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A Simple Method for Determining Residual *p*-arsanilic Acid in Aquatic Products using EDTA-Assisted Solvent Extraction and LC-MRM

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Abstract : A simple method was developed to determine residual *p*-arsanilic acid (ASA), an organo-arsenic compound used as a feed additive, in aquatic products (eel, halibut, and shrimp) using EDTA-assisted solvent extraction and LC-MRM. The method was successfully validated in terms of specificity, linearity (coefficient of determination ≥ 0.995), accuracy (recovery or R, 72.72-78.73%), precision (the relative standard deviation of R, 2.08-6.98%), and sensitivity (the lower limit of quantitation, 5 ppb) according to CODEX guidelines (CAC-GL 71-2009). The use of EDTA in the extraction solvent and water with a suitable pH modifier as the reconstitution solvent may be the key factors for successful results. This is the first method that can be used for monitoring residual ASA in aquatic products using LC-MRM and could contribute to establishing a better aquatic product safety management system.

Keywords : *p*-arsanilic acid, MRM, EDTA, solvent extraction, halibut, eel, shrimp, aquatic products

Introduction

p-Arsanilic acid (ASA, Figure 1) is an organo-arsenic compound used as a feed additive for growth promotion and dysentery prevention or treatment in the livestock industry for decades.[1,2] While ASA was approved by the U.S. Food and Drug Administration (FDA) for use in poultry and swine in 1960s, there had been concerns about environmental and health issues arising from arsenic compounds that may have originated from it.[3] Organo-arsenic compounds, including ASA, are excreted without metabolism and have low bioaccumulation.[4,5] However, these compounds can be converted to more toxic inorganic arsenic compounds, including arsenite (As (III)) and arsenate (As (V)), through biotransformation in the environment.[6,7] The toxicity of inorganic arsenic compounds to humans is extensive and includes skin diseases, cancer, cardiovascular disorders, gastrointestinal disorders, and neurological disorders.[8-10] Thus, Cross Vet Pharm Group Ltd. voluntarily withdrew ASA approval from the U.S. FDA in 2014 due to its potential adverse effects on human health and the environment.[11] Similarly, many other countries have restricted or banned its use in animal feed. Moreover, the need to regulate residual ASA in aquatic products is also emerging due to their increasing consumption worldwide.[12,13] To effectively regulate residual ASA in foods, it is necessary to develop accurate and sensitive methods for determining ASA in foods without interference from food matrices. According to Zhao *et al.*'s report, 8.06-39.3 ppb of ASA was confirmed in ASA-contaminated chicken collected from local markets in China.[2] Thus, high pressure liquid chromatography interfaced with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) is a reliable method for monitoring ASA in foods due to its separation and sensitivity capabilities. For instance, Yang *et al.* reported an ASA assay applicable to chicken using solvent extraction and reversed phase LC-ICP-MS.[14] While it showed good separation features and high sensitivity (the lower limit of quantitation, LLOQ of

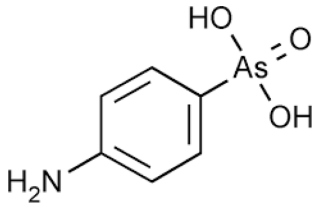

Figure 1. Chemical structure of *p*-arsanilic acid.

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1 ppb), ICP-MS is not commonly available in most laboratories. As an alternative to ICP-MS, atomic fluorescence spectrometry in combination with hydride generation (HG-AFS) can also be connected to HPLC for ASA analyses in foods. Cui *et al.*'s work is a good example of this approach.[15] They employed accelerated solvent extraction (ASE) and reversed phase LC-HG-AFS to determine ASA in pork and chicken. While the unique efficiency of ASE was observed in their study, there was still a substantial margin for improvement in sensitivity (LLOQ of 100 ppb). In South Korea, the Ministry of Food and Drug Safety regulates ASA residues in livestock products using the ASA assay (the assay number of 8.3.46) in the Food Code of South Korea.[16] The ASA assay is based on solvent extraction and reversed phase LC-MRM (multiple reaction monitoring), which have advantages of simple sample preparation procedures and high sensitivity (LLOQ of 5 ppb). However, aquatic products are not included in its subject of application.

