ANALYSIS OF SUCROSE, RAFFINOSE, BETAINE, AND INVERT IN MOLASSES AND IN THE EXTRACT AND RAFFINATE OBTAINED FROM ION EXCLUSION

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ABSTRACT

In conventional analysis of factory process streams, sucrose concentration and purity is determined by refractometry and polarimetry. Brix measurements by refractometry do not give the true weight percent dissolved solids except for pure sucrose solutions. Polarimetric measurements are affected by all optically active compounds, including raffinose, invert, and amino acids. The error introduced is normally accepted since the error is relatively small with high purity streams. For very accurate sugar accounting, (especially with low purity streams such as those involved in recovery of sucrose from beet molasses via ion exclusion) high performance liquid chromatography (HPLC) offers an improved technique. A procedure for determining the concentrations of sucrose, raffinose, betaine, and invert has been developed using HPLC. The procedure also provides semi-quantitative information on the (salt + organic acids) component. The operation is quite simple. The only sample preparation is quantitative dilution, followed by filtration through a 0.22 or 0.45 micron membrane filter. The data manipulation and integration of the chromatographic data were found to be a crucial part of the analytical procedure, which is often neglected. Two different modes of operation are described. One is useful for ordinary daily analysis. The other mode applies a very simple retention time correction to the numerical results, resulting in greater accuracy. The precision of the analysis for sucrose in an extract sample from our pilot plant [as measured by the relative standard deviation of replicate sampling, preparation, and analysis of the same sample] is 2.1% for the uncorrected procedure and 0.6% for the results corrected for retention time variation.

Component recoveries in artificial syrups ranged from 98% to 101%. Analyses of a spiked pilot plant sample gave recoveries of 99% to 101%. Surprisingly, the HPLC analytical procedure can give an estimate of syrup purity and %RDS without any other measurement. With the use of some simple precautions to avoid microbiological fouling, column lifetime has been excellent. Analysis of sugar samples by HPLC is a very valuable tool for the improved understanding, process analysis, and control of the ion exclusion process in the beet sugar factory.

INTRODUCTION

Although the ion exclusion process was invented in the 1950's, it saw limited commercial application for over three decades (1-7). Ion exclusion is now becoming an important process in the beet sugar industry for increasing the sucrose extraction in the factory (7-20). Ion exclusion (Fig. 1) is a process which separates molasses into two diluted fractions, a high purity fraction from which additional sucrose can be recovered, and a low-purity, high-ash secondary molasses (7-13,15-17,19,20). Analysis and troubleshooting of this process, especially doing a mass balance, requires accurate analytical data on the streams involved in the process. Numerous workers have observed the difficulties in obtaining reliable analytical data on low-purity process streams by conventional procedures (21-28). Polarimetric methods are extremely precise and repeatable (29), but respond to all optically active species (21,24,27,28), which leads to difficulty in obtaining accurate results on low-purity streams. In addition, low-purity streams are usually high in color, requiring the use of clarifying agents, which could affect the results and which often pose environmental concerns (30,31).

Derivatization of sugars followed by gas chromatography avoids many of these problems, but depends on the quantitative reaction with the derivatizing agent. It is more time-consuming, and requires a skilled analyst. High performance liquid chromatography (HPLC) offers the potential of specific, accurate, precise, and relatively rapid analysis of molasses and other low-purity streams. It can be done by personnel with little training and can be automated. Much work has been published on the HPLC analysis of sucrose and other components of molasses using reverse-phase (21-23,32,33), amino-bonded (23,37), and ion exchange resin based columns (21,23-28,38-40). All of these procedures require a sample cleanup step; some cleanups are more complicated than others.

This paper focuses on the development of a simple, reliable, HPLC procedure useful for analysis of low-purity syrups, especially the analysis of the molasses feed and the extract and raffinate product streams of a beet molasses ion exclusion process. The column used is based on an ion exchange resin in the potassium form, which results in three significant advantages. First, the cleanup procedure is quite simple (although extremely important). Second, the mechanism involved in the analysis is the same as in the ion exclusion process itself, which helps in the understanding of the process by the factory operating personnel. Finally, when analyzing these high potassium streams, the column lifetime is maximized due to the chemical nature of the column packing. The procedure is examined critically with regard to linearity, precision, recovery, data handling, and the operator time required.

Some Methods of Sucrose Analysis

Many different methods for the analysis of sucrose have been used. Some general characteristics of four common techniques are given in Table 1. Each technique has its unique advantages and

disadvantages. The potential advantages of high performance liquid chromatography are as proposed in Table 2. The operating principles of an HPLC system have been well described previously (21,24,32).

Modern HPLC instruments separate the components in the sample, then quantitate the amounts present, and print out the results by comparison to standards previously separated. A chromatogram obtained from a fairly typical beet molasses sample shows the separation achieved (Fig. 2). Quantitative results on the concentration of sucrose and other components of the molasses are produced in less than 20 minutes. But, how good are the results? Very few workers have commented on some of the pitfalls involved in leaving the quantitation to a "black box" containing proprietary peak integration software (22,39). This paper will attempt to answer some of the questions raised in Table 1 regarding the HPLC analysis of sucrose in factory streams.

METHODS AND MATERIALS

Materials

Chemicals for use as standards were obtained from either Sigma Chemical or Aldrich Chemical. In all cases, reagent grade materials were used. Compounds were stored in a dessicator prior to use. Eluent (Table 3) was made up using filtered, deionized water from a Millipore Milli-Q(R) water purification system.

Procedures

Eluent was degassed using vacuum and heat prior to use and kept at 65 degrees C to keep dissolved gases to a minimum. Samples were diluted with the eluent mixture, then filtered immediately before

analysis using 0.22 or 0.45 micron Gelman Acrodisc(R) syringe filters. Disposable 1cc or 3cc plastic syringes from Becton-Dickinson were used for sample uptake and for the filtration operation. Using disposable syringes and filters eliminated the possibility of cross-contamination prior to the injection system. The equipment comprising the HPLC system is shown in Table 4. A guard column packed with the same resin as the analytical column was installed in the line ahead of the main column. Operating conditions for the HPLC are detailed in Table 5. The Waters R-410 Refractive Index Detector was held at an internal oven temperature of 38 degrees C. Sample preparation is outlined in Table 6. How the chromatographic data is handled is extremely important. The complete integration program file is shown in Figure 3.

Carbohydrates can cause microbiological fouling of the HPLC system. Several precautions minimized the problem. First, at the end of each set of sample runs, two vials containing denatured ethanol were inserted into the sampling system. The first is treated as a sample and injected onto the analytical column. The second vial is programmed as an injection loop flush. This alcohol is left in the injection loop until analyzing the next set of samples. This kept the injection loop clean. Once a week, the HPLC tubing from the inlet to the refractive index detector was disconnected and the detector was flushed with 7N nitric acid. This removed any fouling of the detector, which would cause losses in sensitivity, calibration, and analytical precision. The final precaution taken to avoid microbiological fouling was to keep the eluent flowing. When finished with the HPLC for the day, the pump was left running at 0.1 ml/min. Using these precautions, no significant fouling or deterioration of the column has been observed. As this is a methods paper, further discussion of some elements of the analysis will be found in the "Results and Discussion" section.

RESULTS AND DISCUSSION

Linearity

The areas reported by the integrator versus the amount for each component in the standards is plotted in Figures 4-7. In each case, what appears to be a single data point is actually 3 to 5 separate injections of that particular standard. As can be seen, the repeatability is excellent. The linearity is also extremely good for raffinose and for betaine. However, the component of greatest interest, sucrose, seems to have a non-linear response with regard to concentration (Fig. 7). And for the glucose and fructose areas, the response is linear, except for the highest concentration standard, where the integrator reported a much higher than expected area for the glucose and a lower than expected area for the fructose (Fig. 6).

Careful examination of the chromatograms revealed that integration parameters were causing the non-linearities. The non-linearity was not inherent in the chromatographic process itself. In Figure 8, the entire sucrose peak, including the late tail, is being included. In Figure 9, the sucrose is at a lower concentration. As shown by the peak stop mark, some of the area is not being included in the area the integrator is using to calculate the concentration. By forcing the integrator to include this area (using the solvent peak mode of this integrator - integration function # 3), much improved results were obtained (Fig. 10).

A related problem existed with the glucose and fructose results. The chromatogram of the highest concentration standard (Fig. 11) revealed that at the highest concentration, the glucose peak was treated as a solvent peak, which means that the integrator performs "peak skimming," treating the fructose peak as a small peak riding on the tail of the glucose peak, even though it is of

similar size to the glucose peak. As a result, area is transferred from the fructose peak to the glucose peak, causing the results seen in Figure 6. Once again, changing integration conditions to instruct the integrator to not perform this skimming (integration function # 4 - disable automatic tangent peak skimming) resolves the problem (Figs. 12 and 13).

Originally, calibration was not attempted for the peak eluting at the void volume. Since this peak will contain a mixture of salts, strong organic acids, higher oligosaccharides, and any high molecular weight material not rejected by the membrane filter, no single response factor will be correct. However, later data indicated it still might carry some meaning. So, a calibration was done using K2SO4 as the standard component. If the mixture of components has the same response factor as K2SO4, the results will represent the concentration of this mixed component. The more the response factors differ from that of the K2SO4, the less accurate the results. The response of the (detector + integrator) system was not linear for K2SO4. Since the eluent used in this work has a base level of a salt component in it, a sample with no salt in it results in a negative peak (Fig. 9). With the default parameters of most chromatographic integrators, a negative peak is treated as a drop in the baseline, rather than representing a component eluting at that time. Note the large area reported for raffinose in Figure 9. This large area was due to a chromatographic baseline being set at the most negative point of the negative peak. For work subsequent to the time of Figure 9, integration function # 8 was used to invert negative peaks. This made the calibration curve for the "salts" linear, however it does not pass through the origin. Therefore, for this component, a multi-level calibration is necessary (and is recommended for the other components as well, for best results). Hereinafter, this pseudo-component will be referred to as "salts." The relative response factors found in this work were close enough to each other to conclude that some value exists in calibrating the analysis for the "salt" component (Table 7).

Recovery

Artificial Samples:

In order to assess the quantitative validity of the procedure, known solutions of the compounds being analyzed were subjected to the analysis procedure. The recoveries found of the known amounts in the artificial solutions varied from 98 to 100% (Table 8, first half). During the period between December 18, 1990 and January 3, 1991, no samples were analyzed. The power to all equipment was turned off. Upon return to the laboratory on January 3, the power was restored, the oven and detector were heated up, the pump was started, and the calibration program was typed into the integrator. The artificial syrup which had been stored in the laboratory refrigerator was then analyzed, without re-calibrating the instrument. The results were surprisingly good. The recoveries ranged from 99 to 101% (Table 8, second half).

Spiked Samples:

Real samples can have unknowns which interfere with the analysis and make it less quantitative than would be concluded from pure mixtures. A recovery study was performed with one of the less pure extract samples obtained from Dow Chemical USA's 45 liter ion exclusion pilot plant. The sample was analyzed ten times and the concentrations of each component computed as an average. A portion of the extract sample was then spiked with known quantities of each component by a colleague. The spiked sample was analyzed ten times, and the average concentrations calculated for each component. The averaged recovery data ranges from 98% to 108% (Table 9). The highest recoveries, 108% and 106% were obtained on fructose and glucose, respectively. This excess may also be due to the specifics of the integration software. The integrator was set to ignore peaks below a specified peak area. If glucose and fructose were present in the unspiked sample (Fig. 15), but below the amount required to exceed this threshold, the integrator would not report a concentration. However, in the spiked sample, the area measured by the integrator

was large enough to be reported, and the area reported includes both the area from the glucose spiked into the sample plus the area due to the glucose originally present in the unspiked extract sample. The same phenomenon would affect the fructose recovery, making it also higher than 100%.

Precision

Many workers report a precision as measured by replicate injection of the same sample into a chromatograph, ignoring the fact that difficult or even simple sample preparations can cause errors in the reported results. The repeatability of the analysis procedure was determined using the following protocol. Since sample carryover can cause the results of one analysis to be affected by the composition of the previous sample analyzed, analysis of the test sample, a pilot plant extract sample, was alternated with analysis of a raffinate sample from the pilot plant. The composition of the raffinate (high salt, low sugar, low purity) is dramatically different from the extract (low salt, high sugar, high purity). The autosampler was programmed to use the even-numbered vials as a sample flush, just as when analyzing samples. A similar recommendation has been published previously (26). When analyzing samples, the flush is the sample next to be analyzed (in this case, raffinate or extract). The vial sequence was thus -

1: extract (precision data point #1)
2: water (sample injection loop flush)
3: raffinate (to affect results, if it would)
4: extract (flush)
5: extract (precision data point #2)
6: water (flush)
7: raffinate (repeat of #3)
etc.

Five samples were analyzed [starting from pipetting the original sample for dilution] on each of two separate days. This was repeated by a different analyst. No recalibration was done during the one week timeframe of the precision experiment. The twenty individual results on each of the four components are presented in Table 10. Measurement precision, as represented by relative standard deviations were as follows: sucrose, 2.1%; raffinose, 3.5%; "salts", 5.5%; and betaine, 3.0% (Table 11). Although the precision was reasonable, a significant correlation was noticed between the day of analysis and the results. Further examination uncovered the reason for the variation. The sucrose retention time on each chromatogram is also indicated in Table 10. As can be seen, the concentrations reported by the integrator was higher when the retention time was higher. The retention times of the other components were higher in the same proportion as the sucrose retention time. It was logical to conclude that the flow rate was slightly slower in these runs than when the original calibration was done. At a slower flow rate, the peak stays in the detector longer, leading to a higher measured area and therefore, a higher reported concentration. Correcting the raw concentration data by dividing by the sucrose retention time and multiplying by the average sucrose retention time for the calibration runs gave the data indicated in the second column under each component identification in Table 10. The relative standard deviations obtained when applying this simple linear retention time correction were more satisfactory (Table 11). In particular, the relative standard deviation of the sucrose analysis was found to be 0.6%.

