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The determination of budesonide and fluticasone in human sputum samples collected from COPD patients using LC–MS/MS

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ABSTRACT

A bioanalytical method for the quantitative determination of budesonide and fluticasone in human sputum was developed. Sputolysin® Reagent was added to the sputum samples. After incubation (37 °C; 60–70 min under shaking) and automated solid phase extraction the extracts were analysed using LC–MS/MS. Budesonide and fluticasone showed good linearity ($r > 0.99$) over the range 0.1–100 nM in the first and second validation batch, and over the range 0.25–10,000 nM in the third and fourth validation batch. The lower limit of quantification (LLOQ) achieved was 5 nM for budesonide and fluticasone in 100 μL human sputum. Intra-run and inter-run RSD for four quality control levels (5–100 nM) were within 6.9% (budesonide) and 8.0% (fluticasone). The accuracy ranged from −11.4% to −1.6% (budesonide), and from −11.8% to 0.4% (fluticasone). The validated method was applied to clinical sputum samples from COPD patients.

1. Introduction

Budesonide and fluticasone are synthetic corticosteroids used in the treatment of asthma, rhinitis, chronic obstructive pulmonary disease (COPD), inflammation in the colon (budesonide), eczema and psoriasis (fluticasone). To support (pre)clinical studies budesonide and fluticasone have been analysed in several matrices, such as plasma, serum and urine using (automated) extraction followed by LC–MS/MS [1–6]. A great many clinical studies have been performed studying and comparing the bioavailability and absorption of different corticosteroids after inhalation. However, no studies where pharmacokinetic properties such as lipophilicity and patient characteristics have been performed until now [7]. High or low lipophilicity of an inhaled corticosteroid would be expected to influence both systemic availability and the speed of removal from the airways by mucociliary clearance and cough. This effect was studied for the first time in a clinical comparative study between budesonide and fluticasone [7], where budesonide is several times more water soluble than fluticasone and thus dissolves much faster in the lung lumen. As the drug is removed from the lung by absorption or excretion by mucociliary clearance, there is a competition between solubility/absorption and excretion, depending on lipophilicity. The effect of removal from the lung may also be severely affected by the patient status, as mucociliary clearance is very different for a COPD patient as compared to a healthy volunteer. To be able to study such a difference, methods need to be available also for analysing directly in sputum from COPD patients.

Sputum or bronchial secretion from COPD patients is a complex matrix, which varies in composition both between patients and with time. To quantify an analyte in sputum, the matrix must be liquefied as the viscosity is quite high and differs from sample to sample. Furthermore, the analyte may not be homogeneously distributed in the sample. For the determination of ciprofloxacin in sputum [8] the sample was diluted with potassium phosphate (pH 1.5) and subjected to sonication. The proteins were precipitated (phosphoric acid) and liquid–liquid extraction was performed (methylene chloride) [8]. Grebski et al. [9] investigated several methods for liquefying sputum including mechanical (ultrasound treatment) and chemical homogenization (dithiothreitol; DTT). Recently, Hagan et al. [10] described the LC–MS determination of fluticasone in sputum extracts after protein precipitation (acetonitrile) followed by liquid–liquid extraction (methylene chloride).

This paper describes the analytical method for the quantification of two synthetic corticosteroids, budesonide and fluticasone, in human sputum; the analytical method was not only developed but also thoroughly validated and successfully applied to clinical sputum samples from COPD patients. The sample pre-treatment consisted of addition of Sputolysin® Reagent [11], incubation at 37 °C (60–70 min under shaking) and cooling to room temperature. Sputolysin® Reagent is a concentrate of Dithiothreitol (DTT) in

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phosphate buffer (pH 6.5–7.5), intended for the isolation of epithelial cells, pathogenic or saprophytic bacteria, fungi, and yeasts from sputum. After automated solid phase extraction, the extracts were analysed using LC–MS/MS. The method validation included the determination of the linearity, LLOQ, accuracy, precision, carry-over, selectivity, incurred sample reproducibility, recovery, short term stability in sputum, freeze and thaw stability in sputum, extract stability after completed sample treatment and long term stability in sputum.