In this study, a novel method for determining residual ASA in aquatic products using EDTA-assisted solvent extraction and LC-MRM was developed and validated. The present method is the first measure to regulate residual ASA in aquatic products using LC-MRM and could contribute to establishing a better aquatic product safety management system.

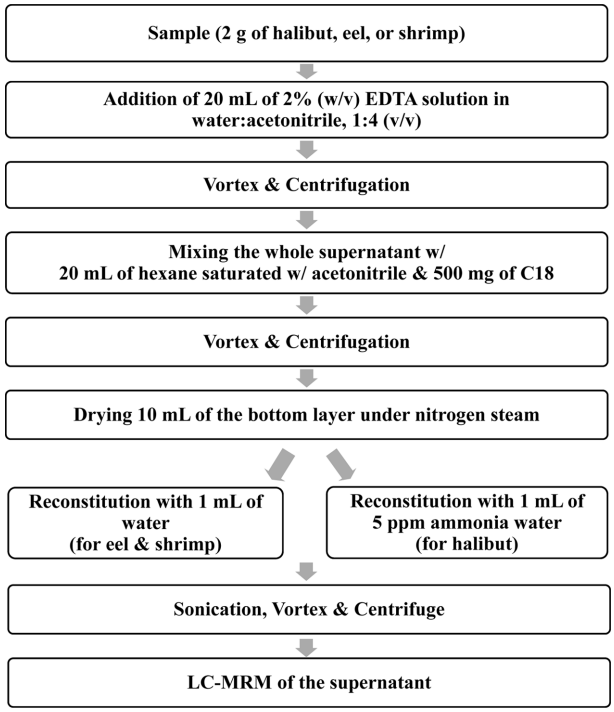

Figure 2. Schematic diagram of the present method to determine residual *p*-arsanilic acid in aquatic products using solvent extraction and LC-MRM.

Experimental

Chemicals and reagents

4-Aminophenylarsonic acid (ASA), ammonium hydroxide solution, and formic acid were purchased from Sigma Aldrich (St. Louis, MO, USA). Acetonitrile, hexane, water, and methanol were obtained from J. T. Baker (Phillipsburg, NJ, USA). Ethylenediaminetetraacetic acid (EDTA) was supplied from SAMCHUN (Pyeongtaek-si, Gyeonggi-do, Korea). All reagents were used without any additional purification process.

Preparation of ASA solutions

To prepare the ASA stock solution, ASA was dissolved in methanol at 1 mg/mL. Working standard solutions were prepared by appropriate dilution of the stock solution with methanol. The stock solution and working solutions were stored at -20°C until use.

Sample preparation

Eel, shrimp, and halibut samples were purchased from local food markets. Individual samples were homogenized, and 2 g of each sample was transferred to a 50 mL conical tube. Then, 4 mL of 10% (w/v) aqueous solution of EDTA and 16 mL of acetonitrile were added to the tube. The mixture was vortexed for 5 minutes and centrifuged at 4°C and 4,000 × g for 10 minutes. The supernatant was mixed with hexane saturated with acetonitrile and 500 mg of C18. The mixture was vortexed for 5 minutes and centrifuged at 4°C and 4,000 × g for 10 minutes. The obtained solution was dried at 40°C under the nitrogen stream and reconstituted in either 1 mL of water (eel, shrimp) or 0.0005% ammonia water (halibut). The final solution was sonicated for 5 minutes, vortexed for another 5 minutes, and then centrifuged at 4°C and 4,000 × g for another 5 minutes. Finally, the supernatant (the final extract) was filtered through a 0.22 μm filter and analyzed by LC-MS/MS (Figure 2). In addition, a matrix-matched standard (MMS) and a standard-spiked sample (SSS) were prepared by adding an appropriate volume of an ASA working standard solution to the final extract of a blank sample and to a blank sample prior to sample preparation, respectively.

Liquid chromatography and tandem mass spectrometry (LC-MS/MS)

For LC-MS/MS, a Shimadzu Nexera UPLC system (Tokyo, Japan) and a Shimadzu LCMS 8050 triple quadrupole mass spectrometer were interfaced through electrospray ionization (ESI) in positive ion mode. Sample extracts were separated for ten minutes at gradient mobile phase conditions (mobile phase A of 0.1% formic acid in water and mobile phase B of 0.1% formic acid in acetonitrile, Table 1) with a flow rate of 0.2 mL/min using a Phenomenex Luna Omega C18 column (2.1 × 100 mm, 3 μm, Torrance, CA, USA). The column oven and the

Table 1. Gradient mobile phase program.

