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Standard Practice for Identification of Chemicals in Water by Fluorescence Spectroscopy[1]

This standard is issued under the fixed designation D4763; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This practice allows for the identification of 90 chemicals that may be found in water or in surface layers on water. This practice is based on the use of room-temperature fluorescence spectra taken from lists developed by the U.S. Environmental Protection Agency and the U.S. Coast Guard (**1**). Ref (**1**) is the primary source for these spectra. This practice is also based on the assumption that such chemicals are either present in aqueous solution or are extracted from water into an appropriate solvent.[2]

1.2 Although many organic chemicals containing aromatic rings, heterocyclic rings, or extended conjugated double-bond systems have appreciable quantum yields of fluorescence, this practice is designed only for the specific compounds listed. If present in complex mixtures, preseparation by high-performance liquid chromatography (HPLC), column chromatography, or thin-layer chromatography (TLC) would probably be required.

1.3 If used with HPLC, this practice could be used for the identification of fluorescence spectra generated by optical multichannel analyzers (OMA) or diode-array detectors.

1.4 For simple mixtures, or in the presence of other non-fluorescing chemicals, separatory techniques might not be required. The excitation and emission maximum wavelengths listed in this practice could be used with standard fluorescence techniques (see Refs (**2-6**)) to quantitate these ninety chemicals once identification had been established. For such uses, generation of a calibration curve, to determine the linear range for use of fluorescence quantitation would be required for each chemical. Examination of solvent blanks to subtract or eliminate any fluorescence background would probably be required.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]
D1129 Terminology Relating to Water
D1193 Specification for Reagent Water
E131 Terminology Relating to Molecular Spectroscopy
E275 Practice for Describing and Measuring Performance of Ultraviolet and Visible Spectrophotometers

3. Terminology

3.1 *Definitions*—For definitions of terms used in this practice, refer to Terminology D1129, Specification D1193, and definitions under the jurisdiction of Committee E13 such as Terminology E131 and Practice E275.

4. Summary of Practice

4.1 This practice uses well tested fluorescence techniques to detect and identify (or determine the absence of) 90 chemicals that have relatively high fluorescence yields. Table 1 lists for each chemical an appropriate solvent (either cyclohexane, water, methyl or ethyl alcohol, depending on solubility), a suggested excitation wavelength for maximum sensitivity, a wavelength corresponding to the emission maximum, the number of fluorescence peaks and shoulders, the width (full width at half of the maximum emission intensity) of the strongest fluorescence peak and the detection limit for the experimental conditions given. Detection limits could be lowered, following identification, by using broader slit widths.

[1] This practice is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.06 on Methods for Analysis for Organic Substances in Water.

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[2] The boldface numbers in parentheses refer to a list of references at the end of this standard.

[3] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

A list of corrected fluorescence spectra for the chemicals included in this practice are also available (**1**).

4.2 Identification of the sample is made by comparison of the obtained spectra with information in Table 1 and by direct visual comparison of appropriate spectra with positions of principal peaks in agreement to ±2 nm and ratios of peak heights in agreement to ±10 % if corrected spectrofluorometers are used.

4.3 Spectral distortions due to self-absorption or fluorescence quenching or dimer formation may occur at higher concentrations (for example, 100 ppm or µg/mL). If this is suspected, the solution should be diluted and additional fluorescence spectra generated. If a suspected chemical is not detected on excitation at the appropriate wavelength, it usually can be assumed that it is not present above the detection limit, barring interference effects due to absorption or quenching that can usually be anticipated.

5. Significance and Use

5.1 This practice is useful for detecting and identifying (or determining the absence of) 90 chemicals with relatively high fluorescence yields (see Table 1). Most commonly, this practice will be useful for distinguishing single fluorescent chemicals in solution, simple mixtures or single fluorescing chemicals in the presence of other nonfluorescing chemicals. Chemicals with high fluorescence yields tend to have aromatic rings, some heterocyclic rings or extended conjugated double-bond systems. Typical chemicals included on this list include aromatics, substituted aromatics such as phenols, polycyclic aromatic hydrocarbons (PAH's), some pesticides such as DDT, polychlorinated biphenyls (PCB's), some heterocyclics, and some esters, organic acids, and ketones.

