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Separation and quantitative determination of nanogram quantities of maltodextrins and isomaltodextrins by thin-layer chromatography

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ABSTRACT

Relatively fast solvent-systems have been developed for TLC separation of maltodextrins and isomaltodextrins containing 1–20 glucose residues. The primary solvent contains methyl cyanide (acetonitrile)–ethyl acetate–1-propanol–water in volume proportions of 85:20:50:X, where the water component, X, is varied from 50–70 for maltodextrins and 90–100 for isomaltodextrins. Separation of the α-(1 → 6) branched maltodextrins, including the two branched hexasaccharide isomers, resulting from the hydrolysis of amylopectin by alpha amylase, was achieved using 3 ascents of the solvent with 50 parts water. Separation of the α-(1 → 3) branched isomaltodextrins, resulting from the hydrolysis of dextran by dextranase, was achieved using the solvent with 70 parts water. A detection technique in the nanogram range has been developed by dipping the plate into ethanol containing 0.5% α-naphthol and 5% sulfuric acid, followed by heating for 10 min at 120°C. Saccharides appear as brown to black spots on a white background. Densitometric scanning of the TLC plate gives a linear relationship for 50 to 2000 ng of glucose. Identical plots on a weight basis are obtained for glucose, maltodextrins, and isomaltodextrins, showing that the densitometric response is independent of the saccharide structure. Thus, the relative weight percent of a series of malto- or isomalto-dextrins may be obtained by dividing the density of each saccharide by the sum of the densities of the saccharides. Malto- and isomalto-dextrins, down to dp 15–16, can be quantitatively determined by densitometric scanning of the TLC plate. These results, along with the increased sensitivity of the detection method, greatly widens the scope of the type of quantitative experiments that can be performed, which heretofore have been dependent on the use of radioactive compounds.

INTRODUCTION

The separation and quantitative analysis of monosaccharides and oligosaccharides are important in a wide variety of applications such as food analysis[1], clinical analysis[2,3], the analysis of enzymatic synthesis of oligosaccharides[4–6], and the analysis of the products from the hydrolysis of polysaccharides[7,8]. In recent years,

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these analyses have tended to be performed by HPLC[9–11]. The separation and quantitative determination of carbohydrates by HPLC, however, have many limitations and drawbacks. Thin-layer chromatography presents itself as an important alternative that can overcome the limitations and drawbacks of HPLC. Quantitative determinations by TLC, however, have been viewed as being difficult. In some applications, it has been possible to use radioactive materials[4,5,12] and quantitatively determine the radioactive compounds directly on the TLC plate by the use of a TLC radioactive autoscanner[4,5] or a PhosphorImager[12].

The quantitative determination of carbohydrate "spots" directly on the TLC plate by densitometry has occasionally appeared in the literature[1–3,13–15]. One of the earliest reports of the use of TLC densitometry was the quantitative determination of the oligosaccharides formed from the hydrolysis of pullulan by pullulanase[13]. Recently, the development of computerized TLC densitometric instrumentation has become available (Analtech, Newark, DE and Bio-Rad Laboratories, Hercules, CA) giving increased sensitivity, precision, and ease of use for such analyses.

In this study, we present relatively fast TLC solvent systems for the separation of maltodextrins and isomaltodextrins, having dp values from 1 to 20. We also present the development of a sensitive detection method in the nanogram range that is not dependent on the chain length of the saccharide, allowing us to quantitatively determine low amounts of maltodextrins and isomaltodextrins down to dp 15–16 directly on the TLC plate by densitometry.

EXPERIMENTAL

Materials.—TLC Plates. Whatman K5, K6, and Diamond K6 silica gel plates of various sizes (5 × 20 cm, 10 × 20 cm, and 20 × 20 cm) were obtained from Fisher Scienfific (Chicago, IL).

Maltodextrins and isomaltodextrins.—Homologous series of maltodextrins were obtained by hydrolysis of corn starch with alpha amylase (EC 3.2.1.1, Thermamyl, Novo Industries, Copenhagen), followed by hydrolysis by isoamylase (Sigma Chemical Co.). Branched maltodextrins were obtained by porcine pancreatic alpha amylase (EC 3.2.1.1, Sigma Chemical Co.) hydrolysis of potato amylopectin. Maltodextrin series were also obtained by debranching of shellfish glycogen by isoamylase (EC 3.2.1.68).

Homologous series of isomaltodextrins were obtained by acid hydrolysis[16] of *Leuconostoc mesenteroides* B-512F dextran. Branched isomaltodextrins were obtained by hydrolysis of B-512F dextran by *Penicillium funiculosum* dextranase (EC 3.2.1.11). Pure individual maltodextrins and isomaltodextrins were obtained by preparative charcoal column chromatography[17].

Methods.—Separation and quantitative determination of the dextrins by TLC. Aliquots (1–3 μL) of the solutions of the various samples were applied 15 mm from the bottom of the TLC plate with a 25-μL Hamilton microsyringe pipet. The

size of the spot was kept between 2 and 3 mm by adding 1 μL at a time with drying in between. The plates were irrigated to the top at 20–22°C, using various solvents and multiple ascents (see Results and Discussion section for details). After development, the carbohydrates were visualized by dipping the TLC plate into an EtOH solution containing 0.5% (w/v) α-naphthol and 5% (v/v) H_2SO_4. After air-drying the TLC plate, it was placed into a forced-air oven for 10 min at 120°C. The densities of the carbohydrate spots on the TLC plate were determined by using an Uniscan densitometer (Analtech, Inc., Newark, DE).