Application of the Technique to Various Sample Types

Ion Exclusion Process Samples

Close monitoring of the ion exclusion process requires a mass balance on solids and a mass balance on sucrose, and

advantageously, a mass balance on other components. The HPLC has been valuable in determining the operating performance of a pilot plant, including sucrose recovery and extract purity. The technique is faster than any other sucrose-specific technique. The sample preparation is simple and good results have been obtained even by pilot plant operators. The HPLC has also analyzed samples from applications research pulse tests of ion exclusion resins (Figs. 16-18). Early in the experiment, the material eluting is mostly comprised of salts and organic acids (Fig. 16). Later, the samples contain much sucrose as well as salt, raffinose, and betaine (Fig. 17). Near the end of the test, the sucrose is contaminated mainly by betaine (Fig. 18). Of course, a pseudo-moving bed separator is more efficient and produces a cleaner product than a simple one-pass pulsed column. If the extract sample from an ion exclusion process is found to be low in purity, HPLC will be able to identify what the impurities are, which helps to indicate what will improve the operation. A simple pulse test, although inefficient from a process standpoint, allows us to isolate resin differences without the complicating effects of process variables. The analytical data from chromatograms similar to Figures 16-18 are used to develop elution profiles of the components of molasses on commercial and experimental resins (Fig. 19).

The Amalgamated Sugar Company provided samples of the feed and the concentrated extract and raffinate products from a continuous pseudo-moving bed pilot plant ion exclusion trial. Our analytical results and data obtained by the more conventional techniques, as supplied by Amalgamated, are shown in Table 12. The HPLC results are much closer to the results obtained by derivatizing the sugars and analyzing by gas chromatography than the results obtained from a direct pol measurement.

Beet Molasses

Beet molasses samples obtained from factories in various growing regions of the country were obtained. They were not necessarily from the same part of the slicing season, or even from the same year and are not meant to be representative of regional differences in molasses composition. Our results of analyzing these samples are shown in Table 13.

Cane Molasses

Two different cane molasses samples were diluted, filtered, and analyzed on the HPLC system. The HPLC chromatogram obtained on one of the samples is shown in Figure 20. The invert (the peaks at 11.73 minutes and 12.78 minutes) is much more prominent than in beet molasses. The fructose is present at a higher concentration than the glucose. There is an unknown peak eluting just prior to the glucose peak which interferes somewhat with the quantitation of the glucose peak. The filtration of the sample prior to injection into the HPLC was more difficult, presumably due to the higher level of suspended solids present in cane molasses. Each separate injection vial required the use of a new disposable filter. In analyzing beet molasses, at least four replicate vials could be made up using the same syringe filter.

Crystalline Sucrose

Recent work at SPRI has demonstrated the ability to distinguish between crystalline sucrose derived from cane and from beets using ion chromatography (41). By setting the sensitivity of the detector at 264 (vs the 16 used in the sucrose assay work) and by using a higher Brix sample for injection, some impurity peaks were found in HPLC chromatograms of crystalline sucrose purchased from a local retail food distribution chain. A sample of "cane sugar" had recognizable peaks at the retention time of glucose and fructose. The impurity eluting just prior to the sucrose peak,

which is believed to be due to kestose, has a slightly lower retention time than the retention time of raffinose in beet molasses. A sample of crystalline beet sugar had a small peak present at the retention time of betaine. Glucose and fructose were not detectable under these conditions. The retention time of the impurity eluting before the sucrose matched the retention time of raffinose in our assay work. These characteristics would allow identification of crystalline sucrose as being derived from cane or beets, provided the impurity levels are not lower than the levels in the samples tested in this experiment.

Interferences, Unknowns, and Co-elution

With any analytical technique there exists the possibility of interference from other components of the mixture. Compounds known to be present in beet molasses were injected into the chromatograph (13,44). A match in retention time does not prove the identity of an unknown, but does provide some evidence as to the identity of some of the unknown peaks present in some of our chromatograms. Retention times which match the retention times of the components being analyzed warn of the distinct possibility of reporting a value in excess of the amount actually present, and hiding the unreported compound. Salts, most organic acids (which will be present as salts at the pH of the eluent), and high molecular weight compounds elute early in one peak (Table 14). Mellibiose was resolved from sucrose, but followed immediately after sucrose. In some samples the integrator detected a small peak riding on the tail of the sucrose, which may be mellibiose (Fig. 18). Glutamine, if present, will co-elute with betaine and would be reported as betaine. Aminobutyric and other less hydrophilic amino acids eluted in the same relative area of the chromatogram as glucose and fructose, but at the low concentrations found in molasses samples, were reasonably well resolved. The highly hydrophobic amino acids, particularly those with phenyl groups, did not elute early enough to yield a detectably sharp peak.

Coping with Microbiological Activity in Sugar Samples

Even at refrigerator temperatures, standards and samples will harbor microbiological activity, changing the concentration of sugar. Long-term stability of a stock standard solution is desirable. By weighing out a standard, filtering through a 0.22 micron membrane filter into an HPLC autosampler vial, then freezing the contents of a vial, long-term (>6 months) stability of standards has been achieved. Since some stratification of solids may have taken place during the freezing process, the vial is shaken thoroughly after thawing to mix the solution well. A guard column helps to prevent chemical, physical, and microbial fouling of the more expensive analytical column.

Microbiological growth in the detector and in the injection system could cause operating difficulties. At the end of each day of operation, two vials of denatured ethanol are put through the injection system. The first is injected onto the column as a sample to flush the sealing surfaces in the injection valve. The second vial of alcohol is used to flush the injector sample loop and the flush line. Once a week, the column outlet is disconnected from the detector and the detector cleaned with 7N nitric acid.

The analytical column has shown a long lifetime when used regularly. The shortest column lifetimes have been obtained when using the system intermittently. Leaving the HPLC pump on at a slow flow rate (0.1 ml/min) has helped extend the column life. The column lifetime has not been limited yet by its separating ability. The column has needed replacement only due to an increase in the operating pressure drop.

CONCLUSIONS AND RECOMMENDATIONS

HPLC of sugar factory samples can be done simply, reliably, and with acceptable accuracy in a routine lab setting. The HPLC analysis yields more information than polarization and does not require the use of environmentally undesirable chemicals. Column lifetime has been very satisfactory, given special attention to column hygiene. HPLC can be used to get a rough gauge of sample DS, purity, and specific concentrations of individual components. A critical item often neglected is the importance of integrator-specific data manipulation parameters. Knowing how the peaks are being integrated requires visualization of the baseline. Having the integrator show peak start and stop tics is necessary to easily visualize the peak areas measured and should appear on all chromatograms. An integration method specific to one manufacturer's equipment which incorporates these recommendations has been developed and is presented here. This software yields good results for the range of samples investigated. For applications which require the most accurate results, such as performance tests of ion exclusion processes, a quantitative correction is highly recommended to account for retention time variation due to flow rate variations. The rapid, precise, HPLC analysis of extract, raffinate, and feed will be a very valuable factory tool if an ion exclusion process is being used.

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TABLE 1

CHARACTERISTICS OF SOME PROCEDURES FOR SUCROSE ANALYSIS

REFRACTOMETRY	POLARIMETRY	DERIVATIZATION/GAS CHROMATOGRAPHY	HPLC
- Precise	- Precise	- Precise	- Precise?
- Repeatable	- Repeatable	- Repeatable	- Repeatable
- Very Easy	- Easy	- Requires Skill and Care	- Easy
- Inexpensive	- Inexpensive	- More Expensive	- Cost?
- Accurate (high purity)	- Accurate (if high purity)	- Accurate	- Accurate?
- Totally Nonspecific	- Nonspecific	- Specific	- Specific
- Extremely Fast	- Very Fast	- Slow	- 20 Minutes
			- Different

TABLE 2

POTENTIAL ADVANTAGES

OF HPLC ANALYSIS

SAMPLE PREPARATION IS SIMPLE

(NO COLOR REMOVAL REQUIRED)

ENVIRONMENTALLY SAFE

ALL CHEMICALS USED ARE NON-HAZARDOUS

ANALYSIS IS SPECIFIC

UNAFFECTED BY RAFFINOSE, INVERT,
AMINO ACIDS, ETC.

MORE INFORMATION IS AVAILABLE

BETAINE

RAFFINOSE

TABLE 3

ELUENT SYSTEM

ELUENT	0.13 G/L K_2HPO_4 IN DEGASSED D.I. WATER
TEMPERATURE	65-70 C
pH	8.5

TABLE 4

HPLC EQUIPMENT

AUTOINJECTOR	MICROMERITICS AN-728
INJECTOR VALVE	VALCO EC6W
PUMP	SHIMADZU LC-6A
OVEN	WATERS
COLUMN	BIO-RAD AMINEX HPX-87K (7.8MM X 300 MM)
DETECTOR	WATERS R-410
INTEGRATOR	HP3396A

TABLE 5

HPLC OPERATING CONDITIONS

COLUMN	AMINEX HPX-87K
ELUENT FLOW RATE	0.6 ML/MIN
TEMPERATURE	85 C
INJECTED AMOUNT	20 u L
DETECTOR	WATERS R-410 REFRACTIVE INDEX AT 16 SENSITIVITY, 38 C INTERNAL T

TABLE 6

HPLC ANALYSIS PROCEDURE

1. DILUTE A KNOWN QUANTITY OF SAMPLE TO 1-2% SUCROSE WITH ELUENT SOLUTION.

2. FILTER DILUTED SAMPLE INTO AN AUTOSAMPLER VIAL OR INTO THE INJECTOR VALVE USING A 0.22 OR 0.45 MICRON MEMBRANE FILTER.

3. INJECT INTO HPLC SYSTEM. QUANTIFY RESULTS BY COMPARING PEAK SIZE (PREFERABLY BY PEAK AREA) TO PEAKS FROM KNOWN STANDARDS INJECTED INTO THE SYSTEM.

4. INTEGRATION PARAMETERS ARE VERY IMPORTANT.

TABLE 7

RELATIVE RESPONSE FACTORS

COMPOUND	$RF/RF_{SUCROSE}$
K2SO4	1.143
RAFFINOSE	1.175
SUCROSE	1.0
BETAINE	1.125
GLUCOSE	1.019
FRUCTOSE	1.039

TABLE 8
RECOVERY - PURE COMPONENTS

		DECEMBER 13, 1990		JANUARY 3, 1991	
COMPOUND	AMOUNT PRESENT*	AMOUNT FOUND*	% RECOVERY	AMOUNT FOUND*	% RECOVERY
K2SO4	0.526	0.517	98	0.521	99
RAFFINOSE	0.104	0.103	99	0.104	100
SUCROSE	1.504	1.480	98.4	1.500	99.7
BETAINE	0.152	0.152	100	0.152	100
GLUCOSE	0.260	0.259	99.6	0.260	100
FRUCTOSE	0.199	0.198	99.5	0.200	100.5

CONCENTRATIONS IN G/100 ML

TABLE 9

RECOVERY DATA - SPIKED EXTRACT

COMPOUND	ORIGINAL AMOUNT	AMOUNT ADDED	EXTRA AMOUNT FOUND*	% RECOVERY
"SALTS" (K2SO4)	0.202	0.0265	0.026	98
RAFFINOSE	0.0496	0.0261	0.028	107
SUCROSE	1.565	0.2585	0.255	99
BETAINE	0.144	0.1069	0.110	103
GLUCOSE	---	0.1994	0.211	106
FRUCTOSE	---	0.2002	0.216	108

CONCENTRATIONS IN G/100 ML

TABLE 10

PRECISION DATA

Analyst	Day	RETENTION Time	SALTS Raw Data	SALTS Corrected	RAFFINOSE Raw Data	RAFFINOSE Corrected	SUCROSE Raw Data	SUCROSE Corrected	BETAINE Raw Data	BETAINE Corrected
A	1									
		8.753	1.37	1.38	0.63	0.64	15.61	15.75	1.46	1.47
		8.750	1.45	1.46	0.65	0.66	15.41	15.55	1.45	1.46
		8.743	1.45	1.46	0.63	0.64	15.57	15.72	1.46	1.47
		8.747	1.48	1.49	0.66	0.66	15.74	15.89	1.47	1.48
		8.739	1.35	1.36	0.63	0.64	15.63	15.79	1.46	1.48
	$\bar{X}$		1.42	1.43	0.64	0.65	15.59	15.74	1.46	1.47
	σ		0.057	0.057	0.014	0.011	0.12	0.124	0.007	0.008
	%RSD		4.0	4.0	2.2	1.7	0.8	0.8	0.5	0.5
A	2	8.860	1.46	1.46	0.68	0.68	15.94	15.89	1.53	1.52
		8.865	1.43	1.42	0.64	0.64	15.95	15.89	1.51	1.50
		8.892	1.45	1.44	0.68	0.68	15.87	15.76	1.50	1.49
		8.911	1.48	1.47	0.67	0.66	15.85	15.71	1.52	1.51
		9.060	1.58	1.54	0.69	0.67	16.26	15.85	1.55	1.51
	$\bar{X}$		1.48	1.47	0.67	0.67	15.97	15.82	1.52	1.51
	σ		0.059	0.46	0.019	0.017	0.17	0.081	0.019	0.011
	%RSD		4.0	3.1	2.8	2.5	1.1	0.5	1.3	0.8
B	1	8.719	1.44	1.46	0.61	0.62	15.56	15.76	1.45	1.47
		8.697	1.37	1.39	0.62	0.63	15.59	15.83	1.49	1.51
		8.730	1.38	1.40	0.63	0.64	15.66	15.84	1.50	1.52
		8.739	1.37	1.38	0.62	0.63	15.73	15.89	1.46	1.48
		8.719	1.36	1.38	0.62	0.63	15.74	15.94	1.49	1.51
	$\bar{X}$		1.38	1.40	0.62	0.63	15.66	15.85	1.48	1.50
	σ		0.032	0.034	0.007	0.007	0.08	0.068	0.022	0.022
	%RSD		2.3	2.4	1.1	1.1	0.5	0.4	1.5	1.4
B	2	9.083	1.57	1.53	0.64	0.62	16.26	15.81	1.55	1.51
		9.090	1.56	1.52	0.66	0.64	16.31	15.84	1.56	1.52
		9.122	1.56	1.51	0.66	0.64	16.42	15.89	1.56	1.51
		9.117	1.54	1.49	0.66	0.64	16.45	15.93	1.58	1.53
		9.127	1.57	1.52	0.66	0.64	16.50	15.96	1.58	1.53
	$\bar{X}$		1.56	1.51	0.66	0.64	16.39	15.89	1.57	1.52
	σ		0.012	0.015	0.009	0.009	0.10	0.06	0.013	0.01
	%RSD		0.8	1.0	1.4	1.4	0.6	0.4	0.9	0.7
OVERALL	$\bar{X}$		1.46	1.45	0.65	0.65	15.90	15.82	1.51	1.50
	σ		0.080	0.057	0.023	0.018	0.34	0.097	0.045	0.022
	%RSD		5.4	3.9	3.5	2.8	2.1	0.6	3.0	1.5

