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Research paper

Decrease of the stage-2 deposition in the twin impinger during storage of beclomethasone dipropionate dry powder inhalers in controlled and uncontrolled humidities[1]

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Abstract

In this study, the respirable fraction of beclomethasone dipropionate in a dry powder formulation for inhalation was tested during storage in various humidity conditions. Samples of Becotide Rotacaps® were stored for 18 months in polypropylene containers at conditions of controlled relative humidities (RHs) (30.8, 56.7 and 72.1%) and uncontrolled humidity (range observed: 46.7 ± 7.0% RH). The temperature was not controlled. Both humidity and temperature were monitored regularly. The respirable fraction was tested after storage for 10 and 18 months with the impaction apparatus Twin Impinger, the deposition in stage 2 represents the respirable fraction. The results showed no significant decrease of the stage-2 deposition during storage at 30.8% RH. However, after storage at conditions of uncontrolled humidity and at conditions of 56.7 and 72.1% RH, a significant loss of the stage-2 deposition was observed. We conclude that the respirable fraction of the tested products decreases during storage at conditions of normal, uncontrolled humidity. The polypropylene container used for this study apparently did not provide adequate protection against the influence of uncontrolled humidity during the shelf life. © 1997 Elsevier Science B.V.

Keywords: Deposition; Twin impinger; Dry powder inhalers; Storage conditions; Humidity

1. Introduction

For the pulmonary administration of drugs, three dosing forms are available: pressurised metered-dose inhalers (pMDIs) or aerosols, dry powder inhalers (DPIs) and nebulisers. The development of DPIs is currently being stimulated due to the world-wide ban on the CFC propellants that constitute aerosols. Administering drugs by the inhalation route has several advantages over the systemic (or oral) route. The drug is delivered directly to its site of action, so that a smaller dose can be given to the patient, and the risk of adverse reactions is diminished.

The clinical efficiency of inhaled drugs depends on the site of deposition in the respiratory tract and the deposition pattern of the drug particles. The deposition of dry powders is influenced by the patient's inhalation technique (for example, the inhalation flow), as well as by the formulation of the powder (for example, the presence of a carrier). A suitable distribution of particles in the respiratory tract enhances the desired clinical effect. Particles in the size range of 1.0–6.0 μm usually settle in the lower respiratory tract [1].

