PNNL-25275

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Analysis of Pharmaceutical and Personal Care Compounds in Wastewater Sludge and Aqueous Samples using GC-MS/MS

March 2016

L Zhong
AV Mitroshkov
TJ Gilmore

Prepared for the U.S. Department of Energy
under Contract DE-AC05-76RL01830

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Pacific Northwest National Laboratory
Richland, Washington 99352

Executive Summary

The Bioenergy Program at Pacific Northwest National Laboratory (PNNL) is evaluating the feasibility of converting wastewater sludge materials to fuels. Wastewater sludge from various municipalities will be used in the evaluation process and as with any municipal waste, there is the potential for residual contaminates to remain in the sludge following wastewater treatment. Many surveys and studies have confirmed the presence of pharmaceuticals in municipal wastewater and effluents (World Health Organization, 2011). Determination of the presence and concentrations of the contaminants is required to define the proper handling of this sludge in laboratory research.

A list of targeted compounds was acquired from the literature and an analytical method was developed for the pharmaceutical and personal care compounds. The presence of organics complicated the analytical techniques and, in some cases, the precision of the results. However, residual concentrations of a range of compounds were detected in the wastewater sludge and the presence and concentrations of these compounds will be considered in identifying the appropriate handling of this material in conduct of research.

Acknowledgments

The authors acknowledge the financial support from the Processing and Manufacturing Technologies Project Management Office to conduct this study.

Acronyms and Abbreviations

MRM	Multiple reactions monitoring
MSTFA	N-Methyl-N-(trimethylsilyl)trifluoroacetamide
MW	Molecular Weight
PNNL	Pacific Northwest National Laboratory
PPE	personal protective equipment
SIM	selected ion monitoring

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1.0 Introduction

Wastewater sludge can be used as feedstock for fuel conversion and the Bioenergy Program at Pacific Northwest National Laboratory (PNNL) is evaluating the feasibility of this conversion. Wastewater sludge from various cities will be used in the evaluation process. As with any municipal waste, there is the potential for residual contaminates to remain in the sludge following wastewater treatment. The determination of the presence and concentrations of the contaminants is required to define the proper handling of this waste water sludge in laboratory research.

Wastewater sludge was received from two different wastewater-treatment plants to determine the presence of pharmaceutical compounds and to quantify their concentrations, an extraction procedure and an analytical method were developed for the pharmaceutical and personal care compounds. Seven sludge samples and three aqueous samples (Table 1) have been processed. The sludge samples S-1 through S-5 were collected from homogenized/well mixed batches (~20 kg batches). Samples S-6 and S-7 were grab sample collected from approximately 20 kg of digested sludge, before homogenization. Samples S-3 and S-4 are duplicate samples. S-6 and S-7 can also be considered independent subsamples collected from the same batch. The aqueous samples were collected from a byproduct stream generated in the fuel conversion process. Within the process, the sludge is heated (350°C) at a pressure that maintains the sludge in a liquid phase. Outputs from the process include a crude oil and an aqueous byproduct.

Table 1. Descriptions of seven sludge sample and three aqueous byproduct samples

Sample No.	Matrix	Approx Mass, g	Sample Date	Descriptions of samples received at PNNL between October 2014 and July 2015.
S-1	Wet Sludge	~30	9/8/15	Autoclaved algae grown on effluent from the primary clarifier.
S-2	Wet Sludge	~30	9/8/15	Autoclaved algae grown on effluent from the primary clarifier.
S-3	Wet Sludge	~30	9/8/15	Autoclaved primary sludge. Sample (S-3) and duplicate (S-4).
S-4	Wet Sludge	~30	9/8/15	
S-5	Wet Sludge	~30	9/8/15	Autoclaved secondary sludge.
S-6	Wet Sludge	~30	9/8/15	Digested sludge, not homogenized. Sample (S-6) and duplicate (S-7).
S-7	Wet Sludge	~30	9/8/15	
A-1	Aqueous	250	9/9/15	Hydrothermal liquefaction aqueous byproduct from conversion on algae grown on primary clarifier effluent.
A-2	Aqueous	250	9/9/15	Hydrothermal Liquefaction aqueous byproduct from conversion on primary sludge. This was ion exchanged before analysis.
A-3	Aqueous	250	9/9/15	Hydrothermal liquefaction aqueous byproduct from conversion of digested sludge.

