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Sensitive simultaneous determination of ciclesonide, ciclesonide-M1-metabolite and fluticasone propionate in human serum by HPLC–MS/MS with APPI

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ABSTRACT

A new and very sensitive analytical method has been developed and validated to jointly determine the anti-inflammatory drug ciclesonide (CIC), its active principle metabolite M1 (CIC-M1) and fluticasone propionate (FP) in human serum, in the low concentration range from 10 to 1000 pg/mL. This was accomplished by high-performance liquid chromatography and tandem mass spectrometry using atmospheric pressure photo ionisation (HPLC–MS/MS with APPI) using 0.5 mL of serum. Serum was mixed with the internal standards (IS) D11-CIC and D11-CIC-M1 and extracted with diisopropylether. A gradient with acetonitrile (containing 10 mM of acetic acid and 10% of acetone) was used. HPLC–MS/MS of the acetic acid adducts of the analytes was performed in negative mode. The novel aspect of this method is that instead of the dopant being introduced directly into the source by means of an external HPLC pump, it was added to the mobile phase. This provided significantly better sensitivity than the usual method of in-source addition of the dopant, and with no loss in HPLC performance. Sensitivity for the analytes was about four times greater than with either APCI or ESI. Validation was performed in three batches. The inter-batch precision (CV) of the quality control samples in human serum ranged from 4.08% to 6.78% for CIC, from 2.57% to 7.74% for CIC-M1, and from 2.38% to 9.61% for FP. The inter-batch accuracy (with reference to the mean value) of the quality control samples in human serum ranged from 99.3% to 110.0% for CIC, from 101.8% to 104.7% for CIC-M1, and from 100.4% to 101.8% for FP. Calibration data and LLOQ data are also presented in this paper. The analytes were stable in human serum over three freeze/thaw cycles, or for 4 h at room temperature, or for at least 18 months when stored at below −20 °C. This method was used for quantifying the analytes after inhalation of low-μg amounts of the drugs by patients.

1. Introduction

Ciclesonide is a new, once-daily inhaled corticosteroid; determination of both the prodrug ciclesonide and the active metabolite M1 is thus necessary. Furthermore, fluticasone propionate is also often used as an inhaled corticosteroid. Determination of these substances – in some cases conjointly – is necessary in very low concentrations down to 10 pg/mL of serum or plasma.

Few, if any, analytical methods for determining ciclesonide (CIC) and the active principle ciclesonide-M1-metabolite (CIC-M1) in human serum have been published in the literature to date.

A number of methods have been published for fluticasone propionate (FP), however. There are two different determination principles which have the capability to reach the low quantitation level which is necessary in order to determine concentrations from bioavailability studies or from patients. One is a RIA with a determination limit of 50 pg/mL plasma [1]. More work has been published with HPLC–MS/MS after solid-phase extraction with positive APCI detection with a LLOQ of 200 pg/mL [2], a LLOQ of 250 pg/mL [3], a LLOQ of 10 pg/mL [4], a LLOQ of 20 pg/mL [5] and HPLC–MS/MS with positive ESI with a LLOQ of 20 pg/mL [6].

All of the HPLC–MS/MS publications used solid-phase extraction, and most of them also employed APCI ionisation.

The method described here uses HPLC–MS/MS with atmospheric pressure photo ionisation (APPI). For more apolar substances, APPI has recently become a powerful tool [7–9]. After liquid–liquid extraction, the method reaches a LLOQ, for all three components, of 10 pg/mL serum by using 0.5 mL. An APPI source has never before been used in a publication for the determination of FP. The dopant for APPI (which is absolutely necessary for charge transfer and high ionisation yield) was not pumped into the source via auxiliary gas line, as usually proposed by the manufacturer, but was part of the mobile phase. This is a novel approach. Significantly less noise resulted from this manner of applying the dopant. Also,

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compared to ESI and APCI, APPI showed significantly more sensitivity for these analytes, by a factor of about 4, even with a high flow rate of 1 mL/min. Significant sensitivity gains for such substances with APPI have been published recently [8–12].

Comparison of various suitable dopants, such as acetone or toluene, at our laboratory demonstrated no significant differences.

2. Experimental

2.1. Instrumentation

The MS/MS system used for these experiments was an API 3000 (PE Sciex, Canada) in combination with atmospheric pressure photo ionisation. Gas for the MS was delivered by a nitrogen generator (Whatman, USA). The auto-sampler was a series 200 auto-sampler from PerkinElmer (Germany) and the liquid chromatography (LC) system consisted of a HP 1100 pump (Agilent Technologies, USA). The column oven was a HP 1100 column oven (Agilent Technologies, USA). The data system consisted of a PC based on Windows NT 4.0 (SP 5) with Analyst 1.4 software. The analytical column was a Luna C18 (50 mm × 2 mm, 5 μm particle size) from Phenomenex (USA).

