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DEPENDENCE OF THE SPLITTING RATIO ON COLUMN TEMPERATURE IN SPLIT INJECTION CAPILLARY GAS CHROMATOGRAPHY

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SUMMARY

The amount of sample entering a capillary column during split injection increases considerably if the major component (*e.g.*, the solvent) recondenses in the column inlet. Owing to the reduction in volume during the recondensation, additional sample vapour is sucked into the column. The resulting true splitting ratio may deviate from the pre-set ratio by a factor exceeding 30. Recondensation occurs at column temperatures below the boiling point of the major component (usually the solvent). It is favoured by a short distance between the tip of the syringe needle and the column entrance, large sample volumes, narrow glass inserts in the injector and other factors that limit the dilution of the sample vapour with carrier gas prior to the splitting process. The deviation of the true from the pre-set splitting ratio is an important source of error in quantitation based on the external standard method because this deviation may depend sensitively on critical parameters. It may cause high standard deviations, and also the true splitting ratio may be different for, *e.g.*, the calibration mixture and the sample, creating systematic errors that are difficult to detect.

INTRODUCTION

We consider it still to be important to investigate the processes involved in split injection because we do not know of a replacement for this injection technique. For many applications split injection is the most convenient sampling method, as it allows one to inject mixtures nearly regardless of the solvent, at any column temperature, with little risk of disturbing solvent effects[1,2] or of band broadening due to slow sample transfer from the injector to the column. For a number of samples it may even be nearly impossible to replace split injection.

The apparent simplicity of the split sampling method conflicts with the many problems that arise as soon as accurate analytical results from other than the easiest samples are required[3]. One of the problems is concerned with the splitting ratio. The pre-set splitting ratio[4], adjusted by the ratio of the carrier gas flow-rates passing by and entering the column, is seldom equal to the true splitting ratio, *i.e.*, the proportion of the sample reaching the column. The true splitting ratio, obtained by division

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of the peak area resulting from a splitless injection by the peak area resulting from a split injection, is generally lower than the pre-set splitting ratio, so that more sample enters the column than expected. This deviation of the true from the pre-set splitting ratio is important for the following reasons:

(1) In general it is not possible to calculate the amount of substance entering the column by division of the total sample size by the pre-set splitting ratio. Errors of factors up to 50 may occur.

(2) As the deviation of the true from the pre-set splitting ratio is often poorly reproducible, quantitation by the external standard method is severely hindered. There is a considerable risk of experiencing systematic errors, *e.g.*, if the true splitting ratio of the calibration mixture is different from the splitting ratio obtained for the sample.

(3) Discrimination, *i.e.*, non-linearity of the splitting process, is partly due to a splitting ratio that fluctuates during the period of time during which the sample is split, thus due to the mechanism which also causes the deviation of the true from the pre-set splitting ratio[3]. When using the internal standard method, the true splitting ratio might be considered to be of little importance. However, it is important as soon as a change in the splitting ratio alters the discrimination pattern of mixtures with a wide range of boiling points.

Although we do not have a solution to offer for these problems, we consider it to be important to describe the sources of errors and their dependence on various parameters in order to keep them under control. Even if using a calibration method that supposedly corrects for all errors, it is important to know about the possible sources of errors in order to know the critical parameters. Reproducibility, the factor which determines the accuracy of results obtained by calibration procedures, is usually tested by re-injecting the same mixture of standards. This method does not give any information about the extent of the deviations involved and it is not known whether these deviations are kept constant when injecting the real sample, possibly including a slight change in a number of unrecognized critical parameters.

In a previous paper[4] we reported on the effects on the splitting ratio caused by the pressure wave initiated upon introducing a large amount of sample vapour into a relatively small injector cavity. The fluctuation of the pressure inside the injector changes all of the gas flow-rates, including the column and the split flow-rates, which determine the true splitting ratio. These flow-rates do not change simultaneously or by the same ratio and therefore they change the splitting ratio in a complex manner. In that paper we did not consider another cause which results in a deviation of the true from the pre-set splitting ratio, *viz.*, recondensation of the sample in the column inlet.

