Determination of cefuroxime in human blood and urine using UHPLC-MS/MS and its application to stability study of cefuroxime over 278 days

Oznaczanie cefuroksymu w ludzkiej krwi i moczu metodą UHPLC-MS/MS i jej zastosowanie do badania stabilności cefuroksymu w ciągu 278 dni

Karolina Nowak[1,4], Marcin Zawadzki[2,4*], Paweł Szpot[3,4]

[1] **Department of Pharmacology, Faculty of Medicine, University of Opole, Poland**
Zakład Farmakologii, Wydział Lekarski, Uniwersytet Opolski

[2] **Department of Social Sciences and Infectious Diseases, Faculty of Medicine, Wroclaw University of Science and Technology, Poland**
Katedra Nauk Społecznych i Chorób Infekcyjnych, Wydział Medyczny, Politechnika Wrocławska

[3] **Department of Forensic Medicine, Faculty of Medicine, Wroclaw Medical University, Poland**
Katedra Medycyny Sądowej, Wydział Lekarski, Uniwersytet Medyczny we Wrocławiu

[4] **Institute of Toxicology Research, Borowa, Poland**
Instytut Ekspertyz Toksykologicznych, Borowa

Abstract

Purpose: Forensically, the widespread use of antibiotics necessitates methods for their detection in biological materials to ascertain their role in adverse reactions or fatalities. Given the need to conduct toxicological studies on materials stored for extended periods under various temperature conditions, research on antibiotic stability in biological matrices over such durations is crucial for accurate toxicological assessments.

Methods: The stability of cefuroxime in blood and urine was determined for 278 days at three different temperatures: -15°C, +4°C, +23°C. The analyses were conducted using ultra-high-performance liquid chromatography coupled with triple-quadrupole tandem mass spectrometry.

Results: The method met all validation requirements. This study also describes the results of the thermal stability of cefuroxime. Cefuroxime showed the greatest stability at -15°C and was highly unstable at room temperature (+23°C) in all types of studied biological matrices.

Conclusions: The study confirms instability of cefuroxime in blood and urine samples. Therefore, the analysis of this antibiotic in biological matrices for purposes such as forensic toxicology should be performed as soon as possible after sampling to avoid decline in concentration. In cases of prolonged material storage, the concentrations should be cautiously interpreted in the prepared expertise.

Keywords

cefuroxime; cephalosporins; long term stability; blood; urine; LC-MS/MS

Streszczenie

Cel: Z medyczno-sądowego punktu widzenia, powszechne stosowanie antybiotyków wymaga opracowania metod ich wykrywania w materiałach biologicznych, aby ustalić ich rolę w reakcjach niepożądanych lub zgonach. W kontekście przeprowadzania badań toksykologicznych na materiałach przechowywanych przez dłuższy czas w różnych warunkach temperaturowych, badania nad stabilnością antybiotyków w matrycach biologicznych przez takie długie okresy są kluczowe dla oceny toksykologicznej w takich przypadkach.

Metody: Stabilność cefuroksymu została określona w krwi i moczu przez 278 dni w trzech różnych temperaturach: -15°C, +4°C, +23°C. Analizy przeprowadzono przy użyciu ultra-wysokosprawnej chromatografii cieczowej sprzężonej z tandemową spektrometrią mas z potrójnym kwadrupolem.

Wyniki: Metoda spełniała wszystkie wymagania walidacyjne. W niniejszym badaniu przedstawiono również wyniki dotyczące stabilności termicznej cefuroksymu. Cefuroksym wykazał największą stabilność w temperaturze -15°C i był wysoce niestabilny w temperaturze pokojowej (+23°C) we wszystkich badanych matrycach biologicznych.

Wnioski: Badanie potwierdza niestabilność cefuroksymu w próbkach krwi i moczu, dlatego analizy tego antybiotyku w matrycach biologicznych, na przykład dla celów toksykologii sądowej, powinny być przeprowadzane jak najszybciej po pobraniu, aby uniknąć spadku stężenia, lub w przypadku długotrwałego przechowywania materiału, należy ostrożnie interpretować stężenia w przygotowanej ekspertyzie.

Słowa kluczowe

cefuroksym; cefalosporyny; długoterminowa stabilność; krew; mocz; LC-MS/MS

Background

Cefuroxime, (6*R*,7*R*)-3-(carbamoyloxymethyl)-7-[[(2*Z*)-2-(furan-2-yl)-2-methoxyiminoacetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, is a second-generation semisynthetic cephalosporin and a commonly prescribed antibiotic with a broad spectrum of activity. It can cause mild to severe immediate hypersensitivity reactions due to its molecular structure, which includes a beta-lactam ring linked to a sulfur-containing dihydrothiazine ring [1,2] Cefuroxime axetil, a pro-drug of cefuroxime, poses a challenge in allergic testing due to structural differences [3].

Antibiotics, particularly beta-lactams like cephalosporins, account for a significant proportion of drug-induced anaphylaxis cases [4]. Studies indicate that the prevalence rates of antibiotic allergies vary, ranging from 5% to 10% in children [5]. While cefuroxime is less commonly implicated in hypersensitivity reactions compared to other antibiotics, it still contributes to adverse drug reactions (ADRs), albeit with varying severity [6-9].

Hypersensitivity reactions to cefuroxime can occur suddenly, even in patients who previously tolerated the drug [10,11]. Adverse reactions range from mild eruptions to severe conditions, such as toxic epidermal necrolysis and anaphylactic shock [12-22]. Merget et al. [11] described the case of work-related occupational urticaria and anaphylaxis after inhalation of cefuroxime by a 53-year-old nurse. Exposure to the antibiotic was due to activities such as crushing tablets or preparing injections. Cefuroxime may also induce organ-specific adverse effects, including kidney and liver injury [23-26].

The use of cefuroxime during pregnancy can lead to adverse effects on the unborn child, as evidenced by cases of congenital fixed drug eruption [27].

Neurological manifestations, such as neurotoxicity and panic attacks, have also been reported following the use of cefuroxime [1,28,29]. In turn, Zahiruddin et al. [30] presented a case of psychosis induced by cefuroxime and metronidazole in a 35-year-old woman, who experienced such symptoms as auditory hallucinations and exhibited abnormal behavior. Additionally, a case of disulfiram-like reactions has been documented [31]. In a rural outpatient clinic, a man received an infusion of cefuroxime sodium and glucose in saline. During infusion the patient presented with sweating, weakness and facial flushing, followed by loss of consciousness. The patient died despite resuscitation efforts. The investigation indicated

that the man had consumed alcohol before he went to the clinic. The aforementioned cases – panic attack, psychosis and cefuroxime induced disulfiram-like reactions – can be mistakenly associated by physicians or police officers with illicit drugs or even new psychoactive substances.

Serious complications of cefuroxime administration include Kounis syndrome, which is characterized by acute coronary syndrome secondary to allergic reactions [2,32]. Forensically, the widespread use of antibiotics necessitates methods for their detection in biological materials to ascertain their role in adverse reactions or fatalities. The authors routinely examine biological material stored in refrigerators for several years. These limitations often restrict analyses to ethyl alcohol or substances routinely screened for, such as psychoactive substances, medications, and their metabolites, while excluding antibiotics. Additionally, new developments in a case after an extended period or the need to reassess results previously obtained by another laboratory can necessitate further analysis. Unfortunately, the authors also examine materials, such as blood, stored at room temperature for several months or longer. Furthermore, due to the lack of legal regulations in our country, biological material collected in hospitals is disposed of shortly after collection, which limits its use in toxicological studies. For this reason, in one case of suspected anaphylaxis following the administration of an antibiotic containing amoxicillin and clavulanic acid in a hospital, the authors were unable to analyse serum or plasma and had to examine organ fragments from the deceased, which were preserved in a formalin container. Another factor motivating these investigations was the death of a child suspected of dying after receiving a cefuroxime-containing preparation. Due to the sensitive nature of the case, the authors do not discuss it in detail. Blood was taken from the child during the autopsy and sent for toxicological analysis approximately one month later. The exhumation of the child's body was conducted about a month after death, during which biological fluids and organ fragments were secured for examination.

Considering the conduct of toxicological studies on materials stored for extended periods under various temperature conditions, research on antibiotic stability in biological matrices, especially whole blood, over such long periods is, in the authors' opinion, crucial for toxicological assessments in these cases.

Among the techniques used for the determination of antibiotics, including cefuroxime, are: capillary zone electrophoresis [33], micellar electrokinetic capillary chromatography [34], screen-printed potentiometric sensors [35], and liquid chromatography with various detectors, such as HPLC-UV-VIS, LC-MS, and LC-MS/MS [36-50].

