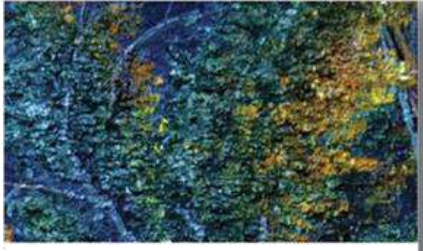

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CONTENT

SCF technologies for chromatography and micronization of drugs

N.G.Bazarnova, M.Yu.Cheprasova, V.N.Tsarev and I.V.Mikushina

Institute of Chemistry and Chemical Pharmaceutical Technologies, Russian Federation
Email: bazarnova@chem.asu.ru

We demonstrate applicability of supercritical fluid (SCF) chromatography for the chiral separation of the substance of salbutamol sulfate (SS) racemic mixture and for production of the solutions with concentration of the active R-isomer greater than 98%. We have developed a SCF chromatography method for simultaneous qualitative determination of vitamins A and E in a mixture, which can be recommended for industrial implementation. We have also developed a method for SS micronization based on Supercritical AntiSolvent precipitation method (SAS). We were able to produce nearly spherical particles. Finally, we propose an optimization of conditions for production of micronized particles, which can be used for development of laboratory procedures for production of micronized substance.

Keywords: SCF technologies, salbutamol sulfate, vitamins A and E, AntiSolvent precipitation method, spherical particles, micronized particles

INTRODUCTION

Supercritical fluid technologies are chemical technologies that use substances, mostly gases, in a supercritical state. In this state the substances have special properties: they combine the solvent capacity of liquids and the high penetrating capacity of gases.

The combination of these properties makes it possible to use supercritical fluids as effective solvents in many technological processes, replacing hazardous organic solvents with safe substances in a supercritical state (water, carbon dioxide, etc.). The most promising applications of supercritical fluid technologies include chromatography, micronization and many others.

The paper presents the results of the chiral separation of the SS racemic mixture and the quantitative determination of vitamins A and E concentrations using supercritical fluid chromatography and dispersion of the drug substance that can be further used for production of an aerosol through supercritical fluid micronization.

MATERIALS AND METHODS

The chiral separation of the racemic mixture of SS and the quantitative determination of vitamins A and E were carried out using the Investigator Supercritical Fluid Chromatography system (Figure 1).

The system includes a pump module FDM-15 that features a CO2 pump and co-solvent pump, a 10-position column thermostat with a horizontal arrangement of the columns Analytical-2-Prep Column Oven, an autosampler Alias, a diode-matrix detector 2998 PDA, an automatic pressure regulator ABPR-20, a post-regulator electric heater and fraction collector featuring a blasting pump AFC Collection Module. The system allows to work with columns with internal diameter of up to 10 mm, and grain of sorbent of 3 m and above.

We used food-grade CO2 of 99.5% purity, compliant with GOST 8050-85, as the main eluant; methanol of "A.C.S. for gradient HPLC" purity (Chimmed, compliant with TU 2636-081-29483781-2015) as the co-solvent; and isopropylamine of "for synthesis" purity (Merck), as the dynamic modifier.

Our system was equipped with Chiralpak IG 5 m, 1504.6 mm chromatographic column and Chiralpak IG 5 m, 104 mm precolumn.

Samples were placed in 2 ml chromatographic vials. The resulting fractions were collected into 100 ml glass jars with plastic lids.

The software allowed us to create a sequence of injections of the SS solution, and to set the time of the sample

