Research article

Development of simple and fast UV-method for the quantitative determination of mometasone furoate in a large number of metered doses of an aqueous nasal spray of mometasone furoate

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ARTICLE INFO

ABSTRACT

Fast and simple spectrophotometric method for quantitative determination of mometasone furoate in a single dose (single actuation) of its nasal spray was developed and validated. This method is based on the spectrophotometric analysis of turbid solution of a single spray in 20 mL isopropanol at 220–310 nm. We further show applicability of this method for analysis of large number of single sprays for the dosing homogeneity. It is based on a new approach to the development of spectrophotometric quantitative determination methods for drug products and possibly other objects, allowing measurements to be carried out on sufficiently turbid test solutions. The proposed approach is not a variant of the derivative spectrophotometry and can replace the methods of derivative spectrophotometry in cases where: derivative of analyte spectrum is not intense enough; derivative of the spectrum of the matrix (turbidity and remaining components of the product) is significant when compared with the derivative of the analyte.

1. Introduction

1.1. About mometasone furoate and its nasal sprays

Mometasone furoate (MF) monohydrate nasal sprays are widely used for treatment of symptoms of seasonal or perennial allergic rhinitis and other illnesses in adults, adolescents, and children aged 3–11 years. MF has been demonstrated to be effective in treatment of inflammatory diseases of the nose and paranasal sinuses; when compared with its alternatives, it shows a greater symptom control; MF's reliability is due to its proven efficacy, as well as to its long-standing presence on the market [1].

1.2. Nasal sprays composition

Nasonex is one of the brand names used for marketing MF aqueous spray formulations for nasal inhalation. At the time of this study, MF was marketed in Ukraine under Nasonex brand name. Also, five other generic formulations were available on the market.

A single dose of Nasonex spray amounts to 100 mg of aqueous suspension that contains 50 μg of MF as well as inactive components [2]. These inactive components are: benzalkonium chloride, citric acid, dispersible cellulose BP 65 cps (carboxymethylcellulose sodium, microcrystalline cellulose), glycerol, Polysorbate 80, purified water, and sodium citrate dihydrate [2].

1.3. Importance of MF average content in spray single doses and dosage uniformity

For the purposes of this study, a "dose" shall mean the amount of drug, namely – mometasone furoate, which is discharged with a single release from the container with the drug. The average content of MF in a single dose and its uniformity is an important indicator of quality of MF aqueous preparations. In view of this, the uniformity of dosage test is one

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of the most important tests designed to ensure quality of such preparations.

The spray discharged from the nasal actuator should be thoroughly analyzed for the drug substance content in multiple doses, from the beginning to the end of an individual container, among containers, and among batches of drug product [3]. This test provides an overall performance evaluation of a batch, assessing the formulation, the manufacturing process, and the pump [3]. Some Pharmacopoeias, e.g. European [4] and American [5], contain specific requirements for selecting containers from a batch, from each of which two doses are taken, namely the first and last doses out of all doses guaranteed by the producer (first test level requires 10 containers, respectively – 20 doses, second level requires 30 containers, respectively – 60 doses). Having analyzed the relevant sections of resources [3, 4, 5], it can be concluded that all of them contain the following basic requirement: a set of results obtained by determining the active substance for all collected doses must not contain a single result going beyond the limits of 75%–125% of the label claim. In other words, this test belongs to the tests with lower and upper limits. In addition, the European Pharmacopoeia 8th edition contains a non-mandatory general article 2.9.47 "Demonstration of Uniformity of Units Using the Large Sample Sizes" [6], which provides guidance on how to use larger samples to characterize uniformity of dosage. The approach described in the mentioned article requires using not fewer than 100 dosage units. It, however, raises the prohibition of any results falling outside the 75% and 125% limits, which can substantially reduce the risk of rejection of a benign series in case where one of the obtained results falls at the rear end of statistical population. Although the European Pharmacopoeia's relevant general chapter says that this approach is applicable to the so-called non-destructive methods of analysis, a draft monograph in Pharmeuropa indicates that the approach is applicable to traditional analytical methods [7]. As assessment "Uniformity of Dosage Units" requires analysis of a large number of individual doses, it is preferable that the most rapid and simple method is used for this purpose. This paper introduces a method that provides an efficient solution to the above-mentioned task.

1.4. Analytical techniques

First, we will touch upon various analytical techniques used for quantitative determination of MF in medicinal preparations.

Apparently, **gas chromatography** cannot be used for these purposes, as the temperature required for transfer of a sufficiently large MF molecule into the gas phase causes destruction of an analyte. Nonetheless, **supercritical fluid chromatography** has many advantages of gas chromatography and, at the same time, does not require high temperature to transfer an analyte to the mobile phase. It is, therefore, suivd for working with MF [8].

Liquid chromatography (HPLC and, to a lesser extent, TLC) is the most commonly used tool for analysis of pharmaceutical products. Also, MF is an excellent object of quantitative determination in various medicinal preparations as its structure indicates the presence of a sufficiently good chromophore (UV detection), moderate hydrophobicity and absence of functional groups with strong acid-base properties. This all makes it easy to use a normal phase version of liquid chromatography (as a rule, TLC), as well as a reverse-phase version (as a rule, HPLC) in our experiments.

There are many excellent works on MF quantitative determination in various pharmaceuticals by HPLC [9, 10, 11, 12, 13, 16] with UV detection. Apparently, HPLC-MS2 (and higher, with APCI, APPI) is the most powerful tool for quantitative determination of MF and its metabolites in blood plasma and other biological objects [14]. In some papers [13, 15], TLC with densitometric UV detection was used for quantification of MF. In most cases [10, 11, 12, 13], chromatographic techniques were used to quantify MF as well as one or more additional analytes in drugs, whereas in paper [9], only MF is defined. The latter could be explained by the fact that the developed technique is universally

applicable to both creams and nasal MF sprays. Moreover, in most cases, the mentioned chromatographic methods are claimed to be stability indicative. HPLC-UV is also mentioned in some pharmacopoeial monographs as a tool for quantitative determination of MF in substances and preparations [16, 17].

For quantitative determination of MF in preparations, **spectrophotometry in the UV spectral range** can be used as well [18]. The mentioned paper describes the use of a two-wave spectrophotometric determination of MF and Salicylic Acid in topical formulations. The turbidity of test solutions was removed by filtration through a membrane filter.

In paper [19], which is devoted to determination of MF and miconazole nitrate in medical creams, two methods were developed based on derivative spectrophotometry and derivative spectrophotometry relationships. Centrifugation was used to remove turbidity of test solutions. In this paper, HPLC technique has also been used, which is stability indicative. Results of spectrophotometric methods and the reference method showed a good match. In papers [18, 19], filtering or centrifuging are used to remove turbidity, which is certainly justified when it comes to objects, in which several analytes are determined. However, in some papers, spectrophotometric determination of a single analyte (not MF) is carried out without removing turbidity [20, 21]. They explicitly or implicitly use derivative spectrophotometry [20] creating pure signal from an analyte in the region where turbidity spectrum can be considered either constant or linear. In the second case [21], the turbidity contribution is taken into account explicitly. In case [21], the turbidity spectrum is calculated based on the knowledge of size distribution of mechanical particles (causing turbidity) using rather complicated calculations [22].

Finally, in British Pharmacopoeia, UV spectrophotometry is used for quantitative determination of MF in its substance [16].

Methods for quantitative determination of MF in pharmaceutical preparations, which are different from chromatography (TLC, HPLC), mass spectrometry (MS) or spectrophotometry (UV), seem to have not been widely used. Nonetheless, some additional methods can be occasionally found (e.g. [23], in which the voltammetry method was developed for MF determination in creams and ointments).

As the goal of this study is to present a time and cost effective, as well as a robust method for quantifying MF in a large number of doses of monohydrate nasal sprays, it seems reasonable to conduct a comparative assessment of the above mentioned techniques in terms of utility for reaching this goal.

Any quantitative determination consists of four main stages: sampling, sample preparation, instrumental analytical operation, and corresponding calculations. The obtained result is a value with uncertainty [24]. The first and the last mentioned stages are not relevant in the present context, as the first one is determined by customer requirements and/or defined by either the corresponding Guidelines or the Pharmacopoeia, and the last one is an easy task for a computer. As a result, only sample preparation with the following instrumental analytical operation is the subject of comparison among various methods.

In terms of execution time of the final analytical operation, UV spectrophotometry in its multi wave variant is, beyond any doubt, the most suitable technique, since, unlike HPLC, it does not require physical separation of an analyte from other components of a preparation. This technique may have competed with TLC with precise automatic application of multiple samples on a plate. However, this TLC technique requires specific equipment and it is not easy to be routinized.

In terms of sample preparation, multi-wavelength spectrophotometry may exceed HPLC and instrumental TLC, as, in some of its variants, it does not require removal of turbidity, i.e. avoids the stage of filtration or centrifugation. Where it is required to rapidly conduct simultaneous analysis of numerous samples, significant complications could be expected both with time and with high probability of gross analytical errors. Considering the aforesaid, we have finally chosen multi-wave spectrophotometry as analytical operation.

2. Experimental

2.1. Materials

Isopropanol (isopropyl alcohol (IPA)) of three brands was used during the experiments. All three solvents meet the requirements of the European Pharmacopoeia for isopropanol reagent *Propan-2-ol R1*:

1. 2-Propanol. Honeywell Specialty Chemicals Seelze GmbH (Honeywell Riedel-de Haën AG TM 24137) (Type 1).
2. 2-Propanol for analysis EMSURE® (Merck KGaA, Frankfurter StraBe 250, 64293 Darmstadt (Germany)) (Type 2).
3. 2-Propanol gradient grade for liquid chromatography LiChrosolv® (Merck KGaA, Frankfurter StraBe 250, 64293 Darmstadt (Germany)) (Type 3).

Milli-Q *water* was obtained from a Millipore Direct-Q 3 UV (Billerica, Massachusetts, USA).

Mometasone furoate (Certified Reference Material) (MF CRM) was purchased from Sigma-Aldrich (certified purity 99.6%, Ucrm = ±0.02%, k = 2).

Benzalkonium chloride (Reference Material, 49.3% in water, courtesy of ALCON-COUVREUR (Belgium)).

Mometasone furoate monohydrate aqueous nasal spray 50 mcg/metered spray (as mometasone furoate) preparations from various manufacturers available on the Ukrainian market were purchased in pharmacies in Kyiv (Table 1).

2.2. Equipment

Specord-202, Analytik Jena AG (Konrad-Zuse-Strasse 1 Jena, Germany) was used for the method development and method validation.

Centrifuge S70 Janetzki (Engelsdorf-Leipzig Heinz Janetzki KG, Germany).

Mechanical Pipettes DRAGON LAB 20–200 μL and 100–1000 μL (DLAB Scientific Co., Ltd., China).

Shaker RO 30 (LABOSHAKE, C. Gerhardt GmbH & Co. KG, Germany).

2.3. Sample preparation

2.3.1. Stock solution, intermediate solutions and reference solutions of MF

Reference solutions of MF with the expected concentration of approximately 2.5 μg/ml were prepared from intermediate solutions of MF with the expected concentration of approximately 50 μg/ml, which, in their turn, had been prepared from a stock solution of MF with the expected concentration of approximately 500 μg/ml.

