Journal of Chromatography, 333 (1985) 269–275
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17 968

Note

Chromatography of radiolabelled anions using reversed-phase liquid chromatographic columns

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(First received May 8th, 1985; revised manuscript received June 13th, 1985)

The development of chromatographic techniques for anions is one of the fastest growing areas in the field of high-performance liquid chromatography (HPLC)[1–3]. Recently, single-column ion chromatographic (SCIC) methods have been devised which have avoided the use of the earlier chemical-suppression techniques[4–15]. Separations of numerous inorganic and organic anions were achieved using either hydrocarbon-bonded phases[4–8] or macroporous polymer phases[9–11]. One approach utilized reversed-phase C_{18} columns with aqueous mobile phases containing ammonium compounds, such as tetrabutylammonium salts[5,6] or octylamine[7]. These techniques do not require special equipment and separations can be achieved on conventional liquid chromatographic (LC) systems using conductivity or ultraviolet (UV) detection. Applications of SCIC to the analysis of radiobromine and radioiodine labelled anions were recently reported[16,17].

This paper reports the adaptation of reversed-phase C_{18} columns using an aqueous mobile phase containing octylamine for separation of numerous radiolabelled anions and demonstrates the applicability of the procedure to several radiochemical determinations. The various anions, examined by low-wavelength UV detection and/or radiochemical (γ) detection, were labelled with either ^{11}C (half-life, $t_{\frac{1}{2}}$ = 20.4 min), ^{13}N ($t_{\frac{1}{2}}$ = 10.0 min), ^{18}F ($t_{\frac{1}{2}}$ = 109.8 min), ^{82}Br ($t_{\frac{1}{2}}$ = 35.3 h), ^{95m}Tc ($t_{\frac{1}{2}}$ = 61 days), ^{99m}Tc ($t_{\frac{1}{2}}$ = 6.0 h) or ^{131}I ($t_{\frac{1}{2}}$ = 8.0 days).

EXPERIMENTAL

Literature procedures were used for the preparation of $^{11}CN^-$ (ref. 18), $^{11}CO_2$ (ref. 18), $O^{11}CN^-$ (ref. 19), $^{13}NO_2^-$ (refs. 20 and 21), $^{13}NO_3^-$ (refs. 20 and 21), $^{18}F^-$ (refs. 22 and 23) and $^{95m}TcO_4^-$ (ref. 24). $^{11}CN^-$ was trapped in 100 μl of ethanol containing 5 μl of 1 *M* potassium hydroxide. The ethanol was removed under vacuum and 100 μl of water was added. $^{11}CO_2$ was collected in dilute sodium hydroxide. $^{82}Br^-$ was prepared by neutron irradiation of dilute hydrobromic acid. Therapeutic $Na^{131}I$ solution (Syncor) was claimed to be "carrier free" and was supplied in phosphate-buffered sodium chloride containing up to 0.16% sodium thiosulfate with a pH of 7.5–9.0, adjusted with sodium hydroxide. $^{131}IO_3^-$ was prepared by Br_2 oxidation of $^{131}I^-$. $^{99m}TcO_4^-$ was obtained from $^{99}Mo/^{99m}Tc$ generators (Union Car-

0021-9673/85/$03.30

bide). Octylamine and Na_2MoO_4 (Aldrich) as well as other chemicals were reagent grade and used as received.

All solvents for HPLC analysis were degassed ultrasonically under vacuum before use. Water was deionized and distilled; acetonitrile was obtained from Burdick and Jackson. Separations were carried out on either a LiChrosorb RP-18 column (Alltech, 10 μm, 25 cm × 4.6 mm I.D.) or an Alltech C_{18} column (10 μm, 25 cm × 4.6 mm I.D.), both equipped with a guard column of Analytichem C_{18} (40 μm, 3 cm × 4.6 mm I.D.). The mobile phase was delivered with an Altex Model 330 solvent delivery system connected to an Altex Model 210 syringe injector having a 20-μl loop. UV detection was performed with an Altex Model 155-40 variable-wavelength detector operated in the absorbance mode. Detection was routinely performed at a range setting of 0.05 a.u.f.s.; limits of detection were determined at a range setting of 0.01 a.u.f.s. (signal-to-noise ratio was 2). The mobile phase was 0.01 *M* octylamine adjusted to a pH of 4.5–6.5 with phosphoric acid. If necessary, acetonitrile was added to the mobile phase to decrease ion retention. Eluates were continuously monitored for radioactivity with a NaI(Tl) detector on-line with a Nuclear Data Model 60A multichannel analyzer operated in the multichannel scaling mode. Gamma spectra were recorded using a Ge(Li) detector equipped with a Nuclear Data Model 600 multichannel analyzer.

