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Research Article

# HPLC method for simultaneous quantification of lumacaftor and ivacaftor bulk and pharmaceutical formulations

Saniye Özcan[1,2], Abeer Elriş[1], Serkan Levent[2,3]

[1]Anadolu University, Faculty of Pharmacy, Department of Analytical Chemistry, Eskişehir, Türkiye.

[2]Anadolu University, Faculty of Pharmacy, Central Analysis Laboratory, Eskişehir, Türkiye.

[3]Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Eskişehir, Türkiye.

✉ Saniye Özcan
saniyeozcan@anadolu.edu.tr

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**ABSTRACT**

In 2015, the Food and Drug Administration granted approval for the use of lumacaftor 200 mg and ivacaftor 125 mg in the treatment of cystic fibrosis patients who possess the F508del mutation, namely those who are 12 years of age or older. Since its approval, the medicine has been implemented in clinical settings, although the presence of numerous disputes, with the aim of mitigating disease symptoms and enhancing the overall quality of life. Given the existing gaps in the literature regarding the analysis of the amalgamation of these two active substances, a straightforward and practical HPLC approach has been devised in adherence to the guidelines outlined in the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q2(R1) document. To accomplish this objective, the process of separation was successfully carried out using a monolithic silica stationary phase (Chromolith High Resolution RP-18e, 100 mm × 4.6 mm i.d., Merck KGaA, Darmstadt, Germany). The separation process was conducted using a gradient mode. The initial composition of the mobile phase consisted of acetonitrile and a phosphate buffer solution with a concentration of 0.030 M and a pH of 3.5. The flow rate was recorded as 1.0 mL/min, and avanafil was used as an internal standard. The improved and verified approach has demonstrated successful application in bulk and pharmaceutical formulation evaluations when utilizing the ivacaftor/lumacaftor combination.

**Keywords:** Bulk form, HPLC, Ivacaftor, Lumacaftor, Pharmaceutical form

## 1. INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive genetic disease and commonual in Caucasians [1]. Although the incidence of the disease is reported to be 1 in 1/2000–3500 live births and the carrier rate is 1/25, it is known that the incidence of the disease varies between populations [2]. Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations affect many organs and tissues, namely lungs, intestines, pancreas, sweat glands, causing organ secretions to become thick and. Even some clinical symptoms may be different, typical signs of cystic fibrosis comprise an increase in sweat salt level, decreased weight gain and growth, persistent cough, and recurrent infections of the lung [3]. The death is the most extreme outcome of the illness, which generally occurs due to respiratory failure; unfortunately, the average survival time is about 46 years for men and 41 years for women [4].

Until recently, symptomatic treatments (e.g., mucolytics, antibiotics, pancreatic enzymes, etc.) focusing on the consequences of the disease were applied. Although 30 years have passed since the discovery of the CFTR gene, there has been no significant development regarding gene therapy yet. However, in recent years, CFTR modulators that improve the activity of the CFTR gene have been developed, and very good clinical results have been obtained [5].

These molecules can partially eliminate the intracellular damage and/or malfunction of the CFTR protein and provide extraordinary improvements in the patient's quality of life. Clinical studies show that the molecules are safe and show mild or moderate side effects. However, these drugs are quite expensive (≈CHF 170,000/year/patient), and they work in a very limited range of patient profiles [6]. Additionally, the patient's age and clinical condition are also important. The mechanism of action of these molecules is not fully known, and they are produced by a single pharmaceutical company. These substances are ivacaftor (IVA), lumacaftor (LUMA), tezacaftor, and elexacaftor.

Thanks to their success in the therapy, agents capable of addressing the underlying CFTR deficiency have become a growing focus [7, 8] CF is a disease that can affect many systems and present with different clinical signs and symptoms in each patient. Clinical findings in CF vary depending on the age of the patient, the genetic mutation he carries, the severity of the disease, and the affected systems [9].