TABLE 1 Summary of Experimental Parameters and Results

Chemical	Code	Concentrations, ppm	Solvent	λ_{exc}, nm	λ^{em}_{max}, nm	Number of Peaks	WHM, nm	Shoulder Number	Detection Limit (DL), ppm	λ_{DL}, nm	Comments
Acenaphthene	ACN	1.03	CH	290	323	4	...	3	0.001	290	
Acetone	ACT	227	CH	290	410	1	...	...	212	290	
Acridine	ACR	96	CH	285/355	386/422	4/2	...	2/0	...	...	
	ACR	9.6	ETOH	290/355	357/415	2/2	...	1/1	0.02/0.04	290/355	
Aniline	ANL	15.5	CH	280	316	1	...	...	0.037	280	
Anthracene	ATH	1.03	CH	355	378	4	...	1	0.001	355	
	ATH	1.55	ETOH	355	380	4	...	1	0.001	355	
Aroclor 1242	PC4	131	CH	270	317	2	35	1	0.3	270	
1254	PC5	129	CH	270	317	2	36	1	2	270	
Atrazine	ATZ	369	CH	290	350	1	...	...	300	290	
Azinphosmethyl	AZP	112	CH	350	410	2	60	...	10	350	
	AZP	122	ETOH	340	420	2	80	...	4	340	
Benz(a)anthracene	BAT	1.1	CH	280	386	4	...	1	0.003	280	
Benzene	BNZ	79	CH	250	279	3	24	1	2/4	250/265	
Benzonitrile	BZN	9.9	CH	260	287	2	28	1	0.1/0.1	260/270	
Benzo(a)pyrene	BAP	0.088	CH	370	405	6	...	2	0.002	370	
Benzyl alcohol	BAL	99	CH	250	284	2	27	1	0.1/0.1	250/260	
Benzyl amine	BZM	118	CH	250	283	1	27	2	3/2	250/260	
Benzyl triethylammonium chloride	BMA	210	H_2O	250	280	1	28	...	59	250	
Bisphenol A	BPA	10.5	ETOH	270	304	1	30	1	0.04/0.02	270/285	
Brucine	BRU	13.5	ETOH	280	327	1	56	...	2/2	280/295	
O-tert-Butylphenol	BOP	21	CH	265	295	1	30	1	0.1/0.1	265/275	
p-tert-Butylphenol	BTP	17.5	CH	260	295	1	31	1	0.6/0.4	260/280	
Carbaryl	CBY	1.0	CH	285	335	2	36	2	0.01	285	
Carnauba wax	WCA	63.5	CH	260	310	1	64	...	42	260	
Castor oil	OCA	390	ETOH	290	328	1	43	2	20	290	
	OCA	286	CH	280/320	...	1	...	...	180/300	280/320	
Catechol	CTC	8.7	H_2O	265	310	1	46	...	0.4/0.2	265/280	
4-Chloroaniline	CAP	17.2	CH	290	328	1	36	1	0.2	290	
1-Chloronaphthalene	CNA	11.3	CH	290	328	3	34	4	0.1	290	
p-Chlorophenol	CPN	101	CH	260	305	1	30	...	1/0.1	260/285	
Chlorpyrifos (Duraban)	DUR	25.3	CH	280	326	1	52	...	1/0.5	280/295	
p-Chlorotoluene	CTN	23.8	CH	265	288	1	29	3	1/0.8	265/275	
p-Chloro-o-toluidine	COT	25	CH	290	328	1	39	1	0.09	300	
Chrysene	CRY	1.0	CH	270	383	5	...	...	0.002	270	
Coconut oil	OCC	286	CH	290	330	...	...	...	100	290	
Cod liver oil	OCL	323	CH	260/280	320/320	1/1	150	...	260,140	260,280	
				330	500	1			65	330	
Copper naphthenate	CNN	98	CH	260	326	1	60	3	3/1	260/280	
Cottonseed oil	OCS	305	CH	280/320	320/380	...	...	...	165,300	280,320	
Coumaphos	COU	11.4	CH	320	377	1	74	...	0.3	320	
o-Cresol	CRO	12.0	CH	265	293	1	30	1	0.04	280	
p-Cresol	CRP	10.3	CH	265	299	1	30	...	0.03	280	
Cumene	CUM	101	CH	250	283	2	28	1	3	250	
p-Cymene	CMP	11.8	CH	260	285	1	28	2	0.4/0.2	260/270	
DDD	DDD	61.0	CH	240	294	1	30	2	4	240	
DDT	DDT	87	CH	245	291	2	28	2	7	245	

