

Effect of the Moisture Content in Aerosol on the Spray Performance of Stmerin[®] D HFA Preparations

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Stmerin[®] D, a pressurized metered dose inhaler (MDI) for treatment of asthma, contains CFCs (chlorofluorocarbons) as a propellant. For the CFC replacement study, two formulations were prepared using hydrofluoroalkanes (HFA-134a and HFA-227) and the effect of storage on the spray performance was investigated under accelerated stress conditions. Drug stability, moisture content and spray performances such as the emitted dose uniformity and aerodynamic particle size distribution were evaluated. Drug content did not change after 3 months storage at 40 °C/75% RH. However, the emitted dose uniformity varied and the respirable fraction (RF) was reduced remarkably. While stored at 40 °C/ambient for 3 months, no change was observed in either drug content or spray performances. This study clarified that the moisture content in the canister played an important role on the spray performance, and it changed not only the emitted dose uniformity but also the particle size distribution. Consequently, in order to improve the stability of the spray performance of aerosol prepared with HFAs, moisture permeation into the canister must be controlled.

Key words metered dose inhaler (MDI); hydrofluoroalkane (HFA) propellant; moisture

The pressurized metered dose inhaler (MDI) is a convenient and promising technology for drug delivery to the respiratory tract. For example, steroid MDIs have been applied to the topical therapy instead of oral administration which causes severe systemic side effects such as notable suppression of the hypothalamic-pituitary-adrenal axis, nephrosis, gastric ulcer, moon face and so on. As the topical therapy with MDI improves the therapeutic index by about 10 to 50 times, *i.e.*, increase the local concentration and decrease the systemic content, the patients can reduce the dose or stop oral intake.¹⁻³⁾

However, these MDIs contain chlorofluorocarbons (CFCs) as a propellant, which are known to contribute to ozone layer depletion.⁴⁾ Therefore, many aerosol products using non-CFC propellants have been developed.⁵⁾ As a propellant for pharmaceutical use, two candidates named hydrofluoroalkanes (HFAs), HFA-134a and HFA-227, are available at this moment.

Meanwhile, Stmerin[®] D (Astellas Pharma Inc., Tokyo, Japan) was a MDI for the treatment of asthma, which contained isoproterenol sulfate (bronchodilator, β -adrenergic stimulant), atropinmethylbromide (bronchodilator, anticholinergic agent) and dexamethasone (anti-inflammatory drug). This commercial product also contained mixed CFCs (CFC-11/CFC-12/CFC-114) as propellants. So the CFC replacement was carried out due to the environmental problem. Generally the CFC replacement is considered to be very difficult because of the physicochemical differences between CFCs and HFAs.^{6,7)} One of the main reasons is CFC-11, which has been used as a solvent in the aerosol formulations, is able to dissolve many suspending agents or surfactants and is also easily mixed with other CFCs, but the HFAs hardly dissolve such suspending agents. This makes it difficult to disperse the drugs in HFAs.

From screening of large amount of suspending agents, we have recently found out that middle chain fatty acid triglyceride (MCT) has potential to suspend the drugs in HFAs. Two preparations with HFA-134a and HFA-227 were pre-

pared.

The objective of this study is to evaluate the stability of suspension type aerosol preparations with HFAs as propellants. For such kinds of aerosol preparations, the moisture content in the canister is known to influence the stability of spray performance.⁸⁾ So the relationship between the moisture content in the canisters and spray performance is also discussed.

Experimental

Materials Isoproterenol sulfate (Boehringer Ingelheim, Germany) and atropine-methylbromide (Boehringer Ingelheim, Germany) are used after pulverized using the jet mill. Dexamethasone micronised is purchased from Roussel uclaf (Paris, France). These active ingredients are of pharmaceutical grade and used without further purification.

HFA-134a (1,1,1,2-tetrafluoroethane, Mistui Dupont Fluoro Chemicals, Japan) and HFA-227 (1,1,1,2,3,3,3-heptafluoropropane, Solvey, Germany) are used as a propellant. Middle chain fatty acid triglyceride (MCT, Miglyol 812, Mitsuba, Japan) is used and functions as both a suspending agent and a lubricant for the valve.

Metering valve (DF10/75 RCU) is purchased from Valois (France). Presspart C386P 10 ml (England) and 30 ml glass bottle (Tokyo Kobunshi, Tokyo) are used as canisters.

