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Short communication

Development and validation of a combined liquid chromatography tandem-mass spectrometry assay for the quantification of aprepitant and dexamethasone in human plasma to support pharmacokinetic studies in pediatric patients

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ABSTRACT

A pharmacokinetic study was set up to investigate the pharmacokinetics of the anti-emetic agents aprepitant and dexamethasone and the drug-drug interaction between these drugs in children. In order to quantify aprepitant and dexamethasone, a liquid chromatography-tandem mass spectrometry assay was developed and validated for the simultaneous analysis of aprepitant and dexamethasone. Protein precipitation with acetonitrile-methanol (1:1, v/v) was used to extract the analytes from plasma. The assay was based on reversed-phase chromatography coupled with tandem mass spectrometry detection operating in the positive ion mode. The assay was validated based on the guidelines on bioanalytical methods by the US Food and Drug Administration and European Medicines Agency. The calibration model was linear and a weighting factor of 1/concentration^2 was used over the range of 0.1–50 ng/mL for aprepitant and 1–500 ng/mL for dexamethasone. Intra-assay and inter-assay bias were within ±20% for all analytes at the lower limit of quantification and within ±15% at remaining concentrations. Dilution integrity tests showed that samples exceeding the upper limit of quantification can be diluted 100 times in control matrix. Stability experiments showed that the compounds are stable in the biomatrix for 25 h at room temperatures and 89 days at −20 °C. This assay is considered suitable for pharmacokinetic studies and will be used to study the drug-drug interaction between aprepitant and dexamethasone in pediatric patients.

1. Introduction

Aprepitant is a selective neurokinin-1 receptor antagonist approved for the prevention of chemotherapy-induced nausea and vomiting (CINV) in adults and pediatric patients from the age of 6 months and for the prevention of postoperative nausea and vomiting in adults.[1] For the prevention of CINV, aprepitant is co-administered with the corticosteroid dexamethasone. Pharmacokinetic (PK) studies in adults have shown a drug-drug interaction between aprepitant and dexamethasone. The area under the plasma concentration time curve (AUC) of dexamethasone increases approximately 2-fold when co-administered with aprepitant[2–4] and hence according to the product information of aprepitant the dexamethasone dose needs to be reduced by 50% when administered together with aprepitant[1]. In children this interaction has not been studied thoroughly.

Aprepitant has been shown to be effective for the prevention of CINV

Abbreviations: AUC, area under the plasma concentration time curve; CINV, chemotherapy-induced nausea and vomiting; CV, coefficient of variation; IS, internal standard; LC-MS/M, liquid chromatography tandem-mass spectrometry; LLOQ, lower limit of quantitation; MF, matrix factor; MRM, multiple reaction monitoring; P, pharmacokinetic(s); QC, quality control; ULOQ, upper limit of quantification.

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in pediatric patients as an adjuvant to ondansetron and dexamethasone [5,6]. However, the complete antiemetic response to triple therapy in children is lower than in adults (approximately 50% and 70–80%, respectively)[7–9], which could be due to an incorrect dose of dexamethasone. It is possible that the influence of aprepitant on the PK of dexamethasone is different in children than in adults. To study the PK of aprepitant and dexamethasone and the drug-drug interaction between these drugs in children, a PK study has been set up.

PK studies in children require a sensitive assay to quantify aprepitant and dexamethasone as the volume of blood available is limited. In addition, a simultaneous quantification is preferred to reduce the amount of blood even more. Several methods to quantify aprepitant or dexamethasone plasma concentrations have been published[11–17], but no simultaneous analysis has been described hitherto. The previously developed methods for quantification of dexamethasone use 50–200 μL human plasma and could be suitable for quantification of dexamethasone in children[14–17], however, for quantification of aprepitant, plasma volumes up to 1 mL were needed[11,12], which is unacceptable in the pediatric population.

The aprepitant quantification method of Wu et al. was used a starting point for the development of a combined liquid chromatography tandem-mass spectrometry (LC-MS/MS) assay for the quantification of aprepitant and dexamethasone[13]. The development and validation of that combined LC-MS/MS assay in small sample volumes from pediatric oncology patients is described here. Its clinical applicability is demonstrated with the analysis of samples of children with cancer undergoing chemotherapy, treated with dexamethasone with or without aprepitant as anti-emetics.

