250- or 500-mg capsules as completely as possible, the recovery appeared to be satisfactory (Table VI). Another advantage of the present method is the ease of performing the assay. The fluorescamine reagent is stable for several weeks. In contrast, the USP XVIII method for the capsules requires freshly prepared sodium nitrite solutions.

REFERENCES

(1) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, p. 542.

(2) *Ibid.*, p. 543.

(3) M. Weigele, J. F. Blount, J. P. Tengi, R. C. Czaijkowski, and W. Leimgruber, *J. Amer. Chem. Soc.*, **94**, 4052(1972).

(4) M. Weigele, S. L. DeBernardo, J. P. Tengi, and W. Leimgruber, *ibid.*, **94**, 5927(1972).

(5) P. Böhlen, S. Stanley, W. Dairman, and S. Udenfriend, *Arch. Biochem. Biophys.*, **155**, 213(1973).

ACKNOWLEDGMENTS AND ADDRESSES

Received September 30, 1974, from the *College of Pharmacy University of Cincinnati, Cincinnati, OH 45221*

Accepted for publication November 29, 1974.

Presented at the Pharmaceutical Analysis and Control Section, APhA Academy of Pharmaceutical Sciences, New Orleans meeting, November 1974.

Supported in part by a Summer Research Fellowship Grant from the University of Cincinnati.

The authors thank E. R. Squibb and Sons, Princeton, N.J., for supplying the procainamide hydrochloride used in this work.

[x] To whom inquiries should be directed.

GLC Determination of 17α-Ethynylestriol 3-Cyclopentyl Ether

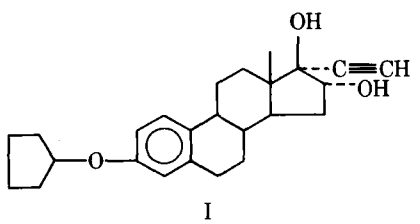
RAFIK H. BISHARA [x], BONNIE S. RUTHERFORD, and ALAN DINNER

Abstract □ A rapid, sensitive, and accurate GLC method of analysis of a new estrogenic drug, 17α-ethynylestriol 3-cyclopentyl ether, was developed. The drug and the internal standard, tetratriacontane, are dissolved in chloroform, and an aliquot is heated with *N*-trimethylsilylimidazole at 80° for 30 min. The silylated sample is chromatographed using a column packed with 1% methyl vinyl silicone gum on Gas Chrom Q. Quantitation is achieved by computer calculation of the peak area ratios. The observed peak is the 16α,17β-bistrimethylsilyl derivative of the new drug substance. The GLC method was applied to the quantitative determination of the estrogenic compound in a tablet formulation containing 25 μg/tablet.

Keyphrases □ 17α-Ethynylestriol 3-cyclopentyl ether—GLC analysis as trimethylsilyl derivative □ Estrogens—GLC analysis of 17α-ethynylestriol 3-cyclopentyl ether □ GLC—analysis, 17α-ethynylestriol 3-cyclopentyl ether

A new potent estrogenic hormone, 17α-ethynylestriol 3-cyclopentyl ether (I), useful in the treatment of spontaneous or induced menopausal syndrome, estrogen deficiency, or conditions when estrogen may be used therapeutically, has been synthesized (1, 2).

In the process of developing a GLC method for analyzing I, the literature concerning the GLC determination of estrogens was screened. Numerous publications deal with the GLC determination of estriol, both as the pure compound (3–6) and in biological materials (7–29). Although there are several reports of the GLC of free estriol (4, 5, 15, 17, 27), the use of estriol derivatives is preferred to prevent irreversible adsorption on the column and/or thermal decomposition of the underivatized hormone (30, 31). Higher volatility of the steroid derivative results in a shorter retention time, sharper peak, and improved sensitivity (31, 32).