RESULTS AND DISCUSSION

Separation of malto- and isomalto-dextrins.—We report here the development of relatively fast solvent systems for the separation of malto- and isomalto-dextrins of dp 1–20 on silica-gel TLC plates. The systems consist of five solvents (A–E) composed of methyl cyanide–ethyl acetate–1-propanol–water in the volume proportions of 85:20:50:X. The water component, X, is varied from 50–100 parts in increments of 10. The maltodextrins are separated when X is 50, 60, and 70 parts, using one, two, three, and four ascents, depending on the number of maltodextrins to be separated and quantitated (see Figs. 1 and 2). Branched maltodextrins are separated from the linear maltodextrins by using three ascents of the solvent with X = 50 (see Fig. 3). The isomaltodextrins are separated when X is 90 or 100 parts (see Figs. 4 and 5). Branched isomaltodextrins are separated from the linear isomaltodextrins by using four ascents of the solvent with X = 70.

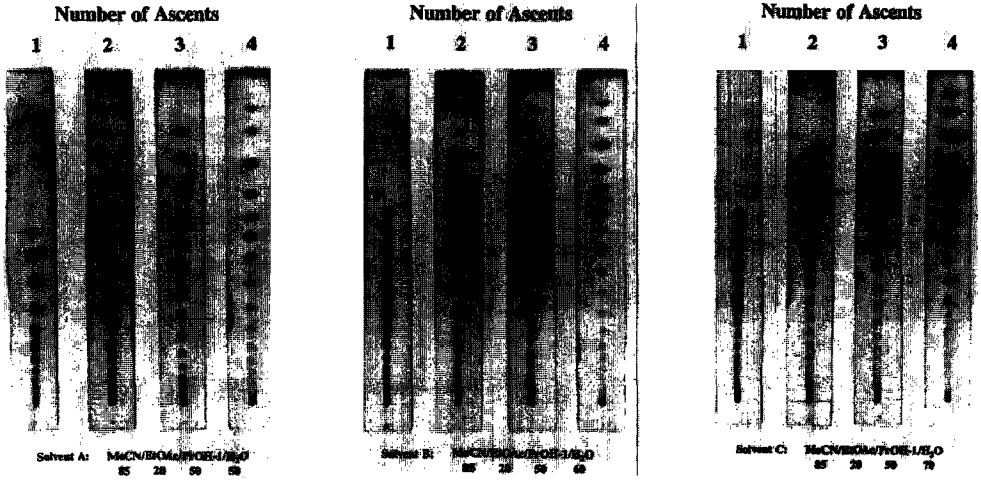


Fig. 1. TLC separation of D-glucose and a homologous series of maltodextrins using 1, 2, 3, and 4 ascents with 3 solvent systems: methyl cyanide–ethyl acetate–propanol–water. Solvent A, 85:20:50:50; solvent B, 85:20:50:60; and solvent C, 85:20:50:70 parts by volume.

