COMPARISONS BETWEEN SUGARBEET NITRATE DETERMINATION BY ION CHROMATOGRAPHY AND ION SELECTIVE ELECTRODE

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I. INTRODUCTION

Nitrate level in sugarbeet is commonly measured as an indicator of beet quality which can be related to the level of nitrogen fertilizer applied to the crop[1]. Nitrate can be rapidly and conveniently measured by ion selective electrode but such determinations may be subject to uncertainties due to unusual electrode behavior or the possible presence in beet samples of interfering substances not present in standards. For these reasons it is desirable to have an alternative method of nitrate determination to perform occasional checks on electrode measurements. Chemical nitrate determination methods are inconvenient or involve the use of very toxic reagents but with the advent of ion chromatography a reasonably convenient method of nitrate determination is now available. Chromatography has the advantage, over electrode methods, of actually separating nitrate ion from other constituents so that potentially interfering materials do not affect the measurement. Ion chromatography requires a longer analysis time (15 minutes/sample) than electrode methods and would not be suitable for routine tare laboratory determinations but is very attractive as a reference method. Accordingly a method for ion chromatographic determination of nitrate in clarified sugarbeet pol samples was developed and comparisons were made for samples from two growing areas.

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[1] McGinnis, R.A., *Beet Sugar Technology*, Third Edition, p. 7, Beet Sugar Development Foundation, Fort Collins, CO (1982).

II. RESULTS AND DISCUSSION

A. Experimental Methods

Nitrate determination by anion chromatography can be monitored by means of a conductivity detector or variable wavelength UV detector. The method described here utilizes UV detection but this evolved from a method for determination of nitrate and nitrite in chloride-containing samples. Chloride is not well-separated from nitrite and peak overlap can be observed with conductivity detection; however, UV detection adds another degree of selectivity to the technique (chloride does not absorb strongly in the UV). If only nitrate is to be measured, there is no reason that conductivity detection cannot be used.

Nitrate comparisons between ion selective electrode and ion chromatography were made using clarified one-half normal weight extracts. Clarified sugarbeet extracts were prepared by blending a mixture of 44 g sugarbeet brei, 289.5 g water, and 10 ml of aqueous aluminum sulfate solution (77.5 g $Al_2(SO_4)_3 \cdot 18H_2O$ per liter of solution). After a five minute blend time samples were filtered. This is a typical one-half normal weight clarified solution such as those prepared in the tare laboratory for polarimetric sucrose determination.

Nitrate by ion selective electrode was measured directly on the filtrate using an Orion Model 93-07 nitrate electrode and Orion Model 90-02 double-junction reference electrode. Standard solutions containing 10 and 100 ppm nitrate were used for electrode standardization. All standards contained 2 ml of 2M $(NH_4)_2SO_4$ per 100 ml of standard solution as an ionic strength adjustor.

Ion chromatographic nitrate determinations were made by diluting clarified filtrates tenfold with water and injecting 20 μl on a Dionex Ionpac AS4A anion exchange column using 14 mM sodium borate eluent at a flow rate of 1.5 ml/minute. Peaks were detected using a Dionex variable wavelength UV detector at a wavelength of 220 nm and integrated using a Hewlett-Packard 3396 electronic integrator.

Precision and recovery tests of the ion chromatographic method were carried out starting from sugarbeet brei, rather than the tare laboratory clarified solutions used for method comparisons. Precision of the ion chromatographic determination alone was determined by preparing a single brei homogenate (20.00 g brei plus 136.1 g water; blended for five minutes) with a concentration of 0.12812 g beet/g homogenate (equivalent to one-half normal weight). The homogenate was filtered (Whatman grade 201 filter paper), diluted by a factor of ten, and passed through a 0.45 μ membrane filter before replicate injection.

Data on the precision of the entire sugarbeet nitrate determination technique as well as the effect of clarifying agent addition was obtained by preparing replicate homogenates from the same sugarbeet sample. Each homogenate sample contained 20.00 g beet in 151.1 g homogenate. Portions (60.44 g) of each homogenate were then blended an additional three minutes with either 2 ml of water or 2 ml of aluminum sulfate solution (concentration given above). The resulting suspensions containing 0.12812 g beet/g homogenate (one-half normal weight) were filtered (Whatman grade 201 filter paper) diluted ten-fold with water, passed through a 0.45 μ membrane filter, and analyzed by ion chromatography.

