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Cubis® II in the Context of Therapeutic Antibodies

Reliable, Consistent and Traceable Preparation of Buffers and Solutions for Antibody Analysis

Antibodies are subject to instability issues due to chemical and physical instabilities. Oxidation, disulfide bond formation, glycation and deamidation are the most frequent chemical instabilities and are caused by oxidants like peroxidases, light or metals, or by sugars via the Maillard reaction. Physical instabilities caused by variables such as temperature, pH-value or UV light can lead to aggregation, denaturation or fragmentation.



All of these effects may change the pharmacokinetics of antibodies (e.g. due to the loss of antigen binding activity, impaired resorption or degradation by proteases). Therefore, according to pharmacopoeias, produced antibodies must be thoroughly tested before they can be applied for treatment of patients.

USP Chapter <129> describes analytic procedures for recombinant therapeutic monoclonal antibodies including murine, chimeric, and humanized IgG isotype monoclonal antibodies and subtypes (e.g., IgG1 and IgG2). The chapter lists analytical methods and procedures such as size-exclusion chromatography (SEC), reduced and non-reduced capillary sodium dodecyl sulfate (CE-SDS) electrophoresis, analysis of N-linked oligosaccharides of monoclonal antibodies using capillary electrophoresis (CE) with laser-induced fluorescence detection, and liquid chromatography (LC) with fluorescence detection for sialic acid analysis.

As described in USP chapter <129>, all methods require special buffers and solutions with defined concentration or composition for analysis. Although, the preparation of solutions and buffers is a routine procedure in analytical laboratories, the process is error-prone as the final concentrations are specified in different units like g/L, mg/mL, mg/μL, mM, M, N, % (w/v) and % (w/w); and furthermore, some of the buffers require multiple components to be weighed. Typical sources of errors include mistakes during unit conversion and the weighing process itself. Furthermore, for sample amounts, it is nearly impossible to precisely match the required target weight and the corresponding adjustment of the solvent volume to compensate the too high or low component weight and to get the correct final concentration. This raises the question of how to avoid calculation and weighing errors when preparing the solutions or buffers and to achieve consistent and reliable results.

Select Components 3/20		
Component name	Molecular mass	Purity
CaCO3	100.09 g/mol	99.7 %
MgCl2	95.21 g/mol	99.5 %
NaCl	58.44 g/mol	99.99 %

Figure 1: Component selection screen

The Cubis® MCA Software Standard Preparation (QAPP001) is designed for the preparation of solutions and buffers and offers a database for components, solvents and recipes (samples). Components are defined by name, molecular weight and purity, and solvents by name and density. Up to 20 components can be selected and combined with a solvent to define a recipe (sample) that is stored in the database (see Figure 1). The right to access the sample management and to edit, create or delete components, solvents and samples is limited to users with

the role right to edit tasks. This user group is also allowed the set permissible tolerances for weight values and the mode for component and solvent weights out of tolerances (accept, accept with password or cancel process). Operators missing these permissions are only allowed to process samples.

After sample selection, the software guides the operator automatically throughout the entire process of solution or buffer preparation. Based on the selected concentration unit, the software automatically calculates the required component weight and displays a tolerance bar to the operator.

