J. Pharm. Pharmacol. 1982: 34: 14–17
Received July 1, 1981

0022–3573/82/010014–04 $02.50/0

A high performance liquid chromatographic method for determination of the radiochemical purity of iodohippuric acid [^{123}I] injection

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A method for the separation of the radiopharmaceutical iodohippuric acid ([^{123}I]IHA) from the two impurities produced in the labelling reaction, iodide (^{123}I$^-$) and iodobenzoic acid ([^{123}I]IBA) has been developed using reversed-phase high performance liquid chromatography (h.p.l.c.). The effect of eluent pH on the separation of the three compounds was studied. An eluent consisting of methanol: 0·1 M acetic acid (30:70) adjusted to pH 4·0 with NaOH gave optimum phase capacity ratios of −0·21 for I$^-$, 0·93 for IHA and 1·54 for IBA. On the chromatograph used, this corresponded to an analysis time of 4 min per sample. To recover ^{123}I$^-$ from the column with a good peak shape, it was found necessary to include sodium iodide (1 mg/100 ml) in the eluent. The recoveries of ^{123}I$^-$ ^{123}IHA and ^{123}IBA from the column were found to be 100·6% (s.d. 1·3), 100·2% (s.d. 2·1) and 98·1% (s.d. 1·2) respectively (n = 10 for each compound). H.p.l.c. was compared with the standard thin layer chromatographic (t.l.c.) method of analysis on 10 preparations of [^{123}I]IHA which gave a mean radiochemical purity of 99·1% (± 0·2 s.e.) by h.p.l.c. and 98·3% (± 0·2 s.e.) by t.l.c. This difference was found to be significant ($P < 0·05$) and an explanation for this difference is proposed. A coefficient of variation of 0·1% on the results from the repeated analysis (n = 20) of one preparation of [^{123}I]IHA, demonstrated that the h.p.l.c. method was capable of very high precision.

o-Iodohippuric acid (^{123}I) injection ([^{123}I]IHA) is a radiopharmaceutical used in the assessment of renal function (Short et al 1973; Zielinski et al 1977). It is prepared by isotopic exchange between the iodine of *o*-iodohippuric acid (IHA) and radioactive iodide (^{123}I$^-$) obtained as sodium iodide (^{123}I) solution. The potential radiochemical impurities in the resulting [^{123}I]IHA are unreacted ^{123}I$^-$ and *o*-iodobenzoic acid ([^{123}I]IBA) which is formed either by exchange labelling of *o*-iodobenzoic acid (IBA) present in the IHA raw material or by decomposition of the [^{123}I]IHA (Kaspersen & Westera 1980).

Determination of the radiochemical purity of [^{123}I]IHA injection should be performed before the product is released for clinical use and when dealing with short half-life radionuclides such as ^{123}I (half-life = 13·3 h), it is desirable to have a rapid method of analysis. The standard method for the analysis of [^{123}I]IHA is thin layer chromatography (t.l.c.) (Bijl et al 1977) which takes approximately 1 h. High performance liquid chromatography (h.p.l.c.) is rapid, capable of high resolution and although brief mention of its use in the determination of the radiochemical purity of [^{123}I]IHA has appeared in the literature (Machulla et al 1976; Westera et al 1978; Kaspersen & Westera 1980), no detailed study has been reported. It was therefore decided to define the parameters for an h.p.l.c. assay of [^{123}I]IHA with a view to the development of a rapid method for routine use.

MATERIALS AND METHODS

Equipment

The liquid chromatograph consisted of: a dual-piston reciprocating pump (Perkin Elmer Series 2/1); a loop-valve injector (Rheodyne 7120) with a 20 µl loop; a radiation detector constructed from a 10 µl coil of PTFE tubing (i.d. 0·3 mm) positioned inside a well-type sodium iodide detector coupled to a nucleonic counting system (J & P Engineering MS 310) with outputs to both a chart recorder and a digital printer; an ultraviolet detector (Pye Unicam LC UV); and a twin pen chart recorder used to record u.v. and radiation chromatograms simultaneously. All work was performed on the reversed phase column packing, Hypersil SAS (Shandon). The chromatographic conditions are shown in Fig. 1.

The thin layer radiochromatogram scanner consisted of a sodium iodide detector with a 2 mm slit collimator, coupled to the nucleonic counting system described above.

Eluent

Eluents consisting of mixtures of methanol and 0·1 M acetic acid were used to investigate the separation of I$^-$, IHA and IBA. The effect of eluent pH was

investigated, 5 M NaOH being used for pH adjustment. The phase capacity ratio (k') for each component was measured at half pH unit intervals between 3·5 and 7·0 by u.v. detection.

