

## Insulin Analysis by LC/MS (ESI)

### 1 Scope

This procedure allows for the analysis of items suspected of containing human, bovine, and porcine insulin, as well as several synthetic insulins. Typical items consist of pharmaceutical preparations, syringe residues, solids, and liquids.

This procedure applies to General Chemistry personnel in the Chemistry Unit that are qualified to examine evidence for the presence of insulin.

### 2 Equipment/Materials/Reagents

- Common laboratory glassware and equipment
- Screw top borosilicate glass test tubes (Fisherbrand Catalog No. 14-959-35AA, or equivalent)
- Screw top glass autosampler vials (inserts removed, MicroLiter part number 09-1220B-101, or equivalent)
- Analytical balance
- pH strips
- Liquid chromatography system with a Vydac 218TP C18 column (or equivalent) coupled to a mass spectrometer (LC/MS) with electrospray ionization (ESI) (e.g., Thermo LTQ, Thermo LTQ OrbiTrap XL, Thermo Exactive OrbiTrap)
- ProMass for Xcalibur (automated ESI deconvolution software)
- Acetonitrile
- Deionized water
- Formic acid
- Glacial acetic acid
- Dilute acetic acid (50 mM)
  - To a 100 mL graduated cylinder add 80 mL deionized water and 250  $\mu$ L glacial acetic acid. Mix well and bring to 85 mL mark with deionized water. Store in glass at room temperature. Stable for at least 3 months.
- Hydrochloric acid
- Dilute hydrochloric acid (0.1 M)
  - To a 25 mL graduated cylinder add 20 mL deionized water followed by 2 mL concentrated hydrochloric acid. Mix well and bring to 25 mL mark with deionized water. Store in glass at room temperature. Stable for at least 6 months.
- Trifluoroacetic acid (TFA)

- Dilute TFA (0.04% by volume)
  - Add 0.4 mL TFA to 1 L deionized water and mix well. Store in glass at room temperature. Stable for at least 2 months.
- Human insulin
- Bovine insulin
- Porcine insulin
- Humalog insulin Lispro injection solution
- Novolog insulin Aspart injection solution
- Levemir insulin Detemir injection solution
- Lantus insulin Glargine injection solution
- Apidra insulin Glulisine injection solution

### **3 Standards and Controls**

#### **3.1 Negative Control**

A Negative Control will be prepared by mirroring the process used to prepare a sample from a questioned item. For example, use the same volume of dilute acetic acid from the same source and lot and within a similar container used to prepare the questioned item(s). It is left to the discretion of the examiner as to what constitutes an adequate Negative Control.

#### **3.2 Human Insulin**

Purchased from United States Pharmacopeia (USP) or another approved vendor. Stability and storage determined by manufacturer.

##### **3.2.1 Human Insulin Stock Solution (1 mg/mL)**

Weigh 10.0 mg Human Insulin into a 10 mL volumetric flask. Add 0.5 mL dilute hydrochloric acid and mix well to dissolve. Bring to the mark with dilute acetic acid. Store refrigerated in borosilicate glass test tubes. Stable for at least six months.

#### **3.3 Porcine Insulin**

Purchased from USP or another approved vendor. Stability and storage determined by manufacturer.

### **3.3.1 Porcine Insulin Stock Solution (1 mg/mL)**

Weigh 10.0 mg Porcine Insulin into a 10 mL volumetric flask. Add 0.5 mL dilute hydrochloric acid and mix well to dissolve. Bring to the mark with dilute acetic acid. Store refrigerated in borosilicate glass test tubes. Stable for at least six months.

### **3.4 Bovine Insulin**

Purchased from USP or another approved vendor. Stability and storage determined by manufacturer.

#### **3.4.1 Bovine Insulin Stock Solution (1 mg/mL)**

Weigh 10.0 mg Bovine Insulin into a 10 mL volumetric flask. Add 0.5 mL dilute hydrochloric acid and mix well to dissolve. Bring to the mark with dilute acetic acid. Store refrigerated in borosilicate glass test tubes. Stable for at least six months.

### **3.5 Humalog Insulin Lispro**

A Humalog Insulin Lispro Injection Solution (100 units/mL) is obtained from Eli Lilly & Company. Stability and storage determined by manufacturer.

#### **3.5.1 Humalog Insulin Working Solution (~52 ug/mL)**

Using a 1 mL disposable plastic syringe, transfer 0.15 mL of the Humalog Insulin Lispro Injection Solution to a 10 mL volumetric flask. Bring to the mark with dilute acetic acid. Store refrigerated in borosilicate glass test tubes. Stable for at least six months, or until expiration of the Humalog Insulin Lispro Injection Solution, whichever comes first.

