CENTRAL INSTITUTE OF PHYSICS

INSTITUTE OF PHYSICS AND NUCLEAR ENGINEERING
Magurele, Bucharest, P.O.Box.MG-6
ROMANIA

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Possible artefacts in thin layer chromatography of tritium-labelled hydrocortisone

Elena Sofronie

Abstract: *Artefacts appearing in thin layer chromatography of tritium labelled hydrocortisone are reported. Evidences are presented that these artefacts cause misleading results concerning radiochemical purity determination. Finally, it is reported a rapid and efficient chromatographic technique allowing the elimination of these artefacts and obtaining of an accurate value for radiochemical purity.*

1. INTRODUCTION

CATCH has described several artefacts which can give misleading results in the paper or thin layer chromatography of radioactive compounds [1]. To all these, it must be added those occuring as a consequence of an incorrect selection of the chromatographic technique. Although it may apparently be suitable for inactive materials, it may lead to artefacts which can be detected by the much more sensitive methods.

This paper reports the artefacts appearing in the thin layer chromatography of tritium-labelled hydrocortisone, even in the recommended technique and systems.

At the same time, it is presented a rapid and efficient method which allows the elimination of these artefacts and the obtaining of a correct value for radiochemical purity.

2. RESULTS AND DISCUSSION

Tritium-labelled hydrocortisone has been synthetized at the Centre of Radioisotope Production in Bucherest-Măgurele. This product is delivered as a benzene-ethanol solution, having a chemical concentration of about $1 - 5 \cdot 10^{-3}$ mg/ml.

For the product radiochemical purity control one used thin layer adsorption chromatography technique, recommended by the producer. In this context, one tried the following chromatographic systems, usually applied to separation of steroids :

- stationary phase : Silicagel G_F and H_F (with and without binder), activated and unactivated;
- mobile phases :

(a) chloroform - acetone (4:1 and 2:1 v/v) ;

(b) methylene chloride - acetone (4 : 1 v/v) ;

(c) chloroform - absolute ethanol - water (9:1:0.1 v/v).

It is to be noted that activation of the adsorbent gave the same result on separations, thus one used only inactivated plates.

After development and drying of the chromatograms, they were scanned for their radioactivity using a scanner for thin layer chromatography, provided with a windowless gas flow proportional counter as a detector.

Figures 1 - 3 illustrates the separations obtained using some of the above mentioned systems.