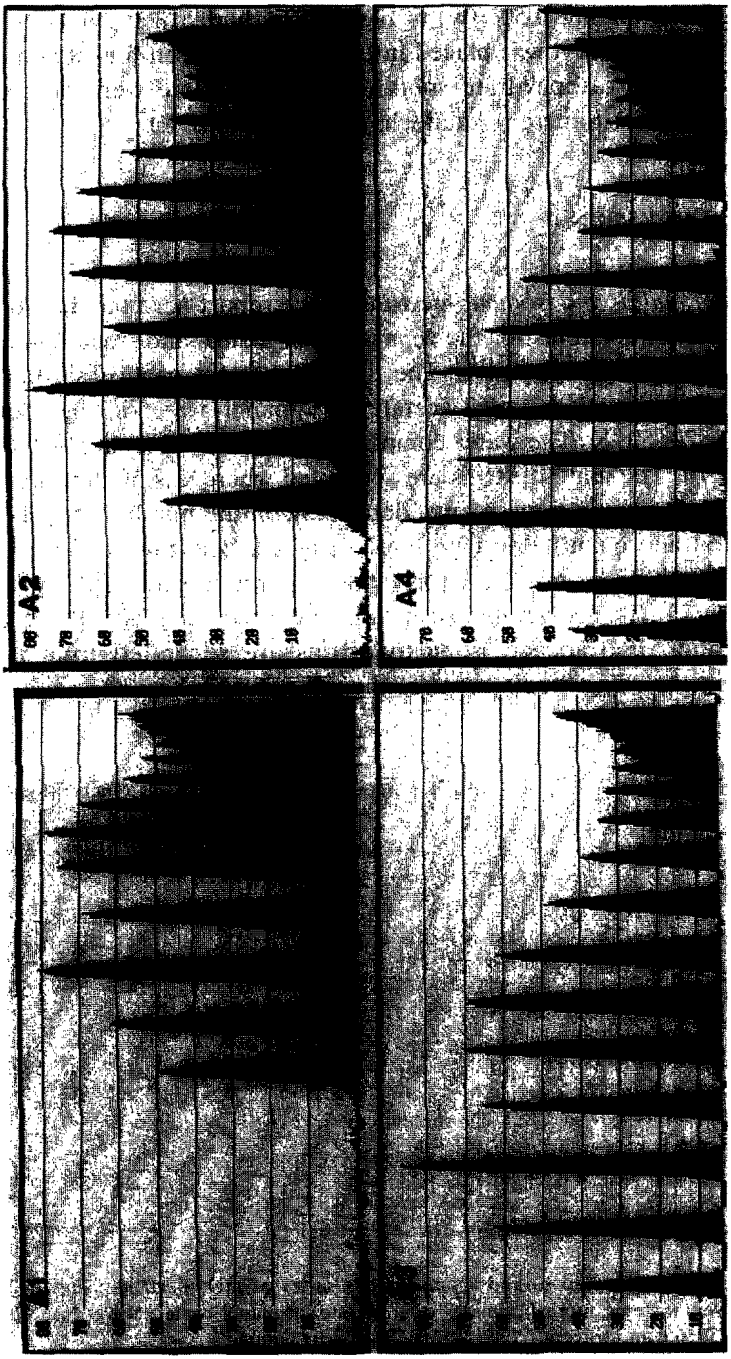
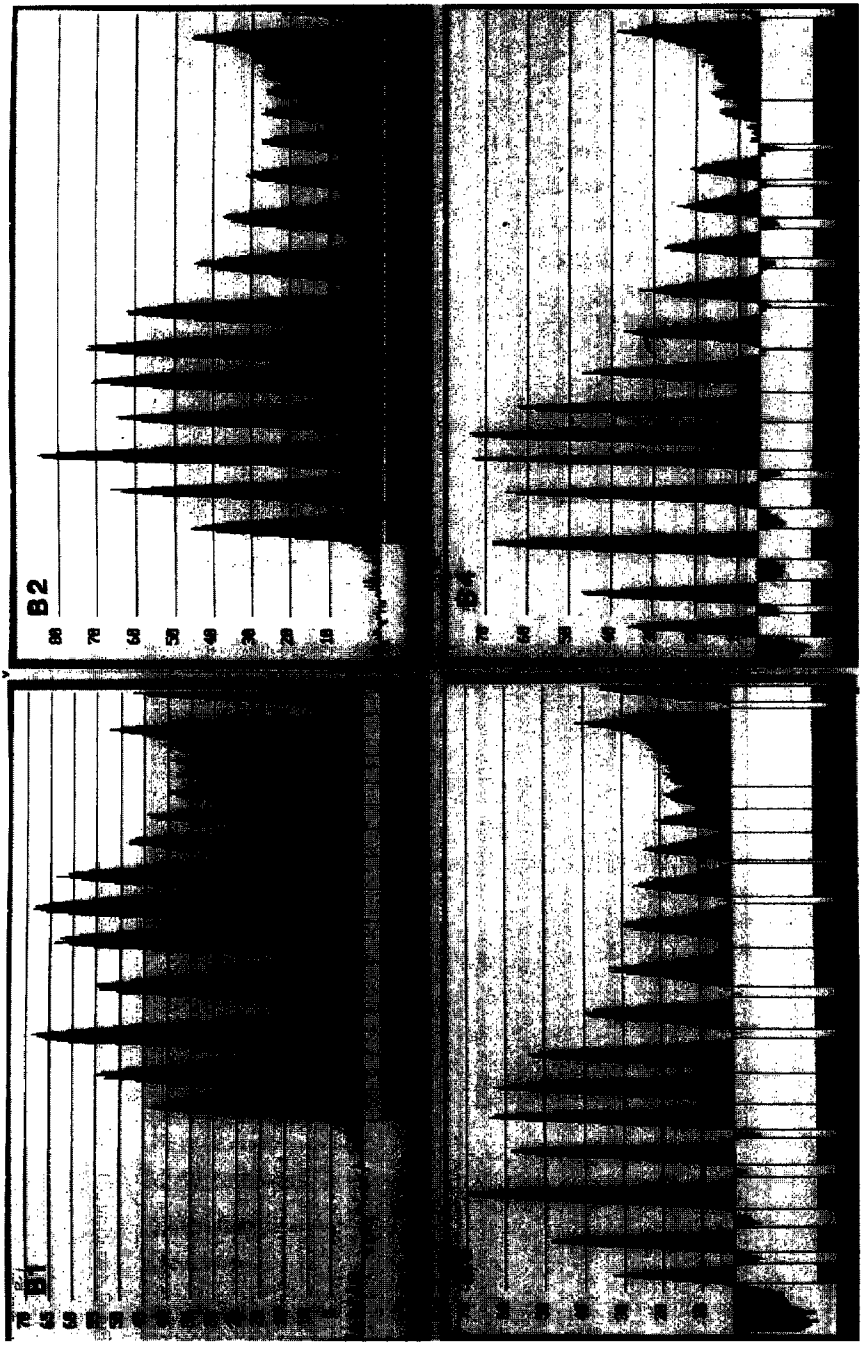


Fig. 2. Uniscan densitometric tracings of the chromatograms in Fig. 1. A: 1, 2, 3, and 4 ascents of solvent A; B: 1, 2, 3, and 4 ascents of solvent B; C: 1, 2, 3, and 4 ascents of solvent C.



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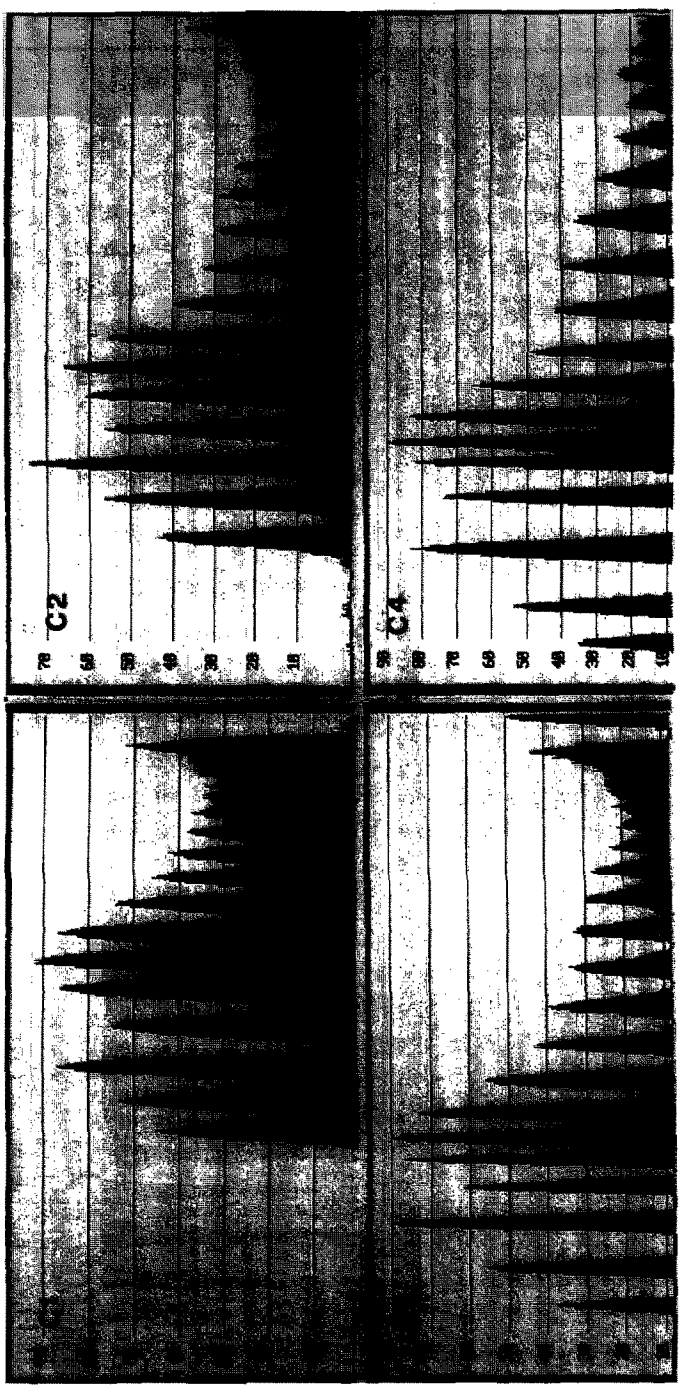