2. Materials and methods

2.1. Chemicals and reagents

Aprepitant (≥98%) and $^{2}H_4$-aprepitant (≥98%, 97.6% $^{2}H_4$) were obtained from Toronto Research Chemicals (North York, ON, Canada). Dexamethasone (100%) was obtained from Sigma Aldrich (Zwijndrecht, the Netherlands) and $^{2}H_4$-dexamethasone (≥98.3%, 97.6% $^{2}H_4$) from Alsachim (Illkirch Graffen-staden, France). Acetonitrile, formic acid, methanol, isopropyl alcohol and water originated from Biosolve Ltd (Valkenswaard, The Netherlands). K_2EDTA plasma was obtained from BioreclamationIVT LLC (Hicksville, NY, USA). The chemical structures of the analytes are depicted in Fig. 1.

2.2. Stock solutions and working solutions

Stock solutions were prepared at a concentration of 1 mg/mL in methanol for aprepitant and dexamethasone. The stock solutions were diluted with methanol to obtain working solutions, containing both analytes. For aprepitant the calibration working solutions were prepared at concentrations of 2, 5, 20, 50, 200, 500, 800, 1,000, 10,000 and 100,000 ng/mL and at concentrations of 20, 50, 200, 500, 2,000, 5,000, 8,000, 10,000 and 100,000 ng/mL for dexamethasone. Two independent stock solutions and working solutions were prepared to spike the calibration standards and quality control (QC) samples. The QC stock- and working solutions were prepared at concentrations of 2, 6, 100, 700, 10,000 and 100,000 ng/mL for aprepitant and at concentrations of 20, 60, 1,000, 7,000 and 100,000 ng/mL for dexamethasone. Stock solutions (1,000 ng/mL) and a working solution (1 ng/mL) containing both internal standards (IS) were prepared in methanol. All stock- and working solutions were stored at −70 °C

