testers offered considerable insight into the performance capability of the instruments studied. The characterization information is fundamental to any developmental work in this area. The study also revealed a sufficiently high correlation among the tablet testers to permit precise prediction of breaking strength values. It is possible now to expand the study to evaluate breaking strength testers of the same kind in interlaboratory crossover experiments.

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Semiautomated UV Analysis of Caffeine in Aspirin–Phenacetin–Caffeine Tablets

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Abstract □ A semiautomated individual tablet assay was developed for caffeine in aspirin–phenacetin–caffeine formulations. Caffeine and phenacetin are extracted from the bicarbonate tablet solution with chloroform. The caffeine and some phenacetin are extracted from the chloroform with acid, and an additional chloroform wash of the acid phase removes any phenacetin. The caffeine is determined by UV spectroscopy in the acid at 266 nm. at a rate of 20 tablets/hr. The coefficient of variation for 10 determinations of one sample solution was 1.72. The difference in results between the NF XIII method and the proposed method, expressed as percent of declared, did not exceed 2.6% when 10 different products were analyzed.

Keyphrases □ Aspirin–phenacetin–caffeine tablets—semiautomated UV analysis of caffeine □ Caffeine in aspirin–phenacetin–caffeine tablets—semiautomated UV analysis □ UV spectrophotometry—analysis, caffeine in aspirin–phenacetin–caffeine tablets

Aspirin, phenacetin, and caffeine in pharmaceutical preparations are determined quantitatively by the official NF procedure (1). Since the monograph employs a partition chromatographic separation on a diatomaceous earth[1] column (2), the content uniformity requirement for caffeine in the tablets is extremely time consuming.

An automated method for caffeine in blood converts the caffeine to an aromatic amine, with subsequent diazotization for color determination (3). This procedure could not be used in the presence of phenacetin.

Caffeine has been reported to fluoresce in chloroform, where the excitation at 280 nm. causes emission that can be measured at 310 nm.[2]. With filter fluorometer equipment normally applied to an automated analyzer[3], excitation energy from the source lamp is rather weak at 280 nm. In addition, the fluorescence is not strong and linearity may be difficult to achieve.

In the method reported here, the caffeine and phenacetin are extracted from the bicarbonate solution with chloroform. The caffeine is extracted from the chloroform with dilute sulfuric acid, which is washed with chloroform to remove any phenacetin from the acid phase. The caffeine is determined by measuring its UV absorbance at 266 nm.

EXPERIMENTAL

Apparatus—The following were used: Liquid sampler II[4] (20/hr.), proportioning pump[4] I, and a spectrophotometer[5] equipped with a 10-mm. flow cell[6].

Reagents—The following were used: 4 *N* sulfuric acid; 0.1 *M* $NaHCO_3$; and chloroform, reagent grade, free of UV-absorbing impurities.

Standard Preparation—Place 82 mg. of caffeine in a 250-ml. volumetric flask with 25 ml. of 0.1 *M* $NaHCO_3$, and heat on a steam bath 4–5 min. to dissolve. Cool and dilute to volume with 0.1 *M* $NaHCO_3$. The concentration of the standard solution is 0.328 mg./ml.

Sample Preparation—For tablets declared to contain 30 mg.

[1] Celite, Johns-Manville Corp., New York, N. Y.

[2] A. Gillespie, Food and Drug Administration, Detroit, Mich., Dec. 1969, personal communication.

[3] Technicon AutoAnalyzer, Technicon, Tarrytown, N. Y.

[4] Technicon, Tarrytown, N. Y.

[5] Beckman DK 2A, Beckman Instruments, Fullerton, Calif.

[6] A. H. Thomas Co., No. 9120-NO 5, Philadelphia, Pa.