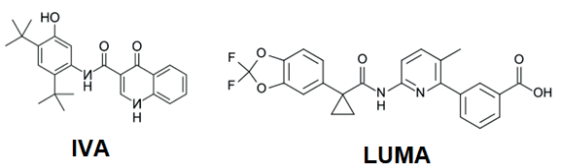


**Figure 1.** Molecular structures of IVA and LUMA

IVA given in Figure 1, is a medication used to treat mainly the G551D mutation which is responsible for 4–5% of CF cases [10]. Additionally, it is incorporated in the combination medications LUMA/IVA, tezacaftor/IVA, and elexacaftor/tezacaftor/IVA, which are administered to individuals with CF for therapeutic purposes [11]. IVA, developed by Vertex Pharmaceuticals in collaboration with the Cystic Fibrosis Foundation, is the pioneering medicine that targets the root cause of the disease rather than only addressing its symptoms. LUMA, given in Figure 1, is a medication used in combination with the fixed-dose combination product Orkambi [DB08820] for the treatment of CF in patients 6 years of age and older. LUMA/IVA, marketed as Orkambi®, is a medication that combines LUMA and IVA. It is prescribed to individuals with cystic fibrosis who possess two copies of the F508del gene.

Both LUMA and IVA are novel drugs that received approval from the FDA in 2015 and 2012, respectively [12, 13]. Phase studies are continuing at various stages to examine their effectiveness against different CF mutations. For this reason, there are not enough studies on IVA/LUMA simultaneous analyses. Table 1 provides an overview of the broad range and various practical applications of the approaches that have been developed and documented in the existing literature for IVA and LUMA analysis. However, today, the right to produce finished products still belongs to Vertex Pharmaceutical. When other pharmaceutical companies are granted production permits in the future, it is obvious that analytically competent methods will be needed for active substance or pharmaceutical formulation production studies. As can be seen in the table we created based on our best knowledge, the method developed for simultaneous quality control analyses of the two is one [18]. So there is a lack of fast, easy, and high accuracy and precision methods for the analysis of LUMA/IVA combination within the scope of pharmaceutical formulation studies. For this purpose, this study aimed to develop an HPLC method that analysts can use easily and conveniently.

**Table 1.** Data on methods developed for IVA and LUMA analysis

| Compound | Linearity (µg/mL) | LOD/ULOQ (µg/mL) | LOQ/LLOQ (µg/mL) | wavelength | m/z | $t_R$ (min) | Method | Sample | Reference |
|---|---|---|---|---|---|---|---|---|---|
| IVA | 15 - 300 | 0.13 | 0.40 | 225 nm | - | | HPLC | Tablet | [14] |
| IVA | 0.01 - 10 | $2.50\times10^{-3}$ | $7.57\times10^{-3}$ | - | 392.49→393 | 6.2 | LC-MS/MS | Saliva and plasma | [15] |
| IVA | 1 -80 | - | - | 309 nm | - | 6.2 | HPLC | | |
| IVA | 62.5–312.5 | - | - | 254 | - | 3.1 | HPLC | Tablet | [16] |
| IVA/LUMA | 0.1-10 | $2.5\times10^{-3}$(IVA) | $7.57\times10^{-3}$ (IVA) | | z 392.49 → 393 | 1.55 (IVA) | LC-MS/MS | Biological fluids | [17] |
| | | $6.08\times10^{-4}$ (LUMA) | $1.84\times10^{-3}$ (LUMA) | | m/z 452.40 → 453 | 2.3 (LUMA) | | | |
| IVA/LUMA | 62.5-312.5 (IVA) | - | - | 254 | | 3.1 (IVA) | HPLC | Bulk and pharmaceutical | [18] |
| | 100-500 (LUMA) | | | | | 4.2 (LUMA) | | | |
| IVA/LUMA | 0.01-10 | 0.01 | 10 | - | 393.20 → 337.10 | 2.64 | LC-MS/MS | Sputum | [19] |
| | | | | | 453.05 →131.10 | 2.56 | | | |
| LUMA | 0.5-20 | 0.08 | 0.2 | 216 | | 5.3 | HPLC | Orkambi® | [20] |
| IVA | 0.55-20.22 | 0.20 | 0.08 | 247 | | 8.3 | | Kalydeco® | [21] |
| | 0.11-20.22 | $0.1\times10^{-6}$ | $1.0\times10^{-6}$ | - | 393.10→ 337.10; 319.15; 172.05 | 8.4 | LC-MS/MS | | |

## 2. MATERIALS AND METHODS

### 2.1. Chemical and Reagents

IVA, avanafil (AVA, as internal standard) and LUMA (99.6%) purity TRC Company, Canada) were purchased. Chromatographically pure $NaH_2PO_4$ (99.8%) and $Na_2HPO_4 \cdot 2H_2O$ (99.6%) were purchased from Sigma-Aldrich Chemie GmbH (Seelze, Germany). Acetonitrile (ACN) and methanol of high chromatographic purity were acquired from Merck KGaA (Darmstadt, Germany).

