III and β-D-glucoheptonic acid γ-lactone. At present only III is commercially available.

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Quantitative Determination of the Stabilizers Octanoic Acid and *N*-Acetyl-DL-tryptophan in Human Albumin Products

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Abstract □ Methods were developed for the determination of octanoic acid and *N*-acetyl-DL-tryptophan, which are used as stabilizers in the human blood-derived therapeutic products normal serum albumin and plasma protein fraction. The method for octanoic acid uses GC; quantitation is achieved using heptanoic acid as the internal standard. The method for *N*-acetyl-DL-tryptophan is based on UV spectrophotometry of the acid-soluble fraction remaining after precipitation of the protein (ϵ_{280} for *N*-acetyl-DL-tryptophan, 5250). The coefficient of variation for replicate determinations of octanoic acid averaged 3.9% (range 2.1–5.5%); that of *N*-acetyl-DL-tryptophan averaged 1.9% (range 0.5–4.0%). Use of these methods for the analysis of 138 lots of commercial products for octanoic acid and 159 lots for *N*-acetyl-DL-tryptophan showed that the stabilizer contents of 132 and 158 of these lots, respectively, were within 20% of the value indicated on the product label.

Keyphrases □ *N*-Acetyl-DL-tryptophan—quantitative determination of stabilizers, octanoic acid, human albumin products □ Octanoic acid—quantitative determination of stabilizers, *N*-acetyl-DL-tryptophan, human albumin products □ Human albumin products—quantitation of stabilizers, octanoic acid and *N*-acetyl-DL-tryptophan

The major therapeutic human albumin products manufactured for intravenous use in the United States are normal serum albumin (prepared as a 5% or 25% protein solution) and plasma protein fraction (prepared as a 5% protein solution). These products are heated at 60°C for 10 h (1) to inactivate hepatitis viruses (2); to minimize changes in the protein during the heating procedure, stabilizers are added (3–5). The stabilizers permitted by federal regulations are the sodium salt of *N*-acetyl-DL-tryptophan (0.16 mmol) or a combination of sodium salts of *N*-acetyl-DL-tryptophan and octanoic acid (0.08 mmol of each) per gram of protein (1); in practice, only the combination is used. Both octanoic acid and *N*-acetyl-DL-tryptophan bind to human albumin (6). The strength of binding varies with the length of the carbon chain of the fatty acids (7, 8) and the steric configuration of tryptophan (9). Depending on the experimental conditions, lowering the concentration of either stabilizer can affect the thermal stability of albumin (5, 10, 11).

Numerous biological effects of octanoic acid have been reported, including inhibition of platelet aggregation (12, 13), hypoglycemia (14, 15), narcotic action in several animal species (16, 17), and suppression of liver clearance of long-chain fatty acids (18). The ability of the sera of certain individuals to agglutinate all human erythrocytes when octanoic acid is present has been described by several investigators (19). Although no untoward responses were elicited by administering octanoic acid-stabilized albumin to a recipient with such fatty acid-dependent ("caprylate-dependent") antibodies (20), some patients who had undergone anaphylactoid reactions to albumin products exhibited positive skin reactions to octanoic acid-treated albumin (21). In contrast, no adverse reactions have been reported for *N*-acetyl-DL-tryptophan. When given intravenously to human recipients, both the D- and the L-form are poorly utilized and rapidly excreted (22).

The present study was undertaken to develop precise and accurate methods for the quantitative determination of these stabilizers in concentrated protein solutions. The methods developed were then used to assess the stabilizer content of commercially manufactured normal serum albumin and plasma protein fraction[1].

[1] All lots of these products were submitted to the Bureau of Biologics before release for marketing; therefore, a sufficient number of samples could be analyzed to assure that the values obtained were representative of products in commercial distribution.