2. Experimental

2.1. Chemicals and materials

All chemicals used in this study were of analytical reagent grade. Acetic acid 100% (Suprapur®) was obtained from Merck (Darmstadt, Germany). Methanol (LCMS) and acetonitrile (HPLC/S) were purchased from Biosolve B.V. (Valkenswaard, The Netherlands). Ethanol was from Baker (Deventer, The Netherlands). Sputolysin® Reagent was obtained from Calbiochem (San Diego, USA). The reference substances budesonide and fluticasone and their deuterated internal standards D_8-budesonide (D_8-BUD; budesonide content <0.1%) and D_5-fluticasone (D_5-FLU; fluticasone content <0.1%) were kindly provided by AstraZeneca (Lund, Sweden). The SPE cartridges (Isolute, MF C18 with "caps" (not endcapped), 100 mg, 1 mL) were from Isolute (International Sorbent Technology LTD, Hengoed, Mid Glamorgan, UK).

2.2. Blank sputum

"Blank" human sputum was collected from COPD patients (with a wash out period of steroids of 5 days before entering the collection period). Normally COPD patients are treated with steroids. The "blank" human sputum was kindly provided by AstraZeneca (Lund, Sweden).

2.3. Preparation of calibration and quality control samples

All sputum samples were ultra-sonicated (MSE Soniprep 150, Wolf Labs; Microprobe; settings: 3 × 10 s at 10 μm) before spiking or any sample treatment. Calibration samples were freshly prepared on each validation occasion by spiking 100 μL sputum with 10 μL spiking solution (budesonide and fluticasone). The calibration levels were approximately 0.10, 0.25, 1.0, 5.0, 25, 50 and 100 nM in the first and second validation batch, and 0.25, 1.0, 5.0, 25, 50, 100, 500, 1000, 5000 and 10,000 nM in the third and fourth validation batch. Quality control (QC) samples were prepared by adding 50 or 100 μL spiking solution (budesonide and fluticasone) to 5 or 10 mL blank sputum, respectively. The QC levels were approximately 0.25 nM (QC LOQ), 5.0 nM (QC LO), 40 nM (QC ME), 80 nM (QC HI), 100 nM (QC ULOQ) and 9000 nM (QC DIL). After preparation, the QC samples were divided in small portions and stored in the freezer (<−18 °C).

2.4. Sample preparation

Micro-centrifuge tubes (polypropylene) were used to add 50 μL internal standard (IS) solution (mixture of D_8-BUD and D_5-FLU) to the sputum sample (to 100 μL blank sputum or 100 μL QC sample, or to 110 μL calibration sample). 900 μL of Sputolysin® Reagent was added to each sputum sample (blank, QC or calibration sample). The mixture was incubated for 60–70 min at 37 °C (±3 °C) under shaking (shaking water bath GFL 1083; Gemini BV). The mixture was then cooled to room temperature. If needed (mixture not transparent), the mixture was ultra-sonicated (MSE Soniprep 150, Wolf Labs; Microprobe; settings: 3 × 10 s at 10 μm). The whole sample was transferred to an automated SPE system (ASPEC XL, Gilson). The SPE cartridges were conditioned with methanol (0.6 mL; 12 mL/min) and water (0.6 mL; 12 mL/min). After application of the sputum sample (1 mL; 1.5 mL/min), the cartridge was washed with water (1.5 mL; 8.0 mL/min), 40% methanol (1.0 mL; 8.0 mL/min) and 40% methanol, 0.1% acetic acid (1.0 mL; 4.0 mL/min). The extract was eluted with 100% acetonitrile, 0.1% acetic acid (0.5 mL; 1.0 mL/min). The extracts were evaporated to dryness (turbo-vap, N_2, 40 °C). Subsequently, 200 μL methanol/water (50/50, v/v) was added to all dried extracts. The reconstituted extracts were vortex-mixed and kept at room temperature for 30 min. The extracts were vortex-mixed again, centrifuged (MSE Mistral 3000i centrifuge (DJB Labcare); 1 min, 500 × *g*) and transferred into autosampler vials for LC–MS/MS analysis.

