Table II—Determination of Sulfamerazine, Sulfamethoxypyridazine, and Succinylsulfathiazole in Tablets by the 9-Chloroacridine Method and the Method of Bratton and Marshall

	9-Chloroacridine Method		Bratton-Marshall Method	
	Mean % of Labeled Amount	*SD* of Mean, %	Mean % of Labeled Amount	*SD* of Mean, %
Sulfamerazine tablets	99.00	0.45	98.75	0.25
Sulfamethoxypyridazine tablets	99.52	0.27	100.63	0.55
Succinylsulfathiazole tablets	99.43	0.40	99.20	0.46

amides containing a primary aromatic amino group in the presence of other sulfa derivatives, such as succinylsulfathiazole, and in the presence of 2,6-diamino-3-phenylazopyridine hydrochloride, tetracycline hydrochloride, sodium penicillin G, and hexamethyleneamine. The latter compounds are found in various combinations with sulfonamides in commercially available dosage forms. It was shown from earlier studies that primary, secondary, and tertiary aliphatic amines, secondary and tertiary aromatic amines, heterocycles, and carbonyl-containing compounds also do not interfere with this method (1).

The analytical method is essentially a micro procedure, and sensitivity is in the range of 10^{-7}–10^{-8} mole ml.$^{-1}$ of sulfonamide, which makes it comparable to other sulfa determinations, particularly the popular diazotization-coupling procedures.

A favorable characteristic of the analysis is that the absorbance of the product formed is stable and does not fade over a 24-hr. period. This is an advantage over the colorimetric method of Bratton and Marshall. In the latter method, absorbance readings must be made within 15 min. after color development, due to precipitation of the azo dyes in the method (3). The 9-chloroacridine method does not involve diazotization. Thus, it eliminates the need for freshly prepared sodium nitrite and ammonium sulfamate solutions required with the Bratton-Marshall technique. Control of pH is required in both methods.

The method of analysis for sulfonamides by the 9-chloroacridine approach was carried out for various sulfonamides, and comparative analyses were performed using the colorimetric procedure of Bratton and Marshall. Assays were performed on sulfamerazine, sulfamethoxypyridazine, and succinylsulfathiazole in tablets. With succinylsulfathiazole, saponification with sodium hydroxide as outlined in NF XI was required to form the primary amine (4). The commercially available sulfamethoxypyridazine tablets used were colored with a yellow dye; but it was found that for the dilutions used, the absorbance from the color was not sufficient to interfere with the assays by either method.

The procedure outlined by Connors was used for the analysis by the Bratton-Marshall method (5).

Four determinations by each method were performed for each sulfonamide. The mean percent of labeled amount and the percent standard deviation of the mean for each sulfonamide are shown in Table II for both methods (6).

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Inclusion Compounds in Pharmaceutical Analysis I: Determination of Dienestrol in Dienestrol Cream

BARBARA J. FORMAN and LEE T. GRADY

Abstract □ Large ratios of monostearin to dienestrol in cream formulations complicate the development of an assay for dienestrol. Monostearin was removed easily in a test-tube procedure by channel-type inclusion in urea. Dienestrol recovery was complete and reproducible analyses were obtained by polarography of the nitrosophenol derivative. Urea inclusion may offer a general approach to analytical methodology where long-chain compounds need be separated from active ingredients.

Keyphrases □ Dienestrol creams—analysis □ Monostearin removal, dienestrol creams—urea inclusion compound □ Column chromatography—separation □ Polarography, organic—analysis

Isolation of dienestrol and related compounds from formulations containing surfactants or hydrocarbons has required troublesome steps such as column chromatography. Gottlieb (1) separated diethylstilbestrol from creams and ointments using toluene at reflux to break emulsions and subsequently isolated the drug on an alumina column. Nevertheless, he reported poor recovery in the presence of monostearin. A more recent illustration (2) was the determination of diethylstilbestrol in a water-dispersibile suppository using an alumina column step prior to quantitative TLC.

In developing an assay for dienestrol in dienestrol cream,[1] the authors separated the drug from at least a thousand-fold excess of monostearin. An existing polarographic method was used for the determinative step. Monostearin interfered with this step by causing gross distortion of the polarograms.

