Quantitative thin layer chromatography on cellulose
II. Selected applications: lower alcohols, tryptophan enantiomers, gold and platinum

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Abstract

The use of videodensitometry in quantitative TLC was examined with four examples: the determination of methanol and ethanol in biological fluids as their xanthate derivatives, the determination of tryptophan enantiomers, the determination of gold and that of platinum.

Keywords: Videodensitometry; Methanol; Ethanol; Tryptophan; Gold; Platinum

1. Introduction

Photometric quantitative evaluation of substances separated by planar chromatography has been carried out in numerous ways and by a splendid array of more or less sophisticated instruments. These have been reviewed and discussed repeatedly [1–3]. In one of the thorough discussions [1] it is evident that usually the chromatogram is moved relative to the detector to obtain a quantitative scan. Only in one technique employing photocopies of the chromatogram [1], and in autoradiochromatography, are spots evaluated at the same instant avoiding changes which may occur if zones are processed after each other.

With the advent of DNA fingerprinting, instrumentation for the evaluation of numerous zones in electropherograms has been developed. We found the approach using videodensitometry (as opposed to scanning densitometry) especially attractive in TLC

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when using reagents which yield relatively unstable coloured spots. It must be noted that only by evaluating the analyte with a series of standards simultaneously on the same thin layer is quantitation possible.

Part I of this manuscript [4] dealt with the determination of perbromate and bromate after thin layer chromatographic separation using starch–KI reagent.

In this paper we report four quantitative evaluations where the same approach produced good results. The alcohols were analysed with possible forensic applications in mind, in the hope of finding an alternative method to gas chromatography of measuring ethanol and methanol in blood, alcoholic beverages and other aqueous media.

Various articles have appeared in the literature regarding the paper and thin layer chromatography of alcohols in the form of their non-volatile derivatives, and examples include 3,5-dinitrobenzoates and xanthates (carbonodithioic acid, O-alkyl esters) [5–

7]. The latter were considered suitable for the derivatization of alcohols in various aqueous media like alcoholic beverages and biological fluids including blood, according to the following equation [1]:

$$CS_2 + KOH + R\text{-}OH \longrightarrow \begin{array}{c} K^+\text{-}S \\ \diagdown \\ C = S \\ \diagup \\ R\text{-}O \end{array}$$
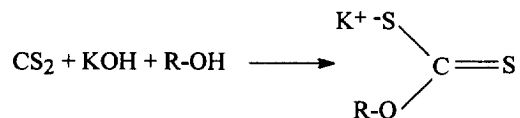
The enantiomers of tryptophan have been separated by several thin layer methods [8–11]. In the systems employing microcrystalline cellulose as support and α-cyclodextrin as eluent the largest separation factors were obtained, and thus the conditions for a good quantitation seemed to be given, which are also valid for the methyl- and fluoro-derivatives of tryptophan [11].

We have tried here to analyse mixtures of D- and L-tryptophan with ratios of 1:1 to 1:100. The determinations of gold and platinum were investigated as they represented an approach not requiring expensive equipment. Such determinations were considered for use in Vietnam by one of the authors (TKXH), who felt that there would be a wide application for it there.

2. Experimental

2.1. Lower alcohols

About 1 ml of the aqueous solution was placed in a test tube with 1 pellet of KOH (or NaOH) and 1 ml of CS_2, and the resulting mixture magnetically stirred for 1.5 h. The solution turned orange slowly. Tests were carried out with the test tube capped and uncapped, and the results were the same, indicating that concentrations of alcohols of less than 5% do not evaporate considerably. The top layer (aqueous) turned orange, while the bottom layer (CS_2) stayed colourless. The sample to apply was taken from the top layer without first separating them. The eluent used, for both paper and thin layer chromatography was butanol–30% ammonia in the ratio 1:1. Thin layers used were Merck 5577 microcrystalline cellulose thin layers (Merck, Darmstadt, Germany). The thin layers were impregnated with 5% K_2CO_3 and thoroughly dried before eluting. This is because

preliminary results without impregnation showed a double spot per analyte, thought to be the two different xanthate salts (K^+ and NH_4^+, respectively from the derivatizing agent and from the eluent) with slightly different mobilities, which means quantitative evaluation has a low precision at low concentrations. Detection was accomplished with 5% $NiSO_4$, which gave a yellow spot for methyl xanthate, and an orange spot for ethyl xanthate. The colour faded after ≈ 10 min.