Other equipment used during sample preparation included a water bath (Julabo, Germany), a vortex mixer (Vibrofix VF1, Janke & Kunkel, Germany), a centrifuge (Megafuge 1.0, Heraeus, Germany), an evaporator (TurboVap, Zymark, Switzerland), and a deep-freezer (−60 °C, ULT 1090–7 VBA, Revco, USA).

2.2. Chemicals

All chemicals used in this study were of analytical reagent grade. Diisopropylether and glacial acetic acid (by Merck, Germany) were of p.a. quality. Acetone (by Merck, Germany) and dimethylsulfoxide (DMSO) (by Riedel de Haen, Germany) were of HPLC grade. Methanol and acetonitrile (both provided by Merck, Germany) were of gradient grade. Water was cleaned by reversed osmosis and ultraviolet-radiation at our lab (Type: SG 2000, Clear UV, by Wasseraufbereitung und Regenerierstation GmbH, Barsbüttel, Switzerland).

CIC, its active metabolite CIC-M1, and the internal standards D11-ciclesonide and D11-ciclesonide-M1 were provided by Altana Pharma AG (Germany). FP was from Promochem (Germany). Molecular structures of the analytes are displayed in Fig. 1a–c. Human serum was provided by "Blutplasmazentrum Koblenz", Germany.

2.3. Preparation of calibration standards, quality control samples and other control samples

To calibrate each batch, calibration standards were made at several concentration levels by adding defined volumes of methanolic analyte solutions (to Standards 3–8), or by adding a more highly concentrated calibration standard (with reference to Standards 1 and 2 where the highest calibration standard was taken) to blank human serum samples. To control each analysed batch, quality control samples (QC samples) were made at three concentration levels, by adding either defined volumes of methanolic analyte solutions (medium level = QC-B and high level = QC-C) or a more highly concentrated QC sample (for low level = QC-A) to blank human serum samples. Additionally, six LLOQ samples were prepared within each validation batch. As a minimum, each analytical batch consisted of freshly prepared calibration standards and QC samples. Calibration standards were spiked at: 10.0, 20.0, 40.1 (40.0 for CIC), 80.1, 150, 251, 501, and 1001 (1000 for CIC) pg/mL of human serum. Quality control samples were spiked at 25.0, 115, and 851 (850 for CIC) pg/mL of human serum.

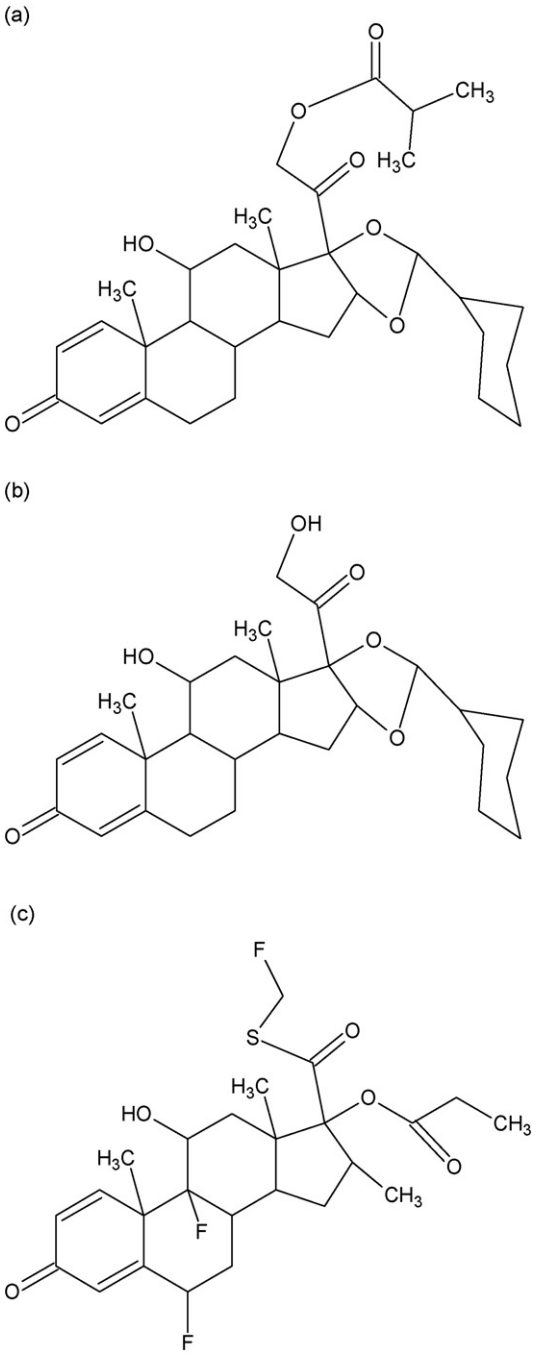

Fig. 1. Structures of the three analytes ciclesonide (a), ciclesonide-M1 (b) and fluticasone propionate (c).

After these samples had been prepared, they were fast-frozen to below −60 °C until they were completely frozen (due to larger serum sample quantities and therefore a slower freezing process at only −20 °C). Later on, a "long-time" storage temperature of below −20 °C was sufficient.