Objectives

In light of data about the possible occurrence of anaphylaxis after cefuroxime administration, we decided to implement a method for the determination of cefuroxime and to study the stability of this drug in blood and urine at three different temperatures over 278 days.

Methods

Chemicals

Water (Chromasolv® LC–MS), acetonitrile (Chromasolv® LC–MS), methanol (Chromasolv® LC–MS), ethyl acetate, and formic acid were purchased from Sigma-Aldrich (Steinheim, Germany); ammonium formate was purchased from Sigma-Aldrich (Bangalore, India); cefuroxime sodium salt was purchased from Supelco (Bellefonte, PA, USA), and diazepam-d_5 was purchased from Cerilliant (Round Rock, TX, USA). Standard solutions of internal standard (IS) – diazepam-d_5, were prepared in methanol and a standard solution of cefuroxime was prepared in water. The working solutions of different concentrations were prepared by diluting the standard solution with methanol/water. The stock solution and standard solutions were stored at –20°C.

Biological materials

For the development and validation of the method, blank (drug-free) blood samples were obtained from a regional blood donation center. Blank urine samples were obtained from a healthy volunteer (one of the authors).

For the stability study, we used blank blood samples obtained from a regional blood donation center (with addition of CPD formula, containing i.a. citrate salts, Maco Pharma, Mouvaux, France), stored in glass tubes, and blood and urine samples obtained from a healthy volunteer (one of the authors). Blood from the volunteer was collected in 5-mL glass tubes containing sodium fluoride, NaF – 4 mg/mL, and sodium heparin – 28 IU/mL, BD Vacutainer, Plymouth, UK). The urine sample was stored without any preservatives, also in a glass tube. Cefuroxime sodium was mixed with all analysed matrices. Samples were stored at three different temperatures: -15°C, +4°C and +23°C. In order to examine cefuroxime stability, quantitative analyses were repeated in duplicates after 8 hours, and 1, 2, 3, 7, 14, 30, 60, 90, 182, and 278 days. The initial analysis concentration was 10,000 ng/mL for all matrices (blood with citrates, blood with NaF, and urine). The stability graphs were presented as exponential curves, and extrapolation of these curves was used to estimate the half-live for each sample.

In the case of urine samples, pH was additionally measured. On day 0, the pH was 6. After 30 days, urine samples across all three tested temperatures maintained a pH of 6. However, after 120 days, pH measurements were only taken for samples

Table I. Multiple reaction monitoring (MRM) conditions used in the UHPLC-ESI-MS/MS analysis of cefuroxime and internal standard;
[a] *ions selected for quantitative analysis*

Compound	Precursor Ion [m/z]	Product Ion [m/z]	Dwell (msec)	Q1 Pre Bias [V]	Collision Energy [V]	Q3 Pre Bias [V]	Retention time [min]
Cefuroxime	423.0 (neg)	207.1[a] 318.0 284.1	17.0	20 11 16	16 9 16	14 21 17	3.77
Diazepam-d_5	290.0 (pos)	198.1[a] 154.1 227.2	13.0	14 14 14	34 28 19	19 15 22	7.64

pos – positive mode, neg – negative mode

*Table II. Validation results; * n = 5*

Parameter		Blood	Urine
The linear concentration range [ng/mL]		100-10,000	100-10,000
The coefficient of determination (R^2)		>0.9997	>0.9997
LOD [ng/mL]		10	10
Intra-day precision [%]*	100 ng/mL	10.6	8.7
	1,000 ng/mL	4.9	0.6
	10,000 ng/mL	4.2	2.1
Intra-day accuracy [%]*	100 ng/mL	10.9	4.3
	1,000 ng/mL	6.1	3.7
	10,000 ng/mL	2.1	4.8
Inter-day precision [%]*	100 ng/mL	5.7	2.6
	1,000 ng/mL	4.2	1.9
	10,000 ng/mL	5.5	1.9
Inter-day accuracy [%]*	100 ng/mL	6.5	7.5
	1,000 ng/mL	5.8	5.1
	10,000 ng/mL	3.8	6.2
Recovery [%]	100 ng/mL	81.3	93.6
	1,000 ng/mL	81.7	105.3
	10,000 ng/mL	76.9	102.7
Matrix effect [%]	100 ng/mL	101.5	105.1
	1,000 ng/mL	89.6	86.3
	10,000 ng/mL	99.7	76.6
Process efficiency [%]	100 ng/mL	82.5	98.4
	1,000 ng/mL	73.2	90.8
	10,000 ng/mL	76.6	78.7

stored at freezing temperature and in the refrigerator, with pH remaining at 6.

Sample procedure

0.2 mL of whole blood/urine was spiked with 0.02 mL of IS (diazepam-d_5, 1 µg/mL) and precipitated with 0.4 mL of acetonitrile. The samples were centrifuged at 2540 × *g* at +4°C for 10 min. The organic phase was placed into 2 mL Eppendorf tubes and evaporated at +40°C to dryness under a stream of nitrogen gas. The dry residues were dissolved with methanol (0.05 mL). The solution was transferred into inserts of autosampler glass vials.

Working solutions and quality control samples

Standard solutions were diluted with methanol. The final concentrations of calibrators were: 10, 25, 50, 100, 250, 500, 1000, 2500, 5000, and 10,000 ng/mL. QC samples were prepared by spiking blank blood/urine to yield final concentrations of 100, 1,000 and 10,000 ng/mL for cefuroxime.

Chromatographic and spectrometric conditions

Chromatographic analyses were performed using an ultra-high performance liquid chromatograph (UHPLC Nexera X2, Kyoto, Japan). The separation was achieved using a Kinetex XB-C18, 2.6 µm, 2.1 × 150 mm column (Phenomenex, Torrance, CA, USA). The injected volume was 2 µL. The analytical conditions were identical to those presented previously in a study by [information deleted to maintain the anonymity of the review process].

Detection of compounds was performed using a triple-quadrupole mass spectrometer (LCMS-8050, Shimadzu, Kyoto, Japan). The spectrometer was equipped with an electrospray ionization (ESI) source, and the determination of the investigated substances was conducted in the multiple reaction monitoring (MRM) mode. The following MS parameters were fixed: nebulizing gas flow, 3 L/min; heating gas flow, 10 L/min; drying gas flow, 10 L/min; interface temperature, 250°C; Desolvation Line (DL) temperature, 200°C; heat block temperature, 350°C. A summary of precursor and product ions, collision energies, dwell time, Q1-Q3 prebias voltages, and retention time for each compound are presented in Table 1.

Results

The method employed enabled the determination of cefuroxime with high sensitivity and selectivity. Table 2 shows the validation results of the method. Figures 1, 2 and 3 show the results of ***in vitro*** cefuroxime stability at three different temperatures in blood (with citrates and NaF) and in urine.

Cefuroxime exhibited the highest stability ***in vitro*** at -15°C, with a gradual decrease in concentration observed over time. In blood samples preserved with citrates and NaF, a decrease of over 20% was noted by the second day, with subsequent moderate declines. By day 278, concentrations decreased by 18% in citrate-preserved blood, 15% in NaF-preserved blood, and 21% in urine. The estimated ***in vitro*** half-lives ($T_{1/2}$) were

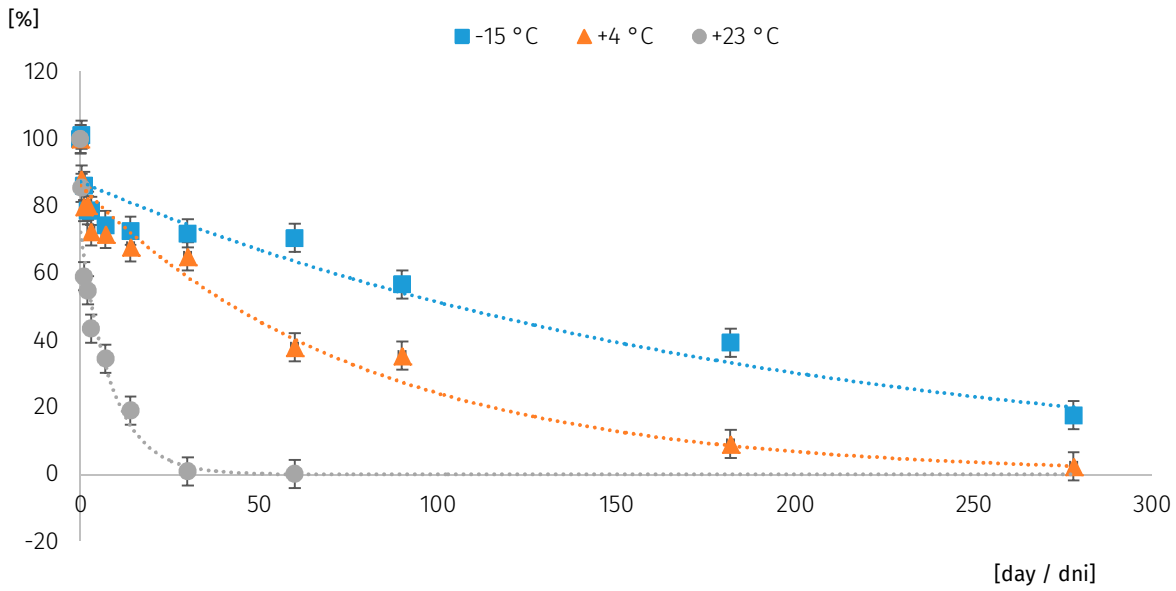

Fig. 1. The stability of cefuroxime in blood with citrates at three different temperatures: -15°C, +4°C, +23°C