I

The trimethylsilyl (21, 22, 25, 28, 33), acetate (19, 23, 24, 29, 33), heptafluorobutyrate (14, 34), and trifluoroacetate (6, 35, 36) derivatives are the most frequently used. The advantages of the trimethylsilyl derivatives relative to the acetate derivatives were discussed (37, 38). Other derivatives that were also reported for the GLC determination of estriol include the 3-methyl ether (39, 41), monochloroacetate (42), chloromethyldimethyl ether (43), chlorodifluoroacetate (43), 3-methyl-16,17-diacetate (44–46), 3-methyl-16,17-diheptafluorobutyrate (7), and 3-methyl-16,17-ditrimethylsilyl (13, 20, 39, 44) and iodomethyldimethylsilyl ethers (47). Several reported procedures were tried with no success for the analysis of I in this study. The purpose of this paper is to report the trimethylsilyl derivatization and GLC conditions for the quantitation of I.

EXPERIMENTAL

Equipment—A gas chromatograph[1] equipped with a flame-ionization detector was used. The detected signals were fed to a computer[2] for peak area integration and to a potentiometric recorder[3] with a chart speed of 38.1 cm (15 in.)/hr and a 1-sec full-scale response. Helium, with a flow rate of 60 ml/min, was the carrier gas. The flow rates of hydrogen and oxygen were adjusted to optimum sensitivity and low noise background in the electronic integrator and were found to be 40 and 300 ml/min, respectively.

[1] F&M model 402, Hewlett-Packard, Avondale, Pa.
[2] Hewlett-Packard 2100.
[3] Honeywell Electronic 16, Honeywell, Philadelphia, Pa.

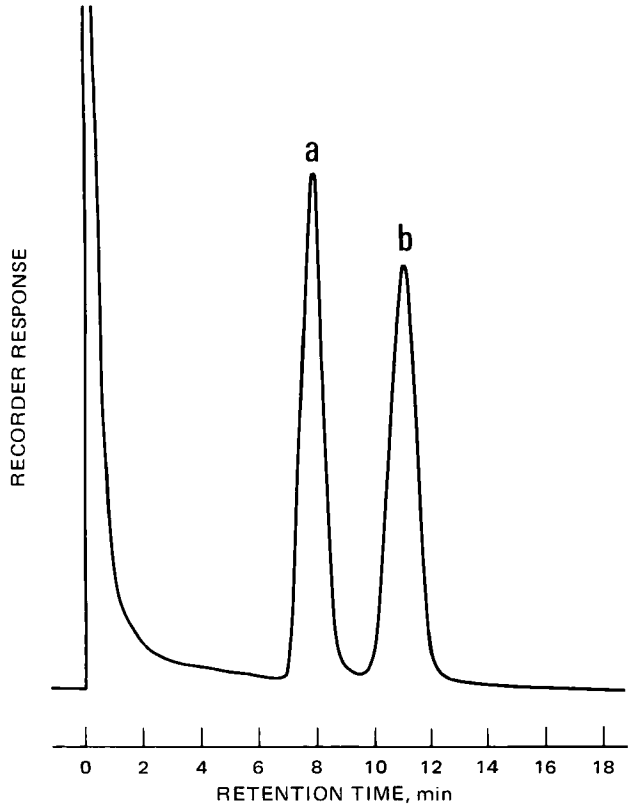


Figure 1—*Typical gas chromatogram of trimethylsilyl-derivatized Compound I. Key: a, 3-(cyclopentyloxy)-16α,17β-bis[(trimethylsilyl)oxy]-19-nor-17α-pregna-1,3,5(10)-trien-20-yne; and b, tetratriacontane.*

The U-shaped glass column (61 cm × 3 mm i.d.) was packed with 1% methyl vinyl silicone gum (UC-W98) on 80–100-mesh Gas Chrom Q[4]. The operating temperatures were: injection port, 260°; column, 225° (isothermal); and detector, 280°. All injections were made using a 10-μl syringe[5].