2.5. LC–MS/MS analysis

The autosampler and LC pump used were from ThermoFisher Scientific (Surveyor LC system). The guard column and the LC column were from Agilent (Zorbax C8 SB, 5 μm, 12.5 mm × 4.6 mm and Zorbax C8 SB, 3.5 μm, 30 mm × 4.6 mm, respectively). Mobile phase A was prepared by mixing 300 mL methanol with 700 mL purified water; 1.0 mL glacial acetic acid was added. Mobile phase B was prepared by mixing 900 mL methanol with 100 mL purified water; 1.0 mL glacial acetic acid was added. The flow rate was 1400 μL/min. The run time was 8.5 min. The gradient was 100% A/0% B at time 0.0 until 0.65 min, 20%A/80% B at 4.0 min until 7.0 min and 100% A at 7.1 min until 8.5 min. The injection volume was 125 μL. The sample tray temperature was 10 °C. The column oven was set at 30 °C. All experiments were performed using a TSQ Quantum Ultra AM mass spectrometer (ThermoFisher Scientific) with XCalibur software (version 2.0, ThermoFisher Scientific). The ionization mode was APCI (negative mode). The MS/MS settings were: budesonide (*m*/*z* 489.2; *m*/*z* 357.1; CE: 17), D_8-budesonide (*m*/*z* 497.3; *m*/*z* 357.2; CE: 17), fluticasone (*m*/*z* 559.2; *m*/*z* 413.2; CE 21) and D_5-fluticasone (*m*/*z* 564.2; *m*/*z* 417.2; CE 21). The vaporizer temperature was 400 °C. The capillary temperature was set at 215 °C. The sheath gas pressure was 30–31 psi. The auxiliary gas pressure was 5 arb. unit. The scan width was 0.1 amu. The CAD was 1.2–1.3 mTorr Ar. The resolution was Unit 0.3 FWHM (precursor resolution) and Unit 0.5 FWHM (product resolution).

3. Results and discussion

3.1. Method validation

The developed method for the quantitative analysis of budesonide and fluticasone in human sputum was validated in three batches. After 9.5 months, the fourth validation batch was run to investigate the long term stability of budesonide and fluticasone in the sputum. Each validation batch consisted of freshly prepared calibration samples to assess the linearity of the method, blank sputum samples to investigate the selectivity and carry-over, and quality control samples (which were stored at <−18 °C, and thawed just before each validation batch) to investigate the accuracy and precision of the method. In addition, each validation batch contained samples to determine the recovery, the short term stability in sputum, the freeze and thaw stability in sputum, the extract stability after completed sample treatment or the long term stability in sputum.

3.2. Linearity

The linearity of the methods was investigated by the analysis of calibration samples spiked with budesonide and fluticasone. In

Table 1
Summary statistics of QC results (budesonide) in human sputum. The results were obtained from three validation batches.

Parameter	QC LOQ (0.250 nM)	QC LO (4.99 nM)	QC ME (39.9 nM)	QC HI (79.9 nM)	QC ULOQ (99.8 nM)	QC DIL[a] (9030 nM)
N (number of QCs)	18	18	18	18	18	18
Mean concentration (nM)	0.120	4.91	39.2	70.7	95.3	8252
Bias (%)	−50.0	−1.6	−1.8	−11.4	−4.5	−8.6
Precision (intra-run) (%)	28.2	3.3	2.4	2.5	3.4	3.4
Precision (inter-run) (%)	140.3	4.9	4.8	6.9	6.1	4.7

[a] On the first, second and third validation occasions the QC sample spiked at 9030 nM was analysed after 100-fold dilution with blank sputum.

each validation batch, the calibration samples were freshly prepared, and extracted and analysed in duplicate. Linear regression was applied with weighting factor $1/x^2$. The acceptance criterion for calibration was: accuracy within ±15% at each calibration level (within ±20% at the LLOQ level). In the first and second validation batch the calibration range was from 0.1 to 100 nM (7 calibration levels). In the third and fourth validation batch the calibration range was from 0.25 to 10,000 nM (10 calibration levels) because the lowest level (0.1 nM) did not fulfil the acceptance criterion. Budesonide and fluticasone showed good linearity ($r > 0.99$) over the range 0.1–100 nM in the first and second validation batch, and over the range 0.25–10,000 nM in the third and fourth validation batch.

