

Development and Validation of SPE-HPLC-MS/MS Method for Determining Cyclophosphamide in Surface Waters

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Abstract

A rapid and selective method for trace amounts determination of cyclophosphamide in surface water samples has been developed. A solid phase extraction SPE method for extraction and clean-up procedure has been optimized for determination by reversed-phase high-performance liquid chromatography with tandem mass spectrometry. The analyses proceed in the positive ion mode by means of the electrospray ionization method (ESI). Clean up was accomplished using a polymeric surface modified styrene – divinylbenzene SPE column.

The final method was validated according to international chemical harmonization (ICH) standards. High selectivity of assay procedure was obtained by choosing optimal columns (most columns contained a bed modified with C18 groups but varied in the amount of bed: 50-500 mg, and grain size: 33-80 µm) and setting separation conditions, such as flow rate during sorption (2 and 6 mL/min), sorbent type, pH of the sample (samples at pH 3 and 7 were examined), solvent strength during desorption (methanol and dichloromethane), time of evaporation (10, 20, and 30 minutes), type of solvent used for injection (HPLC) (water, methanol and methanol-water mixture, 1:1, v/v), matrix effect (tap water and river water), and time “sample preparation-analysis” on recovery (3, 6, and 9 days).

After optimization of sample preparation procedure and analytical conditions environmental water samples were collected from five sampling sites situated in Gdańsk (Pomerania, Poland) and its outskirts and subjected to validated methodology. In four samples cyclophosphamide has been quantified.

Keywords: cytostatics, cyclophosphamide, high performance liquid chromatography, tandem mass spectrometry, surface water

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Introduction

Cyclophosphamide (CPA) is important and one of the most commonly used cytostatic drugs in chemotherapy, often in combination with other antineoplastic agents in management of a wide spectrum of solid tumors and hematological malignancies. Use of CPA with other anticancer drugs is intended to obtain a synergistic or additive anticancer effect resulting from complementary mechanisms of action. CPA is also applied in the treatment of autoimmune diseases [1].

Mechanisms of action involving metabolic activation and unspecific alkylation of nucleophilic compounds also accounts for adverse effects on living organisms, including mutagenic, carcinogenic, teratogenic, and embryotoxic, and therefore is reason for environmental concerns. Not only improper disposal of expired medicines but also through hospital effluents, CPA partially transformed or even unchanged via urine and feces of patients under medical treatment, resulting in transport to sewage system and finally to surface waters.

CPA exhibits high resistance to biodegradation and low adsorption ability in the traditional activated sludge process. This indicates CPA's extreme persistence in an aqueous environment. Therefore, cyclophosphamide is assumed to be an environmentally relevant compound.

During the past years, the growing use of antineoplastic drugs in cancer therapy is an emerging issue in environmental research and it can be expected that consumption will increase due to a developing health care system and a higher life expectancy and result in exceptional occurrences of higher concentrations in wastewater effluent, in drinking water sources, and even in some treated drinking waters. German analysts have been mostly active in monitoring the fate of cytostatics in the environment after administration to patients. The concentrations of the antineoplastic cyclophosphamide in the effluents of domestic waste water treatment plants (WWTPs) in Germany were determined on approximately 10 ng/L [2, 3], which is several orders of magnitude lower than the levels at which acute ecotoxicological effects have been reported in the literature [4].

Recent years brought a substantial amount of publications considering the phenomena of different sorts of pharmaceuticals occurring in environmental compartments, namely wastewater, surface waters, STP effluents, and ground waters. In 1999 the EPA published a special report [5] concerning the fate and occurrence of pharmaceuticals and personal care products (PPCPs) in the environment. Over 50 have been listed as "identified in environmental samples – or having significance with respect to aquatic life." Considering the polar nature of most compounds involved, high performance liquid chromatography coupled with mass spectrometry (HPLC-MS) plays an important role. Obviously, a wide variety of compound classes are involved, including cytostatics [6, 7], immunosuppressants [8], antidepressants [9], illicit drugs [10], and other pharmaceuticals. Very often these compounds exist in trace or ultra-trace amounts. Nevertheless, pharmaceuticals are designed in such a way that even at small amounts of biologically

active compound, drugs can exert a desired biological effect. These small amounts can be neither detected nor quantified by means of classical instrumental analyses, like liquid (LC) or gas chromatography (GC). For the analysis of CPA the HPLC [2, 3, 11-14] or gas chromatography (GC) [15-18] coupled with either mass spectrometry (MS) or tandem mass spectrometry (MS/MS) are recommended. However, it must be stated that the use of GC usually requires a derivatization step. Nowadays there appear publications considering the use of ultra performance liquid chromatography (UPLC) as an alternative for HPLC [19, 20].

