



<http://www.diva-portal.org>

This is the published version of a paper published in *Talanta: The International Journal of Pure and Applied Analytical Chemistry*.

Citation for the original published paper (version of record):

Lindberg, R., Fedorova, G., Blum, K., Pult-Prociak, J., Gillman, A. et al. (2015)

Online solid phase extraction liquid chromatography using bonded zwitterionic stationary phases and tandem mass spectrometry for rapid environmental trace analysis of highly polar hydrophilic compounds – Application for the antiviral drug Zanamivir.

Talanta: The International Journal of Pure and Applied Analytical Chemistry, 141: 164-169

<http://dx.doi.org/10.1016/j.talanta.2015.03.066>

Access to the published version may require subscription.

N.B. When citing this work, cite the original published paper.

Permanent link to this version:

<http://urn.kb.se/resolve?urn=urn:nbn:se:umu:diva-104589>



Online solid phase extraction liquid chromatography using bonded zwitterionic stationary phases and tandem mass spectrometry for rapid environmental trace analysis of highly polar hydrophilic compounds – Application for the antiviral drug Zanamivir

Richard H. Lindberg^a, Ganna Fedorova^{a,b}, Kristin M. Blum^a, Jolanta Pulit-Prociak^c, Anna Gillman^{d,e}, Josef Järhult^{d,e}, Patrik Appelblad^f, Hanna Söderström^{a,*}

^a Department of Chemistry, Umeå University, 901 87 Umeå, Sweden

^b University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zatisi 728/II, 389 25 Vodnany, Czech Republic

^c Cracow University of Technology, Faculty of Engineering and Chemical Technology, Warszawska 24, Strasse, 31-155 Cracow, Poland

^d Section for Infectious Diseases, Department of Medical Sciences, Uppsala University, Sweden

^e Zoonotic Science Center, Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden

^f Merck Millipore, Frosundaviks Alle 1, SE-16970 Stockholm, Sweden

ARTICLE INFO

Article history:

Received 15 December 2014

Received in revised form

23 March 2015

Accepted 31 March 2015

Available online 8 April 2015

Keywords:

Antivirals

Zanamivir

Online solid phase extraction

Liquid chromatography

ZIC-HILIC

Tandem mass spectrometry

ABSTRACT

Zanamivir (Za) is a highly polar and hydrophilic antiviral drug used for the treatment of influenza A viruses. Za has been detected in rivers of Japan and its environmental occurrence has the risk of inducing antiviral resistant avian influenza viruses. In this study, a rapid automated online solid phase extraction liquid chromatography method using bonded zwitterionic stationary phases and tandem mass spectrometry (SPE/LC–MS/MS) for trace analysis of Za was developed. Furthermore, an internal standard (IS) calibration method capable of quantifying Za in Milli-Q, surface water, sewage effluent and sewage influent was evaluated. Optimum pre-extraction sample composition was found to be 95/5 v/v acetonitrile/water sample and 1% formic acid. The developed method showed acceptable linearities ($r^2 \geq 0.994$), filtration recovery ($\geq 91\%$), and intra-day precisions ($RSD \leq 16\%$), and acceptable and environmentally relevant LOQs ($\leq 20 \text{ ng L}^{-1}$). Storage tests showed no significant losses of Za during 20 days and $+4/-20 \text{ }^\circ\text{C}$ ($\leq 12\%$) with the exception of influent samples, which should be kept at $-20 \text{ }^\circ\text{C}$ to avoid significant Za losses. The applicability of the method was demonstrated in a study on phototransformation of Za in unfiltered and filtered surface water during 28 days of artificial UV irradiation exposure. No significant ($\leq 12\%$) phototransformation was found in surface water after 28 days suggesting a relatively high photostability of Za and that Za should be of environmental concern.

© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Antiviral drugs are widely used to treat influenza A viruses but their fate and effects in the environment are not well studied [1]. Water living birds are the natural hosts of influenza A virus [2] and all known human pandemic viruses have contained genetic elements from avian influenza strains [3,4]. If influenza-infected birds are exposed to the antiviral drugs neuraminidase inhibitors (NAIs) in their water environment, resistance can develop [5,6], and could eventually transmit to humans as a resistant pandemic, which

would be of major public health concern [7]. The NAI oseltamivir (Tamiflu[®], Roche) has been mostly studied as it is the predominantly used anti-influenza drug [8], and also stockpiled worldwide in case of a severe influenza pandemic [9,10]. The active metabolite oseltamivir carboxylate (OC) is largely excreted from treated humans, is not removed or degraded by traditional sewage treatment [11,12], nor by direct phototransformation [11,13], and has repeatedly been measured in sewage effluents and river water, see for instance [14–19]. Zanamivir (Za) (Relenza[®], GlaxoSmithKline), is the second most used NAI [8]. It is used primarily when there is resistance to oseltamivir [20], has been less stockpiled, and much less environmentally studied. Additionally, the high polarity and hydrophilicity of Za (predicted values: pK_{a1}

* Corresponding author. Tel.: +46 90 786 6664.

