

PAPER CHROMATOGRAPHIC STUDY OF THE RADIOACTIVE CONTAMINANTS OF ¹³¹I-HIPPURAN

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The simplicity of the procedure, its quickness and, last but not least, the possibility of examining the right and left kidney separately, all promoted the wide acceptance of nephrography performed with ¹³¹I-hippuran (OI*H). Except for the intravenous injection, this diagnostic procedure represents a painless intervention for the patient.

In both nephrography and the determination of renal clearance, all authors have emphasized the importance of the radiochemical purity of the product used (1-6). This is based on their own experiences because in the case of a high ¹³¹I-iodide content lower clearance values were obtained than with a purer product. When they studied the purity of the hippuran product, most authors tried to detect contamination with inorganic iodide. To this end, separation by paper chromatography, electrophoresis or an organic solvent were used (5-14). According to recent hypotheses, the product may also be contaminated by iodinated organic compounds. Using two-dimensional paper chromatography, Anghileri (7) was the first to find a spot originating from some unknown substance which he thought to be some compound akin to hippuran. Using butanol ascending paper chromatography, Hosick *et al* (11) also succeeded in isolating a component which, according to them, was the unknown compound isolated by Anghileri. Brown *et al* (15) purified ¹³¹I-hippuran by DEAE Sephadex gel filtration. In addition to iodide and hippuran, an unknown contaminating substance was also found in the eluate which they designated as Fraction A. They failed to analyze the fraction containing the unknown substance.

When examining the radiochemical purity of OI*H, we also found the contamination formerly designated as some unknown compound by many authors (8,11,15). According to our own hypothesis, this substance is due to some iodinated intermediate of OIH synthesis, possibly OIB. To confirm our hypothesis, we performed various paper chromatographic procedures to determine the R_f values of compounds occurring in the synthesis of OIH, OIB and hippuric acid.

MATERIALS AND METHODS

By combining the methods described in the literature (18-22), we prepared rather pure OIB and OIH. The melting point for OIB was found to range from 158 to 160°C while the melting point for OIH was between 172 and 174°C. Both products were labeled with ¹³¹I by isotope exchange. The hippuran content of the OI*H product was 1 mg/ml, and its radiochemical concentration was 500 μCi/ml.

To the fresh OI*H product different amounts of OI*B and carrier-free ¹³¹I-iodide were added. The experiments were performed with the following mixtures (in which % represents percentage of total radioactivity):

- A. OI*H only
- B. OI*B + 10% ¹³¹I-iodide
- C. OI*H + 10% OI*B
- D. OI*H + 30% OI*B
- E. OI*H + 20% OI*B + 10% ¹³¹I-iodide.

ABBREVIATIONS USED IN TEXT

OIH: orthoiodohippuric acid or its sodium salt.
OI*H: ¹³¹I-labeled orthoiodohippuric acid or its sodium salt.
OIB: orthoiodobenzoic acid or its sodium salt.
OI*B: ¹³¹I-labeled orthoiodobenzoic acid or its sodium salt.

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TABLE 1. SOLVENT MIXTURES USED FOR DEVELOPMENT AND R_f VALUES OBTAINED FOR O-iodohippuran, o-iodobenzoic acid and sodium iodide

Solvent mixture	Procedure	R_f			Reference
		iodide	o-iodohippuran	o-iodobenzoic acid	
1. n-butanol:2N-acetic acid 1:1	Ascending	0.23	0.92-0.94	0.94-0.95	10, 14
2. n-butanol:acetic acid:water 78 : 5 : 17	Ascending	0.20	0.82	0.88	23
3. n-butanol saturated with N-acetic acid	Ascending	0.20	0.90	0.95	5
4. n-butanol:acetic acid:water 4 : 1 : 1	Descending	0.09-0.14 0.16-0.18	0.84	0.89	7, 17
5. benzene:acetic acid:water 2 : 2 : 1	Descending	0.0 -0.05	0.48-0.52	0.88-0.92	13
6. benzene:acetic acid:water 2 : 2 : 1	Ascending	0.0 -0.05	0.48-0.52	0.88-0.92	

We used one-dimensional chromatography on Whatman No. 1 paper at room temperature with the solvent mixtures indicated in Table 1. The paper strips were dried at room temperature. Activity distribution was recorded with an automatic chromatogram scanner (Friezeke-Hoepfner 452). The size of the area beneath the curves is expressed as percentage of applied activity. Following radioactivity measurement, a properly sensitive chemical reaction was used to identify the site of the stains with the compounds. Details are given in Table 2.