Nitrate addition and recovery tests were carried out by preparing replicate blended homogenates containing 40 g beet/300.20 g suspension. Background nitrate determination was carried out by blending 50.03 g of each original suspension with 2 ml water for 2 minutes (final concentration: 0.12812 g beet/g suspension). A spiked homogenate was prepared by blending 150.09 g of each original suspension with 1.00 ml of a solution containing 7.69 mg NO_3^-/ml. Clarified and unclarified final homogenates were prepared by stirring 60.44 g of spiked homogenate with either 2 ml of aluminum sulfate solution of 2 ml of water. This gives a final added nitrate concentration equivalent to 384 ppm in sugarbeet. All final homogenates (background, spiked and clarified, spiked and unclarified) were diluted by a factor of ten with water, passed through a 0.45 μ membrane filter, and the nitrate level determined by ion chromatography.

B. Results

Replicate injections of a single unclarified one-half normal weight beet homogenate showed the ion chromatographic nitrate determination to be very precise. Nine injections gave a mean homogenate nitrate content of 5.11 ± 0.06 ppm (mg/liter). Individual values converted to ppm in sugarbeet (mg NO_3^-/kg beet) gave a mean value of 393 ± 4 ppm (1% relative standard deviation). This relative standard deviation is lower than what would be expected with ion selective electrodes under the best of conditions[2,3].

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[2] Harris, D.C., Quantitative Chemical Analysis, Third Edition, W. H. Freeman & Company, New York, 1991, p. 377.

[3] Orion Research Incorporated, manual for Model 93-07 nitrate electrode, p. 23

Results for replicate beet samples carried through the entire procedure are given in Table 1.

TABLE 1

Replicate Ion Chromatographic Nitrate Results (with and without clarification)

Sample	Nitrate (ppm/beet)	
	Unclarified	Clarified
1	254	232
2	265	239
3	242	225
4	246	228
Mean	**252 ± 10**	**231 ± 6**

Note that for quadruplicate samples, carried from weighing of the beet sample through chromatographic analysis, the relative standard deviation is 4% (unclarified) or 2.6% (clarified). It appears from this test that aluminum sulfate clarification does cause some loss of free nitrate in solution. Note that the mean nitrate value decreases from 252 ppm to 231 ppm which is an approximate 8% decrease in nitrate level. Even though this decrease is statistically significant at the 99% level (t test) it would also be expected to apply to ion selective electrode determinations on clarified filtrates and would thus have no effect on a method comparison. The 21 ppm decrease for nitrate in beet arises from a 2.7 ppm decrease in clarified filtrate nitrate levels (from 32.8 to 30.0 ppm) and, at a typical electrode calibration (100 ppm nitrate in solution set at 100 mV), this change would produce only a 2 mV difference in readings. Such a difference would probably be

difficult to detect under normal nitrate electrode operating conditions.

A second set of replicate beet samples was analyzed for "background" or initial nitrate level, then nitrate equivalent to 384 ppm/beet was added. Ion chromatographic nitrate determination was again made with and without aluminum sulfate clarification. Results are given in Table 2.

TABLE 2

Nitrate Addition Tests

Sample	Nitrate (ppm/beet)		
	Initial Level	Spiked, Unclarified	Spiked, Clarified
1	262	620	593
2	262	637	585
3	266	636	616
4	265	656	608
Mean	**264 ± 2**	**637 ± 15**	**600 ± 14**
Rel. Std. Dev.	**0.8%**	**2.4%**	**2.3%**

Note that for this set of samples precision was good, with relative standard deviations all less than 2.5%. The difference between the initial and spiked, unclarified sample means is 373 ppm or 97% of the added nitrate. If the value for added nitrate (384 ppm/beet) is added to each initial nitrate level in Table 2, the mean of values obtained (648 ppm) is not significantly different (t test) than the measured mean for spiked, unclarified samples. Again for this sample set, the addition of aluminum sulfate lowers the measured nitrate level by a small (5.8%) but statistically significant amount. Due to the low magnitude of this effect it was not studied further.

Comparisons of ion chromatographic nitrate measurements with nitrate electrode values were made using tare laboratory clarified filtrates. However, all nitrate electrode values given in this report were measured in the Research Laboratory in parallel with ion chromatographic determination to eliminate any possible differences due to sample changes on storage or freezing. Two typical chromatograms of high and low nitrate samples are shown in Figure 1. Note the difference in the nitrate peak height relative to the other three anions originating in sugarbeet (chloride, phosphate, and oxalate). The large sulfate peak, from aluminum sulfate clarification, does not interfere with nitrate determination.

