Correspondence case report

Drug Testing and Analysis

Received: 11 May 2016 Revised: 7 June 2016 Accepted: 7 June 2016 Published online in Wiley Online Library: 8 July 2016

(www.drugtestinganalysis.com) DOI 10.1002/dta.2029

Identification of epidermal growth factor (EGF), in an unknown pharmaceutical preparation suspected to contain insulin like growth factor 1 (IGF-1)

Celine Vanhee,* Steven Janvier, Goedele Moens, Séverine Goscinny, Patricia Courselle and Eric Deconinck

Introduction

The last 30 years are marked by a tremendous evolution in the comprehension of human physiology. Although many physiological processes remain enigmatic, our increased understanding has led to a considerable development of medicines. A small, but yet a more progressively important sub fraction of this drug market consists of recombinant proteins and peptides. Although in most western countries a prescription is required for the injection of these compounds, some of them can easily be acquired from illicit internet pharmacies whether or not disguised as 'research companies'. Some of these drugs can not only be used to cure ourselves but also to improve our sports performance, deepen our tan, and strive to the ideals imposed by society. Reports originating from controlling agencies residing in Germany, the UK, Denmark, Norway, Italy, the USA, Australia, and Belgium show that monoclonal anti-cancer antibodies, insulins, growth hormones, human chorionic gonadotropin, *Clostridium botulinum* toxin type A, potential doping peptides, putative anti-obesity drugs, skin tanning peptides, neuropeptides and a putative anti-cancer polypeptide were identified in seized samples.[1–16] The danger of these illegal polypeptides resides in the fact that these polypeptide products are not produced under controlled environment and therefore may contain the wrong ingredient, the wrong dosage, bacteria, viruses, heavy metals, etc., which could result in severe health issues. In some cases these illegal acts had a deadly outcome.[4,17] Furthermore, for many of these polypeptide drugs, there is a limited knowledge of their effects and/or side-effects (long and short term) hence clinical studies have not been terminated or even worse were given a negative advice due to overall potential adverse health effects. Global actions are on-going to protect the sometimes unaware public from these malignant activities. In Belgium, the Federal Agency for Medicines and Health Products (FAMHP) is responsible for this task and upon their request unknown pharmaceutical preparations suspected to contain illegal drugs are regularly subject to analysis in our official medicines control laboratory (OMCL). At the end of 2014, an unknown pharmaceutical preparation, claimed to contain insulin like growth factor 1 (IGF-1) was submitted for analysis by immunoblotting against IGF-1 and analogues, prior to liquid chromatography-tandem mass spectrometry (LC-MS/MS). Based on the interpretation of the western blot and the LC-MS data we were not able to identify this substance as being any form of insulin like growth factor (IGF), including IGF-1, IGF-2 or synthetic analogues potentially used as doping agent.[18] Further analysis by LC-MS/MS and subsequent peptide sequencing showed that the substance present in the sample corresponds to epidermal growth factor (EGF). EGF plays a major role in the regulation of cell growth, proliferation and differentiation and is therefore frequently used in cell culture. This polypeptide, consisting of 53 amino acids, with sequence NSDSECPLSHDGYCLHDGVCMYIEALDKYACNCVVGYIGERCQYRDLKWWELR and 3 intramolecular disulfide bonds has an average Molecular weight of 6215.9 Da. This polypeptide is known to interact with the EGF-receptor (EGFR) and can promote tumour cell motility and invasion. Therefore the blocking of EGF binding to the EGFR and further downstream signalling pathway is currently marked as a target for anti-cancer therapy for certain cancers (reviewed in Yewale *et al.*[19]). Indeed, a vaccine targeting EGF, named CimaVax-EGF, results in the production of antibodies directed against EGF and this vaccine is being used as cancer therapy against non-small-cell lung carcinoma in Cuba. The CimaVax vaccine consists of recombinant EGF chemically conjugated to the protein P64K from Neisseria meningitides. It induces the production of antibodies directed against EGF and results in the prevention of EGF to attach to EGFR and thus inhibiting the signal that tells cancer cells to grow and divide.[20] Nowadays, the product is undergoing further trials for possible licensing in Europe and the United States.[21] Although no injectable legal medicine is available on the market, the product is used in the cosmetic industry. It is thought that the topical application of EGF would enhance wound healing and stimulate skin rejuvenation.

Taken together, we believe that the purchaser was unaware of the presence of EGF in the sample. This case of potential dangerous wrong labelling clearly demonstrates the peril of purchasing potential doping agents via the black market.

* *Correspondence to: Celine Vanhee, Division of Food, Medicines and Consumer Safety, Section Medicinal Products, Scientific Institute of Public Health (WIV-ISP), J. Wytsmansstraat14, B-1050 Brussels, Belgium. E-mail: Celine.Vanhee@wiv-isp.be*

Division of Food, Medicines and Consumer Safety, Section Medicinal Products, Scientific Institute of Public Health (WIV-ISP), J. Wytsmansstraat14, B-1050 Brussels, Belgium

Experimental

Chemicals and reagents

Acetonitrile was UPLC-MS grade and formic acid, ethanol, methanol and acetic acid were analytical grade. All those reagents were purchased from Biosolve (Valkenswaard, the Netherlands). Water was obtained using a milliQ-Gradient A10 system (Millipore, Billerica, MA, USA). Buffer solutions used for SDS-PAGE and immunoblotting were acquired from BioRad (Hercules, CA, USA).