E-mail address: Hanna.Soderstrom@umu.se (H. Söderström).

3.25; pK_{a2} 11.93; water solubility 7.31 g L^{-1} ; and $\log P$ -2.3 and -5.8 [21]) have made reliable quantification in water a challenge [1,22,23]. At present, Za has been measured in rivers in Japan [14–16] and photodegradation after 18 days in surface water, with the formation of four photoproducts, has been reported [22]. However, much of its fate and effects in the environment needs to be studied, which is a highly relevant task especially in the light of increasing Za usage.

Since Za is highly polar and hydrophilic, low retention is obtained on conventional reversed phase liquid chromatography (RP-LC) columns such as the most commonly used octadecyl carbon chain (C18)-bonded silica column. This was also observed in this study. Hydrophilic interaction liquid chromatography (HILIC), in contrast to RP-LC unless ion-pairing is used, has however the ability to significantly retain highly polar and hydrophilic analytes (HPHAs) [24–26]. Today, there are many commercially available HILIC columns with different stationary phase chemistries. Most of the columns carry ionic charges therefore it is recommended to use buffer salts in the mobile phase to control the ionic interactions between the analytes and the stationary phase, in addition to keeping a controlled mobile phase environment. Polar partitioning is the main retention mechanism but the presence of ionic interactions can have a substantial impact on retention. A column with charged functional groups has much greater interaction with charged analytes than that of an uncharged stationary phase and these electrostatic interactions provide the possibility of changing retention time and controlling selectivity by altering pH and/or buffer strength. Truly zwitterionic HILIC stationary phases, such as the ZIC-HILIC, provide sites for weak electrostatic interactions due to the close proximity of ion and counter-ion in balanced proportions within their functional groups. A pleasant side-effect using HILIC is that analytes usually elute with a high content of organic solvent which in turn may improve ionization efficiency and detection limit with mass spectrometry detection [27].

In environmental trace analysis, the extraction of polar analytes (e.g. pharmaceuticals) from aqueous samples is usually made by offline solid phase extraction [28]. In terms of HPHAs in aqueous samples, a “wide selection” of ion-exchange SPE sorbents have been used, for instance: OASIS HLB [29]; OASIS WCX [30]; and Strata X-CV [31]. However, in order to improve sample throughput, and minimize manual labor, automated time effective online SPE has become increasingly popular [32]. Although most of the method development involving HILIC has been in the area of bioanalysis [33], online SPE of environmental samples in combination with HILIC has been reported [34]. For instance Roen et al. successfully quantified nerve agent degradation products in water by the use of a porous graphite carbon online SPE in combination with HILIC [35]; Zhang et al. developed a cation-exchange restricted access material online SPE-HILIC method to determine melamine and cyromazine in milk [36]; and van Nuijs et al. used OASIS HLB and OASIS MCX SPE in the analysis of drugs of abuse (including metabolites) in sewage waters [37].

The aim of this study was to develop and evaluate a rapid automated ZIC-HILIC based online solid phase extraction liquid chromatography tandem mass spectrometry (SPE/LC-MS/MS) method, to our knowledge the first method using ZIC-HILIC in both the enrichment of the analyte (online SPE) and in the analytical column, capable of analysing Za in Milli-Q, surface water, sewage effluent and sewage influent. As the environmental fate of Za has been rarely studied, the method applicability was tested in a phototransformation study where Za in filtered and unfiltered surface water was exposed to simulated sunlight irradiation for 28 days, where the percentage of transformation was determined using the developed method.

2. Experimental

2.1. Chemicals

Za and ^{13}C - $^{15}\text{N}_2$ -Za standards were classified as analytical grade ($>98\%$) and were obtained from GlaxoSmithKline (Stevenage, Hertfordshire, UK). The stock solutions of Za and ^{13}C - $^{15}\text{N}_2$ -Za, and dilutes, were prepared in Milli-Q water and stored at -18°C . Calibration standards of 10 ml were prepared in acetonitrile/Milli-Q water (95/5 v/v, 1% formic acid).

LC/MS grade quality of acetonitrile (ACN) was purchased from Merck Millipore (Lichrosolv-hypergrade, Darmstadt, Germany) and the purified water was prepared using a Milli-Q Advantage, including a UV radiation source, ultrapure water system (Millipore, Billerica, USA). Formic acid (FA) (Sigma-Aldrich, Steinheim, Germany) was used to acidify mobile phases.

2.2. Optimization of sample pre-treatment

Due to the nature of HILIC, aqueous samples need to be diluted with a high content of organic solvent in order for the analytes to be retained on the SPE and analytical columns. In this study, ACN was used to dilute the samples, and FA was used to make the Za molecules positively charged to better interact with the distal charge of the ZIC-HILIC stationary phase.