Reagents—The silylating reagent was *N*-trimethylsilylimidazole[6]. For the tablet assay, the reagent was diluted 1:5 with methylene chloride[7] previously dried over anhydrous sodium sulfate. The reference standard solution was prepared in chloroform[8]. Spectroquality carbon tetrachloride[9] was used for extracting the tablets.

Internal Standard Solution—*n*-Tetratriacontane[10], 2.4 mg/ml in chloroform, was used.

Reference Standard Solution—A I solution containing 2.0 mg/ml of chloroform was prepared. One-milliliter aliquots of the reference standard and internal standard solutions were pipetted and mixed together in a small screw-capped vial sealed with a Teflon septum. One milliliter of the silylating reagent was added by a tuberculin syringe[11], and the vial was heated for 30 min at 80° in a constant-temperature heating block[12]. The vials were then removed from the heating block, cooled to room temperature, and submitted for GLC.

Raw Material Solution—The raw material sample was prepared exactly as the reference standard solution.

Tablet Solution—The average tablet weight of 20 tablets was determined. A portion of the powdered tablet material, equivalent to 25 μg of I, was suspended in 10 ml of 0.05 *N* hydrochloric acid and extracted with three 30-ml portions of carbon tetrachloride.

[4] Custom-made nontested packing, Applied Science Inc., State College, Pa.
[5] Hamilton No. 701, Reno, Nev.
[6] Pierce Chemical Co., Rockford, Ill.
[7] ACS reagent, Matheson, Coleman and Bell, Norwood, Ohio.
[8] Analytical reagent, Mallinckrodt, St. Louis, Mo.
[9] Matheson, Coleman and Bell, Rockford, Ill.
[10] Analabs, North Haven, Conn.
[11] B-D Yale tuberculin, Becton, Dickson and Co., Rutherford, N.J.
[12] Temp-Block module heater, holes with 1.5-cm diameter, Lab-Line Instruments, Melrose Park, Ill.

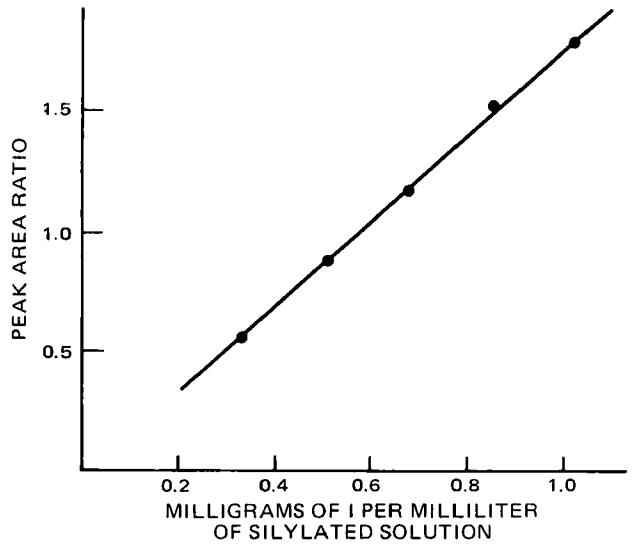


Figure 2—*Calibration curve demonstrating linear response of different amounts of trimethylsilyl Compound I to a constant concentration of tetratriacontane.*

One milliliter of internal standard solution (25 μg/ml) was added, and the solution was evaporated to near dryness. Then the remaining solution was transferred quantitatively to a small glass vial[6], 3.5-ml capacity, and evaporated.

A 0.25-ml aliquot of the silylating mixture was added to the residue, and the vial was sealed and heated as described for the reference standard. The reference standard solution used for the tablet assay was 25 μg/ml. One milliliter of this solution was mixed with 1 ml of the internal standard solution (25 μg/ml) in a small glass vial. After evaporation, 0.25 ml of the silylating mixture was added; then the vial was sealed and heated.

