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THE SIMULTANEOUS ESTIMATION OF POLYHYDRIC ALCOHOLS AND SUGARS BY GAS–LIQUID CHROMATOGRAPHY

APPLICATIONS TO PERIODATE OXIDISED POLYSACCHARIDES*

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SUMMARY

A method has been developed whereby the following compounds may be determined quantitatively and simultaneously by the separation of their trimethylsilyl derivatives on an 8 ft. column containing 20 % SF 96 and using a thermal conductivity detector: ethylene glycol, glycerol, erythritol, threitol, arabinose, xylose, mannose, galactose and glucose. Model systems corresponding to the products expected by the periodate oxidation, borohydride reduction and hydrolysis of a variety of polysaccharides have been examined. Satisfactory separation of all components was obtained thus permitting their quantitative estimation. The need for using standardised conditions and molar response factors is stressed. Glycol aldehyde from C-1 and C-2 is shown not to interfere with the estimations.

A method is proposed for the estimation of the degree of polymerisation of oligosaccharides in general, and, in the case of hetero-oligosaccharides, simultaneously determining the nature of the reducing group.

A computer program for calculating molar response factors is given in an appendix.

One method of investigating the structure of a polysaccharide involves periodate oxidation, borohydride reduction and complete hydrolysis[1]. In the case of a linear 1–4 linked pentosan, for example, the products will be ethylene glycol from the nonreducing end and glycerol from the interior units. When any of the interior units carry side chains these units are immune to periodate oxidation and thus subsequent hydrolysis gives monosaccharides as well as polyhydric alcohols. Much useful information can be obtained from a knowledge of the ratio between nonreducing end groups, interior units and branch points. Two different analytical methods have normally been used in this connection after separation of the components by paper or thin-

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layer chromatography. Thus, polyhydric alcohols have been estimated by chromotropic acid[2] and monosaccharides by the phenolsulfuric acid[3] or other colorimetric method. The necessity of using two distinct analytical procedures in addition to the separations involved is tedious as well as introducing possibilities of error. The present paper reports a method using gas–liquid chromatography (GLC) whereby mixtures of polyhydric alcohols and monosaccharides may be analysed simultaneously and accurately.

TABLE I

POSSIBLE REACTION PRODUCTS FROM PERIODATE OXIDISED POLYSACCHARIDES

Polysaccharide	*Products*
Arabinoxylan	Ethylene glycol, glycerol, xylose arabinose
Glucomannan	Glycerol, erythritol, glucose, mannose
Galactoglucomannan	Glycerol, erythritol, threitol, glucose, mannose, galactose
Arabinogalactan	Ethylene glycol, glycerol, threitol, arabinose, galactose

This investigation arose from the need to have a simple method for the analysis of polysaccharides occurring in wood and Table I illustrates the possible products from four such typical polysaccharides.

The proposed method involves transformation of the mixture of sugars and polyhydric alcohols arising from the periodate oxidation of a polysaccharide into derivatives sufficiently volatile for separation by GLC. In order to test the viability of the method several test mixtures were prepared corresponding to the possible products shown in Table I. For each system studied the relative proportions of the components were varied over wide limits thus covering the different situations encountered in various naturally occurring polysaccharides. The components were separated as their trimethylsilyl derivatives[4] on a column of SF 96.

The separation of the products arising from an arabinogalactan is shown in Fig. 1a and the data obtainable from an arabinoxylan are given in Table II with the separation shown in Fig. 1b. Similarly the simulated glucomannan is represented by Table III and Fig. 2a. In like manner the simulated galactoglucomannan system is shown in Table IV and Fig. 2b. These results need little explanation except in the last case. According to the current view[5] of the structure of a wood galactoglucomannan one would not expect galactose to survive the periodate oxidation nor to give threitol. The model experiments reported here were an opportunity to test the validity of the proposed method and therefore the most general cases were studied. It is clearly seen from the figures that the separations are excellent and the agreement between calculated and found values is good. The worst separation in the present study is that between threitol and erythritol in the galactoglucomannan system but even here it is possible to calculate the relative amounts[6] in fair agreement with theory (Table V). This problem will only commonly arise where a polysaccharide contains galactose units linked 1–4 together with glucose and/or mannose similarly linked.

