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STUDY OF PRESSURISED METERED DOSE INHALERS FOR THE PURPOSE OF STANDARDIZATION OF QUALITY ATTRIBUTES CHARACTERIZING UNIFORMITY OF DOSING

Elena Bezuglaya, Nikolay Lyapunov, Vladimir Bovtenko, Igor Zinchenko, Yurij Stolper

Aim. *The purpose was to provide the rationale of test in regard to uniformity of fine particles dose for pressurised metered dose inhalers (pMDIs).*
Materials and methods. *The pMDIs containing suspensions of salbutamol sulfate (SS) or solutions of beclometasone dipropionate (BD) were studied by laser diffraction and high performance liquid chromatography (HPLC). The particle size distribution of SS, the average dose mass and uniformity of dose mass, the average delivered dose and the uniformity of delivered dose, the average fine particles dose and uniformity of fine particles dose were determined. Apparatus A was used for assessment of fine particles dose.*
Results. *The two analytical procedures for the quantitative determination of SS and BD by HPLC were validated in the ranges with low concentrations of these substances. The 5 medicinal products in pMDI dosage form were studied: 3 preparations were with SS and 2 ones contained BD. It was shown that three products with SS were very similar in regard to particle size distribution in containers and the average values of delivered dose were almost the same, but these products were different in the average dose mass and fine particle dose. According to the research results, the expediency of determining the average dose mass and the tests concerning uniformity of dosing of preparations by dose mass and by fine particle dose was substantiated. It was shown that in the case of pMDI the dosing of solutions of BD was more uniform compared to suspensions of SS. The approaches of leading and other pharmacopoeias concerning uniformity of dosing for pMDIs were critically discussed. The expediency of determination of uniformity of fine particle dose at the stage of pharmaceutical development was substantiated, as the therapeutic effect depends on fine particle dose. Issues concerning standardization pMDIs in regard to uniformity of fine particle dose were discussed.*
Conclusions. *The expediency of standardization and quality control of pMDIs in regard to such attributes as the average dose mass, which characterizes the volume of the metering chamber of the valve as well as the uniformity of the dose mass and the uniformity of fine particle dose, which assure the therapeutic effect of each dose of the product was substantiated*
Keywords: *pressurized metered dose inhaler, particle size, dose mass, delivered dose, fine particle dose, uniformity, procedure, validation*

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1. Introduction

The main regulatory and guidance documents, which outline the requirements for the quality of pressurized metered dose inhalers (pMDI), are the European Pharmacopoeia [1], in particular, the general articles "Preparations for inhalation", "Pressurized pharmaceutical preparations" and "2.9.18. Preparations for inhalation: aerodynamic assessment of fine particles" as well as document EMEA/CHMP/QWP/49313/2005 Corr. "Guideline on the Pharmaceutical Quality of Inhalation and Nasal Products" [2]. According to these documents several types of dose can be distinguished for pMDI. The dose is the quantity of the active substance to be administered at one time. The metered dose (MD) is the quantity of an active substance contained in the metering chamber of the valve. The delivered dose (DD) is the quantity of an active substance that is available to the user, ex-device, on a per dose basis; this dose is the part of the metered dose except for that part which has deposited on the actuator. Delivered dose is also the dose delivered from the actuator to the apparatus used to characterize pMDI. [1, 3–6]. The fine particle dose (FPD) is the part of delivered dose that is consisted from small particles of an active substance (≤ 5 μm) that are capable to penetrate into the lung during inhalation and provide therapeutic effect. The other part of delivered dose contains larger particles of an active substance that deposit in the mouth and throat of the patient and do not provide therapeutic effect [7, 8]. The small and larger particles contained in these two parts of delivered dose settle on different stag-

es of apparatuses for determination of fine particle dose and particle size distribution [9, 10]. Metered dose, delivered dose and fine particle dose are determined on a per one actuation basis.

The metered dose is attributable to the formulation, which is developed taking into account the nominal dose of an active substance and the volume of the metering chamber of the valve. Therefore, the metered dose is very close to the nominal dose stated in the label, and the variability of the MD is due to formulation variability from batch to batch as well as the tolerance on the nominal volume of the metering chamber of valves. Usually metered dose is used for quantitative determination of an active substance. For this purpose it is necessary to remove actuator and discharge 10 deliveries below the surface of the solvent maintaining the pressurised container in the vertical plane and discharging the preparation by actuating the valve [3].

The delivered dose depends on the metered dose and the design of the actuator, on the inner surface of which a certain part of the active substance deposits. The amount of the active substance in each of the 10 doses collected from one container, as well as in each of the 10 doses collected from 10 containers should be determined. The average value of the delivered dose and the uniformity of delivered dose should be calculated; the results should meet requirements stated in the specification. According to pharmacopoeial requirements large variability is permitted in regard to uniformity of delivered dose: the preparation complies with the test if 9 out of 10 results are between 75 % and 125 % of the average value and all are in range 65-135 % [1, 6].

Test concerning uniformity of delivered dose is mandatory, and when it is conducted, the content of the active substance in the test solutions is the lowest compared to reference solutions for other tests. Taking into account the risks in relation to the correctness of the analysis results in testing of uniformity of delivered dose, validated procedures for the quantitative determination of active substances during this test were set out for the first time by the British Pharmacopoeia 2020 in the specific monographs [3, 4].

It is necessary to ensure that each dose received by the patient has a therapeutic effect. The therapeutic effect of pMDIs depends on fine particle dose. However, according to the general articles 2.9.18 of the European Pharmacopoeia and the State Pharmacopoeia of Ukraine in order to determine fine particle dose on the relevant apparatus it is permitted to use more than one dose (but not more than 10), and the determined value should be divided by the number of actuations [1, 6]. Leading pharmacopoeias and State Pharmacopoeia of Ukraine do not provide validated procedures for quantitative determination of active substances for this test and do not set out requirement to determine the uniformity of fine particle dose (UFPD). In general, the lower limit or lower and upper limits for fine particle dose are laid down in the specifications for particular preparations. Results for the individual doses and their uniformity are not estimated. Criteria for assessment of uniformity of fine particle dose are not established.

But there are much more factors that can affect the fine particle dose than the factors that affect delivered dose. These factors include the type of dispersed system (solution or suspension), the size of the suspended particles of the active substances, the aggregation of these particles, the content of propellant, ethanol and water, the nozzle length and actuator orifice diameter, the plume geometry, etc. [8, 10, 11]. The UFPD for the different pMDIs available on the market has not yet been studied. However, it is obvious that at a limit of ≥ 35 % and a result obtained of 35 % (which was determined as the total value for 10 doses divided by 10) the individual fine particle doses are less than 35 %.

Another problem should be identified. The test results for uniformity of delivered dose, obtained by the quantitative determination of active substance, do not characterize the volume of the metering chamber and the performance of the valve. For this purpose the test "Average dose mass and uniformity of dose mass" is necessary, which is not provided in the general article "Preparations for inhalation" of the European Pharmacopoeia [1] and in the guidelines on the pharmaceutical quality of inhalation and nasal preparations [2].

Previously, it was proposed to study of UFPD as the performance characteristic of pMDIs during their pharmaceutical development [12].

However, the rationale for the UFPD testing was limited only by the results of the study of model pMDIs containing suspensions; the advisability of this test for pMDIs with solutions was not considered. The choice of acceptance criteria for UFPD at the different stages of the preparation life cycle (during pharmaceutical development and full-scale manufacture) is still a problem.

In view of the above, the aim of the work was to substantiate the advisability of testing in regard to UFPD based on the results of pMDIs studies concerning different quality attributes that characterize the uniformity of dosing.

