J. Soc. Cosmet. Chem. **25**, 535–544 (1974) © *1974 Society of Cosmetic Chemists of Great Britain*

The thin layer chromatographic detection and determination of an imidazolidinyl urea antimicrobial preservative

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Synopsis—An ANTIMICROBIAL PRESERVATIVE of the IMIDAZOLIDINYL UREA type is detectable in a variety of complex cosmetic and toiletry formulations by TLC. The method is specific for the above type of preservative, even in the presence of a number of other antimicrobials. A ten-fold increase in sensitivity of the ninhydrin reacted zones is achieved when using TRANSMITTED UV LIGHT as compared to visible daylight colours. The antimicrobial is quantitatively determined in a moisturizing lotion with a relative standard deviation of $\pm 10\%$ using DENSITOMETRY.

Introduction

Cosmetics provide an ideal medium for the growth of microorganisms. The manufacturer must guard against the microbial contamination of his product during manufacture and storage and also against contamination by the consumer. Thus antimicrobial preservatives are added which are designed to be effective against a wide spectrum of microorganisms over a long period of time.

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Many non-polar preservatives are in common use and several authors have reported tlc methods for their detection. König (1) separated and identified 16 halogenated aromatics. Karlskind, Valmalle and Wolff (2) quantitatively estimated six halogenated aromatics in soap by means of tlc and spectroscopic methods. Graber, Domsky and Ginn (3) identified zinc omadine and five halogenated aromatics in personal care products. Hexachlorophane, tribromosalicylanilide and trichlorocarbanilide have been detected and quantitatively determined by Schwarze (4) using tlc and ion exchange procedures. In addition, Porcaro and Shubiak (5) developed a uv/liquid chromatographic method for the estimation of hexachlorophane in nanogram quantities using a dianisate ester derivative. Wolf and Senionow (6) detected six halogenated aromatics in soap using High Pressure Liquid Chromatography, although Irgasan CF_3 and trichlorocarbanilide were not separated. Little has been reported of the more difficultly-detected and quantified non-aromatic, polar, hydrophilic antimicrobials, particularly of the imidazolidinyl urea type.

The first commercial member of this family is Germall 115.* This preservative is claimed to be a broad spectrum antimicrobial which is non-toxic, non-irritating and is particularly effective in emulsions and protein-containing formulations. It concentrates in the microbial supporting aqueous phase and its efficiency is not impaired by the presence of non-ionic emulsifiers and proteins.

For quality control purposes and for the identification of antimicrobials in unknown formulations, it was necessary to develop a rapid and specific identification and quantification procedure for imidazolidinyl urea in a wide variety of personal care products. Because of the complexity of the formulations to which this preservative may be added, i.e. lotions, creams, hair conditioners, shampoos, deodorants etc., a comprehensive colorimetric method was impracticable. Thus tlc was used, since no sample preparation was necessary and the method was applicable to new products without change in the procedure.

EXPERIMENTAL

Apparatus

Thin layer plates: 20×20 cm 0.25 mm Silica gel F_{254} (Merck) used as received without further activation.

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Separating chambers: For 20 × 20 cm plates (Desaga).
UV viewer for transmission: Blak-Ray Transilluminator (Shandon Southern Instruments Ltd).
UV viewer for reflectance: Blak-Ray Chromato-Vue (Shandon Southern Instruments Ltd).
Chromoscan densitometer with thin layer attachment: Joyce Loebl and Co. Ltd.

Reagents

Germall 115 (Imidazolidinyl urea).
Flow solvent: chloroform : methanol : acetic acid : water; 50 : 30 : 10 : 10.
Spray reagent: Ninhydrin—0.3 g ninhydrin in 95 ml n-butanol and 5 ml acetic acid.