The xanthate solutions were stable for at least a week when stored in hermetic sample tubes. All reagents were analytical grade and were obtained from Fluka (Buchs, Switzerland).

2.2. Tryptophan enantiomers

Tryptophan and its derivatives (1-methyl, 4-methyl, 5-methyl, 6-methyl, 7-methyl, 4-fluoro, 5-fluoro, 6-fluoro) were obtained from Sigma (Buchs, Switzerland), α-cyclodextrin and NaCl were from Fluka (Buchs, Switzerland). The thin layers used were Merck 5577 microcrystalline cellulose thin layers and Polygram Cel 300 native cellulose thin layers.

The best separation of enantiomers was obtained with microcrystalline cellulose thin layers, 1 M NaCl and 40 mM α-cyclodextrin solution as eluent and a temperature of 7°C (for tryptophan the separation factor a was 1.64 at room temperature and 1.87 at 7°C) [12].

Detection is accomplished by placing the humid thin layer in a container saturated with iodine vapours for 60 s, which produces black or dark brown spots on a white background, due to a stable complex formed between α-cyclodextrin and iodine in presence of tryptophan.

2.3. Gold and platinum

The thin layers were Merck 5577 microcrystalline cellulose thin layers. All chemicals were obtained from Fluka (Buchs, Switzerland). The samples were applied with 1 ml micropipettes, and the metal spots were revealed with a solution of 1 M Na_2S. The solutions of gold and platinum are made by dissolving the pure metals in aqua regia at 200°C. The solution is evaporated at 150°C, and the solutions of

$(AuCl_4)^-$ and $(PtCl_6)^{2-}$ were diluted with 2.5 M HCl to avoid the hydrolysis of the chlorocomplexes.

2.4. Densitometer set-up

The densitometer set-up is made up of a CCD camera with an acquisition device (Cybertech CS-1, Cybertech, Germany) which numerizes images in 480×374 pixels with 256 grey shades, linked to an IBM PC running the Cybertech Wincam 2.1 data treatment program under Windows 3.1. Diffuse ambient lighting was used throughout.

3. Results

3.1. Lower alcohols

In order to analyse samples in their linear concentration response range, two different ranges were investigated: 0–1.5% and 2–10%. Whereas for the range 2–10%, the calibration was linear ($r=0.99815$), that for the 0–2% region was not. In an attempt to find the detection limit and the "lowest linear range", the results shown in Fig. 1 were obtained, which also show that the use of peak areas is better than peak heights at this concentration range.

Analysis of fresh blood solutions with added EtOH (0.1%) were conducted together with a series

of standards. When the blood was derivatized with added MeOH and EtOH, the solution turned dark green, frothed up, and heated up considerably. The great advantage of this system is that only the xanthates, of all blood derivatives, are revealed on the thin layer at R_f values other than zero. Unfortunately the detection limit of the system is just less than 0.1% for ethanol, making quantitative evaluations close to this value very uncertain.

Low concentrations of methanol in water and blood (0.05–0.20%) were also analysed, and a linear relation between peak area and methanol was found ($r=0.99342$). It must be noted that when the same concentration of MeOH in blood and in water were derivatized and chromatographed the densitometric signals varied less than 5% between the different media for triplicates of 0.2% concentration. There is no effect on the ethanol determination if methanol is present and vice versa (Fig. 2). The detection limits for the two analytes with this chromatographic system is of the order of 0.05%, which corresponds to 12.4 mM for methanol (0.5 μl application) and 8.6 mM for ethanol (0.5 μl application). Some analytical results are presented in Table 1.

The alcohol derivatization was also undertaken in micro analysis sample tubes, and quantities as small as 50 μl could be successfully reacted, important in forensic analysis where the sample is often limited.

3.2. Tryptophan enantiomers

We have reported the parameters of the separation of tryptophan enantiomers in a previous paper [11]. Fig. 3 shows the best results obtained at room temperature. In this study only D- and L-tryptophan were quantitatively evaluated, but the procedure is also applicable to the enantiomers shown, given their excellent separations.

