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Analysis of Several Micro-Organic Pollutants on the Phosphorus Recovery from Urine

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1. Introduction

At present the scarcity of natural resources is a major problem in the world. Phosphorus (P) is one of the important natural resources, which is essential for all life forms, including plants, animals, and human, because it involves in many basic processes, *e.g.*, photosynthesis. P is used as fertilizers, especially in agriculture for food production and the limiting factor in crop yields. Moreover, P cannot be substituted by other elements and will be exhausted in 50-100 years later. [1,2)]

Recently, several researchers are interested in P recovery from urine. From various methods, struvite formation is one of the promising ways to recover P from urine in the form of crystals. Struvite has a potential use as a fertilizer[3)] as an effective source of N, Mg, and P for plant growths and soil applications.[4)] However, for actual applications, P recovery through struvite formation is interrupted by micro-organic pollutants contained in urine, such as pharmaceuticals, hormones, *etc.* The micro-organic pollutants can cause serious problems to aquatic organisms and wildlife, such as hormonal disruption, feminization in male fish at concentrations as low as 1 ng/L.[5)] Thus, the amount of micro-organic pollutants remain in struvite and how to minimize the micro-organic pollutants in struvite are very important issues.

Various kinds of pharmaceuticals and hormones are used around the world and many of them are excreted *via* urine. This research focused on ten pharmaceuticals (amoxycillin, carbamazepine, atenolol, erythromycin, furosemide, ibuprofen, norfloxacin, trimethoprim, tetracycline and acetylsalicylic acid) and one hormone (17β-estradiol) as representatives. These pharmaceuticals and the hormone were selected according to their therapeutic class, structure, and physic-chemical properties. The selection of the representatives was based on following two steps. For the first step, therapeutic and structure classifications were considered. The second step was cluster analysis, which pharmaceuticals were classified by using pKa, molecular weight, partition coefficient (logP), distribution coefficient (logD), water solubility, and Henry constant. Finally, ten pharmaceuticals and one hormone were representatively selected from all classified groups.

In overall research, we will find the optimum condition for struvite production and minimizing pharmaceuticals and the hormone in struvite from synthetic and natural urines, which will not be harmful to human or in the acceptable level. With these results, the application will lead to the better sanitation, improving phosphorus recovery from urines and agricultural development not only in megacities but also other areas in Asian countries.

2. Experimental Methods

2.1 Materials

All pharmaceutical compounds, including amoxycillin, carbamazepine, erythromycin, furosemide, atenolol, acetylsalicylic acid, norfloxacin, trimethoprim, tetracycline, ibuprofen, and 17β-estradiol were high purity grade (>97%). Isotopically labeled compounds used as internal standards were norfloxacin-d_5, furosemide-d_5, tetracycline-d_6, atenolol-d_7, acetylsalicylic acid-d_4, amoxycillin-$^{13}C_6$, carbamazepine-d_{10}, ibuprofen-$^{13}C_3$, erythromycin-$^{13}C_2$, 17β-estradiol-$^{13}C_6$, and trimethoprim-$^{13}C_3$. All chemical compounds used for synthetic urine and pH adjustment were NaCl, Na_2HPO_4, KCl, $MgCl_2{\cdot}6H_2O$, $CaCl_2{\cdot}2H_2O$, NH_4Cl, NaOH, formic acid 99%, and phosphoric acid 85%. Filter paper and syringe filter were1μm of pour size glass fiber acrodisc syringe filter, the 0.2 μm pour size Phenex-NY 25 mm syringe filters, and 1 μm pour size glass fiber filters from. LC/MS graded methanol and acetonitrile were used.

2.2 Analytical Methods

The preparation of synthetic urine samples and struvite production was reported by Harada *et al.*[6,7] The compositions of synthetic urine are described in Table 1. The concentration of pharmaceuticals which spiked in this research was calculated from the concentration of pharmaceuticals in sewage treatment plant in Kyoto and Shiga, Japan[8], and the concentration of sex hormone was applied from Escher *et al.*[9] Average concentrations of pharmaceuticals in urine are shown in Table 2. The synthetic urine samples were mixed with rapid speed at 300 rpm for 10 min, while the slow mixing was maintained at 50 rpm for 12 hours.

Table 1. Synthetic urine.[6,7]

Component	Concentration (mM)	Reagent
Na	69.6	NaCl, Na_2HPO_4
K	21.3	KCl
Mg	1.85	$MgCl_2 6H_2O$
Ca	1.21	$CaCl_2 2H_2O$
NH_4-N	20.18	NH_4Cl
PO_4-P	13.45	Na_2HPO_4
Cl	90.3	

Table 2. Average concentrations of pharmaceuticals in urine.[8,9]

Compound	Average concentrations in urine (μg/L/cap./day)
Ibuprofen	45
Estradiol	2
Erythromycin	81
Carbamazepine	13
Trimethoprim	18
Tetracycline	7
Atenolol	81
Norfloxacin	31
Furosemide	51

The natural urines were collected using cleaned stainless steel barrels from 5-7 healthy males aged between 22 and 35 in 24 hrs. The concentrations of pharmaceuticals which were spiked in natural urine and mixing steps were similar to synthetic urine.

