Analysis of Oil-Based Pharmaceuticals[1]

EDWARD SMITH, Division of Drug Chemistry, Office of Pharmaceutical Research and Testing, Bureau of Drugs, Food and Drug Administration, Washington, D.C. 20204

ABSTRACT

Oils of animal, mineral and vegetable origin are used in the formulation of pharmaceuticals. In most cases the analysis of these pharmaceuticals requires the separation of the drug substance from the oil components prior to its quantitation, the required degree of separation depending on the specificity of the quantitation method. When the physical properties of the drug substance are quite similar to those of components of the oil, the separation of the drug becomes complex. Column partition chromatography provides an excellent means of separating steroids from the vegetable oil vehicles used in injectables. Both reverse phase and direct phase techniques are applied to the separation of the steroids from the glyceride, sterol and triterpenoid fractions of the oil. Illustrations are provided to demonstrate the application of thin layer chromatography, paper chromatography, gas chromatography, gel permeation chromatography and adsorption chromatography in the analysis of oil solutions, oil-based dermatological preparations and suppositories.

Oils and related substances are used in pharmaceutical preparations because of their solvent properties and their ability to serve as an effective means of administration of the drug for a specified activity in the body. Such pharmaceuticals include depot-injections of steroids, dermatological preparations and suppositories. In addition, volatile oils such as peppermint oil are used as flavors and as active ingredients of pharmaceuticals. These are beyond the scope of this presentation.

Oils of animal, mineral and vegetable origin and products derived from them, e.g., ethyl oleate, glyceryl monostearate and hydrogenated glycerides, are used in the formulation of pharmaceuticals. The analysis of these pharmaceuticals requires, in most cases, separation of the drug substance from the oil components prior to its quantitation. The degree of separation needed will depend on the specificity of the quantitation method. When the physical properties of the drug substance are similar to those of components of the oil, the separation of the drug becomes quite involved.

The FDA, as a regulatory agency, must devise meaningful assay procedures suitable for regulatory control. The procedures should include the following: (a) separation of the drug from any substance which interferes in the quantitation step; (b) a suitable specific identification or differentiation test, or both; and (c) a suitable test for synthesis precursors or degradation products.

The analysis of steroids in oil solution has been a problem for many years. The vehicles commonly used in these preparations are castor, corn, cottonseed, olive, peanut and sesame oils, together with adjuvants such as benzyl benzoate and benzyl alcohol. These oils consist, of course, essentially of the glycerides of fatty acids, with minor amounts of sterols and triterpenoids.

A biological assay was specified in the USP XIV (1) Testosterone Propionate Injection monograph because of the lack of a suitable chemical analysis. The assay in USP XV-XVII (2-4) was a gravimetric measurement of the semicarbazone derivative, based on the procedure of Madigan et al. (5). For samples containing less than 10 mg/ml of oil, a preliminary partition between 90% alcohol and petroleum ether is used to separate the testosterone propionate from the bulk of the oil prior to conversion to the semicarbazone (5). Dry heat sterilization is usually employed in the preparation of these oil injections. During an investigation of this procedure it was found that heated samples yielded higher values due to increased coprecipitation of unidentified material (6). The melting point of the semicarbazone is used as the criterion of identification in this monograph; this does not differentiate between the semicarbazone of testosterone propionate and that of unesterified testosterone.

Quantitative procedures for progesterone and testosterone propionate have been described (7-9) based on colorimetric determination of the 2,4-dinitrophenylhydrazone prepared from the ketosteroid after preliminary liquid-liquid extraction using 90% alcohol and *n*-heptane. Umberger used an adsorbent magnesium silicate (Florisil) for the chromatographic separation of testosterone propionate and progesterone from their oil solution prior to colorimetric determination of their isonicotinylhydrazones. Tappi and coworkers (11) also used the adsorbent magnesium silicate to separate steroids from olive oil.