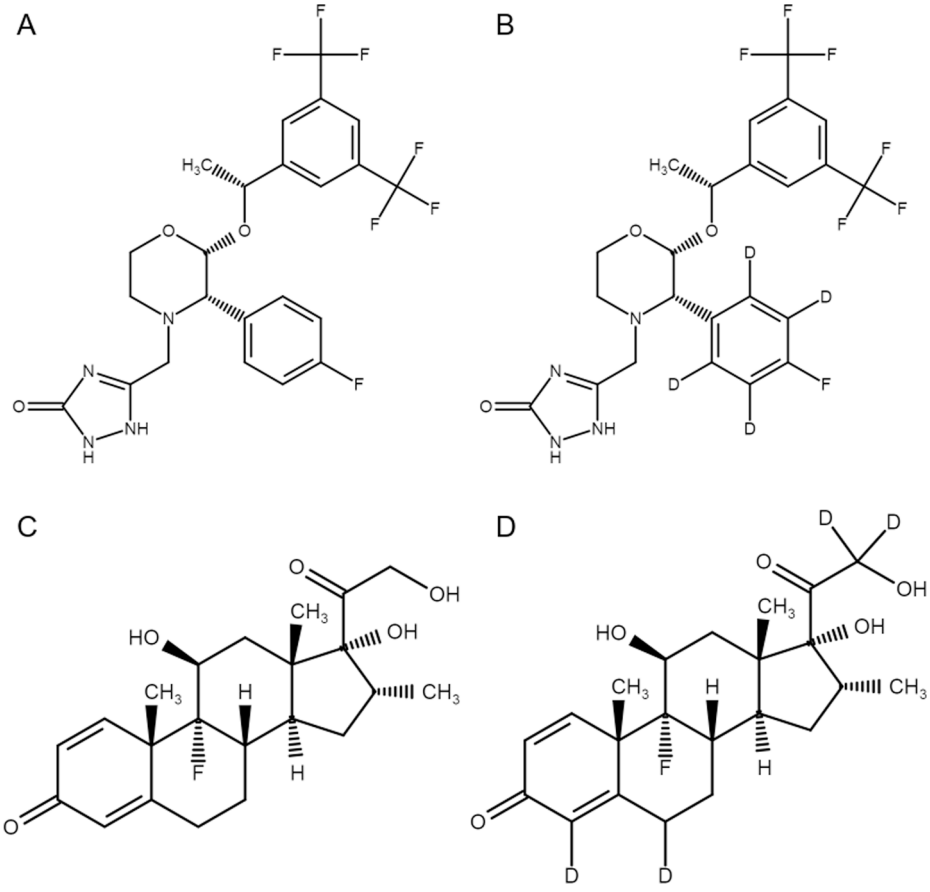

Fig. 1. Chemical structures of aprepitant (A), $^{2}H_4$-aprepitant (B), dexamethasone (C) and $^{2}H_4$-dexamethasone (D).

2.3. Calibration standards, quality control samples

A volume of 380 μL control human K_2EDTA plasma was spiked by 20 μL of the working solution to obtain the calibration standards in the range of 0.1 to 50 ng/mL for aprepitant and 1.0 to 500 ng/mL for dexamethasone. QC samples were prepared in control human K_2EDTA plasma at the lower limit of quantitation (LLOQ), at 3 times the LLOQ (LOW), at approximately midway between the high and low QC samples (MID) and at 75 to 90% of the highest calibration standard (HIGH). The QC samples were prepared by spiking either 150 or 300 μL of the separately prepared working solutions to either 2850 or 5700 μL control human K_2EDTA plasma (depending on the amount of volume needed) to obtain the final QC LLOQ, LOW, QC MID and QC HIGH concentrations. Both calibration standards and QC samples were subsequently stored in aliquots of 100 μL at −20 °C. Back-calculated concentrations of the calibration standards were used for the determinations of the linearity of the calibration model, using the reciprocal of the squared analyte concentrations ($1/x^2$) as the weighting factor.

2.4. Sample preparation

A maximum of 7 whole blood samples of 1 mL were collected from each patient treated with dexamethasone with or without aprepitant. Directly after collection, samples were centrifuged for 5 min at 2000g at room temperature. Thereafter, plasma was obtained and stored at −70/-80 °C until analysis. Before sample pretreatment, samples were thawed at room temperature and vortex-mixed for 10 s. To 100 μL of plasma, a volume of 10 μL IS working solution was added, except for the double blank samples. A volume of 200 μL acetonitrile-methanol (1:1, v/v) was used for protein precipitation (PP) to extract the analytes from plasma. Samples were vortex-mixed for 10 s and centrifuged at 21,500g for 3 min at room temperature. The supernatant was transferred to an autosampler vial with insert.

2.5. Liquid chromatography-mass spectrometry equipment and conditions

An Acquity I class UPLC system with binary pump, integrated degasser, column oven and I class autosampler were used (Waters, Milford, MS, USA). The temperature of the autosampler and column were kept at 8 °C and 40 °C, respectively. Mobile phase A consisted of 0.1% (v/v) formic acid in water and mobile phase B consisted 0.1% (v/v) formic acid in acetonitrile. Gradient elution was applied at a flow of 300 μL/min through an Acquity UPLC BEH C18 column (50 × 2.1 mm ID, 1.7 μm particle size) with an additional Acquity UPLC BEH C18 guard column (5 × 2.1 mm ID, 1.7 μm particle size) (both Waters). The applied gradient program was 30% B (0–1.0 min); 30–98% B (1.0–2.5 min); 98% B (2.5–4.0 min); 98–30% B (4.0–4.01 min); 30% B (4.01–6.0 min).

A QTRAP5500 triple quadrupole mass spectrometer (MS) equipped with a turbo ion spray interface, operating in positive ion mode was used (Sciex, Framingham, MA, USA). Multiple reaction monitoring (MRM) chromatograms were acquired and processed using Analyst™ software (Sciex, version 1.6.2). The MS operating parameters are summarized in Table 1.

2.6. Validation procedures

The validation of the assay was conducted in compliance with the most recent edition of the OECD Principles of Good Laboratory Practice, and based on the FDA and EMA guidelines on bioanalytical method validation[18–20].

2.7. Ethical considerations

In compliance with ethical standards, patients were included after written informed consent was acquired. Ethical approval by the institutional Medical Ethics Committee of the Erasmus Medical Center was obtained under protocol number 2018–1578.