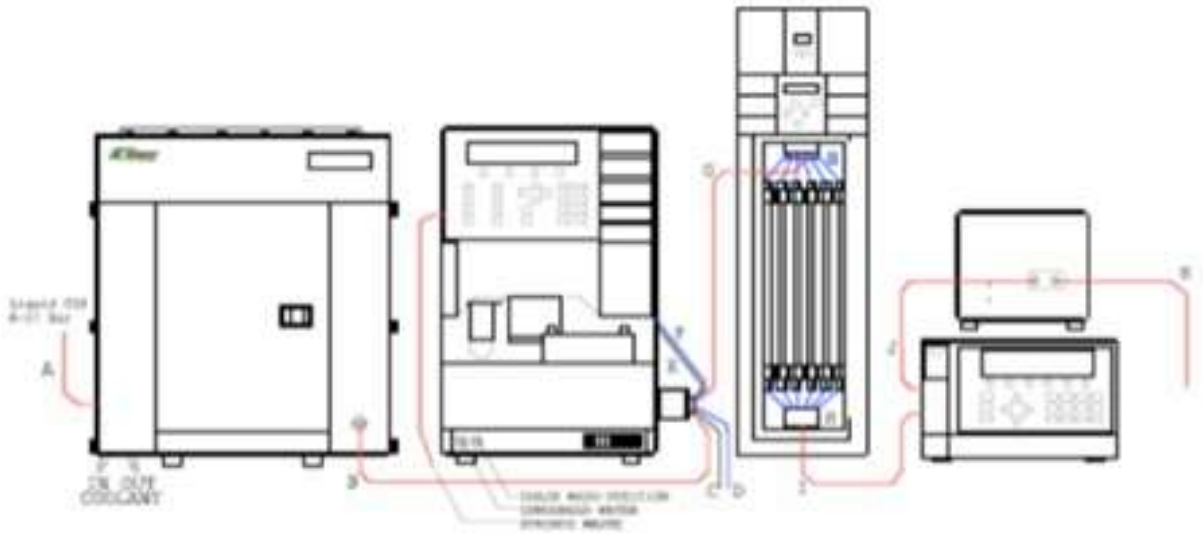

FIGURE 1. . Overview of the Investigator Supercritical Fluid Chromatography system

injection, the collection conditions, the time lag between sample insertions, and the collector number. Then we ran the sequence of injections. Upon the sequence completion, the fractions were retrieved from the collectors.

The chromatograph was then switched to analytical mode, and enantiomeric purity of the fraction samples was determined.

For development of the method of analysis of vitamins we used the drug Aevit (Altavitaminy) containing 0.1 ml (100000 ME) of 55% retinol palmitate oil and 0.1 g of -tocopherol acetate.

The qualitative determination of vitamins was carried out using the using the Investigator Supercritical Fluid Chromatography system (Waters Corporation). The parameters of the method were as follows: pressure in the system 120 bar, CO2 (food-grade) as the mobile phase, isopropyl alcohol (high grade purity) as the co-solvent, flow rate 3 ml/min, thermostat temperature 35 oC, co-solvent concentration 10%, XBridge C18 5 m 4.6x150 mm as the column, UV detector, detection lengths 292 nm and 325 nm.

The hardware schematic diagram for micronization (SAS) by the Waters Corporation company is shown in Figure 2.

RESULTS AND DISCUSSION

0.1. The chiral separation of salbutamol sulfate using supercritical fluid chromatography

Supercritical fluid chromatography (SFC) is a type of chromatography related to high-performance liquid chromatography (HPLC) that uses subcritical or supercritical fluid (SCF) as the main component of the mobile phase (MPh), most often supercritical carbon dioxide (SC-CO2). Supercritical fluids have lower viscosity and high diffusion rates than liquids. Also, supercritical fluids possess some special properties, such as controllable solvent capacity, special heat transfer mechanisms, etc. This combination makes SCF an attractive environment for chromatographic separation. Due to the low viscosity, SCF elution in the SFC can be done at the flow rates that are 2-4 times higher than in the HPLC. Due to the high diffusion rates, the mass exchange processes in the sorbent pores are able to progress at the appropriate speed, and chromatographic efficiency is maintained at a high level. The advantage in speed and productivity is particularly relevant in chromatography of the structurally close, hard-to-separate compounds, including enantiomeric substances and their racemic mixtures, such as salbutamol (2-tert-butylamino-1-(4-oxy-3-oxymetilphenyl)-ethanol).

Salbutamol in the form of salt is a component of modern drugs for bronchial asthma and chronic bronchitis. This drug product for chronic obstructive lung diseases exhibits broncholytic and tocolytic action and is used as a long-acting prophylactic drug and as a relief of bronchial asthma attacks. Drug products based on 2-tert-butylamine-1-(4-oxy-3-oxymethylphenyl)-ethanol are in stable demand on the pharmaceutical market, as in most cases they have long-term, often life-time application. The most effective and most popular form of the drug products on the market is aerosol.