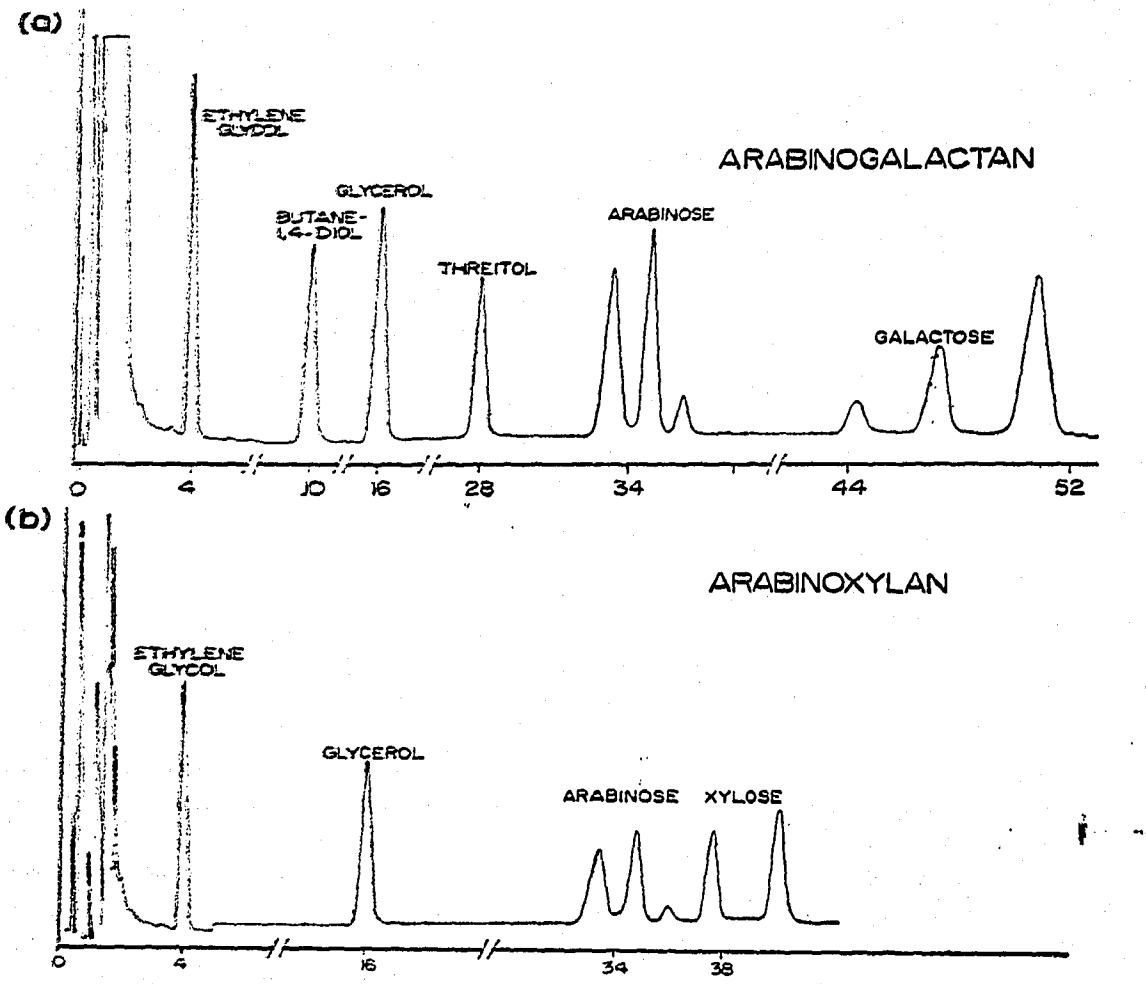

Fig. 1. Separation of products as trimethylsilyl derivatives from (a) an arabinogalactan, and (b) an arabinoxylan.

TABLE II

ANALYSIS OF REACTION PRODUCTS FROM AN ARABINOXYLAN

	% composition by weight			
	Ethylene glycol	*Glycerol*	*Arabinose*	*Xylose*
Found	15.6	18.8	29.3	36.0
Calculated	15.8	19.0	29.9	35.5
Found	30.5	38.1	29.2	3.15
Calculated	30.8	37.7	28.9	3.44
Found	42.9	5.20	3.31	48.5
Calculated	42.8	5.14	4.07	47.8