3. Results and discussion

3.1. Method development

Starting point of the method development was the aprepitant method of Wu et al[13]. Detector settings for all compounds were established by infusion of both analytes and internal standards. As organic component of the mobile phase, acetonitrile proved to have a far lower background than methanol. Variations in gradient compositions were tested to optimize elution times, peak shapes and the separation of the analytes from interferences. Subsequently sample processing was tested and protein precipitation with 200 μL acetonitrile-methanol (1:1, v/v) proved to give the lowest variation and best signal to noise. Chromatograms of a double blank sample, blank sample, plasma sample spiked at QC MID levels, blank patient sample and patient sample are shown in Fig. 2. The method was developed using a small sample volume of 100 μL human plasma. The resulting method proved to be linear from 0.1 to 50 ng/mL for aprepitant and from 1 to 500 ng/mL for dexamethasone.

3.2. Method validation

3.2.1. Accuracy and precision

In order to assess intra- and inter-assay accuracies and precisions, five replicates of QC samples were analyzed in three analytical runs at the LLOQ, 3 times the LLOQ, midrange and high concentrations. The accuracy (bias) was determined as relative difference between the mean measured concentration (per run for intra-assay bias and overall for inter-assay bias) and the nominal concentration and coefficient of variation (CV, %) were used to assess the intra-run precision. Analysis of Variance (ANOVA) was applied to assess the inter-run precision. The

Table 1
Mass spectrometry settings for the analytes and their internal standards.

Parameter					
Run duration	6.00 min				
Ion spray voltage	4000 V				
Collison gas	8 au				
Curtain gas	30 au				
Temperature	500 °C				
Dwell time	75 ms				
Specific Parameters Analyte	Parention (*m/z*)	Production (*m/z*)	Collison energy (V)	Collision exit potential (V)	Declustering potential (V)
Aprepitant	535.3	277.2	25	8	61
$^2H_4$-Aprepitant	539.0	281.1	27	10	86
Dexamethasone	393.0	355.2	17	12	96
$^2H_4$-Dexamethasone	397.0	359.3	17	6	116

