Achieving 21 CFR Part 11 Compliance with the iCE3

Chantal Felten, PhD, Principle Consultant, Alpine Analytical Academy, Whistler, BC, Canada
Susan Darling, Director of Marketing, Biologics and Humphrey Li, Software Engineer, ProteinSimple, Santa Clara, CA, USA

Introduction

Rapid analysis, platform methods and easy method development make the iCE3 ideal for analyzing biologics. In fact, these benefits have made iCE™ cIEF assays one of the key assays for biologics characterization and release today. As electronic data authenticity and integrity are now an integral part of GMP manufacturing for pharmaceutical companies, all analytical instruments must be compliant with the FDA Title 21 Code of Federal Regulations (CFR) Part 11. This guidance defines the requirements for GMP compliant electronic records and signatures including procedural controls such as training and standard operating procedures as well as software technical controls to maintain data security. To support the use of iCE3 in QC and GMP environments, all required functionality is integrated in iCE CFR software to ensure compliance with 21 CFR Part 11. iCE CFR software includes the following 21 CFR Part 11 technical controls:

- User defined login function to limit system access
- Electronic signatures are required throughout run execution, processing and exporting
- A secure, computer-generated, time-stamped audit trail records the date and time of all operator entries and actions that create or modify electronic records
- Accurate and complete copies of records in both printed and electronic format
- Operational system and network domain features that ensure data authenticity and integrity are maintained
- File string encryption and industry standard checksum algorithm application for verification of data integrity
- Operational restrictions limit inadvertent or unauthorized changes to strengthen GMP compliant batch execution

In this application note we will review the iCE3 data workflow and the 21 CFR Part 11 tools in iCE CFR software, with a specific focus on the embedded controls in the batch execution, data processing, audit trail and electronic signatures functions

iCE3 Data Workflow

The iCE3 performs imaged capillary IEF with whole column imaging. A protein sample mixed with ampholytes and pI markers is first flushed through the capillary. During focusing, voltage is applied, a pH gradient is generated, and the proteins focus at their isoelectric point (pI). After focusing, the iCE3 images the entire capillary. iCE3 data differs from traditional capillary electrophoresis (CE) or HPLC systems with fixed detection windows: its imaging detection generates data in pixels rather than the time-based points used in traditional CE or HPLC. In order to analyze iCE3 data with traditional chromatography based analysis software, iCE3 data must be processed to convert the x-axis to pI units. Once the data is processed, it is imported into Chromperfect (iCE analysis software) or another third-party software

(Empower, Chromeleon) and then analyzed. Throughout this process, iCE CFR software provides the tools for compliance.

User Login

iCE CFR software includes tools to manage user accounts, specify user privileges and document user actions. User accounts and permissions are managed in the User Administration window. iCE CFR software utilizes the Windows operating system local or domain login information. User login authority is defined by the type of end user.

There are three user levels in iCE CFR software:

- **Administrator** — Full access
- **Scientist** — Execute /Process/ Review /Export
- **Operator** — Execute /Review (QC function only)

User Administration is controlled only by the Administrator. The Administrator controls the setup and system access configuration for all users for the specific local iCE3 system. The Administrator account should be a member of the Windows local administrators group to ensure full compliance. The iCE3 system computer is shipped with a local administrator account and unless updated during software installation, this account will be the High Administrator. The High Administrator is a special Administrator, the purpose of this user is to manage the software and system. This user will never

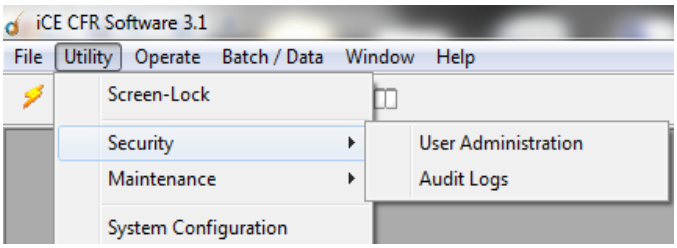
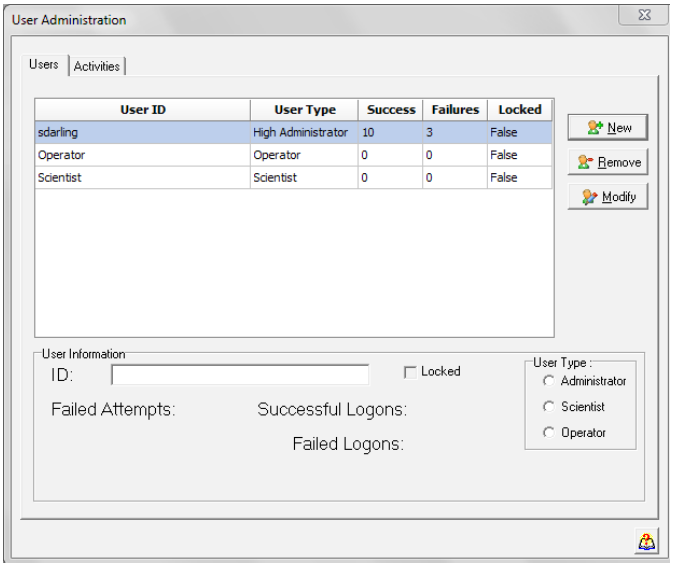


FIGURE 1. Accessing the User Administration window.

be locked out. It is assumed that the user account installing the software will be the High Administrator, so the High Administrator is required to re-install, patch, or uninstall iCE CFR software. If your lab requires 21 CFR Part 11 compliance, you will need to designate the High Administrator and determine user types and permissions as part of your internal 21 CFR Part 11 compliance requirements. Please note that on any given installation, there can be only one user assigned as the High Administrator.

To access the User Administration window in iCE CFR software, select *Utility* > *Security* > *User Administration* as shown in **Figure 1**. The User Administration tabs are only available to the Administrator/High Administrator.

