K17 Comparison of Calibration Approaches for the Quantitative GC/MS Analysis on Secobarbital

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After attending this presentation, attendees will be familiar with the characteristics of the calibration curves resulting from the use of isotopic analogs of the analyte as the internal standards (ISs). Specific parameters studied include (a) ion cross-contribution and (b) column temperature programming conditions that may affect the use of calibration approaches.

This study was placed on practically evaluating the calibration approach by using ^{2}Hand ^{13}C-analogs as ISs for the quantitative determination by GC/MS in urine. An automatic well-established solid-phase extraction and methylation procedures were used prior to the GC/MS measurement. The cross-contribution of ions designated for the analyte and its IS were evaluated by the "direct normalized measurement" method through selected ion monitoring (SIM) mode. The spiked IS magnitude and reconstitute volume were also evaluated for the appropriate GC/MS determination at low concentration level. To decrease the cross-contribution, 50 ng/mL $^{2}H_{5}$-analog and 25 ng/mL $^{13}C_{4}$-analog ISs were respectively added into each standard solution. One-point, linear, hyperbolic and polynomial calibration approaches were used to investigate the quantitative effectiveness based on the comparison of theoretical and observed concentrations of standard solutions containing 10 to 800 ng/mL secobarbital. Two GC column temperature programming conditions, 20 ºC high ramp rate and 2 ºC low ramp rate, were adopted to generate different degrees of peak-overlap and ion cross-contribution for the purpose of evaluating the most appropriate application for each calibration approach.

Ion cross-contribution and the "over-all non-proportional change in ionization efficiency" phenomenon have been regarded as the underlying causes to change the theoretical analyte/IS ratios. Data shown in Table 1 indicate that cross-contribution deriving from IS to the analyte leads to the positive observed concentration at low concentration levels by one-point calibration. This phenomenon obviously shows that 13 % m/z 195 ion contributed from IS generates higher observed concentration values at low concentration levels than that of 1.9 % m/z 196 ion. Thus, the ion-pair with the less amount of ion cross-contribution should be the most appropriate candidate for the quantitation by using the one-point calibration approach. This trend also reveals that the more intensity of ion-pair ratios resulting from ion cross-contribution increases, the more peak-overlapping under 20 ºC high ramp rate does as well. The ratios at low concentration levels become farther to the "expected" values based on the ion crosscontribution resulting from the IS. Temperature programming is the other interference factor in the quantitative determination by one-point calibration. To determine the lower concentration levels, the lower ramp rate is a better temperature programming. The deviation obtained in comparison the theoretical with the observed concentration in standard solutions at low concentration levels

Table 1. Comparison of quantitation results using different ion pairs and calibration approaches—
Analyte/IS: Secobarbital/$^2H_5$-analog.

m/z	Program	Conc. ng/mL	Ratio	Dev.% by One Point	Dev.% by Linear	Dev.% by Hyperbolic	Dev.% by Polynomial
196/201	2 ºC ramp rate	10	0.1909	9.843(-1.6)	9.610(-3.9)	7.654(-23.5)	9.859(-1.4)
		20	0.4016	20.71(3.5)	20.36(1.8)	18.74(-6.3)	20.12(0.6)
		50	0.9697	Calibrator	49.35(-1.3)	48.59(-2.8)	48.09(-3.8)
		100	2.070	106.8(6.8)	105.5(5.5)	106.2(6.2)	103.4(3.4)
		200	3.895	200.8(0.4)	198.6(-0.7)	201.2(0.6)	197.9(-1.1)
		400	7.702	397.1(-0.7)	392.8(-1.8)	397.1(-0.7)	400.6(0.1)
		800	15.73	810.9(1.4)	802.3(0.3)	800.5(0.1)	797.8(-0.3)
	20 ºC ramp rate	10	0.2206	11.16(11.6)	10.57(5.7)	9.232(-7.7)	10.44(4.4)
		20	0.4036	20.42(2.1)	20.06(0.3)	18.92(-5.4)	19.72(-1.4)
		50	0.9884	Calibrator	50.36(0.7)	49.83(-0.3)	49.56(-0.9)
		100	1.974	99.84(-0.2)	101.4(1.4)	101.8(1.8)	100.4(0.4)
		200	3.877	196.1(-1.9)	200.0(0.0)	201.8(0.9)	199.9(-0.0)
		400	7.645	386.7(-3.3)	395.2(-1.2)	398.1(-0.5)	399.9(-0.0)
		800	15.49	783.7(-2.0)	801.8(0.2)	800.3(0.0)	801.0(0.1)
195/200	2 ºC ramp rate	10	0.4298	13.18(31.8)	8.568(-14.3)	7.971(-20.3)	9.968(-0.3)
		20	0.7075	21.70(8.5)	18.01(-9.9)	17.50(-12.5)	18.86(-5.7)
		50	1.631	Calibrator	49.41(-1.2)	49.17(-1.7)	48.72(-2.6)
		100	3.347	102.6(2.6)	107.8(7.8)	108.0(8.0)	105.4(5.4)
		200	6.010	184.3(-7.8)	198.4(-0.8)	199.1(-0.4)	196.2(-1.9)
		400	11.84	362.9(-9.3)	396.5(-0.9)	397.7(-0.6)	401.8(0.5)
		800	23.73	727.8(-9.0)	801.2(0.1)	800.5(0.1)	802.4(0.3)
	20 ºCramp rate	10	0.7008	19.68(96.8)	12.28(22.8)	10.72(7.2)	11.40(14.0)
		20	0.9275	26.05(30.2)	20.18(0.9)	18.84(-5.8)	19.04(-4.8)
		50	1.780	Calibrator	49.90(-0.2)	49.32(-1.4)	47.98(-4.0)
		100	3.346	93.98(-6.0)	104.5(4.5)	105.1(5.1)	102.0(2.0)
		200	6.084	170.9(-14.6)	199.8(-0.1)	202.2(1.1)	198.8(-0.6)
		400	11.62	326.4(-18.4)	392.8(-1.8)	396.8(-0.8)	399.0(-0.3)
		800	23.35	655.7(-18.0)	801.3(0.2)	800.6(0.1)	798.9(-0.1)