Au: arbitrary units

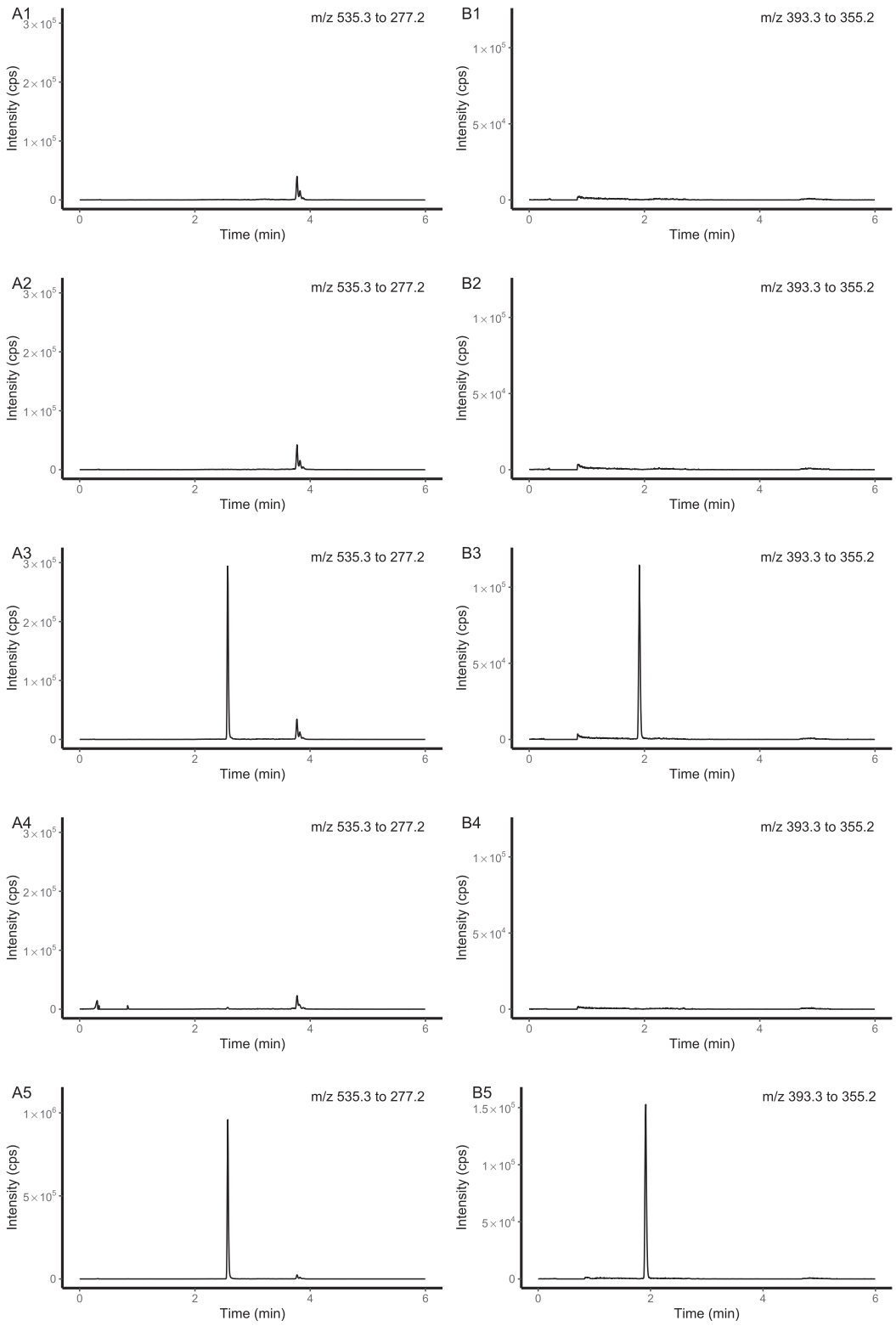

Fig. 2. Chromatograms of aprepitant (A-series) and dexamethasone (B-series) for a double blank sample (1), blank sample (2), plasma sample spiked at QC MID levels (3) (aprepitant: 5 ng/ml, dexamethasone: 50 ng/ml), blank patient sample (4) and patient sample (5) (aprepitant: 1590 ng/mL (diluted 100-fold), dexamethasone: 90.4 ng/mL). The traces of the internal standards are not shown (compounds co-eluted with the analytes).

inter- and intra-assay accuracy and precision were $\leq$ 20% for the LLOQ and $\leq$ 15% for the other concentrations and thus within the acceptance criteria for all analytes. Details on the assay performance data are listed in Table 2.

3.2.2. *Dilution integrity*

High and variable concentrations in patient samples were expected for aprepitant based on previously published pharmacokinetic studies [21]. In order to extend the range for both analytes, a 100-fold dilution factor was validated. Five replicates of a plasma sample spiked with

Table 2
Assay performance data for the analysis of aprepitant and dexamethasone, assessed in 3 different analytical batches, tested at 4 concentration levels analyzed in 5-fold.

Analyte	Nominal concentration (ng/mL)	Intra-assay		Inter-assay	
		Bias (%)	CV, %	Bias (%)	CV, %
Aprepitant	0.100	Within ± 4.2	Within + 7.5	+2.3	+2.1
	0.300	Within ± 7.7	Within + 3.8	+4.0	+3.8
	5.00	Within ± 5.3	Within + 4.2	+3.6	+0.9
	35.0	Within ± 8.5	Within + 1.4	+5.5	+3.3
Dexamethasone	1.00	Within ± 4.6	Within + 9.4	+0.9	+1.8
	3.00	Within ± 6.0	Within + 1.9	+4.4	+1.6
	50.0	Within ± 3.4	Within + 3.8	+2.2	- [a]
	350	Within ± 5.8	Within + 1.6	+3.0	+3.4

[a] No significant additional variation was observed due to the performance of the assay in different analytical runs (mean square within groups is larger than mean square between groups).

aprepitant and dexamethasone concentrations above the upper limit of quantification (ULOQ) (10,000 and 1000 ng/mL, respectively), were 100-fold diluted in control human plasma, prior to sample pretreatment. Bias and CV for aprepitant were −0.1% and 3.4%, respectively. For dexamethasone, bias was 0.4% and CV was 1.4%, within the requirements of ± 15% and a CV ≤ 15%.