Although, there have been many scientific papers describing analytical methods applied for the analysis of CPA in different media, there is no paper that would suggest optimized methodology for the analysis of surface waters from sample pretreatment to concentrate analysis. The authors managed to develop an analytical method that has far more decreased limit of detection and quantization (LOD and LOQ) and much higher analyte recoveries.

Experimental

Chemicals and Materials

Cyclophosphamide standard was purchased from Merck (Warsaw, Poland). The distilled water was obtained from a water distiller HPL 5 (HydroLab Poland). Methanol (MeOH) (lot 0806537002) was purchased from Baker (Witko, Łódź, Poland). The formic acid (HCOOH), hydrochloric acid (HCl), and dichloromethane (CH₂Cl₂) (Batch No. 1167/04/09) were bought from Polish Chemical Reagents (Polskie Odczynniki Chemiczne, Gliwice, Poland). Ethyl acetate (No. WE/205-500-4) was bought from Chempur (Piekary Śląskie, Poland). Other chemicals were purchased from standard sources and were of the highest quality available. The SPE cartridges used were as follows: J.T.Baker Octadecyl (USA), Chromabond C18 EC (MACHEREY-NAGEL GmbH & Co. KG, Germany), Strata-X (Phenomenex, Shim-Pol, Poland), Merck LiChrolut RP-select B (Germany), Waters Oasis HLB (Poland), Agilent Zorbax SPE C18 (USA), Chromabond Easy (MACHEREY-NAGEL GmbH & Co. KG, Germany), J.T. Baker Speedisc (USA).

Chromatographic Conditions

Before analysis, extracts were concentrated in an evaporation chamber (TurboVap® LV, Caliper LifeSciences) assisted by a nitrogen stream and redissolved in 1 mL of MeOH/H₂O mixture (1:1, v/v), transferred into glass vials, and 5 µL were injected with an auto sampler. A LiChroCART 125-4 RP C18 column (Merck Chemicals, Germany) was used for chromatographic separation. For analysis in the positive ion mode eluent A was formic acid buffer solution and eluent B was methanol. The elution started with 20% of eluent A, followed by a 7-min linear gradient to 20% of eluent B, 2-min isocratic elution and a



1-min linear gradient to 20% of eluent A, which was maintained for 4 min to equilibrate the column.

Mass Spectrometry Conditions

The triple quadrupole mass spectrometer equipped with a Linear Ion Trap Systems 4000 QTrap (Applied Biosystems Sciex Instruments, USA) was used. The analyses were done in the positive ion mode by the use of electrospray ionization method (ESI). The mass spectrometer was interfaced with a computer workstation running Aria® OS software and Analyst software (Version 1.5 Applied Biosystems) for data acquisition and processing. Mass spectrometry analyses were done in the multiple reaction monitoring (MRM) mode, measuring the fragmentation products of the protonated or deprotonated pseudo-molecular ions of cyclophosphamide.

Sample Collection, Storage, and Preparation

Optimization of SPE procedure was carried out on the basis of three different sample media that were not supposed to contain an analyte of interest. Tap water for each analysis was collected fresh directly from the tap in one of the University rooms. River water was collected from the Elk and Biebrza rivers and stored in deemed glass containers at room temperature conditions. Urine samples with no probable content of CPA were collected from staff and students working in the laboratory and stored in plastic containers cooled in the refrigerator to 5°C. Since the CPA in real water samples exists in trace amounts it is necessary to use larger amounts of water. In usual laboratory practice 300 mL had been applied to a single SPE cartridge. Nevertheless, in situations of limited amounts of samples it was necessary to use smaller amounts (100 mL).

Cyclophosphamide's Stability Examination

In order to examine CPA stability we analyzed immediately after preparation and at three-day intervals, i.e. in 3, 6, and 9 days after preparation with regard to different SPE cartridges. After each analysis samples were stored at 5°C.

Matrix Effect

The matrix effect on recovery of CFA was examined by spiking three different media (urine, lake, and tap water) with CFA standard. Each sample has been pretreated on different SPE cartridges.

Optimization of Clean-Up Procedure

This part of the experimental work concerns the problem of adjustment of solid-phase extraction (SPE) conditions necessary for obtaining proper recoveries of cyclophosphamide (CPA) in tap and river water. The evaluation of the influence of the investigated parameters on analysis results is done by calculating percentage recoveries of each of the CPA extracts. The formula upon which each of the calculations had been made is:

$$\%R = \frac{\text{peak area of analyzed CPA extract}}{\text{peak area of CPA standard solution}} \times 100\%$$

Each analyzed water sample contained 5 µL of 0.936 µg/mL of CPA. The same amount had been injected into CPA standard solution that did not undergo clean-up procedure. A concise description of SPE optimization procedure was put into Table 1.

