stability of the drugs in the formulation. None of these degradation products was detected in a formulation subjected to high-temperature stability testing.

The linearity of the detector response was established for hydrochlorothiazide in the range of 15–30 mg/capsule (r = 1.000, y-intercept = 0.002) and for triamterene in the range of 30–70 mg/capsule (r = 1.000, y-intercept = 0.04). Standard addition–recovery experiments performed in two different placebos at various drug levels showed recoveries of 98.4–101.7% for hydrochlorothiazide (n = 12, CV = ±1.2%) and 99.5–102.0% for triamterene (n = 12, CV = ±0.9%).

The reproducibility of the method was demonstrated by performing replicate analyses on a commercial formulation[9] and another experimental formulation with different excipients. The statistical data generated from these analyses are presented in Table I.

In conclusion, HPLC provides a rapid and precise method for the quantitative determination of hydrochlorothiazide and triamterene in a combination oral dosage form. The method is sensitive to the degradation products of these drugs and is free from interference due to excipients in the products examined.

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Test for Reproducibility of Metered-Dose Aerosol Valves for Pharmaceutical Solutions

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Abstract □ Seven independent testing sites received metered-dose aerosols containing a standard test solution to assess a newly designed protocol for studying the reproducibility of metered-dose aerosol valves. In accordance with the rather small sampling protocol design, the amount of product dispensed per actuation was measured over the life of the aerosol at designated regions of actuation. A statistical analysis of the data collected at each testing site clearly indicated that the testing protocol is sufficiently reliable and workable for assessing the reproducibility of valve delivery for a given lot of metered-dose valves.

Keyphrases □ Aerosols—testing procedure for establishing the reproducibility of metered-dose valves □ Inhalation products—testing procedure for establishing the reproducibility of metered-dose aerosol valves □ Valve delivery—metered-dose aerosols, testing procedure for establishing reproducibility

Test methods, including specific gravity, net contents, vapor pressure, moisture content, spray patterns, and particulate-size determinations, have been established for determining the acceptance of metered-dose aerosol valves for pharmaceutical use (1–4). Reproducibility of the dosage delivered may be determined by assay techniques which establish the amount of active (5). Although USP XX (6) includes metered-dose inhalation products in the mono-

graphs and establishes some standards with which these metered-dose products must comply, little has been done in establishing a uniform test procedure for all metered-dose products.

The Aerosol Specification Committee[1] elected to establish a simple and uniform test method for metered-dose aerosol valves by examining the uniformity of valve delivery (amount of solution delivered by the valve) within a valve and between individual valves in any given lot. The procedure outlined in this study provides a means for manufacturers to assess the reproducibility of valve delivery for a given lot of metered-dose valves throughout the life of the product.

EXPERIMENTAL

Aerosol Test Solutions—Pharmaceutical test solutions were prepared by taking 0.10% by weight of isopropyl myristate[2] and adding it to a mixture of 24.90% by weight of trichloromonofluoromethane (Propellant

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[1] Of the Industrial Pharmacy Technology Section, Academy of Pharmaceutical Sciences, American Pharmaceutical Association.
[2] Givaudan Corp., Clifton, N.J.

Table I—Means and Ranges of the Amount of Material Delivered per Actuation for Each Site (21- and 22-Actuation Study)