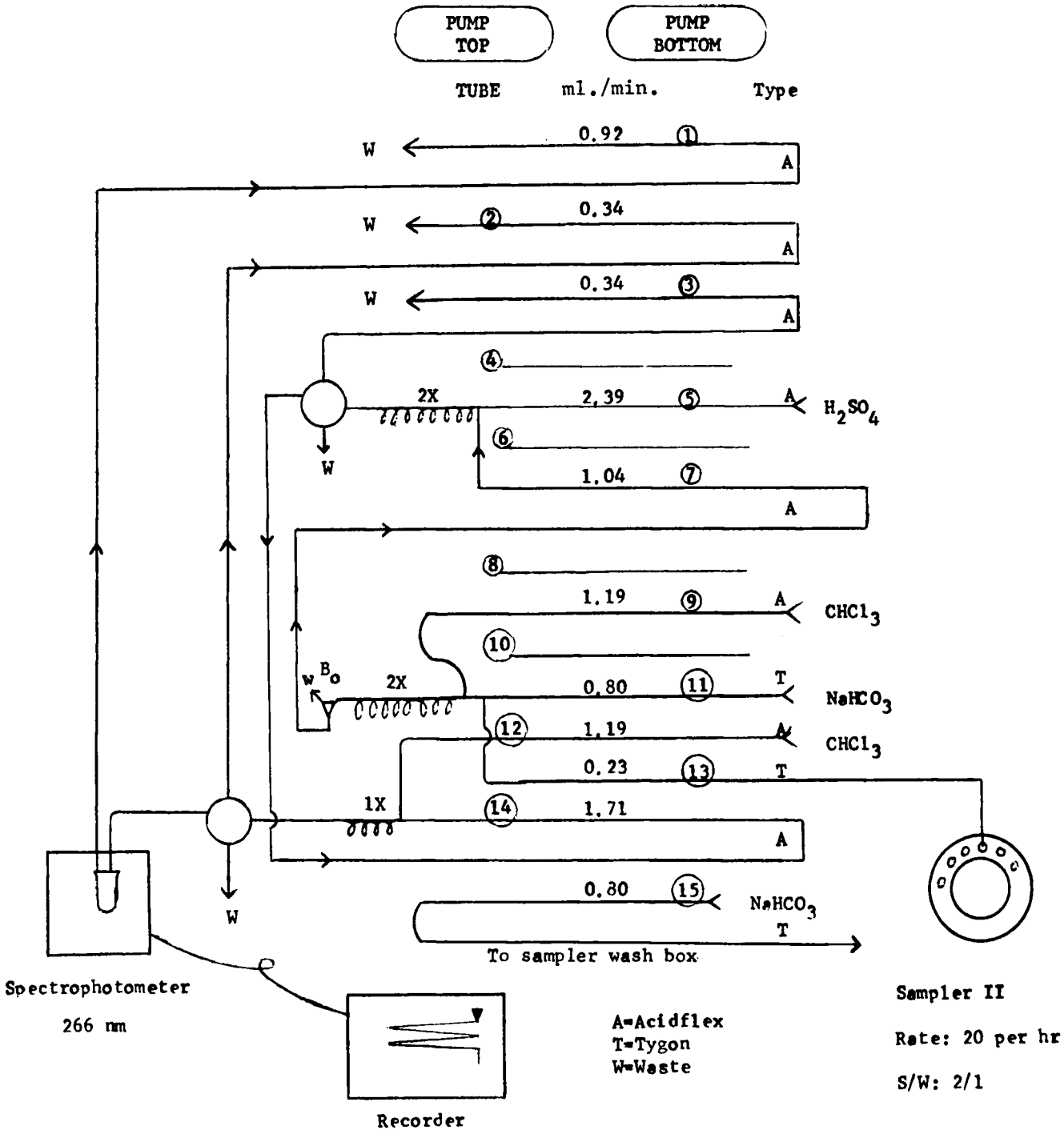

Figure 1—*Automated analyzer flow diagram for caffeine in aspirin–plenacetin–caffeine tablets.*

(1/2 gr.) caffeine, place them separately in a series of 100-ml. volumetric flasks. Add 25 ml. of 0.1 *M* $NaHCO_3$, heat on the steam bath 4–5 min., cool, dilute to volume with 0.1 *M* $NaHCO_3$, and mix (much of the phenacetin will not dissolve, even in warm 0.1 *M* $NaHCO_3$). For tablets declared to contain 15 mg. (1/4 gr.) caffeine, place them separately in a series of containers and add 25.0 ml. of 0.1 *M* $NaHCO_3$ from an automatic delivery pipet. Heat 4–5 min., cool, add another 25.0 ml. of 0.1 *M* $NaHCO_3$, and mix. Filter all tablet solutions through filter paper before placing in sample cups.

