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Note

Analysis of the radiochemical purity of [^{14}C]chloroform and dibromo[1,2-$^{14}C_2$]ethane by radiomonitored high-performance liquid chromatography

DAVID A. WELLS
Graduate Center for Toxicology, University of Kentucky, Lexington, KY 40536-0078 (U.S.A.)
and
GEORGE A. GARBOLAS and GEORGE A. DIGENIS*
Division of Medicinal Chemistry, College of Pharmacy, University of Kentucky, Lexington, KY 40536-0081 (U.S.A.)
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The determination of the radiochemical purity of a synthesized radioactive compound has traditionally involved a separation by thin-layer chromatography, column chromatography or gas chromatography (GC), with subsequent quantitation of radioactivity. In recent years, high-performance liquid chromatography (HPLC) has been recognized as a sensitive and rapid method for the separation and quantitation of radiolabeled compounds[1–5].

In order to examine the radiochemical purity of two commercially obtained products, [^{14}C]chloroform and dibromo[1,2-$^{14}C_2$]ethane, a chromatographic system of first choice would be GC, since methods for the separation of these compounds are well documented in the literature. However, a radioactivity detector on-line with a GC system was not available to us. As an alternative method, an HPLC assay was developed which separated chloroform from the expected chemical contaminants arising from its synthesis, namely dichloromethane and carbon tetrachloride, and which also separated 1,2-dibromoethane from bromoethane. The HPLC assay involved UV detection of the effluent at 205 nm followed by quantitation of radioactivity using a flow-through radioactivity detector. This paper details the use of radiomonitored HPLC in the determination of the radiochemical purity of commercially obtained [^{14}C]chloroform and dibromo[1,2-$^{14}C_2$]ethane.

EXPERIMENTAL

Chemicals and reagents

[^{14}C]Chloroform (2.5 mCi, 4.6 mCi/mmol; lot No. 1844-056) and dibromo[1,2-$^{14}C_2$]ethane (2.5 mCi, 3.8 mCi/mmol; lot No. 2157-031) were obtained from New England Nuclear (Boston, MA, U.S.A.). Dichloromethane, chloroform and methanol were of HPLC grade and purchased from Fisher Scientific (Fairlawn, NJ, U.S.A.). Carbon tetrachloride, bromoethane and 1,2-dibromoethane were of Gold Label grade and were purchased from Aldrich (Milwaukee, WI, U.S.A.). Solvents were filtered through a 0.45-μm Millipore filter and degassed before use.

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Instrumentation and chromatographic conditions

Identification of chemicals and evaluation of radiochemical purities were determined using an HPLC system consisting of an Altex 110A solvent metering pump (Altex, Berkeley, CA, U.S.A.), a Varian Vari-Chrom analytical UV detector (Varian, Palo Alto, CA, U.S.A.) and a Linear Instruments dual-channel recorder (Linear, Reno, NV, U.S.A.). Samples were injected via a Rheodyne Model 7125 injector (Rheodyne, Cotati, CA, U.S.A.) fitted with a 20-μl loop onto an Altex 5-μm Ultrasphere ODS column (25 cm × 4.6 mm I.D.). The mobile phase consisted of methanol–water (75:25, v/v), delivered at a flow-rate of 1.0 ml/min with UV detection at 205 nm. The effluent passed through the UV detector and then into a Flo-One HP flow-through radioactivity detector (Radiomatic, Tampa, FL, U.S.A.). The programmable detector mixed scintillation cocktail (Flo-Scint III, Radiomatic) with effluent in a ratio of 3:1 (v/v). The UV output was recorded on one channel of the recorder and the radioactivity output was recorded simultaneously on the second channel.

Sample preparation

The sealed glass ampule containing the neat sample of [^{14}C]chloroform (65 mg) or dibromo[1,2-$^{14}C_2$]ethane (123.5 mg) was cooled in dry ice and then broken open, and the contents were transferred to an empty glass ampule by using a micropipet. The glass ampule containing the transferred liquid sample was cooled in dry ice and then sealed by using a gas flame. The residue remaining adsorbed to the glass of the original ampule was dissolved in methanol and the successive rinses were combined into a volumetric flask (5 or 10 ml) to make a stock solution. An aliquot of each stock solution was added to 15 ml of scintillation cocktail (Scintiverse II, Fisher Scientific) and counted for radioactivity in a Packard Model 3255 liquid scintillation counter (Packard, Downers Grove, IL, U.S.A.). Another aliquot was injected into the HPLC system for the determination of radiochemical purity.

RESULTS AND DISCUSSION

Retention times of unlabeled standards

Stock solutions of the chemical standards were prepared in methanol (1, 2 or 10 mg/ml) and analyzed by HPLC (for conditions, see Experimental). The chromatogram representing chloroform and its derivatives is shown in Fig. 1 and the retention times are as follows: dichloromethane (a), 3.7 min; chloroform (b), 4.5 min; and carbon tetrachloride (c), 8.3 min. Fig. 2 illustrates the chromatogram of bromoethane (a) and 1,2-dibromoethane (b), having retention times of 4.8 and 5.4 min, respectively. Note that in each chromatogram the first two mass peaks (UV detection) are from the methanol solvent used. This was confirmed by injecting methanol alone and observing the identical peaks on the chromatogram.

