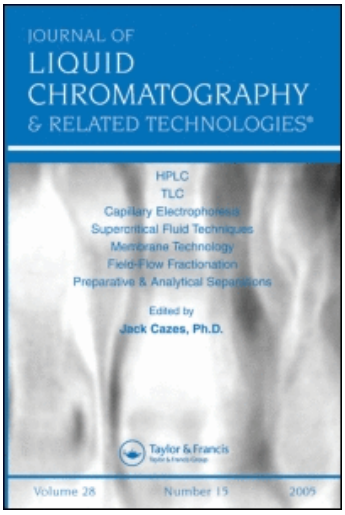


**Journal of Liquid Chromatography & Related Technologies**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# Monitoring of D-Penicillamine in Clinical Practice by Ionexchange TLC

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**To cite this Article** Kiss, Péter and Kovács, Judit(1982) 'Monitoring of D-Penicillamine in Clinical Practice by Ionexchange TLC', Journal of Liquid Chromatography & Related Technologies, 5: 8, 1531 — 1540
**To link to this Article: DOI:** 10.1080/01483918208062849
**URL:** http://dx.doi.org/10.1080/01483918208062849

JOURNAL OF LIQUID CHROMATOGRAPHY, 5(8), 1531-1540 (1982)

# MONITORING OF D-PENICILLAMINE IN CLINICAL PRACTICE BY IONEXCHANGE TLC

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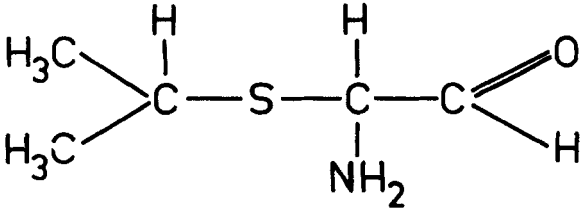
D-Penicillinamine /Fig. 1/, dimethylcysteine, a metabolite of penicilline is a comparatively stable thiol compound, which has been first identified as a component of the penicilline molecule.




D – PENICILLAMIN

* Presented at the First Symposium on Advances of TLC and HPLC, May 14-15, 1982, Szeged, Hungary.

The biological action of D-Pa is attributed to its amino-thiol properties, such as chelation of metals, reaction with carbonyl groups etc. Its therapeutic application dates back to the late fifties when it was used with success in the treatment of Wilson disease, a copper storage disorder. Since then the D-PA proved to be a very efficient drug in various diseases.

In clinical practice, D-PA is used in the following cases:

Newborn period: hyperbilirubinemia, prevention of retrolental fibroplasia.

Metabolic diseases:Wilson disease, cystinuria.

Chronic diseases: rheumatoid arthritis, scleroderma, chronic hepatitis.

Metal poisoning: Hg, Pb.



Pharmacology - toxicity

1. Only the D form is effective and it is less toxic.

2. The toxicity is both dosage and time dependent.

3. A number of side effects may occur during long-term treatment, such as: bone marrow damage /anaemia/, renal failure /proteinuria/, loose of taste and smell sensing, gastro-intestinal symptoms, allergic reactions. All this unfavourable symptoms disappear rapidly after cessation of the therapy.

## Mode of treatment

There are two ways of D-Pa administration:

1. in acute diseases, e.g. in hyperbilirubinemia of newborns intravenous mode of administration is preferred.

2. in chronic illnesses for long-term treatment oral application is employed.

The wide range of possibilities of clinical applications as well as the control of long-term treatment require a careful and reliable drug monitoring. Since the drug causes sometimes severe side effects and the dosage schedule is based upon only empirical data, a sensitive and selective method is needed for the determination of D-Pa in body fluids.

The aim of the present work was to develop a procedure suitable for the determination of D-Pa in small quantities in blood among others such as filter paper eluates.

The D-Pa which is actually an amino acid containing a thiol group could be well separated on Fixion sheets and could be visualized by means of the usual ninhydrin reagent.

## Method

Blood samples in 50-100 µl quantities are drawn by fingertip or heel puncture and are collected either in heparinized tubes or dried on filter paper.