Evaluation of Spray Performance of Aerosol Employing the stored sample, 1) drug stability, 2) moisture content, and spray performances such as 3) the emitted dose content uniformity and 4) aerodynamic particle size distribution were evaluated as follows.

Drug Stability The canister was cooled in methanol/dry-ice. After opening the valve, the content and all parts of MDI were transferred into 50 ml beaker, and propellant was evaporated. Suitable volume of 33% CH₃CN aqueous solution and internal standard solution were added. They were placed in an ultrasonic bath for 5 min to assure the complete extraction and dissolution of drugs. The solution was filtrated through Millipore filter (GV 0.22 μ m) and injected into the HPLC system described below.

Moisture Content The moisture content in the canisters was measured using Karl Fisher coulometric titration (Type: CA-02, Mitsubishi-kasei, Japan). After shaking the MDI, the first 5 puffs were discarded, and then five puffs were delivered into the equipment. The assay value was divided by dosing weight and expressed as a unit of parts per million (ppm). All experiments were carried out at least in triplicate.

Emitted Dose Content Uniformity After the MDI was shaken and the first 5 puffs were discarded, another clean actuator was attached. During the puff number 6—15, 26—35 and 46—55, the MDI was fired into a 100 ml beaker filled with 70 ml of 33% CH₃CN solution containing internal stan-

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Table 1. Drug Stability in HFA Preparations under Various Conditions (Mean±S.D., *n*=3)

Propellant	Storage condition	Storage period	Residual %		
			IPS	DEX	AMB
HFA-134a	40 °C/75% RH	1 month	97.1±0.3	98.6±0.3	103.5±6.3
	40 °C/75% RH	3 month	98.3±2.6	96.0±2.1	105.9±0.7
	40 °C/ambient	3 month	99.0±0.8	101.3±1.8	103.5±2.9
HFA-227	40 °C/75% RH	1 month	95.1±0.9	95.6±1.5	102.2±3.6
	40 °C/75% RH	3 month	95.4±1.9	96.0±2.1	105.9±0.7
	40 °C/ambient	3 month	95.5±0.8	98.3±0.6	101.9±3.5

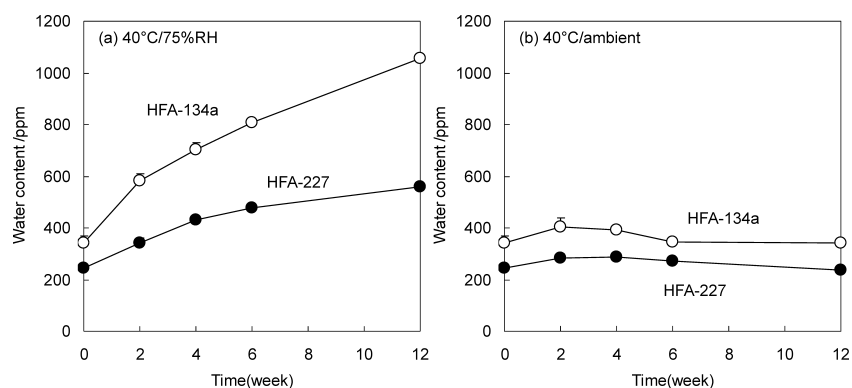


Fig. 1. Time Courses of Moisture Permeation into the Canisters of HFA Preparations Stored at 40 °C/75% RH (a) and 40 °C/Ambient (b)

ard. The MDI was shaken well before each puff. Delivered weight was measured every 10 times. The content of each beaker was transferred to a 100 ml volumetric flask. Each beaker was rinsed with 33% CH₃CN solution, and rinses added to the corresponding flasks. They were assayed using an HPLC method.

Aerodynamic Particle Size Distribution Eight stage andersen cascade impactor (Model: AV-100, Dylec, Japan) was used to measure the aerodynamic particle size distribution of three drugs. Each stage was filled with 7 ml of 33% CH₃CN solution containing internal standard. The air flow rate was adjusted and maintained at 28.3 l/min. After the MDIs were shaken and the first 5 puffs were discarded, another clean and dry actuator was attached.

The first puff was delivered into the cascade impactor. After 15 s, another puff was delivered. This process was repeated 50 times. The delivered weight was measured every 10 times. The actuator, the throat, the 8 stages (00 through 6) and the filter were rinsed with appropriate volume of 33% CH₃CN solution. The drugs in each fraction were assayed using an HPLC method. Mass median aerodynamic diameter (MMAD) was calculated from the aerodynamic particle size distribution.

