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CHROMATOGRAPHIC DETECTION LIMITS IN PHARMACEUTICAL METHOD DEVELOPMENT

EUGENE L. INMAN* and EUGENE C. RICKARD
Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, IN 46285 (U.S.A.)
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SUMMARY

A procedure for the determination of chromatographic method detection limits that incorporates the variabilities associated with sample preparation, measurement, and calculations is described. Theoretical considerations lead to an equation for the calculation of a method detection limit that evaluates the precision of the method near the detection limit and incorporates the number of sample and standard replicates used for a determination. Guidelines for the experimental determination of the method detection limit and for subsequent method procedure limitations were developed from the assumptions employed. This procedure is general and can be applied to chromatographic and non-chromatographic techniques. Several examples which demonstrate the effectiveness of this procedure for chromatographic methods are given. Method detection limits determined in this way should provide consistency for methods developed for pharmaceutical applications.

INTRODUCTION

The method detection limit is often used to characterize a method's effectiveness in the determination of a desired analyte. In general, the concept of a detection limit is one that is familiar to most analytical chemists. However, a diversity of views related to its significance and to the correct procedure for its determination have been discussed[1–3]. Its evaluation is especially important when the analyte is a minor or trace component in the sample matrix[4–6]. This presentation will focus on the definition and determination of the detection limit for analytical methods developed to evaluate pharmaceutical products, where detection limits take on real, rather than theoretical, significance.

Generally, the detection limit is defined as the lowest concentration or amount of an analyte in a given matrix whose response can be distinguished from the response of a blank[7]. The ability to distinguish between two response levels suggests a statistical basis for the definition of the detection limit. Historically, the precise statistical definition of the detection limit has been thoroughly debated. Furthermore, translation of the theoretical principles to practical application has led to confusion in experimental implementation. This paper outlines the experimental design considerations needed for the determination of the detection limit during method validation.

The importance of the detection limit is dependent upon the biological significance of the analyte itself. Analytes, often present as trace impurities that demonstrate significant toxicity, such as a carcinogen or mutagen, frequently require a decision as to their presence or absence at very low levels. The maximum sensitivity of these methods will define the control specification, *i.e.*, the control specification will be set at the method detection limit. Thus, the experimentally estimated detection limit will determine the level which will be reported as "none detected" in a regulatory sense. An evaluation of previously described approaches will provide a perspective for the design of an acceptable procedure for pharmaceutical products.

REVIEW OF APPROACHES TO DETECTION LIMIT DETERMINATION

A common experimental approach for estimating the detection limit is the measurement of serial dilutions of a stock analyte solution. An estimate of the detection limit is obtained by noting the concentration where the responses from the analyte solution and solvent blank are indistinguishable. However, this approach neglects the effects of many sources of variance, such as sample matrix. Thus, this approach produces estimates of the detection limit which are generally lower than those actually observed.

A second common experimental approach includes the effect of variance in the measured response. The detection limit is defined as a specific signal-to-noise ratio, usually a value of two or three depending on the desired confidence level. The limit of quantitation (LOQ) provides for a greater degree of confidence with the signal-to-noise ratio frequently set equal to ten[5]. When the signal-to-noise approach is selected, one must determine an appropriate procedure to measure the noise level. The noise level can be determined from measurements on a number of blanks or from peak-to-peak noise. For chromatographic determinations, the noise can be determined from a region of the chromatogram where no components elute[8,9]. Details of these approaches are described in the literature[10].

Long and Winefordner[7] reviewed the statistical basis for the IUPAC definition of detection limits, which recommends a signal-to-noise ratio of three and the noise is determined from the blank signal. The IUPAC model was evaluated and compared to alternate approaches, including graphical and propagation of errors models. These models allowed for errors in the calibration curve slope or slope and intercept, respectively in addition to errors in measurement of the blank signal. Application of these approaches to a number of spectroscopic examples demonstrated that the calculated detection limit can vary significantly. They concluded that the IUPAC approach is appropriate only when the major source of error is in the blank measurement and, in most cases, it yields artificially low detection limits.