A quantity as low as 5 ng of analyte can be detected, but for quantitative evaluations the working range is between 10 and 1000 ng per spot applied (0.05–50 nmole). It is possible to detect one enantiomer in presence of the other down to concentrations of 1% (Fig. 4).

Both enantiomers can be analysed on the same thin layer, as the presence of one enantiomer does not influence the determination of the other. Fig. 5 shows the case for an equimolar (racemic) mixture of
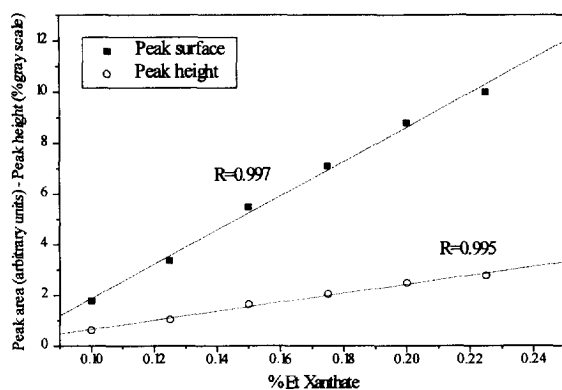

Fig. 1. Calibration curves for peak height and peak area of Et Xanthate spots on Merck no. 5577 microcrystalline cellulose thin layers impregnated with 5% K_2CO_3, eluted with BuOH–30% NH_3 (50:50) and revealed with 5% $NiSO_4$ solution.
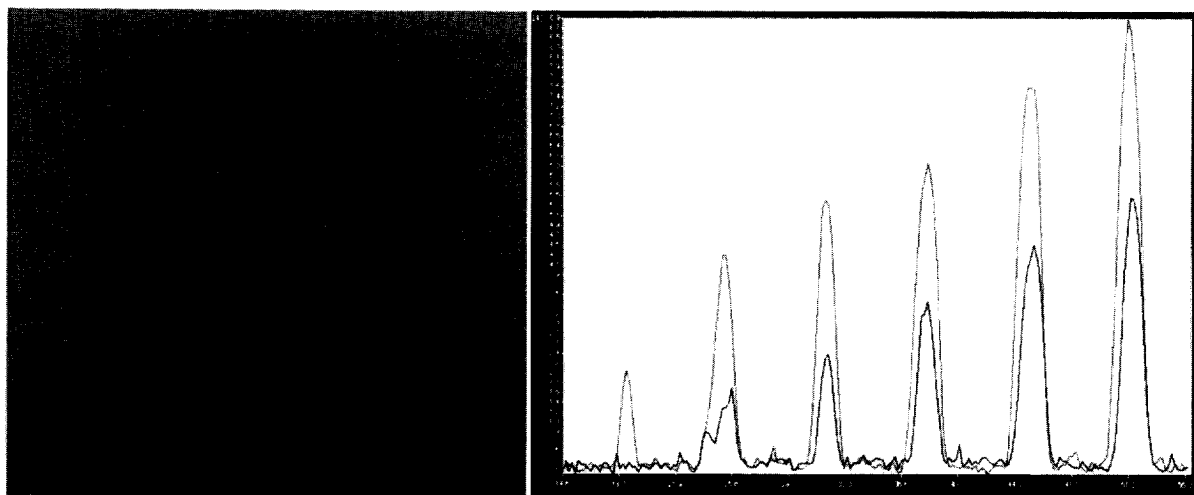
Fig. 2. Left: Chromatogram of Me and Et Xanthate (0.05%–0.2%) eluted on Merck no. 5577 microcrystalline cellulose thin layers impregnated with 5% K_2CO_3 using 0.5 μl application and BuOH–30% NH_3 (50:50) as eluent, revealed with 5% $NiSO_4$. Right: Densitometric analysis of the same chromatogram.

D- and L-tryptophan (which also shows that the two isomers give essentially the same response). Similarly, 1% D-trytophan could be detected in the presence of L-tryptophan, and it was also possible to detect even less than 1% of 6-Me and 6-F-tryptophan enantiomers due to their excellent enantiomeric separation (Fig. 3).