The first set of sample comparisons carried out were on beets from Amalgamated Sugar's Magic Valley growing area in south central Idaho. Values obtained by both ion selective electrode and ion chromatography are given in Table 3 and plotted against each other in Figure 2. For these samples the correlation coefficient between nitrate by electrode and chromatography is 0.9875 and a paired t comparison shows a mean difference of only 13 ppm (ion chromatography lower) that is <u>not</u> statistically significant.

Although several pairs of values vary by as much as 100 ppm the correlation is good considering the accuracy necessary in a routine electrode nitrate determination. Basically all that is necessary is a fast approximate method that distinguishes good and poorer quality sugarbeet samples. The highest differences obtained are at high nitrate levels where, because nitrate levels increase exponentially with electrode millivolt readings, small changes in

TABLE 3

Nitrate Value Comparisons
Magic Valley Sugarbeet Samples - 1990

Sample	Nitrate (ppm/beet)	
	Nitrate Electrode	Ion Chromatography
1	170	160
2	130	118
3	140	108
4	310	280
5	460	340
6	320	301
7	110	71
8	560	528
9	350	338
10	130	116
11	130	134
12	770	815
13	70	58
14	160	152
15	110	73
16	830	946

ion selective electrode measurements affect the calculated nitrate level strongly.

A second set of 19 comparison samples from the Treasure Valley growing area (southwest Idaho) gave results with lower individual differences between electrode values and ion chromatography (maximum difference was 70 ppm) and a mean difference that was low (17 ppm), but statistically significant at the 99% level. Again these slightly lower values obtained by ion chromatography are not very important considering what is expected from the ion-selective electrode measurement and the fact that the difference is only 6% of the mean nitrate (290 ppm) for the set. Figure 3 shows individual values for this sample set in the form of a linear regression plot (correlation coefficient: 0.9956).

During the 1991 harvest, sets of 50 samples from each of the two growing areas were analyzed by both methods. Results from the Magic Valley set are shown in Figure 4. These samples with an average ion chromatographic nitrate content of 460 ppm and a range of 60 to 1180 ppm show the poorest agreement in individual values of the study with a mean difference of 92 ppm (ion chromatography higher) that is statistically significant at the 99% level. As shown in Figure 4, however, the correlation is still good (coefficient: 0.9806).

Results on a Treasure Valley sample set from 1991 are shown in Figure 5. Here again a statistically significant difference was obtained but with ion chromatography only high by an average of 41 ppm (range 180 to 1300 ppm). Correlation is worse for this set of samples (r=0.9066) but still the two methods give reasonably good agreement considering the requirements of the test.

III. CONCLUSIONS

Conclusions reached in this study are:

(1) Nitrate levels in clarified half normal weight sugarbeet sample solutions can be measured quite precisely by ion chromatography. Some evidence indicates that clarification with aluminum sulfate lowers measured nitrate levels by 5-8%. This effect was considered to be unimportant with respect to ion selective electrode-ion chromatography comparisons and was not studied further.

(2) Ion selective electrode nitrate values correlate well with ion chromatographic values, taking into account the requirements and expectations for a routine electrode nitrate

method. Correlation coefficients for all sample series were above 0.907 with three of the four series above 0.98.

(3) In some cases statistically significant differences between the two methods were obtained. These differences were not always in the same direction and are thought to be related to nitrate electrode calibration changes. In spite of these absolute differences, the correlation of nitrate by the two methods over a fairly high range (100 to 1000 ppm/beet) indicates that ion selective electrode nitrate measurement gives a rapid, reasonably reliable indication of sugarbeet quality.