A review of the concept of the detection limit applied to chromatographic techniques was presented by Foley and Dorsey[11]. A number of inconsistencies in common usage were pointed out and a set of definitions and models were proposed to provide standardized evaluation and reporting of detection limits. The detection limit calculations were based on the IUPAC and error propagation models, with special emphasis placed on the determination of baseline noise, analogous to the determination of the blank signal. They recommended a peak-to-peak noise measurement over a region of the chromatogram that is free of analyte peaks. This

standardization of chromatographic and statistical parameters for the detection limit determination allows direct comparisons of differing chromatographic methods.

All of the statistical models described above are based on the assumption that the sample preparation variability is negligible so that the detection limit is determined by the measurement process. In this way, an instrument detection limit (IDL) is described rather than a method detection limit (MDL). That is, the variances introduced by the separation, detection, and measurement processes are included in the IDL detection limit calculation, but not variances associated with the preparation of samples and standards. For most methods used to determine analytes near the detection limit, these other variances are significant and the IDL approach will yield a lower estimate for the detection limit than that which is actually observed. The method detection limit should include these additional sources of variability associated with processing the samples and standards through all of the steps in the analytical method. Furthermore, the assay design (*e.g.*,levels of replication) affects the method detection limit but cannot be accommodated by the IDL approach.

METHOD DETECTION LIMIT

The approach used in this paper will assume that the analyte must be quantitated. That is, that the level of the analyte (usually *versus* some regulatory limit) is important, rather than just a qualitative ability to detect the analyte. This definition has sometimes been referred to as a "limit of determination" in the literature, but an IDL approach is frequently used for its evaluation. The quantitative approach described below defines the MDL to incorporate all the variances associated with the assay. It also accounts for the increase in precision due to replication of samples and standards, *i.e.*, assay design.

The evaluation of the MDL must incorporate variability from the sample preparation, separation, detection, and measurement processes. It also should allow for day-to-day variability in the method performance. That is, one should be confident that an analyte present at the MDL level can be reliably detected and quantitated any time an assay is performed. Finally, the variability associated with a background correction or comparison to a blank sample must be incorporated.

One procedure for the determination of a MDL for trace analyses of wastewaters has been described[6]. The entire analytical procedure was included in the evaluation of precision and the MDL for a single sample replicate was calculated from:

$$\text{MDL} = t(n-1,\ 1-\alpha=0.99)S_m \tag{1}$$

where $t(n-1,\ 1-\alpha=0.99)$ is the Student's t value for a one-tailed test at the 99% confidence level with $n-1$ degrees of freedom, and S_m is the standard deviation obtained from a number of replicate measurements (n). The value for t accounts for the limited number of measurements used to determine S_m. A minimum of seven replicates on a single sample with a concentration of one to five times the estimated MDL was proposed for the initial estimate of S_m. An iterative procedure with seven additional replicates on a solution with the concentration at the MDL estimated from the initial seven replicates is suggested to prevent overestimates of the MDL. Estimates of the MDL from measurements at high concentrations are frequently

incorrect. Glaser *et al.*[6] also estimate confidence limits on the MDL using the chi-square distribution. This concept has been adapted to the procedure proposed below for the evaluation of pharmaceutical methods.

MDL DERIVATION

MDL equation

The calculated method detection limit for a routine assay includes two separate contributions: the inherent method precision (which can be estimated) and the design of the routine assay procedure. That is, the experimental design of the validation procedure can have a dramatic effect on the accuracy of the estimated method precision, while the number of sample replicates and the number of standards used to define the calibration curve affect the expected precision of routine assay results. An effective expression for the MDL must provide for these terms.

