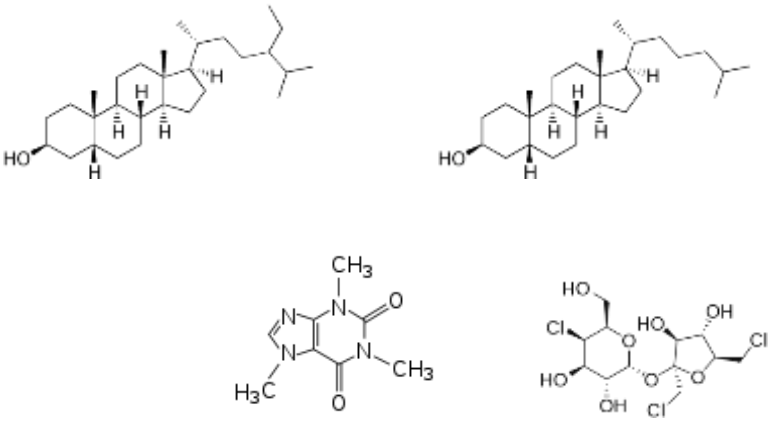
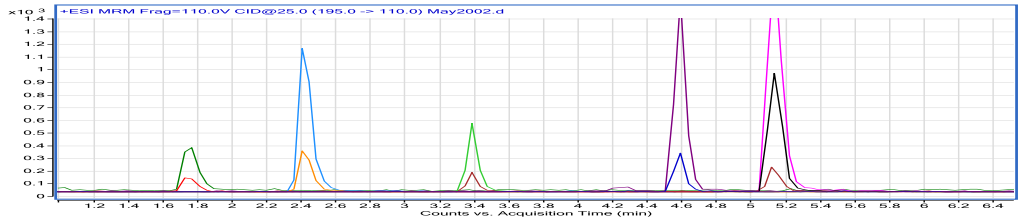
Selection and Evaluation of Chemical Indicators for Waste Stream Identification





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Acknowledgements

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Abstract

Human and animal waste poses a threat to the quality of groundwater, surface water and sources of drinking water. This is especially of concern for private and public water supplies in agricultural areas of Wisconsin where land spreading of livestock waste occurs on thin soils overlaying fractured bedrock. Current microbial source tracking methods for reliable source identification requires the use of expensive and time consuming testing using polymerase chain reaction (PCR) techniques. Due to cost, these tests are often not an option for homeowners, municipalities or state agencies with limited resources. The Water and Environmental Analysis Laboratory (WEAL) sought to develop a method to provide a lower cost analytical technique to determine source of fecal waste using fecal sterols, pharmaceuticals (both human and veterinary), and human care/use products in ground and surface waters using solid phase extraction techniques combined with triple quadrupole mass spectrometry (LC/MS/MS). The combined techniques will allow the detection of fecal sterols and other chemical markers in the sub part per billion levels. The presence and ratios of select fecal sterols can indicate fecal contamination from point sources such as sewage treatment plants, septic leachate or livestock waste. Sterols of interest include; stigmastanol, stigmasterol, sitosterol, 24-ethylcoprostanol and coprostanol. While there are numerous fecal sterols, these five compounds have been previously identified as reliable candidates to identify the source of fecal contamination. Fecal samples were analyzed from known point sources (bovine and swine slurries, human septic systems and municipal wastewater treatment facilities) to establish a baseline sterol profile for each species of interest. Pharmaceuticals and personal care products (PPCPs) indicative of human waste include: acetaminophen, caffeine and its major metabolite paraxanthine, cotinine (a nicotine metabolite) sulfamethoxazole (human antibiotic), carbamazepine (anti-seizure medication) and the artificial sweeteners acesulfame, sucralose and saccharin. The bovine antibiotic sulfamethazine was also a target analyte. Well water samples where suspected contamination was present were analyzed for fecal sterols and PPCPs. These

results were compared to traditional microbiological source tracking results from the Wisconsin State Laboratory of Hygiene. Chemical indicators were found in 6 of 11 groundwater samples, and 5 of 11 were in support of MST results. Lack of detection of chemical indicators in samples contaminated with bovine or human *Bacteroides* supports the need for confirmatory methods and advancement of chemical indicator detection technologies.

Introduction

Groundwater and surface water contamination by landspreading of animal waste and human septage poses a serious risk to human health. Current methods for the source tracking of contaminated well water are not adequate to protect public health. Existing methods utilize indicator (coliform, *E. coli*) bacteria to determine if there is contamination (Sinton 1998). There are several inherent problems with utilizing a method such as this; (1) the use of indicator bacteria does not distinguish between anthropogenic and non-anthropogenic sources, (2) These tests require up to 48 hours to complete causing a delay in warning of potentially harmful exposure to pathogenic contamination, (3) test methods do not test for pathogenic organisms, only indicators of their possible presence (Glassmeyer 2005), and (4) does not evaluate chemical contamination.

While more sophisticated methods of tracking human waste exist, they are often prohibitively expensive and time consuming. Methods such as polymerase chain reaction (PCR) can distinguish between different species of enteric indicator organisms, but cost for these analyses are often over $1000 per sample. Applications of methods such as PCR are not readily available for widespread use (Glassmeyer 2005). The Wisconsin State Laboratory of Hygiene (WSLH) offers these advanced methodologies for Wisconsin Department of Natural Resources (WDNR) related investigations regarding fouled water.

There are multiple sources that can contribute to the microbial contamination of groundwater and surface waters; (1) land spreading of animal waste, (2) failing or inadequate septic systems, (3) land application of sludge from municipal waste water treatment plants (WWTP) (Gourmelon 2010, Smith 2002). These problems are often exacerbated in areas where bedrock fractures are exposed at the surface or where a thin layer of soil overlays fractured bedrock. Private and municipal water wells that

have boreholes intercepting these fractures can be contaminated from distant sources. Identification of the source of fecal contamination is critical to finding a remedy to the problem.

A two-pronged approach to waste identification through chemical analysis was developed using: (1) select fecal sterol analysis and analysis of concentration ratios and (2) select pharmaceutical and personal care/use product analysis. Both techniques utilize solid phase extraction (SPE) and high performance liquid chromatography combined with 3-stage quadrupole mass spectrometry (LC/MS/MS). Differences in physical properties of these analyte groups necessitated development of separate methodologies for each.

Fecal Sterols

The term 'fecal sterols' is used collectively for both sterols and stanols and comprise a family of lipid compounds with a steroidal ring structure base. Stanols are (hydrogen) saturated forms of sterols. These compounds occur naturally in both plants (phytosterols) and animals (zoosterols). Cholesterol is the major zoosterol found in animal tissue while campesterol and sitosterol represent major phytosterols. Hepatic induced metabolic processes result in hundreds of different sterols and these vary in composition and concentration depending upon diet and intestinal flora (Hagedorn 2011). Coprostanol is the major sterol produced in the digestive tract of humans accounting for approximately 60% of the sterol profile. Pig feces are also dominated by coprostanol, but to a lesser degree (Jarde 2007). 5β-campestanol and 5β-stigmastanol represent major fecal sterols metabolic products in excrement from ruminants. Metabolism of sitosterol by herbivores results in preferential production of 24-ethyl coprostanol over coprostanol (Morrison 2013).

Several researchers have proposed the use of fecal sterol ratios to determine a source of contamination in either groundwater or surface water. These ratios are summarized in Table 1.