Procedure

Sample application

5 μl of a 10% solution or suspension of the sample in methanol-water (70 : 30) is applied using a Drummond Microcap pipette. When the samples examined are of a viscous nature, it is necessary to use the rubber bulb in order to fill and dispel the solutions. The size of the applied spot is normally 0.5 cm diameter. Sample and reference solutions are spotted alternately along the plate at a height of 2 cm from the edge of the plate. The spots are well dried after application using a warm-air dryer.

Reference solutions

Solutions are prepared of the product under test without imidazolidinyl urea and the product containing varying known quantities of imidazolidinyl urea from 0.1% to 0.6%.

Chamber

A Desaga chamber for 20 × 20 cm plates is lined with filter paper, saturated in the flow solvent and allowed to equilibrate for 30 min before use.

Development

The chromatogram is allowed to develop for 50 min, in which time the solvent front travels approximately 9 cm.

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Visualization

The plate is briefly dried using a warm-air dryer and sprayed with the ninhydrin spray until well wetted. The plate is heated at a temperature of 150°C for 20 min, allowed to cool and then viewed in uv light of 366 nm by transmission. The imidazolidinyl urea preservative is seen as two pale yellow fluorescent zones at Rfs of 0.27 and 0.35.

Densitometric measurements

These are performed on a Chromoscan densitometer with a thin layer attachment. The following operating conditions are used. Chromoscan: light source 12 V, 100 W, tungsten halide lamp, filter 3.0 O.D., optical wedge 0–0.5 O.D., gain 5, cam A.

Thin layer attachment

Light source uv mercury lamp type ST 75, aperture 1 mm × 17 mm, uv filter 300–400 nm between the light source and specimen, Kodak Wratten uv filter No. 2E between the specimen and detector, specimen expansion ratio 1 : 1. The measurements are carried out using the reflectance mode.

RESULTS AND DISCUSSION

During the optimization of the method, various alternative systems were attempted. Laboratory-prepared plates were compared with precoated plates. The laboratory coated plates were prepared by mixing 52 g of Merck, Silica gel 'G' F_{254} with 110 ml of distilled water in a Waring blender and ten glass plates were then coated using a Camag automatic spreader set at a wet layer thickness of 300 μm. The plates were left at room temperature for 20 min and finally dried for 1 h at 105°C in an air-blown oven. It was found that although satisfactory for samples containing 0.5% imidazolidinyl urea, the laboratory-prepared plates did not display the necessary sensitivity at the lower concentrations of preservative. This may in part be due to the increased initial size of the applied spot on laboratory-prepared plates, which more readily absorb the viscous sample solutions than do the polyvinyl alcohol bound precoated layers. Alumina laboratory-prepared plates were also examined but these failed to produce a satisfactory separation.

On investigating various sample solutions, water alone usually yielded an excessive quantity of bubbles and a solution which was too viscous to pipette conveniently. The urea compound was found to be insufficiently soluble in methanol alone to be certain of recovery from emulsified products,

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but as the preservative is up to 50% aqueous soluble, the relatively small proportion of water in the mixture, methanol-water (70 : 30) was found to be satisfactory and was used for all the products examined.

It was shown to be essential to dry the spots well, after application, otherwise distortion and lack of sensitivity resulted. No decomposition of the imidazolidinyl urea zones was detected on heating the applied spots with a warm-air dryer, even when heated to a considerably greater extent than that required for a good chromatogram.

As imidazolidinyl urea was almost insoluble in any solvent other than water, in which it was very soluble, the correct flow solvent required a certain water content but this needed to be modified by a non-polar solvent, chloroform was chosen as a convenient solvent after considering its position in the eluotropic series. These two solvents were rendered homogeneous by the mutually-miscible solvent methanol.

A mixture of chloroform-methanol-water (45 : 45 : 10) was found to give satisfactory Rf values but rather diffuse spots. As a change in pH will often produce a 'sharpening up' of diffuse spots, the following solvents were investigated.