2.4. Sample preparation

Aliquots of all samples needed for a validation sequence (calibration standards, quality control samples) were thawed at approximately 20–25 °C in a water bath. Within a former validation (separate methods for the analytes), we proofed the freeze/thaw

stability in human serum (three cycles) and stability over 4 h at room temperature (about 25 °C).

Thereafter, all samples used within a validation sequence were prepared for analysis as follows.

0.5 mL of sample was transferred into a glass vial. After 25 μL of the internal standard working solution (D11-CIC at 44 ng/mL and D11-CIC-M1 at 33 ng/mL; in 50% methanol; same working solution used for all samples of a batch) and 2 mL of diisopropylether had been added, samples were shaken vigorously for about 2 min.

After centrifugation at 4000 rpm for 2 min, samples were stored for about 10 min in a deep-freezer (<−60 °C). The liquid organic phase was then separated from the frozen aqueous phase by decanting the organic phase into conical centrifuge glass vials (100 mm × 16 mm i.d., approx. 10 mL). After this, 50 μL of DMSO was added. The volatile part of the organic phase was evaporated using dry compressed air (Turbovap at 50 °C for 7 min), after which the samples were vortexed (for about 4 × 5 s). After the addition of 50 μL of 65% acetone (in water), the samples were vortexed again (for about 4 × 5 s). Finally, the samples were transferred into conical auto-sampler vials, sealed with an aluminium crimp cap and injected into the HPLC–MS/MS system within 20 h, or stored at below −20 °C prior to analysis.

2.5. Chromatographic conditions

The mobile phase 'A' used was 10 mM of acetic acid in a mixture of 90% water and 10% acetone vs. the mobile phase 'B' which was 10 mM of acetic acid in a mixture of 90% acetonitrile and 10% acetone. The gradient was from 25% B to 90% B in 1.7 min, then isocratic at 94% B for 0.7 min and a re-equilibration at 25% B for 0.9 min. The column used was a Luna C18, 50 mm × 2 mm at 50 °C. The flow rate was 1 mL/min, and the injection volume used was 25 μL. Approximate retention times were 1.8 min for CIC-M1 and its internal standard (D11-CIC-M1), 1.9 min for FP (evaluated with D11-CIC-M1 as the internal standard), and 2.4 min for CIC and its internal standard (D11-CIC).

2.6. Mass spectrometric conditions

An APPI source was used in negative ion mode at −2.3 kV on an API 3000. The auxiliary gas was set at 400 °C, the nebulizer gas was set at 15 device units, and the curtain gas was set at 10 device units. Lamp gas was set at 4 device units. MRM transitions of the acetic acid adducts of the analytes were 529.4 → 357.2 m/z for CIC-M1, 540.4 → 357.2 m/z for D11-CIC-M1, 559.4 → 413.2 m/z for FP, 599.4 → 339.2 m/z for CIC, and 610.4 → 339.2 m/z for D11-CIC.

2.7. Method validation

The analytical method was validated in three batches (including demonstration of linearity, accuracy, precision, specificity, recovery and LLOQ). A minimum of one set of calibration curves and five sets of quality control samples were analysed within these three different batches.

2.8. Method linearity

The calibration range was from 10.0 to 1000 pg/mL for CIC and from 10.0 to 1001 pg/mL for both CIC-M1 and FP in human serum. The coefficient of variation (CV) was defined as <15% for precision, and the accuracy target was <±15%. However, at LLOQ level, 20% was acceptable for both precision and accuracy. If the calibration curve was rejected, the batch also had to be rejected. A linear regression was used with a weighting factor of $1/x$, and the coefficient of correlation (R) had to achieve a degree of certainty (R^2) of $R^2 > 0.98$, i.e. R^3 0.99.

2.9. Precision and accuracy

Five replicates of quality control samples were each analysed at three concentration levels. Quality control samples were prepared at three concentrations (≤3× LLOQ, mid-range and at least 80% of the highest calibration concentration) and were incorporated into each sequence (as a minimum in triplicate). A sequence was either accepted or rejected according to the results of the QC samples. At least six of the nine QC samples had to be within ±15% of their respective nominal values; three of the nine QC samples (but not at the same concentration) were allowed to be outside ±15% of their respective nominal values.

If a batch did not adhere to these criteria, it was rejected.

2.10. Specificity

At least six specificity samples were analysed with and without internal standards in one validation batch.

The signal response ratios of possible interferences between the analyte and the IS were correlated with those of Std. 1 (at LLOQ level). Their quotients had to be <1/3 for at least five of six specificity samples, or else specific measures were taken.

The signal-responses of possible interferences of the IS measured in samples without IS were correlated with those of the respective sample with IS. The resulting quotients had to be <5% for at least five of six specificity samples, or else specific measures were taken.

2.11. Stability

All three analytes were tested with regard to stability at −20 °C for up to 18 months. Also stability of all three analytes in serum was tested for 4 h at room temperature (about 25 °C), and three freeze/thaw cycles in serum were performed.