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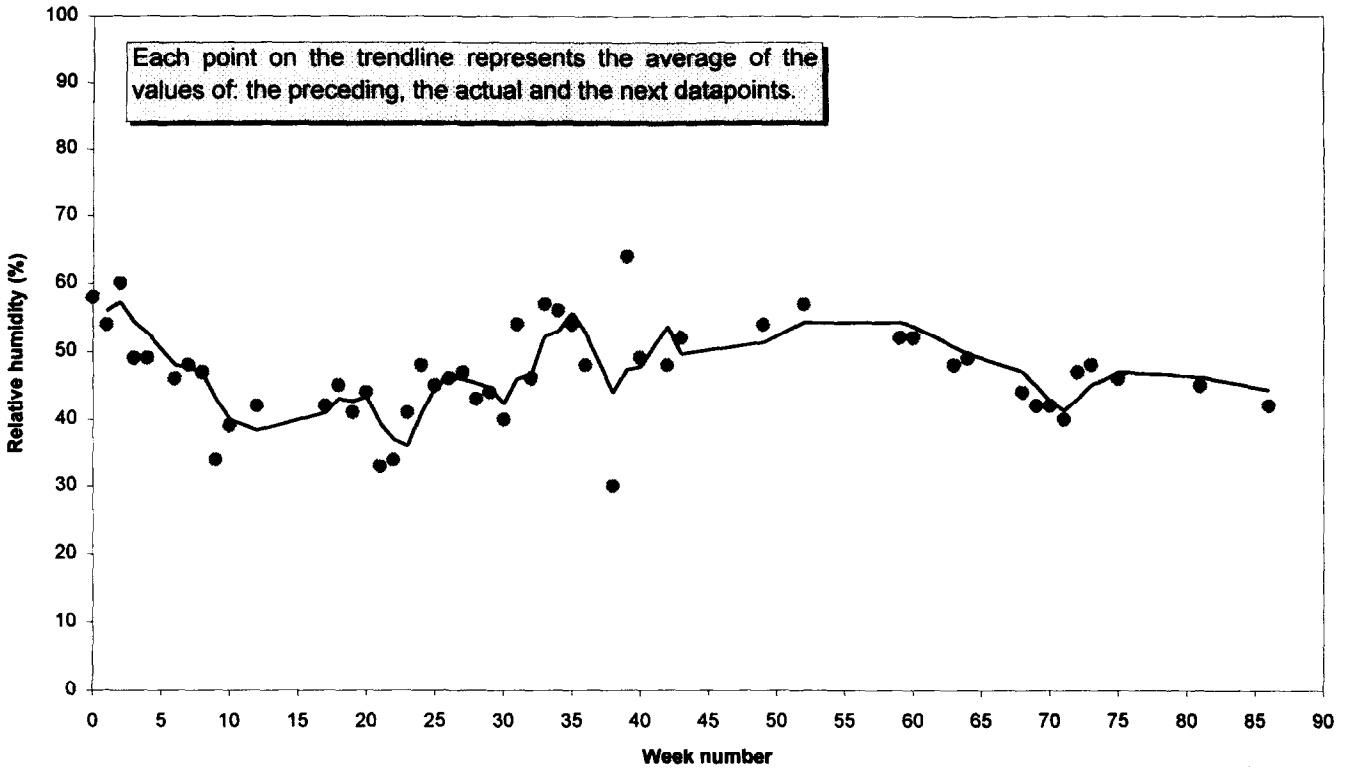

Fig. 1. The relative humidity in the storage facility (uncontrolled conditions) during the period of investigation.

Although the distribution of the particle size is not the exclusive factor that influences the deposition pattern in the human lung, it is a relevant parameter that can be monitored rather easily for quality control purposes. Several techniques for measuring the distribution of particles by size are available and have been described in the literature [2].

Moisture is known to affect the particle size distribution of powders for inhalation. For example, Vidgren et al. studied the in-vitro inhalation behaviour of mixtures of disodium cromoglycate and lactose stored at various humidities and found a significant deterioration of the inhalation properties at relative humidity (RH) values greater than 60% [3]. Two recent publications focus on this phenomenon in more detail. Braun et al. report that the selection and the quality of the excipients in combination with the storage humidity may influence the deposition of DPIs. This was tested on mixtures of disodium cromoglycate and lactose or dextrose [4]. Jashnani and Byron studied the aerodynamic characteristics of powders composed of three salts and the free base of salbutamol after storage at varying conditions of temperature and humidity [5].

However, in this field, little is known about preparations for inhalation containing beclomethasone dipropionate as the active ingredient. Hiller et al. studied the influence of storage conditions on the particle size distribution of a beclomethasone dipropionate aerosol [6]. Therefore, we decided to investigate the respirable fraction of a beclomethasone dipropionate DPI during storage in various humidities, using the impaction apparatus Twin Impinger to establish the in-vitro deposition.

Table 1
Test protocol for the samples of Becotide Rotacaps® stored at various humidities

Dose	Relative humidity (%)			
	32	59	75	Uncontrolled
100 μg/dose	x		x	
200 μg/dose		x		x

Table 2
Summary of the mean values of relative humidity (RH) and temperature and σ_{n-1} in the desiccators measured during the storage period (18 months)

	Relative humidity	rsd	Temperature (°C)
Desiccator $CaCl_2.6H_2O$	30.8 ± 0.8	2.6	17.1 ± 0.5
Desiccator NaBr	56.7 ± 0.4	0.7	17.2 ± 0.5
Desiccator NaCl	72.1 ± 0.2	0.3	17.1 ± 0.5
Uncontrolled	46.7 ± 7.0	15.0	18.5 ± 0.4

Relative humidity and rsd are both in percentages.