Reference standards for IGF-1 (lot 2659615), IGF-2 (lot 1987452) and recombinant human EGF (lot 2637007) were bought from Millipore (Billerica, MA, USA). LongR^3-IGF-1 (lot SLBH8302V) and R^3-IGF-1 (lot SLBK1662V) were purchased from Sigma Aldrich (St Louis, MO, USA) while Des1-3-IGF-1 (lot 414PDES1) was manufactured by PROSPEC (Rehovot, Israel). All reference standards were solubilised in 1% formic acid in water at a final concentration of 0.5 mg/mL.

Sample preparation

An unknown pharmaceutical preparation was submitted for analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Upon reception, the preparation consisted of a white, flocculent powder contained in a glass vial marked with *IGTROPIN* and claimed to contain 'reconbinant IGF-1 long R^3' which probably reflects a spelling mistake or typo of recombinant IGF-1 long R (Figure 1A). An aqueous solution was prepared by adding 500 µL of water to the vial. The powder dissolved almost immediately upon brief swirling.

SDS-PAGE and immunoblotting

A 30 µL aliquot of the solution was subjected to SDS-PAGE according to the method of Laemmli.[22] Positive controls for the immunoblotting and Coomassie staining included 30 µL of the standard stock solutions of IGF-1, IGF-2, LongR^3-IGF-1, R^3-IGF-1 and Des1-3-IGF-1. The Precision Plus Protein™ Dual Xtra Standard was used as a molecular weight marker. The samples were run as described in Vanhee *et al.*[8]

The polyclonal antibody against IGF-1 (lot 038K1674) and the alkaline phosphatase-coupled anti-goat secondary antibody (lot 061M4849) were purchased from Sigma-Aldrich (St Louis, MO, USA). For Western blotting, anti-IGF-1 and the anti-goat secondary antibody were used at 1:5000. After electrophoresis, the proteins were transferred to a nitrocellulose membrane (BioRad, Hercules, CA, USA) by standard wet transfer,[23] followed by colorimetric detection of the presence of alkaline phosphatase by SIGMAFAST™ BCIP© tablets (Sigma Aldrich, St Louis, MO, USA). The membrane was air-dried before imagining.

Low resolution LC-MS/MS of intact polypeptide

Prior to LC-MS/MS analysis of the intact polypeptide, the sample was diluted 10 x in water and acidified with 1% formic acid. The acidified solution of the intact polypeptide and the reference standards digest were both subjected to analysis on a Dionex UltiMate 3000 Rapid Separation LC (RSLC) system (Thermo Scientific, Sunnyvale, CA, USA) coupled to an amaZon™ speed ETD mass spectrometer (Bruker Daltonics, Bremen, Germany). The instrument system was calibrated using the manufacturer's calibration mixture, and the mass accuracy was determined to be <0.1 Da during the period of analysis. A sample volume of 1 µL

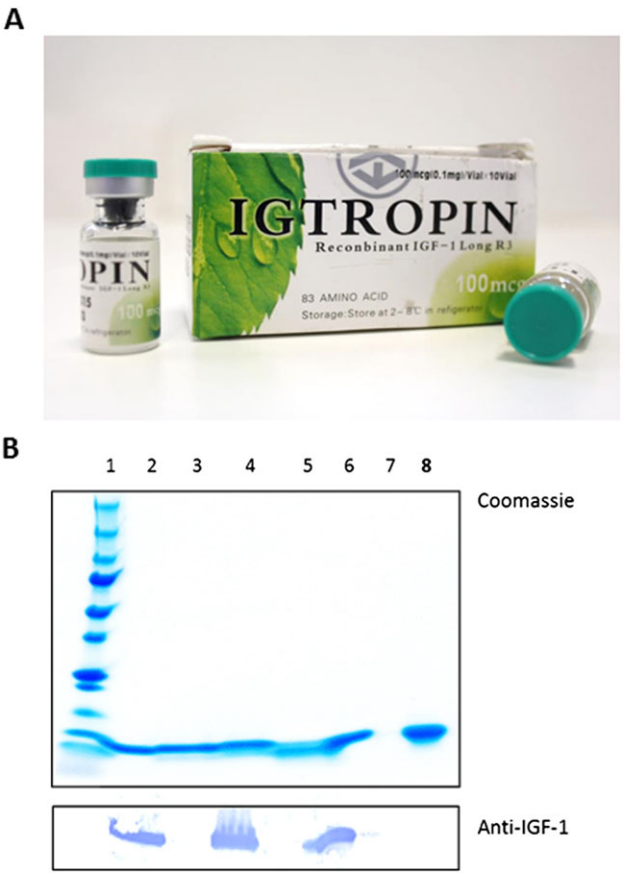

Figure 1. Picture of the box and vials intercepted by the FAMHP (A). It is interesting to note that the labelled ingredient, 'reconbinant IGF-1 long R^3' is misspelled and should be 'recombinant IGF-1 long R'. The content of the intercepted vial was analyzed by SDS-PAGE followed by Coomassie staining or western blot against IGF-1(B). Standards of IGF-1 (lane 2), IGF-2 (lane 3), longR^3-IGF-1 (lane 4), Des1-3-IGF1 (lane 5), and R^3-IGF-1 (lane 6) were used as a control reference. Lane 1 represents the molecular weight marker, lane 7 was loaded with sample buffer and lane 8 was loaded with the unknown sample. [Colour figure can be viewed at wileyonlinelibrary.com]

was injected onto the system. The chromatographic separation was performed at 50°C on an Acquity UPLC CSH C18 Column (150×2.1 mm, 1.7 µm particle size) (Waters, Milford, MA, USA) with a mobile phase consisting of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The optimized elution method, with a constant flow rate of 0.35 ml/min, employed an isocratic run at 10% B for 1 min, followed by a linear gradient to 45% B at 11 min, an increase to 98% B for 2 min and a recalibration at 10% B for 2 min. The longer elution LC method, used to compare the retention time of the reference standard of recombinant human EGF (rhEGF) and the suspected EGF polypeptide present in the unknown sample, employed a 3-times longer linear gradient to 45% B.