Stability data with regard to stability under auto-sampler conditions, as well as freeze and thaw after sample preparation, were assessed. The criterion for all stability measurements was 85–115% for mean accuracy.

2.12. Recovery

The recovery values of the analytes were calculated at each level by the following formula:

$$\text{mean}\left(\frac{\text{area analyte QC samples}}{\text{mean(area analyte non-matrix samples)}}\right)$$

Overall recovery was calculated by taking the mean of the recoveries of each of the three levels. Non-matrix samples were spiked at the same concentration level as the analytes found in QC samples after sample preparation for analysis, and were analysed in triplicate at each of these three levels. These non-matrix samples were analysed without sample preparation. QC samples were analysed five times, at three levels, as standard.

2.13. Interference check of CIC and CIC-M1 with regard to FP, and vice versa

If the mean concentrations of spiked QC samples were within 85–115% of the corresponding mean of the regular QC-samples, then the drug was considered not to have had any impact on the quantitation of the other two analytes of interest. For this purpose,

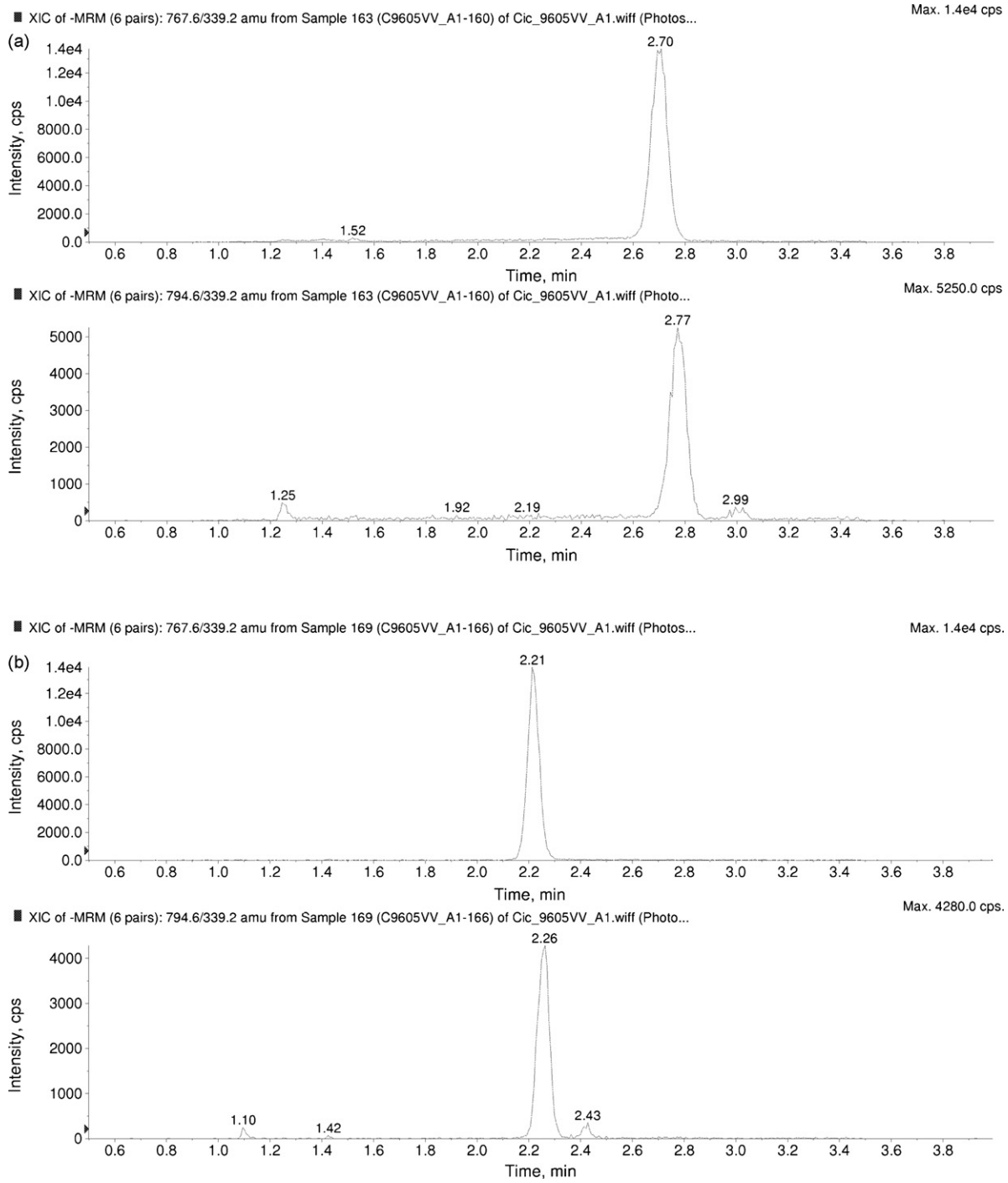

Fig. 2. (a) Two other ciclesonide metabolites (fatty acid conjugates) with a far more disturbed baseline (by about a factor of 5) when using toluene at 0.05 mL/min pumped into the auxiliary gas line by an external pump (about 10% of mobile phase flow). (b) The same compounds, using 10% acetone added into the mobile phase.