Time (minutes)	0.1% (v/v) Formic acid in water (%, v/v)	0.1% (v/v) Formic acid in acetonitrile (%, v/v)
0.0	100.0	0.0
3.0	100.0	0.0
3.1	5.0	95.0
5.0	5.0	95.0
5.1	100.0	0.0
10	100.0	0.0

autosampler were kept at 4°C and 40°C, respectively. MRM, a sensitive and selective MS/MS scan, was used to determine ASA sensitively through mass spectrometry and two transitions (the screening transition of 218.0 *m/z* (precursor ion) / 109.0 *m/z* (product ion)/ -15 V (collision energy); the confirmatory transition of 218.0 *m/z* / 65.1 *m/z* / -26 V) were employed (Table 2). Additional mass spectrometer conditions were as follows: drying gas flow at 10 L/min, heating gas flow at 10 L/min, nebulizing gas flow at 3 L/min, DL temperature at 250°C, interface temperature at 380°C, and heating block temperature at 400 °C. All data were acquired and analyzed using Lab Solutions (version 5.93, Shimadzu), and especially, screening transition peak area values from sample extract analyses were compared with calibration curves obtained from MMS analyses for quantitation. Additionally, three prerequisites were necessary for quantitation: (1) both transition peaks should have the same retention time; (2) the ion ratio (the percentage ratio of the area value of the confirmatory transition peak to that of the screening transition peak) should be between 21 and 35%; and (3) the signal-to-noise ratio (S/N) of the screening transition peak and confirmatory transition peak should not be less than 10 and 3, respectively.

Validation

The present method was validated at three aquatic product matrices (eel, halibut, and shrimp) in terms of specificity, linearity, accuracy, precision, and sensitivity following Codex guidelines (CAC-GL 71-2009).[17] First, we tested the specificity by comparing individual blank matrices with their conjugate SSSs (5 ppb). Second, we evaluated linearity as coefficient of determination (r^2) values of individual calibration curves built from six MMS (5, 10, 20, 30, 40, and 50 ppb) analyses (n=3). Thirdly, we evaluated accuracy and precision as recovery (R) and its relative standard deviation (RSD), respectively. The R was calculated by dividing the screening transition peak area of a SSS by that of its counter MMS. R tests for each matrix were carried out at 5, 10, and 20 ppb for three consecutive days (n=5). Finally, sensitivity was tested as LLOQ, which is the smallest concentration point that satisfies all validation parameters.

Results and Discussion

Applicability test of the current ASA assay in Food Code of South Korea to aquatic products

The purpose of the current ASA assay (the assay number of 8.3.46) in Food Code of South Korea is to determine residual ASA in livestock products.[16] This method extracts ASA using acetonitrile, reconstitutes its dried extract with the same solvent, and analyzes the reconstituted extract using reversed phase LC-MRM. However, since aquatic products are not included in its subject of application, we tested its applicability to aquatic products in terms of retention time and S/N from eel and halibut SSS analyses (10 ppb). According to the results, ASA doesn't retain well on the column (retention time of 1.05) and the S/N values are less than 10 (eel, 6.15; halibut, 5.72), which is the least requirement for quantitation (data not shown). Thus, the current ASA assay in Food Code of South Korea was found to be inapplicable to aquatic products. Incomplete extraction/dissolution of ASA (logP of -0.26) in acetonitrile (the polarity index of 5.8) might be the major cause of this issue.[18,19] Therefore, it is necessary to develop a novel method to determine ASA residues in aquatic products.

Method development

Liquid chromatography and multiple reaction monitoring

New MRM transitions for ASA were developed as the first step to determine residual ASA in aquatic products. The $[M+H]^+$ ion (218.0 *m/z*) of ASA was selected as the precursor ion for its MRM transitions. The ion with 109.1 *m/z* (the strongest fragment ion from the product ion scan

Table 2. Properties of *p*-arsanilic acid.

