International Journal of Medical, Medicine and Health Sciences
ISSN: 2517-9969
Vol:2, No:8, 2008

Use of Caffeine and Human Pharmaceutical Compounds to Identify Sewage Contamination

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***Abstract*—**Fecal coliform bacteria are widely used as indicators of sewage contamination in surface water. However, there are some disadvantages in these microbial techniques including time consuming (18-48h) and inability in discriminating between human and animal fecal material sources. Therefore, it is necessary to seek a more specific indicator of human sanitary waste. In this study, the feasibility was investigated to apply caffeine and human pharmaceutical compounds to identify the human-source contamination. The correlation between caffeine and fecal coliform was also explored. Surface water samples were collected from upstream, middle-stream and downstream points respectively, along Rochor Canal, as well as 8 locations of Marina Bay. Results indicate that caffeine is a suitable chemical tracer in Singapore because of its easy detection (in the range of 0.30-2.0 ng/mL), compared with other chemicals monitored. Relative low concentrations of human pharmaceutical compounds (< 0.07 ng/mL) in Rochor Canal and Marina Bay water samples make them hard to be detected and difficult to be chemical tracer. However, their existence can help to validate sewage contamination. In addition, it was discovered the high correlation exists between caffeine concentration and fecal coliform density in the Rochor Canal water samples, demonstrating that caffeine is highly related to the human-source contamination.

***Keywords*—**Caffeine, Human Pharmaceutical Compounds, Chemical Tracer, Sewage Contamination.

I. Introduction

TO protect public health, it is required to monitor drinking water quality to ensure the pathogens are not present. Therefore, it is necessary to select suitable markers to locate the source of pollution [1]. An ideal marker should unambiguously identify the source of pollution and quantify the magnitude of pollution [1]. In the past, microorganisms, such as coliform, *E. coli* and enterococci, etc, have traditionally been employed as indicators [2-3]. However, these microbial indicators have many disadvantages and limitations such as relative long time for analysis (18-48 h), lacking specificity, etc. To address the above disadvantages, a series of chemical markers such as human endogenous metabolites, pharmaceuticals, personal care products, etc, have recently been suggested to identify human sewage contamination in water bodies [1, 4-12].

Caffeine is employed as a potential chemical tracer for domestic water because of its relative high concentration detected in surface water [1,4,9]. In addition, clear anthropogenic origin of caffeine makes it as a good indicator for human sewage because caffeine is present in beverages, foods and probably the mostly widely consumed drug in the world [4]. Caffeine has been employed for tracking anthropogenic inputs in rural freshwater and urban marine systems [5], being used as an anthropogenic marker for wastewater contamination of surface waters in Switzerland [1], as marker for untreated domestic wastewater [9], etc.

Human pharmaceuticals are also potential chemical tracers because of their relatively high water solubility and low natural background levels [6]. Human pharmaceuticals have been used as indicators in an urban estuary during dry and wet-weather conditions in USA [6]. They were also employed for evaluating wastewater discharges [10].

The aim of this work was to evaluate the feasibility of using caffeine and human pharmaceuticals to identify human-source contamination, as well as correlation between caffeine and fecal coliform.

II. Experimental Procedures

A. Standards and Reagents

The standard compounds of caffeine, ketoprofen, naproxen, diclofenac, gemfibrozil and ibuprofen were purchased from Aldrich (Milwaukee, WI, USA). Isotope standards including ^{13}C-caffeine-d_3, ^{13}C-naproxen-d_3, ^{13}C-diclofenac-d_4, ^{13}C-gemfibrozil–d_6 and ^{13}C-ibuprofen-d_3 were provided by ISOTEC (Miamisburg, OH, USA). HPLC-grade methanol, acetone and acetonitrile were supplied by TEDIA (Fairfield, OH, USA). Acetic acid (HAc), ammonium acetate (NH_4Ac), hydrogen chloride (HCl), sodium ethylenediaminetetraacetateand (Na_4EDTA), formic acid (FA), potassium dihydrogen phosphate (KH_2PO_4) and magnesium chloride ($MgCl_2$) were purchased from Fluka (Buchs,

Manuscript received August 15, 2008. This work was supported by the Incentive for Research & Innovation Scheme (IRIS) under Grant IRIS-031-00 from Environment & Water Industry Development Council (EWI), Singapore.

