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GAS CHROMATOGRAPHIC QUANTITATION OF RESIDUAL IMPURITIES IN RAW MATERIAL MATRIXES

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SUMMARY

To quantitate accurately impurities such as crystallization solvents, isomers, or other residual substances in chemical raw materials, the effect of the matrix on those residuals must be accounted for. This paper describes criteria for evaluation of matrix effects on gas chromatographic quantitation of some residual compounds. Typical method development including sample handling, linearity and precision evaluations, and a practical approach to the decision as to whether standard addition is necessary, is described.

INTRODUCTION

In pharmaceutical as well as other types of analyses it is often necessary to determine trace amounts of solvents, isomers or synthetic impurities in chemical substrates. Spectrometric, colorimetric, titrimetric, chromatographic, electrochemical and other types of assays for residual substances may be in error if the matrix or substrate effect has not been evaluated. The matrix, serving as a foreign substance in a sample, may alter the response of a constituent causing it to be higher or lower than the true value.

The technique of standard addition is widely accepted as an analytical tool for overcoming matrix effects[1]. Net instrument readings are obtained on several solutions: solution A, containing an aliquot of the unknown, and solutions B, C and D each containing the same quantities of unknown solution plus accurately measured amounts of a standard solution of the residual substance at various levels to yield a calibration curve. The quantity of test substance is then determined from its measured instrument responses (R) and the standard calibration curve. The content of residual material (Q_A) originally present in the unspiked sample is given by the intercept of the extrapolated line $R_A R_B R_C \ldots$ on the Q axis as shown in Fig. 1. The value of Q_A may be determined also from $Q_A = R_A/a$ where a is the slope of the line. This calculation may easily be handled by a computer program.

Gas chromatography (GC) has, by virtue of its high selectivity and sensitivity, established itself as a very reliable technique for quantitation of a variety of compounds at trace levels[2–5]. Standard addition is routinely applied in GC[6–8]. In the

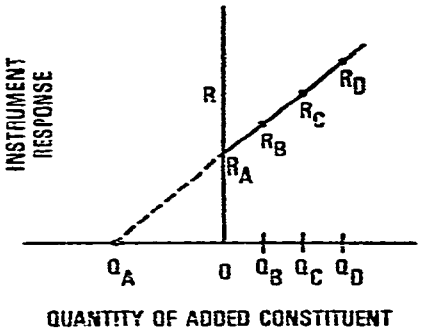


Fig. 1. Illustration of the principle of standard addition.

development of a GC analytical method for a residual substance, it must be decided whether standard addition will be necessary for all future assays of that residual in its substrate. A format is outlined here for use in making that decision. Example GC determinations of various residual compounds in their matrixes are described, and determination of precision of the method is discussed.

EXPERIMENTAL

Reagents

Distilled-in-glass isopropyl alcohol, methyl alcohol and acetonitrile were obtained from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). Acetophenone and methylcyclopentane were purchased from Aldrich (Milwaukee, WI, U.S.A.). Dimethylsulfoxide was "analyzed reagent" grade from J. T. Baker (Philipsburg, NJ, U.S.A.). Matheson, Coleman & Bell (Norwood, OH, U.S.A.) supplied the ACS reagent grade toluene and chloroform used in these experiments. Other chemical materials were obtained from in house sources such as the pilot plant or process development.

Column packing materials were obtained already prepared from several sources. Chromosorb 101 was supplied by Johns-Manville, Celite Division (Denver, CO, U.S.A.). Porapak Q was supplied by Waters Assoc. (Milford, MA, U.S.A.). The Carbowax 20 M-TPA and OV-coated packing materials were supplied by Ohio Valley Specialty (Marietta, OH, U.S.A.).