Method Validation

According to Konieczka [21] and Pawlaczyk [22], the principal purpose of analytical validation is to confirm that a select analytical procedure employed for a specific examination will give reproducible and credible results that are adequate for its intended purpose. Results from validation can be implemented in order to judge the quality, reliability, and consistency of analytical results. Thus it is necessary to define properly both the conditions in which the procedure is to be used and the purpose of the method. These principles apply to all chemical procedures, an integral part of any good analytical practice [23]. The validation characteristics specified for analytical procedures are listed in the following subchapters and defined with a brief interpretation instruction. In compliance with the Commission of the European Communities [24] and International Chemical Harmonization critical factors which should be evaluated in order to fulfill quantitative analysis validation include: accuracy, specificity, precision, linearity and range, quantization limits, and limit of detection.

Results and Discussion

Fragmentation and MS Spectrum

Assays quantifying CPA using high-performance liquid chromatography (HPLC) with tandem mass spectrometry (MS) detection have been described previously. The precursor-to-product ion transitions monitored for cyclophosphamide were m/z 261→140, monitored in the positive multiple reaction monitoring (MRM) mode. Cyclophosphamide fragmentation ions are represented in Fig. 1 together with mass spectrum.

CFA's Stability Examination

Data presented in Figs. 2 and 3 show that CPA has a very fast rate of degradation, as after three days after being prepared CPA concentrations decreased, on average, by 50%. In the next three-day period a decrease in degradation rate was observed. Such manner of reaction rate of decomposition is characteristic for second-order reactions where the rate of product formation strongly depends on the square of the concentration of a single substrate, which is CPA. Summarizing the above results, it should be noted that samples need to be analyzed immediately after collection. If the sample has to be stored since the next stage of the procedure cannot be carried out straight away due to

Table 1. SPE optimization procedure description.

Conditioning	While conditioning, 3 mL of MeOH were poured through a cartridge two times. As methanol flows through the bed, water, with pH corresponding to that of the sample, is applied in the same amounts as MeOH.
Sample application	500 mL of water sample is forced through the sorbent material by vacuum manifold. Flow rate of a sample while it's being sorbed onto the bed is a parameter of a great importance and needs to be strictly controlled.
Washing	Cartridges with analytes deriving from water samples (tap and river water) did not need to be washed as matrix content hadn't much influenced results of the analysis. Nevertheless, real sample clean-up procedures involved washing the sorbent with 1 mL of water with pH adjusted to the pH of the sample.
Drying	Initially cartridges had been dried for 10 minutes under the conditions of the vacuum chamber. However, such a method came to be insufficient and tended to leave cartridges wet. Proper drying of cartridges was performed under nitrogen gas stream. Each SPE tube was dried for about 5 minutes or till the moment when the SPE cartridge was visibly dry.
Elution	Two solvents – methanol and dichloromethane – had been examined in order to determine their ability of efficient desorption of CPA.
Evaporation	Heat and pressure applied during evaporation may exert a substantial influence on CPA stability with respect to its high rate of decomposition even at room temperatures. In order to check the influence of evaporation conditions two sets of samples with 1, 2, and 3 mL of MeOH and 5 µL of CPA standard solution was analyzed.
Reconstitution	Determination of the most suitable solvent used for injection into the chromatographic system comprised applying flow injection analysis (FIA). Carrying out the procedure required disconnecting the HPLC column and injecting prepared samples directly into a continuous flow of a carrier solution. There were 5 kinds of solvents (1 mL) applied into the chromatographic system: MeOH/H ₂ O (v/v, 1:1), H ₂ O, MeOH, urine+acidic H ₂ O, urine+buffer. Recoveries were calculated with respect to CPA standard dissolved in 1 mL of MeOH.

lack of equipment, staff availability or because the sample must be transported over a large distance, samples need to be stored in proper containers. In case of CPA those are deemed glass bottles near 0°C.

Matrix Effect

Matrix effects result from co-eluting matrix components that affect the ionization of the target analyte, resulting either in ion suppression or, in some cases, ion enhancement.