RESULTS AND DISCUSSION

The retention times of various radiolabelled anions were determined on C_{18} reversed-phase columns using 0.01 *M* octylamine as the mobile phase (Tables I and II). The pH was adjusted to less than 7 by phosphoric acid. The retention times of most of the ions were established using appropriate standards. Direct UV detection in the range of 200–235 nm was found to be a convenient method for the determi-

TABLE I

ANION ANALYSIS ON A LICHROSORB RP-18 REVERSED-PHASE COLUMN

Mobile phase was 0.01 *M* octylamine adjusted to pH 4.7 with phosphoric acid at a flow-rate of 2.0 ml/min. Number in parentheses is the wavelength (nm) at which the limit of detection was determined.

Anion (radiolabel)	*Retention time (min)*	*Lower limit of detection (μg/ml)*
$CN^-(^{11}C)$	2.5	*
$HCO_3^-(^{11}C)$	4.3	*
$OCN^-(^{11}C)$	8.9	1.05 (210)
$NO_2^-(^{13}N)$	9.7	0.05 (210)
$NO_3^-(^{13}N)$	12.4	0.03 (210)
$F^-(^{18}F)$	6.7	*
$Br^-(^{82}Br)$	9.1	0.08 (200)
$IO_3^-(^{131}I^-)$	7.0	0.60 (205)
$I^-(^{131}I)$	16.0	0.12 (235)
$TcO_4^-(^{95m}Tc, ^{99m}Tc)$	6.0**	*
$TcO_4^-(^{95m}Tc)$	12.8**	*

* No determination performed.

** 10% and 20% acetonitrile added to mobile phase for 12.8 min and 6.0 min retention times, respectively.

TABLE II

ANION ANALYSIS USING AN ALLTECH C_{18} REVERSED-PHASE COLUMN

Anion	*Retention time (min)*
Mobile phase pH = 4.7*	
NO_2^-	22.3
NO_3^-	40.1
Br^-	21.0
IO_3^-	12.6
Mobile phase pH = 6.2*	
NO_2^-	7.1
NO_3^-	12.2
Br^-	5.0
IO_3^-	3.3
I^-	17.5
MoO_4^{2-}	8.0**
TcO_4^-	10.2**

* Mobile phase was 0.01 *M* octylamine; pH adjusted with phosphoric acid. Flow-rate was 2 ml/min.

** Mobile phase contained 20% acetonitrile; pH 5.7. The limit of detection for MoO_4^{2-} was 0.1 μg/ml at 200 nm and 0.9 μg/ml at 254 nm.

nation of many of the ions and resulted in excellent sensitivity as indicated by the lower limit of detection (Table I). The limit of detection could be increased by increasing the injection volume. Calibration curves for NO_2^-, NO_3^-, I^-, IO_3^- and MoO_4^{2-} indicated a linear detector response as a function of mass over the range investigated.

The ions F^-, HCO_3^- and CN^- are in a group of ions considered to be optically transparent[1–3]. These ions were examined by radiochemical detection. Standard samples of $^{99}TcO_4^-$ can be used for quantitation of pertechnetate by UV detection[25–27]; however, a standard sample was not available to us. Nevertheless, mass amounts of pertechnetate were observable at 254 nm in this study following the decay of ^{99m}Tc to ^{99}Tc.

Skelly[7] recently reported variations in ion retention and efficiency for different reversed-phase packing materials when using octylamine. We observed significant differences between the LiChrosorb RP-18 column and the Alltech C_{18} column (Tables I and II). The retention of the ions on the Alltech C_{18} column was much greater than on the RP-18 column when using a pH of 4.7. Decreasing the amount of acid (pH 6.2) resulted in less retention of the ions on the Alltech C_{18} column (Table II). The addition of acetonitrile to the mobile phase has been shown to decrease retention[7]. This is illustrated in Tables I and II for pertechnetate on both columns.

An investigation was performed using $^{131}I^-$, $^{131}IO_3^-$ and $^{99m}TcO_4^-$ to determine if these ions were retained on the HPLC column or ancillary equipment (Table III). The percent recovery was determined by comparison of the amount of radioactivity injected and ultimately eluted from the column, to standard samples of radioactivity of identical injection volume. None of the ions adhered to the stainless-steel injector or connective tubing. To study adsorption on the column, the percent re-

covery of ions injected with no-added-carrier was compared to injections with added-carrier. The only ion which was retained to any significant extent at no-carrier-added levels was $^{131}I^-$ (Table III). The no-carrier-added $^{131}I^-$ was not irreversibly absorbed; the injection of 10 μg of iodide resulted in co-elution of the radioactivity with the iodide.