CIC and CIC-M1 were spiked at 851 pg/mL to human serum whereas FP was spiked at only 25.1 pg/mL. Another sample was spiked with FP at 851 pg/mL to human serum whereas CIC and CIC-M1 were spiked at only 25.1 pg/mL.

QC-F and QC-G: the signal response ratios of possible interferences between the analyte (CIC and CIC-M1; FP) and the interference drug were correlated with that of Std. 1 (at LLOQ level). Their quotients had to be <1/3. QC-F was spiked with FP only at 850 pg/mL whereas QC-G was spiked with CIC and CIC-M1 at 850 pg/mL of human serum.

3. Results and discussion

3.1. Method development

Recent advances in the development of ionisation techniques, such as atmospheric pressure photo ionisation (APPI) mass spectrometry (MS), have made possible specific detection of particularly lipophilic drugs in serum and plasma at low concentrations. CIC, its active metabolite CIC-M1, or FP were determined after inhalation of low amounts (160 or 320 μg of CIC and 250 or 500 μg of FP,

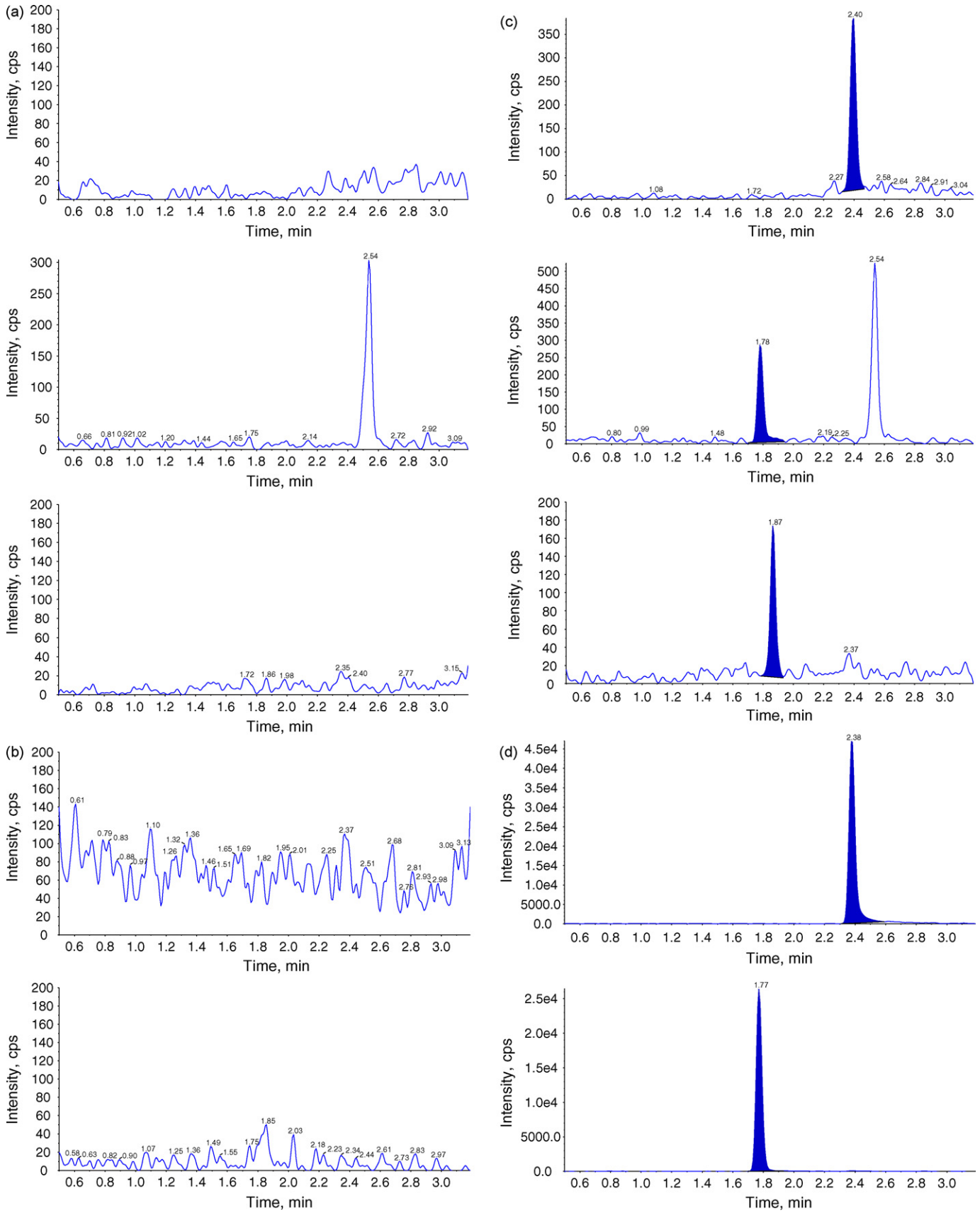

Fig. 3. (a, b) Blank sample without internal standards; (c, d) chromatograms serum Std. 1 at 10.0 pg/mL per analyte; (e, f) chromatograms serum Std. 8 at 1000 pg/mL CIC and 1001 pg/mL for both CIC-M1 and FP, top down chromatograms of: CIC, CIC-M1, FP; D11-CIC, D11-CIC-M1).

