NOTE: Appropriate calibrators for screening assays should consist of at least one positive calibrator. If only one calibrator is used, it must be at the declared cutoff value(s).

Laboratories may use historical calibrations; however, controls must be run with each batch to verify the calibration. In addition, the laboratory must have validated the stability of the calibration, and have a record of the validation.

Evidence of Compliance:
- ✓ Records of calibration

FDT.21130 Analytical Data **Phase II**

The analytical data are presented to permit scientific review of the data for calibrators, controls and unknowns by the analyst.

FDT.21430 Spectrophotometer Calibration **Phase II**

Spectrophotometers, if part of an immunoanalyzer, are calibrated at the frequency and as directed by the instrument manufacturer, and results recorded.

Evidence of Compliance:
- ✓ Records of calibration at defined frequency

FDT.21680 Reanalysis/Secondary Screening **Phase II**

The laboratory defines situations when reanalysis and secondary screening are indicated.

LIQUID CHROMATOGRAPHY (LC)

This section covers the LC inlet system of LC/MS and LC/MS/MS instruments.

Inspector Instructions:

	• Sampling of LC policies and procedures • Sampling of LC control, calibration/standards records • Sampling of column verification records
	• How does your laboratory ensure appropriate extracted calibrator(s) are analyzed? • How does your laboratory evaluate potential carryover? • When are reinjection or reanalysis procedures required?

FDT.22150 Calibration and Calibration Verification **Phase II**

Appropriate calibration or calibration verification is performed on each day of testing or following the manufacturer's instructions.

NOTE: For qualitative assays, an appropriate calibrator should be run at normal and abnormal levels. For quantitative assays, a multipoint calibration may be required if the measurement has a non-linear response. For some assays, a level near the assay's limit of detection (LOD) or at critical decision point(s) is needed. For measurement systems that have a linear response

verified by periodic multipoint calibration verification and AMR verification protocols, a calibration procedure that uses a single calibrator at an appropriate concentration is acceptable. Analyses based on a single point calibration must be controlled by appropriate quality control samples. In addition, inclusion of a negative control (reagent blank) is good laboratory practice.

Evidence of Compliance:
- ✓ Records of calibration/calibration verification

FDT.22230 Column Performance **Phase II**

The performance of the column is monitored on each day of use.

NOTE: Unextracted standards and extracted calibrators or controls typically containing the target compound(s) may be analyzed each day of use to monitor critical aspects of LC performance. Criteria for evaluating such parameters as retention time, relative retention time, separation of closely eluting compounds of interest, plates, chromatography quality and detector response should be established and monitored. Records must be retained.

Evidence of Compliance:
- ✓ Records for column monitoring

FDT.22280 Extracted Calibrators **Phase II**

An appropriate extracted calibrator(s) is analyzed with each batch of samples.

NOTE: At least one extracted calibrator at the commonly accepted cut-off for single-point calibration, or multiple calibrators above and below the commonly accepted cut-off for multipoint calibration, must be analyzed with each run.

Laboratories may use historical calibrations; however, controls must be run with each batch to verify the calibration. In addition, the laboratory must have a record of the validation of the stability of the calibration.

FDT.22330 Daily QC - LC **Phase II**

Appropriate controls are extracted and analyzed with each batch of specimens.

NOTE: See General Quality Control section for specific controls required.

Evidence of Compliance:
- ✓ QC records

FDT.22430 Internal Standard **Phase II**

Internal standards are used as appropriate.

NOTE: An internal standard is not required for FDA-cleared/approved kits where an internal standard is not used. For a qualitative assay, the use of an internal standard is appropriate if sample preparation includes an extraction step(s), there is low or variable analyte recovery, and/or an accurate sample injection volume is important.

Evidence of Compliance:
- ✓ Records for use of internal standards **OR** written justification for not using an internal standard in assay

FDT.22830 LC Records **Phase II**