Evershed and Bethell (1996) proposed a ratio of coprostanol to 5β-stigmastanol to separate human and ruminant pollution, with ratios above 1.5 considered positive for human fecal contamination. Leeming et al. (1997) suggested that if coprostanol/(coprostanol + 5 β -stigmastanol) was greater than 0.73, pollution may be as much as 100% human in origin; if the ratio was less than 0.28, then herbivores could be responsible for up to 100% of the fecal pollution. Shah et al. (2007) reported that fecal sterol ratios were effective at identifying which mixtures contained a human contribution, but could not accurately determine the percent contributions of the different sources. Gourmelon (2010) analyzed 19 samples including animal feces and effluent from municipal waste water treatment plants (WWTP). This study found that sitostanol and 24-ethylepicoprostanol were the dominant sterols in bovine manure, while (in order of concentration – high to low) coprostanol, 24-ethylcoprostanol, and sitosterol were most abundant in swine manure. WWTPs sterol profile was dominated by coprostanol, cholesterol, 24-ethylcoprostanol, and sitosterol. The Gourmelon report supports other claims that the sterol profile in swine manure is somewhat similar to humans. The crux of the Gourmelon study suggested ratios to segregate bovine manure sources from humans and livestock (specifically bovine and swine). Note that in Table 1, the ratio R1 is expressed by Gourmelon is indicative of the dominant source of fecal sterols while R6 (Grimalt 1990) expressed these same ratios differently. Grimalt reports R6 values >0.70 to be exclusively human and <0.30 as herbivore while values in-between are considered mixed waste.

Table 1. Fecal sterol ratios used to assess source of fecal contamination.

Ratio ID	Fecal Sterols	Ratio value	Source Implied	Reference
R1	Coprostanol/Coprostanol + 24-ethylcoprostanol	>0.60	Human	Gourmelon
		<0.60	Bovine/Swine	
R2	Stigmastanol[1]/Coprostanol	>1.0	Bovine	Gourmelon
		<1.0	Swine or Human	
R3	Coprostanol/Stigmastanol	>1.5	Human	Evershed
		<1.5	Herbivore	
R4	Coprostanol/Coprostanol + Stigmastanol	>0.73	Human	Leeming
		<0.28	Herbivore	
R5	Coprostanol/24-ethylcoprostanol	≥1.0	Human	Hagedorn
		<1.0	Herbivore	
R6	Coprostanol/Coprostanol + 24-ethylcoprostanol[2]	>0.70	Human	Grimalt
		<0.30	Herbivore	

[1]expressed as sitostanol in Gourmelon 2010
[2]expressed as 24-ethyl-5β-chlolestan-3β-ol in Grimalt 1999

Pharmaceuticals and personal care products

The goal of evaluating pharmaceuticals and personal care products for this project was to identify, through literature review and analytical processes, those chemicals that are unique to a given waste stream. With trained laboratory personnel and modern instrumentation such as LC/MS/MS, the analytical process provides detection limits in the parts per trillion range and can effectively determine those compounds unique to human and animal waste. Ideal markers should allow for the clear identification of a pollution source (Buerge 2003). The markers unique to human wastes are exemplified by certain pharmaceuticals, artificial sweeteners, caffeine and cotinine (Van Stempvoort 2011, Buerge 2008).

Tracing human waste streams is well documented with studies showing several chemical markers that are relatively stable and mobile in surface water and groundwater. Caffeine (1,3,7-trimethylxanthine) has been used for many years (Buerge 2003, Seiler 1999, Burkardt 1999) as a tracer of human waste, although undergoes degradation to its metabolite, paraxanthine (1,7-dimethylxanthine). Caffeine was found in 70% of surface water samples downstream of wastewater treatment plants in a 2000 USGS reconnaissance (Kolpin 2002) and similar frequency of detection was reported by Glassmeyer et al. (2005) again downstream of wastewater treatment facilities. Paraxanthine was detected at a 29% frequency (Kolpin 2002). Cotinine, the major metabolite of nicotine was reportedly found in 38% of samples in the USGS study and 92% in the Glassmeyer et al. study (2005). Inconsistent use and degradation rates of some of these compounds may lend concern to their utility to quantify human waste, but detection of these compounds confirms the presence of human wastes to the matrix evaluated.

Artificial sweeteners have recently been proposed for use as human waste tracer as they appear to have much greater stability. Sucralose, marketed as "Splenda®" in the U.S., is a polar chlorinated sugar that is 600 times sweeter than sucrose and passes through the human digestive system 95- 98% intact (Loos 2009, Buerge 2009). It has been used as a tracer of human waste in surface water across Western Europe (Buerge 2009) and Canada (Stempvoort 2011). It has been used as a sweetener more widely in the U.S., and has been reported in drinking water supplies (Mawhinney 2011). Acesulfame, (acesulfame potassium) is a widely used sweetener reportedly 200 times sweeter than sucrose and is 100% excreted from the human digestive system (Buerge 2009). While acesulfame has been used as a surface water tracer, it has also been effective as a tracer in groundwater. It has been found in groundwater in Zurich, Switzerland where aquifers are recharging with river water carrying sewage treatment plant effluent (Buerge 2009). In addition, LC/MS/MS detection limits for acesulfame are

reported to be approximately 0.01 µg/L. Sucralose conversely, has LC/MS/MS detection limits in the range of 1.0 µg/L (Buerge 2009).

Other artificial sweeteners have been used as tracers, but have issues of degradation, detection and availability. Saccharin is used worldwide in many beverages and personal care products and is excreted at a rate of 90-100%. Saccharin is often used in products in the US, but not as frequently as sucralose and acesulfame. Cyclamate was banned from use in the U.S. in 1969 but has found widespread acceptance in European countries. Aspartame degrades quickly during wastewater treatment (Buerge 2009) and is therefore discounted as a reliable tracer of human waste contaminated water. In the Swiss study previously noted, acesulfame and sucralose concentrations were not significantly altered by wastewater treatment whereas saccharin and cyclamate were eliminated at a rate of 90 and 99% respectively in treatment facilities with activated sludge processes. The utility of these artificial sweeteners is dependent upon markets that consume diet soft drinks or other artificially sweetened products which represent major inputs into aquatic systems.

The pharmaceuticals carbamazepine, (antiepileptic/mood stabilizer) and acetaminophen (antipyretic/analgesic) were found in 82% and 50% of surface water samples respectively (Glassmeyer 2005)), were added to the WEAL's current list of wastewater tracers as these compounds are unique to a human waste stream. The sulfanilamide antibiotics sulfamethoxazole and trimethoprim are exclusively human antibiotics sold in combination as Bactrim® and Septra®. Sulfamethoxazole has been reported as a common organic wastewater and in literature (Glassmeyer 2005, Kolpin 2002, Barnes 2008) and has also been found in related studies (yet unpublished) in Central Wisconsin. Sulfamethazine is registered as a veterinary antibiotic used extensively for therapeutic and sub-therapeutic disease control.

The antimicrobial triclosan was also found frequently in surface water studies 58% (Kolpin 2002) and 63% (Glassmeyer 2005) however its use as a tracer in groundwater, especially fracture flow, is not well defined. Hunt et al. (2010) reported testing for triclosan (an analyte in USGS Schedules 1433 and 4433) in 33 wells from unconsolidated sand and gravel aquifers in Wisconsin without a single detect. With uncertainty regarding its mobility in fracture flow groundwater, the analyte was considered as a possible tool for identification of human waste streams due to its widespread usage.

Methods

The initial goal was to have one method that could determine both analyte groups at sub-part per billion levels. This was set aside with trials that determined fecal sterols have very limited ability to ionize in the LC/MS/MS. Standard methodologies listed in the literature and EPA Method 1694 (Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS, December 2007) rely on electrospray ionization (ESI) LC/MS/MS as the mechanism for most of the organic wastewater contaminant detection. While fecal sterols can be ionized with this mechanism, detection limits are enhanced over 100-fold by an atmospheric chemical ionization (APCI) process. In addition, solid phase extraction methods are more efficient when the analyte groups were separated.

Analytes were selected from information above and are listed in Table 2. Method development proceeded along the path of developing separate methods for each analyte group.

Table 2. Selected analytes for waste stream identification.