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EXPERIMENTAL

Materials—The column packing used for gas chromatography (GC) was 80/100 Chromosorb W AW coated with 10% SP-1000[2]. Octanoic acid (>99.5% pure)[3], heptanoic acid (96% pure)[3], isoamyl acetate[3], perchloric acid (60%)[4], chloroform[4], *N*-acetyl-DL-tryptophan[5], and [1-^{14}C]octanoic acid (sodium salt, 1.628×10^{11} Bq/mol)[6] were obtained commercially and used without further purification. The human albumin solutions analyzed were normal serum albumin and plasma protein fraction prepared by 14 different manufacturers (designated A–N)[7]. The primary stabilizer-free albumin used was human fraction V powder prepared by manufacturer K. Other stabilizer-free albumin powders employed are described in the pertinent table. All albumin powders were held at −20°C until use.

Gas Chromatographic Conditions—A chromatograph[8] equipped with a flame-ionization detector, an integrator[9], and an automatic sampler[10] was used. A 1.22- or 1.83-m coiled-glass column (2-mm i.d.) was packed as indicated above and initially conditioned at 225°C by carrier gas (nitrogen) flow for 24 h. The operating conditions for the 1.22-m column were as follows: injection port temperature, 225°C; column, 160°C; detector, 300°C; nitrogen carrier gas flow rate 22 mL/min; detector pressures were hydrogen, 193 kPa (28 psig) and compressed air, 207 kPa (30 psig); automatic sampler pressure was compressed air, 552 kPa (80 psig); the volume of sample injected was ~0.5 μL. For the 1.83-m column, the conditions were the same except that the column temperature was 190°C and the nitrogen carrier gas flow rate was 35 mL/min. The retention times for heptanoic acid (the internal standard) and octanoic acid were, respectively, ~3.0 and ~4.6 min for the 1.22-m column and ~3.5 and ~5.0 min for the 1.83-m column.

Assay Procedure for Octanoic Acid—The method described by Kupferberg (23) for the determination of valproic acid in plasma was used. Protein solution (0.5 mL, 5%), 30 μL of internal standard solution (heptanoic acid, 11.52 mg/mL in chloroform), and 0.1 mL of 1.5 M perchloric acid were stirred vigorously with a vortex mixer for 1 min in a glass-stoppered 13-mL centrifuge tube. Chloroform (4 mL) was added, and the mixture was again stirred with a vortex mixer for a few minutes. The tubes were then centrifuged at 2800 rpm, and the aqueous layer was removed by aspiration. The chloroform layer was removed with a pipet (carefully so as not to remove any of the interphase, which contained mostly albumin) and transferred to a new centrifuge tube which contained 100 μL of isoamyl acetate. The chloroform was then removed at 30°C under reduced pressure[11]. The remaining isoamyl acetate solution was analyzed directly (GC). When 20 or 25% albumin[12] preparations were analyzed, 0.125- and 0.1-mL portions, respectively, were used and 0.9% NaCl was added to bring the volume to 0.5 mL.

A five-point standard curve for octanoic acid was prepared for each analysis by placing 10, 20, 30, 40, or 50 μL of octanoic acid standard solution (11.56 mg/mL in chloroform) plus 30 μL of the heptanoic acid internal standard solution, 4 mL of chloroform, and 100 μL of isoamyl acetate in glass-stoppered centrifuge tubes and evaporating as described above. This simplified procedure for preparing the standard curve was found to yield results identical to those obtained when stabilizer-free albumin was added and the entire extraction procedure (including acidification with perchloric acid) was performed or when perchloric acid was added in the absence of albumin. The octanoic acid standard solution could be kept at room temperature for as long as 2 months.

A typical GC profile of heptanoic and octanoic acids is shown in Fig. 1. With the equipment used in the present study[8,9], the area of each peak was integrated and printed automatically. Therefore, the ratio between the areas of the octanoic acid and heptanoic acid peaks was calculated for each sample and each point on the standard curve. The latter was obtained by plotting the area ratio against the amount of standard octanoic acid added (Fig. 2A, upper line), and the octanoic acid contents of the albumin samples were determined from their peak area ratios. However, a reliable standard curve can be constructed from the peak height ratios (Fig. 2A, lower line) and the octanoic acid contents of the samples determined accordingly.