### **3.6 Novolog Insulin Aspart**

A Novolog Insulin Aspart Injection Solution (100 units/mL) is obtained from Novo-Nordisk. Stability and storage determined by manufacturer.

#### **3.6.1 Novolog Insulin Working Solution (~52 ug/mL)**

Using a 1 mL disposable plastic syringe, transfer 0.15 mL of the Novolog Insulin Aspart Injection Solution to a 10 mL volumetric flask. Bring to the mark with dilute acetic acid. Store refrigerated in borosilicate glass test tubes. Stable for at least six months, or until expiration of the Novolog Insulin Aspart Injection Solution, whichever comes first.

### **3.7 Levemir Insulin Detemir**

A Levemir Insulin Detemir Injection Solution (100 units/mL) is obtained from Novo-Nordisk. Stability and storage determined by manufacturer.

#### **3.7.1 Levemir Insulin Working Solution (~142 ug/mL)**

Using a 1 mL disposable plastic syringe, transfer 0.1 mL of the Levemir Insulin Detemir Injection Solution to a 10 mL volumetric flask. Bring to the mark with dilute acetic acid. Store refrigerated in borosilicate glass test tubes. Stable for at least six months, or until expiration of the Levemir Insulin Detemir Injection Solution, whichever comes first.

### **3.8 Lantus Insulin Glargine**

A Lantus Insulin Glargine Injection Solution (100 units/mL) is obtained from Sanofi-Aventis. Stability and storage determined by manufacturer.

#### **3.8.1 Lantus Insulin Working Solution (~55 ug/mL)**

Using a 1 mL disposable plastic syringe, transfer 0.15 mL of the Lantus Insulin Glargine Injection Solution to a 10 mL volumetric flask. Bring to the mark with dilute acetic acid. Store refrigerated in borosilicate glass test tubes. Stable for at least six months, or until expiration of the Lantus Insulin Glargine Injection Solution, whichever comes first.

### **3.9 Apidra Insulin Glulisine**

An Apidra Insulin Glulisine Injection Solution (100 units/mL) is obtained from Sanofi-Aventis. Stability and storage determined by manufacturer.

#### **3.9.1 Apidra Insulin Working Solution (~52 ug/mL)**

Using a 1 mL disposable plastic syringe, transfer 0.15 mL of the Apidra Insulin Glulisine Injection Solution to a 10 mL volumetric flask. Bring to the mark with dilute acetic acid. Store refrigerated in borosilicate glass test tubes. Stable for at least six months, or until expiration of the Apidra Insulin Glulisine Injection Solution, whichever comes first.

### 3.10 Positive Control Solutions

#### 3.10.1 Human, Porcine, and Bovine Insulins (5 ug/mL)

Add 125 uL of the applicable Insulin Stock Solution(s) (1 mg/mL) to a 25 mL volumetric flask. Bring to the mark with dilute acetic acid. Store refrigerated in borosilicate glass test tubes. Stable for at least six months.

#### 3.10.2 Humalog, Novolog, Levemir, and Lantus Insulins (~5 ug/mL)

To a 5 mL volumetric flask, add the below indicated volume of the applicable Insulin Working Solution(s). Bring to the mark with dilute acetic acid. Store refrigerated in borosilicate glass test tubes. Stable for at least one month.

- Humalog Insulin Working Solution (~52 ug/mL)- 480 uL
- Novolog Insulin Working Solution (~52 ug/mL) - 480 uL
- Levemir Insulin Working Solution (~142 ug/mL) - 175 uL
- Lantus Insulin Working Solution (~55 ug/mL) - 450 uL

#### 3.10.3 Apidra Insulin (~10 ug/mL)

Add 960 uL of the Apidra Insulin Working Solution (~52 ug/mL) to a 5 mL volumetric flask. Bring to the mark with dilute acetic acid. Store refrigerated in borosilicate glass test tubes. Stable for at least one month, or until expiration of the Apidra Insulin Glulisine Injection Solution, whichever comes first.

## 4 Sampling

Statistical sampling is performed according to the General Chemistry *Sampling Guidelines for Bulk Materials and Multi-Unit Populations* (GenChem 21).

When non-statistical sampling is utilized on a heterogeneous item, the results of examinations will be clearly limited to the sample(s) that were selected and examined.