EXPERIMENTAL AND RESULTS

Recondensation of the sample

Some experimental results showing the dependence of the splitting ratio on the column temperature during the injection are given in Fig. 1. We injected a test mixture containing *n*-octadecane diluted 1:5000 in various solvents at a pre-set splitting ratio of 100:1, adjusted at a column temperature of 30°C. The temperature of the column during the injection was varied between 30 and 200°C. Ten seconds after the injection the oven was heated to elute the sample at 200°C.

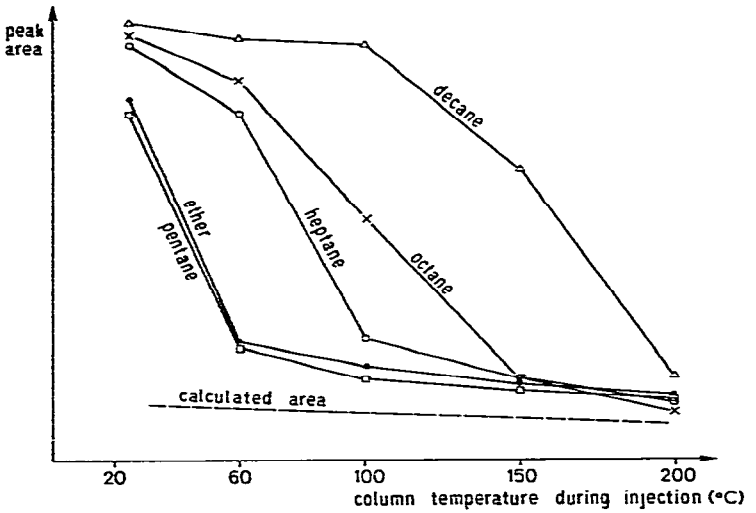

Fig. 1. Dependence of peak areas obtained for *n*-octadecane in the various solvents indicated at a constant pre-set splitting ratio on column temperature during sampling. The peak area expected from the pre-set splitting ratio was calculated by dividing the peak area obtained from a splitless injection of the same sample by the pre-set splitting ratio of 100:1. As the carrier gas flow-rate is reduced at increased column temperature, the pre-set splitting ratio increased by about 25% between 30 an 200°C (broken line). At column temperatures near or below the b.p. of the solvent the peak areas increase, *i.e.*, the true splitting ratio decreases owing to the recondensation of the solvent in the column inlet. The conditions chosen moderately favour recondensation.

The instrument was a Model 4160 (Carlo Erba, Milan, Italy) with an injector at 300°C, equipped with a glass liner of 2 mm I.D.; 2 μl of sample were injected by a syringe with a needle of length 7.5 cm, leaving 2 cm between the needle exit and the column entrance.

The peak area obtained by a splitless injection of the above mixture was divided by the pre-set splitting ratio (100:1) to calculate the peak area expected from the pre-set splitting ratio. As shown in Fig. 1, the peak areas determined for injections at high column temperatures were relatively close to this calculated area. The deviations, *i.e.*, the differences between the true and the pre-set splitting ratio, of a factor of 1.8–2 were assumed to be due primarily to the pressure wave. However, at column temperatures decreasing below the boiling point (b.p.) of the solvent, the peak areas increased many-fold. When using decane as a solvent (b.p. 175°C), the peak areas increased between 200 and 100°C and reached a moderately stable value below 100°C.

The decrease in the splitting ratio (or the increase in the peak area) with decreasing column temperature is explained by the recondensation of the solvent in the cool column inlet. The recondensation greatly reduces the vapour volume of the sample, which creates a zone of reduced pressure in the column inlet, thus sucking in further amounts of sample vapour. Recondensation becomes important at a column temperature close to the b.p. of the solvent. At a column temperature 50–80°C below the b.p. of the solvent, the recondensation is virtually complete and a further decrease in the column temperature hardly alters the splitting ratio.

As recondensation is the cause of the decreasing splitting ratio with decreasing column temperatures, it might be expected that the splitting ratio would be influenced by the coating of the column inlet with stationary phase. To investigate this aspect we used an extremely thick-filmed OV-73 column (film thickness 2 μm) with the following inlet sections:

(a) the complete inlet section coated with 2 μm of OV-73;

(b) with a 1-m length of persilylated but uncoated column connected to the front of the column;

(c) with a 1-m length of column coated with 0.1 μm of Carbowax 1000.