Matrix effects can be highly variable and difficult to control or predict. The severity and nature of suppression or enhancement may be a function of the concentration of the co-eluting matrix components. Method development in SPE is similar to that in HPLC. However, in the case of SPE the sample matrix must be taken into account. The matrix complicates method development because there are four interactions that may occur (solvent, analyte, matrix, stationary phase). In order to optimize an analyte's recovery, conditions should be chosen in such a way that the matrix-solvent interactions and analyte-stationary phase interactions are maximized, whereas matrix-stationary phase and analyte-solvent are minimized. Fig. 4 visibly shows the relationship between recovery and type of sample matrix with respect to five chosen SPE cartridges. Lowest ones have been obtained for urine with buffer at pH=7 and acidified urine, which might have been caused by co-eluting urine components like salts and creatinine. Highest recoveries, on the other hand, have been obtained for samples whose matrix is relatively simple, which is river and tap water.

Optimization of Clean-Up Procedure

The process of SPE optimization leads towards such conditions where CPA recoveries will reach its maximum. The process required changing elements at each of the SPE steps. Among others, the most important were: sorbents, solvents used for desorption and its flow-rate, and sample matrix along with its pH.

Sample Application

It could have been observed that flow rate has a substantial influence on analyte recovery. Much higher recovery values ($R \approx 98\%$) were obtained with smaller flow rate (2 mL/min), whereas faster flow (6 mL/min) caused more than a 50% loss in analyte quantity ($R \approx 40\%$) due to decreased ability to adsorb and desorb analytes of interest.

Elution

The effect of eluting solvent strength with respect to given sorbents is compared in Table 2. When using methanol and dichloromethane the CPA recoveries had been comparable with no favor to any of the solvents. Laboratory practice indicates that it is enough to perform two subsequent elutions of an analyte. Recoveries of any further elution are regarded as insignificant and, from an economical point of view, pointless.

Evaporation

Results of examination of the influence of time of evaporation in recovery of CPA are shown in Table 3. The results show there is almost no relation between time of evaporation and recovery. Owing to this information, it is possible to gather larger amounts of an eluate, thus possibly raising the amount of the eluted analyte and recovery in the end.

Table 2. Influence of solvent strength during desorption.

Name	R(%) Elution with MeOH	R(%) Elution with CH ₂ Cl ₂
Chromabond C18 EC	30	16
Oasis HLB	102	99
J.T.Baker speeddisc	75	78

Table 3. Influence of time of evaporation on recovery of CPA.

	Time [min]	%R
MeOH 1 mL	10	78
MeOH 2 mL	20	91
MeOH 3 mL	30	93

Reconstitution

Results of FIA are contained in Table 4. Recoveries of CPA obtained for MeOH/H₂O (1:1) mixture and H₂O showed much higher recoveries when compared to that of MeOH, whereas the lowest ones could have been observed

Analytical Validation Criteria and Statistic Assessment

The objective of validation of an analytical procedure was to demonstrate that it is suitable for its intended purpose. A tabular summation of the characteristics applicable

for samples containing urine, which indicates the phenomena of ion suppression that may have been caused by the presence of electrolytes and ionizable species in urine samples.

Results, gathered from each part allowed for designing a scheme for optimum sample clean-up and preconcentration procedure. The procedure is presented in Table 5. The cartridge that showed best recoveries (90-102%) in every stage of the optimization process was Oasis, and this sorbent type was primarily chosen for analysis of real samples. Having relatively small grain sizes allows for fast extraction, additionally giving reproducible results of the analysis of parent drugs and its polar metabolites. Unfortunately, for economical reasons Oasis cartridges couldn't have been applied for further analysis. For this reason, the second cartridge, having similar parameters, has been chosen: Strata from Phenomenex. Recoveries for strata varied between 80% and 95%. By using a variety of retention mechanisms like hydrophilic, hydrophobic interactions, and π - π bonding, it came to be reliable when analyzing acidic, basic, and neutral compounds.