The application of partition chromatography to the separation of drugs from oil dosage forms was investigated in our laboratories. Our initial work considered only those dosage forms in vegetable oils other than castor oil, because the latter, which consists primarily of the glycerides of hydroxy fatty acids, has properties quite different from those of other vegetable oils. Jones and Stitch (12) previously reported separation of mixtures of steroids in biological material by partition chromatography, using the polar organic solvent nitromethane as immobile phase on a silicic acid support and 3% $CHCl_3$ in petroleum ether as the mobile phase. Wolff (13) in our laboratories isolated progesterone from its oil injection with nitromethane as the immobile phase, using Celite 545 as support and *n*-heptane as the mobile phase. The glycerides and sterols emerged virtually with the solvent front, and the progesterone eluted

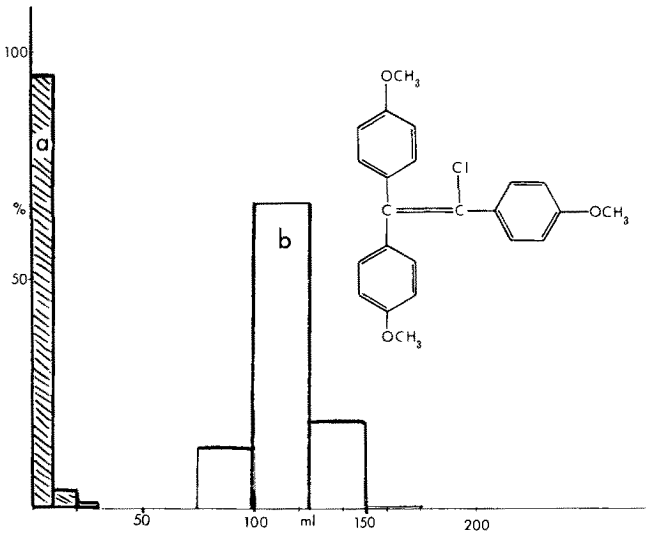

FIG. 1. Separation of chlorotrianisene in corn oil. Column 25 x 300 mm. 7.0 g Celite 545 A.W., 8.0 ml nitromethane; mobile phase *n*-heptane. (a) Sterol and glyceride fractions; (b) chlorotrianisene

[1]One of five papers in the symposium "Fats and Oils in Cosmetics and Pharmaceuticals," presented at the AOCS Meeting, Atlantic City, October 1971

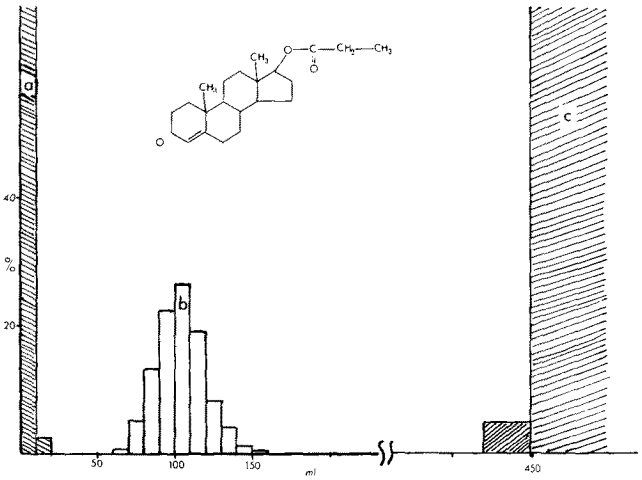

FIG. 2. Separation of testosterone propionate from sesame oil. Column 25 x 300 mm. 8.0 g Celite 545 A.W., 9.0 ml nitromethane; mobile phase *n*-heptane. (a) Sterol fractions; (b) testosterone propionate; (c) sesamin.

after a significant interval. He found that the background UV absorbance of the progesterone fraction from the sterols in the oil was reduced to negligible proportions. This procedure was modified in our laboratory and applied to the assay of chlorotrianisene, a nonsteroidal estrogen, in oil capsules (E. Smith, unpublished data); the modified procedure was adopted as official in NF XII (14). In these separations there is an interval of at least 40 ml between the complete removal of the glyceride and sterol fractions of the oil and the emergence of the desired substance, as shown in Figure 1. Because of lesser differences in polarities, this procedure cannot be adapted directly to the determination of testosterone propionate in oil injectables. Instead of having the large interval, the testosterone propionate follows the elution of the sterol fraction so closely that sharp separation may not be achieved. In chromatographing injections at lower concentrations of testosterone propionate, the large amount of glycerides in the sample required by the assay tends to tail and affect the separation.