These three configurations were tested by injections of a 0.1% solution of *n*-octadecane in *n*-octane at a constant pre-set splitting ratio (100:1), varying the column temperature between 30 and 230°C. The results are summarized in Fig. 2. Conditions were chosen such that the peak areas changed drastically in the range of the b.p. of the solvent (125°C). However, these changes depended little on the characteristics of the column inlet. Thus the stationary phase seems to be of little importance for the recondensation. Recondensation is just a matter of the volatility of the solvent in its own environment, as was observed for recondensations creating the solvent effect during splitless injections.

The increase in the carrier gas flow-rate into the column by recondensation has been observed previously. In a recent paper[5] we reported on the increase in the flow-rate into the column during splitless injections when choosing conditions that favoured the recondensation of the solvent, thus creating a solvent effect. Especially

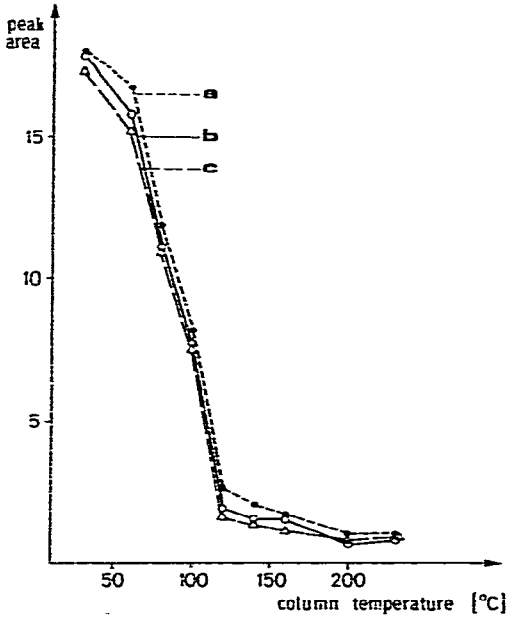

Fig. 2. Recondensation effects on a capillary column with different inlet sections. Injections of 3 μl of 0.1% of *n*-octadecane in *n*-octane; pre-set splitting ratio 1:100; injector temperature, 300°C; glass insert of 2 mm I.D.; long syringe needle to release the sample 2 cm above the column entrance. (a) Column inlet coated with 2 μm of OV-73; (b) column inlet uncoated; (c) inlet coated with 0.1 μl of Carbowax 1000. It is concluded that the recondensation is hardly dependent on the stationary phase in the column inlet.

during the first few seconds of the splitless period far more sample entered the column than would have been possible with the normal flow-rate, even when the dilution of the sample vapour with carrier gas is neglected. In this instance the recondensation in the column inlet is visible.

Factors that influence recondensation

Some factors that influence the recondensation and hence determine the deviation of the true from the pre-set splitting ratio were investigated. These are considered below in some detail to show which parameters have an important influence on the true splitting ratio. As contradictory arguments do not allow simple general working rules to be derived, the reader will have to draw his own conclusions for his samples on how to minimize and to control the recondensation effect.

Width of the injector cavity

We determined the amount of sample (*n*-pentadecane diluted 1:5000 in *n*-octane) entering the column at a pre-set splitting ratio of 100:1 at various column temperatures, using injectors with different inner diameters. Table I gives the ratios of the peak areas obtained at 30 and 200°C, *i.e.*, factors by which the recondensation decreased the true splitting ratio ("recondensation effect").

TABLE I

RATIOS OF PEAK AREAS AT 30 AND 200°C ("RECONDENSATION EFFECTS") FOR DIFFERENT WIDTHS OF THE INJECTOR CAVITY

Distance of the syringe needle from the column entrance 2 and 6 cm, with or without a dense packing of glass-wool in the injector cavity; pre-set splitting ratio, 100:1.