Automated Sample Assay—The flow system and the manifold settings are shown in Fig. 1. Coils labeled 1X are 14 turns, 2 mm. i.d.; those labeled 2X are 28 turns, 2 mm. i.d.

Special Connector for Automated System—To eliminate gas bubbles which formed at the interface of two immiscible liquids in the automated system, a special connector was developed (Fig. 2). The two immiscible liquids are pumped into the connector where the heavier liquid settles to the bottom (D) and is pushed to waste by the excess liquid being pumped in. Some less dense liquid is pumped out the top (B) through the pump to waste, along with any gas released from the interface. This eliminates air bubbles from the flow cell compartment. In addition, if any droplets of the denser liquid are attached to the gas bubble, it falls through D to waste. The liquid, free of gas bubbles, goes out the connector at C.

RESULTS AND DISCUSSION

A warm bicarbonate solution was selected for tablet preparation because caffeine dissolved completely, while much of the phenacetin

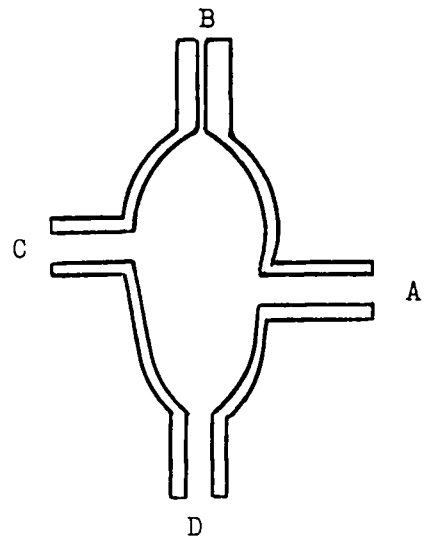

Figure 2—*Special connector for automated analyzer system. Scale, 2 mm. = 1 mm. A, C, and D = 2 mm. i.d., 4 mm. o.d. B = 0.5 mm. i.d., 4 mm. o.d.*

remained at the bottom of the flask. This greatly reduced the interference of phenacetin in the automated UV procedure. Heating on the steam bath seemed to effect solution more completely than did the sonic vibrator.

During this study, the tubing employed in the automated system seemed particularly subject to fatigue and failed after approximately

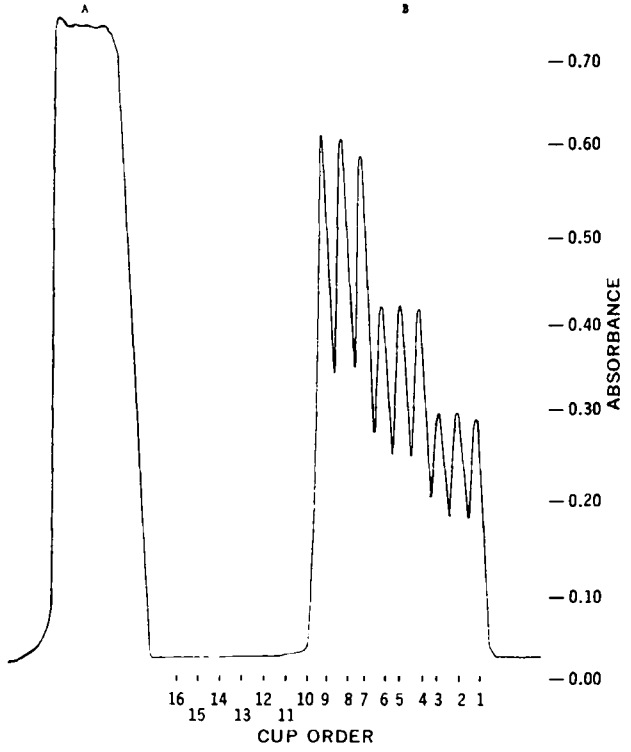

Figure 3—*Automated analyzer recordings of: (A) steady state of caffeine, 0.582 mg./ml.; and (B) linearity for caffeine at 266 nm. at 20 cups/hr.*

