Assay of Simvastatin in Tablets by HPLC

The measurand: mean Simvastatin content of the 8 tablets that were taken for analysis.

The prototype of the method is based on the monographs of Simvastatin in the European Pharmacopoeia (Ph.Eur. 5th Ed.) and the United States Pharmacopoeia / the National Formulary (USP 27 / NF 22).

Scope of use and validation: Quantitative determination of Simvastatin in drug products.

Principle: The drug substance is extracted from the tablets by using solution of the 0.01M phosphate buffer pH4.0 and acetonitrile in the ratio 20:80 (V/V). The samples are filtered and the resulting solution is injected into HPLC. Separation is achieved at room temperature in a Phenomenex Prodigy 3 u ODS (end-capped) column (30 x 4.60 mm), detection wavelength is 238 nm. A Single standard solution or a series of calibration solutions (depending on the calibration procedure) of Simvastatin standard substance is used for calibration.

Model Equation:

{ The main equation

Single point calibration method is used for quantitation (Calibration sample No 3 is used)

The result is given Recovery-corrected (R)

The sample solution is prepared by dissolving several tablets to achieve averaging

}

$C_{SVT} = A_{sample} * C_3 * V_{100} / (A_3 * n_{tab} * R);$

{ Chromatographic measurements

Peak areas are used as the basis for quantification.

We assume that the uncertainty of the chromatographic peak area of the sample solution has 4 components

- Repeatability (contained in A_{sample_rep})

- Uncertainty due to detector drift (A_{sample_drift})

- Uncertainty due to imperfection of peak integration (A_{sample_integr})

- Uncertainty due to the slight nonlinearity of the calibration graph (A_{sample_nonlin})

The A_{sample_rep} carries the value and the repeatability uncertainty. The other components carry the respective uncertainty component only, their value is 0.

}

{ Sample peak area }

$A_{sample} = A_{sample\_rep} + A_{sample\_drift} + A_{sample\_integr} + A_{sample\_nonlin};$

{ Recovery

The value of Recovery (R_0) has been determined from spiking studies.

The R_0 also contains the sample-to-sample variability uncertainty component

The A_{R_eff} is the effective peak area during recovery determination and it takes into account the uncertainty in determining the recovery value.

The division by the const() function reforms A_{R_eff} into a coefficient with vaule 1 and uncertainty

equal to the relative uncertainty of $A_{R\_eff.}$

}

$R = R_0 * A_{R\_eff} / const(A_{R\_eff})$;

$A_{R\_eff} = A_{R\_eff\_integr} + A_{R\_eff\_nonlin}$;

{ Peak area of the calibration standard solution

The area has the same components as the sample peak area, except that the nonlinearity uncertainty contribution is not included.

}

$A_3 = A_{3rep} + A_{3drift} + A_{3integr}$;

{ Preparation of the standard solution }

$C_3 = m_3 * P_{std} / (V_{3\_50} * 100)$;

{ Uncertainity of weighing

}

$m_3 = m_{3\_rep} + m_{3\_buoyancy} + m_{3\_drift} + m_{3\_round} + m_{3\_electrost}$;

$m_{3\_buoyancy} = m_{3\_rep} * f_{buoyancy}$;

{ Volumes of 50 ml volumetric flask }

$V_{3\_50} = V_{3\_50\_cal} + V_{3\_50\_fill} + V_{3\_50\_temp}$;

$V_{3\_50\_temp} = V_{3\_50\_cal} * \Delta t * \gamma_w$;

{ Volume of the sample solution }

$V_{100} = V_{100\_cal} + V_{100\_fill} + V_{100\_temp}$;

$V_{100\_temp} = V_{100\_cal} * \Delta t * \gamma_w$;

List of Quantities:

Quantity	Unit	Definition
A_3	AU	Peak area of the 3. calibration standard solution
A_{3drift}	AU	Drift uncertainty component of the peak area of the 3. calibration standard solution
$A_{3integr}$	AU	Integration uncertainty component of the peak area of the 3. calibration standard solution
A_{3rep}	AU	Value and the repeatability uncertainty component of the peak area of the 3. calibration standard solution
A_{R_eff}	AU	The effective peak area during the Recovery determination (difference of the peak areas of spiked and unspiked solutions)