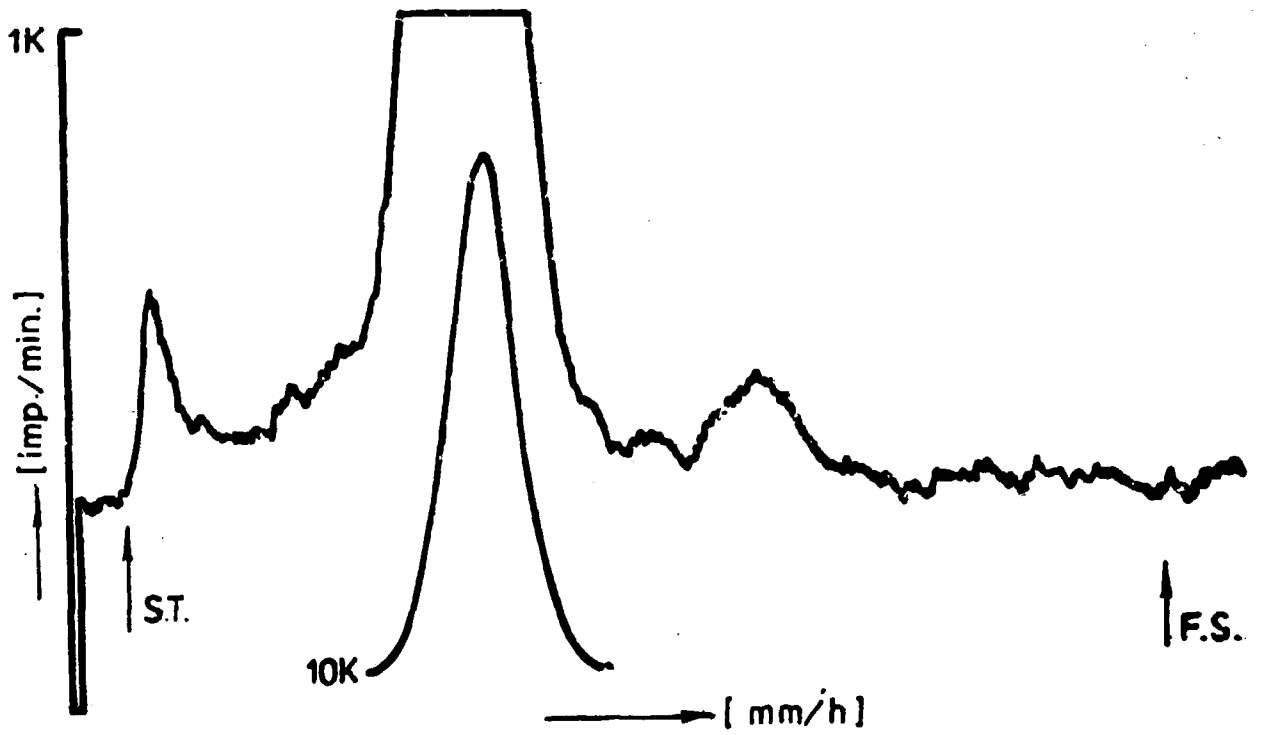

Figure 1 : Stationary phase : Silicagel G_F ; mobile phase: chloroform-acetone (2:1 v/v); R_F hydrocort. : 0.356; radiochem. purity: 91.5%.

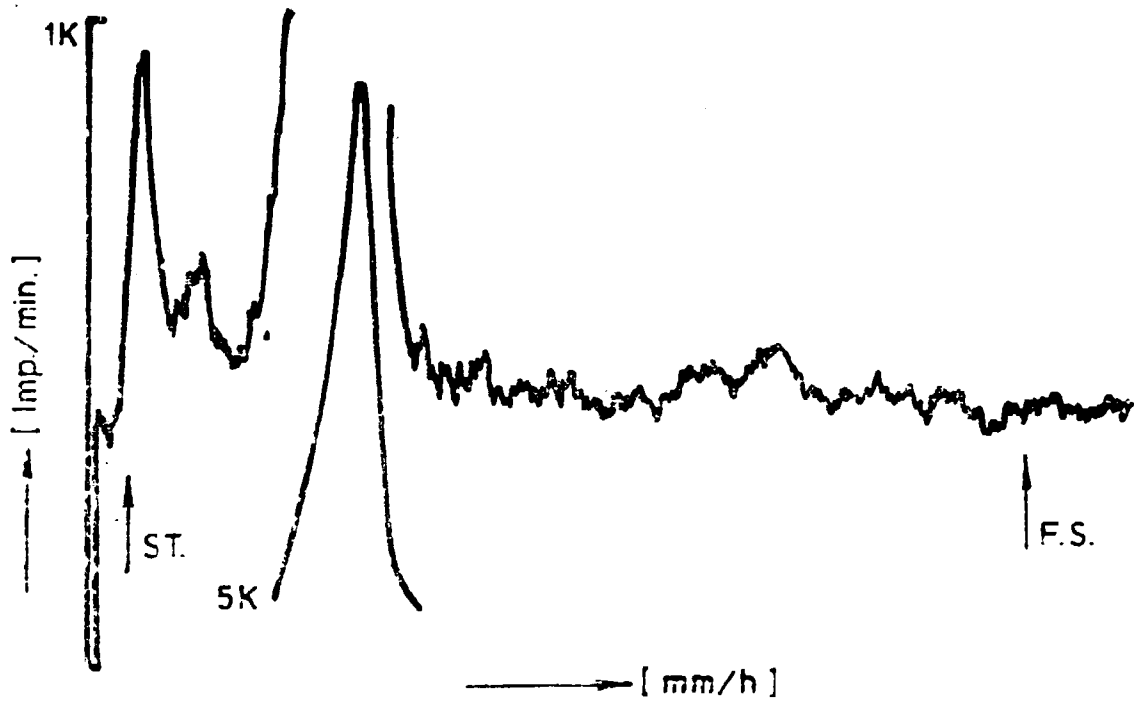

Figure 2 : Stationary phase: Silicagel G_F; mobile phase : methylene chloride - acetone (4:1 v/v); R_F hydrocort. 0.214; radiochemical purity : about 84%.

