MS310[(a)]

An Indicator Strip-Based Colorimetric Test for Nitrate Ions (NO_3^-) in Water and Soil

1.0 Scope and Application

This is a field screening method to be used to test aqueous samples and soils for the presence of nitrate {Merckoquant® 10020 (E. Merck, Frankfurter Strasse 250, D-6100 Darmstadt 1, Germany); EM Science - supplier (E. M. Science, 480 Democrat Road, Gibbstown, New Jersey 08027)}. It is also a useful method for laboratory use to determine an appropriate dilution of test water samples to be used for their analysis by more standard laboratory methods, thus eliminating costly reruns.

2.0 Summary of Method

The outer reaction zone of the test strip contains a reducing agent that reduces nitrate to nitrite. In the presence of an acid buffer, the nitrite is then converted to nitrous acid, which diazotizes an aromatic amine (sulfanilic acid). The diazotized sulfanilic acid couples with N-[naphthyl(1)]-ethylenediamine (NNEDDC) to produce a red-violet azo dye. In practice, the reaction zone of the test strip is wetted in the test sample, removed, and "blotted" to remove excess liquid. The pale yellow-colored reaction zone changes to white for negative samples and to varying shades of red-violet in the range of 5 to 500 μg/mL (ppm) nitrate. After 1 min, the nitrate content of the sample is estimated by comparing the color of the strip with a standard color chart. The intensity of the color is proportional to the nitrate concentration in the range of 5 to 500 μg/mL (ppm).

Soils are tested by extracting the soil with an equal amount of deionized water and testing the extract as described for aqueous samples.

3.0 Interferences

Merck (Rapid Test Handbook, Merck, pp. 191, 192. 1987) has examined a number of chemicals for their interference in the nitrate test.

Nitrite: As little as 0.5 μg/mL (ppm) nitrite produces a false-positive reaction. The test strip contains two reaction zones. The zone at the very end indicates both nitrate and nitrite, while the other reacts only to nitrite. A pink to red-violet coloration in the latter zone indicates the presence of nitrite, which interferes with the nitrate determination. Interference by nitrite can be eliminated by mixing 1 mL of test sample solution (pH <10) with one drop of an aqueous 10%

(a) This method was supplied by L. C. Waters, R. A. Jenkins, R. R. Smith, R. W. Counts and J. H. Stewart (Oak Ridge National Laboratory, Oak Ridge, Tennessee).

amidosulfonic acid solution. The solution is shaken several times and then tested for nitrate after 2 min as described in the procedure (section 7.0). This method allows the detection of 10 µg/mL (ppm) nitrate in the presence of 1000 µg/mL (ppm) nitrite.

Other anions: At concentrations less than 1000 µg/mL (ppm) Br-, BrO_3^-, Cl^-, ClO_3^-, ClO_4^-, CN^-, F^-, I^-, $Mo_7O_{24}^{6-}$, N_3^-, OCN^-, PO_4^{3-}, SO_4^{2-}, SeO_3^{2-}, WO_4^{2-}, acetate, ascorbate, citrate, oxalate, succinate, and tartrate do not interfere. In the presence of more than 100 µg/mL (ppm) $[Fe(CN)_6]^{4-}$, 25 µg/mL (ppm) S^{2-}, 100 µg/mL (ppm) SCN^-, 500 µg/mL (ppm) SO_3^{2-}, 250 µg/mL (ppm) $S_2O_3^{2-}$, 100 µg/mL (ppm) $S_2O_4^{2-}$, or 250 µg/mL (ppm) $S_2O_5^{2-}$, the nitrate concentration indicated is less than that actually present. Oxidizing anions turn the reaction zone light brown to orange brown. This type of interference is caused by more than 20 µg/mL (ppm) CrO_4^{2-}, 100 µg/mL (ppm) $[Fe(CN)_6]^{3-}$, 500 µg/mL (ppm) IO_3^-, 10 µg/mL (ppm) MnO_4^-, 100 µg/mL (ppm) $S_2O_8^{2-}$, or 25 µg/mL (ppm) VO_3^-. These interferences can be largely overcome by adding approximately 50 mg of hydrazine sulfate to 5 mL of test sample solution, mixing and testing according to the procedure (section 7.0). The quantity of nitrate indicated will, however, be less than is actually present.