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Switzerland). Difco™-M-FC-Agar and Difco™ rosolic acid were from BD (Sparks, MD, USA). Digestion solution for chemical oxygen demanding (COD) was from HACH (Loveland, CO, USA). Oasis HLB cartridge (6ml, 150mg) was provided by Waters (Milford, MA, USA). Deionized water was produced on a USF Maxima (Vivendi Water, UK) water-purification system.

B. Sample Collection

Samples were collected in amber bottles from three locations of Rochor Canal and eight locations of Marina Bay of Singapore (Fig. 1) and stored at 4 °C after collection. They were filtered through a 0.45-μm Nylon membrane filter (Millipore, Billerica, MA, USA) prior to extraction.

C. Fecal Coliform Analysis

All samples were collected in sterile glass bottles and immediately put into cooling boxes (~4ºC) until analysis next day (within 24 h). The water samples were diluted with phosphate buffered solution (PBS) and plated onto the M-FC-Agar plate. After incubation at 44.5 ºC for 24 h, typical blue colonies, with different shades of blue, were enumerated as fecal coliforms.

D. Chemical Oxygen Demand (COD) Analysis

COD was determined by using HACH-Spectrophotometer DR/2010 (Loveland, CO, USA) after digestion with HACH-COD reactor.

E. Extraction and Instrumental Analysis

For the analysis of human pharmaceutical products, water samples were extracted with solid phase extraction (SPE). Conditioning of the cartridges was carried out with 5mL of methanol followed by 5 mL of deionized water. After loading of 1L water sample at 10 ml/min (in which pH value was adjusted to 2.0 with HCl followed by the addition of 10 ng isotope surrogates including ^{13}C-naproxen-d_3, ^{13}C-diclofenac-d_4, ^{13}C-gemfibrozil–d_6 and ^{13}C-ibuprofen-d_3, and then stabilized with 500 mg Na_4EDTA) with AutoTrace SPE workstation (Caliper, Hopkinton, MA, USA) and subsequent washing with 10 ml deionized water, the cartridge was dried for 5 minutes under nitrogen flow. The cartridge was then eluted with methanol (2×6 mL) followed by 6 mL of acetone:methanol (1:1). The extracts were then blown down to 0.7mL with TurboVap® II concentration workstation (Caliper, Hopkinton, MA, USA) under nitrogen, reconstituted with 0.1% FA (in methanol) to a finial volume of 1.0 mL, and filtrated through 0.2-μm Nylon membrane filter (Millipore, Billerica, MA, USA). Analysis was performed on a Waters Acquity ultraperformance liquid chromatography (UPLC) system equipped with a Waters Quattro Premier XE triple-quadrope mass spectrometer with an electrospray ionization source working in negative ionization mode. Separation was carried out on an Acquity BEH C18 (50mm×2.1mm i.d., 1.7-μm) column with a binary mobile phase at a flow rate of 0.5 mL/min. The optimized separation conditions were as follows: solvent (A) 0.1%NH_4Ac/HAc; solvent (B) acetonitrile/methanol (1:1). The gradient mobile phase programme was: 0-0.2 min, 90%A; 0.2-1.2 min, 90→1%A; 1.2-3 min, 1%A; 3-3.1 min, 1→90%A; 3.1-5 min, 90%A. The injection volume was 10 μL. Acquisition was carried out in multiple reaction monitoring (MRM). Source conditions were as follows: 0.53 kV of capillary voltage, 0.8 V of lens voltage, 120 ºC of source temperature, 400 ºC of desolvation temperature, 16 L/h of cone gas flow rate, 900 L/h of desolvation gas flow rate. Dwell time of 0.02 s and an interscan delay time of 0.01 s were employed. Cone energy voltages, collision energy voltages and MRM transitions were established for each analyte (see Table I).

