Food and Environmental

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CCα and CCβ automatic calculation and flagging in SCIEX OS-Q Software

Yoann Fillâtre,[1] Loïc Beyet,[1] Jianru Stahl-Zeng[2]
[1]SCIEX, France, [2]SCIEX, Germany

Veterinary drugs are commonly used in livestock breeding to prevent or treat infections of the animals and to ensure their optimal growth. LC-MS/MS is the method of choice to routinely control the concentration of their residues in food of animal origin. As some of the veterinary drugs are banned substances, the traceability of the methods performance is critical. Laboratories must follow the European Decision no. 657/2002/EC concerning the performance of analytical methods and the interpretation of results and calculate two statistical limits:[1]

- The decision limit CCα means the limit at and above which it can be concluded with an error probability of α (1% for substances with non-permitted limits and 5% for substances with a permitted limit such as the Maximum Residue Limit (MRL) that a sample is non-compliant[1]

- Detection capability (CCβ) means the smallest content of the substance that may be detected, identified and/or quantified in a sample with an error probability of β (5% for all substances with a permitted or no permitted limit)[1]

Based on the recommendation of CD657/2002/EC, CCα and CCβ can be calculated with either one of the two following approaches. The first method is based on the calculation of the signal-to-noise ratio of 20 blank samples for banned substances and 20 blank samples spiked at the MRL for permitted substances. The second approach, based on the calibration curve procedure defined in the ISO 11843,[2] is often preferred by the laboratories because of its greater ease of implementation for routine analyses. The calculation is based on day-to-day calibration of the method analyzing the batch of interest including the unknown samples to be controlled.[3]

The complexity of the calculation requires the use of a spreadsheet software such as Excel to determine CCα and CCβ. This procedure requires, at minimum, the manual step of copying/pasting the area of each analyte peak into the spreadsheet. Therefore for each analyte and each batch of analyses, CCα and CCβ must be first calculated before the analyst can then compare and review the analyte concentration versus these decision limits. This means that the procedure is very time consuming for a laboratory to perform routinely.

Thanks to the inclusion of advanced and flexible calculation capabilities in SCIEX OS Software as shown in Figure 1, this calculation can now be executed automatically in the software, saving a lot of time and minimizing the risk of error for laboratories.

Key features

- Automatic calculation of CCα and CCβ for both non-permitted and permitted substances at the same time
- Automatic flagging of samples with analyte concentrations above CCα and/or CCβ

Sample Name	Component Name	Calculated Concentration	MRL	CCalpha_1%	Flag CCalpha_1%	CCalpha_5%	Flag CCalpha_5%	CCbeta	Flag CCbeta
Unknown_2	Diclofenac_Auth 1	< 0	5.000	5.693		5.394		5.789	
Unknown_2	Flunixin_Auth 1	< 0	20.000	23.564		22.028		24.056	
Unknown_2	Ketoprofen_Ban 1	9.277	0.000	0.552	8.725	0.341	8.936	0.683	8.595
Unknown_2	Mefenamic Acid_Ban 1	1.036	0.000	0.615	0.421	0.380	0.656	0.760	0.276
Unknown_2	Meloxicam_Auth 1	< 0	20.000	21.401		20.797		21.594	
Unknown_2	Naproxen_Ban 1	< 0	0.000	0.683		0.422		0.844	
Unknown_2	Oxyphenbutazone_Ban 1	< 0	0.000	0.352		0.218		0.435	
Unknown_2	Phenylbutazone_Ban 1	< 0	0.000	0.481		0.298		0.595	
Unknown_2	Tolfenamic Acid_Auth 1	< 0	50.000	53.644		52.073		54.146	
Unknown_2	Vedaprofen_Ban 1	< 0	0.000	1.987		1.228		2.457	

Figure 1. Automatic analysis. Automatic calculation of CCα and CCβ for both non-permitted and permitted substances with defined MRL + automatic flagging of samples with concentration above those decision limits.