Testosterone propionate is effectively separated from the bulk of the oil in these preparations by reverse phase partition chromatography (6). In this system the support, which is rendered hydrophobic by silanization, retains the nonpolar solvent as the immobile phase. A polar solvent, 90% alcohol, may be used as the mobile phase. The glycerides, which constitute the major portion of the oil, are retained in the immobile phase, while the eluate contains the sterols and triterpenoid fractions of the oil, together with the testosterone propionate. Any free testosterone which may be present in the sample would also appear in this eluate. The final separation of the testosterone propionate from the sterols and triterpenoids of the oil, as well as free testosterone, is achieved with the Celite-nitromethane column, using *n*-heptane as mobile phase. Figure 2 illustrates this separation. The sterols are removed completely by the first 15 ml of *n*-heptane. A distinct interval of ca. 30 ml then follows before the appearance of testosterone propionate in the eluate. The triterpenoids, such as sesamin in sesame oil, are not eluted with the volume of *n*-heptane specified, but are retained on the Celite-nitromethane column. These polar compounds would emerge only after 400 ml of eluant. Unesterified testosterone is also retained on this column.

The reverse phase chromatographic separation has been incorporated in the USP XVIII (15) assays of androgenic steroids in oil injections. The steroid fractions are quanti-

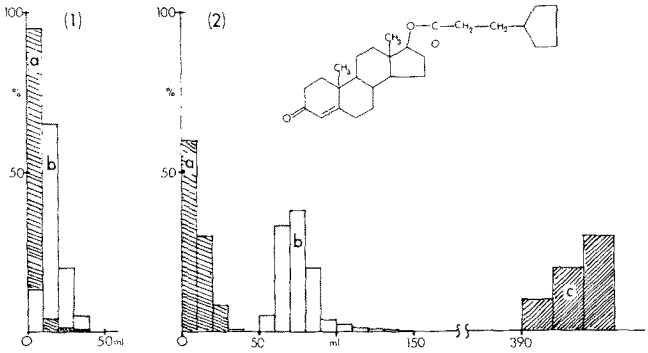

FIG. 3. Separation of testosterone cypionate from sesame oil. Column 25 x 300 mm. (1) 15.0 g Celite 545 A.W., 17 ml nitromethane; (2) 15.0 g Celite 545 A.W., 17 ml nitromethane-90% methanol 1:1, mobile phase *n*-heptane. (a) Sterol and glyceride fractions; (b) testosterone cypionate; (c) sesamin.

of the sterol and triterpenoid fractions nor adjuvants is necessary.

Testosterone cypionate and testosterone enanthate are not amenable to separation from oil by the Celite-nitromethane column procedure. From their structure one could predict that they would be relatively more nonpolar than testosterone propionate and close in polarity to the sterols. They are eluted together with the bulk of the oil from a Celite-nitromethane column. By changing the stationary phase to a mixture of nitromethane and 90% methanol 1:1 and employing a longer Celite column (15 g), these esters of testosterone are completely separated from all fractions of the oil (Fig. 3). Because these two esters are administered in relatively high doses (50-200 mg/ml), an analytical sample will contain only a small amount of oil; therefore the preliminary reverse phase clean-up is not required.

The esters of estradiol which are administered as oil injections have the 17-hydroxy or the 3-phenolic hydroxy, or both, esterified. Only the benzoate and propionate phenol esters are marketed, the latter being the di-ester. The estradiol esters are relatively more polar than the androgenic and progestrogenic esters. The effect on the polarity of the compound by the change in the identity of the fatty acid moiety of the 17-hydroxy ester is predictable; the longer the chain the greater the decrease in polarity with resultant increased rate of elution from a nitromethane column. The optimum separation of the estrogenic esters from their oil solutions by partition chromatography is illustrated in Figures 4-7. As with the androgen esters, a stationary phase of nitromethane-90% methanol is required for the least polar esters (E. Smith, unpublished data). Compounds of like polarity, i.e., estradiol valerate and estradiol isovalerate, and, as previously mentioned, testosterone cypionate and testosterone enanthate, will show no difference in their elution behavior.

The estrogenic steroid esters are administered at much lower levels than the androgens, usually at levels of 0.1-10 mg/ml, compared to 10-200 mg/ml for the androgens (although one product, estradiol valerate, is available at the 20 and 40 mg/ml levels). Therefore most analytical methods for these incorporate colorimetry, fluorimetry or gas chromatography for the determinative step, rather than a UV quantitation.