Beclomethasone dipropionate is a corticosteroid drug that is often used in the treatment of (chronic) respiratory diseases such as bronchitis, asthma and lung emphysema; aerosol and dry powder forms are both available on the market.

2. Materials and methods

2.1. Samples

The drug products investigated were Becotide 100 Rotacaps®, inhalatiepoeder in patronen 100 microgram, and Becotide 200 Rotacaps®, inhalatiepoeder in patronen 200 microgram (Glaxo, UK), registered in The Netherlands under the marketing authorisation numbers RVG 09208 and RVG 09209, respectively. Samples of both were obtained from a local pharmacy. On receipt all samples were packed in polypropylene containers ('securitainers'). The inhaler device used was the Rotahaler®.

2.2. Storage conditions

Before being stored, the samples were dispensed in commercially available polypropylene containers with a polyethylene closure (Type 6977, 120 ml, Spruyt-Hillen, Utrecht, The Netherlands). The samples were stored for 18 months in these containers under controlled conditions of various humidities. This was done by placing the containers in three desiccators in which the humidity was kept constant by a suitable mixture of an inorganic salt and its aqueous solution at a given temperature [7]. The RHs selected were (theoretically) 32% at 20°C ($CaCl_2.6H_2O$), 59% at 20°C (NaBr) and 75% at 20°C (NaCl). The test protocol is shown in Table 1.

For adjustment and regular checks of the humidity in the desiccators, calibrated hair hygro-thermometers (No. 7542, Sato Keiryoki, Tokyo, Japan) were used. The temperature was not controlled, but monitored regularly. We used the same hygro-thermometers each time. A fourth container was stored in conditions of uncontrolled humidity and temperature in the storage facility for test objects at our laboratory. The mean values of RH and temperature in the desiccators and in the storage facility (uncontrolled conditions), measured during the period of storage in the desiccators, are summarised in Table 2. The course of the humidity in the storage facility is shown in Fig. 1.

2.3. Assessment of the respirable fraction

The Twin Impinger, described in the British Pharmacopoeia [8], was selected as the impaction method (Erweka, Heusenstamm, Germany). The apparatus is shown in Fig. 2. For determination of the respirable fraction, 10 ml of methanol was used in the upper impingement chamber D and 20 ml of methanol, in the lower impingement chamber H. A pump was connected to the outlet F of the apparatus. The air flow through the apparatus, as measured at the inlet to the throat with a calibrated flow meter (Model 1307, Brooks Instruments, Veenen daal, The Netherlands), was adjusted to 60 ± 5 l/min.