HPLC Assay Isoproterenol (IPS) and dexamethasone (DEX) were detected at UV 270 nm, and atropin methylbromide (AMB) at UV 210 nm using an HPLC system (Shimadzu Class-10A, detector: SPD-M10A) equipped with a column of Nucleosil 5C8 (150×4 mm, Chemco Scientific Co., LTD., Japan). *p*-Hydroxybenzoic acid isopropyl ester (Tokyo Kasei, Japan) was used as an internal standard. The mixture of acetonitrile and 20 mM phosphate buffer solution pH 3 (33/67 v/v) containing 5 mM sodium lauryl sulfate (Nacalai Tesque, Kyoto, Japan) was used as a mobile phase. Flow rate was maintained at 0.9 ml/min. Thirty μl of sample was injected into the HPLC system.

Storage Condition Aerosol preparations were stored at 40 °C/75% RH and at 40 °C/ambient.

Results

Drug Stability In Table 1, residual percents of three active ingredients after storage are summarized at two conditions, 40 °C/75% RH and 40 °C/ambient. All of three compounds were stable in HFA-134a and HFA-227 preparations for 3 months.

Moisture Content Figure 1 shows the moisture permeation profile of HFA preparations. The moisture permeation

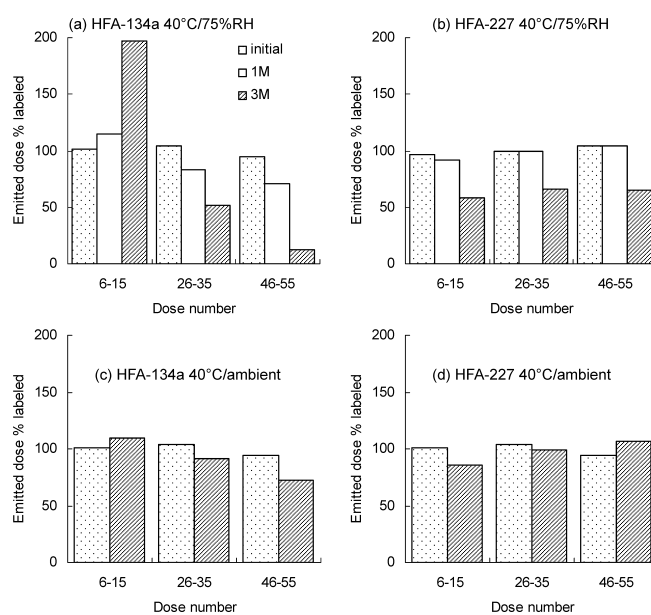


Fig. 2. Effect of Storage on the Emitted Dose Content Uniformities of HFA Preparations

(a): HFA-134a at 40 °C/75% RH, (b): HFA-227 at 40 °C/75% RH, (c): HFA-134a at 40 °C/ambient, (d): HFA-227 at 40 °C/ambient.

rate was faster in HFA-134a than in HFA-227 at 40 °C/75% RH. At 40 °C/ambient, however, there was no increase of moisture content in either preparation.

Emitted Dose Content Uniformity Since the emitted dose exhibited exactly the same patterns for the three active ingredients (data not shown). Figure 2 shows the emitted dose content uniformity of IPS through the life for the two preparations.

At initial, emitted dose slightly decreased as the dosing

numbers increased in the HFA-134a preparation (Fig. 2a, dotted column). On the other hand, the emitted dose slightly increased with dosing numbers in HFA-227 preparation (Fig. 2b, dotted column).

At 40 °C/75% RH (white column), these tendencies were more marked after 1 month storage. And after 3 month storage at 40 °C/75% RH, the emitted dose patterns (striped column) of both preparations drastically changed in comparison with the initial patterns. In the HFA-134a preparation, the average IPS dose during the first dose period (6—15 puff) became about 2-fold larger (196.9% of target value) than the initial value (101.4%). And during the final dose period (46—55 puff), an extremely small value (12.3%) was obtained. In the case of the HFA-227 preparation, every average emitted dose during 6—15, 26—35 and 46—55 puff, became about 65% compared to the initial.

At 40 °C/ambient, however, there was observed no remarkable difference between the initials and the stored samples for both the HFA-134a and HFA-227 preparations.