The mass spectrometer settings were similar to what has been described in Vanhee *et al.*[11] Small alterations were made to the MS mass range (700–1200 *m/z*) and the smart parameter setting (1050 *m/z*). The LC and MS data were analysed by Compass Data Analysis 4.2 (Bruker Daltonics, Bremen, Germany).

Low resolution LC-MS/MS on digested polypeptide

A 20 µL aliquot of the solution of the intact polypeptide was subjected to digestion with trypsin, chymotrypsin or chemical cleavage

with formic acid at elevated temperatures. The intact peptide was first diluted 5 x in 50 mM ammonium bicarbonate, reduced with Dithiothreitol (DTT) and alkylated with iodoacetamide in anticipation of the enzymatic overnight digestion at 30°C with either 1 µg sequencing grade modified trypsin (Promega, Madison, WI, USA) or with 10 µg sequencing grade chymotrypsin (Promega, Madison, WI, USA). Additionally we also incubated the treated polypeptide with 2% formic acid at 105°C.[24] Next, the digests were acidified prior to analysis on a Dionex UltiMate 3000 Rapid Separation LC (RSLC) system (Thermo Scientific, Sunnyvale, CA, USA) coupled to an amaZon™ speed ETD mass spectrometer (Bruker Daltonics, Bremen, Germany). The same conditions as mentioned in[8] were used and also here due to possible interferences of "small molecules" or matrix, single charged masses were not withheld for further MS and MS/MS searches.

The MS and MS/MS queries were performed using Compass Data Analysis 4.2 and BioTools 3.2 (Bruker Daltonics, Bremen, Germany) software working with the Matrix Science Ltd MASCOT® Database search engine v2.1 (Boston, USA). The National Center for Biotechnology Information database was used. A 1.00 Da precursor tolerance for MS spectra and a 1.50 Da fragment tolerance for MS/MS spectra were allowed. Additional MS and MS/MS queries against the amino acid sequence of human EGF were performed using Compass Data Analysis 4.2 and PEAKS 7.5 (Bioinformatics Solutions Inc., Waterloo, Canada). A 0.20 Da precursor tolerance for MS spectra and a 1.00 Da fragment tolerance for MS/MS spectra were allowed. No non-specific cleavage and modifications other than cysteine carbamidomethylation, due to the treatment with iodoacetamide, were allowed. Furthermore only those peptides with a minimum length of 6 amino acids and an -10logP > 20 were withheld as recommended for searches against a very small database.[25]

High resolution LC-MS

High resolution LC-MS (LC-HRMS) analyses were carried out as described in Vanhee *et al.*[10] by using a Waters Acquity UPLC system in combination with the Synapt G2-S High Definition Mass Spectrometer from Waters (Milford, MA, USA), equipped with electrospray ionization. Data acquisition, instrument control and data analysis were performed by MassLynx software (version 4.1, Waters, Milford, MA, USA).

Results

Prior to LC-MS/MS analysis we subjected the sample to SDS-PAGE and performed either a Coomassie staining or a western blot against IGF-1. The migration pattern of the sample on SDS-PAGE post Coomassie staining reveals the presence of a polypeptide, below 10 kDa, which could correspond to an IGF (Figure 1B). The immunoblotting on the other hand showed a signal for IGF-1, LongR^3-IGF-1and R^3-IGF-1, while no signal for Des1-3-IGF-1 and IGF-2 or the unknown sample could be observed. Interestingly only a positive signal was obtained for those polypeptides with N-terminal amino acid sequences containing GP[R/E]TL.[18] This could indicate that some of these amino acids must be present in order to be recognised by the antibodies.

Although the unknown polypeptide in our sample most likely did not correspond to IGF-1, LongR^3-IGF-1, R^3-IGF-1, it could still correspond to IGF-2 or truncated version of IGF-1 and its analogues, including Des1-10-longR^3-IGF-1. Subsequent LC-MS/MS analysis, used for rapid screening of IGF-1 and analogues, of the unknown peptide shows the presence of multiple charged envelopes at a retention time of 5.9 min (Figure 2). The multiple charged envelopes displayed precursor ions with an average m/z=889.10 and an average m/z=1036.86 (Figure 3A). The highest intensity was obtained for the average m/z=889.10, which does not correspond to any of the m/z values obtained with IGF-2, IGF-1 and analogues used in this study (Figure 2) or described in the literature.[18] In order to accurately calculate the mass of this polypeptide we subjected the unknown sample to high resolution LC-MS (Figures 3B and 3C). The MS spectra revealed that the m/z 888.998 and the m/z=1036.996 correspond to a respectively seven- and six-fold protonated precursor, resulting in an average mass of 6215.9 Da. Next, we performed LC-MS/MS on the tryptic digest of the unknown polypeptide. The MS/MS generated spectra of these

