8. For maximum accuracy, a profilometer could be used for inspecting all finishes to the nearest micro-inch.

9. An optical comparator would also be of value since it would permit the precision inspection of all intricately shaped or hobbed punch faces.

10. A Rockwell or similar hardness tester can be used for checking punch and die hardnesses.

RECORDING TOOL LIFE HISTORY

The establishment of specifications and subsequent quality control of the tools should promote the purchasing of tools with a uniform high quality, But, at the present level of knowledge in this area, one cannot be certain that the quality of tooling now used, however uniform, is the most satisfactory for uninterrupted production. So that such information might be obtained, a system for recording the life of the various tools is suggested which will enable one to know the total number of tablets compressed with any given set of tools (3). In addition, information on the products prepared, machines the tools were used with, and the reasons that the tools were eventually discarded is recorded. The careful evaluation of the facts obtained from these records will be useful in purchasing tools with optimum wear characteristics and will result in a better finished product at reduced cost.

SUMMARY

An appropriate set of dimensional specifications and tolerances as well as an incoming inspection program for tableting tools can be considered the keys to an efficient tableting operation. The efforts of the IPT Committee on Specifications in establishing standard punch and die dimensions to suit the majority of pharmaceutical applications is a valuable contribution toward this goal. It is hoped that wide-spread acceptance of these standards will be forthcoming from the pharmaceutical industry since their advantages are self-evident.

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Automated Determination of Ascorbic Acid in Multivitamin Preparations

M. GELLER, O. W. A. WEBER, and B. Z. SENKOWSKI

Abstract □ A specific colorimetric method for the determination of ascorbic acid in multivitamin preparations has been automated. The method is based on the coupling reaction of diazotized 4-methoxy-2-nitroaniline with ascorbic acid. A bathochromic shift in the chromophore is effected by the addition of alkali. The stable blue color developed is measured at 570 mμ. Application of the automated procedure for the determination of ascorbic acid in multivitamin and mineral preparations is described.

Keyphrases □ Ascorbic acid in multivitamin products—automated analysis □ Automated procedure—ascorbic acid analysis □ Schematic diagram—ascorbic acid automated analysis □ Colorimetric analysis—spectrophotometer

Automated modified reactions of ascorbic acid with 2,6-dichlorophenol-indophenol, measured in aqueous media or an organic solvent extract, have been reviewed by Khoury (1). The indophenol reaction lacks specificity and is subject to interferences from reducing substances. Pelletier and Morrison (2) removed interfering ferrous and stannous ions with preliminary oxidation followed by chelation with EDTA before reaction of ascorbic acid with 2,6-dichlorophenol-indophenol. Robinson and Stotz (3) eliminated the interference of the reducing substances by peroxide treatment and the sulfhydryl groups by formaldehyde condensation.

Roe and Kuether (4) oxidized ascorbic acid to dehydroascorbic acid and then condensed it with 2,4-dinitrophenylhydrazine. This reaction is not subject to interference from reducing substances, however, difficulties arise from oxidizing substances such as ferric ions and hydrogen peroxide. Thiourea was used to maintain a reducing environment.

The high specificity of the coupling reaction of diazotized 4-methoxy-2-nitroaniline with ascorbic acid, reported by Schmall *et al.* (5, 6), along with the simplicity of the colorimetry, made it particularly suitable for an automated procedure. Dehydroascorbic acid, all other vitamins, and reducing agents such as ferrous and stannous ions do not interfere when present in quantities normally encountered in pharmaceutical preparations. The excipients commonly encountered in multivitamin preparations not reported by Schmall *et al.* (5) such as mannitol, talc, stearic acid, and magnesium

Table I—Tabulation of Automation Train

Tube Operation Number[a]	Type of Operation	Flow Rate, ml./min.	Solvent (reagent)
1	Wash	1.6	0.2% Oxalic acid
2	Sampling	2.0	Sample
3	Segmentation	0.6	Air
Continuous filter A[b]	Filtration	—	—
4[c]	Dilution	0.23	0.2% Oxalic acid
4I	Resampling and further	0.42	Sample
4II	serial dilution (500-mg.	1.2	0.2% Oxalic acid
4III	samples)	0.42	Air
5	Sampling	0.32	Sample
6	Segmentation	0.42	Air
7		3.39	Anhydrous 3A alcohol
8	Color development	0.23	Amino reagent
9		0.23	0.2% Sodium nitrite
Mixing coil B	Color dilution	—	—
10		3.9	Water
Mixing coil B		—	—
11	pH shift	1.2	10% Sodium hydroxide
Two double mixing coils *C*	—	—	—
Extraction coils *D*	—	—	—
B-O Electrolyte trap *E*	Debubbling	—	—
12	Reading	3.9	Sample from flow cell