The USP XVIII (16) assay for Estradiol Valerate Injection does not incorporate a separation step. It applies differential UV directly to the sample solution. The quantitation depends on the difference in absorbance of the phenolate and the free phenol forms of the compound. The NF XIII (17) method for Estradiol Cypionate Injection utilizes a shakeout procedure with 85% ethanol and hexane to separate the steroid from the bulk of the oil prior to

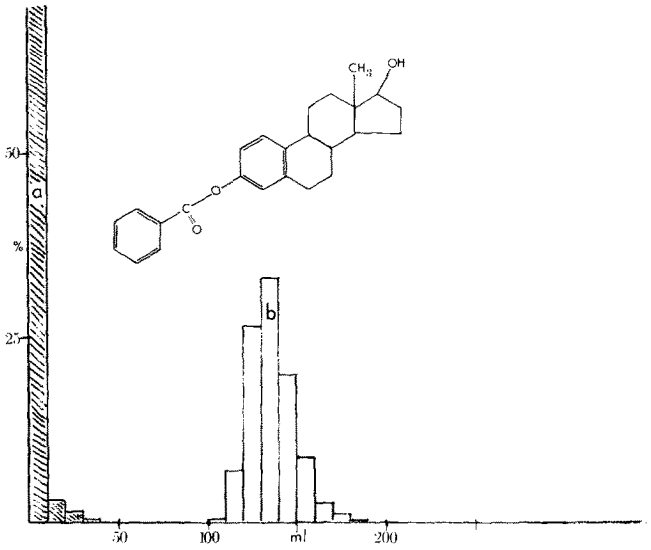

FIG. 4. Separation of estradiol benzoate from sesame oil. Column 25 x 300 mm. 8.0 g Celite 545 A.W., 9.0 ml nitromethane, mobile phase *n*-heptane. (a) Sterol and glyceride fractions; (b) estradiol benzoate.

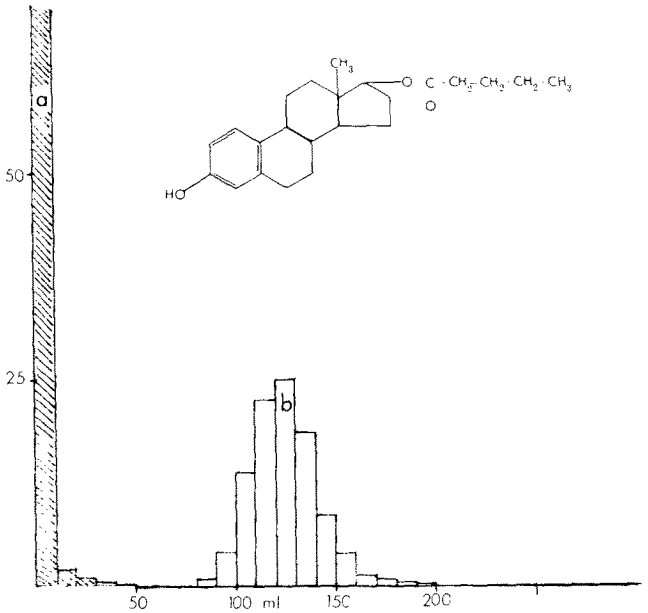

FIG. 5. Separation of estradiol valerate from sesame oil. Column 25 x 300 mm. 10.0 g Celite 545 A.W. 11.0 ml nitromethane, mobile phase *n*-heptane. (a) Sterol and glyceride fractions; (b) estradiol valerate.

Injection (18), the sample is treated with base to hydrolyze the phenolic ester prior to colorimetric measurement. In the Estradiol Benzoate Injection monograph (19) the steroid is separated from the vehicle by adsorption chromatography on dry Silica Gel G with benzene-ethyl acetate as the mobile solvent, prior to hydrolysis. Banes (20) separated diethyl stilbestrol, a synthetic estrogen, from its oil solutions as its phenolate by partitioning it between isooctane and 1N NaOH, prior to quantitation as its photochemical isomerization product.

The NF XIII procedure for Progesterone Injection (21) is simply a gravimetric determination of the progesterone dinitrophenylhydrazone formed without prior separation from the oil. The USP XVIII procedure for Hydroxyprogesterone Caproate Injection (22), and the NF XIII monographs for Nandrolone Deconate (23) and Nandrolone Phenpropionate (24) Injections are based on the colorimetric measurement of the isonicotinylhydrazone, again formed without prior separation from the oil.

