Single-Puff Particle-Size Analysis of Albuterol Metered-Dose Inhalers (MDIs) by High-Pressure Liquid Chromatography with Electrochemical Detection (HPLC-EC)

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INTRODUCTION

Albuterol,1-(4-hydroxy-3-hydroxymethylphenyl)-2-(t-butylamino) ethanol (1), and other bronchodilators are widely used as aerosols in the therapy of obstructive lung diseases. Although the USP currently does not require measurement of particle size and amount of drug delivered in a single puff from metered-dose inhalers (MDIs) (2), there is considerable interest in such testing methodologies (3–8). Methods for the determination of particle size in MDIs, include light scattering, cascade impaction, liquid impingement, and image analysis. Because of low analytical sensitivity, most of these methods need several puffs (5 to 40) per determination, and one may question the usefulness of multipuff measurement profiles to evaluate a product when a patient inhales a single puff (6). In this work, we developed a liquid chromatographic method with electrochemical detection sensitive enough to allow particle-size measurements on a single puff of a commercial albuterol MDI. We measured two size-distribution parameters: the mass median aerodynamic diameter (MMAD) and the geometric standard deviation (GSD) using an Andersen cascade impactor. The results are compared to literature values.

MATERIALS AND METHODS

Reagents. Phosphoric acid (85%) was certified ACS grade (Fisher); ammonium dihydrogen phosphate (99.999%) was obtained from Aldrich; methanol was Optima grade (Fisher); bamethane sulfate (α-[(butylamino)methyl]-*p*-hydroxybenzyl alcohol sulfate salt) was obtained from Sigma; and albuterol was USP reference standard (RS). Ultrapure water was prepared by passing MilliQ water through a C18 solid-phase extraction column (Waters Sep-Pak).

Solutions. Stock ammonium phosphate buffer was prepared by adding 0.25 M phosphoric acid to a liter of 0.25 *M* dihydrogen ammonium phosphate until the pH was adjusted to 2.8; the solution was then filtered through a 0.2-μm nylon membrane filter. Internal standard solution was prepared by dissolving an accurately weighed quantity of bamethane sulfate in methanol to make a solution of about 100 μg/mL; further dilutions were made with the mobile phase to obtain final concentrations of 0.25, 0.50, and 1.0 μg/mL. Standard albuterol solution was prepared by dissolving an accurately weighed quantity of USP Albuterol RS in methanol to obtain a solution having a concentration of about 100 μg/mL; further dilutions were made with the mobile phase to obtain final concentrations of 1.0, 0.1, and 0.01 μg/mL. Working standard solutions, in which the concentration of internal standard was kept at 50 ng/mL and the albuterol concentrations varied from 0.5 to 100 ng/mL, were prepared by diluting the calculated amounts of the internal standard solution and the standard albuterol solution with mobile phase.

Mobile Phase. Stock ammonium phosphate buffer (300 mL) and methanol (160 mL) were mixed, diluted to 1.5 L with ultrapure water, and filtered through a 0.2-μm nylon membrane filter.

Chromatographic System. A modification of the method of Emm *et al.* (9) was used. A Phenomenex Bondclone C18 column, 300 × 3.9 mm, 10-μm particle size, was operated at ambient temperature. The flow rate was 1.5 mL/min. An automatic sample injector (Waters, 712 WISP) was used with an injection volume of 100 μL. The HPLC pump was a Waters Model 590 Programmable Solvent Delivery Module. The detector was a Coulochem II, ESA, coulometric electrochemical detector equipped with a guard cell (Model 5020) operated at 1000 mV and a dual-electrode sensitive analytical cell (Model 5011). The detectors were set at 525 and 800 mV, for Channels 1 and 2, respectively. The signal from detector 2 (Channel 2) was amplified (current range, 500 nA) and recorded at an attenuation of 9 with a Hewlett-Packard 3396A integrator. To minimize electrode equilibration time, the flow of mobile phase was kept at 1.5 mL/min during analysis and overnight. Retention times were: albuterol, 6.5 to 7.0 min; internal standard (IS) (bamethane), 10.0 to 10.7 min. Figure 1 shows an example of a typical HPLC-EC chromatogram.