[a] All tubing except 7 is standard; 7 is solvaflex. [b] For liquid samples, substitute a modified B-O trap. [c] Additional diluent steps and sampling necessary for 500-mg. solid samples.

stearate were tested in concentrations up to 10 times the quantity normally used. No interferences were observed at these levels. Those components reported by Schmall *et al.* are B-complex vitamins, thiamine, riboflavin, pyridoxine, pantothenate, folic acid, niacin, niacinamide, vitamin A, vitamin D, vitamin E, dehydroascorbic acid, 2,3-diketogulonic acid, pantoyl lactone, phenol, glycerol, propylene glycol, nonionic surfactants,[1] ferrous sulfate heptahydrate, stannous chloride dihydrate, and sodium sulfite. Oxidative decomposition of ascorbic acid results initially in the formation of dehydroascorbic acid, and since it was previously (5, 6) determined that neither dehydroascorbic acid nor 2,3-diketogulonic acid, a further decomposition product, interferes with the color development, the described method is indicative of ascorbic acid stability. Mechanistically, the reaction demands the presence of an ene-diol structure (6) which is not present in either of the potential degradation products. Sample preparation merely requires dilution with aqueous oxalic acid prior to colorimetry. This analytical procedure has been automated and utilized for the determination of ascorbic acid in a wide variety of pharmaceutical combinations. Analyses can be conducted at a rate of 20/hr. A typical recording of the time-absorbance plot is shown in Fig. 1. A schematic flow diagram of the manifold is shown in Fig. 2, and the applicable legend appears in Table I.

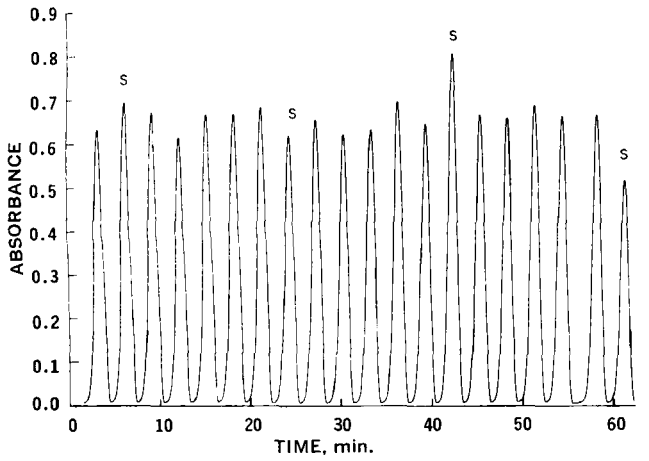

Figure 1—*A typical recording of the time-absorbance plot. S, denotes standards.*

[1] Tweens, Atlas Chemical Co., Wilmington, Del.

REAGENTS

Amino Reagent—Dissolve 1 g. of 4-methoxy-2-nitroaniline[2] in 250 ml. of glacial acetic acid and dilute to 500 ml. with 10% w/v sulfuric acid. This reagent is stable at room temperature for 2 months. Also used were the following solutions: 0.2% aqueous sodium nitrite, prepared fresh daily; anhydrous 3A alcohol (95% ethanol plus 5% methanol); 10% aqueous sodium hydroxide; 5% aqueous oxalic acid; and 0.2% aqueous oxalic acid.

PREPARATION OF STANDARDS AND SAMPLES

Standards—Prepare standard stock solutions of ascorbic acid containing 85–110% of the theoretical product content in 5 ml. of 5% oxalic acid. Transfer 5-ml. aliquots to the sample cups in alternating sequence.

Samples—Introduce one tablet into the sample cups and grind to a powder with a glass rod.[3] Introduce 5 ml. of 5% oxalic acid. For capsules, place one capsule in the sample cup, add 5 ml. of 5% oxalic acid, and allow the capsule to disintegrate. One-milliliter aliquots of liquid samples are introduced into the sample cups and diluted with 4 ml. of 5% oxalic acid solution.

PROCEDURE

Place the standard and sample cups on the turntable. Activate the system and record the standard and sample absorbances at 570 mμ. Determine the quantity of ascorbic acid per dosage form

[2] Eastman Organic Chemical No. 2094.

[3] It was found that coated ascorbic acid in whole tablets was not completely solubilized during the period of homogenization in the Solid Prep unit. This difficulty was overcome by addition of the sample grinding step.

