

Determination of Radioiodide Contamination of Radio-Hippuran and Other Radioiodinated Organic Salts

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Previously, ¹³¹iodide contamination of sodium o-¹³¹iodohippurate (radio-Hippuran) and other radioiodinated pharmaceuticals has been determined by time consuming chromatographic procedures, followed by strip scanning, strip-segment counting, or autoradiographic procedures for quantitating the spots (1, 2).

A simple technique has been developed for the assay of radioiodide contamination of water-soluble, radioiodinated organic salts. A brief description of the preliminary development of this technique was made earlier (3). Several modifications have been made subsequently to increase accuracy and speed.

The new method is based upon the following physical and mathematical considerations: When a saturated solution of elemental iodine in carbon tetrachloride (CCl₄) is allowed to come into equilibrium with water, only a very small per cent of the iodine dissolves in the water as iodide and hypoiodite ions. A continual exchange of ions occurs between the two layers. If a relatively very small amount of radioiodide ions are added to the aqueous layer, after equilibration, the radioactive ions will become evenly distributed throughout the system. However, since only a very small number of iodine atoms exist as iodide ions in the aqueous phase relative to the large number of iodine atoms present in the whole system, statistically almost all of the radioiodine atoms will have become distributed into the CCl₄ layer. Therefore, if these radioiodide ions were added to the aqueous phase of the system along with a water-soluble CCl₄-insoluble radioiodinated organic compound, the radioactivity due previously to radioiodide will pass into the CCl₄-I₂ layer, leaving the radioactive compound in the aqueous layer. The procedure becomes inaccurate when gross iodide concentrations in the aqueous phase become significant (> 0.1%).

The new test was checked quantitatively against a known chromatographic procedure (2), using autoradiographic techniques of spot localization.

EXPERIMENTAL

The assay of radioiodide in radio-Hippuran (or other radiodinated organic salts) is carried out as follows: Samples are prepared just before use by diluting 0.1 to 0.5 μ c of the radioiodinated organic salt to 0.5 ml with a solution containing 0.001 per cent of the corresponding nonradioactive organic salt and 0.001 per cent of nonradioactive sodium iodide to act as carriers. A glass vial containing

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the sample is counted in a well counter,¹ then weighed, and 2 ml of an iodine-saturated carbon tetrachloride solution is added. The vial is closed and swirled for one hour to effect equilibration. After reweighing, one ml of the lower carbon tetrachloride layer is removed by means of a glass syringe or pipette, care being taken not to transfer any of the aqueous layer. The material is placed in an intermediate transfer vial or beaker, and a 0.5 ml aliquot is transferred by means of a clean syringe or pipette to a tared counting vial. (A single transfer procedure can be substituted for the double transfer with slightly lessened reproducibility, because of enhanced possibility of aqueous layer contamination of the aliquot). The aliquot weight is determined by reweighing the counting vial after aliquot addition. The aliquot is counted in the well counter. The per cent radioactivity originally present as radioiodide is obtained by the formula:

Formula I

$$\text{Per cent radioiodide} = 100 \times \frac{(\text{Counts in aliquot}) (\text{Wt of I}_2\text{-CCl}_4 \text{ added})}{(\text{Counts in original sample}) (\text{Wt of aliquot})}$$

(A correction factor of 1.013 is discussed later)

The new analytical procedure was checked in the following ways:

- (1) Analyses of solutions containing 100 per cent of the radioactivity as sodium ¹³¹I iodide were studied for reproductibility.
- (2) Analyses of solutions of sodium o-¹³¹I iodohippurate containing known per cents of the radioactivity as sodium ¹³¹I iodide were studied for accuracy of the results and checked against the chromatographic procedure.
- (3) The effect of nonradioactive carriers was studied.
- (4) The optimum time of swirling was determined.

The results of these studies are summarized in Tables I-V. When radio-Hippuran solutions of known ¹³¹I iodide contamination were prepared, the original contamination of the commercial radio-Hippuran had to be determined. This value was obtained by the usual chromatographic procedure (1, 2).

Swirling was accomplished by placing the counting vials in a vial holder clamped to a heavy-based ring stand. A small motor with an eccentric weight affixed to its shaft was clamped to the top of the ring stand rod. Motor speed was varied by means of a variable transformer until a vigorous swirling action was observed in the tubes. True shaking caused undesirable emulsification. The apparatus is illustrated in Fig. 1. Preliminary studies of commercial Hippuran ¹²⁵I indicate levels of ¹²⁵I iodide contamination to be less than one per cent.

DISCUSSION

Table I compares results of tests run under optimum conditions upon solutions containing a known concentration of 100 per cent radioactivity as ¹³¹I iodide with the erratic, inaccurate results obtained if no isotopic dilution by carriers is used.