[1] To be official in NF XIII.

Aylward and Wood (3, 4) converted monostearin into the urea inclusion compound and identified the adduct as a channel-type inclusion. Formation of such compounds between urea and straight-chain hydrocarbons has been known many years but has been applied extensively only in petroleum and fat chemistry. Schlenk (5) prepared an early review of fatty acid inclusion compounds, and Swern gave an excellent (6) review of inclusion compounds in general. In essence, urea includes straight-chain rather than branched and saturated rather than unsaturated compounds—chains of 6–7 carbon atoms are required and cyclic compounds are not included. Of pharmaceutical interest is the preparation of liquid lanolin from USP semisolid by removing the solid wax fraction, 6–8%, as urea-inclusion compounds (7).

The application of urea inclusion to an analytical problem presented by a cream formulation is reported here.

MATERIALS AND METHODS

All chemicals were reagent grade. Ether–hexane solvent was prepared by mixing equal volumes of these solvents. The electrolyte solution was made by dissolving 54 g. ammonium chloride in 450 ml. 28% aqueous ammonia and diluting to 1 l. with distilled water. Samples of commercial creams[2] and silica gel, 60-80 mesh, chromatographic grade,[3] were used. All concentrative steps were performed at reduced pressure,[4] with mixing, at temperatures below 35°. Polarograms were uncompensated, d.c. runs at room temperature using a silver-saturated potassium chloride reference electrode. Mercury drop-times of 0.24 sec. were obtained with a forced-drop electrode.[5] Nitrogen sparging was used.

Standard Preparation—Dissolve a suitable quantity, accurately weighed, of dienestrol NF Reference Standard in methanol and dilute, quantitatively and stepwise, to obtain a solution containing about 50 mcg./ml. Pipet 2.0 ml. of this solution into a glass-stoppered test tube and evaporate to dryness at reduced pressure. Continue as directed under *Procedure* beginning with "Dissolve the residue in 5.0 ml. 80% acetic acid . . ."

Silica Gel Column—Pack a pledget of glass wool in the base of a 1.5-cm. o.d. chromatograph column. Fill the column nearly to the top with ether–hexane. Slowly pour 5.0 g. silica gel as a slurry with 10 ml. ether–hexane into the column, open the stopcock, allow the gel to settle by gravity, and tamp to achieve a uniform bed. Allow a 5-ml. head of solvent to remain above the bed until ready for use, then allow the meniscus to descend to the top of the bed. The slurry should be prepared the day before use to ensure uniformity.

Procedure—See Fig. 1. Weigh accurately about 1 g. of the commercial cream into a 30-ml. glass-stoppered test tube. Add 5.0 ml. 0.025 *M* pH 6.5 phosphate buffer and 15 ml. ether to each tube. Shake gently 2 min., centrifuge, and transfer the ether layer to another tube. Repeat the extraction with two 10-ml. portions of ether, and evaporate the combined extracts to dryness at reduced pressure.

Add 10 ml. absolute ethanol to each tube, stopper loosely, and heat in a water bath at 80° until a clear solution is obtained. Add 1.3 g. urea and continue heating with occasional shaking until dissolved. Let stand at room temperature 2 hr.,[6] add 5 ml. ether–hexane, and keep at 0–4° for 30 min. Centrifuge, decant the clear supernatant into a 30-ml. stoppered test tube, shake the residue twice with 5.0-ml. portions of ether–hexane, centrifuge, and combine the supernatants. Concentrate to about 1 ml. at reduced pressure.[6]

Apply this concentrate to the top of a silica gel column, open the stopcock, and allow the sample to enter the column bed. Wash the sample test tube with 5 ml. ether–hexane. add the wash to the column, wash the wall of the chromatographic tube with 5 ml. ether–hexane, and allow the washings to enter the bed. Elute the column with 75 ml. ether-hexane and evaporate the eluate under reduced pressure until dry.