2. Research planning (methodology)

To achieve this goal, the main objects of the study were to be preparations in the form of pMDIs available on the market. Three preparations containing salbutamol sulfate (SS) in the form of a suspension, differing in the composition of excipients and the volume of the valve metering chamber were included in the study. Two of these preparations are listed in the Orange Book as Proair HFA aerosol, metered and Ventolin HFA aerosol, metered [13, 14]. Also the preparation which contained beclometasone dipropionate (BD) 250 μg/dose as a solution and a similar model preparation which was equipped with the metering valve and the actuator from another manufacturer were chosen as the objects of the study. For these preparations, the average dose mass and uniformity of dose mass, average delivered dose and uniformity of delivered dose, as well as average fine particle dose and UFPD had to be determined. The sequence of dose sampling for all tests should correspond to that provided for testing of uniformity of delivered dose within one container (intra-inhaler) [1, 6]. To determine delivered dose and fine particle dose it was necessary to use the appropriate analytical procedures for the quantitative determination of each active substance by high performance liquid chromatography (HPLC). Therefore, it was necessary to validate the analytical procedures for quantitative determination of SS and BD in the

appropriate ranges [6, 15]. For different preparations, it was also necessary to compare the particle size distribution of SS, which for suspension pMDIs might also significantly affect the fine particle dose [11, 16]. At this step of the study, apparatus A (glass impinger) was chosen to determine the fine particle dose for one inhaler actuation. Total fine particle dose of active substance deposits in the lower chamber of the apparatus A that is more suitable for determination UFPD than multistage impactors. In the use of apparatus C, D, or E the fine particles dose is apportioned at the several collection plates/cups [1, 6], which could significantly complicate the experiments required for this research.

Thereafter, it was necessary to perform the comparative assessment of the uniformity of dose mass, uniformity of delivered dose and UFPD and to compare these attributes regarding various preparations. According to the research results, the advisability of UFPD test during pharmaceutical development of pMDIs and the possibility of their standardization with regard to this attribute had to be substantiated.

3. Materials and methods

The commercially available pressurised metered-dose inhalers such as Salbutamol-Teva 100 μg/dose (Teva, batches AFB40A, AET46A, AFC39A, hereinafter the pMDI No. 1) [13, 14, 17], Ventolin™ Evohaler™ 100 μg/dose (GlaxoSmithKline, batch VJ8K, hereinafter the pMDI No. 2) [13, 14, 18], Salbutamol 100 μg/dose (Multispray, batch 30518, hereinafter the pMDI No. 3) [18], Beclazon-Eco 250 μg/dose (Norton Waterford/Teva, batch AF A69A, hereinafter the pMDI No. 4) were used in order to substantiate the approach to the assessment of UFPD [14, 17, 18]. Also the model preparation Beclometasone 250 μg/dose (hereinafter the pMDI No. 5), which is similar to Beclazone-Eco 250 μg/dose (pMDI No. 4) was studied. The pMDI No. 5 had to provide 200 doses, so minimum fill was 240 doses. The aluminum containers with a nominal volume of 19 ml (art. C0128, Presspart Manufacturing Ltd.) fitted with the metering valves DF30 PLUS/63 RCU CS20 ARGENT (Aptar Pharma) and the actuators with caps NM200 DIS ØT3.16A ØS0.25 JL0.5 (H&T Presspart) were used as the container closure components. The active substance and excipients for the formulation of the pMDI No. 5 met the requirements of the European Pharmacopoeia [1].

The pMDI No. 1 and the pMDI No. 3 were the suspension of micronized SS (120.5 μg/dose which is equivalent to 100 μg/dose of salbutamol) in the mixture of norflurane (26.46 mg/dose and 26.05 mg/dose, respectively) with ethanol (3.42 mg/dose and 1.13 mg/dose, respectively); the nominal volumes of the metering chambers were 28 μl and 25 μl, respectively. The pMDI No. 2 was the suspension of micronized SS (equivalent to 100 μg/dose of salbutamol) in norflurane liquefied under pressure (up to 75 mg/dose); the nominal volume of the metering chamber was about 61 μl. The pMDI No. 4 and the pMDI No. 5 were solutions of BD (250 μg/dose) in mixture of norflurane (71.75 mg/dose and 68.40 mg/dose, respectively) with anhydrous ethanol (6.00 μg/dose); the nominal volumes of the metering chambers were about 63 μl.

Weighing of samples was performed using analytical balances AUW 120D (Shimadzu, Japan). The solutions were prepared by mass-volume technique using volumetric amber glass flasks (class A, Simax, Czech Republic).

Dose mass was determined according to the procedure: the protective cap was removed from the actuator, the container was shaken for about 30 seconds and one dose was discharged, after not less than 5 seconds container was shaken again and one dose was discharged. The container was removed from the actuator, valve stem was dried by filter paper (inside and outside). The container was weighed with the accuracy 0.1 mg (m_1). The container and the actuator were reassembled and shaken for about 5 seconds and one dose was discharged again. The container was removed from the actuator, the valve stem was dried by filter paper and the container was weighed again (m_2). The difference between m_1 and m_2 is the dose mass. The masses of the 1st, 2nd, 3rd, 99th, 100th, 101st, 102nd, 198th, 199th and 200th doses were determined sequentially. The average dose mass and the deviation (Δ, %) of the mass of each dose from the average dose mass were calculated.

Uniformity of delivered dose was determined according to the procedure described in the general article "Preparations for inhalation" of the European Pharmacopoeia [1] using the dose collection apparatus ERWEKA DUSA-MDI and the vacuum pump ERWEKA HBP 1000 with the flow rate meter DMF 2 (Erweka). The filter MN GF-4 Ø 25 mm (art. 414 0025, Macherey-Nagel GmbH & Co. KG) was used for dose collecting, which was placed on a filter-support base with an open-mesh filter support.

Fine particle dose was determined according to the to the procedure described in the general article "Preparations for inhalation" of the European Pharmacopoeia [1] using apparatus A (glass impinger). The mass balance was determined to ensure that test results were valid; the total mass of salbutamol in the both chambers should be not less than 75 % and not more than 125 % of the average delivered dose determined during testing for uniformity of delivered dose.

Water, carbon dioxide-free R was used as a solvent when preparations with SS were tested and *methanol R* was used in the case of preparations with BD.

HPLC studies were performed using Shimadzu Prominence-i LC-2030C 3D liquid chromatograph with a diode array detector (software: LabSolutions Lite version 5.82). Shimadzu Prominence-i LC-2030C 3D Plus liquid chromatograph with diode-array detector (software: LabSolutions version 5.93) was also used in the study of intermediate precision.

The content of salbutamol in the solutions was determined according to the analytical procedure described in the section "Related substances" of the monograph "Salbutamol Pressurized Inhalation" of the British Pharmacopoeia 2016 [4]. This procedure was validated in regard to quantitative determination of salbutamol in the appropriate range for such tests as uniformity of delivered dose and UFPD.

Analytical procedure.

Test solutions (TS). TS 1. Filtered solution of SS collected in the dose collection apparatus per discharge, in *water, carbon dioxide-free R* (~1.7 μg/ml).

TS 2. The solution of SS collected in the lower chamber of the apparatus A per discharge, in 50 ml of *water, carbon dioxide-free R*.

Reference solutions (RS). *RS 1*. The solution of *Salbutamol sulfate BP CRS* (cat. No. VP302) in *water, carbon dioxide-free R* 2.0 μg/ml. *RS 2*. The solution of *Salbutamol sulfate BP CRS* in *water, carbon dioxide-free R* 0.7 μg/ml.