3.2.3. Carry-over

The carry-over was determined in three analytical runs and no analyte peaks or IS peaks were observed in the first blank sample injected after an ULOQ sample. As a result the carry-over was considered acceptable (≤20% of the analyte peak area of the LLOQ sample and ≤ 5% of the peak area of the IS).

3.2.4. Specificity and selectivity

The selectivity of the method was determined by the analysis of six different batches of control human plasma. Double blank samples and LLOQ samples of each batch were processed and analyzed. The mean measured concentrations at LLOQ level were ± 9.0% for aprepitant and ± 16.1% for dexamethasone (requirement: within ± 20% of the nominal concentrations) and no interferences were detected at the retention times for the analytes or IS. The cross analyte/IS interferences were determined by separately spiking the analytes and IS to control human plasma at their ULOQ levels and IS levels, respectively. The interferences from aprepitant, dexamethasone or IS at the other transitions were ≤ 20% of the peak area of the analytes at the LLOQ level and ≤ 5% of the peak area of the IS: 0.3% for dexamethasone in $^{2}H_4$-dexamethasone and 0% for all other components.

3.2.5. Matrix effect and recovery

Six batches of individual control human plasma at low and high concentrations in singular were prepared to determine the matrix effect. For both the analyte and IS, the matrix factor (MF) was calculated for each matrix lot by calculating the ratio of the peak area in the presence of matrix to the peak area in absence of matrix (working solution of the analyte). Furthermore, the IS normalized MF was calculated by dividing the MF of the analyte by the MF of the IS. At both tested QC concentration levels the CV of the IS-normalized matrix factor from the 6 batches ranged from 0.87 to 0.99 and CV was 3.0% for aprepitant and 5.2% for dexamethasone and were thus within the required ≤ 15% for both analytes.

The overall recovery was calculated by dividing the peak area of a processed sample by the peak area in absence of matrix. The overall recovery was 91.1 ± 5.2% and 90.7 ± 1.8% for aprepitant and its IS, respectively, and was 56.5 ± 2.0% and 61.7 ± 1.3% for dexamethasone and its IS, respectively.

3.2.6. Stability

The analytes were considered stable in the biomatrix when 85–115% of the nominal concentration was found. For stock solutions acceptance criteria of 95%-105% were applied. Plasma samples were stable for at least up to 25 h at 20–25 °C, 89 days at −20 °C and after three freeze (-20 °C)/thaw (20–25 °C) cycles for both analytes at QC LOW and HIGH levels. The processed samples were stable for at least 15 days at 2–8 °C. The stock solutions of dexamethasone were stable for at least 24 h at 20–25 °C. For aprepitant stock solutions, an increase in concentration of 5.6% was observed after 24 h at 20–25 °C, most likely explained by evaporation of solvent. The stock solutions of both analytes were stable at −70 °C for at least 8 months and after three freeze (-70 °C)/thaw (20–25 °C) cycles.

3.3. Clinical application

The assay was used to determine plasma concentrations of two pediatric patients receiving dexamethasone with aprepitant. The patients (patient 1: male, 8.9 years; patient 2: female, 16.4 years) were treated with 1dd 3 mg/kg aprepitant (with a maximum 125 mg on day 1) and 4dd 3 mg/m^2 dexamethasone. Samples were taken at six time points: 0.5, 2, 4, 6, 12 and 24 h after the first administration of the agents. Samples were processed as described in section 2.4. The plasma concentration time curves of two patients receiving both aprepitant and dexamethasone are displayed in Fig. 3.

To our knowledge, this is the first study to describe a LC-MS/MS assay to simultaneously quantify aprepitant and dexamethasone in small volumes of human plasma (100 μL). This makes it possible to simultaneously study the pharmacokinetics of aprepitant and dexamethasone for instance in the pediatric population using low sample volumes. In addition, this assay allows to determine plasma concentrations of aprepitant at a lower level than previously described in literature (LLOQ of 0.1 ng/mL compared to 1 or 10 ng/ml in human plasma) [11–13], which might enable quantification of aprepitant in alternative matrices like cerebrospinal fluid. Since this method was validated for a 100-fold dilution, it will be even possible to use lower samples volumes than 100 μL for the quantification of aprepitant, which makes this method even more suitable for the quantification of aprepitant in the pediatric population. Previous published methods on the quantification of aprepitant report higher upper limits of quantification (ULOQ = 1000 or 5000 ng/mL in human plasma)[11–13]. However, with this described method it is possible to successfully measure higher aprepitant plasma concentrations in human plasma samples by diluting the plasma.