Equipment

The gas chromatograph was a Hewlett-Packard (Avondale, PA, U.S.A.) Model 5711A dual flame instrument equipped with a Model 5702 oven temperature programmer. For the determination of moisture the chromatograph was fitted with a Model 18723A thermal conductivity detector. In all cases a Model 7671A Hewlett-Packard automatic liquid sampler was used for injections from a 10-μl syringe (Hamilton Model 701N; Reno, NV, U.S.A.). For control of the helium carrier gas flow at 60 ml/min a calibrated Brooks dual GC mass flow controller Model 5840 was used (Brooks Instrument Division, Emerson Electric, Hatfield, PA, U.S.A.). Peak areas or heights were measured using an on-line calculation program from the expanded RTE 2100 computer system (Hewlett-Packard). Statistical evaluation of

data was accomplished using programs available through a time-shared DEC-10 computer system (Digital Equipment, Marlborough, MA, U.S.A.).

Sample preparation

In preparation of solutions used for method development, this general scheme was followed. Into each of six separate 100-ml volumetric flasks were accurately weighed authentic residual material ranging between 0 mg and a convenient upper limit, in many cases 250 mg. These were dissolved in and diluted to volume with an appropriate solvent (to yield stock solutions). Next, two sets of six volumetric flasks (5 ml) were assembled. To the first set were added aliquots (4.0 ml) of the six stock solutions, and each volumetric was diluted to volume with solvent. Each of the second set of 5-ml volumetrics was made to contain nearly identical, accurately known quantities of the matrix compound (250 mg). To these volumetrics were added 4.0-ml aliquots of the six stock solutions, and they were diluted to volume and mixed. The linearity range in each case tested covered zero to two times the nominal residual material limit; *i.e.*, if a 0.5% residual material limit was set for the raw material being assayed, the sample preparation was done in such a manner that the range covered was 0–1.0% (by weight of the matrix). Experimental samples are described in Table I.

Procedure

GC operating conditions are described in Table II for the samples tested. Temperature programming is used where necessary to remove solvents, impurities, or the matrix compound itself from the column. Both sets of solutions described above are chromatographed under identical conditions and peak areas and/or heights are measured. No internal standard is used in these experiments since the reproducibility of the automatic injector is adequate for the residual levels studied.

RESULTS AND DISCUSSION

Table III describes some linearity, precision and matrix evaluation data for the experimental substrate-residual cases listed in Table I.

Using a computer program[9] (available on request), the linearity of response of the residual substance with and without matrix is examined. The percent deviation from at least squares line is evaluated for each of the six points. Linear slope, y-intercept, coefficient of determination and log–log slope are also determined for both sets of solutions. The ratio of the slopes of the lines non-matrix/matrix is then calculated to evaluate the matrix effect on the response for the residual material. If this slope ratio is 0.95–1.05 ("equivalent slopes") the matrix effect is considered to be insignificant and all samples are subsequently assayed by simple comparison to solutions of the authentic residual material. Should the ratio be <0.95 or >1.05, the matrix effect is considered to be important and not just normal assay variation. Consequently all samples are assayed using standard addition. In both cases, the assay reproducibility or precision must be known. For the case of equivalent slopes, all normalized chromatographic responses from the two linearity data sets may be combined to evaluate precision. In the case where nonequivalence is observed however, the method precision is determined from the matrix solutions only, or a

TABLE I

EXPERIMENTAL SAMPLES

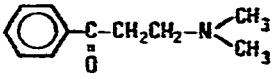
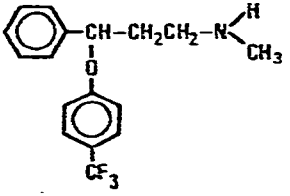
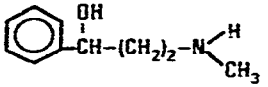
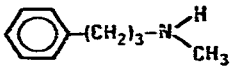
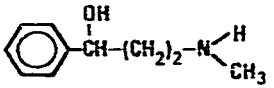
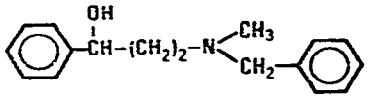
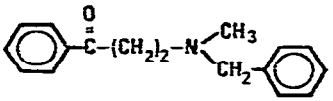
Sample	*Matrix*	*Residual substance*	*Sample solvent*
A		Acetophenone	Methanol
B		Acetonitrile	Methanol
C			Methanol
D			Chloroform
E		Acetophenone	Methanol
F	A synthetic cannabinoid	2-Propanol	Toluene
G	A synthetic cannabinoid	Methylcyclopentane	Dimethylsulfoxide
H	A pentapeptide, acetic acid salt	Water	Methanol

