SEPARATION OF INORGANIC IODIDE IN THE ANALYSIS OF SOME IODOORGANIC RADIOPHARMACEUTICALS

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SUMMARY

A paper chromatography technique using 33% w/v aqueous solution of ammonium sulphate as a solvent has been applied for fast separation of inorganic radioactive iodide from labelled organic compound in the analysis of some iodoorganic radiopharmaceuticals of common use.

L-triiodothyronine, L-thyroxine, Rose Bengal and bromosulphthalein are retained at the starting spot while iodide migrates at R_f 0.5. The separation is obtained at a development path of 5cm in about 10min.

Sodium o-iodohippurate, however, migrated at R_f 0.4 and its separation from inorganic iodide was not achieved under the conditions employed.

Key Words: Radiochemical Purity, Radiopharmaceuticals, Radioactive Iodine

INTRODUCTION

Inorganic iodide is a major radiochemical impurity of iodoorganic radiopharmaceuticals. The amount of iodide present may increase with storage due to autoradiolytic decomposition of the labelled organic compound. For most medical applications the level of inorganic iodine impurity in organic preparations should not exceed 5%.

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Various techniques of paper chromatography, paper electrophoresis and thin layer chromatography are used for testing of iodoorganic radiopharmaceuticals /1-4/. All these methods are based on the separation of inorganic iodine from organic compound.

In the routine analysis of preparations of radioiodinated proteins the paper chromatography technique utilizing aqueous solution of ammonium sulphate as solvent /5/ is used in our Laboratory. The purpose of the present study was to check if that separation procedure could be used for quick quality control of some radioiodinated pharmaceuticals in common use, viz L-triiodothyronine, L-thyroxine, Rose Bengal, bromosulphthalein and sodium o-iodohippurate labelled with iodine-125.

EXPERIMENTAL

$/NH_4/_2SO_4$, NaH_2PO_4 and Na_2HPO_4 were of analytical grade. Phosphate buffer of pH 7.5 contained 0.3% w/v bovine serum albumin and 0.1% w/v sodium azide.

The radioiodine labelled organic preparations were manufactured in our Centre. L-triiodothyronine and L-thyroxine were prepared in a solution containing 50% v/v of propylene glycol /in order to reduce decomposition/ and phosphate buffer without albumin. Rose Bengal and bromosulphthalein were prepared in 0.9% w/v aqueous sodium chloride solution. Sodium o-iodohippurate was supplied in the same solution but containing 1% v/v benzyl alcohol. $Na^{125}I$ was obtained from the Radiochemical Centre, Amersham.

The chromatographic separation was carried out using Whatman 3MM paper and 33% w/v aqueous $/NH_4/_2SO_4$ solution of pH 7.5 as solvent. The chromatogram was developed using ascending technique for a distance of 5cm during 10-12min. After drying the strips were cut into 0.8cm sections and measured in a well type automatic gamma counter.

RESULTS

It has been established previously /5/ that when aqueous solution of ammonium sulphate is used as solvent, iodide and iodate migrate on paper chromatograms at R_f 0.5 and 0.9, respectively whereas iodinated protein remains at the starting spot at R_f 0.0. It was found in the present study that similarly as in the case of iodinated proteins the R_f value for L-triiodothyronine, L-thyroxine, Rose Bengal and bromosulphthalein was 0.0. Sodium o-iodohippurate, however, migrated under these conditions at R_f 0.4, which is too close of the R_f value of iodide to achieve satisfactory resolution during the applied time of chromatographic development.

The separation of organic compound and inorganic iodide was therefore studied for the preparations of L-triiodothyronine, L-thyroxine, Rose Bengal and bromosulphthalein. Since the content of inorganic iodine in fresh preparations of labelled organic compounds is usually in the range 1-5% in order to achieve better visualization of the separated peaks on the chromatograms some amounts of $Na^{125}I$ were added to the analyzed solutions of commercial preparations. Iodination mixtures of the organic compounds containing unreacted iodide were also utilized.

The separation of inorganic iodide from the labelled organic compound in aqueous solutions of L-triiodothyronine, L-thyroxine, Rose Bengal and bromosulphthalein is illustrated in Fig.1. It can be seen from the presented radiochromatograms that for these compounds the obtained resolution is satisfactory for fast quality control. No peak was observed at R_f 0.9 which corresponds to iodate.