GLC—Four 3-μl portions of the standard solution were injected into the chromatograph to prime the column, and the instrument parameters were adjusted to obtain optimum response of the two peaks. The retention time was approximately 8.0 min for silylated I and 11.0 min for the internal standard, tetratriacontane.

When optimum conditions were obtained, duplicate injections were made of the standard solution, the sample solution, and the standard solution again. The peak areas obtained from the electronic integrator were used for the calculation of the percent purity of the raw material sample as shown in Eq. 1. Tablet potency was calculated as in Eq. 2.

GLC–Mass Spectrometry—A 70-ev electron-impact mass spectrum of the observed GLC peak was obtained[13].

Calculation—The ratio of the sample peak to the internal standard peak is calculated for each injection, and the duplicate ratios are averaged:

$$\%\ \text{purity} = \frac{R_{sa}}{R_{std}} \times \frac{\text{standard weight (mg)}}{\text{sample weight (mg)}} \times \text{standard purity} \times 100 \quad \text{(Eq. 1)}$$

where R_{sa} is the ratio of the silylated I peak area to tetratriacontane peak area in the sample solution, and R_{std} is the average ratio of the silylated I peak area to the tetratriacontane peak area of the standard solution before and after sample injections.

The potency of I in micrograms per tablet is calculated as follows:

$$\text{micrograms per tablet} = \frac{R_{sa}}{R_{std}} \times \frac{\text{standard weight } (\mu g)}{\text{sample weight (mg)}} \times \text{standard purity} \times \text{average tablet weight (mg)} \quad \text{(Eq. 2)}$$

where R_{sa} and R_{std} are defined as in Eq. 1.

[13] LKB-9000S gas chromatograph–mass spectrometer, LKB-Produkter AB, Bromma, Sweden.

Table I—Identity and Chemical Names of Some Closely Related Steroids to Compound I

Compound Identity[a]	Chemical Name
P	3-Hydroxyestra-1,3,5(10)-trien-17-one (estrone)
P	Estra-1,3,5(10),16-tetraene-3,17-diol diacetate
P	16α,17α-Epoxyestra-1,3,5(10)-triene-3,17β-diol diacetate
P	3,16α-Dihydroxyestra-1,3,5(10)-trien-17-one diacetate
P	19-Nor-17α-pregna-1,3,5(10)-trien-20-yne-3,16α,17-triol
P	19-Norpregna-1,3,5(10)-trien-20-yne-3,16α,17α-triol
P	16α,17α-(Isopropylidenedioxy)-19-norpregna-1,3,5(10)-trien-20-yn-3-ol
PDP	3-(Cyclopentyloxy)-17β-hydroxyestra-1,3,5(10)-trien-16-one
PDP	3-(Cyclopentyloxy)-2',3'-dihydroxyestra-1,3,5(10)-trieno-(16α,17α-*b*)-furan-17β-ol
RFS	3-Methoxy-19-nor-17α-pregna-1,3,5(10)-trien-20-yn-17-ol
RFS	19-Nor-17α-pregna-1,3,5(10)-triene-3,16α,17-triol
RFS	3-(Cyclopentyloxy)-16α,17α-(isopropylidenedioxy)-19-norpregna-1,3,5(10)-trien-20-yne

[a] P = synthetic precursor, PDP = probable degradation product, and RFS = related foreign steroids.

RESULTS AND DISCUSSION

A typical gas chromatogram is shown in Fig. 1. The first peak is the trimethylsilyl derivative of I, which is identified as 3-(cyclopentyloxy)-16α,17β-bis[(trimethylsilyl)oxy]-19-nor-17α-pregna-1,3,5(10)-trien-20-yne (II) by GLC–mass spectrometry. The mass spectrum of II shows a molecular ion at *m/e* 524, indicating silylation of the two hydroxy groups on ring D at the 16- and 17-positions. The observed fragmentation pattern is analogous to that described for the trimethylsilyl ether derivatives of estriol and other related steroids (48, 49). The second peak in the chromatogram is tetratriacontane, the internal standard. Relative to the internal standard peak, the retention time of II is 0.73.