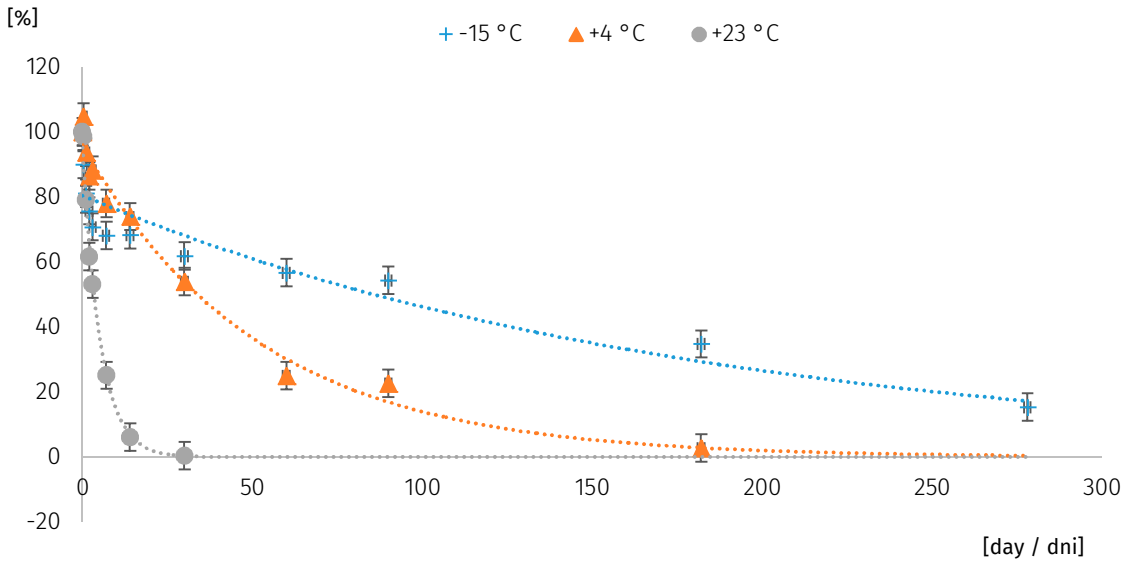

Fig. 2. The stability of cefuroxime in blood with NaF at three different temperatures: -15°C, +4°C, +23°C

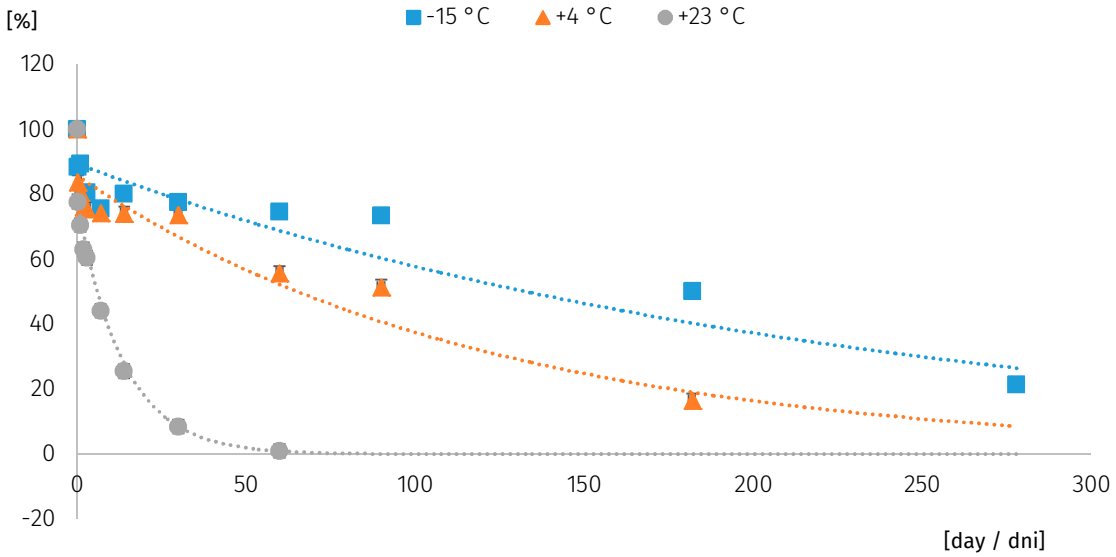

Fig. 3. The stability of cefuroxime in urine at three different temperatures: -15°C, +4°C, +23°C

approximately 105 days in citrate-preserved blood, 86 days in NaF-preserved blood, and 132 days in urine at -15°C.

Storage at +4°C resulted in more rapid degradation, with concentrations decreasing by 62% in citrate-preserved blood, 75% in NaF-preserved blood, and 44% in urine after 60 days. The estimated *in vitro* $T_{1/2}$ values were approximately 42 days in citrate-preserved blood, 34 days in NaF-preserved blood, and 66 days in urine at +4°C.

At room temperature (+23°C), cefuroxime demonstrated high instability *in vitro*. In citrate-preserved blood, the antibiotic became undetectable after 90 days, with concentrations dropping to 19% after 14 days and 1% after 30 days (estimated $T_{1/2}$ ~ 3 days). Stability was even poorer in NaF-preserved blood, with concentrations decreasing to 25% after one week and 6% after two weeks (estimated $T_{1/2}$ ~ 3 days). Urine samples showed slightly better stability, with concentrations of 8% and 1% remaining after 30 and 60 days, respectively (estimated

$T_{1/2}$ ~6 days). Notably, significant decreases in concentration *in vitro* occurred within the first day at room temperature.

Our study also revealed that cefuroxime stability differed between blood samples preserved with citrates and NaF, with the latter exhibiting lower stability for the antibiotic.

Graphical comparisons of cefuroxime stability across different matrices and temperatures are provided in supplementary materials (Figures S2-S7).

Discussion

The stability of cephalosporins depends, i.a. on the pH of the solution, solvent temperature and the addition of sodium dodecyl sulfate [52]. To our knowledge, we have conducted the longest study on thermal stability of cefuroxime in biological materials. Additionally, the stability of this substance in whole blood, which is a primary biological fluid in forensic toxicology, has not been previously studied. This is especially important from a forensic medicine point of view, due to the time difference between death and the discovery of the body, and then the collection of biological materials during autopsy (which may even be counted in weeks) [53]. If the autopsy is performed shortly after death or the discovery of the body, there is no guarantee that the samples will be delivered to the laboratory in a timely manner (without losses in antibiotic concentration). The transport and storage of biological samples, especially under uncontrolled conditions, may also affect the stability of xenobiotics.

There are cases with an undetermined cause of death where routine determinations are initially performed, such as tests for ethyl alcohol and illicit drugs. Only after new circumstances arise, (which may take several months) are toxicological analyses oriented to exclude possibilities like drug-induced anaphylaxis. An unnecessarily extended period can make the detection of xenobiotics impossible and complicate the interpretation of whether cefuroxime was administered before death and affected the cause of death.

Hu et al. [54] conducted stability studies of cefuroxime in human plasma at three concentrations: 84.2, 1680 and 16,800 ng/mL. The samples were stored under various conditions: short-term (+25°C for 6h), autosampler (+4°C for 20 h), and three freeze-thaw cycles (form +25°C to -20°C). Cefuroxime was stable under those conditions. In addition, researchers evaluated the long-term stability of this antibiotic at -20°C for 145 days. Contrary to our results, Hu et al. showed the stability of cefuroxime over the studied time period. However, it is worth to pointing out that our study did not include the analysis of human plasma. From a forensic medicine point of view, whole blood is a more important biological matrix because postmortem plasma or serum samples are rarely analysed. Processes that occur after death lead to changes at the macro- and microscopic level. Serum collected postmortem is, in most cases hemolysed, which may affect the determined xenobiotic concentration, especially when blood-to-serum ratios decrease with increasing plasma protein binding [55,56].