The most general expression for the method detection limit can be written as:

$$\mathrm{MDL} = kS_m \tag{2}$$

where k is a constant of proportionality and S_m is the method standard deviation. The determination of S_m must incorporate any background correction needed. Note that eqn. 1 fits this general form with the Student's t value introduced for k, accounting for the limited measurements used to estimate S_m. As a historical database is generated, S_m becomes more accurately determined and k can be set equal to 2, 3 or any other value corresponding to the z value of the normal distribution for the desired confidence level. The determination of S_m, however, is critical and errors in its experimental determination can become significant.

Eqn. 2 can be expanded by considering the contributions to S_m. For example, sample responses are generally compared to a calibration curve generated from one or more standard measurements. During the routine implementation of a method, one generally assays multiple sample replicates and a mean result is reported. The standard deviation of the mean of n independent measurements is inversely proportional to the square root of the replicate number. Introducing these relationships into eqn. 2 yields:

$$\mathrm{MDL} = k(S_{std}^2/n_{std} + S_{sam}^2/n_{sam})^{1/2} \tag{3}$$

where S_{std} is the standard deviation of the standard measurement, S_{sam} is the standard deviation of the sample measurement, n_{std} is the number of standards used to generate a calibration curve, and n_{sam} is the number of sample replicates. As described, k is defined by the t-distribution value when the number of replicates used is small and the z statistic is appropriate when the number of replications used in the validation process is increased so that S_m becomes reasonably well known. Eqn. 3 may be appropriately modified to incorporate additional sources of variances. However, experimental errors and/or inappropriate assumptions greatly influence the reliability of the estimate for S_m.

Assumptions

The first assumption in eqn. 3 is that the method standard deviation is concentration independent near the detection limit. This is in contrast to the assumption of a constant relative precision observed at higher concentrations and implies a constant rather than a proportional error. The key to the validity of this assumption is that the standard concentration is approximately equal to the sample concentration. The second assumption is that the random error associated with a measurement is approximated by a Gaussian distribution. These assumptions are routinely met over small concentration ranges for many analytical techniques.

EXPERIMENTAL CONSIDERATIONS

The accuracy of the estimated method detection limit is most critically influenced by the experimental determination of S_m. From the definition of S_m and the assumptions given above, three general guidelines can be provided. First, the experimental determination of S_m must incorporate all contributions to the method variability, and therefore should include complete replication of sample preparation, measurement, and calculations. One must not limit the evaluation to the separation and detection steps only. Secondly, the analyte should be present in the sample matrix. Finally, variance contributions such as those due to multiple samples, instruments, analysts, days, or labs may need to be included. These variance components are typically evaluated using analysis of variance approaches from multiple levels of each variable[12,13].

Although these techniques are well known and will yield experimental designs which minimize variance, in some cases it may be sufficient to design the evaluation of S_m so that all relevant variables are included, *e.g.*, multiple samples on multiple days using available people and equipment. Thus, the specific experimental design to determine S_m will be unique for each method.

The experimental design used to determine S_m is dependent upon the major sources of method variability. If the sample and standard preparation and measurement procedures are identical, then S_{std} and S_{sam} are approximately equal, and eqn. 3 can be simplified to:

$$\mathrm{MDL} = kS_{sam}(1/n_{sam} + 1/n_{std})^{1/2} \tag{4}$$

When standards are prepared as solutions of analyte spiked into the sample matrix, this assumption is generally valid. If, however, the standard and sample preparation procedures differ significantly, then S_{std} and S_{sam} must be determined separately. For example, standard solutions that are prepared in the absence of sample matrix often yield conditions where $S_{std} < S_{sam}$.

