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Matrix effects during standard addition quantitation of a trace volatile impurity in a drug substance sample

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Abstract

Matrix effects during standard addition analysis were studied through the determination of trace amounts of butyric acid (a reagent in the synthesis of an experimental drug substance and a residual component affecting the drug quality). By studying the calibration curves with the same concentrations of added component (butyric acid) and different concentrations of drug matrix, it was found that the y-intercept in standard addition analysis is comprised of three factors: (1) y-intercept from a pure analyte calibration curve (without matrix substance), (2) matrix effect from the matrix substance and (3) analyte in the matrix substance. As the matrix effect was quantitatively determined, the absolute value (without matrix effect) of butyric acid in the drug sample could be obtained. Use of an internal standard greatly improved the linearity of the calibration curve and was necessary in this determination. The combination of internal standard and standard addition approaches yielded high accuracy in the determinations.

1. Introduction

One of the most important and difficult analytical problems in pharmaceutical analysis as well other types of chemical analysis is to determine trace amounts of residual components, such as impurities, solvents and reagents, in a large excess of sample matrix substances [1–4]. Standard addition is the most common and useful quantitative method for trace analysis [2,3]. It is widely used in gas chromatography (GC) [2,5], headspace determination [6,7], absorption measurement [8], potentiometric and polarographic measurements [9], etc. The basis for using the standard addition method is to eliminate or minimize matrix effects on the quantitative results. A typical standard addition method is as follows: a series of sample solutions of the same concentration, which contain the analyte of interest, are spiked with increasing amounts of standards of the analyte. The instrumental response of these solutions are plotted *versus* the concentrations of added standards. A linear curve is obtained and extrapolated to the y-axis (see Fig. 1). The y-intercept K and slope A' are obtained ($y = A'x + K$). K is commonly accepted as the response of the analyte of interest. The concentration of the analyte (OX) is determined as K/A'.

A simple calibration curve of a series of standard solutions of a pure analyte is adequate for quantitation of the analyte without matrix substance. The linear equation $y = Ax + B$ is obtained from standard solutions, in which A is

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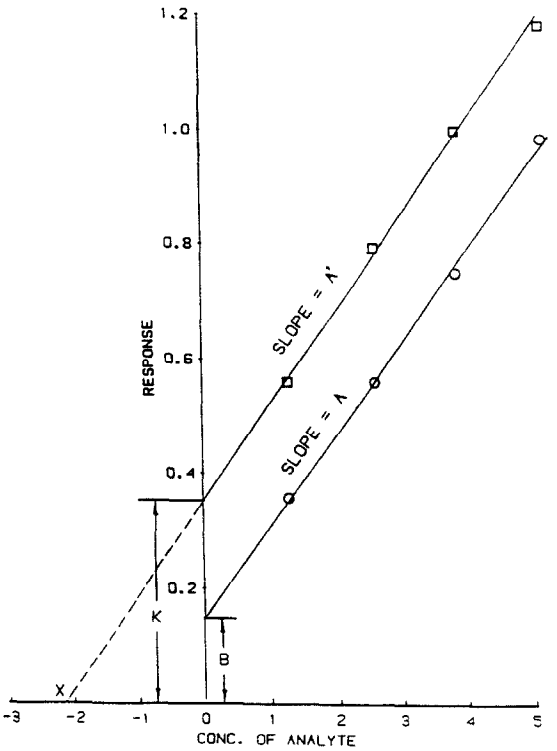

Fig. 1. Calibration curves with and without matrix substance.

the slope of the linear curve and B is the y-intercept (see Fig. 1). The unknown concentration of the analyte can be determined easily by this equation. The calibration curve does not pass through the origin (0) necessarily and, therefore, B cannot be neglected in quantitative analysis. The instrumental response from the analyte of unknown concentration is the sum of two parts: B and response from the analyte. B could be positive, negative or zero.

Compound SC-49483 [1,5-(butylimino)-1,5-dideoxy-D-glucitol tetrabutanoate] is an experimental drug substance being developed at Searle for the treatment of AIDS. Butyric acid is one of the reagents in synthesis. Because of its noxious smell, trace amounts of butyric acid in the drug will affect the drug quality. During the determination of residual butyric acid in drug substance samples by capillary GC, the problem of accurate quantitation was addressed and extensively studied. Internal standard and standard addition methods were studied and combined for accurate determinations at ppm levels. The matrix effect from the drug was also studied and quantitatively determined. The present study has shown that K in standard addition analysis is comprised of three factors: (1) B from the pure analyte calibration curve (without matrix substance), (2) matrix effect from matrix substance and (3) analyte in the matrix substance. This paper has also shown the ways to determine matrix effects quantitatively and to determine the absolute value (without matrix effect) of trace components in a large amounts of matrix.