TABLE II

GAS CHROMATOGRAPHIC CONDITIONS

Sample	*Conditions*
A	4 ft. 3% OV-17 on 100–120 mesh Gas-Chrom Q at 110 °C for 4 min, then 32°/min to 250 °C; hold at 250°C for 2 min
B	4 ft. Porapak Q 80–100 mesh at 130 °C
C	3 ft. 3% OV-225 on 100–120 mesh Chromosorb G AW DMCS at 120 °C for 4 min, then 32°/min to 220°C; hold at 220°C for 2 min
D	3 ft. 3% OV-101 on 100–120 mesh Chromosorb G AW DMCS operated at 190 °C
E	6 ft. 10% Carbowax 20 M-TPA on 80–100 mesh Gas-Chrom Q at 145 °C for 8 min, then 32°/min to 230°C, held at 230 °C for 8 min
F	4 ft. 80–100 mesh Chromosorb 101 at 140 °C for 4 min, then 32°/min to 250 °C
G	4 ft. 80–100 mesh Chromosorb 101 operated at 150 °C for 8 min, then 32°/min to 250 °C, hold at 250 °C for 2 min
H	4 ft. Chromosorb 101 at 80 °C

TABLE III

SUMMARY TEST DATA FOR GAS CHROMATOGRAPHIC RESIDUAL MATERIAL QUANTITATION

Calculations for cases B, D, and F were from peak areas; others were from peak heights.

Case	*Linear slope ratio (non-matrix/matrix)*	*Standard addition required?*	*Precision for two replicates (%)**
A	0.71	yes	± 8.6
B	1.01	no	±20
C	0.99	no	±12
D	1.24	yes	± 4.6
E	0.95	no	±14
F	1.01	no	± 2.0
G	1.00	no	±16
H	0.93	yes	±17**

* Expresses the reliability of the assay result if only two sample replicates are assayed as described in the discussion.

** For four replicates.

separate set of matrix solutions (at least 10) may be prepared (as in this work) to evaluate precision if desired. The computer program used in our laboratory[9] determines the number of experimental replicates necessary for an assay. To obtain this precision in an assay where standard addition is not required, preparation of n standard residual solutions and n sample solutions is necessary. Where standard addition is used n spiked sample solutions (besides an unspiked solution A as in Fig. 1) are required. The confidence range (C.R.) for any convenient number of replicates may also be calculated. In case G, two standard solutions of methylcyclopentane must be compared to two sample solutions of cannabinoid using the described chromatographic conditions to arrive at a result for residual methylcyclopentane in the cannabinoid with a confidence of ± 16%. In case A, residual acetophenone may be determined in the matrix with a confidence of ± 8.6% if an unspiked and two spiked (at different levels with acetophenone) samples are prepared and evaluated as described.

Unless the assay values for residual materials are particularly important, analyst and instrument time may be saved by accepting higher relative standard deviation (R.S.D.) values. A C.R. of 2% is typically required in our laboratories for raw material assays while at the ppm level for some residual impurities ± 50% may be acceptable. Table III describes determinations at 1–3% residual levels and the precision associated with two replicates (*i.e.*, C.R. projected for assay involving only two replicates) is acceptable.

For assays using standard addition the quantity of residual substance (Q_A) originally present in the substrate may easily be calculated after the slope and intercept are determined either graphically or by use of a least-squares program:

$$Q_A = \frac{y\text{-intercept}}{\text{slope}}$$

$$\% \text{ residual substance} = \frac{Q_A}{\text{avg. wt. sample, mg}} \times 100$$

CONCLUSIONS

A simple format is described for evaluating the effect of a sample matrix on the GC quantitation of residual materials. A slope variation of more than 5% between matrix and non-matrix lines indicates a slope nonequivalence and requires, in our laboratories, that standard addition be used in all assays for the residual substance in its particular matrix. In the case of slope nonequivalence, replicates to determine assay precision must contain the matrix.

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