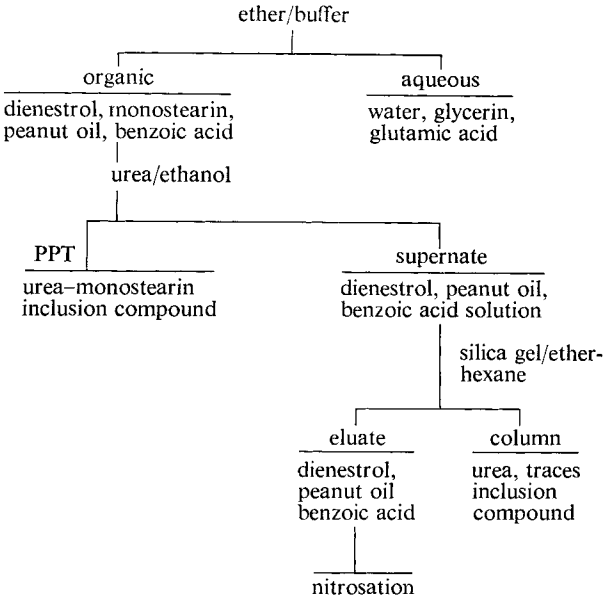

Figure 1—*Flow diagram for assay of Dienestrol Cream. Dienestrol 0.01%, monostearin, glycerin, water, glutamic acid, peanut oil, benzoic acid.*

Dissolve the residue in 5.0 ml. 80% acetic acid and mix until a clear solution is obtained. Transfer 4.0 ml. by pipet to a 25-ml. volumetric flask, add 3 drops concentrated sulfuric acid and 5 drops freshly prepared saturated aqueous sodium nitrite. Allow to stand 30 min. to complete the nitrosation, immerse in an ice bath, and add slowly 20 ml. of the electrolyte solution. When the fuming subsides, remove the flask from the bath and make to volume with electrolyte solution, mixing well. Allow this solution to stand 30 min., but not longer than 2 hr., at room temperature before proceeding. Filter a portion of the solution through a small pledget of cotton into the polarographic cell and sparge 10 min. Record the polarogram between about −0.25 and −1.25 v. Measure the diffusion current between −0.45 and −0.95 v., corrected for solvent blank, against a saturated calomel reference electrode. Determine the amount of dienestrol in the sample by the formula:

$$\left(\frac{i(x)}{i(\text{std.})}\right)\frac{(\text{mcg. std.})(10^{-4})}{Wx} = \%\ \text{dienestrol}$$

RESULTS AND DISCUSSION

Analysis for dienestrol in the commercially available cream[7] was complicated by the presence of a large ratio of monostearin to drug, possibly 2000:1. The exact ratio was unknown as the manufacturer will not supply formulation details. Loss on drying of samples placed an upper limit of about 21% on nonvolatiles. Because the method chosen would likely constitute the official assay for dienestrol cream in the next revision of the NF, a procedure was sought which would allow some variation in cream formulations while preserving specificity and sensitivity for dienestrol. A ratio of monostearin to urea of 4000:1 was decided upon.

It was not possible either to nitrosate or polarograph dienestrol in the presence of such a mass of material, so a complete separation was essential. Attempts to separate dienestrol from monostearin or from the commercial formulation by partition between ether and dilute alkali or alkaline buffers resulted only in the formation of stable gels. Column chromatography can be unreliable for routine analytical methods, especially with such a mass-ratio, so an alternate approach was sought.

[2] Purchased locally.
[3] Matheson Coleman & Bell.
[4] Rotary Evapo-Mix, Buchler Instruments, Fort Lee, N. J.
[5] Metrohm Herisau, Brinkmann Instruments, Polarecord E-261 with E-354 electrode.
[6] Samples may be maintained overnight at these points.
[7] Dienestrol cream, Ortho Pharmaceutical Corp., Raritan, N. J., 0.01% active ingredient; see Fig. 1 for remaining declared ingredients.

Table I—Assay of Dienestrol Cream and Synthetic Mixtures[a]

Sample	% Recovered
Mixtures	96.7
	100.0
	95.6
	95.2
	105.0
	107.9
Av. = 100.1%	
CV = 5.3%	
Creams	99.5
	92.3
	103.8
	87.2
	96.9
	90.0
	90.5
	93.6
Av. = 95.0%	
CV = 5.6%	

[a] Mixtures run at various times; cream samples all from one lot; results read from standard curve.