Table II—Comparison of Automated and Manual Methods

Product	Theoretical mg./Tablet (Capsule)	Automated Average[a] mg./Tablet (Capsule)	Manual Iodometric Titration,[b] mg./Tablet (Capsule)
Soft gelatin capsule			
A	165	156	156
B	82.5	77.4	80.6
C[d]	75	78.6	80.5
Multivitamin tablets			
A	73.2	75.0	73.7
B[d]	75	76.6	79.0
C[d]	50	51.9	53.4
D[c]	73.2	73.2	73.7 Composite sample
Vitamin C tablets			
A	575	558	548
B[d]	500	513	510
	mg./ml.		
Multivitamin drops			
A	91.7	92.4	92.5
B	108.3	100.4	105
C	104.2	102.5	103
D	108.3	103.7	106
E[d]	100	117.9	120

[a] Average of ten sample runs. [b] Average of duplicates of composite sampling. [c] Experimental results are averages of multiple sample runs. Different colored tablets caused no interference in the $\lambda_{max.}$ nor adversely affected the analytical results. [d] Exact theoretical (other than label claim) not available.

by the following calculation.

$$A + \frac{(B - A)(E - C)}{D - C} = \text{mg. ascorbic acid per sample}$$

where: A = mg. ascorbic acid in Standard A; B = mg. ascorbic acid in Standard B; C = absorbance of Standard A; D = absorbance of Standard B; E = absorbance of sample.

RESULTS

Various dosage forms were analyzed by the automated method. These results were compared with those obtained by manual iodometric titrations and the data are presented in Table II. The values obtained by the automated method for tablets and soft gelatin capsules are averages of determinations on individual units. The manual titration results are the average of two determinations on composite samples. Satisfactory agreement was obtained by the two methods.

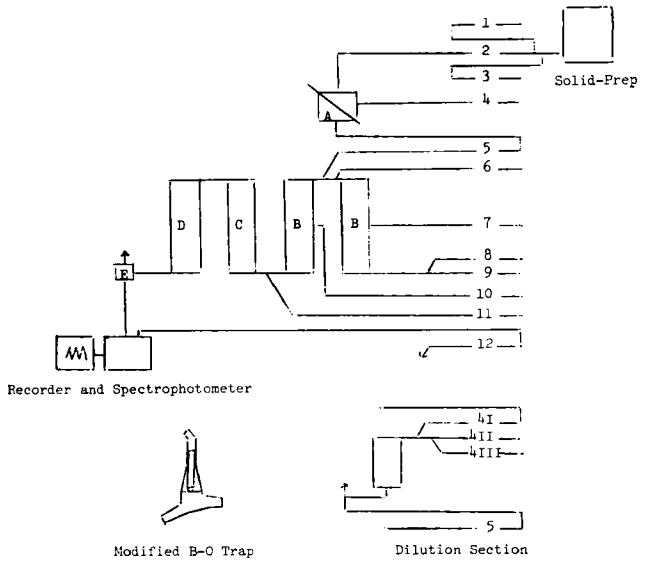

Figure 2—*Schematic flow diagram.*

The assay, normalized assay ranges, and averages of single dosages are presented in Tables III and IV.

The coefficients of variation were calculated based on the normalized values. Since it was difficult to obtain representative individual weights for the soft gelatin capsules, assay values may be subject to greater variation. The coefficient of variation among batches is in good accord with the coefficient of variation for the analytical procedure, indicating uniformity in manufacturing.

The results obtained for various multivitamin drops are presented at the bottom of Table II and show reasonable conformance to expected values indicating the reliability of the automation to liquid samplings as well as solid samplings. The composition of the various vitamin preparations, as declared on the label, are presented in Table V.

DISCUSSION

The wide use of ascorbic acid in pharmaceuticals and related products made an automated assay procedure highly desirable. The system used is simple and specific for ascorbic acid, and is not subject to interference from reducing or oxidizing substances. In addition there is a bathochromic shift in alkaline medium to 570 mμ, eliminating interferences from any yellow color which may be attributable to discoloration or decomposition. Furthermore, the reaction does not require careful buffering.

Although a plot of concentration *versus* absorbance does not pass through the origin, a linear relationship is obtained from 10 to