Further examination of eqn. 4 offers another guideline for method design. If S_{std} and S_{sam} are equal, one can not lower the detection limit significantly by increasing the number of sample replicates without increasing the number of standards. The most efficient approach includes an equivalent number of standard and sample replicates. Using this condition, eqn. 4 can be reduced to:

$$\mathrm{MDL} = k\sqrt{2}\, S_{sam} / \sqrt{n_{sam}} \tag{5}$$

While the conditions required for use of this equation seem restrictive, they are routinely met if standards are prepared by spiking analyte into the blank sample matrix. In trace analysis, standards are often prepared in this way. Note that k in eqns. 4 and 5 can be chosen specifically for each method according to the confidence level required.

With these guidelines, the following procedure is outlined for the determination of S_m: (i) obtain a sample with little or no analyte present; (ii) spike in a quantity of analyte estimated to be near the detection limit; (iii) confirm that this amount of analyte is detectable; (iv) spike two to five times this level into multiple lots of sample, multiple replicates each; (v) if a background correction is needed, samples should be run as sample-blank pairs; (vi) for multiple data sets, pool the variance obtained for each lot, using the following equation:

$$S_p = \left\{ \sum_{i=1}^{j} [n_{(i)} - 1] S^2_{sam(i)} \Big/ \sum_{i=1}^{j} [n_{(i)} - 1] \right\}^{1/2} \quad (6)$$

The pooled standard deviation, S_p, is calculated from the variances, $S_{sam(i)}$, obtained from $n_{(i)}$ measurements on j lots. S_p is then used in place of S_m in eqn. 2 and S_{sam} in eqn. 5.

The guidelines require that several limitations be placed on the method procedure. These recommendations should be incorporated into the method and followed each time the method is run. First, the number of replicate samples should be considered in conjunction with the number of standard replicates depending on the values of S_{sam} and S_{std}. Secondly, the concentration range of the standards should be small, probably within a factor of 10 of the estimated detection limit. Finally, the lowest standard concentration should be at or just above the MDL calculated for a single replicate. This standard provides a system suitability check.

An examination of these guidelines and the detection limit equation results in several observations and conclusions. The determination of S_m is critical for the derivation of the MDL and careful experimental design is required. The number of measurements used to determine S_m should be as large as practical. With the inclusion of the term for n, the detection limit is dependent upon the number of replicate determinations made on a sample. With sufficient replication of a single sample, it is possible for the MDL to be lower than the IDL. However, this generally has only theoretical significance since the required number of sample replicates is likely to be quite large. Also note that under many circumstances, increasing the sample replication number has little effect on the MDL if the standard replication number is not increased as well.

This procedure for the determination of the limit of detection is general and can be applied to chromatographic and non-chromatographic techniques. Foley and Dorsey[11] recommend that chromatographic detection limits (IDL) be reported as analyte amounts. However, the amount of analyte is related back to an original sample amount. Thus, the MDL can be reported in terms of concentration for a specific procedure. Since the MDL is dependent upon the initial sample size, sample size must be specified in the method procedure.

EXAMPLES

To demonstrate the effectiveness of the outlined procedure, several examples were selected in high-performance liquid chromatography (HPLC), gas chromatography (GC) and thin-layer chromatography (TLC). The details of these methods will not be presented, rather, the validation results will be reviewed. Three examples are representative of methods used to monitor process-related impurities in bulk drug characterizations, and one example focuses on degradation products and impurities in the dosage form. In each case, the development of experimental conditions for sample preparation, analyte separation, and measurement was completed and defined before the method was validated. Final MDL calculations were based on the use of eqn. 4 and the number of standard and sample replicates are the same for each method. For pooled data, eqns. 5 and 6 were used.