Viberg et al. [57] investigated the stability of cefuroxime in human plasma. The authors spiked fresh heparinized blood with cefuroxime at concentration of 1,200, 8,000 and 42,000 ng/mL and placed into glass vials (stored at room temperature and at +4°C). Analyses were conducted at 0, 40 min, 1.5, 3, 4.5, 6 and 24 h. Studies by Viberg et al. demonstrated the stability of cefuroxime in blood for 4.5 h at room temperature, and over 24 h at +4°C. Partani et al. [58] evaluated the stability of cefuroxime in plasma at two concentrations: 240.3 and 12,012.8 ng/mL (low and high concentration of QC sample) under different conditions: three freeze-thaw cycles (from around -20°C to room temperature), bench-top stability (room temperature for ~7 h), autosampler stability (+10°C for ~48 h), long-term stability (around -20°C for 107 days). Cefuroxime in plasma was stable under all aforementioned conditions. The authors also evaluated the stability of the working and stock solutions of cefuroxime and IS (cefoxitin) stored for 12 h at room temperature and for 15 days at refrigerator temperature (between 1 and +10°C). The solutions were stable under these thermal conditions. Lecaillon et al. [52] performed a stability study of cefuroxime in plasma and urine stored in autosampler and frozen at -20°C. Both matrices were stable in the autosampler over 5 h. Frozen plasma was stable over 3 months, and frozen urine, over 2 months. Researchers also evaluated calibrator solutions stored at +5°C. Cefuroxime solutions were stable for 14 days.

Conclusions

The authors demonstrated instability of cefuroxime in whole blood preserved with NaF and citrate salts, and urine samples stored under three different temperatures over 278 days. Stability studies of this substance in whole blood provide new information, particularly for forensic toxicologists. For determination of cefuroxime in biological materials, it is suggested to perform analyses as soon as possible after sampling or store samples frozen until analysis, especially in cases suspected of being associated with anaphylaxis after the administration of an antibiotic.

Supplementary data

- Chromatograms of cefuroxime in blood (with NaF) and urine (Fig. S1).
- Comparison of the cefuroxime stability in blood with citrates (A) and with NaF (B) at -15°C (Fig. S2), +4°C (Fig. S3) and +23°C (Fig. S4).
- Comparison of the cefuroxime stability in blood with citrates (A), in blood with NaF (B), and in urine (C) at -15°C (Fig. S5), +4°C (Fig. S6) and +23°C (Fig. S7).

Limitations of the study

For clinical purposes, it would be valuable to conduct studies on the stability of cefuroxime in serum and/or plasma. However, in forensic toxicology, whole blood and urine are the most frequently analysed biological materials, hence the authors limited their studies to these biological matrices.

An additional limitation of the study was monitoring cefuroxime stability in biological matrices at only a single concentration level (10,000 ng/mL).

Additionally, based on the authors' experience, as mentioned in the manuscript, samples collected from patients in the hospital are typically disposed of 24 hours after collection. Consequently, these samples are often unavailable for subsequent analysis in forensic toxicology laboratories.

References | Piśmiennictwo

1. Gu J., Liu S., Zhi Y.: Cefuroxime-inducted anaphylaxis with prominent central nervous system manifestations: a case report. J Int Med Res. 2019, 47(2), 1010–1014. doi:10.1177/0300060518814118
2. Tiglis M., Mester A., Hurmuzache T., et al.: Intraoperative anaphylactic shock following cefuroxime-axetil routine test care report and literature review. Rev Chim. 2019, 70(3), 925–928
3. Wurpts G., Aberer W., Dickel H., et al.: Guideline on diagnostic procedures for suspected hypersensitivity to beta-lactam antibiotics: Guideline of the German Society for Allergology and Clinical Immunology (DGAKI) in collaboration with the German Society of Allergology (AeDA), German Society for Pediatric Allergology and Environmental Medicine (GPA), the German Contact Dermatitis Research Group (DKG), the Austrian Society for Allergology and Immunology (ÖGAI), and the Paul-Ehrlich Society for Chemotherapy (PEG). Allergol Select. 2020, 4, 11–43. doi:10.5414/ALX02104E
4. Scadding GW.: Providing an antibiotic allergy service. Clin Pulm Med. 2019, 26, 146–153. doi:10.1097/CPM.0000000000000325
5. Stepanovic B., Sommerfield D., Lucas M., von Ungern-Sternberg BS.: An update on allergy and anaphylaxis in pediatric anesthesia. Paediatr Anaesth. 2019, 29(9), 892–900. doi:10.1111/pan.13703
6. Azevedo J., Gaspar Â., Mota I., et al.: Anaphylaxis to beta-lactam antibiotics at pediatric age: six-year survey. Allergol Immunopathol (Madr). 2019, 47(2), 128–132. doi:10.1016/j.aller.2018.07.008
7. Sachs B., Dubrall D., Fischer-Barth W., Schmid M, Stingl J.: Drug-induced anaphylactic reactions in children: a retrospective analysis of 159 validated spontaneous reports. Pharmacoepidemiol Drug Saf. 2019, 28(3), 377-388. doi:10.1002/pds.4726
8. Arulappen AL., Danial M., Sulaiman SAS.: Evaluation of reported adverse drug reactions in antibiotics usage: a retrospective study from a Tertiary Care Hospital, Malaysia. Front Pharmacol. 2018, 9, 809. doi:10.3389/fphar.2018.00809
9. Yang MS., Kang DY., Seo B., et al.: Incidence of cephalosporins-induced anaphylaxis and clinical efficacy of screening intradermal tests with cephalosporins: a large multicenter retrospective cohort study. Allergy. 2018, 73(9), 1833–1841. doi:10.1111/all.13435
10. Del Villar-Guerra P., Vincente-Arche BM., Bustamante SC., Rodríguez CS.: Anaphylactic reaction due to cefuroxime axetil: a rare cause of anaphylaxis. Int J Immunopathol Pharmacol. 2016, 29(4), 731–733. doi:10.1177/0394632016664529
11. Merget R., Sander I., Fartasch M., et al.: Occupational generalized urticarial and anaphylaxis after inhalation of cefuroxime in a nurse. Am J Ind Med. 2018, 61(3), 261–266. doi:10.1002/ajim.22788
12. Utku ÖG., Ergül B., Balci M., Oğuz D.: A rare case of ischemic colitis: cefuroxime related anaphylactic shock. Gastroenterol Nurs. 2019, 42(5), 448–450. doi:10.1097/SGA.0000000000000474
13. Menon AR., Kumar B., Vasavi K., et al.: A case report on linezolid and cefuroxime induced leucocytoclastic vasculitis. J Young Pharm. 2019, 11(2), 227–229. doi:10.5530/jyp.2019.11.47
14. Kumar D., Niveditha G., Kumar BS., et al.: Stevens-Johnson syndrome/toxic epidermal necrolysis overlap syndrome due to oral cefuroxime. J Clin Sci Res. 2019, 8, 155–158. doi:10.4103/JCSR.JCSR_23_18
15. Kiran TA., Hiremath SRR., Jaiprakash SV., Pais N.: Study of cefuroxime/oxcarbazepine induced Stevens-Johnson syndrome. Indian J Pharm Pract. 2008, 1, 58–59
16. Saeed SAM., Bazza M., Zaman M., Ryatt KS.: Cefuroxime induced lymphomatoid hypersensitivity reaction. Postgrad Med J. 2000, 76(899), 577–579. doi:10.1136/pmj.76.899.577
17. Xiao H., Liu X., Guo X.: Macular edema with serous retinal detachment post-phacoemulsification followed by spectral domain optical coherence tomography: a report of two cases. BMC Res Notes. 2015, 8, 647. doi:10.1186/s13104-015-1639-1
18. Grgurević I., Pejša V., Morović-Vergles J., et al.: N. Fatal toxic epidermal necrolysis and severe granulocytopenia following therapy with cefuroxime. Acta Dermatovenerol Croat. 2008, 16(3), 133–137