Cations: At concentrations less than 1000 µg/mL (ppm), the following cations do not interfere: Al^{3+}, As^{3+}, Ba^{2+}, Ca^{2+}, Co^{2+}, Cr^{3+}, K^+, Li^+, Mg^{2+}, Mn^{2+}, Na^+, Ni^{2+}, Pb^{2+}, Rb^+, Sb^{3+}, Sn^{2+}, Ti^{4+}, and Zn^{2+}. The color is less intense in the presence of more than 500 µg/mL (ppm) Fe^{2+}, 50 µg/mL (ppm) Fe^{3+}, 50 µg/mL (ppm) VO^{2+}, or 500 µg/mL (ppm) Zr^{4+}. More than 50 µg/mL (ppm) Ag^+, 50 µg/mL (ppm) Hg^+, or 100 µg/mL (ppm) Hg^{2+}, interferes by turning the reaction zone grey.

pH: The test is independent of pH in the range of 1 to 12. More strongly acidic solutions should be buffered with sodium acetate and more strongly basic solutions with tartaric acid.

4.0 Apparatus and Materials

- Timer: Capable of measuring in seconds
- Pipettes: Plastic 5- or 10-mL disposables with filler bulb to measure water sample volume
- Test tubes, plastic, disposable, 13 x 100 mm (Falcon 2027 or equivalent)
- Permanent ink marking pen: For labeling test strips and tubes
- pH paper (0 to 14 range): For measuring pH of test sample
- Merckoquant® 10020 Nitrate Test Kit (E. Merck, Frankfurter Strasse 250, D-6100 Darmstadt 1, Germany E. M. Science, 480 Democrat Road, Gibbstown, New Jersey 08027):

- Instructions: Supplied with kit.

- Test strips (100): Impregnated with nitrate-specific color reagents. A separate reaction zone indicates whether nitrite is present.

- Color chart: This chart, permanently affixed to the test strip container, provides reference hues for values of 0, 10, 25, 50, 100, 250, and 500 µg/mL (ppm). A separate chart provides an indication of the amount of nitrite present.

5.0 Reagents

Except in cases of strongly acidic or basic samples, all required reagents are included in the test kit (section 4.0). In these cases, the samples should be adjusted as indicated in section 3.0.

6.0 Sample Collection, Preservation, and Handling

The test is designed to be performed in the field. Because the samples are not modified, they may be disposed of at their collection source.

7.0 Procedure (Merckoquant® 10020 Nitrate Test Instructions)

7.1 Single Samples

7.1.1 Two to three milliliters of the solution to be tested are added to a clean test tube.

7.1.2 The pH is tested with pH paper. If outside the 1 to 12 range, an adjustment should be made to that range with sodium acetate or tartaric acid (section 3.0, paragraph 5).

7.1.3 The test strip is immersed into the sample until the reaction zone is thoroughly moistened (about 1 sec). The excess liquid is removed by wiping the test strip against the test-tube rim.

7.1.4 After 1 min, the color of the reaction zone at the very end of the strip should be compared with the color chart. Coloration of the second reaction zone indicates nitrite, which must be removed from the sample to obtain an accurate measure of nitrate (section 3.0, paragraph 2). (If significant sample dilution has occurred during pH adjustment, this should be taken into account in the final estimate of the nitrate concentration in the sample.)

7.2 **Multiple Samples**: In situations where multiple samples or replicates of single samples are to be tested, an alternate procedure is suggested.

7.2.1 An appropriate number of disposable, plastic tubes and a corresponding number of test strips should be labeled using a permanent-ink marker.

7.2.2 Two to three milliliters of the solutions to be tested are added to the tubes.

7.2.3 The pH is tested and adjusted as required.

7.2.4 The strip test is performed as indicated in sections 7.1.3 and 7.1.4. The strip should be read as quickly as possible after the 1-min reaction period. (Consistent readings are obtained when made within a 30-min time period.)

8.0 Quality Control

Samples of test strips from each batch should be tested using solutions of known nitrate concentration. Batches that do not produce true colors should not be used. Likewise, test samples that do not produce true colors should be suspected of containing interferants.

9.0 Method Performance

9.1 Spiked and naturally contaminated water samples were used to evaluate this method. A stock solution of sodium nitrate, at a nominal concentration of 1000 µg/mL (ppm), was made in simulated groundwater (SGW) {deionized water to which 0.165 g/L of sodium chloride and 0.148 g/L of sodium sulfate has been added (contains 100 µg/mL [ppm] each of SO_4^{2-} and Cl^-) (Rocky Mtn. 1989)}. Nitrate in the stock solution was determined by ion chromatography to be 1050 µg/mL (ppm). Results of tests made using deionized water were essentially identical to those presented below using SGW.