The Design of Experiment (DOE) method was used initially in the optimization of the amount of ACN and FA used in sample pre-treatment to obtain the highest utility of information. A DOE method is characterized by controlled (independent) input variables, and observed and measured output variables. The input variables in this study were the proportion of water sample/ACN and FA, respectively, used in the sample pre-treatment, and the output variable was the signal area of Za measured with the online SPE/LC-MS/MS analysis system and the method described in 2.3 and 2.4, respectively. The experiments were planned based on a mixed experiment plan where variables were tested at two and three diversity levels. The experimental plan and the results are shown in the Supplementary material (Table S1).

Milli-Q and sewage treatment influent/effluent waters were included in the optimization of the sample pre-treatment. Influent and effluent waters were collected from Umeå STP and Za was not present above its LOQ (20 ng L^{-1} and 15 ng L^{-1} , respectively) in these waters. In the DOE method, constant variables were injection volume and Za concentration at 1 mL and 1000 ng L^{-1} , respectively. After the initial optimization using DOE, 5 ml injection volume was used in order to improve the LOQ.

2.3. Analytical system

In this study an autosampler from PAL HTC autosampler was used and it was equipped with cooled sample trays (CTC Analytics AG, Zwingen, Switzerland). The LC pumps (Surveyor and Accela), mass analyzer (TSQ Quantum Ultra EMR, triple stage quadrupole MS/MS) and software (Xcalibur) were made by Thermo Fisher Scientific (San Jose, CA, USA). The online SPE is based on a column switching system using a 6- and a 10-port valve described in details by Khan et al. [38].

2.4. Online SPE/LC-HESI-MS/MS method

As presented in Section 3.1, the sample dilution in the pre-treatment was decided to be 95% ACN and 1% FA. In total, the sample pre-treatment in the evaluated on-line SPE/LC-MS/MS method included filtration of 0.5 mL aqueous sample ($0.45 \mu\text{m}$ Filtropur S polysulfone, Sarstedt, Germany), dilution by 9.5 mL ACN (5/95% v/v), addition of $100 \mu\text{L}$ FA (1% v/v) and addition of Za-IS at a concentration

corresponding to 500 ng L^{-1} in the water sample. The injection volume was 5.0 mL using a 5 mL loop. The online extraction column used was a ZIC-HILIC (20 mm \times 2.1 mm i.d., 5 μm particle size, Merck Millipore, Darmstadt, Germany) and the analytical column used was a ZIC-HILIC (50 mm \times 2.1 mm i.d., 5 μm particle size, Merck Millipore, Darmstadt, Germany), following a corresponding guard column (14 mm \times 1.0 mm i.d., 5 μm particle size). The total time of the online SPE and the LC-MS/MS analysis was 15 min and the loading of the sample to the SPE was made the first 5.10 min. Detailed information of the mobile phase gradients and flows are shown in Table 1.

Heated electrospray (HESI) in positive ion mode was used for ionization of Za, and the HESI properties were as follows: ionization voltage, 3.5 kV; sheath gas, 35 (arbitrary unit); auxiliary gas, 15 (arbitrary units); vaporizer temperature, 200 $^{\circ}\text{C}$; capillary temperature, 325 $^{\circ}\text{C}$; and collision gas (argon) pressure, 1.5 mTorr. A resolution of 0.7 FMWH was used for the mass analyzing quadrupoles. Details including precursor/product ions, collision energies, tube lens values, quantify/qualify ions and their relative abundance, are provided in Table 2. Although the ^{13}C - $^{15}\text{N}_2$ -Za 63.3 m/z ion was in most abundance it was to a greater extent, in comparison to the 121.1 m/z ion, influenced by matrix effects. Quantification was made by internal standard calibration (ISC).

2.5. Method performance tests

In all of the method performance tests, Milli-Q, surface, and sewage treatment influent/effluent waters were included. Surface water and influent/effluent waters were collected from Umeå

Table 1
Online SPE and LC-mobile phase gradients and flows.

Time	H ₂ O (%) 0.1% FA	ACN (%) 0.1% FA	Flow (mL min ⁻¹)
A			
0.00	5.0	95.0	0.05
0.01	5.0	95.0	1.10
5.00	5.0	95.0	1.10
5.20	95.0	5.0	0.30
10.00	95.0	5.0	0.30
10.10	5.0	95.0	1.00
14.99	5.0	95.0	1.00
15.00	5.0	95.0	0.05
B			
0.00	15	85	0.40
7.00	15	85	0.40
10.00	50	50	0.40
11.00	95	5	0.40
13.00	95	5	0.40
13.01	15	85	0.40
15.00	15	85	0.40

A, Online SPE (Surveyor pump); B, Analytical LC (Accela pump).

Table 2
MS/MS parameters.

Analytes	Precursor	Product	CE (V) ^a	Tube lens (V)	Type ^b	RA Q/q (%) ^c
Za	333.0	60.2	18	98	Q	100
Za	333.0	121.1	33	98	q	58
^{13}C - $^{15}\text{N}_2$ -Za	336.0	63.3	19	105	q	100
^{13}C - $^{15}\text{N}_2$ -Za	336.0	121.1	31	105	Q	68

^a Collision energy.