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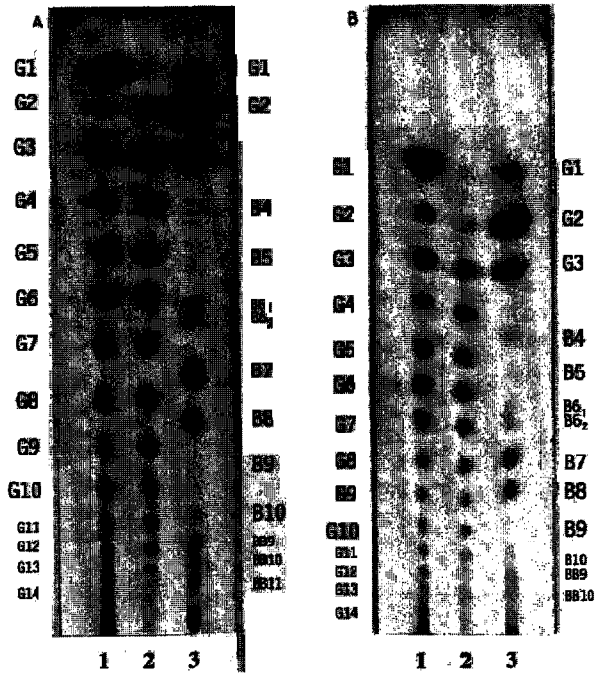

Fig. 3. TLC separation of maltodextrin series (lane 1), and the products from isoamylase hydrolysis of shellfish glycogen (lane 2) and porcine pancreatic alpha amylase hydrolysis of potato amylopectin (lane 3) using 4 ascents of solvent A, 85:20:50:50 methyl cyanide–ethyl acetate–1-propanol–water; A is a K5 plate and B is a Diamond K6 plate. G*n* represents maltodextrins containing *n* glucose residues; B*n* represents branched maltodextrins containing *n* glucose residues and one α-(1 → 6)-branch linkage; BB*n* represents multiply branched maltodextrins containing *n* glucose residues and two or more α-(1 → 6)-branch linkages.

When the water content was increased, a greater mobility and a greater number of dextrins were separated. A higher water content is needed to separate the isomaltodextrins as dextrins containing α-(1 → 6) linkages migrate more slowly than dextrins containing α-(1 → 3) or α-(1 → 4) linkages. Solvents D and E separate the α-(1 → 4)-linked maltodextrins, but with mobilities so high and migrations so close that resolution was inadequate for quantitative analyses. Although the number of dextrins that could be separated was increased when the amount of water was increased, the time per ascent also increased. The solvent with X = 50 took 60 min; the solvent with X = 60, 75 min; the solvent with X = 70, 100 min; and the solvents with X = 90 and 100 each took 120 min. The solvents give round, distinct spots with no distortions (see Figs. 1 and 4). Other solvent systems for separating malto- and isomalto-dextrins have been reported[18] (e.g., 5:5:4 1-butanol–ethanol–water, for maltodextrins and 5:5:6 for isomaltodextrins), but the times for one ascent are prohibitively long, taking 11 and 13 h, respectively.