Chloroform-methanol-acetic acid-water (20 : 30 : 40 : 10), although compacting the spots, produced an increase in Rf's and failed to separate the preservative from other components in the formulations. Chloroform-methanol-ammonia (50 : 40 : 10) gave very low Rf's and even after the carefully-attempted removal of the ammonia, a poor background colour reduced the limits of detection considerably. Chloroform-methanol-acetic acid-water (50 : 30 : 10 : 10) having a pH of 2.5 was found to yield compact zones separated from all other visible components. This solvent also gave a good separation of the imidazolidinyl urea components in Germall itself.

A number of spray reagents were investigated, several being general reagents, whilst the remainder were intended to be more specific for the imidazolidinyl urea grouping. Of the twelve reagents tried, few successfully detected less than 25 μg of imidazolidinyl urea (*Table I*). Only Erlich's reagent and ninhydrin are sufficiently sensitive to detect 0.5% imidazolidinyl urea in a product, i.e. 2.5 μg for the loading used. The sensitivity of the ninhydrin spray is increased by a factor of ten when viewed by transmitted uv light of 366 nm. There seems to be little record of compounds which react with ninhydrin being more sensitively detected using uv light rather than colours visible in daylight. It seems likely that the difference in sensitivity of detection found when viewed by reflected and transmitted uv light may be mainly due to the difference in intensity of light on the plate,

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Table I. The limits of detection of imidazolidinyl urea type (*Germall* 115) using various reagents

Reagent	Colour of reaction	Limit of detection
Ninhydrin (daylight)	Red	2.5 μg
Ninhydrin (reflected UV)	Yellow	1 μg
Ninhydrin (transmitted UV)	Yellow	0.25 μg
Dragendorff's reagent	No reaction	—
Blue Salt Irga B	No reaction	—
Cobalt thiocyanate	Blue	25 μg
Erlich's reagent	Yellow	2.5 μg
Silver nitrate	No reaction	—
Iodine	No reaction	—
Fluorescein	No reaction	—
Rhodamine 6G	Yellow in UV	10 μg
Prochazka reagent	No reaction	—
Chromic acid	No reaction	—
Pinacryptol yellow	Blue in UV	10 μg

i.e. 450 μW cm^2 at 18 in for reflectance and 1900 μW cm^2 at the surface for transmission.

The extent to which plates were sprayed was found to be critical, thus in order to establish optimum conditions, plates were sprayed until varying states of wetness were obtained.

Plates which were observed to be not wetted, i.e. not darkened, plates which were just wetted and even plates which were fairly well wetted, gave poor results. Well-wetted plates and those which were soaked, i.e. of a shiny appearance were satisfactory. The correct spray was therefore judged to be well wetted until the first shiny appearance was observed, which normally required 20 ml of reagent for a 20 × 20 cm plate, although due to variance in spraying techniques the appearance of the plate rather than the volume used is the better criterion.

Chromatograms heated at 105°C, the normally recommended temperature for ninhydrin failed to give good sensitivity even when heated for up to 90 min. At 150°C little reaction was noted for up to 10 mins heating but the maximum sensitivity was achieved after 15 min and no change was detected after further heating for 30 min. Thus heating at 150°C for 20 min is preferred. The spots once developed are stable for several days if protected from daylight.

As when examined at a higher loading, the preservative revealed six components, i.e. two major and four minor, the possibility of decomposition

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due to chromatography was examined. A two-dimensional chromatogram was carried out using the solvent chloroform-methanol-acetic acid-water (50 : 30 : 10 : 10) in both directions. All the components were found to lie in a diagonally straight line, showing that no new components were formed during the chromatographic run. In addition, solutions kept for several days showed no detectable decomposition compared to freshly-prepared solutions. The detection of imidazolidinyl urea when added to several cosmetic and toiletry products was attempted. These products included a moisturizing lotion containing fifteen ingredients, some of which were themselves complex mixtures and known to include an aliphatic amine, a mixture of parabens, two dyes, a lanolin product, an emulsifier, long chain alcohols and esters, poly hydroxy compounds and aloe, polysaccharides, proteins, amino acids and vitamins. An egg shampoo was also tested, as unlike some antimicrobials, the efficiency of imidazolidinyl urea is not impaired by the presence of proteins. The other products tested were, a deodorant foam bath, a hand cream, a squeeze-on deodorant, a roll-on deodorant and an aerosol deodorant (Fig. 1).