Fecal Sterols:	Compound	
	Coprostanol	
	24-Ethylcoprostanol	
	Sitosterol*	
	Stigmasterol	
	Stigmastanol	

PPCPs:	Compound	Use
	Acesulfame*	artificial sweetener
	Acetaminophen	analgesic
	Caffeine*	stimulant
	Carbemazepine*	anti-seizure, mood stabilizer
	Cotinine*	nicotine metabolite
	Paraxanthine	caffeine metabolite
	Saccharin	artificial sweetener (added in year 2 of study)
	Sucralose*	artificial sweetener
	Sulfanilic Acid	food dye additive (added in year 2 of study)
	Sulfamethazine*	bovine antibiotic
	Sulfamethoxazole*	human antibiotic (added in year 2 of study)
	Triclosan*	antimicrobial

(*plus deuterated analog used as an internal standard)

Sample preparation and analysis – fecal sterols

Fecal sterol sample were collected in one-liter amber bottles and stored at 4^{o}C. Samples received through WSLH were collected in one quart mason jars and frozen. Prior to extraction, samples were filtered through Whatman glass microfiber filters to remove any suspended particulates. Samples were modified with 3.0 mL/L of a pH 4.3 acetate buffer. Waters HLB SPE (200 mg) cartridges were used for extraction fecal sterols from the modified sample. Extractions cartridges were conditioned with 5 mL of a 4:1 mixture of dichloromethane (DCM) and ethyl ether (EE) then dried under nitrogen gas for five minutes. This process was repeated. The cartridge was further conditioned with pH 7 phosphate buffer, and the cartridge was dried for an additional 10 minutes. After conditioning, 250 mL of sample was loaded onto the cartridge then dried for 20 minutes. Samples were eluted with 5 ml of the 4:1 DCM:EE mixture. Following elution, samples were dried in a Turbovap sample concentrator to near dryness.

Internal standard (sitosterol-D7) was added at a concentration of 200 µg/L. The sample extract was reconstituted to a final volume of 500 µL in 95% methanol 5% reverse osmosis (RO) water. The process results in a 500-fold concentration factor from raw sample to sample extract.

Samples were analyzed on an Agilent 6430 QQQ LC/MS/MS system. Details of this method are listed in Appendix A. Briefly, 20 µl of sample is injected into a high performance liquid chromatograph (HLPC) equipped with a Poroshell 120 EC-C18 column and eluted with 95% methanol 5% water. A slight gradient elution is used to enhance separation and analytes are transported to the APCI for ionization and analysis by the 3-stage quadrupole mass spectrometer. One selected ion per analyte (precursor ion) is allowed to pass through the first set of quadrupoles (Q1), and is re-ionized in the second set (collision cell or Q2). The third set of quadruples (Q3) allows selected ion fragments (product ions) to pass through to the electron multiplier for detection. The same principles of analysis apply to PPCPs although ESI is used as the ionization source.

Isotopically labelled (deuterated) internal standards are added to sample extracts prior to analysis. The purpose of this addition is to correct for ion suppression which occurs in both APCI and ESI LC/MS/MS. Analyte recovery is measured with externally spiked samples and surrogate standards.

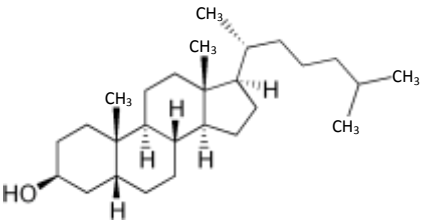
Fecal sterol analyses present a challenge in the analytical process. This is due to the similar structures (Figure 1), physical characteristics, and the use of APCI which tends to ionize a wider range of organic compounds than ESI.

Sample preparation and analysis – PPCPs

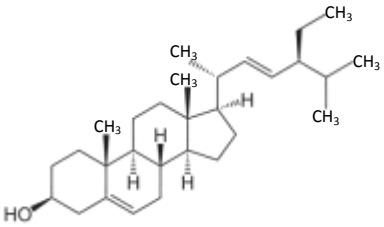
PPCP samples were collected in one-liter amber bottles and stored at 4^{o}C. Samples received through WSLH were collected in one quart mason jars and frozen. Prior to extraction, samples were filtered through Whatman glass microfiber filters to remove any suspended particulates. Samples

analytes were extracted from water using Waters HLB (200 mg) SPE cartridges. Cartridges were conditioned with 5 mL of methanol, 5 ml of RO water, and then another 5 ml methanol. One hundred ml of sample was pumped through the SPE cartridge and eluted with 5 ml methanol. Sample extracts were dried in a Turbovap sample concentrator to near dryness. Deuterated internal standards (as indicated in Table 2) were added at varying concentration depending upon an analytes response. The sample extract was reconstituted to a final volume of 500 µL of 90% RO water and 10% methanol in 15 mM acetic acid. This represents a 200-fold concentration factor.

Samples were analyzed on an Agilent 6430 QQQ LC/MS/MS system. Details of this method are listed in Appendix B. Briefly, 20 µl of sample extract is injected onto an Agilent XDB C-18 column and eluted with 90% RO water and 10% methanol, both modified with 15 mM acetic acid. A gradient elution is employed to enhance chromatographic separation (Figure 4) and is detailed in Appendix B. Ion formation and detection are described above.



Coprostanol – $C_{27}H_{48}O$
MW = 388.4



Stigmasterol – $C_{29}H_{48}O$
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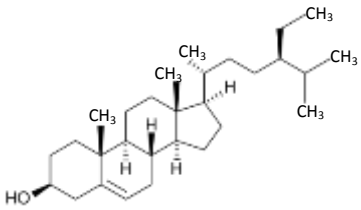
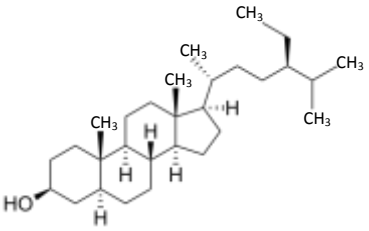
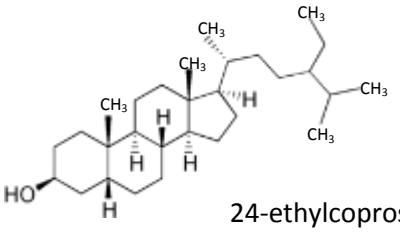


Sitosterol – $C_{29}H_{50}O$
MW = 414.4



Stigmastanol – $C_{29}H_{52}O$
MW = 416.4



24-ethylcoprostanol – $C_{29}H_{52}O$
MW = 416.4

Figure 1. Molecular structure of selected fecal sterols used for waste stream identification.

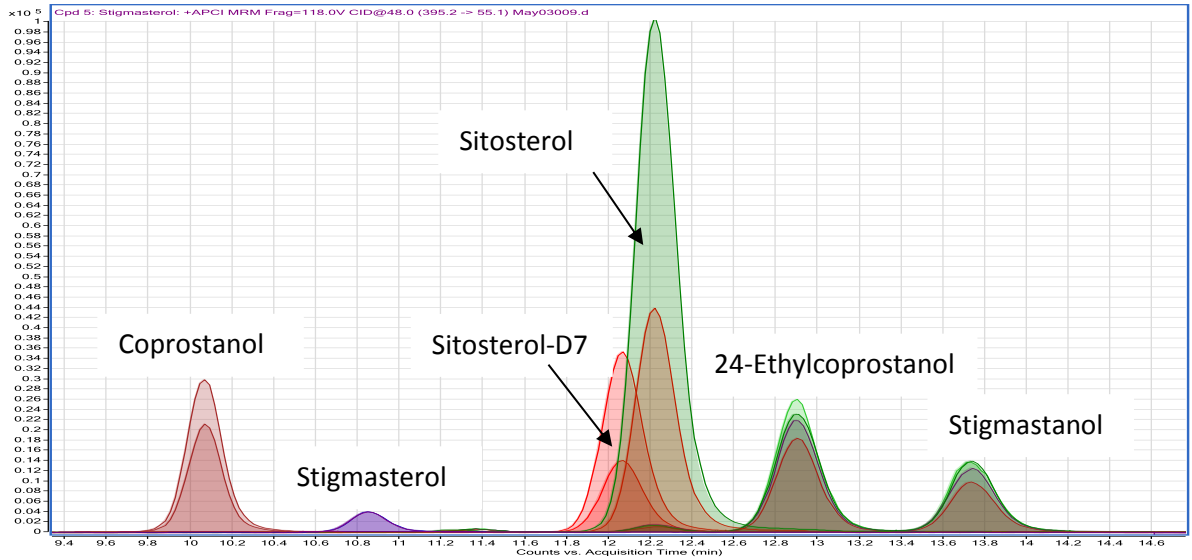
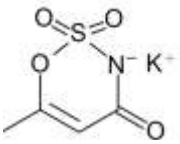
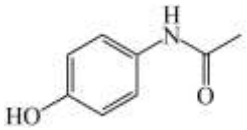


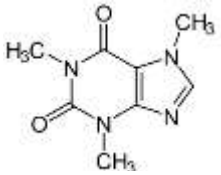
Figure 2. Ion chromatogram of selected fecal sterols.