The User Administration window has two tabs, Users and Activities, as shown in **Figure 2**. The Users tab is where the Administrator can add, remove or modify users and specify the user type. The Activities tab is where global preferences and user permissions are set. E-signature and



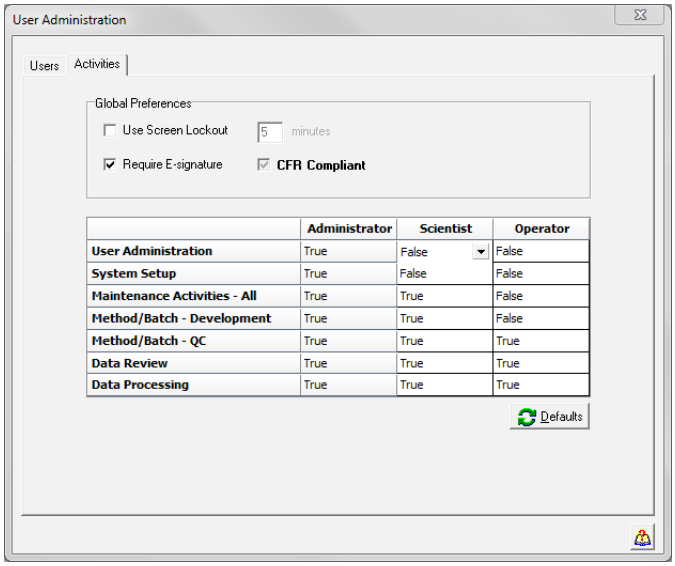


FIGURE 2. User Administration Users tab (left) and Activities tab (right).

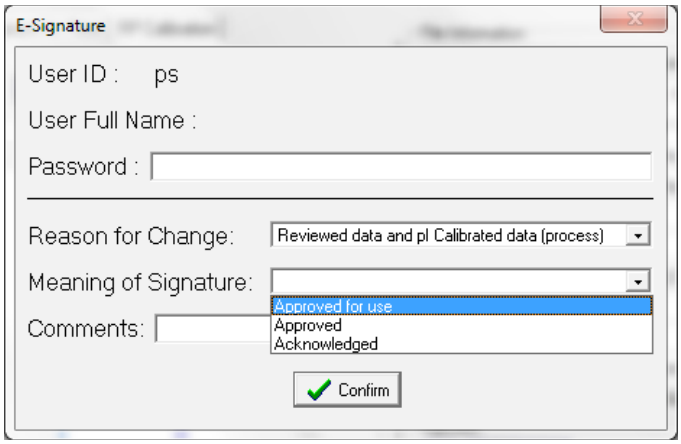


FIGURE 3. E-signature dialog box.

CFR compliance features are enabled by default in the Activities tab (**Figure 2**, right). These features are under electronic signature control and can only be disabled by the Administrator.

For complete 21 CFR compliance, the CFR Compliant and Requires E-signature boxes must be checked. Another valuable security tool is Use Screen Lockout. When this is checked, iCE CFR software will lock the screen after the specified time and require users to login to re-access the software.

You can also specify permissions for user types in the Activities tab. For example, in **Figure 2** (right) the Operator cannot access the Method/Batch-Development module, but they can access the Method/Batch-QC module. In this example, any user designated as an Operator is limited to running the system via the Method/Batch-QC module and is not allowed to create a Development Batch. This safeguard ensures that QC personnel do not accidently operate the system in a non-GMP compliant fashion. The Administrator can change these settings to fit user-specific requirements. Creating and modifying user access should be documented either in your company-specific 21 CFR Part 11 compliance documents, or your instrument operation, use and maintenance SOP.

Electronic Signatures

iCE CFR software tracks and confirms user actions via electronic signature. The software's electronic signature is a unique identification code (username) and password. *(Note: Password expiration is defined in your End User IT policy).* All user actions in the Method/Batch-QC module require electronic signatures. An example of the E-signature dialog box is shown in **Figure 3**. In order to complete the intended action, the user needs to enter their password and select the meaning of the signature.

The software will record the action and e-signature in the audit trail along with the reason that triggered the e-signature. All user changes are tracked in the audit trail in this manner.

When several e-signature functions are carried out in a continuous session, the password must be entered each time. iCE CFR software automatically locks a user account after three repeated invalid login attempts and records this event into the audit log. A locked user account can only be unlocked by the Administrator.

Audit Trail

Another key 21 CFR Part 11 feature in iCE CFR software is the audit trail. The software generates secure, time- and date-stamped audit trails for the following actions:

- Instrument — Records all changes to the system configuration, including Instrument Set-Up procedures such as cartridge calibration or autosampler tray changes.
- File — Contains audit entries for changes or creation of files such as Sample Processing or Batch table files. For each injection ID, the audit trail records **all events** from batch initiation to export of data to third party software.
- Security — Contains audit entries for User administration of program, such as the User Login Audit Trail and Changes to User Access Configuration.

All audit logs are secure and cannot be altered. Audit logs include: Date/Time Stamp, User ID, Event, Req. Sig (electronic signature requirement), and Reason and Meaning description. See **Figure 4** for an example audit log.