Imidazolidinyl urea was successfully detected down to at least 0·1% in all the products tested and only the roll-on deodorant and the aerosol deodorant exhibited any distortion of the preservative zones.

The preservative was easily detected in the presence of other antimicrobials (*Table II*), including parabens with which it is recommended for joint use, as a synergistic effect in efficiency has been observed.

The detection system was specific for the imidazolidinyl urea type of antimicrobial when compared with other antimicrobials and additives, as none of those tested revealed a similar distinctive coloration in uv light, even though a number reacted with ninhydrin to form colours visible in daylight.

Quantitative

Although for routine quality control, a simple, visual comparison of spots with the appropriate standard spots is both rapid and sufficiently accurate, a more precise densitometric determination was also investigated. For all quantitative work, the sample was run with replicate spots alternately spaced with standard solutions, which consisted of the 'blank' product doped with known quantities of imidazolidinyl urea. The chromatographic conditions were as for the previous qualitative work.

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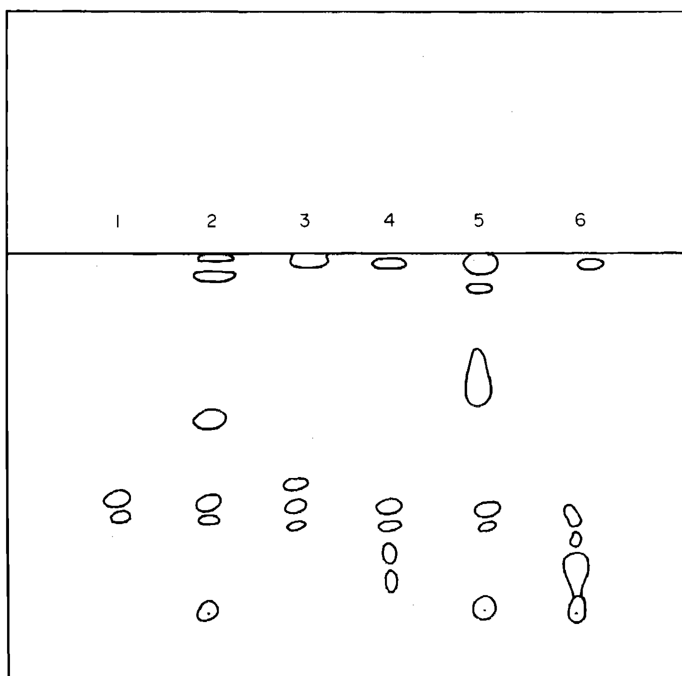

Figure 1. Diagram of the detection of imidazolidinyl urea type (Germall 115) added to personal care products. System: pre-coated silica gel; flow solvent= chloroform-methanol-acetic acid-water (50 : 30 : 10 : 10); detection = ninhydrin, viewed by transmitted uv light 366 nm. 1 = Germall 115; 2 = hand cream; 3 = foam bath; 4 = moisturizing lotion; 5 = egg shampoo; 6 = deodorant.

The main difficulty encountered in achieving reproducible results is in obtaining a regular and easily definable baseline. This in turn is due to the background colour on the plate which varies in the direction of the solvent flow.

It was thought that adding ninhydrin to the flow solvent might improve the uniformity of the background colour and also remove errors due to uneven spraying. Unfortunately the reverse was found to be the case, the background intensity and variance being increased using this procedure.