The first example represents a method developed for the detection of a fluorescent component in a bulk drug. The analyte is separated from the main component by TLC and is detected with a scanning densitometer operated in the fluorescence mode. Sample preparation includes dissolution of the drug substance in an appropriate solvent and spotting the solution on the TLC plate. During the development of the method, it appeared that a major source of variability was due to differences in the TLC plates. The MDL evaluation included sixteen lots of bulk drug run on five different plates. It was estimated that the variability of the standard measurement was approximately equivalent to the variability of the sample measurement. The IDL was estimated at a concentration equivalent to 5 ppm in the bulk drug, so a quantity of analyte equivalent to 10 ppm was spiked into each sample. A standard deviation was calculated for each plate and pooled to yield a method standard deviation of 2.7 ppm. Using a value of 2 for k, the results shown in Table I were generated, assuming that the number of standard and sample replicates are the same. A replicate number of three was selected with a detection limit of 5 ppm. The method procedure was written to include a standard curve for each TLC plate with standard concentrations of 5, 20 and 50 ppm. Detection of the 5-ppm standard is used as a system suitability check.

The basic assumption of concentration independence for the standard devia-

TABLE I

EXAMPLE 1 RESULTS FOR TLC–FLUORESCENCE DETECTION METHOD

$S_{sam} = S_{std} = 2.7$ ppm.

n	*MDL (ppm)*
1	8
2	6
3	5
4	4
7	3
15	2
59	1

TABLE II

EXAMPLE 2 RESULTS FOR GC–NITROGEN-SELECTIVE DETECTION METHOD

$S_{sam} = S_{std} = 1.6$ ppm.

n	*MDL (ppm)*
1	5.7
2	4.0
3	3.3
4	2.8
8	2.0
32	1.0

tion is true only over a limited concentration range. That is, the analyte level has a great effect on the estimated MDL. To illustrate this point, the MDL of the first example (actual MDL = 5 ppm) was estimated from a sample which contained 500 ppm of analyte. It yielded a method detection limit of about 50 ppm. This poor estimate confirms that detection limit measurements should be made near the detection limit.

The second method was developed for the detection of an impurity in a bulk drug, where chemical derivatization is needed for separation and detection. After derivatization, the analyte is separated from the main component by GC and detected with a nitrogen-selective detector. Variability includes major contributions from the derivatization step and changes in detector sensitivity from day-to-day. A method standard deviation of 1.6 ppm was determined from a pooled set of 31 determinations of several lots of bulk drug, with each determination including three sample and three standard preparations. Assuming S_{sam} and S_{std} are equal, S_{sam} was calculated to be equal to 2.0 ppm. Using a value of 2 for k, the results shown in Table II were generated. A replicate number of three was selected with a MDL of 3.3 ppm. The use of analyte standards is significant in this determination since the derivatization is not selective for the analyte, but also includes the bulk drug substance. Therefore, standards are prepared by spiking analyte into an acceptable lot of bulk drug. This procedure accounts for incomplete reaction and matrix effects. In this method, standard preparation variability is significant.

Another method was developed for the detection of sodium bicarbonate in a vial of formulated antibiotic. The method procedure includes the injection of a volume of hydrochloric acid into a vial, with the headspace above the solution assayed for carbon dioxide by GC with a thermal conductivity detector. Measurement of the bicarbonate detection limit was important to evaluate the acceptability of this simple assay scheme. A typical chromatogram is shown in Fig. 1. Note that an atmospheric level of carbon dioxide is found in the blank. For this method, one is not interested in the absolute detection limit of the chromatographic procedure, but the ability to distinguish between sample and blank. The IDL was estimated to be about 0.01 to 0.02%. Therefore, a spiked concentration of 0.1% was selected for the determination of S_{sam}. The results are shown in Table III. Five separate lots were assayed with ten replicates each to determine an S_p of 0.018%. Note that S_{sam} for the individual lots ranges from 0.011% to 0.026% demonstrating that a sufficient number of samples

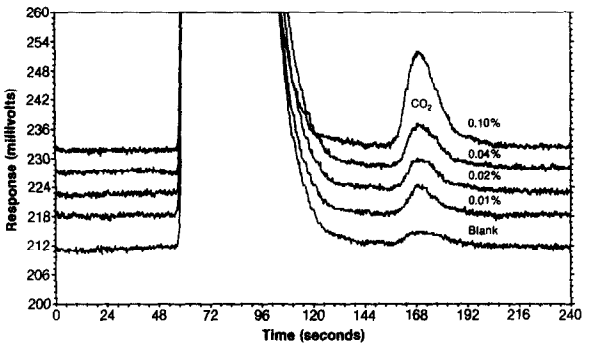

Fig. 1. Example 3, determination of sodium bicarbonate in a vial of formulated antibiotic by GC.

should be used for this evaluation and that the determination of S_{sam} is critical to the final method detection limit assignment. The corresponding MDL table is shown in Table IV.

