Application of the FFCA-reaction for the detection of thyroid hormones and iodinated derivatives to thin-layer chromatography

The colour reaction for iodide and iodinated compounds described in 1959 by GMELIN AND VIRTANEN and proposed by these authors for use in paper chromatography[1] can be applied equally well to thin-layer chromatography, provided some modifications are made in order to adapt it to the conditions of the stationary phase. In this laboratory it has been currently used for some years for the identification of circulating thyroid hormones[2–4]. This reaction is based on the catalytic properties of iodide in bringing about the reaction of the ferri–ferro and ferricyanide–ferrocyanide systems with the system As(III)-As(V) and the subsequent formation of Prussian blue (FFCA-reaction). It is very simple in its performance and highly sensitive, quantities of about 0.05 μg of each monoiodo-, diiodo-, triiodo- and tetraiodothyronine as well as monoiodo- and diiodotyrosine being revealed (Fig. 1).

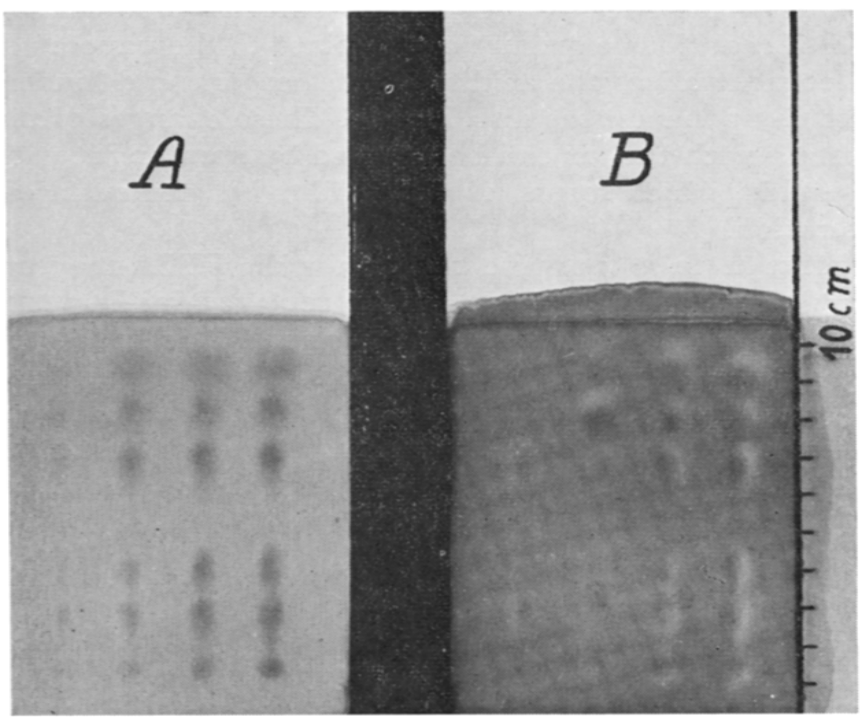

Fig. 1. Chromatograms of a mixture of iodoamino acids 2 h after spraying with FFCA-reagent. The cellulose layer (MN 300 G, Macherey, Nagel & Co.) was divided in two fields A and B. In each field was spotted an aqueous mixture of monoiodotyrosine, monoiodo-, diiodo-, triiodo- and tetraiodothyronine in different concentrations, corresponding from left to right to 0.01, 0.05, 0.10 and 0.15 μg of each substance. The chromatograms were run in acetone–0.5 N acetic acid (2:8) and sprayed with FFCA-reagent. Field A was exposed to ammoniacal vapours. Quantities of about 0.05 μg of each substance are clearly visible in this field. In each vertical column, from top to bottom, are monoiodotyrosine, monoiodo-, diiodo-, triiodo- and tetraiodothyronine. In field B (non-exposure to ammonia), the results are already greatly altered.

The FFCA-reagent is prepared according to the former instructions by mixing immediately before use 5 parts of solutions A and B with 1 part of solution C. A vol./vol. dilution of the freshly prepared reagent with water before the application was found, however, to be more convenient as it permits an intensive and even im-

pregnation of the stationary phase without bringing about an excess of the reactive substances in the layer which cannot afterwards be washed according to the original technique.

Solution A: 2.7 g $FeCl_3 \cdot 6H_2O$ dissolved in 100 ml 2 *N* HCl.

Solution B: 3.5 g $K_3Fe(CN)_6$ dissolved in 100 ml distilled water.

Solution C: 5 g $NaAsO_2$ are dissolved in 30 ml *N* NaOH at 0° and mixed under vigorous stirring with 65 ml 2 *N* HCl. If the temperature is allowed to rise, or if the stirring is interrupted, arsenious oxide is precipitated. The solutions should be stored in the dark.

After spraying the reagent, the thin-layer chromatogram is covered with a glass plate and kept in the dark for 15 min, bright blue spots appearing in the presence of the iodinated compounds against a white-yellowish background. Careful drying of the plates at room temperature or in an oven (not over 50°) previous to the application of the FFCA-reagent is important in order to obtain good results. The spots are stable for at least half an hour, then slowly fade due to progressive spreading and simultaneous development of the colour of the background. A very suitable modification in order to retard this process is the cautious exposure of the plate to the vapours of an 0.5 *N* ammonia solution which neutralizes the acidity of the reagent and hinders the subsequent interaction of its components (see also Fig. 1). These changes of the chromatogram so treated in contact with the air can be avoided by spraying it with Neatan® neu, Merck AG. Darmstadt, which forms a plastic film on the surfaces[5] and preserves them without alteration.

Tyrosine and thyronine give a false positive reaction with FFCA-reagent, depending presumably on their phenolic groups; this was also reported by BOWDEN *et al.* concerning the classical ceric sulphate–arsenious acid reaction[6]. This handicaps the usefulness of these reactions for the identification of thyroid hormones and their iodinated derivatives but can be overcome owing to the high water-solubility of thyronine and tyrosine which permits a separation of these substances, at least from the physiologically more important iodinated thyronines, previous to chromatography[7].

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