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THE RELIABILITY OF PEAK AREA MEASUREMENTS

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SUMMARY

The reliability of various methods of area measurement commonly employed is assessed, for peaks of varying symmetry. Some quantitative data on the use of the new technique of interval programmed integration are presented, and the value of the technique is compared with existing methods. A comparison is also made between the reliability of quantitative data based on peak areas using a differential detector and step heights using an integral detector.

INTRODUCTION

The majority of gas chromatographic detectors have a differential response, and the composition of a mixture can be estimated either by measuring peak heights or peak areas, after calibration of the detector for the compounds under analysis. The reliability of a quantitative analysis will depend a great deal on the accuracy and precision to which the measurements can be made. A number of papers have been published¹⁻⁴ in which various methods of area measurement have been compared. It is not always possible to select the most suitable method for the problem in hand, based on the information presented in these papers. Most comparisons have been carried out on Gaussian or near Gaussian peaks, where several techniques are found to be fairly satisfactory. It is the purpose of this paper to compare several methods of area measurement with a new technique, for the common situations in which all peaks are resolved and fairly symmetrical, and for chromatograms exhibiting drifting baselines. A comparison is also made between the reliability of measurements of peak areas resulting from a differential detector response, and step heights arising from an integral detector.

DISCUSSION

Using a differential detector the composition of a mixture can be estimated either by measuring peak heights or peak areas. It is more fundamental to measure peak areas in that the total area is proportional to the total amount of material present: this method is normally used where the detector response is a simple function of a stoicheometric property of the components. However, in cases where the detector

response is not predictable it is often adequate to use peak height measurements. The use of peak height which represents the concentration of the component at a given time, demands constant column operating conditions. For example, changes in the operating temperature during an analysis will affect retention times, and hence peak heights are affected, while the corresponding peak areas remain constant.

Commonly used methods of peak area measurement are: (1) cutting out and weighing, (2) planimetry, (3) triangulation, (4) peak height \times width at half height, (5) peak height \times retention distance, and (6) automatic integration. Of these, method 1 has the obvious disadvantages of destroying the chromatogram and of depending on the uniformity of the chart paper. Methods 2, 3, and 4 have been assessed by SCOTT AND GRANT², who concluded that method 4 was the most precise for symmetrical peaks. A comparison has been made³ between methods 1, 2, and 3 for Gaussian peaks of varying height and width at half height ratios. The relative error was greatest for very sharp peaks, broad flat peaks, and those of small area. Minimum error was found for values lying between 1 and 4. It was shown that triangulation offered no advantages, and planimetry was less precise than height/width measurements for small areas but was capable of giving better precision for large areas. It was also found that a significant improvement in precision was obtained when peak width measurements were made much below the half height position. The peaks were not derived from a gas chromatographic system, but were manually constructed.

Method 5 assumes that column performance is independent of the substances under analysis, and that the calibration constants for the detector are proportional to retention volumes.

Method 6, which includes electromechanical and electronic methods of integration, produces results rapidly and demands a minimum of operator time. Electronic methods are costly, particularly when designed to cope with baseline drift.

The precision of an electromechanical and an electronic method of area measurement was compared by EMERY⁴. Most painstaking replicate analyses were carried out, and the resulting peak areas measured using a ball and disc integrator and a relatively expensive electronic integrator. The variation of the areas measured with the electronic integrator was about half that of the corresponding areas measured by the ball and disc integrator. The precision of manual peak height measurements carried out on the same chromatograms lay between the precision of these two methods. The effect of relative component concentration on precision, using only the electronic integrator was measured. Not unexpectedly precision, expressed as the coefficient of variation, improved with increasing proportion of component in the mixture.

DEANS⁵ has examined the reliability of using peak height measurements alone to determine the percentage composition of mixtures. The results are compared with those obtained using electronic integrators, on a six-component mixture which included a partially resolved pair of components. All integrators tested, embracing the simplest to those incorporating drift correction devices, gave similar results which were significantly worse than the precision of the height measurements. The measurements were made on peaks of large height/width ratios, and using a system incorporating gas pressure controllers.