I.D. of injector cavity (mm)	*2 cm distance, glass-wool*		*2 cm distance, no glass-wool*		*6 cm distance, glass-wool*	
	2 µl	*4 µl*	*2 µl*	*4 µl*	*2 µl*	*4 µl*
2	28	20	14	8	2	2.5
3.6	3	9	4	6	3	2.5
6	4	7	4	8	2.5	3

The recondensation was most effective for the narrow (2 mm) glass insert, especially if combined with the use of the long syringe needle, leaving a distance of 2 cm between the tip of the needle and the column entrance. The true splitting ratio was decreased by factors of up to 30, which means that the true splitting ratio was close to 2:1 instead of 100:1 according to the pre-set ratio (the true splitting ratio at a column temperature of 200°C was about 60:1). This is nearly splitless injection. In fact, the reconcentration effect may be used to introduce a large proportion of a sample without creating a band broadening effect (at a pre-set splitting ratio of 2:1 the sample enters the column so slowly that the peaks are broadened). The recondensation effect is markedly reduced when using an injector wider than 2 mm. However, there was a surprisingly small difference whether the glass insert of 3.6 mm I.D. was used or the glass liner was taken out of the injector, leaving the metal body of 6 mm I.D. For the 6 mm injector cavity, the recondensation still decreased the splitting ratio by factors of between 2.5 and 7.

Distance between the needle tip and the column entrance

The ratios of the areas at column temperatures of 30 and 200°C in Table I show that the distance between the needle tip of the syringe and the column entrance is a dominating parameter influencing the recondensation effect. When using the 2 mm glass insert, the 2 cm distance gave ratios between 20 and 30 whereas for the 6 cm distance they were only between 2 and 2.5. For the wider injectors the differences between the recondensation effects determined by using the long and the short distance were only of the order of a factor of 2.

Both the width of the glass insert and the distance between the needle and the column influence the dilution of the sample vapour with carrier gas. Increasing dilution of the vapour leads to a double reduction of the recondensation effect. Under critical conditions (column temperature) the dilution influences the extent of the recondensation (depending on the partial vapour pressure). At the same time the recondensation of the vapour from a diluted gas phase creates only a weak reduction in the pressure, sucking in only small amounts of additional vapour (which is again diluted). Both parameters are well known also to influence the maximal column temperature for creating a solvent effect.

Sample volume

Table II shows peak areas (average integration counts divided by 1000) obtained for different sample volumes. The test sample was *n*-pentadecane diluted 1:5000 in *n*-octane. The experimental conditions favoured recondensation as the long syringe needle (giving the 2 cm distance) and the 2 mm glass liner were used. The glass insert was filled with glass-wool. At low column temperatures the peak areas show an unregular dependence on the sample volume. Instead of being doubled on changing from a 1- to a 2-μl sample size (always considering the needle volume of the syringe), the peak area was multiplied by a factor close to 15. However, again doubling the sample volume from 2 to 4 μl increased the peak area by only 30%. Above the b.p. of the solvent (125°C) the relationships normalized, although not yielding the accurate data as desirable.

The data in Table II show that the strong recondensation effect requires a concentration of sample vapour in the carrier gas stream which exceeds a critical limit. This is confirmed by the ratio of the peak areas at 30 and 200°C. This ratio was relatively small for the 1-μl sample size (with the small peak area at 30°C), which is in

TABLE II

DEPENDENCE OF PEAK AREAS (INTEGRATOR COUNTS × 10^{-3}) ON SAMPLE VOLUME AT VARIOUS COLUMN TEMPERATURES

Temperature (°C)	*Sample volume*			
	1 μl	*2 μl*	*3 μl*	*4 μl*
30	35	510	640	690
60	27	360	490	530
100	21	180	330	420
140	15	26	48	76
200	11	19	28	32

agreement with relatively little recondensation. The many-fold larger peak area obtained for 2 μl of sample at 30°C was due to a concentration of sample vapour in the carrier gas which exceeded the critical limit to cause strong recondensation. This is confirmed by the fact that the difference in the peak areas obtained at 30 and 200°C was large.

Pre-set splitting ratio

When considering the influence of the sample size on the recondensation effect (Table II), it is not surprising that the deviation of the true from the pre-set splitting ratio also depends on the pre-set splitting ratio itself. The data given in Table III show that the true splitting ratio may change by a factor of 15 when the pre-set splitting ratio is changed by a factor of only 5 (6 cm distance at 30°C). Apparently the reduced splitting ratio promoted the recondensation effect. The recondensation effect involved is confirmed by the fact that the change in the true splitting ratio between a column temperature of 30 and 200°C is increased when decreasing the pre-set splitting ratio. However, as the data in Table III show, this observation cannot be generalized as the strong deviations occur only under critical conditions.