19. Sahoo HB., Rath B., Behera JP., Moharana CS.: Cefuroxime axetil associated oral eruption: a case report. Apollo Medicine. 2016, 13(2), 133–134. doi:10.1016/j.apme.2016.05.003
20. Akman C., Duran A., Kalafat UM., Ocak T.: Uveitis attack and drug reaction due to cefuroxime axetil. Cutan Ocul Toxicol. 2016, 35(3), 254–256. doi:10.3109/15569527.2015.1067817
21. Pathak A., Pandey AK., Mishra AK., Mishra AK.: Cefuroxime axetil induced glossitis: a case report. Int J Basic Clin Pharmacol. 2014, 3(4), 729–731. doi:10.5455/2319-2003.ijbcp20140809
22. Vucicevic Boras V., Brailo V., Vidovic Juras D., et al.: Oral adverse reaction to cefuroxime axetil. Annu Res Rev. 2014, 4(15), 2471-2475. doi:10.9734/ARRB/2014/10103
23. Trucker A., Hegarty P., Magill PJ., et al.: Acute kidney injury after prophylactic cefuroxime and gentamicin in patients undergoing primary hip and knee arthroplasty- a propensity score-matched study. J Arthroplasty. 2018, 33(9), 3009–3015. doi:10.1016/j.arth.2018.04.044
24. Ekiz F., Üsküdar O., Şimşek Z., et al. Cefuroxime axetil-induced liver failure. Ann Hepatol. 2010, 9(3), 306. doi:10.1016/S1665-2681(19)31645-X
25. Nirella MA., Kumarasena RS., Dassanayake AS., et al.: Worsening cholestasis and possible cefuroxime-induced liver injury following "successful" therapeutic endoscopic retrograde cholangiopancreatography for a distal common bile duct stone: a case report. J Med Case Rep. 2016, 10, 371. doi:10.1186/s13256-016-1123-0
26. Kunze W., Streidl J., Klemm T., Lutze J.: Cefuroxime-induced hepatocellular-cholestatic hepatitis with pancytopenia. OALib J. 2019, 6, 1–7. doi:10.4236/oalib.1105036
27. Hossain D.: Cefuroxime-induced congenital fixed drug eruption: a case report. J Pakistan Assoc Dermatologists. 2012, 22(4), 379–383
28. Van Dam DGHA., Burgers DMT., Foudraine N., et al.: Treatment of cefuroxime-induced neurotoxicity with continuous venovenous haemofiltration. Neth J Med. 2017, 75(1), 32–34
29. Jain M., Aggarwal A.: A probable association of panic attack with cephuroxime (cefuroxime) axetil administration. Prog Neuropsychopharmacol Biol Psychiatry. 2012, 36(1), 211. doi:10.1016/j.pnpbp.2010.11.017 [letter to the editor]
30. Zahiruddin O., Shanooha M., Mohd Azhar MY.: Psychosis induced by cefuroxime and metronidazole. Med J Malaysia. 2014, 69(1), 33–34
31. Dong H., Zhang J., Ren L., Zhu S.: Unexpected death due to cefuroxime-induced disulfiram-like reaction. Indian J Pharmacol. 2013, 45(4), 399–400. doi:10.4103/0253-7613.114991
32. Gao J., Gao Y., Ma J.: Cefuroxime-associated Kounis syndrome with unique peculiarity in perioperative prophylaxis. J Infect Public Health. 2018, 11(6), 889–892. doi:10.1016/j.jiph.2018.02.009
33. Al-Attas A., Nasr JJ., El-Enany N., Belal F.: A green capillary zone electrophoresis method for the simultaneous determination of piperacillin, tazobactam and cefepime in pharmaceutical formulations and human plasma. Biomed Chromatogr. 2015, 29(12), 1811–1818. doi:10.1002/bmc.3500
34. Šestáková N., Theurillat R., Sendi P., Thormann W.: Monitoring of cefepime in human serum and plasma by micellar electrokinetic capillary chromatography: improvement of sample preparation and validation by liquid chromatography coupled to mass spectrometry. J Sep Sci. 2017, 40(8), 1805–1814. doi:10.1002/jssc.201601446
35. Kulapina EG., Kulapina OI., Ankina VD.: Screen-printed potentiometric sensors based on carbon materials for determining cefotaxime and cefuroxime. J Anal Chem. 2020, 75, 231–237. doi:10.1134/S1061934820020100
36. Alonso JM., Peccinini RG., Campos ML., et al.: Plasma and peritoneal fluid concentration of ceftriaxone after intravenous and intraperitoneal administration in horses. J Equine Vet Sci. 2021, 96, 103310. doi:10.1016/j.jevs.2020.103310
37. Cox S., Martin A., Hamill M., et al.: Development of a method for the determination of cefovecin in plasma by HPLC. Biomed Chromatogr. 2014, 28(8), 1126–1130. doi:10.1002/bmc.3129
38. Beaudoin ME., Gangl ET.: Bioanalytical method validation for the simultaneous determination of ceftazidime and avibactam in rat plasma. Bioanalysis. 2016, 8(2), 111–122. doi:10.4155/bio.15.233
39. Dorn C., Kratzer A., Schießer S., et al.: Determination of total or free cefazolin and metronidazole in human plasma or interstitial fluid by HPLC-UV for pharmacokinetic studies in man. J Chromatogr B Analyt Technol Biomed Life Sci. 2019, 1118-1119, 51–54. doi:10.1016/j.jchromb.2019.04.025
40. Kratzer A., Schießer S., Matzneller P., et al.: Determination of total and free ceftolozane and tazobactam in human plasma and interstitial fluid by HPLC-UV. J Pharm Biomed. 2018, 163, 34–38. doi:10.1016/j.jpba.2018.09.044
41. Ezquer-Garin C., Ferriols-Lisart R., Alós-Almiñana M., et al.: Validated HPLC-UV detection method for the simultaneous determination of ceftolozane and tazobactam in human plasma. Bioanalysis. 2018, 10(7), 461–473. doi:10.4155/bio-2017-0257
42. Grégoire M., Leroy AG., Bouquié R., et al.: Simultaneous determination of ceftaroline, daptomycin, linezolid and rifampicin concentrations in human plasma by on-line solid phase extraction coupled to high-performance liquid chromatography-tandem mass spectrometry. J Pharm Biomed. 2016, 118, 17–26. doi:10.1016/j.jpba.2015.10.008
43. Wang L., Zheng X., Zhong W., et al.: Validation and application of an LC-MS-MS method for the determination of ceftizoxime in human serum and urine. J Chromatogr Sci. 2016, 54(5), 713–719. doi:10.1093/chromsci/bmv243

44. Sutherland CA., Nicolau DP.: Development of an HPLC method for the determination of ceftolozane/tazobactam in biological and aqueous matrixes. J Chromatogr Sci. 2016, 54(6), 1037–1040. doi:10.1093/chromsci/bmw047
45. Rigo-Bonin R., Cobo-Sacristán S., Padullés A., et al.: Measurement of ceftazidime concentration in human plasma by ultra-performance liquid chromatography-tandem mass spectrometry. Application to critically ill patients and patients with osteoarticular infections. Biomed Chromatogr. 2016, 30(3), 410–418. doi:10.1002/bmc.3563
46. Patil K., Tambe H., Zope V., et al.: Simultaneous determination of zidebactam and cefepime in dog plasma by LC-MS/MS and its application to pre-clinical pharmacokinetic study. Biomed Chromatogr. 2018, 32(8), e4249. doi:10.1002/bmc.4249
47. Paal M., Zoller M., Schuster C., et al.: Simultaneous quantification of cefepime, meropenem, ciprofloxacin, moxifloxacin, linezolid and piperacillin in human serum using an isotope-dilution HPLC-MS/MS method. J Pharm Biomed. 2018, 152, 102–110. doi:10.1016/j.jpba.2018.01.031
48. Jin HE., Kim IB., Kim YC., et al.: Determination of cefadroxil in rat plasma and urine using LC-MS/MS and its application to pharmacokinetic and urinary excretion studies. J Pharm Biomed. 2014, 947–948, 103-110. doi:10.1016/j.jchromb.2013.12.027
49. Kano EK., dos Reis Serra CH., Mori Koono EE., et al.: An efficient HPLC-UV method for the quantitative determination of cefadroxil in human plasma and its application in pharmacokinetic studies. J Liq Chromatogr Relat. 2012, 35(13), 1871–1881. doi:10.1080/10826076.2011.627607
50. Khan A., Iqbal Z., Khn MI., et al. Simultaneous determination of cefdinir and cefixime in human plasma by RP-HPLC/UV detection method: method development, optimization, validation, and its application to a pharmacokinetic study. J Chromatogr B. 2011, 879, 2423–2429. doi:10.1016/j.jchromb.2011.06.040
51. Information deleted to maintain the anonymity of the review process.
52. Lecaillon JB., Rouan MC., Souppart C., et al.: Determination of cefsulodin, cefotiam, cephalexin, cefotaxime, desacetyl-cefotaxime, cefuroxime and cefroxadin in plasma and urine by high-performance liquid chromatography. J Chromatogr. 1982, 228, 257–267. doi:10.1016/s0378-4347(00)80438-7
53. Nowak K., Szpot P., Zawadzki M.: The stability of 4-chloromethcathinone in blood and vitreous humor. J Forensic Sci. 2020, 65(5), 1784–1790. doi:10.1111/1556-4029.14454
54. Hu X., Huang M., Liu J., et al.: Simple and robust analysis of cefuroxime in human plasma by LC-MS/MS: application to a bioequivalence study. Adv Pharmacol Sci. 2014, 981624. doi:10.1155/2014/981624
55. Nowak K., Jurek T., Zawadzki M.: Postmortem determination of short-term markers of hyperglycemia for the purposes of medicolegal opinions. Diagnostics. 2020, 10, 236. doi:10.3390/diagnostics10040236
56. Jantos R., Schuhmacher M., Veldsra JL., et al.: Determination of blood/serum ratios of different forensically relevant analytes in authentic samples. Arch Kriminol. 2011, 277(5-6), 188–203
57. Viberg A., Sandström M., Jansson B. Determination of cefuroxime in human serum or plasma by liquid chromatography with electrospray tandem mass spectrometry. Rapid Commun Mass Spectrom. 2014, 18, 707–710. doi:10.1002/rcm.1396
58. Partini P., Gurule S., Khuroo A., et al.: Liquid chromatography/electrospray tandem mass spectrometry method for the determination of cefuroxime in human plasma: application to a pharmacokinetic study. J Chromatogr B. 2010, 878, 428–434. doi:10.1016/j.jchromb.2009.12.025

