Preparation and Analysis of 4-Iodoantipyrine-(I\textsuperscript{131})

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Abstract:
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- 4-Iodoantipyrine was prepared from antipyrine according to the reaction equation:
  \[3C_{12}H_{12}N_2O + 2KI + KIO_3 + 3HCl \rightarrow 3C_{12}H_{12}N_2OI + 3KCl + 3H_2O\]
- The product was separated from the reaction mixture and purified by recrystallization. The iodoantipyrine was labeled with I\textsuperscript{131} by means of an exchange reaction with NaI\textsuperscript{131}. It was separated from the unbound radioactivity by anion exchange chromatography.
- The purity of the product was determined by cellulose thin layer chromatography using ethanol-chloroform-water (45:45:10) as the solvent system. The purity of the 4-iodoantipyrine-I\textsuperscript{131} decreased with time due to a splitting off of the I\textsuperscript{131} atom and subsequent formation of NaI\textsuperscript{131}. This spontaneous deiodination was best minimized by storage in methanol at low temperatures.

Introduction
Although 4-iodoantipyrine was initially used for the measurement of total body water,\textsuperscript{1} it is no longer generally employed for this purpose. Several investigators\textsuperscript{2-4} have demonstrated that it is rapidly metabolized in the organism with the release of free inorganic iodide. On the other hand, 4-1\textsuperscript{131}-antipyrine has been found to be a valid tool for determination of total and regional cerebral blood flow in human beings as well as in animals.\textsuperscript{5-7} By virtue of its lipid solubility, iodoantipyrine readily crosses the blood-brain barrier. It enters the brain at a rate parallel to that of the whole body as measured from the time of injection to analysis. The fractional tissue uptake of the I\textsuperscript{131} indicator thus, in theory, becomes equivalent to the flow fraction of cardiac output.\textsuperscript{5}

4-I\textsuperscript{131}-antipyrine is presently available commercially on a limited basis. However, it is costly. A survey of the English literature revealed little information on the preparation and stability of this compound. It is our present purpose to describe simple techniques for the preparation, purification and labeling of iodoantipyrine, and the subsequent analysis and storage of the labile I\textsuperscript{131}-antipyrine product.

Methods
Iodoantipyrine was prepared\textsuperscript{8} according to the reaction equation:
\[3C_{12}H_{12}N_2O + 2KI + KIO_3 + 3HCl \rightarrow 3C_{12}H_{12}N_2OI + 3KCl + 3H_2O\]
In order to prepare approximately 150 gm of iodoantipyrine, 94 gm (0.5 M) antipyrine, 35.6 gm KIO\textsubscript{3} and 55.4 gm of KI were dissolved in 1,250 ml of distilled H\textsubscript{2}O. The reaction mixture was heated to boiling; at a low boil, 73 gm of 25% HCl (w/v) solution was added dropwise with a separatory funnel. Care was taken to avoid the formation of organic periodides which may arise in the form of tarry brown precipitates; periodide reactions are dependent on the concentration of both the KI and the HCl.\textsuperscript{9}

White crystalline needles appeared when the reaction mixture was allowed to cool in an ice bath. These were separated from the mixture,
PREPARATION AND ANALYSIS OF 4-iodoantipyrine

The iodooantipyrine was recrystallized using 90% ethanol, washed with distilled water, and oven-dried at 70°C. The purity of the iodooantipyrine was estimated by melting point determination (162 to 162.5°C). The iodooantipyrine was labeled with I131 by means of an exchange reaction with NaI131. Ten milligrams of carrier-free NaI131 were added to 3 ml of normal sodium acetate buffer, pH 4.6, containing 0.4 mg/ml iodooantipyrine.

This mixture was heated in a low-boiling water bath for 50 to 60 minutes and then cooled to ambient temperature. Immediately after cooling 5 drops of 0.1N NaHSO3 were added in order to bind the free iodine. Two methods were tried to separate the I131-antipyrine from the reaction mixture—chloroform extraction and column chromatography. Anion exchange chromatography was found to be the more advantageous.

Dowex 1-X8 (mesh size 100 to 200) was washed with 0.1 NaOH followed by 0.1N HCl, and stored in distilled water. For each milliliter of reaction mixture, approximately 15 cc of Dowex (packed column volume) was used. The Dowex was equilibrated with normal saline just prior to the application of the reaction mixture. In this manner, more than 99% of the material adhered to the column. The I131-antipyrine was eluted from the column with anhydrous methanol. With 30 ml of methanol, better than 90% of the radioactivity was recovered. The methanol solution was centrifuged to remove Dowex particles. The I131-antipyrine was stored in methanol. Prior to its injection into animals the methanol was evaporated and the material redissolved in saline.

Purity of the I131-antipyrine product was analyzed by ascending Cellulose Thin Layer Chromatography (Eastman Kodak No. 6064) with ethanol-chloroform-water (45:45:10) as the solvent. After spotting and subsequent solvent migration, the dried chromatogram was cut into one-fourth-inch sections. The radioactivity peaks on the chromatogram were associated with the migration of the product compounds, I131-antipyrine and NaI131. The area under each identified peak became the quantitative measure of concentration. Figure 1 demonstrates a typical chromatographical analysis of the methanol-stored I131-antipyrine product eight days after its initial preparation.

antipyrine stored in water, but only 3.0% of the radioactivity if stored in methanol.

Discussion

I-131-antipyrine is relatively unstable. Inorganic iodine in the form of NaI131 is lost during its storage.

Chromatographic Analysis of Methanol Stored I131-Antipyrine (8 Days After Preparation)

FIGURE 1

An example of a typical chromatographical analysis of the I131-antipyrine product stored in methanol eight days after its initial preparation.

FIGURE 2

The results of a daily chromatographical analysis of the methanol-stored and water-stored I131-antipyrine products.
This decomposition may be due to natural isotopic decay, interaction of the radioactive emission (alpha, beta, gamma) with molecules of the compound, and/or thermodynamic instability. The instability is probably due to radiation decomposition as evidenced by the significantly higher decomposition rate of $^{131}$I compounds as compared with $^{128}$I-labeled compounds.\textsuperscript{3} Other factors affecting the rate of $^{131}$I splitting include temperature, pH, solvent choice, specific activity, exposure to light and the presence of other substances in the solution. In TLC, the ethanol-toluene (50:50) solvent system, sometimes recommended in the literature, was found to be inadequate for a quantitative analysis because the $^{131}$I-antipyrine trailed into the Na$^{131}$I peak, giving poor separation of compounds. A better solvent system was ethanol-chloroform-water (45:45:10), giving an $^{131}$I-antipyrine Rf of 9.5 and Na$^{131}$I Rf of 7.0. It should be noted that the relatively greater degradation of $^{131}$I-antipyrine reported by Bayle and Evans may be, in part, due to poor resolution of the TLC obtained with the ethanol-toluene solvent system.

Our methanol and water storage studies revealed a slight logarithmic increase in the rate of Na$^{131}$I formation from $^{131}$I-antipyrine, probably related to the effect of an increasing Na$^{131}$I concentration at room temperature. Storage in methanol at $-60^\circ$C further reduced decomposition and Na$^{131}$I formation by 40%.

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