Chromatographic conditions:

– stainless-steel chromatographic column 150×3.9 mm packed with *end-capped octylsilyl silica gel for chromatography R* (5 μm) (Symmetry C8, "Waters", cat. No. WAT046970, or YMC-Pack C8, "YMC", cat. No. OC12S05-1546WT);

– isocratic elution;

– mobile phase: *acetonitrile for chromatography R* – solution containing 0.287 % w/v of *sodium heptanesulfonate* and 0.25 % w/v of *potassium dihydrogen phosphate* (22:78) adjusted to pH 3.65 with *phosphoric acid dilute R*;

– flow rate 1 ml/min;

– detection wavelength of 220 nm;

– column temperature 25 °C;

– inject 20 μl of each of the 10 test solutions and reference solution;

– allow the chromatography to proceed for about 12 min.

System suitability. The test is valid provided that:

– chromatographic column performance (apparent efficiency) calculated by the peak due to salbutamol in the chromatogram obtained with the reference solutions, is at least 4000 theoretical plates;

– for the peak due to salbutamol in the chromatogram obtained with the reference solutions the symmetry factor is 0.8 to 1.5;

– the maximum permitted relative standard deviation for the areas of peaks due to salbutamol in the chromatogram obtained withe the reference solutions does not exceed 2.11 % for three injection.

The potentiometric determination of pH (*2.2.3*) [9, 12] of the buffer solution and the mobile phase were conducted using a pH meter Metrohm 827 lab (Metrohm, Switzerland) with an electrode Porotrode (Metrohm, Switzerland; cat. No. 6.0235.200).

The quantitative determination of BD in the solutions was performed according to the analytical procedure which was validated in the appropriate range for such tests as uniformity of delivered dose and UFPD.

Analytical procedure.

Test solutions (TS). *TS 1*. Filtered solution of BD collected in the dose collection apparatus per discharge, in *methanol R* (~ 10 μg/ml).

TS 2. The solution of BD collected in the lower chamber of the apparatus A per discharge, in 50 ml of *methanol R* (about 2.5 μg/ml).

Reference solutions (RS). *RS 1*. The solution of *Beclometasone dipropionate BP CRS* (cat. No. 030) in *methanol R* 10 μg/ml. *RS 2*. The solution of *Beclometasone dipropionate BP CRS* in *methanol R* 1.25 μg/ml.

Chromatographic conditions:

– stainless-steel chromatographic column 250×4.6 mm packed with *end-capped octylsilyl silica gel for chromatography R* (5 μm) (Hypersil BDS C18, "Agilent", cat. No. 79926BD-585, or Zorbax Ec C18, "Agilent", cat. No. 7995118-585);

– isocratic elution;

– mobile phase: *water for chromatography R* – *acetonitrile for chromatography R* (40:60);

– flow rate 2 ml/min;

– detection wavelength of 238 nm;

– column temperature 40 °C;

– inject 20 μl of each of the 10 test solutions and reference solution;

– allow the chromatography to proceed at least 15 min.

System suitability. The test is valid provided that:

– chromatographic column performance (apparent efficiency) calculated by the peak due to BD in the chromatogram obtained with the reference solutions, is at least 5000 theoretical plates;

– for the peak due to BD in the chromatogram obtained with the reference solutions the symmetry factor is 0.8 to 1.5;

– the maximum permitted relative standard deviation for the areas of peaks due to BD in the chromatogram obtained with the reference solutions does not exceed 2.11 % for three injection.

Validation of procedures for quantitative determination was carried out according to the generally accepted methodology [6, 15]. Acceptance criteria for validation characteristics were calculated in accordance with the requirements of general article *5.3.N.2* of State Pharmacopoeia of Ukraine [6].

Determination of the particle size of SS was performed by laser diffraction [1, 6] using the laser particle diffraction analyzer "Shimadzu SALD-2201" (Shimadzu; software: WingSALD-II, version 2.1.0). 20 ml of anhydrous ethanol was placed into the beaker, the container with preparation was shaken and the dose was discharged below the surface of ethanol; these actions were repeated 4 more times and the particle size in obtained suspension was immediately determined.

4. Research results

The performance characteristics of preparations for inhalation which are suspensions depend on the particle size distribution [8, 10, 19]. The data on the size particles distribution for SS in the pMDIs No. 1–3 are presented in Fig. 1 and Table 1.

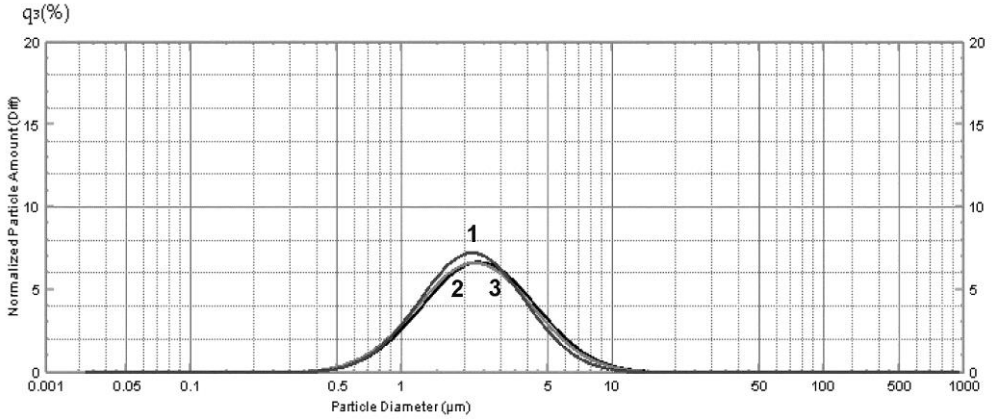

Fig. 1. Particle size distribution for SS in the pMDI No. 1, pMDI No. 2 and pMDI No. 3

Table 1

The maximum size of SS particle (D) in different fractions containing from 10 % to 90 % of particles from their total number in samples of suspensions from the pMDIs No. 1–3

No.	The maximum size (D, μm) of particles in fractions, the content of which is:								
	10 %	20 %	30 %	40 %	50 %	60 %	70 %	80 %	90 %
1	0.930	1.217	1.469	1.731	2.019	2.351	2.774	3.355	4.387
2	1.005	1.315	1.608	1.900	2.228	2.612	3.090	3.778	4.947
3	1.037	1.333	1.606	1.870	2.167	2.502	2.933	3.530	4.555

As can be seen from the data presented in Fig. 1 and in Table 1, the particle size distribution for SS was almost the same in the case of all three pMDIs.

The size of 99 % particles of SS should be not more than 10 μm and 90-95 % − not more than 5 μm. The content of particles with size up to 4.936 μm and up to 10.231 μm was (respectively): in the pMDI No. 1 – 93,350 % and 99,684 %, in the pMDI No. 2 – 90,016 % and 99,306 %, in the pMDI No. 3 – 92,623 % and 99,688 %. Therefore, the particle size in the case of all pMDIs under the study met the well-accepted requirements.

The concentration range of the model solutions of SS for validation of the analytical procedure for quantitative determination was from 0.2 μg/ml to 2.8 μg/ml (equivalent to salbutamol). The concentration 0.7 μg/ml corresponds to the concentration in the test solution if the part (35 %) from the nominal dose of salbutamol (100 μg) deposits in the lower chamber of the apparatus A; the reference solution with the content of salbutamol 2.0 μg/ml was used when determination of delivered dose was performed.