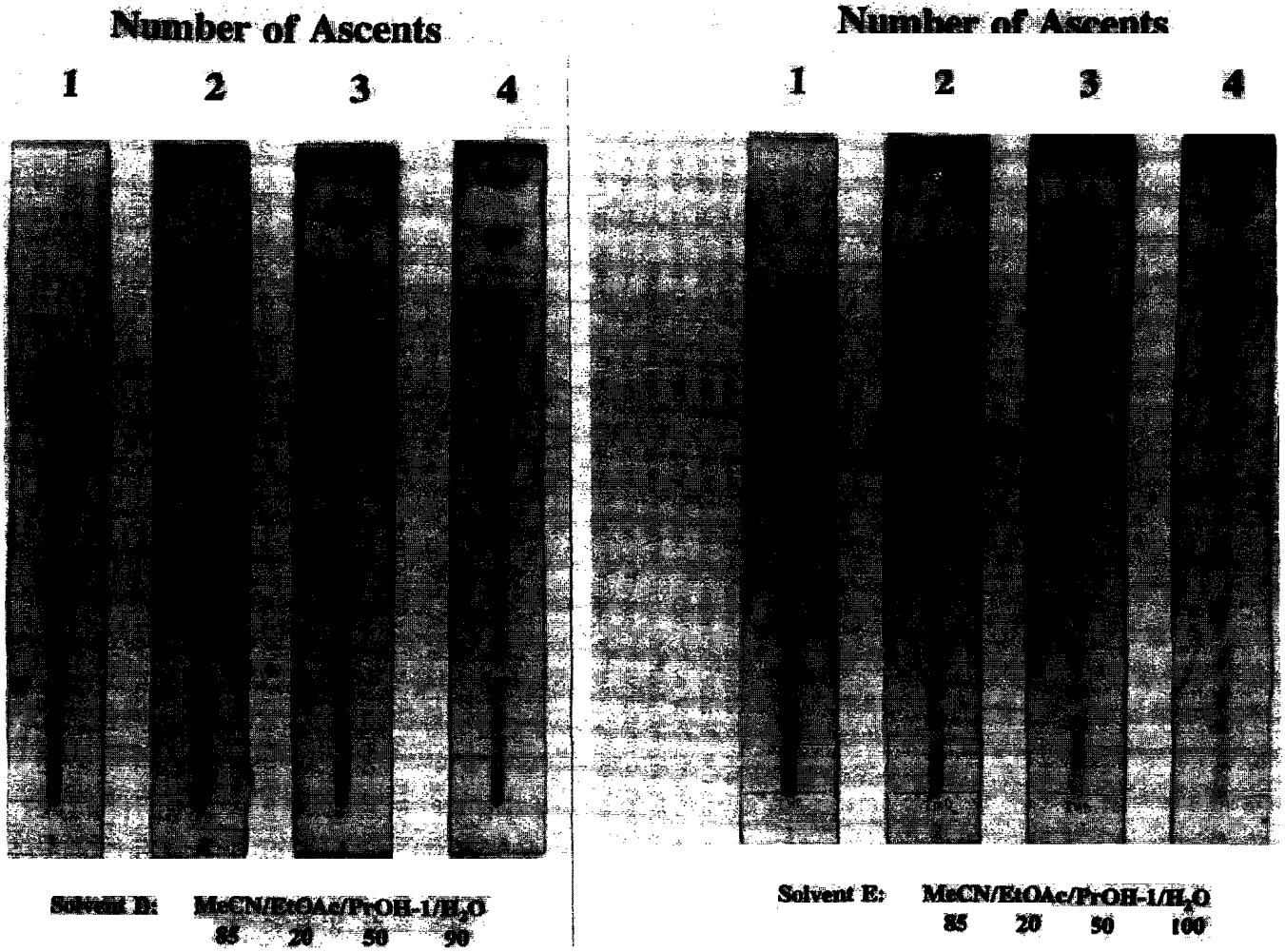

Fig. 4. TLC separation of D-glucose and a homologous series of isomaltodextrins using 1, 2, 3, and 4 ascents with 2 solvent systems: methyl cyanide–ethyl acetate–propanol–water. Solvent D, 85:20:50:90 and solvent E, 85:20:50:100.

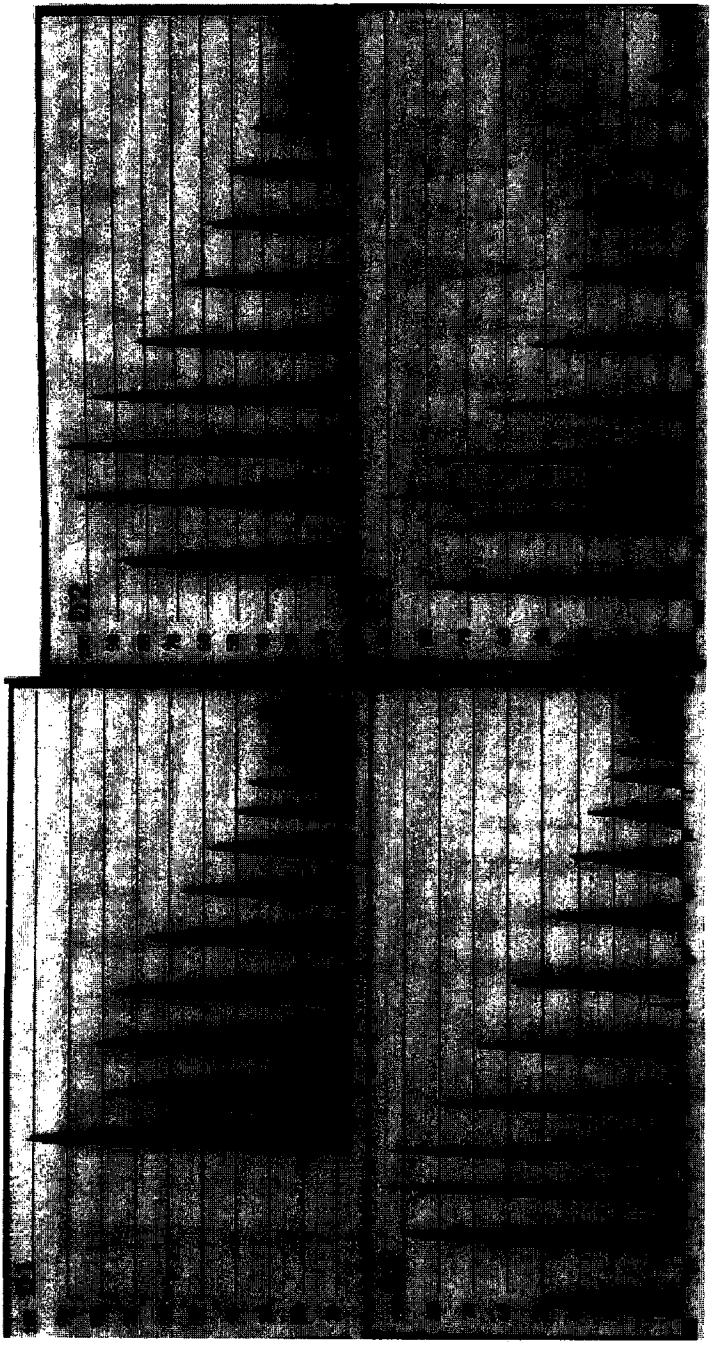

Fig. 5. Uniscan densitometric tracings of the chromatograms in Fig. 4. A: 1, 2, 3, and 4 ascents of solvent D; B: 1, 2, 3, and 4 ascents of solvent E.