FIGURE 1

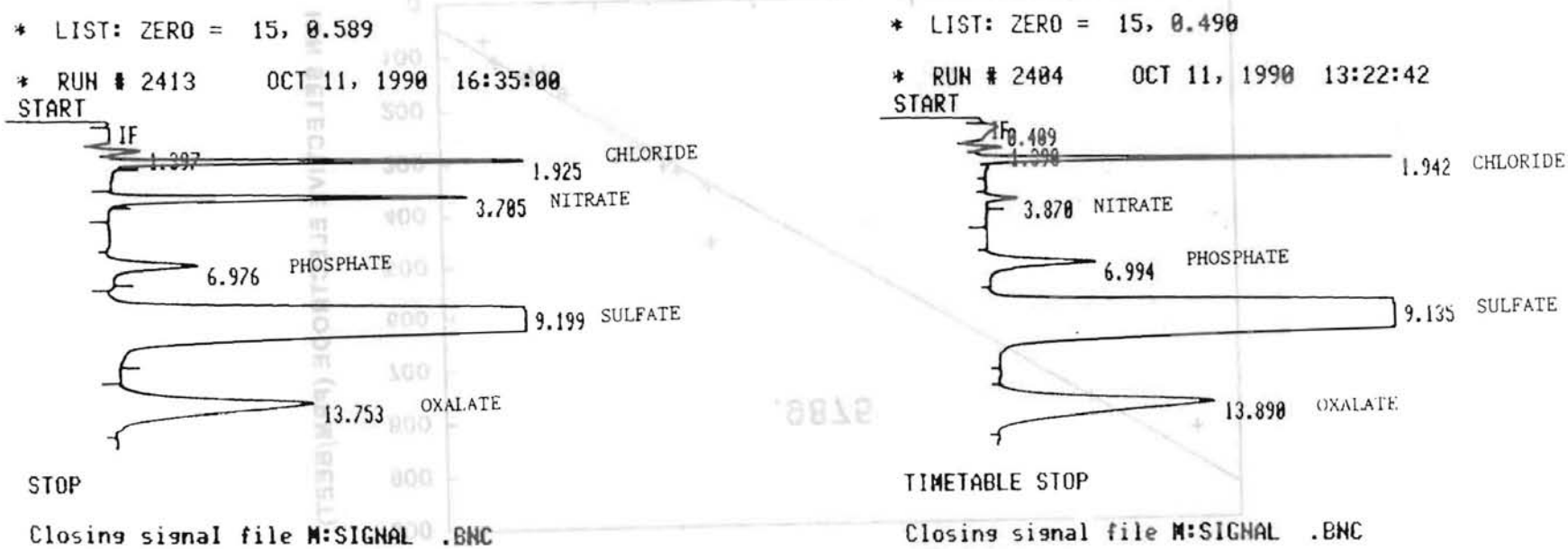
* LIST: ZERO = 15, 0.589
* RUN # 2413 OCT 11, 1990 16:35:00
START
IF
1.397
1.925 CHLORIDE
3.785 NITRATE
6.976 PHOSPHATE
9.199 SULFATE
13.753 OXALATE
STOP
Closing signal file M:SIGNAL .BNC
* LIST: ZERO = 15, 0.490
* RUN # 2404 OCT 11, 1990 13:22:42
START
IF
0.409
1.942 CHLORIDE
3.870 NITRATE
6.994 PHOSPHATE
9.135 SULFATE
13.890 OXALATE
TIMETABLE STOP
Closing signal file M:SIGNAL .BNC