Assay of a Single Puff From Albuterol MDI. A commercial sample of an albuterol MDI (Schering Proventil inhaler; control No. 1BBS47; expiration date, 9/93) was used; this MDI is labeled as providing 90 μg per puff. The MDI was shaken, and the first three puffs were discarded in order

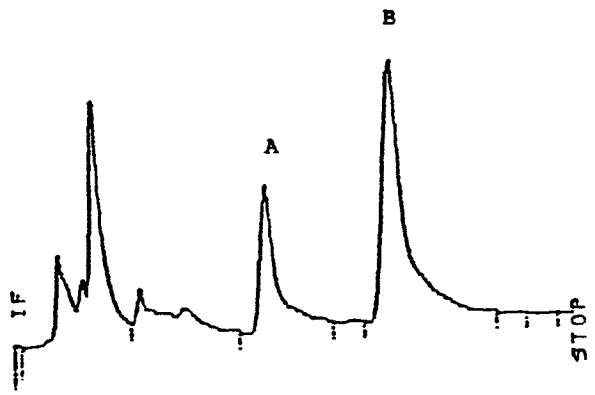

Fig. 1. A typical HPLC-EC chromatogram. (A) Albuterol, 2 ng; 6.79 min; (B) internal standard, 5 ng; 10.19 min.

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Table I. Data for Accuracy and Precision Obtained from the Calibration Curves on 3 Days

Concentration (ng/mL)			
	Measured (mean, %CV)		
Actual	Day 1	Day 2	Day 3
0.5	0.53, 11.3	0.48, 10.4	0.53, 9.4
1.0	0.96, 2.1	1.02, 5.9	1.03, 2.9
5.0	4.96, 0.8	5.00, 1.6	5.01, 0.8
10.0	10.02, 0.2	10.00, 0.4	9.99, 0.2
20.0	19.72, 0.2	19.92, 1.2	19.98, 0.2
40.0	40.45, 0.7	40.65, 0.4	40.47, 0.5
60.0	59.81, 1.1	59.26, 0.4	60.32, 0.5
80.0	80.18, 0.4	79.94, 0.9	78.25, 0.4
100.0	99.84, 0.1	100.23, 0.5	100.91, 0.4

to eliminate any possibility of a "first-spray effect" reported recently (6). The valve of the pressurized pack was rinsed with the mobile phase and dried. The vacuum pump was started, and after 10 sec one puff was delivered into an Andersen Cascade Impactor (Mark II) equipped with a 90° aluminum throat; after 5 sec the pump was turned off. The impactor was then disassembled; all plates (0 through 7) and the filter (Whatman GF/A filter) were transferred to individual 800-mL beakers. Appropriate volumes of the mobile phase were added to each beaker. The actuator, the valve of the pressurized pack, and the throat were all rinsed with the mobile phase. All 12 beakers and their contents were placed in an ultrasonic bath for 5 min to extract the albuterol, the appropriate amounts of the internal standard were added to maintain a constant concentration of 50.0 ng/mL, and all fractions were diluted before injection into the liquid chromatograph.

RESULTS AND DISCUSSION

Calculations and Validation of Albuterol Assay. A calibration curve was generated by plotting peak-height ratios (albuterol/IS) vs albuterol concentration and linear-regression analysis was applied. The accuracy and precision of the assay were determined by injecting known concentrations of albuterol, measuring peak-height ratio responses vs the internal standard, and calculating the concentrations with the established calibration curve. Four replicate injections were made for each assay (intraassay coefficient of variation was 3% or less for concentrations of 1 ng/mL or higher) and each determination was repeated on three different days. Results are provided in Table I.

The albuterol content in the 12 solutions prepared in each cascade experiment was calculated after applying linear regression to a newly constructed calibration curve. An example of the analytical results of a typical cascade experiment is outlined in Table II.