With an eluent consisting of methanol −0·1 M acetic acid (30:70) adjusted to pH 4·0, and radiation detection, sodium iodide (^{123}I) solution (AERE Harwell) diluted to 4MBq ml^{-1} with 0·02 M NaOH was injected onto the column. A very poor peak shape was obtained. In an attempt to improve the shape of this peak, the injection was repeated using eluents containing various concentrations (0·01, 0·1 and 1·0 mg/100 ml) of sodium iodide (NaI).

Preparation of iodohippuric acid (^{123}I)

A modifiction of the method of Wanek et al (1977) was used. 3·0 ml of a sterile solution containing 20 mg iodohippuric acid (Aldrich) and 2 mg cupric sulphate ($5H_2O$) (BDH Analar) was added to a 10 ml sterile vial containing 1–2 ml of sodium iodide (^{123}I) in 0·02 M NaOH (300–450MBq) (AERE Harwell). Labelling was then achieved by placing the vial in a boiling water bath for 20 min. A 4MBq ml^{-1} dilution of the labelled product was used for chromatographic studies. A similar method was used for the preparation of iodobenzoic acid (^{123}I) except that: (a) the 20 mg IBA (Aldrich) was dissolved in 1·5 ml of ethanol to which 1·5 ml of an aqueous solution containing 2 mg $CuSO_4$, $5H_2O$ was added, and (b) only 25MBq of sodium iodide (^{123}I) in 2·0 ml 0·02 M NaOH was used.

Recovery studies

To ensure that no radioactive species were being retained on the column, ten 20 µl samples of each radioactive solution (^{123}I$^-$, [^{123}I]IHA and [^{123}I]IBA) were injected onto the column and the eluates collected. An eluent consisting of methanol–0·1 M acetic acid (30:70) containing 1 mg NaI/100 ml and adjusted to pH 4 was used. Using a 20 µl micropipette, ten 20 µl standards of each radioactive solution were prepared. The samples and standards were assayed for radioactivity in an automatic gamma sample counter (Wallac 80000) and the recoveries from the column calculated.

Fig 1

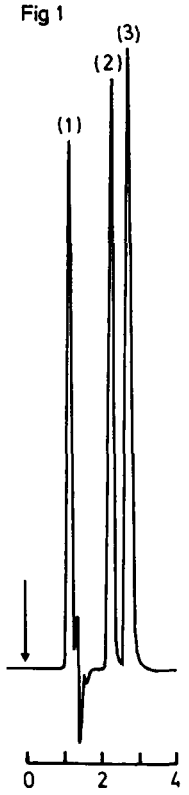

FIG. 1. The chromatogram of I$^-$ (1), IHA (2) and IBA (3). Column: packing Hypersil SAS 5 µm; length 100 mm; diam. 5 mm. Eluent: see text. Flow rate 1 ml min^{-1}. Pressure 1000 p.s.i. Detector: u.v. at 240 nm, AUFS.

Fig 2

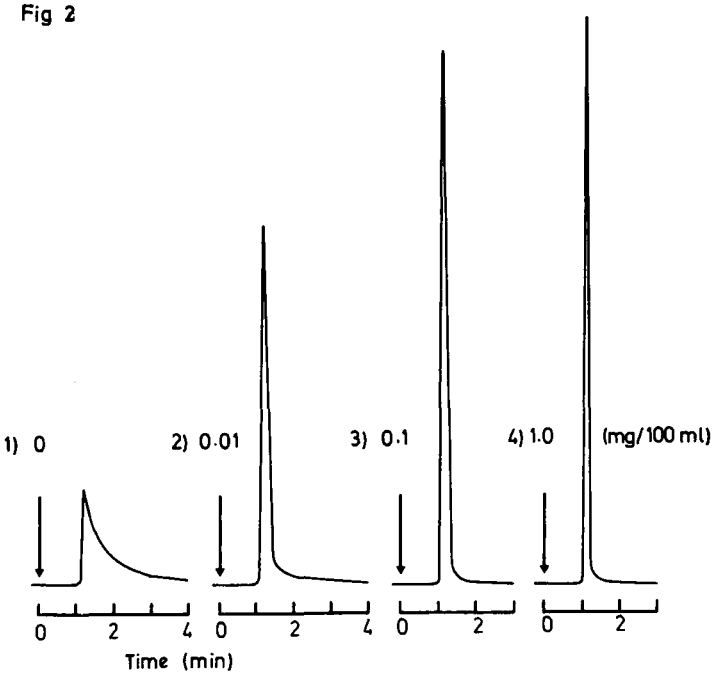

FIG. 2. The effect of different eluent concentrations of sodium iodide on the shape of the ^{123}I$^-$

Comparison with t.l.c.