A detailed procedure for the preparation and storage of these solutions is presented below.

2.3.1.1. Stock solution of MF. A precisely weighed portion of MF CRM (measured out on an analytical balance) within the range of 0,045 to 0.055 g was transferred to a 100 ml volumetric flask and about 50 ml of

IPA was added. The flask was closed with a stopper and shaken on a shaker with a frequency of 150 cycles per minute for 1 h to ensure that MF has been completely dissolved. Volume of the solution in the flask was brought up to the mark with isopropyl alcohol and thoroughly mixed. The obtained solution was poured into a 100 ml amber glass bottle with a screw cap and was stored at a temperature of 2–8 °C (shelf life is 6 months). Before use, the solution was kept for 2 h to get the ambient temperature.

2.3.1.2. Intermediate solution of MF. Using a volumetric pipette, 10.0 ml of MF CRM stock reference solution was transfered into a 100 ml volumetric flask and was brought up to the mark with IPA. The obtained solution was poured into a 100 ml amber glass bottle with a screw cap and was stored at a temperature of 2–8 °C (shelf life is 6 months). Before use, the solution was kept for 2 h to get the ambient temperature.

2.3.1.3. Reference solution of MF. Using a volumetric pipette, 5.0 ml of intermediate MF CRM solution was transferred into a 100 ml volumetric flask and was brought to the mark with *the IPA that was used to obtain the test solutions,* and was thoroughly mixed (about 2.5 μg/ml). The obtained solution was poured into a 100 ml amber glass bottle with a screw cap. The shelf life is 14 days at room temperature and 2 months at 2–8 °C.

$$C_1^R = 2.455 \ \mu g/ml, C_2^R = 2.829 \ \mu g/ml$$

2.3.1.4. Solutions of MF for the preparation of validation solutions, etc. (solution of MF). These solutions were prepared from the *stock solution of CRM 1*. Their calculated concentrations (in μg/ml) were: $C_{50\%} = 1.228$, $C_{75\%} = 1.841$, $C_{100\%} = 2.455$, $C_{125\%} = 3.069$, $C_{150\%} = 3.683$. They were prepared in greater amounts than the reference solutions, stored in amber glass bottles at 2–8 °C (shelf life is 3 months); before use, they were allowed to get the ambient temperature.

2.3.2. Test solutions

One dose of the preparation (single activation of the metering valve of a drug container) was collected into a 50 ml pre-labeled and pre-weighted amber glass bottle with a screw cap (Fig. 1). After the second weighing, the mass of dose was determined as the weight difference. This operation was carried out for the entire set of doses intended to be assayed in the same run. A 20.0 ml of IPA was added to each collection-bottle using a volumetric pipette. The bottles were capped and placed on a shaker with a shaking frequency of 150 cycles per minute for 60 min.

2.3.3. Validation solutions

2.3.3.1. Placebo. The ideal way for carrying out validation of a method for quantitative determination of an active substance (analyte) in a medicinal preparation is the preparation of model mixtures based on "placebo" and known quantities of the active substance. Placebo is a matrix of preparation completely or substantially devoid of the active substance. If it is impossible to prepare a "placebo", the preparation itself is used. In this case, however, the method of additives has certain metrological difficulties. For instance, it might be necessary to determine concentration of an analyte, which is significantly higher than the target concentration and goes beyond the analytical measurement range.

Therefore, it always makes sense to try to get a placebo. In our case, placebo-based validation solutions provide basic information; model mixtures based on an aqueous suspension of the drug product provide supporting information that serves only to compare these two approaches to validation.

The drugs in question are MF suspensions in an aqueous system (see Section 1.2) that contain dispersible cellulose BP 65 cps (carboxymethylcellulose sodium, microcrystalline cellulose), an excipient known for its ability to impart high viscosity to water solutions [25, 26]. It was shown in [27] that the preparations in question at rest have a very high

Table 1
Preparations used in the study.

#	Name, manufacturer and country of origin	Abbreviated name used hereinafter
1	Nasonex, Schering-Plough Labo N.V. (Belgium)	NA
2	Forinex, Farmak, Joint-Stock Company (Ukraine)	FO
3	Flix, Orhan Gazi Mahallesi (Turkey)	FL
4	Mometasone-Teva, Teva Czech Industries (Czech Republic)	MT
5	Glenspray, Glenmark Pharmaceuticals Ltd (India)	GL
6	Allertec Nazo, Farmea (France)	AL

Volume: 50 ml.
Neck diameter: 18 mm.
Material: brown medical glass
Quality and application: for cosmetic and pharmaceutical products
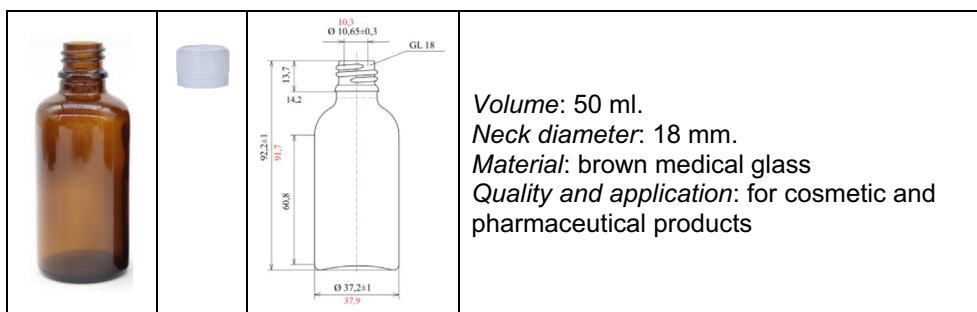
Fig. 1. Amber glass bottle with a screw cap.

viscosity.

This feature casts doubt on the possibility to separate the suspended MF from the matrix using conventional filtering methods. However, centrifugation for 30 min with 3000 revolutions per minute (about 2000 g) after a slight (8:2) dilution of the preparation with water made it possible to precipitate MF from all preparations to a sufficiently low residual content. In this regard, it should be noted that the residual concentration of MF is limited by the solubility of MF in aqueous media, which is about 20 μg/ml [2], making it impossible to obtain a placebo with zero concentration of MF. The minimum achievable content of MF in placebo is about 5% of the nominal content; most likely, the finest fraction of solid MF cannot be removed.

With the account of the above dilution (8 ml of the drug and 2 ml of water were mixed and placed in a centrifuge tube), validation solutions were prepared as follows: instead of 100 mg of undiluted placebo, we took 100 (8 + 2)/8 = 125 mg of placebo diluted with water etc.

For validation of dosage uniformity techniques, as a rule, a range of contents of at least 70–130% of the target concentration is recommended, unless a wider more appropriate range is justified based on the nature of the dosage form (e.g. metered dose inhalers) [28].

2.3.3.2. Range. We chose a range of MF concentrations of 50–150% and the same range of placebo contents with the account of two possible limiting cases:

a) the first one is a bigger mass emission of the MF-depleted sample: 150% placebo (150/0.8 ≈ 188 mg) and 50% MF.
b) the other one is a smaller mass of MF-enriched sample: 50% placebo (50/0.8 ≈ 63 mg) and 150% MF.

Since the placebo for six preparations (see Section 2.1) might differ slightly, each placebo was prepared for each of the drugs. For each of 6 preparations, validation solutions were prepared using its placebo according to Table 2. In each case, 20.0 ml of solution of MF was added to the portion of placebo by means of a volumetric pipette. For estimation of the variation of results between the prepared validation solutions, which could take into account uncertainty of sample preparation, solutions at each level of contents were prepared in five repetitions.

Preparation of the validation solutions was carried out as close as

possible to the procedure of preparation of the test solutions:

The target placebo mass was transferred into a 50 ml pre-labeled and pre-weighted amber glass bottle with a screw cap (Fig. 1) using a suitable mechanical pipette. The bottle with placebo was weighed. The mass of placebo was determined by the weight difference. This operation was carried out for the entire set of validation solutions intended to be assayed in the same run. 20.0 ml of a proper solvent (see Table 2) was added to each bottle using a volumetric pipette. The bottles were capped and placed on a shaker with a shaking frequency of 150 cycles per minute for 60 min.

2.3.3.3. Drug product. Similarly to validation solutions with placebo, we prepared validation solutions with drug product (see Table 3). For this purpose, we used weighed quantities of drug product instead of weighed quantities of placebo.[1]

2.3.4. Preparation of benzalkonium chloride solutions

Benzalkonium chloride (BC) is not an analyte in the current context. However, it is the only excipient in the examined preparations, which has a very characteristic but low-intensity absorption spectrum in the region used in the method. Therefore, its absorption must be taken into account. Instructions of drugs producers, such as Nasonex, do not indicate the quantitative content of excipients. However, the approximate content of benzalkonium chloride can be found in the analytical literature. According to [29], it is about 25 μg per dose. We used several concentrations of BC in isopropyl alcohol to calculate an average spectrum of BC responding to a concentration of 1 μg/ml, which we used for developing our procedure.

After shaking, spectrophotometry is carried out. It is imperative to use a two-beam instrument, in which a monochromatic beam falls on the cuvettes. Therefore, the use of diode-matrix spectrophotometers, in

Table 2
Information regarding the preparation of validation solutions with placebo.

#	Placebo content, % to nominal mass	Target sample placebo diluted with water, mg	20.0 ml MF solution[1] added	The number of prepared validation solutions
1	150	188	IPA	5
2	50	188	150	5
3	75	156	125	5
4	100	125	100	5
5	125	94	75	5
6	150	63	50	5

Table 3
Information regarding the preparation of validation solutions with drug product.

#	Drug product content in % to nominal mass	20.0 ml MF solution added	The number of prepared validation solutions
1	50	IPA	2
2	75	IPA	2
3	100	IPA	2
4	125	IPA	2
5	150	IPA	2
6	150	IPA	2
7	50	150	2
8	75	125	2
9	100	100	2
10	125	75	2
11	150	50	2

[1] Concentration of added MF solution, % of target concentration. Preparation of the corresponding solutions is described in section 2.3.1.4. above.

which the monochromator is located behind the cuvette compartment, must be excluded. This is a consequence of the use of test solutions with a relatively high level of turbidity (Fig. 2).

Spectrophotometry parameters

Mode: absorbance, the wavelength range: 220–310 nm; scanning speed: 50 nm/s; optical slit width - 1 nm; discreteness of data: 1 nm.

3. Results and discussion

3.1. Method development

The purpose of this study was to develop a simple and highly productive (in terms of a number of samples that can be investigated during a working day) spectrophotometric method for determination of content of MF in a single dose of drugs such as Nasonex. Below, selection of the method basic parameters is described, in particular: volume and type of sample solvent, sample collection bottle, procedure of sample preparation and spectrophotometer parameters.