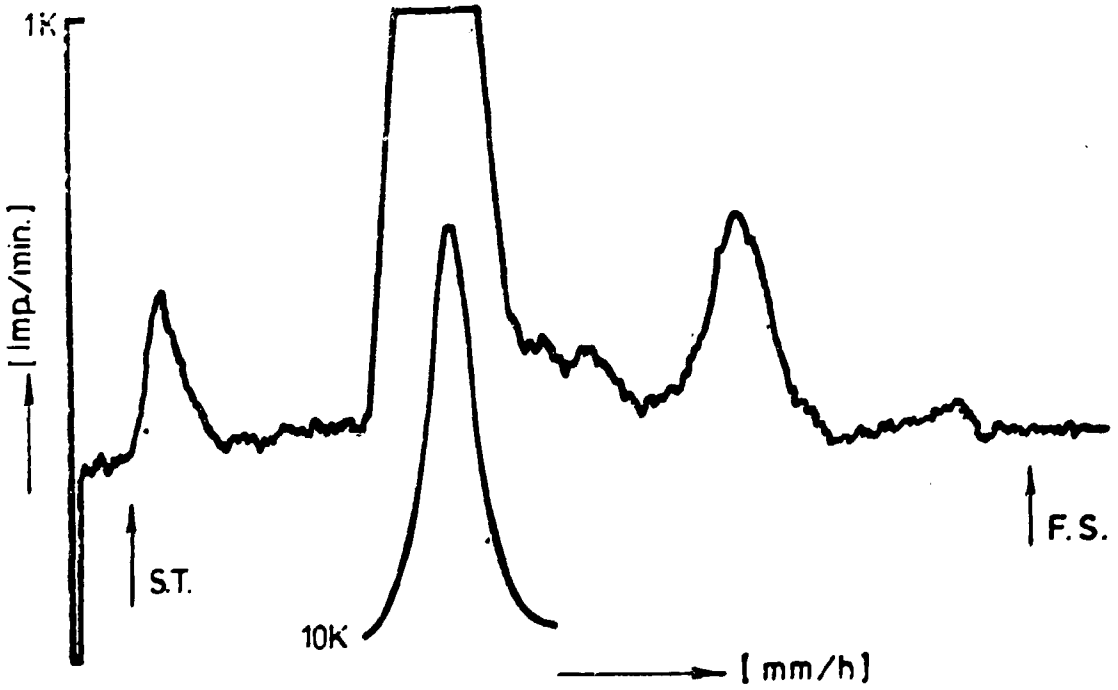

Figure 3 : Stationary phase : Silicagel G_F; mobile phase : chloroform-absolute ethenol - water (9:1:0.1 v/v); R_F hydroc. 0.355; radiochem. purity : 85%.

Looking over the experimental results obtained one could evidence the following artefacts :

1) Part of radioactivity (about 4-10%) was retained immediately after the chromatogram start.

Two facts would be responsible for this appearance:

(a) retention of hydrocortisone in the layer because it is practically a carrier free product;

(b) some degradation of the product during the elution of the chromatogram.

To clerify this question one carried out another series of experiments:

(a) addition of inactive carrier to the sample prior to spotting and development of the chromatogram;

(b) effectuation of a preparative chrometogram, elution of the spot corresponding to cortisol, and rechromatographing them into the same system;

(c) multiple developments of the sample chromatographed.

The separations obtained are illustrated in Figures 4-6.

As one can see the results are rather worse than the first ones since the retention was not solved but increased and moreover, an increased degradation of the sample appeared in all circumstances.

Conclusion is drawn that degradation during the chromatogram development is the reason both of the initial retention and of the additional degradation of the sample following the later experiments.

(2) Being a very strong polar compound, hydrocortisone is physically adsorbed in the layer and the result is a high activity background along the chromatograms.

(3) Loss of radioactivity after development of the chromstograms which, in some circumstances, attained even 90% from initial peak measured immediately after sample spotting. It is so called " disparition