The application of inclusion chemistry to the removal of unwanted compounds in pharmaceutical analysis has not been reported previously. The authors suggest that this may constitute one general approach to the analysis of ointments, creams, lotions, suppositories, and certain cosmetics. Further work is in progress to estimate the limits of this technique.

Commercial creams and synthetic mixtures containing 0.1 mg. dienestrol, 400 mg. monostearin, 0.1 ml. peanut oil, and 0.2 ml. glycerin were carried through the final procedure (see Table I). The commercial cream assayed with no evidence of interference. Variability was caused equally by the separative and determinative steps.

Isolation of Dienestrol—An extraction step was used to remove water-soluble constituents (see Fig. 1). No emulsions were encountered with 0.025–0.1 *M* pH 6.5 phosphate buffers with either mixtures or commercial cream. Lower dienestrol recoveries (88–96%) were found with 0.025 *M* pH 7.2 buffer and synthetic mixtures of monostearin and drug. For this reason, the dilute pH 6.5 buffer was included in the procedure to extend the method to any neutral formulation. Due largely to the glutamic acid in the commercial samples, pH 4.45 was observed in the aqueous phase after extraction. Formulations for intravaginal application usually are acid-buffered.

Aylward and Wood (3, 4) prepared the monostearin inclusion compound with urea by precipitation from methanol, the most common solvent for the preparation of urea adducts. Monostearin solubility in methanol, however, was low and would have required larger volumes in the procedure, so ethanol was tried and found suitable. The inclusion compound, precipitated from ethanol, melted at 132–133° as is common for these adducts; the IR spectrum confirmed it as the channel-inclusion compound by comparison to the spectrum of a mixture of urea and monostearin in the 17.4:1 mole ratio that was reported as maximal by Aylward. Swern (6) has noted that a 3:1 w/w ratio of urea to included compound appears to apply for a variety of examples. The procedure developed in this study was designed to remove included long-chain compounds representing up to 40% of formulation weight. All of the synthetic mixtures used in these experiments contained 400 mg. monostearin, *i.e.*, a 4000:1 w/w ratio to dienestrol.

To test the yield of adduct, inclusion compounds were made from 0.28- and 0.4-g. samples of monostearin by the usual ethanol-urea step, the supernatants separated and evaporated to dryness; water was added to the residues to dissolve urea, and the insoluble monostearin collected in ether and weighed after evaporation: <3% of the 0.28-g. samples had escaped inclusion, as opposed to 10–20% of the larger samples. Washing the precipitate with ethanol instead of ether–hexane led to lower recoveries of the inclusion compound (89%, for samples averaged).

Recovery of dienestrol separated through the formation of the urea-monostearin inclusion compound was 106 ± 3%. The addition of the silica gel column to the sequence improved the recovery data to 100 ± 2%. Excess urea and any remaining inclusions were retained on the column and no residue was obtained from eluates of synthetic mixtures of drug and monostearin. The column step is not strictly necessary and can be eliminated for samples containing low ratios of monostearin.

The commercial cream also contains benzoic acid. Separate experiments determined that the benzoic acid passes through the entire procedure into the final eluate. Separation from dienestrol could be achieved by extraction at this stage or by neutralizing the solution used to charge the column. However, because benzoic acid was not nitrosated and was polarographically inactive under the conditions of this procedure, no effort was made to remove it.

Peanut oil is an ingredient in the commercial cream, and is not removed by the present procedure. Initial experiments with a longer, 10-g., silica gel bed allowed peanut oil to be eluted preferentially by hexane. Synthetic mixtures containing up to 0.1 ml., or 10% of the sample, of peanut oil were carried through the procedure with no attempt to preferentially elute oil and drug—no loss of dienestrol recovery was observed and slight warming of the nitrosation medium kept the oil in solution. Any formulation containing larger proportions of triglycerides would require a longer column or preliminary hydrolysis. The behavior of triglycerides on silica gel columns is well known and standard procedures are available (8, 9).