3.1.1. Sample solvent selection

As described in paper [2], MF is practically insoluble in water (0.02 mg/mL), slightly soluble in methanol, ethanol, and isopropanol (4–8 mg/mL), soluble in acetone and chloroform (59–74 mg/mL), and freely soluble in tetrahydrofuran (>100 mg/mL). It would be tempting to use water as a sample solvent as thermodynamic solubility of about 20 μg/ml requires only 2.5 ml of water to dissolve 50 μg of MF. However, our experimental studies have shown that kinetic limitations make it difficult to achieve complete dissolution of MF without preliminary adding some organic solvent, which requires additional time, creates additional uncertainty etc. Among the above-mentioned organic solvents, only isopropanol is not legally restricted, does not belong to excise goods and is not toxic. Therefore, isopropanol (IPA) was chosen as the solvent for samples. The selected IPA meets the requirements of the European Pharmacopoeia for isopropanol reagent Propan-2-ol R1 (Propan-2-ol R1 Transmittance not less than 25% at 210 nm, 55% at 220 nm, 75% at 230 nm, 95% at 250 nm and 98% at 260 nm, determined using water in the reference cell). The spectral range, in which the spectrum is measured, is chosen to be 220–310 nm. The lower limit is set at 220 nm as at shorter wavelengths contribution of benzalkonium chloride to the total absorption increases dramatically, IPA has a fairly high transmittance.

The upper limit was set at 320 nm as for work with long wavelengths it would be necessary to use a halogen lamp. Therefore, use of absorbances at wavelengths longer than 320 nm would create great uncertainty of the obtained results.

3.1.2. Selection of bottle for sample collection

As the method should be easily applied for analysis of a sequence of several dozens to several hundreds of samples, this imposes certain restrictions on containers to be used for collecting samples. As practice shows, volumetric flasks are rather problematic to use in this type of experiment as bringing a bottle to the mark requires considerable time. Therefore, it should be an inexpensive 20–100 ml bottle with a screw cap; dark glass is required as, according to the available information, MF solutions decompose under the influence of light [30].

In this case, the only method of obtaining a traceable dilution is the addition of an aliquot of a solvent with a measuring pipette or another dosing equipment. The volume of aliquot to be added should be optimal as follows. On the one hand, it should not be too small, otherwise the relative uncertainty of this volume would be too large and the uncertainty of the total volume, because of mixing a water sample with IPA, would be too large. On the other hand, it should not be too high because of considerable expense of a solvent and the resulting low optical density. In view of the aforesaid, we chose the volume of an IPA to be 20.0 ml, added with a volumetric pipette.

3.1.3. Selection of sample preparation procedure

After about 100 mg of an aqueous sample and 20.0 ml of IPA were added to a bottle, MF is transferred to the solution by shaking for 1 h with a frequency of 150 cycles per minute. (For the preparation of test solutions, the same lot of IPA, which is used to prepare the reference solutions, must be used). The resulting test solution has a noticeable turbidity. It may be eliminated by filtration, centrifugation, or long-term sedimentation. All these operations performed for dozens or hundreds of test solutions significantly increase time and possibility of gross analytical errors and make the procedure cumbersome. Therefore, we set a goal to carry out the final analytical operation on turbid test solutions. In this case, the sample preparation is confined to four steps: introduction of doses into vials, addition of IPA aliquots, tightening bottle caps, shaking during 1 h. After these steps, the test solutions are ready for spectrophotometry.

3.1.4. Selection of a procedure for isolating pure signal from the analyte

As shown earlier in this paper (see Section 1.4.3), two approaches can be used to isolate pure signal from the analyte in the presence of turbidity. We have chosen the second one - using the turbidity spectrum in an explicit form. However, unlike in paper [21], the turbidity spectrum is determined experimentally.

Analytically, our task is to develop a stable spectrophotometric quantitative determination of MF in the system, which is a true solution of MF and BC in isopropanol (only those substances that have noticeable spectra in the target concentrations are indicated) and suspension of solid substances from the preparation (most likely carboxymethylcellulose sodium and microcrystalline cellulose). In view of this, we have chosen to treat the system as composed of three components: MF (1) as the only analyte, BC (2) and turbidity (TU) (3). The spectra of all three components together with the total spectrum are shown in Fig. 3.

3.1.5. Selection of spectrophotometer parameters

Spectrum should be registered quickly for at least two reasons:

- Implementation of the technique should take as little time as possible.
- Suspended particles tend to settle and, if a measurement takes too much time, the beginning of spectrum and its end belong to "somewhat different" systems.

Fig. 2. Cells with IPA (left) and with the test solution (100 mg of the drug +20.0 ml IPA) (right).
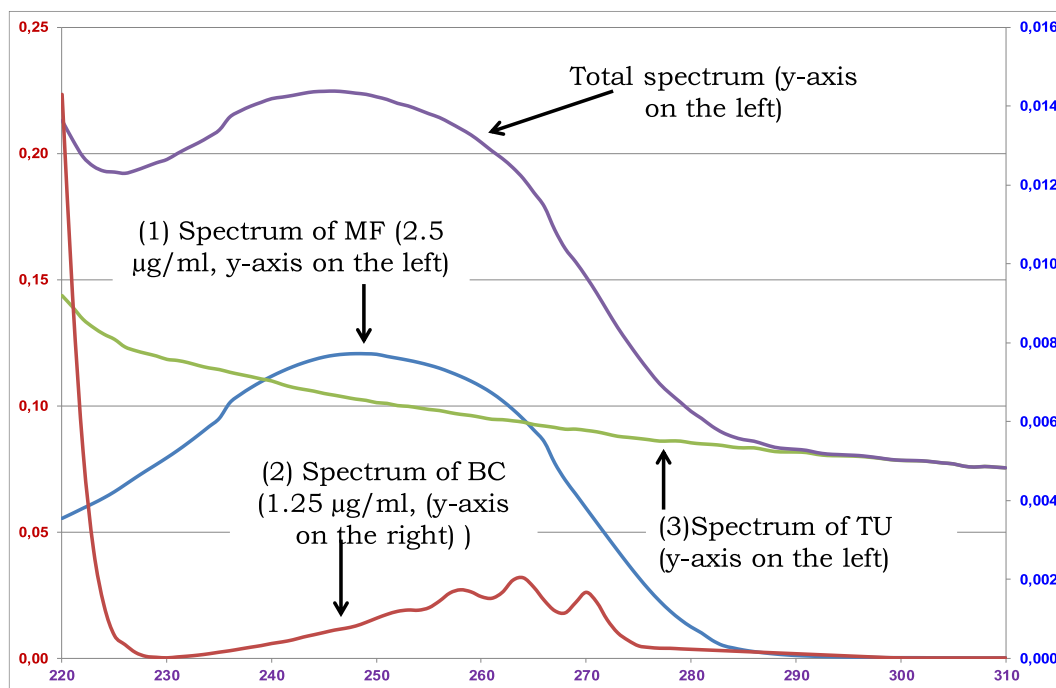
Fig. 3. Spectra of the components and the total spectrum of the drug (as test solution).

Therefore, we chose a spectrum measurement rate of 50 nm/s. Since the entire spectral range is 90 nm, the measurement of a single spectrum occurs in about 2 s. For better quality of results, we took 10 spectra and averaged the data for each wavelength. The width of optical slit should not be large as there would be negative phenomena associated particularly with the presence of turbidity otherwise. Therefore, we have chosen the width of optical gap to be 1 nm. Absorbance values were taken with an interval of 1 nm.

3.1.6. Finding TU spectrum

As our task is to look for concentration of only one analyte (MF), the spectra of remaining light-absorbing components of the system, in particular, the turbidity spectrum, can be represented in a normalized form. To obtain a normalized averaged (for four drugs) turbidity spectrum, the following experiment was conducted: 5 test solutions were prepared for four drug products (NA, FO, GL and AL), in which target masses of about 188 mg of placebo were placed instead of the drug. After shaking on a shaker, the resulting solutions were allowed to stand for 2 h in order to obtain supernatants free from turbidity. A supernatant spectrum was taken for each test solution. As the next step, spectra of intensely mixed solutions were also taken. For each test solution, the turbidity spectrum was calculated as difference between the last and first spectra. Finally, operations of normalization and averaging of the spectra were carried out (see supporting materials). As a result, a normalized and
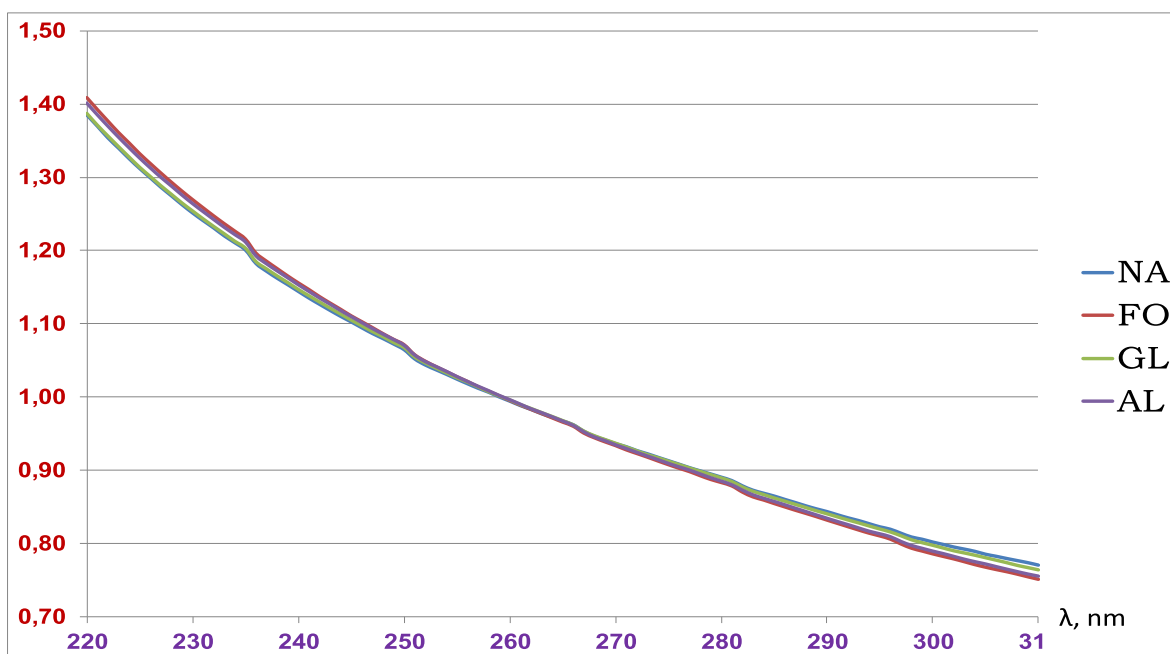

Fig. 4. Obtained average spectra of TU. As these are reduced spectra, units are not indicated on the ordinate axis.

averaged turbidity spectrum of four products was found (see Fig. 4). Additionally, a set of weights for each analytical wavelength was obtained, which are calculated based on the formula presented in Fig. 5 below.

3.1.7. Finding equation to calculate concentration of MF in the test solutions

As the TU spectra for all four drugs were close to each other, the simplest least squares method, which is widely used in analytical chemistry and in spectrophotometry, can be used to find concentration of MF [31].

However, an attempt to use the TU spectrum calculated as an average for these four drug products leads to unacceptable systematic errors for some of all six drug products, i.e. the developed technique loses its versatility and requires calculations for each preparation using spectrum of its own placebo. To overcome this problem, we used the weighted least squares method, in which the weight for each data point of the averaged TU spectrum was defined as:

$$W_i = \left(\frac{1}{RSD_i}\right)^2,$$ (1)

where: RSD_i are calculated values for a given wavelength for the reduced TU spectra of four preparations), % (see Fig. 5).