^b Quantify ion (Q), qualify ion (q).

^c Relative abundance between the product ions.

River and Umeå STP, and Za was not present above its LOQ in any of the environmental samples.

In order to evaluate linearity (r^2), four nine point ISC curves were made in the concentration range 0.1–2000 ng L^{-1} . The LOQs of the antiviral in the various matrices were based on the lowest point within the linear range in the calibration curves. Milli-Q water was injected following calibration points to assess potential memory effects. Intra-day precision tests to evaluate the precision of the extraction and the instrumental response were made by consecutive injections of fortified samples at concentration levels 50 ng L^{-1} ($n=5$) and 1000 ng L^{-1} ($n=3$).

Ion suppression/enhancement was studied by means of calibration curves (based on analyte peak area) fortified to (in ng L^{-1}): 0, 1, 10, 50, 100, 250, 750, 1500, and 2000. The calibration curve slopes of Za in the environmental samples were compared to the Milli-Q equivalent. The accuracy of quantification (AOQ) was established by comparisons of results obtained from ISC curves (peak area ratios) and from standard addition calibration (SAC) curves (peak area) to nominal values. The quantification range was between 50 ng L^{-1} and 750 ng L^{-1} . Relative recoveries of Za, in relation to the IS, in the surface and influent/effluent water samples were calculated by dividing the slopes of the IS based calibration curve with the slope obtained from the Milli-Q samples.

The stability of Za during storage at +4 $^{\circ}\text{C}$ and -20 $^{\circ}\text{C}$ was evaluated by fortification of the four sample types with Za (800 ng L^{-1}). Samples were analyzed immediately and after being stored for up to 20 days. The stability of Za was evaluated by the comparison of Za quantities in stored samples to day 0 samples. The recovery of Za during syringe filtration was assessed using samples fortified to a level of 1000 ng L^{-1} . The IS was added post filtration.

2.6. Method applicability – phototransformation

Two matrices, unfiltered and filtered surface water, were included in the artificial UV irradiation exposure experiment. The surface water used was collected from Umeå River, had a pH of 5 and a concentration of Za below LOQ. The water chemistry of the collected surface water was not analyzed in this study. However, an environmental monitoring performed by the Society for water conservation in Ume- and Vindel River one week later, about 300 m from the site, detected a TOC concentration of 1.7 mg/L and a total concentration of nitrate and nitrite of 0.065 mg/L [39]. The surface water was not sterilized prior to the artificial UV irradiation exposure. The filtered surface water matrix was prepared by filtration through a 0.45 μm MFTM-membrane filter. To each matrix, Za was then added to get a final water concentration of 100 $\mu\text{g L}^{-1}$, and the initial concentration of each matrix was measured. For each matrix and exposure time point, 10 mL of each matrix was prepared in triplicates in 12-mL Pyrex tubes. Transformation of Za caused by other reactions than photolysis was controlled by covering additional pyrex tubes by several layers of aluminum foil (referred to as dark controls). All samples of one matrix were placed underneath four mercury UV-lamps (Philips TLK 40 W/09N). The irradiation spectrum of the artificial UV-light source was recorded at the exposure site in 1 nm steps with an ILT 900-R spectroradiometer (International Light Technologies, Massachusetts, USA). The intensity of the UV-lamps in the range 200–460 nm together with the absorbance of Za is shown in the Supplementary material (Fig. S1). In summary, the total irradiance in the UV range (300–460 nm) was 25 W/m^2 , and the maximum irradiance (average of six scans) of 0.58 $\text{W/m}^2/\text{nm}$ was recorded at 364 nm. The absorbance values were also used to calculate the molar absorptivity values of Za (according to the Beer-Lambert law) which gave a maximum molar absorptivity of 526 $\text{L mol}^{-1} \text{cm}^{-1}$ at 232 nm. The samples were constantly rotated using an RM5 “rocking/rolling action” and a fan cooled the samples to keep the temperature between 24 and 25 $^{\circ}\text{C}$. The irradiated samples were

collected after 16, 40, 64, 112, 208 and 672 h of exposure. The dark control samples of unfiltered and filtered water were collected after 16 (filtered dark controls only), 40 (unfiltered dark controls only), 208 and 672 h.