2. Experimental

2.1. Chemicals

The major drug component SC-49483 was synthesized by the Chemical Sciences Department of Searle Research and Development. Butyric acid, valeric acid and all other solvents were purchased from Aldrich (Milwaukee, WI, USA). The solvent methylene chloride was HPLC grade (99.9% pure).

2.2. Apparatus and conditions

All GC analyses were performed on a Hewlett-Packard HP 5890 gas chromatograph equipped with a flame ionization detector and an HP 7673 autosampler. A fused silicone capillary column DB-EEAP of 15 m × 0.53 mm I.D. and 1 μm thickness (J & W, Folsom, CA, USA) was used for analysis. A piece of 90 cm of phenylmethyl siloxane deactivated uncoated fused-silica tubing with 0.53 mm I.D. (J & W) was used for protection of major column. Carrier gas (helium) flow-rate was 5 ml/min, hydrogen and air flow-rates were 30 and 350 ml/min, respectively. The split ratio is 1:12. The GC conditions were as follows: the initial temperature was 110°C, held for 1 min, then raised at 8°C/min to 175°C, then raised at 70°C/min to 250°C, held for 10 min. The injection and detection temperatures were 180 and 260°C, respectively. All the data and chromatogram were recorded and processed with an in-house chromatographic data system.

2.3. Procedure for calibration curves with internal standard and drug matrix

A diluting solution (about 5 μg/ml) was made by accurately weighing about 5 mg valeric acid into a 1000-ml volumetric flask, filling with methylene chloride to the volume and shaking well. The concentration of valeric acid can be accurately calculated. This diluting solution was used for preparing all the calibration standards and drug samples. A stock solution of matrix sample (about 150 mg/ml) was made by accurately weighing about 1500 mg SC-49483 into a 10-ml volumetric flask, filling with diluting solution to the volume and shaking well until SC-49483 was completely dissolved. Then a series of calibration standards was made with five different concentrations of butyric acid and the same concentration of drug matrix (15 mg/ml, 1.00 ml stock solution of matrix sample solution in each of the standards). These standards were then injected into the GC system. The run was duplicated and the data were averaged. A calibration curve could be made by plotting the peak area ratio of butyric acid to valeric acid of these five standards *versus* the concentration of added butyric acid.

For quantitative determination of the matrix effect, five calibration curves were made with different matrix concentrations as 0, 15.07, 30.07, 45.05 and 60.02 mg/ml SC-49483 (see Table 3). The concentrations of added butyric acid for each of these calibration curves were the same, *viz.* 1.29, 2.58, 3.87, 5.16 and 6.45 μg/ml (not listed in Table 3). The concentration of internal standard valeric acid was 4.37 μg/ml. The results in Table 3 are the average of two runs.

3. Results and discussion

3.1. Internal standard in the determination of butyric acid

Because of the nature of GC, especially under split injection mode, an internal standard can greatly improve the linearity of the calibration curve for quantitation. The highest precision for quantitative chromatography is obtained by use of an internal standard because the uncertainties introduced by sample injection are avoided [10]. The internal standard approach is widely used in GC [11,15], headspace GC [12], GC–mass spectrometry [13], vapor measurement [14], liquid chromatography [16], etc. In the present analysis of trace amounts of butyric acid in the drug substance, the evaporation mode of the matrix substance is complicated. The evaporation rate of matrix substances and the exact split ratio of this vapor are unpredictable. Although an autosampler is used and the injected sample volume is constant, the amounts of butyric acid entering the separation column are different from injection to injection with the same sample solution. Therefore, an internal standard, valeric acid, was used since it has chemical properties similar to butyric acid and a suitable retention time. A chromatogram of butyric acid in drug matrix SC-49483 spiked with the internal standard valeric acid is shown in Fig. 2.