4. Conclusion

We successfully developed a sensitive LC–MS/MS assay for the simultaneous quantification of aprepitant and dexamethasone in small volumes of pediatric human plasma. The validated linear assay ranges are 0.1–50 ng/mL for aprepitant and 1–500 ng/mL for dexamethasone. Stability showed that both analytes were stable in human K_2EDTA plasma at room temperature for longer than 24 h. This assay is considered suitable for pharmacokinetic studies and will be used to study the drug-drug interaction between aprepitant and dexamethasone in pediatric patients.

CRediT authorship contribution statement

A. Laura Nijstad: Conceptualization, Investigation, Visualization,

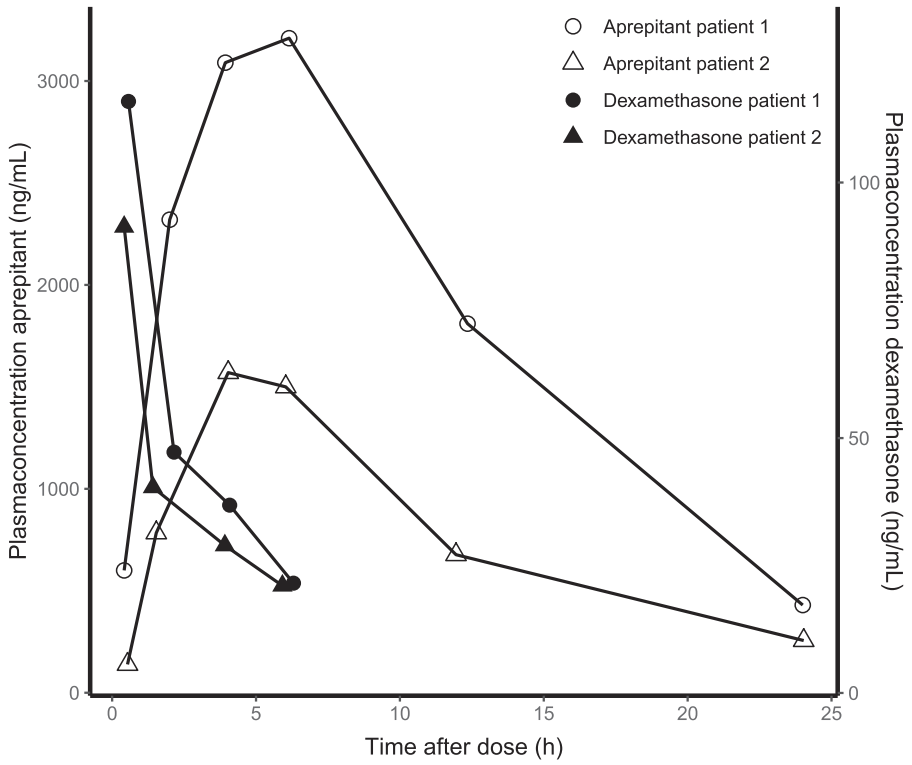

Fig. 3. Plasma concentration time curves of two patients treated with aprepitant (first day: 1dd 3 mg/kg, maximum 125 mg) and dexamethasone (4dd 3 mg/m^2).

Formal analysis, Writing - original draft. **Matthijs M. Tibben:** Investigation, Validation, Formal analysis, Writing - review & editing. **Abadi Gebretensae:** Investigation, Validation, Formal analysis, Writing - review & editing. **Hilde Rosing:** Methodology, Supervision, Project administration, Writing - review & editing. **Evelien Vos-Kerkhof:** Conceptualization, Writing - review & editing. **C. Michel Zwaan:** Conceptualization, Writing - review & editing. **Alwin D.R. Huitema:** Conceptualization, Writing - review & editing. **Jos H. Beijnen:** Conceptualization, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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