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Verified Effect of pCure Towards Oxytetracycline in Sewage Water

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The release of pharmaceutical compounds to water resources has become a major and world-wide environmental concern. This kind of organic molecules are difficult targets for the wastewater treatment plants. New solutions that can remove or reduce the concentration of pharmaceutical residues at the source of contamination are desired. The continuous human use of drugs results in secretion of pharmaceutical residues to toilet and household sewage water. pCure is a new type of household product, developed to treat contaminated household sewage water. The active ingredient in the pCure products is enzymes. The enzymes are released from the product into the sewer system, where the enzymes act on target pharmaceutical compounds. Here, the effect of pCure towards the pharmaceutical compound Oxytetracycline is verified.

Introduction

The asset of pharmaceuticals for treatment of diseases is a cornerstone for global health and human development. However, pharmaceuticals have spread in Nature and become an alarming environmental concern. Today, the major release of drugs and pharmaceuticals into aquatic systems is through consumption.[1] To facilitate the elimination of the pharmaceutical from the body, metabolic enzymes located mainly in the gut and liver tissues are converting the chemical structure into metabolites to different degree. However, there are pharmaceuticals that are known to be unmetabolized. Both metabolites and unmetabolized pharmaceuticals are removed from the body by excretion, mainly through urine and feces.[1,2] From the toilet sewage water, the pharmaceutical residues are transported to the wastewater treatment plant. Most wastewater treatment plants are not designed for and cannot remove pharmaceuticals efficiently. Consequently, the contaminated water effluent is spread to various water resources.[1] The most commonly detected pharmaceutical compounds in water environments are analgesics, antibiotics, β-blockers, hormones and antidepressants.[3-11]

As one solution to this environmental concern, Pharem Biotech has developed pCure.[12] pCure is a new type of household product developed to treat contaminated sewage water at the source of contamination. The active ingredient in the pCure products is an enzyme blend. pCure contains a blend of enzymes that are carefully selected to remove pharmaceutical residues released into toilet sewage water. The selection of target pharmaceutical compounds is based on the occurrence in Nature and in sewage water in combination with compound bioaccumulation, biohazard and risk level.

The effect of pCure is based on the activity of the added enzyme blend. Enzymes are essential proteins (biological molecules) present in all living organisms. Cellular metabolism contains networks of chemical reactions, in distinct catabolic and anabolic pathways, which are catalysed and controlled by enzymes. Enzymes possess unique catalytic power. They can accelerate reaction rates by providing alternative reaction pathways, compared to other catalysts.[13] Since enzymes are proteins, which are biodegradable molecules, they are considered as environmentally friendly catalysts.[14] Nowadays, enzymes are applied in various industry sectors, including pharmaceutical industry and bioremediation.[15-21] In pCure, carefully selected enzymes are applied to reduce the concentration of target pharmaceutical compounds based on their catalytic activity.

To verify the effect of pCure, standardized methods developed according to ISO 14034:2016 Environmental Technology Verification (ETV) and ISO/IEC 17025:2017 - General requirements for the competence of testing and calibration laboratories were prepared.[22,23] The methods aim at showing on the effect of pCure towards various pharmaceutical compounds.

In this report, the effect of pCure towards Oxytetracycline is analysed. Oxytetracycline is categorized as an antibacterial agent. The compound is (for example) found in Terracortril and Terracortril + Polymyxin B.[24-27] Oxytetracycline is considered to have high environmental impact from being suspected to cause antibiotic resistance.[28-31] The concentration of oxytetracycline in urban wastewater are varying dependent on region. The average concentration for Oxytetracycline has been measured to 70 ng/L.[6,11,32-34]

Results

To verify the effect of pCure and the pCure enzyme blend ENZ-B.GEN-1811 towards different pharmaceutical compounds, standardized method protocols[22,23] were followed. The protocols were repeated 6-7 times and performed by qualified personnel and selected instruments.