## 5 Procedure

- a. Verify that the LC/MS is in proper working condition by analyzing the applicable Insulin Positive Control(s) using the instrumental operating conditions in section 8.
- b. Visually examine each item and record relevant information. If statistical sampling is required, refer to *Sampling Guidelines for Bulk Materials and Multi-Unit Populations* (GenChem 21).
- c. Prepare items within screw top borosilicate glass test tubes with an appropriate amount of dilute acetic acid. For suspected pharmaceutical preparations, a 200-fold dilution is recommended (e.g., 50 uL of sample to a final volume of 10 mL). For fluids recovered from intravenous (IV) drip bags, a 10-fold dilution is recommended (e.g., 200 uL of sample added to 1.8 mL dilute acetic acid). For syringe residues, rinse the syringe with 500 uL of dilute acetic acid; no further dilution is necessary at this point. Other dilution factors may be used based on case history and professional judgment.
- d. Use an empty, labeled screw top borosilicate glass test tube as a Negative Control. Add the same volume of dilute acetic acid that was used to prepare the items. If multiple volumes were used, use the largest volume.
- e. Transfer aliquots of the Negative Control and item solutions to labeled MicroLiter screw top autosampler vials without inserts. Analyze the Negative Control(s), questioned item(s), and applicable Positive Control(s) by LC/MS (ESI) using the instrumental operating conditions in section 8. Incorporate dilute acetic acid blanks between each sample.
- f. Process applicable mass spectra using ProMass for Xcalibur ESI deconvolution software:
  1. Export the desired mass spectrum from the Xcalibur Qual Browser (right click, Export – Clipboard – Exact Mass).
  2. Open ProMass for Xcalibur software and click on the “Build Params” icon.
  3. Load the “INSULIN.PARAMS” file.
  4. Click on the clipboard icon, which will run the deconvolution program. A report containing the Base Peak Mass, Intensity, and Spectral Quality will be generated. Print a copy of the report for the case notes.

## 6 Calculations

Not applicable

## 7 Measurement Uncertainty

Not applicable

## 8 Instrumental Conditions

The following instrumental conditions are not intended to be prescriptive nor exhaustive. Minor modifications to the conditions may be used as needed and without authorization, provided the same conditions are used for all applicable solvent blanks, control samples, and questioned items; and the Positive Control(s) provide acceptable data. The utilized conditions will be recorded and retained with the case notes.

### 8.1 Liquid Chromatography/Mass Spectrometry (LC/MS)

#### 8.1.1 Liquid Chromatography Parameters

Mobile Phase Compositions		Flow Parameters			Column Parameters	
A: 0.04% TFA (v/v) in deionized water		total flow = 0.3 mL/min			type	C-18
		time (min)	% A	% B	length	150 mm
B: Acetonitrile		0	75	25	internal diameter	2.1 mm
<b>Autosampler</b>		2.0	75	25	particle size	5 um
temperature	20 °C	5.0	71	29	temperature	30 °C
injection volume	10 uL	12.0	70	30		
		16.0	20	80		
		18.0	20	80		
		19.0	75	25		
		24.0	75	25		
		total run time = 24 min.				

### 8.1.2 Mass Spectrometer Parameters

Duration = 24.00 min; Source parameters are set through the tune file and should be optimized on each instrument. Retain a copy of the tune parameters with the case notes.	
<b>Scan Event #1</b>	
<b>Ionization mode</b>	ESI (+)
<b>Scan mode</b>	Full scan MS
<b>Scan range (LTQ)</b>	950-2000 <i>m/z</i>
<b>Scan range (OrbiTrap)</b>	1400-2060 <i>m/z</i> at 30,000 resolution

Table 1: Insulin +5, +4, and +3 Ions at Unit Mass Resolution [(\*) +6, +5, and +4 ions for Lantus]

Type of Insulin	Ions Observed ( <i>m/z</i> )
Human Insulin and Humalog (insulin lispro)	1162-1164, 1452-1454, 1936-1938
Porcine Insulin	1156-1158, 1444-1446, 1926-1928
Bovine Insulin	1147-1149, 1433-1435, 1911-1913
Apidra (insulin glulisine)	1165-1167, 1456-1458, 1941-1943
Lantus (insulin glargine)	1011-1013, 1213-1215, 1516-1518(*)
Levemir (insulin detemir)	1183-1185, 1479-1481, 1972-1974
Novolog (insulin aspart)	1165-1167, 1456-1458, 1942-1944