3.3. Gold

The R_f value of gold is reproducible and independent of the concentration used. In this study a linear relationship was observed for the concentration range 0.04–200 nmole per spot, meaning a quantity of 8 ng could be detected.

The R_f value of gold when eluted with 2.5 M HCl is low (0.49) compared with values for other metals ($\geq$0.70) [12,13]. Fig. 6 shows a simultaneous analy-

sis of pure gold and gold mixed with Pt and Pd on the same thin layer. The concentration of these metals is 250 times that of gold. Table 2 shows the analytical results for the analysis of gold in a mixture with different metals. The standard deviations shown are calculated from 3 replicates.

3.4. Platinum

Direct separation works well for gold but not for platinum. The R_f value of platinum is high and too close to that of several other metals. The complexation of Pt with a solution of $SnCl_2$ can change the R_f of Pt in a useful way. Amongst the complexes of Pt, those with the $SnCl_3^-$ ligand (formed by $SnCl_2$ in HCl) are important from an analytical point of view. Several separation methods of platinum metals are based on the formation of these complexes [14].

Table 1
Analysis of lower alcohols: analytical results

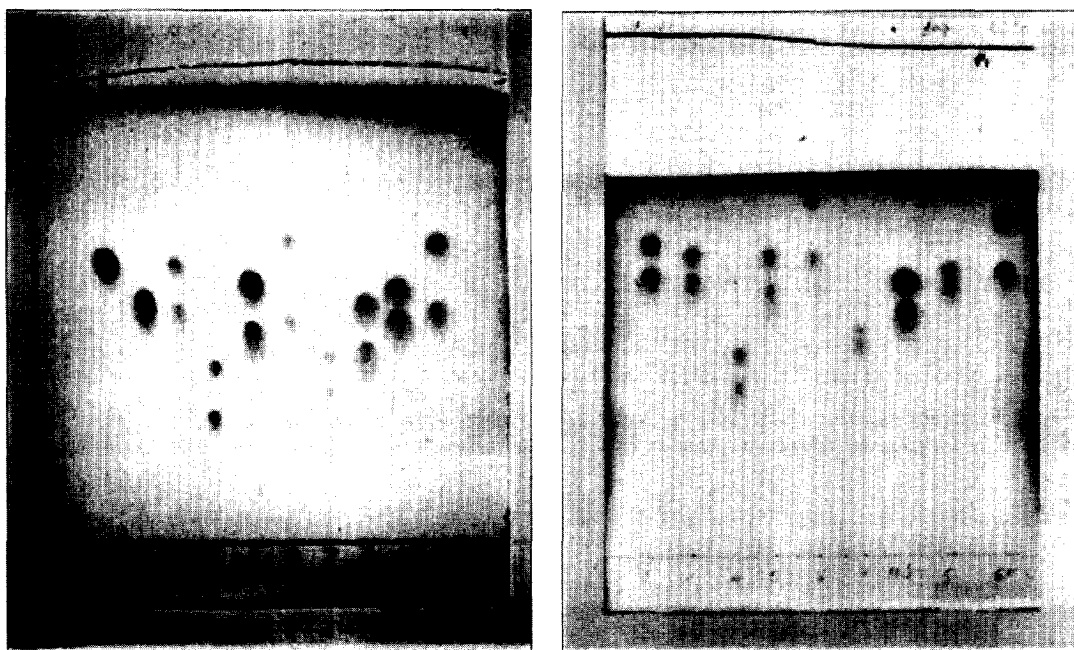| Analyte (concentration range)	Concentration found (concentration applied) (%)	Relative standard deviation (%) (number of replicates)
EtOH (2–10%)	6.04 (6.00)	5.6 (5)
EtOH (0–1.5%)	1.13 (1.20)	2.5 (3)
MeOH (0.05–0.2%)	0.99 (1.00)	6.7 (3)