### 2.2. Instruments

The analyses were conducted using a Prominence series HPLC system manufactured by Shimadzu (Kyoto, Japan). The system includes a DGU-20A5 online degasser, an LC-20AT tandem double submersible pump with a low-pressure gradient unit, and a SIL-20A autosampler. The CTO-10ASVP column oven, CBM-20A communication bus module, and SPD-M20A diode array detector are being used. The system's holding volume was determined to be 0.50 mL. The system underwent inspection, and chromatograms were processed using LCSolutions 1.11 SP1 software (Kyoto, Japan). The equipment includes an Explorer E12140 analytical balance manufactured by Ohaus in Nänikon, Switzerland, an RK 100H ultrasonic bath produced by Bandelin in Berlin, Germany, a RO 15 multi-point mixer made by IKA in Staufen, Germany, and an Eppendorf device from Hamburg. A centrifuge type 5810R, manufactured in Hamburg, Germany, was utilised for the processing of samples and solutions.

### 2.3. Chromatographic Parameters

The study employed a mobile phase gradient elution method. The initial composition of the mobile phase consisted of a 30 mM phosphate buffer with a pH of 3.5 and acetonitrile at a ratio of 3:97 (volume/volume). The gradient elution programme is shown in Table 2.

Liquid chromatographic separation was conducted using a second-generation C18-bonded monolithic silica column (Chromolith High Resolution RP-18e, 100 mm × 4.6 mm i.d., Merck KGaA, Darmstadt, Germany). The temperature of the column oven was adjusted to 30 °C, while the injection volume was set to 5 µL. The rate of flow is 1.0 millilitres per minute. The photodiode array detector was configured to operate at a specific wavelength of 220 nm. Real-time spectra were captured within the range of 190 to 380 nm. The data sampling frequency was set at 6.25 Hz, and a time constant of 0.080 was applied.

### 2.4. Preparation of Solutions

The buffer solutions were created by dissolving 1503.0 mg of $NaH_2PO_4$ and 503.5 mg of $Na_2HPO_4.2H_2O$ in 500 mL of water. The mixture was then sonicated for 5 minutes and filtered using a nonsterile membrane filter with a diameter of 47 mm and a pore size of 0.45 µm, manufactured by Sartorius in Germany.

The solvent utilised in all dissolving and dilution processes for standard solution preparation was a mixture of acetonitrile:water (25:75, *v*/*v*), by volume. For the preparation of stock solutions, precisely measured quantities of 5 mg IVA and 5 mg LUMA were individually placed into 10 mL volumetric flasks and then diluted to the desired volume. The proportion of IVA and LUMA quantities in both the samples and working solutions was established using this stock solution. To prepare the AVA solution, 5.0 mg of AVA standard was added to a 25 mL volumetric flask and then diluted with enough solvent to reach a final concentration of 100.0 µg/mL.

**Table 2.** The applied flow gradient elution program

| Time (min) | ACN (%) |
|---|---|
| 1.00 | 30 |
| 2.00 | 60 |
| 2.50 | 50 |
| 3.00 | 40 |
| 4.00 | 15 |
| 4.50 | 30 |
| 5.00 | 40 |
| 6.00 | 65 |
| 6.01 | 30 |
| 8.00 | Stop |

