

SECTION OF ANALYTICAL CHEMISTRY
COMMISSION ON MICROCHEMICAL TECHNIQUES*

**RECOMMENDED TEST SUBSTANCES FOR THE
MICRODETERMINATION OF NITROGEN IN
ORGANIC COMPOUNDS**

A number of compounds are recommended for use as reference substances for the microdetermination of nitrogen in organic substances. 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 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:

- (i) those which have extreme values (high as well as low) for nitrogen;
- (ii) those in which nitrogen occurs in a variety of ways (different nitrogen linkages);
- (iii) compounds representing a variety of structural types; and
- (iv) compounds containing elements which may cause interferences in the nitrogen determination.

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

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Table I. List of substances †

No.	Compound and class	Empirical formula	Molecular weight	N (%)	C (%)	H (%)	O (%)	Hal (%)	S (%)	As (%)	P (%)
1	(A) NO_2 Picric acid	$\text{C}_6\text{H}_3\text{O}_7\text{N}_3$	229.114	18.34	31.45	1.32	48.88				
2	1-Chloro-2,4-dinitrobenzene	$\text{C}_6\text{H}_3\text{O}_4\text{N}_2\text{Cl}$	202.563	13.83	35.58	1.49	31.60	17.50 Cl			
3	(B) NO 1-Nitroso-naphthol-2	$\text{C}_{10}\text{H}_7\text{O}_2\text{N}$	173.174	8.09	69.36	4.07	18.48				
4	(C) CN Cyanoacetic acid, ethyl ester	$\text{C}_3\text{H}_7\text{O}_2\text{N}$	113.119	12.38	53.09	6.24	28.29				
5	(D) $>\text{N} - \text{NH}_2$ 2,4-Dinitrophenylhydrazine	$\text{C}_6\text{H}_6\text{O}_4\text{N}_4$	198.146	28.28	36.37	3.05	32.30				
6	(E) $-\text{N} = \text{N} -$ Azobenzene	$\text{C}_{12}\text{H}_{10}\text{N}_2$	182.228	15.37	79.09	5.53					
7	(F) $>\text{NH}$, $-\text{NH}_2$ Diphenylamine	$\text{C}_{12}\text{H}_{11}\text{N}$	169.228	8.28	85.17	6.55					
8	<i>o</i> -Arsanilic acid	$\text{C}_6\text{H}_5\text{O}_3\text{NAs}$	217.048	6.45	33.20	3.72	22.11			34.51	
9	(<i>o</i> -Aminophenylarsonic acid)	$\text{C}_6\text{H}_5\text{O}_3\text{NAs}$	140.194	39.97	51.40	8.63					
10	Hexamethylenetetramine	$\text{C}_4\text{H}_{12}\text{N}_4$	76.125	36.80	15.78	5.30			42.12		
11	Thiourea	CH_4NS	60.039	46.65	20.00	6.71	26.64				
12	Urea	CH_4ON_2	202.715	13.82	47.40	5.47		17.49 Cl	15.82		
	S-Benzylthiuronium chloride	$\text{C}_8\text{H}_{11}\text{N}_2\text{ClS}$									

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13	Acetanilide	C_8H_9ON	135-168	10-36	71-09	6-71	11-84		
14	<i>p</i> -Bromoacetanilide	C_8H_8ONBr	214-076	6-54	44-88	3-77	7-47	37-33 Br	
15	Trifluoroacetanilide	$C_8H_6ONF_3$	189-144	7-41	50-80	3-20	8-46	30-14 F	
16	Chloroacetamide	C_2H_4ONCl	93-519	14-98	25-69	4-31	17-11	37-91 Cl	18-62
17	Sulphanilamide	$C_{10}H_{10}N_2S$	172-212	16-27	41-85	4-68	18-58		
18	Melamine	$C_3H_6N_6$	126-129	66-61	28-57	4-80			
19	5-Chloro-4-hydroxy-3-methoxy- benzyl isothiourea phosphate*	$C_8H_{14}O_4N_2ClSP$	344-725	8-13	31-36	4-09	27-85	10-29 Cl	9-30
(G) Ring-N									
20	Nicotinamide	$C_6H_6ON_2$	122-130	22-94	59-01	4-95	13-10		
21	8-Hydroxyquinoline	C_8H_7ON	145-163	9-65	74-47	4-86	11-02		
22	5,7-Dibromo-8-hydroxyquinoline	$C_8H_5ONBr_2$	302-979	4-62	35-68	1-66	5-28	52-75 Br	
23	Tryptophan	$C_{11}H_{12}O_2N_2$	204-233	13-72	64-69	5-92	15-67		
24	Caffeine (1,3,7-Trimethylxanthine)	$C_8H_{10}O_2N_4$	194-200	28-85	49-48	5-19	16-48		

* W. H. Smith, *Anal. Chem.*, **30**, 149 (1958)† *Notes*

For the calculation of molecular weights and percentages, the atomic weights used are those proposed by the Commission on Atomic Weights of the IUPAC, 1957. 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, 0.29). The substances numbered 1, 2, 5, 7, 10, 12, 17, 19 and 24 were included in the list entitled "Recommended Test Substances for the Microdetermination of Carbon and Hydrogen", *Pure and Applied Chemistry*, **1**, 143 (1960).