Analysis of Pharmaceuticals by Ultraviolet Densitometry on Thin-Layer Chromatograms I

Parabens in Gels and Creams

By HERBERT SCHRIFTMAN

Precision of about 1 percent and excellent recovery are attainable by quantitative densitometry of ultraviolet chromophores separated by thin-layer chromatography on plates containing a phosphor. Quenching of the fluorescent background affords a sensitive measure of concentration. Substrates with native or derived fluorescence can be determined on plates by fluorometry using either a nonfluorescent background or a phosphor that fluoresces at different wavelengths. This report describes application of these and similar techniques to the determination of parabens in pharmaceutical and cosmetic products. The densitometry technique requires simple solvent extraction of the paraben-preserved product and chromatography on silica gel thin layers containing phosphor. Use of a 4:1 pentane–acetic acid system provided resolution of the parabens from each other, and no interference was encountered with hydrocortisone acetate, partial sodium salt of *n*-lauryl β-imino diproprionic acid, EDTA, polyethylene glycol, hydroxyethyl cellulose, hydroxypropyl methylcellulose, D & C Green No. 5 dye, and various waxes and fats. Preliminary investigations for applying direct ultraviolet densitometry to other types of thin layers are mentioned.

IN A RECENT comprehensive review (1) on the use of TLC in pharmaceutical analysis, Comer and Comer attributed the major disadvantages of quantitative TLC to the "lack of precision and accuracy of direct measurement instrumentation or the tedious nature of removal of absorbent containing the desired compounds." This report describes direct, accurate, and precise determination of paraben mixtures on TLC plates by UV densitometry, which eliminates the aforementioned difficulties. The procedures for the parabens are exemplary, for the general technique may be extended to many other mixtures containing UV chromophores.

Mixtures of methyl and propyl parabens are widely used as preservatives in foods, drugs, and cosmetics, and several procedures have been reported for the separation and determination of these esters. Paper (2), column (3), and thin-layer (4) chromatographic methods have been reported in which the separated parabens are quantitated by spectrometry after elution. Donato (5) described a method combining UV spectrometry for determining total parabens with gas chromatography for determining their ratio. A subcommittee of the PMA Quality Control Section has recently evaluated a gas chromatographic assay for parabens which makes use of the internal standard technique (6).

In the method described here, the parabens are spotted and separated by ascending TLC using Brinkmann silica gel plates with phosphor and a pentane–acetic acid (4:1) solvent system, after a relatively simple solvent extraction from the product. The separated parabens are analyzed *in situ* by photoelectric densitometry on a Photovolt recording TLC densitometer. The total absorbance of each spot expressed as the total area under the density curve is measured by K & E planimeter or alternately by a Photovolt recording integrator, and compared by direct proportion to a reference spot concomitantly analyzed.

Application of this procedure to a pharmaceutical gel is evaluated and found to be comparable in precision, speed, and accuracy to Donato's (5) method.

Besides having applicability to other products containing parabens (cosmetic creams, pharmaceutical ointments, and antacid suspensions), the technology described here has been extended to other mixtures containing UV chromophores. Investigations for applying direct UV densitometry to the assay of other drug products as well as using other types of TLC plates and sheets (*i.e.*, cellulose) and other media possessing UV transparency (*i.e.*, agarose—a new agar gel used for thin-layer electrophoresis) are in progress.

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EXPERIMENTAL

Reagents—ACS grades of absolute methanol, diethyl ether, anhydrous sodium sulfate, and glacial acetic acid and chromatoquality grade of pentane (Matheson) were employed.

Equipment—Brinkmann Silica Gel GF TLC plates (20 × 20 cm.) with fluorescent indicator, micropipet[1] (0.001 and 0.002 ml.), Photovolt densitometer model 530 with recording and integrator attachments, 15-ml. screw-cap vials, and appropriate chromatographic containers were used.