SUPPLEMENTARY MATERIALS

Determination of cefuroxime in human blood and urine using UHPLC-MS/MS and its application to a stability study of cefuroxime over 278 days.

I. Chromatograms of cefuroxime in blood (with NaF) and urine (Fig. S1)

II. Comparison of the cefuroxime stability in blood with citrates (A) and with NaF (B) at -15°C (Fig. S2), +4°C (Fig. S3) and +23°C (Fig. S4)

III. Comparison of the cefuroxime stability in blood with citrates (A), in blood with NaF (B), and in urine (C) at -15°C (Fig. S5), +4°C (Fig. S6) and +23°C (Fig. S7)

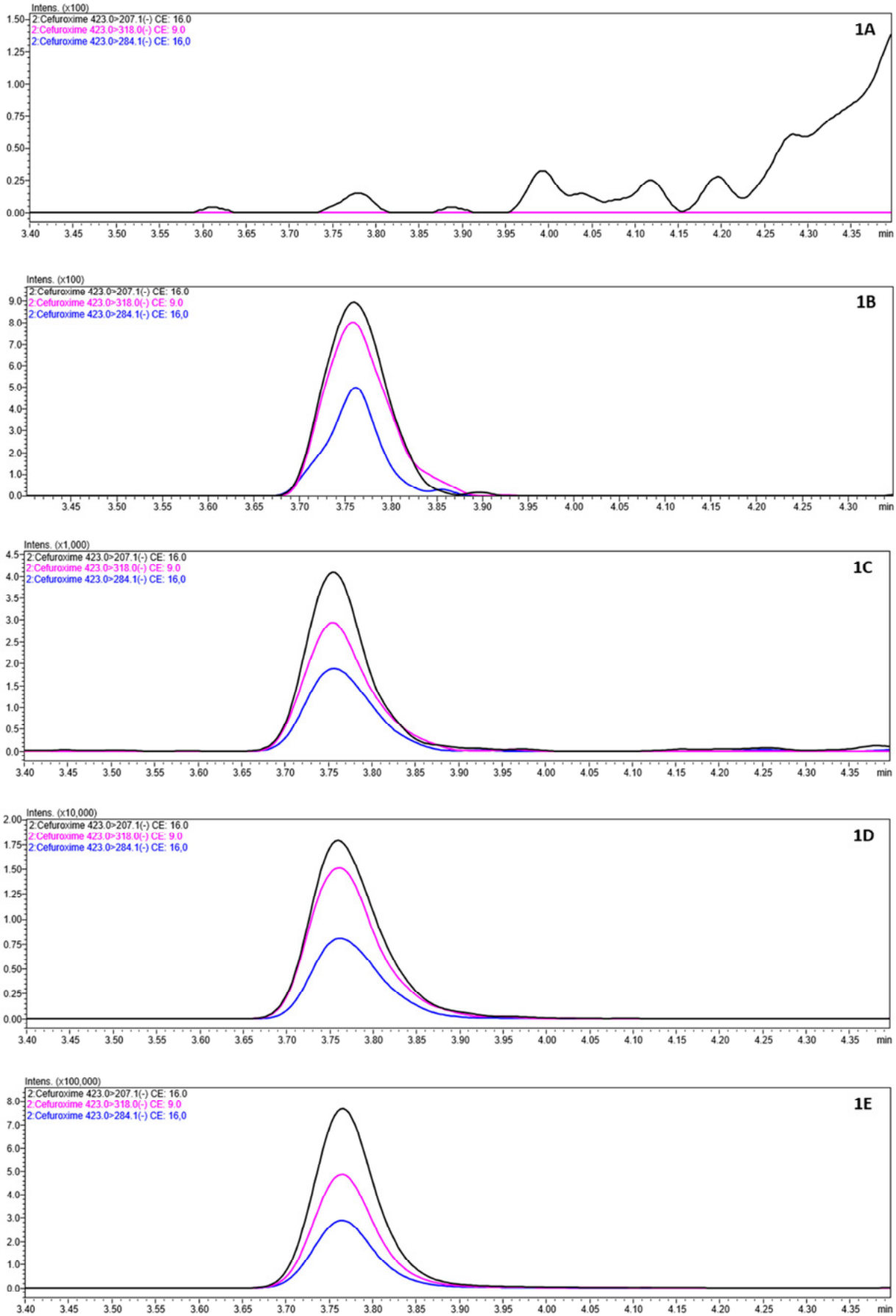

Fig. S1. Chromatograms of cefuroxime in blood with NaF (1) and urine (2); A- blank sample; B – 10 ng/mL; C – 100 ng/mL; D – 1,000ng/mL; E – 10,000 ng/mL