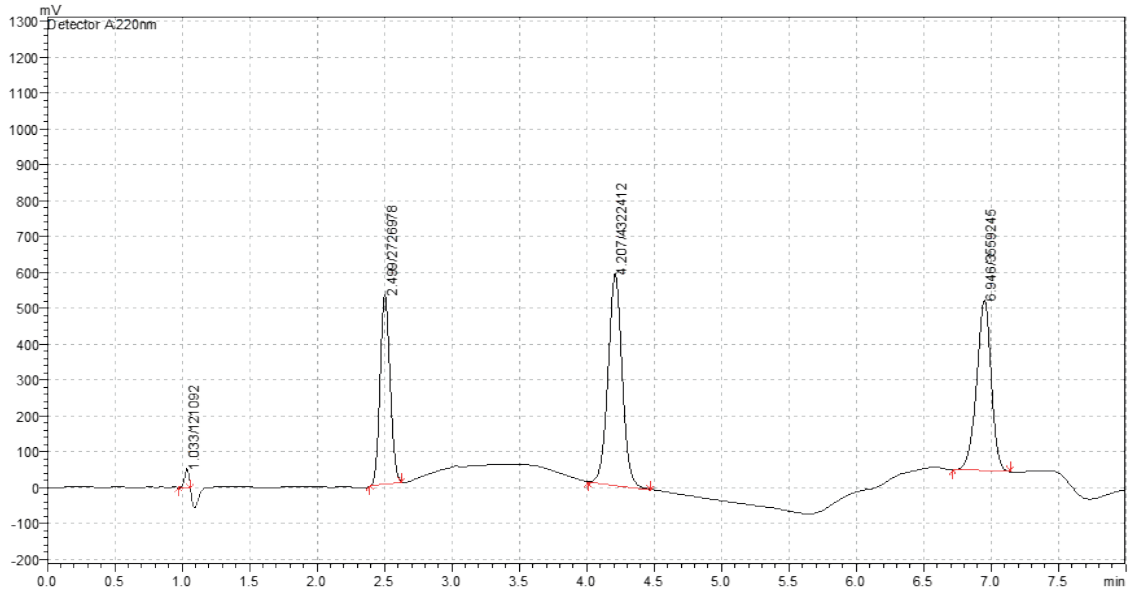


**Figure 2.** The chromatogram of standard solutions (C=150 µg/mL for IVA and LUMA, 100 µg/mL for AVA)

In addition, viable remedies for recuperation investigations were formulated using a pseudo composition of Orkambi® [20]. The needed dilutions were made with standard solution of active substance or IS.

### 2.5 Method validation

#### 2.5.1. System Suitability Test

An evaluation of system compatibility was deemed essential in the development of the HPLC method to analyse the chromatographic performance of the HPLC apparatus. The asymmetry factor (As) and tailing factor (T) were calculated following the requirements of the United States Pharmacopoeia (USP) using the Shimadzu LCsolution v1.11 SP1 software.

#### 2.5.2. Specificity

According to the ICH Q2(R1) guideline, it is advisable to employ an additional analytical process in order to compare the test outcomes for samples that are anticipated to contain impurities or degradation products. Hence, an analysis was conducted on the chromatograms and peaks of interest to ascertain the absence of any discernible positive or negative response to IVA, LUMA, and AVA. Furthermore, the assessment of peak purities was conducted by employing a photodiode array detector, which allowed for the examination of both the analyte and internal standard peaks. It was shown that these peaks could not be ascribed to multiple compounds simultaneously.

#### 2.5.3. Linearity and Range

A linearity chart was constructed to encompass five different levels of IVA and LUMA concentrations, specifically 90, 120, 150, 175, and 200 µg/mL. The injection of each solution was performed in triplicate, and the resulting average values were deemed to be representative. The assessment of linearity was conducted using linear regression analysis, which included both intraday and interday repeats. The slope, intercept, correlation coefficient, confidence intervals for the slope, and the intercept at a 95% confidence level were computed. All statistical computations were performed using GraphPad Prism v6.0b (trial version).

#### 2.5.4. Precision

Recovery tests were conducted in order to ascertain the precision of the methodology. The tablet samples were subjected to the addition of predetermined quantities of LUMA and IVA solutions, which were

carefully selected to represent low, medium, and high levels. Three sets of parallel items were prepared for each level. The spiked samples underwent reanalysis, and the mean recovery with standard deviation, expressed as a percentage of the blank spike solution (%RSD), was determined.