The distribution of obtained weights can be characterized by the following values: the minimum value is 0.8, the maximum value is 184, and the average value is 22. Now, we have all the data for finding concentrations of MF in the tested solutions by means of the weighted least squares method in matrix form [32]:

$$C = \left[\left(A^T \times W \times A\right)^{-1} \times A^T \times W\right] \times B = a \times B,$$ (2)

where:

- A is the matrix consisting of three columns (spectra MF (at a concentration of 1 μg/ml), BC and TU) and 91 rows (wavelength range 220–310 nm, one nanometer step);
- W is the diagonal weights matrix;
- b is the vector of optical densities of the test solution (column representing spectrum of this solution in the range 220–310 nm, one nanometer step).

A row of this matrix $[(A^T \times W \times A)^{-1} \times A^T \times W]$, when multiplied by the column representing vector of the spectrum of the corresponding solution, results in a concentration of MF in this solution $\left(C_{MF} = \sum_{i=220}^{310} a_{MF,i} \times B_i, \frac{\mu g}{mL}\right)$ has the form.

Matrices $A, W, a = \left[\left(A^T \times W \times A\right)^{-1} \times A^T \times W\right]$ along with all the calculations are presented in supporting materials.

3.1.8. Necessary adjustments

It should be noted that formula (2) merely allows calculation of analyte concentration in test solutions. The following nuances should however be taken into account.

1. It is reasonable to use advantages of the comparison method with external standards, i.e., to register spectra for the reference MF solutions alongside with the test solutions and to correct the measured MF concentration in test solutions taking into account data for the reference standards (P_1).
2. Volume of solution is slightly different from 20.0 ml as an IPA aliquot is added to a certain amount of aqueous sample.
3. Not all doses that leave the container with preparation get into the bottle that serves to collect the sample (P_2).
4. MF in the preparation may contain some amount of related compounds(P_3).

3.1.8.1. Adjustment accounting for the reference solution. We have two quantities as follows:

- the calculated ("true") value of MF concentration in the reference solution;
- the measured value of MF concentration in the reference solution obtained by spectrophotometry with subsequent calculation using formula (2).

It is reasonable to assume that the true and measured concentrations of MF in the test and reference solutions are related as follows:

"True" concentration of MF in the reference solution, C_R	-	Measured concentration of MF in the reference solution C_R^1
"True" i.e. adjusted concentration of MF in the test solution, C_T	-	Measured concentration of MF in the test solution, C_T^1

Therefore:

$$C_T = \frac{C_R}{C_R^1} C_T^1 = P_1 \times C_T^1,$$ (3)

where: $P_1 = \frac{C_R}{C_R^1}$.

λ nm	220	221	222	223	224	225	226	227	228	229	230	231	232
	-0.8749	-0.6398	-0.4375	-0.3400	-0.2445	-0.2019	-0.1836	-0.1545	-0.1375	-0.1193	-0.1036	-0.0897	-0.0749

λ nm	233	234	235	236	237	238	239	240	241	242	243	244	245
	-0.0516	-0.0294	-0.0055	0.0540	0.0915	0.1246	0.1765	0.2103	0.2369	0.3095	0.3518	0.4118	0.5322

λ nm	246	247	248	249	250	251	252	253	254	255	256	257	258
	0.5497	0.5981	0.8329	0.8408	0.7846	1.0258	1.2112	1.5121	1.9088	2.4081	2.3000	2.9614	4.7636

λ nm	259	260	261	262	263	264	265	266	267	268	269	270	271
	4.2448	3.8087	3.6128	2.3919	-0.4571	-1.6379	-1.6630	-1.5478	-1.4217	-2.1027	-2.4416	-2.8665	-1.7884

λ nm	272	273	274	275	276	277	278	279	280	281	282	283	284
	-2.0275	-1.7331	-1.4321	-1.1575	-1.2679	-1.4925	-1.0602	-0.8809	-0.9183	-0.9391	-0.6425	-0.6571	-0.6365

λ nm	285	286	287	288	289	290	291	292	293	294	295	296	297
	-0.5559	-0.5392	-0.5039	-0.5021	-0.4643	-0.3896	-0.3681	-0.3464	-0.2995	-0.2880	-0.3134	-0.2712	-0.2169

λ nm	298	299	300	301	302	303	304	305	306	307	308	309	310
	-0.2000	-0.1813	-0.1757	-0.1731	-0.1708	-0.1478	-0.1329	-0.1384	-0.1302	-0.1287	-0.1227	-0.1126	-0.1112

3.1.8.2. Volume adjustment. The main target value of the method is MF mass in a dose drug, which is calculated by means of the following formula:

$$m_T^{MT} = V_T \times C_T, \tag{4}$$

where V_T represents the volume resulting from mixing 20.0 ml of IPA and the part of aqueous suspension, which was put into the bottle (we denote its mass as m_T; it is about 100 mg); C_T represents MF concentration in the solution. It is necessary to estimate the total volume (the density of MF aqueous suspension can be taken equal to 1 g/ml).

At first glance, this volume is merely a sum of a big volume of isopropyl alcohol (20 ml) and a small volume of MF aqueous suspension, which is 0.5% of the volume of isopropyl alcohol in the case of a target dose of 100 μl.

However, to ensure maximum accuracy, we should consider effects of total volume depression when mixing alcohol and water. We will carry out our assessments in the temperature range, which corresponds to the definition of the European Pharmacopoeia "Room Temperature" (15–25

^{0}C) [33].

The weight fraction of water (w) in a solution containing 20.0 ml of IPA and 0.1 ml of water can be expressed by the following formula:

$$w = \frac{0.1 \times \rho_{H_2O}^t}{20 \times \rho_{IPA}^t + 0.1 \times \rho_{H_2O}^t} \tag{5}$$

where $\rho_{H_2O}^t$; ρ_{IPA}^t are densities of water and IPA at specified temperatures [34]. Using data for density of water solutions in IPA from [35], densities can be calculated at different temperatures. On the other hand, knowing density of IPA and water, we can calculate masses of these solutions formed at the indicated temperatures [36]. Knowing density and mass of these solutions, one can calculate their volumes. All these calculations are presented in Table 4.

As it can be seen from Table 4, adding 0.1 ml of water to 20 ml of IPA leads to formation of approximately 20.08 ml of solution. In view of this, m_T grams of MF aqueous suspension added leads to an increase of sample solution volume by $0.8 \times m_T$ ml. All data together with all the corresponding calculations for obtaining this result are presented in
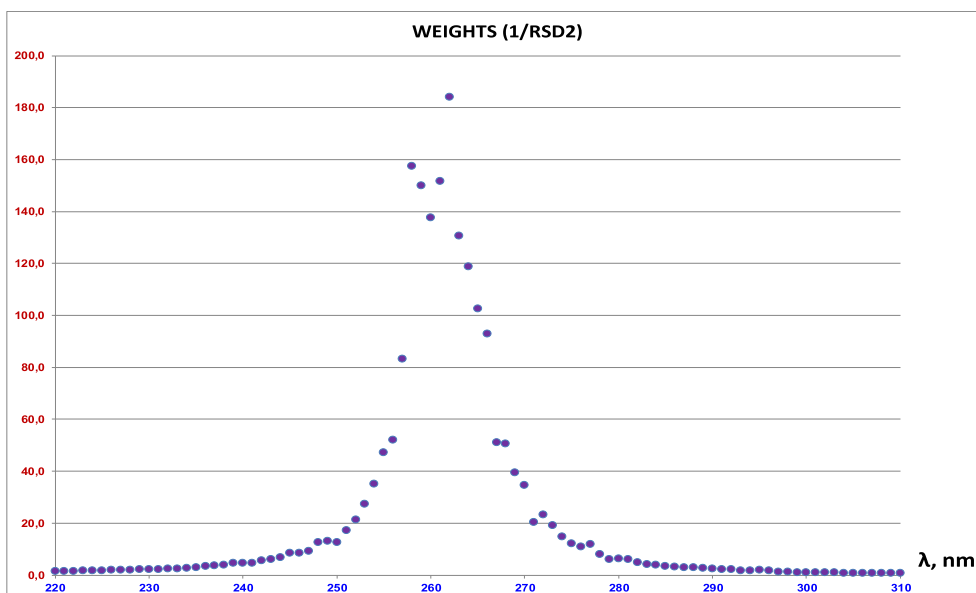

Fig. 5. Weights for finding the matrix for calculations.

supporting materials.

Thus, Eq. (4) can be rewritten explicitly:

$$m_T^{MT} = (20 + 0.8 \times m_T) \times C_T. \tag{6}$$

3.1.8.3. Adjustment to the part of sample that made its way into the bottle. Based on our experience, this adjustment needs to be made for each type of bottle/container used to collect a sample. We present the corresponding data for the bottles we have used (Fig. 1). The ratio of ample mass that was put into the bottle to the mass that was taken from the container with the preparation was very close for all six preparations: 97.6% with the standard deviation 0.4%. Therefore, if there was a need to recalculate sample mass that was taken from the container, recalculation coefficient $P_2 = \frac{100}{97.6} = 1.025$ may be used. All data together with all the corresponding calculations for obtaining this result are presented in supporting materials.

3.1.8.4. Adjustment to MF related compounds. The only Pharmacopoeia known to us, which contains a monograph for MF aqueous nasal spray, is the British Pharmacopoeia [16]. In the corresponding monograph, the limits for content of MF related compounds obtained by TLC are provided: none more than 2%, no more than one from 1% to 2%, any other not more than 0.5%. This data is not very helpful for making conclusions about the amount of related compounds. In view of this, we will be using some information from the American Pharmacopoeia [17] and will be referring to our experience with medicinal drugs. A rule of thumb is that the totality of ordinary impurities (related compounds) in preparation should not exceed 2%. In the case of MF preparations, this can be confirmed by rationing the amount of related compounds for such preparations in the American Pharmacopoeia, in which related compounds are determined by HPLC: Mometasone Furoate Cream ≤1%, Mometasone Furoate Ointment ≤1%, Mometasone Furoate Topical Solution ≤2%. Thus, it is reasonable to take the limiting content of the totality of related compounds in the preparation concerned as 2%. Then, the "average" content (from 0 to 2%) is 1%. As we do not know how these related compounds affect the result of determination (i.e., we do not know their correction factors), it is reasonable to assume that the technique will perceive them as MF (correction factor is 1 for all). Taking into account all the above reasoning, we can conclude that the adjustment is obtained by multiplying the amount of MF in a dose by $P_3 = 0.99$.

3.1.8.5. Summary of all adjustments. The summary of all adjustments discussed in sections 3.1.8.1 reads

$$m_T^{MT} = P_1 \times P_2 \times P_3 \times (20 + 0.8 \times m_T) \times C_T, \tag{7}$$

After substituting numerical values, we get:

$$m_T^{MT} = P_1 \times 1.025 \times 0.99 \times (20 + 0.8 \times m_T) \times C_T, \tag{8}$$

and finally, for the mass of the sample that made its way into the bottle equal to 0.1

$$m_T^{MT} = 20.4 \times P_1 \times C_T \tag{9}$$

3.2. Method validation

3.2.1. Validation concept

To validate a method of quantitative determination of an active

ingredient content in a drug, which is being proposed hereby, ICH [37] suggests evaluating the following validation characteristics: ***accuracy, precision (repeatability, intermediate precision), specificity, linearity, range and robustness***.