MS Spectrum

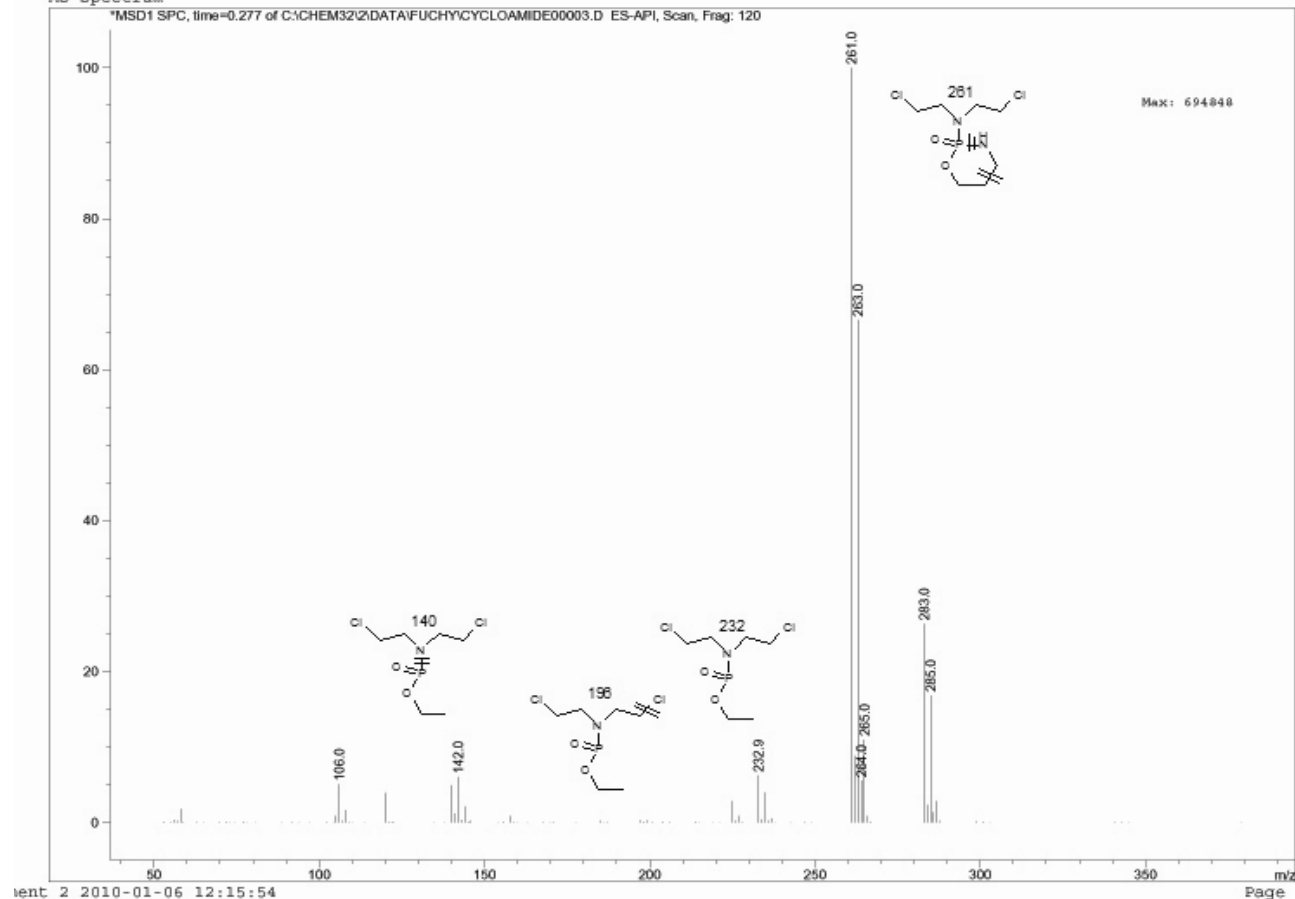


Fig. 1. CPA mass spectrum.

Table 4. Influence of the type of solvent used for injection.

No.	Type of solvent used	%R
1	CPA in MeOH/H ₂ O 1:1 1 mL	132
2	CPA in H ₂ O 1 mL	110
3	CPA in MeOH 1 mL	92
4	CPA in urine+acidic H ₂ O 1 mL	59
5	CPA in urine+buffer 1 mL	58

to identification, control of impurities, and assay procedures is presented in Table 6. It was possible to design the experimental work so that the appropriate validation characteristics were considered simultaneously to provide a sound, overall knowledge of the capabilities of the analytical procedure: specificity, linearity, range, accuracy, precision and limits of detection and quantitation. In case of cyclophosphamide assay in surface water samples by means of HPLC/MS/MS analysis, the procedure fulfills all International Conference of Harmonization criteria.

Determination of Cyclophosphamide in Environmental Water Samples

Five sampling sites situated in Gdańsk have been chosen with regard to their characteristic neighborhood. Three of them are near hospitals, clinics (having oncology departments), and scientific centers where chemotherapeutics are a subject of research studies. Results of the analysis gathered in Table 7 are based upon each sample peak area read from chromatograms. Cyclophosphamide concentrations had been calculated by applying formula obtained from the examination of a method's linearity and recalculated with respect to its concentration in water reservoirs.

Cyclophosphamide has been found in each water sample. Samples with the highest concentration of CPA came to be samples collected from sampling site No. 2. Such high concentrations of CPA can be partially explained by its location (vicinity of two large hospitals). However, it should be a subject of further investigation. Samples with the lowest quantity of CPA were those collected from sampling site No. 4. The amount of CPA present in sampling site No. 5 was below LOD value.

