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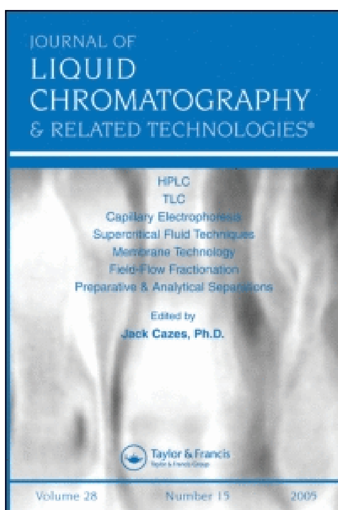

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THE IMPORTANCE OF EFFECTIVE PREDEVELOPMENT PLATE WASHING TECHNIQUES IN THIN-LAYER CHROMATOGRAPHY

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ABSTRACT

Comparative studies were made of the two methods commonly used for predevelopment TLC plate cleaning. In separate studies TLC plates were washed by the two methods: ascending development and dip washing using several solvent and solvent combinations. Residues remaining on the surface of plates after washing were visualized by fluorescence techniques and scanned by densitometry. Evaluation of the two washing methods showed that plate dipping gave superior results in every case over ascending development. The most effective solvent for predevelopment TLC plate washing was found to be methanol.

INTRODUCTION

Many aspects of thin-layer chromatography have been studied in great detail in attempts to improve the performance and reliability of this technique. However, one important parameter that invariably influences chromatographic results, thin-layer plate cleaning, has not been system-

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atically addressed. There are many techniques in the literature for plate cleaning, and investigators use many mixtures of developing solvents as well as solvents specifically prepared for this purpose (1-4). Further complicating the choice of plate cleaning methods is the problem of deciding whether ascending development or immersion in a solvent bath (plate dipping) yields a cleaner surface. In the course of a study on the use of the vapor-phase fluorescence (VPF) for TLC visualization (5) with certain antibiotic classes, we found that conventional ascending development cleaning methods left sufficient material on the plates so as to interfere with the detection of compounds migrating at or near the solvent front. A study was therefore initiated to develop an efficient method for TLC plate cleaning for use when fluorescent detection techniques are employed. Both ascending development and plate dipping methods of cleaning were studied with several single and binary solvent systems. Plate dipping was found to be superior to ascending development for removing surface residue. A dipping protocol was developed, which yielded a uniformly clean adsorbent surface and eliminated background contaminants revealed when scanning with fluorescence densitometry. The method, although developed for fluorescence detection, has proven to be applicable to all of our plate cleaning needs, regardless of the type of visualization employed.

MATERIALS AND METHODS

HPTLC silica gel 60 plates were from three sources: Analtech HPTLC-GHL 56527 (Newark, DE); Merck silica gel G 5633 (Cherry Hill, NJ) and Whatman HP-K 4807-400 (Clifton, NJ). All solvents were HPLC grade from Burdick & Jackson (Muskegan, MI). Both plate dipping and

ascending development experiments were carried out in CAMAG Twin-trough tanks (Wrightsville Beach, NC). Induced vapor phase fluorescence (VPF) by the method of Segura and Gotto (5) was performed in a heating device developed in this laboratory (6). Backround fluorescence was measured by densitometry using a CAMAG TLC Scanner II (Wrightsville Beach, NC) with a 366 nm excitation wavelength (mercury lamp), 400 nm emission cut-off filter, 0.2 x 3 mm slit, and a 0.5 mm/sec scan speed.

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Fluorescence Backround Visualization Techniques

TLC plates were scanned by fluorescence densitometry after each of the following steps in this sequence: as received from the supplier (Step A); after dipping in the solvent tank (Step B); after ascending development (Step C); and after heating in the VPF chamber (Step D) (6).

Enhanced induction (5) of fluorescent background residue (step D of above sequence) was carried out in the VPF heating chamber as follows: approximately 2 g of ammonium bicarbonate was placed in the heating chamber (6), the chamber was sealed with a glass cover and clamp, and the unit was placed in a forced air oven set at 130°C for 90 min. At the end of this period, the heating chamber was cooled, and the plates were then removed and scanned by densitometry.