RESULTS AND DISCUSSION

When examining products A and B after chromatographing with the first four solvent mixtures in Table 1, R_f values 0.82-0.92 were obtained for OIH, 0.88-0.95 for OIB and 0.09-0.23 for sodium iodide (Table 1, Fig. 1A,B). These values agree fairly well with those in the literature. However, with the same solvents the two organic compounds hardly separated or did not separate at all on the paper. Only the broadening of the curve near the

frontline or the distortion of the descending branch showed the presence of OIB in addition to OIH. The splitting of the second peak (Fig. 1C) could be observed only at a rather high OI*B content (20-30%). We therefore searched for more suitable solvent mixtures. With benzene:acetic acid:water (2:2:1) we obtained an optimum separation for the three compounds with the following R_f values: iodide 0.00-0.05; OIH 0.48-0.52; OIB 0.88-0.92 (Fig. 2A,B).

As a matter of fact, this method is given in the *XVIIth American Pharmacopoeia* and is generally recommended for use with descending chromatography to test radiochemical purity of ^{131}I -hippuran.

In our experiment the method was altered inasmuch as chromatograms were developed to a front distance of about 30 cm instead of the time recommended (16 hr). The standard deviations of the R_f values of the parallels were $\pm 5\%$. When performing ascending chromatography with the same mixture—benzene:acetic acid:water—we obtained an identical distribution (Fig. 2C).

TABLE 2. R_f VALUES OF COMPOUNDS USED FOR OIH SYNTHESIS BENZENE:ACETIC ACID:WATER (2:2:1)

Substance	R_f	Detection reaction
Anthranilic acid	0.85-0.88	p-dimethyl amino benzaldehyde dissolved in anhydrous acetic acid
Glycine	0.08-0.12	Ninhydrine dissolved in n-butanolic acetic acid
^{125}I -sodium iodide	0.0 -0.05	Potassium iodate dissolved in acetic acid starch; activity measurement
Sodium-iodate	0.0 -0.05	Potassium iodide dissolved in acetic acid starch
^{125}I -o-iodobenzoic acid	0.88-0.92	Bowden reaction (Ce-IV sulphate-Na-arsenite) (24); activity measurement
^{125}I -o-iodohippuran	0.48-0.52	Bowden reaction (24); activity measurement; ninhydrine dissolved in n-butanolic acetic acid; p-dimethyl amino benzaldehyde dissolved in anhydrous acetic acid

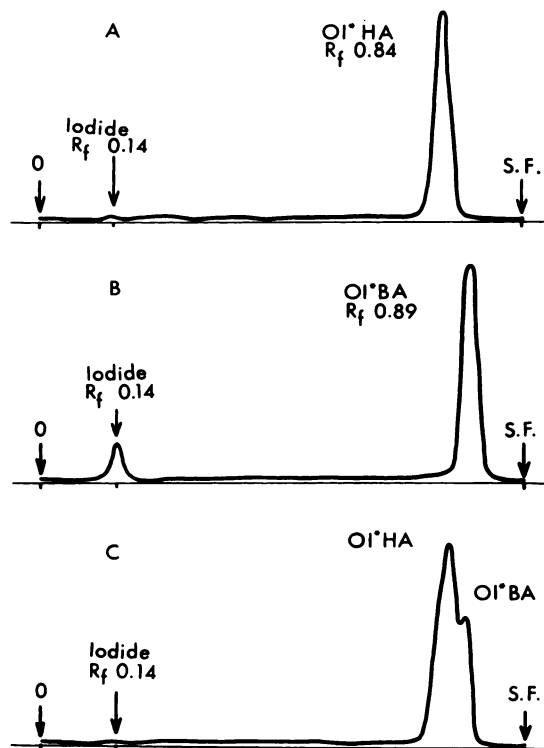


FIG. 1. Paper chromatograms of ^{125}I -o-iodohippuran, ^{125}I -o-iodobenzoic acid and Na^{125}I . Mixture used for development: n-butanol:acetic acid:water (4:1:4). Descending chromatography is used. A shows product "A", B shows product "B", and C shows product "D".