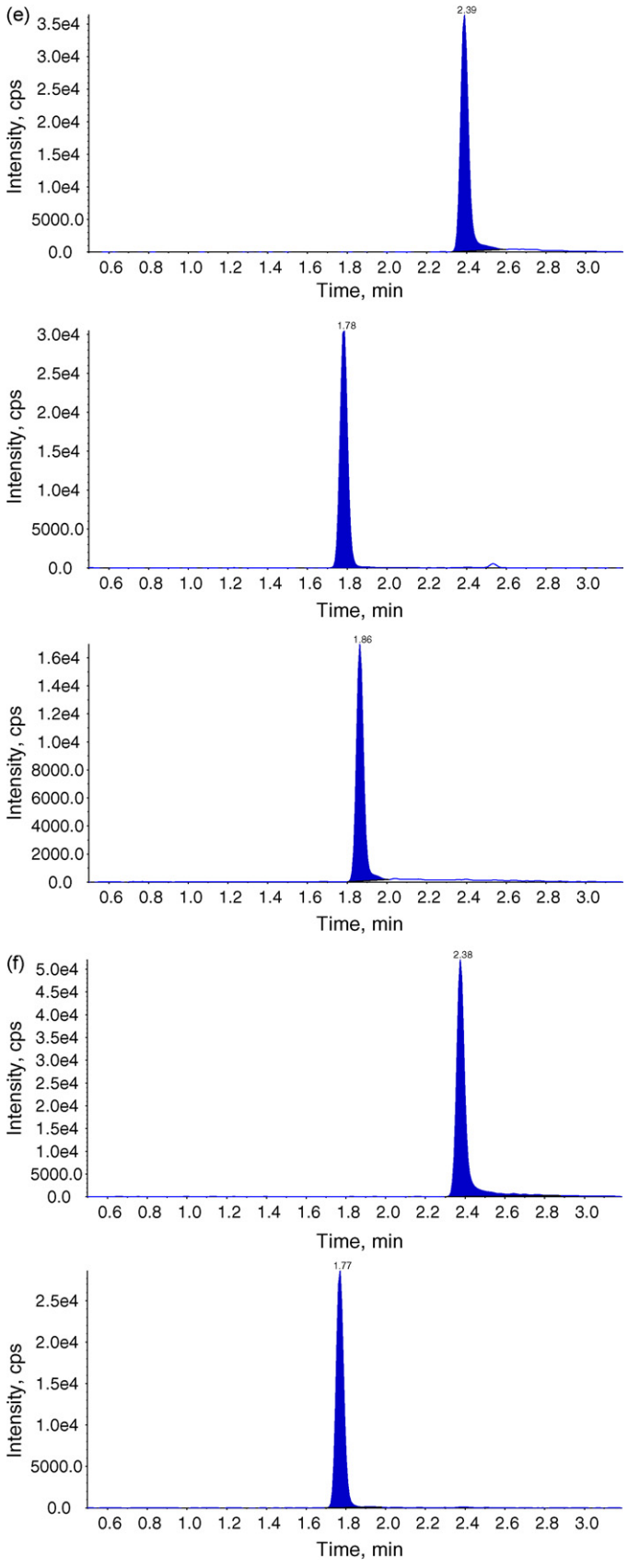

Fig. 3. (*Continued*).

Table 1
Linear regression parameters and carryover of CIC in human serum

Intercept	Slope	*R*	Cal. range (pg/mL)	*n* of Stds.	w.F.	Carryover (%)
0.00484	0.000671	0.9998	10.0–1000	8	1/*x*	0.56
0.000676	0.000714	0.9991	10.0–1000	8	1/*x*	0.19
0.00179	0.000669	0.9992	10.0–1000	8	1/*x*	0.22

Table 2
Linear regression parameters and carryover of CIC-M1 in human serum

Intercept	Slope	*R*	Cal. range (pg/mL)	*n* of Stds.	w.F.	Carryover (%)
0.00177	0.00102	0.9992	10.0–1001	8	1/*x*	0.00
0.00111	0.00107	0.9997	10.0–1001	8	1/*x*	0.00
0.00166	0.00108	0.9999	10.0–1001	8	1/*x*	0.00