For deproteinization as well as for elution from the filter paper a 10% aqueous trifluoroacetic acid solution is used.

After deproenization the excess TFA is removed, since it may interfere with the chromatographic procedure.

The chromatography is carried out on Fixion 50X8 chromato-sheets, using citrate buffer pH 4.4 /$Na^+$ - 0.3M/:

| | |
|---|---|
| citric acid.$2H_2O$ | 14.1 g |
| NaOH | 8.0 g |
| NaCl | 5.85 g |
| HCl /37%/ | 5.0 ml |
| deionized water ad | 1000.0 ml |



The 200 x 200 mm chromatosheets were developed in standard TLC tanks at +4°C in a refrigerator. Drying and staining with ninhydrin was carried out as described previously.

D-Pa appears as dark pinkviolet spot between leucine and valine /Fig. 2 and 4/.

Quantitative evaluation was performed by a Telechrom video-densitometer type OE-976.

## Results

D-Pa /100 mg/kg bodyweight/ was administered intravenously to hyperbilirubinemic newborn infants.

At first the drug was administrated as a single intravenous injection. Fig 2 shows the developed chromatogram and it could be established that after two hours the drug completely disappears from the blood stream. The densito-

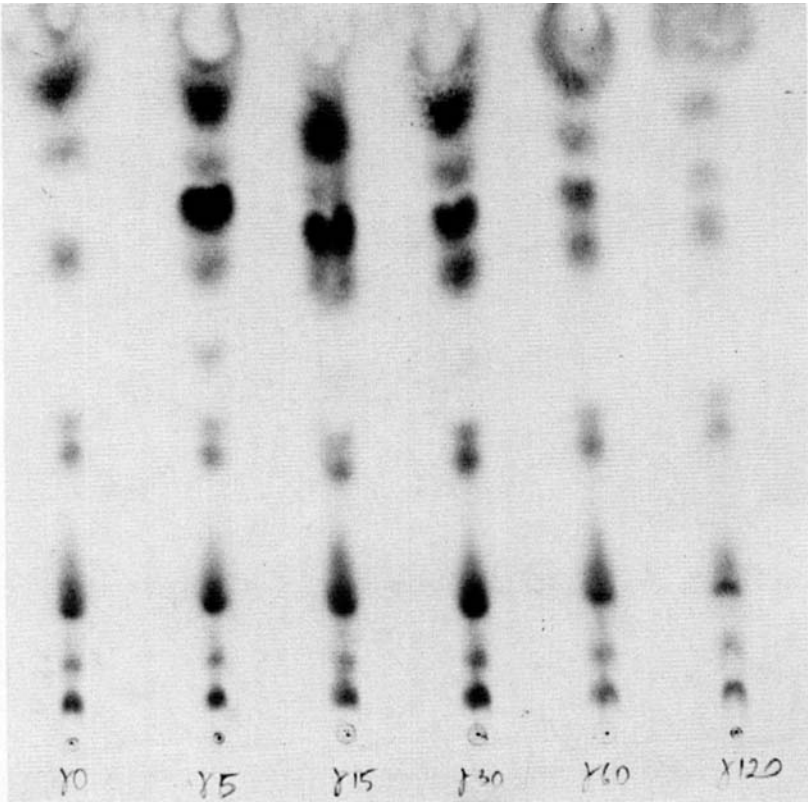



Fig. 2

Distribution of D-Pa in blood serum after administration of 100 mg/kg i.v.