Fig. 3. Images of two chromatograms of Merck 5577 microcrystalline cellulose thin layers (left) and of Cel 300 native cellulose thin layers (right), developed with α-cyclodextrin (40 mM) and NaCl 1 M as mobile phase. The spots are (left to right): D- and L-tryptophan, 1-Me-tryptophan, 4-Me-tryptophan, 5-Me-tryptophan, 6-Me-tryptophan, 7-Me-tryptophan, 4-F-tryptophan, 5-F-tryptophan, 6-F-tryptophan. The chromatograms were revealed two years previously with iodine vapours. For Merck 5577 D- and L-tryptophan were spotted separately.
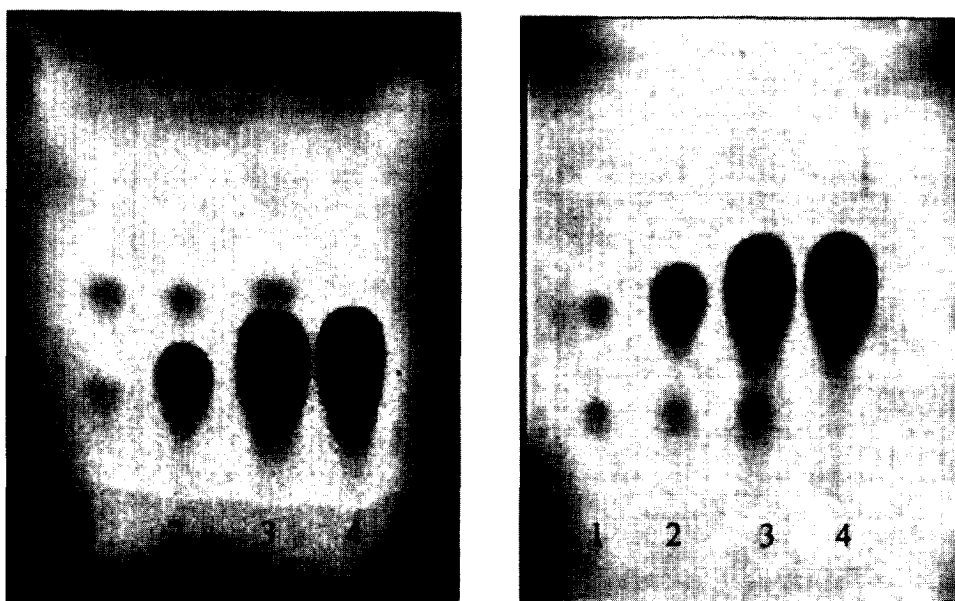