Hydroxyprogesterone caproate and estradiol valerate injections in which castor oil is the vehicle are also marketed. Castor oil, comprised primarily of the glycerides of hydroxy fatty acids, has quite different properties from those of other vegetable oils commonly used as the vehicle. Consequently it is not amenable to the partition chromatographic procedures described. Because castor oil is soluble in methanol, the isonicotinylhydrazone and differential UV procedures in the official compendia can be applied directly to solutions of the injections.

Paper chromatography has been applied to the separation of steroids from oil solutions. Roberts and coworkers (25) utilized this technique to isolate estradiol valerate from castor oil; the isolated steroid was determined spectrophotofluorimetrically. Hydroxyprogesterone caproate in castor oil and testosterone enanthate in sesame oil were colorimetrically quantitated as their isonicotinylhydrazones after isolation from their chromatogram (26). Paper chromatography has been applied to the separation of nandrolone decanoate (27) from its oil solutions prior to colorimetric determination as its isonicotinylhydrazone. Talmadge and coworkers (28) used paper chromatography to separate quingesterone from its decomposition products, progesterone and 6α- and 6β-hydroxyprogesterone, in its oil solutions. In all of these paper chromatographic methods, the separate spots were cut out and the steroid was

thin layer chromatography (TLC) to separate steroids from their oil solutions. Bican-Fister (29) colorimetrically determined progesterone and testosterone propionate after extracting the isolated zones from the TLC plate. For testosterone propionate, progesterone, 19 nor-testosterone propionate and estradiol cypionate at levels of 10 mg/ml or greater, Cavina and Moretti (30) subjected the sample to continuous ascending chromatography for up to 8 hr. The area of the separated steroid is extracted with chloroform and then quantitated by UV or colorimetrically as the isonicotinylhydrazone. For lower level preparations of estradiol dipropionate and estrone-3-benzoate, the isolated spots are saponified without prior removal from the adsorbent (31). The steroid is then extracted and determined colorimetrically or by gas liquid chromatography (GLC) as its trimethylsilyl ether (31). The same TLC procedure was also applied by these workers (32) to two monoesters of estradiol, estradiol-3-benzoate and estradiol-17 β-cypionate, at levels of 2 mg/ml of oil. In a recent paper (33) they omitted the thin layer chromatographic separation for samples at levels of 10 mg/ml or greater; they separated the steroids by partition between 80% alcohol and hexane and applied their GLC procedure directly to the extract. In a later paper these workers (34) utilized gradient elution on a silicic acid column to separate the steroids from their oil solutions. This was followed by a UV or GLC quantitation. They observed effects of the ester structure change on the order of elution similar to those we observed in our partition procedures.

Talmadge and coworkers (35) quantitated ethinyl estradiol by GLC after extracting it from its oil solution by first partitioning it between heptane and NaOH solution and then back extracting it with chloroform from the acidified extract.

Penner et al. (36) recently reported a procedure which provides selective adsorption of 17α-ethynyl steroids on silver nitrate-impregnated Florisil to separate it from its oil vehicle. After displacement with ethanolic ammonium chloride the 17α-ethynyl steroid, quingestanol acetate, was determined spectrophotometrically.

The vehicles employed for the formulation of ointments, creams and similar preparations are usually vegetable and

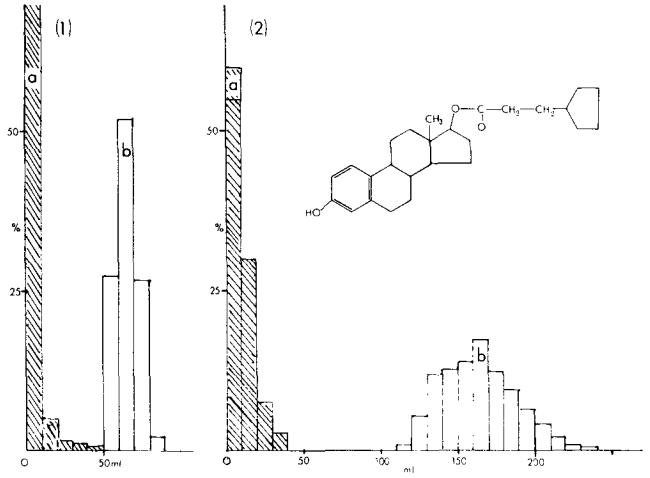

FIG. 6. Separation of estradiol cypionate from sesame oil. Column 25 x 300 mm. (1) 8.0 g Celite 545 A.W., 9.0 ml nitromethane; (2) 10.0 g Celite 545 A.W., 12.0 ml nitromethane-90% methanol 1:1, mobile phase *n*-heptane. (a) Sterol and glyceride fractions; (b) estradiol cypionate.