Actuations	Site I		Site II		Site III		Site IV	
	X	Range	X	Range	X	Range	X	Range
1	71.5	60.7–84.8	26.8	9.1–77.9	23.6	14.9–30.5	28.6	13.0–64.0
2	73.2	57.3–99.9	74.9	70.3–84.7	74.3	70.2–77.1	74.1	68.5–78.6
3	74.8	61.9–87.0	76.1	72.3–77.6	74.2	71.8–76.5	74.0	65.5–78.2
4	71.0	60.6–76.9	76.4	75.1–77.5	75.5	72.1–77.7	80.0	74.5–77.3
5	76.0	75.5–77.3	76.5	74.9–78.0	75.0	73.2–75.8	72.2	56.8–77.3
6	75.4	72.5–78.0	77.2	75.3–80.0	74.4	72.7–75.6	76.3	74.5–77.7
7	77.1	72.0–86.7	76.8	75.6–77.7	75.6	74.1–77.1	77.6	74.8–77.3
8	73.7	67.5–76.1	76.7	75.4–77.6	75.1	72.4–77.5	75.7	72.2–78.9
9	74.8	72.2–76.7	76.6	75.3–76.8	74.6	73.1–76.8	74.6	74.2–75.9
10	76.6	71.6–87.2	76.7	75.6–77.2	74.5	71.4–77.1	75.1	73.0–76.8
26	74.1	72.2–76.5	75.5	74.5–76.3	70.5	63.8–75.1	72.5	64.0–76.5
27	77.4	72.7–86.0	76.5	74.9–79.2	73.1	71.5–74.0	74.7	73.5–75.7
28	69.9	62.4–75.3	75.2	74.1–75.9	73.9	72.2–75.4	74.1	72.9–75.4
29	73.6	71.5–76.5	75.1	73.4–77.1	73.2	71.7–74.6	47.7	72.8–76.1
76	74.5	73.2–74.8	72.5	74.1–75.7	72.5	71.2–75.3	73.3	71.8–75.2
77	74.3	72.7–76.1	73.6	74.7–75.1	73.6	72.0–75.5	73.9	73.3–75.1
78	74.4	73.0–75.7	73.7	73.2–75.4	73.7	73.1–76.3	73.9	71.7–76.5
79	74.0	73.0–74.7	73.7	74.4–76.2	73.7	72.8–75.6	73.8	73.0–75.0
117	72.8	71.7–75.3	73.8	66.6–82.1	73.8	72.8–75.1	72.4	68.6–73.7
178	74.1	74.0–75.9	74.1	60.7–76.0	74.1	72.9–75.8	71.2	62.4–73.6
179	72.4	70.0–75.5	72.8	67.8–75.5	72.8	70.7–75.7	69.3	56.0–74.5
180	68.4	63.2–76.5	70.3	63.7–80.0	70.3	58.1–75.4	65.1	33.6–73.7
Mean	73.1		72.8		71.4		71.6	

Table II—Means and Ranges of the Amount of Material Delivered per Actuation for Each Site (14-Actuation Study)

Actuations	Site I		Site II		Site III		Site IV		Site V	
	X	Range	X	Range	X	Range	X	Range	X	Range
7	74.2	73.6–77.6	75.6	75.2–76.2	75.2	74.1–76.6	76.0	73.1–77.3	75.4	73.1–78.6
8	75.8	72.3–77.9	75.4	74.6–75.9	76.2	75.0–77.9	76.0	74.6–76.9	76.6	75.3–80.2
9	75.5	74.5–76.9	75.5	74.8–76.3	75.2	74.6–76.3	76.6	75.6–77.8	77.2	76.2–80.1
10	75.3	73.9–77.3	74.8	74.0–75.7	75.8	74.0–76.5	75.9	74.9–77.0	76.0	75.4–77.4
81	76.6	73.7–79.3	75.0	74.2–76.7	75.8	74.1–77.2	75.8	75.0–76.9	75.4	73.3–79.1
82	78.1	73.6–90.0	75.3	74.5–76.3	75.1	75.1–76.4	74.5	73.7–75.1	75.5	74.7–79.2
83	75.4	74.1–78.0	75.0	73.8–75.8	76.0	74.6–78.8	75.0	74.4–75.8	86.4	74.6–129.1
84	75.7	74.2–78.5	74.0	72.2–75.3	75.7	74.1–78.4	75.3	75.0–75.8	75.2	71.7–80.1
85	75.4	73.9–77.7	75.1	74.4–75.6	76.1	74.2–77.9	75.1	74.9–76.2	74.8	74.9–78.1
166	76.8	74.8–78.4	74.8	74.1–75.5	74.8	74.2–75.5	74.0	70.5–75.9	75.0	74.1–77.1
167	75.1	73.8–76.7	74.6	73.1–76.1	74.6	73.4–75.2	74.5	72.5–75.0	75.2	74.2–78.2
168	75.0	72.2–76.4	75.3	74.6–75.9	74.9	74.2–76.2	74.9	74.3–76.0	75.2	73.3–78.4
169	75.6	74.2–76.5	75.1	73.8–76.6	75.1	73.9–76.5	74.9	74.0–75.8	75.6	73.4–78.3
170	75.9	74.6–78.3	75.9	73.6–76.6	75.3	74.4–75.8	75.1	74.0–76.0	75.4	73.1–78.5
Mean	75.7		75.1		75.4		75.2		76.4	

11[3]), 50.00% by weight of dichlorodifluoromethane (Propellant 12[4]), and 25% by weight of dichlorotetrafluoroethane (Propellant 114[5]). This solution was prepared by the cold fill process, and a suitable metered container was used.