It must be recognised that a digital integrator, used conventionally, cannot begin counting until there has been a finite baseline shift, typically 0.2% full-scale deflection (f.s.d.) for a completely noise free signal. As a result the integrator will not

take into account any fraction of the peak area below this value. This is insignificant for symmetrical peaks, but could lead to a substantial error in the case of a peak exhibiting a long tail. For a simple digital integrator it is necessary to maintain a completely stable baseline throughout a run, and any drift in baseline above the 0.2% level will contribute to the peak areas. Recently a method has been described by YEEND⁶, in which the integrator is deliberately set above zero when there is zero signal from the gas chromatographic detector. By means of a simple modification, described below, the number of integrator counts at fixed time intervals is recorded. It is thus possible to follow any drift in baseline by observing the change in count rate when no components are being eluted. On elution of a component the count rate of the integrator will change in the normal manner, but it will continue to print out at the same time interval. The peak area is obtained from the sum of the counts, above the zero signal count rate. The method has several advantages over the conventional operating procedure:

- (i) detector baseline drift and integrator zero drift can be taken into account in peak area calculations;
- (ii) there is no threshold below which the integrator does not count;
- (iii) the integrator will count negative peaks without the need for a signal polarity reversal switch, provided that the zero count rate is set sufficiently high. In the conventional operating mode the integrator will not count negative peaks, and a polarity reversing switch can only be used satisfactorily if peaks are well separated;
- (iv) the performance of the device should not be significantly affected by peak shape, and should not deteriorate for peaks exhibiting excessive tails.

Some results using this technique (interval programmed integration) are presented below.

Many of the difficulties in the integration of a differential signal would be avoided by the use of an integral detector, but few practical designs are commercially available. The precision and accuracy attainable with the mass detector have been the subject of recent publications⁷⁻⁹. For the purposes of comparison some further measurements were made and the results are presented below (Table IV).

EXPERIMENTAL

Experiments were undertaken to compare the reliability of measurement of peak areas by two methods commonly regarded as fairly satisfactory, namely peak weight, and peak height \times width at half height measurements. In addition a digital and mechanical integrator were used. The initial study was limited to a comparison of the reliability of various methods for completely resolved peaks, and peaks where only a small amount of distortion was observed. It is necessary to separate effects not caused by errors in area measurement. The results must be independent of errors caused by syringe delivery etc. This can be accomplished by injecting nominally the same amount of material of a two-component mixture several times, and expressing peak areas as percentage composition. The true composition of the mixture is not required to calculate the standard deviation of the results (precision), but it is required to find the bias (accuracy). The accuracy and precision of step height measurements, using the mass detector were measured under the same conditions. Mixtures of known

composition were prepared, the details of which are given in Table III. The first series of runs was performed using a Shandon KG2 Chromatograph fitted with a Martin gas density balance and mass detector. The output of the gas density balance was connected to a potentiometric recorder in the usual manner, and in addition to a digital integrator.* A total of 31 runs were performed, each using a nominal sample size of $3\ \mu\text{l}$. The peak areas of all runs were calculated from peak height and width measurements, and the results are given in Table IV. Peak heights, and step heights were of the order of 5 cm. The areas of fourteen of the runs were measured by the digital integrator used in the conventional manner. In this mode the integrator cannot begin counting until there is a finite baseline shift, which was set for this experiment at 0.5% f.s.d. Fifteen of the runs were carried out using the integrator in the interval programmed mode. The integrator was set above the zero count position when no signal was received from the gas density balance, and by means of a micro-switch printed out the number of counts at fixed intervals of time. The device used to trip the print-out mechanism of the integrator was made in the laboratory. To the shaft of a synchronous motor, geared to give a speed of rotation of 2 r.p.m. at 50 cycles sec^{-1} , was attached a $2\frac{1}{2}$ in. diameter disc. Near the circumference of the disc were attached, at equal distances apart, small protrusions made from 4BA screw heads. A wiping contact was positioned such that each screw head in turn was touched by the wiper as the disc rotated, thus momentarily completing an electrical circuit and causing the integrator to print. With four contacts spaced at intervals of 90° , print out will occur every 7.5 sec. Provision was made for print out at other time intervals by changing the number of contacts, and by using a 1 r.p.m. motor. For the device to be satisfactory the following conditions must be fulfilled: (i) mains frequency must not fluctuate significantly, (ii) the distance between each contact must be identical, and (iii) the wiper must always make contact at the same point on each head.