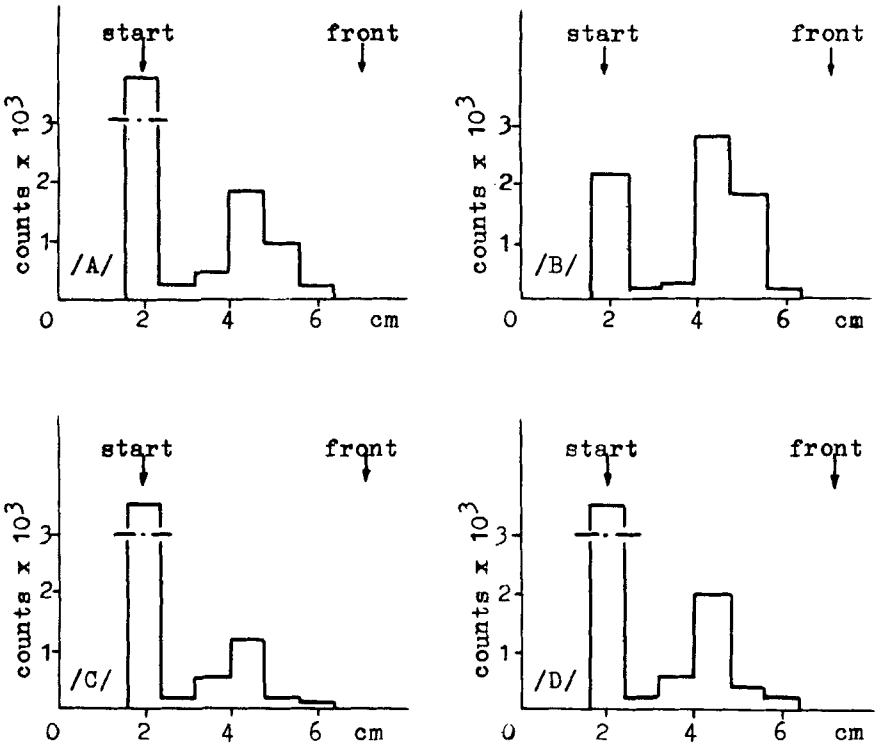

Fig. 1 Chromatographic separation of inorganic iodide from iodoorganic compound in aqueous solutions

A - iodination mixture of L-triiodothyronine
B - L-thyroxine in phosphate buffer without albumin
C - commercial preparation of Rose Bengal
D - commercial preparation of bromosulphthalein

However, the separation of inorganic iodide in the commercial preparations of L-triiodothyronine and L-thyroxine in the solution containing 50% v/v of propylene glycol required a modification of the separation conditions because the spot at the origin was diffused. In order to retain the organic compound 0.002cm^3 of phosphate buffer containing albumin had been placed on the starting spot before the sample was applied. The developed chromatographic strips were cut into narrow /0.4cm/ sections. The resolution of the modified technique is illustrated in Fig.2.

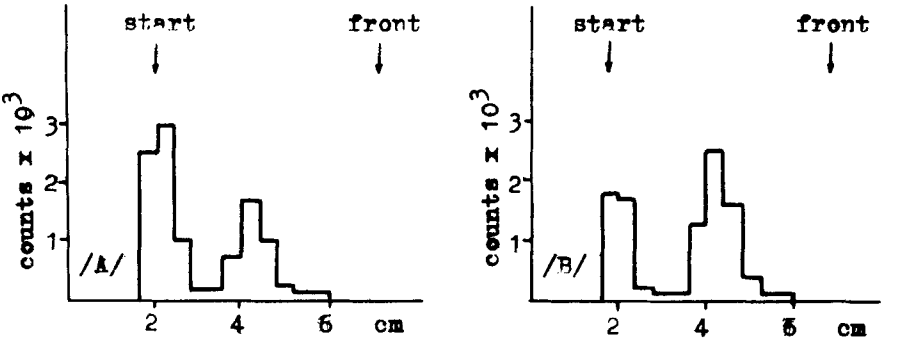

Fig. 2 Chromatographic separation of inorganic iodide from iodoorganic compound in 50% v/v propylene glycol solutions
A - commercial preparation of L-triiodothyronine
B - commercial preparation of L-thyroxine

The chromatograms presented in Fig.1 and Fig.2 pertain to samples containing relatively large percentage of inorganic iodide. However, the chromatographic pattern is essentially the same for iodoorganic preparations containing only about 1% of inorganic iodide. The chromatograms of the fresh

preparations of L-triiodothyronine and Rose Bengal are presented in Fig.3 as examples. The mean values of the iodide determinations were in agreement, within the limits of the experimental error, with the data obtained by paper electrophoresis in a routine quality control procedure /4/. However, the standard deviation was about 0.2% as compared to 0.1% obtained by paper electrophoresis.

In a control experiment it was shown that when $Na^{125}I$ is applied to the chromatogram 5% of the activity is adsorbed to the paper. Hence, the background between the peaks is negligible if the content of inorganic iodide is about 1% of the total activity of the preparation.

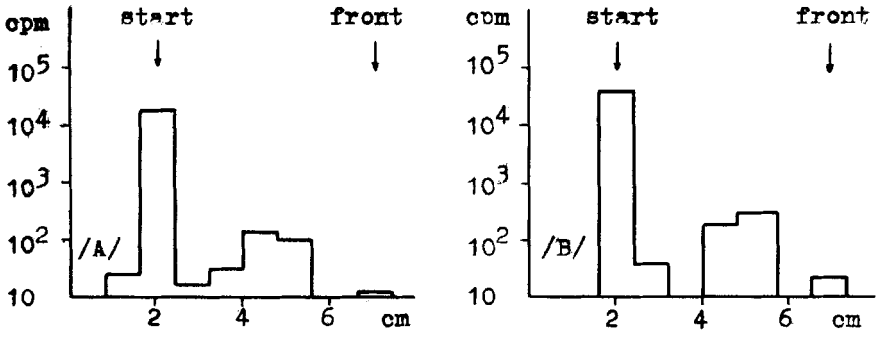

Fig. 3 Chromatographic separation of inorganic iodide impurities from iodoorganic compound /notice the semi-logarithmic scale/
A - fresh preparation of L-triiodothyronine
B - fresh preparation of Rose Bengal

CONCLUSIONS

The chromatographic technique described above is a useful method for fast and easy separation of inorganic iodide from organic compound in radioiodinated proteins /5/ as well as in major iodine labelled radiopharmaceuticals in common use, viz L-triiodothyronine, L-thyroxine, Rose Bengal and bromosulphthalein. The method can be utilized in semi-quantitative quality control of labelled preparations as well as for the determination of unreacted iodide in iodination mixtures.

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