Thin-layer chromatography of Captan and Captax

Captan (N-trichloromethyl-thio-4-cyclohexene-1,2-dicarboximide) and Captax (2-mercaptobenzothiazole) have both extensive application. The former being utilized primarily as a general fungicide for the treatment of a wide variety of foliar soil and seed-borne diseases[1–4], and the latter as a fungicide[5–7] and accelerator for rubber vulcanization[8,9].

The analytical determinations of both have been mainly carried out by colorimetric techniques, *viz.* for Captan: procedures utilizing resorcinol[10–12], pyridine-alkali[13,14], and pyridine-tetraethylammonium hydroxide[15]; for Captax: bismuth nitrate[16] and selenious-sulfuric acid[17]. Chromatographic analyses, however, have been limited to both paper[18,19] and silicic acid column[20] procedures for Captan and paper techniques for Captax[21,22].

Our studies with Captan and Captax involve a variety of metabolic and feeding studies. This initial investigation was to determine the feasibility of thin-layer chromatography with its inherent advantages of speed, enhanced sensitivity and high resolving power for both residue analysis and elaboration of purity of standard and commercial samples of these compounds. Concomitantly it was of interest to compare two quantitation techniques, *viz.* densitometry and that of PURDY AND TRUTER[23] as to their efficacy with thin-layer chromatography.

Experimental

Detecting reagents. For Captan: 25% resorcinol in glacial acetic acid[24]. For Captax: (1) Cupric chloride reagent[25]: solution I—aqueous solution of 3% cupric chloride, 6% ammonium chloride and 6% ammonium hydroxide; solution II—20% aqueous hydroxylamine. Solutions I and II are mixed (1:2) prior to use. (2) Sulfuric acid–butanol reagent: (1:1) solution of conc. sulfuric acid and *n*-butanol, prepared daily.

Solvent systems. For Captan: chloroform; for Captax: isopropanol–ammonium hydroxide–carbon tetrachloride (50:10:40)[26].

Preparation of chromatoplates. The chromatoplates* utilized were 8 × 8 in. plates coated to a thickness of 250 μ with Camag DF-5 silica gel (for Captax) and TLC-7G Mallinckrodt silica gel for Captan determinations respectively. The plates were washed by ascending chromatography with chloroform–methanol (1:1), then activated in an oven at 75° for 30 min. Acetone solutions of Captax and chloroform solutions of Captan were spotted, chromatographed by the standard ascending technique, the developed plates sprayed with the indicated detector, and dried at room temperature in a hood for color development for Captax, and dried in an oven at 140° for five minutes for Captan.

Preparation of channel chromatoplates. The method consisted of dividing the plate into strips or channels a few mm wide, extending from several mm below the point of origin to the line marking the solvent front. One μl of a chloroform solution of Captan** (0.5, 1, 2, 4, 6, 8, 10, 16 and 20 μg/μl) was applied at the origin within each channel, developed by the ascending method and made visible by the indicated

* Pre-coated plates were obtained from Analtech, Wilmington, Del., U.S.A.

** Analytical standard obtained from California Chemical Co., Ortho Division, Richmond, Calif., U.S.A.

detecting reagent. Captax* standard solution in acetone (2, 4, 6, 8, 10, 16 and 20 μg/μl) was applied and treated in analogous manner as that described above.

Extraction of Captan from mouse tissue. An adult mouse (*ca.* 20 g) after sacrifice was homogenized in a Waring blender with 50 ml hexane and 1 ml of 5 N hydrochloric acid and spiked with Captan. The homogenate was filtered through 1 × 15 cm column of 1:1 (v/v) celite–sodium sulfate and then refiltered with an additional 20 ml hexane. The filtrate was then partitioned with 2× 50 ml hexane–acetonitrile (2:1). The hexane–acetonitrile extract was backwashed with 10 ml of hexane and then concentrated to dryness under vacuum utilizing a Rinco evaporator. The final dilutions were made in chloroform.

Results and discussion

The resorcinol reagent detected Captan at R_F of 0.35 as a yellow spot (bright yellow fluorescence under U.V. of 3660 Å) with the lower limit of detection being 1 and 0.5 μg, respectively.

The cupric chloride detecting reagent revealed Captax at R_F 0.25 as a pale yellow spot (bright orange fluorescent spot under U.V. of 3660 Å) on a white background, the lower limit of detection being 2 μg. The sulfuric acid–butanol reagent also detected Captax as a pale yellow spot on a white background but the lowest detectable limit was 4 μg.

Densitometry and the technique of PURDY AND TRUTER[23] (plot of the log sample weight *vs.* the square root of the spot area) were evaluated for the quantitation of both Captax and Captan on channel chromatoplates.

For Captax. Fig. 1 is a plot of the log sample weight *vs.* maximum optical density obtained by utilizing both the cupric chloride** (Curve I) and sulfuric acid–butanol (Curve II) reagents. Measurements were accomplished at 420 λ utilizing a Photovolt Densitometer.***

Linearity from 2–16 μg is obtained utilizing the cupric chloride reagent (Curve I).