TABLE 1 *Continued*

Chemical	Code	Concentrations, ppm	Solvent	λ_{exc}, nm	λ^{em}_{max}, nm	Number of Peaks	WHM, nm	Shoulder Number	Detection Limit (DL), ppm	λ_{DL}, nm	Comments
1,2,5,6-Dibenzanthracene	DBA	0.015	CH	300	396	4	...	2	0.001	300	
Dicamba	DIC	22.2	H_2O	310	420	1	70	...	0.9	310	
Dichlorobenil	DIB	108	CH	285	312	1	30	...	0.6	285	
2,4-Dichlorophenoxy-acetic acid	DCA	159	CH	270	310	1	46	1	30	270	
Diethylbenzene	DEB	100	CH	255	283	1	28	2	0.2/0.1	255/270	
Diethylene glycol	DEG	202	CH	265	310	2	...	...	202	265	
Diethylphthalate	DEP	145/289	CH	260/280	300/320	1/1	...	...	...	280	
2,4-Dimethylphenol	DMH	10.5	CH	265	300	1	31	1	0.2/0.04	265/280	
3,5-Dimethylphenol	DPM	10.5	CH	265	295	1	28	1	0.07/0.03	265/280	
Diphenylamine	DAM	11.2	CH	290	333	1	37	2	...	290	photochemical change
		1.2	CH	290	333	1	37	2	...	290	
Diphenyldichlorosilane	DDS	157	CH	260	285	2	30	...	3/2	260/270	
Diquat dibromide	DQD	35.5	H_2O	310	348	1	41	1	0.055	310	
Dodecylbenzene	DDB	116	CH	250	285	3	30	...	*	250	* strong impurity
		116	CH	220	285	3	30	...	13.6	220	
Dowtherm A	DTH	10.8	CH	260	305	2	33	2	0.035	260	
Ethylbenzene	ETB	103	CH	250	283	2	26	...	3.1/1.5	250/260	
Fluoranthene	FLA	1.0	CH	360	465	2	91	3	0.005	360	
Gallic acid	GLA	103	H_2O	290	346	1	77	...	0.70	290	
Hydroquinone	HDQ	1.1	H_2O	290	326	1	38	1	0.025	290	
Indene	IND	175	CH	260	309	2	32	3	0.12	260	
Lard	OLD	340	CH	270	330	...	...	...	...	270	
	OLD	287	CH	280	330	1	...	...	...	280	
Linseed oil	OLS	355	CH	300	418	1	105	...	32	300	
Methoxychlor	MOC	95	CH	270	299	1	30	1	1.3/0.8	270,280	
Methylaniline	MAN	10.8	CH	290	325	1	35	...	0.01	290	
Methyl isobutyl ketone	MIK	358	CH	290	400	1	...	...	...	290	
Methyl styrene	MSR	105	CH	255	307	1	35	2	0.12	255	
Naphthalene	NPT	10.5	CH	280	323	2	24	3	0.02	280	
1-Naphthylamine	NAD	1.85	CH	325	377	1	55	1	0.0012	325	