For the analysis of caffeine, the water sample was directly injected into UPLC-MS-MS system after filtration through 0.2-μm Nylon membrane filter followed by the addition of isotope surrogate of ^{13}C-caffeine-d_3. The optimized separation conditions were as follows: solvent (A) 0.1% FA in water; solvent (B) acetonitrile with a flow rate of 0.5 mL/min. The gradient elution was: 0-0.5 min, 95%A; 0.5-3.0 min, 95→10%A; 3-3.1 min, 10→95%A; 3.1-5 min, 95%A. ESI was in positive mode and source conditions were as follows: 0.50 kV of capillary voltage, 0.5 V of lens voltage, 120 ºC of source temperature, 380 ºC of desolvation temperature, 46 L/h of cone gas flow rate, 800 L/h of desolvation gas flow rate. Dwell time of 0.11 s and an interscan delay time of 0.03 s were employed. Cone energy voltages, collision energy voltages and MRM transitions were established for each analyte (also see Table I).

TABLE I
LC-MS-MS PARAMETERS FOR THE MRM ACQUISITION MODE[A]

Compound	Precursor ion (m/z) $[M+H]^+$	CV (V)	Quantitation product ion (m/z)	CE (V)	Confirmation product ion (m/z)	CE (V)
ESI-:						
ketoprofen	252.8	14	209.0	8		
naproxen	228.8	14	170.0	15	185.0	6
^{13}C-naproxen-d_3	232.0	16	173.0	15	188.0	7
diclofenac	293.7	18	214.0	20	250.0	12
^{13}C-diclofenac-d_4	297.8	19	217.0	20	254.0	11
gemfibrozil	248.9	17	121.0	16	127.0	10
^{13}C- gemfibrozil–d_6	255.0	23	121.2	15	133.0	12
ibuprofen	204.8	18	161.0	6		
^{13}C-ibuprofen-d_3	208.0	18	164.0	8		
ESI+:						
caffeine	194.8	25	138.0	18	110.0	24
^{13}C-caffeine-d_3	198.0	35	140.0	20	112.0	25

[A]CV, cone voltage; CE, collision energy.

III. RESULTS AND DISCUSSION

Establishment of Sensitive Methods for Caffeine and Human Pharmaceutical Compounds

Gas chromatography-mass spectrometry (GC-MS) was usually employed for the analysis of caffeine [1,4-5,9]. To increase the determination sensitivity, sample preparation was necessary. SPE, one of modern sample preparation methods has been carried out for the extraction of caffeine from water samples [1,5,9], and the limit of detection was as low as 2 ng/L combined with GC-MS [1]. Continuous liquid-liquid extraction was also conducted for the extraction of caffeine with a