Firstly, the effect of the pCure enzyme blend ENZ-B.GEN-1811 was measured towards target compounds. For all tested compounds, 100 µM of target pharmaceutical and internal standard was applied. A relatively high concentration of pharmaceutical compound was used to avoid or reduce possible instrumental variations and to maintain the analytical detection range. All reactions were performed six times using the pCure enzyme blend (enzyme-catalysed reaction), heat-inactivated enzyme (control reactions) and without enzyme (reference reactions) in separate reaction vessels. The reactions were followed for 4 hours and samples were regularly taken out at pre-determined timepoints for HPLC measurement. The removal of the pharmaceutical compound was determined in percent compared to control reaction. In average, ENZ-B.GEN-1811 removed 95% of Oxytetracycline from the reaction solution as measured after 2 h (Figure 1 and 2).

Thereafter, the effect of the pCure block towards the target pharmaceutical compound was tested in sewage water (in triplicates). Sampled sewage water[35] was spiked with the target pharmaceutical compound according to an estimated average compound concentration found in sewage water[6,11,32-34] and the analytical limit of quantification values[36,37]. pCure was added in a concentration corresponding to previously measured release of block mass in flush tests.[38] Samples of the spiked sewage water treated with pCure or using a pCure control block (starch instead of enzyme blend) were regularly removed for measurement. Following ISO 14034:2016, the sample measurement was performed by external analytical companies holding ISO 17025 accreditation. The obtained measurement data was analysed by regression statistics to determine the effect of pCure towards target pharmaceutical compound.[39]

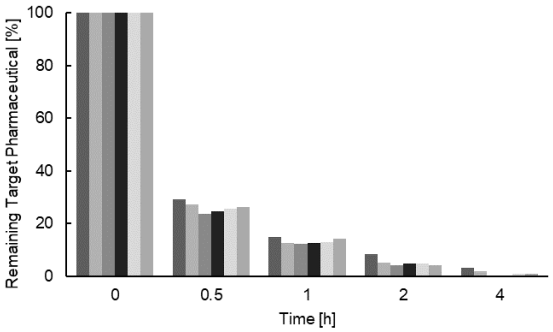

Figure 1. Remaining target pharmaceutical compound [%] in reaction solution after treatment with ENZ-B.GEN-1811 shown for six protocol repeats (various grey colours) over time. Values are normalized towards time point zero and shown relative to control reaction.

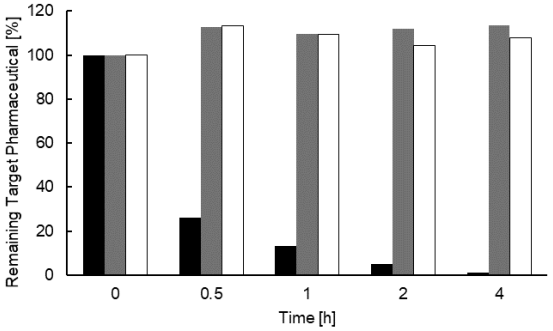

Figure 2. Remaining target pharmaceutical compound [%] in reaction solution after treatment with ENZ-B.GEN-1811 (black) in comparison to control (grey) and reference (white) reactions. The figure shows the mean values of 6 protocol repeats over time. Values are normalized towards time point zero.

Table 1. Effect trend (β_1), probability (*P*) value and confidence level (CI) of measurement data obtained using pCure[a] and pCure Control block (pCure without enzymes) towards Oxytetracycline[b][c] in sewage water based on regression analysis.

Values	pCure	pCure Control
β_1	.04	.09
P value	.054	.007
CI-95% upper	0.081	0.15
CI-95% lower	-0.00085	0.039

[a] pCure Home (KW8/2019,14/4058/B/239A)
[b] Measured at IVL Swedish Environmental Research Institute
[c] Measured at Eurofins

Table 2. Effect of pCure[a] towards Oxytetracycline[b][c] in sewage water based on regression analysis.

