Internal standard calibration in the case of nonlinear calibration dependencies

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Summary

An algorithm of calculations for internal standard calibration is described that allows to get adequate results in the case of nonlinear calibration dependencies. The algorithm is implemented in the «MultiChrom» software (Ampersand LTD, Russia).

The algorithm is based on the assumption that calibration dependency of the internal standard component is known a priori or obtained from the set of calibration experiments by external standard method. Calculations with the help of this algorithm may be especially effective for the variants of detection with typical nonlinear dependencies, e.g. in thin-layer chromatography.

Introduction

One of the most important steps in quantitative chromatographic analysis is the construction of calibration dependencies. These dependencies are used for calculation of analyte concentrations in initial sample based on area or height of corresponding component peak.

To increase the accuracy of calculations Ray [1] offered internal standard method aimed to compensate dosing errors and sample loss during sample pretreatment.

The essence of the method is in addition of the internal standard substance to the analyzed sample and in accounting of the area of corresponding peak on the chromatogram while calculation of concentration of other components of the mixture. Known concentration of the substance together with detector response data really improve reproducibility of the results obtained to the fraction of percent.

The reason of increased reproducibility is in accounting of additional information internal standard component. This information can be used on the step of concentration calculation as well as on the step of obtaining calibration dependency.

In the literature in many cases internal standard method is implicitly treated only as a method of concentration calculation [2] and a question of calibration while using this method is not discussed. It should be noted that such interpretation has a good background, as calculations using internal standard method is possible and gives reasonable results in the case of calibration by external standard method or using data about relative coefficients of detector response from literature.

Usually calculation of the i-th component Ci concentration in internal standard method is performed using formula

$$C_i = C_{(st)} \frac{K_i R_i}{K_{(st)} R_{(st)}} = C_{(st)} K'_{(st)} \frac{R_i}{R_{(st)}}, \tag{1}$$

where K is the absolute detector response coefficient, K' – normalized detector response coefficient (detector response coefficient to standard component is accepted to be equal to one), R - detector response (area or height of corresponding peak), subscript (st) refers to corresponding values of the standard component.

In the case when internal standard calibration graph construction is offered [3], it is constructed in coordinates ($Q_i/Q_{(st)}$, $R_i/R_{(st)}$) or (Q_i, $Q_{(st)}R_i/R_{(st)}$), where Q is the quantity of the component equal to product of its concentration to injected volume.

Described calculation scheme is used quite widely, but doesn't provide adequate results in the case of nonlinear calibration dependencies. Really, notions of detector response coefficient or relative coefficient are no longer valid.

The curve, describing nonlinearity of the detector, can be constructed only in the case when one of axes is the absolute value of the detector response, i.e. in coordinates (Q, R). Using anamorphosis, changing detector response value R by multiplication to coefficient $Q_{(st)}/R_{(st)}$ is not legal, as this value is not constant for nonlinear calibration dependency of the standard component.

Theory

In the algorithm offered regression coefficients $W(R)$ are calculated in coordinates (Q, R), where Q is the substance quantity in the peak and R – area or height of the peak. Quantity of the i-th component is calculated taking into account known concentration of standard in k-th calibration solution, detector response to internal standard R and calibration dependency W of the internal standard component and equals

$$Q_{ik} = C_{ik} \cdot \frac{W_{(st)}(R_{(st)k})}{C_{(st)k}}, \qquad (2)$$

where C_{ik} – concentration of i-th component in k-th calibration solution. Formula (2) corrects position of calibration points along Q axis of component quantity, leaving detector response R as it was measured in the experiment.

In the case if calibration dependency of internal standard component is linear and its coefficient is accepted to be equal to one, calculations are equivalent to traditional scheme of relative response coefficients calculations by the method of internal standard.

While calculations of unknown concentration of the component C_i formula

$$C_i = C_{(st)} \frac{W_i(R_i)}{W_{(st)}(R_{(st)})} \qquad (3)$$

Is used, where $C_{(st)}$ – concentration of internal standard component in the mixture being analyzed. In the case of linear calibration dependencies formula (3), as expected, turns into formula (1).

Calibration process

Calibration process consists of two steps. On the first step calibration dependency of the internal standard component is obtained, on the second – of all other components. Calibration of standard component is made by external standard method. The two calibration steps utilize different sets of calibration data (in some cases these sets may coincide). On the first step increased demands to the accuracy of sample dosing, typical for calibration by external standard method, are made. On the second step less attention may be focused on accuracy of dosing, as calibration curve of the standard component is already known and is used for improving accuracy of injected dose.

Discussion

The difference between internal standard calibration and quantification should be emphasized. Reproducibility of results is mostly effective by quantification step (formula 3) even if calibration is obtained by external standard method.

Internal standard calibration is used for obtaining more accurate values of coefficients of calibration dependency, calculated accounting known area and concentration of the standard component in every calibration run.

Resulting calibration dependency is drawn in the same coordinates as external standard dependency, and less dosing and measurement errors, both dependencies would coincide.

To get concentration of the component in the analyzed sample using the algorithm offered one needs two calibration dependencies: that of analyzed component and standard. In the case of calculations without computer it may cause some inconveniences. Using computer for calculation of calibration dependencies allows to hide from the user complexity of calculations and provides appropriate convenience of work.

It should be noted, that in the case of single-point calibration using internal standard calibration dependency gives absolute no advantages compared to external standard method, as all dependencies are linear and all the difference between methods is in using of absolute or relative detector response coefficients.

Conclusions

Offered calculation scheme will give the most appreciable effect by simultaneous realization of two conditions:

1) Nonlinear calibration dependency of standard component.
2) Variation of peak area of standard component from run to run.

The most perspective is using of scheme offered in thin-layer chromatography, as here, as a rule, calibration dependencies are nonlinear.

LITERATURE

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