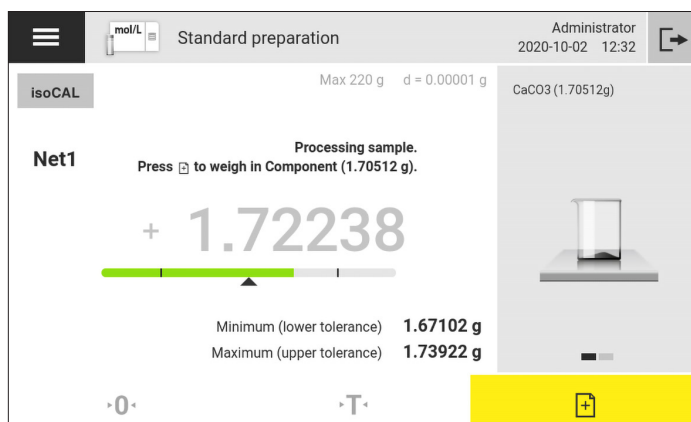


Figure 2: Weighing of components with operator guidance by tolerance bar

Depending on the task settings, the operator has to confirm weight values out of tolerance and is asked to get the confirmation by a user with the right edit task (usually lab managers) or the process is cancelled. For the preparation of mixed solutions or buffers, the operator has to weigh in multiple components. For each measured component, the software calculates the required solvent volume to get to the desired final concentration and, for mixed solutions or buffers, applies the mean value. Using the entered solvent density, the required volume value is transformed to a weight value. Also, during solvent addition, the software displays a tolerance bar with the required liquid weight to the operator. The applied solvent weight is gravimetrically

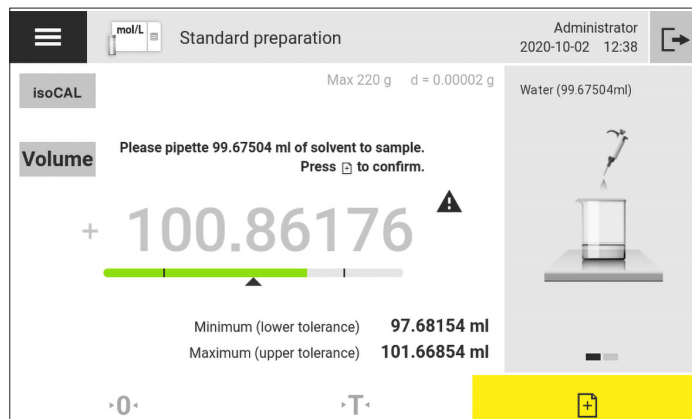


Figure 2: Addition of solvent with operator guidance by tolerance bar

checked by the balance and used to calculate the verified concentrations of the components and final volume of the solution.

The automatic calculations integrated in the software help to avoid unit conversion errors and save the operator working time. Based on the entered volume, the required component amounts are displayed to the operator before starting the weighing process (see Figure 4). This helps the operator to arrange the necessary components and to concentrate on the weighing process. Through the task tolerance settings, the lab manager has the complete assurance that the solution or buffer prepared by the operator matches with the recipe. At the end of the buffer or solution preparation, the software application creates comprehensive GLP reports. In the report, all weighing results, calculated concentrations and volume, user name, date and time, used equipment and climate data is recorded. Via the implemented calculations, control mechanisms and reporting, the software helps to create reliable, consistent, and traceable results when preparing buffers and solutions for analytical purposes (see Figures 5 & 6).

Process preparation	
Sample name	Buffer 12345
Total required volume	100.00 ml
Calculated component amount:	
CaCO3	1705.11535 mg
MgCl2	3417.08543 mg
NaCl	5100.51005 mg

Figure 4: Component amounts are calculated according to entered volume

Components	
Comp1	CaCO3
Lot	
Molecul.	100.09 g/mol
Purity	99.70 %
TarConc	17.000 mg/ml
VerConc	17.025 mg/ml
Comp2	MgCl2
Lot	
Molecul.	95.21 g/mol
Purity	99.50 %
TarConc	34.000 mg/ml
VerConc	33.336 mg/ml
Comp3	NaCl
Lot	
Molecul.	58.44 g/mol

Figure 5: Preview of report created by the software application

TarConc	34.000 mg/ml
VerConc	33.336 mg/ml
Comp3	NaCl
Lot	
Molecul.	58.44 g/mol
Purity	99.99 %
TarConc	51.000 mg/ml
VerConc	50.440 mg/ml
Solvent	Water
Lot	
Density	1.000 g/ml
Temp.	20 °C
Sample	Buffer 12345
RefNo	1
Volume	100.00 ml
VerVol	+100.86172 ml


Figure 6: Preview of report created by the software application

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