TABLE III

RECOVERY OF RADIOLABELLED ANIONS AFTER INJECTION ON HPLC SYSTEM

The determinations were performed using conditions listed in Table I.

Anion	*Mass injected*	*Recovery (%)*
$^{131}I^-$	0.3–0.6 ng*	96.6 ± 2.5
$^{131}I^-$	30 μg	99.4 ± 0.5
$^{131}IO_3^-$	1–2 ng*	99.6 ± 1.1
$^{99m}TcO_4^-$	**	99.4 ± 1.1

* The mass amounts injected are based on the specific activities of the anions: $^{131}I^-$ was 491 ± 13 mCi/μmol; $^{131}IO_3^-$ was 178 ± 8 mCi/μmol.

** A comparison was made of a sample of pertechnetate immediately after elution from a $^{99}Mo/^{99m}Tc$ generator and after a significant time period for decay of ^{99m}Tc to ^{99}Tc (24 h).

Applications to radiochemical determination

The separations which were developed are sufficiently applicable to a variety of radiochemical determinations.

^{11}C. Excellent resolution of the ^{11}C labelled anions CN^-, HCO_3^-, and OCN^- was achieved (Table I). $H^{11}CO_3^-$ was produced by collection of $^{11}CO_2$ [specific activity *ca.* 40 mCi/μmol at end of bombardment (EOB)] in dilute base or by oxidation of $^{11}CN^-$ (ref. 19). The retention time of the ^{11}C species produced from oxidation of $^{11}CN^-$ was found to vary on occasion as a function of carrier-added ions. For example, on one occasion the no-carrier-added peak eluted at 11.5 min; the addition of carrier Na_2CO_3 resulted in elution at 9.4 min and the addition of carrier $NaHCO_3$ resulted in elution at 4.3 min. Normally, the no-carrier-added peak was observed at 4.3 min and was, therefore, assumed to be HCO_3^-. No-carrier-added $^{11}CN^-$ usually resulted in a peak with considerably more trailing than carrier-added $^{11}CN^-$. The specific activities of the no-carrier-added $^{11}CN^-$ and the $H^{11}CO_3^-$, produced from $^{11}CN^-$, were 2–3 Ci/μmol at EOB[28]. During a typical injection, less than 0.1 ng of material was applied to the column. Different ionic species can exist depending on the pH of the injection. Trace (μg) amounts of carrier species were usually added to insure adequate resolution during analytical determinations.

In a recent investigation $^{11}CN^-$ was oxidized to $O^{11}CN^-$ then subsequently transformed into [^{11}C]urea[19]. The kinetics of several steps of the conversion were determined using an LC technique and radiochemical detection[19]. For example, the rate of basic hydrolysis of $O^{11}CN^-$ to $^{11}CO_3^{2-}$ was determined to be $(1.0 \pm 0.1) \cdot 10^{-3}$ min^{-1} at 113°C. Using the system developed in this study, the rate of the hydrolysis was determined to be $(0.91 \pm 0.11) \cdot 10^{-3}$ min^{-1} which is in agreement with our previous value and the literature[29].

^{13}N. The proton irradiation of water results in the formation of $^{13}NO_2^-$, $^{13}NO_3^-$, and $^{13}NH_4^+$ as the primary products. This irradiation has been investigated thoroughly including HPLC ion-exchange separations of the species formed[20,21]. To study the applicability of the reversed-phase system, we investigated the ions formed during proton irradiation of water. The target had an internal volume of 5.4 cm^3. Circulation of 15 ml of aerated water through the target during irradiation resulted in a NO_3^-–NO_2^- ratio of 9.2:1 with a specific activity of 28.5 mCi/μmol at EOB. Irradiation of a target in the static mode containing 5.4 ml of water gave a $^{13}NO_3^-$–$^{13}NO_2^-$ ratio of 19:1 and a specific activity of 127 mCi/μmol at EOB. The sensitivity of detection for NO_3^- is sufficient to allow determination of specific activities >2 Ci/μmol. Another product which was observed was $^{13}N_2$ which eluted at the void volume of the column. $^{13}NH_4^+$ was not eluted from the column; the addition of 20% acetonitrile to the mobile phase resulted in elution of a very broad $^{13}NH_4^+$ peak at 3.2 min.