¹Nuclear—Chicago Model No. C-120.

Table II compares the results of the I_2 - CCl_4 test with those obtained by chromatographic procedures upon samples of radio-Hippuran containing known amounts of free ^{131}I iodide contamination. The I_2 - CCl_4 test results compare favorably with those obtained by the older, more laborious procedure.

TABLE I
EXPERIMENTAL RESULTS VERSUS 100 PER CENT KNOWN ^{131}I IODIDE CONTENT
WITH AND WITHOUT CARRIER

	<i>With Carrier*</i>	<i>Without Carrier*</i>
Number of determinations	54	54
Mean per cent ^{131}I iodide	98.2%	93.7%
Standard deviation	1.34%	10.73%

*Carrier is 0.001% NaI and 0.001% nonradioactive hippuran.

TABLE II
COMPARISON OF I_2 - CCl_4 TEST AND CHROMATOGRAPHIC PROCEDURE FOR
DETERMINATION OF RADIOIODIDE IN RADIO-HIPPURAN SOLUTIONS
OF KNOWN CONTAMINATION (VIA SINGLE TRANSFER
PROCEDURE UNLESS OTHERWISE INDICATED)

Exp	Number of Determinations		Per Cent Mean ^{131}I Iodide Contamination			Variation of I_2 - CCl_4 Test from		Variation of Chrom from calc'd
	via Chrom	via I_2 - CCl_4	Calc'd	via Chrom	via I_2 - CCl_4	Calc'd	Chrom	
1	3	6	—	5.2	6.2	—	+1.0	—
2	3	6	—	4.9	6.0	—	+1.1	—
3	3	6	—	4.7	5.6	—	+0.9	—
4	3	6	—	8.7	9.0	—	+0.3	—
5	3	6	14.1	15.6	15.1	+1.0	-0.5	+1.5
6	3	6	14.1	14.6	14.8	+0.7	+0.2	+0.5
7	—	6	14.1	—	15.2	+1.1	—	—
8	3	4	14.1	13.6	14.3	+0.2	+0.7	-0.5
9a	3	3	12.4	13.2	13.8	+1.4	+0.6	+0.8
b	3	3	12.4	13.2	13.3	+0.9	+0.1	—
				(double transfer)				
10	—	1	2.7	—	3.7	+1.0	—	—
11	—	1	6.2	—	4.5	-1.7	—	—
12	—	1	2.9	—	3.4	+0.5	—	—
13	—	1	20.8	—	20.7	-0.1	—	—
14	—	1	18.2	—	19.8	+1.6	—	—
15	—	1	17.0	—	16.5	-0.5	—	—

Table III demonstrates the effect of varying nonradioactive sodium iodide concentrations as a carrier system for determinations of 100 per cent radioactivity as Na¹³¹I. It would be anticipated that as NaI concentration is increased, the experimental results would decrease in value, due to the increased solubility of elemental iodine in aqueous solutions of iodide ions. The results indicate, though not conclusively at the concentrations studied, that such is true. However, since

TABLE III
EFFECT OF CONCENTRATION OF NAI AS A CARRIER (100 PER CENT RADIOACTIVITY AS Na¹³¹I)

	0.001% NaI	0.01% NaI	0.1% NaI
Number of determinations	18	18	18
Mean per cent ¹³¹ iodide	98.9%	98.8%	97.4%
Standard deviation	1.45%	1.33%	1.77%

TABLE IV
EFFECT OF ADDITION OF NONRADIOACTIVE HIPPURAN (OIHA) TO 0.001 PER CENT NAI AS CARRIERS IN SOLUTIONS CONTAINING 100 PER CENT RADIOACTIVITY AS Na¹³¹I
(Time Studies)

	0.001% OIHA 0.001% NaI		0.01% OIHA 0.001% NaI		0.1% OIHA 0.001% NaI	
	Freshly Prepared	5 days old	Freshly Prepared	5 days old	Freshly Prepared	5 days old
No. of determinations	18	12	18	12	12	18
Mean per cent ¹³¹ Iodide	98.5%	95.5%	98.8%	93.8%	97.7%	84.4%
Standard deviation	1.23%	1.03%	1.64%	1.74%	1.43%	1.76%

TABLE V
EFFECT OF SWIRLING TIME ON I₂-CCl₄ TEST
(ANALYSES OF 100 PER CENT RADIOIODIDE WITH 0.001 PER CENT NAI AND 0.001 PER CENT OIHA AS CARRIERS(1))

	0 min	Swirling Time			
		15 min	30 min	1 hr	2 hr
No. of determinations	18	21	18	18	18
Mean per cent ¹³¹ iodide	92.2%	92.6%	97.6%	98.2%	98.2%
Standard deviation	4.29%	0.73%	1.94%	1.63%	1.62%

the 0.001 per cent concentration gives accurate results, no need for the use of higher concentrations is indicated.