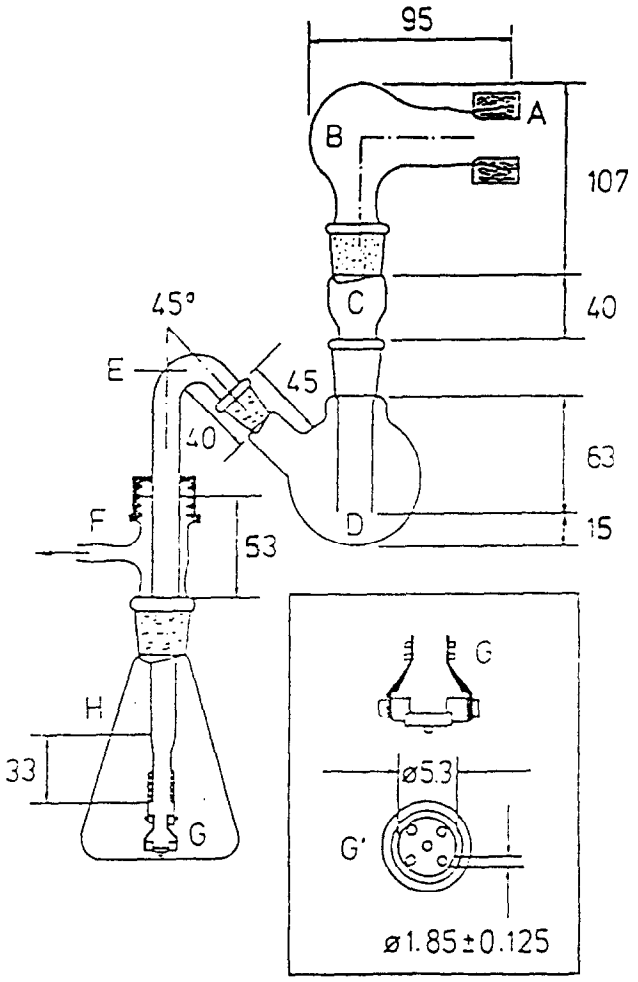

Fig. 2. The Twin Impinger, the impaction apparatus used for determining the respirable fraction. Dimensions are in ml.

2.4. Delivery of the inhalation doses

The inhaler device was placed on the mouthpiece adapter A of the apparatus. The pump was switched on for 3 s. After the pump was switched off, the inhaler was removed and loaded with the next capsule or dose. This procedure was repeated a further nine (100 μg capsules) or four (200 μg capsules) doses.

After having discharged the final dose, the apparatus was dismantled. The following steps were:

1. The emptied capsules were each washed with 8 ml of methanol. The washings were collected in a volu-

Table 3
Summary of the results for the deposition in the successive stages of the Twin Impinger

	Start		10 months		18 months	
	$\mu g \pm \sigma_{n-1}$	rsd%	$\mu g \pm \sigma_{n-1}$	rsd%	$\mu g \pm \sigma_{n-1}$	rsd%
Stage T	15.1 ± 8.5	56.3	7.2 ± 1.4	19.4	12.7 ± 1.7	13.4
Stage F	23.1 ± 0.8	3.5	21.7 ± 1.3	6.0	25.5 ± 6.6	25.9
Stage 1	66.4 ± 7.8	11.7	73.4 ± 3.4	4.6	65.2 ± 5.0	7.7
Stage 2	9.3 ± 1.2	12.9	10.6 ± 0.9	8.5	9.1 ± 0.4	4.4
Recovery (mass balance)	113.9 ± 3.5	3.1	112.9 ± 0.3	0.3	112.5 ± 3.0	2.7
Average content	119.4 ± 2.1	1.8	114.8 ± 1.1	1.0	112.3 ± 1.7	1.5

Samples tested, Becotide 100 Rotacaps® (RVG 09208).
The average deposition results ($n = 3$) were obtained at 30.8% RH.

Table 4
Summary of the results for the deposition in the successive stages of the Twin Impinger

	Start		10 months		18 months	
	$\mu g \pm \sigma_{n-1}$	rsd%	$\mu g \pm \sigma_{n-1}$	rsd%	$\mu g \pm \sigma_{n-1}$	rsd%
Stage T	29.3 ± 3.7	12.6	13.9 ± 1.6	11.5	24.9 ± 2.5	10.0
Stage F	69.4 ± 15.5	22.3	63.9 ± 5.8	9.1	57.5 ± 4.9	8.5
Stage 1	97.4 ± 6.7	6.9	117.4 ± 6.0	5.1	104.6 ± 5.8	5.6
Stage 2	37.1 ± 1.0	2.7	21.5 ± 3.1	14.4	13.8 ± 2.1	15.2
Recovery (mass balance)	233.2 ± 11.7	5.0	216.7 ± 13.1	6.0	200.8 ± 1.0	0.5
Average content	241.7 ± 7.2	3.0	228.4 ± 3.8	1.7	206.6 ± 2.7	1.3

Samples tested: Becotide 200 Rotacaps® (RVG 09209).
The average deposition results ($n = 3$) were obtained at 56.7% RH.

metric flask marked 'T' (for stage T) and diluted with methanol water.

2. The inhaler device, the mouthpiece adapter A and throat B were washed with methanol water. The washings were collected in a volumetric flask marked 'F' (for stage F) and diluted with methanol water.

3. The content of the upper impingement chamber D was transferred to a volumetric flask marked '1' (for stage 1). The chamber and neck C were washed with methanol water. The washings were collected in flask 1 and diluted with methanol water.