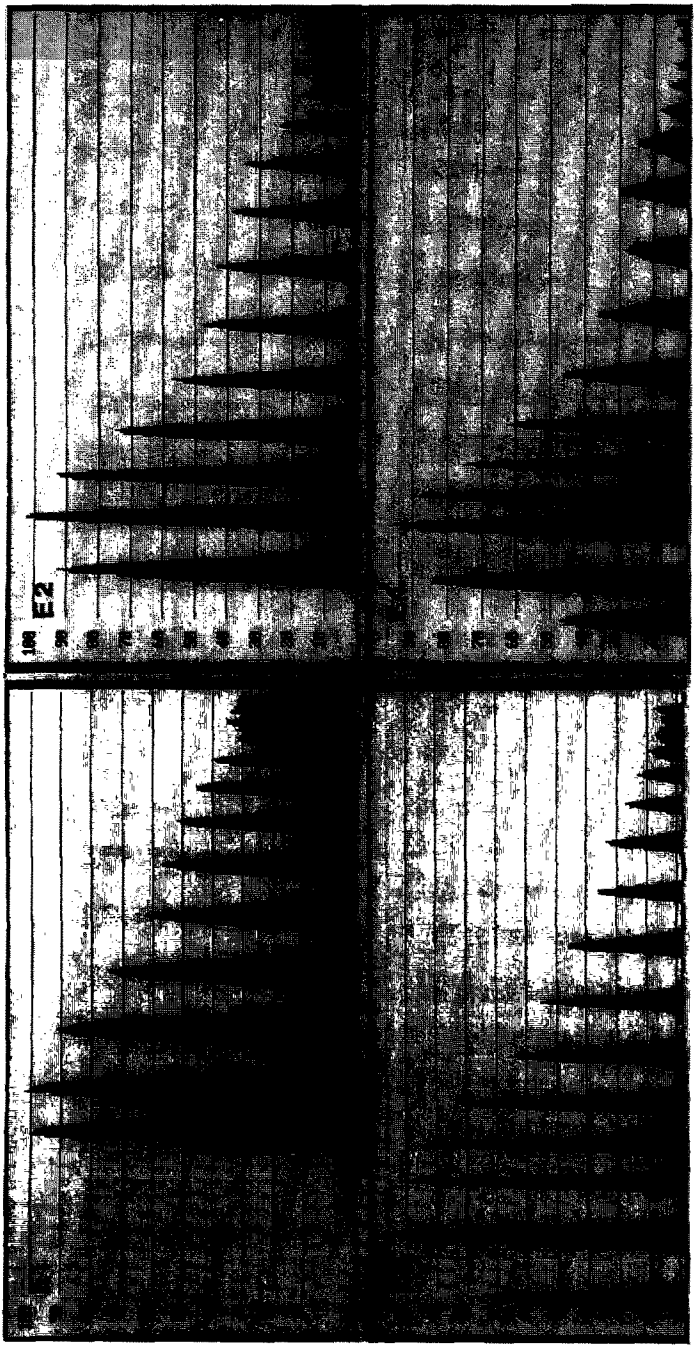

Fig. 5. (continued)

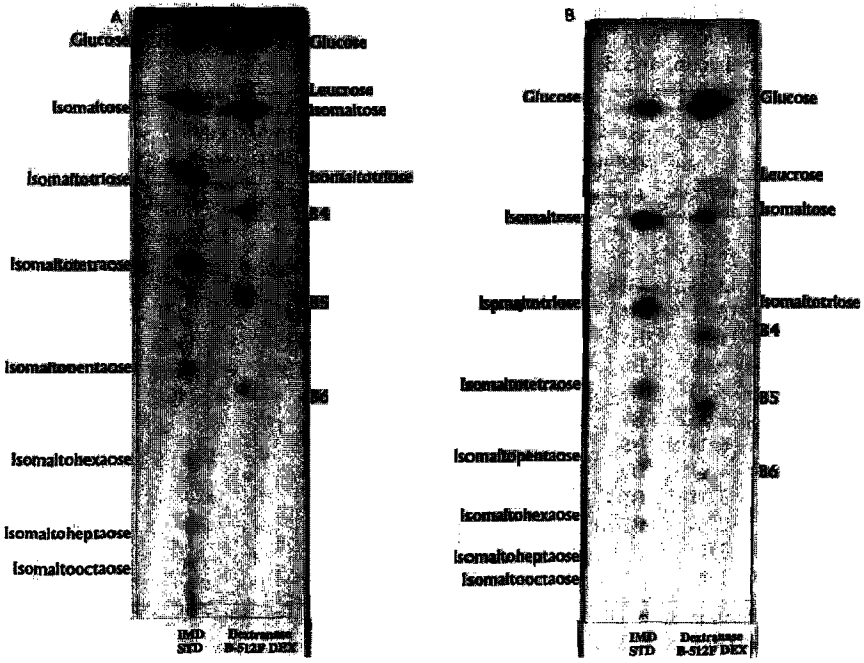

Fig. 6. TLC separation of the products from *P. funiculosum* dextranase hydrolysis of B-512F dextran using four ascents of solvent C, 85:20:50:70 methyl cyanide–ethyl acetate–1-propanol–water, A is a K5 plate and B is a Diamond K6 plate.

The number of ascents also gave an increase in the number of dextrins that could be separated and resolved. For a qualitative analysis of the dextrins, one or two ascents gave sufficient separation of dextrins of dp 1–15 (see Figs. 1 and 4). For quantitative analysis, three or four ascents gave the best resolution so that a quantitative densitometric analysis of dp 1–15 could be achieved (see Figs. 2 and 5).

A maltodextrin containing a single α-(1 → 6) linkage migrates more slowly than an equivalent dextrin containing only α-(1 → 4) linkages. Likewise, the migration of an isomaltodextrin containing a single α-(1 → 3) or α-(1 → 4) linkage, is faster than an equivalent dextrin containing only α-(1 → 6) linkages (see Figs. 3 and 6). Thus, the singly α-(1 → 6) branched maltodextrins, resulting from the alpha amylase hydrolysis of amylopectin (B4, B5, B6, and B7)[19], are resolved from the linear, nonbranched maltodextrins. Further, the two branched hexasaccharides ($B6_1$ and $B6_2$) were separated, although not completely resolved (see Fig. 3).