The Batch File

The iCE CFR software batch file also includes tools to track, record and, if desired, lock operational parameters during programming and execution. Unlike

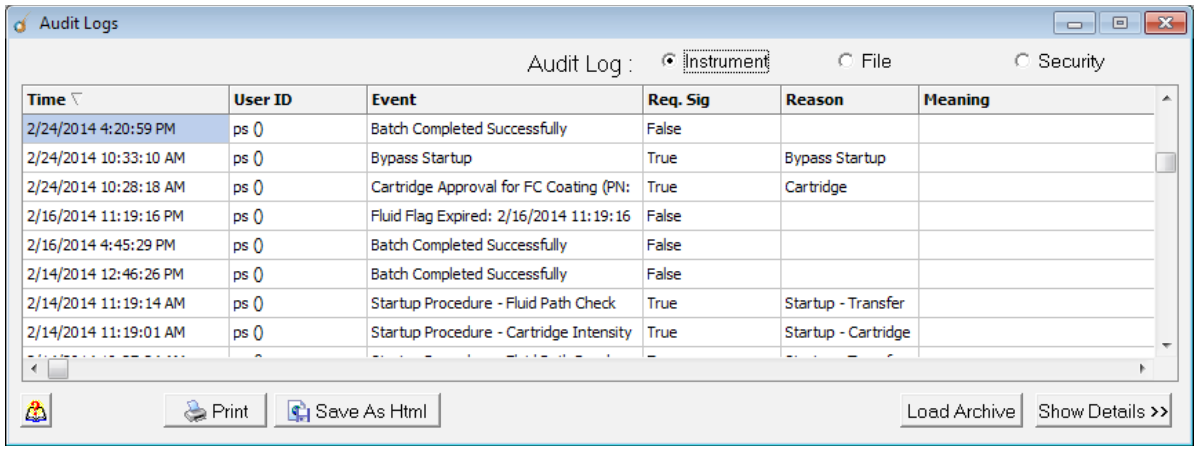


FIGURE 4. The audit trail function logs all changes and system events in the audit log.

conventional chromatography software which uses a combination of a method file containing instrument parameters and a sequence file to support running a batch of samples, iCE CFR software requires only one batch file. This batch file includes instrument parameters, run sequence and sample information, the data file name and pl marker details.

There are two types of batch files, a Development Batch and a QC Batch. The Development Batch gives you full access to both operational parameters and sample information. A QC Batch locks in the operational parameters and allows you only to specify number of samples, sample names and filenames. The QC Batch provides added confidence that operational parameters cannot be changed and is ideal for routine operation or for QC labs where operational parameters don't need to be changed.

Both the Development and QC Batch include all the technical controls for 21 CFR Part 11 compliance and are by default under electronic signature.

CREATING A BATCH

Whenever you create or save a batch file, the software automatically creates a folder structure and restricts visibility to only authorized users. The Development and QC Batch files are stored in different folders and each folder is only visible in the specific module. You must begin batch programming with a Development Batch. Once a Development Batch is created, a single injection can be converted into a QC Batch. The Development Batch programming window usually has three tabs: iCE Parameters, Autosampler Parameters and Injection Conditions. A fourth tab, Mixing Parameters, will be available if the External Sample Preparation mode is enabled. In the iCE Parameters tab shown in **Figure 5**, the sample ID, data injection file name, vial location and operational parameters such as focusing time are entered. iCE CFR software will not allow duplicate file names. If you attempt to program duplicate names,

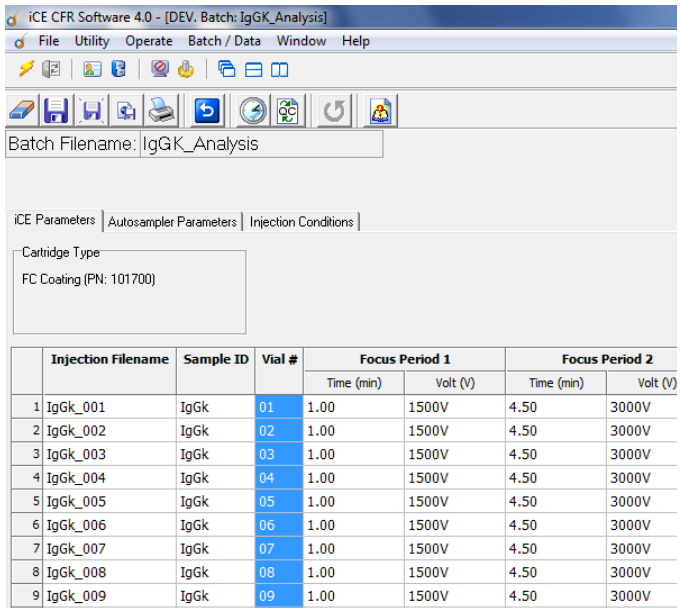


FIGURE 5. iCE Parameters tab in the Development Batch window.

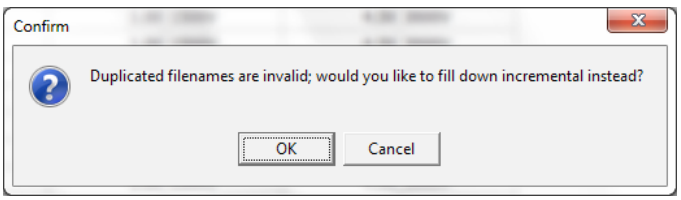


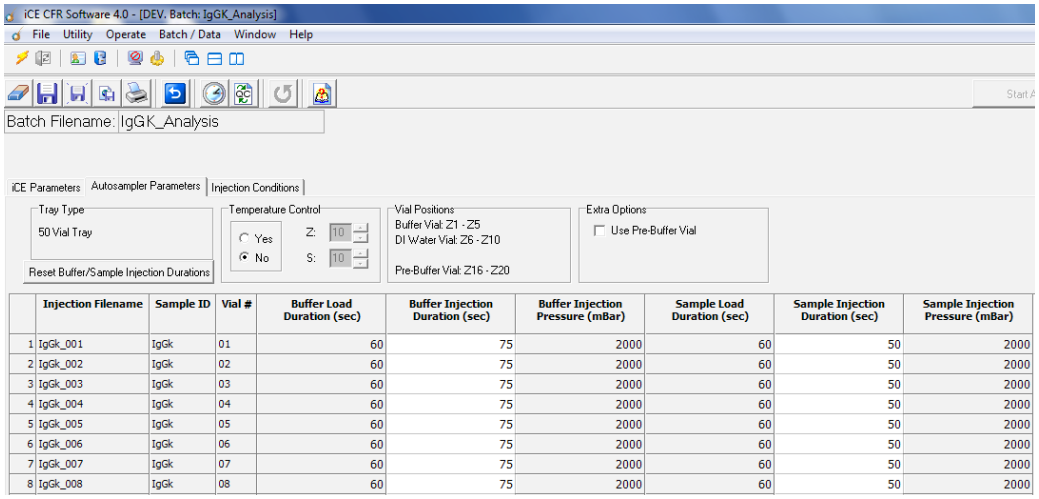
FIGURE 6. Duplicate file name error.