2-tert-butylamine-1-(4-oxy-3-oxymethylphenyl)-ethanol has chirality characteristics. Enantiomers (the structural formulas are shown in Figure 3) are biologically active. The R-isomer provides the necessary therapeutic effect, the

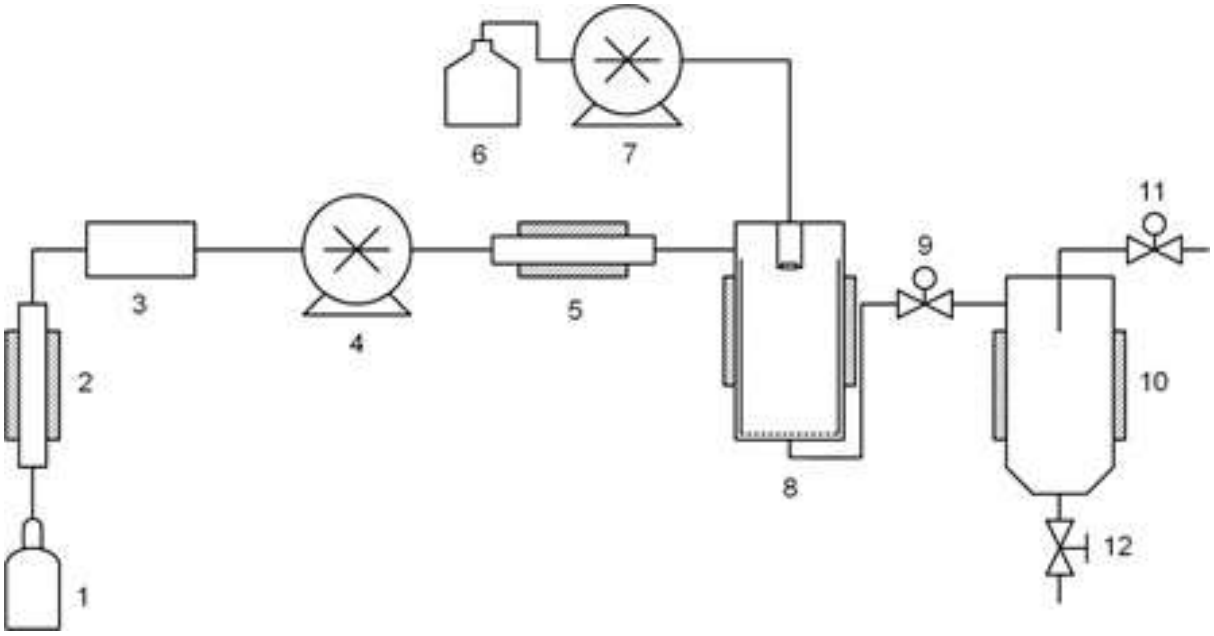

FIGURE 2. . Schematic diagram of the laboratory facility for supercritical anti-solvent precipitation: 1 – CO2 supply, 2 – cooling heat-exchanger, 3 – mass flow meter based on Coriolis effect, 4 – CO2 pump, 5 – electric heating heat-exchanger, 6 – initial solution supply, 7 – solution pump, 8 – precipitation chamber, 9 – automatic back pressure regulator, 10 – cyclone separator, 11 – manual back pressure regulator, 12 – drain valve

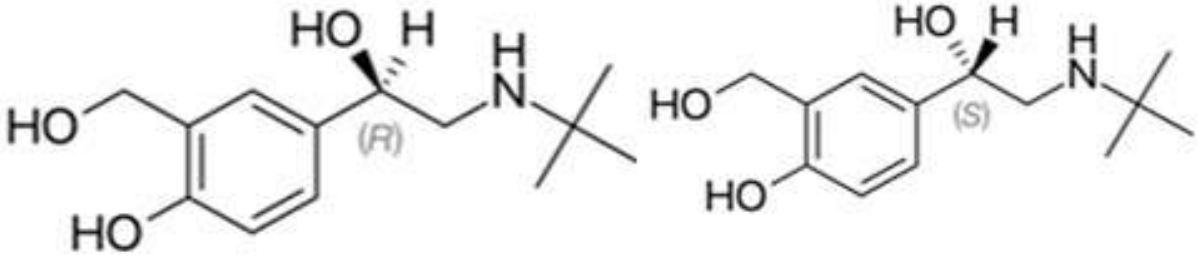

FIGURE 3. Structural formulas for enantiomers of 2-tert-butylamino-1-(4-oxy-3-oxymethylphenyl)-ethanol