The synthetic and natural urine samples were obtained in a 500 mL glass beaker for the preparation of struvite. For the separation of struvite from supernatant, we used filter paper GF/B 1 μm. The prepared samples were spiked with the internal standard and adjusted its pH into below pK_a value of each pharmaceuticals and the hormone by phosphoric acid. All pharmaceuticals and the hormone were analyzed by liquid chromatography-tandem mass spectrometry (LC/MS/MS). Prior to analysis, the samples were filtered by syringe filter with pour size of 0.4 μm. Samples were concentrated with solid phased extraction by using Strata X cartridge (30 mL, 1 mL). The pre-condition of cartridges were rinsed by 1 mL methanol and followed by 1 mL of milliQ water. The samples (1 mL) were loaded through cartridges by gravity and washed by 1 mL of 5% methanol in water. Then, cartridges were dried under vacuum for 5 min, to remove excess of water and finally elute with 1 mL methanol. The extracts obtained were evaporated under a gentle nitrogen stream at 37°C and reconstituted to 100 μL in 30% acetonitrile in MilliQ water.

3. Results and Discussion

To investigate the optimum parameters in the chemical analysis by LC/MS/MS; full, daughter ion, and multiple reaction monitoring (MRM) scans were carried out. The injection volume was set at 10 μL. The optimum parameters for ten pharmaceuticals and one hormone obtained are summarized in Table 3. Concentrations of all pharmaceuticals and the hormone were calculated by using the ratio of peak area of standard compound to internal standard and calibration curves (Table 4) which obtained from internal standards with fixed concentrations as shown in Table 2 were mixed with various concentrations of standards compound in the ratio of standard to internal standard of 0:1, 0.5:1, 1:1, and 2:1. The calibration curves show excellent linearity, with satisfactory correlation coefficients (r^2>0.99).

Table 3. Optimum parameters for the LS/MS/MS analysis of various pharmaceuticals and the hormone.

No.	Compound	Ionization mode	Cone voltage (V)	Collision Energy (eV)	Precursor ion (m/z)	Product ion (m/z)	RT (min)
1	Ibuprofen	ESI+	35	15	161	119	16.4
2	Estradiol (E2)	ESI+	35	15	255	159	9.7
3	Erythromycin	ESI+	35	36	735	158	12.8
4	Carbamazepine	ESI+	35	18	237	194	16.1
5	Trimethoprim	ESI+	35	25	291	123	9.3
6	Tetracycline	ESI+	35	28	445	154	10.3
7	Atenolol	ESI+	35	23	267	145	7.0
8	Norfloxacin	ESI+	35	16	320	276	9.7
9	Furosemide(-)	ESI-	35	18	329	285	16.4
10	Amoxycillin	ESI+	35	20	366	114	6.5
11	Aspirin(-)	ESI-	35	18	137	93	13.8
12	Ibuprofen-$^{13}C_3$	ESI+	35	15	163	121	16.4
13	Estradiol-$^{13}C_6$ (E2)	ESI+	35	15	261	159	9.7
14	Erythromycin-$^{13}C_2$	ESI+	35	36	737	160	12.8
15	Carbamazepine-d_{10}	ESI+	35	18	247	204	16.2
16	Trimethoprim-$^{13}C_3$	ESI+	35	25	294	126	9.3
17	Tetracycline-d_6	ESI+	35	28	451	160	10.3
18	Atenolol-d_7	ESI+	35	23	274	145	7.1
19	Norfloxacin-d_5	ESI+	35	16	325	281	9.7
20	Furosemide-d_5 (-)	ESI-	35	18	334	290	16.4
21	Amoxycillin-$^{13}C_6$	ESI+	35	20	372	114	6.6
22	Aspirin-d_4	ESI-	35	18	141	94	13.7

Table 4. Concentrations of ten pharmaceuticals and one hormone in synthetic and natural urines and calibration curves.