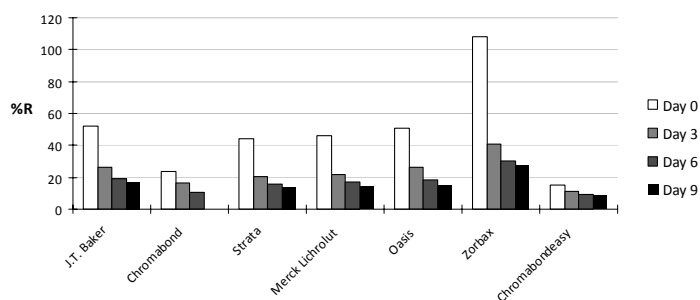


Fig. 2. Influence of CPA stability at pH=3 on its recovery.

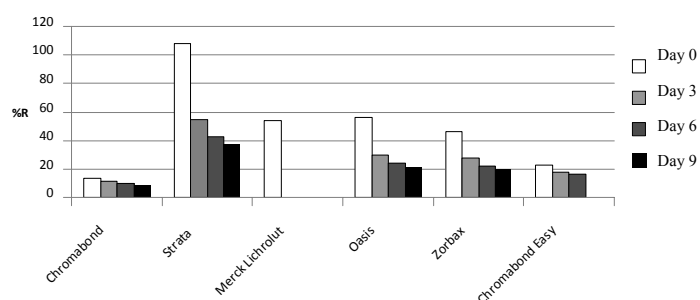


Fig. 3. Influence of CPA stability at pH=7 on its recovery.

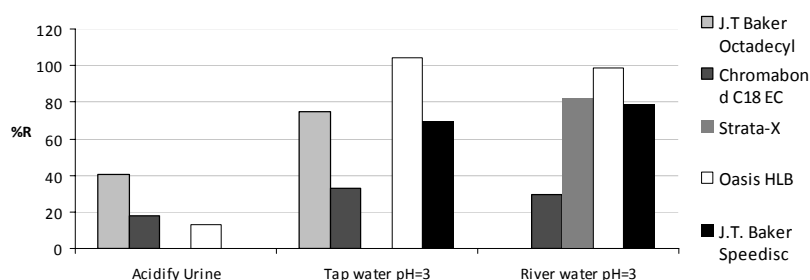


Fig. 4. Influence of the sample matrix on an analyte's recovery.

Table 5. The suggested solid-phase extraction procedure of CPA from water matrix for HPLC-MS/MS analysis.

SPE procedure element	Suggested materials and methods
Cartridge	Strata-X from Phenomenex or Oasis
Conditioning	2×3 mL of MeOH
	2×2 mL of water (pH=3/pH=7)
Load	300 mL of water sample (pH=3/pH=7)
Washing	1×1 mL of water (pH=3/pH=7)
Drying	Under N ₂ stream for 5 min or until it's completely dried
Elution	3×1 mL of CH ₂ Cl ₂
Evaporation	Evaporation chamber
Reconstitution	1 mL of MeOH:H ₂ O (v:v, 1:1)

Conclusions

The subject of the paper was to design the analytical methodology for the analysis of inland surface water samples and to determine, if possible, the presence of CPA. The main aim of optimization and validation procedure was to develop a sensitive and precise method of analysis and to obtain analyte recoveries as high as possible.

Our paper has been divided into three major parts concerning: optimization of solid-phase extraction method for selective pre-concentration and purification of CPA, validation of the HPLC-MS/MS method for the analysis of CPA, and analytical examination of real water samples for the presence and quantization of CPA.

Optimization of SPE focused on increasing percentage ratio of recovery by enhancing such parameters as: flow rate of water sample during sorption, sorbent type, degree of influence of matrix on analysis, strength and volume of solvent used for desorption, time of solvent evaporation, type of solvent used for injection (HPLC), and stability of CPA. SPE had been proven to be an effective extraction method when compared to liquid-liquid extraction. Strata-X SPE columns had been proven to give reproducible results of the analysis.

Validation parameters confirm that the reported method can provide the necessary sensitivity, linearity, precision, accuracy, and specificity to allow the determination of CPA in surface water samples. Small values of standard deviation along with confidence interval and quite significant dispersion confirms that the method is precise and repeatable. Also, no interference from endogenous substances was observed, proving that the developed method is specific.

Analysis of real water samples with designed and approved analytical methodology indicated that CPA exists in concentrations high enough to establish regular monitoring of not only this cytostatic but also other species of pharmaceutical origins present in surface waters, especially water intakes and others influencing human health and environmental degradation.

Table 6. Summary of performed validation of CPA HPLC/MS/MS assay method in water samples.