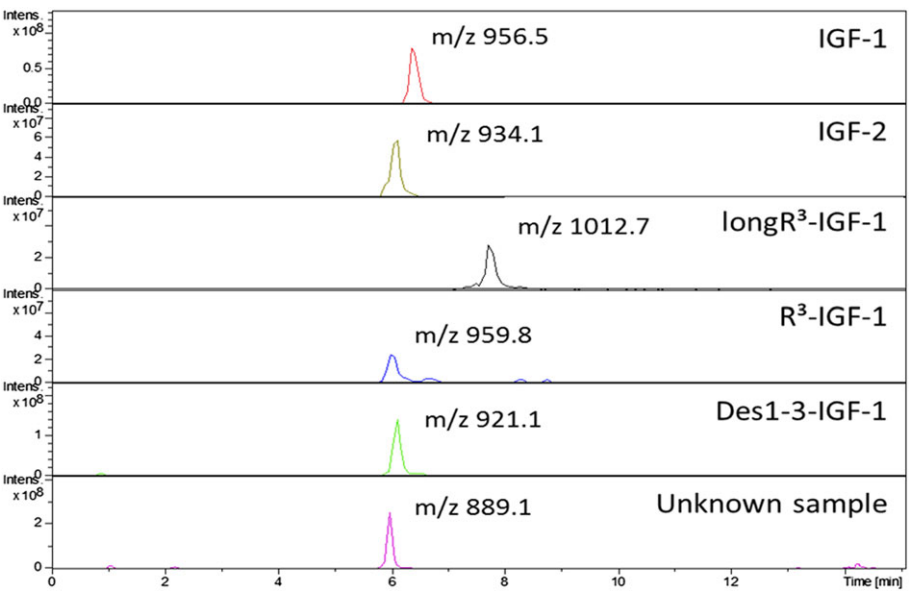

Figure 2. Total ion chromatograms of a full scan mass spectrum of IGF-1, IGF-2, Des1-3-IGF1, R^3-IGF-1, longR^3-IGF-1 and the unknown sample. Each m/z value corresponds to the measured precursor ion with the maximum intensity. [Colour figure can be viewed at wileyonlinelibrary.com]