Quantity	Unit	Definition
$A_{R\_eff\_integr}$	AU	The value and integration uncertainty of the effective peak area during the recovery determination
$A_{R\_eff\_nonlin}$	AU	The uncertainty of the effective peak area due to nonlinearity of the calibration graph during the recovery determination
A_{sample}	AU	Peak area of the sample solution
A_{sample_drift}	AU	Drift uncertainty component of the sample peak area
A_{sample_integr}	AU	Integration uncertainty component of the sample peak area
A_{sample_nonlin}	AU	Uncertainty component of the sample peak area that takes into account the slight non-linearity of the calibration graph
A_{sample_rep}	AU	The value of the sample solution peak area together with its repeatability uncertainty
C_3	mg/ml	Concentration of the 3. calibration standard solution
C_{SVT}	mg/tab	Content of simvastatin in Simvastatin 10 mg tablets
$f_{buoyancy}$	unitless	Correction factor for air buoyancy
m_3	mg	Mass of the Simvastatine CRS taken for preparation of the 3. calibration standard solution
$m_{3\_buoyancy}$	mg	Air buoyancy component of uncertainity of mass m3
m_{3_drift}	mg	Drift component of uncertainity of mass m3
$m_{3\_electrost}$	mg	Electrostatic component of uncertainity of mass m3
m_{3_rep}	mg	Value of the mass of the Simvastatine CRS taken for preparation of the 3. calibration standard solution together with repeatability component of uncertainity
m_{3_round}	mg	Rounding component of uncertainity of mass m3
n_{tab}	tab	Number of the tablets taken for preparation of the sample solution
P_{std}	%	Purity of the Simvastatin CRS
R	unitless	Recovery
R_0	unitless	The value of R and its uncertainty component that is due to the imperfection of the sample preparation procedure
V_{100}	ml	Volume of the sample solution
V_{100_cal}	ml	Value of the volume of the sample solution together with calibration component of uncertainity
V_{100_fill}	ml	Filling component of uncertainity of volume V_{100}
V_{100_temp}	ml	Uncertainity component of volume V_{100} due to temperature difference from 20°C
V_{3_50}	ml	Volume of the 3. calibration standard solution
$V_{3\_50\_cal}$	ml	Value of the volume of the 3. calibration standard solution together with calibration component of uncertainity
$V_{3\_50\_fill}$	ml	Filling component of uncertainity of volume V_{3_50}
$V_{3\_50\_temp}$	ml	Uncertainity component of volume V_{3_50} due to temperature difference from 20°C
Δt	°C	Diference of solution temperature from 20°C

Quantity	Unit	Definition
γ_w	1/°C	Thermal expansion coefficent of water

A_3: Interim Result

A_{3drift}: Type B rectangular distribution
Value: 0 AU
Halfwidth of Limits: 48000 AU

The drift per hour is maximum 12 000 peak area units. Assuming that the solution will be kept no longer than 4 hours between preparation and measurement we arrive at this uncertainty estimate. This uncertainty estimate is essentially independent of the peak area.

$A_{3integr}$: Type B rectangular distribution
Value: 0 AU
Halfwidth of Limits: 38500 AU

This uncertainty contribution has been obtained by integrating the peak in two ways - one somewhat underestimating (Area(min)) and the other somewhat overestimating (Area(max)) the peak area. The uncertainty contribution has been taken equal to [Area(max)-Area(min)]/2. See text for more detail. According to our data this uncertainty contribution is proportional to the peak area.

A_{3rep}: Type A summarized
Mean: 8987202 AU
Standard Uncertainty: 12000 AU
Degrees of Freedom: 12

The repeatability uncertainty has been estimated using the pooled standard deviation of triplicate injections (altogether 3 x 6 injections) of a standard solution subjected to different stability tests. See the text for more detail. According to our data this uncertainty contribution is essentially independent of the sample peak area.

A_{R_eff}: Interim Result

The systemati influences are practically limited to the systematic effects in peak areas (repeatability can thus be excluded). Also, it is necessary to keep in mind that calculation of Recovery by spiking experiments leads to differences of areas of peaks that have been obtained within short time intervals (the drift component can thus be excluded). Therefore the components to be included are the integration uncertainty and the uncertainty due to nonlinearity.