S-isomer has no broncholytic activity [1], and also has a negative effect on the human body [2-4], such as arrhythmia, tachycardia, inflammatory processes. The metabolism of the S-isomer is 10 times slower than that of the R-isomer, it accumulates in the lungs and bronchus, which can lead to bronchial disfunction [3,4]. The use of enantiomerically-pure R-isomer of 2-tert-butylamino-1-(4-oxy-3-oxymethylphenyl)-ethanol or its salt is preferable in clinical practice to the use of a racemic mixture.

Obtaining enantiomerically-pure drugs is an important sphere of modern pharmaceutics. However, separating enantiomers from the same compound is a technologically complex task. It is usually solved by development of an enantioselective synthesis, through a biotechnological process, or through liquid chiral chromatography, which is a labor-intensive and costly process with low productivity. There is a series of drugs based on enantiomerically pure 2-tert-butylamino-1-(4-oxy-3-oxymethylphenyl)-ethanol produced by an Indian pharmaceutical company Cipla Ltd under the brand Levolin in the form of sulfate, by an American pharmaceutical company Sunovion Pharmaceutical Inc. under the brand Xopenex NFA in the form of tartrate. The 2-tert-butylamino-1-(4-oxy-3-oxymethylphenyl)-ethanol substance used in their production is obtained through multi-stage enantioselective synthesis [5] followed by subsequent recrystallization in order to get the required polymorphic modification [6], or recrystallization of racemates from solutions with optically active acids, such as tartaric [7].

We have developed a technique for the chiral separation of the substance of the racemic mixture of SS on the semi-preparative supercritical fluid chromatograph Investigator SFC System produced by Waters Corp.

As a result of a series of experiments, we have obtained the solution of an R-isomer of SS from a racemic mixture using supercritical fluids (figure 4).

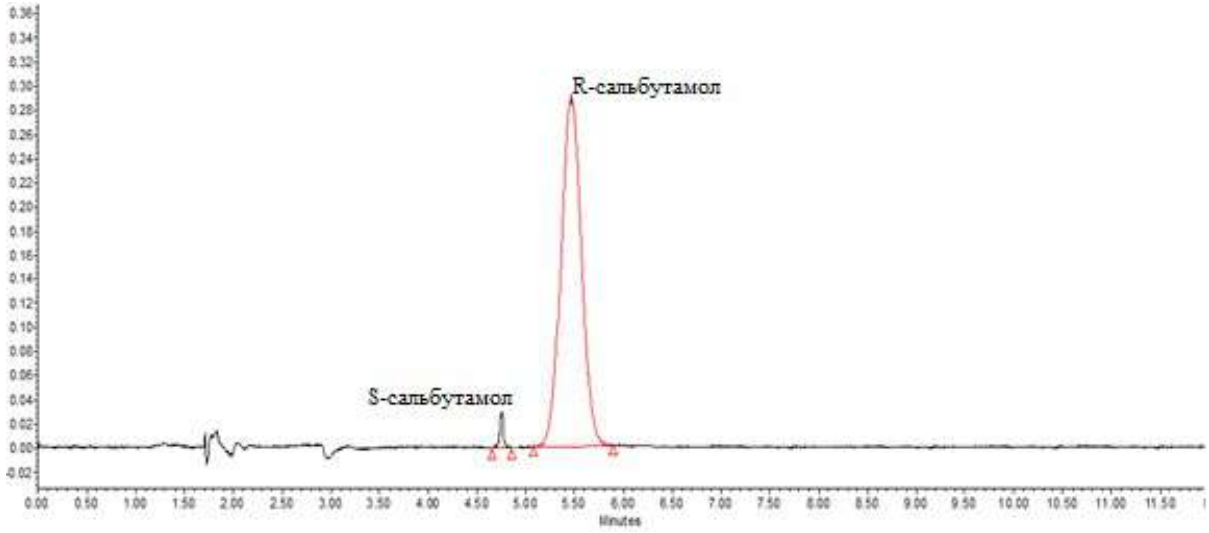

FIGURE 4. Chromatogram of a sample of the collected fraction of the SS R-isomer solution