Methods

A set of matrix-spiked calibrants must be analyzed with at least three levels of equally-distant concentrations. The critical concentration can be calculated as followed:

- For non-permitted substances

$$CC_\alpha = t_{(\alpha,IJ-2)} \frac{\hat{\sigma}}{\hat{b}} \sqrt{\frac{1}{K} + \frac{1}{IJ} + \frac{\bar{x}^2}{\sum(x_{ij} - \bar{x})^2}}$$

$$CC_\beta = \delta_{(IJ-2,\alpha,\beta)} \frac{\hat{\sigma}}{\hat{b}} \sqrt{\frac{1}{K} + \frac{1}{IJ} + \frac{\bar{x}^2}{\sum(x_{ij} - \bar{x})^2}}$$

- For permitted substances with a defined MRL

$$CC_\alpha = x_{MRL} + t_{(\alpha,IJ-2)} \frac{\hat{\sigma}}{\hat{b}} \sqrt{\frac{1}{K} + \frac{1}{IJ} + \frac{x_{MRL}-\bar{x}^2}{\sum(x_{ij} - \bar{x})^2}}$$

$$CC_\beta = x_{MRL} + \delta_{(IJ-2,\alpha,\beta)} \frac{\hat{\sigma}}{\hat{b}} \sqrt{\frac{1}{K} + \frac{1}{IJ} + \frac{x_{MRL}-\bar{x}^2}{\sum(x_{ij} - \bar{x})^2}}$$

Table 1. Definition of the calculation parameters.

Parameters	Definition
I	Number of calibrating levels of concentration
J	Number of replicates per calibrating level of concentration
K	Number of unknown replicates
x_{ij}	Concentration of calibration levels
$\bar{x}$	Average concentration of the calibration levels
x_{MRL}	Referenced MRL value of the analyte
$\hat{\sigma}$	Residual standard deviation of the regression function
$\hat{b}$	Slope of the calibration curve
$t_{(\alpha,IJ-2)}$	t Student at risk α and for degree of freedom (dof) equal to (I.J)-2
$\delta_{(\beta,IJ-2)}$	Statistical function that can be approximated by using 2 $t_{(\alpha,IJ-2)}$

The following constant values I, J, K, t student at confidence level of 95 and 99 % and the MRL are manually entered in the corresponding custom column. Some of these values can be different from one analyte to another and can be modified accordingly.

By using $\hat{b}$, the slope of the calibration curve and $\hat{\sigma}$, a custom calculated column of formulae $\hat{\sigma} = \sqrt{\frac{1}{n-2}\sum_{i=1}^{n}(yi - \hat{y}_i)^2}$, the critical limits CC_α and CC_β can be calculated for each analyte and displayed (with or without the other columns) in the results table. This calculation procedure includes, if necessary, the use of internal standards.

CC_α and CC_β calculation

For non-permitted substances:

Figure 2 displays the results comparison of CC_α and CC_β between the automatic calculation with SCIEX OS Software and the classical calculation with a spreadsheet software (bottom right) for the non-permitted substance Mefenamic Acid.

In this case, the constant values have the following values: I = 4, J = 2, K = 2 (only one repetition is shown in the results table), MRL = 0, t student (1%) = 3.143 and t student (5%) = 1.943. SCIEX OS Software is then able to calculate $\delta_{(IJ-2,\alpha,\beta)}$ (Delta), $\hat{\sigma}$ (Sigma) and finally $CC\alpha$ (1%) and $CC\beta$ (5%).

As shown in Figure 1, the results obtained with SCIEX OS Software (CC_α = 0.615; CC_β = 0.760) are strictly identical as those obtained by the spreadsheet software.

For permitted substances with a defined MRL:

Figure 3 displays the results comparison of CC_α and CC_β between the automatic calculation with SCIEX OS Software and the classical calculation with a spreadsheet software (bottom right) for the permitted substance Tolfenamic Acid.