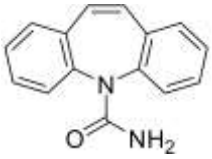
Acesulfame-K - $C_4H_4KNO_4S$
W = 201.2



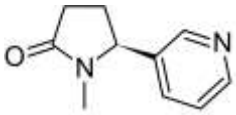
Acetaminophen – $C_8H_9NO_2$
MW = 151.2



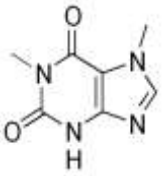
Caffeine – $C_8H_{10}N_4O_2$
MW = 194.2



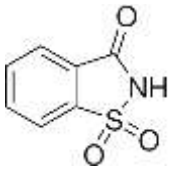
Carbamazepine – $C_{15}H_{12}N_2O$
MW = 236.3



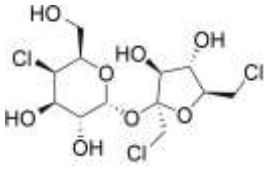
Cotinine – $C_{10}H_{12}N_2O$
MW = 176.2



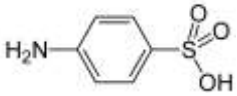
Paraxanthine – $C_7H_8N_4O_2$
MW = 180.2



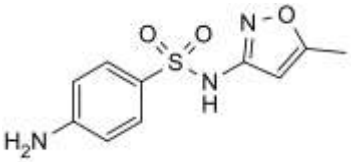
Saccharin – $C_7H_5NO_3S$
MW = 183.2



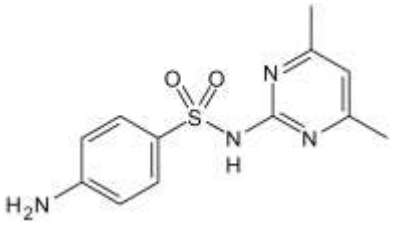
Sucralose – $C_{12}H_{19}Cl_3O_8$
MW = 397.6



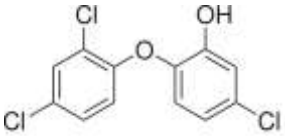
Sulfanilic acid – $C_6H_7NO_3S$
MW = 173.2



Sulfamethoxazole – $C_{10}H_{11}N_3O_3S$
MW = 253.3



Sulfamethazine – $C_{12}H_{14}N_4O_2S$
MW = 278.3



Triclosan – $C_{12}H_7Cl_3O_2$
MW = 288.0

Figure 3. Molecular structure of selected compounds used for waste stream identification.

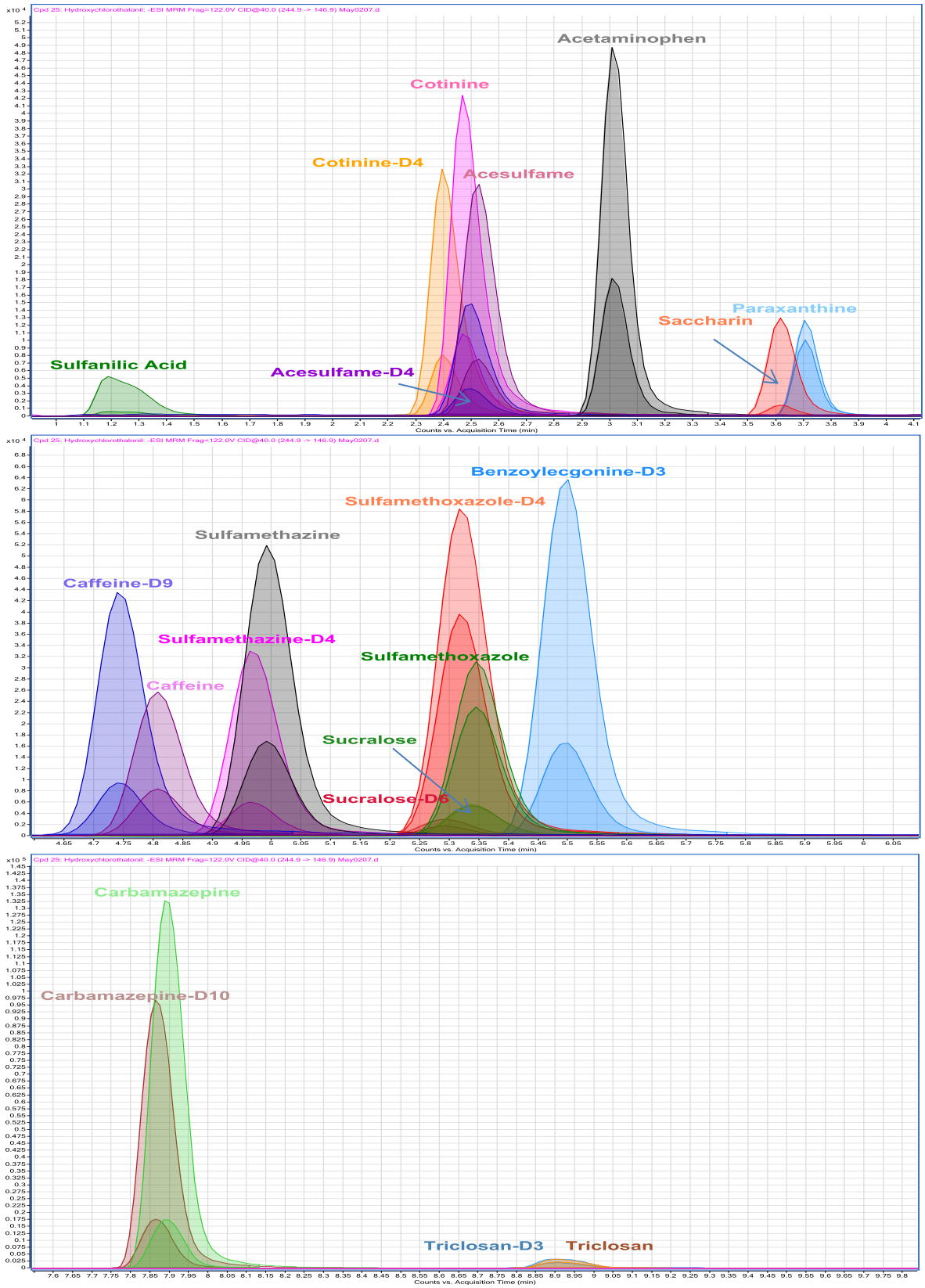


Figure 4. Ion chromatograms of selected PPCPs.

Microbiologic Methods

Microbiologic analyses were conducted by the WSLH and reports sent to the WEAL. Analytes, methods and references are list in Table 3.

Table 3: Microbial source tracking analytes and methodologies conducted at the Wisconsin State Laboratory of Hygiene.