the software displays an error message and will not allow you to proceed until the naming conflict is corrected (**Figure 6**). The Autosampler Parameters tab shown in **Figure 7** includes items such as rinse and sample loading information. The Injection Conditions tab (**Figure 8**) includes information about the sample preparation including ampholytes, additives, and most importantly — pI marker information. The pI marker information is used for calibration of data files after data acquisition is complete.

Once the Development Batch is created, you can execute it by selecting ***Start Acquisition***, or you can convert it to a QC Batch if desired. All actions are captured in the audit trail.

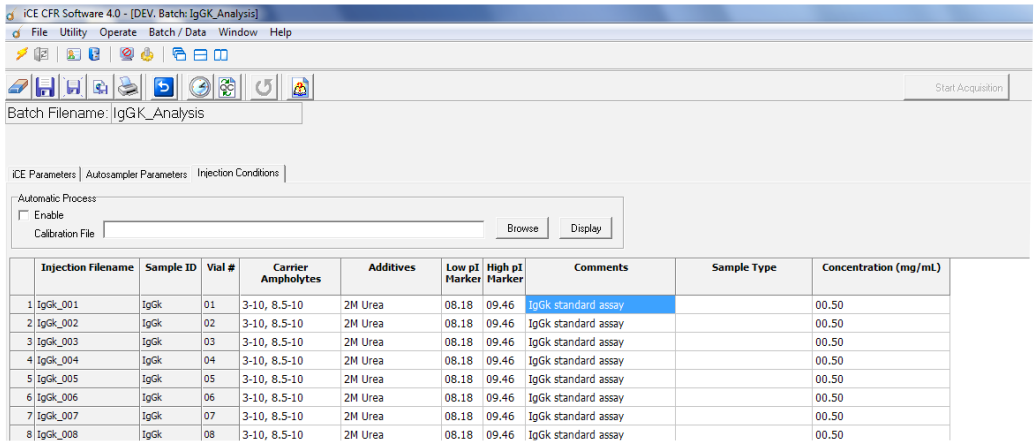
CONVERTING TO A QC BATCH

To create a QC Batch, select one row in the Development Batch and then click the QC Batch icon (**Figure 9**). This will prompt the E-signature dialog box to display



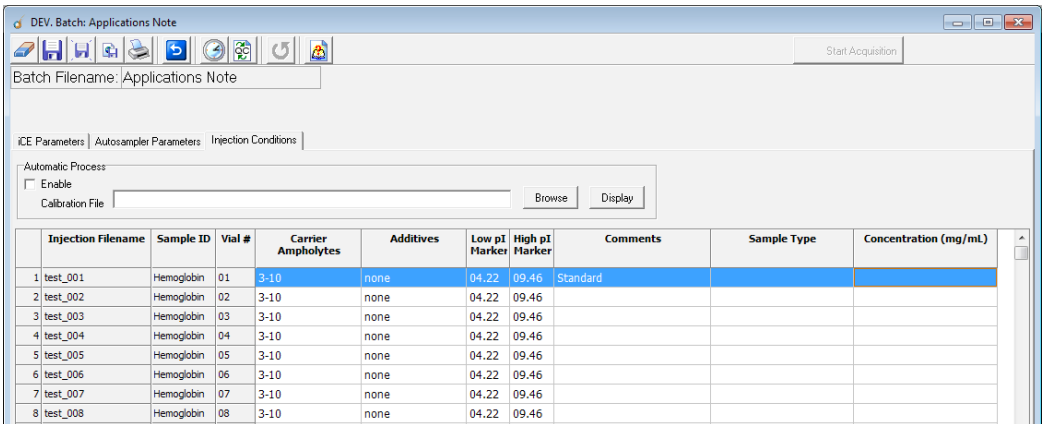
	Injection Filename	Sample ID	Vial #	Buffer Load Duration (sec)	Buffer Injection Duration (sec)	Buffer Injection Pressure (mBar)	Sample Load Duration (sec)	Sample Injection Duration (sec)	Sample Injection Pressure (mBar)
1	IgGk_001	IgGk	01	60	75	2000	60	50	2000
2	IgGk_002	IgGk	02	60	75	2000	60	50	2000
3	IgGk_003	IgGk	03	60	75	2000	60	50	2000
4	IgGk_004	IgGk	04	60	75	2000	60	50	2000
5	IgGk_005	IgGk	05	60	75	2000	60	50	2000
6	IgGk_006	IgGk	06	60	75	2000	60	50	2000
7	IgGk_007	IgGk	07	60	75	2000	60	50	2000
8	IgGk_008	IgGk	08	60	75	2000	60	50	2000

FIGURE 7. Autosampler tab in the Development Batch window.