2.0 Materials and Methods

2.1 Selection of Compounds

Thousands of compounds with a wide range of chemical structures are used in pharmaceutical products (Halling-Sorensen et al. 1998). Based on literature-reported gas chromatography–mass spectrometry (GC-MS) analysis of pharmaceutical compounds (Togola and Budzinski 2007; Mottaleb et al. 2015) and the relevant compounds listed in a U.S. Environmental Protection Agency report on Targeted National

Sewage Sludge Survey (EPA 2009), the chemicals listed in Table 2 were selected as the target compounds in this study.

Table 2. Target compounds for analysis

Compound Name	CAS No.	Formula	MW	Class
17-alpha-Ethynylestradiol	57-63-6	$C_{20}H_{24}O_2$	296.40	Hormone
17-beta-Estradiol	50-28-2	$C_{18}H_{24}O_2$	272.38	Hormone
4-para-Nonylphenol	84852-15-3	$C_{15}H_{24}O$	220.35	Steroid
4-tert-Octylphenol	140-66-9	$C_{14}H_{22}O$	206.32	Other drugs
Acetaminophen	103-90-2	$C_8H_9NO_2$	151.16	Other drugs
Bis-phenol A	80-05-7	$C_{15}H_{16}O_2$	228.29	Hormone-like
Caffeine	58-08-2	$C_8H_{10}N_4O_2$	194.19	Other drugs
Carbamazepine	298-46-4	$C_{15}H_{12}N_2O$	236.27	Other drugs
Ciprofloxacin HCL	86393-32-0	$C_{17}H_{18}FN_3O_3 \cdot HCl$	385.82	Antibiotics
Diclofenac sodium salt	15307-79-6	$C_{14}H_{10}Cl_2NNaO_2$	318.13	Other drugs
Erythromycin USP	114-07-8	$C_{37}H_{67}NO_{13}$	733.9	Antibiotics
Estrone	53-16-7	$C_{18}H_{22}O_2$	270.37	Hormone
Fluoxetine HCl	56296-78-7	$C_{17}H_{18}F_3NO \cdot HCl$	345.79	Other drugs
Gemfibrozil	25812-30-0	$C_{15}H_{22}O_3$	250.3	Other drugs
Ibuprofen	15687-27-1	$C_{13}H_{18}O_2$	206.28	Other drugs
Naproxen	22204-53-1	$CH_3OC_{10}H_6CH(CH_3)CO_2H$	230.26	Other drugs
Ofloxacin	82419-36-1	$C_{18}H_{20}FN_3O_4$	361.37	Other drugs
Primidone	125-33-7	$C_{12}H_{14}N_2O_2$	218.25	Other drugs
Progestrone	57-83-0	$C_{21}H_{30}O_2$	314.46	Hormone
Sulfamethoxazole	723-46-6	$C_{10}H_{11}N_3O_3S$	253.28	Antibiotics
Testosterone	58-22-0	$C_{19}H_{28}O_2$	288.42	Hormone
Triclosan	3380-34-5	$C_{12}H_7Cl_3O_2$	289.54	Antibiotics
Trimethoprim	738-70-5	$C_{14}H_{18}N_4O_3$	290.32	Antibiotics

2.2 Sample Extraction

Methylene chloride (CH_2Cl_2) (>99.9 percent) was used as the extractant for the pharmaceutical compounds from the sludge and water samples. For the sludge samples, 5 g of sludge was extracted using 20 ml of CH_2Cl_2 for 48 hours. For the aqueous samples, 100 ml of sample was extracted using 10 ml of CH_2Cl_2 for 48 hours. In each case, the extractant and sample were mixed using a rotational mixer (Figure 1) and then centrifuged to separate the extractant. The extractant from each sample was then transferred to a vial for further processing and analysis (Figure 2).