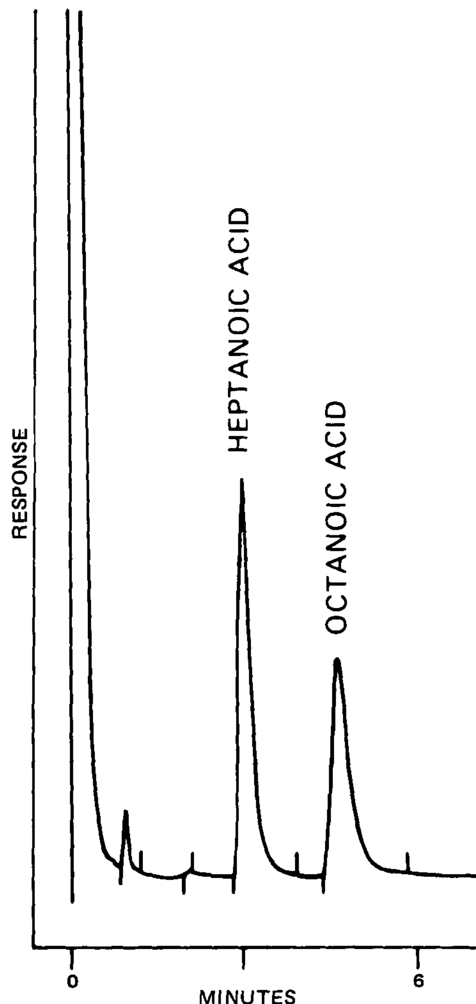

Figure 1—*Typical chromatogram obtained by GC analysis of a commercial human albumin. A 1.22-m column was used. In this experiment the retention times of the heptanoic acid internal standard and the octanoic acid from the albumin sample were 3.05 and 4.61 min, respectively.*

Assay Procedure for *N*-Acetyl-DL-tryptophan—*N*-Acetyl-DL-tryptophan was quantitated spectrophotometrically by precipitating the protein with perchloric acid and subsequently measuring the UV absorbance of the acid-soluble fraction. Virtually all of this absorbance was shown to be due to the *N*-acetyl-DL-tryptophan in the products analyzed (see below). In this procedure 0.1 mL of 5% protein solution, 0.1 mL of 0.9% NaCl, and 1.8 mL of 0.3 M perchloric acid were mixed and held for 10 min at room temperature before centrifuging at 2800 rpm for 10 min. The supernatant was decanted, and the absorbance was measured at 280 nm with a spectrophotometer[13]. When 20 or 25% albumin[12] solutions were analyzed, they were diluted to 5% protein before beginning the assay.

The molar absorption coefficient of *N*-acetyl-DL-tryptophan at 280 nm was determined by spectrophotometric analysis of solutions prepared from the compound dried to constant weight in a vacuum oven (maximum temperature 60°C). The dried compound was also analyzed for nitrogen by a semimicro-Kjeldahl procedure (expected 11.375%, found 11.33%). An 8-mM *N*-acetyl-DL-tryptophan stock solution was then prepared by dissolving the dried compound in 1 M NaOH and diluting with water (final concentration 0.01 M NaOH). The absorbance of this solution after further 100-fold dilution with water corresponded to a molar absorption coefficient of 5250. This figure was not altered by making the final dilution with 0.27 M perchloric acid. It agreed closely with the ϵ_{279} value of 5300 reported by McMenamy and Oncley (9).

As initially performed, the analysis involved a standard curve which was prepared by mixing 20, 40, 60, 80, or 100 μL of the 8-mM *N*-acetyl-DL-tryptophan stock solution (stable for at least 6 months when refrigerated and protected from light), 0.1 mL of 5% stabilizer-free albumin,

[2] Supelco, Bellefonte, Pa.
[3] Aldrich Chemical Co., Milwaukee, Wis.
[4] J. T. Baker Chemical Co., Phillipsburg, N.J.
[5] California Foundation for Biochemical Research, Los Angeles, Calif.
[6] New England Nuclear, Boston, Mass.
[7] Received by the Bureau of Biologics, FDA, before June, 1980.
[8] Model 5830A; Hewlett-Packard, Avondale, Pa.
[9] Model 18850A; Hewlett-Packard, Avondale, Pa.
[10] Model 7671A; Hewlett-Packard, Avondale, Pa.
[11] Evapomix; Buchler Instruments, Fort Lee, N.J.
[12] Normal serum albumin may also be prepared as a 20% protein solution (1). At present, this product is manufactured only for export.