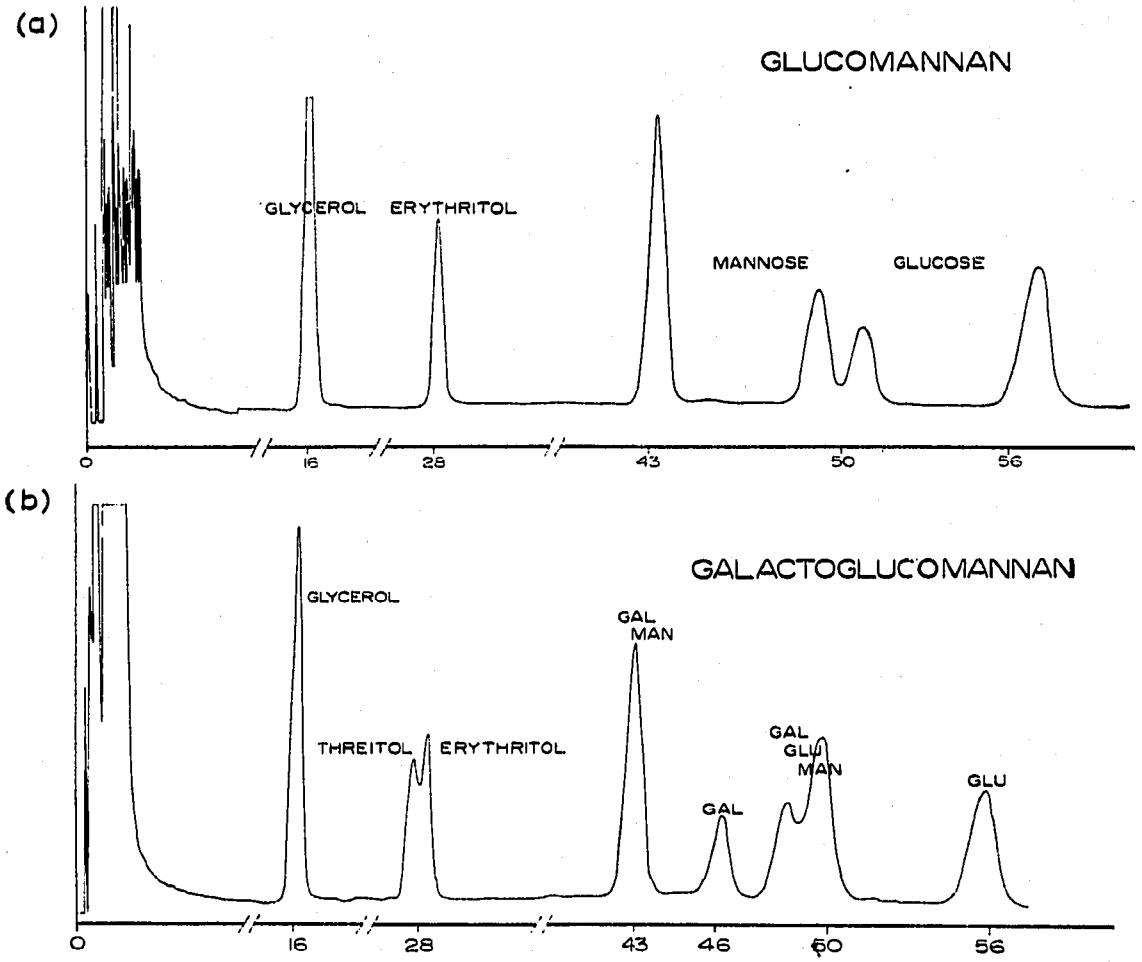

Fig. 2. Separation of products as trimethylsilyl derivatives from (a) a glucomannan, and (b) a galactoglucomannan.

TABLE III

ANALYSIS OF REACTION PRODUCTS OF A GLUCOMANNAN

	% composition by weight			
	Glycerol	*Erythritol*	*Mannose*	*Glucose*
Found	19.2	9.20	35.5	36.4
Calculated	19.2	8.70	36.0	36.4
Found	31.4	1.48	65.1	2.22
Calculated	33.2	1.46	62.8	3.11
Found	6.78	29.6	6.20	57.5
Calculated	6.34	28.6	5.85	59.2

TABLE IV

ANALYSIS OF REACTION PRODUCTS OF A GALACTOGLUCOMANNAN

	% composition by weight			
	Glycerol	*Galactose*	*Mannose*	*Glucose*
Found	17.6	25.7	29.38	28.2
Calculated	15.3	27.8	28.4	28.5
Found	4.12	53.2	39.1	3.71
Calculated	4.02	54.8	37.4	3.74
Found	4.08	2.85	38.6	54.7
Calculated	3.94	3.62	36.8	55.7
Found	—	47.5	2.61	50.2
Calculated	—	47.2	4.81	48.4