4. The content of the lower impingement chamber H was transferred to a volumetric flask marked '2' (for stage 2). The chamber and coupling tube E were washed with methanol water. The washings were collected in flask 2 and diluted with methanol water. Note that when we speak of 'methanol water', it is always 72:28 v/v. Each determination was repeated twice.

The content of beclomethasone dipropionate in each flask was determined by chromatographic or UV techniques. Before they were assayed, samples of the solutions were filtered through a 0.45 μm filter (Type Spartan 30/B, Schleicher and Schuell, s-Hertogenbosch, The Netherlands).

Additionally, the average content of beclomethasone dipropionate was determined by dissolving the content of four capsules in methanol water. The solution obtained was transferred to a volumetric flask and diluted with methanol water. The content of this solution was determined with the techniques mentioned; the average content was calculated as an average of three results.

2.5. Beclomethasone dipropionate assay

Two methods were used for beclomethasone dipropionate assay: a reversed-phase liquid chromatography with UV detection was used at the start and after storage for 10 months, and UV spectrophotometry was used after storage for 18 months. Both methods are described briefly.

2.5.1. High-performance liquid chromatography (HPLC)

The chromatographic system employed was a liquid chromatograph equipped with an autosampler (Type AS-950, Jasco, Tokyo, Japan), a pump (Type PU-980, Jasco, Tokyo, Japan), a reversed phase column (250 mm × 4.6 mm, 10 μm packings, Hypersil ODS)

Table 5
Summary of the results for the deposition in the successive stages of the Twin Impinger

	Start		10 months		18 months	
	$\mu g \pm \sigma_{n-1}$	rsd%	$\mu g \pm \sigma_{n-1}$	rsd%	$\mu g \pm \sigma_{n-1}$	rsd%
Stage T	15.1 ± 8.5	56.3	5.3 ± 0.8	15.1	17.5 ± 7.5	42.9
Stage F	23.1 ± 0.8	3.5	21.7 ± 1.6	7.4	30.3 ± 1.7	5.6
Stage 1	66.4 ± 7.8	11.7	80.1 ± 1.4	4.6	65.4 ± 5.2	8.0
Stage 2	9.3 ± 1.2	12.9	5.0 ± 0.3	6.0	6.7 ± 0.9	13.4
Recovery (mass balance)	113.9 ± 3.5	3.1	112.1 ± 1.2	1.1	119.9 ± 4.6	3.8
Average content	119.4 ± 2.1	1.8	113.2 ± 4.0	3.5	112.8 ± 6.6	5.9

Samples tested: Becotide 100 Rotacaps® (RVG 09208).
The average deposition results ($n = 3$) were obtained at 72.1% RH.

Table 6
Summary of the results for the deposition in the successive stages of the Twin Impinger

	Start		10 months		18 months	
	$\mu g \pm \sigma_{n-1}$	rsd%	$\mu g \pm \sigma_{n-1}$	rsd%	$\mu g \pm \sigma_{n-1}$	rsd%
Stage T	29.3 ± 3.7	12.6	20.1 ± 2.0	10.0	27.4 ± 1.7	6.3
Stage F	69.4 ± 15.5	22.3	56.2 ± 4.8	8.5	63.4 ± 6.3	9.9
Stage 1	97.4 ± 6.7	6.9	122.2 ± 7.5	6.1	120.0 ± 4.0	3.3
Stage 2	37.1 ± 1.0	2.7	29.6 ± 1.5	5.1	17.6 ± 1.6	9.1
Recovery (mass balance)	233.2 ± 11.7	5.0	228.1 ± 1.6	0.7	228.4 ± 8.1	3.5
Average content	241.7 ± 7.2	3.0	223.3 ± 2.8	1.3	218.9 ± 2.7	1.2

Samples tested: Becotide 200 Rotacaps® (RVG 09209).
The average deposition results ($n = 3$) were obtained at conditions of uncontrolled humidity.