TABLE 11

RELATIVE STANDARD DEVIATIONS OF THE ANALYSIS

	"SALTS"	RAFFINOSE	SUCROSE	BETAINE
NUMBER OF DATA POINTS	20	20	20	20
AVERAGE CONCENTRATION (G/100 ML)	1.46	0.65	15.90	1.51
STANDARD DEVIATION	0.080	0.023	0.34	0.045
% RELATIVE STANDARD DEVIATION	5.5	3.5	2.1	3.0
AVERAGE CONCENTRATION (G/100 ML)	1.45	0.65	15.82	1.50
STANDARD DEVIATION	0.057	0.018	0.097	0.022
% RELATIVE STANDARD DEVIATION	3.9	2.8	0.6	1.5

TABLE 12

COMPARISON OF HPLC RESULTS TO CONVENTIONAL ANALYSES

SAMPLE [1]	HPLC DS	RDS [2]	HPLC PURITY	PURITY DIRECT POL [2]	PURITY BY GC % SUCROSE/RDS [2]
FEED	63.7	63.75	63.2	66.98	63.12
CONCENTRATED EXTRACT	66.8	67.93	94.9	93.48	93.73
CONCENTRATED RAFFINATE	60.7	61.67	10.8	18.19	9.6

[1] SAMPLES FROM A PILOT PLANT ION EXCLUSION RUN AT THE AMALGAMATED SUGAR COMPANY, TWIN FALLS, IDAHO

[2] DATA PROVIDED BY TOM HENSCHEID AT AMALGAMATED

TABLE 13

MOLASSES ANALYSES - PERCENT ON DS

	MICHIGAN	OHIO	IDAHO	RED RIVER VALLEY
SUCROSE	60.4	60.1	63.2	58.6
"SALTS"	30.0	30.7	24.4	29.2
BETAINE	5.7	5.6	6.3	8.1
RAFFINOSE	1.9	2.4	3.8	1.3
INVERT	1.0	0.6	1.3	1.2

TABLE 14

SPECIFICITY CHECK - RELATIVE RETENTION TIMES OF OTHER COMPOUNDS

COMPOUND	R_T/R_T, SUCROSE
[SALTS]	0.72
LACTIC ACID	0.72
PYROGLUTAMIC ACID	0.72
ACONITIC ACID	0.72
[RAFFINOSE]	0.88
[SUCROSE]	1.00
MELLIBIOSE	1.08
GLUTAMINE	1.24
[BETAINE]	1.24
[GLUCOSE]	1.34
AMINOBUTYRIC ACID	1.40
ALANINE	1.41
VALINE	1.41
GALACTOSE	1.44
[FRUCTOSE]	1.46
INOSITOL	1.49
MANNOSE	1.51
GLYCINE	1.57
I-LEUCINE	1.58
PROLINE	1.63
LEUCINE	1.66

FIGURE 1

SUCROSE RECOVERY FROM MOLASSES

VIA ION EXCLUSION

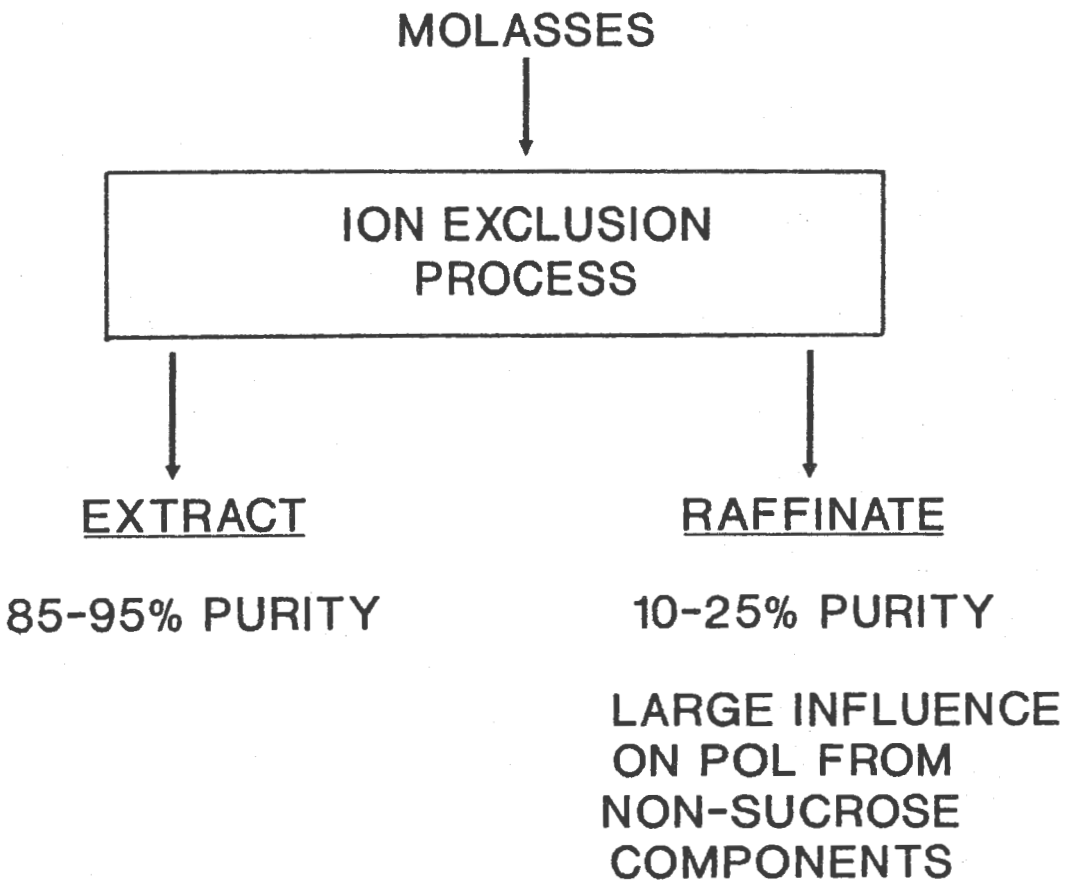
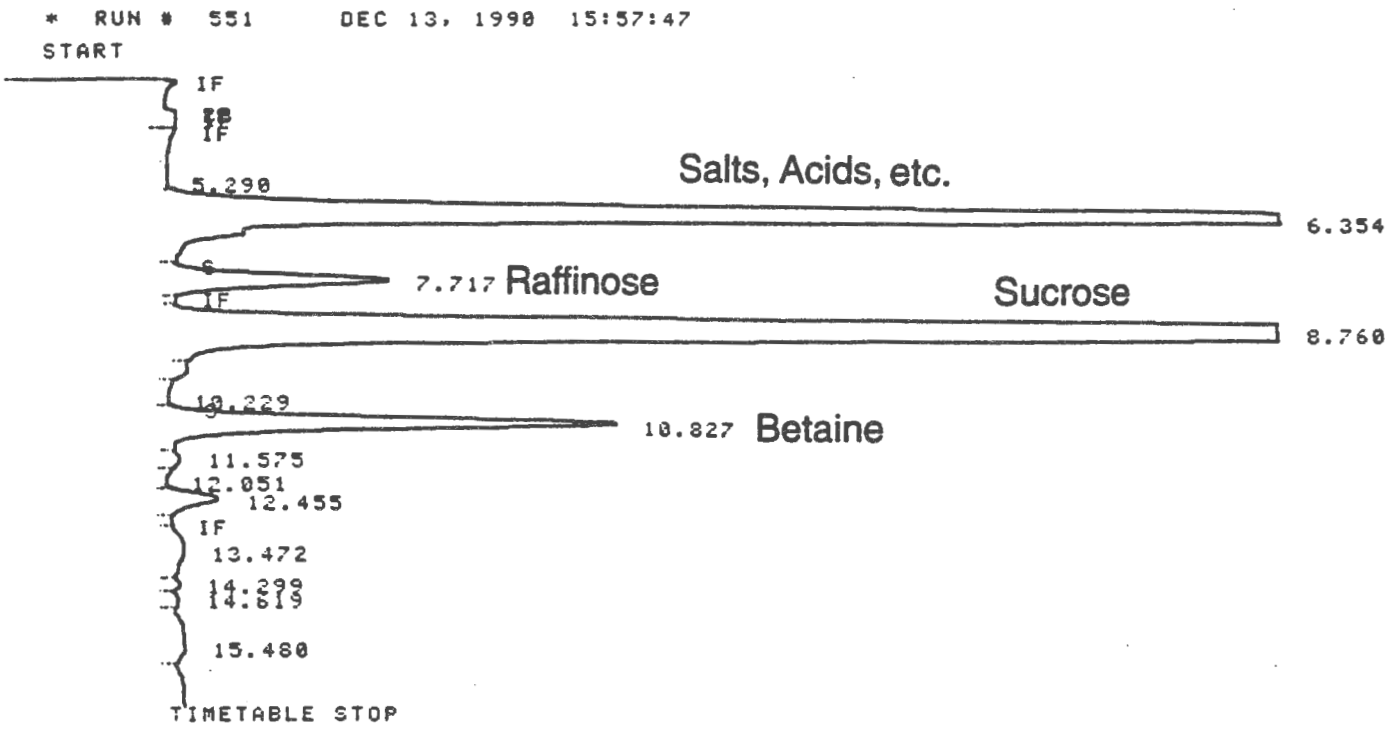


FIGURE 2

HPLC CHROMATOGRAM OF TYPICAL BEET MOLASSES



RUN# 551 DEC 13, 1990 15:57:47

IDENTIFIER : DOW CHEMICAL

MOLASSES ANALYSIS ON AMINEX HPX-87K LC COL

ESTD-AREA

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	g/100ml	NAME
5.290	N PH	934462	1.119	13924		.021	
6.354	BHB	35169152	.268	2134034	1	.935	Salts, acids, et
7.717	BP	3219672	.284	189136	2	.075	Raffinose
8.760	BPB	80325760	.279	4800595	3R	1.873	Sucrose
10.827	BP	6600864	.277	397861	4	.173	Betaine
12.455	VP	651391	.267	40705		.015	
13.472	N VV	406292	.629	10774		.009	
15.480	N VV	819658	.846	12203		.014	

TOTAL AREA=1.2793E+08
MUL FACTOR=1.0000E+00

FIGURE 3

PRINTOUT OF THE HEWLETT-PACKARD 3396A INTEGRATION PROGRAM FOR MOLASSES ANALYSIS

```
*  LIST: METH  @
METHOD NAME: M:ES911.MET

RUN PARAMETERS
ZERO   =  2
ATT 2^ =  7
CHT SP =  0.1
AR REJ =  100000
THRSH  =  4
PK WD  =  0.30

TIMETABLE EVENTS
   0.010 INTG # =  9
   4.050 INTG # =  8
   4.100 CHT SP =  0.6
   4.500 INTG # =  11
   5.900 INTG # = -9
   6.000 INTG # =  3
   8.700 INTG # =  3
   9.500 INTG # =  4
  13.000 INTG # =  13
  17.000 STOP

CALIBRATION
ESTD
REF % RTW:   2.000   NON-REF % RTW:   2.000

LEVEL: 1              RECALIBRATIONS: 1
LEVEL: 2              RECALIBRATIONS: 1
LEVEL: 3              RECALIBRATIONS: 1

CAL#      RT      LV     AMT        AMT/AREA
  1      6.582     1 2.4040E-02   1.5454E-08
                   2 9.6160E-02   2.1431E-08
                   3 4.8080E-01   2.5408E-08
  2      8.019     1 5.4000E-03   2.2670E-08
                   2 2.1640E-02   2.2888E-08
                   3 1.0820E-01   2.1854E-08
  3R     9.112     1 7.2720E-02   2.3152E-08
                   2 2.9090E-01   2.2769E-08
                   3 1.4544E+00   2.2484E-08
  4     11.212     1 7.5000E-03   2.5633E-08
                   2 3.0020E-02   2.4828E-08
                   3 1.5010E-01   2.4793E-08
  5     12.224     1 1.2460E-02   2.3496E-08
                   2 4.9840E-02   2.2756E-08
                   3 2.4920E-01   2.2650E-08
  6     13.299     1 9.9600E-03   2.4239E-08
                   2 3.9840E-02   2.3219E-08
                   3 1.9920E-01   2.3050E-08

CAL#      NAME
  1  Salts, acids, et
  2  Raffinose
  3  Sucrose
  4  Betaine
  5  Glucose
  6  Fructose

INTEGRATION PLOT TYPE ..... FILTERED

RUN DATA STORAGE
Store signal data ........... NO
Store processed peaks ....... NO

CALIBRATION OPTIONS
RF of uncalibrated peaks .... 2.2500E-08
Calibration fit ............. L
Disable post-run RT update .. NO
SAMPLE AMT .................. 0.0000E+00
MUL FACTOR .................. 1.0000E+00

REPORT OPTIONS
Suppress local report ....... NO
HEIGHT% report .............. NO
Report title:
MOLASSES ANALYSIS ON AMINEX HPX-87K LC COL
Amount label ................ g/100ml
Report uncalibrated peaks ... YES
Extended report ............. YES

POST-RUN LIST OPTIONS
Store post-run report ....... NO
External post-run report .... NO
List run parameters ......... NO
List timetable .............. NO
List calibration table ...... NO
List remote method .......... NO
Form-feed before report ..... NO
Form-feed after report ...... YES
Skip perforations in report . NO
Skip perforations in plot ... NO