Plate Cleaning Techniques

Plate dipping (Step B in above sequence). Two CAMAG Twin-through tanks (labeled as 1 and 2) were each filled to a depth of 10 cm with the same cleaning solvent used in each experiment. Each plate was

first immersed in tank No. 1 for 5 min, removed from the tank and air dried. At this point the plate was immersed in tank No. 2 for an additional 1 min, removed from the tank, air-dried, and heated in a gravity oven at 80°C for 15 min.

Ascending one-dimensional development (Step C in above sequence). Solvent was placed in one side of a CAMAG Twin-trough tank to a height of 8 mm (approximately 5 ml). Plates were placed in the chamber and developed to a line one cm from the top of the plate. Plates were then removed from the tank, air dried for 10 min and then dried in a gravity oven at 80°C for 15 min.

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RESULTS AND DISCUSSION

Our interest in predevelopment plate cleaning methods began as a result of our experiences using the induced fluorescence visualization technique of Segura and Gotto (5) to detect the polycyclic ether antibiotics: lasalocid, monensin, narasin and salinomycin. This technique, unlike most other fluorescent indicators, induces fluorescence in a wide range of compounds having no native fluorescence. Fluorescence is induced by heating the spotted TLC plates in a sealed chamber containing ammonium bicarbonate. Moreover, it was found that this treatment also induces fluorescence in surface contaminants on TLC plates. Plates precleaned by ascending development and subjected to this treatment showed a broad band at the solvent front as detected by fluorescence densitometry. Analytes of interest that migrate at or near the solvent front were partially obscured by the overlapping fluorescence of the surface contaminants compressed into this area; a phenomenum also observed by other investigators (7).

To improve TLC plate adsorbents for fluorescence densitometry, our initial efforts involved testing various solvent systems with the ascending development precleaning method. In each series of experiments, with individual solvent systems, nine HPLTC plates (2.5 x 10 cm) were compared, three each from the following manufactures: Analtech, Merck and Whatman. Although the results from the three plate sources were similar, Merck plates had the lowest initial background fluorescence response and are represented here exclusively. In the ascending development experiments the following solvent systems were compared: ethyl acetate, ethyl acetate/ methanol (50/50), isopropanol, methanol, and chloroform/methanol (50:50). These solvents represent a wide polarity range and are indicative of some of the solvent systems that have been used for plate precleaning (1-4,7). The plates after ascending development, VPF treatment and densitometric scanning showed a large peak area in the densitogram at or near the solvent front with all the solvents tested. The largest backround residue peak area was observed with methanol (Figure I) indicating the greater solvating capacity of this solvent. From this Figure we see that the debris on the plate was not moved into a narrow band at the solvent front. Instead, the plate material is distributed over a large region in the upper 10-25% of the plate. Compounds of interest with Rf valves in this region would be difficult to detect because of the concentration of backround contamination. From these results it is apparent that ascending development is inefficient as a plate precleaning technique when fluorescence detection is used.

Plate dipping methods were investigated next using the same solvents as those employed in the ascending development experiments.

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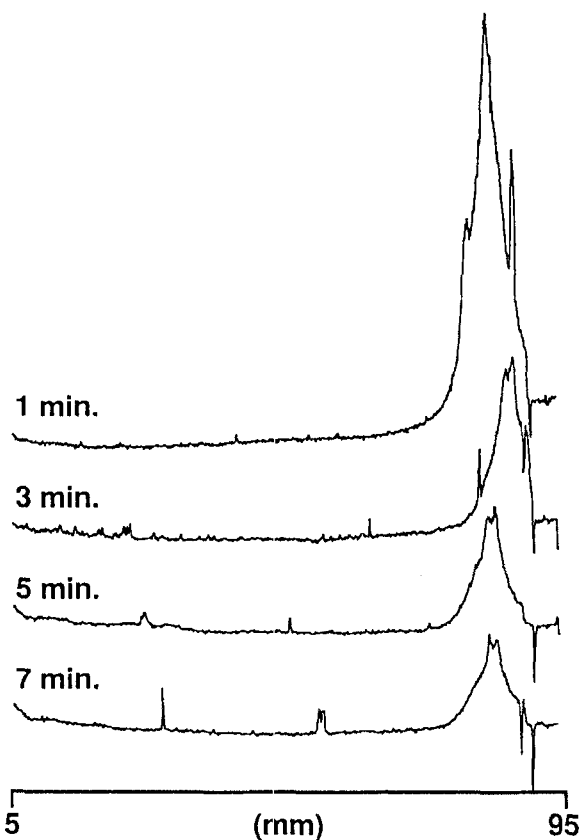

FIGURE 1. Densitogram of Merck Silica Gel 60 HPTLC plate after ascending development only in methanol and heating to 130° with NH_4HCO_3 (Step D as shown).