Tables 1 and 2 summarize the data for the responses of five concentrations of butyric acid, the internal standard valeric acid and respective peak area ratios. Table 1 was obtained from standards without drug matrix, Table 2 from

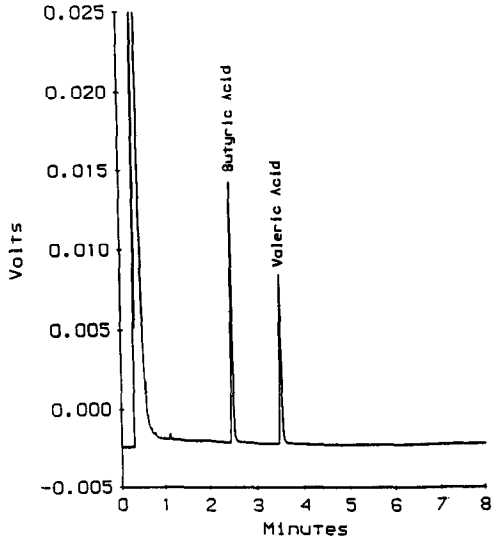

Fig. 2. Chromatogram of determination of butyric acid.

Table 1

Linearity without drug matrix

Concentration of butyric acid (μg/ml)	Peak area		Peak area ratio butyric acid/ valeric acid
	Butyric acid (t_R = 2.44 min)	Valeric acid (t_R = 3.46 min)[a]	
1.91	0.0000653	0.0005057	0.12913
3.83	0.0000885	0.0003612	0.24502
5.74	0.0002163	0.0006028	0.35883
7.65	0.0001401	0.0002951	0.47475
9.56	0.0002223	0.0003790	0.58654
Average		0.0004338	
R.S.D. (%)		0.3058	
Correlation coefficient	0.8053		0.9999
y-Intercept	0.0003677		0.01537
Slope	0.00001912		0.05986

[a] The concentration of valeric acid is 5.56 μg/ml.

standards with drug matrix. The internal standard significantly improved the linearity of the calibration curve for butyric acid with and without drug matrix. Without using internal standards, the linear correlation coefficients for butyric acid are 0.9212 and 0.8053, with and without drug matrix, respectively. However, they are larger than 0.999 when using the internal standard valeric acid. It is obvious that use of an internal standard greatly improved the response linearity for butyric acid in the SC-49483 drug substance samples.

Table 2

Linearity with drug matrix SC-49483[a]

Concentration of butyric acid (μg/ml)	Peak area		Peak area ratio butyric acid/ valeric acid
	Butyric acid (t_R = 2.44 min)	Valeric acid (t_R = 3.46 min)[b]	
3.83	0.0003044	0.0004044	0.75272
5.74	0.0003948	0.0004547	0.86826
7.65	0.0005360	0.0005368	0.99851
9.56	0.0006228	0.0005527	1.12683
Average		0.0004996	
R.S.D. (%)		0.1333	
Correlation coefficient	0.9212		0.9996
y-Intercept	0.0002083		0.5087
Slope	0.00004065		0.06412

[a] The concentration of SC-49483 is 15.024 mg/ml.
[b] The concentration of valeric acid is 5.56 μg/ml.

3.2. Standard addition to minimize matrix effect

Previous authors have suggested that the standard addition method should be used to minimize errors when the difference in slopes of analyte calibration curves with and without sample matrix is larger than 5% [1]. Tables 1 and 2 compare slope values for butyric acid in a range of 1.91 to 9.56 μg/ml, with and without drug matrix. The slope value is 0.06412 and the linear correlation coefficient is 0.9997 with drug matrix, while it is 0.05986 without drug matrix. The difference between these two slopes is 7.1%, indicating that the standard addition method should be used for this analysis.

3.3. Quantitative determination of matrix effect

Although the matrix affects quantitative analysis, the quantitative study of matrix effects has not been reported. Usually, attempts are made to approximate the concentrations of analyte and matrix substance in standard addition analysis to minimize the matrix effects. In the present paper, a study was conducted to determine the quantitative effects of the matrix substance in trace analysis.

Data in Table 3 and Fig. 3 show the information of calibration curves with different concentrations of drug matrix. The slopes of these calibration curves are different (7.2 to 11.1%) from the slope determined without matrix drug. The average of four slopes with drug matrix (concentrations from 15.07 to 60.02 mg/ml) is 0.1640 with 1.63% R.S.D. Although the matrix concentration increases 400%, the slopes of the calibration curves have only small changes in the range from 0.1616 to 0.1649. However, the y-intercept values exhibited marked changes from 0.1126 to 0.4615 as the matrix concentration increases. As shown in Table 3, the pure butyric acid calibration curve has an intercept of 0.01235, which means there was a chromatographic response at zero concentration of butyric acid although no peak was detected at this point. The response is not contributed by butyric acid but by unknown system factors. The zero-butyric acid response (y-intercept, 0.01235), of course, is also included in the y-intercepts of calibration curves with drug matrix since they are under the same experimental conditions. This is an im-