Compound	Molar mass (Da)	Retention time (minutes)	MRM transition		
			Precursor ion (*m/z*)	[a]Product ion (*m/z*)	[b]CE (V)
p-Arsanilic acid	217.05	1.93	218.0	109.0	-15
				65.1	-26

[a]The product ion of the screening transition; and the product ion of the confirmatory transition
[b]Collision energy; the CE of the screening transition; and the CE of the confirmatory transition

of the precursor ion) and the ion with 65.1 *m/z* (the second strongest fragment ion from the product ion scan of the precursor ion) were chosen for the screening transition and the confirmatory transition, respectively. Among various analytical columns (Agilent HILIC, Luna HILIC, Gemini C6-phenyl, Agilent EC C18, Luna Omega C18, and Kinetex C18), Luna Omega C18 was selected as the column of choice due to its best values in both retention time and screening transition peak area (data not shown).

Sample preparation

As mentioned above, the current ASA assay in Food Code of South Korea was found not to be applicable to aquatic products probably due to incomplete extraction/dissolution of ASA (logP of -0.26) in acetonitrile (the polarity index of 5.8) used as an extraction solvent and a reconstitution solvent of the method.[18,19] To address this issue, we developed a new extraction and purification (E/P) method based on that of an assay of multiple veterinary drugs (the assay number of 8.3.1) in Food Code of South Korea.[20] The new method employs a smaller amount of sample (2 g *vs.* 5 g), an extraction solvent consisting of a mixture between water and acetonitrile, and water as a reconstitution solvent. First, since there is a possibility of ASA sensitivity reduction by adsorption between ASA and metal oxides such as Al_2O_3 and MgO, we attempted to suppress the adsorption to enhance ASA sensitivity by removing metal ions from a sample through chelation with EDTA, a chelating agent. We compared four different percentages (0, 1, 2, and 3%, w/v) of EDTA in the extraction solvent in terms of ASA screening transition peak area from eel SSS analyses (10 ppb, n=3). As shown in Figure 3, we found that 2% (w/v) EDTA was optimal due to its highest peak area value (relative area value of 100.0 ± 1.0%).[21,22] The dramatic sensitivity reduction observed with 3% (w/v) EDTA may be due to EDTA-ASA adduct formation during ESI. Non-volatile electrolytes in sample solutions generally negatively affect the sensitivity of mass spectrometry by forming adducts with analytes during ESI; EDTA, a non-volatile electrolyte, seemed to have similar effects.[23] Also, four different volumes (10, 20, 30, and 40 mL) of extraction solvent were compared in terms of ASA screening transition peak area from eel SSS analyses (10 ppb, n=3). Interestingly, 20 mL of extraction solvent showed a significantly larger peak area (100.0 ± 1.1) than the others probably due to EDTA in the extraction solvent (Figure 4). The amount of EDTA added to the sample was optimized in previous experiments; therefore, adding smaller amounts (by 10 mL) or larger amounts (by 30 and 40 mL) might induce sensitivity reduction by ASA-metal oxides adsorption formation or by ASA-EDTA adduct formation. Finally, four different reconstitution solvents with various pH modifiers (0.1% (v/v) formic acid in water, 100% water, 5 ppm ammonia water, and 10 ppm

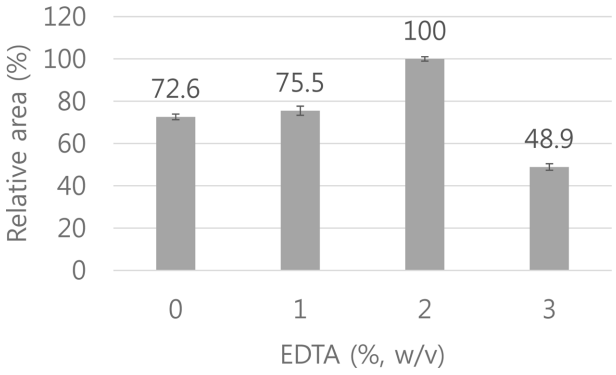

Figure 3. Effect of different EDTA percentages (w/v) in the extraction solvent on MRM screening transition peak area of *p*-arsanilic acid.