Figure 2 shows the linear relationship of various amounts of I (0.34–1.02 mg/ml) to a constant amount of tetratriacontane (0.8 mg/ml).

Twelve related steroids were chromatographed using this method. Table I shows the chemical names of these related compounds and identifies them as precursors, possible degradation products, or related foreign steroids. All 12 tested compounds showed shorter retention times than that of II. Thus, the synthetic precursors and the known degradation products of I do not interfere with its GLC analysis.

Duplicate injections of five different raw material samples by a single analyst give a standard deviation of 0.004 and a relative standard deviation (*SD*/mean × 100) of 0.322%. The accuracy and precision of the tablet assay were determined by analyses of five replicate samples. A relative standard deviation of 0.80% and a relative error of −0.20% were obtained.

Aged and degraded samples of tablets containing I were evaluated by this method. The assay values show a decrease on aging for 1 year at high temperature (50°). No interferences or extraneous peaks were observed on the chromatograms.

REFERENCES

(1) N. J. Bach and E. Farkas, U.S. pat. 3,697,558 (1972).

(2) R. J. Kraay and E. Farkas, U.S. pat. 3,790,605 (1974).

(3) H. H. Wotiz and H. F. Martin, *J. Biol. Chem.*, **236,** 1312(1961).

(4) J. C. Touchstone, A. Nikolski, and T. Murawec, *Steroids,* **5,** 423(1965).

(5) M. W. Ruchelman and C. D. Howe, *J. Chromatogr. Sci.*, **7,** 340(1969).

(6) W. Voelter, G. Jung, E. Breitmaier, G. Bouchon, E. Bayer, and D. Gupta, *Anal. Chim. Acta,* **53,** 185(1971).

(7) E. Alsat, C. Corpechot, C. Ego, E. Richard, and L. Cedard, *J. Chromatogr.*, **44,** 383(1969).

(8) S. J. Richardson, *Clin. Chim. Acta,* **29,** 473(1970).

(9) S. Kushinsky, J. Coyotupa, K. Honda, M. Hiroi, K. Kinoshita, M. Foote, C. Chan, R. Y. Ho, W. Paul, and W. J. Dignam, *Mikrochim. Acta,* **1970,** 491.

(10) P. C. Lee and P. J. Wood, *Clin. Chim. Acta,* **30,** 221(1970).

(11) J. Heikkila and H. Adlercreutz, *J. Steroid Biochem.*, **1,** 243(1970).

(12) G. H. Nelson, *Amer. J. Obstet. Gynecol.*, **106,** 649(1970).

(13) W. Cooper, M. G. Coyle, and J. A. Mills, *J. Endocrinol.*, **51,** 447(1971).

(14) H.-G. Kaplan and M. M. Hreshchyshyn, *Amer. J. Obstet. Gynecol.*, **111,** 386(1971).

(15) A. Sanghvi, L. Taddeini, R. Nagarajan, and C. Wight, *Clin. Chim. Acta,* **31,** 255(1971).

(16) S. Tanaka, M. Satake, and K. Sakai, *Horumon To Rinsho,* **19,** 955(1971).

(17) J. C. Touchstone, T. Murawec, D. K. O'Leary, L. Luha, and G. Mantell, *Clin. Chem.*, **17,** 119(1971).

(18) P. D. Smith and S. R. Stitch, *Clin. Chim. Acta,* **36,** 439(1972).

(19) H. R. M. Alink, *ibid.*, **39,** 449(1972).

(20) M. J. Tikkanen and H. Adlercreutz, *J. Steroid Biochem.*, **3,** 807(1972).

(21) R. M. Bryan, P. T. Florendo, J. R. Elliott, and R. T. O'Kell, *Amer. J. Med. Technol.*, **38,** 250(1972).