	Injection Filename	Sample ID	Vial #	Carrier Ampholytes	Additives	Low pI Marker	High pI Marker	Comments	Sample Type	Concentration (mg/mL)
1	IgGk_001	IgGk	01	3-10, 8.5-10	2M Urea	08.18	09.46	IgGk standard assay		00.50
2	IgGk_002	IgGk	02	3-10, 8.5-10	2M Urea	08.18	09.46	IgGk standard assay		00.50
3	IgGk_003	IgGk	03	3-10, 8.5-10	2M Urea	08.18	09.46	IgGk standard assay		00.50
4	IgGk_004	IgGk	04	3-10, 8.5-10	2M Urea	08.18	09.46	IgGk standard assay		00.50
5	IgGk_005	IgGk	05	3-10, 8.5-10	2M Urea	08.18	09.46	IgGk standard assay		00.50
6	IgGk_006	IgGk	06	3-10, 8.5-10	2M Urea	08.18	09.46	IgGk standard assay		00.50
7	IgGk_007	IgGk	07	3-10, 8.5-10	2M Urea	08.18	09.46	IgGk standard assay		00.50
8	IgGk_008	IgGk	08	3-10, 8.5-10	2M Urea	08.18	09.46	IgGk standard assay		00.50

FIGURE 8. Injection Conditions tab in the Development Batch window.



	Injection Filename	Sample ID	Vial #	Carrier Ampholytes	Additives	Low pI Marker	High pI Marker	Comments	Sample Type	Concentration (mg/mL)
1	test_001	Hemoglobin	01	3-10	none	04.22	09.46	Standard		
2	test_002	Hemoglobin	02	3-10	none	04.22	09.46			
3	test_003	Hemoglobin	03	3-10	none	04.22	09.46			
4	test_004	Hemoglobin	04	3-10	none	04.22	09.46			
5	test_005	Hemoglobin	05	3-10	none	04.22	09.46			
6	test_006	Hemoglobin	06	3-10	none	04.22	09.46			
7	test_007	Hemoglobin	07	3-10	none	04.22	09.46			
8	test_008	Hemoglobin	08	3-10	none	04.22	09.46			

FIGURE 9. QC Batch window.

(**Figure 10**). The QC Batch only allows you to edit sample-specific parameters — all other operational parameters are locked. In a QC Batch, you can only specify injection file name, sample ID, vial location, replicates and comments. A comparison of a Development and QC Batch is shown in **Figure 11**. The QC Batch only has one programming window. All operational parameters are listed in the upper right corner of the QC Batch window but they cannot be edited. Utilizing the QC Batch guarantees operational parameters will not be accidentally or deliberately changed and is an excellent tool for QC labs.

RUNNING A BATCH

When you run a batch, the completed runs are saved as individual Injection IEF data files which are embedded with secure information on data acquisition specifics such as: Injection ID-Time/Date, Run Started-Time/Date and Run Ended-Time/Date. Additional information on batch execution can be found in the audit log.

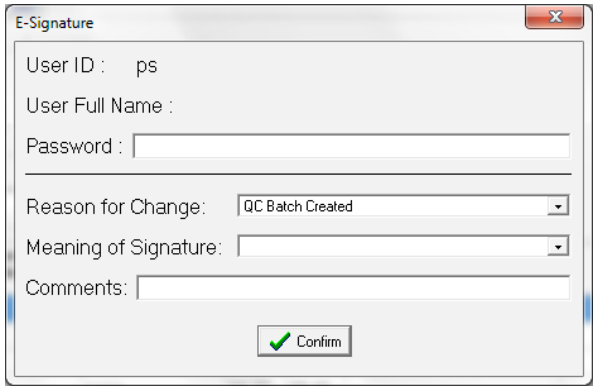


FIGURE 10. QC Batch E-signature dialog box.

PAUSING, STOPPING OR ABORTING A BATCH

During execution of a Development Batch, you can Pause, Stop or Abort its execution. When running a QC Batch, Pause is not available - once a QC Batch starts it must run to completion or be aborted. Pause, Stop and Abort actions are also under e-signature control.

MODIFYING A BATCH

iCE CFR software allows a previously executed batch file to be modified so it can be used again. Modified batches can only be run after changing the batch file name. In fact, when modifying a previously executed batch file, the Save option is unavailable. This function ensures executed batch information will never be overwritten, and all executed batches contain unique identifiers. Another important function to note is that batch files cannot be deleted within the iCE CFR software.

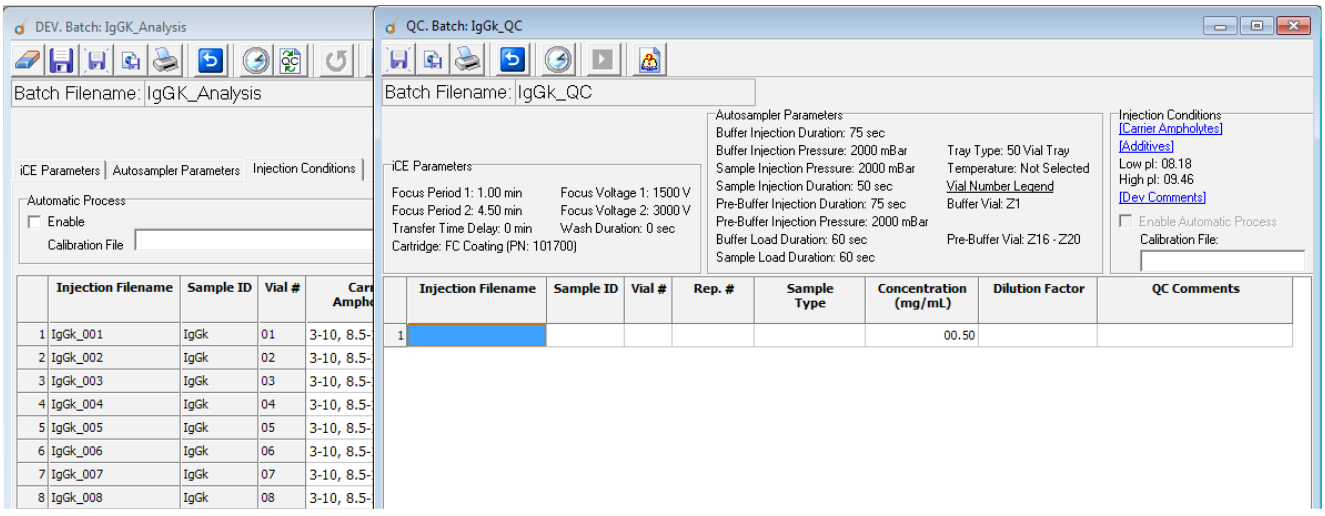


FIGURE 11. Comparison of Development Batch and QC Batch windows.