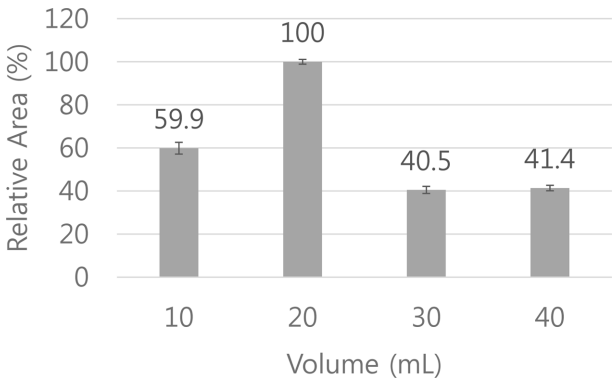

Figure 4. Effect of different volumes of the extraction solvent on MRM screening transition peak area of *p*-arsanilic acid.

Table 3. Effect of different acid/base modifiers in the reconstitution solvent (water) on the recovery of *p*-arsanilic acid from halibut, eel, or shrimp standard-spiked samples (10 ppb, n=3).

Reconstitution solvent	Recovery (%) of *p*-arsanilic acid from standard-spiked samples (10 ppb, n=3)		
	Halibut	Eel	Shrimp
0.1% (v/v) Formic acid	64.60 ± 4.58	69.80 ± 4.12	64.59 ± 1.26
100% Water	65.66 ± 3.05	73.56 ± 1.55	82.65 ± 3.02
5 ppm Ammonia water	75.46 ± 1.31	65.96 ± 2.59	81.75 ± 3.03
10 ppm Ammonia water	66.07 ± 3.25	57.99 ± 5.69	61.31 ± 1.54

ammonia water) were compared in terms of R from three matrices SSS (10 ppb, n=3) to optimize the final step of reconstituting the extract residue. While pure water without pH modifier showed the best R in eel and shrimp analyses (73.56 ± 1.55% from eel; 82.65 ± 3.02% from shrimp), 5 ppm ammonia water produced the best R (75.46 ± 1.31%) in halibut analyses (Table 3). The efficiency to be $[M+H]^+$ during ESI may be altered by the pH of the sample extract solution since ASA is a polyprotic acid with two acidic sites and a basic site: the composition ratio among its four different acidic/basic forms ($ASAH_3^+$ (or $[M+H]^+$), $ASAH_2$, $ASAH^-$, and ASA^{2-}) in a solution is determined by its pH. Thus, the difference in optimal pH modifier condition of the reconstitution solvent for halibut from that for eel and shrimp may be explained by their difference in acidity/basicity at their extract level.

Method validation and simple applications

The present method was validated in terms of specificity, linearity, accuracy, precision, recovery, and sensitivity following Codex guidelines (Table 4).[17] Eel, halibut, and shrimp were selected as matrices for validation because fat is considered a major component that can interfere with residual analyses in food. These matrices have high (eel, 17.1%), medium (halibut, 3.3%), and low (shrimp, 0.7%) fat content levels among aquatic products. Validation with these matrices can test the applicability of the present method over a broad range of aquatic products. Specificity was confirmed by the absence of a peak at the retention time of ASA (1.93 minutes) in blank matrices results (Figure 5). Linearity was confirmed by all r^2 values of calibration curves with the concentration range of 5 to 50 ng/mL being at least 0.9951. Accuracy and precision were evaluated as R and RSD of R, respectively. All results were good enough to satisfy CODEX guideline criteria: intra-day accuracy between 72.25% and 80.06%; inter-day accuracy between 72.72% and 78.73%; intra-day precision not more than 7.12%; and inter-day precision not more than 6.98%. All ASA MRM peaks obtained over validation studies produced S/N values of at least 10 (at screening transition peaks) or at least 3 (at confirmatory transition peaks). Thus, the smallest concentration point that satisfied the criteria of all validation parameters was determined as the LLOQ of the present method, which was found to be 5 ppb. This is the same LLOQ as that of the current regulatory ASA assay actively used in South Korea for monitoring residual ASA in livestock products.[16] Therefore, the developed method was successfully validated through CODEX guidelines and is the first method that can be used for monitoring residual ASA in aquatic products using LC-MRM.