In this case, the first calibration level is removed thus changing the value of I (I = 3) and t student (1 %: 3.747; 5 %: 2.132) which can be done directly in the result table. Additionally, the regulatory MRL of Tolfenamic acid must be set in the MRL column. The calculation of CC_α (5%) and CC_β (5%) return the exact same results as the spreadsheet software (CC_α = 52.073; CC_β = 54.146) confirming the validity of the calculations.
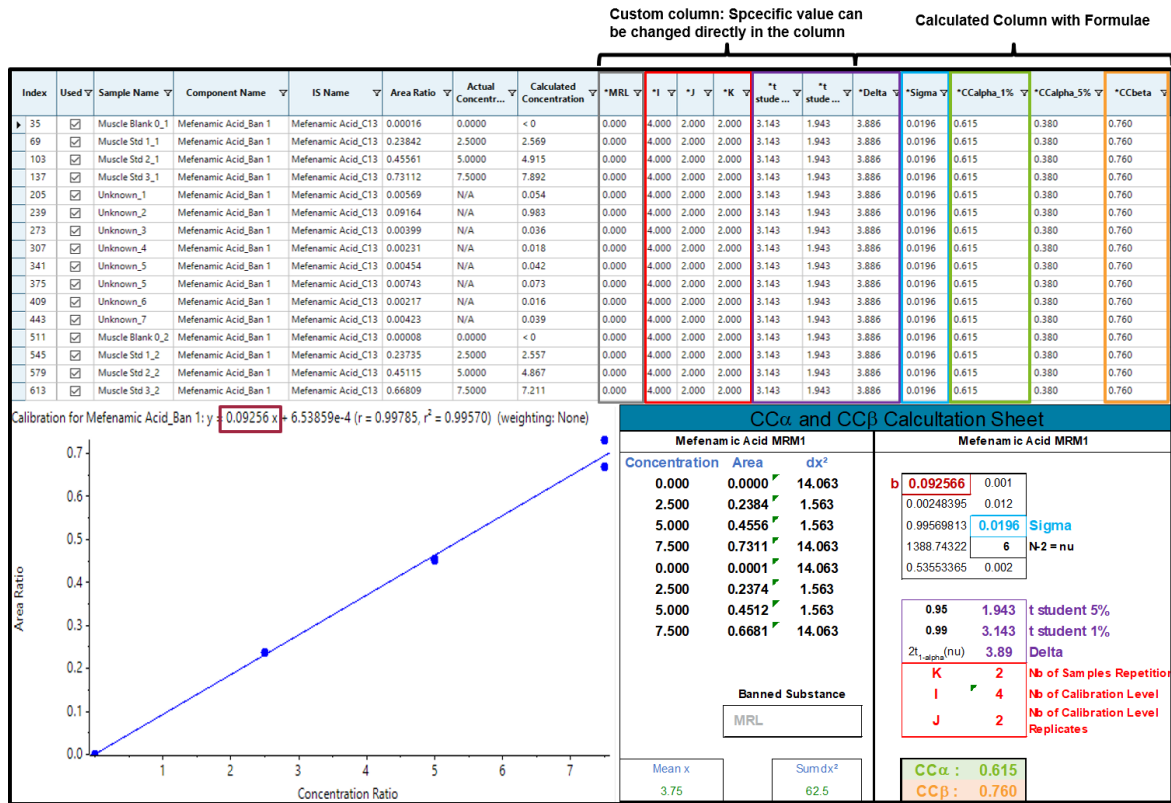
Custom column: Spcecific value can be changed directly in the column **Calculated Column with Formulae**