Analyte	Method	Reference
Rhodococcus coprophilus	WSLH Internal	
Total *Bacteroides* species	Layton et al.	Applied and Environmental Microbiology June 2006, pp. 4214-4224
Human *Bacteroides* species	Layton et al.	Above
Bovine *Bacteroides* species	Layton et al.	Above
Enterococci	Enterolert-MPN QT	Federal Register - July 2003
Total coliform	Colilert 18-MPN QT	Standard Methods for the Examination of Water and Wastewater - SM9223B
E. coli	Colilert 18-MPN QT	Above

Experimental Design

Samples during this study were either manufactured from known sources or came from the Wisconsin Department of Natural Resources field agents who investigate fouled water complaints from homeowners. The intent of the study was to acquire samples directly from the field agents, however, many of the samples were sent directly to WSLH and frozen for several weeks before delivery to WEAL. The effect of this delay in analysis and storage by freezing lends some uncertainty to possible analyte degradation. Over, the duration of this study, 11 samples were completely analyzed by MST, fecal sterols and PPCPs. Additionally, 12 samples were manufactured and analyzed for fecal sterols and PPCPs only.

Manufactured samples were taken from known sources of waste including, swine and bovine slurry tanks, septic systems (conventional and mound), and municipal waste water treatment plants. These samples were not subjected to MST. Samples, analyzed in replicate, were diluted and underwent SPE and analysis by LC/MS/MS. All samples were tested for fecal sterols and human waste indicators. Those samples were extracted and analyzed by methods outlined above.

A determination of fecal sterol ratios was compared to published ratios (Table 1) in literature sources. Water samples collected by WDNR were split between the WSLH and WEAL for independent analysis.

Results and Discussion

Comparison of fecal sterol ratios from published sources were made to those from samples collected, and tabulated along with MST results from WSLH. The emphasis of this study was reliant upon MST methodologies as the 'gold standard' and with this consideration, 9 of the 11 samples were determined to contain both human and bovine waste and render the use of sterol ratios to identify a sole source as problematic. Table 4 summarizes sample fecal sterol ratios in samples and compares them to ratios described in peer-review literature sources (ratios defined in Table 2).

Table 4. Ratios of sterols and predicted source of fecal contamination.

Sample ID	R1	Result	R2	Result	R3	Result	R4	Result	R5	Result	R6	Result	WSLH
123480001	0.50	B/S	2.00	B	0.50	E	0.33	M	1.00	H	0.50	M	BH
124394001	0.50	B/S	2.10	B	0.48	E	0.32	M	1.00	H	0.50	M	BH
125559001	0.20	B/S	4.00	B	0.25	E	0.20	E	0.25	E	0.20	E	BH
125560001	0.26	B/S	3.06	B	0.33	E	0.25	E	0.35	E	0.26	E	H
123394001	0.52	B/S	2.62	B	0.38	E	0.28	E	1.10	H	0.52	M	BH
124262001	0.50	B/S	2.50	B	0.40	E	0.29	M	1.00	H	0.50	M	BH
124151001	0.55	B/S	2.09	B	0.48	E	0.32	M	1.22	H	0.55	M	BH
124151001rep	0.52	B/S	2.27	B	0.44	E	0.31	M	1.10	H	0.52	M	BH
99894001	0.33	B/S	2.00	B	0.50	E	0.33	M	0.50	E	0.33	M	H
132999001	0.48	B/S	2.04	B	0.49	E	0.33	M	0.94	E	0.48	M	BH
132999001rep	0.48	B/S	1.97	B	0.51	E	0.34	M	0.94	E	0.48	M	BH
63931001	Fecal sterol analysis incomplete												BH
Bovine Slurry	0.48	B/S	2.26	B	0.44	E	0.31	M	0.94	E	0.48	M	---
Pig Slurry	0.68	S	0.32	S/H	3.16	H	0.76	H	2.09	H	0.68	M	---
Human	0.49	B/S	0.16	S/H	6.11	H	0.86	H	0.98	E	0.49	M	---
Septic A (conv.)	0.68	H	0.12	H	8.53	H	0.90	H	1.83	H	0.68	M	---
Septic B (mound)	0.34	B/S	0.38	H	2.62	H	0.72	H	0.51	E	0.34	M	---
WWTP influent	0.33	B/S	0.09	H	10.6	H	0.91	H	0.49	E	0.33	M	---
WWTP effluent	0.83	H	0.05	H	18.8	H	0.95	H	4.87	H	0.83	H	---

B= Bovine E= Herbivore M=Mixed
S=Swine H=Human (Indicated by authors)

It should be noted that the Wisconsin State Lab of Hygiene did not present test results as swine contamination, only bovine and human. The method referenced by WSLH (Layton et al., 2006) asserts a 100% positive identification (0% false positive) for the bovine-associated *Bacteroides* 16S-rRNA gene sequence by real time PCR. However, the human-associated *Bacteroides* analysis for the same gene sequence is apparently similar to that of swine and the authors state a 32% false positive detection to this process. It is uncertain if any advancements have been made is selectivity of the human-associated *Bacteroides* gene since publication of this reference.

Analyses were conducted for PPCPs (listed in Table 2) to determine if there was a human or livestock waste component to these samples. While it was expected most of these compounds are unique to human wastes, sulfamethazine is clearly identified as a livestock antibiotic. In addition,

subsequent literature review found reference for the approved use of saccharin in piglet feed (Buerge 2011) and in manure slurries from swine operations in Canada. This is noted for use to encourage the intake of solid food and build body mass in piglets. An ensuing review of piglet feed product labels for use in the United States confirms the presence of sodium saccharin in swine starter products that are formulated for early development. Feed labels for calves were investigated and while most contain a natural sweetener such as sucrose or molasses, there were none found that contain sodium saccharin as the flavor modifier to encourage feed intake. There were no feed labels either for swine or bovine found to contain acesulfame or sucralose, two popular artificial sweeteners in products marketed for human consumption. The working assumption is that a human waste impacted water sample would likely contain acesulfame, sucralose, and/or saccharin, while a pure livestock contaminated sample may contain only saccharin. The antibiotics also provide indication of the waste source. Table 5 presents results of those compounds detected in this analytical process. Sulfanilic acid, carbamazepine and triclosan were eliminated from this table as they were not routinely detected in the course of this study. All data are presented in Appendix C. Triclosan was eliminated due to inconsistencies related to chromatography.

Six of the 11 groundwater samples and all of the manufactured samples were analyzed in replicate to evaluate the reproducibility of the PPCP method. Relative percent differences were determined for the analytes detected and are listed with the data summary in Appendix C.

Table 5. PPCP data summary.

		Acesulfame	Sucralose	Saccharin	Cotinine	Acetaminophen	Caffeine	Paraxanthine	Sulfamethazine	Sulfamethoxazole
Sample ID	MST	All concentrations in ng/L (parts per trillion) unless otherwise noted.								
Limits of Detection (LOD) ==>		7.0	25	5.0E	3.0	35.0	12.0	5.0	1.0	1.0E
123480001 (n=2)	B/H	53.4	193	13.3	<LOD	<LOD	13.6	<LOD	<LOD	2.1
124394001 (n=2)	B/H	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.1
125559001 (n=4)	B/H	24.7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.8
125560001	H	<LOD	<LOD	<LOD	<LOD	INT.	<LOD	<LOD	2.4	<LOD
1255561001	B/H	8.7	<LOD	<LOD	<LOD	<LOD	7.3	<LOD	27.8	<LOD
125562001	B/H	<LOD	<LOD	13.5	<LOD	<LOD	<LOD	<LOD	26.3	<LOD
123394001 (n=3)	B/H	<LOD	<LOD	153.8	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
124262001 n=2)	B/H	<LOD	<LOD	11.7	<LOD	<LOD	<LOD	<LOD	13.0	1.4
124151001 (n=2)	B/H	<LOD	<LOD	134.1	<LOD	<LOD	<LOD	<LOD	37.1	<LOD
99894001-A	H	<LOD	<LOD	34.8	54.2	<LOD	<LOD	<LOD	<LOD	<LOD
63931001	B/H	2628	5679	NA	120	80	151	157	79	NA
pig slurry (n=2)	---	interference	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1279	<LOD
cow slurry (dairy, n=2)	---	interference	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
WWTP Influent (n=2)	---	1357	27.6 ug/L	3386	1121	38.0 ug/L	89.0 ug/L	15.9 ug/L	65.8	732
WWTP Effluent (n=2)	---	1994	26.6 ug/L	247	39.2	6.8	320	109	25.7	551
Septic A (conv. n=2)	---	323	582	6235	141	538	5352	9672	<LOD	9.0
Septic B (mound n=2)	---	202	21.1 ug/L	3712	10.2	695	33.3 ug/L	30.0 ug/L	<LOD	5.8