The performance of the device was checked by timing ten contacts starting at each contact point in turn. The results are given in Table I. The performance was regarded as satisfactory.

The peak areas of fifteen of the runs were obtained by cutting out the peaks and weighing them on an ordinary laboratory four-place balance. The repeatability of weighing a single peak was measured: no variation of results measured to 0.1 mg

TABLE I

VARIATIONS IN PRINT OUT TIMES

<i>Contact No.</i>	<i>Time for ten contacts (sec)</i>	<i>Time per contact (sec)</i>
1	74.8	7.48
2	74.9	7.49
3	75.0	7.50
4	75.0	7.50
1	75.1	7.51
2	75.1	7.51

* Honeywell Precision Integrator, Honeywell Controls Ltd., Brentford, Middlesex, Great Britain.

TABLE II

VARIATIONS IN CHART PAPER WEIGHT

 σ = standard deviation; V = coefficient of variation.

No. of squares	Mean weight (mg)	σ (mg)	V (%)
6	12.00	0.36	3.0
6	11.62	0.21	1.8

(1% of the total weight) was observed. The variation of weight of the chart paper over the length containing the runs was measured by cutting out small squares of equal size, about the weight of a typical peak. The results are given in Table II.

Published data³ on the coefficient of variation of recorder chart paper weight gives a value of 2.5% for the same area of paper.

Variations in chart speed during a run will affect peak areas obtained by all methods except the digital integrator: no measurable variations in chart speed were observed, and the accuracy of the speed on each setting was quite satisfactory.

The performance of a ball and disc integrator* was assessed. The integrator was attached to a potentiometric recorder, and a similar mixture to that used above was analysed using a Pye 104 chromatograph fitted with a flame ionisation detector. Sample details are given in Table III and results in Table IV.

Data published by SCOTT AND GRANT² have been recalculated in the form used in the present work and are given at the foot of Table IV.

TABLE III

SAMPLE DETAILS

GDB = gas density balance; MD = mass detector; FID = flame ionisation detector.

Series No.	Peak area (cm ²)	Sample composition		Area correction factors		
		Compound	True % weight	GDB	MD	FID
1	1.7	<i>n</i> -Propyl alcohol	44.94	1.87	1.00	—
	2.8	Methyl <i>n</i> -propyl ketone	55.06	1.48	1.00	—
2		<i>n</i> -Propyl alcohol	47.07	—	—	1.64
		Methyl <i>n</i> -propyl ketone	52.93	—	—	1.41

The most satisfactory peak area measurements were obtained using the digital integrator, but contrary to expectations, better results were obtained using the integrator in the conventional mode. The peak areas under study were "typical" rather than ideal peaks. For a very broad and low peak it would be expected that the results obtained using the integrator conventionally would become poorer, but that the performance of the integrator using the fixed interval print-out technique would

* Disc Series 200 Integrator, Disc Instruments Ltd., Hemel Hempstead, Hertfordshire, Great Britain.

TABLE IV

RELIABILITY OF AREA MEASUREMENTS

n = No. of determinations; \bar{x} = mean % weight of *n*-propyl alcohol; σ = standard deviation; V = coefficient of variation.

Method of area measurement	Series	n	\bar{x}	σ	V	Bias	% bias
Peak height/width	1	31	43.41	1.4	2.9	-1.53	3.4
Peak weight	1	15	44.90	2.1	4.7	-0.04	0.1
Digital integrator: conventional	1	14	45.12	1.2	2.5	+0.18	0.4
fixed interval print out	1	15	46.20	1.1	3.0	+1.26	2.8
Step height (mass detector)	1	8	45.36	0.9	2.0	+0.43	1.0
Ball and disc integrator	2	18	45.54	1.0	2.2	+2.25	4.8
Peak width and height	ref. 2	20	23.37	0.28	1.2		
Triangulation	ref. 2	20	22.56	0.60	2.7		
Planimetry	ref. 2	16	23.49	1.23	5.2		

be unaffected. A similar result would occur for a peak with a long tail, irrespective of its height. The precision of the results obtained using the ball and disc integrator was equally satisfactory. Peak weight determinations gave a very accurate result, but the coefficient of variation was high due in part to variations in paper weight (Table II). The mass detector gave results as good as the best peak area results.