Nonyl phenol	NNP	17.1	CH	265	298	1	28	...	0.09	265	
Olive oil	OOL	237	CH	260	320	1	...	...	...	360	
	OOL	290	CH	310	...	...	...	...	...	310	
Palm oil	OPM	300	CH	260	320	1	60	...	218	260	
			CH	350	500	1	140	...	300	350	
Peanut oil	OPN	249	CH	260,290	120,320	1	...	...	...	...	
Phenol	PHN	11.9	CH	265	288	1	30	2	0.011/0.007	265/275	
Phenyl ether	DPE	20.4	CH	265	291	1	36	1	0.10	265	
Phthalic acid	PHA	97	H_2O	280	330	1	100	...	84	280	
	PHA	228	H_2O	270	340	1	100	...	114	270	
Piperazine	PPZ	235	CH	280	350	1	...	...	...	...	
Polyethoxylated non-ylphenol	PEN	9.5	CH	265	297	1	30	...	0.08/0.03 17	265/280	
Pyrogallol	PGA	152	H_2O	270	335	1	86	1	30	270	
Quinoline	QNL	113	ETOH	275	321	5	...	2	...	...	photolyzes
		113	ETOH	355	420	1	70	0	...	...	photolyzes
		95	CH	275	336	3	...	2	0.37	275	photolyzes
		95	CH	350	...	2	57	1	...	...	
Resorcinol	RSC	10.1	H_2O	265	303	1	39	1	0.135/0.05	265/280	
Salicylic acid	SLA	1.5	H_2O	300	409	1	64	...	0.005	300	
Sodium dodecylbenzenesulfonate	SDB	90	CH	290	347	1	52	2	0.90	290	
Soya bean oil	OSB	290	CH	270,320	...	...	...	...	0.300	270,320	
Styrene	STY	1.1	CH	270	306	2	32	2	0.03	270	
Tanaic acid	TNA	13	H_2O	280	340	1	100	...	0.63	280	
1,2,3,4-Tetrahydro-naphthalene	THN	12.3	CH	260	284	1	27	2	0.21/0.13	260/270	
p-Toluidine	TLI	14.1	CH	290	325	1	34	...	0.03	290	
Toluene	TOL	107	CH	250	284	2	27	1	2.1/1.6	250/215	
p-Toluene sulfonic acid	TAP	120	H_2O	260	285	1	28	1	2.1/1.5	260/265	
Tricresylphosphate	TCP	123	CH	260	288	1	66	1	0.55/0.35	260/270	
1,3,5-Triethylbenzene	TEB	122	CH	250	292	1	28	3	12.5/1.5	250/270	
Turpentive	TPT	301	CH	260	283	1	34	3	31/13	260/270	
Undecylbenzene	UDB	87.3	CH	250	284	2	33	2	6.0	250	
Uranyl nitrate	UAN	61.0	H_2O	290	520	3	56	2	6.1/10.5	290/330	
m-Xylene	XLM	114	CH	260	285	1	28	1	2.0/1.4	260/270	
o-Xylene	XLO	92	CH	260	285	1	30	...	1.5/1.3	260/270	

