A TLC Method for Identification of Germicides in Personal Care Products[1]

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Abstract

Inherent difficulties in the common ultraviolet (UV) analytical methods for determining germicide mixtures in personal care products have made desirable that another technique, thin layer chromatography (TLC), be applied for the identification of specific components. A variety of germicides can be identified on a TLC plate using silica gel as a substrate, a benzene-ether developing solvent, and a UV light for observing the separate fractions. The R_f values obtained make it possible to distinguish between such germicidal classes as salicylanilides, carbanilides and phenolics.

Introduction

The need for a procedure capable of determining germicides in admixture, especially useful for soaps, cosmetics and other household products prompted the development of the thin layer chromatography (TLC) method described herein.

In the chemical specialties' area many methods have been used for the determination of germicides in personal care products. The many methods and procedures written for the identification and separation of germicides in personal care products have incorporated the use of extractive procedures to separate the germicide, followed by ultraviolet (UV) spectrophotometry (1–11), colorimetric analysis (12–20), gas liquid chromatography (21,22), and polarography (23) to identify the germicides. In recent years the chemical specialty area has become more sophisticated in the formulation of germicidal preparations. It has been found that a combination of germicides rather than a single germicidal system is more effective. With the advent of new products containing a combination of germicides, the analyst's job has been complicated by the fact that many of the procedures used in the past can no longer be adapted to mixed germicidal systems.

This procedure describes a separation and identification scheme capable of identifying six germicides: hexachlorophene, a bisphenol; 3,4,4′-trichlorocarbanilide (TCC) and 4,4′-dichloro-3-trifluoromethylccarbanilide (Irgasan CF_3), two carbanilides; 3,4′,5-tribromosalicylanilide (TBS), and 3,5-dibromo-3′-trifluoromethylsalicylanilide (Fluorophene), two salicylanilides; and zinc omadine, an unusual metal

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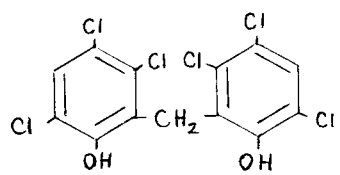
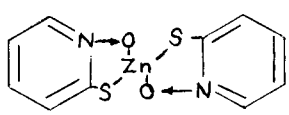
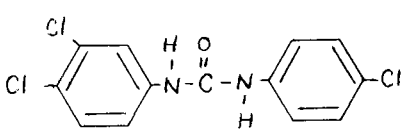


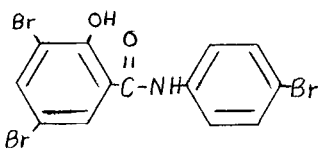
HEXACHLOROPHENE



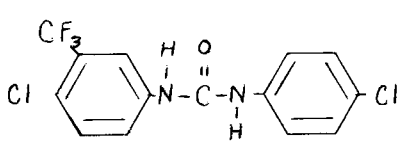
ZINC OMADINE



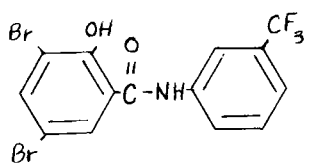
3,4,4'-TRICHLOROCARBANILIDE (TCC)



3,4',5-TRIBROMOSALICYLANILIDE (TBS)



4,4'-DICHLORO-3-TRIFLUOROMETHYLCARBANILIDE (IRGASAN CF_3)



3,5-DIBROMO-3'-TRIFLUOROMETHYLSALICYLANILIDE (FLUOROPHENE)

FIG. 1. Structures of common germicides.

TABLE I
Identification of Antibacterial Agents

Agent	R_f value	Black light	Color reagent	Short wave UV
Hexachlorophene	0.25–0.36	(−)	Red (+)	(+)
Zinc Omadine	0.26–0.32	SL. (+)	(−)	(+)
TCC	0.35–0.46	(−)	(−)	(+)
Irgasan CF_3	0.32–0.46	(−)	(−)	(+)
TBS	0.79–0.86	(+)	Orange (+)	(+)
Fluorophene	0.75–0.82	(+)	Orange (+)	(+)

containing germicide. Chemical structures of these six germicides are shown in Figure 1.

A relatively simple procedure has been developed for the identification and separation of these germicides using TLC. The method, shown schematically below, involves extraction of the germicidal system with the appropriate solvent, i.e., acetone, alcohol, or dimethylformamide, "spotting" a portion of the extract onto the TLC plate (Silica Gel F_{254} precoated, prepared by E. Merck, distributed by Brinkmann Instruments Co.) and separating the germicides using a benzene-ether (80:20) solvent mixture.

TLC Separation Scheme

1. Dry Sample
2. Solvent Extraction
3. Filter
4. Spot on TLC Plate
5. Develop with Benzene/Ether
6. Examination of Plate

The germicides are separated according to their chemical class, i.e., carbanilides, (TCC and Irgasan CF_3) and salicylanilides (TBS and Fluorophene). Zinc Omadine and hexachlorophene do not separate from each other.

