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Thin Layer Chromatographic Separation and Quantitation of L-Dopa and L-Tyrosine in Mixtures

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A new TLC separation and quantitation method for L-tyrosine and L-Dopa mixtures was developed. The minimum tyrosine and Dopa quantities which can be measured by this technique are 0.7 and 1.5 μg respectively. The method may be used to measure the kinetic parameters of polyphenoloxidase as well as to trace the enzyme catalyzed conversion of L-tyrosine into L-Dopa. For a set of 20 measurements the maximum difference between any two measurements of spot areas was found to be 3 mm^2 (4.5%) for L-Dopa (4 μg) and 2mm^2 (5.6%) for L-tyrosine (1μg) .

Key words: TLC Separation, L-Dopa-L-Tyrosine mixtures.

Introduction

Enzymatic hydroxylation of L-tyrosine seems to be the best method for the synthesis of L-Dopa, an effective drug for the treatment of Parkinsons disease. Therefore many investigations using immobilized polyphenoloxidase (PPO) or entrapped cells rich in PPO have been carried out[1−5]. In these studies as well as in enzyme kinetic measurements, quantitative determination of L-Dopa in the presence of excess L-tyrosine is necessary. Since the UV absorption spectra of L-tyrosine and L-Dopa are very similar, spectrophotometric methods are not suitable. The colorimetric method based on chemical procedures[6] is time consuming, requires specific reagents and is influenced by substances such as ascorbate which are likely to be present in the medium. HPLC, currently the most common method, requires sophisticated instruments and expensive reagents[7].

In this paper a rapid and sensitive quantitation method based on TLC is presented.

Materials and Methods

Desaga TLC set wás used for the preparation of plates. TLC plates (20 × 20 cm) were covered with silica gel G (1mm thick) and activated before use according to the method of Stahl[8].

Spray reagent: 3% ninhydrin in n-butanol. (3ml acetic acid was added to 100 ml spray solution)

Developing Solution: Phenol - Water (75:25 w/w) containing 20 mg NaCN in 100g.

Spray reagent: Standard L-Dopa (10 mM) and L-tyrosine (2mM) solutions were prepared weekly. All chemicals used were of analytical grade.

Procedure:

Precisely known volumes of standard L-Dopa, L-tyrosine and mixture solutions (2-30 μL) were spotted onto the plates, 1.5 cm apart. Applications were done in small increments and solvents were allowed to dry between consecutive spottings.

Spotted plates were developed in preequilibrated tanks to a height of 15 cm in a phenol-water solvent system. After the evaporation of the solvents, plates were sprayed with ninhydrin reagent and heated at 110 $^{\circ}$C for ten minutes to produce coloured products.

For quantitative analysis, either area or weight measurements were carried out as follows.

After the compounds were located as coloured spots, a sheet of transparent paper was laid on the plate and spots were copied onto the paper. Following magnification (4 times), spot areas were measured both with a planimeter and by putting the copied spot areas on millimetric graph paper and counting. As an alternative, magnified spots were cut from graph paper and weighed with an analytical balance.

Results and Discussion

Among the different solvent systems tested, phenol-water gave the most satisfactory results. L-Dopa and L-tyrosine were completely separated giving well defined spots. The development time was 4 hours for a height of 15 cm. R_f values were determined to be 0.39 and 0.53 for L-Dopa and L-tyrosine respectively, perfectly satisfactory results for the two compounds differing only by one OH group.

For quantitative analysis spot areas (A) were used according to the graphical method of Purdy and Truter[9]. In Fig. 1 and Fig. 2 $\sqrt{A}$ - log w plots are given for L-tyrosine and L-Dopa, respectively. As seen from these figures, straight lines were obtained in the range of 0.7-7 mg for tyrosine and 1.5- 12 mg for Dopa. Smaller quantities did not give visible spots whereas larger quantities caused tailing.