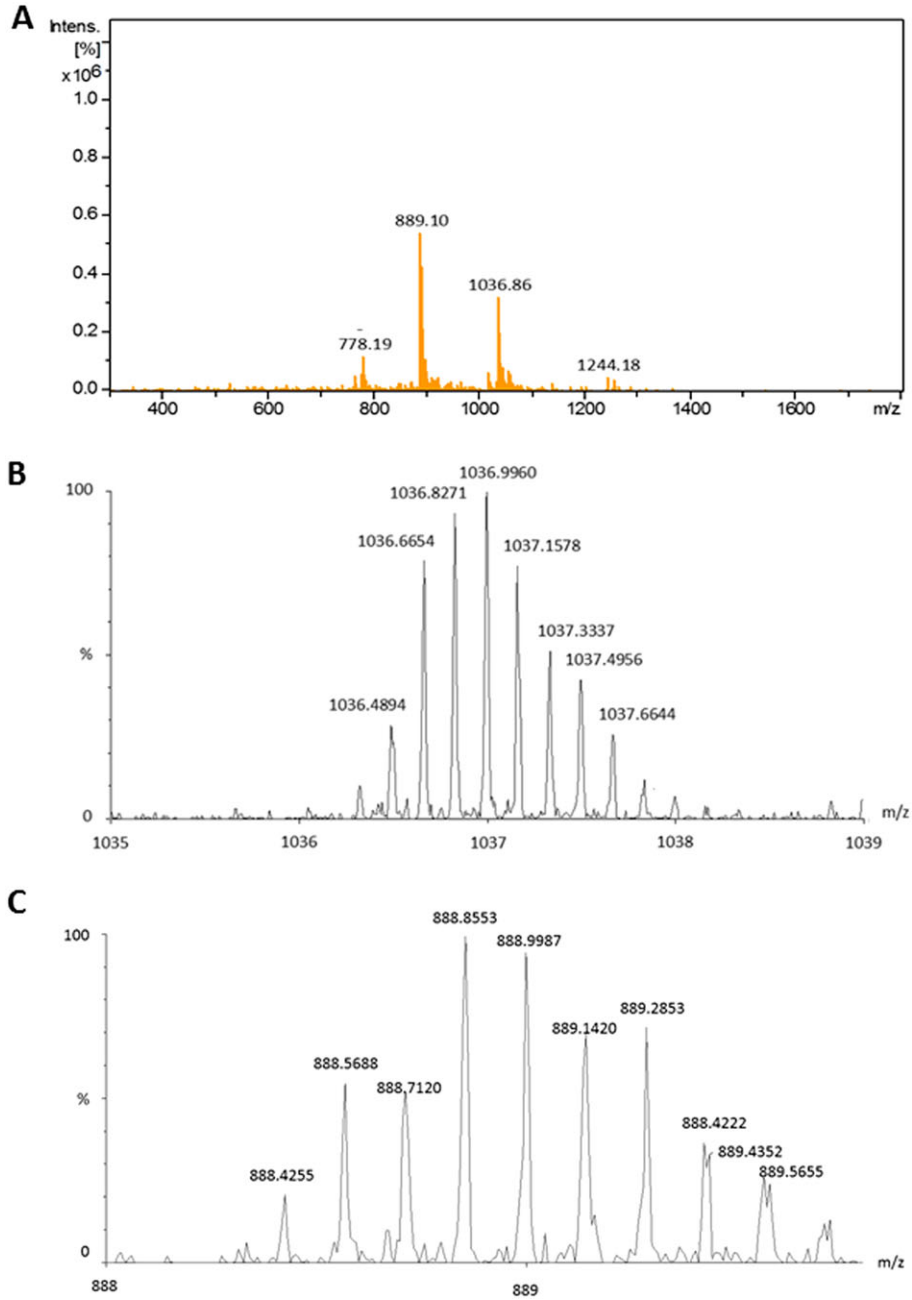

Figure 3. Full scan low resolution MS spectrum of the component present in the unknown sample and eluting at 5.9 min (A) and high resolution MS spectrum to attribute the charge state to the six-fold (B) and seven-fold (C) protonated precursor ion. [Colour figure can be viewed at wileyonlinelibrary.com]

compounds were loaded into MASCOT™ and in addition to trypsin, used for digestion, also human epidermal growth factor (hEGF) gave a significant hit (protein score of 97 > 40) but with only one non-redundant significant peptide match, for which almost the full series of y- and b-type ions were detected (Figure 4A). This 13 amino acid peptide has a calculated mono-isotopic molecular mass of 1559.69 Da and could reflect the measured mono-isotopic mass of 1559.86 Da (error < 0.20 Da) of the digested polypeptide.

Although the masses of the unknown sample and theoretical mass of hEGF are almost identical, we judged that the presence of only one positive non-redundant peptide hit, was not sufficient to conclude that the sample indeed contained hEGF. Therefore we subjected this peptide to complementary digestion with chymotrypsin and treatment with formic acid. Indeed LC-MS/MS and subsequent queries against the sequence of hEGF, gave two positive significant non redundant peptide hits, with almost the full series of y- and b-type ions present, for both treatments. In case of digestion with chymotrypsin, two different peptides, matching the peptides YIEADKY and IGERCQY were detected (Figures 4B, 4C, and 4F). The first peptide has a calculated mono-isotopic molecular mass of 1013.51 Da and the second peptide has a mono-isotopic molecular mass of 924.41 Da, reflecting the measured mono-isotopic masses of 1013.51 and 924.53 Da (error < 0.20 Da). In case of chemical cleavage, two different peptides, matching the N-terminal peptide SECPLSHD and the C-terminal LKWWELR were detected (Figures 4D, 4E, 4F). The first peptide has a calculated mono-isotopic molecular mass of 943.37 Da and the second peptide has a mono-isotopic