$A_{R\_eff\_integr}$: Type B rectangular distribution
Value: 3000000 AU
Halfwidth of Limits: 12600 AU

$A_{R\_eff\_nonlin}$: Type B rectangular distribution
Value: 0 AU
Halfwidth of Limits: 40000 AU

A_{sample}: Interim Result

A_{sample_drift}: Type B rectangular distribution
Value: 0 AU
Halfwidth of Limits: 60000 AU

The drift per hour is maximum 12 000 peak area units. Assuming that the sample solution will be kept no longer than 5 hours between preparation and measurement we arrive at this uncertainty estimate. This uncertainty estimate is essentially independent of the peak area.

A_{sample_integr}: Type B rectangular distribution
Value: 0 AU
Halfwidth of Limits: 35000 AU

This uncertainty contribution has been obtained by integrating the peak in two ways - one somewhat underestimating (Area(min)) and the other somewhat overestimating (Area(max)) the peak area. The uncertainty contribution has been taken equal to [Area(max)-Area(min)]/2. See text for more detail. According to our data this uncertainty contribution is proportional to the peak area.

A_{sample_nonlin}: Type B rectangular distribution
Value: 0 AU
Halfwidth of Limits: 82000 AU

This uncertainty contribution has been estimated from the behavior of the residuals of the calibration line. The uncertainty corresponds to the maximum possible devaition (in terms of peak area) of the calibration line from the actual concentration vs peak area relationship. This uncertainty contribution is assigned only to the peak area of the sample and not to the peak areas of the standard solutions. According to this approach this uncertainty countribution is assumed to be independent of the peak area of the sample.

A_{sample_rep}: Type A summarized
Mean: 8349089 AU
Standard Uncertainty: 19000 AU
Degrees of Freedom: 28

The repeatability uncertainty has been estimated using the pooled standard deviation of triplicate injections (altogether 3 x 14 injections) of a sample solution subjected to different stability tests. See the text for more detail. According to our data this uncertainty contribution is essentially independent of the sample peak area.

C_3: Interim Result

C_{SVT}: Result

$f_{buoyancy}$: Type B rectangular distribution
Value: 0.0010 unitless
Halfwidth of Limits: 0.0002 unitless

The buoyancy factor is the part (around one thousandth under our conditions) of the mass that is added to the balance reading in order to get the physical mass of the weighed substance. The uncertainty estimate is deliberately very conservative.

m_3: Interim Result

$m_{3\_buoyancy}$: Interim Result

m_{3_drift}: Type A summarized
Mean: 0 mg
Standard Uncertainty: 0.0097 mg
Degrees of Freedom: 2

$m_{3\_electrost}$: Type B rectangular distribution
Value: 0 mg
Halfwidth of Limits: 0.21 mg

When weighing the standard substance, intereference from electrostatics (instability of the reading) was quite serious and is thus taken into account as an uncertainty component. This uncertainty componen has been estimated as 0.5% of the mass of the substance.

m_{3_rep}: Type A summarized
Mean: 41.82 mg
Standard Uncertainty: 0.0196 mg
Degrees of Freedom: 18

m_{3_round}: Type B rectangular distribution
Value: 0 mg
Halfwidth of Limits: 0.005 mg

n_{tab}: Constant
Value: 8 tab

P_{std}: Type B rectangular distribution
Value: 99.4 %
Halfwidth of Limits: 0.6 %

R: Interim Result

R_0: Type A summarized
Mean: 1.002068 unitless
Standard Uncertainty: 0.002295 unitless
Degrees of Freedom: 2

Recovery value and sample-to-sample variability uncertainty. The standard uncertainty is the standard deviation of the single mavlue, not the standard deviation of the mean

V_{100}: Interim Result

V_{100_cal}: Type B rectangular distribution
Value: 100.00 ml
Halfwidth of Limits: 0.1 ml

V_{100_fill}: Type B rectangular distribution
Value: 0 ml
Halfwidth of Limits: 0.09 ml

V_{100_temp}: Interim Result

V_{3_50}: Interim Result

$V_{3\_50\_cal}$: Type B rectangular distribution
Value: 50.00 ml
Halfwidth of Limits: 0.06 ml

$V_{3\_50\_fill}$: Type B rectangular distribution
Value: 0 ml
Halfwidth of Limits: 0.09 ml