Fig. 4. Left: 1, 50% D- and 50% L-tryptophan; 2, 10% D- in L-tryptophan; 3, 1% D- in L-tryptophan; 4, L-tryptophan. Right: 1, 50% D- and 50% L-tryptophan; 2, 10% L- in D-tryptophan; 3, 1% L- in D-tryptophan; 4, D-tryptophan.
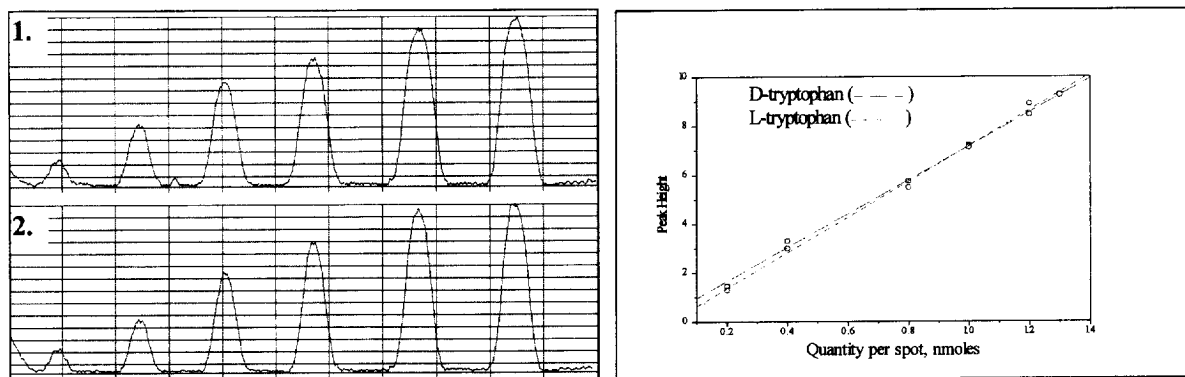
Fig. 5. Left: Densitometric peaks of D- (1) and L-tryptophan (2) in an equimolar mixture. Quantity per spot 0.2–1.3 nmole. Right: Calibration curve for D-tryptophan (correlation coefficient $R = 0.998$); calibration curve for L-tryptophan (correlation coefficient $R = 0.998$).
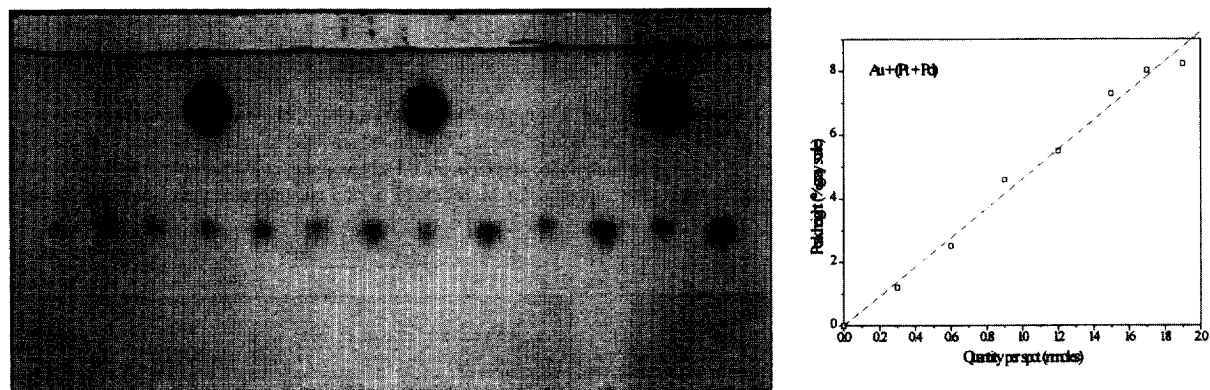

Fig. 6. Analysis of pure gold and 0.7% gold in a solution of Pt and Pd (250 times more concentrated). Thin layer Merck 5577 microcrystalline cellulose, eluent 2.5 M HCl, revelation 1 M Na_2S. Spots 2, 6 and 10 are pure Au, spots 4, 8 and 12 are Au + (Pt + Pd), and the other are standards (0.3–1.9 nmole). Left: Photo of the chromatogram. Only the spots of palladium and gold are visible, as platinum is not visible under these conditions. Right: Calibration curve ($r = 0.995$).

Table 2
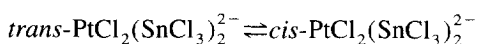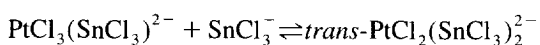Analysis of gold in presence of other metals: analytical results

Molar ratio of gold in a mixture with other metals	Quantity of gold per spot (nmole)	Relative standard deviation ($n = 3$)
Au:Cu = 1:1000	0.34	0.029
Au:Ni = 1:1000	0.35	0.028
Au	0.77	0.04
Au:(Pt + Pd) = 1:250	0.79	0.03
Au:Cd = 1:1000	1.60	0.03
Au:Co = 1:1000	3.96	0.04
Au:Fe = 1:1000	4.03	0.04

Table 3
R_f values of some $SnCl_3^-$ complexes chromatographed on Merck 5577 microcrystalline cellulose thin layers using 2.5 M HCl as eluent and revealed with 1 M Na_2S

Element	Pt	Pd	Rh	Os	Ru	Ir
R_f	0.4	0.85	0.62–0.72	0.85	0.8–0.94	0.07–0.4–0.65

Young et al. [15] have identified two trichlorostannocomplexes of Pt:

$$PtCl_4^{2-} + SnCl_3^- \rightleftharpoons PtCl_3(SnCl_3)^{2-} + Cl^-$$

$$PtCl_3(SnCl_3)^{2-} + SnCl_3^- \rightleftharpoons trans\text{-}PtCl_2(SnCl_3)_2^{2-}$$

$$trans\text{-}PtCl_2(SnCl_3)_2^{2-} \rightleftharpoons cis\text{-}PtCl_2(SnCl_3)_2^{2-}$$