Ten preparations of [^{123}I]IHA were analysed for radiochemical purity by both t.l.c. and h.p.l.c. T.l.c. was performed on silica gel plates (Merck 5554) with a solvent consisting of the upper layer of a mixture of benzene–glacial acetic acid–n-butanol–water (70:70:20:30) (Bijl et al 1977). The distribution of radioactivity on the plate was then measured using the radiochromatogram scanner. The R_F values for ^{123}I$^-$, [^{123}I]IHA and [^{123}I]IBA are 0·1, 0·5 and 0·9 respectively.

The h.p.l.c. analyses were performed using the eluent described above. To assess the precision of the h.p.l.c. analysis, one preparation of [^{123}I]IHA was analysed 20 times using the conditions described above.

RESULTS AND DISCUSSION

Methanol–0·1 M acetic acid (40:60) gave a good separation of IHA, IBA and I$^-$, but column life was rather short, probably because of the low pH of the eluent. Raising the pH of this eluent however resulted in the loss of separation of IHA and IBA. When the eluent was changed to methanol–0·1 M acetic acid (30:70) and pH adjustment performed, retention of the IHA and IBA was found to decrease as the pH was increased. No retention of the I$^-$ was observed at any pH. pH 4·0 was chosen as the optimum for the separation, giving phase capacity ratios (k′) of 1·54, 0·93 and −0·21 for IBA, IHA and I$^-$ respectively. Fig. 1 shows the chromatogram obtained using this eluent, the three peaks being well resolved.

When the sodium iodide (^{123}I) solution was chromatographed using the methanol–0·1 M acetic acid (30:70) eluent (pH 4) and radiation detection, a very poor peak shape was obtained (see Fig. 2·1). It was thought that this might be due to the fact that the 'carrier free' iodide (^{123}I), was being adsorbed onto the unreacted silanol sites of the reversed phase column packing. This artifact of reversed-phase h.p.l.c. column packings has been previously reported by Knox & Jurand (1977) who demonstrated the degradation of the peak shapes of paracetamol and its metabolites in the presence of unreacted silanol sites. Fig. 2 shows the effect of adding different concentrations of 'carrier' sodium iodide to the eluent. As the concentration of NaI in the eluent was increased, the peak shape improved and at a concentration of 1 mg NaI/100 ml, the peak shape was excellent. This concentration of NaI was then included in all eluents.

In an h.p.l.c. assay for radiochemical purity, the counts in the peaks are compared directly and not by the more common internal standard method used in non-radioactive assays. It is essential therefore that each component of the mixture to be chromatographed is totally recovered from the column and that no impurities remain bound to the column packing, leading to an overestimate of radiochemical purity. The recoveries of ^{123}I$^-$, [^{123}I]IHA and [^{123}I]IBA from the Hypersil SAS column used in this work were: ^{123}I$^-$ 100·6% (s.d. 1·3); [^{123}I]IHA 100·2% (s.d. 2·1); [^{123}I]IBA 98·1% (s.d. 1·2) (n = 10 for each compound). Since the three possible components of an [^{123}I]IHA injection are efficiently recovered from the column, h.p.l.c. should be a suitable method for assaying the radiochemical purity of [^{123}I]IHA.

T.l.c. is the standard technique by which the radiochemical purity of [^{123}I]IHA is assayed. When the h.p.l.c. and t.l.c. methods were compared, the mean radiochemical purity of ten samples of [^{123}I]IHA was found to 98·3% (±0·2 s.e.) by t.l.c. and 99·1% (±0·2 s.e.) by h.p.l.c. Analysis of the data by a paired Student's *t*-test demonstrated a significant difference ($P < 0·05$) between the results obtained by the two techniques. A possible explanation for the t.l.c. result being consistently lower than the h.p.l.c. result is the poor resolution of the t.l.c. system where the main [^{123}I]IHA peak (R_F 0·5) merges with both the impurity peaks and contributes to the counts in these peaks. This would lead to an overestimate of the impurities.

The results of the repeated h.p.l.c. assay of one preparation of [^{123}I]IHA showed a coefficient of variation of 0·1% (mean radiochemical purity = 99·1%), demonstrating that the h.p.l.c. method is capable of very high precision.

H.p.l.c. has therefore been shown to be a suitable method for determination of the radiochemical purity of *o*-iodohippuric acid (^{123}I) injection, with excellent precision and reasonable agreement with the standard t.l.c. technique. When it is necessary to measure the radiochemical purity of a short half-life radiopharmaceutical before it is released for clinical use, it is desirable to have an analysis method which is as rapid as possible, to ensure efficient use of the radionuclide. The h.p.l.c. assay of the radiochemical purity of [^{123}I]IHA described in this paper, takes 4 min per sample compared with approximately one hour for the standard t.l.c. method. The great speed and high resolution of h.p.l.c. should lead to its increased use in the development and quality control of other short half-life radiopharmaceuticals.

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