[13] Model 250; Gilford Instrument Laboratories, Oberlin, Ohio.

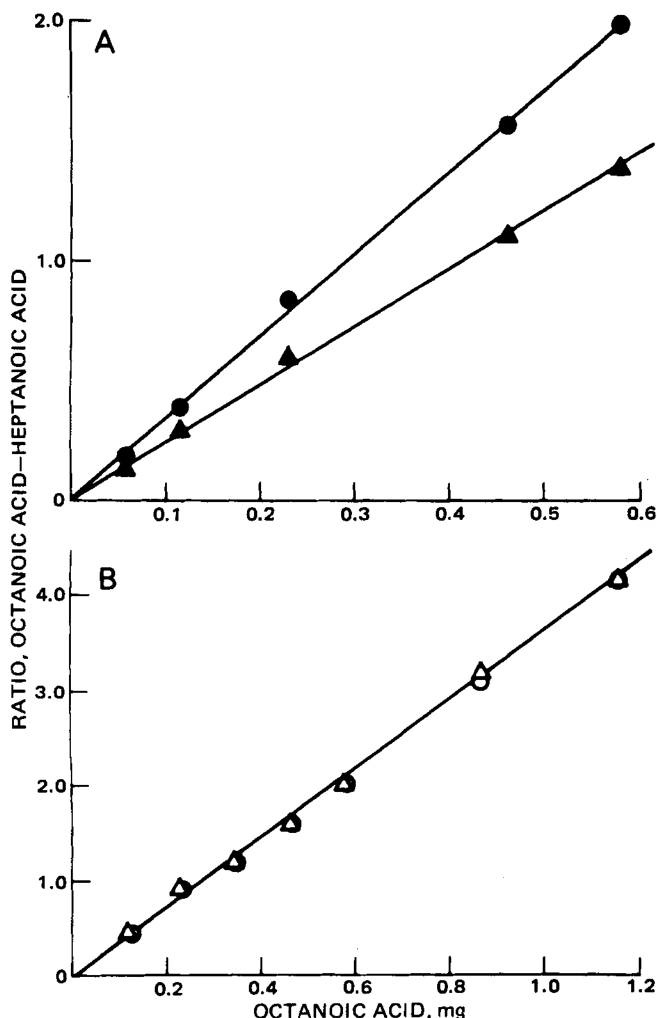

Figure 2—*Effect of experimental variants on the determination of octanoic acid. (A) Ordinate shows peak area (●) or peak height (▲) ratios; abscissa, milligrams of octanoic acid in the sample. A 1.22-m column was used. (B) Ordinate shows peak area ratios; abscissa, milligrams of octanoic acid in the sample. Portions of the same isoamyl acetate layers were chromatographed on 1.22-m (○) and 1.83-m (△) columns.*

sufficient 0.9% NaCl to bring the volume to 0.2 mL, and 1.8 mL of 0.3 M perchloric acid, and measuring the absorbance of the supernatant (after centrifugation) at 280 nm. It was found subsequently that an identical linear standard curve was obtained when 0.9% NaCl was used in place of the stabilizer-free albumin solution and, later, that the standard curve was so reproducible that the determination could be based on the molar absorption coefficient alone (see *Appendix* for calculations). Therefore, for routine analyses, no standard curve is used. When the same samples were analyzed with and without the use of a standard curve, the values for *N*-acetyl-DL-tryptophan content agreed within 2%.