Aerosol Components—In the initial study (Protocol I), an experimental 63-μl aerosol valve[6] was used. In each subsequent study (Protocol II), a valve with a 50-μl metering chamber[6] and stainless steel stem was used. Fifteen-milliliter aluminum aerosol containers[6] and standard button actuators[6] were employed in all studies.

Preparation of Samples[7]—In a thermostatically controlled chill tank maintained at $-32°$, isopropyl myristate was mixed with trichloromonofluoromethane. By using gravity-fed metering equipment with an electrically controlled solenoid shutoff, this solution was filled by volume and time into the aluminum container. The filling machine reservoir was maintained at a constant volume and temperature, thus allowing for precise volume fills in the container *via* a predetermined timed opening of the filler tube. At a second similar filling station, at 2:1 blend of Propellants 12 and 114, maintained at $-37°$, was metered into the aluminum container to the appropriate volume. Aerosol valves (without diptubes) were placed on the aluminum cans and crimped. The completed cans then were tested for leaks by immersing each for 3–5 min in a water bath maintained at 55°. Cans showing evidence of leaks (production of gas bubbles) were removed and discarded.

Sampling Procedure—After filling, leak testing, and spray testing, the containers were packed in the inverted position (valve down) into nested cardboard shippers. In a random manner, one shipper (containing 100 cans) was sent by parcel post to each testing site[8] where the cartons were stored at ambient conditions with the cans inverted (valve down).

Testing Procedure—To minimize possible variation due to the multicentric nature of the studies, the following protocols were utilized at each site.

Protocol I—Five cans were selected from the 100 cans supplied. With the actuator kept in place, the container was weighed accurately to ±0.1 mg. The valve was actuated once, and then the container was reweighed and the weight loss was recorded. Single actuations were repeated, and the weight loss was recorded for each individual actuation up to and including 10 actuations. The time interval was recorded between each actuation. The valve was actuated again for Actuations 11–25, with 3 sec between each actuation. The container was weighed, and single actuations were repeated. The weight loss of each individual actuation was recorded up to and including Actuation 29. The valve was actuated again for Actuations 30–75, with 3 sec between each actuation. The container was weighed, and single actuations were repeated. The weight loss was recorded for each individual actuation up to and including Actuation 79. The valve was actuated again for Actuations 80–176, with 3 sec between each actuation and 1 min before dispensation of Actuations 80, 91, 101, and 111. The container was weighed, and single actuations were repeated. The weight loss of each individual actuation up to and including Actuation 180 was recorded.

Protocol II—Five cans were selected from the 100 cans supplied. With

[3] Freon 11, E. I. du Pont de Nemours & Co., Wilmington, Del.
[4] Freon 12, E. I. du Pont de Nemours & Co., Wilmington, Del.
[5] Freon 114, E. I. du Pont de Nemours & Co., Wilmington, Del.
[6] Riker Laboratories, Northridge, Calif.
[7] Prepared by Armstrong Laboratories, Division of ATI, West Roxbury, Mass.

[8] Designated by the Aerosol Specification Committee.

Table III—Analysis of Variance for 22-Actuation Study

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square	F	Probability
Between cans					
Sites	190.20	3	63.40	<1.00	NS[a]
Cans within sites	1745.76	16	109.11		
Within cans					
Actuation	1303.17	1	1303.17	17.59	$p < 0.01$
Actuations × sites	456.42	3	152.14	2.05	NS[a]
Actuation × cans within sites	1185.60	16	74.10		

[a] Not significant.