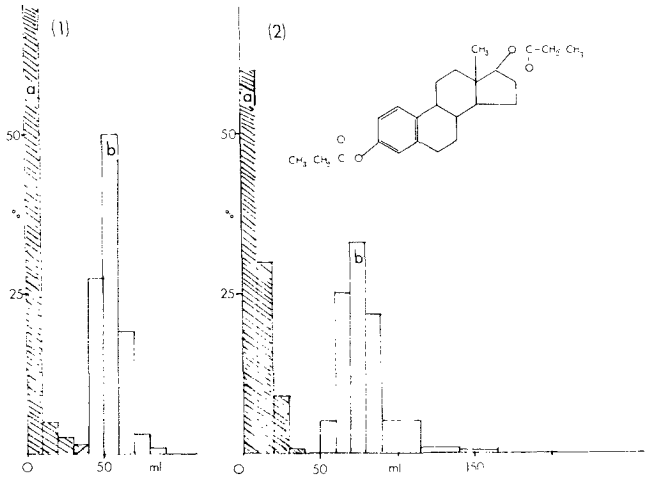

FIG. 7. Separation of estradiol dipropionate from sesame oil. Column 25 x 300 mm. (1) 8.0 g Celite 545 A.W., 9.0 ml nitromethane; (2) 15.0 g Celite 545 A.W., 17.0 ml nitromethane-90% methanol 1:1, mobile phase *n*-heptane. (a) Sterol and glyceride fractions; (b) estradiol dipropionate.

forms is predicated upon the nature of the vehicle (hydrophilic or hydrophobic) and upon the chemical nature of the drug substance. As would be expected, the separation of drugs with either basic or acidic characteristics from the vehicle is relatively straightforward. Basic drugs can be separated from lyophilic vehicles by partitioning between aqueous acid and an immiscible solvent. The drug is extracted as its salt into the aqueous acid and the oil base into the solvent layer. This mode of separation is employed in the NF XIII assays for such products as benzocaine ointment, dibucaine cream and tetracaine ophthalmic ointment (37). In the case of a hydrophilic ointment the process is reversed: the basic drug is extracted as the free base by an immiscible organic solvent while the vehicle remains in the aqueous phase. This process is the basis of the USP XVIII assay of lidocaine ointment and lidocaine jelly (38).

More complex separation is required for drug combinations. The AOAC procedure for benzoic and salicylic acid ointment (39) utilizes a two column partition chromatographic system in which ferric chloride-urea and sodium bicarbonate solutions are used as the stationary phases. A $CHCl_3$ solution of the ointment is passed over these columns; salicylic acid is retained as its ferric-phenol complex on the first column and benzoic acid is retained as its sodium salt on the second column, while the ointment base passes through. The salicylic and benzoic acids are recovered from the individual columns after acidification of the stationary phases in situ with a solution of acetic acid in chloroform.

Two general analytical schemes are used for the analysis of antibiotics in oil bases. If the antibiotic is in a hydrophilic vehicle it is blended with a buffer or aqueous acid with the acid of polysorbate 80. If it is in a lyophilic vehicle a preliminary liquid-liquid extraction step is used whereby the ointment or oil vehicle is retained in the organic phase and the antibiotic is extracted into the appropriate buffer or acidic aqueous phase. The official procedures apply a microbiological assay to the extracts (40). Van Giessen and Tsuji (41) recently reported a GLC method for neomycin in petrolatum-based ointments. The ointment base is dissolved in chloroform and the neomycin is removed by centrifugation.

As in the case of oil injectables of neutral compounds, separation procedures for corticosteroids in ointments, creams and related preparations are more complex. As with monographs for Hydrocortisone and Hydrocortisone Acetate (42) the ointment or cream is heated with alcohol to dissolve the steroid and part of the base. The solution is cooled, which congeals the base, and the alcohol solution is decanted. The steroid in the alcohol solution is measured colorimetrically with blue tetrazolium. This procedure cannot be applied to mastitis preparations because they usually contain other drugs, including antibiotics and sulfonamides, together with the corticosteroid. Bracey et al. (43) applied partition chromatography to isolate hydrocortisone and hydrocortisone acetate from these preparations. The sample mixed with dry Celite is packed on a column employing a methanol-water stationary phase over a sodium bicarbonate solution trap layer. The interfering oil fractions are eluted first with methylene chloride-isooctane (1-9). The steroid is then eluted with methylene chloride, leaving trapped on the sodium bicarbonate the other substances which would cause interference in the blue tetrazolium quantitation step.