RESULTS AND DISCUSSION

Octanoic Acid Analysis—*Development and Validation of the Method*—Before analysis, a 0.5-mL sample of 5% normal serum albumin was mixed with sodium [1-^{14}C]octanoate (3700 Bq). Counts obtained after acidification and extraction showed that >97% of the radioactivity was extracted into the chloroform layer. Because radiolabeled heptanoic acid was not available, recovery experiments were performed by mixing measured quantities of unlabeled heptanoic acid with stabilizer-free albumin or saline solutions and then adding a fixed amount of octanoic acid either before or after the acidification and extraction. The mean recovery of heptanoic acid was >97%. Subsequently, a series of samples were subjected to replicate analyses in which 2, 4, 6, or 8 mL of chloroform was used for the extraction step. The volume of chloroform used had no discernible effect on the value obtained for octanoic acid, indicating that any of these volumes can be used. For routine analyses, however, 4 mL of chloroform was always employed. Similarly, the 1.22- and the 1.83-m columns gave identical results over a wide range of octanoic acid concentrations (Fig. 2B). Because of the greater ease in packing, the shorter column was routinely used.

Table I—Assessment of Precision of Octanoic Acid Determination by GC [a]

Lot No. [b]	Mean ± *SD*, mM [c]	*CV* [d]
1	3.57 ± 0.15	4.2
2	3.57 ± 0.078	2.2
3	7.64 ± 0.31	4.1
4	3.50 ± 0.089	2.5
5	3.55 ± 0.073	2.1
6	20.4 ± 1.12	5.5
7	20.4 ± 0.75	3.7
8	20.0 ± 0.55	2.8
9	20.7 ± 0.98	4.7
10	20.5 ± 0.83	4.0
11	20.9 ± 0.76	3.6

[a] A 1.22-m column was used. [b] Lots 1–5 were 5% normal serum albumin from manufacturer G; lots 6–11 were 25% normal serum albumin from manufacturer N. [c] Standard deviation for five separate determinations; 0.08 mmol/g protein corresponds to concentrations of 4 mM and 20 mM in 5% and 25% protein solutions, respectively. [d] Coefficient of variation = *SD* × 100/mean.

To verify the accuracy of the analysis, the octanoic acid standard and the heptanoic acid internal standard were chromatographed individually. At the loads used in the present study, each showed only a single peak in addition to those produced by the solvent system. The octanoic acid standard solution was then titrated by the method of Marvel and Rands (24)[14]: nominal concentration 80 mM, by titration 78.7 mM. A solution of nominal concentration 80 mM was then prepared by dissolving sodium octanoate[15] in water. It was analyzed for sodium by flame photometry and for octanoic acid by the GC method described above: concentration by flame photometry 78.5 mM, by GC 79.3 mM, mean deviation 1.0%.

To assess the precision of the method, five lots of 5% normal serum albumin and six lots of 25% normal serum albumin were each subjected to five replicate analyses for octanoic acid. The coefficient of variation for replicate determinations ranged from 2.1 to 5.5% and averaged 3.9% (Table I).

Recovery was estimated by adding a series of incremental quantities of octanoic acid to replicate samples of normal serum albumin such that the largest amount approximately doubled the octanoic acid concentration. Analysis of these samples indicated that the recovery of added octanoic acid was 95.5 ± 4.2% (*SD*).

Analysis of Commercial Products—The octanoic acid concentrations of 138 lots of product were determined; of these, only six deviated >20% from the concentration given on the product label (Table II). Three of these lots were produced by manufacturer M, who used both venous plasma and placentas as source material. Preparation of albumin from placentas utilized octanoic acid in the purification procedure (25) as well as for stabilization and may account for the higher levels found in these samples. (Placentas are no longer used for the manufacture of albumin and globulin products in the United States.) One of the lots from manufacturer G had an octanoic acid concentration almost twice that of the others (Tables I and II), suggesting inadvertent repetition of stabilizer addition during manufacture. There is no obvious explanation for the low octanoic acid concentration in two lots from manufacturer K.

Comparison with Other Methods—Several methods for the determination of fatty acids involve extraction and subsequent titration in a two-phase (aqueous/nonpolar) system (24, 26, 27). Although these do not require preliminary evaporation of the solvent, the titration step does not readily lend itself to automation. Several other methods involve GC analysis after preparation of the methyl ester derivatives of the extracted fatty acids (28–30). One of these methods was specifically designed for the determination of octanoic acid and utilized sodium [1-^{14}C]octanoate as an internal standard to correct for losses during the procedure (29). The GC step in this analysis, like that described by Burnett *et al.* (30), was performed on a 1.83-m column; the packings employed were 80/100 mesh Gas-Chrom P coated with 15% diethylene glycol succinate (29) and 100/120 mesh Chromosorb W AW coated with 10% SP-2330 (30), respectively.