Particle-Size Analysis of Single Puff from Albuterol MDI. Table III provides an illustration of the particle-size distribution of a single puff from an albuterol MDI entering the cascade impactor. The total amount of albuterol collected in a typical cascade experiment was 98.8 μg (±1.6), whereas the albuterol entering the impactor was only 33.9 μg (±1.9); the amount of respirable albuterol (sum of stages 2 through filter or albuterol amounts with an aerodynamic diameter <5.5 μm) was 33.41 μg (±1.9). Our cascade-impactor experiments have shown that about 5% of the albuterol was left on the valve of the MDI, about 19% was deposited on the actuator, about 41% was deposited in the cascade inlet throat, and about 34% entered the impactor.

Data interpretation was done in two ways. First, particle-size diameter graphs were generated by plotting effective cutoff diameters (ECDs) as the ordinate and cumulative percentage greater than stated size as the abscissa on log-probability paper (10,11) (Fig. 2). Next the best straight line was drawn through five data points (ECD = 1.10 through 5.80) (10,11). The mass median aerodynamic diameter (MMAD) is represented by the value of ECD at a cumulative percentage greater than stated size of 50%; the geometric standard deviation (GSD) equals the quotient of the MMAD divided by the value of ECD at a cumulative percentage greater than stated size of 84.13% (10,11). Replicate results of five experiments yielded a mean MMAD of 1.78 ± 0.01 with a mean GSD of 1.72 ± 0.04.

Table II. Example of Albuterol Determination in a Single-Puff Particle-Size Separation Experiment

Std. albuterol conc. (ng/mL)	Height ratio[a]	Fraction	Height ratio[a]	Albuterol conc. (ng/mL)	Volume (mL)	Diln. factor	Albuterol found (μg)	Albuterol found (%)
0.00	0.0000	1	1.9012	71.4	50	5	17.9	18.0
5.00	0.1349	2	1.8548	69.7	50	1	3.5	3.5
10.0	0.2790	3	1.0966	41.0	100	10	41.0	41.3
20.0	0.5517	4	0.2172	7.8	25	1	0.2	0.2
40.0	1.0802	5	0.3477	12.7	25	1	0.3	0.3
60.0	1.6070	6	0.6368	23.6	25	1	0.6	0.6
100.0	2.6443	7	2.0463	76.9	50	1	3.8	3.8
		8	1.2763	47.8	50	5	12.0	12.1
		9	1.3317	49.9	50	5	12.5	12.6
Slope = 37.82		10	1.5980	60.0	50	1	3.0	3.0
Int. = −0.45		11	0.5907	21.9	50	1	1.1	1.1
r = 0.9999		12	1.8011	67.7	50	1	3.4	3.4

[a] Albuterol/internal standard.

Table III. Particle-Size Analysis of a Single Puff From an Albuterol MDI

Part or stage	ECD (μm)[a]	Albuterol (μg)[b]	Cum. mass (μg)[c]	Cum. %[d]
Actuator	—	17.86	—	—
Valve	—	3.48	—	—
Throat	—	41.02	—	—
Stage 0	9.00	0.19	0.19	0.52
Stage 1	5.80	0.32	0.51	1.38
Stage 2	4.70	0.59	1.10	2.99
Stage 3	3.30	3.85	4.95	13.43
Stage 4	2.10	11.95	16.90	45.86
Stage 5	1.10	12.48	29.38	79.73
Stage 6	0.65	3.00	32.38	87.87
Stage 7	0.43	1.09	33.47	90.83
Filter	—	3.38	36.85	100.00
Total		99.21		
Total Andersen[e]		36.85		

[a] Effective cutoff diameter (ECD) for each stage is determined using fractional-efficiency curves (a plot of particle size at each stage versus probability of impaction) (10).
[b] Micrograms of albuterol determined, as described in Table II.
[c] Cumulative mass of albuterol at each stage of Andersen cascade impactor.
[d] Cumulative percentage of albuterol at each stage.
[e] Total micrograms of albuterol collected (stage 0 through the filter).