Adsorption chromatography is used in the monograph for flurandrenolide cream and ointment (44). The ointment is extracted with hot alcohol as above. The alcohol is diluted with water and the steroid is extracted with chloroform. The cream is simply dissolved in chloroform and filtered over sodium sulfate. The respective chloroform solutions are then passed over a chromatographic magnesium silicate column which retains the steroid while the vehicle is eluted. Finally the steroid is eluted with a 1:19 solution of alcohol in chloroform.

Another technique, gel permeation chromatography, was applied by Cosi and Bichi (45) to separate fluocinolone acetonide from its ointment base. They utilized a Sephadex LH 20 column with chloroform as the solvent. Levorato (46) separated corticosteroids from their vehicles in creams, ointments and lotions by thin layer chromatography on Silica Gel GF prior to UV or colorimetric determination.

Most drugs formulated in oil base suppositories are either acidic or basic in nature; therefore the problems of isolation are identical with those in lyophilic ointments. Monographs for amines such as aminophylline (47), chlorpromazine (48), bisacodyl (49) and prochlorperazine (50) utilize the same extraction procedure which is applied to ointments, wherein the drug is separated from the vehicle by partition between ether and aqueous acid. The phenolic compound diethylstilbestrol is separated from oleaginous suppositories with NaOH as its phenolate from an isooctane

is passed over a partition chromatographic column containing sodium bicarbonate solution as the stationary phase. The vehicle is eluted, while aspirin is trapped as the sodium salt on the column. The aspirin is then eluted after acidification of the column in situ with a solution of acetic acid in chloroform.

Again, just as with the ointments, the separation of drug combinations in suppositories requires more complex operations. The procedure for the analysis of ergotamine tartrate and caffeine suppositories (53) applies partition between ether and a tartaric acid solution to separate the alkaloids from the suppository vehicle. The alkaloids are then extracted with chloroform and subjected to partition chromatography over a column containing citric acid solution on Celite. The caffeine is eluted with chloroform while the ergotamine is retained on the column. The latter is extracted with chloroform as the free base from the extruded Celite mass.

Cometti and coworkers (54) recently reported on gas chromatographic analysis of multicomponent suppositories. Depending on the composition of the vehicle, absolute ethanol, chloroform or a mixture of chloroform and alcohol-containing internal standards is added to the melted suppository to dissolve the sample. The hot solution is injected onto the gas chromatograph.

ACKNOWLEDGMENTS

L. Wong, C.J. Reamer, M. Sharkey and B. Ross helped in the study of the analysis of steroids in oil injectables; and J. Levine contributed valuable suggestions. All are members of the Division of Drug Chemistry.