The effect of a drifting baseline on the precision and accuracy of several methods of area measurement was measured, using a Pye 104 chromatograph fitted with a katharometer and using helium as carrier gas. Each area was measured manually using the peak height/width method, and by two digital integrators connected in parallel, one used in the conventional mode* and the other in the interval programmed mode**. The interval timer was made from a commercially available timer*** fitted with a synchronous motor geared to give 6 r.p.m. A single wiping contact on the motor shaft tripped a micro-switch once per revolution, giving a print out interval of 10.0 sec.

TABLE V

SAMPLE DETAILS

Series No.	Peak area (cm ²)	Sample composition		Drift % f.s.d./min)	Area correction factor
		Compound	True % composition		
3	4.5	<i>n</i> -Propyl alcohol	42.69	zero	0.72
	5.0	Isoamyl alcohol	57.31	zero	0.81
4	4.5	<i>n</i> -Propyl alcohol	42.69	zero	0.72
	5.0	Isoamyl alcohol	57.31	0.38	0.81
5	4.5	<i>n</i> -Propyl alcohol	42.71	1.3	0.72
	5.0	Isoamyl alcohol	57.29	0.23	0.81

* Kent Chromalog I Integrator, Kent Instruments Ltd., Luton, Bedfordshire, Great Britain.

** Honeywell Precision Integrator, Honeywell Controls Ltd., Brentford, Middlesex, Great Britain.

*** Constant cycle sequence timer type 221-1, motor type 392, Crouzet (England) Ltd., Brentford, Middlesex, Great Britain.

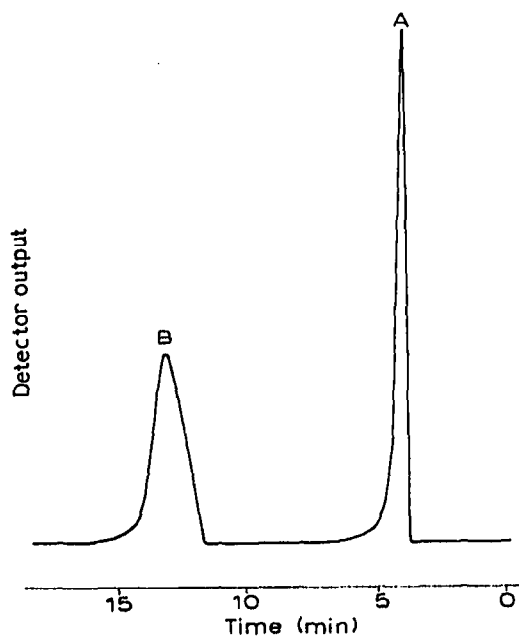


Fig. 1. Chromatogram of a two-component mixture (series 3). A = *n*-propyl alcohol; B = isoamyl alcohol.

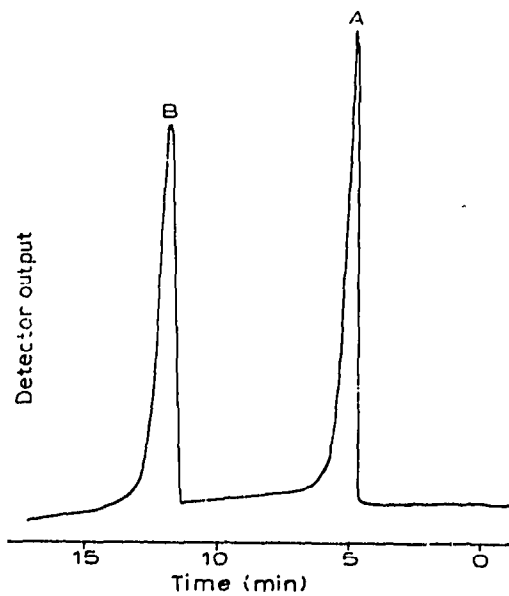


Fig. 2. Chromatogram of a two-component mixture (series 4). A = *n*-propyl alcohol; B = isoamyl alcohol.