Table III—Individual Assay[a] and Variation Normalization of Tested Products

Dosage Number	Assay, mg./Dosage: Capsule *A* (165)	Capsule *B* (82.5)	Capsule *C* (75)	Tablet *A* (73.2)	Tablet *B* (75)	Tablet *C* (50)	Tablet *D*[b] (575)	Tablet *E*[b] (500)
1	156	74.6	81.3	77.2	72.1	50.5	553	511
2	170	76.5	84.3	72.2	76.3	55.6	569	525
3	155	71.4	77.8	75.9	80.1	51.6	544	495
4	143	88.2	80.7	74.9	77.1	51.1	573	511
5	174	77.9	77.1	73.5	75.7	49.4	576	515
6	149	73.7	73.8	76.3	77.5	56.3	563	513
7	169	75.1	76.3	75.5	77.8	52.6	539	532
8	141	77.5	79.0	75.8	78.6	52.7	568	517
9	161	78.9	76.1	75.9	77.1	50.8	547	502
10	145	79.8	79.2	72.8	73.5	48.8	547	509
Range	141–174	71.4–88.2	73.8–84.3	72.2–77.2	72.1–80.1	48.8–56.3	539–576	495–532
Average	156	77.4	78.6	75.0	76.6	51.9	558	513
Normalized[c] coefficient of variation, %	±5.8	±4.6	±3.9	±1.9	±2.3	±4.2	±1.6	±2.0

[a] Theoretical values in mg./dosage are given in parentheses. [b] Tablets *D* and *E* contained only ascorbic acid. [c] Individual tablet weight adjusted to average tablet weight.

Table IV—Individual Assay and Variation Normalization of Multivitamin Tablets[a]

Tablet No.	Assay, mg./Tablet				
	Batch *A*	Batch *B*	Batch *C*	Batch *D*	Batch *E*
1	73.5	70.5	76.4	71.0	73.9
2	70.9	74.7	75.1	76.1	70.7
3	71.5	76.8	75.1	76.5	74.4
4	70.4	74.2	70.5	77.5	71.4
5	72.8	74.8	75.1	71.8	72.1
6	72.9	73.1	71.9	76.1	69.6
7	72.2	77.5	72.9	73.9	70.1
8	75.4	71.9	71.2	73.7	70.3
9	74.7	71.1	76.6	74.2	71.5
10	72.1	74.0	70.9	71.2	72.6
Range	70.4–75.4	70.5–77.5	70.5–76.6	71.0–77.5	69.6–74.4
Average	72.6	73.9	73.6	74.2	71.4
Normalized[b] coefficient of variation, %	±1.8	±2.0	±3.1	±2.3	±1.3

[a] The theoretical value was 73.2 mg./tablet in each batch. Batches *A* through *E* are the same formulation differing only in color. [b] Individual tablet weight adjusted to average tablet weight.

Table V—Labeled Components

Product	Components
Soft gelatin capsule	
A	Vitamin A, B_1, B_2, B_6, B_{12}, C, D_2, E, niacinamide, *d*-panthenol
B	Vitamin A, B_1, B_2, B_6, B_{12}, C, D_2, E, niacinamide, *d*-panthenol
C	Vitamin A, B_1, B_2, B_6, B_{12}, C, D, distilled tocopherols, niacinamide, pantothenic acid
Multivitamin tablets	
A	Vitamin A, B_1, B_2, B_6, B_{12}, C, D, E, niacinamide, calcium pantothenate, *d*-biotin, ferrous fumarate
B	Vitamin A, B_1, B_2, B_6, B_{12}, C, D, niacinamide, calcium pantothenate, biotin, mannitol, cellulose, magnesium stearate, salt, artificial flavor, and color. Artificial flavor includes sodium cyclamate and sodium saccharin
C	Vitamin A, B_1, B_2, B_6, B_{12}, C, D, niacinamide
D	Identical to A except no ferrous fumarate
Vitamin C tablets	
A	Ascorbic acid
B	Ascorbic acid
Multivitamin drops	
A	Vitamin A, calciferol, ascorbic acid, *dl*-α-tocopherol acetate
B	Identical to A plus sodium fluoride
C	Vitamin A, B_1, B_2, B_6, C, E, niacinamide, *d*-panthenol, *d*-biotin
D	Identical to C plus sodium fluoride
E	Vitamin A, B_1, B_2, D, C, niacinamide

90 mg. of ascorbic acid per cup. The deviation of the plot from the origin may at first seem unusual, but can be readily explained if one analyzes the mechanism of the color development (6). The active intermediate in this reaction is the limiting species in the color development. This intermediate must be involved in a ring cleavage before color development (6), however the process is reproducible. The initial reaction may involve a second minor interaction which would shift the absorbance concentration curve from passing through the origin. By bracketing the samples between a high and low standard and employing the calculation shown under *Procedure* the concentration can readily be obtained. Alternatively, a plot of standard concentration *versus* absorbance can be constructed and the content per unit obtained directly.

The coefficient of variation for the procedure was ±1.5% and was calculated from the measurement of 28 replicates on a homogeneous standard solution. By subtracting this value from the variation of the sample being analyzed one can draw conclusions as to the uniformity of the manufacturing process.

This method, which has been used manually for over 10 years with little or no modifications, has been successfully adapted to automation.

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