TABLE V

ANALYSIS OF THREITOL AND ERYTHRITOL MIXTURES

	% composition by weight	
	Threitol	*Erythritol*
Found	54.5	45.5
Calculated	51.5	48.5
Found	83.2	16.8
Calculated	84.1	15.9
Found	20.8	79.2
Calculated	17.5	82.5

TABLE VI

ANALYSIS OF SIMULATED CRUDE HEMICELLULOSE HYDROLYSATES

	% composition by weight				
	Arabinose	*Xylose*	*Galactose*	*Mannose*	*Glucose*
Found	17.9	23.1	20.4	16.95	22.0
Calculated	18.4	21.6	21.1	17.25	21.7
Found	17.75	22.6	21.6	34.0	4.07
Calculated	18.2	21.7	21.3	34.4	4.37
Found	3.15	39.8	3.03	16.62	39.0
Calculated	3.28	38.7	3.81	15.3	39.0
Found	33.8	4.07	38.5	3.64	20.0
Calculated	33.6	3.85	38.9	3.94	19.92

Comparisons of Figs. 1 and 2 will show that the pentoses have a lower retention time than galactose or mannose, the fastest of the hexoses studied. This means that the same column and procedure may be used to separate all of the polyhydric alcohols and sugars mentioned in Table I. More importantly it means that this same system may be used to separate and estimate all five of the sugars commonly occurring in wood polysaccharides. This separation has now been described by others whose results appeared while our work was in progress[7–10]. Particularly notable is the excellent Swedish paper to which further reference will be made[11]. The results presented in Table VI are further confirmation of the accuracy of this method.

The results quoted in Table II–VI were obtained using an instrument with a thermal conductivity detector and the systems studied contained free sugars. Each

TABLE VII

MOLAR RESPONSE FACTORS OF POLYOLS AND SUGARS

Compound	*Molar response factor*[a]
Ethylene glycol	0.832 ± 0.033
Glycerol	1.26 ± 0.014
Threitol	1.78 ± 0.088
Erythritol	1.72 ± 0.061
Arabinose	1.74 ± 0.061
Xylose	1.77 ± 0.064
Galactose	2.08 ± 0.126
Glucose	2.02 ± 0.091
Mannose	2.02 ± 0.059

[a] Butane-1,4-diol = 1.

of these points requires brief comment. Different substances cause such a detector to respond to a varying degree, thus for identical molar amounts of different compounds the areas under the peaks are not the same. It is therefore necessary to determine the molar response factor (m.r.f.) for each compound to be determined. This is done relative to an internal standard, which in the present work was butane-1,4-diol. This compound is readily available and has a convenient retention time, as Fig. 1a shows. The view has recently been expressed that an inert compound is preferable[12] and the terphenyl suggested would appear to be satisfactory here also. When working with compounds of similar molecular complexity it may be acceptable to assume that all the response factors are identical but when the compounds examined range from C_2 and C_6 this is incorrect[13], as shown by Table VII. These values are presented here to show the variation which may be expected. It is our experience that the exact m.r.f. applicable to any analysis depends on the precise experimental conditions used and that the value is influenced by such factors as the rate of temperature programming. A contrary view has been expressed[14] but at this time it would seem advisable for each worker to determine suitable response factors using exactly the same experimental conditions as in the analytical determinations. It would be most unwise to assume without verification that the figures quoted in Table VII are valid for other systems.

The second comment concerns the fact that in solution any one sugar exists as an equilibrium mixture of the anomers of the furanose and pyranose forms. When

certain peaks overlap it will be necessary to use a peak corresponding to one anomeri form as a measure of the total amount of that particular sugar. This has been clearly discussed in the Swedish paper cited previously[11]. Since the relative equilibrium concentrations of the different forms are highly dependent on the solvent used[15] it follows that the figures quoted in that paper[11] are only valid for that particular system. For accurate results it is thus necessary for each worker to adopt a standardised procedure for the treatment of polysaccharide hydrolysates. The composition of the sugar peaks obtained in the present work is given in Table VIII and the equilibrium composition of the solutions is shown in Table IX.

The analysis of a periodate oxidised glucomannan by gas–liquid chromatography of the derived erythritol and glycerol acetates was reported in 1960 by BISHOP AND COOPER[16] and more recently ZINBO AND TIMELL[17] have used the silyl derivatives for the analysis of a xylan. In neither case has the fate of glycol aldehyde, obtainable from carbons one and two, been studied, although the former authors noted the existance of an unidentified peak with a greater retention time than glycerol triacetate and suggested this might be due to glycol aldehyde diacetate.