(Hewlett-Packard, Amstelveen, The Netherlands), an integrator (Type SP 4400 Chromjet, Spectra-Physics Analytical, San Jose CA, USA) and an UV detector (Type UV-975, Jasco, Tokyo, Japan), set at a detection wavelength of 239 nm. Methanol water was used as the mobile phase at a flow rate of 1.2 ml/min. The column was kept at 25°C ± 2°C. The injection volume was 50 μl.

2.5.2. UV spectrophotometry

An UV spectrophotometer (Type Lambda 16, Perkin-Elmer, Nieuwerkerk a/d IJssel, The Netherlands) set at a detection wavelength of 239 nm was used. Methanol water was used as the solvent.

Both methods were validated for variability (within the day and day to day), recovery, specificity, selectivity, linearity, detection limit and range. They proved to be suitable according to internal (GLP) standards and could therefore be considered interchangeable. In the final stage of the study application of the UV method was preferred due to its ease of operation.

Simultaneously with each assay, a calibration curve was established by dissolving an appropriate amount of beclomethasone dipropionate CRS (Sigma, St. Louis MO, USA) in methanol water. The solutions obtained were assayed as already described. The content of beclomethasone dipropionate of each solution was calculated from the peak heights of the chromatograms.

For each determination, the total amount of recovered drug (or mass balance) was also calculated. The amount of beclomethasone dipropionate in each flask or stage was calculated in μg/dose and as the percentage of the total amount of drug recovered. The average amount of beclomethasone dipropionate was calculated in μg/dose.

Table 7
Summary of the stage-2 deposit values, as μg/dose, with σ_{n-1}

Storage conditions	Start	10 months	18 months
	$\mu g \pm \sigma_{n-1}$	$\mu g \pm \sigma_{n-1}$	$\mu g \pm \sigma_{n-1}$
30.8	9.3 ± 1.2	10.6 ± 0.9	9.1 ± 0.4
56.7	37.1 ± 1.0*	21.5 ± 3.1*	13.8 ± 2.1*
72.1	9.3 ± 1.2*	5.0 ± 0.3*	6.7 ± 0.9
Uncontrolled humidity	37.1 ± 1.0*	29.6 ± 1.5*	17.6 ± 1.6*

*$P = 0.05$ (one-tailed F-test).

3. Results

The results obtained for the deposition of beclomethasone dipropionate in the successive stages of the Twin Impinger after storage in various humidities are summarised in Tables 3–6. The results for the stage-2 depositions in the Twin Impinger are summarised in Tables 7–9 and are shown graphically in

Table 8
Summary of the stage-2 deposit values in percentages of the start values (= 100%)

Storage condition: relative humidity (%)	Start (%)	10 months (%)	18 months (%)
30.8	100.0	114.0	97.8
56.7	100.0	58.0	37.2
72.1	100.0	53.8	72.0
Uncontrolled humidity	100.0	79.8	47.4

Table 9
Summary of the stage-2 deposit and σ_{n-1} values in percentages of the total amount of drug recovered

Storage condition: relative humidity (%)	Start (%)	10 months (%)	18 months (%)
30.8	8.2 ± 1.1	9.4 ± 0.8	8.1 ± 0.4
56.7	15.9 ± 0.4	9.9 ± 1.4	6.9 ± 1.0
72.1	8.2 ± 1.1	4.4 ± 0.3	5.6 ± 0.8
Uncontrolled humidity	15.9 ± 0.4	13.0 ± 0.7	7.7 ± 0.7

Fig. 3. In Table 7, the stage-2 deposition values are summarised as μg/dose. In Table 8, the stage-2 deposition values are given in terms of the percentage of the start value (= 100%), and in Table 9, in terms of the percentage of the recovery or mass balance.

After storage at 30.8% RH, no significant decrease of the deposition in stage 2 of the Twin Impinger was observed (one-tailed F-test, $P = 0.05$). However, the stage-2 deposition decreased significantly during storage at conditions of 56.7, 72.1% RH and at uncontrolled humidity (one-tailed F-test, $P = 0.05$).