Processing Raw Data

After data acquisition, the raw IEF data must be processed to create a pI-calibrated analysis file. An example of raw IEF data is shown in **Figure 12**. All data file calibration functions are under e-signature control and once the calibration is reviewed and confirmed, the data file is locked (**Figure 13** and **Figure 14**). To reanalyze the data file, it must be unlocked before it can be reprocessed, and once re-analysis occurs, the original calibration information is overwritten. This process requires confirmation at all steps by e-signature. Besides the information that is already securely embedded in the original raw IEF file, the pI-calibrated data file also includes secure information on processing such as time, date, operator and processing details.

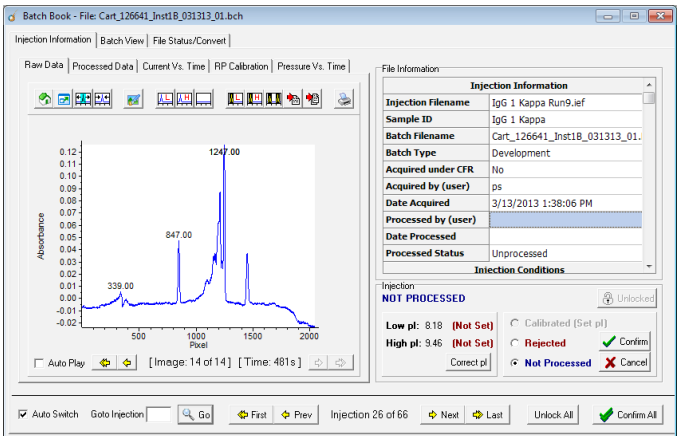


FIGURE 12. Raw IEF data prior to processing.

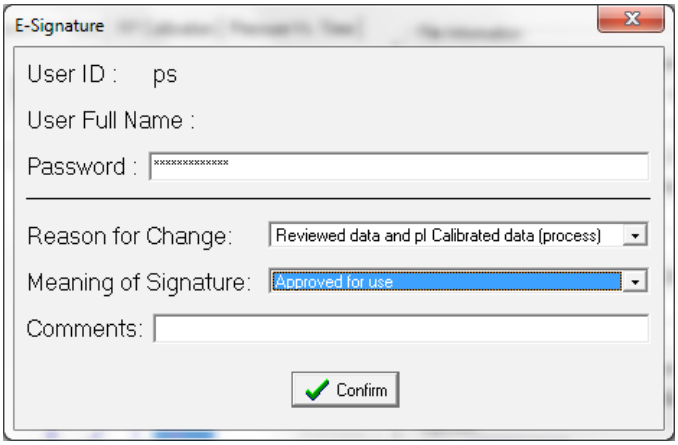


FIGURE 13. Data processing E-signature dialog box.

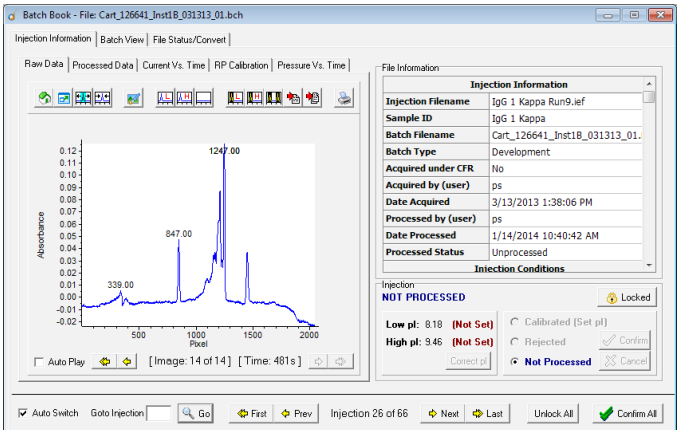


FIGURE 14. Locked, processed, pI-calibrated data.

Converting and Exporting Data for Analysis in Third-party Quantitation Software

For quantitative analysis, the processed, pI-calibrated IEF data must be converted to ANDI (Analytical Data Interchange) format for export to third-party chromatographic analysis software such as Chromperfect® (Justice Laboratory Software), Empower (Waters Corporation), Chromeleon™ (Thermo Fisher Scientific, Inc.) or ChemStation (Agilent Technologies). The ANDI file format supports export of the data embedded with the secure sample-specific information. The amount of sample-specific information embedded in the ANDI file is dependent on the specific third-party software. For example, due to differences in data files and formats, the conversion to Empower vs. conversion to Chromperfect may contain different amounts of information.

Chromperfect is customized for iCE CFR software. The batch information is exported to the ANDI file for Chromperfect, and Chromperfect can display the iCE-defined fields (see **Table 1**). For Empower, the file export program has been modified specifically for Empower to ensure proper mapping of exchanged data and parametric information. Empower cannot display all iCE-related information. **Table 2** shows how iCE-related fields are mapped to Empower fields.