TABLE VIII

COMPOSITION OF PEAKS IN GAS PHASE CHROMATOGRAM OF A MIXTURE OF ARABINOSE, XYLOSE, GALACTOSE, GLUCOSE AND MANNOSE

Major component	*Minor component(s)*
Arabinose 1,2,3	Xylose 1 + 2
Xylose 3 + 4	—
Mannose 1	Galactose 1
Galactose 2	—
Galactose 3	Mannose 2 Glucose 1
Glucose 2	—

TABLE IX

NUMBER OF SUGAR ANOMERS DETECTED BY GAS–LIQUID CHROMATOGRAPHY AND THE PERCENTAGE COMPOSITION OF EQUILIBRIUM SOLUTIONS

Sugar	*No. of anomers*	*Percentage composition*[a]	
Arabinose	3		
Xylose	4	1 + 2	3.58 ± 0.28
		3 + 4	96.4 ± 0.28
Galactose	3	1	8.19 ± 0.80
		2	29.3 ± 1.27
		3	62.6 ± 1.02
Mannose	2	1	74.5 ± 1.21
		2	25.5 ± 1.21
Glucose	2[b]	1	38.3 ± 0.33
		2	61.7 ± 0.36

[a] Anomers numbered in order of elution.
[b] It is possible that glucose shows a third anomer present in very small concentration.

In an attempt to discover what happens to the glycol aldehyde a solution of this compound in pyridine was silylated and examined using the same chromatographic conditions as before. There resulted a single peak indistinguishable from glycerol and attributed to the dimer[18]. When an aqueous solution of glycol aldehyde was concentrated to dryness and silylated two peaks were obtained. One of these was indistinguishable from glycerol and the other had a shorter retention time, presumably the monomer. If an aqueous solution of glycol aldehyde was first reduced with sodium borohydride only one peak corresponding to ethylene glycol was obtained. This suggested one way of dealing with the problem. When, however, synthetic mixtures of glycol aldehyde and the neutral products listed in Table I were analysed, several new and unexpected peaks were obtained. These peaks were assumed to be due to acetals formed between glycol aldehyde and the polyhydric alcohols[18]. These observations did not accord with those made on periodate oxidised amylose, where the amount of glycol aldehyde should equal the sum of glycerol and erythritol. Furthermore, the gas chromatographic analysis of the products from mesquite gum gave results in good agreement with those obtained by paper chromatographic separations[19].

The difference in treatment of the model systems and the polysaccharides was an acid hydrolysis step. When the model systems containing glycol aldehyde were heated at 100° for 24 h with 1 *N* sulfuric acid and then analysed no unidentifiable peaks were obtained. There was no change in the ratio of the sugars present and the solutions were dark brown and contained a brown precipitate. There was a small increase in the glycerol concentration and a small decrease in ethylene glycol. The former is no doubt due to traces of glycol aldehyde dimer since a separate experiment in which glycol aldehyde alone was treated with acid gave a small peak in the glycerol region. The aparent loss of ethylene glycol cannot at present be explained since it was verified that this compound is not lost on ion exchange resins nor on concentration of its aqueous solutions.

It is concluded that the glycol aldehyde is almost entirely destroyed in the hydrolysis of the polyalcohol and is thus not a serious factor in such analyses. Where a highly accurate value for glycerol is necessary this could be obtained by reduction of the hydrolysate with borohydride before analysis to convert any remaining glycol aldehyde dimer to ethylene glycol.

The first applications of gas–liquid chromatography to polysaccharide chemistry were reported for methylated sugars[20] because of their volatility. This technique is now widely applied and has also been used in conjunction with periodate degradation to facilitate the resolution of a complex mixture of isomeric sugars[21–23]. With the introduction of trimethylsilylation by SWEELEY *et al.*[4] this technique has been extended to the separation of sugars[7–11] and the glycosylalditols obtained by Smith degradation (ref. 23 and refs. therein). The present paper completes the types of polysaccharide analysis which may conveniently be studied by this method. Although the work reported here arose out of our interests in wood polysaccharides it is clear that the procedure is a general one and may be used for many other types of polysaccharides. It has already been applied with success to a study of sapote gum[24] and mesquite gum[19]. Work is now in progress to extend the method to systems containing deoxy and amino sugars thus permitting the study of a wider variety of polysaccharides.