TABLE III

PEAK AREAS AND DIFFERENCES IN PEAK AREAS AT LOW AND AT HIGH COLUMN TEMPERATURES AS A FUNCTION OF PRE-SET SPLITTING RATIO

Distance between the needle tip and column entrance, 2 and 6 cm; sample size, 2 μl; 2-mm glass insert packed with glass-wool; injector at 300°C.

Column temperature (°C)	*Splitting ratio*			
	6 cm distance		*2 cm distance*	
	100:1	*20:1*	*100:1*	*20:1*
30	41	700	410	2100
200	22	70	14	100
Difference	×2	×10	×30	×20

Injector packed with glass-wool

For some of the experiments the glass insert of the injector was packed with silanized glass-wool. Glass-wool hinders the large droplets of sample from passing the splitting point without prior evaporation or at least fragmentation into small droplets. Large droplets may fall on to the capillary entrance on one occasion or they may pass it on another, thus not providing reproducible results. Further, large droplets are not likely to be split according to the gas flow-rates.

The true splitting ratio and the reproducibility of the results were little dependent on whether the injector was packed or empty, provided that volatile solvents were used (pentane, hexane, diethyl ether; injector at 300°C as throughout this work). However, with *n*-octane as the solvent the recondensation effect differed with and without glass-wool by a factor of up to 2. Such differences were found to be related to

the absolute peak areas. In those instances where the glass-wool caused an increased amount of sample to enter the column, the recondensation effect was reinforced.

n-Octane is only partially evaporated on being introduced into an empty injector at 300°C. The absolute peak areas were mostly smaller than for injections into a glass insert packed with glass-wool. Further, the reproducibility of the peak areas was sometimes extremely poor. Table IV gives the first few peak areas for a series of injections obtained under conditions giving pronounced differences (narrow glass liner, sample volume 4 μl and high column temperature). When using the short syringe needle leaving a distance of 6 cm to the column entrance, the peak areas varied by a factor of 20, between 30 and 1000% of the area obtained with glass-wool (ten replicate injections). The peak areas give the impression that sometimes a large droplet fell into the column, giving exceedingly high peak areas, but more often the droplets passed by the column, resulting in too small a peak. The peak areas varied by less than by a factor of 2 for the long syringe needle (2 cm distance). Considering the reproducibility of the absolute peak areas for the sample dissolved in *n*-octane, the use of glass-wool in the injector was shown to be of great advantage.

TABLE IV

TYPICAL PEAK AREAS FROM INJECTIONS INTO AN INJECTOR WITH A 2 mm I.D. GLASS LINER WITH AND WITHOUT GLASS-WOOL

Short and long syringe needles were used to release the sample 6 and 2 cm from the column entrance; column at 200°C.

Distance (cm)	*With glass-wool*	*No glass-wool*
2	3260, 3209, 3298, 3251	5915, 6216, 6912, 4873, 5502
6	6075, 6009, 6063, 6105	5658, 1444, 13,850, 1367, 4028

DISCUSSION

The user of the split injector may aim for two different goals:

(a) to eliminate the deviation of the true splitting ratio from the pre-set ratio; and

(b) (the far more modest goal) to establish working rules that allow one to work with today's deficient systems with the intention of minimizing deviations and to use conditions that allow one to reproduce them (non-reproducibility of results is a consequence of non-reproducibility of the deviations).

Obviously it would be most useful to eliminate all mechanisms that cause deviations of the true from the pre-set splitting ratio or, more precisely, to avoid fluctuations of the splitting ratio during sampling. However, during the study reported here we could not find conditions that allowed a general elimination of the recondensation effects. Recondensation was reduced by diluting the sample vapour with carrier gas. The mixing of the sample vapour with carrier gas required by Ettre and Purcell[6] to achieve linear splitting probably also aims for a stable splitting ratio during the splitting process. The dilution with carrier gas is improved by increasing the effective injector volume, *i.e.*, by an enlarged injector cavity and by an increased

distance between the needle and the column. However, our attempts to eliminate recondensation by dilution of the vapour had only limited success. For a distance of 6 cm between the needle and the column and for an injector cavity of 6 mm I.D., the splitting ratio still increased by factors exceeding 2 on increasing the column temperature from below to above the b.p. of the solvent. The difference between the true and the pre-set splitting ratio easily reached a factor of 3. Further, a comparison of the results obtained with an injector of 3.6 and 6 mm I.D. did not indicate that a substantial improvement could be achieved by a further enlargement of the injector. During these experiments we found no evidence that glass-wool would improve the mixing of sample vapour with carrier gas.