CHROMPERFECT ANDI FILE AND ICE FIELD	EXAMPLE INFORMATION
Injection Filename:	Hb1.ief
Sample ID:	Hb1-Hbstd
Batch Filename:	0 hour_011714_01
Acquired by:	cbl (CBL)
Injection Acquired Date:	1/17/2014 12:01:56 PM
Sample Type:	NOT GIVEN
Analyzer iCE Analyzer, Model:	iCE3, SN: JW 1013, Firmware Version: 2.07
Sample Information	
Batch Type:	Development
Acquired under CFR:	No
Processed by (operator):	cbl (CBL)
Processed Date:	1/17/2014 4:38:59 PM
Processed Status:	Calibrated
[Sample Conditions]	
Carrier Ampholytes:	8% pH3-10Pharmalytes
Additives:	no MC
pI Marker Low:	4.22
pI Marker High:	9.46
Condition Comments:	DI in washing
Concentration (mg/mL):	
Tray Temp (C):	17.5
Analyzer Settings	
Cartridge Type:	FC Coating (PN: 101700)
Cartridge SN:	200108
Cartridge CN:	0
Focus Period 1:	1500 V for 1.00 min
Focus Period 2:	3000 V for 4.50 min
Sample Transfer Time (sec):	10
Wash Duration (sec):	0
Scans Averaged:	16
Exposure Time (ms):	169
Desalt Current (uAmp):	101
Transfer Time Delay (min):	0.00
Lamp Type:	Deuterium
Lamp Run Time (hr):	91.54
Software Ver:	4.0
Autosampler Settings	
Model:	PrinCE Next
SN:	60-20-00-0-003
Firmware Version:	7635.0100.01.02 (0001)
Refrigeration Option:	Yes

TABLE 1. Chromperfect ANDI File iCE Field Display.

ICE FIELD	EMPOWER MAPPED FIELD
Sample Information	
Injection Filename	source_file_reference
Sample ID	sample_name (partial)
Batch Filename	raw_data_table_name
Batch Type	sample_id_comments (partial)
Acquired under CFR	sample_id_comments (partial)
Acquired by (user)	operator_name
Date Acquired	injection_date_time_stamp
Processed by (user)	sample_id_comments (partial)
Date Processed	sample_id_comments (partial)
Processed Status	sample_id_comments (partial)
(Raw Data)	ordinate_values
Sample Conditions	
Carrier Ampholytes	sample_id_comments (partial)
Additives	sample_id_comments (partial)
pI Marker Low	sample_id_comments (partial)
pI Marker High	sample_id_comments (partial)
Condition Comments	sample_id_comments (partial)
Sample Type	sample_type
Concentration	sample_id_comments (partial)
Tray Temperature	sample_id_comments (partial)
Dilution Factor	(no)
Replication Number	(n/a)
Analyzer Settings	
Cartridge Type	detector_name

ICE FIELD	EMPOWER MAPPED FIELD
Cartridge S/N	detector_name
Focus Period 1	sample_id_comments (partial)
Focus Period 2	sample_id_comments (partial)
Sample Transfer Time	sample_id_comments (partial)
Wash Duration	sample_id_comments (partial)
Scans Averaged	sample_id_comments (partial)
Exposure Time	sample_id_comments (partial)
Desalt Current	sample_id_comments (partial)
Transfer Time Delay	sample_id_comments (partial)
Lamp Type	sample_id_comments (partial)
Lamp Run Time	sample_id_comments (partial)
Model	detector_name
Serial Number	detector_name
Software Version	detector_name
Firmware Version	detector_name
Autosampler Settings	
Model	sample_id_comments (partial)
Serial Number	sample_id_comments (partial)
Firmware Version	sample_id_comments (partial)
Refrigeration Option	sample_id_comments (partial)
Buffer Vial Duration	sample_id_comments (partial)
Buffer Vial Pressure	sample_id_comments (partial)
Sample Vial Duration	sample_id_comments (partial)
Sample Vial Pressure	sample_id_comments (partial)
Pre-Buffer Vial Duration	sample_id_comments (partial)
Pre-Buffer Vial Pressure	sample_id_comments (partial)
Drying Vial Duration	sample_id_comments (partial)
Drying Vial Pressure	sample_id_comments (partial)

TABLE 2. Mapping of iCE fields to Empower.

At minimum, the ANDI file will contain this embedded secure sample information: Injection Date/Time, Sample Date/Time, System Configuration and Operator. Data export is also under e-signature control to keep your data secure. Once you select a data format for export, an e-signature dialog box will display, requiring you enter your password and confirm the action for the audit trail (**Figure 15**).

In the case of multiple exports of the same data file, iCE CFR software will not overwrite previously exported files. It will check for an existing file name and will add a sequential identifier if the file has been previously exported. The export function is also under electronic signature so each time the data is exported, a user will need to confirm the export action. The software will save all converted files and track all data conversions in the audit trail.

Importing ANDI Files into Third-party Quantitation Software

Once the data is exported, you can import it into your third-party quantitation software for analysis. The import of ANDI files and the subsequent audit trail is defined by both the third-party software and the end user's or company's IT security protocols. The amount of embedded sample detail recorded after import is also third-party software-dependent.

IMPORTING ANDI FILES CHROMPERFECT

Chromperfect was designed and developed for iCE3 data, and all data information is directly transferred. An example report from Chromperfect is shown in **Figure 16**.

IMPORTING ANDI FILES INTO EMPOWER

As shown in the example in **Figure 17**, Injection Date/Time, Sample Date/Time, System Configuration and Operator details are securely imported to Empower. As noted in the previous section, the terminology is different between the two software packages. Please see **Table 2** for information on how the iCE fields are mapped to Empower.