The fourth example illustrates a common problem, detection of an isomeric impurity. This example involves the detection by HPLC of small levels of the *S* isomer in the *R* isomer. Since the activity of the compound resides in the *R* isomer, but the toxicity may be increased by the presence of the *S* isomer, a 0.1 to 1% detection limit goal was established. The compound was first derivatized to the corresponding monoester diastereomers with (*S*,*S*)-O,O-dibenzoyltartaric acid anhydride, dried down, and reconstituted with sample solvent. Analyte quantitation was estimated by an external standard approach where the response of the impurity peak in a concentrated sample solution was compared to the response of the main peak of a diluted sample solution, with a 1 to 10 dilution factor.

This example is described more extensively to demonstrate the actual steps involved in the evaluation of the MDL. Numerous data were collected for unspiked samples and additional data were obtained by spiking four levels of the *S* isomer into the sample (Table V). Since the *R* isomer was used as the standard, no standard curve

TABLE III

EXAMPLE 3 SUMMARY

Estimated IDL = 0.02%; spiked concentration = 0.1%.

Sample lot	S_{sam}
1 ($n=10$)	0.020%
2 ($n=10$)	0.026
3 ($n=10$)	0.016
4 ($n=10$)	0.011
5 ($n=10$)	0.012
	S_p = 0.018%

TABLE IV

EXAMPLE 3 MDL TABLE

n	*MDL (%)*
1	0.05
2	0.04
3	0.03
7	0.02
26	0.01

TABLE V

EXAMPLE 4 DATA

S Isomer spike level	*Day*	*S Isomer found (%)*	*Within day*	
			Average	S_{sam}
0	1	0.19	0.19	–
0	2	0.21, 0.24, 0.23, 0.28, 0.27	0.25	0.03
0	3	0.10, 0.09, 0.14	0.11	0.03
0	5	0.10	0.10	–
0	6	0.16, 0.06, 0.10	0.11	0.05
0.3%	5	0.19, 0.23, 0.37	0.26	0.09
0.5%	4	0.31, 0.23, 0.26	0.27	0.04
0.5%	5	0.48, 0.37, 0.44	0.43	0.06
1.0%	3	1.02, 0.64, 1.12	0.93	0.25

was needed and the data are reported directly in percentage *S* isomer. Although the diastereomer peaks were well resolved, there were other trace contaminants present in the sample which contributed to the difficulty in establishing baselines and integrating peaks (Fig. 2). Thus, differences were observed between days and sometimes within the same day (*e.g.*, 1% spike level in Table V). The data at each spike level were combined to give the averages and standard deviations shown in Table VI.

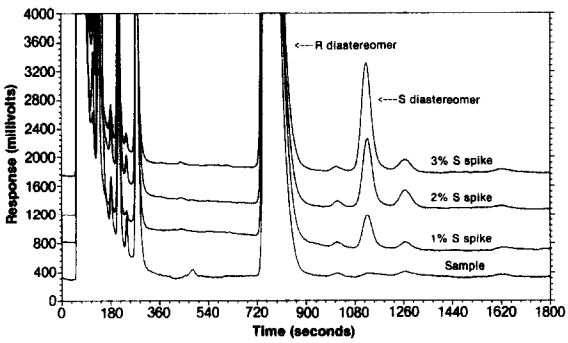

Fig. 2. Example 4, determination of *S* diastereomer in *R* diastereomer by HPLC.