Aerodynamic Particle Size Distribution Aerodynamic

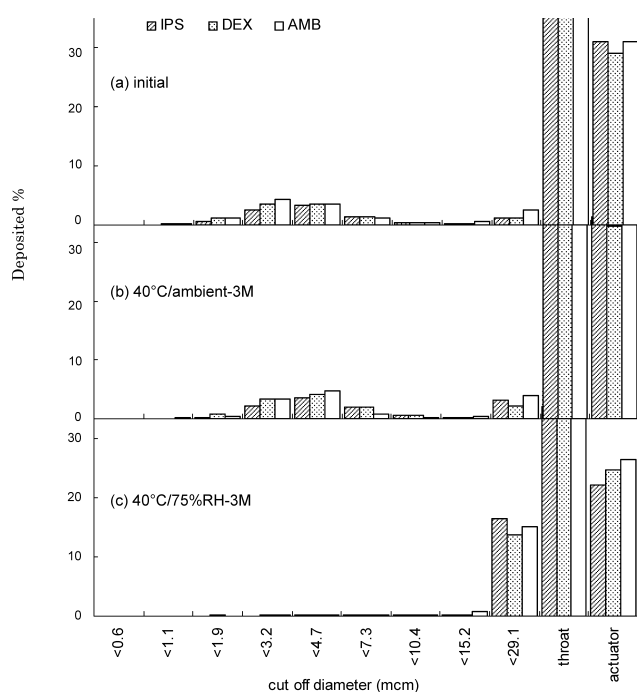


Fig. 3. Aerodynamic Particle Size Distributions of HFA-134a Preparation at Initial (a), after Storage at 40 °C/Ambient for 3 Months (b), and at 40 °C/75% RH for 3 Months (c)

particle size distribution after firing the HFA-134a and HFA-227 preparations are described in Figs. 3 and 4, respectively. And respirable fractions (Fine particle fraction, FPF) and mass median aerodynamic diameters (MMADs) are summarized in Table 2, where the FPF was calculated as a ratio of the fraction less than 4.7 μm diameter to the total amount of drugs recovered.

In both preparations, the values of FPF became very small after 3 months storage at 40 °C/75% RH. Namely, FPF of IPS was reduced from 6.4% to 0.2% in HFA-134a preparation and from 9.4% to 0.6% in HFA-227 preparation, respectively. MMADs became slightly larger and the drug deposition on the throat and the stage 00 (15.3—24 μm) largely increased. MMADs of IPS became 5.7 μm from 3.6 μm in HFA-134a and 5.1 μm from 3.5 μm in HFA-227 preparation, respectively.

While stored at 40 °C/ambient, no influence of storage on FPF and MMAD was observed for HFA-134a and HFA-227 preparations, similarly to the emitted dose content uniformity.

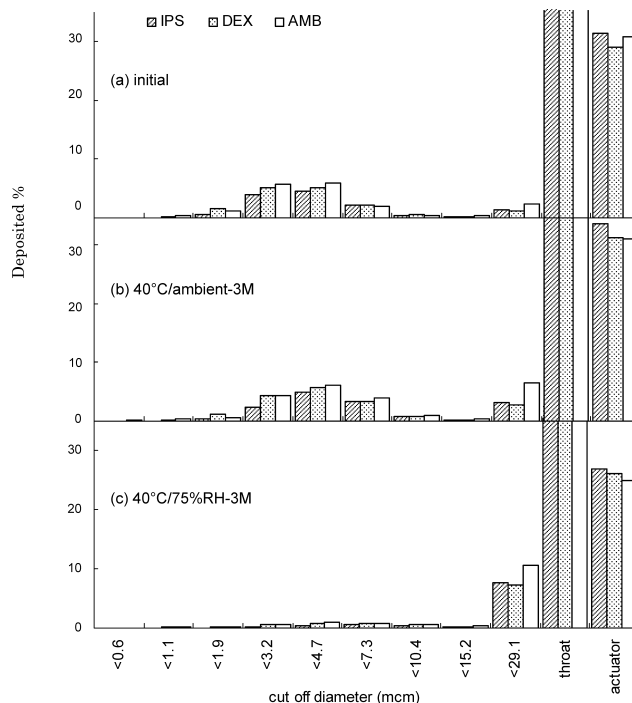


Fig. 4. Aerodynamic Particle Size Distributions of HFA-227 Preparation at Initial (a), after Storage at 40 °C/Ambient for 3 Months (b), and at 40 °C/75% RH for 3 Months (c)