3.3. Quality control

The accuracy and precision of the methods were investigated by analysis of quality control samples. As the initial calibration range was from 0.1 to 100 nM, the QC levels were selected at approximately 0.25 nM, 5 nM, 40 nM, 80 nM and 100 nM. QC samples at approximately 9000 nM were prepared to investigate the (100-fold) dilution of QC samples. The QC samples were extracted and analysed in six-fold in each validation batch. The acceptance criterion for accuracy was a maximum bias of ±15% at each QC level (±20% at QC LOQ). The acceptance criterion for intra-batch and inter-batch precision, expressed as the coefficient of variation (CV), was 15% at each QC level (20% at QC LOQ). The results are presented in Table 1 (budesonide) and Table 2 (fluticasone). For budesonide, the accuracy and the (intra-batch and inter-batch) precision results were acceptable for the QC LO, QC ME, QC HI and QC ULOQ. The accuracy and the precision results at the QC LOQ level (0.250 nM) were not acceptable, i.e. the accuracy and the precision were outside the 20% limit for defining the LOQ. For fluticasone, the accuracy and the (intra-batch and inter-batch) precision were acceptable for the QC LO, QC ME, QC HI and QC ULOQ. At the QC LOQ level (0.230 nM) not all accuracy and precision results were acceptable, i.e. the accuracy and the precision were outside the 20% limit for defining the LOQ. Sputum samples (QC DIL) spiked with budesonide and fluticasone (at 9030 nM and 8280 nM, respectively) were diluted 100-fold with blank sputum in all three validation batches. In addition, the QC DIL samples were diluted 100-fold with NaCl (0.9%) solution in one of the three validation batches. For the accurate quantification of budesonide (9030 nM) sputum samples can be diluted 100-fold with either blank human sputum or with NaCl (0.9%) solution prior to sample pre-treatment. Sputum samples containing a high fluticasone concentration (8280 nM), however, cannot be quantified very accurately after 100-fold dilution with either blank human sputum or NaCl (0.9%) solution before sample treatment (bias: −27%); instead sputum samples containing a high fluticasone concentration (8280 nM) may only be analysed without prior dilution (mean accuracy: −17%; $n = 6$), which limits the range of analysis for fluticasone. Representative chromatograms of budesonide and fluticasone (5 nM) and the internal standards in human sputum are presented in Fig. 1.

3.4. Selectivity and carry-over

The selectivity of the method was investigated by the analysis of blank human sputum samples from 6 different individuals with and without the addition of the internal standards. The acceptance criterion for the selectivity was a maximum signal of 20% in the blanks relative to the LLOQ of the method. If compared to the calibration samples spiked with budesonide at approximately 5 nM (same run), the budesonide signal obtained from blank human sputum was 7–8% (with and without internal standard). The fluticasone signal obtained from blank human sputum was 6–8% (with and without internal standard), relative to the calibration samples spiked with fluticasone at approximately 5 nM. The background signal did not come from the deuterated internal standards. With other words, a background level of both budesonide and fluticasone was observed in the "blank" sputum. Similar results were obtained when monitoring additional MRM transitions. The background levels of budesonide and fluticasone in blank sputum can be explained by the fact that the "blank" human sputum was collected from COPD patients (treated with steroids) with a wash out period of steroids of 5 days before entering the collection period.

The carry-over of the method was investigated by the analysis of QC ULOQ samples (100 nM) each followed by three blank human sputum samples. Hardly any carry-over was measured; an estimate for carry-over of budesonide, based on the D_8-BUD signal, was approximately 0.2%. An estimate for carry-over of fluticasone, based on the D_5-FLU signal, was approximately 0.05%. Representative chromatograms of blank human sputum spiked with the internal standards are shown in Fig. 2.

3.5. LLOQ

For budesonide and fluticasone, the LLOQ was set at approximately 5 nM with a 100 μL sputum volume. Budesonide and fluticasone concentrations below 5 nM could be measured in

Table 2
Summary statistics of QC results (fluticasone) in human sputum. The results were obtained from three validation batches.