General Procedure—Aliquots of the sample containing the equivalents of 2.5 to 15 mg. of esters are transferred to a separator adapted for centrifugation where the esters are extracted with several portions of 5-ml. volumes of ether. The ether phases are collected, washed with distilled water, and filtered through sodium sulfate and Whatman No. 1 paper into a 50-ml. beaker. Another 5-ml. ether wash of the filter is collected and the combined ether solution is evaporated gently on a steam bath with a stream of air. The residue is dissolved in exactly 1.0 ml. of methanol and transferred into a 15-ml. screw-cap vial. The appropriate quantity of the solution is spotted on an activated and dried Brinkmann plate. The quantity spotted ranged from three times 0.001 to five times 0.002 ml. Concomitantly, a standard containing equivalent quantities is spotted. Due to uncontrollable high humidity conditions in this laboratory, it was found necessary to redry the activated plates in a vacuum desiccator after the spotting operation. Special care was also required for maintaining a low water content in the solvent systems, *i.e.*, keeping a beaker of silica gel (indicating type) in the chromatography tank. As a matter of course, all ensuing experiments were conducted in this manner. The dried plate is placed in the tank containing a mixture of 160 ml. of pentane and 40 ml. of acetic acid; and the solvent is permitted to ascend the plate about 15 cm. (in about 30–45 min.). The plate is air-dried and scanned face down with the recording TLC densitometer using a short-wave UV light (254 mμ) and a phototube detector fitted with a 485-mμ color filter. The total absorbance of each spot expressed as the total area under the density curve is measured and compared by a K & E planimeter or alternately by an integrator. Propylparaben migrates faster than methylparaben, and their relative migrations in comparison with other parabens are illustrated in Fig. 1. Figure 2 shows a typical separation of the methylparaben and propylparaben spots with their corresponding recorded and integrated density curves.

Specific Assays—*Pharmaceutical Gels*—About 5 g. of gel accurately weighed (equivalent to about 7.5 and 2.5 mg. of methyl and propylparaben, respectively) is dissolved in a beaker with 5 ml. of a methanol–water mixture (1:1). The solution is quantitatively transferred with two additional 5-ml. portions of solvent into a separator adapted for centrifugation. Four 5-ml. volumes of ether are employed for the subsequent extraction and centrifugation steps, discarding the lower aqueous phases and collecting the upper ether layers into a regular separator. The collected extracts are washed twice with water and filtered through sodium sulfate and Whatman No. 1 paper into a 50-ml. beaker. Another 5-ml. ether wash of the filter is collected and the combined ether solution is evaporated gently on a steam bath with a stream of air. The residue is dissolved in exactly 1.0 ml. of methanol and transferred into a 15-ml. screw-cap vial. Three times 0.001 ml. of this solution is spotted on a Brinkmann TLC plate and analyzed as described above in the *General Procedure*. Concomitantly, a standard containing 75 and 25 mg. of methyl and propylparaben, respectively, per 10 ml. of methanol is spotted and compared.

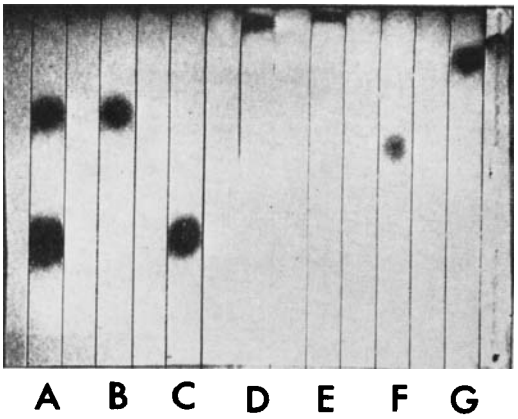

Fig. 1—Relative migration of parabens. Key: A, *methyl and propylparaben;* B, *propyl;* C, *methyl;* D, *hexyl;* E, *heptyl;* F, *ethyl;* G, *butyl paraben.*