To assess accuracy of quantitative determination of an active substance in a drug, ICH [37] proposes the following approaches:

1. The use of model (synthetic) mixtures of the excipients, to which the known quantities of the drug substance are added, i.e. the use of placebo and some variant of the standard addition method.
2. Using a variant of the standard addition method, but, instead of placebo, the drug product itself is used.
3. Comparison of the results of analysis of the drug product by the proposed method with the results of its analysis by the second, well-characterized procedure.
4. Accuracy can be inferred from precision, linearity and specificity.

In our case, we cannot have a placebo as required in the first of the above approaches, i.e. true placebo is completely free of MF. However, as described in Section 2.3.3.1 above, we can get a "placebo" that is very close to it, which contains some small residual amount of MF and which, in this aspect, is much closer to the true placebo than it is to the drug product. For more confidence, we also used the second of the above approaches as an auxiliary.

Acceptance criteria for validation characteristics can be selected based on the target uncertainty of the method [38]. So, the acceptance criteria for accuracy and specificity can be chosen as follows:

Accuracy. The difference between the true value and the value obtained using this method for all model mixtures (validation solutions), except for the most unfavorable situations from an analytical point of view, must not exceed the target uncertainty of the method.

$$\left| m_{MF}^{TRUE} - m_{MF}^{FOUND} \right| \leq u^{tg} \tag{10}$$

Specificity. The difference between the true value and the value obtained using this method for the analytically most unfavorable ratio analyte/interfering component, in our case – placebo, must not exceed the extended target uncertainty of the method (this provision complements the accuracy requirement).

$$\left| m_{MF}^{TRUE} - m_{MF}^{FOUND} \right| \leq U^{tg} \tag{11}$$

Repeatability. In our method, the turbidity of solutions may vary. Since this should not interfere with the results, three measurements of validation solutions are carried out:

1. After a solution has settled to a degree when sufficient amount of a practically transparent test solution (upper layer) can be transferred into a cuvette, a spectrum is taken. The solution is practically free from turbidity.
2. After the first measurement, the liquid from the cuvette is returned to the bottle with the solution, the content of the bottle is slightly mixed, the cuvette is filled, and the spectrum is taken. The solution has an intermediate turbidity.
3. After the second measurement, the liquid from the cuvette is returned to the bottle, the cap is closed, the bottle is vigorously shaken for 30 s, the cap is open, the cuvette is immediately filled with turbid liquid, and the solution is measured. The solution has the highest turbidity.

Table 4
Data for calculating the volume of test solutions.

t, ^{0}C	ρ_{H2O}, g/ml	ρ_{IPA}, g/ml	V_{H20}, ml	V_{IPA}, ml	m_{H20}, g	m_{IPA},g	w	$\rho(w)$,g/ml	m,g	V_{sol}, ml
15	0.9991	0.7892	0.1	20	0.0999	15.78	0.006290	0.7910	15.8839	20.082
25	0.997	0.7808	0.1	20	0.0997	15.62	0.006344	0.7826	15.7157	20.081

The criterion for precision can be selected as follows:

The combined standard deviation for the above three measurements must not exceed u^{tg}, and any individual standard deviation must not exceed U^{tg}:

$$S_{MF}^{POOLED} \leq u^{tg} \tag{12a}$$

$$S_{MF}^{i} \leq U^{tg} \tag{12b}$$

Intermediate precision. The intermediate precision criterion can also be based on the extended target uncertainty. The maximum difference between measurements taken on different days by different analysts and using different grades of isopropyl alcohol should not exceed U^{tg}:

$$\Delta_{day, person, IPA}^{MAX} \leq U^{tg} \tag{13}$$

Linearity, range. The range chosen above is 50–150% of the nominal amount of MF or, respectively, the nominal concentration (see 2.3.3.2 *Range*).

The requirements of linearity can be formulated as follows: the dependence of amount (concentration) of MF obtained using this method on the entered amount (concentration) of MF must be linear in the whole range; the correlation coefficient is no less than 0.999.

Robustness. We chose the size of optical slit and the scanning speed, within the limits of device used as the intentionally variable parameters of the technique. The absolute value of difference between the values obtained under conditions described in the method (m1) and under conditions with modified parameters (m2) must not exceed the extended target uncertainty:

$$\left| m_{MF}^{m1} - m_{MF}^{m2} \right| \leq U^{tg} \tag{14}$$

3.2.2. Completeness of extraction of MF from the sample

Before taking samples, the content of container with the drug was transferred to a bottle and intensively mixed and, as homogeneity is assumed, the concentration of MF in samples can serve as a marker of degree of extraction. The same is the case for our "placebo". The sample that was placed into the bottle contains MF mainly in the form of a solid substance, and during the sample preparation, it stays in contact with IPA when shaken on a shaker for 1 h. During this time, the entire MF should go into solution. To verify this assumption, exhaustive extraction tests were carried out. Forinex was selected as the study drug product. Time points of 15, 30, 60, 90 and 180 min were selected. For each time period, five placebo samples and five drug samples were prepared (the exact weight (about 0.1 g) of placebo sample or drug was added to each bottle; see Section 2.3.3.1). All bottles were filled with 20.0 ml of IPA and shaken for the corresponding period of time.

After that, all solutions were analyzed by the proposed method. The results are presented in Table 5. All data along with all the calculations for obtaining these results are presented in supporting materials.

As it can be seen from Table 5, after 15 min of shaking, no significant changes in the detectable concentration of MF in the preparation or in "placebo" are observed. On the other hand, 60 min of shaking under the proposed conditions ensures complete extraction of MF from the preparation.

3.2.3. Target uncertainty of the method

Before assessing basic validation characteristics of the proposed method, one is supposed to estimate the target uncertainty for this method.

For the quantitative determination method with upper and lower tolerance limits, Q^{max}, Q^{min} respectively, *Eurachem/CITAC Guide* [38, see p. 5] suggests the following formula for calculating the extended target uncertainty (U^{tg}):

$$U_r^{tg} = \frac{Q_r^{max} - Q_r^{min}}{8} = \frac{125\% - 75\%}{8} = 6.25\%, \tag{15a}$$

or:

$$U^{tg} = \frac{Q^{max} - Q^{min}}{8} = \frac{(125\% - 75\%)*50\mu g}{100\%*8} = 3.13\mu g \tag{15b}$$

In view of this, as extended uncertainty equals uncertainty multiplied by factor 2,

$$u^{tg} = \frac{U_r^{tg}}{2}\% = 1.56\mu g \tag{16}$$

3.2.4. Evaluation of accuracy, precision (repeatability, intermediate precision), specificity, linearity, range, robustness

3.2.4.1. Values extracted from experimental data. Repeatability.
From the data presented in Table 9 (see values of S in column 5), we calculate an estimated value of the pooled standard deviation using formula [39]:

$$S_{C_{MF}}^{POOLED} = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + \ldots + (n_k - 1)s_k^2}{n_1 + n_2 + \ldots + n_k - k}} \tag{17a}$$

As we have three determinations in each series ($n_i = 3$) and there are 25 series ($k = 25$), we obtain the following formula:

$$S_{C_{MF}}^{POOLED} = \sqrt{\frac{2 \times \sum_{i=1}^{25} s_i^2}{3 \times 25 - 25}} = \sqrt{\frac{\sum_{i=1}^{25} s_i^2}{25}} = 0.015, \quad \frac{\mu g}{ml}. \tag{17b}$$

As this represents an estimate of a combined standard deviation of MF concentration, we calculate an estimated value of MF mass taking into account that sample volume is approximately 20 ml:

$$S_{m_{MF}}^{POOLED} = 0.015 \times 20 = 0.3 \quad \mu g \tag{17c}$$

$$Max\left(S_{C_{MF}}^{i}\right) = 0.038 \frac{\mu g}{ml} \rightarrow Max\left(S_{m_{MF}}^{i}\right) = 0.76\mu g < 3.13\mu g \tag{17d}$$

The results of an additional assessment of repeatability of measurement of the test solution together with an assessment of repeatability of measurement of the reference solution are presented in Table 6. In this case, 30 spectra of the test solution and 30 spectra of the reference solution were taken, from which the concentrations were calculated.

As it can be seen from Table 6, these results are very close to those presented above. In view if this, the standard deviation of concentration measurement can be considered equal to 0.3 μg both for measurement of the test and the reference solution, and, therefore, the total standard deviation that characterizes repeatability equals to $\sqrt{2} \times 0.3 = 0.42$. Accordingly:

$$S_{m_{MF}}^{T} = 0.42 \quad \mu g < 1.56\mu g \tag{17e}$$

As it can be seen from (17d) and (17e), the requirements for repeatability are met.

Intermediate precision.

The study of intermediate precision is divided into two parts:

1. Different analysts and different working days.

Table 5
Completeness of extraction of MF from drug product doses and placebo.

Time, min	MF concentration found in the drug, μg/g			MF concentration found in "placebo", μg/g		
	Mean	S	RSD, %	Mean	S	RSD, %
15	479.2	5.5	1.1	66.0	2.5	3.8
30	480.9	6.8	1.4	66.7	2.1	3.2
60	484.1	5.7	1.2	65.4	1.7	2.7
90	481.0	4.8	1.0	65.4	3.5	5.6
180	481.2	6.4	1.3	64.3	3.4	5.2

2. Various grades of IPA for preparation of test and reference solutions (see Section 2.1. Materials).

Part 1. Test solutions of the complete sequence of doses of drug product FLIX (151 doses in total) were prepared. Four analysts conducted analysis of the test solutions on four different working days. Each analyst analyzed the entire sequence of doses once. Consequently, four results were obtained for each dose (mass of MF in each dose, μg), after which the maximum and minimum results were chosen and their differences were calculated. These differences are shown in Fig. 6 against the number of the corresponding dose.

As it can be seen from Fig. 6, not a single difference exceeds the critical value of 3.13 μg.

Part 2. Liquid suspension of MF from one container of Nasonex was transferred into a bottle of brown glass (see Fig. 1). After intensive shaking, portions of about 50%, 75%, 100%, 125% and 150% of the nominal value of 0.1 g (exact weights) were taken. These portions were used to prepare three series of test solutions using three different brands of IPA (see 2.1. Materials). MF reference solutions were prepared using the same brands of IPA. As intensive shaking of bottle was performed prior to sampling of each portion of MF aqueous suspension, it can be assumed that concentration of MF in all portions was approximately the same. Therefore, measurements of all three series are expected to give the same concentration of MF in this aqueous suspension. The averaged results of these determinations are presented in Table 7.

As it can be seen from Table 7, the average values of MF concentration in aqueous suspension, which has been found by means of the proposed method using three brands of IPA, have RSD 0.6%, which indicates the absence of significant systematic deviations.

Taking into account the results presented under part 1 and part 2, it can be concluded that the method is validated regarding the parameter "intermediate precision".