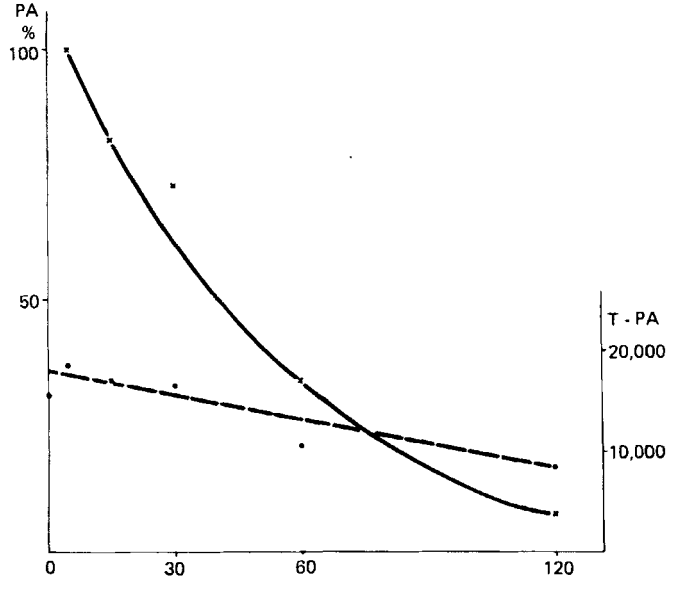



Fig. 3

Videodensitometric readings of the chromatogram shown on Fig. 2



metric evaluation confirms quantitatively these observations /Fig. 3/.

Secondly, the administration was followed by continuous drop infusion. This mode of employment ensures a steady drug level throughout the time of infusion, usually 5 hours long /Fig. 4 and 5/.

These findings correspond with our clinical observations, that especially in the treatment of hyperbili-

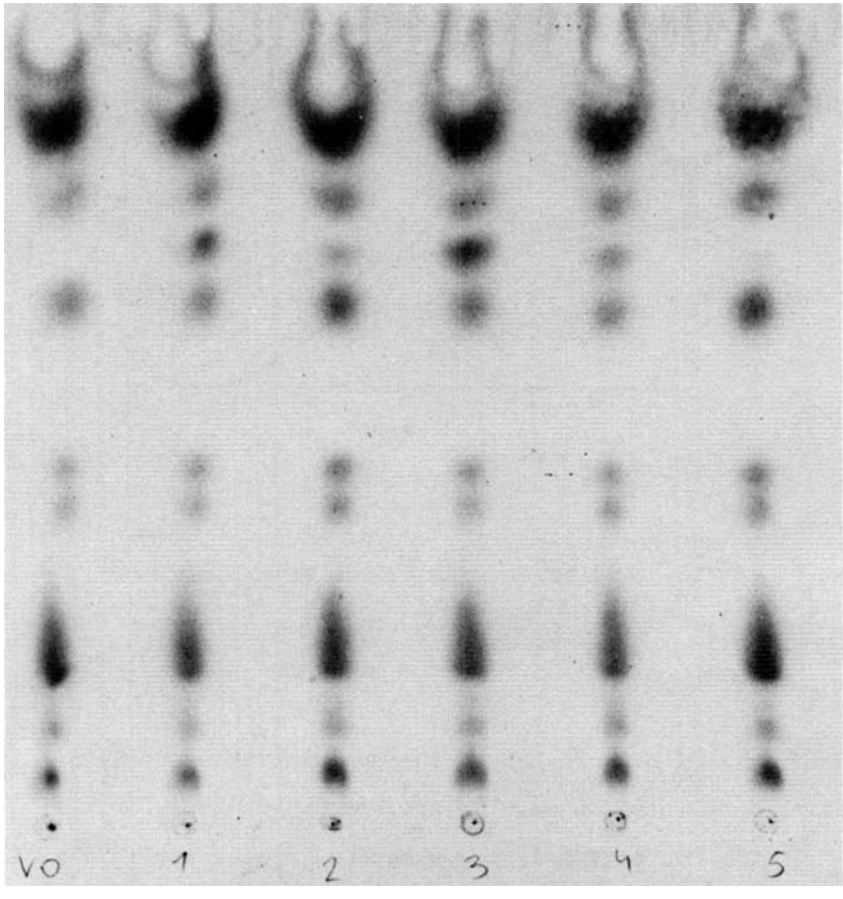



Fig. 4

Distritubiton of D-Pa in blood serum after administration of 100 mg/kg in drop infusion