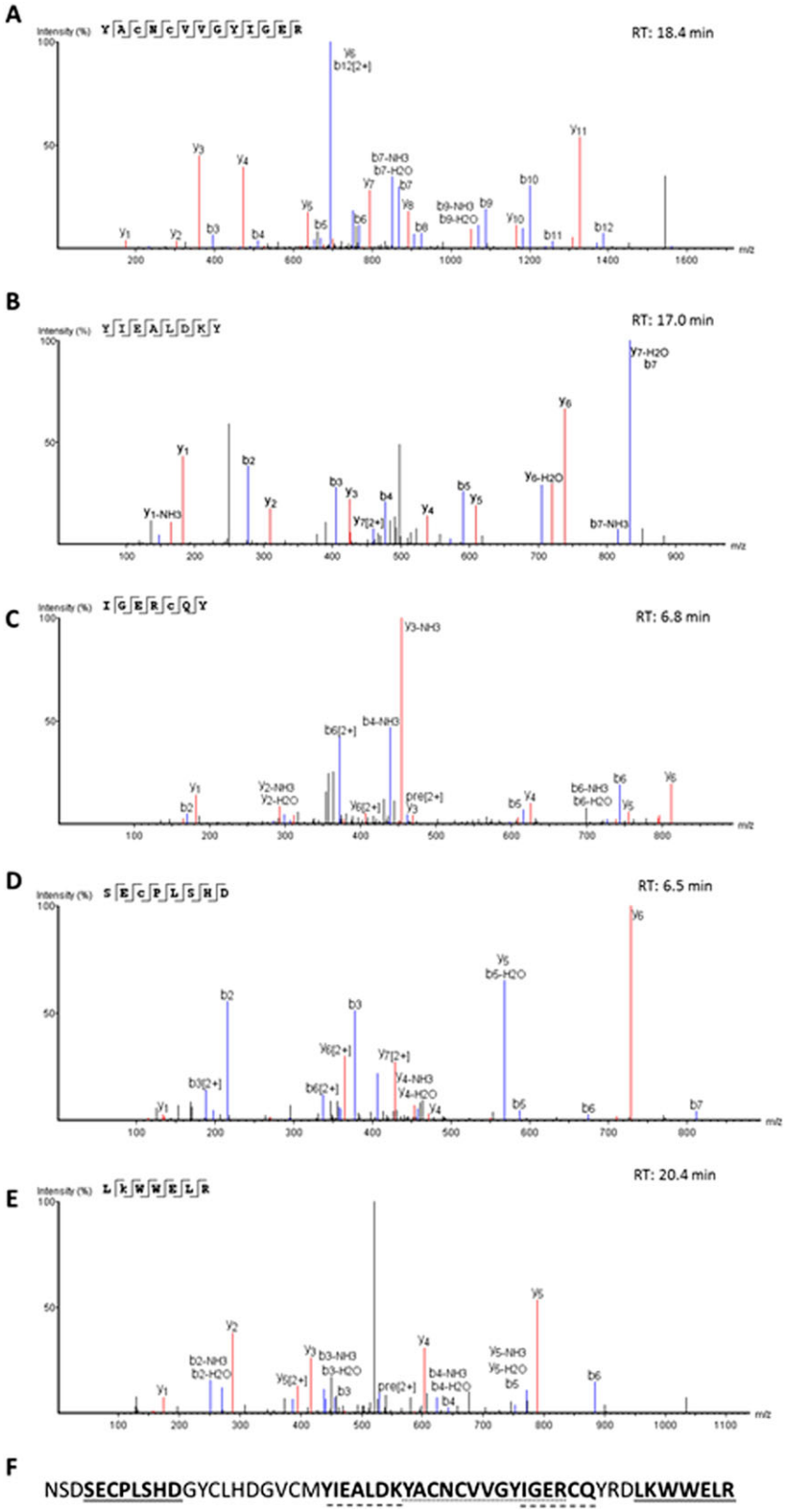

Figure 4. Assigned product ion spectra of *m/z*=780.3 (A) obtained after tryptic digest which most likely corresponds to a 13 amino acids long peptide originating from EGF, sequence marked with a dotted underline (F). This *m/z* elutes at 18.4 min and has a peptide score that equals 83.01 (>40). Assigned product ion spectra of *m/z*=507.8, eluting at 17.0 min (B) and *m/z* 463.3 eluting at 6.8 min after digestion with chymotrypsin(C). The peptide scores (−10lgP) are respectively 54.6 and 22.6 (>20) and the their error corresponds to 93.3 and 107.2 ppm. Both peptides, marked with a dashed underline show non-redundant EGF sequence coverage of 28% (F). Assigned product ion spectra of *m/z*=472.7, eluting at 6.5 min (D) and *m/z* 515.9 eluting at 20.4 min, obtained after heated acid treatment (E). The peptide scores (−10lgP) are respectively 81.6 and 73.86 (>20) and the their error corresponds to 11.6 and 139.4 ppm. Both peptides, marked with a solid underline show non-redundant EGF sequence coverage of 28% (F). [Colour figure can be viewed at wileyonlinelibrary.com]

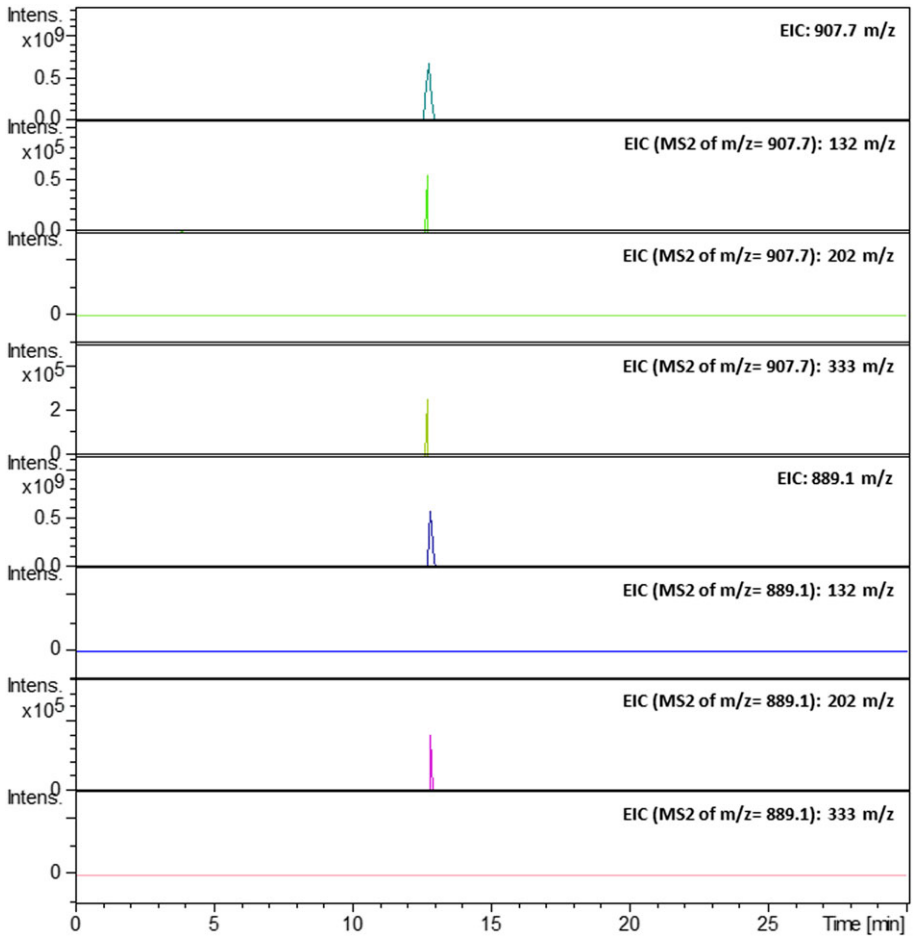

Figure 5. Extracted ion chromatograms of rhEGF with *m/z*=907.7 and the suspected sample with *m/z*=889.1 (tolerated error 0.5) and their specific corresponding extracted ion chromatograms (*m/z*=132, *m/z*=333 and *m/z*=202; tolerated error 0.5) of the MS2 product ions originating from the respective precursor ion. [Colour figure can be viewed at wileyonlinelibrary.com]