Table 2. Fine Particle Fraction (FPF) and Mass Median Aerodynamic Diameter (MMAD) of HFA Preparations Stored under Various Conditions (Mean \pm S.D., $n=3$)

Propellant	Storage condition	Storage period	FPF (%) <4.7 mcm			MMAD (mcm)		
			IPS	DEX	AMB	IPS	DEX	AMB
HFA-134a		Initial	6.4 \pm 1.5	8.7 \pm 1.4	9.2 \pm 1.3	3.6 \pm 0.1	3.2 \pm 0.1	3.0 \pm 0.3
	40 °C/75% RH	1 month	1.7	3.1	3.1	4.5	4.0	3.9
	40 °C/75% RH	3 month	0.2 \pm 0.0	0.7 \pm 0.2	0.4 \pm 0.3	5.7 \pm 0.5	3.9 \pm 0.3	4.1 \pm 1.0
HFA-227	40 °C/ambient	3 month	6.0 \pm 0.5	8.2 \pm 0.3	8.4 \pm 1.1	3.9 \pm 0.1	3.6 \pm 0.0	3.5 \pm 0.2
		Initial	9.4 \pm 1.8	12.2 \pm 1.6	13.3 \pm 2.5	3.5 \pm 0.1	3.3 \pm 0.2	3.2 \pm 0.2
	40 °C/75% RH	1 month	5.6	8.8	8.5	4.0	3.7	3.8
	40 °C/75% RH	3 month	0.6 \pm 0.2	1.6 \pm 0.4	1.8 \pm 0.4	5.1 \pm 0.5	4.2 \pm 0.4	3.8 \pm 1.2
	40 °C/ambient	3 month	7.6 \pm 1.0	11.4 \pm 0.9	11.5 \pm 2.2	4.1 \pm 0.0	3.7 \pm 0.1	3.8 \pm 0.0

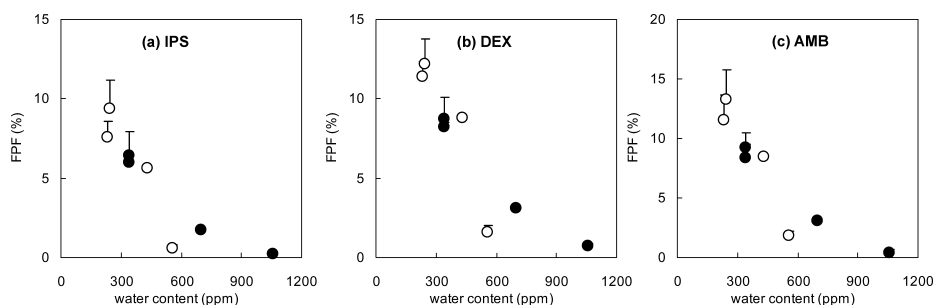


Fig. 5. Relationship between Moisture Content in the Canister and Fine Particle Fraction of IPS (a), DEX (b) and AMB (c) after Actuation of Preparations with HFA-134a (○) and HFA-227 (●)

Discussion

For CFC replacement of Stmerin® D, reformulation with HFA-134a or HFA-227 was conducted. For the success of MDI development, physical stability such as spray performance is recognized as a crucial issue as well as the drug stability. In this study the stability of the preparations was evaluated under the accelerated stress condition.

At first, the drug stability was investigated using two preparations. The drug contents did not change after 3 months storage at 40 °C/75% RH and 40 °C/ambient (Table 1), regardless of the formulation.

Next, the spray performance was characterized by the emitted dose content uniformity and the aerodynamic particle size distribution. In contrast to the drug stability, the spray performance drastically changed with the exception of sample stored at 40 °C/ambient (Figs. 2, 3).