Time-point	Effect [%]
4 h	-15
8 h	-24

[a] pCure Home (KW8/2019,14/4058/B/239A)
[b] Measured at IVL Swedish Environmental Research Institute
[c] Measured at Eurofins

Table 3. Effect, probability (*P*) value and confidence level (CI) of measurement data obtained using pCure[a] towards Oxytetracycline[b][c] in sewage water shown at different time-points.

Time-point	Effect [%]	*P* value	CI-95% [%]	
			Upper	Lower
4 h	n.d.[d]	n.d.	n.d.	n.d.
8 h	-25	.13	23	-109
All measured values	(-30)	.03	4.3	-109

[a] pCure Home (KW8/2019,14/4058/B/239A)
[b] Measured at IVL Swedish Environmental Research Institute
[c] Measured at Eurofins
[d] n.d = no data

Conclusion

The effect of pCure is verified by showing on the specific enzymatic activity of the pCure enzyme blend towards Oxytetracycline in a controlled environment and the removal of Oxytetracycline by pCure block tests in raw sewage water. In average, ENZ-B.GEN-1811 removed 95% of Oxytetracycline after 2 h at the applied conditions (Figure 1 and 2). This is followed up by testing pCure in sewage water. The content of sewage water can vary greatly depending on nearby households and the pCure user behaviour. This can also be seen by the differences in the measured removal (Table 3). In accordance to the product definition, the removal level is not defined. Instead, a removal effect by pCure is claimed. The use of pCure should according to the presented results reduce the amount of Oxytetracycline reaching the environment.

To conclude, the effect of pCure towards Oxytetracycline is verified.

Experimental Section

Preparation of pCure enzyme blend effect analysis

Chemicals: Chemicals were purchased from Sigma-Aldrich and VWR (Sweden). Oxytetracycline (cat. no. PHR1537) and dimethylsulphoxide (DMSO, cat. no.1.02931.0500) were purchased from Sigma-Aldrich (Sweden). Phosphate buffer (200 mM, pH 6.5, cat. no. GENO786-879) was purchased from VWR (Sweden).

Equipment: An innova4230 refrigerated incubator from New Brunswick Scientific was used to control the mixing and temperature of the reaction solutions. An Eppendorf Thermomixer 5436 was used for enzyme heat-inactivation and sample quenching prior to HPLC measurement. For centrifugation of quenched reaction samples, an Eppendorf 5417R benchtop centrifuge was used. An Agilent 1100 HPLC equipped with a DAD UV detector and a ReproSil Gold C18 column (cat. no.

r25.9g.s1546, Dr. Maisch, GmbH) was used for compound identification and reaction measurement.

Enzyme solution: The enzyme blend (ENZ-B.GEN-1811) was prepared by Pharem Biotech AB, from enzymes obtained from external manufacturers. The enzyme blend (powder) was dissolved in sodium phosphate buffer (20 mM, pH 6.5) at the concentration 10 mg/mL (based on powder weight).

Enzyme inactivation: One part of the enzyme solution was heat inactivated (95°C, 30 min) and used as an inactivated control reaction for the calculation of the enzyme activity.

Pharmaceutical compound dissolution: Oxytetracycline was prepared as a stock solution (10 mM) in DMSO. The dissolved pharmaceutical compound stock solution was further diluted 100-fold to 100 µM in reaction solution.

Internal standard solution: The internal standard solution was prepared as a stock solution (10 mM) in DMSO.