molecular mass of 1029.58 Da, reflecting the measured mono-isotopic masses of 943.39 and 1029.73 Da (error < 0.20 Da). Taken together, the data obtained with high resolution LC-MS and low resolution LC-MS/MS data indicate that hEGF might be present in the sample. However, in order to further ascertain the presence of human EGF in the sample, we compared the retention time of the sample and a reference standard consisting of recombinant human EGF (rhEGF) with an N-terminal methionine since to our knowledge no rhEGF without N-terminal methionine was available. Since the length of the optimized LC run was only 15 min, we subjected both samples to a modified LC run with a 3-times slower gradient. As shown in Figure 5, the retention times of both polypeptides are very similar (difference of 0.07 min.). Moreover the most intense precursor ion of rhEGF, with theoretical average molecular mass of 6347.14 Da, has an *m/z*=907.70. Assuming that this value corresponds to the seven-fold protonated precursor, the obtained calculated average molecular mass of rhEGF corresponds to 6346.90 Da. The difference of the observed average molecular masses, 131.25 Da, could be explained by presence of an N-terminal methionine present in rhEGF. Therefore we verified if b_1 fragment ion, with an *m/z*=132, is present in the MS2 spectrum of the respective precursor ions of rhEGF and in the unknown sample. Moreover also an *m/z*=333 corresponding to the b_3 ion of hEGF with N-terminal methionine. No such *m/z* was found for the unknown sample. Additionally we also observed that an *m/z*=202, corresponding to the b_2 ion of hEGF, was present in the unknown sample, while no such mass was obtained in the MS2 spectrum of rhEGF. In conclusion we can state that the data are consistent with the presence of human EGF in the sample.

Conclusion

An unknown pharmaceutical preparation was submitted by the FAMHP for analysis in our laboratory. Based on the interpretation of the immunoblotting, LC-HRMS and LC-MS/MS analysis we were able to attribute a putative 53 amino acids long sequence to the unknown polypeptide. The sequence is consistent with human epidermal growth factor (hEGF).

The injection of this polypeptide has not been approved for therapeutic use by any western government health authority in the world and could potentially have adverse health effects. Although no injectable legal medicine is available on the market, the topical application of this polypeptide is currently being used in the cosmetic industry to accelerate wound healing and stimulate skin rejuvenation. Since the container of the seized sample claimed to contain 'reconbinant IGF-1 long R^{3}', one could imagine that a mix-up occurred at a manufacturing company where both products are being generated. How the product is being made is also questionable hence recombinant expression would in most cases generate a peptide with an N-terminal methionine. Although,

it is theoretically possible to enzymatically remove the N-terminal methionine after recombinant expression or even synthesise the polypeptide, it is also possible that this EGF is purified from human urine.[26] The later production strategy embodies also a potential health risk due to possible co-purification of even prion proteins.[27] Once more, this case study illustrates the potential danger of purchasing illegal drugs.