$V_{3\_50\_temp}$: Interim Result

Δt: Type B rectangular distribution
Value: 0 °C
Halfwidth of Limits: 4 °C

γ_w: Constant
Value: 0.00021 1/°C

Uncertainty Budget:

Quantity	Value	Standard Uncertainty	Degrees of Freedom	Distribution	Sensitivity Coefficient	Uncertainty Contribution	Index
A_{3drift}	0.0 AU	27700 AU	infinity	rectangular	$-1.1 \cdot 10^{-6}$	-0.030 mg/tab	5.6 %
$A_{3integr}$	0.0 AU	22200 AU	infinity	rectangular	$-1.1 \cdot 10^{-6}$	-0.024 mg/tab	3.6 %
A_{3rep}	$8.9872 \cdot 10^{6}$ AU	$0.0120 \cdot 10^{6}$ AU	12	normal	$-1.1 \cdot 10^{-6}$	-0.013 mg/tab	1.0 %
$A_{R\_eff\_integr}$	$3.00000 \cdot 10^{6}$ AU	$0.00727 \cdot 10^{6}$ AU	infinity	rectangular	$-3.2 \cdot 10^{-6}$	-0.023 mg/tab	3.4 %
$A_{R\_eff\_nonlin}$	0.0 AU	23100 AU	infinity	rectangular	$-3.2 \cdot 10^{-6}$	-0.074 mg/tab	34.6 %
A_{sample_drift}	0.0 AU	34600 AU	infinity	rectangular	$1.2 \cdot 10^{-6}$	0.040 mg/tab	10.1 %
A_{sample_integr}	0.0 AU	20200 AU	infinity	rectangular	$1.2 \cdot 10^{-6}$	0.023 mg/tab	3.4 %
A_{sample_nonlin}	0.0 AU	47300 AU	infinity	rectangular	$1.2 \cdot 10^{-6}$	0.055 mg/tab	18.8 %
A_{sample_rep}	$8.3491 \cdot 10^{6}$ AU	$0.0190 \cdot 10^{6}$ AU	28	normal	$1.2 \cdot 10^{-6}$	0.022 mg/tab	3.0 %
$f_{buoyancy}$	0.001000 unitless	0.000115 unitless	infinity	rectangular	9.6	0.0011 mg/tab	0.0 %
m_{3_drift}	0.0 mg	0.00970 mg	2	normal	0.23	0.0022 mg/tab	0.0 %
$m_{3\_electrost}$	0.0 mg	0.121 mg	infinity	rectangular	0.23	0.028 mg/tab	4.9 %
m_{3_rep}	41.8200 mg	0.0196 mg	18	normal	0.23	0.0045 mg/tab	0.1 %
m_{3_round}	0.0 mg	0.00289 mg	infinity	rectangular	0.23	0.00067 mg/tab	0.0 %
n_{tab}	8.0 tab						
P_{std}	99.400 %	0.346 %	infinity	rectangular	0.097	0.034 mg/tab	7.1 %
R_0	1.00207 unitless	0.00229 unitless	2	normal	-9.6	-0.022 mg/tab	3.1 %
V_{100_cal}	100.0000 ml	0.0577 ml	infinity	rectangular	0.096	0.0056 mg/tab	0.2 %
V_{100_fill}	0.0 ml	0.0520 ml	infinity	rectangular	0.096	0.0050 mg/tab	0.2 %
$V_{3\_50\_cal}$	50.0000 ml	0.0346 ml	infinity	rectangular	-0.19	-0.0067 mg/tab	0.3 %
$V_{3\_50\_fill}$	0.0 ml	0.0520 ml	infinity	rectangular	-0.19	-0.010 mg/tab	0.6 %

Quantity	Value	Standard Uncertainty	Degrees of Freedom	Distribution	Sensitivity Coefficient	Uncertainty Contribution	Index
Δt	0.0 °C	2.31 °C	infinity	rectangular	not valid!	$430 \cdot 10^{-21}$ mg/tab	0.0 %
γ_w	0.00021 1/°C						
C_{SVT}	9.644 mg/tab	0.126 mg/tab	2000				

Result: Quantity: C_{SVT}
Value: 9.64 mg/tab
Expanded Uncertainty: ±0.25 mg/tab
Coverage Factor: 2.00
Coverage: 95% (t-table 95.45%)