Because methanol appeared to be the most efficient solvent in the ascending development experiments, this solvent was used as a guide when evaluating the other solvent systems tested. In the procedure employed (Figure 2) each TLC plate was scanned by fluorescence densitometry: as it was received from the supplier (Step A), after dip

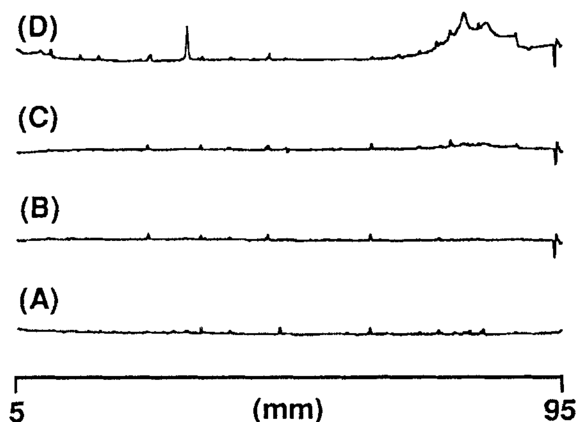

FIGURE 2. Plate cleaning sequence (isopropanol): as received (Step A); dip washed with $(CH_3)_2CHOH$ (Step B); MeOH ascending development (Step C); and 130°/NH_4HCO_3 (Step D).

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washing in the test solvent (Step B), after recleaning the plate in methanol by ascending development (Step C) and after subjecting the plate to VPF treatment to enhance any contaminants left on the plate surface after step C (Step D). Contaminants on the plates exhibit fluorescence after steps A-C. The treatment with ammonium bicarbonate (Step D) in the VPF chamber (5,6) causes further enhancement of the fluorescent backround and is a more sensitive diagnostic than (Steps A-C) for determining the extent of backround residue. Figure 2 shows the results obtained when isopropanol was used as the dipping solvent. After dipping (Step B), little background contamination was observed on the plate; however, when the plate was recleaned by ascending development (Step C) with methanol, a more polar solvent, we see that the plate had not been fully cleaned by the isopropanol dip because

backround residue is now compressed near the solvent front. Vapor phase fluorescence treatment of the plate surface (Step D) revealed even more background contamination in this area. Similar dipping experiments were carried out with the following solvent systems: Chloroform/methanol (50/50); ethyl acetate/methanol (50/50); and ethyl acetate. In each case, results were similar to those obtained with isopropanol (Figure 2); i.e., none of the dipping solvents tested fully removed all the residual material on the plates. Because none of these solvent systems completely removed background contamination, methanol next was investigated as a dipping solvent. In these experiments, methanol was the dipping solvent and also was used in the ascending development washing step to concentrate at the solvent front any debris left on the plate after dipping (Figure 3). The results with methanol were satisfying in that very little surface contamination could be detected on the plates after step (C), indicating that the plate residue was effectively removed during the dipping step B. Subsequent VPF treatment showed only slight residual background in the solvent front area (Step D). On the basis of these results we concluded that, of all solvents tested, methanol was the most effective for predevelopment plate cleaning.