*
```

Figure 4. Linearity of (detector + integrator) response
RAFFINOSE

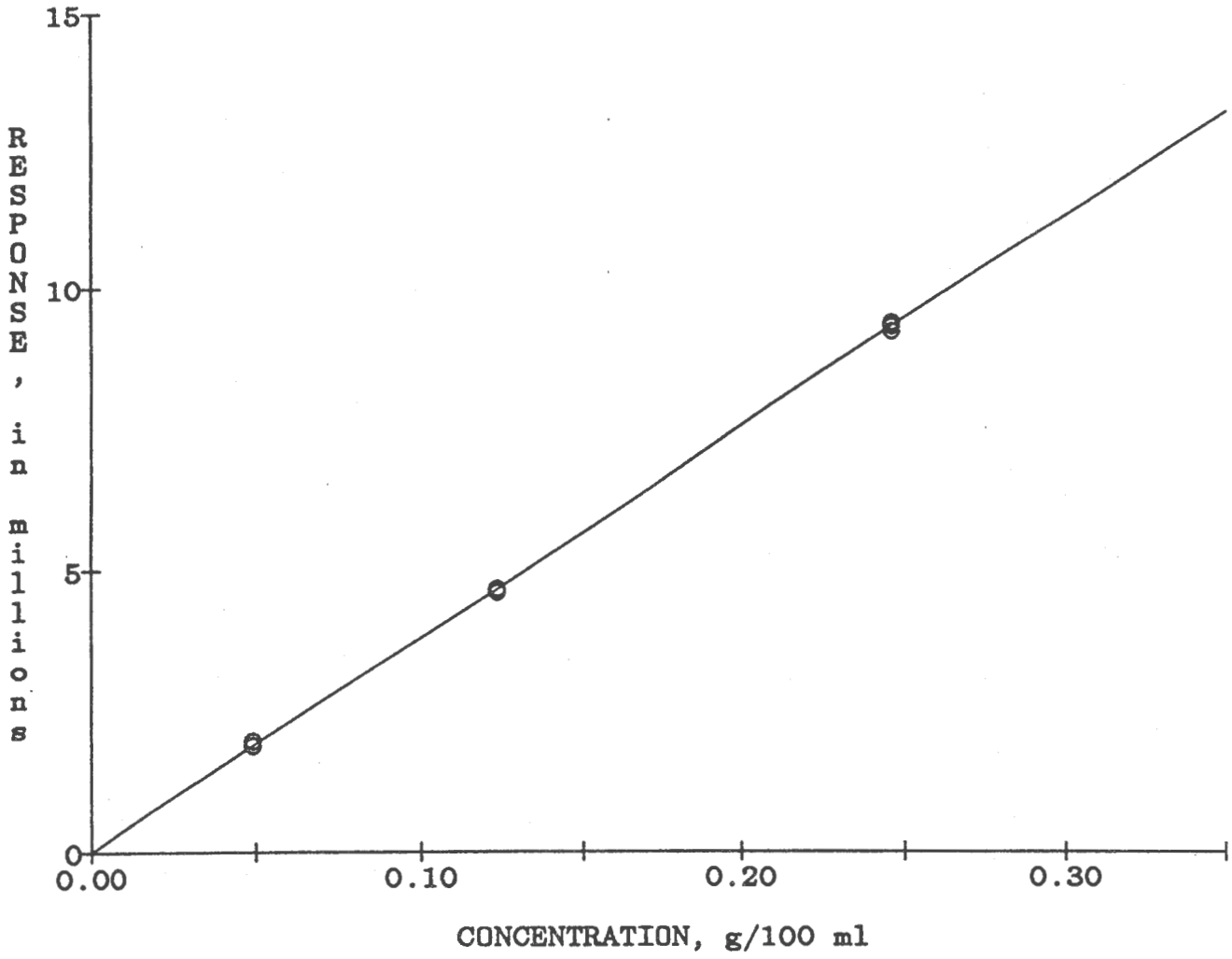


Figure 5. Linearity of (detector + integrator) response
BETAINE

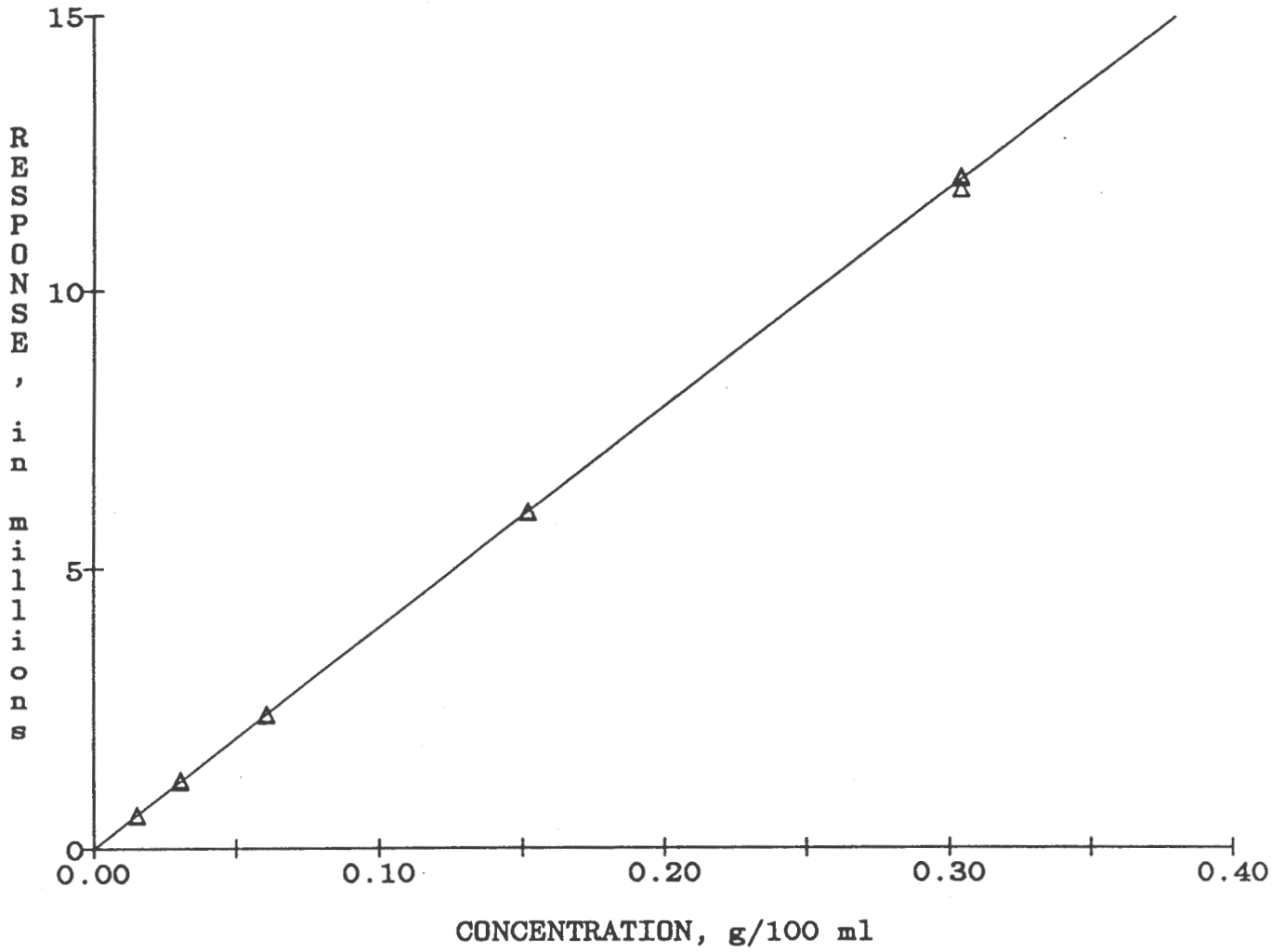


Figure 6. Linearity of (detector + integrator) response
GLUCOSE AND FRUCTOSE

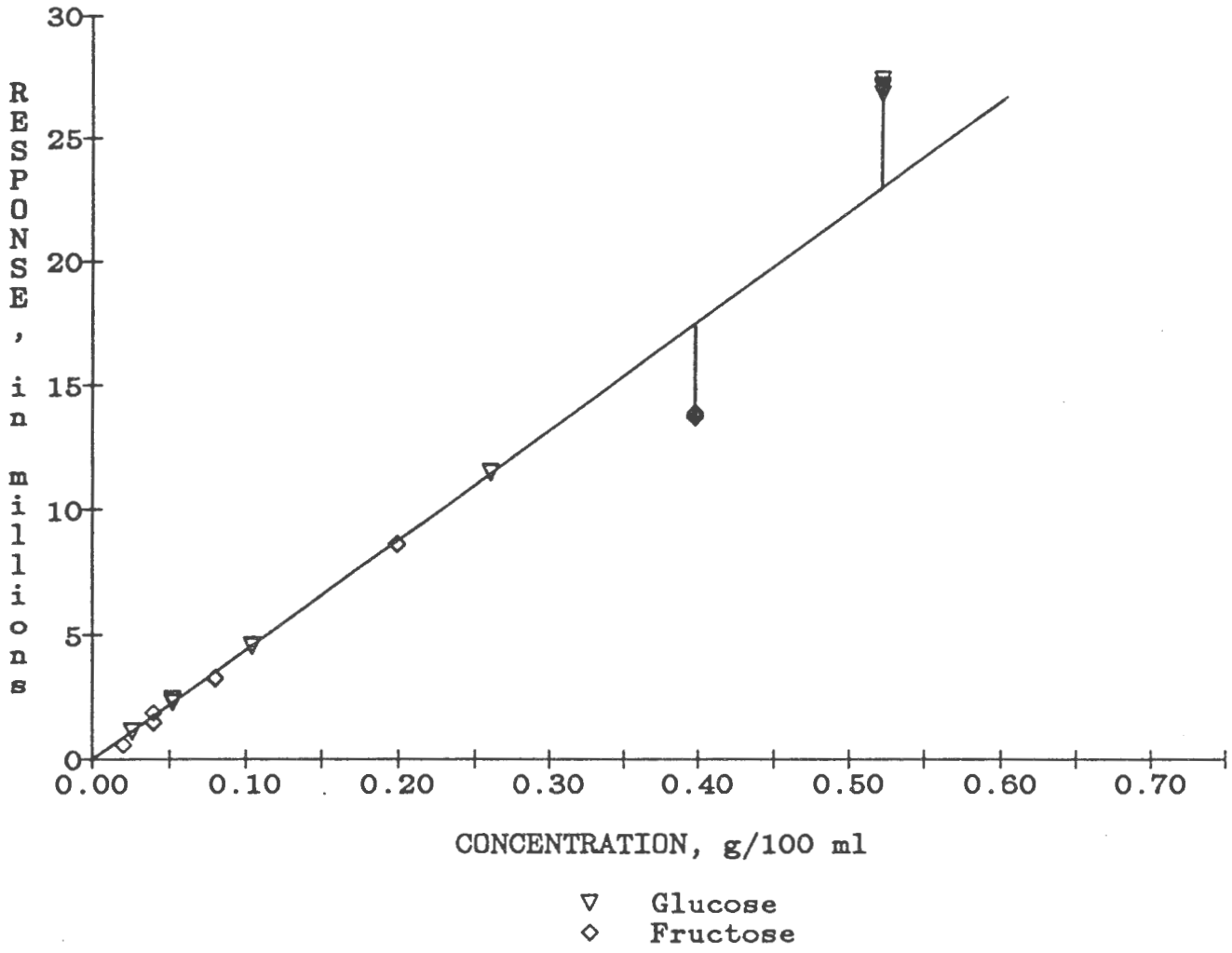


Figure 7. Linearity of (detector + integrator) response
SUCROSE

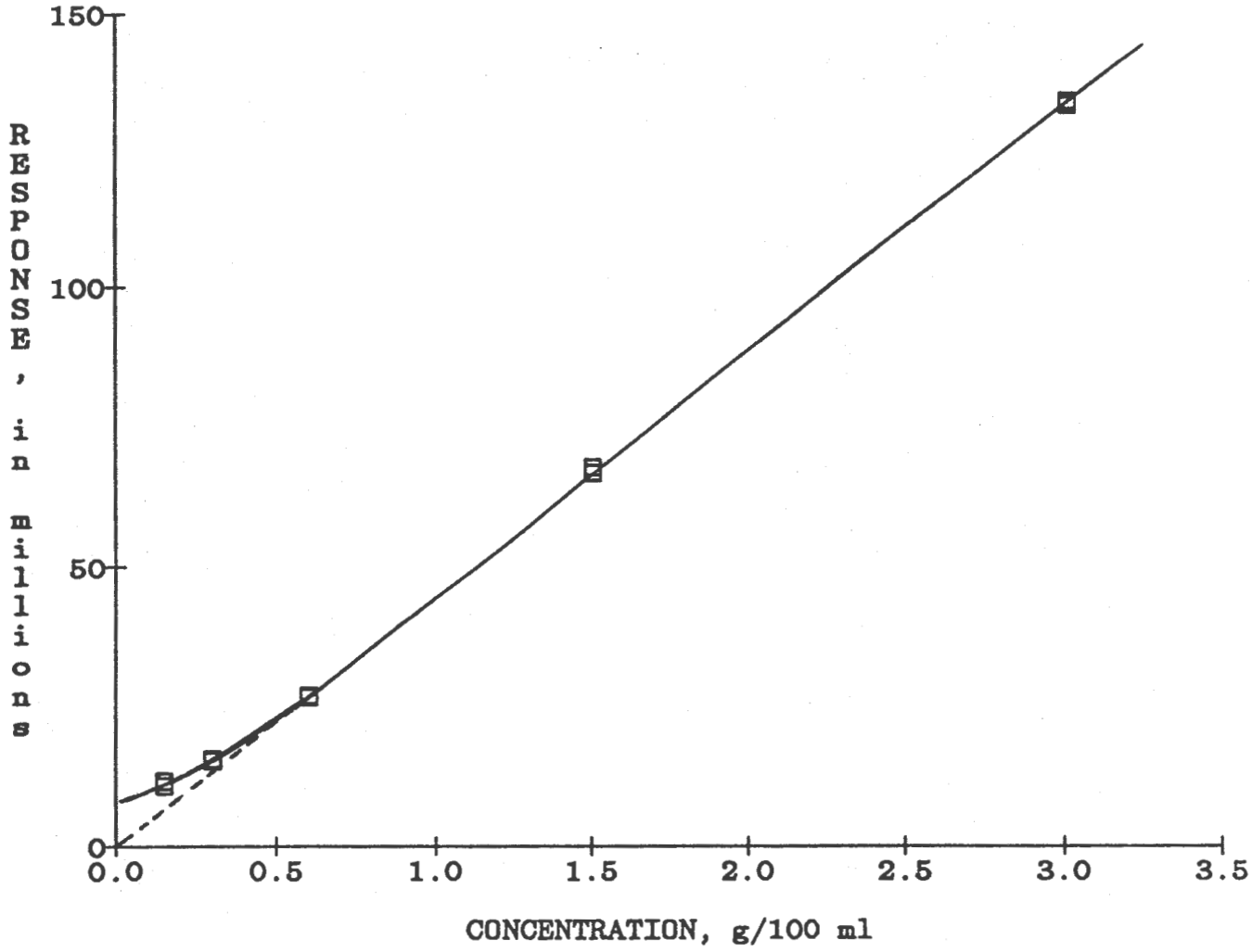
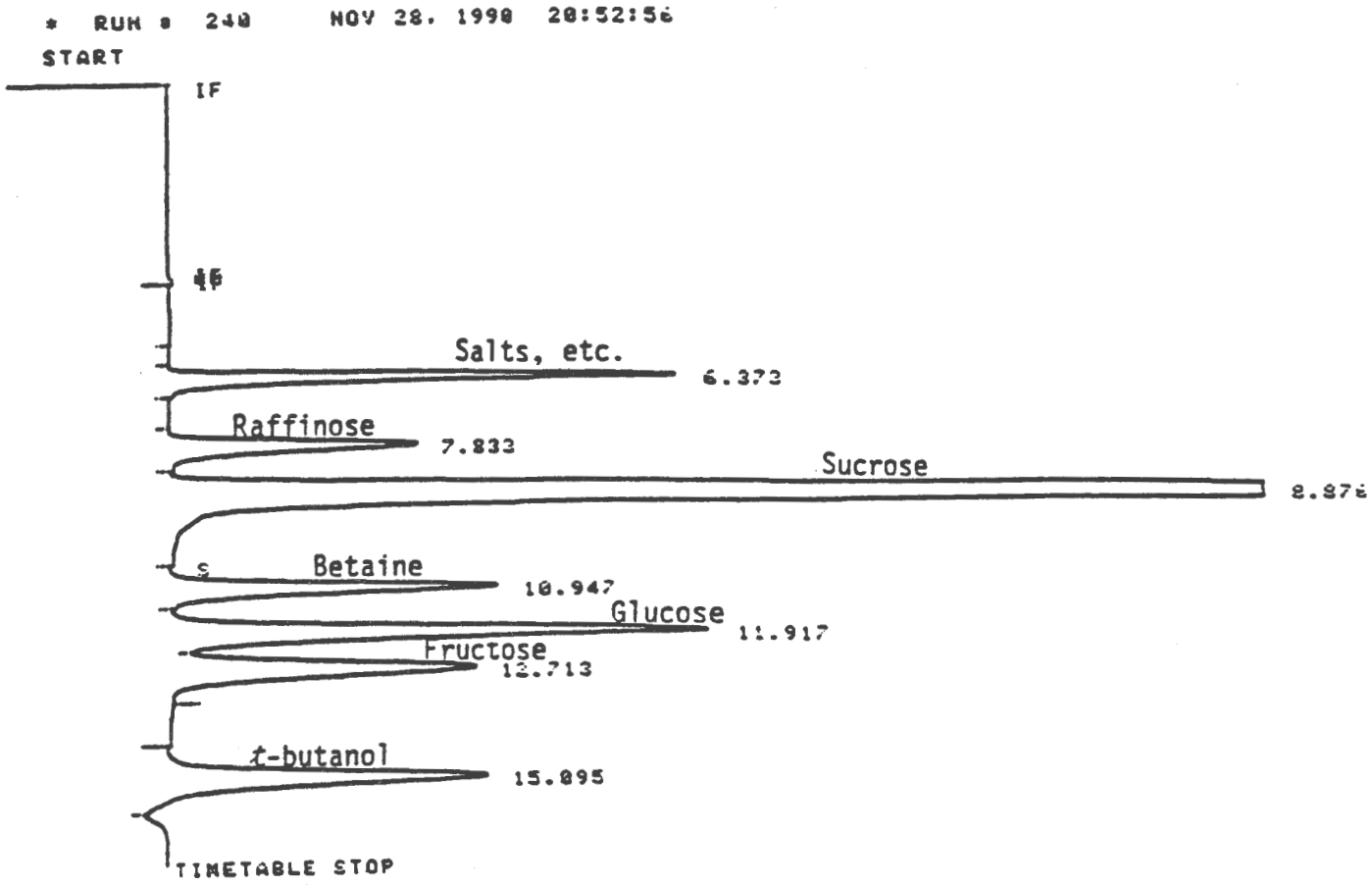


FIGURE 8

HPLC CHROMATOGRAM
OF HIGH CONCENTRATION STANDARD



RUN# 240 NOV 28, 1990 20:52:56

SAMPLE NAME: STANDARD D A
REPORT IMMEDIATELY FOLLOWING 5-LEVEL CALIBRATION

MOLASSES ANALYSIS ON AMINEX HPX-87K LC COL

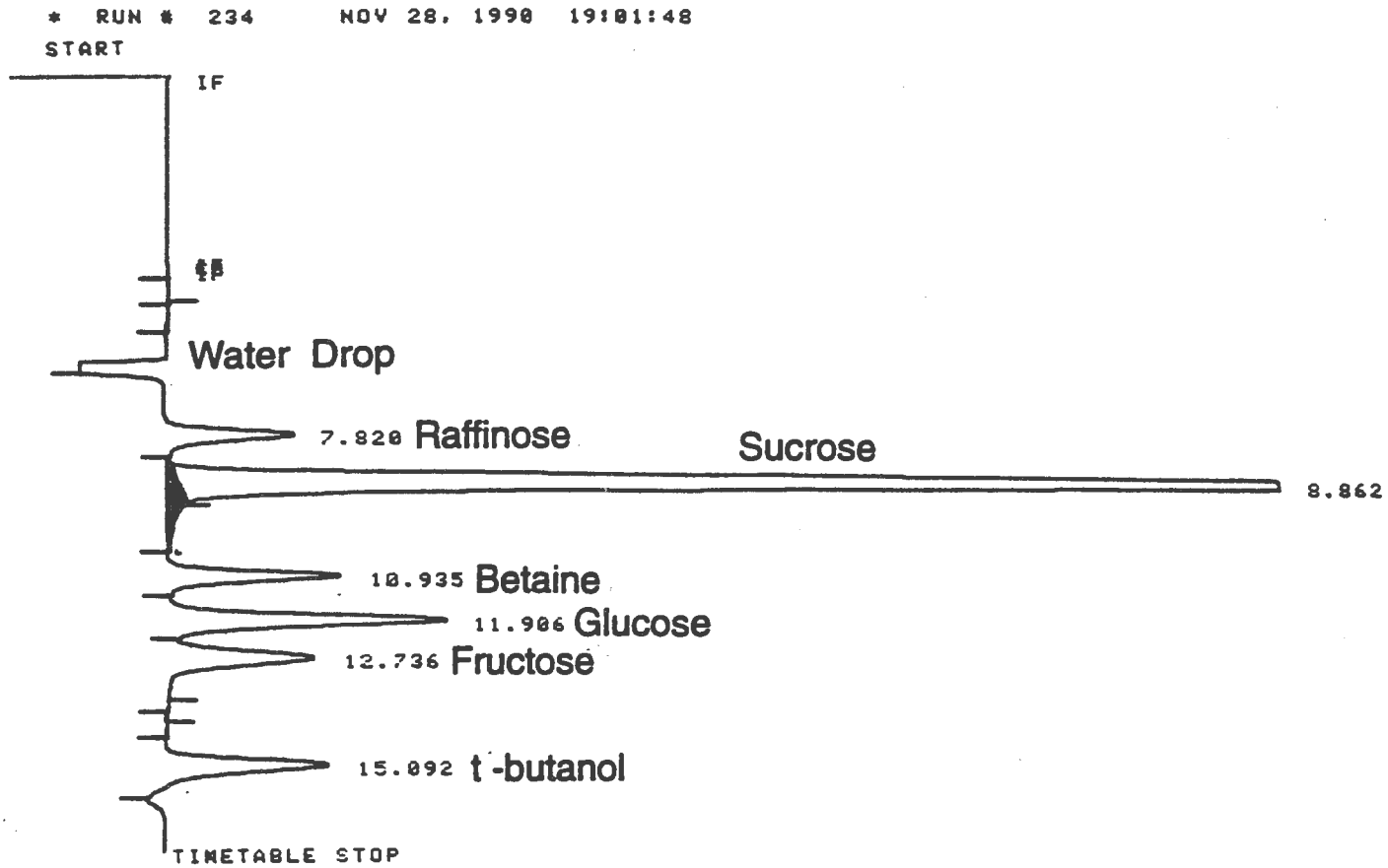
ESTD-AREA

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	g/100ml	NAME
6.373	VV	3675754	.188	236900	1	.061	Salts, acids, et