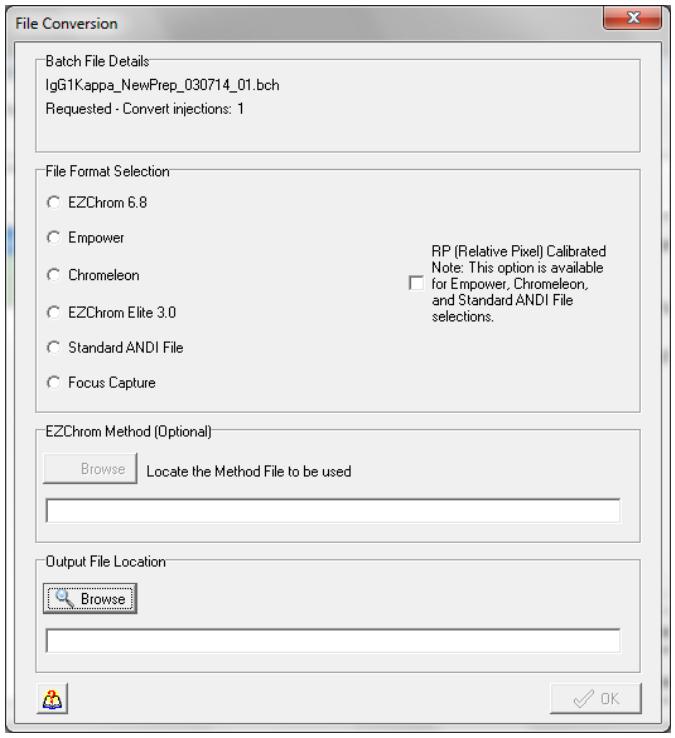


FIGURE 15. File Conversion data export window.

Additional information may be added to the resulting Empower injection file by defining a custom import field which can be populated upon import by the analyst.

Archiving Raw Data

GMP regulations require that all QC laboratory data be retained for as long as the batch record must be kept. This includes the raw data itself. Laboratory data records must be stored as "complete and accurate" copies, which for iCE CFR software includes the raw data files, the processed, pI-calibrated files and audit logs.

There are many different options for iCE CFR software record archiving, and these are generally user-defined and based on the IT capabilities at your facility. A simple yet compliant approach may include instruction in the SOP method for manually archiving the data on a secure network drive. A more complex option could be the building of an automatic push code, which initiates automatic IEF.file backup from the ICE3 computer hard drive to a secure network folder on a daily basis. iCE CFR software employs file encryption and a checksum algorithm to support file integrity during secure data archiving. All current and future iCE CFR software versions are backwards compatible and will allow secure archived data to be read and processed.

-- SAMPLE INFORMATION --
Batch Type: Development
Acquired under CFR: No
Processed by (operator): humphrey (Humphrey Li)
Processed Date: 2/21/2014 5:27:10 PM
Processed Status: Calibrated

-- SAMPLE CONDITION --
Carrier Ampholytes: 8%pH3-10Pharmalytes
Additives: 0.35%MC
pI Marker Low: 4.22
pI Marker High: 9.46
Condition Comments:
Concentration (mg/mL):
Tray Temp (C): 8.0

-- ANALYZER SETTINGS --
Cartridge Type: FC Coating (PN: 101700)
Cartridge SN: 108699
Cartridge CN: 0
Focus Period 1: 1500 V for 1.00 min
Focus Period 2: 3000 V for 4.50 min
Sample Transfer Time (sec): 100
Wash Duration (sec): 0
Scans Averaged: 16
Exposure Time (ms): 84
Desalt Current (uAmp): 101
Transfer Time Delay (min): 0.00
Lamp Type: Deuterium
Lamp Run Time (hr): 115.66

-- AUTOSAMPLER SETTINGS --
Software Ver: 2.3.5
Model: PrinCE cIEF Microinjector
SN: 541807412
Firmware Version: 2005
Refrigeration Option: Yes
Buffer Vial Pressure (mBar): 2000
Buffer Vial Duration (sec): 0
Sample Vial Pressure (mBar): 2000
Sample Vial Duration (sec): 100
Pre-Buffer Vial Duration (sec): 0
Pre-Buffer Vial Pressure (mBar): 0
Drying Vial Duration (sec): 0

FIGURE 16. Example report from Chromperfect.

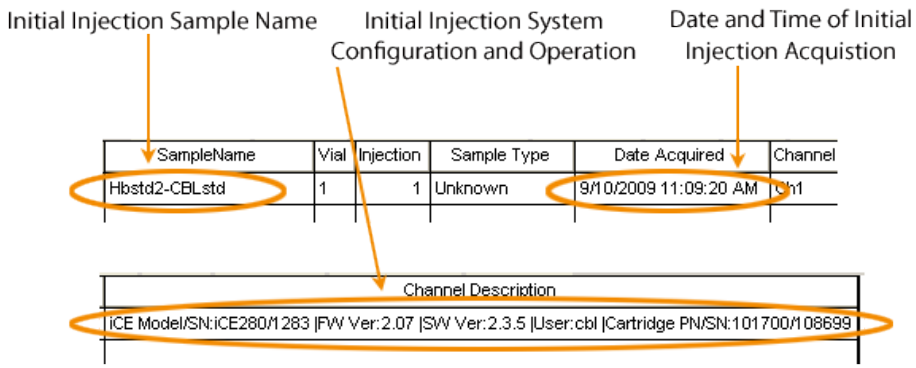


FIGURE 17. Information imported into Empower with audit trail.

Conclusion

iCE CFR software offers many tools to help ensure data authenticity and integrity but complete compliance will require procedural controls (SOP, training and administration throughout). The iCE3 data workflow includes the following 21 CFR Part 11 technical controls:

- Restricted access
- Secure, computer-generated, time-stamped audit trails
- E-signatures throughout run execution, data processing and export
- Controlled sequence of events
- Operational restrictions that ensure data authenticity and integrity

iCE CFR software's 21 CFR Part 11-compliant processes for data file export and import also allow you to transfer files securely to the third-party software of your choice such as Chromeleon, Empower, or Chromperfect.

21 CFR Part 11 compliance features combined with rapid analysis, platform methods and easy method development truly make the iCE3 an ideal system for the analysis of biologics.

proteinsimple

Toll-free: (888) 607-9692
Tel: (408) 510-5500
Fax: (408) 510-5599
orders@proteinsimple.com
proteinsimple.com