Test solutions were made by diluting the stock solution to various concentrations with SGW. Triplicate 2.5-mL samples were added to 13- x 100-mm tubes (a single sample was used to test the 1:10 dilutions of contaminated water). The samples were randomized and tested using the test strips as described in section 7.2. Four or five analysts independently read the strips and recorded the concentrations with reference to the color chart. The nitrate concentrations defined by the color chart are 0, 10, 25, 50, 100, 250, and 500 µg/mL (ppm). Increments between these values were also used. To evaluate the accuracy of the method, numerical values were assigned to the results obtained from each strip as follows: a result of 50 µg/mL (ppm) was given the value 50; a result of 50 to 100 was given the value 75 and so on. The values were summed and averaged to produce the data presented in Tables 1 and 2 and Figures 1 and 2.

Among the conclusions that can be made about the strip-test assay for nitrate in spiked SGW are the following (see Table 1 and Figure 1):

1. False positives occurred at a very low frequency. Two of 52 analyses of blank samples were tested positive (see Tables 1 and 2).

2. Five ppm were consistently distinguished from zero. There were no false-negative results with samples that contained ≥5 ppm (Table 1).

3. Below 100 µg/mL (ppm), good agreement existed between the estimates made with the strip test and the results obtained by ion chromatography (Table 1 and Figure 1).

4. Above 200 µg/mL (ppm), the test appears to overestimate the nitrate concentration (Table 1 and Figure 1).

5. Overall with reference to ion chromatography, the strip-test assay gave a best-fit straight line with an $R^2 = 0.959$ and a slope of 1.188 (Figure 1).

The strip-test assay was very accurate in estimating the nitrate content of 10 contaminated water samples (see Table 2 and Figure 2). Only above 350 µg/mL (ppm) did the results deviate significantly from those determined by ion chromatography. Regression analysis of the strip-test data with reference to ion chromatography (Figure 2) gave a best-fit straight line with an $R^2 = 0.972$ and a slope of 0.898. These results clearly indicate the potential of using this test in the laboratory as a prescreen to determine the appropriate sample dilutions to be used in standard laboratory analysis.

Taken together with the manufacturer's claims concerning the lack of interferences by a wide variety of inorganic ions, this method appears to be a rapid and effective field method for screening water samples that contain nitrate at ≥5 µg/mL (ppm).

9.2 Assay sensitivity and range: Nitrate was consistently detected in samples with ≥5 µg/mL (ppm) nitrate. The working range of the test is 5 to 500 µg/mL (ppm).

10.0 Reference

Rocky Mountain Arsenal. 1989. *Rocky Mountain Arsenal Chemical Quality Assurance Plan*, section 4-7. Commerce City, Colorado 80022-2180.

Table 1. Strip Test Assay for Nitrate in Spiked Water

					Nitrate Concentration[a]					
Sample No.	Test No.	Actual[b] Conc. µg/mL (ppm)	Calculated Conc. µg/mL (ppm)	Average Conc. µg/mL (ppm)	0	0-10 (5)	10	10-25 (17.5)	25	25-50 (37.5)	50
Experiment 1											
1	27	0	0		A,B,C,D[c]						
2	34	0	0		A,B,C,D						
3	36	0	0		A,B,C,D						
4	10	0	1.2		A,C,D	B					
5	23	0	1.2		A,C,D	B					
6	5	0	0	0.4	A,B,C,D						
7	37	5.25	8.8			A	B,C,D				
8	35	5.25	5			A,B,C,D					
9	29	5.25	5	6.3		A,B,C,D					
10	13	10.5	6.2			A,C,D	B				
11	21	10.5	5			A,B,C,D					
12	24	10.5	6.2	5.8		A,C,D	B				
13	26	21	13.8				C,D	A,B			
14	20	21	17.5				C	A,D	B		
15	14	21	17.5	16.3			C	A,D	B		
16	16	31.5	19.4				C	A	B,D		
17	1	31.5	34.4						C,D	A	B
18	39	31.5	21.2	25				A,C	B.D		
19	9	52.5	40.6						D	A	B,C
20	22	52.5	40.6							A,B,D	C
21	6	52.5	34.4	38.5					C	A,B,D	
22	19	78.75	68.8								C
23	18	78.75	65.6							B	C

(a) The nitrate concentration (µg/mL) (ppm) was estimated by comparison of the test strip color with the reference color chart.
(b) Determined by ion chromatography
(c) Each test strip (one strip per water sample) was independently read by four evaluators (A,B,C,D) as indicated.

