Levofloxacin Dry Powder for Inhalation by Spray Drying Method
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Purpose
Cystic fibrosis is a genetic disease, generally affects the lungs, causes dehydrated and viscous mucus layer and decreased pH in the lung. This environment leads to repetitive infections mainly by Pseudomonas aeruginosa (PA), affecting the quality of life of these patients. For treatment of infections antibiotic by inhalation are administered to the lungs as solutions for nebulization and dry powder inhaler (DPI). Mucolytic agents are currently employed as well to manage the disease. Levofloxacin hemihydrate (LV), a fluoroquinolone agent effective against PA was selected as antibiotic, N-acetylcysteine (NAC) and dornase alfa (DNase) as mucolytics. DPI formulations containing LV only (100%), LV-N-acetylcysteine (LV-NAC) (90.5% : 9.5%), and LV-dornase alfa (LV-DNase) (76.5% : 2.5%) (Pulmozyme : 23.5%) were manufactured by spray drying (Buchi B-290) of drug solutions in isopropyl alcohol. X-Ray analysis of DPIs implied that produced powders have amorphous structure. DSC and FTIR analysis showed that production process does not affect physical and chemical structure of LV. Particle size distribution analysis (Malvern Master Sizer 2000) demonstrated that all DPIs have mean particle size smaller than 2 μm. SEM images were correlated with X-Ray and particle size analysis (1). For this study, our aim was to evaluate drug content, water content and aerodynamic parameters of produced DPIs. Mass Median Aerodynamic Diameter (MMAD), Emitted Dose (ED), Fine Particle Dose (FPD) and Fine Particle Fraction (FPF) were determined using Andersen Cascade Impactor (ACI) results.

Methods
For determination of drug content, HPLC method was used for LV and NAC. Bicinchoninic Acid Assay (BCA) method was used for DNase. Produced powders were dissolved in mobile phase (0.025 M phosphoric acid, adjusted with trimethylamine to pH 3.0 and acetonitrile (87:13)) in volumetric flasks and analyzed using an HPLC. In case of DNase containing formulation, BCA reagent mixture prepared regard to instructions, produced powders dissolved in the mixture, and samples measured using ELISA Microplate reader at 562 nm. To detect water content of powders, TGA/DSC1 (METTLER Toledo) was used under 80 mL/min &#36425;ux of nitrogen between 25°C to 250°C at 10°C/min rate. The weight loss was measured between 60°C and 120°C. For ACI analyses, size 0 HPMC capsules were filled with DPIs that have same amount of levofloxacin (28 mg). RS01 device (Plastiape SpA, IT) was used for ACI analyses. Flow rate and time were adjusted to obtain 4 L of air passing through the device. Prior to experiment, ACI plates were coated by 1% (w/v) concentration of Tween 20 solution in ethanol. All parts of ACI and device were washed separately with the mobile phase, and then samples were analyzed by an HPLC method for quantification of LV.

Results
Drug content results showed that, DPIs contain 95.5±1.4%, 83.4±1.0% and 69.9±0.4% of LV for only LV, LV-NAC and LV-DNase, respectively. Also, LV-NAC contains 5.7% of NAC and LV-DNase contains 3% of DNase. TGA results implied that powders include 2.33±0.02%, 2.25±0.01%, 1.32±0.12% and 3.47±0.07% of water in untreated LV, only LV, LV-NAC and LV-DNase DPIs. ACI results demonstrated that the highest ED (>65%) and the lowest FPD and FPF (<70%) values were obtained from the LV-DNase DPI combination. The lowest ED (<65%) and the highest FPD and FPF (>70%) values were obtained from the LV-NAC DPI formulation. All MMADs results were below 3.2 μm.

Conclusion
Drug contents of LV formulations were in agreement with the expected values taking into account water content and impurities of drugs. TGA results showed that produced powders and untreated powder have almost same water content. Amorphous structure of produced powders may cause this result. For ACI results, in case of LV-DNase DPI formulation, although high fraction of formulation can leave the device, only smaller fraction had aerodynamic respirable size. Contrary, LV-NAC DPI formulation showed the best performance. All the formulations have appropriate MMADs to reach the deeper parts of the lung. This project was supported by a grant from The Scientific and Technological Research Council of Turkey (TUBITAK) (SBAG-213S043).

Reference