3. Results and discussions

3.1. Optimization of sample pre-treatment

The results of the DOE method showed that when analysing MilliQ and influent water, the highest amount of FA (10%) and ACN (99%) used resulted in the highest signal area, while the analysis of the effluent showed the highest response when a lower amount of ACN (95%) was used (see Table S1, Supplementary material). When increasing the injection volume to 5 ml, the difference in signal area of Za between 1% and 10% FA was minor, and with the higher injection volume (1 vs 5 mL), acceptable LOQ (Table 3) could be achieved. Thus, the use of 95% ACN and 1% FA in the sample pre-treatment was selected to limit the use of chemicals. The extraction conditions also match the chosen initial mobile phase composition, thus providing a possibility for peak compression/narrow eluting peaks and thereby higher method sensitivity. When the mobile phase pH is controlled by addition of only FA as in this study, and not with a real buffer, the acid acts only as a proton donor, making the Za molecules positively charged (predicted values: pK_{a1} 3.25; pK_{a2} 11.93; [21]) to better interact with the

distal charge of the ZIC-HILIC stationary phase.

3.2. Method performance

The results of the method development are shown in Table 3. Satisfactory linearities (r^2) of the Za ISC curves were obtained, all of them ranged between 0.994 and 0.998. The LOQs show a correlation between quantifiable Za concentration and matrix component density with the highest LOQ value (Table 3) and slightly higher noise (Fig. 1) for the analysis of the influent sample. Compared to the method developed by Azuma et al. [22], their LOQ for Za in methanol solution (1.2 ng L^{-1}) is lower (see Table 3) but on the other hand the method is more time consuming and labor intensive. Intra-day precision was excellent at both low (50 ng L^{-1}) and high (1000 ng L^{-1}) levels considering that variations in both extraction and instrumental determination are included (Table 3).

A negative influence of matrix components on the instrumental analysis was seen for the environmental samples and the Za ion suppression was 37% and 44% in the effluent and influent, respectively. Although ion suppression occurred, the relative recoveries were 95%, and above, thus the $^{13}\text{C}-^{15}\text{N}_2\text{-Za}$ used as IS (Table 2) compensated for the Za peak area variations. The comparison of the ISC and SAC method confirms the high accuracy of the determination using $^{13}\text{C}-^{15}\text{N}_2\text{-Za}$ as an internal standard (Fig. 2). For ISC the deviation from the nominal value was in the range of -14% to 15% , obtained in influent and effluent samples, respectively. Equivalent range for SAC was -21% to 22% . No significant losses of Za (less than 12%) were observed during 20 days storage at $+4^\circ\text{C}$ and -20°C with the exception of Za in the influent sample being stored at $+4^\circ\text{C}$. At this temperature, 39% of the Za amount was lost, most likely due to the relatively high biological activity and/or high particle content. These results suggest that such storage of influent samples is not recommended. Low recoveries of pharmaceuticals during filtration have been reported [40] but in this study no significant losses of Za were seen during syringe filtration.

3.3. Method applicability – phototransformation

Low phototransformation ($\leq 12\%$) of Za was observed in both filtered and unfiltered surface water during 28 days of exposure to

Table 3
Results from the method performance tests.

Parameter	Milli-Q	Surface	Effluent	Influent
Linearity (r^2)	0.996	0.996	0.998	0.994
LOQ (ng L^{-1})	10	15	15	20
IDP ^a (RSD %, $n=5$, 50 ng L^{-1})	8	6	7	8
IDP ^a (RSD %, $n=3$, 1000 ng L^{-1})	10	7	15	16
Filtration recovery (% average/RSD)	95/6	92/1	91/4	94/6
Storage test, Za lost $+4^\circ\text{C}/-20^\circ\text{C}$ (%)	5/3	9/6	5/3	39/12
Ion suppression (%)	–	26	44	37
Relative recovery (%)	–	95	100	95

^a Intra-day precision.

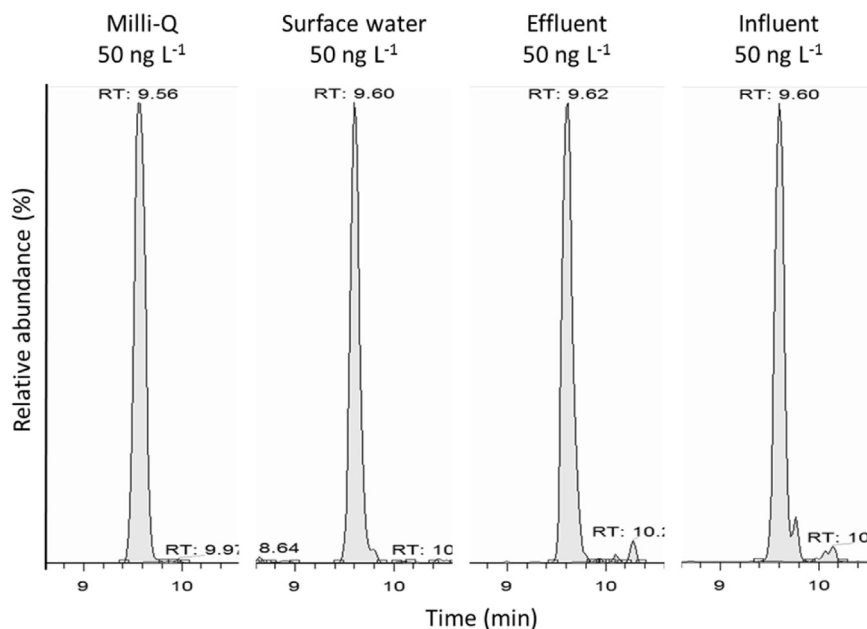


Fig. 1. Chromatograms of Zanamivir in the four tested matrices at a concentration of 50 ng L^{-1} .