No.	Validation criterion	Result
1	Specificity/selectivity	Studied experimental data does not contain interfering species, it emphasizes HPLC method of assay cyclophosphamide and comforts the validation parameter of specificity. The peak area depends 100% on CPA concentration in analyzed solutions. Optimization of SPE cleaning eliminated the other matrix of any interfering components.
2	Accuracy	Accuracy evaluated through the percentage analyte recovery indicates the method is satisfactory in order to assay trace concentrations of cyclophosphamide by HPLC analysis. Mean recovery is 87%. The concentration covered the range of concern.
3	Linearity	$r=1$ indicates a linear relationship in the range of the model solution's concentrations. Method has the ability to produce results, which in the range of specified concentrations are proportional to concentrations in the sample.
4	Precision	Confidence interval for probability of 95% is 0.68% of arithmetic mean. Real value varies between: $1.35 \times 10^6 - 1.19 \times 10^6 > \mu > 1.35 \times 10^6 + 1.19 \times 10^5$. Standard deviation = 1%. Repeatability research in that case shows dispersion RSD%=14%. SD small values, together with confidence interval and quite significant dispersion conforms that method is precise and repeatable.
5	Range	The range of an analytical method examination is establishment at intervals between the highest and lowest values of CPA in the sample. The HPLC/MS/MS assay of CPA method is precise, accurate, and linear in range of 0.00936-0.09360 ng CPA/mL.
6	LOD	LOD= 0.0049 [ng/mL]
	LOQ	LOQ=0.0146 [ng/mL]

Abbreviations

C18	– octadecyl group
CAD	– collision-activated dissociation
CE	– collision energy
CPA	– cyclophosphamide
CUR	– curtain gas
CXP	– collision exit potential
DP	– declustering potential
EP	– entrance potential
ESI	– electrospray ionization
FIA	– flow injection analysis
GC	– gas chromatography

Table 7. Determined concentrations of CPA in real water samples.

Sampling site No.	Type of reservoir	Area characteristics	Concentration of CPA [ng/L]
1	Water intake	<ul style="list-style-type: none"> • an impounding reservoir on Radunia River • area: 72 ths m², total volume: 3.5 mln m³ • water uptake for two districts of Gdańsk since 1986 • hydro-power station 	1.56
2	Artificial reservoir	<ul style="list-style-type: none"> • artificial water reservoir • built on Siedlicki Stream • located in Gdańsk City Center, close to two hospitals 	4.97
3	River	<ul style="list-style-type: none"> • length: 104.6 km • drainage area: 837.1 km² • connections with municipal sewage water system • runs through agricultural areas as well as suburban and rural parts of Gdańsk, thus its waters might contain substantial amounts of pollutants of different origins 	0.94
4	River	<ul style="list-style-type: none"> • length: 13.5 km • drainage area: 35.5 km² • flows through rural and industrial parts of Gdańsk • surroundings: Faculty of Pharmacy (Medical University in Gdańsk) 	0.09
5	Artificial reservoir	<ul style="list-style-type: none"> • artificial water reservoir • built on Royal Stream • surroundings: SwissMed Clinic 	< LOD

HPLC – high performance liquid chromatography
 ICH – International Chemical Harmonization
 i.d. – internal diameter
 IS – ionspray voltage
 LC – liquid chromatography
 LOD – limit of detection
 LOQ – limit of quantization
 m/z – mass-to-charge ratio
 MRM – multiple reaction monitoring
 MS – mass spectrometry
 MS/MS – tandem mass spectrometry
 pH – potential of Hydrogen
 PPCS – pharmaceuticals and personal care products
 RSD – relative standard deviation
 RP – reversed phase
 SD – standard deviation
 SPE – solid-phase extraction
 UPLC – ultra performance liquid chromatography
 WWTP – waste water treatment plant