Fig. 2 shows the representative chromatograms obtained with placebo solution, reference solution 2 and test solution. Fig. 3 illustrates the chromatogram obtained with the model solution of salbutamol 0.23 μg/ml, which corresponds to the concentration in the test solution if the part (11.5 %) from the nominal dose of salbutamol (100 μg) deposits in the lower chamber of the apparatus A. The results in regard to the linearity, repeatability, accuracy and intermediate precision as well as acceptance criteria are summarized in Table 2. The data concerning stability of model solutions of SS are presented in Table 3.

The specificity of the analytical procedure was proved because the retention times (Rt) of the peaks corresponding to salbutamol in chromatograms obtained with the reference solution and the test solution had no difference, and the chromatogram with placebo solution had no peaks with the similar retention time (Fig. 2). The purity of the peaks due to salbutamol in the chromatograms obtained with test solution and the reference solution, which was 1.000000, also confirmed the specificity of the analytical procedure. The signal-to-noise ratio (S/N) for the peak due to salbutamol in the chromatogram obtained with the model solution 0.23 μg/ml was 59.06 while the acceptance criterion for S/N is ≥ 10 (Fig. 3).

According to the results of validation studies, the analytical procedure for the quantitative determination of salbutamol by HPLC in the appropriate range met the requirements for the linearity, repeatability, accuracy and intermediate precision against the acceptance criteria calculated for tolerance B=10 % (Table 2).

The results of validation studies with using two chromatographic columns proved that the test model solutions of SS met the requirements for their stability (Table 3).

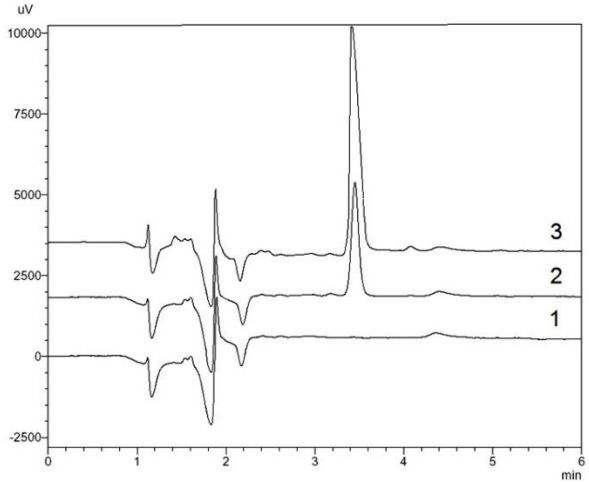

Fig. 2. Representative chromatograms obtained with placebo solution (1), reference solution (2), test solution (3) when determination of the fine particle dose of salbutamol was performed (peaks with Rt=3,521 min and Rt=3,528 min correspond to salbutamol)

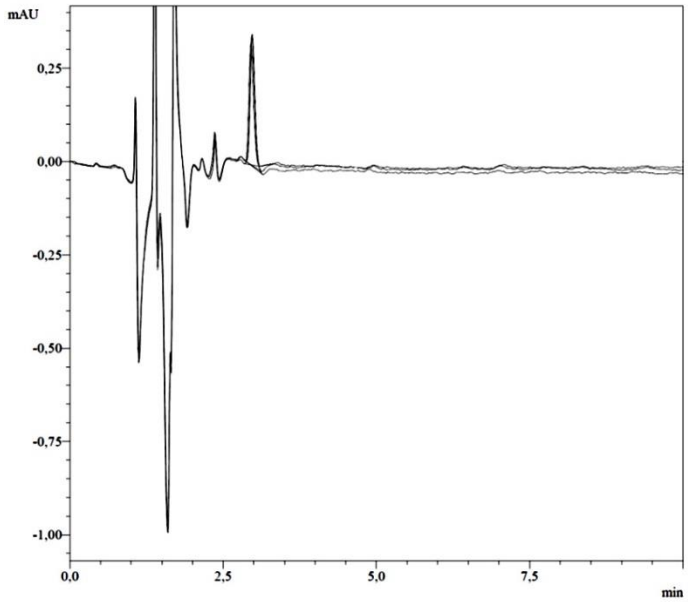

Fig. 3. Chromatograms obtained with the model solution of salbutamol 0.23 μg/ml, the peak with Rt=2.965 min corresponds to salbutamol (S/N=59.06)

Table 2

The results of the study in regard to validation characteristics of the analytical procedure for the quantitative determination of salbutamol sulfate (SS) and their evaluation against the acceptance criteria [6]

Parameter	Value	Criteria (n=9)	Conclusion
Linearity			
b	1.00729		
S_b	0.01157		
α	0.25304	1) $\leq \lvert S_\alpha \bullet 1.8946 \rvert = \lvert 1.64 \rvert$ 2) if it does not meet criterion (1), then $\leq \lvert 1.14 \rvert$	Pass
S_α	0.86805		
SD_0, %	1.31031		
SD_0/b, %	1.30083	$\leq \lvert 1.69 \rvert$	Pass
r	0.99954	$> \lvert 0.99909 \rvert$	Pass
Repeatability			
relative standard deviation RSD_z, %	1.50		
relative confidence interval $\Delta_z = t \bullet (95\ \%, 9–1) \bullet RSD_z$	2.79	<3.20 %	Pass (B=10 %)
Accuracy			
mean Z, %	100.99		
systematic error δ, %	0.99	1) $\leq \Delta_z : \sqrt{9} = 0.93$ % 2) if it does not meet criterion (1), then $\leq 0.32 \bullet 3.2$ % = 1.02 %	Pass according to criterion 2
Intermediate precision			
combined average Z_{intra}, %	100.78		
$SD_{z\text{-}intra}$, %	1.52		
$\Delta_{intra} = t \bullet (95\ \%, 36–1) \bullet SD_z$	2.64	<3.2 %	Pass

Table 3

Evaluation the stability of the model solutions of SS

Column	Z_{first}	Z_{last}	$\lvert \Delta Z_i \rvert$	$\lvert \Delta Z_i \rvert \leq \sqrt{2} \bullet 3.2$ %	Conclusion
Symmetry® C8	98.85 %	99.44 %	0.59 %	0.59 % <4.53 %	Stable
YMC-Pack C8	100.58 %	100.15 %	0.43 %	0.43 % <4.53 %	Stable

Validation of the analytical procedure for the quantitative determination of BD was performed using model solutions in concentration range from 0.5 μg/ml to 14.0 μg/ml. The concentration of BD of 1.25 μg/ml cor-

responds to its content in the test solution if the part (25 %) from the nominal dose 250 μg deposits in the lower chamber of the apparatus A; the reference solution with the content of BD 10.0 μg/ml was used when determination of delivered dose was performed.

Fig. 4 shows the representative chromatograms obtained with placebo solution, reference solution 2 and test solution. Fig. 5 illustrates the chromatogram obtained with the model solution of BD 0.5 μg/ml, which corresponds to the concentration in the test solution if the part (10 %) from the nominal dose of BD (250 μg) deposits in the lower chamber of the apparatus A. The results in regard to the linearity, repeatability, accuracy and intermediate precision as well as acceptance criteria are summarized in Table 4. The data concerning stability of model solutions of BD are presented in Table 5.