### 2.5.5. Accuracy

Precision investigations encompass both intraday and interday (sometimes known as intermediate) tests. The precision of the recommended approach was assessed by analysing standard solutions with a concentration of 120 µg/mL over three consecutive days. The data were subjected to statistical analysis, which involved calculating several measures such as the mean, standard error of the mean, standard deviation, %RSD, and confidence interval at a 95% confidence level. Furthermore, the analysis included an examination of the differences between groups on different days using a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

### 2.5.6. Limitations of Detection and Quantification

The determination of the limit of detection (LOD) and limit of quantification (LOQ) was conducted in accordance with the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) standards, utilizing the standard deviation of the response and slope as key factors. In the context of Limit of Detection (LOD) and Limit of Quantification (LOQ), it is observed that the ratio of the standard deviation (σ) of the y-intercepts of the regression lines to the slope is multiplied by a factor of 3.3 for LOD and 10 for LOQ. These values of σ and slope were determined based on the analysis of combined data obtained from linearity experiments.

## 3. RESULTS AND DISCUSSION

The hydrophobicity profiles of IVA and LUMA molecules exhibit similarities, as indicated by their respective log P values of 5.6 and 5.8. This suggests that their retention in liquid chromatography and partitioning between phases are expected to be comparable. In light of this distinction, ACN was initially evaluated as an adjunctive organic modifier to water in the mobile phase. This choice was motivated by its favourable characteristics, including low absorption and low viscosity in the UV region, which facilitate enhanced mass transfer. The signals were monitored utilising a photodiode array detector operating at a wavelength of 220 nm. Satisfactory absorption was reported for both substances. In contrast, the compounds exhibit distinct molecular ionization properties. Specifically, IVA demonstrates a $pK_a$ value of approximately 11.1, while LUMA exhibits a $pK_a$ value of about 4.6 within the neutral to fundamental range. To enhance the regulation of pH-dependent ionization and retention for both chemicals, the mobile phase was buffered by employing a 0.030 M phosphate buffer that was adjusted to various pH values. Although the smoother peaks and elution within a 10-minute timeframe were seen, it is noteworthy that the two compounds exhibited distinct retention behaviors in response to alterations in the mobile phase's ACN content at a pH of 2. The chromatogram presented in Figure 1 depicts the standard solution.

A comprehensive analysis was conducted to compute the results of all system suitability tests (SST) as prescribed by the ICH Q2 (R1) guidelines for the optimized technique. The detailed findings are presented in Table 3. It is evident that all SST values fall within the prescribed limits and adhere to the parameters for chromatographic separation.

In order to conduct linearity and accuracy tests, the calculation involved determining the ratio between the peak area and retention time of each standard solution and the peak area and retention time of the IS solution contained in that particular solution. The utilization of peak normalization approach is crucial for systems that exhibit high sensitivity towards minor alterations or possess a significant matrix effect. By employing the suggested method, it is possible to achieve results of considerable accuracy and precision. Additionally, it facilitates the execution of method transfer, a crucial aspect in drug analysis, within more suitable ranges of values. Table 4 presents the data on linearity and precision that were acquired throughout the investigation.

**Table 3.** System suitability results (n = 3)

| Parametre | LUMA | IVA | AVA | Recommended value |
|---|---|---|---|---|
| Retention time (min) | 2.5 | 4.2 | 6.9 | - |
| Retention time %RSD | 0.06 | 0.3 | 0.5 | RSD ≤1% |
| Repeatability of the peak area %RSD (n=6) | 1.1 | 1.3 | 1.2 | RSD ≤ 1% |
| Tailing factor (T) | 1.2 | 1.3 | 1.2 | T ≤ 2 |
| Capacity factor (k) | 2.6 | 2.1 | 2.2 | 2<k<10 |
| Number of theoretical plate (N) | 16289 | 30025 | 30258 | N > 2000 |
| USP Width | 0.3 | 0.5 | 0.7 | ≤1 |
| HETP (USP) | 10.45 | 33.3 | 52.1 | - |