The method described in the present study requires neither derivatization of the octanoic acid nor the use of a radiolabeled tracer and utilizes

[14] The titration was performed as described except that 2% Triton X-100 (Rohm and Haas, Philadelphia, Pa.) was used in place of 2% Dreft.
[15] Nutritional Biochemical Corp., Cleveland, Ohio.

Table II—Octanoic Acid Concentration in Commercial Albumin Products

Manu-facturer	Product[a]	No. of Lots Analyzed	Mean ± SD[b]	Lots >20% Beyond Labeled Value[c]
A	PPF	5	3.93 ± 0.08	0
	25%	5	19.68 ± 1.90	0
B	5%	8	3.48 ± 0.22	0
	25%	6	18.33 ± 0.72	0
C	25%	3	19.40 ± 1.04	0
D	5%	6	3.54 ± 0.12	0
	PPF	10	4.00 ± 0.24	0
	25%	5	19.44 ± 1.72	0
E	5%	6	3.98 ± 0.18	0
	PPF	6	4.09 ± 0.12	0
	25%	6	19.86 ± 2.18	0
F	25%	6	19.85 ± 1.65	0
G	5%	5	4.68 ± 2.32	1[d]
	25%	5	17.78 ± 1.19	0
H	25%	5	20.15 ± 1.92	0
I	25%	5	18.48 ± 0.73	0
J	25%	5	18.57 ± 1.04	0
K	5%	4	3.27 ± 0.35	2
	25%	5	18.56 ± 1.43	0
L	5%	5	3.74 ± 0.19	0
	25%	6	18.59 ± 1.35	0
M	5%	6	4.62 ± 0.43	2
	25%	6	22.75 ± 1.53	1
N	25%	9	20.93 ± 1.23	0

[a] PPF = plasma protein fraction (human); numbers refer to normal serum albumin (human) with the nominal protein concentration indicated. [b] Mean ± SD expressed as mM. [c] The labeled values for the octanoic acid concentrations of plasma protein fraction and of 5% and 25% normal serum albumin are 4, 4, and 20 mM, respectively. [d] This lot contained approximately twice the labeled value of octanoic acid; omission of this lot gave a value of 3.90 ± 0.19 (mean ± SD).

Table III—UV Absorbance of Acid-Soluble Fraction from Stabilizer-Free Albumin

Albumin[a]	Manufacturer	Absorbance at 280 nm
Human fraction V	K	0.048
Human fraction V	D	0.024
Human placental	I	0.025
Human fraction V	in-house	0.017
Bovine crystalline	O[b]	0.014
Human purified (from in-house)	in-house	0.014
Human purified (from normal serum albumin)	in-house	0.010

[a] Human albumin fraction V manufactured in-house was prepared from the plasma of two donors by cold ethanol fractionation (31). Purified albumins were prepared by ion exchange chromatography (32) of in-house human albumin fraction V and commercial normal serum albumin, respectively. [b] Distributed by Sigma Chemical Co., St. Louis, Mo.

a shorter (and thus more easily handled) 1.22-m column. Identical results were obtained when the 1.22- and 1.83-m columns (both packed with 80/100 mesh Chromosorb W AW coated with 10% SP-1000) were used to analyze human albumin products for octanoic acid. Moreover, a previous survey of such products involved GC on a 1.83-m column (100/120 mesh Supelcoport[2] coated with 5% diethylene glycol succinate) and yielded results similar to those presented above[16].