Table IV illustrates the detrimental effects of the use of high concentrations of nonradioactive Hippuran as a carrier, especially if the solutions to be tested are stored for any appreciable time with the carriers before testing. It has been found that when solutions of nonradioactive Hippuran and sodium ^{131}I iodide are mixed, a quite rapid exchange of radioactivity into the organic molecule occurs, especially at higher concentrations of the Hippuran (4). A minimal concentra-

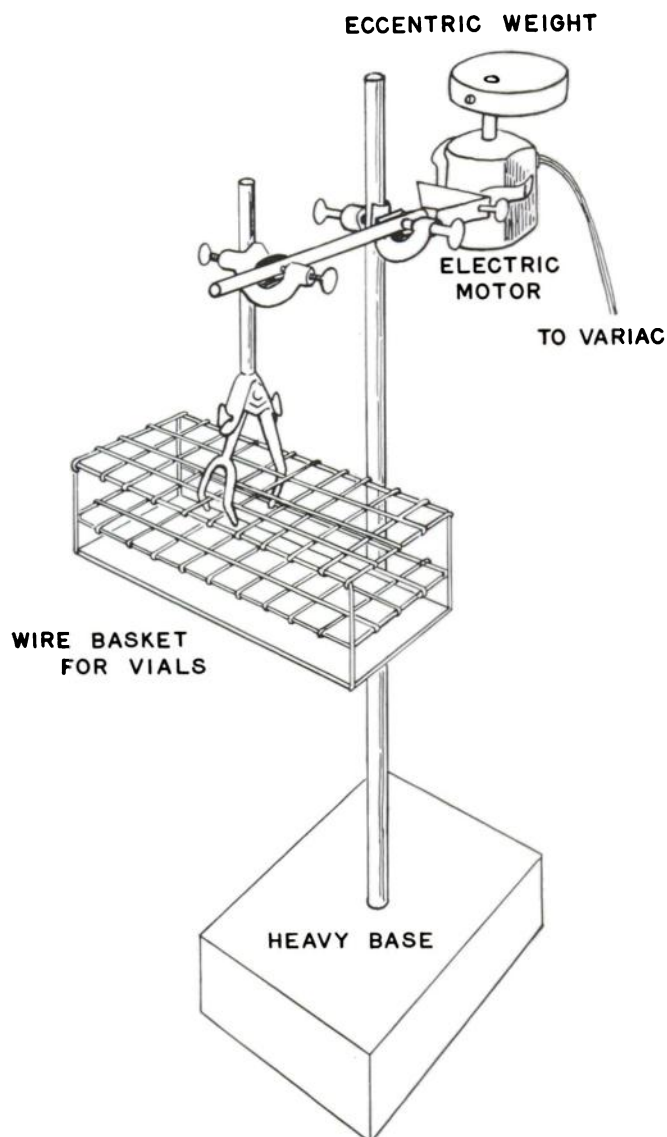


Fig. 1.

tion of carrier Hippuran (0.001%) is recommended. A future study of the effect of eliminating the Hippuran carrier is advised, because commercial radio-Hippuran has a much lower specific activity than does commercial sodium ¹³¹iodide.

By comparison of various swirling times in Table V, it appears that one hour is sufficient for good results.

An average of all optimum experimental results for 100 per cent ¹³¹iodide "contamination" is 98.72 per cent. It probably is justifiable to apply a correction factor of 1.013 (the reciprocal of 0.9872) to the results obtained by Formula I:

Formula II

$$\text{Per cent radioiodide} = 1.013 \times 100 \frac{(\text{Counts in aliquots})(\text{Wt of I}_2\text{-CCl}_4 \text{ added})}{(\text{Counts in original sample})(\text{Wt of aliquot})}$$

SUMMARY

A simple technique for the estimation of radioiodide contamination in water soluble, carbon tetrachloride insoluble, radioiodinated organic compounds is described. Aqueous samples are treated by swirling for one hour with a layer of carbon tetrachloride saturated with nonradioactive elemental iodine. The disproportionation of the original radioactivity between the aqueous and I₂-CCl₄ layers after equilibrium is determined, and the per cent of radioactivity originally present as radioiodide is calculated therefrom. The activity originally present as radioiodide transfers into the I₂-CCl₄ layer, leaving the radio-organic compound in the aqueous layer.

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