Radiohalogens. Excellent resolution of a number of radiolabelled halogens was achieved. $^{18}F^-$ was produced by the proton irradiation of ^{18}O enriched water[22,23]. The no-carrier-added $^{18}F^-$ did not elute from the column; the addition of carrier F^- (μg amounts) resulted in elution at 6.7 min (Table I). A recent investigation demonstrated that the retention times of some high specific activity no-carrier-added compounds vary as a function of the amount of carrier compounds added[30].

The Na^{131}I solution used in this study was claimed to be carrier-free. We recently determined that the use of similar solutions for radioiodination of organic molecules resulted in compounds with specific activities of approximately 1 Ci/μmol, calculated to the calibration time of the solution. The specific activities of two lots of $^{131}I^-$ as determined by conditions in Table II were 469 ± 19 and 491 ± 13 mCi/μmol, respectively. The specific activity was determined by relating the amount of radioactivity eluted from the column to the mass of iodide as determined by UV detection. The sensitivity of detection for I^- could allow determination of much higher specific activities. The results shown in Table III indicate that a problem may be encountered in quantitative elution of high specific activity $^{131}I^-$ from the column. For quality control purposes only, carrier iodide could be added to a sample submitted for HPLC analysis.

A study was also performed on the amount of $^{131}IO_3^-$ in the Na^{131}I solution comparing thin-layer chromatographic (TLC) techniques to HPLC. Using established TLC procedures[31–33], the $^{131}IO_3^-$ was determined to range from 0.2 to 1.8%, whereas analysis using the SCIC techniques reported here indicted no $^{131}IO_3^-$ in the solution (detectable limit >0.05% of $^{131}I^-$ injected). Trace amounts of $^{131}IO_3^-$ should not be retained on the HPLC system (Table III). This was further confirmed by the addition of 10 μg of IO_3^- to the $^{131}I^-$ sample to be analyzed. No $^{131}IO_3^-$ was observed. Oxidation of $^{131}I^-$ to $^{131}IO_3^-$ could be occurring on the TLC surface[34]; nonetheless the results demonstrate the differences inherent to various methods of analysis. The formulation contained up to 0.16% thiosulfate as an antioxidant. It is possible that truly carrier-free $^{131}I^-$ solutions and solutions containing no antioxidant would yield greater discrepencies between TLC and HPLC analyses[31,34]. Previously, Machulla *et al.*[35] developed an ion-exchange HPLC separation for various radioiodine species. At that time they pointed out the problems associated with TLC quality control of carrier-free radioiodide solutions.

Pertechnetate. An HPLC method using an amino-bonded phase column has been reported by Zodda and co-workers[25,26] for analysis of pertechnetate in the eluate from $^{99}Mo/^{99m}Tc$ generators. They reported[26] that MoO_4^{2-} was either completely retained on the column or was too dilute to be detected by UV. In our investigation separation of MoO_4^{2-} and TcO_4^- was achieved using C_{18} columns (Tables I and II). The MoO_4^{2-} could be detected in concentrations as low as 0.1 μg/ml using a UV detector at 200 nm (Table II). Radiochemical detection could also be used for $^{99}MoO_4^{2-}$; none was found during routine analyses of $^{99m}TcO_4^-$ eluates from the generators. This was confirmed by γ-spectra analysis of the generator eluate.

We have recently presented the preparation of ^{95m}Tc from the $^{nat}Mo(p,xn)^{95m}Tc$ reaction[24]. After dissolution of the target in NaOH and extraction into methylethylketone, the chemical form of the ^{95m}Tc was shown to be $^{95m}TcO_4^-$ by simultaneous injection with $^{99m}TcO_4^-$. The concentrations of the MoO_4^{2-} were also monitored before and after elution of the $^{95m}TcO_4^-$ from an alumina column with 0.9% sodium chloride. No MoO_4^{2-} was detected after elution.

CONCLUSIONS

The applicability of readily available reversed-phase HPLC columns, using octylamine in the mobile phase, to the analysis of several radiolabelled anions has been demonstrated. The columns are easily converted back to true reversed-phase character by washing with acetonitrile or methanol. The use of other methods of detection such as electrochemical, conductivity or indirect UV[1–3] should expand the usefulness of the technique.

ACKNOWLEDGEMENTS

The research was supported in part by PHS Grant NINCDS 1 PO1 NS 15639. The authors express their appreciation to Ms. Zohra Khatib and Ms. Jan Holle for aiding in manuscript preparation.

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