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Note

Thin-layer chromatography of isosorbide dinitrate, nitroglycerin and their degradation products

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A number of thin-layer chromatographic (TLC) procedures have been reported for the separation and identification of the organic nitrate ester class of medicinal agents and some of their degradation compounds. Representative among the parent compounds are nitroglycerin[1,2], isosorbide dinitrate[3–5] and pentaerythritol tetranitrate[6,7]. These agents have found widespread use over the years as coronary vasodilators in the treatment of angina pectoris and differ primarily in length of pharmacological activity. The denitro degradation species of these compounds obtained by chemical means have been shown to be similar to the metabolic conversion products in man and animals. The above methods commonly employ silica gel adsorbent and visualization with a spray reagent such as 1–5% diphenylamine in ethanol followed by exposure to UV light.

This communication describes an improved and versatile TLC procedure which utilizes a sensitive metaperiodate–permanganate chromogenic spray reagent for use in the identification of several organic nitrate esters of glycerine and isosorbide and their associated degradation products (or metabolites). The procedure is also useful for the isolation of other related organic nitrate esters including erythrityl tetranitrate, pentaerythritol tetranitrate and mannitol hexanitrate.

EXPERIMENTAL

Materials

All solvents and reagent chemicals were analytical grade and used without further purification. Isosorbide USP Reference Standard was obtained from the U.S. Pharmacopoeial Convention (Rockville, MD, U.S.A.). Isosorbide dinitrate, nitroglycerin and pentaerythritol tetranitrate were supplied by ICI Americas (Wilmington, DE, U.S.A.). Mannitol hexanitrate was furnished by Atlas Powder (Tamaqua, PA, U.S.A.) and erythrityl tetranitrate from Burroughs Wellcome (Research Triangle Park, NC, U.S.A.). Standard solutions of each reference compound were prepared fresh at a concentration of 1 mg/ml in anhydrous diethyl ether. Analtech silica gel G (plate A) and E. Merck silica gel 60 (plate B) pre-coated plates, 250-μm thick and measuring 20 cm × 20 cm were used. The plates were activated at 110°C for 30 min, stored in a desiccator and prior to use, 5 mm of adsorbent was removed from the bottom and sides.

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Thin-layer chromatography

The standard solutions were spotted on plates A and B using capillary micropipettes (1–5 μl) at a distance of 3 cm from the bottom edge of the adsorbent layer at 1-cm intervals. The plate was placed in a metal tank (9 cm × 23 cm × 23 cm) lined with Whatman No. 3MM chromatographic paper and equilibrated by saturation with freshly prepared mobile phase about 30 min before development. Chromatographic development was accomplished using the uni-dimensional ascending technique to a height of 15 cm (about 35–40 min) under ambient conditions employing toluene–ethyl acetate (1:1) as the mobile phase. The plates were air-dried following development and the spots located by applying the chromogenic reagent (0.1% sodium metaperiodate and 0.5% potassium permanganate in a 4% solution of sodium hydroxide) thoroughly and evenly to wet the support. The plates were allowed to air-dry for about 5 min and then placed in a forced-draft oven at 100°C for 1–5 min. The optimal heating time produced distinct yellow spots against a violet background. Pentaerythritol tetranitrate, which is initially observed as a green spot on a violet background on the wet plate, failed to exhibit distinct yellow spots after the drying and heating steps at a volume of up to 25 μl.

RESULTS AND DISCUSSION

The retention data for the twelve compounds included in this study and shown in Table I represent the mean of six values obtained by two participants on three different days. R_F values relative to the migration of nitroglycerin are also included

TABLE I

CHROMATOGRAPHIC RETENTION DATA ON SILICA GEL

Mobile phase: toluene-ethyl acetate (1:1). Plate A: Analtech silica gel G; plate B: E. Merck silica gel 60.