The results obtained for the average content of beclomethasone dipropionate in the capsules are summarised in Table 10.

After storage, the values of all assays decreased significantly (one-tailed F-test, $P = 0.05$), except those of the assay after storage at 72.1% RH. This deviant result is explained by the relatively high value of σ_{n-1}, because the trends are comparable for all assays.

4. Discussion

The aim of this study was to investigate the influence of humidity on the in-vitro deposition or respirable fraction of a beclomethasone dipropionate DPI. Samples of Becotide Rotacaps®, the market-leading product in The Netherlands, were stored for 18 months at various humidities. The deposition was established with the Twin Impinger, which is described in the British Pharmacopoeia. Recently this method was included in the European Pharmacopoeia (EP) too, together with three other methods (single-stage or metal impinger, multi-stage liquid impinger and multi-stage cascade impactor). Since the EP does not mention a preference for any particular method, the Twin Impinger was selected because of its comparative ease of operation. It also compares favourably with other techniques like microscopy or laser diffraction. It has been used extensively since its first description in the literature [9]. The cut-off diameter of the second stage of the Twin Impinger, operated at a flow rate of 60 l/min is 6.4 μm [1,6]. Thus, the fraction of the delivered dose deposited in this stage is considered to be the respirable fraction.

The bracketing design of the test protocol used in this study (shown in Table 1) is common for long-term stability studies of drug products [10]. Additionally to carrying out the procedure described in the British Pharmacopoeia, the depositions in stage 1 and in the throat of the Twin Impinger, as well as in the emptied capsules, were determined. The recovery or mass balance was established to ascertain the appropriateness of each test.

The results of this investigation show that the stage-2 deposition of the products tested remained constant during storage at 30.8% RH, but decreased significantly during storage at 56.7, 72.1% RH and at conditions of uncontrolled humidity ($P = 0.05$). The deposition in the upper stages (1 and throat) increased slightly. Remarkably, the start values of both the strengths tested, expressed in terms of the percentage of recovery, differed almost two-fold, whereas comparable values would be expected. The decrease of the stage-2 deposition was also greater for the 200 μg/dose strength than for the 100 μg/dose strength. At present, we still have no satisfactory explanation for this phenomenon, which will be investigated further in a follow-up study. The results from the stage-2 deposition appear not to be influenced by the relatively small decrease of average content of beclomethasone dipropionate during the storage period, as is shown in Table 9. If the stage-2 deposition values are expressed in terms of percentages of the total drug recovered (taking into account the amount of drug delivered to the apparatus), a decrease