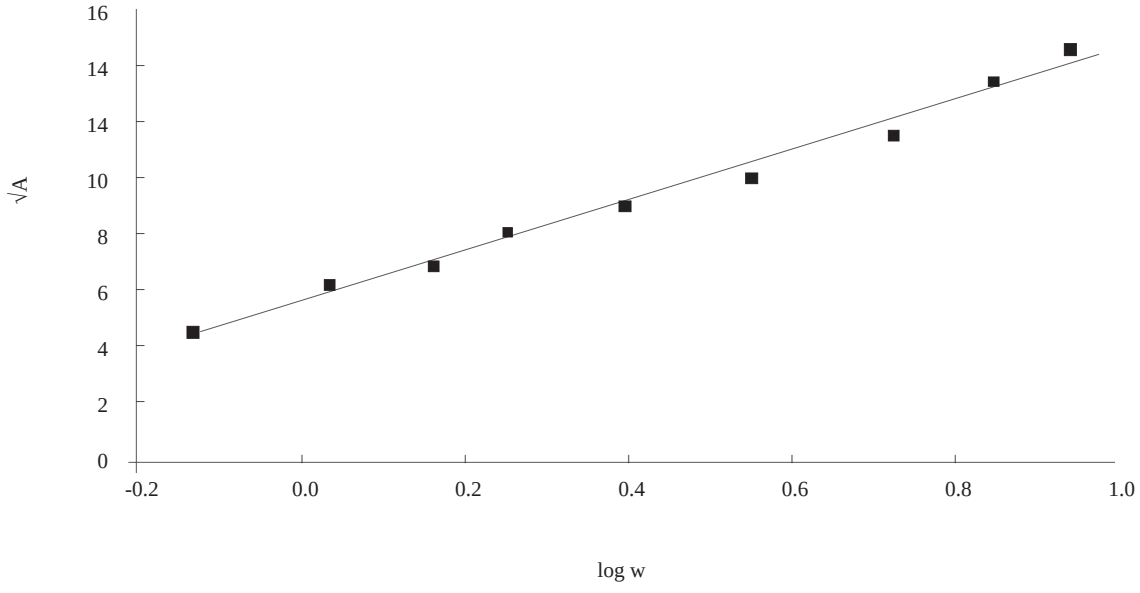

Figure 1. $\sqrt{A}$ - log w plot for L-tyrosine [A = spot area (mm^2) w=weight of tyrosine (μg)]