Parameter	QC LOQ (0.230 nM)	QC LO (4.60 nM)	QC ME (36.8 nM)	QC HI (73.6 nM)	QC ULOQ (92.0 nM)	QC DIL[a] (8280 nM)
N (number of QCs)	18	18	18	18	18	18
Mean concentration (nM)	0.250	4.62	36.5	65.0	88.6	6215
Bias (%)	9.3	0.4	−0.8	−11.8	−3.7	−24.9
Precision (intra-run) (%)	26.6	5.1	3.3	2.2	3.0	8.8
Precision (inter-run) (%)	128.4	7.5	5.4	8.0	5.4	27.2

[a] On the first, second and third validation occasions the QC sample spiked at 8280 nM was analysed after 100-fold dilution with blank sputum.

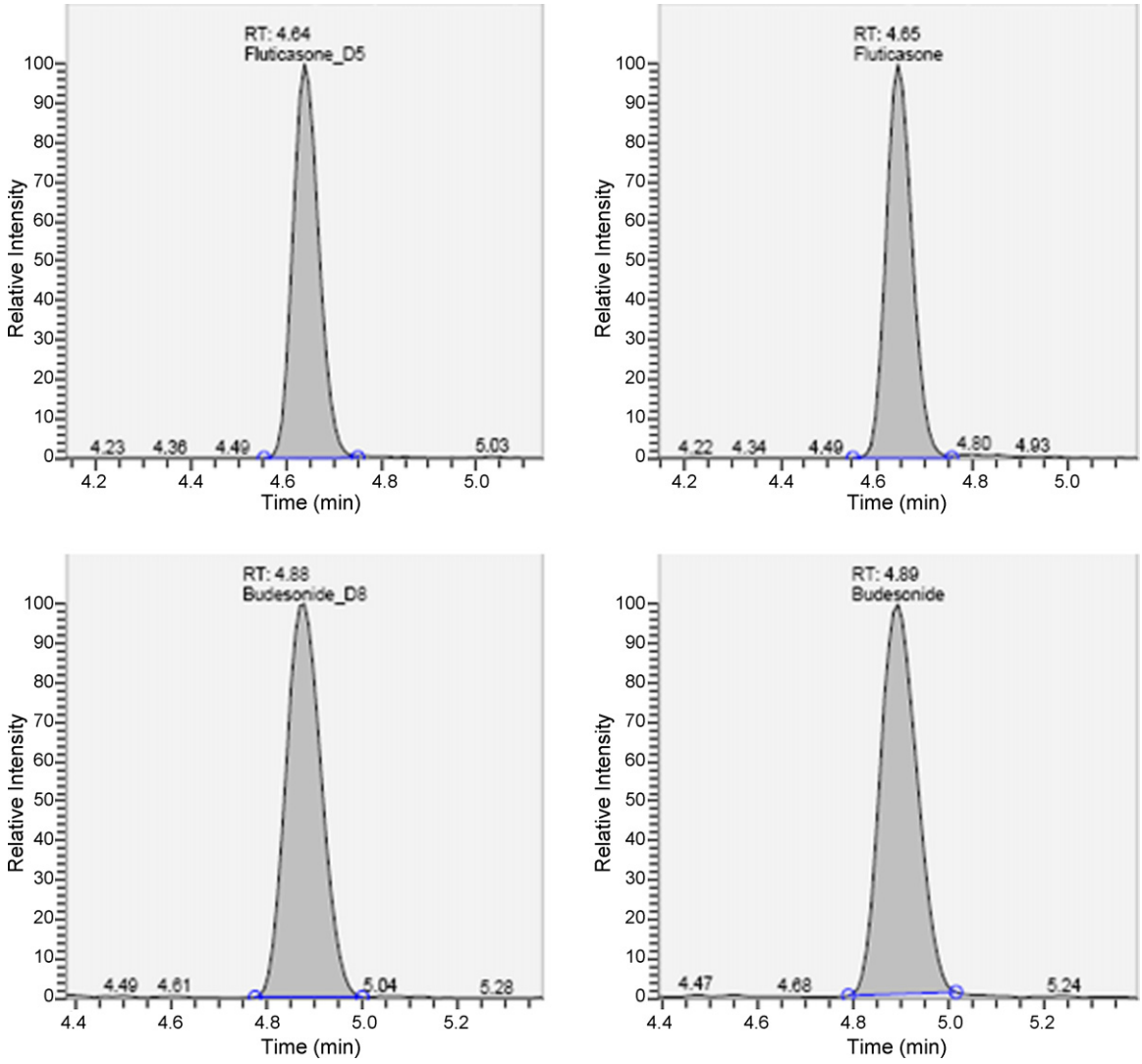

Fig. 1. A representative chromatogram of budesonide and fluticasone in human sputum (QC low, 5 nM).

sputum samples, but because of the observed background levels of budesonide and fluticasone in 'blank' sputum the LLOQ was increased accordingly.