(22) M. A. Mitchell, *Can. J. Med. Technol.*, **34,** 106(1972).

(23) P. A. M. Weiss, *Endokrinologie,* **59,** 279(1972).

(24) N. Montalbetti, U. L. Pellegatta, and G. Vignati, *Scand. J. Clin. Lab. Invest.*, **29,** 8.7(1972).

(25) W. Fischer-Rasmussen, *Dan. Med. Bull., Suppl.*, **19** (1972).

(26) H.-G. Kaplan and M. M. Hreshchyshyn, *Steroids,* **19,** 763(1972).

(27) J. C. Touchstone, T. Murawec, and R. J. Bolognese, *Clin. Chem.*, **18,** 129(1972).

(28) G. Adessi, D. Eichenberger, C. Goutte, and M. F. Jayle, *Clin. Chim. Acta,* **45,** 369(1973).

(29) D. W. Nugent and P. F. Fottrell, *ibid.*, **46,** 201(1973).

(30) H. Adlercreutz and T. Luukkainen, in "Gas Phase Chromatography of Steroids," K. B. Eik-Nes and E. C. Horning, Ed., Springer-Verlag, New York, N.Y., 1968, pp. 72–149.

(31) E. C. Horning, T. Luukkainen, E. O. A. Haahti, B. G. Creech, and W. J. A. VandenHeuvel, *Rec. Progr. Hormone Res.*, **19,** 57(1963).

(32) F. J. Kabot and L. S. Ettre, *J. Gas Chromatogr.*, **2,** 21(1964).

(33) H. H. Wotiz and S. C. Chattoraj, *J. Chromatogr. Sci.*, **11,** 167(1973).

(34) B. F. Maume, G. M. Maume, J. Durand, and P. Padieu, *J. Chromatogr.*, **58,** 277(1971).

(35) W. J. A. VandenHeuvel, J. Sjövall, and E. C. Horning, *Biochim. Biophys. Acta,* **48,** 596(1961).

(36) P. P. Nair, I. J. Sarlos, D. Solomon, and D. A. Turner, *Anal. Biochem.*, **7,** 96(1964).

(37) H. Adlercreutz and T. Luukkainen, in "Gas Chromatography of Steroids in Biological Fluids," M. B. Lipsett, Ed., Plenum, New York, N.Y., 1965, pp. 215–228.

(38) H. H. Wotiz and S. J. Clark, "Gas Chromatography in the Analysis of Steroid Hormones," Plenum, New York, N.Y., 1966.

(39) T. Luukkainen and H. Adlercreutz, *Biochim. Biophys. Acta,* **70,** 700(1963).

(40) G. Charransol and B. Desfosses, *J. Eur. Steroides,* **2,** 423(1967).

(41) J. H. H. Thijssen and W. Veeman, *Steroids,* **11,** 369(1968).

(42) A. Rado, B. Desfosses, and M. F. Jayle, *J. Eur. Steroides,* **2,** 317(1967).

(43) A. Pinelli and P. P. Nair, *J. Chromatogr.*, **43,** 223(1969).

(44) R. I. Cox and A. R. Bedford, *Steroids,* **3,** 663(1964).

(45) E. Menini, *Biochem. J.*, **94,** 15P(1965).

(46) J. S. Biggs, A. Klopper, and G. R. Wilson, *J. Endocrinol.*, **44,** 579(1969).

(47) D. Exley and A. Dutton, *Steroids,* **14,** 575(1969).

(48) T. Luukkainen and H. Adlercreutz, *Ann. Med. Exp. Biol. Fenn.*, **45,** 264(1967).

(49) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Mass

Spectrometry of Organic Compounds," Holden-Day, San Francisco, Calif., 1967, p. 475.

ACKNOWLEDGMENTS AND ADDRESSES

Received October 3, 1974, from the *Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46206*

Accepted for publication December 12, 1974.