Table IV—Analysis of Variance for 21-Actuation Study

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square	F	Probability
Between cans					
Sites	28.56	3	9.52	<1.00	NS[a]
Cans within sites	2420.16	16	151.26		
Within cans					
Actuations	135.26	1	135.26	2.51	NS[a]
Actuations × sites	101.16	3	33.72	<1.00	NS[a]
Actuations × cans within sites	860.96	16	53.81		

[a] Not significant.

the actuator kept in place, the container was weighed accurately to ±0.1 mg. The valve was actuated six times. After 30 sec, the weight of the container was recorded. Single actuations were repeated, and the weight loss was recorded for each individual actuation up to and including Actuation 10. The time interval between each actuation was recorded. The valve was actuated again at a rate of one actuation every 2 sec until one-half of the total labeled actuations minus five. (In this study, Actuation 80 was one-half of the total actuations minus five.) After 5–7 min (to allow temperature equilibrium), single actuations were repeated, and the weight loss was recorded for each individual actuation up to and including Actuation 85. The valve again was actuated at a rate of one actuation every 2 sec until five less than the total labeled actuations. (In this study, Actuation 165 was five less than the total actuations.) The wait of 5–7 min and single actuations were repeated, and the weight loss was recorded for each individual actuation up to and including Actuation 170.

In both protocols, relative humidity, temperature, condensation on can (if any), misdirected sprays, and amount accumulated on actuator (by weighing the actuator before and after rinsing with plenty of water) were reported. All actuations were performed with the cans inverted.

RESULTS AND DISCUSSION

The means and ranges of the amount of material delivered through the metered-dose valve utilizing Protocols I and II are given in Tables I and II, respectively. Both protocols were designed to assess the amount of material actuated by a metered valve under testing during each region of product use. The regions specifically evaluated were the initial doses actuated and actuations when the aerosol container was approximately 10, 30, 50, 70, and 95% empty. No attempt was made to access the valve's performance just prior to container emptying (tail off) since it varied considerably, depending on the contents remaining in the container and the experimenter's technique (container agitation, angle of container, etc.).

Two separate analyses of variance were performed on the data presented in Table I to determine the influence of testing sites (laboratory conditions and technician performance) and actuation number on the amount of material actuated. The analyses were performed using two-factor analysis of variance with repeated measures on one factor (7) and a computer program[9] for repeated-measured analysis of variance. Con-

[9] Bio-Medical Computer Program-P Series, BMDP Computer Center, Pittsburgh, PA 15238.

Table V—Analysis of Variance for 14-Actuation Study

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square	F	Probability
Between cans					
Sites	72.44	4	18.11	0.89	NS[a]
Cans within sites	408.00	20	20.40		
Within cans					
Actuations	22.62	13	1.74	0.008	NS[a]
Sites × actuations	707.72	52	13.61	0.063	NS[a]
Actuations × cans within sites	56,409.60	260	216.96		

[a] Not significant.

servative degrees of freedom were utilized in the statistical analysis of the data because of the repeated, nonindependent nature of the actuations.

The first analysis was a four-site by 22-actuation design (Protocol I) with actuations as the repeated factor. The 22 actuations outlined in Protocol I consisted of measurements of Actuations 1–10, 26–29, 76–79, and 177–180. The results given in Table III clearly indicate no significant effect for the test sites, a significant effect for actuations, and no significant interaction between sites and actuations. Therefore, there were no differences between sites, but there were differences between successive actuations.

Since the significant effect for actuation was thought to be due to the unique effects of the first actuation (valve priming), a second analysis was performed. It was identical to the previous one, except that the data for the first actuation were omitted. This procedure resulted in a four-site by 21-actuation design. The results presented in Table IV clearly indicate no significant effects for sites or actuations and no significant interactions. Therefore, when the unique first actuation was omitted, there were no differences between sites and no differences between successive actuations.

One analysis of variance was performed on the data in Table II. The data collected utilizing Protocol II (14-actuation study) when analyzed statistically indicated no difference in the milligrams of solution actuated between sites, between actuations, or in the site by actuation interaction (Table V).

CONCLUSIONS

The statistical analysis of data clearly indicated that the protocols described are not influenced by the experimenter or the test site at which the study was conducted. The abbreviated procedure involving only 14 actuation measurements outlined in Protocol II also yielded the same results as the 21-actuation study and is practical for assessing the reproducibility of valve delivery for a given lot of metered-dose valves.

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