Compound	Adding amount (μg/L)	Calibration curve	Concentration in synthetic urine (μg/L)		Concentration in natural urine (μg/L)	
			Filtrate	Re-dissolved struvite	Filtrate	Re-dissolved struvite
Ibuprofen	45	y=0.0216x+0.0170	44.92	N.D.	45.02	N.D.
Estradiol (E2)	2	y=0.5131x+0.0414	2.11	N.D.	4.91	N.D.
Erythromycin	81	y=0.0137x+0.0489	104.93	N.D.	68.88	N.D.
Carbamazepine	13	y=0.1123x+0.0018	14.05	N.D.	10.78	N.D.
Trimethoprim	18	y=0.0508x+0.0058	18.13	N.D.	17.99	N.D.
Tetracycline	7	y=0.1687x+0.0424	0.12	7.21	0.46	4.15
Atenolol	81	y=0.0123x+0.0314	83.80	N.D.	107.71	N.D.
Norfloxacin	100	y=0.0104x-0.0056	102.35	5.72	89.09	12.90
Furosemide	51	y=0.0138x+0.0054	51.43	N.D.	63.30	N.D.
Amoxycillin	100	y=0.0078x+0.0050	26.09	N.D.	1.54	N.D.
Aspirin	100	y=0.0089x-0.0052	8.22	N.D.	N.D.	N.D.

After struvite precipitation, the results of synthetic and natural urine samples show that most pharmaceuticals and the hormone remained in the supernatant (filtrate), except tetracycline remained high amount in struvite, which were 103% and 59% in synthetic and natural urine, respectively. The concentration of tetracycline in synthetic urine was higher than natural urine because we added extra Mg in synthetic urine to 10 mM.[6] Another compound which remained in struvite was norfloxacin. It remained 6% and 13% in synthetic and natural urine, respectively.

Although the other pharmaceuticals and the hormone could not detect in both samples but the filtrate concentration of estradiol in natural urine was higher than synthetic urine because estradiol is the predominant sex hormone present in females. It is also present in males, which increases concentration of estradiol in natural urine. On the other hand, amoxicillin and aspirin were low concentration in filtrates, which might be attributed from the degradation during struvite formation due to struvite formation was produced under alkali condition at pH 9.6 and 2 pharmaceuticals, moreover, were in the process more than 12 hrs. Therefore, this circumstance might change the structure or destroy some.

Based on the results, it is very interesting to carry out the further experiments which relate to norfloxacin and tetracycline. Due to their high concentration in struvite, it may affect to person who use struvite as fertilizers or consume the food from struvite fertilizer. The optimum condition for minimum pharmaceuticals and the hormone in struvite from urine will also be investigated. It will lead to the better sanitation, improving phosphorus recovery from urines and agricultural development.

4. Conclusions

Phosphorus was recovered from urine *via* struvite production. The amount of micro-organic pollutants in struvite was investigated. The experiments were carried out with ten compounds of pharmaceuticals and one hormone in synthetic and natural urines. After the production of struvite, the results showed that only tetracycline and norfloxacin remained in struvite, especially, tetracycline remained quite high amount in struvite. The optimum condition for recovered P in struvite with minimizing tetracycline and norfloxacin will further studied.

References

1) Rosmarin, A. (2004): The Precarious Geopolitics of Phosphorous, *Down to Earth (Science and Environment Fortnightly)*, June 30, pp. 27-31.

2) Jasinski, S.M. (2006): Phosphate Rock, Mineral Commodity Summaries. Virginia. *US Geological Survey*, pp. 124-125.

3) Parsons, S. A., Wall, F., Doyle, J., Oldring, K. and Churchley, J. (2001): Assesing the potential for struvite recovery at sewage treatment works, *Environ. Technol.*, Vol. 22, pp. 1279-1286.

4) Booker, N. A., Priestley, A. J. and Fraser, I. H. (1999): Struvite formation in wastewater treatment plants: opportunities for nutrient recovery, *Environ. Technol.*, Vol. 20, pp. 777-782.

5) Kolodziej E.P., Harter, T. and Sedlak D.L. (2004): Dairy wastewater, aquaculture, and spawning fish as sources of steroid hormones in the aquatic environment, *Environ. Sci. Technol.*, Vol. 38, pp. 6377–6384.

6) Harada, H., Shimizu, Y., Miyagoshi, Y., Matsui, S., Matsuda T. and Nagasaka, T. (2005): Prediction of Struvite Formation to Recover Phosphorus from Human Urine Using an Equilibrium Model, *J. Jpn. Soc. Water Environ.*, Vol. 28, pp. 191-196.

7) Harada, H., Shimizu, Y., Miyagoshi, Y., Matsui, S., Matsuda T. and Nagasaka, T. (2006): Predicting struvite formation for phosphorus recovery from human urine using an equilibrium model, *Water Sci. Technol.*, Vol. 54, pp. 247-255.

8) Narumiya, M., Okuda, T., Nakada, N., Yamashita, N., Tanaka, H., Sato, K., Sueoka, M. and Oiwa, T. (2009): Occurrence and Fate of Pharmaceuticals and Personal Care Products during Wastewater Treatments, *Environ. Eng. Res.*, Vol.46, pp.175-186.

9) Escher, B. I., Pronk, W., Suter, M. J. F. and Maurer, M. (2006): Monitoring the removal efficiency of pharmaceuticals and hormones in different treatment processes of source- separated urine with bioassays, *Environ. Sci. Technol.*, Vol. 40, pp. 5095–5101.

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