TABLE 1. Results of experiment 4 in testing the laboratory method for obtaining the solution of the R-isomer of the SS from a racemic mixture using supercritical fluids (Chiralpack IG 5 m 1504.6 mm column, isopropylamine DM)

Sample number	R-isomer content,%	Average	Standard deviation
1	98.74	98.88	0.23
2	98.81	98.88	0.23
3	99.02	98.88	0.23
4	98.93	98.88	0.23
5	98.88	98.88	0.23

We conducted a series of experiments to test the laboratory method of separation using semi-preparative SCF-chromatograph. The sample size of each experiment for a single measurement was 5 injections. The results of one of the experiments are presented in Table 1.

As a result of laboratory method testing, solutions of the R-isomer of the SS are obtained from a racemic mixture using supercritical fluid chromatography containing more than 98% of R-isomer.

The SS content in the solution was confirmed by UV spectroscopy using a method developed earlier [8].

The specific rotation of the solution containing more than 98% of R-isomer of 2-tert-butylamino-1-(4-oxy-3-oxymethylphenyl)-ethanol was -36,9.

0.2. Express method of vitamin A and E determination

Retinol (Vitamin A) is a liposoluble vitamin-like substance of plant origin from the group of retinols. Due to the presence of a large amount of unsaturated bonds, it participates in activation of oxidation-reduction processes (redox), stimulates synthesis of pyrimidine and purine bases. It also participates in the energy supply for metabolism by creating favorable conditions for the synthesis of ATP [9, 10].

Vitamin E (-tocopherol) is a liposoluble vitamin of plant origin from the group of tocol. It is a universal protector of cellular membranes, protecting them from oxidative damage. The antioxidant properties result from the ability of the highly reactive hydroxyl of chromane nuclei to react directly with free radicals of oxygen (O2, NO, NO2) and unsaturated fatty acids (RO, RO2) [10, 11].

At present, the physiological need for vitamin A for adults ranges from 700 to 900 g/day [12], the need for vitamin E for adults is 20 to 30 mg/day [13].

Drugs of plant origin containing liposoluble vitamins A and E are widely used in medical practice. Both the drug products and biologically active food supplements can be found on the market. The 14th edition of the State Pharmacopoeia of the Russian Federation includes articles on -tocopherol acetate [14], retinol, retinol acetate, retinol palmitate [15].

In most cases, the end product contains a mixture of different vitamins rather than individual vitamins. This applies both to the drug products and the biologically active food supplements.

The express method of simultaneous qualitative determination of individual vitamins in a mixture is an important