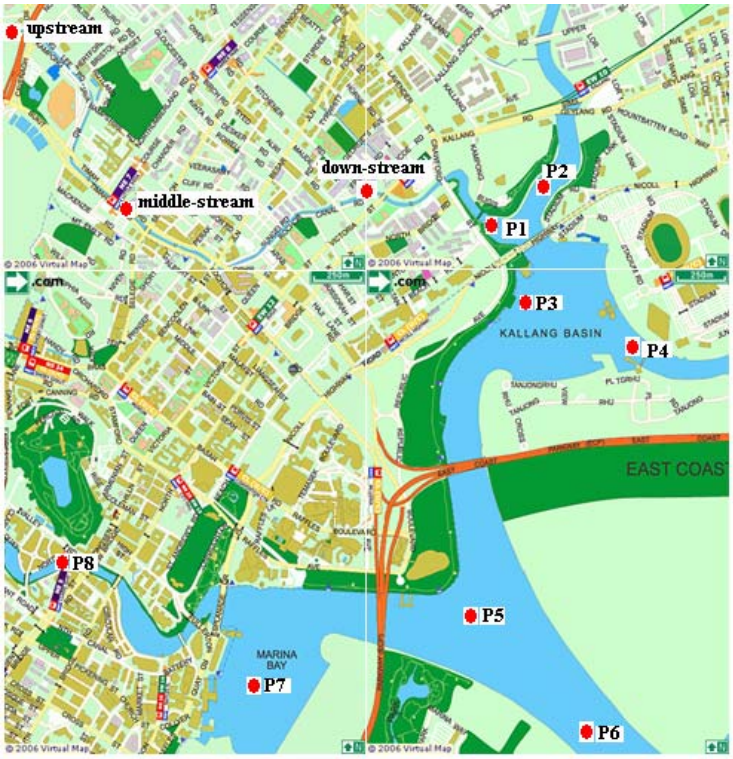

Fig. 1 Map of the sampling areas showing sampling sites

TABLE II
WATER QUALITY DATA FOR SURFACE WATER INVESTIGATED[A]

Station	Fecal coliform (cfu/100 mL)	Caffeine (ng/mL)	Ketoprofen (ng/L)	Naproxen (ng/L)	Diclofenac (ng/L)	Gemfibrozil (ng/L)	Ibuprofen (ng/L)	COD (mg/L)
Marina Bay:								
1	249,000	0.71	23	21	4	4	47	3.0
2	128,000	0.47	9	10	26	BD	39	3.0
3	304,000	0.74	5	24	20	3	60	3.6
4	2,000	0.96	20	19	19	4	41	17.2
5	3,167	0.65	14	13	12	1	46	17.6
6	<500	0.71	19	17	10	4	37	17.7
7	1,000	0.64	15	12	7	BD	41	20.5
8	2,000	0.69	23	15	15	BD	42	24.0
Rochor Canal:								
Down-stream	258,700	1.35	31	21	BD	BD	123	11.1
Middle-stream	205,000	0.68	16	15	BD	BD	100	2.3
Upstream	120,000	0.37	28	24	BD	BD	195	11.0

[A]BD, below detection; the detection limit: diclofenac, 0.5ng/L; gemfibrozil, 0.5ng/L.

detection limit of 0.04 ng/mL [4]. Recently, UPLC-MS-MS has been introduced because high sensitivity of target compounds can be obtained. In this study, the detection limit of 0.1 ng/mL for caffeine with UPLC-MS-MS was achieved by direct injection without sample preparation. It was found that caffeine commonly existed in water samples from Marina Bay and Rochor Canal with the concentration much higher than 0.1 ng/mL. Therefore, UPLC-MS-MS was employed for the analysis of caffeine in water samples after simple filtration step.

LC-MS has widely been carried out for the analysis of human pharmaceuticals compounds [6,10]. Because of low concentration of human pharmaceutical compounds (low ng/L) existing in water samples, an enrichment step is necessary. SPE has usually been employed for the extraction of pharmaceutical residues. In this study, Oasis HLB SPE cartridge combined with UPLC-MS-MS was used for the analysis of five drug residues including ketoprofen, naproxen, diclofenac, gemfibrozil and ibuprofen. Limit of detection (S/N=3) at 0.5 ng/L was obtained for 5 compounds when 1L water sample was used.

Caffeine and Human Pharmaceutical Compounds in Surface Waters

As seen from Table II, the highest concentration of caffeine measured during this study was 1.35 ng/mL at downstream point of Rochor Canal. 0.68 and 0.37 ng/mL of caffeine was determined at middle-stream and upstream points of Rochor Canal, respectively. It clearly shows that from upstream to downstream points, the caffeine concentration in Rochor Canal water increases constantly, following the same trend of human density nearby. At Marina Bay, the concentration of caffeine in the range of 0.41-0.96 ng/mL was determined (shown in Table 2). Obviously, much smaller concentration difference at Marina Bay was observed, compared with that at Rochor Canal (0.37-1.35 ng/mL). The possible reason is the high mobility and solubility of caffeine in water.