3.6. Recovery

The recovery of budesonide, fluticasone and their internal standards from human sputum was determined by comparing the analytical results of extracted human sputum samples (100 μL) at two concentrations (QC LO and QC HI; $n = 6$) with blank extracts spiked with the analytes and the internal standard at concentrations equivalent to a theoretical 100% recovery. For budesonide, the recovery was 98.5% (QC LO) and 96.0% (QC HI). The recovery of the internal standard (D_8-BUD) was 110%. For fluticasone, the recovery was 84.2% (QC LO) and 71.5% (QC HI). The recovery of the internal standard (D_5-FLU) was 96.5%.

3.7. Stability investigation

The performance requirements for all stability measurements were a maximum bias of ±15% at each investigated QC level.

3.7.1. Short term stability in sputum

The short term stability was determined by the single-fold analysis of three QC LO, three QC HI and three QC DIL samples, after storage at room temperature (±20 °C) for up to 24 h. The results are presented in Table 3. The measured budesonide concentration in the stored QC LO, QC HI and QC DIL samples was within 15% of the actual budesonide concentration: budesonide can be regarded stable for the entire range (5–9000 nM) in human sputum when stored at room temperature (±20 °C) for up to 24 h. The measured fluticasone concentration in the stored QC LO and QC HI samples was within 15% of the actual fluticasone concentration level. In the QC DIL samples, however, the bias was −49.0%. Fluticasone can be regarded stable only from approximately 5 nM to 80 nM in human sputum when stored at room temperature (±20 °C) for up to 24 h.

3.7.2. Freeze and thaw stability in sputum

The freeze and thaw stability of budesonide and fluticasone in human sputum was investigated at three QC levels (QC LO, QC HI and QC DIL). After preparation, the QC samples were stored at <−18 °C for at least 24 h and thawed unassisted at room temperature (±20 °C). When completely thawed, the samples were refrozen. The freeze/thaw cycle was repeated, then analysed on the second and third cycle. The results are presented in Table 4. For