B= positive for Bovine *Bacteroides*

H= postitive for Human *Bacteroides*

E= estimated based on signal/noise

Conclusions

Limiting factors to this study were the small number of samples received and the apparent mix of human and bovine waste in 9 of 11 samples as reported by WSLH. Mixtures of waste obstructed the determination of source identification though fecal sterol ratio analysis. Six ratios of fecal sterols from five independent researchers were used in an attempt to identify sources as bovine, human or mixed

waste. Of the 11 samples with MST data, 9 contained both human and bovine *Bacteroides*. If the assumption is made that the MST process is the most reliable method, the ratios R4 (Leeming) and R6 (Grimalt) accurately predicted the waste as being from a mixed source in 64 and 73% of the samples respectively. Comparisons of concentrations of coprostanol indicative of human waste, and β-sitosterol along with stigmasterol as indicators of herbivore waste may be the simplest fecal sterol indicator of the major contributing source. A mixture of waste sources prohibits assigning to waste to any single source regardless of using MST, fecal sterol, or PPCP analyses.

The antibiotic sulfamethazine has a chemical structure that is quite responsive to ESI LC/MS/MS techniques. Sample interferences with the sulfamethazine precursor and product ions are minimal, and the sample concentration factor (200x) results in a reliable 1.0 ng/L (part per trillion) level of sensitivity. However, the presence of sulfamethazine ensures the presence of livestock waste, however, does not segregate bovine from swine wastes. This compound was detected in 6 of 11 groundwater samples, and while in 5 of 11, the presence of bovine waste is confirmed through MST, 1 sample (125560001) contradicts MST analysis.

The presence of human *Bacteroides* and human waste-associated compounds is confirmed in several groundwater samples. Anecdotally, this may be from septage haulers disposing of human septic waste in agricultural slurry tanks rather than the approved method of disposing into municipal waste water treatment plants. However, it remains possible that human waste is from failing septic systems. *Bacteroides* PCR methods cited by WSLH (Layton) expresses 32% false positive due to the similarities of the genetic sequence of 16S-rRNA in swine and humans. Also, published reports have determined fecal sterol analysis having similar levels of coprostanol in swine and humans. With these considerations, it would be ill-advised to rely on the sole use of these techniques for source tracking. Without more swine-specific PCR determinations, the analysis for PPCPs may assist in determining waste sources.

The presence of saccharin in waste warrants more attention. Wastewater samples confirm its presence in untreated waste, and it represents an abundant artificial sweetener in the U.S. However, as evident by the effluent WWTP samples, is subject to degradation and this is confirmed by Buerge, et al. (2009). Consideration must be given to the presence of saccharin in swine feed. In the absence of detectable human-associated artificial sweeteners such as acesulfame and sucralose, saccharin must be investigated as a compound attributable to swine (especially weaning swine) waste.

The presence of acesulfame and sucralose used in soft drinks and a myriad of other products are unique to a human waste stream. The presence of these confirms the presence of human-impacted groundwater and, unlike saccharin, there is absence of evidence these compounds are added to livestock feed. These artificial sweeteners are detected in 4 of 9 fouled water samples and confirmed by MST as having human *Bacteroides*. These compounds are also found in aquifers consisting of unconsolidated materials. Municipal waste water treatment plant influent and effluent samples along with the septic system samples confirms the stability of sucralose and acesulfame even with advanced water treatment. The addition of these analytes to a suite of analytes to confirm the presence of human waste impacted groundwater is advisable.

The determination for human waste indicators is limited by the cleanliness of the sample extract. Waste samples are inherently laden with other co-extracted materials that add complexity to the chromatographic separation. Even with MS/MS as the detector, interferences are evident in suppression of ionization of the isotopically labeled internal standards. This is often true in the early eluting compounds such as sulfanilic acid and acesulfame. The presence of caffeine and its major degradate, paraxanthine, in wastewater illustrates the relative amount of this compound in human waste streams. Based on comparisons from WWTP influent and effluent samples, and the two septic

systems, caffeine is subject to inconsistent degradation to paraxanthine. However, these compounds are indicative of human waste and should be considered as reliable representatives.

When compared to MST using PCR techniques, the scope and sensitivity of chemical analytes selected for waste stream identification were alone inadequate to determine a waste source. With the advancement of lower cost, increasingly sensitive LC/MS/MS technology, and the continued introduction of synthetic organic chemicals into human and livestock diets, chemical indicators that are specific to a waste stream can be continually inserted into these methodologies and evaluated for their effectiveness in identifying a waste source. Chemical techniques should be used in combination with MST technologies as confirmatory methods until technologies advance to ensure reliable identification of waste sources.

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Appendix A. Agilent 6430 LC/MS/MS method for fecal sterol analysis.

Acquisition Method Info

Method Path	D:\MassHunter\methods\Fecal Sterols 103113.m
Method Description	APCI Fecal sterols Proshell 120 EC-C18 2.7um 3.0mm X 100mm

BinPump
Column-SL
MS QQQ

MS QQQ Mass Spectrometer

Ion Source	APCI	**Tune File**	atunes.TUNE.XML
Stop Mode	No Limit/As Pump	**Stop Time (min)**	20
Time Filter	On	**Time Filter Width (min)**	0.12

Time Segments

Index	Start Time	Scan Type	Ion Mode	Div Valve	Delta EMV	Store
1	0	MRM	APCI	To Waste	400	No
2	2	MRM	APCI	To MS	400	Yes

Time Segment 1

Scan Segments

Cpd Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)	Cell Acc	Polarity
Compound1	No	350	Unit/Enh	200	Unit/Enh	200	135	0	7	Positive

Source Parameters

Parameter	Value (+)	Value (-)
Gas Temp (°C)	340	340
APCIHeater	375	375
Gas Flow (l/min)	6	6
Nebulizer (psi)	20	20
Capillary (V)	4000	0
APCINeedlePos	7.2	7.2
APCINeedleNeg	0	0

Scan Segments

Cpd Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)	Cell Acc	Polarity
D7-Sitosterol	Yes	404.4	Unit/Enh	161	Unit/Enh	15	125	18	5	Positive
Stigmastanol	No	399.4	Unit/Enh	109.1	Unit/Enh	15	110	25	5	Positive
24-ECP	No	399.3	Unit/Enh	95.1	Unit/Enh	15	122	29	5	Positive
Sitosterol	No	397.4	Unit/Enh	161	Unit/Enh	15	125	18	5	Positive
Stigmasterol	No	395.2	Unit/Enh	83.2	Unit/Enh	15	118	17	5	Positive
Coprostanol	No	3714	Unit/Enh	109.1	Unit/Enh	15	110	25	5	Positive

Appendix A continued. Agilent 6430 LC/MS/MS method for fecal sterol analysis.