Compound	*Plate A*			*Plate B*		
	R_F	Rel. R_F*	MDQ (μg)**	R_F	Rel. R_F	MDQ (μg)
Isosorbide dinitrate and related compounds						
Isosorbide dinitrate	0.66	0.94	5	0.56	0.93	5
Isosorbide	0.05	0.07	0.5	0.02	0.03	0.5
5-Isosorbide mononitrate	0.22	0.31	0.5	0.14	0.23	0.5
2-Isosorbide mononitrate	0.28	0.40	0.5	0.19	0.32	0.5
Nitroglycerin and related compounds						
Nitroglycerin	0.70	1.00	2	0.60	1.00	2
1-Mononitroglycerin	0.16	0.23	0.5	0.08	0.13	0.5
2-Mononitroglycerin	0.17	0.24	0.5	0.10	0.17	0.5
1,2-Dinitroglycerin	0.47	0.67	0.5	0.36	0.60	0.5
1,3-Dinitroglycerin	0.62	0.89	0.5	0.50	0.83	0.5
Erythrityl tetranitrate	0.73	1.04	2	0.65	1.08	2
Pentaerythritol tetranitrate	0.74	1.06	25	0.67	1.12	25
Mannitol Hexanitrate	0.75	1.07	4	0.68	1.13	4

* Relative to nitroglycerin.

** Minimum detectable quantity.

in this compilation. The volume of the standard solutions spotted ranged from 5 to 40 μl for this retention study which was performed with two brands of pre-coated plates. The minimum detectable quantity (MDQ) was also estimated by spotting 0.5–5 μl volumes of each of the standard solutions with the exception of pentaerythritol tetranitrate. This compound was observed to be relatively insensitive to the mixed spray reagent after the heat treatment step. Consequently, visualization was attained via the intense green spot on the wet plate. This type of mixed spray reagent containing 2% sodium metaperiodate and 1% potassium permanganate at a pH of 7.2 was originally employed with a paper chromatographic method for carbohydrates[8]. The reagent was later reported for use with the TLC identification of pentaerythritol[6]. In general, we have found the mixed spray reagent under strongly basic conditions to be superior in sensitivity to the diphenylamine spray–UV approach for detection.

Plate A readily adsorbs the spray reagent and yields a high contrast permanganate background color relative to the yellow spots of interest. The plates can be heated 2–5 min at 100°C without significant loss of the violet background and will remain color stable for up to 24 h under ambient conditions. Excessive heating results in loss of the permanganate background color providing darker yellow spots on a yellow background. The more impervious coating of plate B resists spray reagent adsorption which causes background color dissipation with heating at 100°C over 2 min. The violet color also fades considerably within 1 h under ambient conditions. Plate B was observed to be slightly more retentive for all compounds.

Preliminary experiments employing acetone as a solvent indicated the presence of an artifact spot having an R_F value in the region of the 2- and 5-mononitrate esters of isosorbide. We have tentatively identified this extraneous spot as 4-hydroxy-4-methyl-2-pentanone (diacetone alcohol), an aldol condensation product formed during the manufacturing process of acetone[9]. The use of this solvent was therefore discontinued in favor of anhydrous diethyl ether.

Mannitol hexanitrate was observed to exhibit a faint secondary spot having an R_F value of 0.70 and 0.63 on plates A and B, respectively, which may be due to an impurity.

In summary, the proposed TLC procedure is simple, sensitive and amenable for use with a number of organic nitrate esters of medicinal interest and their associated degradation products. The retention data presented is indicative of the versatility of the procedure which has recently been applied in our laboratory as a rapid means of assessing the purity of both diluted bulk preparations and dosage forms containing isosorbide dinitrate. Repeated determinations carried out with both types of plates in this study indicate the retention values that are reproducible ($\pm$ 0.02 R_F units). The utility of the procedure might be extended to other formulations containing nitroglycerin, erythrityl tetranitrate, pentaerythritol tetranitrate and mannitol hexanitrate and the related degradation species of these compounds.

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