Table 1. (contd)

Sample No.	Test No.	Actual Conc. µg/mL (ppm) [b]	Calculated Conc. µg/mL (ppm)	Average Conc. µg/mL (ppm)	Nitrate Concentration[a] 50-100 (75)	100	100-250 (175)	250	250-500 (375)	500
Experiment 1										
22	19	78.75	68.8		A,B,D					
23	18	78.75	65.6		A	D				
24	7	78.75	100	78.1	A,C,D		B			
25	33	105	93.8		A	B,C,D				
26	12	105	93.8		A	B,C,D				
27	38	105	93.8	93.8	A	B,C,D				
28	31	210	193.8				A,B,D	C		
29	25	210	293.8				A	B,D		
30	11	210	231.2	239.6			A	B,C,D		
31	28	262.5	387.5				A		D	B,C
32	4	262.5	312.5					B,D	A,C	
33	32	262.5	375	358.3				B	A,D	C
34	30	420	437.5						A,D	B,C
35	3	420	437.5						A,D	B,C
36	8	420	468.8	447.9					A	B,C,D

(a) The nitrate concentration (µg/mL) (ppm) was estimated y comparison of the test strip color with the reference color chart.
(b) Determined by ion chromatography
(c) Each test strip (one strip per water sample) was independently read by four evaluators (A,B,C,D) as indicated.

Table 1. (contd)

Sample No.	Test No.	Actual Conc. µg/mL (ppm) [b]	Calculated Conc. µg/mL (ppm)	Average Conc. µg/mL (ppm)	Nitrate Concentration[a] 0	0-10	10	10-25	25	25-50	50
						(5)		(17.5)		(37.5)	
Experiment 2											
1	8	0	0		A,B,C,D						
2	2	0	0	0	A,B,C,D						
3	7	10.5	8.8			D	A,B,C				
4	12	10.5	6.2	7.5		A,B,D	C				
5	4	52.5	45					D		C	B
6	3	52.5	32.5	38.8				D	C	B	A
7	6	105	131.2								
8	11	105	84.4	107.8						D	

Sample No.	Test No.	Actual Conc. µg/mL (ppm) [b]	Calculated Conc. µg/mL (ppm)	Average Conc. µg/mL (ppm)	Nitrate Concentration[a] 50-100	100	100-250	250	250-500	500
					(75)		(175)		(375)	
Experiment 2										
5	4	52.5	45		A					
6	3	52.5	32.5	38.8						
7	6	105	131.2		D	C	A,B			
8	11	105	84.4	107.8		A,B,C				
9	1	262.5	325				D		A.B.C	
10	5	262.5	406.2	365.6					A,C,D	B

(a) The nitrate concentration (µg/mL) (ppm) was estimated by comparison of the test strip color with the reference color chart.
(b) Determined by ion chromatography
(c) Each test strip (one strip per water sample) was independently read by four evaluators (A,B,C,D) as indicated.