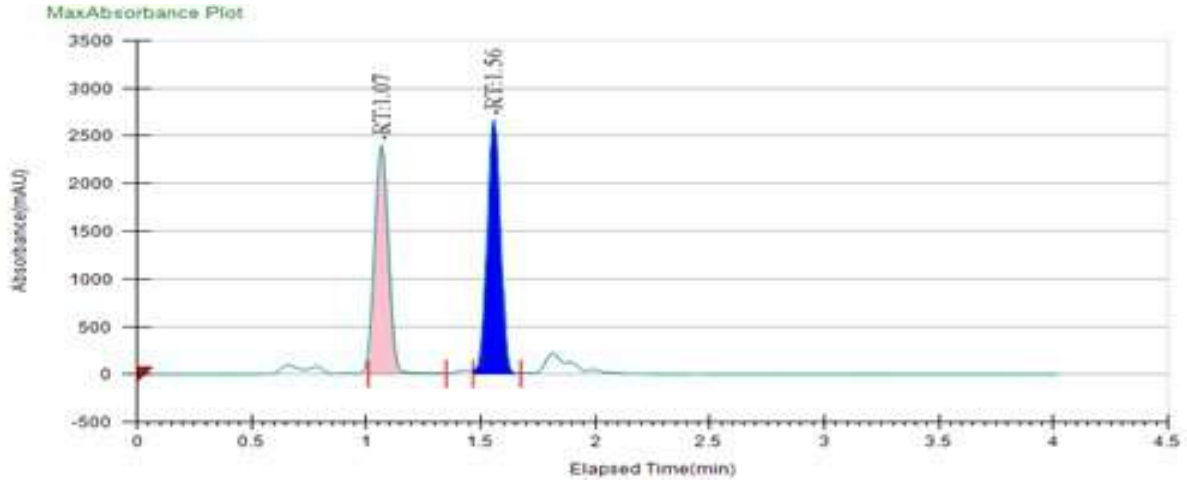

FIGURE 5. Selectivity of separation of the vitamin A and E mixture (RT:1.07 - vitamin A, RT:1.07 - vitamin E)

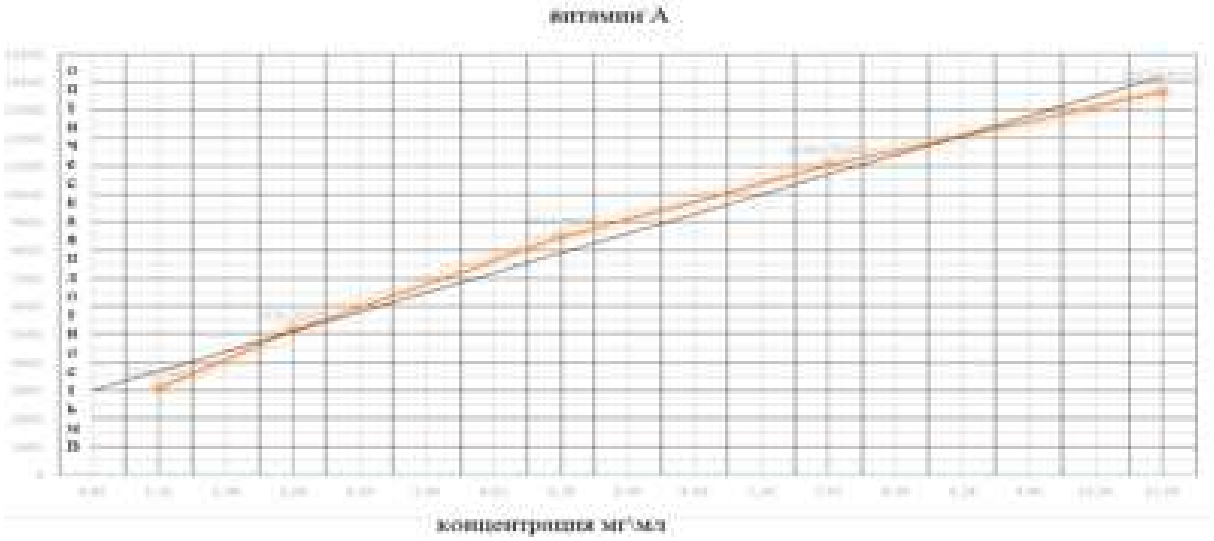

FIGURE 6. . Dependence of the optical density on the vitamin A concentration

condition for determining the quality of pharmacologically active substances as well as different types of products of plant origin. Chromatogram of separation of the vitamin A and E mixture is presented in Figure 5

Measurement range for vitamin A is 1,600 to 40,000 IU (480 – 12,000 mg). Measurement range for vitamin E is 0.16 mg to 20 mg.

Linear relation between the optical density and the concentration can be observed in the whole range of the studied concentrations (Figures 6,7).

At present, UV-detecting HPLC method is used for the qualitative and quantitative determination of vitamins A and E. The movable phase uses isopropyl alcohol, sorbent C18, d-5 m.

The main disadvantage of the method is its duration of 10 to 15 minutes for each measurement and the high cost of the applied eluents.

The method of simultaneous determination of vitamins A and E through supercritical fluid chromatography numerous advantages over the method of determination of vitamins by means of high-performance liquid chromatography. The main advantages of the methodology include: one-time determination takes 3-4 minutes; use of the reagents of the class HP as eluents; extremely simple preparation (dissolution of the test sample). This technique can be modified for isolation of pure vitamins from vegetable extracts.