The emitted dose content uniformity is one of the most important characteristics of aerosol preparations, because it can assure the homogeneity of the suspension as well as the valve function. In the HFA-134a preparation stored at 40 °C/75% RH for 3 months, average IPS emitted dose during the 6—15 puff increased 2 fold higher (196.9%) than the target dose. And the emitted dose decreased in proportion to the number of dosing. During the 46—55 puff, the average emitted dose decreased to 12.3% of the target dose. This emitted dose pattern suggests that the sedimentation of drugs occurred in liquid HFA-134a. Concerning the HFA-227 preparation after 3 months storage at 40 °C/75% RH, each average emitted dose during 6—15, 26—35 and 46—55 puff became about 65% of the initial value. This result indicates that the creaming of drugs occurred in liquid HFA-227. At 40 °C/ambient, no difference was observed between the initial and the stored sample. This suggests that moisture permeation influenced the homogeneity of the suspension.

From the observation of the glass bottle preparation, these kinds of phenomena seemed to be related to the density of propellants and to be promoted by the aggregation of drugs. The density of HFA-134a and HFA-227 are 1.23 g/ml and 1.41 g/ml at 20 °C, respectively. The drug particles settle in liquid HFA-134a and float in liquid HFA-227 after standing still for some minutes. According to Stokes's law, the settling velocity is proportional to the second power of the particle radius described as below equation,

$$V = d^2 \times \frac{(\rho_s - \rho_l)}{18\eta}$$

where V is settling velocity, d is Stokes' diameter, ρ_s and ρ_l are density of solid and liquid, g is acceleration due to gravity, and η is viscosity of liquid.⁹⁾ When stored at 40 °C/75% RH, the drug particles tended to aggregate easily even after sufficient agitation. The moisture permeated into the canister would distribute on the surface of the drug particles and act as a binding agent for liquid bridge formation. These agglomerates lead to larger apparent diameter which results in the higher velocity of sedimentation in liquid HFA-134a and creaming in liquid HFA-227. This reflects on the emitted dose uniformity.

Furthermore, the aerodynamic particle size distribution is another important characteristic of aerosol preparations. Because the fine particle fraction or the respirable fraction, defined as the volume ratio of the particle size less than 5—6.4 μm diameter range, is considered to directly reflect on the pulmonary drug delivery and on the clinical efficacy, nevertheless FPF depends on the apparatus evaluated.^{5,10—11)}

As seen in the emitted dose content uniformity, only for the samples stored at 40 °C/75% RH, reduction of FPFs and increase of MMADs were observed both in the HFA-134a and HFA-227 preparations. In Fig. 5, FPF values were plotted against the moisture content in order to assess the effect of the moisture content on the respirable fraction. FPFs decreased with the moisture content increased in both preparations. Especially beyond 500 ppm, a remarkable reduction of FPFs was observed, nevertheless the emitted dose was still above 65%. The particle size distribution in Figs. 3 and 4 exhibited that most of the emitted dose were deposited on the throat and the stage 00 (15.3—24 μm). In the aerosol system, the aerosol spray mist consists of unevaporated propellant droplets which coat the drug particles with non-volatile suspending agent (MCT). Increase of the moisture content in HFA causes the slower evaporation of a propellant which leads to an increase of the apparent particle size. This results in an increase of deposition on the throat and stage 00 fraction which represent the oropharyngeal region *in vivo*. These *in vitro* data also suggested a reduction of efficacy for the stressed samples because of the remarkable reduction of lung deposition.

This study demonstrated that the moisture content in the formulations increased with time when stored at 40 °C/75% RH, but those were constant when stored at 40 °C/ambient. The moisture was reported to penetrate through the metering valve, especially through the sealing gasket.⁸⁾ The moisture permeation rate would be influenced by the material of the gasket and its thickness. The faster moisture increase in

HFA134a formulation compared to HFA227 formulation seemed to relate to the solubility of water in HFAs, *i.e.* 2200 ppm in HFA134a and 610 ppm in HFA227 at 25 °C, respectively. In order to reduce the water penetration rate, the material of the gasket should be optimized and an appropriate propellant should be selected.

Conclusion

Under the accelerated stress condition, *i.e.*, at 40 °C/75% RH, it was clear that moisture could penetrate into the canister. This moisture drastically changed the emitted dose content uniformity of aerosol preparation due to acceleration of the drug aggregation in liquid HFAs and reduction of the suspension homogeneity. Further, the moisture strongly affects the particle size distribution after actuation because it causes slower evaporation of the propellant and increases the apparent particle size. This suggests a reduction of efficacy for stressed samples because of remarkable reduction of lung deposition.

These results demonstrate that the moisture permeation

into the canister must be controlled in order to improve the stability of suspension type aerosol preparations with HFAs.

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