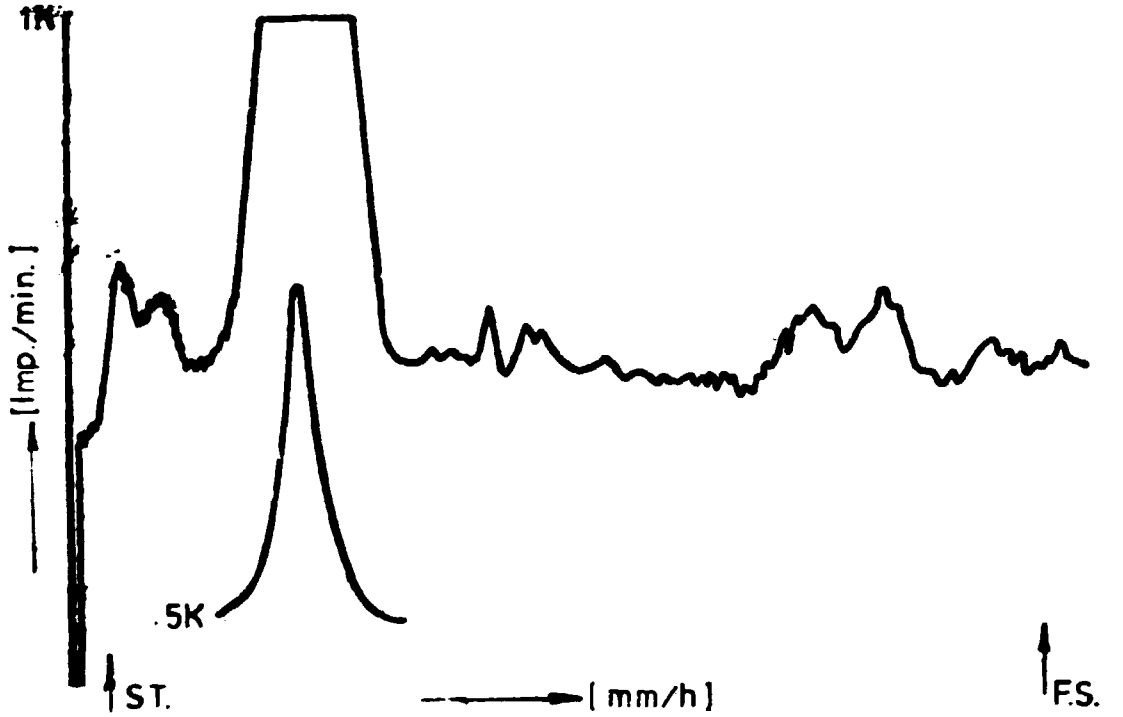

Figure 4 : Addition of inactive carrier ; stationary phase: Silicagel G_F ; mobile phase: methylene chloride-acetone (4 : 1 v/v).