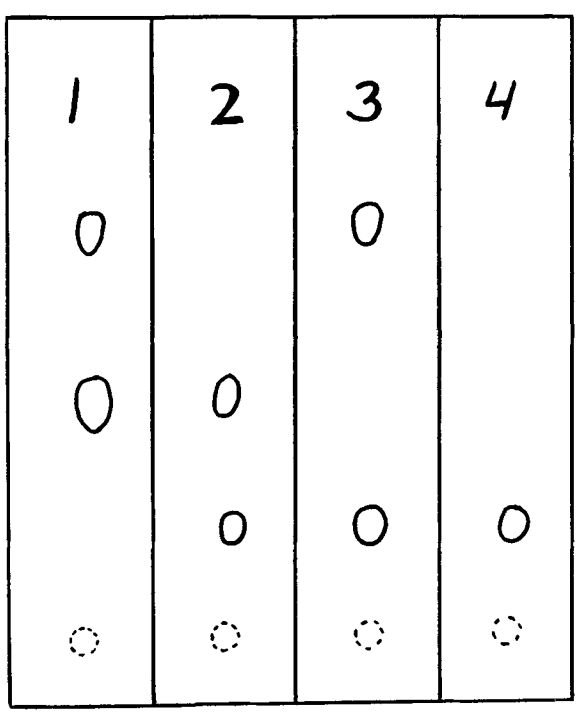


FIG. 2. Typical germicide separations: Sample 1, soap containing TCC, TBS and Irgasan CF_3. Sample 2, soap containing TCC and hexachlorophene. Sample 3, soap containing TBS and hexachlorophene. Sample 4, shampoo containing hexachlorophene.

For the analysis of germicides in soaps, the procedure is slightly different; the drying of the sample is omitted.

Experimental Procedures

A 10 g sample of soap is homogenized for 3 min in a Waring blender with 100 ml of dimethylformamide and immediately filtered through a coarse porosity filter paper into a beaker. Several drops are applied in as small a spot as possible to a Silica Gel F_{254} thin layer plate. The plates are developed with a benzene-ether (80:20) solvent system. The germicides separate from other components in the soap extract, and are located on the TLC plate using a short wave UV light (253 A). Simultaneously, solutions of the six reference germicides are spotted onto the TLC plates and developed in the same solvent mixture.

Using a short wave UV light, only three spots will be seen when a mixture of the six germicides are chromatographed as a mixture. The top spot is a mixture of TBS and Fluorophene, the middle spot is TCC and Irgasan CF_3, and the bottom spot is a mixture of Zinc Omadine and hexachlorophene.

The R_f values obtained for standards are shown in Table I. Using a short wave UV source, the location of all of the germicides can be detected on the TLC plate. Three of the germicides are detectable by long wave UV. Spraying the plate with 4-aminoantipyrine and potassium ferricyanide yielded colored complexes with three of the six germicides. This data is summarized in Table I.

What does this data mean? TBS and Fluorophene have the same R_f value, give the same orange color complex, and are detectable by long wave UV. If either of these is present in a mixture, a positive identification cannot be made without an additional differentiation step. Similarly, TCC and Irgasan CF_3 behave identically.

The data show that if there is a mixture of Zinc Omadine and hexachlorophene in a personal care product, it can be ascertained that the product contains either hexachlorophene, Zinc Omadine or a mixture of the two. Additional steps must then be taken to positively identify which one or both are present. If the thin layer plate is viewed with a long wave UV light there will be no spot visible if only hexachlorophene is present, but if Zinc Omadine is present, it can be clearly seen. If hexachlorophene is present, spraying the plate with 4-aminoantipyrine and potassium ferricyanide will yield a red color. Zinc Omadine does not yield a color.

Carbanilides and salicylanilides are shown to be present or absent.

If carbanilides are present, the spot is scraped off, extracted with acetone, filtered and evaporated to dryness. The residue is hydrolyzed in 1% KOH-ethylene glycol. The solution is extracted with ether and the ether extract is injected into a gas chromatograph. The salicylanilides are identified by scraping off the spot, extracting with acetone, evaporating to dryness and preparing the trimethyl silyl derivative. The sample is then run through a gas chromatograph to separate the TBS and Fluorophene.

Figure 2 shows a sketch of a TLC plate on which germicides extracted from four commercial personal care products were separated. The first three are soaps and the fourth is a shampoo. The first sample contains TCC, TBS and Irgasan CF_3. TCC and Irgasan CF_3 do not separate and must be positively

identified using the gas chromatography procedure. Sample 2 contains a mixture of hexachlorophene and TCC. Sample 3 contains hexachlorophene and TBS. Sample 4 contains only hexachlorophene.

Results and Discussion

The TLC method described in this report is capable of separating certain groups of germicides as chemical types. By this technique, we will not miss one germicide type when it is in combination with another type.

An additional differentiation step must be used to permit positive identification of all these specific compounds. Perhaps two-dimensional TLC, using two different solvent systems would yield complete separations. Alternately, another spray might yield colored complexes with some of the germicides. Or, as has been described, the mixture can be scraped off the plate, dissolved in a solvent and identified by gas chromatography. Additional work to complete the separation and identification of these germicides and to make the procedure quantitative appears warranted. As new germicides become commercially available it is expected that their identification can be incorporated into this procedure.

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