Synthetic Human Growth Hormone (Somatropin) Analysis by LC/MS (ESI)

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1 INTRODUCTION

This procedure allows for the analysis of items suspected of containing Somatropin. Somatropin is a synthetic form of human growth hormone (hGH) protein found in many commercial preparations. These preparations are typically provided in a lyophilized powder form along with a fixed volume of diluent to prepare a specific concentration. A variety of substances are used as diluents including bacteriostatic water and saline. Typical items consist of labeled vials and injection pens. Unlabeled items may also be accepted for analysis on a case-by-case basis.

2 SCOPE

This procedure applies to General Chemistry personnel in the Chemistry Unit who are qualified to examine evidence for the presence of synthetic hGH.

3 EQUIPMENT

- General laboratory supplies
- Analytical balance
- pH strips
- Liquid chromatography system with a Zorbax 300SB-C_3 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) (or equivalent)
- Acetonitrile
- Deionized water
- Formic acid
- Genotropin Miniquick (Pfizer®)
- Trifluoroacetic acid (TFA)

4 STANDARDS AND CONTROLS

4.1 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 deionized water or submitted diluent from the same source and lot and within a similar container used to extract the questioned item(s). It is left to the discretion of the examiner as to what constitutes an adequate Negative Control.

4.2 Genotropin Miniquick (Pfizer®) (~800 ug/mL)

Remove a Genotropin Miniquick device from the freezer and allow it to thaw at room temperature for at least 15 minutes. Prepare the injectable solution by mixing the lyophilized powder with the diluent using the commercial device. This solution results in a concentration of ~800 ug/mL. Transfer any remaining solution to a labeled glass vial and store in a freezer.

4.3 hGH Positive Control (~200 ug/mL)

Dilute the above Genotropin Miniquick solution in deionized water to yield a concentration of ~200 ug/mL. Transfer any remaining solution to a labeled glass vial and store in a freezer. The 200 ug/mL Positive Control will be verified at the time of use.

5 SAMPLING

Statistical sampling is performed according to the GENCHEM-301.

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.

6 PROCEDURE

A. Verify that the LC/MS is in proper working condition by analyzing the hGH Positive Control (~200 ug/mL) using the instrumental operating conditions in section 7.

B. Visually examine each item and record observations. If statistical sampling is required, refer to GENCHEM-301.

C. If necessary, prepare the item solution by dissolving in the associated diluent. Use deionized water if a diluent was not included with the item. Make note of the estimated hGH concentration based on the item packaging. If deionized water was used, add the same volume of deionized water from the same source to an empty, labeled test tube as a Negative Control.

D. Transfer an aliquot of the item solution to a labeled test tube. Dilute the item solution with deionized water to yield a final hGH concentration of ~200 ug/mL. Use an empty, labeled test tube as a Negative Control if one was not prepared in step (C) and add the same volume of deionized water from the same source. Vortex mix the Negative Control and item solutions.

E. Transfer aliquots of the Negative Control and item solutions to labeled autosampler vials. Analyze the Negative Control(s), questioned item(s), and ~200 ug/mL hGH Positive Control by LC/MS (ESI) using the instrumental operating conditions in section 7. Incorporate deionized water blanks between each sample.

F. Process applicable mass spectra using ProMass for Xcalibur ESI deconvolution software:
    - Export the desired mass spectrum from the Xcalibur Qual Browser (right click, Export – Clipboard – Exact Mass).
    - Open ProMass for Xcalibur software and click on the “Build Params” icon.
    - Load the “hGH.PARAMS” file.

- 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. Retain a copy of the report for the case notes.

7 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.

7.1 Liquid Chromatography/Mass Spectrometry (LC/MS)

7.1.1 Liquid Chromatography Parameters

Mobile Phase Compositions		**Flow Parameters**			**Column Parameters**	
A: 0.05% formic acid, 0.01% TFA (v/v) in deionized water (pH ~ 2.5)		total flow = 0.25 mL/min			type	300 SB-C_3
		time (min)	% A	% B	length	150 mm
B: 0.05% formic acid, 0.01% TFA (v/v) in acetonitrile (pH ~ 5)		0	90	10	internal diameter	2.1 mm
		2.0	90	10	particle size	5 um
		21.0	28	72	temperature	30 ºC
Autosampler		22.0	90	10		
temperature	15 ºC	28.0	90	10		
injection volume	5 uL	total run time = 28 min.				

7.1.2 Mass Spectrometer Parameters

Duration = 28.00 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.	
Scan Event #1	
Ionization mode	ESI (+)
Scan mode	Full scan MS
Scan range	1000-2000 *m/z*

Ions observed (*m/z*)	**Adduct**
1844-1845	$[hGH + 12H]^{12+}$
1702-1703	$[hGH + 13H]^{13+}$
1581-1582	$[hGH + 14H]^{14+}$
1475-1476	$[hGH + 15H]^{15+}$
1383-1384	$[hGH + 16H]^{16+}$
1302-1303	$[hGH + 17H]^{17+}$
1230-1231	$[hGH + 18H]^{18+}$

8 LIMITATIONS

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

- Consistent with
- Not identified
- Inconclusive

Refer to GENCHEM-903, GENCHEM-201, and Department of Justice Uniform Language for Testimony and Reports for General Forensic Chemistry and Seized Drug Examinations for examples of reporting examination conclusions and the associated limitations and decision criteria.

Refer to GENCHEM-302 for instrumental limitations and decision criteria.

Refer to GENCHEM-303 for mass spectra comparison decision criteria.

9 REVISION HISTORY

Revision	Issued	Changes
01	08/17/2020	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. Section 9 edited to include conclusion statements and references to ASSTR, ULTR, etc. Updated references in section 11, changed formatting.
02	06/15/2022	Revised to match new format requirements. No substantive changes to content.