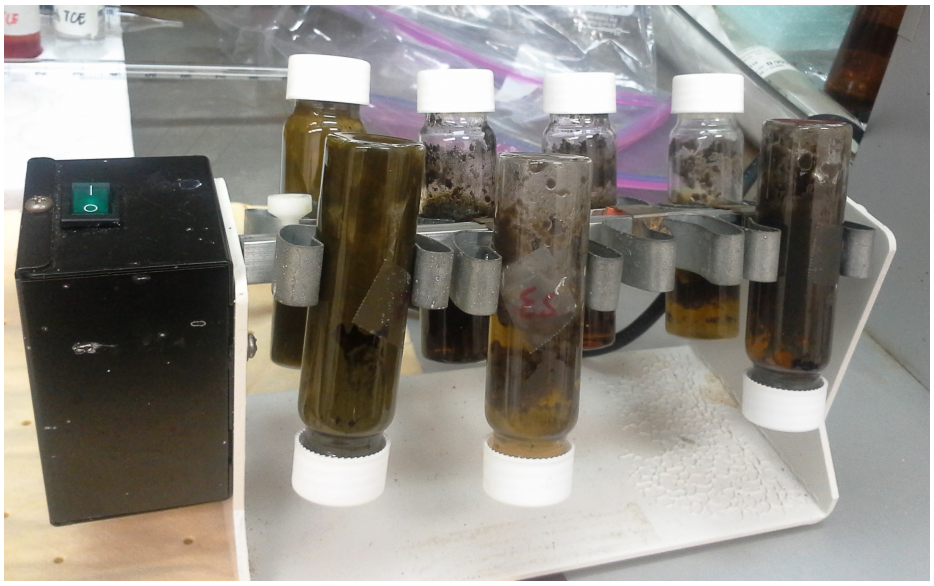

Figure 1. Sludge extraction set up.

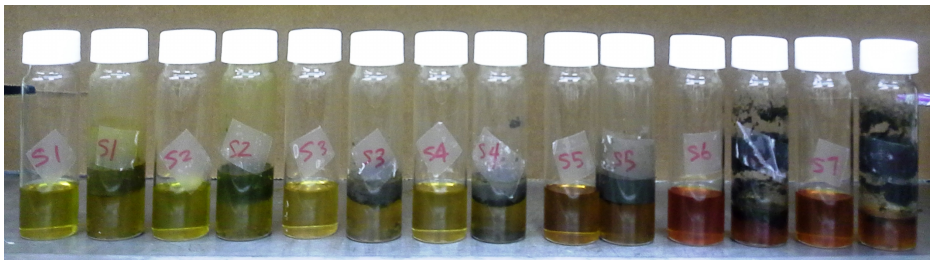

Figure 2. Extracting vials after centrifugation paired with separated extractant (to the left of the extracting vial, S1-S7).

2.3 Standard and Sample Preparation

Standards at 2000 ppm concentration were ordered from Restek Corporation (Bellefonte, PA). The standards were diluted using CH_2Cl_2 to prepare a set of standards used for GC-MS/MS calibration. The extractant CH_2Cl_2 (>99.9%) was used for analysis with no further processing. Blank samples were used during calibration and sample analysis. Internal standards were added to each standard, sample, and blank.

2.4 Standard and Sample Derivatization

Derivatization was needed to better identify and quantify the following six compounds:

1. Ciprofloxacin HCL (86393-32-0)
2. Erythromycin USP (114-07-8)
3. Sulfamethoxazole (723-46-6)
4. Gemfibrozil (25812-30-0)
5. Naproxen (22204-53-1)
6. Ofloxacin (82419-36-1)

N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) (Sigma-Aldrich Inc.) was used as the derivatizing agent. Briefly, the derivatizing process includes the following steps:

1. mix 0.2 mL of MSTFA with 0.2 mL of CH_2Cl_2
2. add 20 mg of a compound to be derivatized
3. mix and place the mixture in an oven set at 60°C for 30 min.