5.2 With appropriate separatory techniques (HPLC, TLC, and column chromatography) and in some cases, special detection techniques (OMA's and diode arrays), this practice can be used to determine these 90 chemicals even in complex mixtures containing a number of other fluorescing chemicals. With the use of appropriate excitation and emission wavelengths and prior generation of calibration curves, this practice could be used for quantitation of these chemicals over a broad linear range.

5.3 Fluorescence is appropriately a trace technique and at higher concentrations (greater than 10 to 100 ppm) spectral distortions usually due to self-absorption, or inner-filter effects but sometimes ascribed to fluorescence quenching, may be observed. These effects can usually be eliminated by diluting the solution. Detection limits can be lowered following identification by using broader slit widths, but this may result in spectral broadening and distortion.

5.4 This practice assumes the use of a corrected spectrofluorometer (that is, one capable of producing corrected fluorescence spectra). On an uncorrected instrument, peak shifts and spectral distortions and changes in peak ratios may be noted. An uncorrected spectrofluorometer can also be used if appropriate data is generated on the instrument to be used.

6. Interferences

6.1 For the identification of compounds with low fluorescence yields and relatively high detection limits, the presence of other chemicals with high fluorescence yields emitting in the same spectral region, for example, anthracene, fluorescein, etc., may interfere unless separatory techniques are employed.

6.2 Some naturally occurring fluorescing materials, such as humic acids from leaf mold, may also interfere with the identification of chemicals with relatively low fluorescence yields especially at dilute concentrations of the hazardous chemicals, especially for emission in the near ultraviolet.

6.3 Since light must be absorbed before being reemitted, colored solutions, or solutions with absorbances greater than 0.02 at the excitation or emission wavelengths of interest will also interfere. Such solutions usually require further dilution.

6.4 Halogenated solvents and other solvents containing possible quenchers are not recommended for this application since they may raise detection limits.

7. Apparatus

7.1 *Scanning Fluorescence Spectrophotometer or Spectrofluorometer,* corrected to give constant emission intensity to ±5 to 10 % for fluorescence spectra over the spectral range scanned, normally from 220 to 600 nm. The spectral correction should be checked using an appropriate chemical such as anthracene for which the peak ratios of the corrected fluorescence peaks are known. The instrument should have an appropriate excitation source such as a high-pressure xenon lamp or other continuum source with at least 150 or 250 W. Band widths should be adjustable to at least 5 nm for excitation slit widths and at least 2 nm for emission slit widths. An appropriate photomultiplier tube with good detection characteristics over the 250 to 700 nm spectral range. For example, tubes with an S-20 response, should be used.

7.2 *Fluorescence Cells*—Standard fluorescence cells, fluorescence-free fused silica cells with a 10-mm path length.

7.3 *Recorder*—Strip chart or x-y recorder.

7.4 *Weighing Pans*—Aluminum, disposable.

8. Reagents

8.1 *Purity of Reagents*—Spectroquality grade chemicals shall be used in all tests. Spectroquality solvents required may include cyclohexane, methanol, and ethanol. Purity of solvents should be checked on running solvent blanks. Anthracene and other appropriate PAH's may be required to check spectral corrections (see Ref (**1**)).

8.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water that meets the purity specifications of Type I or Type II water, presented in Specification D1193. Check the water purity by running water blanks.

9. Sampling and Sample Preparation

9.1 Neat samples (from a surface film or layer on water) only require dilution in an appropriate solvent (after skimming from the surface of the water using perforated TFE-fluorocarbon if on water). An initial concentration for an unknown might be 100 µg/mL for preferably 25 mL of solution, with further dilutions once a fluorescence signal detected, down to 10 or 1 µg/mL. If a particular compound is not soluble in cyclohexane, the following solvents may be tried in order: water, methanol, ethanol, and acetonitrile.

9.2 If an unknown is dissolved in water (assuming no chemicals such as humic acid are present at levels that might interfere with the determination), it can be tested directly with appropriate dilutions or preconcentrations as required. If a chemical is emulsified in water or is sparingly soluble in water or if it is required to know the concentration of the unknown more precisely, it may be necessary to evaporate the solution, or to extract the chemical into a suitable solvent followed by evaporation, weighing, and redissolving in an appropriate solvent.

9.3 If an unknown fluorescent solute is dissolved in an organic solvent or mixture, it may sometimes be measured directly. But more often, if other components of the mixture fluoresce, a separation by an extraction or chromatographic step may be required followed by weighing and dilution in an appropriate solvent.

9.4 Sample bottles must be made of glass, precleaned with dilute nitric acid, with plastic screw caps having TFE-fluorocarbon liners. Solutions must be made up in precleaned volumetric flasks (preferably red). Volumetric flasks and fluorescence cells must be cleaned with dilute nitric acid followed by rinsing with water. Glassware and cells should receive a final rinse with the solvent of choice. Solutions should be prepared fresh each day, but may be held for up to 3 days if stored in a refrigerator.