Cups	*Contents*
1, 2, 3	*caffeine, 0.2910 mg./ml.*
4, 5, 6	*caffeine, 0.4074 mg./ml.*
7, 8, 9	*caffeine, 0.5820 mg./ml.*
10, 11, 12	*$NaHCO_3$, 0.1 M*
13, 14	*aspirin, 2.27 mg./ml.*
15, 16	*phenacetin, 1.62 mg./ml.*

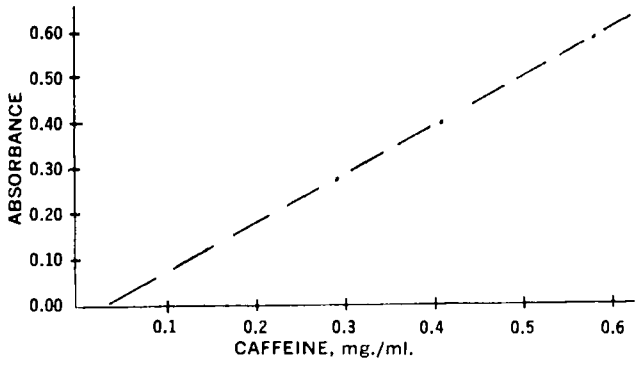

Figure 4—*Automated analyzer linearity of caffeine at 266 nm.*

30–40 hr. of use. This is most readily detected by running at steady state on a daily basis.

Figure 3 demonstrates the automated analyzer response for caffeine, phenacetin, and aspirin standards. Figure 4 shows the linearity of the caffeine in the range investigated. The lack of a zero intercept, however, indicates that some error could be introduced if sample and standard absorbances were significantly different.

The precision of the procedure was determined by analyzing one

Table I—Standard Deviation of One Solution of Aspirin–Phenacetin–Caffeine

Cup Order	Contents	Net Absorbance	Milligrams per Tablet Found	Percent of Declared
1	Standard	—[a]	—	—
2	Standard	—[a]	—	—
3	Standard	0.384	—	—
4	Sample	0.378	32.4	100.0
5	Sample	0.379	32.4	100.0
6	Sample	0.385	33.0	101.8
7	Sample	0.363	31.0	95.8
8	Sample	0.384	32.9	101.6
9	Standard	0.383	—	—
10	Sample	0.381	32.7	100.8
11	Sample	0.384	32.8	101.4
12	Sample	0.383	32.8	101.4
13	Sample	0.381	32.6	100.6
14	Sample	0.382	32.7	101.0
15	Standard	0.384	—	—
16	Standard	—[a]	—	—
Average (10)			32.5	100.4
SD		0.009		
CV		1.720		

[a] The absorbances of the first two cups and the last cup are not used in the calculations.

Table II—Comparison of Official and Automated Procedures for Caffeine in Aspirin–Phenacetin–Caffeine Tablets

Product	Composite Results, Percent of Declared: NF XIII	Composite Results, Percent of Declared: Automated[a]	Composite Results, Percent of Declared: Difference	ITA[b] by Automated, Average (10)	Coefficient of Variation
A	101.7	99.6	−2.10	97.3	3.399
B	97.0	97.5	+0.50	98.8	2.738
C	99.5	100.6	+1.10	98.2	2.612
D	102.8	100.7	−2.10	102.5	4.434
E	98.0	100.6	+2.60	100.6	1.991
F	98.3	96.3	−2.00	98.0	1.727
G	96.0	98.5	+2.50	98.3	3.420
H	99.2	97.4	−1.80	97.5	3.295
I	100.1	98.1	−2.10	99.9	3.793
J	98.0	98.3	+0.30	101.6	5.213

[a] Technicon AutoAnalyzer assay. [b] Individual tablet analysis.

composite solution in 10 separate cups. The usual sequence used in this laboratory is as follows: three standards, five tablets, standard, five tablets, *etc.*, composite, and two standards. The composite is a weighed portion of 20 tablets ground to fine powder, equivalent to one tablet weight. The coefficient of variation for 10 determinations of one composite solution was 1.72 (Table I).