7.833	PH	1947957	.269	114589	2	.036	Raffinose
8.876	SHB	26581856	.271	1637126	3R	.512	Sucrose
10.947	BV	2384320	.261	152102	4	.053	Betaine
11.917	VV	4536621	.306	247328	5	.090	Glucose
12.713	VB	3209758	.379	141229	6	.064	Fructose
15.095	BP	3490872	.390	153248		.071	

TOTAL AREA=4.4727E+07
MUL FACTOR=1.0000E+00

FIGURE 9

HPLC CHROMATOGRAM
OF MEDIUM CONCENTRATION STANDARD



RUN# 234 NOV 28, 1990 19:01:48

SAMPLE NAME: STANDARD D A
REPORT IMMEDIATELY FOLLOWING 5-LEVEL CALIBRATION

MOLASSES ANALYSIS ON AMINEX HPX-87K LC COL

ESTD-AREA

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	g/100ml	NAME
7.820	PV	13015328	1.492	145400	2	.256	Raffinose
8.862	VB	15502728	.304	848757	3R	.299	Sucrose
10.935	BV	1212011	.264	76637	4	.027	Betaine
11.906	VV	2301837	.309	124011	5	.046	Glucose
12.736	VB	1484058	.376	65779	6	.029	Fructose
15.092	BP	1688072	.369	76290		.034	