Radiomonitored HPLC analysis of [^{14}C]chloroform

A 20-μl aliquot of the stock solution of [^{14}C]chloroform in 5 ml methanol (17.3 μCi/ml, 0.45 mg/ml) was analyzed by radiomonitored HPLC and the corresponding chromatogram is shown in Fig. 3. Five significant radioactive peaks (upper tracing on chromatogram) were identified which together account for 99.7% of the total radioactivity present in the sample. The identities of peaks 1 and 4 are unknown and

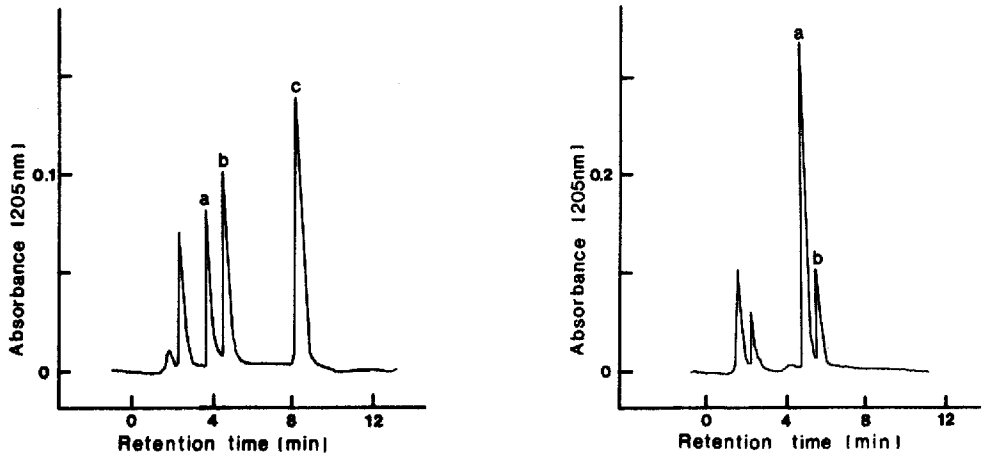

Fig. 1. HPLC chromatogram of a mixture of dichloromethane (a), chloroform (b) and carbon tetrachloride (c). The first two UV peaks are from the methanol solvent used in preparing the stock solutions. See Experimental for chromatographic conditions.

Fig. 2. HPLC chromatogram of a mixture of bromoethane (a) and 1,2-dibromoethane (b). The first two UV peaks are from the methanol solvent used in preparing the stock solutions. See Experimental for chromatographic conditions.

together represent 2.2% of the total radioactivity. Peak 2 was determined to be dichloromethane, based on the retention time of the radioactive peak compared to that of the unlabeled standard, although no definitive UV absorption peak is detectable. This impurity represents 3.5% of the total radioactivity. Peak 5 is also an impurity, determined (in the same manner as described for dichloromethane) to be carbon tetrachloride, present as 0.8% of the total radioactivity. Peak 3 represents [^{14}C]chloroform and is detectable by UV absorption as peak c (lower tracing on chromatogram), having a retention time of 4.5 min. The radiochemical purity of [^{14}C]chloroform was determined to be 93.2%. The two UV peaks designated as a and b are from the methanol solvent used in preparing the stock solutions.

Radiomonitored HPLC analysis of dibromo[1,2-$^{14}C_2$]ethane

A 20-μl aliquot of the stock solution of dibromo[1,2-$^{14}C_2$]ethane in 10 ml methanol (6.8 μCi/ml, 0.34 mg/ml) was analyzed by radiomonitored HPLC and the corresponding chromatogram is shown in Fig. 4. Two major radioactive peaks (upper tracing on chromatogram) were identified which together account for 99.9% of the total radioactivity present in the sample. Peak 1 was determined to be bromoethane, based on the retention time of the radioactive peak compared to that of the unlabeled standard, although no definitive UV absorption peak is detectable. Peak 2 was determined to be dibromo[1,2-$^{14}C_2$]ethane, confirmed by UV absorption as peak c (lower tracing on chromatogram) with a retention time of 5.4 min. The radiochemical purity of dibromo[1,2-$^{14}C_2$]ethane was determined to be 99.6%. Radioactivity peaks 3 and 4 arising at about 9 min are unidentified but are likely to be polybrominated derivatives and are known to collectively contain less than 0.1% of the total radioactivity in the sample. The two UV peaks designated as a and b are from the methanol solvent used in preparing the stock solutions.