2.5 Analytical Methods

A GC-MS/MS system (Agilent 7000C) was used for the analysis (see Figure 3). Scan method was used to identify compounds by their mass spectra and to determine the retention times of the compounds in the samples. The selected ion monitoring (SIM) method was then used to measure a set of compounds. The multiple reactions monitoring (MRM) method was used to determine concentrations of compounds hard to determine using the SIM method.

Figure 3. GC-MS/MS system used for analysis.

2.5.1 Scan Method

Scan method is the least sensitive, but most informative method. Every compound can be identified by its mass spectrum even without standards for those compounds. One sample (i.e., S-5) was screened using this method to evaluate the content of the samples and the sufficiency of chromatographic separation. The significant number of compounds was identified and the results are presented in Appendix A. The precise quantitation of those compounds cannot be determined without the standards. However, most were in the region of 1 to 100 ppm in screened solution.

2.5.2 SIM Method

In this method the GC/MS registers only the characteristic ions for certain compound with retention times previously determined during the standards run. SIM method, which is 100 times more sensitive than scan method is sufficient for most of the compounds. In this work, samples were heavily loaded with organic materials, which produced significant interferences. In general, compounds with smaller molecular weights (MW) and shorter retention times (e.g., 4-tert-octylphenol) were reliably quantified. Compounds with larger MW in the second part of chromatogram had more significant interferences and MRM method was necessary.

2.5.3 MRM Method

In this method the first mass spectrometer selects characteristic ions for certain compounds and the second mass spectrometer registers only the fragment ions for this selected characteristic ions after they are breaking in the collision cell, which is located between the two mass spectrometers. Thus, this method is more selective than SIM method. However, samples were complex and significant interferences were observed even using the MRM method.

3.0 Results

Results are presented in Table 3. Results highlighted in green are those with the highest degree of certainty. In some cases those results were confirmed by another method; however, in other cases another method was not necessary because the results were already reliable. Results highlighted in yellow are those where compounds were identified in the mixture, but the presence of significant interferences made quantification less certain than results with green highlights. Where there is no highlighting target compounds were not detected.

Duplicate samples were analyzed using complementary methods, i.e. SIM and MRM. The concentrations of quantifiable compounds from these methods correlated reasonably well, as indicated by the results from samples S3, S4, S6, and S7.

Ofloxacin was initially analyzed; however, after careful consideration, results were removed for this compound. The trimethylsilyl (TMS) derivative of 2,6-Bis(tert-butyl) phenol was observed in the product of Ofloxacin derivatization and in the samples—indicating this phenol could have been present in the original samples. Moreover, a pathway for how Ofloxacin could produce this phenol in derivatization process could not be determined.

The water samples (i.e., A-1 to A-3) were heavily loaded with organic matter, and the extractant of these samples was visually black—indicating the presence of organics. Only a few target compounds could be observed and quantified in these three samples. Most of the other target compounds could not be analyzed because of strong interferences from unidentified non-target compounds. However, this does not imply that the target compounds were not present.

Future analysis may require an alternate analytical approach for these kinds of samples. In addition, liquid chromatography–mass spectrometry (LC-MS) may be a better analytical tool for many of the target compounds.

Table 3a. Sample number and description.

Sample	Type
S-1	Wet Sludge
S-2	Wet Sludge
S-3	Wet Sludge
S-4	Wet Sludge
S-5	Wet Sludge
S-6	Wet Sludge
S-7	Wet Sludge
A-1	Aqueous
A-2	Aqueous
A-3	Aqueous

Table 3b. Compound concentrations in sludge and aqueous samples. Concentration units: Sludge (S1-S7) -- µg/g (mg/kg) (ppm); aqueous sample (A1-A3) -- µg/ml (mg/L) (ppm)