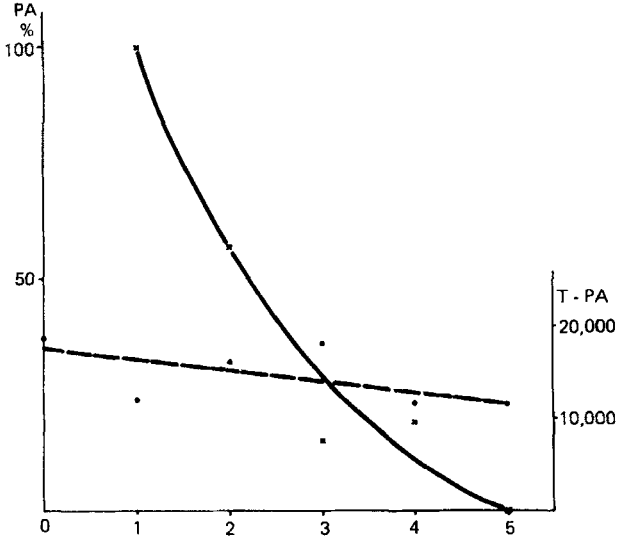



Fig. 5

Videodensitometric readings of chromatogram shown on Fig. 4



rubinemia of newborn babies the administration of D-Pa using drop infusion must be preferred.

The conventional Fixion method is also applicable detecting D-Pa in urine. It is especially indicated in the therapy control of cystinuric patients. D-Pa is excreted unaltered in the urine. The urine sample may be applied onto the chromatosheet without previous deproteinization or desalting. D-Pa be well distinguished even in the presence of cystine /Fig. 6 /.

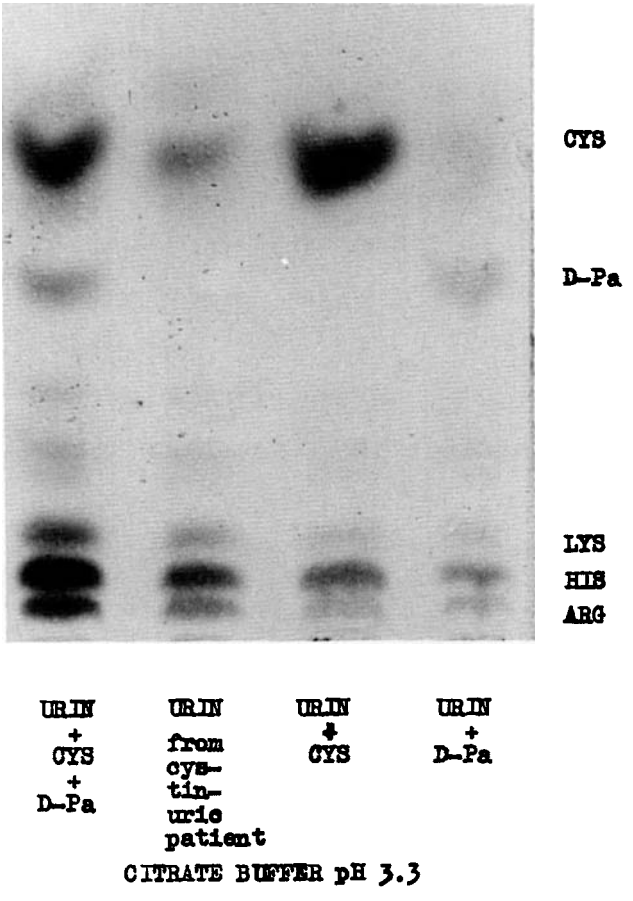



Fig. 6

Detection of D-Pa in urine

Summarizing:

The ion exchange TLC method developed by us is suitable for regular monitoring as well as for therapy control during short or long-term D-Pa administration. It is a convenient tool for appropriate dosage schedule, which is very important regarding the prevent ion of the possible side effects.

The method is simple, very sensitive and reproducible and its application means a significant help in the every day clinical practice.



Thanks are due to Drs. T. Dévényi and S. Pongor /Enzymology Department, Institute of Biochemistry, Hungarian Academy of Sciences/ for their help and assistance.

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