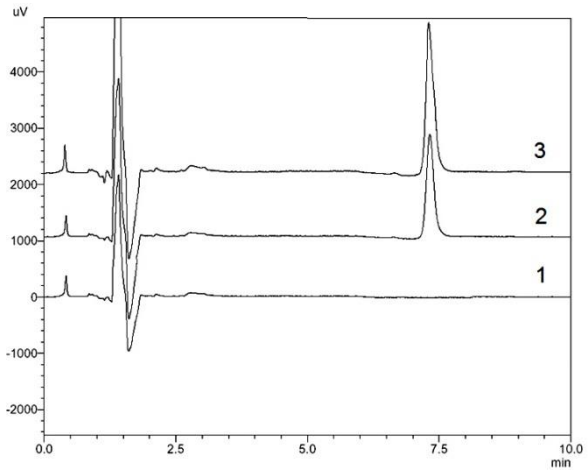

Fig. 4. Representative chromatograms obtained with placebo solution (1), reference solution (2), test solution (3) when determination of the fine particle dose of BD was performed (peaks with Rt=7.395 min and Rt=7.402 min correspond to BD)

The specificity of the analytical procedure was demonstrated because the retention times (Rt) of the peaks corresponding to BD in chromatograms obtained with the reference solution and the test solution did not differ, and in the chromatogram obtained with placebo solution there was no peak with the similar retention time (Fig. 4). The purity of the peaks corresponding to BD in the chromatograms obtained with test solution and the reference solution, which was 1.000000, also confirmed the specificity of the analytical procedure. The signal-to-noise ratio (S/N) for the peak corresponding to BD in the chromatogram obtained with the model solution 0.5 μg/ml was 30.81 while the acceptance criterion for S/N is ≥10 (Fig. 5).

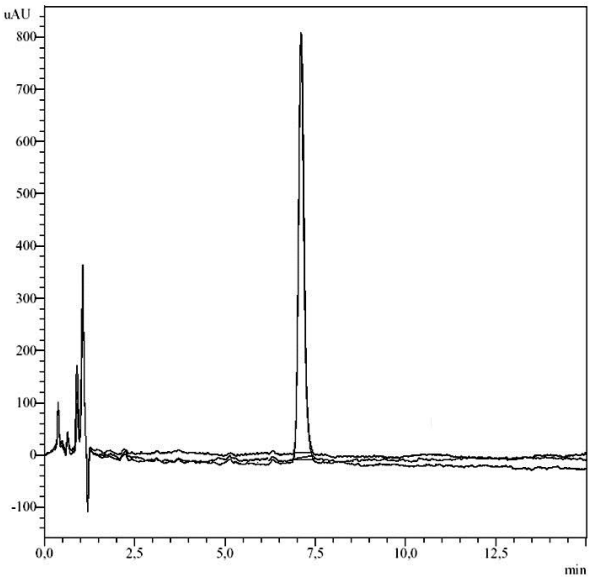

Fig. 5. Chromatograms obtained with the model solution of BD 0.5 μg/ml, the peak with Rt=7.059 min corresponds to BD (S/N=30.81)

Table 4

The results of the study in regard to validation characteristics of the analytical procedure for the quantitative determination of beclometasone dipropionate (BD) and their evaluation against the acceptance criteria [6]

Parameter	Value	Criterion (n=18)	Conclusion
Linearity			
b	0.99590		
S_b	0.00249		
α	0.29870	1) $\leq \lvert S_\alpha \bullet 1.8946 \rvert = \lvert 0.38 \rvert$ 2) if it does not meet criterion (1), then ≤ 0.54	Pass
S_α	0.20183		
SD_0, %	0.32856		
SD_0/b, %	0.32991	$\leq \lvert 0.84 \rvert$	Pass
r	0.99998	$> \lvert 0.99993 \rvert$	Pass
Repeatability			
relative standard deviation RSD_z, %	0.6353		
relative confidence interval $\Delta_z = t \bullet (95\ \%, 18 - 1) \bullet RSD_z$	1.1813	<1.6 %	Pass
Accuracy			
mean Z, %	100.11		
systematic error δ, %	0.11	1) $\leq \Delta_z : \sqrt{9} = 0.39$ % 2) if it does not meet criterion (1), then $\leq 0.32 \bullet 1{,}6$ % = 0.51 %	Pass
Intermediate precision			
combined average Z_{intra}, %	100.08		
$SD_{z\text{-}intra}$, %	0.5886		
$\Delta_{intra} = t \bullet (95\ \%, 36 - 1) \bullet SD_z$	1.0240	<1.6 %	Pass

According to the results of validation studies, the analytical procedure for the quantitative determination of BD by HPLC in the appropriate range met the requirements for the linearity, repeatability, accuracy and intermediate precision against the acceptance criteria calculated for tolerance B=5 % (Table 4).

The results of validation studies using two chromatographic columns proved that the test model solutions of BD met the requirements for their stability (Table 5).

In the experiments using both analytical procedures the chromatographic systems met the requirements to their suitability and minor changes of the chromatographic conditions did not affect the parameters of the suitability; thus the robustness of both procedures was proved (Tables 6, 7).

The results of the tests in regard to dose mass and uniformity of dose mass for the pMDIs with SS are presented in Table 8. Table 9 shows the results of the same tests for the pMDIs with BD.

Table 5

Evaluation the stability of the model solutions of BD

Column	Z_{first}	Z_{last}	$\lvert\Delta Z_i\rvert$	$\lvert\Delta Z_i\rvert \le \sqrt{2} \cdot 1{,}6$ %	Conclusion
Hypersil BDS C18	99.11 %	99.76 %	0.65 %	0.65 % ≤ 2.26 %	Stable
Zorbax Eclipse XDB-C18	99.96 %	100.51 %	0.55 %	0.55 % ≤ 2.26 %	Stable

Table 6

The results of the study in regard to the robustness of the analytical procedure for the quantitative determination of SS

Conditions	Column efficiency	Symmetry factor	RSD, %
	≥ 4000 t. p.	from 0.8 to 1.5	≤ 2.11 % (3 injections)
Conditions stipulated by the procedure: mobile phase 22 : 78, pH 3.65; temperature 25 °C, flow rate 1.0 ml/min, column Symmetry C8	6165	1.280	0.110
mobile phase pH=4,0	6864	1.224	0.249
mobile phase pH=3,0	6412	1.229	0.291
mobile phase 19 : 81	9966	1.078	0.554
mobile phase 25 : 75	4120	1.490	0.337
temperature 20 °C	5600	1.256	0.013
temperature 30 °C	6181	1.274	0.151
flow rate 0.8 ml/min	5367	1.264	0.104
flow rate 1.2 ml/min	7437	1.264	0.074
column YMC-Pack C8	4462	1.255	0.047

Table 7

The results of the study in regard to the robustness of the analytical procedure for the quantitative determination of BD

Conditions	Column efficiency	Symmetry factor	RSD, %
	≥ 5000 t. p.	from 0,8 to 1,5	≤ 2,11 % (3 injections)
Conditions stipulated by the procedure: mobile phase 40 : 60; temperature 40 °C; flow rate 2.0 ml/min, column Hypersil BDS C18	8521	1.193	0.219
mobile phase 43 : 57	6937	1.020	0.158
mobile phase 37 : 63	8826	0.992	0.090
temperature 35 °C	8381	1.184	0.258
temperature 45 °C	8282	1.159	0.230
flow rate 1,8 ml/min	9029	1.188	0.301
flow rate 2,2 ml/min	7889	1.159	0.230
column Zorbax Eclipse XDB-C18	12881	1.071	0.259

Table 8

The results of the tests in regard to dose mass (m) and uniformity of dose for the pMDIs with salbutamol sulfate (SS)