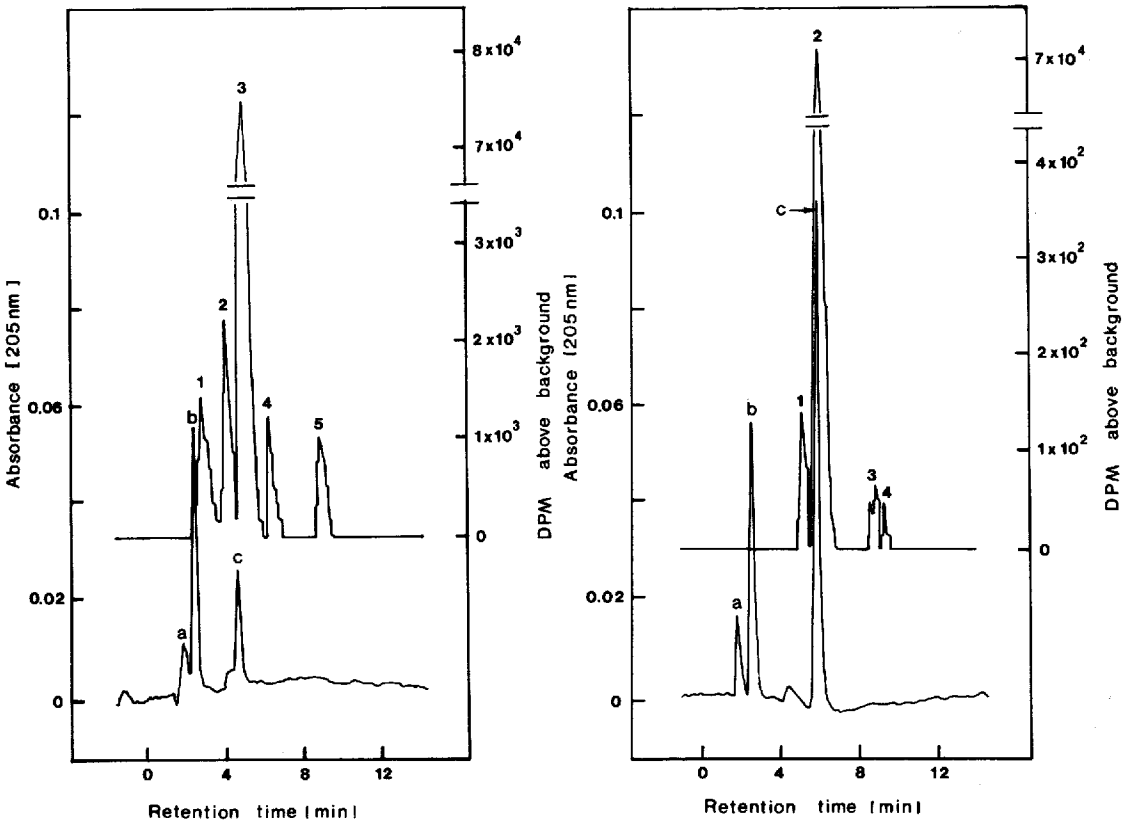

Fig. 3. Lower tracing: HPLC chromatogram of [^{14}C]chloroform (c) in methanol (a, b) by UV detection at 205 nm. Upper tracing: Radiochromatogram; ^{14}C-detection by direct analysis of column effluent using a flow-through radioactivity detector (see Experimental for details and chromatographic conditions). Peaks: 1 = unidentified; 2 = dichloromethane; 3 = chloroform; 4 = unidentified; 5 = carbon tetrachloride.

Fig. 4. Lower tracing: HPLC chromatogram of dibromo[1,2-$^{14}C_2$]ethane (c) in methanol (a, b) by UV detection at 205 nm. Upper tracing: Radiochromatogram; ^{14}C-detection by direct analysis of column effluent using a flow-through radioactivity detector (see Experimental for details and chromatographic conditions). Peaks: 1 = bromoethane; 2 = 1,2-dibromoethane; 3, 4 = unidentified.

CONCLUSIONS

[^{14}C]Chloroform and dibromo[1,2-$^{14}C_2$]ethane were commercially obtained and their radiochemical purities were evaluated. In the case of [^{14}C]chloroform, the manufacturer reported a radiochemical purity of 97.4% using GC with flame ionization detection, with the system coupled to an undescribed radioactivity detector. A radiomonitored HPLC analysis of the same sample determined that the radiochemical purity was only 93.2%. The results for the dibromo[1,2-$^{14}C_2$]ethane sample were more closely comparable between the GC and HPLC radiomonitored systems. By GC analysis, also with flame ionization detection and an undescribed radioactivity

detector, the manufacturer reported 99% radiochemical purity, while HPLC analysis determined the purity to be 99.6%.

In the determination of the radiochemical purity of synthesized radiolabeled products, it has been shown in this report that radiomonitored HPLC is a very sensitive method and can yield more accurate results than those obtainable by radio-gas chromatography. It is suggested that HPLC be used more routinely in evaluations of the radiochemical purities of labeled compounds. A flow-through radioactivity detector can easily be added to any existing HPLC system and its usefulness can be extended to radioactivity quantitation of biological samples from radiotracer experiments.

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