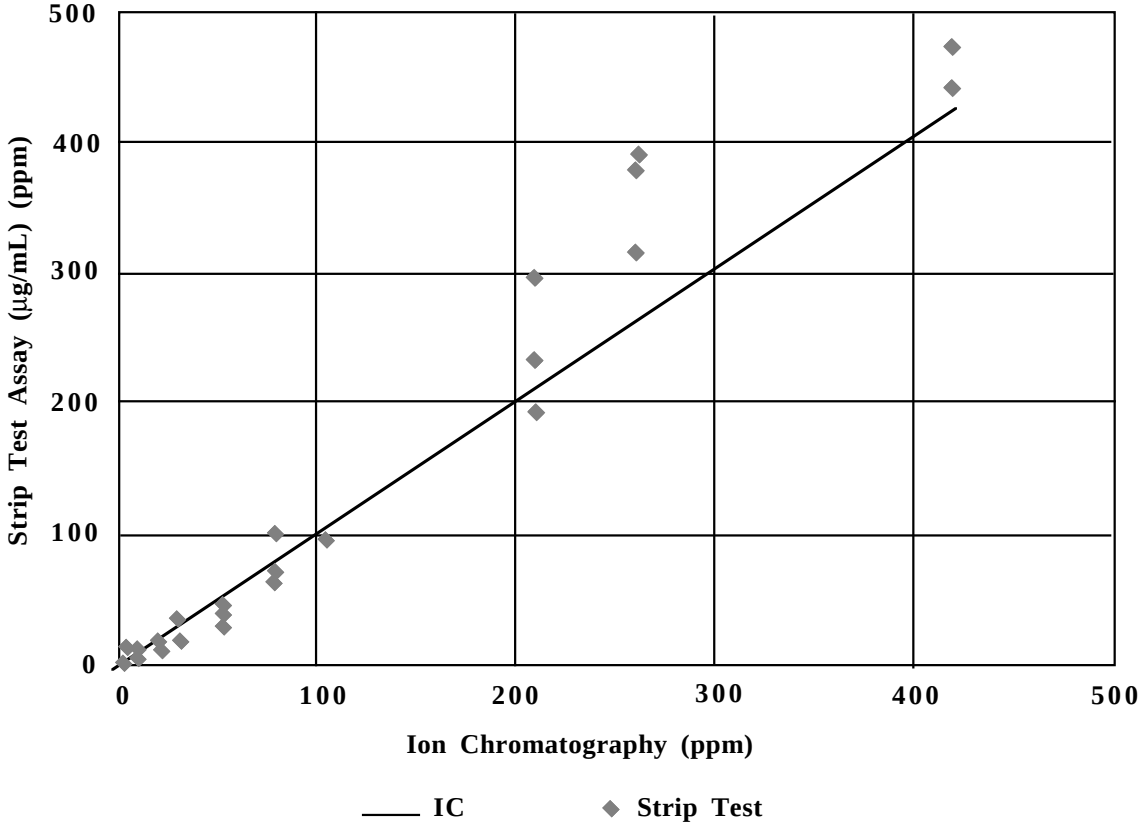

Figure 1. Strip-Test Assay for Nitrate in Spiked Water. Strip test versus ion chromatography.

Table 2. Strip Test Assay for Nitrate in Contaminated Water

Sample No.	Sample ID	Test No.	Actual Conc. (b) µg/mL (ppm)	Calculated Conc. µg/mL (ppm)	Average Conc. µg/mL (ppm)	Nitrate Concentration(a) 0	0-10 (5)	250-500 (17.5)	500 (37.5)
1	SGW36	36	0			A,B,C,D,E (c)			
2	SGW	4	0			A,B,C,D,E			
3	SGW	21	0	0		A,B,C,D,E			
4	125	10	2835	0			A		B,C,D,E
5	125	14	2835	475			A		B,C,D,E
6	125	33	2835	475	475				
7	126	3	3082	500					A,B,C,D,E
8	126	15	3082	450				A,D	B,C,E
9	126	24	3082	475	475			A	B,C,D,E
10	127	18	3545	450				A,D	B,C,E
11	127	30	3545	450				A,C	B,D,E
12	127	13	3545	475	458			A	B,C,D,E
13	128	1	2722	475				A	B,C,D,E
14	128	23	2722	475				A	B,C,D,E
15	128	9	2722	475	475			A	B,C,D,E
16	129	17	2738	475				A	B,C,D,E
17	129	29	2738	475				A	B,C,D,E
18	129	12	2738	425	458			A,C,D	B,E
28	133	19	2.24	0		A,B,C,D,E			
29	133	5	2.24	0		A,B,C,D,E			
30	133	16	2.24	0	0	A,B,C,D,E			
42	SGW	42	0	0	0	A,B,C,D,E			

(a) The nitrate concentration (µg/mL) (ppm) was estimated by comparison of the test strip color with the reference color chart.
(b) Determined by ion chromatography
(c) Each test strip (one strip per water sample) was independently read by four evaluators (A,B,C,D) as indicated.

Table 2. (contd)