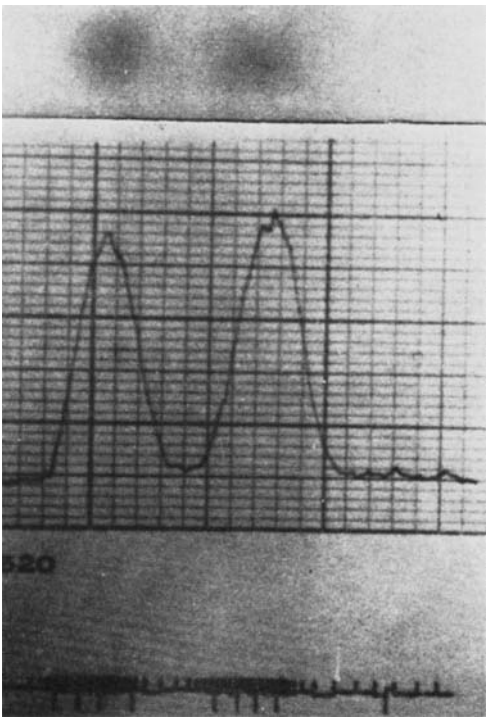

Fig. 2—Typical separation of the methylparaben and propylparaben spots with their corresponding recorded and integrated density curves.

[1] Microcaps, Drummond Scientific Co.

TABLE I—ANALYSIS OF GEL

Sample	Wt., g.	% of Declared Value—Methyl Paraben	% of Declared Value—Propyl Paraben
1	5.3405	98.8 (98.3, 99.2)	101.9 (102.6, 101.1)
2	6.1025	99.2 (98.8, 99.6)	99.5 (98.1, 100.8)
3	5.0380	102.2	100.0
4	5.2115	102.6	100.7
5	5.1450	99.7	99.9
6	5.4061	101.2	100.6

Statistical Evaluation

Methyl Paraben		Propyl Paraben	
average	= 100.5	average	= 100.3
σ	= 1.6	σ	= 0.8
3σ	= 4.8	3σ	= 2.4

Cosmetic Cream—Approximately 10 g. of sample accurately weighed into a beaker is well mixed and stirred with 25 ml. of hot methanol on a steam bath. The hot mixture is filtered through glass wool into a 50-ml. conical flask. Additional 5- to 10-ml. volumes of hot methanol are used for transferring the contents of the beaker and washing the filter. The solution is chilled in refrigerator or ice bath and filtered cold through Whatman No. 1 paper. Flask and filter paper are washed with additional quantities (2 × 5 ml.) of chilled methanol. The combined filtrate is concentrated to one-half its volume on a Rinco rotary vacuum evaporator. The cooling, filtering, and evaporation steps are repeated and the final residue is dissolved in methanol and transferred quantitatively into a 10-ml. volumetric flask. Five times 0.002 ml. is applied to Brinkmann plates and analyzed as described above. The standard solution in this example contains 15 and 10 mg. of methylparaben and propylparaben, respectively, per 10 ml. of methanol.

RESULTS AND DISCUSSION

The analytical data for the gel was comparable in precision and accuracy with the values previously reported in other chromatographic methods (2–5). For example, our σ values of 1.6 and 0.8 (see Table I) are comparable to Donato's (5) results of 1.2 and 3.1 (calculated from his data) for methylparaben and propylparaben. In addition, Donato's GLC method requires the formation of the trimethylsilyl ether derivatives, determination of their ratio, and finally UV spectrophotometric measurement of the total parabens. In the method presented here, the separated parabens are still intact and available for further study or identification. In addition, the recovery data for a gel placebo formulation, to which known quantities are added, is in the range of 97.2–101.6% of theory (see Table II). Similar precision and accuracy were obtained with the cosmetic creams.

TABLE II—RECOVERY DATA[a]

Sample	Recovery, % Methyl Paraben	Recovery, % Propyl Paraben
1	97.2	100.3
2	99.1	101.6

[a] 7.5 mg. and 2.5 mg. of methyl and propyl paraben, respectively, were added for each 5 g. of gel placebo.

No interferences or difficulties were encountered from the excipients in the above formulations which included partial sodium salt of *n*-lauryl β-imino dipropionic acid (Deriphat 160), EDTA, polyethylene glycol, hydroxyethyl cellulose (Natrosol, Hercules), hydroxypropyl methylcellulose NF (Methocel), D & C Green No. 5 dye, and various waxes and fats.