Enzyme activity assay: In total, 6 protocol repeats were performed. Initially, pharmaceutical and internal standard stock solutions were mixed and diluted to 200 µM in Milli-Q water. For the enzyme activity assay, enzyme solution (10 mg/mL in 20 mM sodium phosphate buffer pH 6.5) and the pharmaceutical compound solution (200 µM) was mixed 1:1. The enzyme reaction solution contained: enzyme (5 mg/mL), pharmaceutical (100 µM) and internal standard (100 µM) in sodium phosphate buffer (10 mM, pH 6.5). The total volume of the reaction was 1.5 mL. One control and one reference reaction were performed for comparison. The control reaction contained heat-inactivated enzyme (5 mg/mL, Control reaction). The other reference reaction contained no enzyme, instead the corresponding volume of buffer was added (Reference reaction). The Enzyme, Control and Reference reactions were performed in Eppendorf tubes (2 mL). For temperature and mixing control, a refrigerated incubator shaker (160 rpm/min) set at 21°C was used. Samples were regularly taken at pre-determined time points (0, 0.5, 1, 2 and 4 h). The reaction samples were stopped (quenched) by incubation at 95°C for 5 min. All samples were quenched and stored in the fridge prior to HPLC measurement.

HPLC measurement: The quenched reaction samples were centrifugated (14.000 rpm, 2 min, 4°C), transferred to HPLC tubes and further analysed on an Agilent 1100 HPLC equipped with G1315B DAD UV detector. The HPLC was operated at HPLC measurement: The quenched reaction samples were centrifugated (14.000 rpm, 2 min, 4°C), transferred to HPLC tubes and further analysed on an Agilent 1100 HPLC equipped with G1315B DAD UV detector. The HPLC was operated at room temperature. Samples were injected (30 µL) by an Agilent autosampler. Chromatographic separation was achieved on a ReproSil Gold C18 column (5 µm, 200A, 150 x 4.6 mm). The mobile phase consisted of Solvent A (water + 0.1% Trifluoro acetic acid (TFA)) and Solvent B (Acetonitrile + 0.1% TFA). A solvent gradient method was applied. Solvent B was increased from 15-95% during 13 min and then kept constant at 95% for the remaining 2 min. The total HPLC method time was 15 min, using a flowrate of 1 mL/min. The retention time of Oxytetracycline was 9.3 min (detected at 254 nm).

Calculation of pCure enzyme blend effect: Identification of the target pharmaceutical peak was performed by running the reference reaction solution containing only buffer and pharmaceutical compound. The obtained peak areas of pharmaceutical and standard in all reaction samples were exported using the software Clarity 6.1.0.130 (Agilent Technologies). Removal of pharmaceutical in percent were calculated by exporting the data to Microsoft Excel. The mean area values were compared between time points and relative to control and reference samples. Due to the increased reaction rates of the enzyme-catalysed reactions, correct time point zero data were not possible to obtain. Therefore, the control reaction time point zero data were used also for the enzyme-catalysed reactions.

Preparation of pCure block effect analysis

Chemicals: All pharmaceuticals (Table 4), hydrochloric acid (HCl, cat. no. 25814), dimethylsulphoxide (DMSO, cat. no. 1.02931.0500) and phosphoric acid (cat. no. P5811) were purchased from Sigma-Aldrich (Sweden). Methanol (MeOH, cat. no. 20864.320) and phosphate buffer (500 mM, pH 6.5, cat. no. J60845) were purchased from VWR (Sweden).

Equipment: An Infors Multitron refrigerated incubator was used to control the mixing and temperature of the reaction solutions. A shaking water bath (Julabo SW20) was used for sample (enzyme) heat-inactivation (sample quenching).

Sewage water: Sewage water was collected from five different pump stations by SYVAB in Södertälje.[35] The water was used immediately or stored in the freezer and thawed at room temperature before use.

Preparation of sewage water reaction medium: The sewage water was buffered (10-40 mM, pH 6.2) using phosphate buffer. pH adjustment was made using HCl or phosphoric acid.

Preparation of pharmaceutical compound solutions: The pharmaceutical compounds were dissolved, prepared as stock solutions and further diluted in Milli-Q water to 1000-fold the reaction concentration (Table 4).