Sample No.	Sample ID	Test No.	Actual Conc. µg/mL (ppm) [b]	Calculated Conc. µg/mL (ppm)	Average Conc. µg/mL (ppm)	Nitrate Concentration[a] 25-50 (37.5)	50	50-100 (7.5)	100	100-250 (175)	250	250-500 (375)	500
19	130	11	179.4	185		A				D,E	B,C		
20	130	7	179.4	170				C,D,E	B				
21	130	2	179.4	190	179					A,C,D,E	B		
22	131	28	350.9	335			A					C,D,E	B
23	plus sed(d)	6	350.9	360						A		C,D,E	B
24	okys sed(d)	8	350.9	335	343					A	C	D,E	B
25	132	31	456.9	360						A		C,D,E	B
26	132	26	456.9	385						A		C,E	B,D
27	132	35	456.9	385	379					A		C,D	B,E
31	134	22	142	155				A	D	C,E	B		
32	134	25	142	140				A	D	B,C,E			
33	134	34	142	140	145			A	D	B,C,E			
34	131	27	350.9	340				A				C,D,E	B
35	minus sed(4)	32	350.9	360						A		C,D,E	B
36	minus sed(d)	20	350.9	375	358							A,B,C,D,E	
37	125 dil1:10	37	141.7	140	140			E	D	A,B,C			
38	minus sed(4)	38	154.1	175	175				D	A,C,E	B		
39	127 dil 1:10	39	177.2	190	190					A,C,D,E	B		
40	128 dil 1:10	40	136.1	125	125			E	A,D	B,C			
41	129 dil 1:10	41	136.9	160	160				A,D	C,E	B		

(a) The nitrate concentration (µg/mL) (ppm) was estimated by comparison of the test strip color with the reference color chart.
(b) Determined by ion chromatography
(c) Each test strip (one strip per water sample) was independently read by four evaluators (A,B,C,D) as indicated.

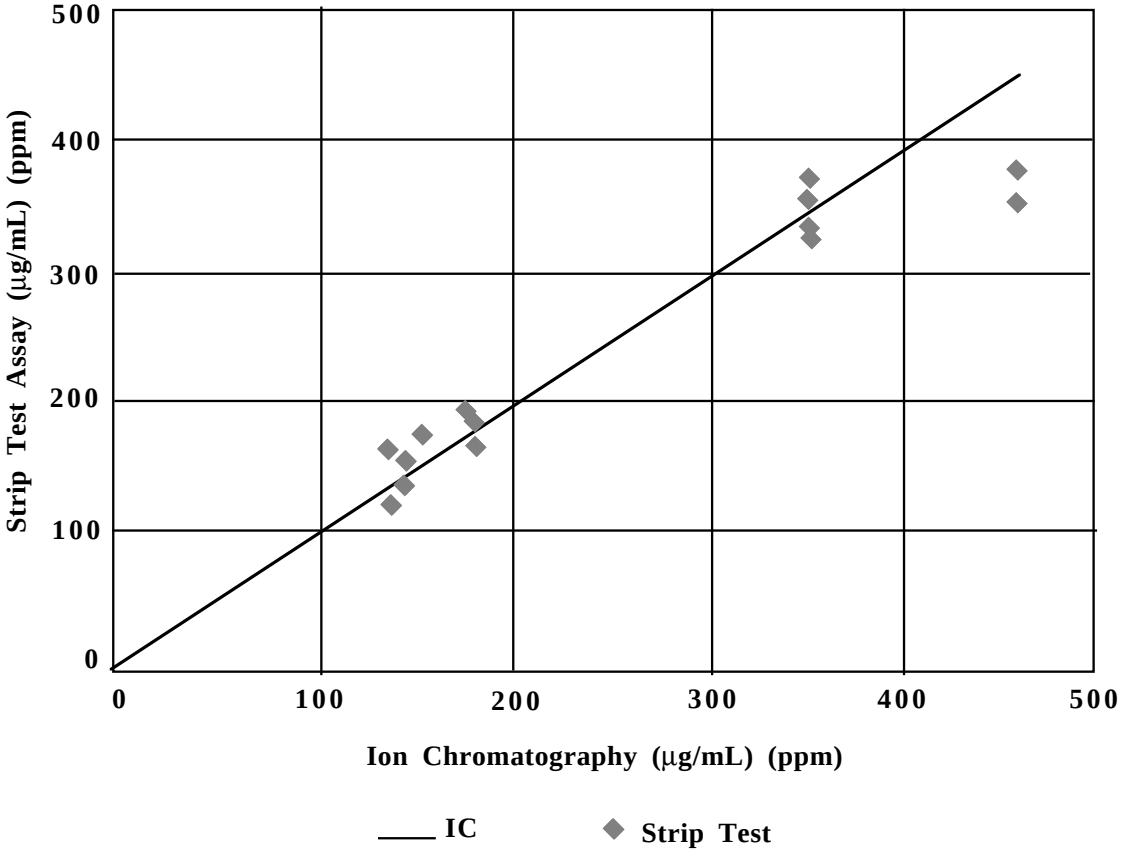

Figure 2. Strip-Test Assay for Nitrate in Contaminated Water. Strip test versus ion chromatography.