Determinative Step—The NF XII assay for dienestrol in tablets uses the polarographic procedure reported by Summa and Graham (10). Dienestrol is nitrosated in a diluted acetic acid and the nitrosophenol subsequently rearranged in ammoniacal buffer to the quinone form which is reduced at the dropping mercury electrode. Gottlieb (11) previously applied this reaction sequence to the spectrophotometric determination of dienestrol, hexestrol, diethylstilbestrol, and related estrogens. Lykken (12) originated the sequence in order to determine total phenols in hydrocarbons and organic solvents by extraction into alkali and he found that it applied to phenols in general. Lykken's 80% acetic acid–1.5% potassium hydroxide nitrosation solvent was retained in the later methods even though the alkali was superfluous.

In this laboratory, this determination gave coefficients of variation of 1.5% at 16 mcg./ml. and 1.8% at 5 mcg./ml. using NF reference standard dienestrol. Diffusion current was linear with respect to dienestrol samples in the range of interest, 1.5–6.0 mcg./ml. in the final solution. Summa and Graham (10) reported linearity at 8–90 mcg./ml. and 1% precision. As sample size was not limiting in this study, the procedure was not adjusted either to smaller volumes or to fewer manipulations.

SUMMARY

Formulations of drugs such as creams, lotions, ointments, and suppositories contain long-chain hydrocarbons, esters, alcohols, fatty acids and monoglycerides. Drugs such as synthetic estrogens, not extractable into water at neutral or acid conditions, present difficult assay problems. For the particular case of dienestrol in the presence of monostearin, urea inclusion has been successfully applied to a test-tube separation of these compounds. As urea includes a variety of long-chain compounds, this may constitute a general approach to the analytical methodology of these formulations.

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TECHNICAL ARTICLES

In Vitro Assessment of Dissolution Kinetics: Description and Evaluation of a Column-type Method

F. LANGENBUCHER

Abstract □ A new method is described for the assessment of the dissolution behavior of solid dosage forms. The method, which is based on the mass transfer between solid and liquid phase in an exchange column, is shown to avoid some disadvantages of the commonly used beaker methods employing fixed liquid volumes. Its usefulness is demonstrated by results obtained with nondisintegrating and uniform granules of benzoic acid in water. The influence of various external parameters, such as liquid flow rate, cell cross-sectional area, amount of material, and particle diameter, is found to agree with theory and literature data. Because of its reproducibility and the absence of arbitrary external parameters, the method seems to be useful for a meaningful study of dissolution kinetics.

Keyphrases □ Dissolution method—column-type apparatus □ Diagram, dissolution apparatus—column-type □ Kinetics, dissolution—nondisintegrating granules □ Parameters affecting—dissolution kinetics

Most *in vitro* studies reported in the literature on the subject of the dissolution behavior of solid drugs involve modifications of the beaker or stirred-tank model, where the drug is dissolved in a fixed volume of solvent liquid, the agitation being accomplished by means of a stirrer or some rocking or shaking action. These methods in general suffer from several important disadvantages.

1. The flow conditions in the liquid medium depend on a great number of external parameters such as diameter and height of the vessel, liquid volume, speed, position, and form of the stirrer, *etc.* Some of these are difficult to standardize and to reproduce in different laboratories. In addition, the influence of these parameters on the dissolution kinetics is difficult to interpret.

2. The liquid volume must be fixed *a priori*, since it essentially determines the dissolution kinetics. It should be chosen as a standard and in accordance with the *in vivo* conditions. Nevertheless, depending on the solubility and dosage of the drug, variable volumes have been used by different authors: whereas Levy (1) originally proposed 300 ml. as standard, volumes as low as 100 ml. (2, 3) and as large as 2 l. (4, 5) or 20 l. (6) have been used.

3. In all beaker methods the drug concentration in the liquid increases from zero up to either the saturation limit or the concentration which corresponds to the completely dissolved drug amount. This concentration buildup is different from the *in vivo* process in which the dissolved material is removed continuously from the liquid by absorption. Gibaldi and Feldman (7) pointed out that dissolution-limited absorption phenomena must be studied by methods in which the liquid acts as a perfect sink, *i.e.*, the concentration never exeeds 10 to 20% of the saturation. To obtain this, the authors use a sufficiently large reservoir of a water-immiscible organic liquid, which permits the dissolved drug to be removed from the aqueous phase. Other authors attempted to achieve the same objective by means of specific adsorbents in the dissolving liquid (8). Although these modifications reduce the critical role of the aqueous volume, they introduce other arbitrary parameters such as volume