Table 2: Insulin +4 and +3 Ions at 30,000 Resolution

Type of Insulin	Theoretical Mass ( $m/z$ )		Theoretical Ion Ratio (%)
	+4 charge	+3 charge	
Human Insulin and Humalog (insulin lispro)	1452.167	1935.887	48.6
	1452.418	1936.222	82.7
	1452.668	1936.556	100
	1452.919	1936.889	95.5
	1453.169	1937.223	76.3
	1453.420	1937.557	52.7
Porcine Insulin	1444.665	1925.884	48.9
	1444.915	1926.218	83.0
	1445.166	1926.552	100
	1445.416	1926.886	95.2
	1445.667	1927.220	75.8
	1445.917	1927.554	52.2
Bovine Insulin	1433.658	1911.209	49.5
	1433.909	1911.543	83.5
	1434.159	1911.877	100
	1434.410	1912.211	94.6
	1434.660	1912.544	74.9
	1434.910	1912.878	51.4
Apidra (insulin glulisine)	1455.917	1940.887	48.4
	1456.168	1941.221	82.5
	1456.418	1941.555	100
	1456.669	1941.889	95.7
	1456.919	1942.223	76.6
	1457.170	1942.557	53.1
Lantus (insulin glargine)	1515.963	2020.948	45.3
	1516.213	2021.282	80.1
	1516.464	2021.616	100
	1516.714	2021.950	98.4
	1516.965	2022.284	80.9
	1517.215	2022.617	57.4
Levemir (insulin detemir)	1479.455	1972.271	46.1
	1479.706	1972.605	80.7
	1479.956	1972.939	100
	1480.207	1973.273	97.6
	1480.457	1973.607	79.6
	1480.707	1973.941	56.1
Novolog (insulin aspart)	1456.661	1941.879	48.8
	1456.912	1942.213	82.8
	1457.162	1942.547	100
	1457.412	1942.881	95.4
	1457.663	1943.215	76.2
	1457.913	1943.549	52.7

## 9 Limitations

The following conclusions apply to the analysis of items for the presence of insulin:

- Consistent with
- Not identified
- Inconclusive

Refer to *Chemistry Unit (CU) FBI Approved Standards for Scientific Testimony and Report Language for General Chemistry* (GenChem 32, ASSTR), *General Approach to Report Writing in General Chemistry* (GenChem 27), and *Department of Justice Uniform Language for Testimony and Reports for General Forensic Chemistry and Seized Drug Examinations* (GenChem ULTR) for examples of reporting examination conclusions and the associated limitations and decision criteria.

Refer to *General Chemistry Instrument Parameters* (GenChem 34) for instrumental limitations and decision criteria.

Refer to *General Chemistry Guidelines for Comparison of Mass Spectra* (GenChem 33) for mass spectra decision criteria.

## 10 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. Some of the chemicals may be carcinogenic. Refer to the *FBI Laboratory Safety Manual* for the proper handling and disposal of all chemicals. Personal protective equipment should be used when handling any chemical and when performing any type of analysis.

## 11 References

Darby, S.M., Miller, M.L., Allen, R.A., and LeBeau, M.A., "A Mass Spectrometric Method for Quantitation of Intact Insulin in Blood Samples", *Journal of Analytical Toxicology* 25 (2001), pp 8-14.

*Sampling Guidelines for Bulk Materials and Multi-Unit Populations*; FBI Laboratory Chemistry Unit – General Chemistry SOP (GenChem 21)

*Chemistry Unit (CU) FBI Approved Standards for Scientific Testimony and Report Language for General Chemistry* – General Chemistry SOP (GenChem 32)

*General Approach to Report Writing in General Chemistry*; FBI Laboratory Chemistry Unit –  
General Chemistry SOP (GenChem 27)

*Department of Justice Uniform Language for Testimony and Reports for General Forensic  
Chemistry and Seized Drug Examinations* (GenChem ULTR)

*General Chemistry Instrument Parameters*; FBI Laboratory Chemistry Unit – General Chemistry  
SOP (GenChem 34)

*Guidelines for Comparison of Mass Spectra*; FBI Laboratory Chemistry Unit – General  
Chemistry SOP (GenChem 33)

*FBI Laboratory Safety Manual*

Rev. #	Issue Date	History
0	11/08/12	New document derived from previous toxicology SOP titled, <i>Insulin Analysis by LC/MS(ESI)</i> .
1	09/01/20	Minor edit made to title. Removed previous sections 1 (Introduction), 3 (Principle), 4 (Specimens), 7 (Calibration), and 11 (Decision Criteria); renumbered sections accordingly. Edited new section 1 for clarity and to include personnel. Changed lettered listing in new section 2 to bullets and revised the list. Edited new section 3 to add detail; changed formatting. Added content to section 4 (Sampling) and updated reference. Edited content of section 5 for clarity. Changed new section 7 title from 'Uncertainty of Measurement'. Added first paragraph in section 8; edited remainder of section for formatting and clarity; Tables 1 and 2 incorporated (were in "Decision Criteria" section previously). Section 9 edited to include conclusion statements and references to ASSTR, ULTR, etc. Removed sections a. through d. (retained with validation binder). Updated references in section 11, changed formatting.

**Approval**

Redacted - Signatures on File

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