In contrast, the sulfuric acid–butanol reagent produced erratic results over the range from 4–20 μg.

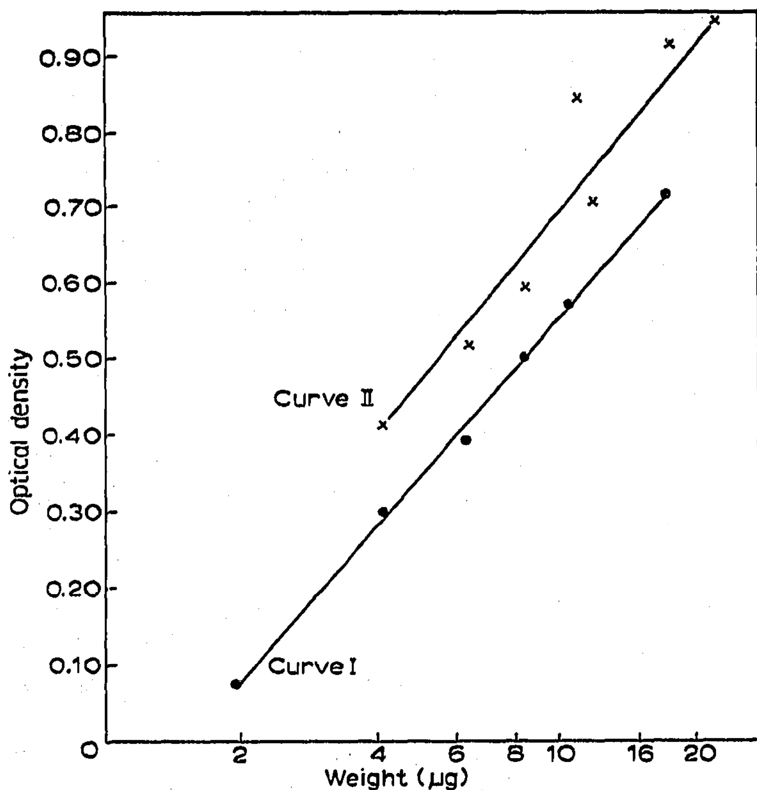
Fig. 2 depicts the results of triplicate runs utilizing the measurement technique of PURDY AND TRUTER and is a plot of the log sample weight *vs.* the square root of the spot areas (width of channel × height of streak) determined both as a yellow streak after spraying with the cupric chloride reagent and as a quench at 2537 Å on a fluorescent background. The linearity obtained was, as in the densimetric procedure above, 2–16 μg.

For Captan. Fig. 3 is a plot of the log sample weight *vs.* maximum optical density obtained in an analogous manner to that described above for Captax. Linearity from 1–10 μg was obtained; however, a wide deviation was found to occur at concentrations from 14–20 μg. Fig. 4 depicts the results utilizing the technique of PURDY AND TRUTER. It can be seen that linearity occurs with greater precision and is extended to 1–20 μg

* Obtained from R. T. Vanderbilt Co., New York, N.Y., U.S.A.

** The utility of the cupric chloride reagent was limited to approx. 20–30 min after initial spraying due to the onset of yellowing of the chromatograms, and dictates the speed in which the densitometric measurements must be obtained.

*** Model 52-C with a motorized TLC stage, Model 520-A photomultiplier and a linear/log varicord recorder, Model 43, all obtained from Photovolt Corp., New York, N.Y., U.S.A.



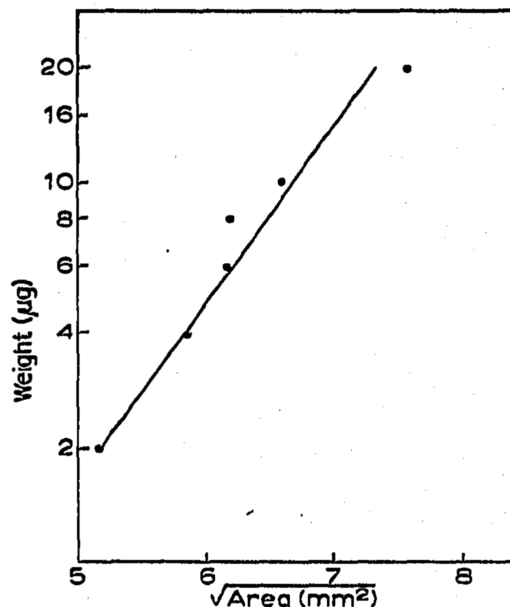
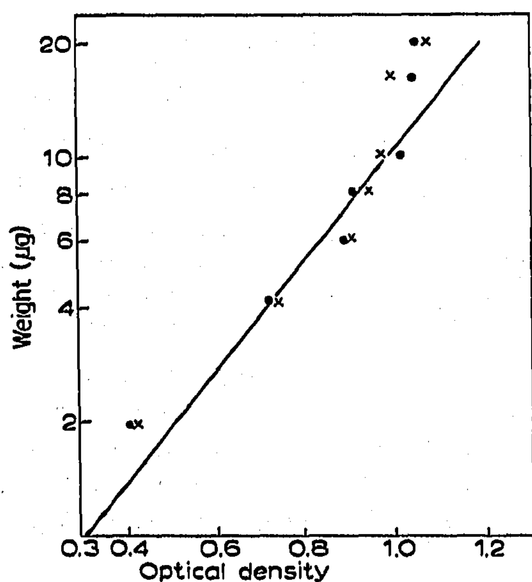


Fig. 1. Quantitation of Captax by densitometry.