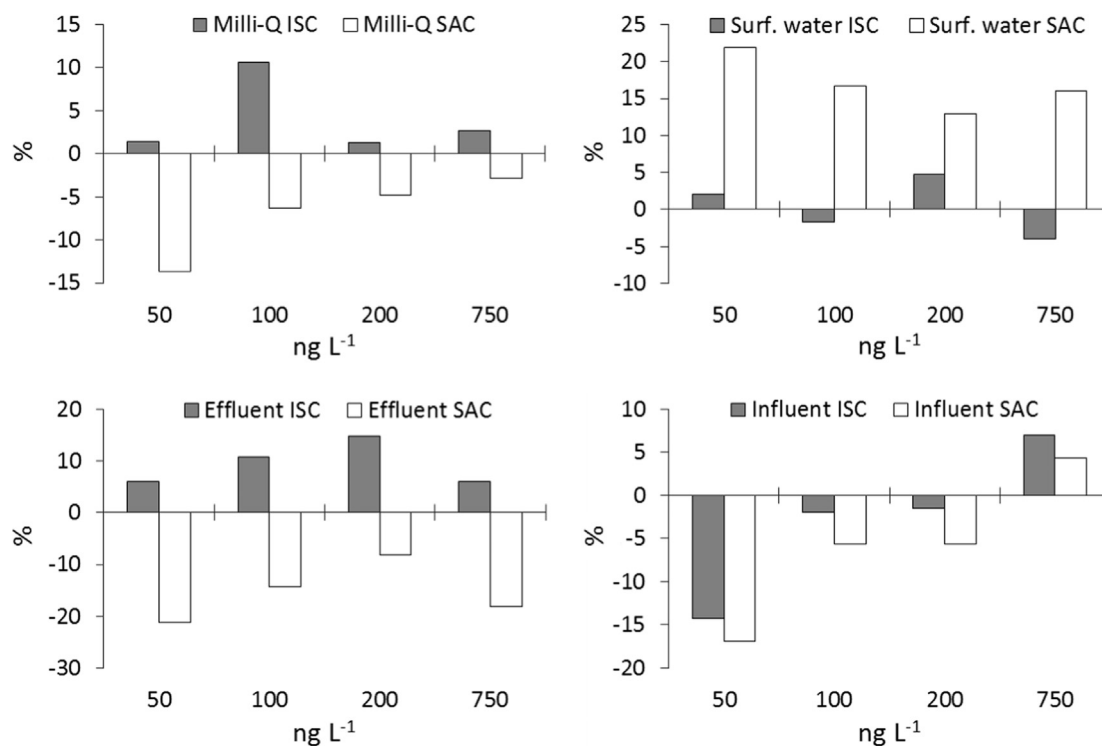


Fig. 2. Comparison of calibration methods, internal standard (ISC) and standard addition (SAC). Y-axis is the difference of quantified concentrations, using the two different calibration methods, in relation to nominal values.

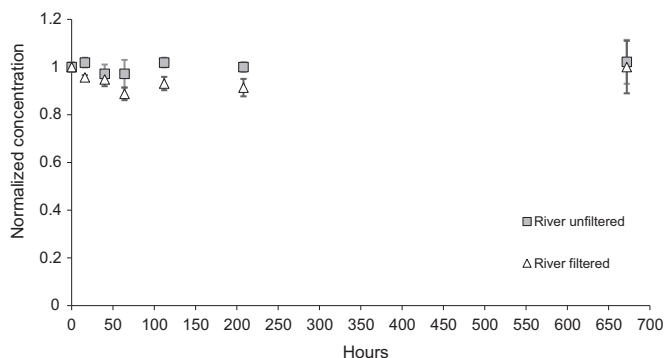


Fig. 3. Average concentration of zanamivir in the tested matrices during artificial UV irradiation exposure (Concentrations were normalized to initial concentration, RSD of triplicates were in the range 1–13%).

artificial UV irradiation (Fig. 3). Similar transformation rate ($\leq 8\%$) was also observed in the dark controls indicating that mainly other reactions than phototransformation caused the transformation of Za. Furthermore, no overlap between the absorption spectra of Za and the irradiation spectrum of the artificial UV-light source shows that the phototransformation pathway of Za was indirect (see Fig. S1, Supplementary material).