Linearity, range

The range, in which linearity of MF is expected, has been already determined in this study (see 2.3.3.2); it is 50–150% of the nominal concentration. For illustrative purposes, Fig. 7 shows a diagram of the found concentrations of MF in the reference solutions from the "true" (calculated) concentrations in a wider range 5–150%. The fact that MF behaves similarly in validation solutions and test solutions is demonstrated below in the Accuracy section.

Specificity. The specificity will be checked in the accuracy section.

Accuracy. We have six drug products, for each of which it is necessary to check the accuracy of the results obtained. As the technique claims that turbidity of the test solution is not a disturbing factor, measurements were carried out as usual in three modes (See 3.2.1. Validation concept, point "Repeatability).

Typical spectra obtained in this way are shown in Fig. 8.

We shall consider in detail the results obtained for the product Nasonex. The results for other products will be presented in an abbreviated form.

Nasonex. First, the concentration of MF in placebo is determined $\left(\frac{\mu g}{g}\right)$ with the purpose of finding the concentration of residual MF in placebo. All the operations included into the method were carried out for five bottles, in which precise weights (about 0.188 g) of Nasonex placebo were transferred. Table 8 presents the results obtained:

Then, placebo-based model mixtures (validation solutions) with addition of MF solutions with known concentrations were analyzed.

The following conclusions can be drawn from the results presented in Table 9:

1. For all validation solutions, the standard deviation for three measurements does not exceed 0.04 μg/ml, i.e. for solution volume of approximately 20 ml it would amount to 0.8 μg, which is substantially less than any criteria selected in Section 3.2.1. Thus, it can be concluded that the method allows tested solutions to be used without any removal of turbidity.

2. Accuracy of the method for Nasonex preparation satisfies a predetermined criterion (see formula (10)).

$$\left| m_{MF}^{TRUE} - m_{MF}^{FOUND} \right| \leq u^{tg} \rightarrow 0.43 \leq 1.56$$

3. For the case of the worst MF placebo ratio, i.e. NA-B188R50 validation solutions, the result meets the acceptance criteria for accuracy and specificity (11)

$$\left| m_{MF}^{TRUE} - m_{MF}^{FOUND} \right| \leq U^{tg} \rightarrow 0.59 \mu g < 3.13 \mu g$$

4. Linearity in the claimed range is demonstrated by the dependence of the found MF mass transferred from the MF solution with a known concentration to the validation solution on the "true" mass of MF introduced by this method; see Fig. 9.

Similar studies have been carried out for model mixtures based on the Nasonex preparation itself. Table 10 presents determined concentrations of MF in aqueous suspension of the drug Nasonex.

Column 10: mass of MF introduced to this validation solution from aqueous suspension of the drug product, calculated using formula $m_{MF}^{DP} = C_{MF}^{DP} \times m^{DP}$, where C_{MF}^{DP} is the concentration of MF in this suspension found above (see Table 10); m^{DP} is the sample of suspension from column 8, g.

Column 11: found mass of MF transferred to the validation solution from the solution of MF of known concentration, calculated using formula $m_{MF}^{FOUND} = m_{MF}^{*} - m_{MF}^{DP}$, μg.

All other explanations are the same as for Table 9.

The following conclusions can be drawn from the results presented in Table 11:

1. As expected, the results for validation solutions containing the drug are significantly worse than those for validation solutions containing placebo. The worst result in Table 10 for NA-P150R50-2 shows a deviation of 2.45 μg. Nevertheless, the requirement for specificity is fulfilled (see formula (11)

$$\left| m_{MF}^{TRUE} - m_{MF}^{FOUND} \right| \leq U^{tg} \rightarrow 2.45 \mu g < 3.13 \mu g.$$

2. The requirement for accuracy (11), however, is no longer met.

$$\left| m_{MF}^{TRUE} - m_{MF}^{FOUND} \right| \leq U^{tg} \rightarrow 1.60 \mu g \leq 1.56 \mu g$$

It should be recalled (see Section 2.3.3) that the use of drug product for preparation of validation solutions is auxiliary.

The aggregated results for the remaining 5 drug products are presented below in Tables 12 and 13.

Table 6
Characterization of repeatability.

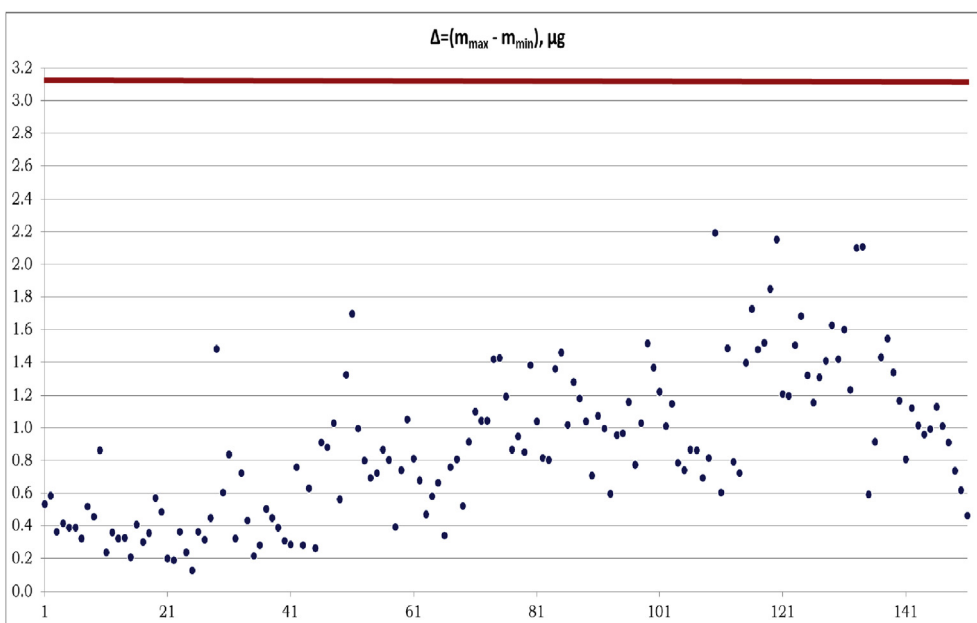| #	Number of repeated measurements	Mean, μg/ml	S, μg/ml	S (m) = 20.4*S, μg	RSD, %
NA-P100-1	30	2.650	0.015	0.30	0.55
RS-1-100%	30	2.470	0.016	0.32	0.64

Fig. 6. Differences between the maximum and minimum results.

Table 7
Results of quantitative determination of MF in aqueous suspension obtained using different brands of IPA.

Name of test solution	Content of MF in aqueous suspension, µg/g Brands of IPA			MEAN	S	RSD, %
	1	2	3			
NA-P50	523	520	508			
NA-P75	511	504	501			
NA-P100	511	497	491			
NA-P125	500	504	502			
NA-P150	498	509	512			
Average	508.6	507.0	502.5	506	6.1	0.6

1. For the case of the worst MF placebo ratio, i.e. NA-B188R50 validation solutions, the result meets acceptance criteria specificity (11)

$$\left| m_{MF}^{TRUE} - m_{MF}^{FOUND} \right| \leq U^{tg} \rightarrow 3.13\mu g \leq 3.13\mu g$$

2. The requirement for accuracy is not fulfilled in one case (B156R75-2) for the drug product Forinex

$$\left| m_{MF}^{TRUE} - m_{MF}^{FOUND} \right| \leq U^{tg} \rightarrow 1.98\mu g > 1.56\mu g$$

However, as it is the only case out of 120 cases (100 in Table 12 and 20 in Table 9), this fact is unlikely to affect the decision that the method meets the requirements for accuracy.
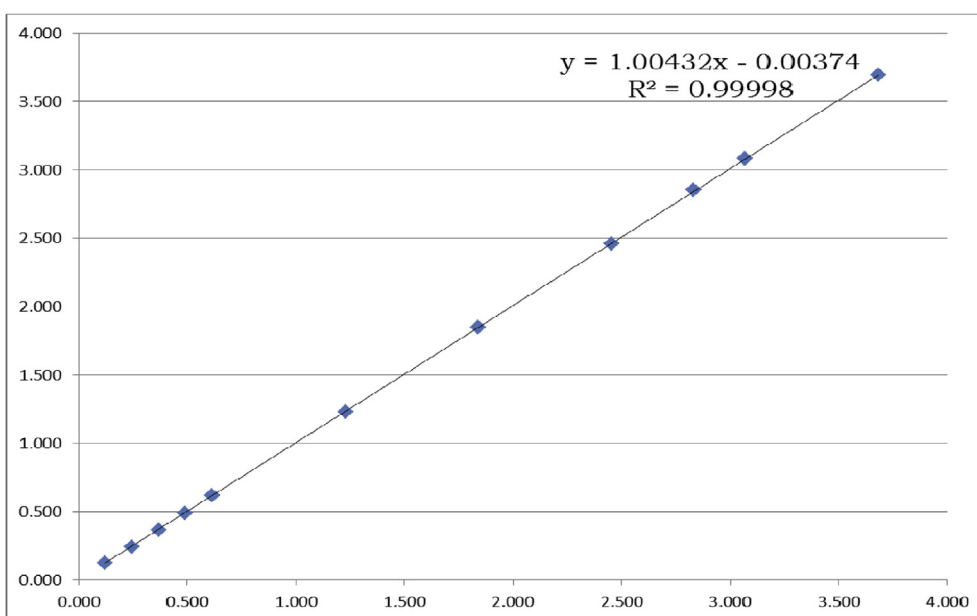