Table 4. Compiled information regarding the pharmaceutical compounds, their catalogue numbers, solvents for dissolution and the spiked concentrations of pharmaceutical compounds for measurement at IVL or Eurofins.

Pharmaceutical compound	Catalogue number[a]	Solvent	Conc. IVL VO-1032 [ng/L]	Conc. Eurofins VO-1033 [ng/L]	Conc. Eurofins VO-1037 [ng/L]
Diclofenac sodium	93484	MeOH	1700	1700	1700
Doxycycline	D9891	MeOH	1400	1400	1600
Estradiol	PHR1480	MeOH or DMSO	170	170	180

Estriol	E1253	MeOH or DMSO	1300	1300	1300
Felodipine	1269389	DMSO	1300	1300	3200
Ketoconazole	PHR1385	DMSO	200	200	200
Oxytetracycline	PHR1537	DMSO	1300	1300	4500
Paracetamol	P0300000	Water	73000	73000	73000
Tetracycline	31741	MeOH	1200	1200	1600

[a] All pharmaceuticals were purchased from Sigma-Aldrich.

Preparation of pCure solution: pCure block and pCure Control block (contains starch instead of enzymes) were obtained from the manufacturer (Buck-Chemie GmbH). From each block, 100 mg of block material was weighed and transferred to separate glass flasks (100 mL). The block materials were dissolved (1.2 g/L) in 83.2 mL of sewage water reaction medium.[38] The pH of the pCure solutions were adjusted if needed.

Reaction: All reactions were performed in 7 repetitions and in glass flasks. For comparison, control reactions containing pCure control block solution were prepared. Various pharmaceutical compounds (Table 4) were added to each reaction vessel to be able to verify the simultaneous effect of pCure towards them. The final concentration of the individual pharmaceutical compounds in the reaction solution is shown in Table 4 (right column).

For pCure reactions (1 L): 20 mL of pCure solution (1.2 g/L), 1 mL of each of the 20 different pharmaceutical stock solutions (20 mL) and sewage water reaction medium up to 1 L.

For control reactions (1 L): 20 mL of pCure control block solution (1.2 g/L), 1 mL of each of the 20 different pharmaceutical stock solutions (20 mL) and sewage water reaction medium up to 1 L.

The reactions were incubated in a refrigerated incubator shaker set at 18°C and 80 rpm.

Reaction samples and quenching: Samples (110 mL to IVL and 220 mL to Eurofins) were regularly withdrawn from the reactions into glass flasks (250 mL) at different time-points. The reaction samples were stopped (quenched) by an incubation at 90-95°C for 15 min in a shaking water bath. After quenching, the samples were cooled down for 1-2 h at room temperature. Samples for measurement at IVL were transferred to plastic storage containers (cat. no. 216-2405, Kartell, VWR) and stored in the freezer until delivery. Samples for measurement at Eurofins were collected, transferred to dark glass bottles and stored in the fridge prior to delivery on the next day.

External measurement: All samples (pCure and control) were measured externally using LCMS at Eurofins and IVL Swedish Environmental Research Institute. At Eurofins, the following accredited method and instruments were applied: Pharmaceuticals – EPA 1694 (HPLC/MS/MS). At IVL, the following non-accredited method and instrument were applied: Pharmaceuticals (HPLC-HRMS).

Calculation of pCure block effect: The measurement data was received from Eurofins and IVL. A collective statistical analysis was performed.[39]

Keywords: Clean water • Enzyme technology • Enzyme-based bioremediation • Pharmaceutical residues • Municipal wastewater treatment

Acknowledgement

We are grateful to all collaborators, investors and personnel that have aided the development, production and verification of pCure.

References

[1] Patel, M., Kumar, R. Kishor, K., Mlsna, T., Pittman, Jr.C.U., Mohan, D. (2019) Pharmaceuticals of emerging concern in aquatic systems: Chemistry, occurrence, effects, and removal methods. *Chem. Rev.* 119, 3510-3673.