Simple applications of the validated method to monitor

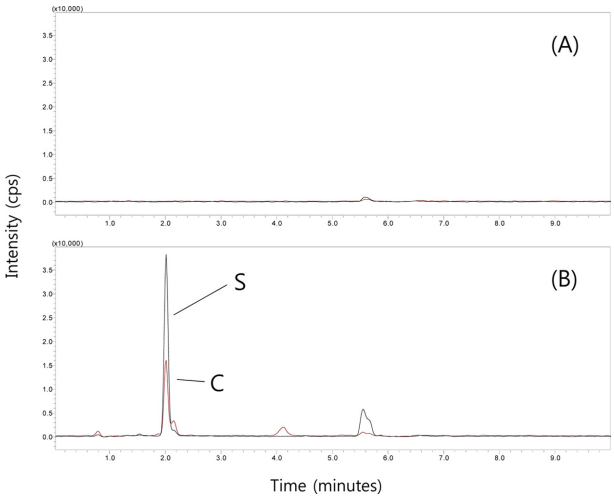

Figure 5. MRM chromatograms from blank shrimp (A) and 5 ppb standard-spiked shrimp (B) analyses. S and C stand for the screening transition peak and the confirmatory transition peak, respectively.

Table 4. Method validation information.

Matrices	Linearity ([a]r^2)	Fortified concentration (ppb)	Intraday (n=5)		Interday (n=5, 3 days)		[c]LLOQ (ppb)
			Recovery (%)	[b]RSD (%)	Recovery (%)	[b]RSD (%)	
Halibut	0.9983	5	80.06	7.12	78.63	5.48	5
		10	76.24	6.45	76.96	6.98	
		20	79.66	6.35	78.73	5.94	
Eel	0.9951	5	75.88	3.14	75.69	4.89	
		10	74.88	4.78	74.17	3.84	
		20	73.48	3.88	72.90	3.53	
Shrimp	0.9983	5	74.17	2.40	73.45	3.88	
		10	72.25	1.54	73.96	2.16	
		20	72.91	2.15	72.72	2.08	

[a]Coefficient of determination
[b]Relative standard deviation of recovery
[c]Lower limit of quantitation

residual ASA in aquatic products were carried out. Three samples per species of eel, halibut, and shrimp were purchased from local food markets. Each sample was prepared and analyzed in triplicate using the validated method to monitor residual ASA. No ASA peak was observed in any of the chromatograms, indicating that there was no contamination of ASA in any of the tested samples.

Conclusions

A simple method to determine residual ASA in aquatic products (eel, halibut, and shrimp) using EDTA-assisted solvent extraction and LC-MRM was developed and validated. In this method, two key factors were optimized: the concentration of EDTA in the extraction solvent was optimized to enhance ASA with an intention to suppress the formation of ASA-metal oxides adsorption and ASA-EDTA adducts; water with a suitable pH modifier was chosen as the reconstitution solvent for complete dissolution of ASA from the extract residue as well as for higher ionization efficiency of ASA during ESI. To the best of our knowledge, this is the first method that can be used to monitor residual ASA in aquatic products using LC-MRM. Therefore, this method could contribute to establishing a better aquatic product safety management system.