Second, a computer program was used to calculate the probit (12) utilizing Microsoft's Excel spreadsheet (13). Figure 3 shows a plot of the calculated probit versus log ECD (ECD = 1.10 through 5.80). Applying linear regression to the plot in Fig. 3, produced the values of 1.79 and 1.70 for the MMAD and GSD, respectively. The spreadsheet calculated values (MMAD = 1.79 ± 0.01 and GSD = 1.70 ± 0.03) were very close to those calculated using particle-size diameter graphs (Fig. 2).

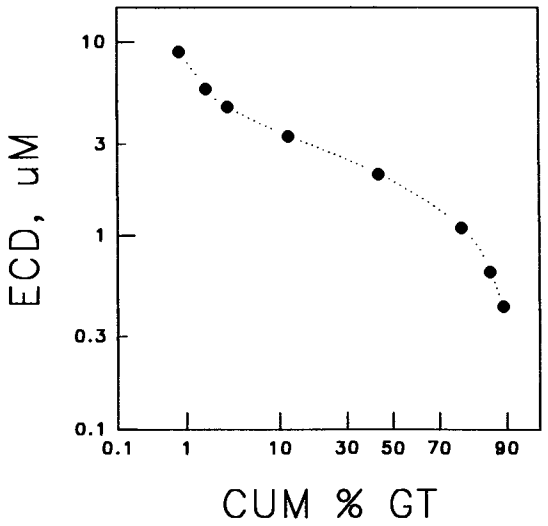

Fig. 2. Particle-size diameter graph of one puff from an albuterol MDI. Effective cutoff diameter (ECD) vs cumulative percentage greater than stated size (CUM%GT).

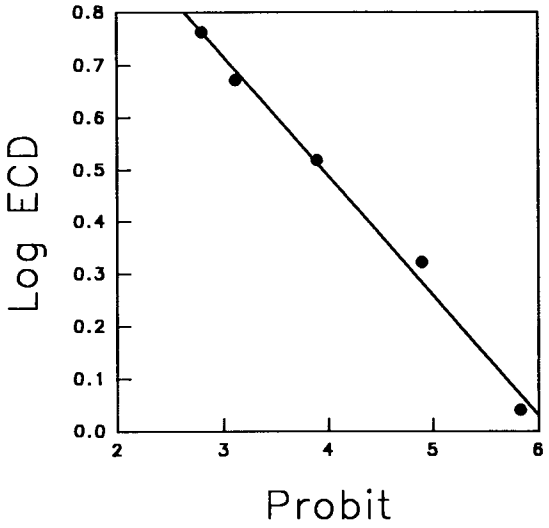

Fig. 3. A plot of the calculated probit (12) versus log ECD (ECD from 1.10 through 5.80).

The HPLC-EC analytical method combines a high sensitivity (50 pg minimum detectable amount) and a good precision (CV of 3% or less for concentrations of 1 ng/mL or higher). The method for collecting and extracting albuterol in the cascade-impactor experiments, although tedious, apparently provided complete recovery of delivered albuterol.

We compared our measured values of MMAD (1.8) and GSD (1.7) with the values for albuterol MDIs previously reported by Fults *et al.* (7) (MMAD, 2.0 to 2.8; GSD, 1.7 to 2.4) and Kim *et al.* (14) (MMAD, 2.4; GSD, 1.7). Our lower value of the MMAD may arise from the single-puff measurements. In the previous studies (7,14) 5 to 40 puffs were used. The small amounts of albuterol particles collected in the single-puff experiment reduce the formation of artifactual aggregates that may result from overloading the collection surfaces of the cascade impactors in multipuff experiments (15). An analytical method with the requisite sensitivity, such as the one reported here, could be used to study the effect of the number of puffs on the particle size measured with cascade impactors. It also allows determination of content uniformity of single puffs from MDIs.

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