TOTAL AREA=3.5204E+07
MUL FACTOR=1.0000E+00

Figure 10. Linearity of response to sucrose after forcing solvent skimming

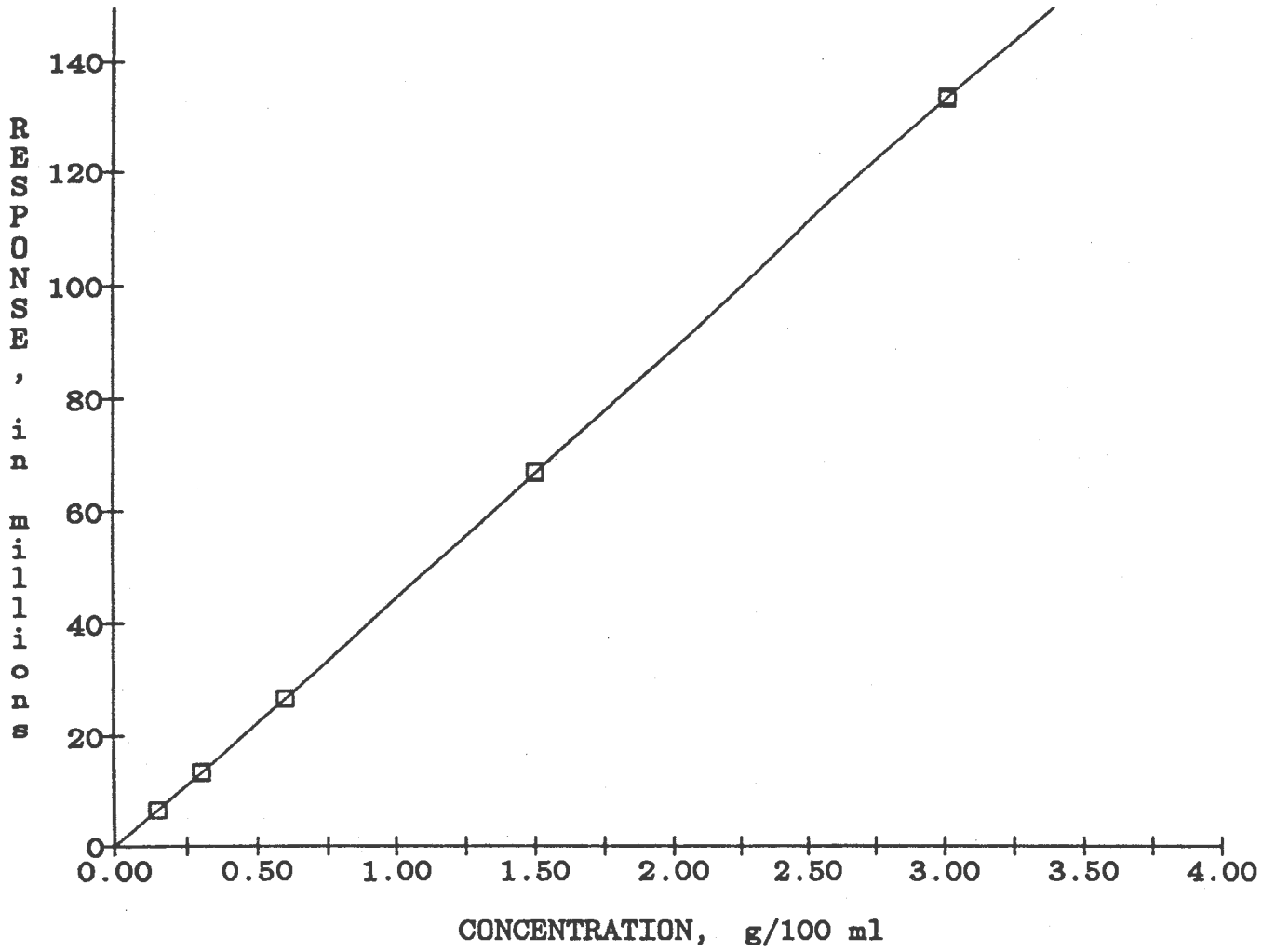
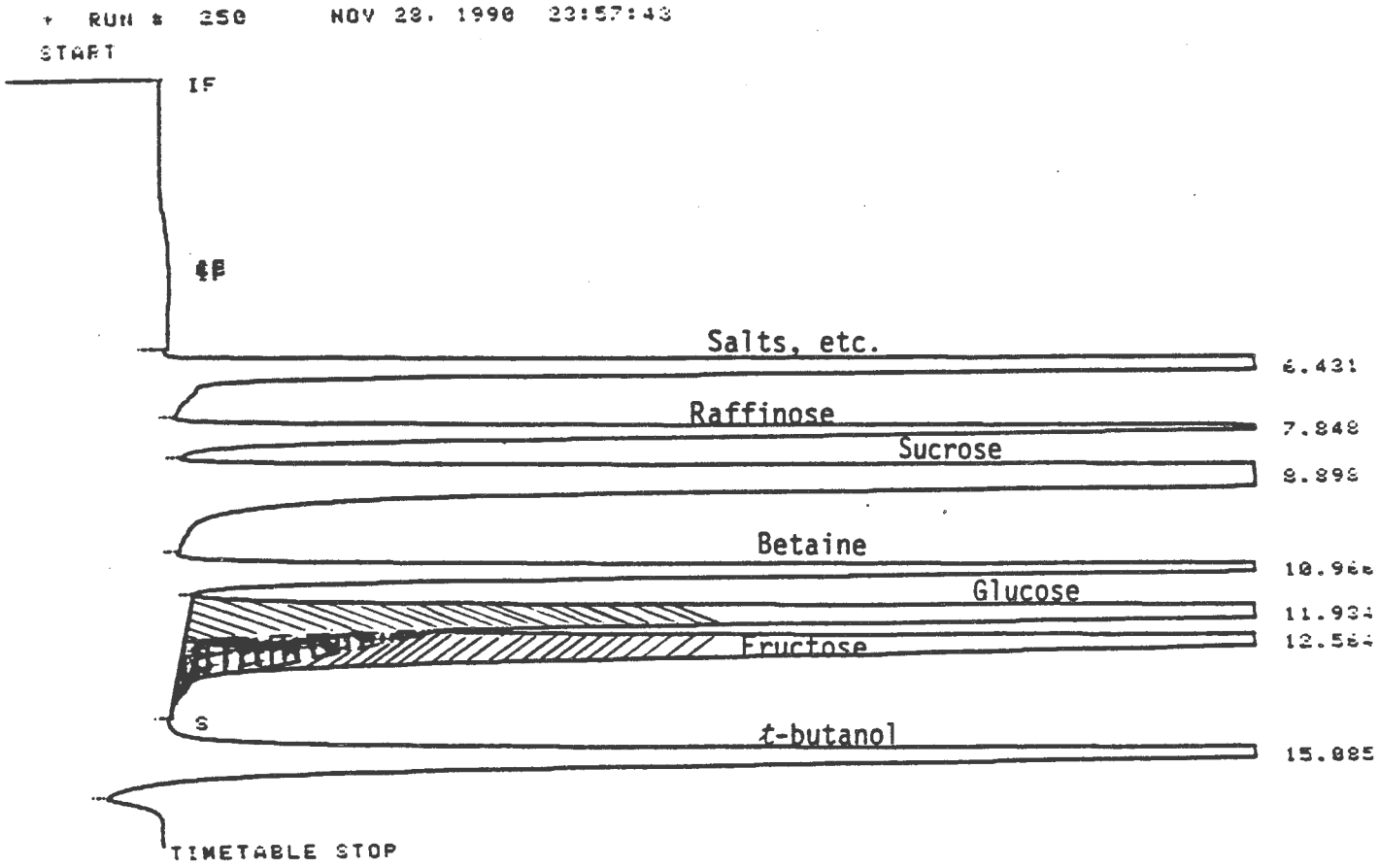


FIGURE 11

HPLC CHROMATOGRAM
OF HIGHEST CONCENTRATION STANDARD



RUN# 250 NOV 28, 1990 23:57:43

SAMPLE NAME: STANDARD D A
REPORT IMMEDIATELY FOLLOWING 5-LEVEL CALIBRATION

MOLASSES ANALYSIS ON AMINEX HPX-87K LC COL

ESTD-AREA

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	g/100ml	NAME
6.431	BBH	40895968	.192	3557400	1	.925	Salts, acids, et
7.848	TBB	9202778	.267	574624	3	.181	Raffinose
8.898	BHH	134263440	.272	8230925	3R	1.586	Sucrose
10.966	TBB	11833128	.259	762445	4	.361	Betaine
11.934	BHB	27371520	.364	1252736	5	.543	Glucose
12.564	TBB	13813200	.413	557230	6	.273	Fructose
15.085	BP	17341424	.381	758820		.354	

TOTAL AREA=2.5472E+08
MUL FACTOR=1.0000E+00

Figure 12. Linearity of response to glucose after disabling automatic peak skimming for the glucose peak.

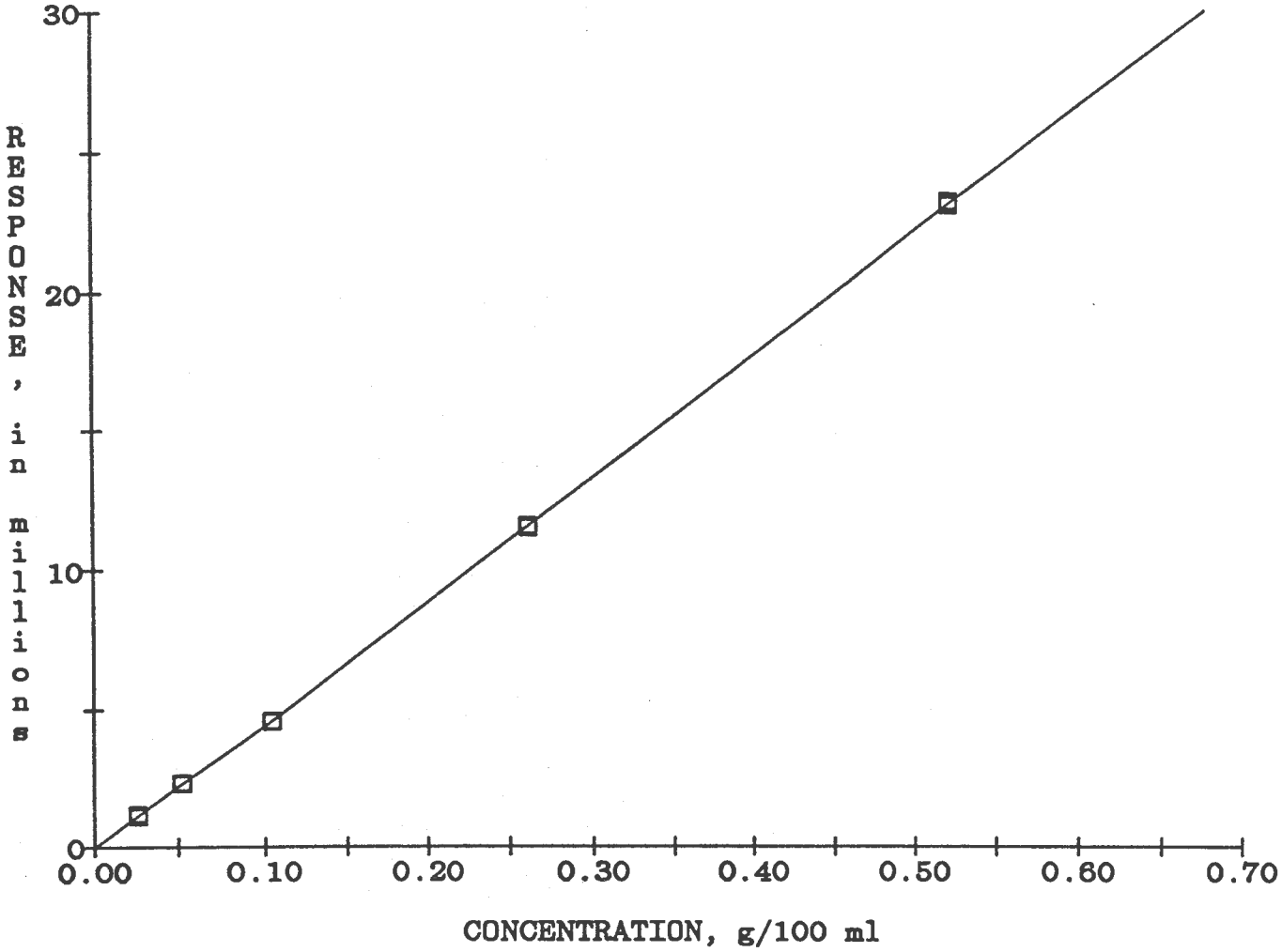


Figure 13. Linearity of response to fructose after disabling auto peak skimming for the glucose peak.