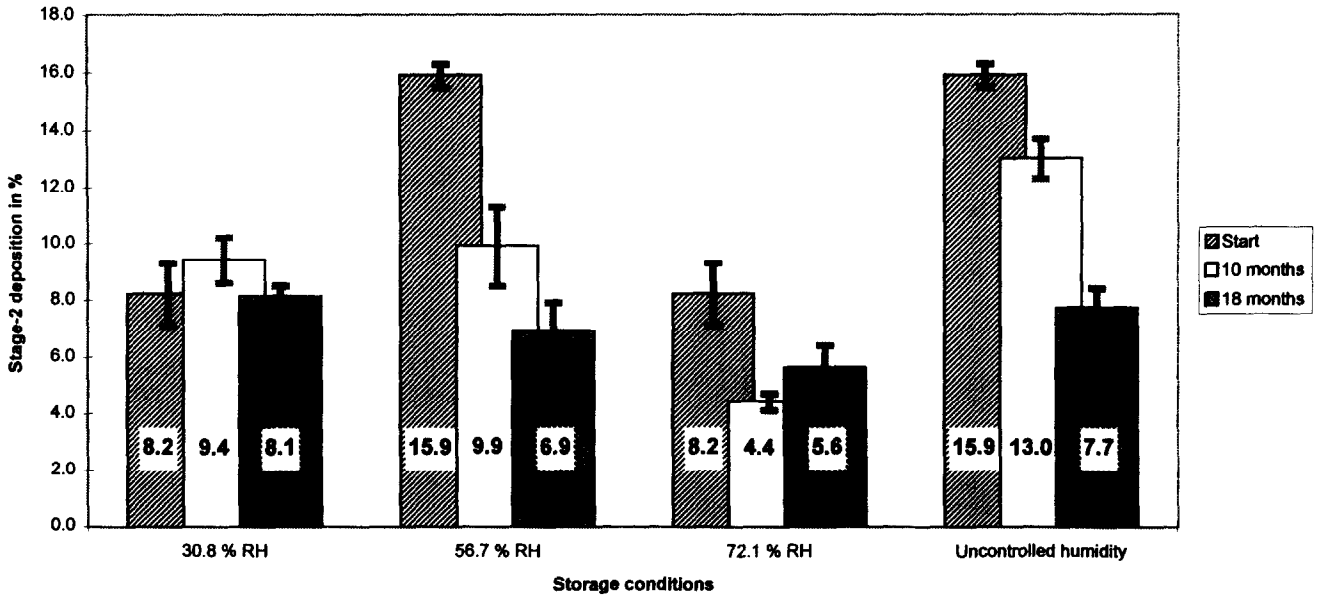

Fig. 3. Graph of the stage-2 deposition of the tested products during the period of investigation, in terms of percentage of the total amount of drug recovered (data from Table 9).

Table 10
Summary of the results for the average content of beclomethasone dipropionate in the capsules in percentages of the declared content (110 and 220% for the 100 μg/dose strength and 200 μg/dose strength, respectively), with σ_{n-1}

Storage condition: relative humidity (%)	Start (%)	10 months (%)	18 months (%)
30.8	108.4 ± 1.9*	104.4 ± 1.0*	102.1 ± 1.5
56.7	109.9 ± 3.3*	103.8 ± 1.7*	93.9 ± 1.2*
72.1	108.4 ± 1.9	102.9 ± 3.6	102.5 ± 6.0
Uncontrolled humidity	109.9 ± 3.3*	101.5 ± 1.3*	99.5 ± 1.2

*$P = 0.05$ (one-tailed F-test).

in the respirable fraction is still observed. The results of the assays after storage, summarised in Table 10, show a decrease for all test conditions except 72.1% RH. However, the latter result can be attributed to the relatively large standard deviation.

Although the original container was not used for this study, the same packaging materials were used (polypropylene, with a polyethylene closure). In practice, a retail pharmacy often sells medicines in smaller doses for which the registered container cannot be used. Therefore, our study can be considered as a simulation of a storage situation for medicines at the patient's home. We will consider the suitability of the original packaging for this form of dosing in a follow-up study.

Clearly, moisture affects the in-vitro aerodynamic characteristics of the tested products during storage in the test container. As Braun et al. have demonstrated, the selection of the excipient (the carrier material) for the formulation of a DPI can be very determinative for the in-vitro deposition [4]. We will seek confirmation of this possibility for beclomethasone dipropionate DPIs in a follow-up study.

According to the ICH Guideline for Stability Testing of New Active Substances and Medicinal Products [10], long-term stability testing of drug products should be carried out at 25 ± 2°C and 60% RH ± 5%. This requirement is considered satisfactory in view of the results, since a significant decrease of the stage-2 deposition was observed after storage at uncontrolled humidity and 56.7% RH. Testing of the beclomethasone dipropionate DPIs used in this study according to the ICH Guideline probably would yield comparable results. Therefore, the storage test conditions required can be considered appropriate for testing DPIs.

The currently registered storage condition for the Becotide Rotacaps® products, stated in the Summary of Product Characteristics (SPC), is 'store dry below 30°C'. In view of the results of this study, it is clear that the word 'dry', which is often mentioned in storage instructions for medicines, can no longer be considered suitable for assuring proper storage during the shelf life. Not only do we suggest that an additional warning for storing DPIs may be necessary, we also recommend international harmonisation of the description of the humidity conditions for storing medicines. Alterna-

tively, addition of a desiccant to the container could be considered.

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