OTHER APPLICATIONS

Preliminary investigations for applying direct UV densitometry to other multicomponent drug products and other types of layers are listed below. Detailed and comprehensive reports of these applications are in preparation and will be available at a later date.

1. Antacid suspensions containing parabens were assayed using the method outlined above except that cellulose thin layers were employed and in most instances the extraction technique was further simplified.

2. Gel chromatography and electrophoresis techniques were developed in which multicomponent products were separated and analyzed *in situ* in a specially designed gel tray. Using these trays the analyst could also observe periodically or continuously the progress of the migration and separation of the compounds.

3. Cellulose thin layers were substituted for paper in the various chromatographic and electrophoretic analyses of pharmaceutical products reported by this author (7–10). In those instances, the papers were developed by various chromogenic reagents, and then analyzed by color densitometry. In the current study, the thin-layer chromatograms and electrophoretograms are evaluated by UV densitometry without any prior treatment.

4. Oxolinic acid (a new synthetic antimicrobial agent specially effective against *Proteus* in urinary tract infections) and its various analogs were analyzed by the technology described here, based on their fluorescent properties.

SUMMARY

TLC and UV densitometry have been successfully applied for analyzing the paraben contents of pharmaceutical gels and cosmetic creams. The procedure compares favorably in precision, accuracy, and speed with present acceptable technology, *i.e.*, GLC. Application of UV densitometry to the

assay of other products and drugs as well as the use of other thin-layer media is indicated and will be reported at a later date.

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Keyphrases

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UV light, phototube detector—scanning

3-Substituted-2-benzoxazolinones

By JOSEPH SAM, J. L. VALENTINE, and C. W. RICHMOND

The preparation of 2-amino-5-trifluoromethylbenzoxazole, 5-trifluoromethyl-2-benzoxazolinone and their chemical precursors are described. The reaction of appropriately substituted 2-benzoxazolinones with substituted alkyl halides provided the 3-substituted 2-benzoxazolinones.

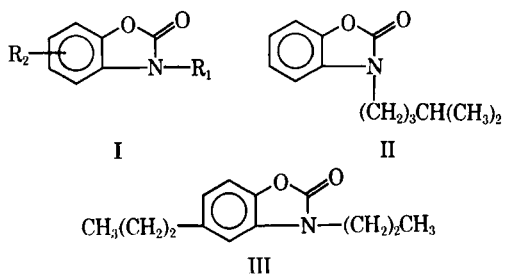
PREVIOUS INVESTIGATIONS (1–4) on the medicinal value of 3-substituted-2-benzoxazolinones (I, Table I) prompted the present work on other 3-substituted derivatives. Lespagnol and co-workers (5, 6) studied the time required for 2-benzoxazolinone and some 3-substituted derivatives to produce immobilization in fish. Swinyard and associates (7) demonstrated that the 3-substituted derivatives prepared by Lespagnol were active in antagonizing maximal electroshock states. Subsequently, Close and co-workers (2) studied a number of 3-substituted derivatives for their analgesic activity. Two of these derivatives (II and III) were tested clinically and proved to be less active than indicated by the animal studies

The 2-benzoxazolinones (XI) utilized in this study were prepared according to procedures described in earlier reports; however, additional studies were conducted on the preparation of 5-trifluoromethyl-2-benzoxazolinone (VI).

Previously, the preparation of VI *via* two routes was reported (1). One of these routes involved the condensation of phosgene with 2-amino-4-trifluoromethylphenol (V), whereas the alternate route involved the use of the intermediates 2-nitro-4-trifluoromethylphenyl ethyl carbonate (VII) and the corresponding amino derivative (VIII) *in situ*. Compounds VII and VIII now have been isolated and the structures elucidated. (Scheme I.)

The preparation of VII was accomplished by condensing IV with ethyl chloroformate. Catalytic reduction of VII gave VIII which upon treatment with aqueous hydrochloric acid yielded VI.

The pronounced biological properties of 5-chloro-2-aminobenzoxazole (8) prompted the authors to investigate the preparation of 5-trifluoromethyl-2-aminobenzoxazole (X). The con-



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