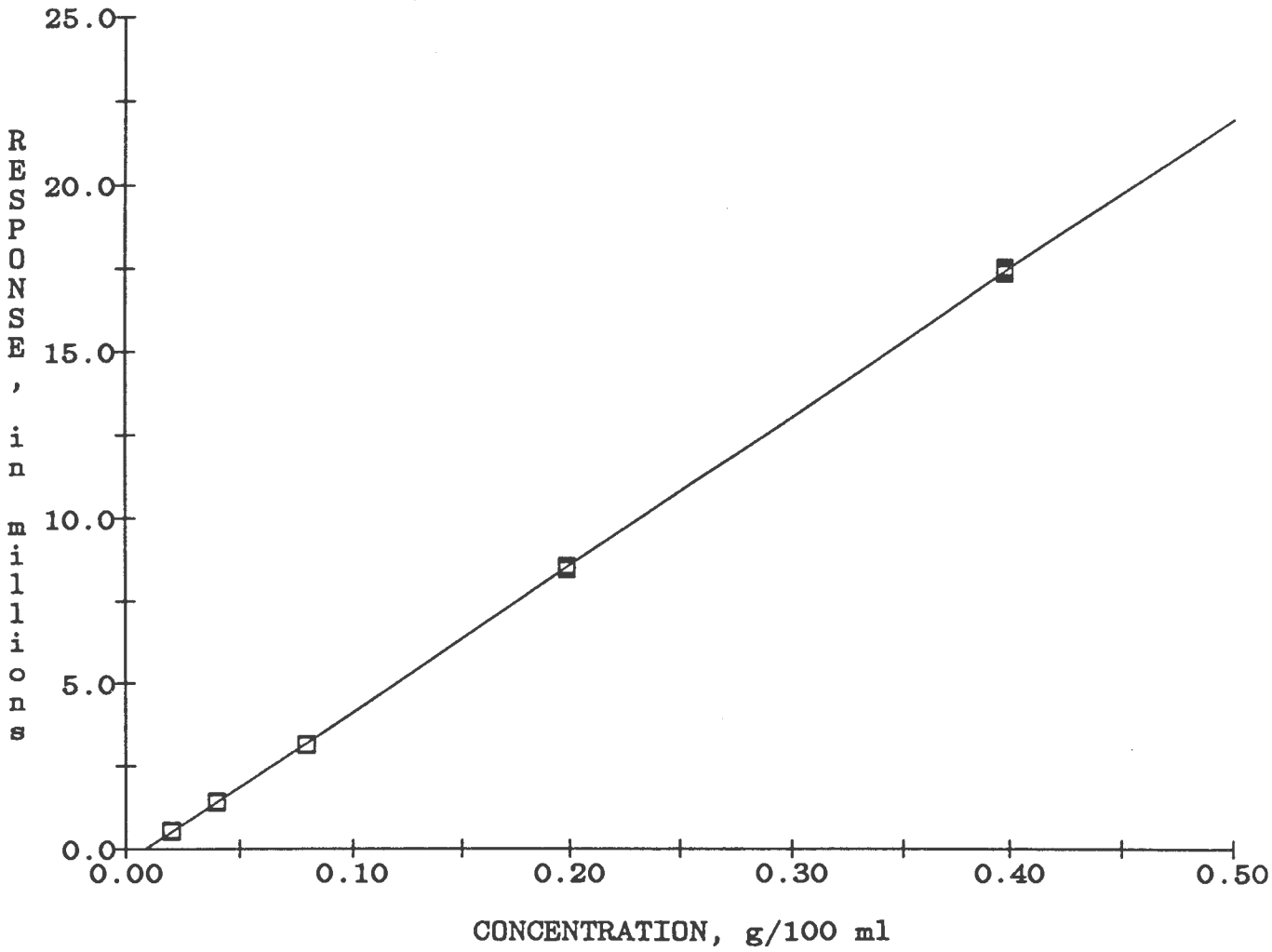


Figure 14. Linearity of (detector + integrator) response "SALTS," as K2S04

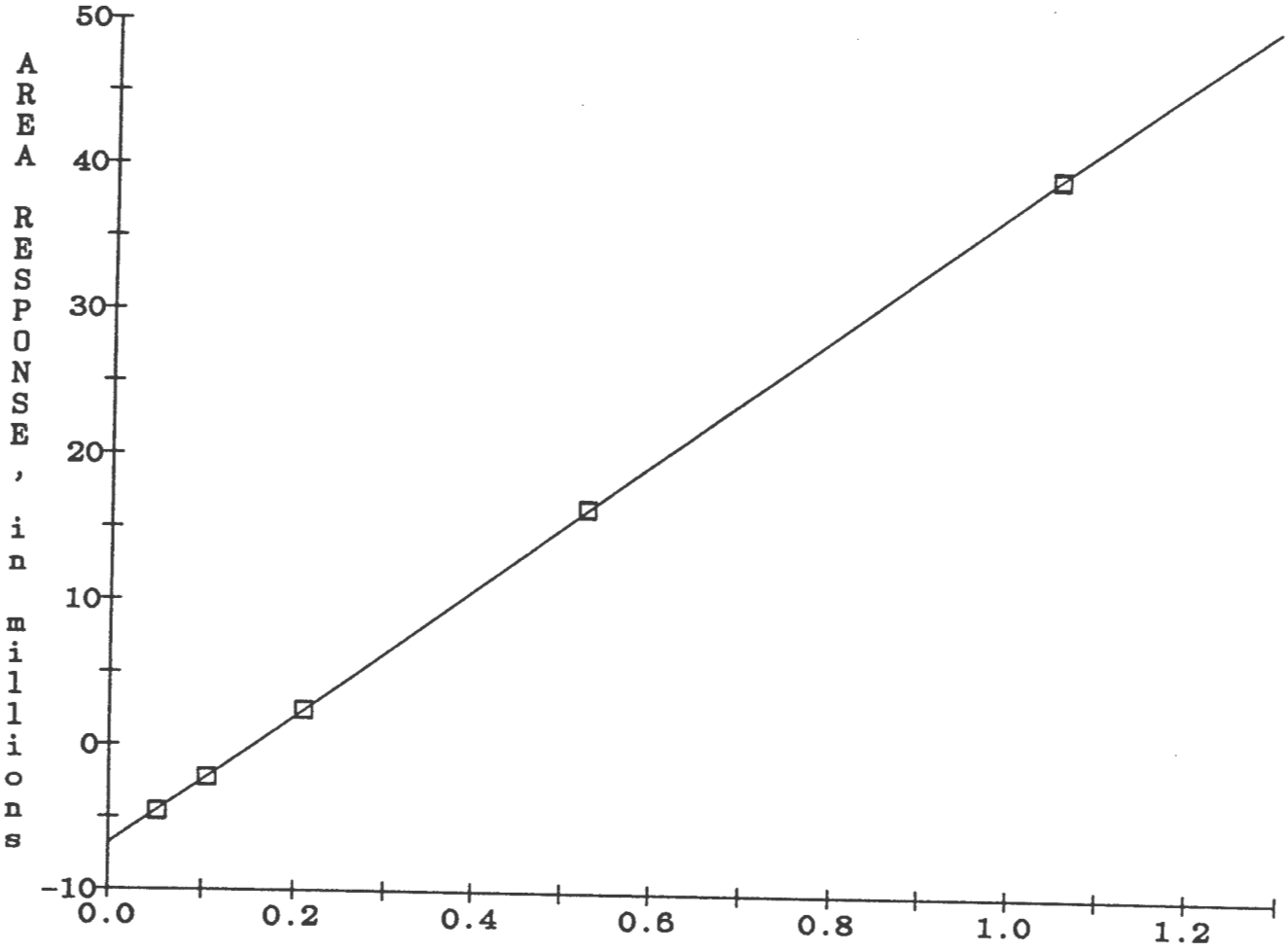
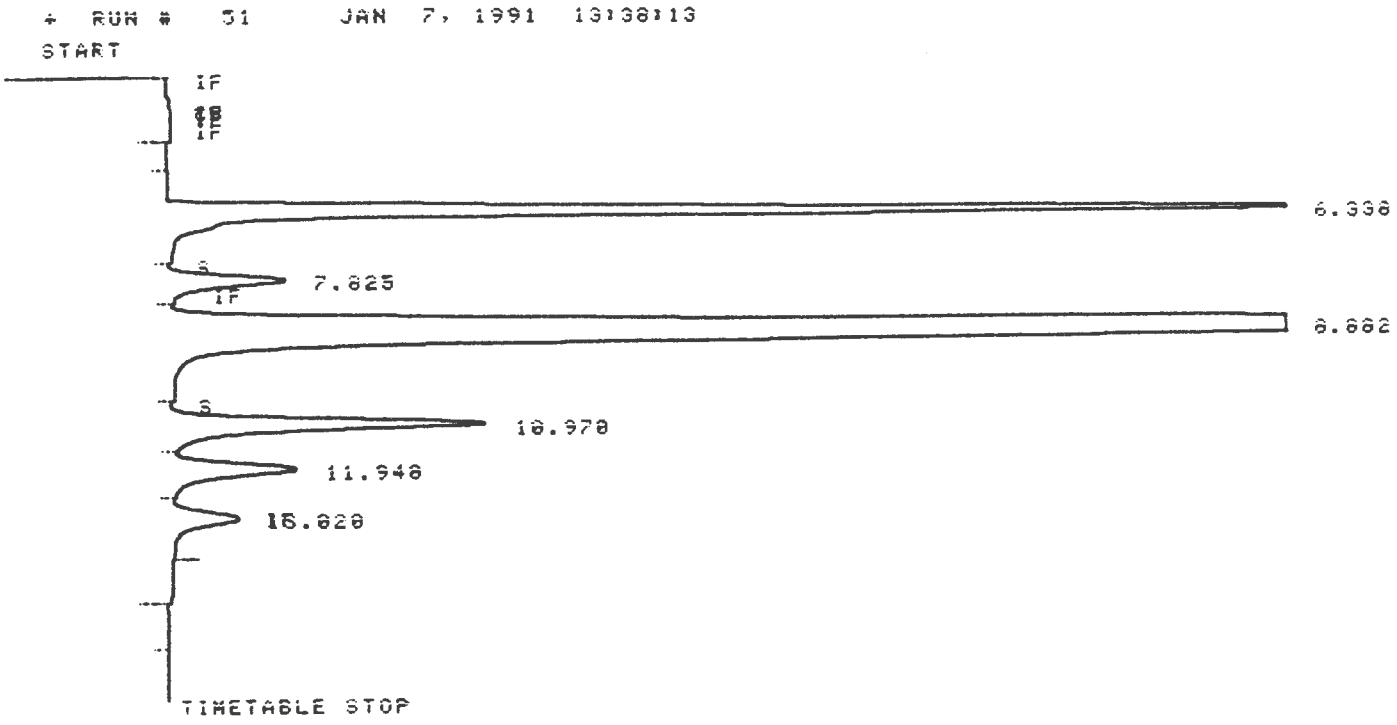


FIGURE 15A

HPLC STANDARD WITH GLUCOSE ELUTING AT 11.97 MINUTES



RUN# 51 JAN 7, 1991 13:38:13

MOLASSES ANALYSIS ON AMINEX HPX-87K LC COL

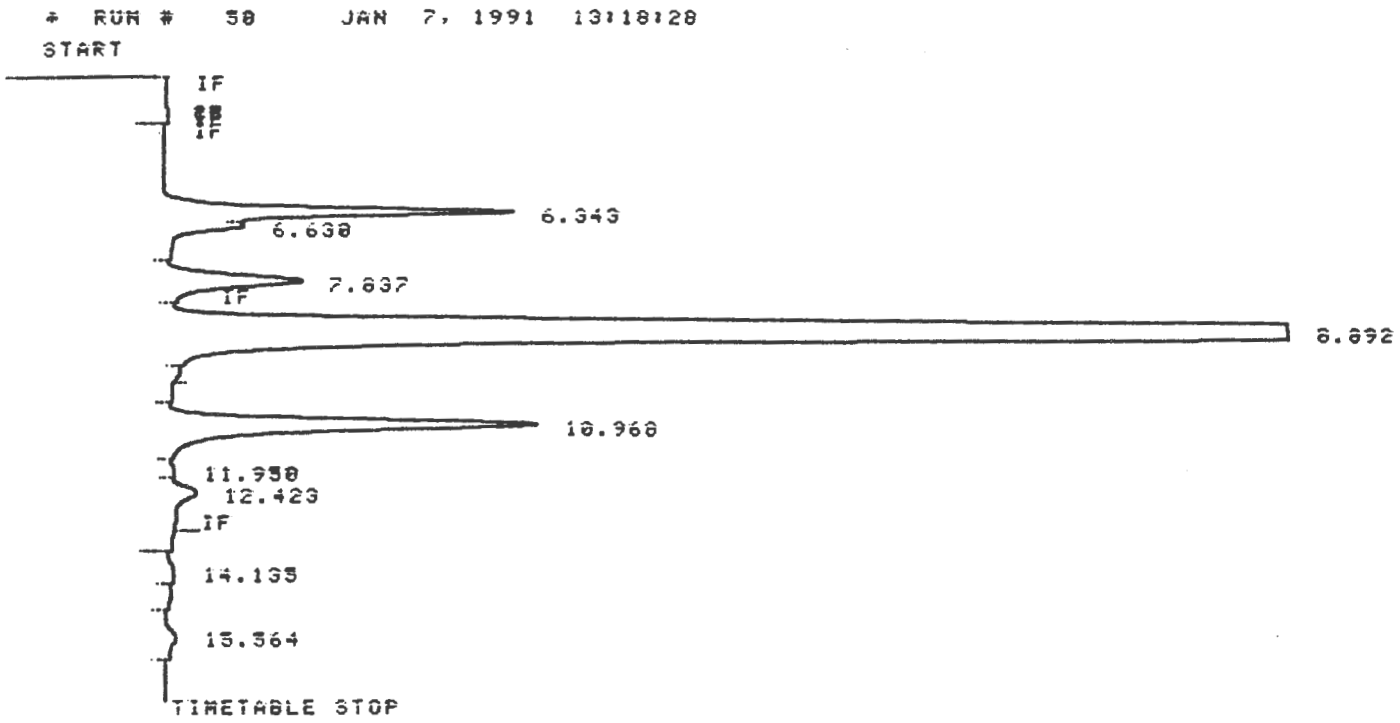
ESTD-AREA

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	g/100ml	NAME
6.338	SHB	13898448	.209	1107970	1	.377	Salts, acids, et
7.825	BH	1830539	.290	105229	2	.050	Raffinose
8.882	SHB	69326848	.290	3981360	SR	1.617	Sucrose
10.970	BV	4808269	.290	276079	4	.126	Betaine
11.948	VV	2356758	.348	113012	5	.055	Glucose
13.020	VB	1346620	.358	62652	6	.033	Fructose

TOTAL AREA=9.3567E+07
MUL FACTOR=1.0000E+00

FIGURE 15B

UNSPIKED PILOT PLANT EXTRACT DETECTED BUT UNREPORTED PEAK AT 11.95 MINUTES



RUN# 50 JAN 7, 1991 13:18:28

MOLASSES ANALYSIS ON AMINEX HPX-87K LC COL

ESTD-AREA

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	g/100ml	NAME
6.343	BV	4199114	.226	309594	1	.122	Salts, acids, et
6.638	VV	1020516	.237	71848		.023	
7.637	VH	2500981	.337	123764	2	.068	Raffinose
8.892	SHB	66028608	.292	3883101	3R	1.587	Sucrose
10.968	TBV	5722874	.293	325521	4	.158	Betaine
12.423	TVB	478811	.345	23146		.011	
14.135	BV	184638	.421	7305		.004	
15.364	PV	271986	.413	10973		.006	