by linear calibration was obviously lower than that by one-point approach. Some figures even reduce to negatives. This trend presents that ion cross-contribution generating higher ion-pair ratios at low concentration levels can be adjusted based on the lower ion-pair ratios deriving from the “non-proportional over-all change in ionization efficiency” phenomenon at high concentration levels. Thus, the linearity of the calibration curve increases, especially at low concentration range. Due to the slight increase of ion cross-contribution along with the increasing peak-overlap, the temperature programming with high ramp rate will also bring about the higher observed concentration by linear calibration at low concentration levels Quantitation results using hyperbolic calibration show the different phenomenon. The ion-pair with the higher ion cross-contribution leads to the lower observed concentration and deviation. This trend indicates that the characteristic of the hyperbolic curve is suitable for standard solutions with the higher ion crosscontribution. Thus, the quantitative effectiveness for low concentration levels using high ramp rate are better than those using low ramp rate on GC temperature program. Resulting data using polynomial calibration demonstrate that all of ion-pairs generate ideal quantitation without interference caused by ion cross-contribution and GC temperature programming. Polynomial curves can appropriately fit in ion-pair ratio of each standard solution. The only defect is the complicated procedure used to solve the equations obtaining from polynomial regression.

Ion cross-contribution is the underlying cause to interfere with the quantitative determination by one-point and linear approaches at low concentration range. This situation can be improved by GC temperature programming. On the contrary, hyperbolic calibration can be used for the ion-pair containing high ion crosscontribution. Polynomial calibration is an ideal approach because there is no need to select an ion-pair via the time-consuming evaluation.

§Ion cross-contribution–m/z: 196/201 (1.9 % contributed by IS; 0.33 % contributed by analyte); m/z 195/200 (13 % contributed by IS; 0.59 % contributed by analyte).

*Regression equations—For m/z 196/201: $y=0.0196\ x + 0.0025$($r^2=0.9998$) under 2 ºC ramp rate, $y = 0.0193\ x + 0.0165$ ($r^2=0.9999$) under 20 ºC ramp rate; for m/z 195/200: $y = 0.0294\ x + 0.1779$($r^2=0.9998$) under 2 ºC ramp rate, $y = 0.0286\ x + 0.3719$($r^2=0.9999$) under 20 ºC ramp rate.

†Regression equations—For m/z 196/201: $y = (2.3995 + x)/(-0.0020\ x + 52.68)$ ($r^2=0.9999$) under 2 ºC ramp rate,

$y = (2.4480 + x)/(-0.0014 x + 52.97)$ ($r^2=1.0000$) under 20 ºC ramp rate; for m/z 195/ 200: $y = (6.7870 + x)/(-0.0004 x + 34.34)$ ($r^2=0.9998$) under 2 ºC ramp rate, $y = (14.383 + x)/(-0.0012 x + 35.84)$ ($r^2=1.0000$) under 20 ºC ramp rate.

‡Regression equations—For m/z 196/201: $y = 6x10^{-9}X^3-6x10^{6}X^2+0.0207X-0.0126$ ($r^2=1.0000$) under 2 ºC ramp rate, $y = 3x10^{-9}X^3-3x10^{-6}X^2+0.0198X-0.0142$ ($r^2=1.0000$) under 20 ºC ramp rate; for m/z 195/200: $y = 8x10^{-9}X^3-9x10^{-6}X^2+0.0315X-0.1167$ ($r^2=0.9999$) under 2 ºC ramp rate, $y = 7x10^{-9}X^3-7x10^{-6}X^2+0.0299X-0.3608$ ($r^2=1.0000$) under 20 ºC ramp rate.

Calibration, GC/MS Analysis, Isotopic Analogs