Table 3
linear regression parameters and carryover of FP in human serum

Intercept	Slope	*R*	Cal. range (pg/mL)	*n* of Stds.	w.F.	Carryover (%)
0.00574	0.000446	0.9985	10.0–1001	8	1/*x*	0.84
0.000222	0.000604	0.9999	10.0–1001	8	1/*x*	0.00
0.000361	0.000573	0.9997	10.0–1001	8	1/*x*	0.00

respectively) in the course of a clinical study on asthma treatment. Experiments with more frequently used ESI and APCI ionisation techniques for an API 3000 or an API 4000 (both Sciex, Canada) showed insufficient detection limits. Changing the ion source to APPI (for an API 3000) permitted a sensitivity gain of about factor 5. One remarkable discovery is that with APPI, the crucial aspect is the selection of gas-flows and the correct method of adding the dopant for charge-transfer. This means that usually, under optimised conditions, the baseline is extremely low compared to ESI and APCI, even though the signals may be comparable. Addition of the dopant acetone into the mobile phase at about 10% was the best way of providing the charge-bearing transmitter into the ionisation region. Adding the dopant directly into the gas supply of the source usually made results worse, irrespective of whether acetone, toluene or another dopant was used. At the time of method development, no APPI source for an API 4000 was available which could provide even better sensitivity. Sample clean-up was effected with a very efficient liquid–liquid extraction for these relatively lipophilic substances using diisopropylether. However, reconstitution after evaporation of extraction solvent was a problem, with low and varying rates of recovery. Addition of a small amount of DMSO before evaporation eliminated these difficulties. FP was evaluated with the same internal standard as CIC-M1, without difficulty.

3.2. Dopant delivery

Sensitivity was tested under different conditions (external vs. internal dopant delivery). The best sensitivity (signal-to-noise ratio) was obtained after adding the dopant acetone directly into the mobile phase (10%). Fig. 2a and b shows comparable signal heights of the same sample injected twice for two other ciclesonide metabolites (fatty acid conjugates) with a far more disturbed baseline (by about a factor of 5) when using toluene at 0.05 mL/min pumped into the auxiliary gas line by an external pump (about 10% of mobile phase flow, Fig. 2a) instead of using 10% acetone added into the mobile phase (Fig. 2b). With these two substances, we undertook more systematic testing on dopants. This knowledge was applied to the three analytes in a short-test run, and yielded a comparable increase in sensitivity. Acetone has comparable elution power to acetonitrile. Previous experiments with acetone, toluene, cyclohexane and several other proton-transferring solvents showed no significant difference between them with regard to ionisation yield.

3.3. Precision, accuracy and linearity

The calibration curve was linear in the range from 10.0 to 1000 pg/mL for CIC, and from 10.0 to 1001 pg/mL for both CIC-M1 and FP in human serum (Fig. 3: blank sample without internal standards; chromatograms serum Std. 1 at 10.0 pg/mL per analyte; chromatograms serum Std. 8 at 1000 pg/mL CIC and 1001 pg/mL for both CIC-M1 and FP, top down: CIC, CIC-M1, FP; D11-CIC, D11-CIC-M1). Regression parameters are presented in Tables 1–3. The intra-batch precision (CV) and the intra-batch accuracy (with reference to the mean value) of the quality control samples in human serum are presented in Table 4. The respective inter-batch data are presented in Table 5. LLOQ summary data are presented in Table 6.

Several hundred human serum samples derived from clinical studies have been analysed with this method.

3.4. Specificity

The specificity of the method was determined by screening six samples of both blank human serum (no analytes added and no internal standards during sample preparation) and zero human serum (no analytes added, internal standards added during sample preparation), taken from different individuals. This was done to show chromatographic differences between different individuals, and interferences between chromatographic areas of analytes com-

Table 4
Intra-batch precision (CV) and accuracy

Batch no.	QC-A1 (at 25.0 pg/mL)			QC-B1 (at 115 pg/mL)			QC-C1 (at 850 pg/mL)		
	Mean (pg/mL)	CV (%)	Accuracy (%)	Mean (pg/mL)	CV (%)	Accuracy (%)	Mean (pg/mL)	CV (%)	Accuracy (%)
Ciclesonide									
1	27.3	10.02	109.2	113	5.96	98.2	856	2.65	100.7
2	27.4	5.89	109.7	112	1.89	97.5	812	1.82	95.5
3	27.9	4.16	111.5	117	2.44	102.1	882	2.18	103.8
Batch no.	QC-A1 (at 25.0 pg/mL)			QC-B1 (at 115 pg/mL)			QC-C1 (at 851 pg/mL)		
	Mean (pg/mL)	CV (%)	Accuracy (%)	Mean (pg/mL)	CV (%)	Accuracy (%)	Mean (pg/mL)	CV (%)	Accuracy (%)
Ciclesonide-M1									
1	26.8	5.66	107.1	121	3.62	105.5	865	0.49	101.5
2	25.2	9.31	100.7	119	3.82	103.1	862	3.74	101.2
3	24.7	6.84	98.6	121	4.21	105.4	875	2.75	102.7
Fluticasone propionate									
1	26.6	10.58	106.1	116	3.53	100.6	868	1.92	101.9
2	25.5	7.89	101.7	112	10.50	97.8	849	2.23	99.7
3	23.4	5.69	93.7	123	6.19	107.1	880	1.76	103.3