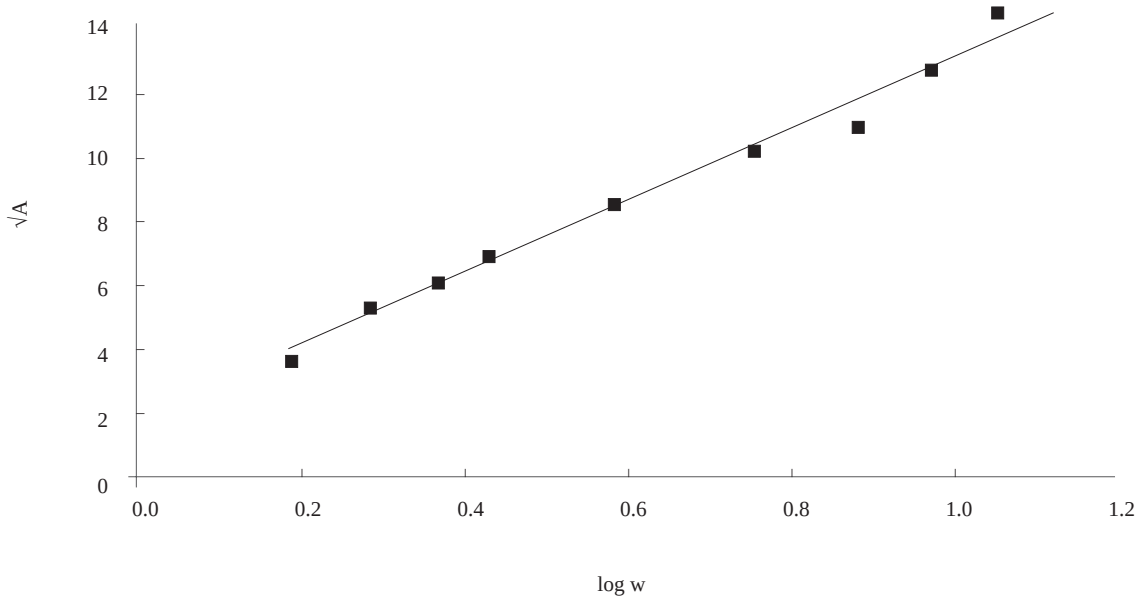

Figure 2. $\sqrt{A}$ - log w plot for L-Dopa

Different techniques used in measuring spot areas did not affect the results appreciably. Spot areas measured by planimetry and millimetric counting for L-tyrosine (0.72 μg) are given in Table 1. The mean spot areas calculated from a set of 20 experiments were found to be 20.57 ± 0.61 mm^2 and 20.42 ± 0.64 mm^2 for planimetric and millimetric measurements respectively.

Spot areas (A) and weights (m) of cut papers belonging to the spots were found to be proportional as expected (Fig. 3). Therefore $\sqrt{m}$ - log w plots are also linear (Fig. 4) and can be used for quantitative analysis.

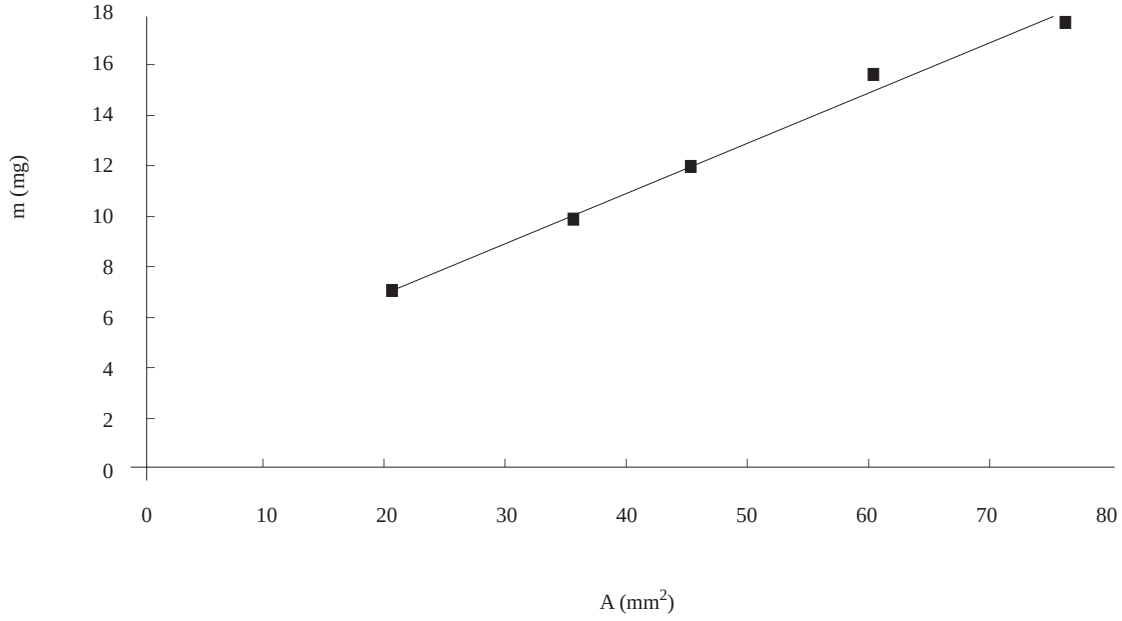

Figure 3. m-A graph for L-tyrosine [m: weight of paper corresponding to spot area].