In comparison, a study on degradation of Za in surface water found 100% (half-life 3.6 h) and 30% degradation after 18 days of artificial and natural sunlight exposure, respectively [22]. The differences in degradation rate could be explained by different light intensity, and types and concentrations of inducers/inhibitors in the surface waters. About twenty times higher artificial sunlight irradiance (500 vs 22 W m^{-2}) was, for instance, used in the study by Zonja et al., compared to this study. This comparison shows that the photostability of Za varies significantly with sunlight irradiation intensity which in turn can show high global, seasonal and daily variation. Furthermore, the TOC (7.5 vs approximately

1.7 mg/L) and nitrate concentration (<5 vs approximately 0.065 mg/L), respectively, were higher in the surface water used in the study by Zonja et al. Thus, the TOC and nitrates could have worked as inducers and hence caused higher indirect photodegradation in the study by Zonja et al. than in this study. In summary, the results of Zonja et al.'s and this study indicate that the half-life of Za can be > 18 days depending on environmental conditions. Hence Za can show a rather high photostability; a stability depending on the environmental conditions such as sunlight intensity, and type and concentration of inducers and/or inhibitors in the surface water.

4. Conclusions

Emerging pollutants today are becoming more and more diverse in their chemical properties with, for instance, a large variety in their polarity. In this study, an online SPE method based on bonded zwitterionic stationary phases, for rapid trace determination of the highly polar and hydrophilic compound Za in surface waters, sewage water treatment influent and effluent samples, was successfully developed. 95% ACN and 1% FA were identified as sample pre-treatment which extracted Za effectively from the aqueous samples. The linearities ($r^2 \geq 0.994$), and intraday precisions (RSD $\leq 16\%$) were acceptable, and the LOQ values ($\leq 20 \text{ ng L}^{-1}$) were acceptable and environmentally relevant.

Although ion suppression of Za ion occurred (maximum 44%), the IS used ($^{13}\text{C}-^{15}\text{N}_2\text{-Za}$) was able to compensate Za peak area suppression (relative recoveries above 95%). The ISC determinations of Za in the environmental matrices were compared to SAC. Both of the calibration methods showed acceptable accuracy in relation to nominal concentrations (especially ISC, less than 15% deviation). Storage tests revealed that influent samples should be kept at -20°C to avoid significant Za losses. The filtration recovery was excellent and above 91%.

No significant transformation of Za was observed in surface water after 28 days of artificial sunlight irradiation exposure suggesting a relatively high photostability of Za. There is a risk that Za can induce antiviral resistance in the environment; a relatively high photostability of Za should therefore be of environmental concern.

Acknowledgments

The authors of this paper gratefully acknowledge the Swedish Research Council Formas (Grant no. 211-2013-1320), the Swedish Foundation for Strategic Environmental Research (Mistra) and the Family Olander-Nielsen's Foundation for financial support, and GlaxoSmithKline and Merck Millipore Sweden for material support.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.talanta.2015.03.066>.