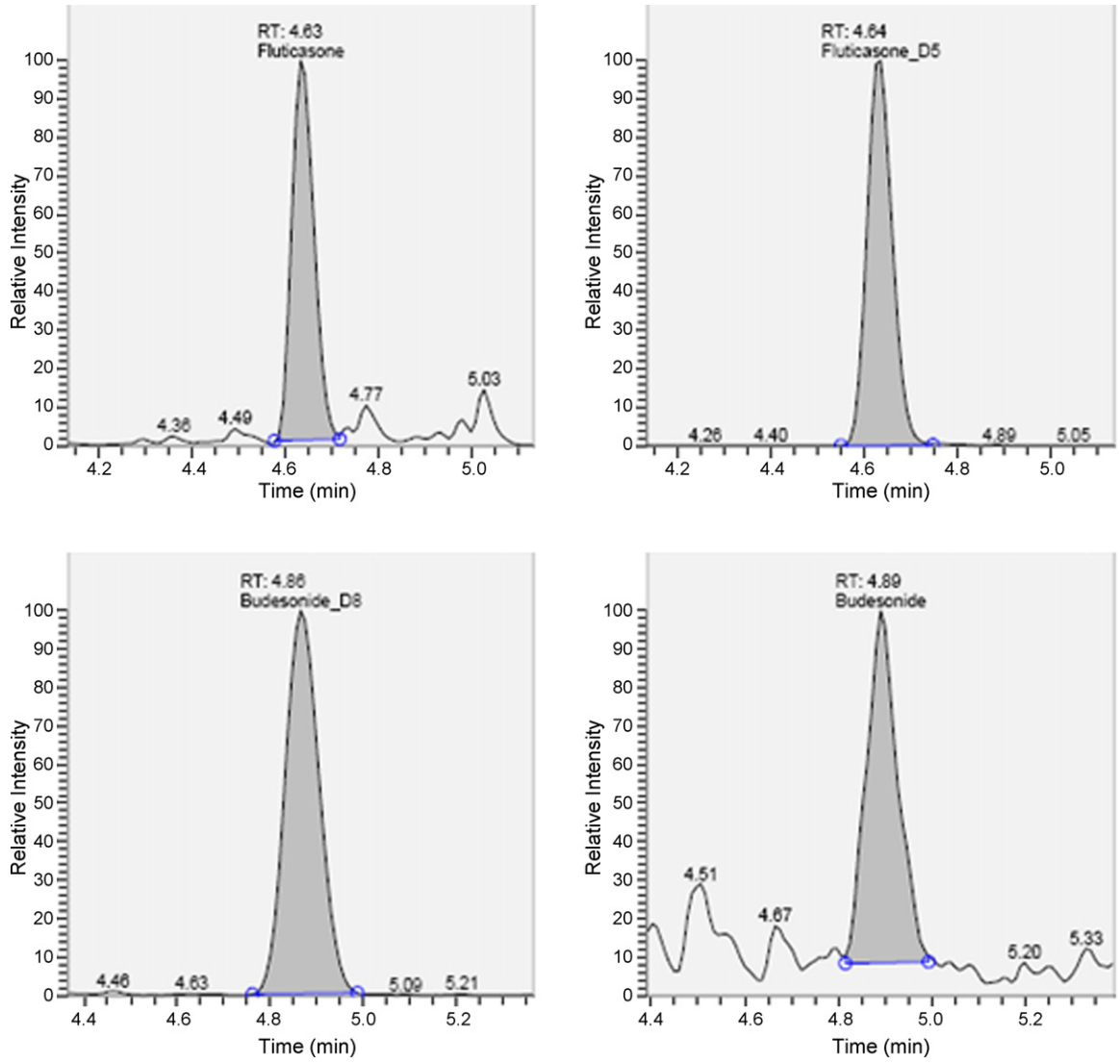

Fig. 2. A representative chromatogram of blank human sputum spiked with internal standards D_8-budesonide and D_5-fluticasone.

Table 3
Results of short term stability investigation for budesonide and fluticasone in sputum. The sputum samples were stored at room temperature (±20 °C) for 24 h before sample preparation.

Parameter	Budesonide QC LO (4.99 nM)	Budesonide QC HI (79.9 nM)	Budesonide QC DIL (9030 nM)	Fluticasone QC LO (4.60 nM)	Fluticasone QC HI (73.6 nM)	Fluticasone QC DIL (8280 nM)
Mean conc. (nM)	5.02	70.5	8233	4.80	64.0	4219
N	3	3	3	3	3	3
Bias (%)	0.5	−11.7	−8.8	4.2	−13.1	−49.0
SD (nM)	0.231	1.78	27.8	0.0568	2.38	402
CV (%)	4.6	2.5	0.3	1.2	3.7	9.5

Table 4
Results of the freeze and thaw stability investigation for budesonide and fluticasone in sputum (measured concentrations expressed as nM).

Freeze/thaw cycles	Budesonide QC LO (4.99 nM)	Bias (%)	Budesonide QC HI (79.9 nM)	Bias (%)	Budesonide QC DIL (9030 nM)	Bias (%)	Fluticasone QC LO (4.60 nM)	Bias (%)	Fluticasone QC HI (73.6 nM)	Bias (%)	Fluticasone QC DIL (8280 nM)	Bias (%)
2 cycles	5.16	3.4	70.7	−11.5	9385	3.9	4.71	2.3	66.7	−9.4	7103	−14.2
	4.86	−2.6	71.6	−10.3	[a]	–	4.62	0.3	66.3	−10.0	[a]	–
3 cycles	5.41	8.3	74.6	−6.6	8501	−5.9	4.75	3.1	64.3	−12.7	6646	−19.7
	5.06	1.4	71.9	−10.0	[a]	–	4.21	−8.5	60.8	−17.4	[a]	–

[a] Due to an instrumental error, one of the two QC DIL results was missing.

Table 5
Results of the extract stability investigation of budesonide and fluticasone after completed sample treatment. After sample preparation, the extracts were stored at 2–10 °C for one week before LC–MS/MS analysis.