Fig. 2. Quantitation of Captax by channel technique.



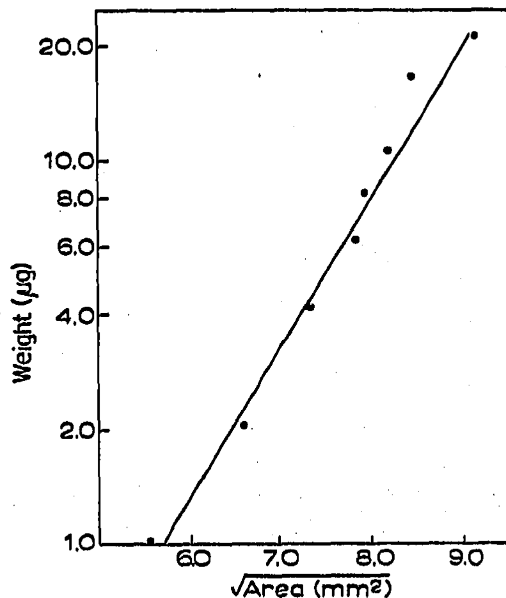


Fig. 3. Quantitation of Captan by densitometry.

Fig. 4. Quantitation of Captan by channel technique.

TABLE I

RECOVERY OF CAPTAN FROM MOUSE TISSUE

Replicate no.	*Extract No. 1**			*Extract No. 2**		
	Captan added (μg/ml)	*Captan recovered (μg/ml)*	*Recovery (%)*	*Captan added (μg/ml)*	*Captan recovered (μg/ml)*	*Recovery (%)*
1	40	38	95	40	41	102
2	40	48	120	40	41	102
3	40	40	100	40	45	110

* Extracts 1 and 2 were spiked with 200 and 800 μg respectively. The final dilutions in chloroform were 5 and 20 ml respectively, resulting in a final Captan conc. of 40 μg/ml for each solution. Fifty μl of sample was applied per spot and compared with Captan standards.

utilizing the latter method of quantitation which is dependent only upon spot area and not color density.

Table I depicts the recovery of Captan from mouse tissue extracts following addition of 200 and 800 μg amounts and demonstrates the utility both of the extraction procedure and the channel technique for the quantitation of Captan.

Conclusions

The quantitative determination of Captax and Captan on channel chromatoplates, both by densitometry and measurement of spot area (technique of PURDY AND TRUTER), was found to be feasible. The primary advantage of the latter procedure is both the speed and the inexpensive nature of the analysis.

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A new spray reagent replacing sulphuric acid in thin-layer chromatography

Concentrated sulphuric acid is one of the most widely used general spray reagents in thin-layer chromatography. In addition to many advantages this reagent presents well known difficulties. The irritating action of its vapours on mucous membranes and their corrosive effects makes it impossible to use it outside well ventilated hoods. The use of special equipment such as chambers for spraying and visualisation of chromatograms is also indispensable. The spraying itself requires special precautions as oversprayed chromatograms give erroneous results.

The use of an aqueous solution of ammonium sulphate as a new spray reagent for TLC has been developed; it has all the advantages of sulphuric acid without its shortcomings. The thermal dissociation of this salt liberates adequate amounts of sulphuric acid, the excess of which is neutralised with ammonia from the dissociated salt after its decomposition.

The use of this reagent does not result in the formation of irritant and corrosive vapours, and does not require special equipment, or the use of separate rooms.

A 20 % aqueous solution of ammonium sulphate can be used, but better results are obtained with a 1:1 mixture of ammonium sulphate and ammonium hydrogen sulphate. The latter reagent is prepared by dissolving 20 g of ammonium sulphate in 100 ml water and adding 4 ml sulphuric acid ($d = 1.84$). This solution is then ready for use and can be stored for any period of time.

The chromatograms have to be sprayed quite heavily until they become transparent. No special precautions concerning the spraying technique are undertaken and overspraying is not critical.

The sprayed chromatograms are dried with hot air and visualised at elevated temperature. The minimal temperature required is different for various compounds, usually some 25° higher than when a sulphuric acid spray is used.

A practical method of heating the chromatograms is the use of ordinary hot plates (with metal cover) on which the dried chromatograms can be laid directly. This