References

- ZHANG J., TIAN Q., ZHO S. Clinical Pharmacology of Cyclophosphamide and Ifosfamide, *Current Drug Therapy* **1**, 55, **2006**.
- TERNES T.A. Occurrence of drugs in German sewage treatment plants and rivers. *Water Res.* **32**, 3245, **1998**.
- BUERGE I.J., BUSER H.R., POIGER T., MULLER M.D. Occurrence and fate of the cytostatic drugs cyclophosphamide and ifosfamide in wastewater and surface waters. *Environ. Sci. Technol.*, **40**, 7242, **2006**.
- LORAINE G.A., PETTIGROVE M.E. Seasonal variations in concentrations of pharmaceuticals and personal care products in drinking water and reclaimed wastewater in Southern California. *Environ. Sci. Technol.*, **40**, 687, **2006**.
- DAUGHTON CH.G., TERNES T.A. Pharmaceuticals and Personal Care Products in the Environment: Agents of Subtle Change?, *Environ. Health Persp.* **107**, Supplement 6, **1999**.
- BAI F., FRAGA CH. H., TAGEN M., SCHAIQUEVICH P., HAGEDORN N., STEWART C.F. Simultaneous determination of cyclophosphamide and carboxyethylphosphoramide mustard in human plasma using online extraction and electrospray tandem mass spectrometry (HTLC–ESI-MS/MS). *J. Chromatogr. B*, **877**, 1709, **2009**.
- STOKVIS E., ROSING H., BEIJNEN J.H. Liquid chromatography–mass spectrometry for the quantitative bioanalysis of anticancer drugs. *Mass Spectrom Rev* **24**, 887, **2005**.
- VOGESER M., SPÖHRER U. Automated processing of whole blood samples for the determination of immunosuppressants by liquid chromatography tandem–mass spectrometry. *Clin. Chem. Lab. Med.*, **44**, 1126, **2006**.

9. CONSTANZER M, CHAVEZ C, MATUSZEWSKI B. Development and comparison of high-performance liquid chromatographic methods with tandem mass spectrometric and ultraviolet absorbance detection for the determination of cyclobenzaprine in human plasma and urine. *J Chromatogr B* **666**, 117, **1995**.
10. MAUERER H.H. Current role of liquid chromatography–mass spectrometry in clinical and forensic toxicology. *Anal Bioanal Chem* **388**, 1315, **2007**.
11. ZUCCATO E., CALAMARI D., NATANGELO M., FANELLI R. Presence of therapeutic drugs in the environment, *The Lancet*, **355**, 1789, **2000**.
12. TURCI R., SOTTANI C., RONCHI A., MINOIA C. Biological monitoring of hospital personnel occupationally exposed to antineoplastic agents. *Toxicol. Lett.* **134**, 57, **2002**.
13. SACHER F., LANGE F.T., BRAUCH H.J., BLANKENHORN I. Pharmaceuticals in groundwaters Analytical methods and results of a monitoring program in Baden-Württemberg, Germany, *J. Chromatogr. A* **938**, 199, **2001**.
14. CASTIGLIONI S., BAGNATI R., CALAMARI D., FANELLI R., ZUCCATO E. A multiresidue analytical method using solid-phase extraction and high-pressure liquid chromatography tandem mass spectrometry to measure pharmaceuticals of different therapeutic classes in urban wastewaters, *J. Chromatogr. A* **1092**, 206, **2005**.
15. STERGER-HARMANN T., KUMMERER K., SCHAKER J. Trace analysis of antineoplastics isofamide and cyclophosphamide in sewage water by two step solid phase extraction and GC/MS. *J. Chromatogr A*, **726**, 179, **1996**.
16. MOLDOVAN Z. Occurrences of pharmaceutical and personal care products as micropollutants in rivers from Romania. *Chemosphere* **64**, 1808, **2006**.
17. CATASTIN C., MULLOT J.U., BOUKARI S., MAZELLIER P., LEVI Y., CERVANTES P., ORMSBYA J.N. Assessment of antineoplastic drugs in effluents of two hospitals. *Eur. J. Water Qual.* **39**, 171, **2008** [In French].
18. MOLDOVAN Z., CHIRA R., ALDER A.C. Environmental exposure of pharmaceuticals and musk fragrances in the Somes River before and after upgrading the municipal wastewater treatment plant Cluj-Napoca, Romania. *Environ. Sci. Pollut. R.* **16**, (Suppl 1), 46, **2009**.
19. MAGNÉR J. A., ALSBERG T. E., BROMAN D. Bag-SPE – a convenient extraction method for screening of pharmaceutical residues in influent and effluent water from sewage treatment plants, *Anal. Bioanal. Chem.*, **395**, 1481, **2009**.
20. YIN J., SHAO B., ZHANG J., LI K. A Preliminary Study on the Occurrence of Cytostatic Drugs in Hospital Effluents in Beijing, China. *B. Environ. Contam. Tox.* **2009**.
21. KONIECZKA P., NAMIEŚNIK J. Assessment and control of chemical analyses data, WNT Warsaw, **2007** [In Polish].
22. PAWLACZYK J., ZAJĄC M. Validation of chemical analysis method, A.M. Poznań, **2001** [In Polish].
23. Validation of an analytical method – Polfa seminar materials, Book 9, Warsaw **1995** [In Polish].
24. Commission of the European Communities. The rules governing medicinal products in the European community, vol III Addendum. Note for guidance; analytical validation July 1989/Luxemburg: The Commission, **1990**.