Index	Used	Sample Name	Component Name	IS Name	Area Ratio	Actual Concentr...	Calculated Concentration	*MRL	*I	*J	*K	*t stude...	*t stude...	*Delta	*Sigma	*CCalpha_1%	*CCalpha_5%	*CCbeta
35	☑	Muscle Blank 0_1	Mefenamic Acid_Ban 1	Mefenamic Acid_C13	0.00016	0.0000	< 0	0.000	4.000	2.000	2.000	3.143	1.943	3.886	0.0196	0.615	0.380	0.760
69	☑	Muscle Std 1_1	Mefenamic Acid_Ban 1	Mefenamic Acid_C13	0.23842	2.5000	2.569	0.000	4.000	2.000	2.000	3.143	1.943	3.886	0.0196	0.615	0.380	0.760
103	☑	Muscle Std 2_1	Mefenamic Acid_Ban 1	Mefenamic Acid_C13	0.45561	5.0000	4.915	0.000	4.000	2.000	2.000	3.143	1.943	3.886	0.0196	0.615	0.380	0.760
137	☑	Muscle Std 3_1	Mefenamic Acid_Ban 1	Mefenamic Acid_C13	0.73112	7.5000	7.892	0.000	4.000	2.000	2.000	3.143	1.943	3.886	0.0196	0.615	0.380	0.760
205	☑	Unknown_1	Mefenamic Acid_Ban 1	Mefenamic Acid_C13	0.00569	N/A	0.054	0.000	4.000	2.000	2.000	3.143	1.943	3.886	0.0196	0.615	0.380	0.760
239	☑	Unknown_2	Mefenamic Acid_Ban 1	Mefenamic Acid_C13	0.09164	N/A	0.983	0.000	4.000	2.000	2.000	3.143	1.943	3.886	0.0196	0.615	0.380	0.760
273	☑	Unknown_3	Mefenamic Acid_Ban 1	Mefenamic Acid_C13	0.00399	N/A	0.036	0.000	4.000	2.000	2.000	3.143	1.943	3.886	0.0196	0.615	0.380	0.760
307	☑	Unknown_4	Mefenamic Acid_Ban 1	Mefenamic Acid_C13	0.00231	N/A	0.018	0.000	4.000	2.000	2.000	3.143	1.943	3.886	0.0196	0.615	0.380	0.760
341	☑	Unknown_5	Mefenamic Acid_Ban 1	Mefenamic Acid_C13	0.00454	N/A	0.042	0.000	4.000	2.000	2.000	3.143	1.943	3.886	0.0196	0.615	0.380	0.760
375	☑	Unknown_5	Mefenamic Acid_Ban 1	Mefenamic Acid_C13	0.00743	N/A	0.073	0.000	4.000	2.000	2.000	3.143	1.943	3.886	0.0196	0.615	0.380	0.760
409	☑	Unknown_6	Mefenamic Acid_Ban 1	Mefenamic Acid_C13	0.00217	N/A	0.016	0.000	4.000	2.000	2.000	3.143	1.943	3.886	0.0196	0.615	0.380	0.760
443	☑	Unknown_7	Mefenamic Acid_Ban 1	Mefenamic Acid_C13	0.00423	N/A	0.039	0.000	4.000	2.000	2.000	3.143	1.943	3.886	0.0196	0.615	0.380	0.760
511	☑	Muscle Blank 0_2	Mefenamic Acid_Ban 1	Mefenamic Acid_C13	0.00008	0.0000	< 0	0.000	4.000	2.000	2.000	3.143	1.943	3.886	0.0196	0.615	0.380	0.760
545	☑	Muscle Std 1_2	Mefenamic Acid_Ban 1	Mefenamic Acid_C13	0.23735	2.5000	2.557	0.000	4.000	2.000	2.000	3.143	1.943	3.886	0.0196	0.615	0.380	0.760
579	☑	Muscle Std 2_2	Mefenamic Acid_Ban 1	Mefenamic Acid_C13	0.45115	5.0000	4.867	0.000	4.000	2.000	2.000	3.143	1.943	3.886	0.0196	0.615	0.380	0.760
613	☑	Muscle Std 3_2	Mefenamic Acid_Ban 1	Mefenamic Acid_C13	0.66809	7.5000	7.211	0.000	4.000	2.000	2.000	3.143	1.943	3.886	0.0196	0.615	0.380	0.760

Calibration for Mefenamic Acid_Ban 1: y = 0.09256 x + 6.53859e-4 (r = 0.99785, r² = 0.99570) (weighting: None)

CCα and CCβ Calcultation Sheet

Mefenamic Acid MRM1

Concentration	Area	dx²
0.000	0.0000	14.063
2.500	0.2384	1.563
5.000	0.4556	1.563
7.500	0.7311	14.063
0.000	0.0001	14.063
2.500	0.2374	1.563
5.000	0.4512	1.563
7.500	0.6681	14.063

Mefenamic Acid MRM1

b	0.092566	0.001	
	0.00248395	0.012	
	0.99569813	0.0196	Sigma
	1388.74322	6	N-2 = nu
	0.53553365	0.002	

0.95	1.943	t student 5%
0.99	3.143	t student 1%
2t₁₋alpha(nu)	3.89	Delta
K	2	No of Samples Repetition
I	4	No of Calibration Level
J	2	No of Calibration Level Replicates

Banned Substance

MRL

Mean x	Sum dx²
3.75	62.5

CCα : 0.615
CCβ : 0.760

Figure 2. CCα and CCβ for the non-permitted substance mefenamic acid.