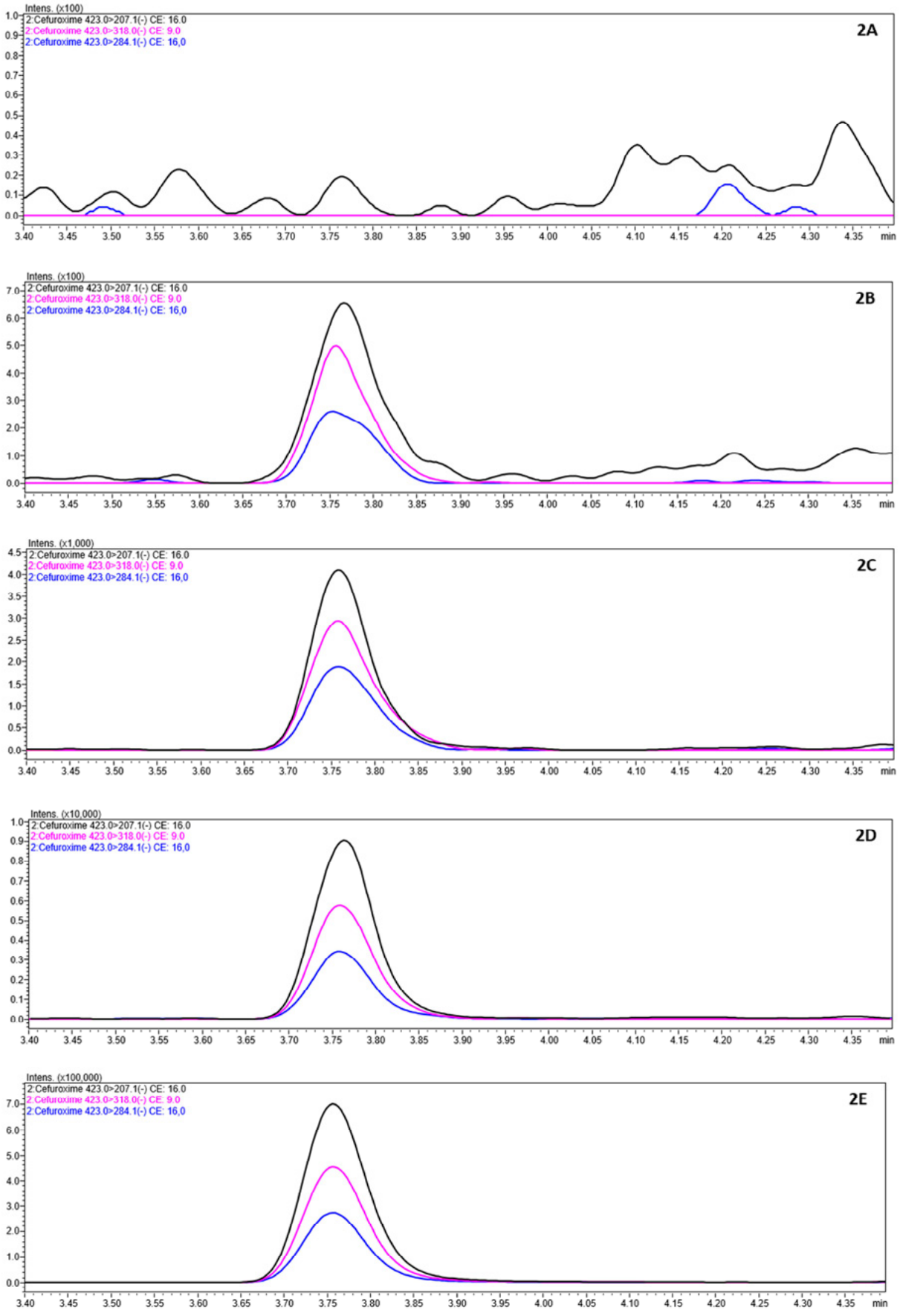

Fig. S1. Chromatograms of cefuroxime in blood with NaF (1) and urine (2); A- blank sample; B – 10 ng/mL; C – 100 ng/mL; D – 1,000ng/mL; E – 10,000 ng/mL

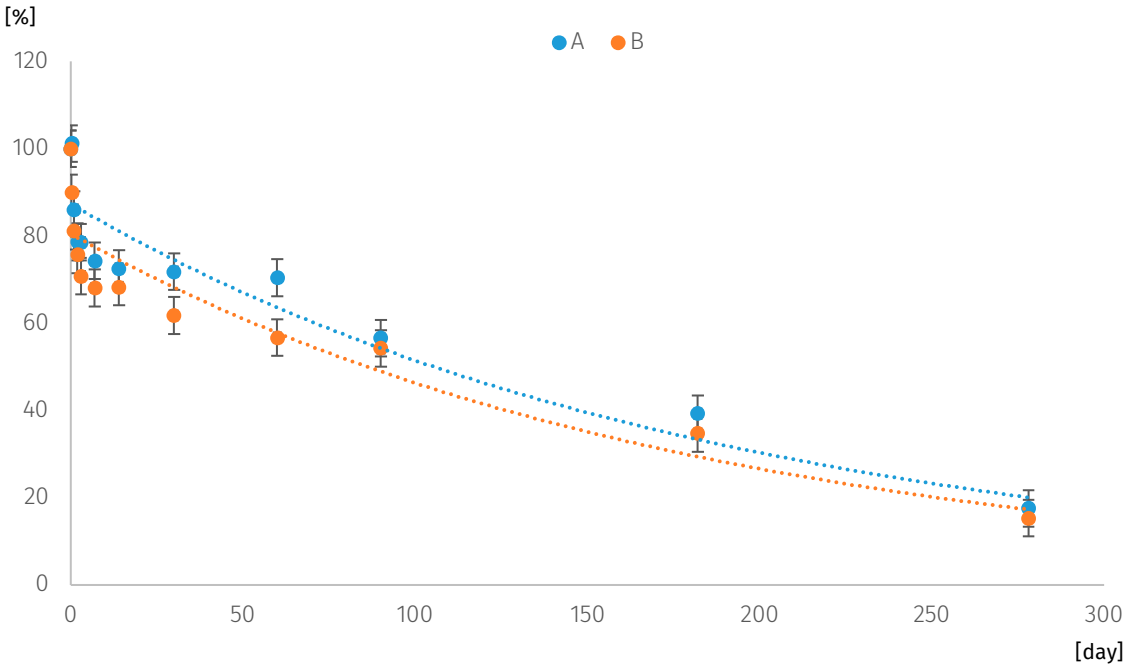

Fig. S2. Comparison of the cefuroxime stability in blood with citrates (A) and with NaF (B) at -15°C

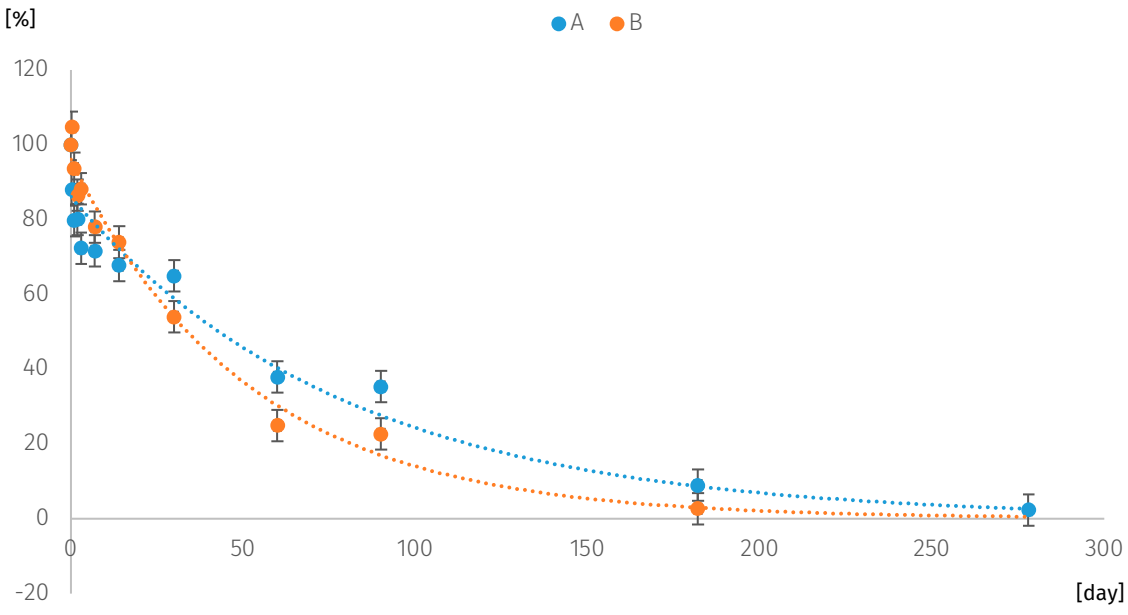

Fig. S3. Comparison of the cefuroxime stability in blood with citrates (A) and with NaF (B) at +4°C

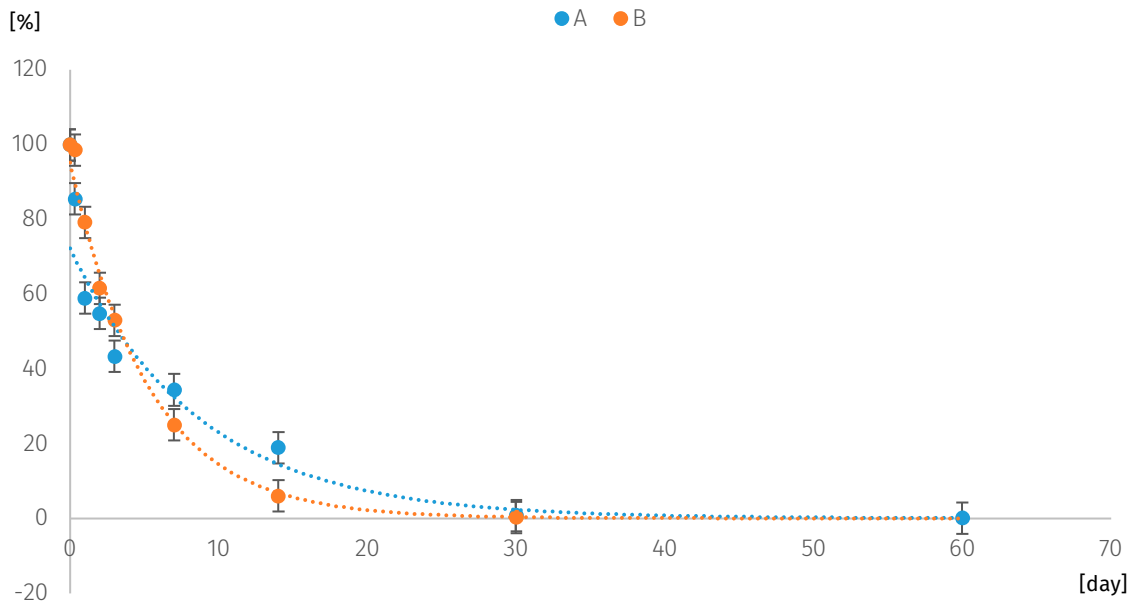

Fig. S4. Comparison of the cefuroxime stability in blood with citrates (A) and with NaF (B) at +23°C