Table 3

Multiple calibration curve with different amounts of drug matrix

Matrix concentration (mg/ml of SC-49483)	Correlation coefficient	y-Intercept	Slope (ml/μg)	Difference of slope (%) compared with pure butyric acid
0	0.9998	0.01235	0.1507	
15.07	0.9991	0.1126	0.1622	+7.6%
30.07	0.9980	0.2198	0.1616	+7.2%
45.05	0.9995	0.3403	0.1674	+11.1%
60.02	0.9919	0.4615	0.1649	+9.4%
Average[a]			0.1640	
S.D.[a]			0.00267	
R.S.D. (%)[a]			1.63	

The sample lot used in this table is different from the one used in Tables 1 and 2; therefore, the concentrations of butyric acid in these two sample lots are different. The concentrations of added butyric acid and internal standard valeric acid are described in the Experimental section.

[a] Average, S.D. and R.S.D. are statistics of four slopes with different concentrations of drug matrix SC-49483.

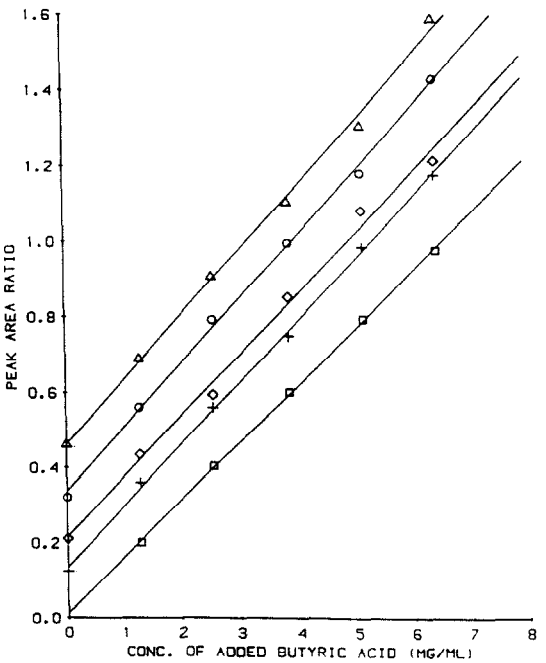

Fig. 3. Calibration curves with different concentrations of drug matrix including 0 (□), 15.07 (+), 30.07 (◇), 45.05 (○) and 60.02 (△) mg/ml.

portant factor which should be considered in standard addition analysis.

Another factor, which is important but usually neglected in standard addition analysis, is the quantitative impact of the matrix concentration on the analysis of the analyte. The *y*-intercept of the calibration curve with 15.07 mg/ml matrix concentration is 0.1126. The difference between 0.1126 and 0.01235 (*y*-intercept without matrix, see Fig. 3 and Table 3) is contributed by two factors: butyric acid in the drug substance and the matrix effect, which could be positive, negative or zero. Since butyric acid always exists in the drug samples, it is difficult to determine quantitative effects of matrix directly. An indirect method has to be used.

Data in Table 4 were used for studying the matrix effect quantitatively. Column 5 is analogous to the equation of K/A' (see Introduction). If the matrix effect is not considered, these values can be assumed to represent the concentration of butyric acid in the sample solution. The assumed unit concentrations of butyric acid in drug matrix (μg/mg) can be calculated by column 5/column 1 and are listed in column 6. The assumed unit concentration of butyric acid (μg/mg) increases as concentrations of drug matrix (mg/ml) increases. The increase is due to the matrix effects since the unit concentration of butyric acid in the drug matrix is constant in the same lot. The values in columns 5 and 6 are contributed by two factors: butyric acid in the drug substance and the matrix effect from the drug substance. A plot of assumed unit concentration *versus* matrix concentration was

Table 4

Matrix effect on chromatographic response

1: Matrix amounts (mg/ml of SC-49483)	2: *y*-Intercept	3: Difference of *y*-intercept with and without drug matrix	4: Slope (ml/μg)	5: Column 3 divided by column 4 (μg/ml)	6: Assumed unit concentration of butyric acid (μg/mg)
0	0.01235				
15.07	0.1126	0.10025	0.1622	0.61806	0.0410
30.07	0.2198	0.20745	0.1616	1.28373	0.0427
45.05	0.3403	0.32795	0.1674	1.95908	0.0435
60.02	0.4615	0.44915	0.1649	2.72377	0.0454
y-Intercept (μg/ml)					0.0396
Slope (μg ml/mg^2)					0.0000934
Correlation coefficient					0.9895

