

ANALYTICAL CHEMISTRY DIVISION
COMMISSION ON MICROCHEMICAL TECHNIQUES†
RECOMMENDED TEST SUBSTANCES FOR THE
MICRODETERMINATION OF VARIOUS
ELEMENTS AND FUNCTIONAL GROUPS IN
ORGANIC COMPOUNDS

About fifty compounds are recommended for use as reference substances for the microdetermination of various elements and functional groups in organic substances. Percentages are shown for the most commonly analysed functional groups, but many other groups are represented, and their theoretical values may be calculated by the reader. All of these substances, or a proper selection from the list, may be used to determine the universal applicability of a given method, either already described or one which might be developed in the future.

The compounds selected are stable over long periods of time and are non-hygroscopic (any exceptions to the latter generalization are so noted). The substances are either commercially available in a sufficiently pure state to be used for test purposes based on the accuracy of present-day methods or may be purified or prepared by conventional laboratory means to meet these standards.

The compounds selected include the following:

- (1) compounds for the determination of the blank value and those which have extreme values (high as well as low) for element and/or group content;
- (2) compounds representing a variety of structural types;
- (3) compounds containing elements which may cause interferences in the various determinations.

These recommendations may be changed or supplemented as the need arises.

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List of substances	Empirical formulae	Molecular weight	C (%)	H (%)	O (%)	N (%)	Hal (%)	S (%)	Element (%)	Functional group (%)
C, H										
Anthracene	C ₁₄ H ₁₀	178.234	94.34	5.66						
Cyclohexane	C ₆ H ₁₂	84.162	85.63	14.37						
Naphthalene	C ₁₀ H ₈	128.174	93.71	6.29						
C, H, O										
Acrylsalicylic acid	C ₉ H ₈ O ₄	180.159	60.00	4.48	35.52					23.89 CH ₃ CO 24.99 COOH 20.40 CH ₃ O 36.86 COOH 15.24 CH ₃ 11.41 COOH 2.80 active H 15.82 COOH 0.35 active H
Anisic acid	C ₈ H ₈ O ₃	152.149	63.15	5.30	31.55					0.56 active H 29.59 COOH
Benzoic acid	C ₇ H ₆ O ₂	122.123	68.85	4.95	26.20					0.83 active H 7.87 CH ₃ O
Bixin	C ₃₅ H ₅₈ O ₄	394.511	76.11	7.67	16.22					0.26 active H
Cholesterol	C ₂₇ H ₄₆ O	386.664	83.87	11.99	4.14					2.80 active H
d-Glucose (dextrose)	C ₆ H ₁₂ O ₆	180.156	40.00	6.71	53.28					
Malic acid	C ₄ H ₆ O ₄	116.072	41.39	3.47	55.13					
Phthalic anhydride	C ₈ H ₄ O ₃	148.117	64.87	2.72	32.40					
Stearic acid	C ₁₈ H ₃₆ O ₂	284.484	76.00	12.75	11.25					0.35 active H
C, H, N										
Azobenzene	C ₁₂ H ₁₀ N ₂	182.226	79.10	5.53		15.37				
Diphenylamine	C ₁₂ H ₁₁ N	169.227	85.17	6.55		8.28				
Hexamethylenetetramine	C ₆ H ₁₂ N ₄	140.190	51.41	8.63		39.97				
Melamine	C ₃ H ₄ N ₆	126.123	28.57	4.80		66.63				
C, H, N, O										
Acetamide	C ₂ H ₅ NO	135.166	71.02	6.71	11.81	10.36				31.85 CH ₃ CO 0.75 active H
Caffeine (1,3,7-trimethylxanthine) (anhydrous)	C ₈ H ₁₀ O ₂ N ₄	194.194	49.48	5.19	16.48	28.85				23.23 CH ₃ attached to N or 44.87 NCH ₃ 39.81 C ₂ H ₅ O
Cyanoacetic acid, ethyl ester	C ₅ H ₇ O ₂ N	113.116	53.09	6.24	28.29	12.38				
2,4-Dinitrophenylhydrazine	C ₈ H ₈ O ₄ N ₄	198.138	36.37	3.05	32.30	28.28				
8-Hydroxyquinoline	C ₈ H ₇ ON	145.161	74.47	4.86	11.02	9.65				
Nicotinamide	C ₆ H ₆ ON ₂	122.127	59.01	4.85	13.10	22.94				
1-Nitroso-naphthol-2	C ₁₀ H ₇ O ₂ N	173.171	69.36	4.07	18.48	8.09				
Picric acid	C ₆ H ₃ O ₇ N ₃	229.104	31.46	1.32	48.88	18.34				
Tryptophan	C ₁₁ H ₁₂ O ₂ N ₂	204.229	64.69	5.92	15.67	13.72				
Urea	CH ₄ ON ₂	60.056	20.00	6.71	26.64	46.65				
C, H, . . . Br										
p-Bromocetanilide	C ₈ H ₈ ONBr	214.067	44.89	3.77	7.47	6.54	37.33 Br			
5,7-Dibromo-8-hydroxyquinoline	C ₈ H ₅ ONBr ₂	302.963	35.68	1.66	5.28	4.62	52.75 Br			
2,4,6-Tribromophenol	C ₆ H ₃ ONBr ₃	330.916	21.78	0.91	4.84		72.47 Br			

Notes

For the calculation of molecular weights and percentages, the atomic weights used are those proposed by the Commission on Atomic Weights of the I.U.P.A.C., 1961.

The percentage figures are given to the second decimal. Where the third decimal is less than 0.005, the last figure has been disregarded. Where the last figure has been rounded off, it has been underlined ($0.375 = 0.38$).

Almost all of the compounds have been included in the four previous lists of recommended test substances, "Recommended Test Substances for the Microdetermination of Carbon and Hydrogen", *Pure Appl. Chem.* **1**, 143 (1960), "Recommended Test Substances for the Microdetermination of Nitrogen in Organic Compounds", *Pure Appl. Chem.* **3**, 513 (1961), "Recommended Test Substances for the Microdetermination of Halogens and Sulphur in Organic Compounds", *Pure Appl. Chem.* **5**, 759 (1962), and "Recommended Test Substances for the Microdetermination of Oxygen in Organic Compounds", *Pure Appl. Chem.* **7**, 707 (1963).