***N*-Acetyl-DL-tryptophan Analysis**—*Development and Validation of Method*—Several different protein precipitants, including isopropyl alcohol, trichloroacetic acid, and perchloric acid, were examined. The latter was ultimately chosen because it afforded essentially complete precipitation, consistent analytical results, and good recovery of *N*-acetyl-DL-tryptophan. No further improvement was achieved by carrying out the precipitation at 4°C; therefore, all operations in the analysis were performed at room temperature. Unlike the GC procedure, in which the retention time provides an internal identity criterion for octanoic acid, the spectrophotometric method for *N*-acetyl-DL-tryptophan includes no qualitative test. The underlying assumption is that virtually all the UV absorbance of the acid-soluble fraction is due to *N*-acetyl-DL-tryptophan. To demonstrate the validity of this assumption a series of stabilizer-free albumin powders were used to prepare 5% protein solutions, which were then analyzed as described above. The absorbances found (Table III) corresponded to 0.95–4.6% of that obtained when 4 mM *N*-acetyl-DL-tryptophan[17] was treated in a similar manner, implying that at least 95–99% of the absorbance measured under the conditions of the method can be ascribed to the compound.

[16] M. A. Gross and J. S. Finlayson, unpublished results. A preliminary sampling of 230 lots of NSA produced by 11 manufacturers and released before April 1979 indicated that 212 had octanoic acid concentrations within 20% of the labeled value.

Table IV—Assessment of Precision of *N*-Acetyl-DL-tryptophan Determination by UV Spectrophotometry[a]

Manu-facturer	Product[b]	Mean ± SD, mM[c]	CV[d]
A	PPF	4.65 ± 0.046	1.0
	25%	22.8 ± 0.47	2.1
B	5%	3.65 ± 0.11	3.0
	25%	20.1 ± 0.32	1.6
C	PPF	4.06 ± 0.11	2.7
	5%	4.08 ± 0.11	2.7
	25%	19.9 ± 0.34	1.7
D	25%	20.7 ± 0.41	2.0
E	PPF	4.36 ± 0.058	1.3
	5%	4.30 ± 0.079	1.8
	25%	21.3 ± 0.40	1.9
G	5%	3.99 ± 0.076	1.9
	25%	22.9 ± 0.19	0.8
F	25%	20.7 ± 0.29	1.4
H	25%	19.0 ± 0.19	1.0
I	25%	21.0 ± 0.84	4.0
J	25%	20.0 ± 0.60	3.0
K	25%	18.1 ± 0.33	1.8
L	5%	4.12 ± 0.069	1.7
	25%	19.0 ± 0.53	2.8
M	5%	3.99 ± 0.067	1.7
J	25%	20.1 ± 0.11	0.5
N	25%	20.1 ± 0.47	2.3

[a] The standard curve procedure was used (see text). [b] PPF = plasma protein fraction; numbers refer to normal serum albumin with the nominal protein concentration indicated. [c] Mean ± SD for five separate determinations. [d] Coefficient of variation = $SD \times 100$/mean.

Table V—*N*-Acetyl-DL-tryptophan Concentration in Commercial Albumin Products

Manu-facturer	Product[a]	No. of Lots Analyzed	Mean ± SD[b]	Lots >20% Beyond Labeled Value[c]
A	PPF	6	4.59 ± 0.05	0
	25%	6	22.18 ± 1.13	1
B	5%	7	3.50 ± 0.10	0
	25%	5	19.00 ± 1.06	0
C	25%	3	20.37 ± 0.06	0
D	5%	6	4.05 ± 0.16	0
	PPF	7	4.06 ± 0.32	0
	25%	5	20.06 ± 1.02	0
E	5%	10	4.35 ± 0.25	0
	PPF	10	4.39 ± 0.12	0
	25%	6	20.27 ± 1.31	0
F	25%	8	19.90 ± 0.75	0
G	5%	5	4.09 ± 0.07	0
	25%	5	21.53 ± 1.25	0
H	25%	6	19.56 ± 1.44	0
I	25%	8	20.59 ± 0.77	0
J	25%	5	20.38 ± 0.70	0
K	5%	5	4.00 ± 0.20	0
	25%	5	20.18 ± 1.51	0
L	5%	5	4.21 ± 0.09	0
	25%	6	19.43 ± 0.63	0
M	5%	6	3.76 ± 0.32	0
	25%	7	17.60 ± 1.70	0
N	25%	17	19.20 ± 0.76	0

[a] PPF = plasma protein fraction (human); numbers refer to normal serum albumin (human) with the nominal protein concentration indicated. [b] Mean ± SD expressed mM. [c] The labeled values for *N*-acetyl-DL-tryptophan content of plasma protein fraction and of 5% and 25% normal serum albumin are 4, 4, and 20 mM, respectively.