**Table 4.** Linearity and precision data

| Parameter | IVA | LUMA |
|---|---|---|
| Linearity (µg/mL) | 0.78 | 1.96 |
| Slope (n=7) | -0.008 | -0.006 |
| Intercept (n=7) | 0.9986 | 0.9968 |
| LOD (µg/mL) | 60 ng/ mL | 50 ng/ mL |
| LOQ (µg/mL) | 200 ng/mL | 200 ng/mL |
| Slope (intra-day. k= 3) | 0.99 | 2.21 |
| Intercept (inter-day. k= 3) | -0.01 | -0.01 |
| Regression coefficient (inter-day. k= 3) | 0.9940 | 0.99623 |
| ANOVA | F (1,13) = 1.04 | F (1,13) = 1.58 |
| | P>0.05 | P>0.05 |

**Table 5.** Recovery results (n=3)

| | | Precision | | Accuracy | |
|---|---|---|---|---|---|
| | Added(µg/mL) | SD | RSD (%) | Recovery (%) | Bias (%) |
| IVA | 120 | 0.2 | 0.9 | 98.6 | -1.4 |
| | 150 | 0.1 | 0.5 | 98.8 | -1.2 |
| | 175 | 0.1 | 0.4 | 98.4 | -1.6 |
| LUMA | 120 | 0.1 | 0.9 | 98.4 | -1.6 |
| | 150 | 0.1 | 0.8 | 96.4 | -3.6 |
| | 175 | 0.3 | 1.9 | 97.8 | -2.2 |

In order to conduct recovery tests, the researchers employed the conventional addition procedure and conducted a total of nine independent determinations at three distinct concentrations that encompassed the desired range. So, to achieve the desired objective, a patented formulation of Orkambi® was created, and subsequent recovery studies were conducted by introducing a specific quantity of a standard solution mixture to these tablets. The obtained results were given in Table 5.

In fact, in previous methods developed for IVA or LUMA analysis (one of the authors also contributed), stability studies have shown that the active ingredients are quite stable [20, 21]. However, in this study, stability studies were carried out meticulously because IVA and LUMA were mixed in the working solutions and ava contributed as IS. According to the results obtained, it can be seen in Table 6 that IVA/ LUMA are quite stable under operating conditions.

**Table 6.** Stability results of active substances (n=3)

| | Added (µg/mL) | Short-term stability (48 h at room temper-ature) | | Long-term stability (3 weeks -20°C) | | Freezing-thawing stability (n=3) | |
|---|---|---|---|---|---|---|---|
| | | Found (Mean) | RSD (%) | Found (Mean) | RSD (%) | Found (Mean) | RSD (%) |
| IVA | 150.0 | 150.2 | 0.5 | 149.2 | 0.6 | 149.1 | 0.8 |
| LUMA | 150.0 | 150.3 | 0.7 | 150.1 | 0.6 | 149.3 | 0.9 |

## 4. CONCLUSION

Drug analysis is the backbone of the pharmaceutical industry. Each method developed contributes to the drug analysis of flour and sheds light on the process. HPLC has become the most important apparatus in this business today and has become indispensable. Although a new high-throughput technique is developed day by day and recommended to analysts, it does not seem possible to give up HPLC due to the convenience, high repeatability, and accuracy it provides.

In this study, a fast, highly accurate, and precise HPLC method was developed for the analysis of IVA/LUMA combinations in bulk and pharmaceutical formulations. Caftors are currently the most important therapeutic agents for CF diseases. They are also pioneer molecules in pharmaceutical chemistry because they contribute to the correction of the defective function of the CFTR gene. The importance of these molecules will increase day by day, and their analysis will be needed in almost all studies on them. In the method developed with this foresight, HPLC was preferred because it is an easily accessible and productive technique. The study is comprehensive, in accordance with the ICH Q2 (R1) guidelines, and will contribute to drug analysis.

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### Ethical approval

Not applicable, because this article does not contain any studies with human or animal subjects.

### Author contribution

Concept: SÖ, AE; Design: SÖ; Supervision: SL; Materials: SÖ; Data Collection and/or Processing: SÖ, SL, AE; Analysis and/or Interpretation: SÖ, AE; Literature Search: SÖ, SL; Writing: SÖ, AE; Critical Reviews: SL.

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### Conflict of interest

The authors declared that there is no conflict of interest.

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