	17-beta-Estradiol	4-tert-octylphenol		Acetaminophen		Bis-phenol A		Caffeine
Sample	SIM	SIM	MRM	SIM	MRM	SIM	MRM	SIM
S-1	ND	0.06	0.06	ND	2.96	0.16	0.28	ND
S-2	ND	0.07	0.06	ND	2.31	0.07	ND	ND
S-3	1.55	0.13	0.18	ND	ND	11.97	2.69	2.11
S-4	1.78	0.18	0.22	ND	ND	2.99	1.65	2.05
S-5	0.50	0.24	0.32	ND	3.47	1.80	2.61	0.88
S-6	ND	2.46	3.15	ND	ND	6.88	7.62	1.32
S-7	ND	2.33	3.09	ND	ND	7.04	8.23	1.28
A-1	ND	ND	ND	ND	ND	ND	ND	ND
A-2	ND	ND	0.01	ND	0.21	ND	ND	ND
A-3	ND	ND	0.05	ND	ND	ND	ND	0.66

	Carbamazepine		Deri. Carmabazepine		Diclophenac		Estrone		Ethynylestrad.	
Sample	SIM	MRM	SIM	MRM	SIM	MRM	SIM	MRM	SIM	MRM
S-1	ND	ND	ND	ND	ND	ND	ND	0.15	ND	ND
S-2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
S-3	ND	ND	ND	ND	ND	ND	ND	1.38	ND	ND
S-4	ND	ND	ND	ND	ND	ND	ND	0.97	ND	ND
S-5	ND	ND	ND	ND	ND	ND	ND	0.45	ND	ND
S-6	ND	ND	ND	ND	ND	0.025	0.64	0.72	ND	ND
S-7	ND	ND	ND	ND		ND	0.16	0.20	ND	ND
A-1	ND	ND	ND	0.11	ND	ND	ND	ND	ND	ND
A-2	ND	ND	ND	0.03	ND	ND	ND	ND	ND	ND
A-3	ND	ND	ND	0.16	ND	ND	ND	ND	ND	ND

	Fluoxetine		Gemfibrozil			Ibuprofen		Naproxen	
Sample	SIM	MRM	SIM	MRM	Derivat.	SIM	MRM	SIM	Derivat.
S-1	ND	ND	ND	ND	0.29	ND	ND	ND	0.92
S-2	ND	ND	ND	ND	ND	ND	ND	ND	ND
S-3	ND	ND	ND	ND	0.07	ND	3.43	ND	1.26
S-4	ND	ND	ND	ND	0.07	ND	4.04	ND	0.93
S-5	ND	ND	ND	ND	ND	ND	3.35	ND	ND
S-6	ND	ND	ND	ND	ND	ND	3.71	ND	ND
S-7	ND	ND	ND	ND	ND	ND	3.87	ND	ND
A-1	ND	ND	ND	ND	ND	ND	ND	ND	ND
A-2	ND	ND	ND	ND	ND	ND	ND	ND	ND
A-3	ND	ND	ND	ND	ND	ND	ND	ND	ND

	Primidone		Progestrone		Testosterone		Triclosan		Trimethoprim	
Sample	SIM	MRM	SIM	MRM	SIM	MRM	SIM	MRM	SIM	MRM
S-1	ND	ND	ND	ND	0.44	ND	0.51	No data	0.14	No data
S-2	ND	ND	ND	ND	0.17	ND	0.35	No data	ND	No data
S-3	ND	ND	ND	ND	ND	ND	5.43	No data	0.20	No data
S-4	ND	ND	ND	ND	ND	ND	5.90	No data	0.24	No data
S-5	ND	ND	ND	ND	ND	ND	7.86	No data	0.31	No data
S-6	ND	ND	1.61	1.93	ND	ND	16.75	No data	0.62	No data
S-7	ND	ND	1.22	1.40	ND	ND	15.65	No data	0.73	No data
A-1	ND	ND	ND	ND	ND	ND	ND	No data	ND	No data
A-2	ND	ND	ND	ND	ND	ND	ND	No data	ND	No data
A-3	ND	ND	ND	ND	ND	ND	ND	No data	ND	No data

ND = not detected.