Thus, the developed method of simultaneous qualitative determination of vitamins A and E in the mixture can be recommended for wide-scale introduction. The SFC method is comparable to the HPLC method for sensitivity and reproducibility.

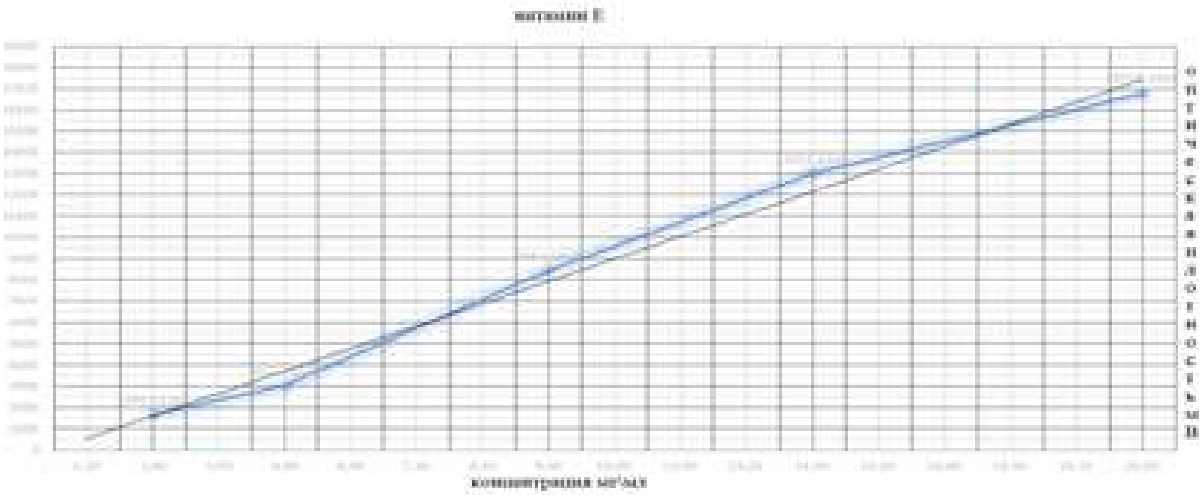

FIGURE 7. .Dependence of the optical density on the vitamin E concentration

0.3. Micronization of the drug substance by supercritical antisolvent deposition

Salbutamol is one of the active pharmaceutical ingredients (API) for which the inhalation dosage form is the most convenient and effective way of administering the drug into the human body [16]. Its key advantages over the oral treatment are the targeted delivery of the API (for example, to the upper respiratory tract), the high rate of absorption of the API into the bloodstream, and the reduced likelihood of undesirable side effects [17]. Control of the size and shape of the API solid particles is critical in the manufacture of inhalation drugs. The size determines the delivery point of the AFI during inhalation. In order to deposit the drug into bronchi, particles of 1 – 5 m, preferably 2-3 m are required; to be deposited in lungs, particles of submicron size. The shape of the particles affects their aerodynamic characteristics and the rate of absorption.

Sphere-shaped particles with the least mechanical risk of soft tissue damage due to lack of sharp edges are generally considered preferable [18–21].

The supercritical antisolvent precipitation (SAS) method belongs to the group of solvent methods using supercritical fluid technologies and is used to produce microparticles of substances insoluble in SC-CO2, the latter being a precipitator, an antisolvent, and causing the crystallization of the target substances from the solution upon contact.

The SAS method allows to control the size and morphology of the microparticles produced.

A number of studies have shown that for crystalline substances the relationship of size and morphology to the values of SAS parameters is often nonmonotonic, and, when developing specific applications, the joint influence of the parameters [22-26] should be taken into account.

Currently, particles of spherical morphology have been obtained in the process of micronization of SS using the SAS method [27, 28]. Experimental studies have been conducted on the SAS-precipitation of SS from various solvents in the SC-CO2 environment, and spherical particles have been obtained.