[2] Ionescu, C., Caira, M.R. (2005) Drug metabolism: current concepts, Netherlands, Springer.

[3] Vieno, N., Tuhkanen, T., Kronberg, L. (2007) Elimination of pharmaceuticals in sewage treatment plants in Finland. *Water Res*. 41, 1001-1012.

[4] Naturvårdsverket (2008) Avloppsreningsverkens förmåga att ta hand om läkemedelsrester och andra farliga ämnen. Rapport 5794. ISBN 978-91-620-5794-5797.

[5] Helmfrid, I., Eriksson, C. (2010) Läkemedelsrester från sjukhus och avloppsreningsverk. Svenskt vatten, Rapport 2010:1.

[6] Wahlberg, C., Björlenius, B., Paxéus, N. (2010) Läkemedelsrester i Stockholms vattenmiljö. Stockholm Vatten. ISBN 978-91-633-6642-0.

[7] Vasquez, M.I., Lambrianides, A., Schneider, M., Kümmerer, K., Fatta-Kassinos, D. (2014) Environmental side effects of pharmaceutical cocktails: What we know and what we should know. *J. Hazard. Mater.* 279, 169-189.

[8] Barsel, C., Cousins, A. P., Hörsing, M., Ek, M., Ejhed, H., Allard, A-S., Magnér, J., Westling, K., Wahlberg. C., Fortkamp, U., Söhr, S. (2015) Pharmaceutical residues and the other emerging substances in the effluent of sewage treatment plants. IVL Swedish Environmental Research Institute, IVL-report B 2226.

[9] aus der Beek, T., Weber, F.A., Bergmann, A., Hickmann, S., Ebert, I., Hein, A., Küster, A. (2016) Pharmaceuticals in the global environment – Global occurrences and perspectives. *Environ. Toxicol. Chem.* 35, 823-835.

[10] Loos, R., Marinov, D., Sanseverino, I., Napierska, D., Lettieri, T. (2018) Review of the 1st Watch List under the Water Framework Directive and recommendations for the 2nd Watch List, EUR 29173 EN, Publications Office of the European Union, Luxembourg, ISBN 978-92-79-81839-4, doi:10.2760/614367, JRC111198.

[11] Database "Pharmaceuticals in the environment" https://www.umweltbundesamt.de/en/database-pharmaceuticals-in-the-environment-1. Accessed 190130.

[12] https://www.pcure.se/en/our-products, Accessed 190324.

[13] Berg, J.M., Tymoczko, J.L., Stryer, L. Biochemistry, International 7th edition, England, W. H. Freeman and Company.

[14] Anastas, P., Eghbali, N. (2010) Green Chemistry: Principles and practice. Chem. Soc. Rev. 39, 301-312.

[15] Dec, J., Bollag, J.M. (2000) Use of enzymes in bioremediation pesticide biotransformation in plants and microorganisms, *Chapter 10, pp. 182.193.* DOI: 10.1021/bk-2001-0777.ch010