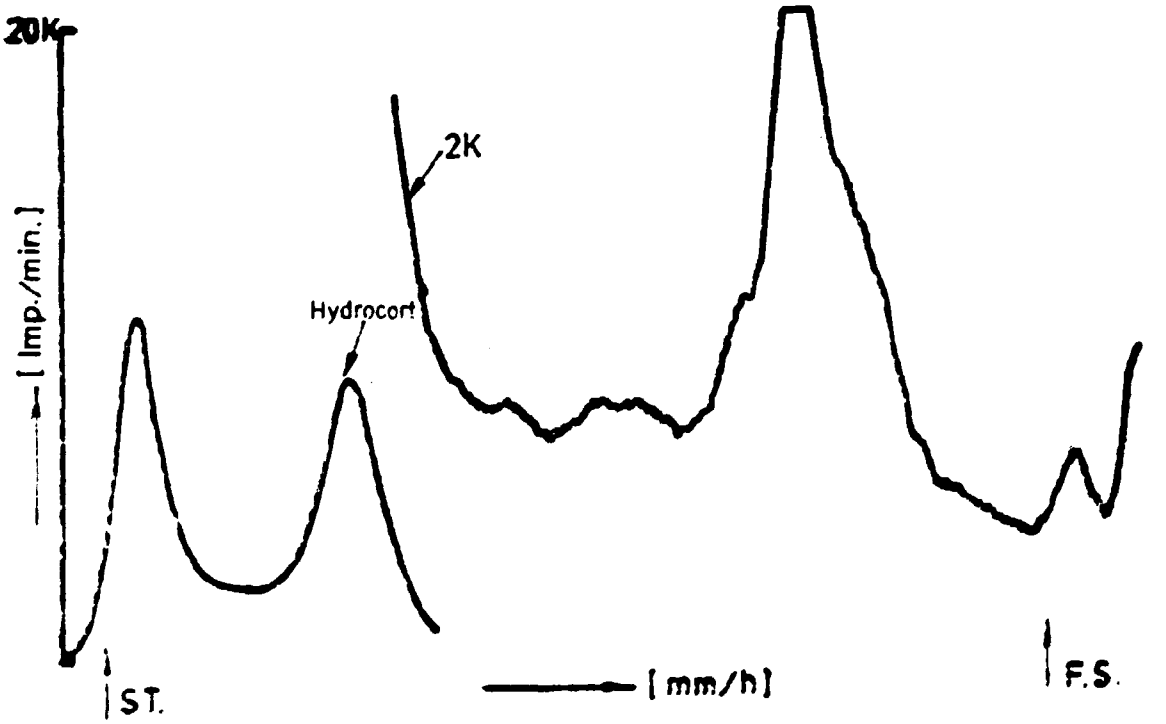

Figure 5 : Hydrocortisone rechromatography; stationary phase: Silicagel G_F ; mobile phase : chloroform - acetone (2 : 1 v/v).

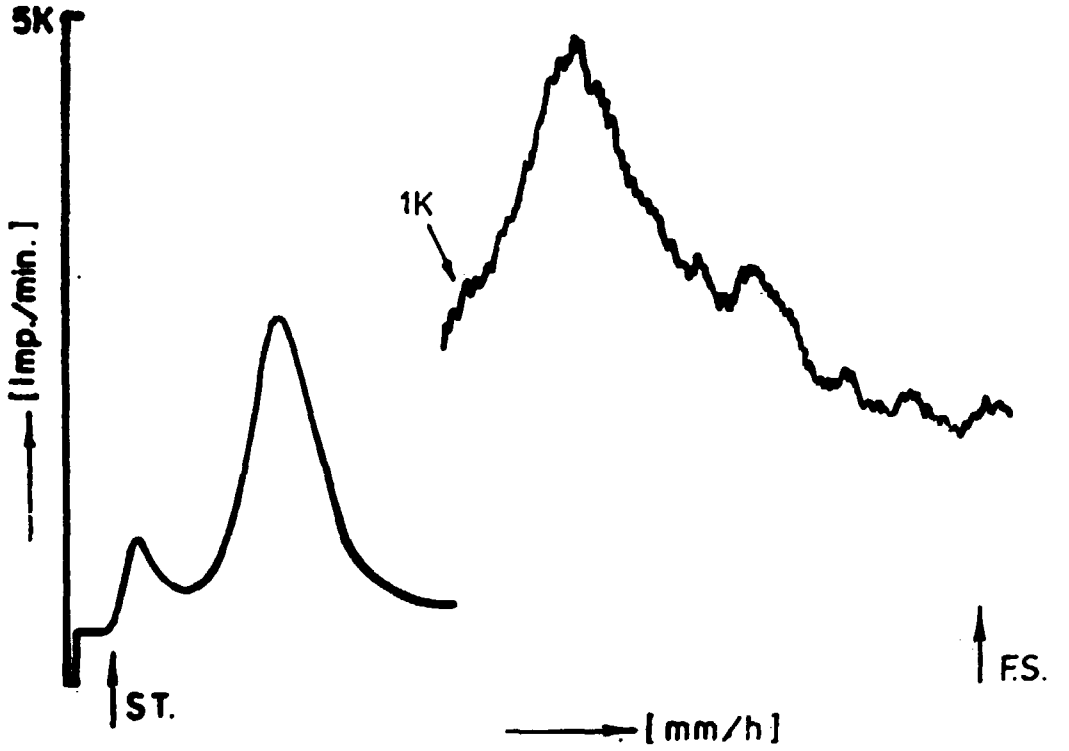

Figure 6 : Double development of chromatogram; stationary phase: Silicagel G_F ; mobile phase: chloroform - acetone (4 : 1 v/v).

of the sample " being already reported in the tritium labelled steroids chromatography [2]. This fact is due to the very soft beta emission of tritium and, as a consequence, nonuniform distribution of the compound throughout the thickness of the adsorbent layer. It may be ameliorated by carefully drying of the chromatogram both after sample spotting and after development of this one [2]. Another solution may be the change of the chromatographic technique.

All the experimental occurences above described and illustrated led to apparent low values of radiochemical purity that ranged from about 84% to 92%, thus being under the admissible limit.

Having in mind that hydrocortisone is a polar compound and that the adsorption chromatography was not the best choice to obtain good results, one considered necessary to change the working technique. Thus, one adopted the partition chromatography [3].
In this context, one used the following system:

- Kiselghur G as a support for stationary phase;
- Dimethyl formamide as a stationary phase;
- Chloroform as a mobile phase.

The separation obtained is presented in Figure 7.