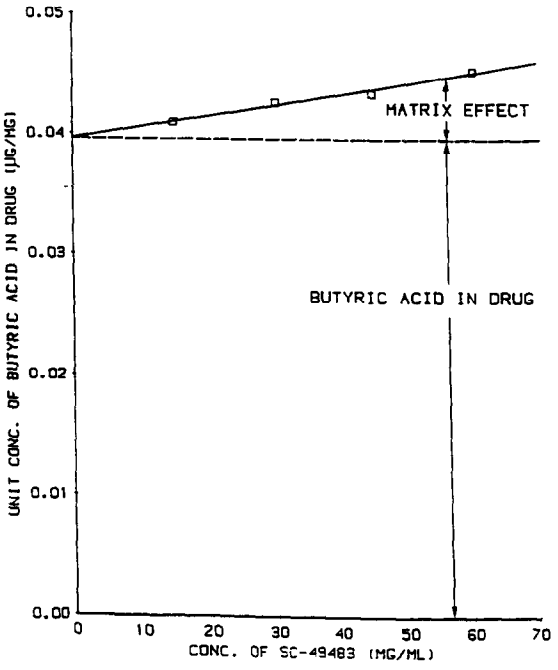

Fig. 4. Unit concentration (μg/mg) of butyric acid with different concentrations of drug matrix (mg/ml).

linear (Fig. 4). The intercept, slope and correlation coefficient were calculated and are also listed in column 6, Table 4. The value of intercept (0.0396 μg/mg) is the actual unit concentration of butyric acid in drug matrix SC-49483 since it is the value at zero concentration of drug matrix. Matrix effects no longer exist at this point. The higher assumed unit concentration of butyric acid observed with the higher concentration of drug matrix is due to the increased matrix effect, which has a positive quantity of 0.0000934 (slope) with a unit (μg/mg per mg/ml =) μg ml /mg^2. This means that 1 mg/ml increase in concentration of drug matrix will result in 0.0000934 μg/mg increase in the experimentally determined butyric acid level. The matrix effects could be negative or zero. If it is negative, the assumed unit concentration of butyric acid would decrease as the concentration of matrix increases. If it is zero, the assumed unit concentration of butyric acid would not change as the concentration of the matrix changes.

The experiments detailed above illustrate the impact of drug matrix concentration on butyric acid determination. It has been illustrated that K (see Introduction and Fig. 1) during standard addition analysis is comprised of three parts: (1) B from the pure butyric acid calibration curve (without drug matrix), (2) matrix effect from drug substance and (3) butyric acid in the drug substance.

3.4. Deviation in routine analysis due to matrix effect

It has been shown that the true value of butyric acid concentration in the matrix drug is accessible. However, it is not practical to analyze routine samples in this manner. It is much more practical in daily analysis to use one calibration curve for multiple samples under the same conditions, although some deviation in analysis will result.

The impact of the matrix concentration dependence illustrated in Table 4 and Fig. 4 on quantitative results can be understood by considering the following example. The deviation can be estimated according to the linear regression data (intercept and slope) in column 6 of Table 4. The slope is 0.0000934 (μg ml/mg^2), which means that a 1 mg/ml increase of matrix concentration causes ($1 \times 0.0000934/0.0396 =$) 0.24% deviation. For example, the deviation at 5.0 mg/ml matrix concentration is about ($5 \times 0.0000934/0.0396 =$) 1.2% higher. In daily analysis, it is difficult to weigh the sample so that it has exactly the same concentration as the matrix drug. But it can be weighed very close. For the concentration of matrix of 15.00 mg/ml, it is easy to weigh 140 to 160 mg sample for a 10-ml volumetric flask. The concentration would be within 14.0 to 16.0 mg/ml, and the error would be within ($\pm 1 \times 0.0000934/0.0396 =$) $\pm$ 0.24%, which is insignificant.

4. Conclusions

Matrix effects during standard addition analysis were extensively studied through the determination of trace amounts of butyric acid in the

matrix drug SC-49483. Both internal standard and standard addition are necessary for quantitation because of the nature of GC and matrix effect. Linearity is greatly improved by the use of internal standard. The difference of slopes with and without matrix drug is larger than 7%. The matrix effect was studied by calibration curves with different concentrations of matrix drug. The slopes have small changes when the concentration of matrix drug increased 400% from 15 to 60 mg/ml. The matrix effect of the drug substance was quantitatively analyzed and the true value of butyric acid concentration in the matrix drug was accurately determined. The deviation using one calibration curve for multiple samples in routine analysis was also discussed quantitatively.

5. References

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