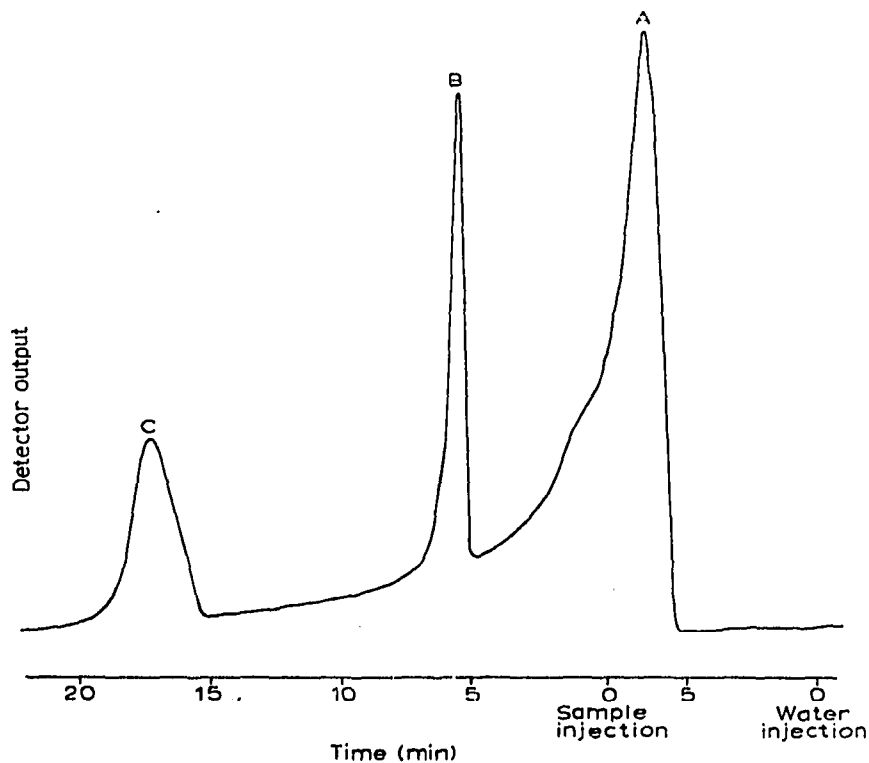


Fig. 3. Chromatogram of a two-component mixture (series 5). A = water; B = *n*-propyl alcohol; C = isoamyl alcohol.

Provision was made for several contacts per revolution and for the interchange of motors of different speeds of rotation. Several sets of runs were performed, each consisting of twenty replicate analyses, the chromatograms of each set exhibiting different degrees of drift (see Figs. 1-3). All peaks exhibited a tail. Reproducibly drifting baselines were induced, in the case of series 4 by temperature programming, and in the case of series 5 by injecting 5 μ l of water 8.0 min before the injection of each sample so that the sample was eluted on the tail of the water peak. A comparison of the reliability of the three methods is given in Table VI. Details of the samples analysed are given in Table V.

TABLE VI

RELIABILITY OF AREA MEASUREMENTS (DRIFTING BASELINE)

Method	Series	\bar{x}	σ	V	Bias	% bias
Peak height/width	3	42.41	0.95	2.18	-0.27	0.63
	4	41.07	1.25	3.03	-1.62	3.79
	5	43.01	0.61	1.42	+0.31	0.73
Integrator-conventional mode	3	43.75	0.18	0.41	+1.07	2.50
	4	45.81	1.33	2.90	+3.13	7.32
	5	49.52	7.17	14.48	+7.82	18.4
Integrator-interval programmed mode	3	44.19	0.31	0.71	+1.50	3.52
	4	42.62	0.78	1.82	-0.06	0.14
	5	45.34	0.46	1.01	+2.64	6.17

The precision of the manual method was significantly worse than that of the integrators for a straight baseline (series 3), but did not deteriorate as drift increased. The results using the integrator in the conventional mode became progressively worse as baseline drift increased and were excessively poor for series 5 runs. Using the interval programmed technique precision was unaffected by baseline drift and in all cases was satisfactory.