On the other hand, dilution of the sample vapour with carrier gas is undesirable from the point of view of the initial band width of the sample deposited in the column. Peak broadening as a result of a slow transfer of a large (diluted) vapour cloud into the column can be avoided only by choosing a high flow-rate in the injector, *i.e.*, by a high splitting ratio. However, for many applications it is desirable to use a pre-set splitting ratio down to 10:1 or 5:1.

There remains the option of injecting small sample volumes. This seems attractive as the pressure wave is weak and the recondensation effects are small owing to the high dilution of the small amounts of vapour. However, small sample volumes cause problems with the syringe because of premature and selective elution out of the needle[7,8] (especially when using a 1-μl syringe).

Recondensation of the sample is avoided if the column temperature is kept at least about 20°C above the b.p. of the major constituents of the sample (*e.g.*, solvent). Unfortunately, for our applications, this is possible only for a few samples.

A split injector that minimizes the deviation of the true from the pre-set splitting ratio would probably have a cavity about 10–12 cm long with an I.D.of 5–6 mm. The pressure wave would be negligible and the recondensation effect would probably cause the true splitting ratio to deviate by less than a factor of 2 from the pre-set ratio. However, the matter becomes complicated when some contradictory requirements for this injector are included:

(a) It should be possible to work at a splitting ratio below 10:1.

(b) Conditions for minimizing discrimination often require a minimal evaporation with the intention of splitting the sample in the liquid (droplet) phase[3]. This calls for a rapid transfer of the sample from the syringe to the column and therefore, for a narrow liner and a short distance between the needle and the column.

(c) It should be possible to carry out splitless injections with the same sampling device. Splitless injections require a minimum of dilution of the sample vapour. The transfer of the vapour does not allow the use of an injector volume exceeding about 1 ml. Further, the syringe should release the sample vapour close to the column entrance, which calls for extremely long syringe needles (the column is bound to be situated on the bottom of the injector to minimize dead volumes).

At present the operator has to work with a split injector which, in general, does not provide a predictable splitting ratio. In this situation it is greatly preferable to quantitate on the basis of internal standards, which renders an accuracy that is fairly independent of the splitting ratio, because the accuracy of the analytical results depends only on the ratio of the sample parts to the internal standard (at least if one does not consider the dependence of the discrimination phenomena on the splitting

ratio). However, if using, *e.g.*, selective detectors or gas chromatography–mass spectrometry and single-ion detection, the operator is often forced to use the external standard method or the method of standard addition[9] based on absolute peak areas. These techniques rely on a contant true splitting ratio, *i.e.*, on the reproducibility of its deviation from the pre-set splitting ratio. As long as the injection of a sample has not been investigated, we recommend that the following working rules should be considered:

(1) The pre-set splitting ratio must be kept constant. A change in this ratio does not always change the true splitting ratio in the same proportion.

(2) The column temperature during the injection must be reproduced, especially if the column temperature is close to the b.p. of the solvent or of the major constituent.

(3) The external standard must be dissolved in the same solvent as the sample in order to reproduce the evaporation characteristics, the pressure wave and the recondensation effect.

(4) The sample volume has to be reproduced. From Table II it may be estimated that a change in the sample volume of 0.1 μl may double the peak area if the sample volume happens to be in a critical range depending on the recondensation effect.

The last rule not only requires a constant sample volume to reduce standard deviations but also disallows some fairly common methods. It is not possible to change the sample volume and to correct the peak area as may be desirable because the column is overloaded or the peaks are too small to be detected. An attractive version of the method of standard addition also is not allowed. It would often be of advantage to add the standard mixture to the sample only in the syringe, *i.e.*, by taking the normal sample volume and adding a known volume of the standard mixture on top of it. However, the total sample volume changes and therefore it cannot be assumed that the splitting ratio remains constant.

There is a single useful aspect of the recondensation effect: it is possible to work at very small true splitting ratios without the risk of obtaining broadened peaks.

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