The fact that the same chromatographic system will separate polyols and sugars

has suggested a further use of this technique. In the investigation of oligosaccharides it is necessary to know the degree of polymerisation and, for hetero-oligosaccharides, the nature of the reducing group. By hydrolysis of the borohydride reduced oligosaccharide and examination of the hydrolysate by gas–liquid chromatography it is possible to identify the polyhydric alcohol, from the reducing group, and to determine the ratio of this compound to the sugar residues and thus the degree of polymerisation[25].

EXPERIMENTAL

Chromatography was carried out on an F and M 720 dual column instrument fitted with a thermal conductivity detector. The two columns were 8 ft. × 0.25 in. coiled copper columns packed with equal weights (to within 20 mg) of 20 % SF 96 on 60–80 mesh Diatoport S. The columns were held isothermally at 130° for 6 min and then programmed at 3° per min to hold at 220°. When analysing for sugars only (Table VI) the program was started at 190° and immediately programmed at 2° per min to hold at 220°. The injection port was 270°, the detector block 295° and the helium flow 88 ml per min (6.8 sec for 10 ml).

For simple systems other columns may be used. Thus the silyl derivatives of ethylene glycol, glycerol and methyl β-D-glucopyranoside may be separated on a 2 ft. × 0.25 in. column of SE 30. This column was run isothermally at 70° until the ethylene glycol emerged and then programmed at 10° per min to hold at 230°.

Peak areas were measured initially with a disc integrator with suitable correction for baseline drift. In later work an Infotronics digital integrator was used.

Sugars were dissolved in water containing a small amount of chloroform and allowed to come to equilibrium. Aliquots were removed and concentrated to dryness at 40° in a rotary evaporator. Solutions of the polyhydric alcohols in anhydrous pyridine were added to give the test solutions reported in Tables II–VI. These solutions were immediately silylated with a large excess of trimethylsilyl chloride and hexamethyldisilazane and after 5 min shaking portions were injected directly. The values quoted in the tables represent the mean of at least five determinations.

For the determination of molar response factors separate solutions of the individual compounds with butane-1,4-diol were prepared. In the case of the sugars these runs also served to determine the relative concentrations of the different forms at equilibrium.

In preliminary experiments it was ascertained that none of the compounds involved were lost on passage through Amberlite IR 120 and Duolite A4 resins nor on concentration of their aqueous solutions.

APPENDIX

Programme for calculating molar response factors (Fortran)

```
1     DIMENSION FMR(10), EXP(10,10), ANS(10,10), AVE(10), AVEDEV(10)
2     READ 106,NSAMPL
3     DO 1 KK=1,NSAMPL
4     READ 100, NPTS,NSETS
5     READ 101, (FMR(K), K=1,NPTS)
6     DO 2 K=1, NSETS
```

```
 7        READ 105, STAND
10        READ 101, (EXP(L,K), L=1, NPTS)
11        PRINT 102, STAND
12        DO 4 L=1, NPTS
13        ANS(L,K) = EXP(L,K)/(STAND*FMR(L))
14        PRINT 103, EXP(L,K), ANS(L,K)
15      4 CONTINUE
16      2 CONTINUE
17        DO 6 K=1, NPTS
20        SUM=0.0
21        DO 8 L=1, NSETS
22      8 SUM= SUM+ ANS(K,L)
23        AVE(K)=SUM/FLOAT(NSETS)
24      6 CONTINUE
25        PRINT 104
26        DO 10 K=1, NPTS
27        SUM=0.0
30        DO 12 L=1, NSETS
31     12 SUM= SUM + (ANS(K,L)- AVE(K))**2
32        AVEDEV(K) = SQRT((SUM)/(FLOAT(NSETS)-1.0))
33        PRINT 103, AVE(K),AVEDEV(K)
34     10 CONTINUE
35      1 CONTINUE
36    100 FORMAT(215)
37    101 FORMAT(8F10.0)
40    102 FORMAT(1HO,10H STANDARD=F15.3,//1X,21H EXPERIMENTAL POINTS,5X,1
          14 H NORMALIZATION)
41    103 FORMAT (//1X, F21.8, F19.8)
42    104 FORMAT (1HO, 20H AVERAGE OF POINTS, 18H AVERAGE DEVIATION)
43    105 FORMAT (F10.8)
44    106 FORMAT (15)
45        STOP
46        END
```

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