Fig. 6 shows the separation of the products from the hydrolysis of *L. mesenteroides* B-512F dextran by *P. funiculosum* dextranase. Seven products are formed, namely, D-glucose, leucrose, isomaltose, isomaltotriose, and three branched dextrins (B4, B5, and B6). The branched dextrins contain primarily α-(1 → 6) linkages with a single α-(1 → 3) branch linkage[20]. Densitometry gives the relative quantita-

TABLE I

Relative amounts of products from isoamylase debranching of shellfish glycogen, alpha amylase hydrolysis of potato amylopectin, and dextranase hydrolysis of B-512F dextran

Isoamylase hydrolysis of shellfish glycogen			Porcine pancreatic amylase hydrolysis of potato amylopectin			Dextranase hydrolysis of B-512F dextran		
Compd [a]	Density	Wt%	Compd	Density	Wt%	Compd	Density	Wt%
G1	321	3.29	G1	1181	9.52	G1	2635	55.13
G2	1180	12.10	G2	2759	22.24	Leu [b]	300	6.28
G3	1275	13.08	G3	2067	16.66	IM2	762	15.94
G4	1278	13.11	B4	455	3.67	IM3	74	1.55
G5	1108	11.36	B5	387	3.12	B4	436	9.12
G6	954	9.78	$B6_1$	572	4.61	B5	427	8.93
G7	800	8.20	$B6_2$	786	6.34	B6	146	3.05
G8	786	8.06	B7	1387	11.18			
G9	593	6.08	B8	1066	8.58			
G10	443	4.54	B9	222	1.79			
G11	296	3.04	BB9	321	2.59			
G12	271	2.78	BB10	1204	9.70			
G13	301	3.09						
G14	145	1.49						

[a] Gn is a maltodextrin containing *n* glucose residues; B*n* is a branched dextrin containing *n* glucose residues with an α-(1 → 6)-branch linkage for the dextrins from amylopectin and an α-(1 → 3)-branch linkage for the dextrins from dextran; BB*n* represents a multiply α-(1 → 6) branched dextrin of *n* glucose residues. [b] Leu is leucrose, α-D-glucopyranosyl-(1 → 5)-D-fructopyranose.

tive amounts of the products from the dextranase reaction (see Table I). From these data, it may be shown that the particular sample of B-512F dextran is 4.6% branched, agreeing well with the known degree of branching (4.5–5%) of B-512F dextran[21].

Types of TLC plates.—We have found that "ordinary" Whatman K5, K6, and Diamond K6 silica-gel plates gave the best separation and resolution of the malto- and isomalto-dextrins. We did not observe any differences in the separation, resolution, and ascent time for these three types of plates, although the Diamond K6 plates did give somewhat lower mobilities.

Detection method and quantitative analysis.—A new visualization reagent is reported that detects carbohydrates in the nanogram range down to 25 ng. This system is one thousand times more sensitive than the common sulfuric acid charring method. It is based on the reaction of phenols with carbohydrates in the presence of sulfuric acid, and is similar to the phenol–sulfuric acid method of quantitatively determining carbohydrates in solution[22], but uses α-naphthol instead of phenol. It is a modification of the time-honored qualitative Molisch test[23] for carbohydrates. Carbohydrates give brown spots on a white background on K5 and K6 plates and blue-black spots on Diamond K6 plates.

The reagent contains relatively low concentrations (5%) of sulfuric acid and involves the dipping of the TLC plate into the reagent. This provides a uniform

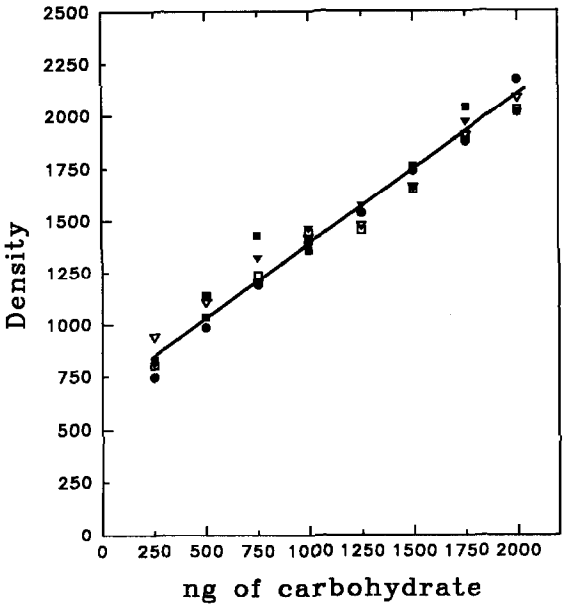

Fig. 7. TLC densitometric analysis of 250–2000 ng of D-glucose (●), maltose (■), maltotriose (▼), maltotetraose (▽), and maltohexaose (□).

application of the reagent to the plate and eliminates the disagreeable and unhealthy environment of spraying high concentrations (20–50%) of sulfuric acid that is commonly used in the sulfuric acid-charring procedure.