Of 5 drugs investigated, ibuprofen was found to be with the highest concentration ranging from 37 to 195 ng/L, followed by ketoprofen (5-31 ng/L) and naproxen (10-24 ng/L). No diclofenac and gemfibrozil was found in Rochor Canal water samples. The occurrence of diclofenac was found to be within the range of 4-26 ng/L in Marina Bay water samples. Gemfibrozil was found in water samples from 5 locations of Marina Bay (ranging from 1-4 ng/L). These data illustrate that concentration of drugs in Marina Bay and Rochor Canal water samples is far lower than that of caffeine. It is obvious that caffeine is more suitable than human pharmaceutical compounds as the chemical tracer because of its relative higher occurrence level, simple and rapid analysis process and persistence in the water bodies.

Fecal Coliform Results and Correlation Analysis

Fecal coliform was analyzed in all water samples collected from 11 locations investigated. At points 1-3 of Marina Bay, which are the estuaries of Rochor Canal and Kallang River, as well as 3 locations along Rochor Canal, fecal coliform was found in high densities (>5000 cfu/100mL).

The relationships between caffeine and other water quality measurements including fecal coliform and COD were evaluated and shown in Fig. 2. It is obvious that positive correlations between caffeine and fecal coliform were significant for 3 locations of Rochor Canal (R^2=0.889; Fig. 2 (top)). However, this positive correlation does not exist in the water samples from 8 points of Marina Bay. The relationships between COD and caffeine for 11 water samples were also investigated. It is found that COD did not correlate with caffeine concentration (shown in Fig. 2 (bottom)).

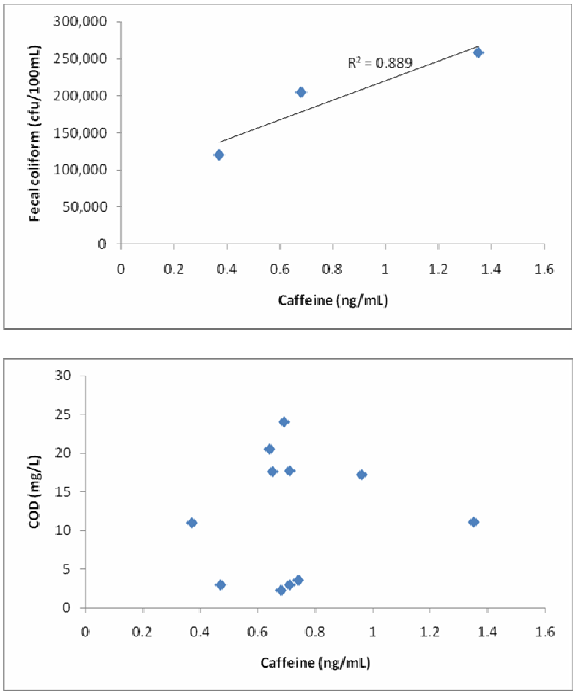

Fig. 2 Relationship between caffeine and fecal coliform in Rochor canal at Singapore (top) and relationship between caffeine and COD in 11 samples tested at Singapore (bottom)

Evaluation of Sewage Contamination Based on Caffeine and Human Pharmaceutical Compounds in Surface Waters

To summarize, in general, the caffeine concentration in Rochor Canal water samples was higher than those in Marina Bay. In water samples from Rochor Canal, the elevated caffeine and fecal coliform levels were significantly correlated, indicating that caffeine is highly related to human-source contamination. Therefore, caffeine measurement in canal indicates the presence of wastewater more directly and more sensitive than reservoir [1]. In addition, the presence of certain level of caffeine indicates that leakage exists in the sewage pipe along Rochor Canal, where rehabilitation needs to be conducted. It is also found that the existence of human pharmaceuticals in Rochor Canal and Marina Bay waters also helps to validate sewage contamination because these compounds are highly related to human-source contamination.