No.	Dose number	pMDI No. 1		pMDI No. 2		pMDI No. 3	
		m, mg	Δ, %	m, mg	Δ, %	m, mg	Δ, %
1	1	31.03	+1.43	74.66	+0.01	27.62	+1.21
2	2	30.84	+0.81	75.19	+0.72	27.56	+0.99
3	3	30.29	–0.99	74.87	+0.29	27.60	+1.14
4	99	30.16	–1.42	75.26	+0.81	27.25	–0.15
5	100	30.58	–0.04	74.02	–0.85	27.23	–0.22
6	101	30.67	+0.25	73.95	–0.94	27.14	–0.55
7	102	30.33	–0.86	75.28	+0.84	27.19	–0.37
8	198	31.01	+1.36	73.96	–0.93	27.35	+0.22
9	199	30.53	–0.21	75.38	+0.97	27.08	–0.77
10	200	30.49	–0.34	73.98	–0.90	26.91	–1.39
Average mass (Z_{av})		**30,59**		**74.66**		**27.29**	
RSD_z		0,97 %		0.83 %		0.87 %	

Note. Δ – *deviation from the average mass*

Table 9

The results of the tests in regard to dose mass (m) and uniformity of dose for the pMDIs with beclometasone dipropionate (BD)

No.	Dose number	pMDI No. 4		pMDI No. 5	
		m, mg	Δ, %	m, mg	Δ, %
1	1	76.04	+0.61	74.82	+0.25
2	2	75.48	–0.13	74.87	+0.32
3	3	75.50	–0.11	74.41	–0.29
4	99	75.56	–0.03	74.49	–0.19
5	100	75.89	+0.41	74.73	+0.13
6	101	75.50	–0.11	74.44	–0.25
7	102	75.47	–0.15	74.62	–0.01
8	198	75.37	–0.28	74.75	+0.16
9	199	75.77	+0.25	74.57	–0.08
10	200	75.24	–0.45	74.61	–0.03
Average mass (Z_{av})		**75.58**		**74.63**	
RSD_z		0.32 %		0.21 %	

Note. Δ – *deviation from the average mass*

The results of the tests in regard to delivered dose and uniformity of delivered dose for the pMDIs with SS are given in Table 10. Table 11 shows the results of the same tests for the pMDIs with BD.

The results of the tests in regard to fine particle dose and uniformity of fine particle dose for the pMDIs with SS are given in Table 12. Table 13 shows the results of the same tests for the pMDIs with BD.

Table 10

The results of the tests in regard to delivered dose (DD) and uniformity of delivered dose for the pMDIs with salbutamol sulfate (SS)

No.	Dose number	pMDI No. 1		pMDI No. 2		pMDI No. 3	
		DD, μg	Δ, %	*DD*, μg	Δ, %	*DD*, μg	Δ, %
1	1	89.67	+3.20	85.57	+2.98	90.82	+6.26
2	2	83.55	–3.85	77.15	–7.15	79.52	–6.96
3	3	91.51	+5.31	94.61	+13.86	82.17	–3.86
4	99	78.81	–9.30	92.2	+10.96	91.19	+6.69
5	100	88.92	+2.34	83.27	+0.21	78.01	**–8.73**
6	101	85.08	–2.08	88.14	–6.07	92.03	+7.68
7	102	94.66	**+8.95**	83.57	+0.57	93.39	**+9.27**
8	198	88.83	+2.24	77.25	–7.03	80.53	–5.78
9	199	90.05	+3.63	73.52	–11.52	82.41	–3.58
10	200	77.80	**–10.46**	75.65	–8.96	84.63	–0.98
Average *DD* (Z_{av})		**86,89**		**83.09**		**85.47**	
RSD_z		6,30 %		8.64 %		6.79 %	

Note. Δ – *deviation from the average DD*

Table 11

The results of the tests in regard to delivered dose (DD) and uniformity of delivered dose for the pMDIs with beclometasone dipropionate (BD)

No.	Dose number	pMDI No. 4		pMDI No. 5	
		DD, μg	Δ, %	DD, μg	Δ, %
1	1	189.42	+3.36	201.53	+3.97
2	2	174.70	–4.67	201.20	+3.80
3	3	181.43	–1.00	188.10	–2.96
4	99	178.41	–2.65	185.47	–4.31
5	100	184.38	+0.61	193.44	–0.20
6	101	192.24	+4.90	187.29	–3.37
7	102	177.23	–3.29	189.47	–2.25
8	198	186.71	+1.89	205.67	+6.11
9	199	182.43	–0.45	185.44	–4.33
10	200	185.63	+1.30	200.67	+3.53
Average *DD* (Z_{av})		**183,.26 (73.30 %)**		**193.83 (77.53 %)**	
RSD_z		3.01 %		3.98 %	

Note: Δ – deviation from the average DD

Table 12

The results of the tests in regard to fine particle dose (FPD) and uniformity of fine particle dose (UFPD) for the pMDIs with salbutamol sulfate (SS)

No.	Dose number	pMDI No. 1		pMDI No. 2		pMDI No. 3	
		FPD, μg	Δ, %	FPD, μg	Δ, %	FPD, μg	Δ, %
1	1	56.71	–9.22	40.98	–13.09	69.08	+6.79
2	2	67.00	+7.26	47.24	+0.19	64.29	–0.62
3	3	63.49	+1.64	48.47	+2.80	60.57	–6.37
4	99	59.59	–4.61	50.14	+6.34	61.10	–5.55
5	100	64.76	+3.67	42.21	–10.48	60.98	–5.73
6	101	57.36	–8.18	53.48	+13.43	60.72	–6.14
7	102	67.22	+7.61	49.36	+4.69	69.75	+7.82
8	198	59.42	–4.88	44.86	–4.86	66.57	+2.91
9	199	71.21	+14.00	45.83	–2.80	62.89	–2.78
10	200	57.91	–7.30	48.93	+3.78	70.94	+9.66
Average *FPD* (Z_{av})		**62,47**		**47.15**		**64.69**	
RSD_z		7.98 %		8.01 %		6.32 %	

Note: Δ – deviation from the average FPD

Table 13

The results of the tests in regard to fine particle dose (FPD) and uniformity of fine particle dose (UFPD) for the pMDIs with beclometasone dipropionate (BD)

No.	Dose number	pMDI No. 4		pMDI No. 5	
		FPD, μg	Δ, %	FPD, μg	Δ, %
1	1	130.41	–2.95	141.84	+4.56
2	2	142.64	+6.15	136.61	+0.71
3	3	131.89	–1.85	123.30	–9.10
4	99	130.85	–2.63	124.30	–8.37
5	100	141.76	+5.49	140.08	+3.27
6	101	142.82	+6.28	137.86	+1.63
7	102	131.46	–2.17	134.49	–0.86
8	198	136.33	+1.45	135.40	–0.18
9	199	126.50	–5.86	139.26	+2.66
10	200	129.14	–3.90	143.38	+5.70
Average FPD (Z_{av})		**134.38 (53.75 %)**		**135.62 (54.25 %)**	
RSD_z		4.51 %		5.03 %	

Note: Δ – deviation from the mean FPD

5. Discussion of research results

The particle size distribution of SS in the case of the pMDIs No. 1, No. 2 and No. 3 differed slightly (Fig. 1, Table 1), so the particle size could not be cause of significant differences in their performance characteristics [19]. Salbutamol sulfate was the dispersed phase of suspensions in the case of the pMDIs No. 1, No. 2 and No. 3, and beclometasone dipropionate was dissolved in the case of the pMDIs No. 4 and No. 5. The different microstructure might have led to some differences in the performance characteristics of the pMDIs [19]. For study of these characteristics, analytical procedures for quantitative determination of SS (equivalent to salbutamol) and BD were used, the correctness of which had been proved by the results of validation studies (Fig. 2–5, Tables 2–7).