The accuracy of the procedure was studied by comparing the automated assay with the NF column method on 10 different product composites. Both analyses were made on the same mixed powdered composites. The results of the automated method were in general agreement with those by the NF procedure (Table II). The deviation, expressed as percent of declared, was less than ±2.6%.

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Dissolution Rate Studies IV: Solvent Flow Patterns in a Column-Type Apparatus

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Abstract □ Solvent flow patterns in a column-type, continuous flow apparatus were determined using a flow visualization technique. A supporting bed of glass spheres in the dissolution chamber ensures laminar flow, and this is preferred over the complex, poorly defined flow found with static beaker methods.

Keyphrases □ Dissolution rate studies—solvent flow patterns and laminar flow conditions, column-type equipment □ Tablet dissolution—solvent flow patterns and laminar flow conditions, column-type equipment □ Solvent flow in column-type dissolution equipment—flow patterns, laminar flow conditions □ Column-type dissolution equipment—solvent flow patterns, laminar flow conditions

The advantages of the column-type, continuous flow method for determining the dissolution characteristics of solids have been repeatedly emphasized (1–4). One important advantage is that solvent flow is columnar as opposed to the complex, poorly defined flow found with static beaker methods (5). However, some problems have been encountered with columnar flow because of turbulence (4), problems that would be minimized under conditions of laminar flow. This report describes, for a column-type apparatus, (*a*) solvent flow patterns under various experimental conditions, and (*b*) the necessary conditions for laminar flow.

EXPERIMENTAL

Equipment—The dissolution apparatus was the column type described previously (3). The light sources were a common laboratory microscope light and an electronic tachometer and motion analyzer[1]. The latter was calibrated with respect to the powerline frequency according to the instruction manual. The reflecting spheres were polystyrene base beads[2] (diameter 90–125 μ; specific gravity 1.06).

[1] Strobotac, General Radio Co., West Concord, Mass.

[2] Duke Standards Co., Palo Alto, Calif.

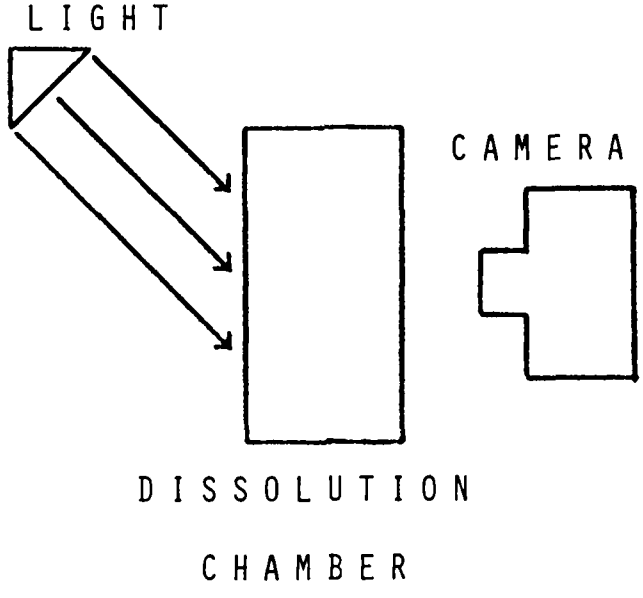

Figure 1—*Arrangement of equipment for photographic and television work.*

A lens hood was used to reduce flare with the still camera[3]. Depending on the tachometer flashing rate, apertures varied between f-11 and f-22 and exposure times between 0.5 and 0.25 sec. All photographs were taken at one-half life size image.

The television camera[4] had a 75-mm. f-1.9 lens and a video tape recorder[5].

Procedure—The experimental procedure was similar to that described by Withey and Bowker (5) but was appropriately modified for the column-type apparatus. The basic arrangement of the equip-

[3] A Nikon FTN with a 105-mm. Novoflex short-mount lens on a Novoflex bellows. The film was Kodak 2475 recording film developed in Acufine to an ASA index of 2400.

[4] Apeco VF-200.

[5] Tele-Tape.