FIGURE 2

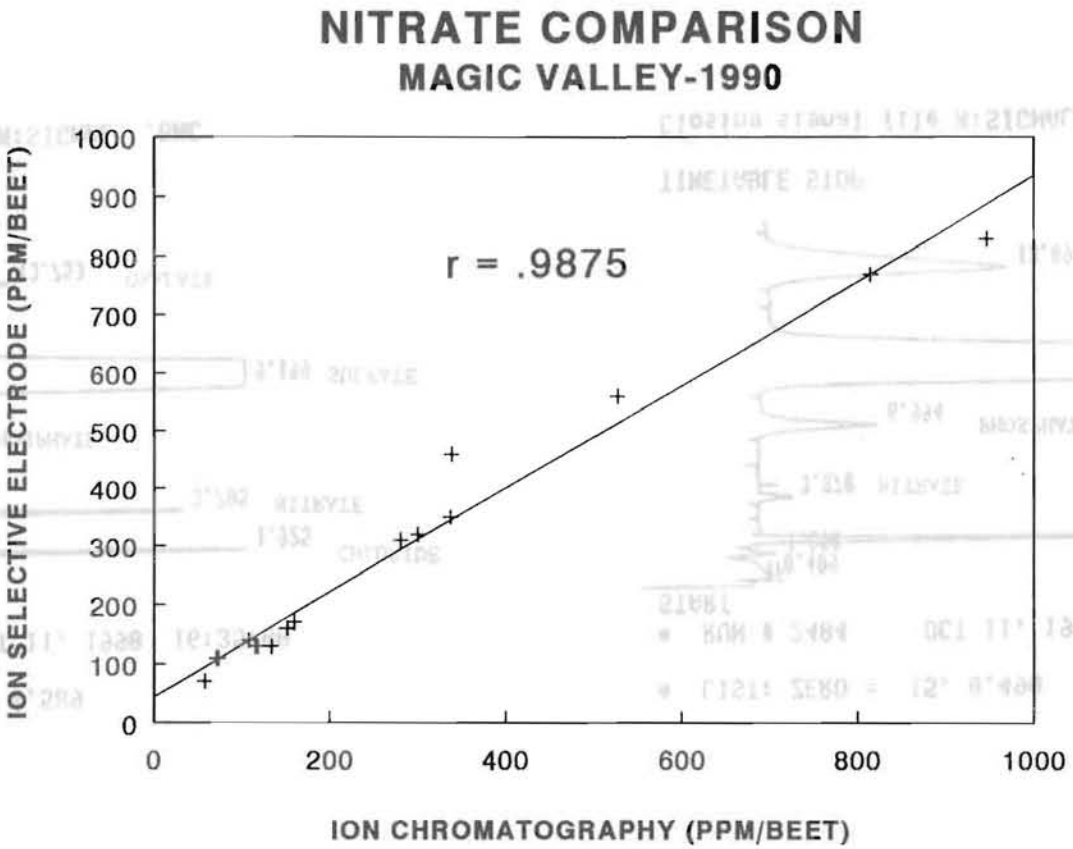
NITRATE COMPARISON
MAGIC VALLEY-1990
r = .9875
ION SELECTIVE ELECTRODE (PPM/BEET)
ION CHROMATOGRAPHY (PPM/BEET)
0
100
200
300
400
500
600
700
800
900
1000
0
200
400
600
800
1000

FIGURE 3

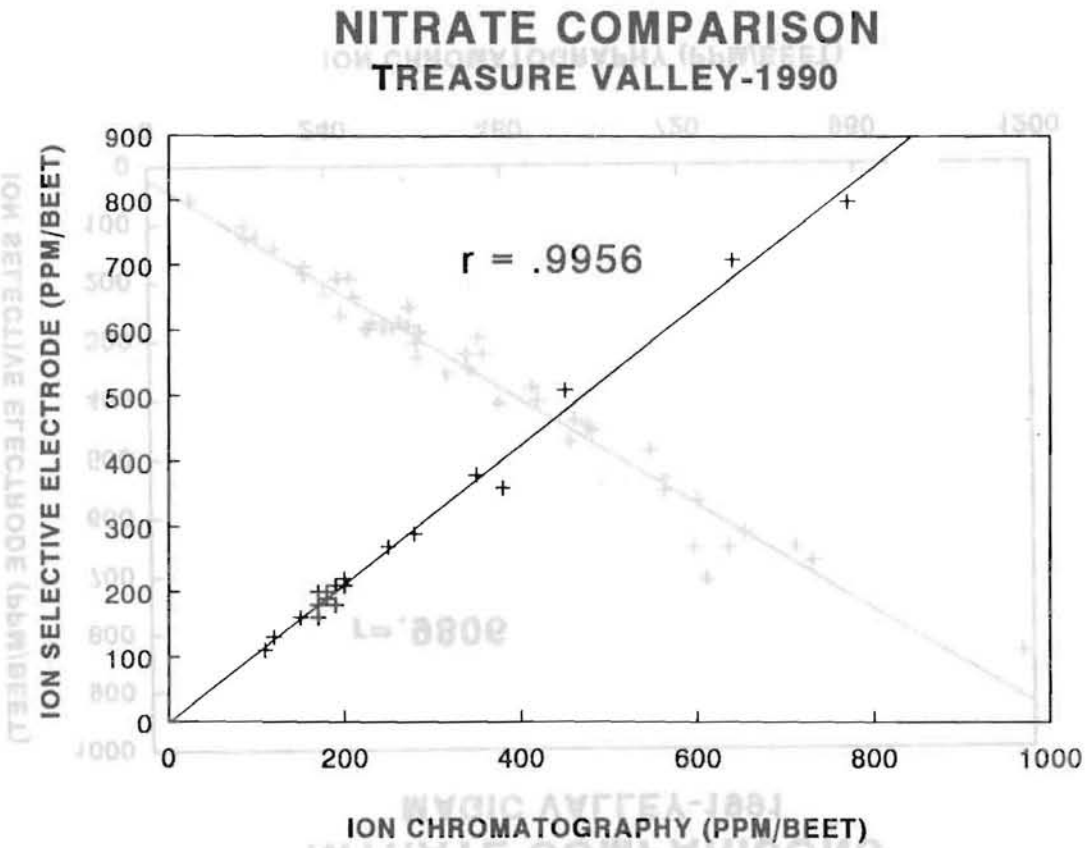
NITRATE COMPARISON
TREASURE VALLEY-1990
r = .9956
ION SELECTIVE ELECTRODE (PPM/BEET)
ION CHROMATOGRAPHY (PPM/BEET)
0
100
200
300
400
500
600
700
800
900
0
200
400
600
800
1000