TABLE VII

SAMPLE DETAILS (SERIES 6)

Compound	% weight	Baseline change (% f.s.d./min)	Area correction factor
Solute 4% {	<i>n</i> -Butyl alcohol	47.65	1.7
	Methyl isobutyl ketone	52.35	2.6
	100.00		1.15 0.86
Solvent 96% {	Acetone	20	
	Water	80	
	100		

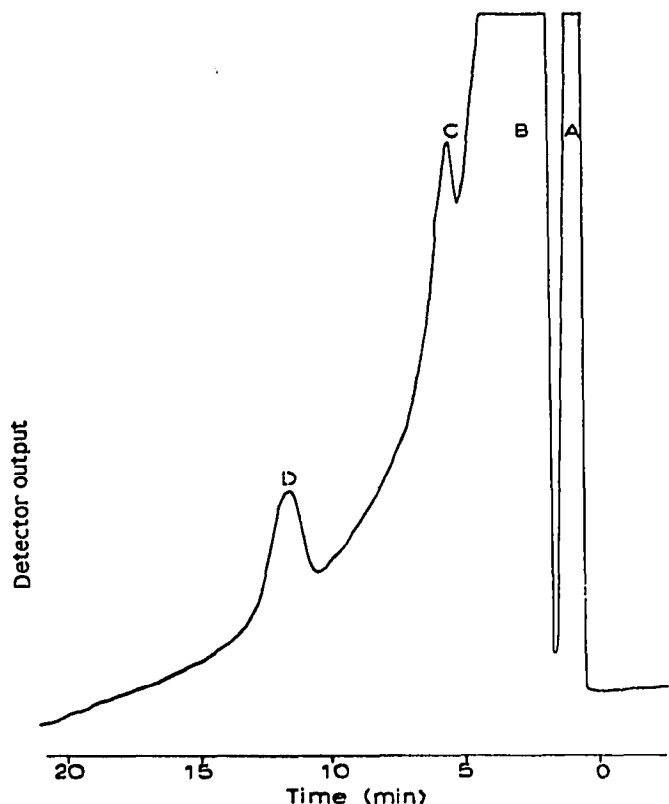


Fig. 4. Chromatogram of a two-component mixture in solvent (series 6). A = acetone; B = water; C = *n*-butyl alcohol; D = methyl isobutyl ketone.

The difficulty of estimating the proportions of components eluted on the tail of a solvent is illustrated in Fig. 4. Clearly it is not possible to use peak height/width measurements since the leading edge of the first peak is only a third of the height of the tailing edge. Peak height alone, or peak weight could be used, bearing in mind the disadvantages previously noted. The new technique of interval programmed integration may offer advantages, and this method was compared with those mentioned above. Sample details are given in Table VII, and results in Table VIII.

Elution of the solutes in the presence of excess solvents will result in a change in response of the detector. Previous results (Table IV) showed that although the peak weight method is not very precise it gives good accuracy. The relative response of the detector was calculated from peak weight measurements assuming zero bias and was

TABLE VIII

RELIABILITY OF MEASUREMENTS (IN THE PRESENCE OF SOLVENT)

Method	σ	V	Bias	% bias
Peak weight	1.58	3.33	—	—
Peak height	0.82	1.40	—	—
Interval programmed integration	4.09	11.42	-2.57	5.39

used to find the bias of the integrator results. Response factors based on height measurements will be different, so that it was not possible to compare the accuracy of the three methods. However, in view of the nature of the chromatograms and the fact that the solute represented only 4% of the solution, the accuracy of the interval programmed method is acceptable. Although precision in this case is significantly worse than using the other methods, in the long-term interval programmed integration may be preferable since the limitations associated with the other methods are not inherent in interval programming.

CONCLUSIONS

No single method of area measurement is satisfactory for all types of gas chromatographic peak. Triangulation and planimetry offer no advantages over the other methods. The peak weight method is useful for grossly asymmetric peaks, and gives good accuracy, although precision is rather poor. Quantitative results obtained using the peak height/width method are satisfactory even for chromatograms exhibiting a small degree of baseline drift. The ball and disc, and digital integrators, were satisfactory for symmetrical peaks with a stable baseline. The performance of a digital integrator (without baseline drift correction facilities) used in the conventional mode becomes progressively worse as baseline drift increases. Interval programmed integration was found to give good results not significantly affected by tailing peaks or drifting baselines, and was able to give acceptable results even for an extreme case in which minor amounts of solute were eluted in the presence of a solvent. The technique offers a means of obtaining good precision and accuracy for a variety of peak geometries, by a simple modification to a basic digital integrator. Quantitative analyses based on step height measurements, using an integral detector, were entirely satisfactory.

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