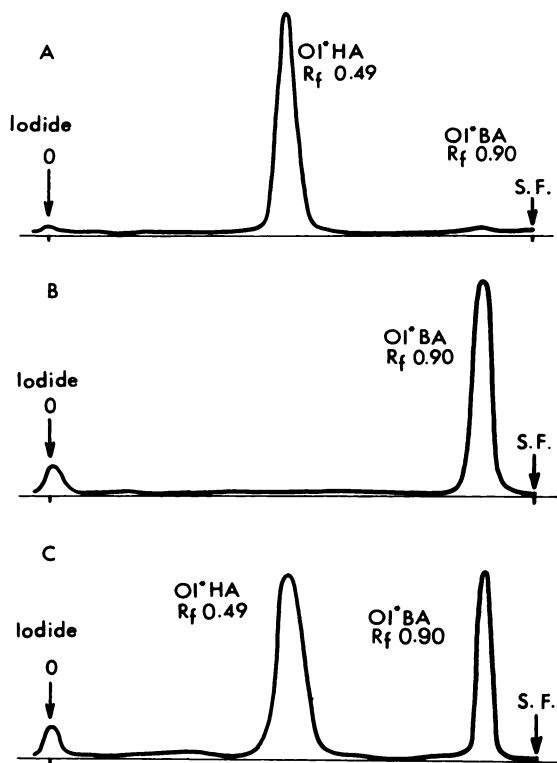


FIG. 2. Paper chromatograms of ^{125}I -o-iodohippuran, ^{125}I -o-iodobenzoic acid and Na^{125}I . Mixture used for development: benzene:acetic acid:water (2:2:1). A shows product "A", descending; B shows product "B", descending; C shows product "E", ascending.

Next we studied the chromatographic separation of compounds used for OIH synthesis (anthranilic acid, glycine, sodium iodide and sodium iodate). The R_f values obtained and the color reactions applied are indicated in Table 2.

With the solvent mixture of benzene:acetic acid:water (2:2:1), only the R_f value of anthranilic acid comes rather close to that of OIB. However, these two compounds are easily distinguished by chemical color reaction and activity measurement.

On the basis of our results, the organic contamination of commercial ^{131}I -o-iodohippuran seems to be OI*B. Figure 3 shows the chromatograms of hippuran products from two different factories. The product in 3A contained 10.3% while the product in 3B contained 32.2% of OI*B. Although this contamination does not occur in the isotope-exchange reaction, in most cases the inactive OIH used as basic material is contaminated with OIB. It is therefore of utmost importance that the melting point of the starting material should not be less than 170–172°C since in that case activity originating from OIB does not exceed 2–3% of total activity.

SUMMARY

The mixture of benzene:acetic acid:water (2:2:1) was found to be the best solvent for separating ^{131}I -labeled o-iodohippuran from other organic and inorganic contaminating compounds in the product. Using this solvent, one-dimensional paper chromatography proved to be suitable both for qualitative and

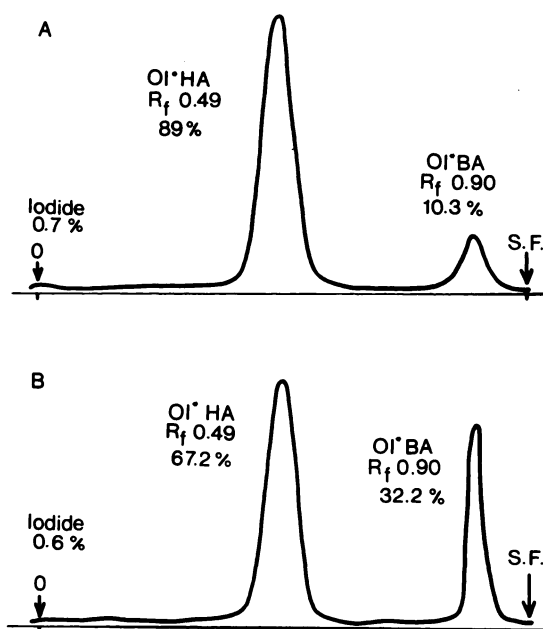


FIG. 3. Chromatograms of two ^{125}I -o-iodohippurans obtained from different producers. Mixture used for development: benzene:acetic acid:water (2:2:1). Descending chromatography is used in this investigation.

quantitative purposes. The hitherto unknown contaminating compound of ¹³¹I-o-iodohippuran could be isolated by this one-dimensional paper-chromatographic procedure and was found to be ¹³¹I-o-iodobenzoic acid.

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