Custom column: Spcecific value can be changed directly in the column **Calculated Column with Formulae**

Index	Used	Sample Name	Component Name	IS Name	Area Ratio	Actual Concentr...	Calculated Concentration	*MRL	*I	*J	*K	*t stude...	*t stude...	*Delta	*Sigma	*CCalpha_1%	*CCalpha_5%	*CCbeta
38	☐	Muscle Blank 0_1	Tolfenamic Acid_Auth 1	Tolfenamic Acid_C13	0.00153	0.0000	< 0	50.000	3.000	2.000	2.000	3.747	2.132	4.264	0.0958	53.644	52.073	54.146
72	☑	Muscle Std 1_1	Tolfenamic Acid_Auth 1	Tolfenamic Acid_C13	2.13552	25.0000	24.444	50.000	3.000	2.000	2.000	3.747	2.132	4.264	0.0958	53.644	52.073	54.146
106	☑	Muscle Std 2_1	Tolfenamic Acid_Auth 1	Tolfenamic Acid_C13	4.19500	50.0000	50.053	50.000	3.000	2.000	2.000	3.747	2.132	4.264	0.0958	53.644	52.073	54.146
140	☑	Muscle Std 3_1	Tolfenamic Acid_Auth 1	Tolfenamic Acid_C13	6.06324	75.0000	73.284	50.000	3.000	2.000	2.000	3.747	2.132	4.264	0.0958	53.644	52.073	54.146
208	☑	Unknown_1	Tolfenamic Acid_Auth 1	Tolfenamic Acid_C13	0.00204	N/A	< 0	50.000	3.000	2.000	2.000	3.747	2.132	4.264	0.0958	53.644	52.073	54.146
242	☑	Unknown_2	Tolfenamic Acid_Auth 1	Tolfenamic Acid_C13	0.02425	N/A	< 0	50.000	3.000	2.000	2.000	3.747	2.132	4.264	0.0958	53.644	52.073	54.146
276	☑	Unknown_3	Tolfenamic Acid_Auth 1	Tolfenamic Acid_C13	0.00081	N/A	< 0	50.000	3.000	2.000	2.000	3.747	2.132	4.264	0.0958	53.644	52.073	54.146
310	☑	Unknown_4	Tolfenamic Acid_Auth 1	Tolfenamic Acid_C13	0.00057	N/A	< 0	50.000	3.000	2.000	2.000	3.747	2.132	4.264	0.0958	53.644	52.073	54.146
344	☑	Unknown_5	Tolfenamic Acid_Auth 1	Tolfenamic Acid_C13	0.00089	N/A	< 0	50.000	3.000	2.000	2.000	3.747	2.132	4.264	0.0958	53.644	52.073	54.146
378	☑	Unknown_5	Tolfenamic Acid_Auth 1	Tolfenamic Acid_C13	0.00167	N/A	< 0	50.000	3.000	2.000	2.000	3.747	2.132	4.264	0.0958	53.644	52.073	54.146
412	☑	Unknown_6	Tolfenamic Acid_Auth 1	Tolfenamic Acid_C13	0.00212	N/A	< 0	50.000	3.000	2.000	2.000	3.747	2.132	4.264	0.0958	53.644	52.073	54.146
446	☑	Unknown_7	Tolfenamic Acid_Auth 1	Tolfenamic Acid_C13	0.00041	N/A	< 0	50.000	3.000	2.000	2.000	3.747	2.132	4.264	0.0958	53.644	52.073	54.146
514	☐	Muscle Blank 0_2	Tolfenamic Acid_Auth 1	Tolfenamic Acid_C13	0.00125	0.0000	< 0	50.000	3.000	2.000	2.000	3.747	2.132	4.264	0.0958	53.644	52.073	54.146
548	☑	Muscle Std 1_2	Tolfenamic Acid_Auth 1	Tolfenamic Acid_C13	2.18243	25.0000	25.027	50.000	3.000	2.000	2.000	3.747	2.132	4.264	0.0958	53.644	52.073	54.146
582	☑	Muscle Std 2_2	Tolfenamic Acid_Auth 1	Tolfenamic Acid_C13	4.27148	50.0000	51.004	50.000	3.000	2.000	2.000	3.747	2.132	4.264	0.0958	53.644	52.073	54.146
616	☑	Muscle Std 3_2	Tolfenamic Acid_Auth 1	Tolfenamic Acid_C13	6.29667	75.0000	76.187	50.000	3.000	2.000	2.000	3.747	2.132	4.264	0.0958	53.644	52.073	54.146

Calibration for Tolfenamic Acid_Auth 1: y = 0.08042 x + 0.16974 (r = 0.99887, r² = 0.99774) (weighting: None)