Fig. 7. Dependence of the found concentrations of MF in the reference solutions on "true" (calculated) concentrations.
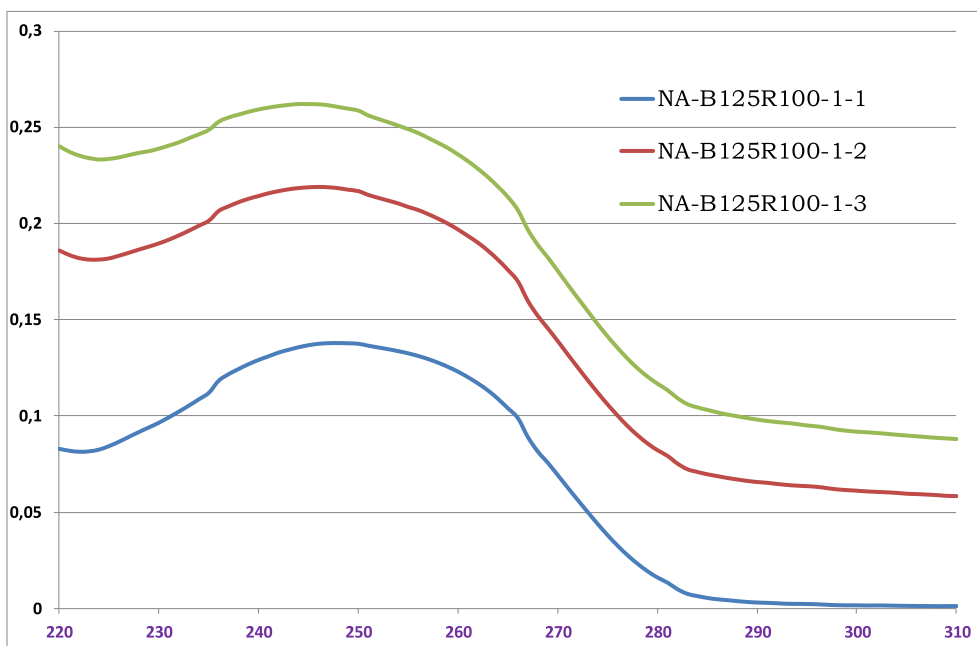
Fig. 8. Typical spectra of the first, second and third determination.
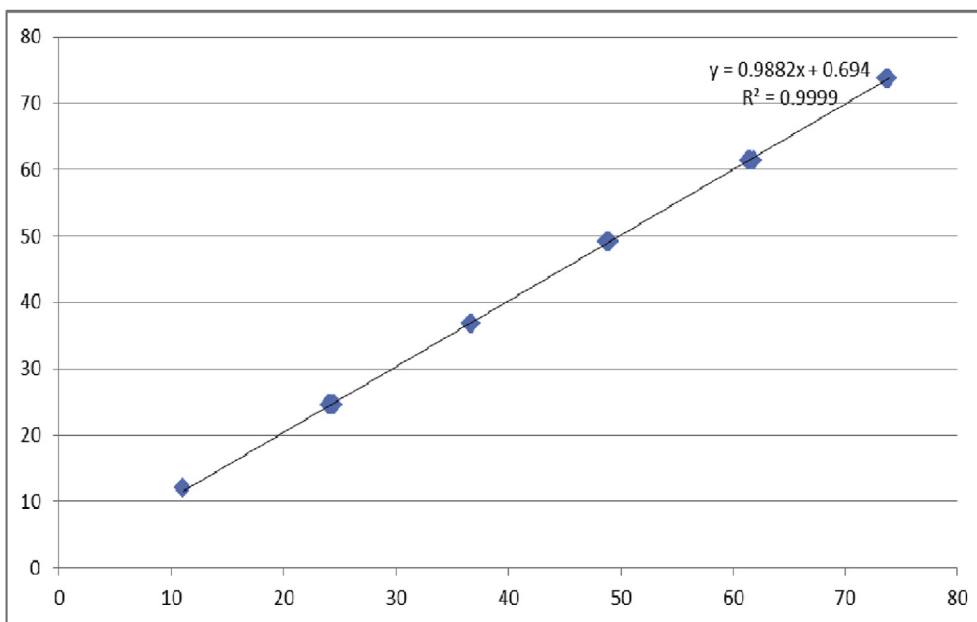

Fig. 9. Dependence of found masses of MF on "true" (calculated) masses.

As it can be seen from Table 13, all results meet the criteria for accuracy and specificity.

The entire set of results presented in Tables 9, 10, 11, 12, and 13 suggests that the method can be considered validated for accuracy and specificity.

Robustness. To test robustness of the method, we used test solutions prepared from an aqueous suspension of Mometasone-Teva, with different target concentrations. The results are presented in Table 14.

The largest deviation is 0.044 µg (MT-125-1), which is significantly less than the acceptance criterion for robustness (see Eq. (14).

$$\left| m_{MF}^{m1} - m_{MF}^{m2} \right| \le Q^{tg} \rightarrow 0.044 \mu g = 3.13 \mu g$$

The entire set of results presented in section 3.2 suggests that the developed method can be considered completely validated.

3.3. Comparison of results obtained using the proposed method and calculations obtained using the second derivative

As spectrophotometric methods for quantitative determination of an analyte in turbid solutions are mainly derivative (see Section 1), it seems reasonable to compare the results obtained using the developed technique with the results obtained using derivative spectrophotometry. As MF has maximum absorbance in the analytical region of the spectrum, it is reasonable, by analogy with [20], to use the second derivative in the form of the following function:

Table 8
Concentrations of residual MF in placebo Nasonex.

#	C_{MF}, µg/ml (Measurement number)			Mean	S	RSD, %	Placebo m, g	m* $_{MF}$, µg	C_{MF}, µg/g
	1	2	3						
NA-B188-1	0.456	0.470	0.427	0.451	0.022	4.9	0.1750	9.079	51.9
NA-B188-2	0.515	0.448	0.495	0.486	0.034	7.1	0.1807	9.787	54.2
NA-B188-3	0.519	0.498	0.490	0.502	0.015	3.0	0.1840	10.121	55.0
NA-B188-4	0.519	0.497	0.500	0.505	0.012	2.3	0.1861	10.184	54.7
NA-B188-5	0.521	0.492	0.500	0.504	0.014	2.9	0.1823	10.164	55.8
							C_{MF}^{P}	MEAN	54.3
								S	1.5
								RSD, %	2.7

* formula (6) is used for calculation.

$$Y = 2A_{\lambda_{MAX}} - (A_{\lambda_{MAX}-5nm} + A_{\lambda_{MAX}+5nm}), \tag{18a}$$

where: $A_{\lambda_{MAX}}, A_{\lambda_{MAX}-5nm}, A_{\lambda_{MAX}+5nm}$ - are the absorbances at wavelengths corresponding to the maximum of the MF spectrum in the region of 220–310 nm.

Since the maximum of MF spectrum is observed at 248 nm, we obtain:

$$Y = 2A_{248nm} - (A_{243nm} + A_{253nm}). \tag{18b}$$

It is natural to calculate MF concentration in a solution using the external standard approach:

$$C_x^{MF} = \frac{Y_x}{Y_{RS}} \times C_{RS}^{MF}, \tag{19}$$

where C_x^{MF}, C_{RS}^{MF} are the determined concentration of MF and the concentration of MF in reference solution, respectively, µg/ml; Y_x, Y_{RS} are the analytical responses (18b) for the solution, in which the concentration of MF is determined, and the reference solution, respectively.

For all validation solutions presented in this study, a comparison was made between the results obtained using the calculation method proposed and the results obtained using the second derivative method (formulas 18 and 19). Concentrations were converted to MF masses by multiplying by 20 (approximate volume of the test solution).

Table 9
The results of determination of MF in validation solutions prepared using placebo Nasonex (designations are explained in the sub-table caption).

1	2	3	4	5	6	7	8	9	10	11	12	13	14
NA-B188R50-1	1.70	1.65	1.67	1.67	0.02	1.4	0.174	33.6	9.5	24.1	24.6	98.2	0.44
NA-B188R50-2	1.77	1.73	1.71	1.74	0.03	1.8	0.200	34.8	10.8	24.0	24.6	97.6	0.59
NA-B188R50-3	1.75	1.73	1.72	1.73	0.02	0.9	0.193	34.8	10.5	24.3	24.6	99.0	0.25
NA-B188R50-4	1.73	1.66	1.70	1.70	0.04	2.2	0.181	34.0	9.8	24.2	24.6	98.6	0.34
NA-B188R50-5	1.73	1.71	1.72	1.72	0.01	0.6	0.186	34.6	10.1	24.5	24.6	99.7	0.07
NA-B156R75-1	2.27	2.23	2.26	2.26	0.02	1.0	0.164	45.5	8.9	36.6	36.8	99.3	0.27
NA-B156R75-2	2.25	2.25	2.24	2.25	0.01	0.4	0.160	45.3	8.7	36.6	36.8	99.5	0.20
NA-B156R75-3	2.29	2.28	2.27	2.28	0.01	0.5	0.169	45.9	9.2	36.7	36.8	99.8	0.08
NA-B156R75-4	2.29	2.26	2.25	2.27	0.02	0.8	0.167	45.7	9.1	36.6	36.8	99.4	0.21
NA-B156R75-5	2.28	2.27	2.27	2.27	0.01	0.3	0.165	45.8	8.9	36.8	36.8	100.0	0.02
NA-B125R100-1	2.75	2.75	2.75	2.75	0.00	0.0	0.117	55.2	6.3	48.9	49.1	99.6	0.22
NA-B125R100-2	2.76	2.75	2.76	2.76	0.01	0.3	0.117	55.3	6.4	48.9	49.1	99.7	0.16
NA-B125R100-3	2.79	2.75	2.79	2.77	0.02	0.9	0.129	55.7	7.0	48.7	49.1	99.1	0.43
NA-B125R100-4	2.80	2.78	2.79	2.79	0.01	0.3	0.131	56.0	7.1	48.9	49.1	99.7	0.16
NA-B125R100-5	2.79	2.78	2.79	2.78	0.00	0.2	0.125	55.9	6.8	49.1	49.1	100.0	0.02
NA-B96R125-1	3.28	3.26	3.28	3.27	0.01	0.3	0.082	65.9	4.5	61.5	61.4	100.2	0.09
NA-B96R125-2	3.29	3.30	3.29	3.30	0.01	0.2	0.090	66.4	4.9	61.5	61.4	100.2	0.09
NA-B96R125-3	3.31	3.32	3.32	3.32	0.01	0.2	0.089	66.8	4.9	61.9	61.4	100.9	0.55
NA-B96R125-4	3.31	3.31	3.30	3.31	0.01	0.2	0.097	66.6	5.2	61.3	61.4	100.0	0.03
NA-B96R125-5	3.33	3.31	3.33	3.32	0.01	0.3	0.099	66.9	5.4	61.6	61.4	100.3	0.19
NA-B63R150-1	3.83	3.83	3.83	3.83	0.00	0.1	0.058	77.0	3.1	73.8	73.7	100.2	0.18
NA-B63R150-2	3.83	3.82	3.84	3.83	0.01	0.3	0.060	77.0	3.3	73.7	73.7	100.1	0.07
NA-B63R150-3	3.85	3.85	3.85	3.85	0.00	0.1	0.067	77.4	3.6	73.8	73.7	100.2	0.12
NA-B63R150-4	3.84	3.81	3.84	3.83	0.02	0.4	0.063	77.0	3.4	73.6	73.7	99.9	0.08
NA-B63R150-5	3.85	3.85	3.86	3.85	0.00	0.1	0.0655	77.5	3.6	73.9	73.7	100.3	0.25

- Column 1: name of validation solution.
- Columns 2–4: concentrations of MF obtained from measurements number 1, 2, and 3.
- Columns 5–7: mean value, S, and RSD % for these three measurements, respectively.
- Column 8: weight of placebo sample used to prepare this validation solution, g.
- Column 9: mass of MF calculated on the basis of the mean value from column 5 and using formula (6), µg.
- Column 10: mass of MF transferred to this validation solution from placebo, calculated using formula $m_{MF}^P = C_{MF}^P \times m^P$, where C_{MF}^P is the concentration of MF in placebo found above (see Table 8), m^P is the sample of placebo from column 8, µg.
- Column 11: calculated mass of MF transferred to the validation solution from the solution of MF of a known concentration, calculated using formula $m_{MF}^{FOUND} = m_{MF}^* - m_{MF}^P$, µg.
- Column 12: the "true" (calculated) mass of MF, introduced into the validation solution by adding 20 ml of MF solution of a known concentration; it is equal to $m_{MF}^{TRUE} = 20* C_{MF}^{RS}$, µg.
- Column 13: the ratio of the mass found to the «true» (calculated) mass of MF introduced into this validation solution $\frac{m_{MF}^{FOUND}}{m_{MF}^{TRUE}} \times 100$,%, i.e. quantity that characterizes the *accuracy* of the method.
- Column 14: the absolute value of the difference between the true (calculated) amount of MF and the amount of MF found, µg.