TABLE VI

EXAMPLE 4 SUMMARY

S Isomer spike level	*Days*	*n*	*Average*	S_{sam}
0	5	13	0.17	0.08
0.3%	1	3	0.26	0.10
0.5%	2	6	0.35	0.10
1.0%	1	3	0.93	0.25
				S_P = 0.11

n	*MDL (%)**
1	0.3
2	0.2
5	0.1

* MDL = $0.30/\sqrt{n}$.

Then, the pooled (across spike levels) standard deviation, S_P, and the MDLs were calculated using a value of 2 for k.

Several observations can be made regarding the results for the fourth example. First, the calculated method detection limit is higher than the instrument detection limit, estimated at less than 0.1%, even though the external standard approach acts as an internal control for the completeness of the derivatization reaction. Secondly, the standard deviations for all data at one dose level are higher than the standard deviation for data obtained within a single day. This indicates a difficulty in reproducing the derivatization reaction, chromatography, and/or the peak integration between days. Thirdly, the standard deviations are relatively independent of spike level except for the one anomalous data point at the 1.0% spike level. Finally, although the average percentage S found increases with spike level, there seems to be a bias which affects samples at low percentage S isomer. Spikes at higher levels were necessary to demonstrate complete recovery and linearity (Table VII), but were not used in calculation of the MDL.

TABLE VII

EXAMPLE 4, HIGH LEVELS

Regression line: slope = 1.03, y-intercept = 0.13, correlation coefficient = 0.989

S Isomer spike level (%)	*S Isomer found (%)*
0	0.2
1	1.2
2	1.9
3	3.4

CONCLUSIONS

The determination of a method detection limit is included in the validation of that method. This determination is greatly influenced by the experimental design used for its evaluation. The equation used to calculate the MDL includes the sample and standard replicate numbers and the standard deviation of each, determined by assaying a sufficient number of samples near the estimated detection limit. All sources of variability should be included, allowing for the complete sample preparation scheme and daily sensitivity differences in the instrumentation used for analyte measurement. A number of guidelines have been presented to aid in this determination. MDLs determined in this way should prove to be acceptable for the long-term application of the method for the analysis of pharmaceutical samples.

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REFERENCES

1 B. L. Karger, M. Martin and G. Guiochon, *Anal. Chem.*, 46 (1974) 1640.
2 L A. Currie, *Anal. Chem.*, 40 (1968) 586.
3 H. Kaiser, *Pure Appl. Chem.*, 34 (1973) 35.
4 S. M. Ficarro and K. A. Shah, *Pharm. Manuf.*, 3 (Sept.) (1984) 25.
5 *Principles of Environmental Analysis*, ACS Committee on Environmental Improvement, L. H. Keith, Chairman, *Anal. Chem.*, 55 (1983) 2210.
6 J. A. Glaser, D. L. Foerst, G. D. McKee, S. A. Quave and W. L. Budde, *Environ. Sci. Technol.*, 15 (1981) 1426.
7 G. L. Long and J. D. Winefordner, *Anal. Chem.*, 55 (1983) 712A.
8 J. E. Knoll, *J. Chromatogr. Sci.*, 23 (1985) 422.
9 R. E. Synovec and E. S. Yeung, *Anal. Chem.*, 57 (1985) 2162.
10 H. C. Smit and H. L. Walg, *Chromatographia*, 8 (1975) 311.
11 J. P. Foley and J. G. Dorsey, *Chromatographia*, 18 (1984) 503.
12 J. C. Miller and J. N. Miller, *Statistics for Analytical Chemistry*, Wiley, New York, NY, 1984, Ch. 3 and 4.
13 G. T. Wernimont and W. Spendley, *Use of Statistics to Develop and Evaluate Analytical Methods*, Association of Official Analytical Chemists, Arlington, VA, 1985, Ch. 3 and 4.