CCα and CCβ Calcultation Sheet

Tolfenamic Acid MRM1

Concentration	Area	dx²
25.000	2.1355	625.000
50.000	4.1950	0.000
75.000	6.0632	625.000
25.000	2.1824	625.000
50.000	4.2715	0.000
75.000	6.2967	625.000

Tolfenamic Acid MRM1

b	0.08042	0.170	
	0.00191553	0.103	
	0.99773572	0.0958	Sigma
	1762.56324	4	N-2 = nu
	16.1682802	0.037	

0.95	2.132	t student 5%
0.99	3.747	t student 1%
2t₁₋alpha(nu)	4.26	Delta
K	2	No of Samples Repetition
I	3	No of Calibration Level
J	2	No of Calibration Level Replicates

Banned Substance

MRL | 50

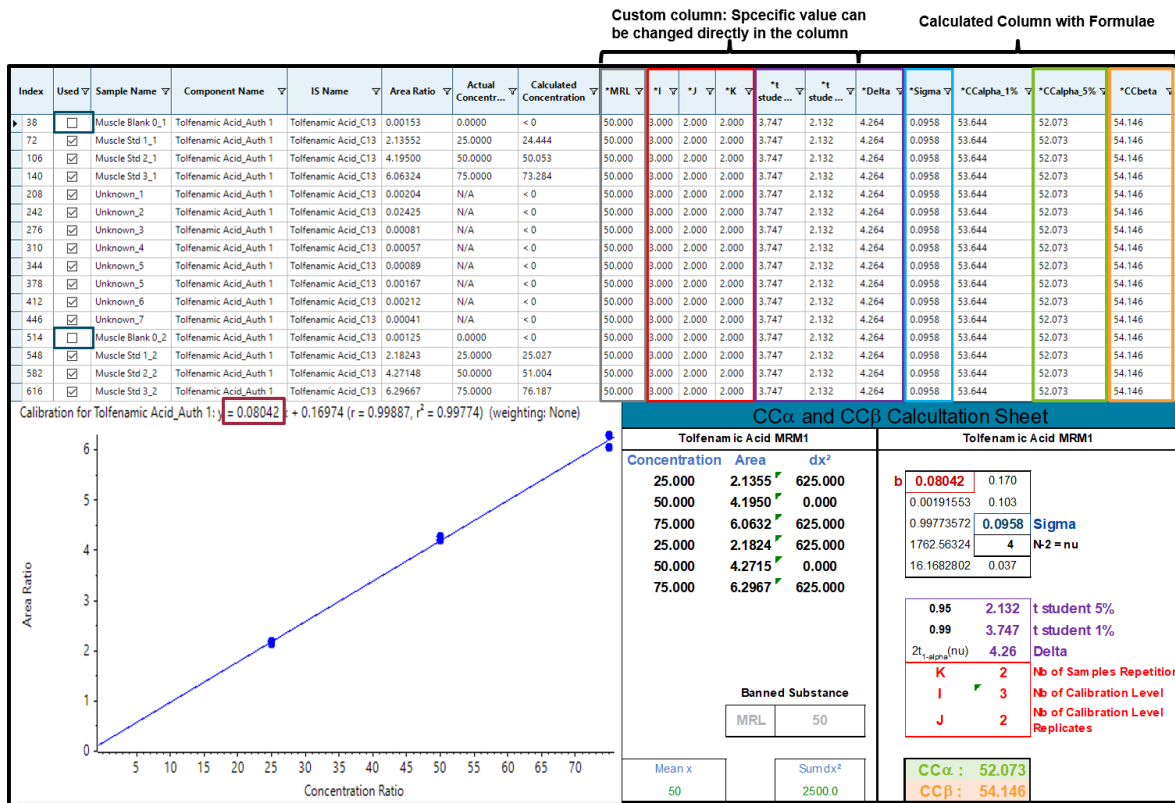| Mean x	Sum dx²
50	2500.0

CCα : 52.073
CCβ : 54.146

Figure 3. CCα and CCβ for the permitted substance tolfenamic acid with a MRL of 50 µg/kg.