Table 10

Determination of concentration of MF in aqueous suspension of the drug product Nasonex.

| # | C_{MF}, µg/ml (Measurement number) | | | Mean | S | RSD, % | Drug Product m, g | m* $_{MF}$, µg | C_{MF}, µg/g |
	2	3	4						
NA-P50-1	1.42	1.40	1.40	1.41	0.01	0.8	0.05	28.2	517.7
NA-P50-2	1.21	1.20	1.20	1.20	0.01	0.6	0.05	24.1	508.9
NA-P75-1	1.94	1.92	1.92	1.93	0.01	0.6	0.08	38.7	512.2
NA-P75-2	1.97	1.95	1.95	1.96	0.02	0.8	0.08	39.3	518.7
NA-P100-1	2.68	2.63	2.62	2.64	0.03	1.2	0.10	53.1	510.1
NA-P100-2	2.57	2.54	2.54	2.55	0.02	0.8	0.10	51.2	512.7
NA-P125-1	2.99	2.94	2.97	2.97	0.03	0.9	0.11	59.6	519.7
NA-P125-2	3.02	2.98	2.98	2.99	0.02	0.7	0.12	60.2	514.4
NA-P150-1	4.06	3.93	3.97	3.98	0.07	1.7	0.16	80.2	502.4
NA-P150-2	3.72	3.67	3.67	3.69	0.03	0.9	0.15	74.2	506.4
							C_{MF}^{DP}	MEAN	512.3
								S	5.6
								RSD, %	1.1

* formula (6) is used for calculation.

Table 11

The results of determination of MF in validation solutions compiled using an aqueous suspension of the drug Nasonex (designations are explained in the sub-table caption to Table 9 and specified in the sub-table caption to this table).

1	2	3	4	5	6	7	8	9	10	11	12	13	14
NA-P150R50-1	5.30	5.24	5.21	5.25	0.05	0.9	0.160	105.2	82.2	23.0	24.6	93.8	1.53
NA-P150R50-2	5.33	5.28	5.24	5.28	0.04	0.8	0.164	105.9	83.8	22.1	24.6	90.0	2.45
NA-P125R75-1	4.80	4.75	4.74	4.76	0.03	0.6	0.118	95.9	60.7	35.2	36.8	95.7	1.60
NA-P125R75-2	5.07	4.98	5.03	5.03	0.04	0.9	0.128	101.2	65.8	35.5	36.8	96.3	1.37
NA-P100R100-1	4.99	4.95	4.96	4.97	0.02	0.4	0.098	99.7	50.3	49.4	49.1	100.6	0.29
NA-P100R100-2	4.95	4.91	4.90	4.92	0.03	0.6	0.098	98.7	50.2	48.5	49.1	98.9	0.56
NA-P75R125-1	4.98	4.95	4.96	4.96	0.02	0.4	0.076	99.8	38.7	61.0	61.4	99.5	0.33
NA-P75R125-2	4.85	4.85	4.86	4.85	0.01	0.1	0.071	97.5	36.4	61.1	61.4	99.5	0.28
NA-P50R150-1	5.03	5.00	5.02	5.01	0.01	0.3	0.049	100.7	25.2	75.5	73.7	102.6	1.89
NA-P50R150-2	4.87	4.85	4.85	4.86	0.01	0.2	0.047	97.5	24.0	73.5	73.7	99.9	0.10

Consequently, two results were obtained for each solution, µg. For each pair of results, the absolute difference was calculated. The obtained differences were compared with the value of the target extended uncertainty 3.13 µg. It turns out that from about 500 such differences, about 16% exceed the expanded target uncertainty. As the developed method, and, thus, the calculation of concentrations, has been validated, it can be concluded that the second derivative method is not reliable in quantification of MF in unit doses of the drug products such as Nasonex. This can be explained by *inter alia* three circumstances:

1. MF has a gentle maximum of UV spectrum in the region of 220–310 nm, and, therefore, the small second derivative.
2. Turbidity of the solution, according to the proposed sample preparation method, is too large.
3. Benzalkonium chloride, which is included as an excipient in the composition of the drug products under consideration, has a very intense spectrum of the second derivative, although with a low intensity of the initial spectrum.

3.4. Position of the proposed method in the toolkit of analytical methods

The proposed approach is applicable for quantitative determination of analyte(s) in tested solutions with turbidity. Let us consider alternative approaches:

- Methods that are based on physical separation of analyte from interfering substances of sample matrix (gone into the mentioned tested solution) [9, 10, 11, 12, 13] have the following disadvantages when compared with the proposed approach:
  - Require a preliminary separation of particles, the presence of which is causing the turbidity (e.g. by filtration or centrifugation);

  - Require by an order more time for running the final analytical operation - chromatography (HPLC) as compared with the final analytical operation - spectrophotometry. This is pertinent in the case of a large number of samples.
- Traditional methods of UV-spectrophotometry [18, 19].
  - Require a preliminary separation of particles, the presence of which is causing the turbidity (e.g. by filtration or centrifugation);
- Methods of UV-spectrophotometry based on calculation of the turbidity spectrum on the basis of data about the quantity and size distribution of particles, which are causing the turbidity [21, 22].
  - Require specific complex equipment and software for determination of quantity and particle size distribution, which are causing the turbidity;
- Methods of spectrophotometry based on the use of derivative of the analyzed solution UV-VIS spectrum [20].
  - It is complicated to apply if the analyte itself does not have a significant derivative spectrum, or one or more matrix components have spectra with high derivative results.

In view of the aforesaid, it can be concluded that the proposed approach may have some advantages if compared with other methods of analytical chemistry for cases where it is necessary to run a quantitative determination of analyte(s) in a large number of tested solutions, which contain particles (turbidity) and there are complications with use of UV spectra derivative.

4. Conclusions

A simple and a very fast spectrophotometric method for quantitative determination of mometasone furoate in separate doses of nasal sprays of an aqueous suspension of mometasone furoate has been developed and

Table 12

The results of determination of MF in validation solutions compiled with placebo using Forinex, Flix, Mometasone-Teva, Glenspray, Allertec Nazo.

#	$\left|m_{MF}^{TRUE} - m_{MF}^{FOUND}\right|$, µg				
	FO	FL	MT	GL	AL
B188R50-1	2.06	1.56	0.89	0.59	0.52
B188R50-2	3.13	1.55	0.77	0.51	0.37
B188R50-3	2.37	1.74	0.60	0.58	0.39
B188R50-4	2.37	1.64	0.77	0.64	0.26
B188R50-5	1.90	1.77	0.98	0.78	0.18
B156R75-1	0.75	0.66	1.09	0.42	0.62
B156R75-2	<u>1.98</u>	0.57	1.02	0.45	0.75
B156R75-3	0.86	0.80	0.87	0.45	0.53
B156R75-4	1.09	0.63	1.04	0.41	0.41
B156R75-5	1.06	0.64	1.04	0.56	0.34
B125R100-1	0.56	0.32	0.26	0.18	0.13
B125R100-2	0.87	0.76	0.42	0.00	0.27
B125R100-3	0.28	0.15	0.23	0.33	0.33
B125R100-4	0.53	0.32	0.33	0.43	0.20
B125R100-5	0.93	0.70	0.21	0.17	0.16
B96R125-1	0.95	0.84	1.50	0.07	0.62
B96R125-2	0.43	0.73	1.17	0.46	0.83
B96R125-3	1.10	0.75	1.24	0.29	0.33
B96R125-4	1.14	0.69	1.15	Gross error	0.44
B96R125-5	1.17	0.81	1.06	1.11	0.51
B63R150	1.14	1.34	1.11	0.56	0.60
B63R150-2	0.89	0.93	1.22	0.02	0.23
B63R150-3	1.10	0.70	1.15	0.05	0.27
B63R150-4	0.79	0.96	1.06	0.08	0.01
B63R150-5	1.04	1.03	0.91	0.02	0.14

validated.

The developed method illustrates in detail one possible approach to development of spectrophotometric quantitative determination methods for drugs and possibly other objects allowing measurements of sufficiently turbid test solutions.

The proposed method is based on the fact that turbidity is perceived as one of the components of analytical system, the spectrum of which can be considered constant during a single measurement. During the entire series of measurements, it remains self-similar, i.e. its change does not affect results of analyte determination.

The proposed method can successfully replace methods of derivative spectrophotometry in cases where:

- derivative of analyte spectrum is not intense enough;
- derivative of spectrum of the matrix (turbidity + the remaining components of the product) is significant if compared with the derivative of an analyte.

Table 13

The results of determination of MF in validation solutions compiled with use of aqueous suspensions of drugs Forinex, Flix, Mometasone-Teva, Glenspray, Allertec Nazo.

#	$\left|m_{MF}^{TRUE} - m_{MF}^{FOUND}\right|$, µg				
	FO	FL	MT	GL	AL
P150R50-1	1.57	0.34	0.41	0.26	1.01
P150R50-2	0.95	0.04	0.98	0.71	1.27
P125R75-1	0.03	0.37	0.57	0.13	0.51
P125R75-2	0.68	0.82	0.42	0.53	0.72
P100R100-1	0.58	0.15	0.53	0.13	0.02
P100R100-2	0.06	0.26	0.00	0.16	0.80
P75R125-1	0.01	0.36	0.55	0.39	0.12
P75R125-2	0.09	0.38	0.51	0.33	0.12
P50R150-1	0.69	0.09	0.01	0.25	0.35
P50R150-2	0.68	0.39	0.23	0.34	0.38

Table 14

The ratio of concentration obtained with intentionally changed instrument parameters to concentration obtained with instrument parameters accepted for the developed method (optical slit 1 nm, scanning speed 50 nm/sec).

#	SLIT 2 nm	SPEED 100 nm/s	SPEED 20 nm/s
MT-50-1	0.010	0.015	0.010
MT-50-2	0.021	0.025	0.021
MT-50-3	0.000	0.008	0.000
MT-50-4	0.018	0.009	0.018
MT-50-5	0.031	0.005	0.031
MT-75-1	0.020	0.015	0.020
MT-75-2	0.011	0.003	0.011
MT-75-3	0.015	0.005	0.015
MT-75-4	0.002	0.003	0.002
MT-75-5	0.009	0.004	0.009
MT-100-1	0.016	0.007	0.016
MT-100-2	0.000	0.021	0.000
MT-100-3	0.031	0.006	0.031
MT-100-4	0.014	0.009	0.014
MT-100-5	0.008	0.007	0.008
MT-125-1	0.044	0.017	0.044
MT-125-2	0.025	0.004	0.025
MT-125-3	0.024	0.025	0.024
MT-125-4	0.022	0.017	0.022
MT-125-5	0.013	0.004	0.013
MT-150-1	0.030	0.036	0.030
MT-150-2	0.029	0.017	0.029
MT-150-3	0.006	0.010	0.006
MT-150-4	0.037	0.012	0.037
MT-150-5	0.043	0.027	0.043

Declarations

o Liudmyla Horshkova, Iryna Niemchinova, Yuriy Barabash, Viktor-iia Netrunenko, Anton Shostak, Liudmyla Chernova, Maria Ten-ditna and German Gurjanov for their support in research activities.
● Company ProPharma (Ukraine) for the provided samples of phar-maceutical preparations.
● Taras Kulbaba for his assistance with editing English text of the paper and his numerous valuable comments.

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