Table 1. Comparison of area measurement techniques for 0.7 μg (2μL) L-tyrosine.
Number of tests: 20

No.	Planimetric Method area (mm^2)	Millimetric Method area (mm^2)
1	20.4	20.0
2	22.0	21.0
3	20.4	20.5
4	20.8	21.0
5	20.0	20.0
6	20.6	20.0
7	20.6	21.0
8	20.1	19.5
9	20.8	21.0
10	21.2	21.0
11	20.2	20.0
12	20.1	20.0
13	20.1	19.0
14	21.9	21.5
15	19.7	20.0
16	20.0	20.0
17	20.3	20.5
18	21.2	21.0
19	20.6	21.0
20	20.5	20.5

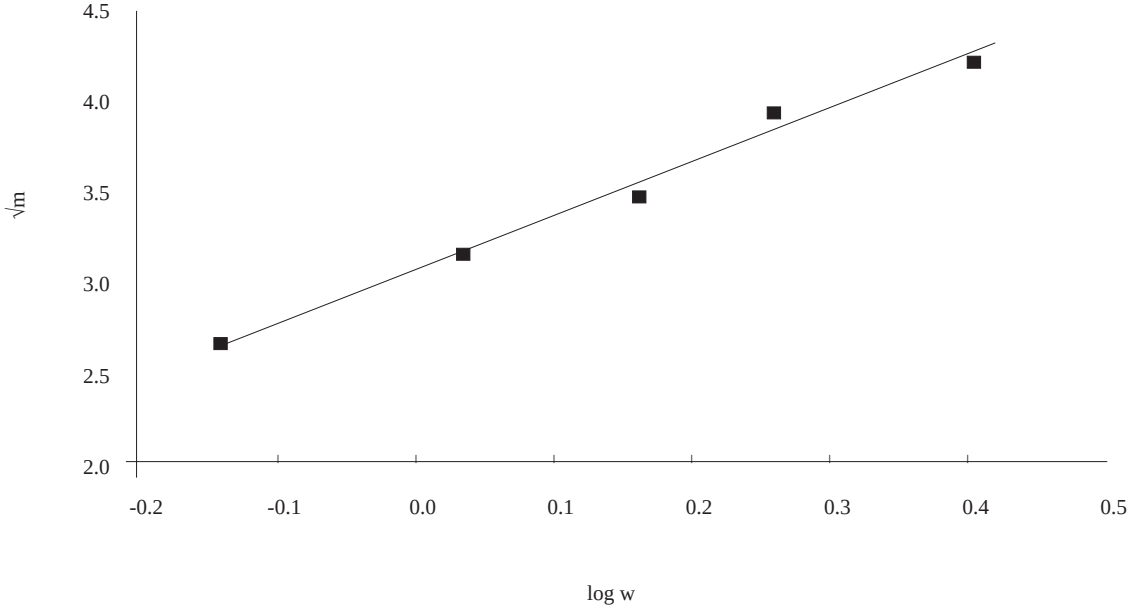

Figure 4. $\sqrt{m}$ log w graph for L-tyrosine

Results of three quantitation methods for different L-tyrosine concentrations are given in Table 2. Each value in the table corresponds to the mean of the 20 separate measurements.

Table 2 . Results of different quantitation methods

L-Tyrosine Amount (μg)	**Planimetric Method Mean area SD.**	**Millimetric Method Mean area SD.**	**Weighing Method Mean weight SD.**
0.7μg (2μL)	20.57 mm^2; 0.61	20.42 mm^2; 0.64	7.1 mg; 0.51
1.0μg (3μL)	35.51 mm^2; 0.66	35.83 mm^2; 0.68	9.98 mg; 0.71
1.4μg (4μL)	45.27 mm^2; 0.58	45.06 mm^2; 0.65	12.1 mg; 0.81
1.8μg (5μL)	60.26 mm^2; 0.74	60.1 mm^2; 0.87	15.07 mg; 1.27

Although spot areas were not as well defined for L-Dopa as for L-tyrosine, reproducible results could nevertheless be obtained. A set of 20 measurements performed for 4 μg (10 mL) L-Dopa gave an average spot area of 67.35 ± 1.17 mm^2. The maximum difference between any two measurements was found to be 3 mm^2 (4.5%).

The method may be used to measure kinetic paremeters of polyphenoloxidase as well as to follow the enzymatic conversion of L-tyrosine into L-Dopa.

On a 20×20 cm plate 13 sample and standard solutions can be separated efficiently. Ascorbic acid, generally used to prevent further oxidation of L-Dopa, does not give interfering spots.

Simply, through the observation of TLC plates, it is also possible to check whether any unwanted oxidation product of L-Dopa exists.

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