-*tert.*-butyl-4-methylphenol (BHT) an antioxidant) can be added to cause the residue to form a fused crust, provided it is separable from the compound sought.

The powder is re-dissolved in 10 ml dry hexane for application to the Sep-Pak cartridge.

Scintillation mixture I

Isolation of compounds less polar than scintillant. The hexane-solution is passed through a silica Sep-Pak cartridge, which has been pretreated with dry hexane (2 ml), PPO binds to the silica, while compounds of low polarity pass through the cartridge. The cartridge can be washed with a further 1–2 ml dry hexane to ensure all of the less polar compound has been washed through. More polar compounds, which are still less polar than the scintillant, may bind to the silica, but these can be eluted with 0.5% acetone in dry hexane, without displacing the scintillant. Two or three Sep-Pak cartridges can also be used in series to improve separation of a compound from the scintillant if necessary.

Isolation of compounds more polar than scintillant. Washing the cartridge with 10 ml 4% acetone in dry hexane removes the scintillant, PPO, leaving behind more polar compounds. These can be eluted with 10 ml acetone.

Scintillation mixture II

Isolation of compounds less polar than scintillant. The low solubility of BBOT in dry hexane enables most to be removed by centrifugation prior to the application of the solution to the Sep-Pak. After the sample has been added to the Sep-Pak cartridges the naphthalene and other non-polar compounds can be washed away with 1–2 ml dry hexane. This solution can then be applied to an alumina B Sep-Pak: the naphthalene passes through, but many other low-polarity compounds, such as β-carotene, may bind to the Sep-Pak. The compounds which are held by the alumina can then be eluted with acetone. It is also possible to remove much of the naphthalene by crystallisation from hexane at 4°C or volatilisation under a stream of nitrogen at 80°C, provided, of course, that the compound required is not volatile.

Any compounds less polar than the scintillant (BBOT) which do not pass through the silica Sep-Pak when applied in dry hexane can be eluted as described for scintillation mixture I.

Isolation of compounds more polar than scintillant. BBOT is eluted with 4% acetone in dry hexane (10 ml), and more polar compounds can then be eluted with acetone (5 ml) as described previously.

TABLE I

RECOVERY OF ANDROSTENEDIONE FROM SCINTILLATION MIXTURE I

Eluting solvent	*Radioactivity (dpm)*	*% Total radioactivity present*	*Major component**
Dry hexane (10 ml)	388	Negligible	Nil
4% Acetone in dry hexane (10 ml)	656	Negligible	PPO
Acetone (5 ml)	2643880	>99%	Androstenedione

* Based on both radioactivity and visual inspection after evaporation.

RESULTS

The following examples were chosen: (i) a more polar compound, the steroid hormone [^{3}H]androstenedione in 10 ml scintillation mixture I; (ii) a less polar compound, the carotenoid [^{14}C]-β-carotene in 10 ml scintillation mixture II.

Table I shows the data for the recovery of radioactive androstenedione from scintillation mixture I. Here, the scintillant PPO has been separated and [^{3}H]androstenedione has been recovered with negligible loss.

Table II shows the data for the recovery of radioactive β-carotene from scintillation mixture II. Obviously, it would be much simpler to recover β-carotene from mixture I, but this example has been included to show that it is possible efficiently to recover a non-polar compound from mixture II, in this case >80%. We see, from Table II, that some radioactivity apparently bound to the silica when the sample was applied in dry hexane.

DISCUSSION

The recovery of samples from scintillation fluids has conventionally been carried out by thin-layer chromatography (TLC) to separate labelled compound from the scintillant after the solvents have been evaporated. Sep-Pak cartridges provide a cheap, alternative and rapid method giving a high percentage recovery. One cartridge

TABLE II

RECOVERY OF β-CAROTENE FROM SCINTILLATION MIXTURE II

Eluting solvent	*Radioactivity (dpm)*	*% Total radioactivity present*	*Major component**
Dry hexane (10 ml)	3959	2	Naphthalene
Acetone (post silica) (10 ml)	29725	16	BBOT
Acetone (post alumina-B) (10 ml)	153841	82	β-Carotene

* Based on both radioactivity and visual inspection after evaporation.

can accomodate the material used in 10 ml of scintillation fluid and the Sep-Pak cartridges can be re-used after thorough washing and reactivation.

The utility of this method, as well as the choice of which scintillation mixture can be determined by prior comparison of the chromatographic properties of the scintillant and compound of interest. For example, a wide range of steroid hormones will all be found to have an R_F value of about 0.03 on Merck 20 × 20 cm glass-backed silica gel plates with 4% acetone in dry hexane as solvent, while PPO and BBOT had R_F values of 0.37 and 0.27, respectively, in this system. This method would therefore be applicable to this class of compounds.

It may not be necessary to chromatograph a valuable compound whose recovery is desired if its chromatographic behaviour is already known, the R_F value only of the scintillant needs to be measured.

Obviously, problems will arise if the compound to be recovered has a similar polarity to the scintillant. However, BBOT, PPO and naphthalene are resistant to borohydride reduction of ketones, diazomethane methylation of carboxyl and some phenolic groups and acetylation of hydroxyl groups by pyridine–acetic anhydride. It may, therefore, be possible to derivatize the compound by one of these reagents so that it can be separated from the scintillant. Most compounds, however, do not fall into this category and can be separated from one or other scintillant with ease.

It is also possible that highly polar compounds could not be eluted from the normal-phase Sep-Pak material, if this occurs C_{18} reversed-phase Sep-Pak cartridges can be used. Samples that retain contaminating traces of scintillant after passage through Sep-Pak can be purified by a final TLC or HPLC run if necessary.

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