The test pMDIs were different in their average dose mass of (Tables 8 and 9) because of the different metering chamber volumes of the valves used for the preparations, as well as the different density of the content of the container, which depended on the formulations of these pMDIs. For the single container, the deviations of the mass of individual doses from the average value were minor and did not exceed 1.5 %, and the relative standard deviation (RSD) did not exceed 1.0 % (Tables 8 and 9). However, there is a risk of variability in the volume of the metering chamber in different batches of valves; it should be taken into account when limits for average dose mass and uniformity of dose mass are established. For example, in the case of the pMDI No. 1 the average dose mass for the batch AET46A was 30.59 mg, for the batch AFB40A it was 32.45 mg and for the batch AFC39A it was 33.15 mg. Therefore, the difference in average dose mass between the first batch and the next two batches was 6.1 % and 8.4 %, respectively. Thus, the limits for the average dose mass and uniformity of dose mass should be established in the specifications for pMDIs, although these tests are not provided as obligatory by the leading pharmacopoeias, State Pharmacopoeia of Ukraine and EMEA guidelines [1–3, 5, 6]. These limits should be established during pharmaceutical development using the appropriate valves of different batches.

In contrast to the average dose mass, delivered dose should be standardized by the content of active substance. The average values of delivered dose in the case of the pMDI with SS were close (Table 10) although the differences in regard to the average dose mass were observed (Table 8). For the single container, the variability of delivered dose was much more significant than the variability of average dose mass, that was evidenced by RSD value, which was the almost 10 times higher, as well as by the deviations of individual delivered doses from the average delivered dose, which were up to 10–15 %. The highest value of RSD for delivered dose was found in the case of the pMDI No. 2 without ethanol in the formulation, and the lowest – for the pMDIs with BD, which were solutions. At the same time, the higher content of ethanol in the pMDIs No. 5 and No. 6 probably led to the decrease in the average delivered dose of BD compared to the pMDIs with SS (Tables 10 and 11) [19, 20].

If the dose mass mainly depends on the volume of the valve metering chamber, then the delivered dose depends on the metered dose and the design of the actuator as well, where part of active substance might deposit. In view of the above, it is clear that even in the case of pMDI-solutions, the test in regard to the uniformity of dose mass cannot replace the test concerning uniformity of delivered dose and vice versa, and limits for the uniformity of dose mass and limits for uniformity of delivered dose cannot be the same (9 out of 10 results should lie between 75 % and 125 % and all should lie between 65 % and 135 %). Unfortunately, according the provisions that are laid down in the general articles “Dosage forms for inhalations” and “Aerosols and sprays” of the State Pharmacopoeia of the Russian Federation (SP RF) it is acceptable to replace the test “Uniformity of delivered dose” by the test “Uniformity of dose mass” in the case of the pMDI which are solutions [21]. For pMDI-solutions it is not acceptable to assess the uniformity of delivered dose by the value of average dose mass and the differences between average and individual masses because these two tests are intended to determine the different attributes that are content of active substance and mass; moreover, these attributes depends on different factors and reasons for non-conformity could be different. Furthermore, according to the general article 2.9.18 of the European Pharmacopoeia [1], during testing for the aerodynamic assessment of fine particle the average value of delivered dose should be used as the acceptance criterion to calculate mass balance, which serves to ensure that test results are valid. However, the mass balance is not stipulated in the general article “Aerodynamic distribution of fine particles” of the SP RF in the case of using the apparatus A, C, D and E for testing of pMDIs [21].

The delivered dose characterizes the suitability of the actuator and does not ensure the therapeutic effect which depends on the fine particle dose. The same nominal doses and almost the same delivered doses do not indicate an equivalent therapeutic effect of the two pMDIs if they differ in fine particle dose [22]. However, the limits in regard to fine particle dose for the pMDI with the various active substances as well as the acceptance criteria for uniformity of fine particle dose are not established by leading pharmacopoeias, State Pharmacopoeia of Ukraine and SP RF [1, 3, 5, 6, 21].

The lower limits for the fine particle dose, which are of $\geq$ 35 % and $\geq$ 25 % of the nominal dose, are established in the specifications for the pMDIs with SS and BD respectively. For the three tested pMDIs with SS it was found that despite the same particle size distribution of SS and almost the same average delivered dose the fine particle doses were significantly higher in the case of the pMDIs No. 1 and No. 3 compared to the pMDI No. 2. This was due to the low ethanol content in the pMDIs No. 1 and No. 3 [19, 20].

It is necessary to ensure that each dose could provide an appropriate therapeutic effect, but delivered dose is not the comprehensive characteristic that could guarantee the therapeutic efficacy of the pMDI. Therefore, uniformity of fine particle dose should be determined and standardized. The data presented in Tables 12 and 13 shows that all tested fine particle doses met the requirements from the first dose to the last one stated on the label. The level of the uniformity of fine particle dose and the level of the uniformity of delivered dose were approximately the same, as it demonstrated by the very close values of RSD. The highest deviations of individual

fine particle doses from the average value did not exceed 10–15 % in the case of the pMDIs with SS and 7–10 % in the case of the pMDIs with BD. The dosing of the pMDIs with solutions of BD was more uniform considering the RSD values.

During pharmaceutical development [2, 23] it should be demonstrated that an average fine particle dose exceeds the established lower limit at least on the value of the highest deviation of individual fine particle doses from the average dose . The testing for uniformity of fine particle dose could be a tool for long-term study of the suspension stability in the containers because the aggregation of particles results the decrease of the fine particle dose [8, 16, 19]. The constant level of the uniformity of fine particle dose within the shelf life is a guarantee of the effectiveness of each dose of the pMDIs during their storage [8, 12].

The values of the 10 results for testing of fine particle dose should be higher than the lower limit for this characteristic. All 5 tested pMDIs met this requirement (Tables 12 and 13). If lower and upper limits for fine particle dose are established in the specification (for example, from 18 % to 33 %), the all 10 results of testing for fine particle dose should be in this range. In the case of the pMDIs with suspensions of SS, the content of the active substance in each of the 10 doses of fine particle was within ±15 % of the average value, and in the case of the pMDIs with solutions of BD the content of BD was within ±10 % of the average value (Tables 12 and 13). These data could be used for the standardization of the uniformity of fine particle dose.

The results of research create a basis for the determination of uniformity of fine particle dose using multistage impactors [1, 6, 9]. It is also rational to conduct test for UFPD in regard to pMDIs if the lower and upper limits for fine particle dose are established in the specifications, as well as using 10 different containers of the same pMDI (inter-inhaler testing).

Highly sensitive analytical methods should be used to determine UFPD. Therefore, it is rational to develop or improve procedures for quantitative determination of active substances, in particular, SS or formoterol fumarate by HPLC using a fluorescence detector, etc. [24, 25].

The obtained results could be used in the development, research and standardization of preparations in the form of pMDI, as well as they could be considered for development of the general articles and monographs of State Pharmacopoeia of Ukraine.

6. Conclusions

The validation of two analytical procedures for the quantitative determination of SS and BD by HPLC in the suitable concentration ranges including low content of these substances was conducted. The 3 pMDIs with SS and the 2 pMDIs with BD were studied. It was shown that for the three pMDIs with SS the particle size distribution in the containers and the average delivered dose were almost the same, but these preparations were different in the average dose mass and fine particle dose. According to the research results, the expediency of the determining of average dose mass and testing for the uniformity of dosing by the dose mass and fine particle dose was substantiated. It was demonstrated that dosing was more uniform in the case of the pMDIs with solutions compared to the pMDIs with suspensions. Approaches of leading and other pharmacopoeias to the uniformity of dosing of pMDIs have been critically discussed. The expediency of the testing for UFPD during pharmaceutical development was substantiated because it is the fine particle dose that determines the therapeutic effect of pMDIs. The issues concerning the standardization of UFPD for pMDIs were discussed.