Acknowledgements

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References

1. Wang, L.; Cheng, H. *Environ. Sci. Technol.* **2015**, 49, 3473, https://doi.org/10.1021/es505358c
2. Zhao, D.; Wang, J.; Yin, D.; Li, M.; Chen, X.; Juhasz, A. L.; Luo J.; Navas-Acien A.; Li H.; Ma, L. Q. *J. Hazard. Mater.* **2020**, 383, 121178, https://doi.org/10.1016/j.jhazmat.2019.121178
3. U.S. Food and Drug Administration, List of Drug Master Files submitted to U.S. Food and Drug Administration.; https://www.fda.gov/media/166951/download.
4. Zhu, X. D.; Wang, Y. J.; Liu, C.; Qin, W. X.; Zhou, D. M. *Chemosphere* **2014**, 107, 272, https://doi.org/10.1016/j.chemosphere.2013.12.060
5. Ye, C.; Deng, J.; Huai, L.; Cai, A.; Ling, X.; Guo, H.; Wang Q.; Li, X. *Sci. Total Environ.* **2022**, 806, 150379, https://doi.org/10.1016/j.scitotenv.2021.150379
6. Geng, A.; Wang, X.; Wu, L.; Wang, F.; Chen, Y.; Yang, H.; Zhang, Z.; Zhao, X. *Ecotoxicol.* **2017**, 137, 172, https://doi.org/10.1016/j.ecoenv.2016.11.030
7. Joshi, T. P.; Zhang, G.; Cheng, H.; Liu, R.; Liu, H.;Qu, J. *Water Res.* **2017**, 116, 126, https://doi.org/10.1016/j.watres.2017.03.028
8. Jomova, K.; Jenisova, Z.; Feszterova, M.; Baros, S.; Liska, J.; Hudecova, D.; Rhodes, C.J.; Valko, M. *J Appl. Toxicol.* **2011**, 31, 95, https://doi.org/10.1002/jat.1649
9. Hunt, K. M.; Srivastava, R. K.; Elmets, C. A.; Athar, M. *Cancer letters.* **2014**, 354, 211, https://doi.org/10.1016/j.canlet.2014.08.016
10. Tchounwou, P. B.; Yedjou, C. G.; Udensi, U. K.; Pacurari, M.; Stevens, J. J.; Patlolla, A. K.; Noubissi, F.; Kumar, S. *Environ. Toxicol*. **2019**, 34, 188, https://doi.org/10.1002/tox.22673
11. U.S. Food and Drug Administration, List of Medically Important Antimicrobial Drugs Affected by GFI #213.; https://www.fda.gov/animal-veterinary/antimicrobial-resistance/list-medically-important-antimicrobial-drugs-affected-gfi-213.
12. Ministry of Food and Drug Safety, South Korea, Pesticides and Veterinary Drugs Information.; http://www.foodsafetykorea.go.kr/residue/vd/mrls/list.do?menuKey=2&subMenuKey=83#none.
13. Hu, Y.; Cheng, H.; Tao, S.; Schnoon, J. L. *Environ. Sci. Technol.* **2019**, 53, 12177, https://doi.org/10.1021/acs.est.9b04296
14. Yang, L. J.; Liu, Y. M.; Cui, F. J.; Guo, W.; Xu, C. G.; Li, Z. J. *Food Sci.* **2011**, 32, 202, https://doi.org/10.7506/spkx1002-6630-201118043
15. Cui, J.; Xiao, Y. B.; Dai, L.; Zhao, X. H.; Wang, Y. *Food Anal. Methods.* **2013**, 6, 370, https://doi.org/10.1007/s12161-012-9533-0
16. Ministry of Food and Drug Safety, South Korea, 8.3.46 Assay of nystatin, metomidate, buparvaquone, and arsanilic acid.; https://various.foodsafetykorea.go.kr/fsd/#/ext/Document/FC?searchNm=ARSANILIC%20ACID&itemCode=FC0A567003004A613.
17. Codex Committee on Residues of Veterinary Drugs in Foods, Guidelines for the Design and Implementation of National Regulatory Food Safety Assurance Programmes Associated with the Use of Veterinary Drugs in Food Producing Animals CAC-GL 71-2009.; https://www.fao.org/input/download/standards/11252/CXG_071e_2014.pdf.
18. Molinspiration, Calculation of Molecular Properties and Bioactivity Score. https://www.molinspiration.com/cgi-bin/properties.
19. Department of Chemistry, University of Massachusetts-Amherst, Physical properties of solvents. https://people.chem.umass.edu/xray/solvent.html.
20. Ministry of Food and Drug Safety, South Korea, 8.3.1 Assay of multiple veterinary drugs.; https://various.foodsafetykorea.go.kr/fsd/#/ext/Document/FC?searchNm=flunixin&itemCode=FC0A567003004A805.
21. Chen, W. R.; Huang, C. H. *J. Hazard. Mater.* **2012**, 227, 378, https://doi.org/10.1016/j.jhazmat.2012.05.078
22. Yang, Y.; Li, Z.; Bai, Y.; Dong, K.; Li, Y.; Zhuang, L. *J. Hazard. Mater. Lett.* **2020**, 1, 100006, https://doi.org/10.1016/j.hazl.2020.100006
23. Choi, Y. S.; Wood, T. D. *Rapid Commun. Mass Spectrom*. **2008**, 22, 1265, https://doi.org/10.1002/rcm.3502