A partial answer to the problem was achieved by drawing a pencil line approximately 2 cm above the line of the imidazolidinyl urea spots and a line parallel to this through the origins of the spots. This enabled a base line for each applied spot to be drawn on the graph, joining the responses of the two pencil lines, i.e. before and after the Germall peaks. The maximum peak

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Table II. Antimicrobial preservatives chromatographed using the conditions required for the imidazolidinyl urea type (*Germall* 115)

Anti-microbial preservative	UV 254 nm	Ninhydrin	Rf × 100
Germall 115 (Imidazolidinyl urea type)	–	+	27 + 35
(traces)			(0 + 15 + 60 + 72)
Sorbic acid	–	+	86
Dichlorophene	+	–	87
Hexachlorophene	+	–	88
Chlorcresol	+	–	90
Chlorxylenol	+	–	91
Trichlorocarbanilide	+	–	88
Irgasan DP 300 (2,4,4[1]-trichloro-2[1]-hydroxy diphenyl ether)	+	–	92
Methyl paraben	+	–	85
Propyl paraben	+	–	88
Propylene glycol	–	–	69
Tribromosalicylanilide	+	–	92
Irgasan CF3 (4,4[1]-dichloro-3-trifluoromethyl-carbanilide)	+	–	89
Cetrimide	–	+	65
Bronopol, (2-bromo-2-nitropropane-1,3-diol)	+	–	80
Phenoxyethanol	+	–	91

Flow solvent, chloroform-methanol-acetic acid-water (50 : 30 : 10 : 10).
+, Detected; –, not detected.

height above this base line was recorded and the results of the standard spots were used to construct a calibration graph. A typical calibration graph of the preservative added to a moisturizing lotion is shown in *Fig. 2*.

Although most plates gave a straight line graph for values from 0.5 μg to 2.5 μg, in some cases the curve 'flattened out' at the higher values giving rise to poor reproducibility. The relative standard deviation for ten determinations of the urea product in a moisturing lotion at the level of 0.5% was found to be ±10%. One determination was considered to be the mean of four replicate sample spots on one plate calculated from a calibration graph of standards run on the same plate. Although the relative deviation was high, it may be considered acceptable, as the percentage of preservative in any formulation will always be at a low level.

Conclusion

Using the tlc method described, it is possible to detect the imidazolidinyl urea type of antimicrobial preservative in a wide variety of cosmetic and

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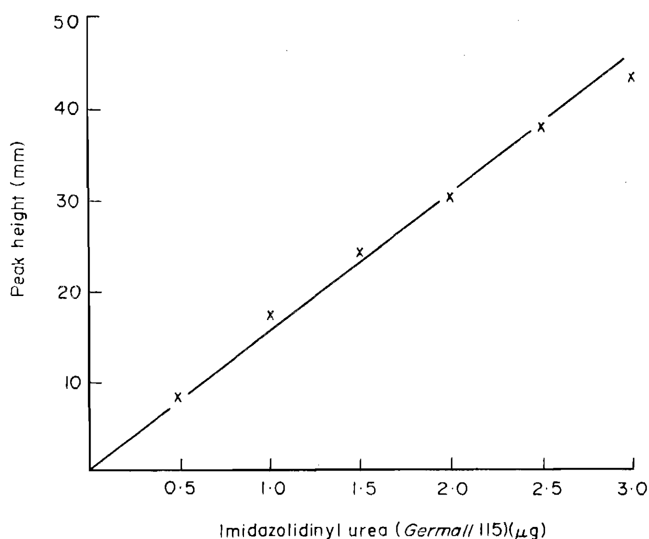

Figure 2. A calibration graph of imidazolidinyl urea type (Germall 115) added to a moisturizing lotion.

toiletry formulations. The uv colour reaction is specific for imidazolidinyl urea, even in the presence of a number of other antimicrobials. The preservative may be quantitatively determined with sufficient accuracy for routine quality control by either visual comparison or densitometric measurements.

(*Received: 12th March 1974*)

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