Parameter	Budesonide QC LO (4.99 nM)	Budesonide QC HI (79.9 nM)	Fluticasone QC LO (4.60 nM)	Fluticasone QC HI (73.6 nM)
Mean conc. (nM)	5.22	73.8	4.79	67.4
N	3	3	3	3
Bias (%)	4.6	−7.6	4.2	−8.5
SD (nM)	0.0383	1.15	0.244	1.38
CV (%)	0.7	1.6	5.1	2.1

Table 6
Results of the long term stability investigation of budesonide and fluticasone in sputum. The sputum samples were stored in the freezer (<−18 °C) for 9.5 months before analysis in six-fold.

Parameter	Budesonide QC LO (4.99 nM)	Budesonide QC HI (79.9 nM)	Budesonide QC DIL[a] (9030 nM)	Fluticasone QC LOW (4.60 nM)	Fluticasone QC HI (73.6 nM)	Fluticasone QC DIL[a] (8280 nM)
Mean conc. (nM)	4.92	69.7	8188	4.38	68.0	5704
N	6	6	6	6	6	6
Bias (%)	−1.5	−12.7	−9.3	−4.8	−7.6	−31.1
SD (nM)	0.0660	3.84	281	0.103	2.65	118
CV (%)	1.3	5.5	3.4	2.4	3.9	2.1

[a] The QC DIL samples were analysed without prior dilution. The calibration range was from 0.25 to 10,000 nM (validation batch 4).

budesonide, the measured concentrations in the QC LO, QC HI and QC DIL samples were within 15% of the actual concentrations after two and three freeze and thaw cycles: budesonide can be regarded stable for the entire range (5–9000 nM) in human sputum samples after two and three freeze and thaw cycles. For fluticasone, the measured concentrations in the QC LO, QC HI and QC DIL samples were within 15% of the actual concentrations after two freeze and thaw cycles. After three freeze and thaw cycles, however, the QC LO results were accepted whereas the measured fluticasone in the QC HI and QC DIL samples seemed to decrease (relative deviation: −15.0% for QC HI, and −19.7% for QC DIL).

3.7.3. Extract stability after completed sample treatment

The stability of the extracts (after SPE, solvent evaporation and reconstitution in mobile phase) was determined by the single-fold analysis of three QC LO and three QC HI sample extracts which were stored in the refrigerator (2–10 °C) for one week. A fresh calibration line served as the reference. The results are presented in Table 5. The measured budesonide and fluticasone concentrations in the sputum extracts were within 15% of the actual concentrations at the QC LO and QC HI level: budesonide and fluticasone (approximately 5–80 nM) can be regarded stable in human sputum extracts when stored in the refrigerator (2–10 °C) for one week.

3.7.4. Long term stability in sputum

The long term stability of budesonide and fluticasone in human sputum samples was determined after 9.5 months storage in the freezer (at <−18 °C) by the analysis of QC LO, QC HI and QC DIL samples in six-fold. For comparison, freshly prepared QC samples were analysed in the same run (data not shown). The stability results are presented in Table 6. Based on the mean accuracy results obtained (within 15% of the nominal concentration) budesonide can be regarded stable in human sputum for the entire range (5–9000 nM) when stored in the freezer (at <−18 °C) for 9.5 months. Fluticasone can be regarded stable in human sputum from approximately 5 nM to 80 nM when stored at <−18 °C for 9.5 months.

3.8. Incurred sample reproducibility (ISR)

After the validation, the method was successfully applied to the analysis of more than 200 clinical sputum samples from COPD patients [7]. The measured budesonide and fluticasone concentrations ranged from below the LLOQ up to approximately 21,000 nM (budesonide) and 10,000 nM (fluticasone). Incurred sample reproducibility was investigated by the reanalysis of 10–20% of the human sputum samples. For both budesonide and fluticasone more than two third of the repeat values was within 80–120% of the original value [12].

4. Conclusions

A bioanalytical LC–MS/MS method for the quantitative determination of budesonide and fluticasone in human sputum from COPD patients was developed and validated. The LLOQ of the methods was set at approximately 5 nM with a 100 μL sputum volume, as a consequence of the background steroid in the "blank" sputum provided. The validated method was successfully applied to the analysis of clinical sputum samples from COPD patients.

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