Source Parameters

Parameter	Value (+)	Value (-)
Gas Temp (°C)	340	340
APCIHeater	375	375
Gas Flow (l/min)	6	6
Nebulizer (psi)	20	20
Capillary (V)	4000	0
APCINeedlePos	7.2	7.2
APCINeedleNeg	0	0

Chromatograms

Chrom Type	Label	Offset	Y-Range
TIC	TIC	10	2500000

Instrument Curves

Actual

Autosampler

Name	ALS	**Model**	G1329B
Ordinal #	1	**Options**	
Stop time (min)	As Pump	**Post Time (min)**	Off
Injection Type	Needle Wash	**Injection Volume (µl)**	20
Overlap Time (min)	Disable Overlapped Injection	**Draw Position (mm)**	0
Draw Speed (µl/min)	200	**Eject Speed (µl/min)**	200
Wash Vessel	91		

Binary Pump

Name	BinPump	**Model**	G1312B
Ordinal #	1	**Options**	
Stop Time (min)	15	**Post Time (min)**	Off
Flow (ml/min)	0.5	**Pressure Min (bar)**	0
Pressure Max (bar)	600	**Max Flow Gradient (ml/min)**	100
Solvent A		**Solvent B**	
Solvent Ratio A	5	**Solvent Ratio B**	95
Solvent Type A1		**Solvent Type B1**	
Solvent Type A2		**Solvent Type B2**	
Compress. A (*10-6/bar)	100	**Compress. B (*10-6/bar)**	115
Stroke A (µl)	Auto	**Stroke B (µl)**	Auto
Stroke Syncronization			

Pump Time Table

	Time	Flow	Pressure	Solv Ratio B
	0	No Change	No Change	95
	3	No Change	No Change	98
	12.75	No Change	No Change	98
	12.77	No Change	No Change	95

Thermostated Column Compartment

Name	Column-SL	**Model**	G1316B
Ordinal #	1	**Options #**	CSV
Stop time (min)	As Pump	**Post Time (min)**	Off
Left Temp. (°C)	20	**Right Temp. (°C)**	Same as left
Left Ready (°C)	When Temp Within Set Point +/- 0.8	**Right Ready (°C)**	When Temp +/- 0.8
Valve Position	1		

Appendix B. Agilent 6430 LC/MS/MS method for PPCP analysis.

Acquisition Method Info

Method Name	HW TMP method.m
Method Path	D:\MassHunter\methods\110113 training\HW TMP method.m
Method Description	MRM using both positive and negative ion mode - xdb c-18 4.6 x 50 mm 1.8u

Device List

ALS
BinPump
Column-SL
MS QQQ

MS QQQ Mass Spectrometer

Ion Source	ESI	**Tune File**	atunes.TUNE.XML
Stop Mode	No Limit/As Pump	**Stop Time (min)**	15
Time Filter	On	**Time Filter Width (min)**	0.07

Time Segments

Index	Start Time	Scan Type	Ion Mode	Div Valve	Delta EMV	Store
1	0	MRM	ESI	To Waste	400	No
2	0.8	MRM	ESI	To MS	400	Yes
3	4.2	MRM	ESI	To MS	400	Yes
4	7.4	MRM	ESI	To MS	400	Yes

Time Segment 1

Scan Segments

Cpd Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)	Cell Acc	Polarity
cmpd1	No	181	Unit/Enh	124	Unit/Enh	20	100	35	5	Positive

Source Parameters

Parameter	Value (+)	Value (-)
Gas Temp (°C)	350	350
Gas Flow (l/min)	10	10
Nebulizer (psi)	45	45
Capillary (V)	4000	4000

Time Segment 2

Scan Segments

Cpd Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)	Cell Acc	Polarity
Trimethoprim-D9	No	300.1	Unit/Enh	264.3	Unit/Enh	40	100	34	1	Positive
Trimethoprim	No	291.2	Unit/Enh	261	Unit/Enh	40	115	23	1	Positive
Paraxanthine	No	181	Unit/Enh	124	Unit/Enh	42	100	35	3	Positive
Cotinine-d4	Yes	181	Unit/Enh	98	Unit/Enh	42	90	20	5	Positive
Paraxanthine	No	181	Unit/Enh	69	Unit/Enh	42	100	35	3	Positive
Cotinine	No	177	Unit/Enh	98	Unit/Enh	42	90	20	5	Positive
Acetaminophen	No	152	Unit/Enh	110	Unit/Enh	42	90	15	3	Positive
Saccharin	No	181.9	Unit/Enh	105.9	Unit/Enh	100	125	28	0	Negative
Sulfanilic Acid	No	172	Unit/Enh	108	Unit/Enh	100	122	28	0	Negative
Acesulfame-D4	Yes	166.2	Unit/Enh	166.2	Unit/Enh	90	80	0	2	Negative
Acesulfame	No	162.1	Unit/Enh	162.1	Unit/Enh	90	80	0	2	Negative

Source Parameters

Parameter	Value (+)	Value (-)
Gas Temp (°C)	350	350
Gas Flow (l/min)	10	10
Nebulizer (psi)	45	45
Capillary (V)	4000	4000

Time Segment 3

Scan Segments

Cpd Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)	Cell Acc	Polarity
Sucralose-D6	Yes	427	Unit/Enh	245	Unit/Enh	40	129	15	2	Positive
Sucralose-D6	Yes	425	Unit/Enh	243	Unit/Enh	40	129	15	2	Positive
Sucralose	No	419	Unit/Enh	239	Unit/Enh	40	129	15	2	Positive
Benzoylecognine-D3	No	293	Unit/Enh	171	Unit/Enh	36	100	24	2	Positive
Benzoylecognine	No	290	Unit/Enh	168	Unit/Enh	36	100	24	2	Positive
Venlafaxine-D6	No	284.3	Unit/Enh	268.3	Unit/Enh	100	93	16	0	Positive
Sulfamethazine-D4	Yes	283	Unit/Enh	190	Unit/Enh	36	90	15	2	Positive
Sulfamethazine	No	279	Unit/Enh	186	Unit/Enh	36	90	15	2	Positive
Venlafaxine	No	278.2	Unit/Enh	260.3	Unit/Enh	100	95	16	0	Positive
Sulfamethoxazole-D4	Yes	258.2	Unit/Enh	160.1	Unit/Enh	36	95	16	1	Positive
Sulfamethoxazole	No	254	Unit/Enh	156.1	Unit/Enh	36	95	16	1	Positive
Caffeine-D9	Yes	204	Unit/Enh	144	Unit/Enh	36	110	25	2	Positive
Caffeine	No	195	Unit/Enh	138	Unit/Enh	36	110	16	2	Positive

Appendix B continued. Agilent 6430 LC/MS/MS method for PPCP analysis.

Source Parameters

Parameter	Value (+)	Value (-)
Gas Temp (°C)	350	350
Gas Flow (l/min)	10	10
Nebulizer (psi)	45	45
Capillary (V)	4000	4000

Time Segment 4

Scan Segments

Cpd Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)	Cell Acc	Polarity
Carbamazepine-D10	Yes	247	Unit/Enh	204	Unit/Enh	65	125	18	2	Positive
Carbamazepine	No	237	Unit/Enh	194	Unit/Enh	65	125	18	2	Positive
Triclosan-D3	Yes	292	Unit/Enh	35	Unit/Enh	50	70	4	2	Negative
Triclosan-D3	Yes	290	Unit/Enh	35	Unit/Enh	50	70	4	2	Negative
Triclosan	No	289	Unit/Enh	35	Unit/Enh	50	70	4	2	Negative
Triclosan	No	287	Unit/Enh	35	Unit/Enh	50	70	4	2	Negative
Hydroxychlorothalonil	No	244.9	Unit/Enh	146.9	Unit/Enh	50	122	40	0	Negative

Source Parameters

Parameter	Value (+)	Value (-)
Gas Temp (°C)	350	350
Gas Flow (l/min)	10	10
Nebulizer (psi)	45	45
Capillary (V)	4000	4000

Chromatograms

Chrom Type	Label	Offset	Y-Range
TIC	TIC	10	2500000

Instrument Curves

Actual

Autosampler

Name	ALS	**Model**	G1329B
Ordinal #	1	**Options**	
Stop time (min)	As Pump	**Post Time (min)**	Off
Injection Type	Needle Wash	**Injection Volume (ul)**	20
Overlap Time (min)	Disable Overlapped Injection	**Draw Position (mm)**	0
Draw Speed (ul/min)	200	**Eject Speed (ul/min)**	200
Wash Vessel	91		