Another question arises as a result of these experiments. Namely, how long should the plates be held in the dipping chamber to insure complete plate cleaning? Some investigators have reported dipping plates for as long as one hr (1) or overnight (2). To answer this question, timed experiments were conducted in which plates separately were dipped in methanol for 1, 3, 5 and 7 min respectively, using the same

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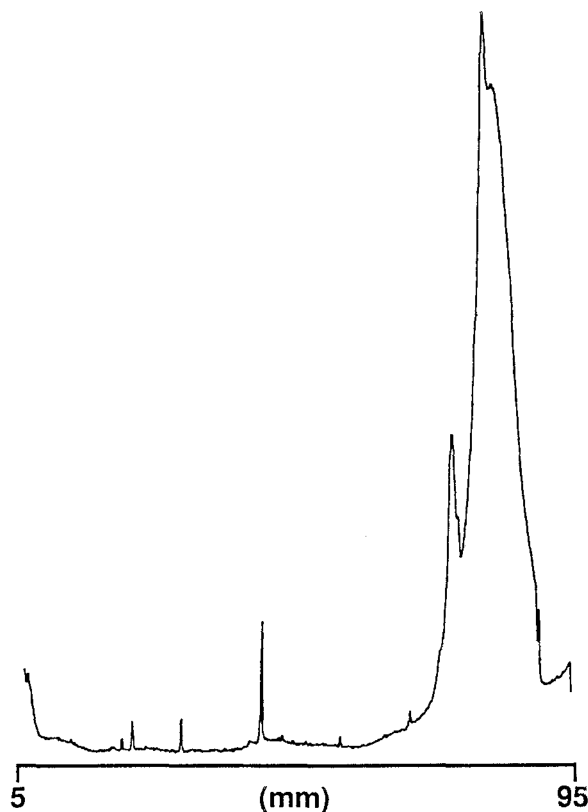

FIGURE 3. Plate cleaning sequence (methanol): as received (Step A); dip washed with MeOH (Step B); MeOH ascending development (Step C); and 130°/ NH_4HCO_3 (Step D).

step sequence (A-D) as depicted in Figure 3. An over-lay of the results only for Step D of the sequence is shown in Figure 4. From the Figure it is apparent that the 1 and 3 min dipping periods were not sufficiently long enough to fully clean the plate surfaces. The plate cleaned for 5 min showed the same residue levels as the one dipped for the 7 min interval.

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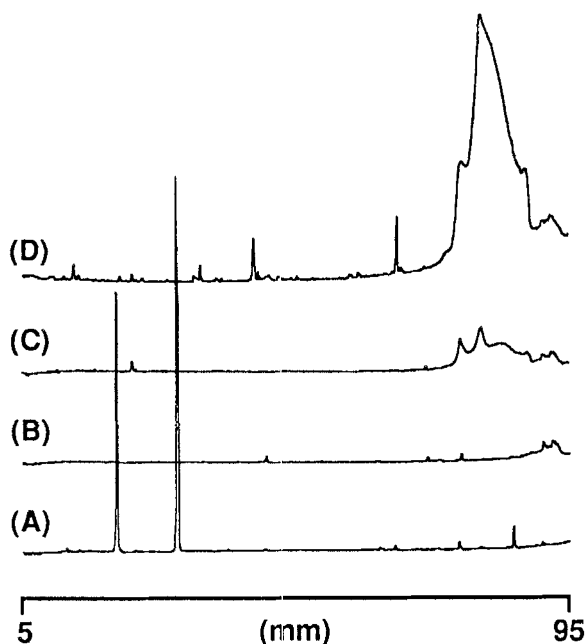

FIGURE 4. Timed dipping experiments with MeOH. Separate plates dipped in MeOH for 1, 3, 5, and 7 min, respectively (Step B), then MeOH ascending development (Step C) and finally heated (130°C) with NH_4HCO_3 (Step D as shown).

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From these results it was concluded that the 5 min immersion period in methanol was sufficient to reduce backround TLC plate residues to acceptable levels.

When large numbers of plates are to be cleaned on a regular basis it is suggested that they be processed using two methanol dipping tanks (Materials and Methods). The methanol solvent in tank 1 may be used for approximately 20 10x10 cm HPTLC plates. At that point it should be discarded. Tank 2 should then be relabeled as Tank 1 which

now becomes the primary dipping chamber. A fresh tank of methanol then should be prepared for use as Tank 2. This second tank is required only for high volume plate cleaning. Alternatively, if only single 10x10 cm plates require cleaning, they may be placed horizontally in 13½x15 cm stainless steel trays or equivalent in sufficient methanol to cover the surface, and removed after 5 min.

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