Presented at the Pharmaceutical Analysis and Control Section, APhA Academy of Pharmaceutical Sciences, New Orleans meeting, November 1974.

The authors are grateful to Mr. G. E. Drummond and Mr. H. F. Hugar for technical assistance.

[x] To whom inquiries should be directed.

PHARMACEUTICAL TECHNOLOGY

Effect of Physical Properties on Compression Characteristics

E. SHOTTON [x] and B. A. OBIORAH [*]

Abstract □ The transmission of force to the die wall was measured by a piezoelectric sensor, and the compression cycles of lactose granules of different shapes were compared. In addition, a nearly spherical fraction of spray-dried lactose was similarly compared with a crystalline sample. Better tablets were formed when the conversion of axial to radial pressure was high and the residual pressure on the die wall remained after removal of the top punch. With acetaminophen and phenacetin, the pressure on the die wall was low, as was the residual pressure, and capping occurred in both cases. With direct compression acetaminophen, higher die wall pressure was produced and capping did not occur. It is considered that these results can be explained by the ease with which the more nearly isodiametric particle can rearrange under pressure and by the elastic properties of the solid.

Keyphrases □ Lactose granules—effect of physical properties on compression characteristics □ Compression of lactose granules—effect of physical properties compared □ Die wall pressures—compared for various lactose granules, effect on tablet compressibility

The effect of crystal habit and particle shape upon the properties of cubic and dendritic crystals of sodium chloride and the tablets produced from these crystals was previously examined (1). For any particular material, the difference between applied and lower punch pressure depends on the coefficient of friction at the die wall and on the radial pressure.

Working with sodium chloride, aspirin, and hexamine (methenamine), Shotton and Ganderton (2) postulated that capping of hexamine was due to the failure of the material induced by axial elastic recovery after compression force was removed, and this capping occurred when the interparticulate bonds were sufficiently strong to resist separation of the crystals.

EXPERIMENTAL

The following materials were used: phenacetin[1], acetaminophen[2], direct compression acetaminophen[3], spray-dried lactose[4], and crystalline lactose[5]. Lactose granules[6] with the following composition by weight were also used: lactose, 50%; sucrose, 33%; maize starch, 16%; and magnesium stearate, 1%.

A vibratory sieving machine was used to obtain 30–40-mesh lactose granules, acetaminophen crystals, and phenacetin crystals, and an air jet sieve was used to obtain a 75-μm fraction of direct compression acetaminophen. The 40–45-μm fraction of the spray-dried and crystalline lactose powders was separated using a zig-zag classifier[7]. All materials were dried at 60° for 4 hr in a hot air oven and stored in wax-sealed screw-capped jars.

Different shape fractions of the lactose granules were obtained from the shape-sorting table described by Ridgway and Rupp (3). In Table I, 1, 4, 8, and 12 refer to the fractions obtained at these stations of this machine. The smaller the number of the station, the more spherical was the shape of the particles; the larger the number of the station, the more angular were the particles. The shape coefficient for the shape fractions of lactose granules was calculated from the average values of the particle volume, surface area, and projected diameter using the method of Heywood (4) as modified by Ridgway and Rupp (3).

Shape factor determinations were not carried out for the lactose powders because it was not possible to count accurately such fine particles. Microscopic examinations, however, showed that the spray-dried lactose was composed of spheroidal particles and that the crystalline lactose was highly angular. The shape-sorting table was unsuitable for the needle-shaped crystals of acetaminophen and phenacetin because these were broken down by vibration. The values of shape coefficient for lactose granules are presented in Table II.

[1] Monsanto Ltd.
[2] Paracetamol crystals BP, Graesser Salicylates Ltd.
[3] Graesser Salicylates Ltd.
[4] McKesson and Robbins Ltd.
[5] Whey Products Ltd.
[6] Thomas Kerfoot and Co., Ltd.
[7] Alpine.