The addition of $SnCl_2$ to the mobile phase allows separation of Pt from a mixture of other metals, but,

on cellulose thin layers, the Pt spots elongate during chromatography. Preliminary trials show that complexation of samples before chromatography with 2.5 M HCl gives good results. The formation of the complex is also done in 2.5 M HCl, and adjusted as to have a final $SnCl_2$ concentration of 1 M. This is allowed to stand 10 min before chromatographing. Under these conditions, only one orange spot is seen for Pt for the concentration range examined (0.1–150 nmole per spot). A complexation time of more than 20 min results in elongated spots and a slight R_f change. Trials with Pt(II) and Pt(IV) complexed with $SnCl_2$ show spots of the same R_f. The results shown in Table 3 show that, excepted Ir(III), the other metals of the Pt group do not influence the determination of platinum.

Fig. 7 shows the determination of platinum in presence of rhodium (same concentration). Table 4
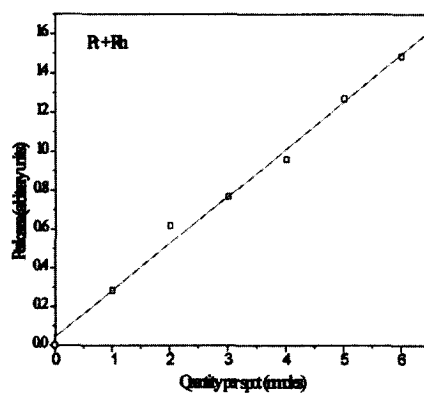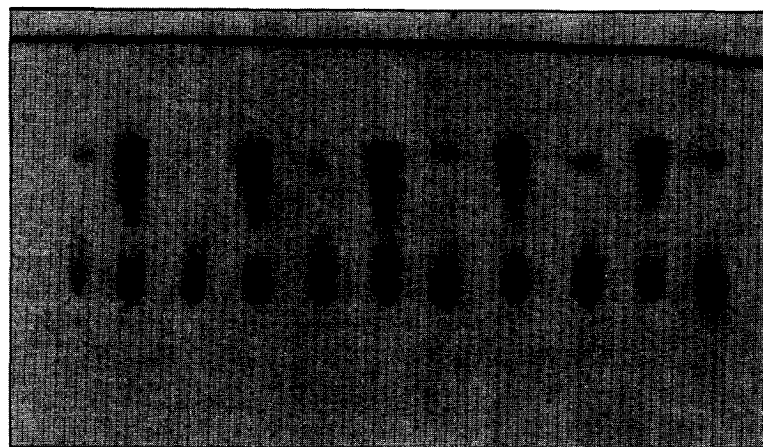

Fig. 7. Analysis of platinum in presence of Rh (same concentration). Thin layer Merck 5577 microcrystalline cellulose, eluent 2.5 M HCl, revelation 1 M Na_2S. Spots 2, 4, 6, 8 and 10 are the analyte (Pt+Rh), the others are the standards (1–6 nmole). Left: Photo of the chromatogram. Right: Calibration curve ($r = 0.996$).

Table 4
Analysis of platinum in presence of other metals: analytical results

Molar ratio of platinum in a mixture with other metals	Quantity of platinum per spot (nmole)	Relative standard deviation ($n = 3$)
Pt:Mo = 1:100	0.08	0.036
Pt:Pd = 1:10	1.99	0.045
Pt:Rh = 1:1	3.87	0.021[a]
Pt:Fe = 1:1000	15.00	0.017
Pt:Co = 1:1000	15.00	0.017

[a] Calculated from 5 replicates.

shows the analytical results for the analysis of platinum in a mixture with different metals. The detection limit observed for platinum is 0.1 nmole per spot applied (≈ 20 ng).

4. Conclusion

Four examples of quantitative estimations are reported using thin layer chromatography followed by videodensitometry of coloured spots. This approach yields results with an accuracy and precision of the same order as with previous quantitative TLC methods, with the added advantage of being able to deal with unstable coloured spots. Since the quantitation is essentially automatic and fast, it seems to be a useful quantitative technique.

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