The pressure, solution flow rate and SS concentration in the solution have a significant influence on the morphology and size of the SS particles produced by the SAS method. By modifying various parameters of the SAS process, it is possible to obtain SS particles ranging 0.7 m to 8.5 m of needle-shaped, spherical or closely related morphology, including particles that meet the size and morphology requirements for inhalation forms of preparations. The dependence of the particle average size on the SS concentration in the solution is nonmonotonic. Of the three solvents – methanol, hexafluoroisopropanol and dimethyl sulfoxide (DMSO) – the latter is the best for synthesis of micron-sized particles. Conditions for the production of micronized SS particles of a size of 1¡d¡5 m and with morphology close to spherical were developed and optimized. Laboratory procedures for the production of micronized SS substance have been developed.

Figure 8 presents the diagram of the SS microparticle production process.

The process consists of 14 operations, including: preparation of the salt crystals for precipitation; dissolution of the crystals of the R-isomer of SS; micronization of the salt of the R-isomer of SS; the system preparation and setup for the operating mode; stabilization of temperature and pressure in the system; the salt solution of the R-isomer of salbutamol sulfate is supplied to the working vessel periodically; collection of the micronized R-isomer of salbutamol salt; shutdown of the solution supply pump; supplying an extra amount of CO2 through the system under pressure

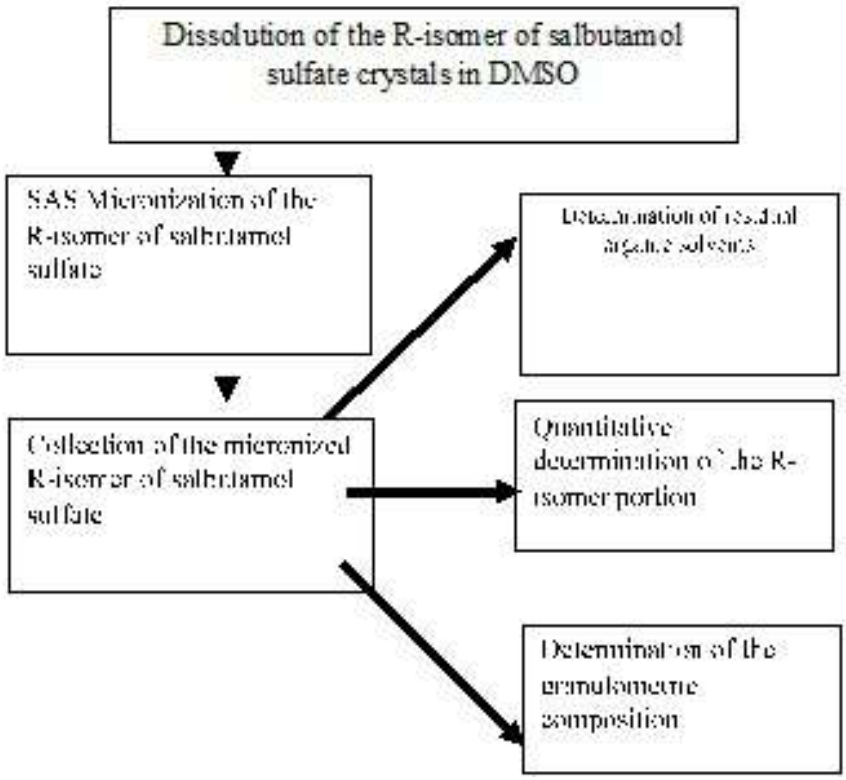

FIGURE 8. .Schematic diagram of SS microparticle production

to flush the residues of the working solution and the solvent from the system; operating pressure reduction in the system; shutting off the CO2 supply; opening of the drain valve of the working vessel; equalization of pressure in the working vessel with the atmospheric pressure; collection of micronized powder of the R-isomer of SS.

The following control measurements are carried out: determination of the yield of the micronized R-isomer of SS; granulometric control of the obtained micronized R-isomer of SS; quantification of the R-isomer of SS in powder; determination of residual organic solvents (methanol, TEA, DMSO, ethyl acetate).

CONCLUSIONS

Thus, a method of synthesis of microparticles of salbutamol sulphate with morphology close to spherical is developed and tested in the course of the study. Data have been obtained for the development of laboratory procedures for the production of micronized substance of salbutamol sulfate

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