References

- [1] S. Jain, P. Kumar, R.K. Vyas, A.K. Dalai, *Water Air Soil Pollut.* 224 (2013) 1–19.
- [2] B. Olsen, V.J. Munster, A. Wallensten, J. Waldenström, A.D.M.E. Osterhaus, R.A. M. Fouchier, *Science* 312 (2006) 384–388.
- [3] N.J. Cox, K. Subbarao, *Annu. Rev. Med.* 51 (2000) 407–421.
- [4] C. Brockwell-Staats, R.G. Webster, R.J. Webby, *Influenza Other Respir. Viruses* 3 (2009) 207–213.
- [5] J.D. Järhult, S. Muradrasoli, J. Wahlgren, H. Söderström, G. Orozovic, G. Gunnarsson, C. Bröjer, N. Latorre-Margalef, J. Fick, R. Grabic, J. Lennerstrand, J. Waldenström, Å. Lundkvist, B. Olsen, *PLoS One* 6 (2011) e24742.
- [6] A. Gillman, S. Muradrasoli, H. Söderström, J. Nordh, C. Bröjer, R.H. Lindberg, N. Latorre-Margalef, J. Waldenström, B. Olsen, J.D. Järhult, *PLoS One* 8 (2013) e71230.
- [7] J.D. Järhult, *Infect. Ecol. Epidemiol.* 2 (2012) 18385.
- [8] A. Meijer, H. Rebelo-de, V. Correia, T. Besselaar, R. Drager-Dayal, A. Fry, V. Gregory, L. Gubareva, T. Kageyama, A. Lackenby, J. Lo, T. Odagiri, D. Pereyaslov, M.M. Siqueira, E. Takashita, M. Tashiro, D. Wang, S. Wong, W. Zhang, R.S. Daniels, A.C. Hurt, *Antivir. Res.* 110 (2014) 31–41.
- [9] A. Patel, S.E. Gorman, *Clin. Pharmacol. Ther.* 86 (2009) 241–243.
- [10] A.L. Wan Po, P. Fardon, N. Palmer, *Emerg. Infect. Dis.* 15 (2009) 1686–1687.
- [11] J. Fick, R.H. Lindberg, M. Tysklind, P.D. Haemig, J. Waldenström, A. Wallensten, B. Olsen, *PLoS One* 2 (2007) e986.
- [12] G.C. Ghosh, N. Nakada, N. Yamashita, H. Tanaka, *Chemosphere* 81 (2010) 13–17.
- [13] P. Bartels, W. von Tumpling Jr., *Sci. Total Environ.* 405 (2008) 215–225.
- [14] T. Azuma, N. Nakada, N. Yamashita, H. Tanaka, *Chemosphere* 93 (2013) 1672–1677.
- [15] T. Azuma, N. Nakada, N. Yamashita, H. Tanaka, *Environ. Sci. Technol.* 46 (2012) 12873–12881.
- [16] R. Takanami, H. Ozaki, R.R. Giri, S. Taniguchi, S. Hayashi, *J. Water Environ. Technol.* 10 (2012) 57–68.
- [17] H. Söderström, J.D. Järhult, B. Olsen, R.H. Lindberg, H. Tanaka, J. Fick, *PLoS One* 4 (2009) e6064.
- [18] A.C. Singer, J.D. Järhult, R. Grabic, G.A. Khan, R.H. Lindberg, G. Fedorova, J. Fick, M.J. Bowes, B. Olsen, H. Söderström, *PLoS One* 9 (2014) e108621.
- [19] A.C. Singer, J.D. Järhult, R. Grabic, G.A. Khan, G. Fedorova, J. Fick, R.H. Lindberg, M.J. Bowes, B. Olsen, H. Söderström, *PLoS One* 8 (2013) e60221.
- [20] D.E. Dulek, J.V. Williams, C.B. Creech, A.K. Schulert, H.A. Frangoul, J. Domm, M.R. Denison, J.D. Chappell, *Clin. Infect. Dis.* 50 (2010) 1493–1496.
- [21] (<http://www.drugbank.ca/drugs/DB00558>) (accessed 12.03.15).
- [22] B. Zonja, C. Gonçalves, S. Pérez, A. Delgado, M. Petrovic, M.F. Alpendurada, D. Barceló, *J. Hazard. Mater.* 265 (2013) 296.
- [23] T. Azuma, N. Nakada, N. Yamashita, H. Tanaka, *Int. J. Environ. Anal. Chem.* 94 (2014) 853.
- [24] A.L.N. van Nuijs, I. Tarcomnicu, A. Covaci, *J. Chromatogr. A* 1218 (2011) 5964–5974.
- [25] P. Hemström, K. Irgum, *J. Sep. Sci.* 29 (2006) 1784–1821.
- [26] B. Buszewski, S. Noga, *Anal. Bioanal. Chem.* 402 (2012) 231–247.
- [27] P. Jandera, *Anal. Chim. Acta* 692 (2011) 1–25.
- [28] M. Seifrtová, L. Nováková, C. Lino, A. Pena, P. Solich, *Anal. Chim. Acta* 649 (2009) 158–179.
- [29] F. Qin, Y.Y. Zhao, M.B. Sawyer, X.F. Li, *Anal. Chim. Acta* 627 (2008) 91–98.
- [30] K.M. Peru, S.L. Kuchta, J.V. Headley, A.J. Cessna, *J. Chromatogr. A* 1107 (2006) 152–158.
- [31] M. Scheurer, F. Sacher, H.J. Brauch, *J. Environ. Monit.* 11 (2009) 1608–1613.
- [32] M. Farre, L. Kantiani, M. Petrovic, S. Perez, D. Barcelo, *J. Chromatogr. A* 1259 (2012) 86–99.
- [33] R.N. Xu, L. Fan, M.J. Rieser, T.A. El-Shourbagy, *J. Pharm. Biomed. Anal.* 44 (2007) 342–355.
- [34] R. Li, Y. Guoa, Q. Yuana, *J. Liq. Chromatogr. Relat. Technol.* 34 (2011) 1112–1132.
- [35] R.T. Roen, S.R. Sellevag, E. Lundanes, *Anal. Chim. Acta* 761 (2013) 109–116.
- [36] Y. Zhang, S. Lin, P. Jiang, X. Zhu, J. Ling, W. Zhang, X. Dong, *J. Chromatogr. A* 1337 (2014) 17–21.
- [37] A.L.N. van Nuijs, I. Tarcomnicu, L. Bervoets, R. Blust, P.G. Jorens, H. Neels, A. Covaci, *Anal. Bioanal. Chem.* 395 (2009) 819–828.
- [38] G.A. Khan, R. Lindberg, R. Grabic, J. Fick, *J. Pharmacol. Biomed. Anal.* 66 (2012) 24–32.
- [39] Society for water conservation in Ume- and Vindel River Recipientkontroll i Ume- och Vindelälven. (<http://www.umevindelvsvf.se/rapporter.html>), 2013 (accessed 18.03.15).
- [40] R.H. Lindberg, M. Östman, U. Olofsson, R. Grabic, J. Fick, *Water Res.* 58 (2014) 221–229.