FIGURE 4

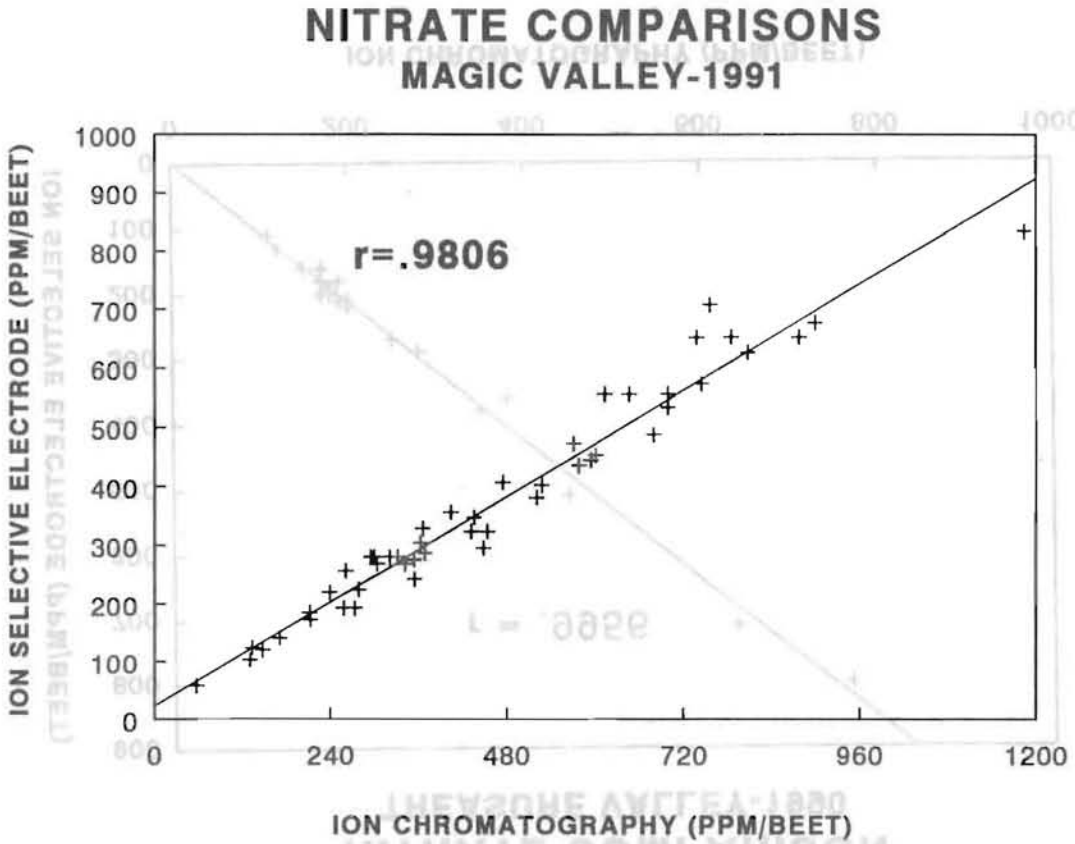
NITRATE COMPARISONS
MAGIC VALLEY-1991
r=.9806
ION SELECTIVE ELECTRODE (PPM/BEET)
ION CHROMATOGRAPHY (PPM/BEET)
0
100
200
300
400
500
600
700
800
900
1000
0
240
480
720
960
1200

FIGURE 5

NITRATE COMPARISONS
TREASURE VALLEY-1990
r=.9066
ION SELECTIVE ELECTRODE (PPM/BEET)
ION CHROMATOGRAPHY (PPM/BEET)
0
260
520
780
1040
1300