[17] A stabilizer/protein ratio of 0.08 mmol/g corresponds to stabilizer concentrations of 4, 16, and 20 mM in 5, 20, and 25% protein solutions, respectively.

The method precision was evaluated by performing five replicate analyses on each of 23 lots of product, including at least one lot from every manufacturer. The coefficient of variation for replicate determinations averaged 1.9% (range 0.5–4.0%) and did not differ among 5% normal serum albumin, 25% normal serum albumin, and plasma protein fraction (Table IV). Recovery was assessed by adding increments of *N*-acetyl-DL-tryptophan to replicate samples of normal serum albumin such that the largest increment more than doubled the concentration of this stabilizer. Analyses of these samples showed that the recovery of added *N*-acetyl-DL-tryptophan was 95.7 ± 2.0% (*SD*).

Analysis of Commercial Products—The *N*-acetyl-DL-tryptophan concentrations of 159 lots of product were determined. Only one of these deviated more than 20% from the value indicated on the label, and the mean concentration of most manufacturers' products was within 10% of the label value (Table V).

Applications of Octanoic Acid and *N*-Acetyl-DL-tryptophan Analyses—The procedures developed in the present study should be useful in the quality control of commercial albumin products. In addition, they may have other applications. For example, in a preliminary study, use of these methods showed that when solutions containing 4 mM *N*-acetyl-DL-tryptophan and 4 mM octanoic acid were heated for 10 h at 60°C, the concentrations of both stabilizers remained constant. This result was not altered by the presence or absence of albumin or by varying the pH between 4.9 and 8.6, indicating that within this range of conditions the stabilizers themselves are stable. In a separate investigation it was found that, as assessed by the present methods, stabilizer-free albumin monomer could be prepared by chromatography on agarose[18] columns at pH 8.0, whereas dialysis proved incapable of rendering normal serum albumin free of stabilizer[19]. Finally, because at least 96% of the protein in normal serum albumin is albumin (1), measurement of the total absorbance (280 nm) of a sample of normal serum albumin and concomitant determination of its *N*-acetyl-DL-tryptophan content may offer a rapid method for estimating the protein concentration[20].

APPENDIX: CALCULATION OF CONCENTRATIONS AND RATIOS

Octanoic Acid Concentration (mM)[17]:

(a) In 5% normal serum albumin or plasma protein fraction
= octanoic acid (mg) in 0.5-mL assay sample × 2 × 1000/mol. wt. of octanoic acid
= octanoic acid (mg) in 0.5-mL assay sample × 13.87

(b) In 20% normal serum albumin
= octanoic acid (mg) in 0.125-mL assay sample × 13.87 × 4
= octanoic acid (mg) in 0.125-mL assay sample × 55.48

(c) In 25% normal serum albumin
= octanoic acid (mg) in 0.1-mL assay sample × 13.87 × 5
= octanoic acid (mg) in 0.1-mL assay sample × 69.35

Octanoic Acid/Protein Ratio (mmol/g of protein):

In all products = octanoic acid (mg) in assay sample × 0.2774

***N*-Acetyl-DL-tryptophan Concentration (mM):**

(a) In 5% normal serum albumin or plasma protein fraction
= A_{280} of acid-soluble fraction × 2/0.1 × 5.25
= A_{280} of acid-soluble fraction × 3.81

(b) In 20% normal serum albumin
= A_{280} of acid-soluble fraction × 15.24

(c) In 25% normal serum albumin
= A_{280} of acid-soluble fraction × 19.05

***N*-Acetyl-DL-tryptophan/Protein Ratio (mmol/g of protein):**

= A_{280} of acid-soluble fraction × 0.0762

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