4.0 Summary

The presence of organics complicated the analytical techniques and, in some cases, the precision of the results. However, residual concentrations of a range of compounds were detected in the wastewater sludge and the presence and concentrations of these compounds will be considered in identifying the appropriate handling of this material in conduct of research.

4.1 Types of Compounds

The types of compounds detected included common pain relievers, hormones and antibiotics, and plasticizers, which can also be endocrine disruptors. Highest concentrations were of Triclosan, which was found in all sludge samples ranging from 0.5 to 16.75 ppm. Triclosan is an antibacterial and antifungal agent found in consumer products, including soaps and detergents. Trimethoprim, a synthetic antibacterial, was detected in the sludge in concentrations ranging from 0.14 to 0.73 ppm with the highest concentrations in S-6 and S-7. The compound, 4-tert-octylphenol, is used to manufacture alkylphenol ethoxylates, which are anionic surfactants used in detergents, industrial cleaners, and emulsifiers. It is also identified, as an endocrine disruptor. Another endocrine disruptor, Bis-phenol A, a plasticizer, was found in all sludge samples except S-2. The female hormone Progestrone was found in some samples, including S-6 and S-7, at concentrations ranging from 1.22 to 1.93 ppm. Estrone, another female hormone, was found in samples S-1, S-5, S-6, and S-7 with concentrations ranging from 0.15 to 1.38 ppm. Ibuprophen and Naproxen, anti-inflammatory pain relievers, were found in some sludge samples in the 3 to 4 ppm range for Ibuprophen and approximately the 1 ppm range for Naproxen. Gemfibrozil, which is used to reduce cholesterol and triglycerides in the blood, was found in some samples in concentrations less than 1 ppm. Caffeine, a central nervous system stimulant, was also found in some samples (i.e., 0.66 ppm in an aqueous sample and from 0.88 to 2.11 ppm in sludge samples).

4.2 Protection

All detected compounds were in the “parts per million” range. To date, no occupational exposure limits have been identified for these compounds. However, the pharmaceutical industry recently developed personal protective equipment (PPE) guidelines. While the concentrations working directly with the nearly pure compound in the pharmaceutical industry are much higher than the residual concentrations in the wastewater sludge, it can be assumed that the exposure pathways for the compounds are similar and that the PPE identified by the pharmaceutical industry would be conservative if used for handling wastewater sludge. The PNNL worker health and safety representative will use the results in this report in combination with the pharmaceutical industry reports to identify the appropriate PPE for this work and will update the worker exposure assessment as applicable.

5.0 References

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Appendix

Compounds Identified During the Screening of S-5 Sample

Appendix

Compounds Identified During the Screening of S-5 Sample

(Siloxanes were excluded because they are mostly coming from the column)