REFERENCES

1. "United States Pharmacopeia," 14th Revision, Mack Publishing Co., Easton, Pa., 1950, p. 609.
2. Ibid., 15th Revision, Mack Publishing Co., Easton, Pa., 1955, p. 717.
3. Ibid., 16th Revision, Mack Publishing Co., Easton, Pa., 1960, p. 738.
4. Ibid., 17th Revision, Mack Publishing Co., Easton, Pa., 1965, p. 700.
5. Madigan, J.J., E.E. Zenno and R. Pheasant, Anal. Chem. 23:1691 (1951).
6. Smith, E., J. Pharm. Sci. 56:630 (1967).
7. Vieira de Abreu, M.M., Rev. Port. Farm. 13:141 (1963).
8. Diding, E., Svensk Farm. Tidskr. 56:1 (1952).
9. Sonderstrom, K., J. Pharm. Belg. 10:379 (1955).
10. Umberger, E.J.,Anal. Chem. 27:768 (1955).
11. Tappi, G., E.M. Andreoli and E. Frea, Pharm. Weekblad 93:231 (1958).
12. Jones, J.K.N., and S.R. Stitch, Biochem. J. 53:679 (1953).
13. Wolff, J., J. Pharm. Sci. 52:93 (1963).
14. "The National Formulary," 12th Edition, Mack Publishing Co., Easton, Pa. 1965, p. 92.
15. "United States Pharmacopeia," 18th Revision, Mack Publishing Co., Easton, Pa., 1970, p. 710.
16. Ibid., 18th Revision, Mack Publishing Co., Easton, Pa., 1970, p. 242.
17. "The National Formulary," 13th Edition, Mack Publishing Co., Easton, Pa., 1970, p. 284.
18. Ibid., 13th Edition, Mack Publishing Co., Easton, Pa., 1970, p. 286.
19. Ibid., 13th Edition, Mack Publishing Co., Easton, Pa., 1970, p. 281.
20. Banes, D., J. Ass. Offic. Anal. Chem. 43:249 (1960).
21. "The National Formulary," 13th Edition, Mack Publishing Co., Easton, Pa., 1970, p. 599.
22. "United States Pharmacopeia," 18th Revision, Mack Publishing Co., Easton, Pa., 1970, p. 320.
23. "The National Formulary," 13th Edition, Mack Publishing Co., Easton, Pa., 1970, p. 68.
24. Ibid., 13th Edition, Mack Publishing Co., Easton, Pa., 1970, p. 471.
25. Roberts, H.R., and M.R. Siino, J. Pharm. Sci. 52:370 (1963).
26. Roberts, H.R., and K. Florey, Ibid. 51:794 (1962).
27. Drug Standards Laboratory, Ibid. 53:98 (1964).
28. Talmadge, J.M., M.H. Penner and M. Geller, Ibid. 53:76 (1964).
29. Bican-Fister, T., J. Chromatogr. 22:465 (1966).
30. Cavina, G., and G. Moretti, Ibid. 22:41 (1966).
31. Cavina, G., G. Moretti and J. Sardi de Valverde, Ann. Inst. Super. Sanita 4:75 (1968).
32. Moretti, G., G. Cavina and J. Sardi de Valverde, J. Chromatogr. 40:410 (1969).
33. Cavina, G., G. Moretti and P. Siniscalchi, Ibid. 47:186 (1970).
34. Cavina, G., G. Moretti, A. Mollica and R. Antonini, Farmaco Ed. Prat. 26:275 (1971).
35. Talmadge, J.M., M.H. Penner and M. Geller, J. Pharm. Sci. 54:1194 (1965).
36. Penner, M.H., D.C. Tsilifonsis and L. Chafetz, Ibid. 60:1388 (1971).
37. "The National Formulary," 13th Edition, Mack Publishing Co., Easton, Pa., 1970, p. 79, 224, 690.
38. "United States Pharmacopeia," 18th Revision, Mack Publishing Co., Easton, Pa., 1970, p. 365.
39. Weber, J.D., J. Ass. Offic. Anal. Chem. 48:1151 (1965).
40. Code of Federal Regulations, Title 21—Food and Drugs, Parts 141-149, U.S. Government Printing Office, Washington, D.C., 1971.
41. Van Giessen, B., and K. Tsuji, J. Pharm. Sci. 60:1068 (1971).
42. "United States Pharmacopeia," 18th Revision, Mack Publishing Co., Easton, Pa., 1970, p. 307, 309.
43. Bracey, A., L. Garrett and P.J. Weiss, J. Pharm. Sci. 55:1113 (1966).
44. "The National Formulary," 13th Edition, Mack Publishing Co., Easton, Pa., 1970, p. 316.
45. Cosi, G., and G.L. Bichi, Farmaco Ed. Prat. 25:248 (1970).
46. Levorato, C., Ibid. 24:227 (1969).
47. "United States Pharmacopeia," 18th Revision, Mack Publishing Co., Easton, Pa., 1970, p. 34.
48. Ibid., 18th Revision, Mack Publishing Co., Easton, Pa., 1970, p. 124.
49. "The National Formulary," 13th Edition, Mack Publishing Co., Easton, Pa., 1970, p. 99.
50. Ibid., 13th Edition, Mack Publishing Co., Easton, Pa., 1970, p. 597.
51. "United States Pharmacopeia," 18th Revision, Mack Publishing Co., Easton, Pa., 1970, p. 189.
52. Ibid., 18th Revision, Mack Publishing Co., Easton, Pa., 1970, p. 54.
53. "The National Formulary," 13th Edition, Mack Publishing Co., Easton, Pa., 1970, p. 268.
54. Cometti, A., G. Bagnasco and N. Maggi, J. Pharm. Sci. 60:1074 (1971).

[Received December 11, 1971]