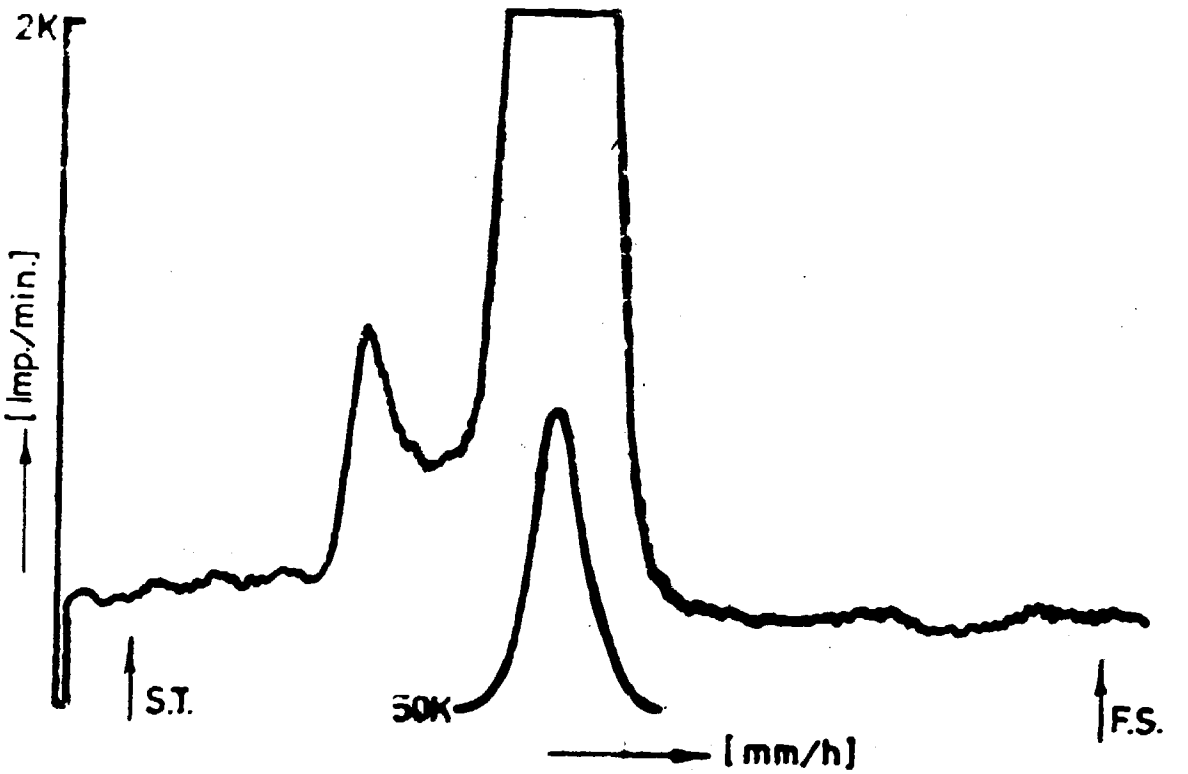

Figure 7 : Partition chromatography on hydrocortisone. Mobile phase: chloroform ; stationary phase: dimethyl formamide; radiochemical purity : 95,7% ; R_F hydrocort. = 0.43 ± 0.05.

It is evident that all the artefacts above mentioned are much reduced. Thus, degradation is lowered because the elution time was shortened from 1.0 - 1.5 hours to 7 - 10 minutes. Radioactivity retention in the layer (background and " sample disparition ") is minimized owing to inertness of the stationary phase solid support.
Using this technique, radiochemical purity values of 96% - 97% were obtained.

To choose the optimum conditions for reasonably good separation and R_F values one tried different proportions between stationary phase

and the solid support. But once established this ratio and in order to obtain reproducible results, it is very important to keep them constant and , moreover, all experimental parameters involved in the fulfilment of the chromatogram.

Finally, it must be pointed out that the apparently low radiochemical purity values were due to above illustrated and demonstrated artefacts which are undetectable in the thin layer or paper chromatography of inactive materials. They were found out as a direct result of the great sensitivity of detection by radio-scanning.

REFERENCES

1. J.R. Catch, Purity and Analysis of Labelled Compounds, Review 8, The Radiochemical Centre, Amersham, 1968, 24 pp.
2. D.T. Phillips and W.R. Waterfield, J. Chromatog., 40, 309 (1969).
3. E. Prescott, J. Chromatog., 9, 345 (1962).