RT, min	Name	CAS	MW
6.05	Phenol	108-95-2	94
7.00	D-Limonene	5989-27-5	136
7.26	Benzeneacetaldehyde	122-78-1	120
7.51	Benzene, n-butyl	104-51-8	134
7.74	p-Cresol	106-44-5	108
7.96	Benzenemethanethiol	100-53-8	124
8.18	1,2,4-Trithiolane	289-16-7	124
8.53	Phenylethyl Alcohol	60-12-8	122
8.76	1-Phenyl-1-butene	1005-64-7	132
8.91	N-(3-Methylbutyl)acetamide	13434-12-3	129
9.35	Benzene, pentyl-	538-68-1	148
9.76	2-Piperidinone	675-20-7	99
11.74	Indole	120-72-9	117
12.85	Naphthalene,1,2,3,4-tetrahydro-1,1,6-trimethyl	475-03-6	174
12.92	n-Decanoic acid	334-48-5	172
13.26	1H-Indole, 4- methyl	16096-32-5	131
13.29	1H-Indole, 2- methyl	95-20-5	131
13.61	Naphthalene, 2,7-dimethyl-	582-16-1	156
13.65	Naphthalenol,-octahydro-4,8a-dimethyl	19700-21-1	182
14.66	1H-Indole,2,3-dihydro-4-methyl	62108-16-1	133
14.84	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl	644-30-4	202
15.02	Pentadecane	629-62-9	212
15.25	Acetamide, N-(2-phenylethyl)-	877-95-2	163
15.62	Benzene,(1-butylhexyl)-	4537-11-5	218
15.75	Benzene, (1-propylheptyl)	4537-12-6	218
16.02	Dodecanoic acid	143-07-7	200
16.51	Hexadecane	544-76-3	226
16.87	Tridecanoic acid	638-53-9	214
17.02	Benzophenone	119-61-9	182
17.05	Benzene, (1-butylheptyl)	4537-15-9	232
17.19	Benzene,(1-propyloctyl)-	4536-86-1	232
17.49	Benzene,(1-ethylnonyl)-	4536-87-2	232
17.67	n-Hexyl salicylate	6259-76-3	222
17.91	Heptadecane	629-78-7	240
18.23	1-Tetradecanamine, N,N-dimethyl-	112-75-4	241
18.35	Benzene,(1-pentylheptyl)-	2719-62-2	246
18.42	Benzene, (1-butyloctyl)	2719-63-3	246
18.58	Benzene, (1-propylnonyl)-	2719-64-4	246
18.85	Tetradecanoic acid	544-63-8	228
19.71	Pentadecanoic acid	1002-84-2	242

RT, min	Name	CAS	MW
19.95	Caffeine	58-08-2	194
20.28	1-Hexadecanol	36653-82-4	242
20.48	Homosalate	118-56-9	262
21.23	Palmitoleic acid	373-49-9	254
21.30	Pyrrolo(1,2-a0hexahydro-3-(2-methylpropyl)	5654-86-4	210
21.56	Hexadecanoic acid	57-10-3	256
22.12	Heptadecanoic acid	506-12-7	270
22.29	Cyclic octaatomic sulfur	10544-50-0	256
23.27	1-Decanamine, N-decyl-N-methyl-	7396-58-9	311
23.61	9-Octadecanoic acid	112-79-8	282
23.82	Octadecanoic acid	57-11-4	284
23.87	Pregnan-20-one(5α)	848-62-4	302
25.10	Tricosane	638-67-5	324
25.22	Eicosylamine, N,N-dimethyl-	NIST#:406305	325
25.41	N-Methyl-N-benzyltetradecanamine	83690-72-6	317
26.04	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	14705-60-3	244
26.14	Tetracosane	646-31-1	338
27.13	Pentacosane	629-99-2	352
27.3	Didodecyldimethylammonium bromide	3282-73-3	461
27.64	Phtalic acid, di(2-propenylpentyl) ester	NIST#:377935	390
28.06	Hexacosane	630-01-3	366
28.85	Octocrylene	6197-30-4	361
29.45	1,4-Benzenecarboxylic acid, bis(2-ethylhexyl)ester	6422-86-2	390
30.18	Squalene	111-02-4	410
30.25	Didecan-2-yl phtalate	28029-89-2	446
30.87	Cholesta-3,5-diene	747-90-0	368
32.35	Cholestan-3-ol	360-68-9	388
32.53	Stigmasta-3,5-diene	79897-80-6	396
32.69	Cholest-5-en-3-ol	NIST#210384	386
32.75	Cholesterol	57-88-5	386
32.83	Cholestanol	80-97-7	388
33.13	Lathosterol	80-99-9	386
33.18	Cholest-14-en-3-ol	20780-35-2	386
33.25	Ergostanol	6538-02-9	402
33.99	Cholest-4-en-3-one	601-57-0	384
34.21	Stigmastanol	19466-47-8	416
34.65	γ-Sitosterol	83-47-6	414
34.98	Hexadecanoic acid, hexadecyl ester	540-10-3	480
36.31	γ-Sitostenone	84924-96-9	412
37.52	Oleyl oleate	3687-45-4	532

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