[16] Kirk, O., Borchert, T.V., Fuglsang, C.C. (2002) Industrial enzyme applications. *Curr. Opin. Biotechnol*. 13, 345-351.
[17] Choi, J-M., Han, S-S., Kim H.S. (2015) Industrial applications of enzyme biocatalysis: Current status and future aspects. *Biotechnol. Adv.* 33, 1443-1454.
[18] Singh, R., Kumar, M., Mittal, A., Meht, P.K. (2016) Microbial enzymes: industrial progress in 21st century. *3 Biotech*. 6, 174.
[19] Yan, J., Li, W., Ng, T.B., Deng, X., Lin, J, Ye, X. (2017) Laccases: Production, expression regulation, and applications in pharmaceutical biodegradation, *Front. Microbiol.,* https://doi.org/10.3389/fmicb.2017.00832.
[20] Chapman, J., Ismail, A.E., Dinu, C.Z. (2018) Industrial applications of enzymes: Recent advances, techniques, and outlooks. *Catalysts* 8, 238; doi:10.3390/catal8060238
[21] Sharma, Dangi, Shukla. (2018) Contemporary enzyme based technologies for bioremediation: A review. *J. Environ. Manage.* 210, 10-22.
[22] Method 101: pCure Sample Preparation in Sewage Water for Analysis of Effect Towards Pharmaceuticals, https://cdn.pcure.se/pcure_labs/verification/method_description_im101_pcure_sample_preparation_in_sewage_water_for_analysis_of_effect_towards_pharmaceuticals.pdf, Accessed 201008
[23] Method 201: Analysis of pCure Enzyme Blend Effect Towards Pharmaceuticals, https://cdn.pcure.se/pcure_labs/verification/method_description_im201_analysis_of_pcure_enzyme_blend_effect_towards_pharmaceuticals.pdf, Accessed 201008
[24] https://www.drugbank.ca
[25] Pharmaceutical products, https://labs.pcure.se, Accessed 190606
[26] Pharmaceutical products, https://www.ema.europa.eu/en
[27] Pharmaceutical products, https://www.fda.gov/
[28] Compound hazard, https://labs.pcure.se, Accessed 190606
[29] Yang, L., Ying, G., Su, H., Stauber, J.L., Adams, M.S., Binet, M.T. (2008) Growth-inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater microalga Pseudokirchneriella subcapitata. *Environ. Toxicol. Chem*. 27, 1201-1208.
[30] Oliveira, R., McDonough, S., Ladewig, J., Soares, A., Nogueira, A., Domingues, I. (2013) Effects of oxytetracycline and amoxicillin on development and biomarkers activities of zebrafish (Danio rerio), *Environ. Toxicol. Pharmacol*, 36, 903-912.
[31] Ferreira, C., Nunes, B., de Melo Henriques-Almeida, J., Guilhermino, L. (2007) Acute toxicity of oxytetracycline and florfenicol to the microalgae Tetraselmis chuii and to the crustacean Artemia parthenogenetica, *Ecotox. Environ. Safe*. 67, 452-458.
[32] Chang, X.S., Meyer, M.T., Liu, X.Y., Zhao, Q., Chen, H., Chen, J.A., Qiu, Z.Q., Yang, L., Cao, J., Shu, W. Q. (2010) Determination of antibiotics in sewage from hospitals, nursery and slaughter house, wastewater treatment plant and source water in Chongqing region of Three Gorge Reservoir in China. *Environ Pollut.* 158, 1444-1450.
[33] Li, B., Zhang, T. (2011) Mass flows and removal of antibiotics in two municipal wastewater treatment plants. *Chemosphere* 83, 1284-1289.
[34] Gao, P., Ding, Y., Li, H., Xagoraraki, I. (2012) Occurrence of pharmaceuticals in a municipal wastewater treatment plant: Mass balance and removal processes. *Chemosphere* 88, 17-24.
[35] Sewage water sampling report, https://cdn.pcure.se/pcure_labs/verification/pcure_urban_sewage_water_sampling.pdf, Accessed 191213
[36] Eurofins LOQ values: https://analyskatalog.eurofins.se/Search/SearchView/gGTKTaX6, Accessed 191212
[37] IVL LOQ values: https://www.ivl.se/sidor/lab--analys/avloppsvatten.html, Accessed 191212
[38] pCure standard dissolving test, https://cdn.pcure.se/pcure_labs/verification/standard-dissolving-test_for_pcure_products.pdf, Accessed 190606
[39] pCure statistical data, https://cdn.pcure.se/pcure_labs/verification/analysis_report_analysis_of_pcure_effect_towards_selected_pharmaceuticals_in_sewage_water.pdf, Accessed 200108