Densitometric analysis shows that 250–2000 ng amounts of D-glucose, maltose, maltotriose, maltotetraose, and maltohexaose gave, within experimental error, an identical linear response that was independent of the structure and dependent on the amount of carbohydrate (see Fig. 7). Likewise, densitometric analysis of D-glucose, isomaltose, isomaltotetraose, isomaltopentaose, and isomaltohexaose also gave a linear response that was dependent on the amount and identical for D-glucose and each dextrin, within experimental error (see Fig. 8). These data show that equal amounts of D-glucose, the maltodextrins, and the isomaltodextrins give the same amount of intensity or density on the TLC plate on a weight basis and that the density is linearly proportional to the weight amount between 250–2000 ng. Thus, D-glucose or maltose can equally serve as standards for determining the amount of any one of the malto- or isomalto-dextrins. This linear and structurally independent response of the method allows the determination of the relative weight percent of the individual dextrins in a mixture by dividing the individual densities by the sum of the densities, without the use of an absolute standard. The use of relative weight percent is important as it reduces the amount of experimental error in the analysis by eliminating the necessity of putting exactly equal amounts of different dextrin samples onto the TLC plates. This is especially

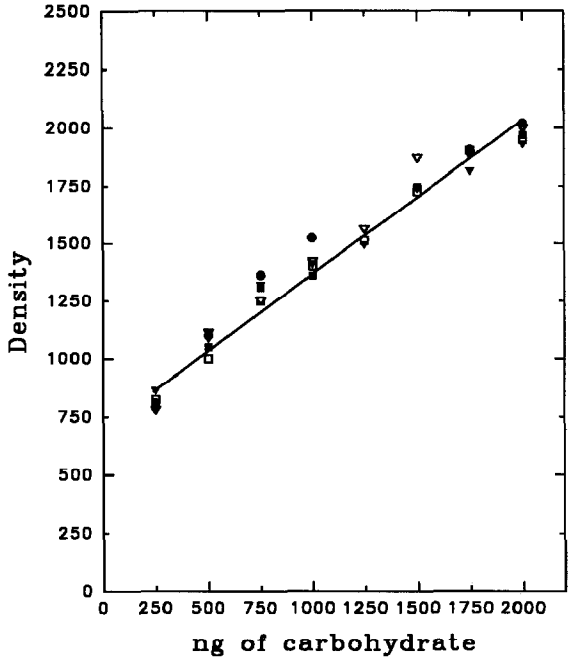

Fig. 8. TLC densitometric analysis of 250–2000 ng of D-glucose (●), isomaltose (■), isomaltotetraose (▼), isomaltopentaose (▽), and isomaltohexaose (□).

important when the amounts of each dextrin in different samples are to be compared.

A typical standard curve for D-glucose, giving a linear response between 50 and 2000 ng, is shown in Fig. 9. The precision of the quantitative TLC analysis by densitometry is illustrated for the analysis of an alpha amylase (Thermamyl alpha amylase) hydrolyzate of corn starch (see Table II). The digest was taken to an average dp of 4 and the products were separated and analyzed in triplicate on three different TLC plates to give average values with standard deviations.

TLC compared with HPLC.—Fifteen to twenty dextrins in a mixture can be separated and quantitatively determined by TLC from an aliquot of 1–3 μL. The TLC detection sensitivity is in the nanogram range and is several orders of magnitude more sensitive than the refractive index method of detecting carbohydrates in HPLC. Further, the detection method is superior to the HPLC pulsed amperometric detection (PAD) method in which there is a variation in the response that is dependent on the dp of the malto- and isomalto-dextrins[24–26], requiring a set of pure dextrin standards for each dextrin in the mixture. This variation in the PAD response, although apparently proportional to the number of HCOH groups in the dextrin, decreases for equal weight amounts as the dp of the dextrins increase[25,26]. This does not permit the determination of the relative weight percent of the dextrins in the sample. The TLC method is quite inexpensive when

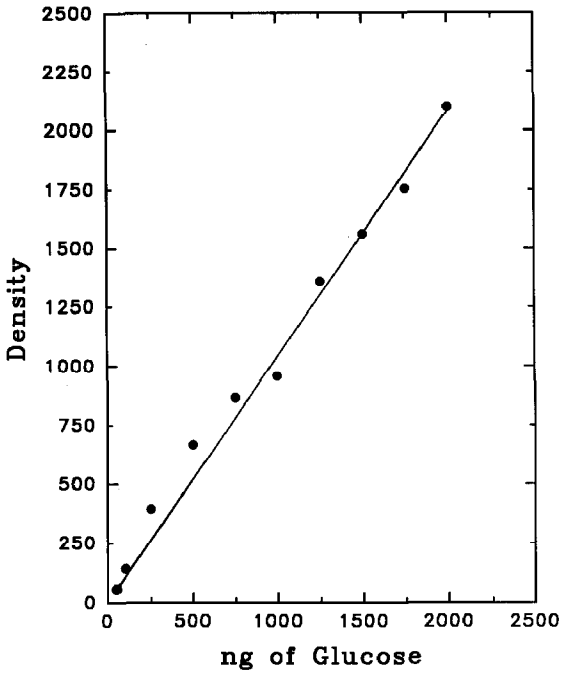

Fig. 9. TLC densitometric standard curve for 50–2000 ng D-glucose.

TABLE II

Precision of TLC densitometric analysis illustrated by a triplicate analysis of the products from an alpha amylase hydrolysis of corn starch

Compound	Wt%	SD [a]
G1	1.96	0.09
G2	8.36	0.25
G3	12.27	0.43
G4	8.88	0.57
G5	11.09	1.18
G6	13.77	0.84
G7	11.94	0.08
G8	7.84	0.54
G9	5.51	0.60
G10	4.95	0.60
G11	4.06	0.68
G12	3.12	0.42
G13	2.72	0.63
G14	1.98	0.55
G15	1.55	0.51

[a] SD, standard deviation.

compared to HPLC, as it does not require the high cost of complicated high pressure equipment and is free of the frustration of frequent episodes of down-time. Sample preparation and application is much simplified in that TLC does not require the elaborate sample filtration and the use of ultrapure solvents that HPLC requires. The TLC method is as fast as or faster than HPLC, even when using multiple ascents, in that 10–12 samples can be analyzed per 20 × 20 cm plate and several plates can be run simultaneously, whereas HPLC can only analyze one sample at a time. Further, samples and standards can be analyzed on the same TLC plate, giving more controlled conditions than can be obtained by the serial analyses required by HPLC. TLC uses a fresh new adsorbent layer for each sample, thus, ensuring reproducible results that are difficult by HPLC due to adsorbent contamination from previous analyses and worn and altered column adsorbent.

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