References

[1] J. Henninge, M. Pepaj, I. Hullstein, P. Hemmersbach. Identification of CJC-1295, a growth-hormone-releasing peptide, in an unknown pharmaceutical preparation. *Drug Test. Anal.* **2010**, *2*, 647.
[2] M. Thevis, W. Schänzer. Analytical approaches for the detection of emerging therapeutics and non-approved drugs in human doping controls. *J. Pharmaceut. Biomed. Anal.* **2014**. DOI:10.1016/j.jpba.2014.05.020.
[3] O. Krug, A. Thomas, K. Walpurgis, T. Piper, G. Sigmund, W. Schänzer, T. Laussmann, M. Thevis. Identification of black market products and potential doping agents in Germany 2010–2013. *Eur. J. Clin. Pharmacol.* **2014**, *70*, 1303.
[4] M. R. Graham, B. Davies, F. M. Grace, J. S. Baker. Exercise, Science and Designer Doping: Traditional and Emerging Trends. *J. Sports Med. Doping Stud.* **2012**. DOI:10.4172/2161-0673.1000113.
[5] R. A. Hartvig, N. B. Holm, P. W. Dalsgaard, L. A. Reitzel, I. B. Müller, K. Linnet. Identification of peptide and protein doping related drug compounds confiscated in Denmark between 2007–2013. *Scand. J. Forensic Sci.* **2014**. DOI:10.2478/sjfs-2014-0003.
[6] T. Breindahl, M. Evans-Brown, B. Hindersson, J. McVeigh, M. Bellis, A. Stensballe, A. Kimergård. Identification and characterization by LC-UV-MS/MS of melanotan II skin-tanning products sold illegally on the Internet. *Drug Test. Anal.* **2014**. DOI:10.1002/dta.1655.
[7] M. C. Gaudiano, L. Valvo, A. Borioni. Identification and quantification of the doping agent GHRP-2 in seized unlabelled vials by NMR and MS: a case-report. *Drug Test. Anal.* **2014**, *6*, 295.
[8] C. Vanhee, G. Moens, E. Deconinck, J. O. De Beer. Identification and characterization of peptide drugs in unknown pharmaceutical preparations seized by the Belgian authorities: case report on AOD9604. *Drug Test. Anal.* **2014**, *6*, 964.
[9] S. Esposito, K. Deventer, P. Van Eenoo. Characterization and identification of a C-terminal amidated mechano growth factor (MGF) analogue in black market products. *Rapid Commun. Mass Spectrom.* **2012**, *26*, 686.
[10] C. Vanhee, G. Moens, E. Van Hoeck, E. Deconinck, J. O. De Beer. Identification of the small research tetra peptide Epitalon, assumed to be a potential treatment for cancer, old age and Retinitis Pigmentosa in two illegal pharmaceutical preparations. *Drug Test. Anal.* **2015**. DOI:10.1002/dta.1771.
[11] C. Vanhee, S. Janvier, B. Desmedt, G. Moens, E. Deconinck, J. O. De Beer, P. Courselle. Analysis of illegal peptide biopharmaceuticals frequently encountered by controlling agencies. *Talanta*. **2015**, *142*, 1.
[12] B. J. Venhuis, P. H. J. Keizers, R. Klausmann, I. Hegge. Operation resistance: A snapshot of falsified antibiotics and biopharmaceutical injectables in Europe. *Drug Test. Anal.* **2015**. DOI:10.1002/dta.1888.
[13] S. Esposito, K. Deventer, P. Van Eenoo. Identification of the growth hormone-releasing hormone analogue [PRO1, VAL14]-hGHRH with an incomplete C-term amidation in a confiscated product. *Drug Test. Anal.* **2014**, *6*, 1155.
[14] M. Thevis, T. Kuuranne, K. Walpurgis, H. Geyera, W. Schänzer. Annual banned-substance review: analytical approaches in human sports drug testing. *Drug Test. Anal.* **2015**. DOI:10.1002/dta.1928.
[15] B. Gevaert, M. D'Hondt, N. Bracke, H. Yao, E. Wynendaele, J. P. C. Vissers, M. De Cecco, J. Claereboudt, B. De Spiegeleer. Peptide profiling of Internet-obtained Cerebrolysin using high performance liquid chromatography – electrospray ionization ion trap and ultra high performance liquid chromatography – ion mobility – quadrupole time of flight mass spectrometry. *Drug Test. Anal.* **2015**. DOI:10.1002/dta.1817.
[16] H. D. Cox, C. M. Hughes, D. Eichner. Detection of GHRP-2 and GHRP-6 in urine samples from athletes. *Drug Test. Anal.* **2015**. DOI:10.1002/dta.1791.
[17] M. M. Cheng. Is the drugstore safe? Counterfeit diabetes products on the shelves. *J. Diabetes Sci. Technol.* **2009**, *3*, 1516.
[18] A. Thomas, M. Kohler, W. Schänzer, P. Delahaut, M. Thevis. Determination of IGF-1 and IGF-2, their degradation products and synthetic analogues in urine by LC-MS/MS. *Analyst*. **2011**, *136*, 1003.
[19] C. Yewale, D. Baradia, I. Vhora, S. Patil, A. Misra. Epidermal growth factor receptor targeting in cancer: a review of trends and strategies. *Biomaterials*. **2013**, *34*, 8690.
[20] E. Neninger Vinageras, A. de la Torre, M. Osorio Rodríguez, M. Catalá Ferrer, I. Bravo, M. Mendoza del Pino, D. Abreu Abreu, S. Acosta Brooks, R. Rives, C. del Castillo Carrillo, M. González Dueñas, C. Viada, B. García Verdecia, T. Crombet Ramos, G. González Marinello, A. Lage Dávila. Phase II randomized controlled trial of an epidermal growth factor vaccine in advanced non-small-cell lung cancer. *J. Clin. Oncol.* **2008**, *26*, 1452.
[21] Cancer Research UK. Can you tell me about the CimaVax lung cancer vaccine? Available at: http://www.cancerresearchuk.org/about-cancer/cancers-in-general/cancer-questions/can-you-tell-me-about-the-cimavax-lung-cancer-vaccine [8 April 2016].
[22] U. K. Laemmli. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*. **1970**, *227*, 680.
[23] C. Vanhee, S. Guillon, D. Masquelier, H. Degand, M. Deleu, P. Morsomme, H. Batoko. A TSPO-related protein localizes to the early secretory pathway in Arabidopsis, but is targeted to mitochondria when expressed in yeast. *J. Exp. Bot.* **2011**, *62*, 497.
[24] A. Li, R. C. Sowder, L. E. Henderson, S. P. Moore, D. J. Garfinkel, R. J. Fisher. Chemical cleavage at aspartyl residues for protein identification. *Anal. Chem*. **2001**, *73*, 5395.
[25] J. Zhang, L. Xin, B. Shan, W. Chen, M. Xie, D. Yuen, W. Zhang, Z. Zhang, G. Lajoie, B. Ma. PEAKS DB: De Novo sequencing assisted database search for sensitive and accurate peptide identification. *Mol. Cell. Proteomics*. **2011**. DOI:10.1074/mcp.M111.010587.
[26] S. Cohen, G. Carpenter. Human epidermal growth factor: isolation and chemical and biological properties. *Proc. Natl. Acad. Sci. U. S. A.* **1975**, *72*, 1317.
[27] A. Van Dorsselaer, C. Carapito, F. Delalande, C. Schaeffer-Reiss, D. Thierse, H. Diemer, D. S. McNair, D. Krewski, N. R. Cashman. Detection of prion protein in urine-derived injectable fertility products by a targeted proteomic approach. *PLoS One*. **2011**. DOI:10.1371/journal.pone.0017815.