VI. Conclusion

In this study, the possibility of using caffeine and human pharmaceutical compounds to identify sewage contamination was investigated. The occurrence of relatively high concentration of caffeine and its persistence in surface water bodies make it a useful marker for domestic wastewater. Furthermore, the existence of pharmaceuticals can be employed for conforming sewage contamination. High correlation was found between caffeine concentration and fecal

coliform density in the Rochor Canal water samples, demonstrating that caffeine is highly related to the human-source contamination.

ACKNOWLEDGMENT

The authors gratefully thank Y. Yang for assistance in COD determination, Ivy Lam for technical assistance in fecal coliform counting, X. Qian for assistance in pharmaceuticals determination. We also thank K.W. Yap and S.H. Eng for water sampling.

REFERENCES

[1] I.J. Buerge, T. Poiger, M.D. Müller, and H-R. Buser, "Caffeine, an anthropogenic marker for wastewater contamination of surface waters," Environ. Sci. Technol., vol. 37, pp. 691-700, 2003.

[2] C.L. Meays, K. Broersma, R. Nordin, A. Mazumder, "Source tracking fecal bacteria in water: a critical review of current methods," Journal of Environmental Management, vol. 73, pp. 71-79, 2004.

[3] K.G. Field, M. Samadpour, "Fecal source tracking, the indicator paradigm, and managing water quality," Water Research, vol. 41, pp. 3517-3538, 2007.

[4] R.L. Seiler, S.D. Zaugg, J.M. Thomas, D.L. Howcroft, "Caffeine and pharmaceuticals as indicators of waste water contamination in wells," Ground Water, vol. 37, pp. 405-410, 1999.

[5] K.A. Peeler, S.P. Opsahl, J.P. ChantonI, "Tracking anthropogenic inputs using caffeine, indicator bacteria, and nutrients in rural freshwater and urban marine systems," Environ. Sci. Technol., vol. 40, pp. 7616-7622, 2006.

[6] M.J. Benotti, B.J. Brownawell, "Distributions of pharmaceuticals in an urban estuary during both dry- and wet-weather conditions," Environ. Sci. Technol., vol. 41, pp. 5795-5802, 2007.

[7] C. Maldonado, J. Dachs, J.M. Bayona, "Trialkylamines and coprostanol as tracers of urban pollution in waters from enclosed seas: the Mediterranean and black sea," Environ. Sci. Technol., vol. 33, pp. 3290-3296, 1999.

[8] K.O. Isobe, M. Tarao, M.P. Zakaria, N.H. Chiem, L.Y. Minh, H. Takada, "Quantitative application of fecal sterols using gas-chromatography-mass spectrometry to investigate fecal pollution in tropical waters: western Malaysia and Mekong Delta, Vietnam," Environ. Sci. Technol., vol. 36, pp. 4497-4507, 2002.

[9] I.J. Buerge, T. Poiger, M.D. Müller, H-R Buser, "Combined sewer overflows to surface waters detected by the anthropogenic markers," Environ. Sci. Technol., vol. 40, pp. 4096-4102, 2006.

[10] S.T. Glassmeyer, E.T. Furlong, D.W. Kolpin, J.D. Cahill, S.D. Zaugg, S.L. Werner, M.T. Meyer, D.D. Kryak, "Transport of chemical and microbial compounds from known wastewater discharges: potential for use as indicators of human fecal contamination," Environ. Sci. Technol., vol. 39, pp. 5157-5169, 2005.

[11] P.D. Anderson, V.J. D'aco, P. Shanahan, S.C. Chapra, M.E. Buzby, V.L. Cunningham, B.M. Duplessie, E.P. Hayes, F.J. Mastrocco, N.J. Parke, J.C. Rader, J.H. Samuelian, B.W. Schwab, "Screening analysis of human pharmaceutical compounds in U.S. Surface," Environ. Sci. Technol., vol. 38, pp. 838-849, 2004.

[12] C. Pratt, J. Warnken, R. Leeming, J.M. Arthur, D.I. Grice, "Detection of intermittent sewage pollution in a subtropical oligotrophic, semi-enclosed embayment system using sterol signatures in sediments," Environ. Sci. Technol., vol. 41, pp. 792-802, 2007.