Table 5
Inter-batch precision (CV) and accuracy of three validation batches

QC-A1 (at 25.0 pg/mL)			QC-B1 (at 115 pg/mL)			QC-C1 (at 850 pg/mL)		
Mean (pg/mL)	CV (%)	Accuracy (%)	Mean (pg/mL)	CV (%)	Accuracy (%)	Mean (pg/mL)	CV (%)	Accuracy (%)
Ciclesonide								
27.5	6.78	110.0	114	4.15	99.3	850	4.08	100.0
QC-A1 (at 25.0 pg/mL)			QC-B1 (at 115 pg/mL)			QC-C1 (at 851 pg/mL)		
Mean (pg/mL)	CV (%)	Accuracy (%)	Mean (pg/mL)	CV (%)	Accuracy (%)	Mean (pg/mL)	CV (%)	Accuracy (%)
Ciclesonide-M1								
25.6	7.74	102.1	120	3.77	104.7	867	2.57	101.8
Fluticasone propionate								
25.1	9.61	100.4	117	7.76	101.8	865	2.38	101.6

Table 6
Summary inter-batch results of LLOQ samples of three validation batches

Analyte	Exp. conc. (pg/mL)	Mean calc. conc. (pg/mL)	CV (%)	Mean accuracy (%)
CIC	10.0	9.75	13.26	97.4
CIC-M1	10.0	10.7	11.45	107.3
FP	10.0	10.6	12.94	106.0

pared to those of internal standards. The specificity criteria were fully met for all three analytes.

3.5. Stability

All three analytes are stable in human serum for at least 18 months at below −20 °C. Stability of all three analytes in serum is at least 4 h at room temperature (about 25 °C). Three freeze/thaw cycles in serum can be performed.

Samples may be frozen in injection solution before analysis. All three analytes were stable for at least 20 h under auto-sampler conditions (about 25 °C).

The criterion for all stability measurements was 85–115% for mean accuracy, and this criterion was met.

3.6. Recovery

Recovery was found to be 100.0% for CIC and 108.4% for metabolite 1. Recovery for internal standards was 114.5% for IS of CIC and 127.9% for IS of metabolite 1. This unexpectedly high recovery is due in part to slight evaporation of the organic solvent acetone (injection solution consisted of 50% DMSO/50% acetone) in quality control samples during sample cleanup. This had no impact on the reliability of any of the presented data, because of internal standardisation (for recovery calculations, no internal standard was used for evaluation). Recovery was found to be 84.3% for FP.

3.7. Interference check

Interference of FP regarding CIC and CIC-M1 and vice versa was determined in one validation batch. This was performed at the highest QC level for CIC and CIC-M1 (or FP, respectively) and at the lowest QC level for the interfering drug. This was also done for zero samples containing only CIC and CIC-M1 or FP. CIC and CIC M1 had no impact on the determination of FP, and vice versa.

3.8. Example of pharmacokinetic figures

A representative pharmacokinetic profile of the three analytes after inhalation of CIC and FP is shown in Fig. 4 (log/lin diagram).

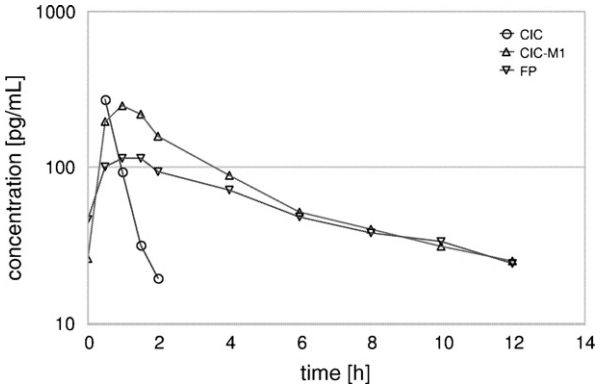

Fig. 4. A representative pharmacokinetic profile of the three analytes after inhalation of CIC and FP (log/lin diagram).

4. Conclusion

Quantitation of ciclesonide, ciclesonide-M1 and fluticasone propionate in samples derived from pharmacokinetic studies after inhalative administration of CIC and/or of FP, respectively, required a very sensitive method (down to 10 pg/mL serum; 0.5 mL serum used). Hardly any analytical methods for determining CIC and its active metabolite CIC-M1 in human serum have been published in the literature. Results show that all three analytes can be quantified with a LLOQ of 10 pg/mL in serum. This has been achieved by the combination of a very efficient and selective sample clean-up with a sensitive MS/MS instrument (API 3000) in conjunction with atmospheric pressure photo ionisation by using 10% acetone as the dopant which was added into the mobile phase. Adding the dopant to the mobile phase in this way, instead of introducing it directly into the source by means of an external HPLC pump, is a new approach which leads to reduced noise and therefore to a better signal-to-noise ratio. As this is a relatively new ionisation technique, compared to electrospray ionisation and atmospheric pressure chemical ionisation, further improvements may be expected in methods for lipophilic substances using APPI.

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