Flagging non-compliant results

By adding calculated columns (Flag CCalpha 1%) and (Flag CCbeta) which compare the calculated concentration of the analyte in the sample with the calculated CC_α and CC_β, SCIEX OS Software is able to automatically flag every concentration above these decision limits. Figure 4 shows the example of the non-permitted substance Ketoprofen. In this example, the concentrations of Ketoprofen found in samples "Unknown_2" (9.277 µg/kg) and "Unknown_7" (0.630 µg/kg) are exceeding CC_α (0.552) and CC_β (0.683) in the first case but only exceeds CC_α (0.552) in the second case.

Therefore, the automatic calculation of CC_α and CC_β in combination with the real-time flagging of samples with concentrations above these decision limits can save a lot of time and minimize the risk of error for laboratories thus improving the efficiency of their workflow.

References

1. European Commission, 2002/657/EC: Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (Text with EEA relevance) (notified under document number C(2002) 3044), 2002. http://data.europa.eu/eli/dec/2002/657/oj/eng (accessed August 28, 2019).

2. ISO, ISO 11843-2:2000, n.d. http://www.iso.org/cms/render/live/en/sites/isoorg/contents/data/standard/02/01/20186.html (accessed August 30, 2019).

3. E. Verdon, D. Hurtaud-Pessel, P. Sanders, Evaluation of the limit of performance of an analytical method based on a statistical calculation of its critical concentrations according to ISO standard 11843: Application to routine control of banned veterinary drug residues in food according to European Decision 657/2002/EC, Accreditation Qual. Assur. 11 (2006) 58–62. doi:10.1007/s00769-005-0055-y.

Index	Used	Sample Name	Component Name	IS Name	Area Ratio	Calculated Concentration	*MRL	*CCalpha_1%	*Flag CCalpha_1%	*CCbeta	*Flag CCbeta
51	✓	Muscle Blank 0_1	Ketoprofen_Ban 1	Ketoprofen_d3	0.00019	< 0	0.000	0.552		0.683	
85	✓	Muscle Std 1_1	Ketoprofen_Ban 1	Ketoprofen_d3	0.39170	2.636	0.000	0.552	2.084	0.683	1.954
119	✓	Muscle Std 2_1	Ketoprofen_Ban 1	Ketoprofen_d3	0.72454	5.003	0.000	0.552	4.451	0.683	4.321
153	✓	Muscle Std 3_1	Ketoprofen_Ban 1	Ketoprofen_d3	1.03878	7.238	0.000	0.552	6.686	0.683	6.555
221	✓	Unknown_1	Ketoprofen_Ban 1	Ketoprofen_d3	0.01352	< 0	0.000	0.552		0.683	
255	✓	Unknown_2	Ketoprofen_Ban 1	Ketoprofen_d3	1.32561	9.277	0.000	0.552	8.725	0.683	8.595
289	✓	Unknown_3	Ketoprofen_Ban 1	Ketoprofen_d3	0.06151	0.288	0.000	0.552	-0.264	0.683	-0.394
323	✓	Unknown_4	Ketoprofen_Ban 1	Ketoprofen_d3	0.02472	0.027	0.000	0.552	-0.525	0.683	-0.656
357	✓	Unknown_5	Ketoprofen_Ban 1	Ketoprofen_d3	0.05455	0.239	0.000	0.552	-0.313	0.683	-0.444
391	✓	Unknown_6	Ketoprofen_Ban 1	Ketoprofen_d3	0.06576	0.319	0.000	0.552	-0.233	0.683	-0.364
425	✓	Unknown_7	Ketoprofen_Ban 1	Ketoprofen_d3	0.10961	0.630	0.000	0.552	0.078	0.683	-0.052
459	✓	Unknown_8	Ketoprofen_Ban 1	Ketoprofen_d3	0.01245	< 0	0.000	0.552		0.683	
527	✓	Muscle Blank 0_2	Ketoprofen_Ban 1	Ketoprofen_d3	0.00036	< 0	0.000	0.552		0.683	
561	✓	Muscle Std 1_2	Ketoprofen_Ban 1	Ketoprofen_d3	0.39946	2.691	0.000	0.552	2.139	0.683	2.009
595	✓	Muscle Std 2_2	Ketoprofen_Ban 1	Ketoprofen_d3	0.75567	5.224	0.000	0.552	4.672	0.683	4.542
629	✓	Muscle Std 3_2	Ketoprofen_Ban 1	Ketoprofen_d3	1.07588	7.501	0.000	0.552	6.949	0.683	6.819

Figure 4. SCIEX OS Software flags results above CC_α and CC_β.

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
sciex.com

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