Conflict of interests

The authors declare that they have no conflicts of interest.

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References

1. The European Pharmacopoeia (2019). European Directorate for the Quality of Medicines & HealthCare of the Council of Europe. Strasbourg, 5224.

2. Guideline on the Pharmaceutical Quality of Inhalation and Nasal Products (2006). EMEA/CHMP/QWP/49313/2005 Corr.

3. British Pharmacopoeia 2020 (2019). London: The Stationery Office. Available at: https://www.pharmacopoeia.com

4. British Pharmacopoeia 2016 (2015). London: The Stationery Office. Available at: https://www.pharmacopoeia.com

5. The United States Pharmacopoeia, 41 – NF 36 (2018). The United States Pharmacopoeial Convention. Rockville. Available at: https://opac.kku.ac.th/catalog/Bibltem.aspx?BiblD=b00420302

6. Derzhavna Farmakopeia Ukrainy. Vol. 1 (2015). Kharkiv: Derzhavne pidpryiemstvo «Ukrainskyi naukovyi farmakopeinyi tsentr yakosti likarskykh zasobiv», 1128.

7. Lippmann, M. (2011). Regional Deposition of Particles in the Human Respiratory Tract. Comprehensive Physiology, 213–232. doi: http://doi.org/10.1002/cphy.cp090114

8. Carvalho, T. C., Peters, J. I., Williams III, R. O. (2011). Influence of particle size on regional lung deposition – What evidence is there? International Journal of Pharmaceutics, 406 (1-2), 1–10. doi: http://doi.org/10.1016/j.ijpharm.2010.12.040

9. Tougas, T. P., Mitchell, J. P., Lyapustina, S. A. (Eds.) (2013). Good Cascade Impactor Practices, AIM and EDA for Orally Inhaled Products. Springer, 442. doi: http://doi.org/10.1007/978-1-4614-6296-5

10. Lyapunov, N. A., Bezuglaya, E. P., Bovtenko, V. A., Stolper, Yu. M. (2018). Comparative assessment of aerodynamic properties of salbutamol pressurized metered dose inhalers. Drug development & registration, 1, 54–61.

11. Sheth, P., Stein, S. W., Myrdal, P. B. (2013). The influence of initial atomized droplet size on residual particle size from pressurized metered dose inhalers. International Journal of Pharmaceutics, 455 (1-2), 57–65. doi: http://doi.org/10.1016/j.ijpharm.2013.07.061

12. Lyapunov, N. A., Bovtenko, V. A., Bezuglaya, E. P., Stolper, Yu. M. (2016). Obosnovanie novogo podkhoda k otsenke kachestva dozirovannykh aerozoley dlya ingalyatsii na etape ikh razrabotki. Nauchnye vedomosti Belgorodskogo gosudarstvennogo universiteta. Meditsina. Farmatsiya, 226 (5), 170–179.

13. Orange Book: Approved Drug Products with Therapeutic Equivalence Evaluations (2020). Available at: www.accessdata.fda.gov/scripts/cder/ob/index.cfm

14. Buckingham, R. (Ed.) (2020). Martindale: The Complete Drug Reference. London: Pharmaceutical Press, 4912.

15. Note for Guidance on Validation of Analytical Procedures: Text and Methodology, Step 5 (1995). CPMP/ICH/381/95 (ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology).

16. Lyapunov, N. A., Bezuglaya, E. P., Bovtenko, V. A., Stolper, Yu. M., Baumer, V. N., Bryleva, E. Yu. (2018). Quality risk management for salbutamol pressurized metered dose inhalers. Drug development & registration, 4, 49–61.

17. Gosudarstvenniy reestr lekarstvennykh sredstv (GRLS). Available at: http://grls.rosminzdrav.ru

18. Derzhavnyi reiestr likarskykh zasobiv Ukrainy. Available at: http://www.drlz.kiev.ua/

19. Sheth, P., Sandell, D., Conti, D. S., Holt, J. T., Hickey, A. J., Saluja, B. (2017). Influence of Formulation Factors on the Aerosol Performance of Suspension and Solution Metered Dose Inhalers: A Systematic Approach. The AAPS Journal, 19 (5), 1396–1410. doi: http://doi.org/10.1208/s12248-017-0095-3

20. Bovtenko, V. A., Bezuglaya, E. P., Stolper, Yu. M., Lyapunov, N. A. (2018). Izuchenie svoystv preparatov salbutamola sulfata v forme dozirovannykh ingalyatorov pod davleniem. Farmakom, 1, 57–70.

21. Gosudarstvennaya Farmakopeya Rossiyskoy Federatsii, XIV izdanie. Vol. 1 (2018). Moscow: Ministerstvo zdravookhraneniya Rossiyskoy Federatsii, 1814.

22. Guideline on the Requirements for Clinical Documentation for Orally Inhaled Products (OIP) Including the Requirements for Demonstration of Therapeutic Equivalence between two Inhaled Products for Use in the Treatment of Asthma and Chronic Obstructive Pulmonary Disease (COPD) in Adults and for Use in the Treatment of Asthma in Children and Adolescents (2009). CPMP/EWP/4151/00 Rev. 1.

23. Note for Guidance on Pharmaceutical Development, Part I (2009). EMEA/CHMP/167068/2004 (ICH Topic Q 8 (R2) Pharmaceutical Development.

24. Murtaza, G., Ahmad, M., Madni, M. A., Asghar, M. W. (2009). A new reverse phase HPLC method with fluorescent detection for the determination of salbutamol sulfate in human plasma. Bulletin of the Chemical Society of Ethiopia, 23 (1), 1–8. doi: http://doi.org/10.4314/bcse.v23i1.21292

25. Patel, N., Parmar, V. K. (2018). A Sensitive High-Performance Thin Layer Chromatography Method for Simultaneous Determination of Salbutamol Sulphate and Beclomethasone Dipropionate from Inhalation Product. Pharmaceutical Sciences, 24 (2), 131–140. doi: http://doi.org/10.15171/ps.2018.20

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Elena Bezuglaya*, PhD, Senior Researcher, Head of Laboratory, Laboratory of Technology and Analysis of Medicinal Products, State Scientific Institution "Institute for Single Crystals" of National Academy of Sciences of Ukraine, Nauky ave., 60, Kharkiv, Ukraine, 61072

Nikolay Lyapunov, Doctor of Pharmaceutical Sciences, Professor, Leading Researcher, Laboratory of Technology and Analysis of Medicinal Products, State Scientific Institution "Institute for Single Crystals" of National Academy of Sciences of Ukraine, Nauky ave., 60, Kharkiv, Ukraine, 61072

Vladimir Bovtenko , PhD, Junior Researcher, Laboratory of Technology and Analysis of Medicinal Products, State Scientific Institution "Institute for Single Crystals" of National Academy of Sciences of Ukraine, Nauky ave., 60, Kharkiv, Ukraine, 61072

Igor Zinchenko, PhD, Junior Researcher, Laboratory of Technology and Analysis of Medicinal Products, State Scientific Institution "Institute for Single Crystals" of National Academy of Sciences of Ukraine, Nauky ave., 60, Kharkiv, Ukraine, 61072

Yurij Stolper, PhD, Senior Researcher , Laboratory of Technology and Analysis of Medicinal Products, State Scientific Institution "Institute for Single Crystals" of National Academy of Sciences of Ukraine, Nauky ave., 60, Kharkiv, Ukraine, 61072

***Corresponding author:** Elena Bezuglaya, e-mail: bezugla.op@gmail.com*