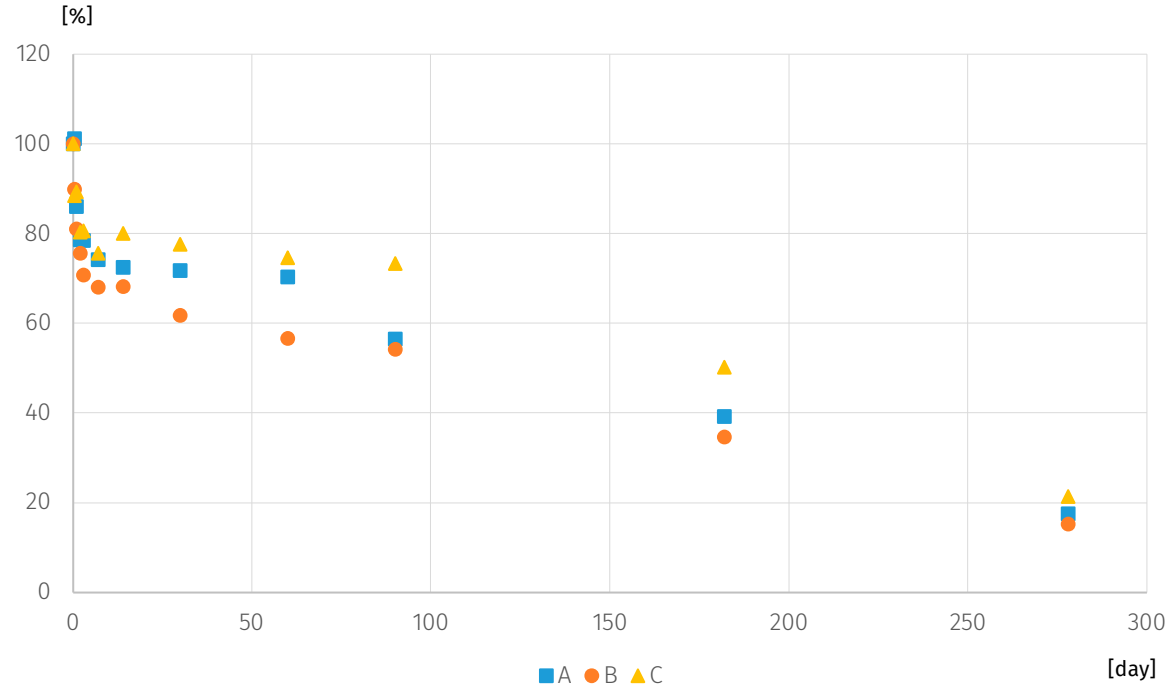

Fig. S5. Comparison of the cefuroxime stability in blood with citrates (A), in blood with NaF (B), and in urine (C) at -15°C

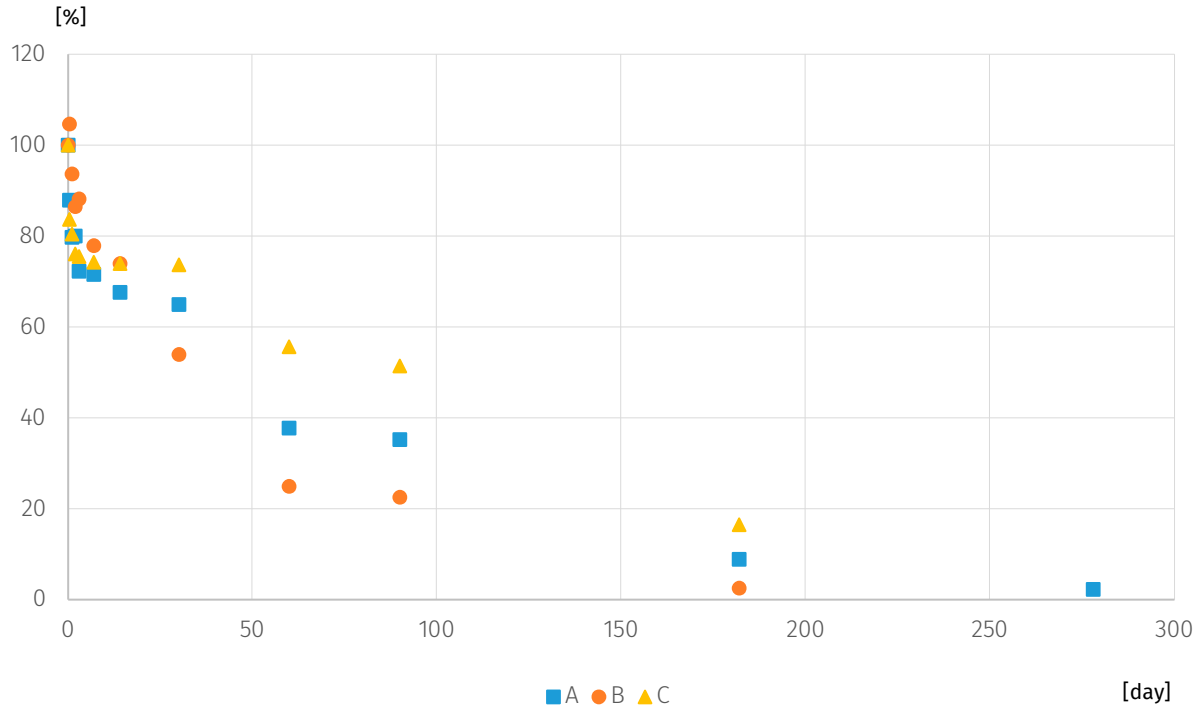

Fig. S6. Comparison of the cefuroxime stability in blood with citrates (A), in blood with NaF (B), and in urine (C) at +4°C

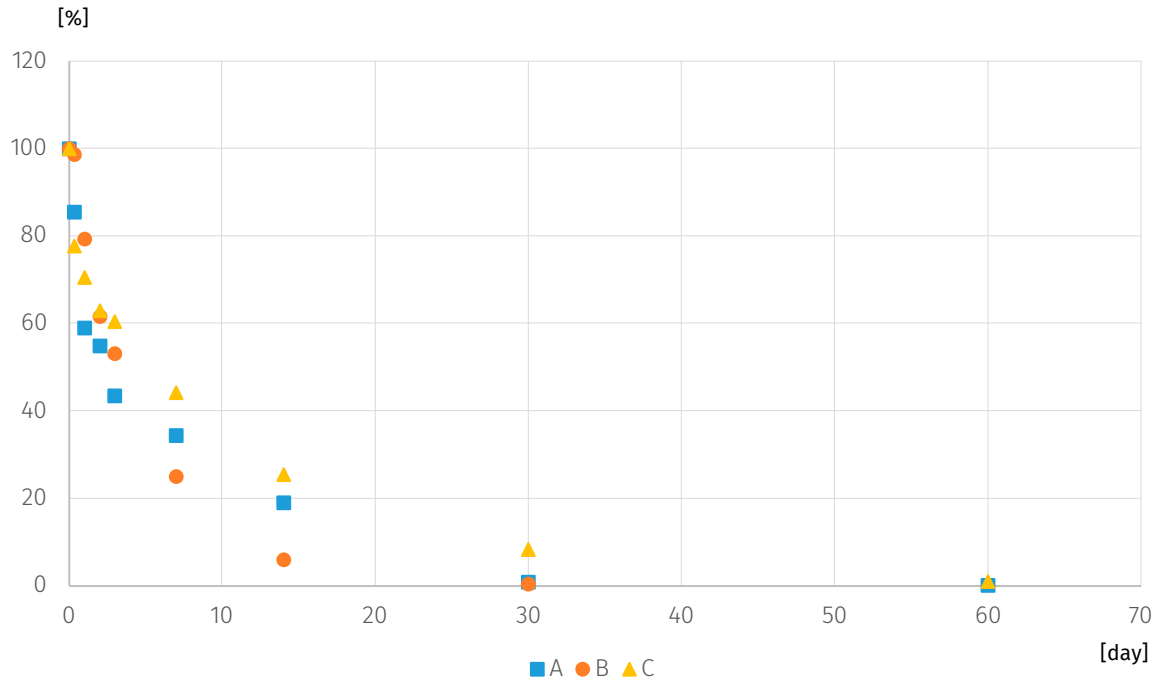

Fig. S7. Comparison of the cefuroxime stability in blood with citrates (A), in blood with NaF (B), and in urine (C) at +23°C