TOTAL AREA=8.2487E+07
MUL FACTOR=1.0000E+00

FIGURE 16
HPLC CHROMATOGRAM FROM
A SAMPLE TAKEN EARLY IN A
PULSED ION EXCLUSION EXPERIMENT

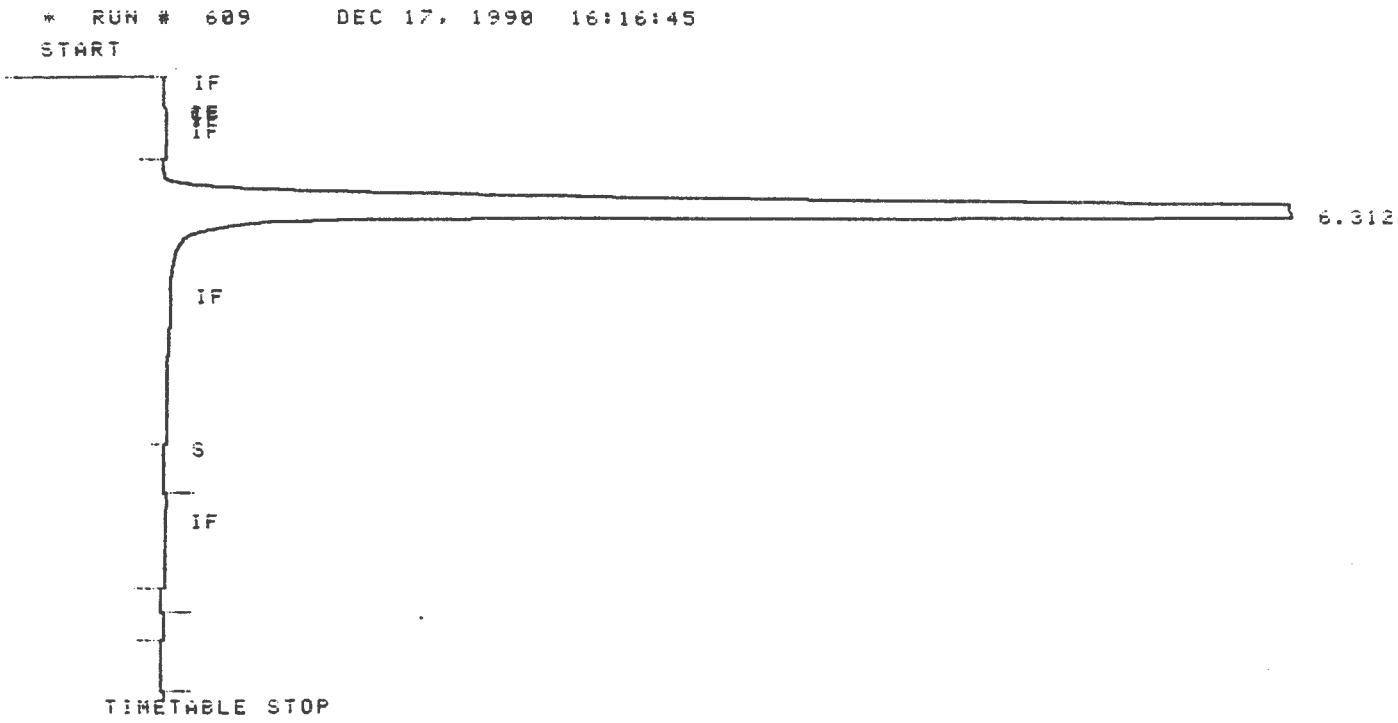


FIGURE 17

HPLC CHROMATOGRAM FROM A SAMPLE TAKEN MIDWAY IN A PULSED ION EXCLUSION EXPERIMENT

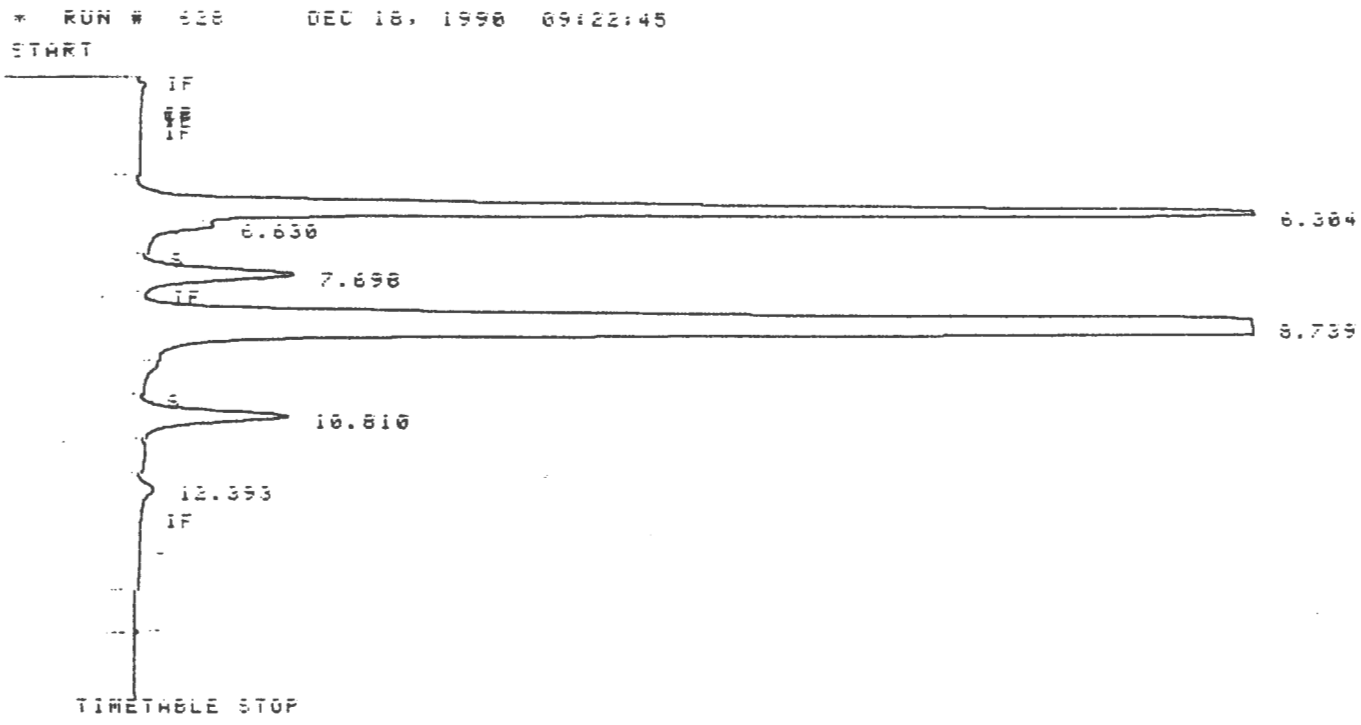


FIGURE 18

HPLC CHROMATOGRAM FROM A SAMPLE TAKEN LATE IN A PULSED ION EXCLUSION EXPERIMENT

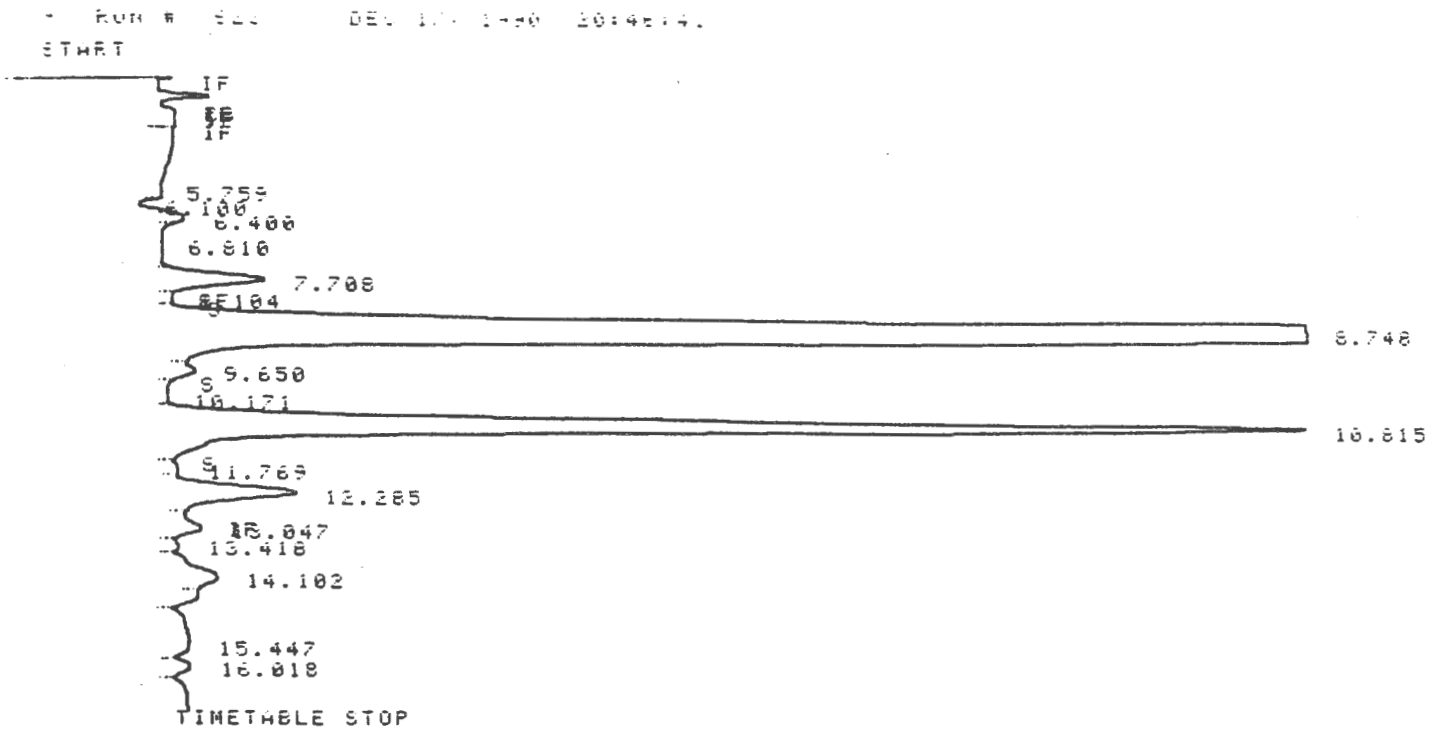


Figure 19. Molasses elution profile from a pulsed ion exclusion resin test experiment.

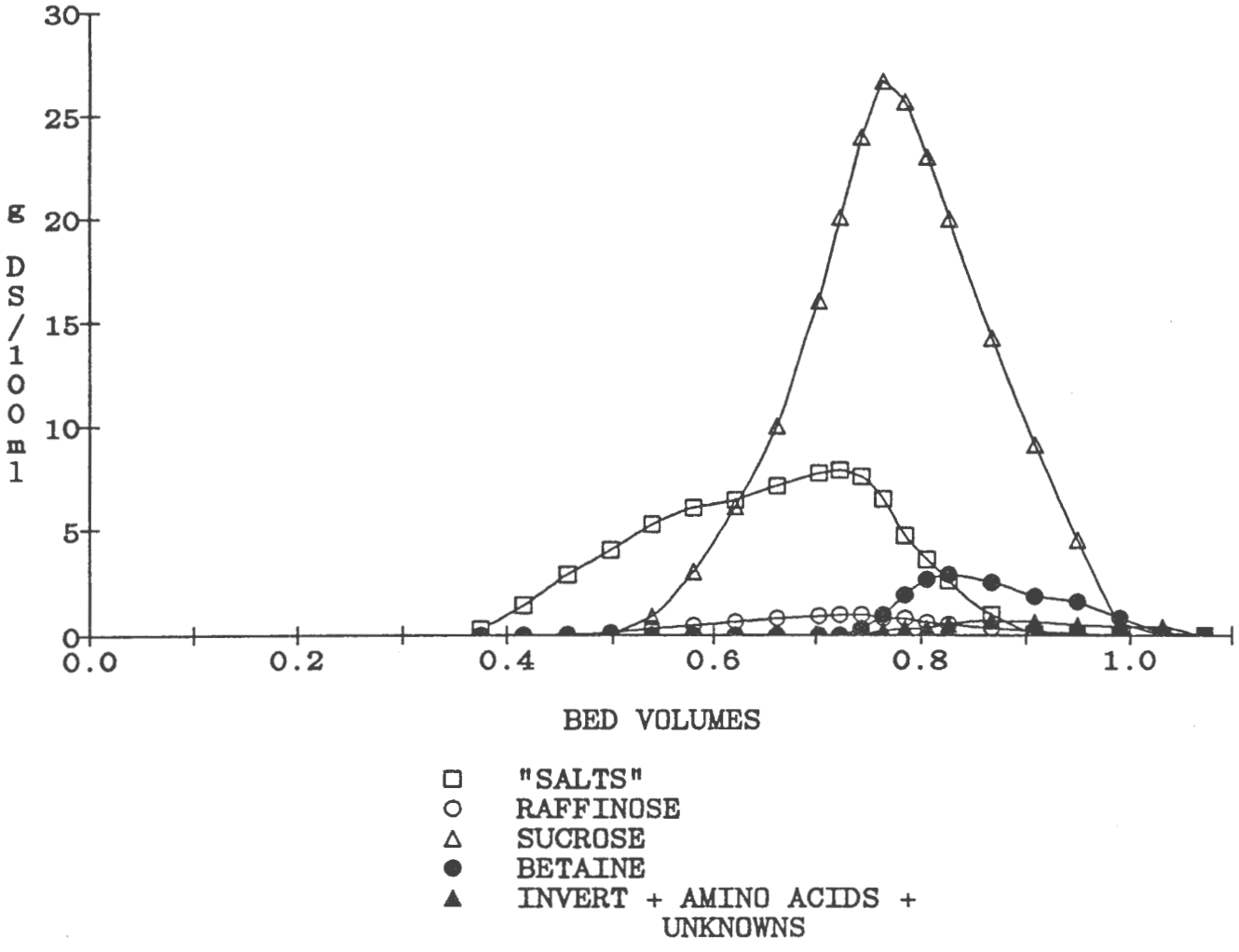


FIGURE 20

HPLC CHROMATOGRAM OF CANE MOLASSES 5.16 G DILUTED TO 250 ML