Binary Pump

Name	BinPump	**Model**	G1312B
Ordinal #	1	**Options**	
Stop Time (min)	16	**Post Time (min)**	2
Flow (ml/min)	0.5	**Pressure Min (bar)**	0
Pressure Max (bar)	600	**Max Flow Gradient (ml/min)**	100
Solvent A	15 mM Acetic acid	**Solvent B**	15 mM Acetic acid in MeOH
Solvent Ratio A	90	**Solvent Ratio B**	10
Solvent Type A1		**Solvent Type B1**	
Solvent Type A2		**Solvent Type B2**	
Compress. A (*10-6/bar)	100	**Compress. B (*10-6/bar)**	115
Stroke A (ul)	Auto	**Stroke B (ul)**	Auto
Stroke Syncronization			

Pump Time Table

Time	Flow	Pressure	Solv Ratio B
0	No Change	No Change	10
5	No Change	No Change	45
6.5	No Change	No Change	95
15	No Change	No Change	95
16	No Change	No Change	10

Thermostated Column Compartment

Name	Column-SL	**Model**	G1316B
Ordinal #	1	**Options #**	CSV
Stop time (min)	As Pump	**Post Time (min)**	Off
Left Temp. (°C)	50	**Right Temp. (°C)**	50
Left Ready (°C)	When Temp Within Set Point +/-0.8	**Right Ready (°C)**	When Temp +/-0.8

Appendix C. Groundwater sample and wastewater PPCP concentrations.

Groundwater samples	HW +/-	AW +/-	MST	Sulfanilic acid	Acesulfame	Sucralose	Saccharin	Cotinine	Acetaminophen	Paraxanthine	Caffeine	Sulfamethazine	Sulfamethoxazole	Carbemazepine
				ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
Limit of Detection (LOD) ==>				5.0E	7.0	25	5.0E	3.0	35.0	5.0	12.0	1.0	1.0E	2.0
123480001-A	+	-	B/H	<LOD	54.8	214.0	13.8	<LOD	<LOD	<LOD	18.1	<LOD	2.0	<LOD
123480001-B	+	-	B/H	<LOD	51.9	172.0	12.8	<LOD	<LOD	<LOD	9.1	<LOD	2.2	<LOD
RPD					6%	22%	8%				66%		8%	
124394001-A	+	-	B/H	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.1	<LOD
124394001-B	+	-	B/H	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Method Blank				<LOD	<LOD	3.6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
125559001-A	+	-	B/H	<LOD	26.0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
125559001-B	+	-	B/H	<LOD	24.1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.4	<LOD
125559001-B	+	-	B/H	<LOD	26.6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.3	<LOD
125559001-C	+	-	B/H	<LOD	22.1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.3	<LOD
RPD					8%/19%								2%	
125560001-A	-	+	H	<LOD	<LOD	<LOD	<LOD	<LOD	INT.	<LOD	<LOD	2.4	<LOD	<LOD
1255561001-A	+	+	B/H	<LOD	8.7	<LOD	<LOD	<LOD	24.8	<LOD	7.3	27.8	<LOD	<LOD
125562001-A	+	+	B/H	<LOD	<LOD	<LOD	13.5	3.0	33.8	<LOD	<LOD	26.3	<LOD	<LOD
123394001-B	+	-	B/H	<LOD	<LOD	<LOD	173.6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
123394001-C	+	-	B/H	<LOD	<LOD	<LOD	143.8	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
123394001-D	+	-	B/H	<LOD	<LOD	<LOD	143.8	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RPD							0%/21%							
124262001-B	+	+	B/H	<LOD	<LOD	<LOD	11.4	<LOD	<LOD	<LOD	<LOD	12.6	1.4	<LOD
124262001-C	+	+	B/H	<LOD	<LOD	<LOD	12.0	<LOD	<LOD	<LOD	<LOD	13.5	<LOD	<LOD
RPD							4%					7%		
Method Blank				<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
124151001-B	+	+	B/H	<LOD	<LOD	<LOD	133.1	<LOD	<LOD	<LOD	<LOD	36.6	<LOD	<LOD
124151001-C	+	+	B/H	<LOD	<LOD	<LOD	135.2	<LOD	<LOD	<LOD	<LOD	37.6	<LOD	<LOD
RPD							2%					3%		
99894001-A	+	-	H	<LOD	<LOD	<LOD	34.8	54.2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
63931001	+	+	B/H	NA	2628	5679	NA	120	80	157	151	79	NA	75

Appendix C, continued. Groundwater and wastewater sample PPCP concentrations.

Manufactured samples				Sulfanilic acid	Acesulfame	Sucralose	Saccharin	Cotinine	Acetaminophen	Paraxanthine	Caffeine	Sulfamethazine	Sulfamethoxazole	Carbemazepine
Type				ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
Limit of Detection (LOD) ==>				5.0E	7.0	25	5.0E	3.0	35.0	5.0	12.0	1.0	1.0E	2.0
pig slurry				<LOD	interference	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1250	<LOD	<LOD
pig slurry				<LOD	interference	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1308	<LOD	<LOD
												5%		
cow slurry				<LOD	interference	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
cow slurry				<LOD	interference	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Blank				<LOD	interference	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
WWTP Eff				<LOD	2537	21843	325	50.9	9.5	145	356	33.1	654	226
WWTP Eff				<LOD	2449	44780	292	47.1	7.5	129	444	31.1	724	220
RPD					4%	69%	11%	8%	24%	12%	22%	6%	10%	3%
WWTP Infl				<LOD	1680	33781	4213	1295	4213	19449	88030	72.6	847	143
WWTP Infl				<LOD	1711	35175	4252	1507	4252	20347	90000	91.8	983	167
RPD					2%	4%	1%	15%	1%	5%	2%	23%	15%	15%
Septic A (conv.)				<LOD	421	504	7810	183	7777	12772	6792	<LOD	10.8	<LOD
Septic A (conv.)				<LOD	387	951	7777	170	7810	11409	6588	<LOD	11.6	<LOD
RPD					8%	61%	0%	7%	0%	11%	3%		7%	
Septic B (mound)				<LOD	299	23125	3980	11.9	3980	30543	33320	<LOD	7.8	<LOD
Septic B (mound)				<LOD	412	29625	5300	13.5	5300	44433	33430	<LOD	6.6	<LOD
RPD					32%	25%	28%	13%	28%	37%	0%		17%	

RPD = Relative percent difference

<LOD = less than limit of detection

HW = human waste

AW = animal waste

Appendix C, continued. Groundwater and wastewater sample fecal sterol concentrations.

	Coprostanol	Stigmasterol	Sitosterol	24-ECP	Stigmastanol
WSLH #	**µg/L in sample**				
123480001	0.03	0.70	0.17	0.03	0.06
124394001	0.10	0.92	0.09	0.10	0.21
125559001	0.01	1.68	0.18	0.04	0.04
125560001	0.18	0.79	0.61	0.51	0.55
123394001	1.15	10.3	1.35	1.05	3.01
124262001	0.02	0.63	0.10	0.02	0.05
124151001	0.11	0.42	0.14	0.09	0.23
124151001	0.11	1.61	0.14	0.10	0.25
99894001	0.01	0.72	0.21	0.02	0.02
132999001	0.15	4.68	0.35	0.16	0.30
Manufactured samples					
Bovine Slurry	83.7	898	82.2	88.9	189
Pig Slurry	137	33.8	43.0	65.5	43.3
Septic A (conv.)	341	305	220	186	40.0
Septic B (mound)	105	79.6	296	207	40.2
WWTP Influent	66.5	56.1	23.4	135	6.30
WWTP Effluent	147	14.4	5.90	30.1	7.80
Human (Combined)	176	171	147	180	28.8

replicate analyses are averaged