Journal of Chromatography B, 877 (2009) 1359–1365

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Absolute ProGRP quantification in a clinical relevant concentration range using LC–MS/MS and a comprehensive internal standard☆

Bjørn Winther[a], Petter Moi[a], Marianne S. Nordlund[b], Niclas Lunder[c], Elisabeth Paus[b], J. Léon E. Reubsaet[a,*]

[a] Department of Pharmaceutical Chemistry, School of Pharmacy, University of Oslo, Oslo, Norway
[b] Central Laboratory, Radiumhospitalet, Rikshospitalet Medical Centre, Oslo, Norway
[c] Department of Psychopharmacology, Diakonhjemmet Hospital, Oslo, Norway

ARTICLE INFO

Article history:
Received 26 June 2008
Accepted 10 December 2008
Available online 24 December 2008

Keywords:
Biomarker
Small cell lung cancer
ProGRP
Tryptic peptide
Signature peptide
LC–MS/MS
Absolute quantification
MRM
Internal standard
NHS-acetylation

ABSTRACT

The objective of this study was to develop a method using liquid chromatography with tandem mass spectrometric detection for the absolute quantification of the small cell lung cancer biomarker ProGRP in human serum, using its tryptic signature peptide NLLGLIEAK. The samples were precipitated for most of its proteins using acetonitrile prior to tryptic digestion. Further sample clean-up and enrichment was achieved by the use of an on-line restricted access media column, followed by separation on a BioBasic C8 column. Detection and quantification was carried out by operating a triple quadrupole MS in the selected reaction monitoring mode. This setup allowed analysis of realistic samples and detections limits in human serum of 150 pg ProGRP on column. Using an internal standard derived from the parent ProGRP after acetylation of the lysine side chain allowed better quantification through variation correction in all sample pretreatment steps, trypsination included.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

Small cell lung cancer (SCLC) is an aggressive disease with rapidly growing neoplasm and early metastasis with low long-term survival. However, the cancer is highly sensitive to early initiated systemic chemotherapy, and thereby makes an early diagnosis crucial. Quantification of protein biomarkers is frequently used for diagnosis and treatment monitoring of SCLC. Pro-gastrin-releasing peptide (ProGRP) is such a biomarker occurring in the very low abundant range for both healthy and diagnosed subjects with a reference value of 58.9 pg/mL [1].

Quantification of ProGRP is at present performed by immunoassays using enzymatic or fluorescence detection [2,3]. Although immunochemical methods provide good sensitivity, efficiency and simplicity, they could be subject to potential cross-reactivity additionally development of one or even more antibodies per novel biomarker to be investigated is required. Liquid chromatography–mass spectrometry (LC–MS) may be an alternative method with an advantage in allowing detection of multiple biomarkers in one run. However, in contrast to immuno-based quantification methods, the quantification of proteins at low concentrations in complex matrices using LC–MS remains, in general, complicated. This is due to high amounts of biomolecules interfering with the analysis, limitations in the chromatographic separation and reduced MS sensitivity utilizing electrospray ionization (ESI) interface, due to the charge distribution of intact proteins [4]. Chelius and Bondarenko [5] showed that the peak areas of tryptic peptides correlated linearly to the concentration of proteins, and that this method could be used for quantitative profiling of proteins in human sera. These aspects have led to the development of methods based on pretreatment in combination with enzymatic digestion of the sample, followed by quantification of the equimolar protein signature peptide as illustrated by Kulasingam et al. [6] in the quantification of prostate-specific antigen.

Biological sample matrices are generally too complex for a direct analysis on ESI-MS and consequently require some sample preparation. Hence often internal standards (IS) are needed, and a widely used approach is the isotope dilution mass spectrometry (IDMS).

☆ This paper is part of the special issue "Quantitative Analysis of Biomarkers by LC–MS/MS", J. Cummings, R.D. Unwin and T. Veenstra (Guest Editors).

* Corresponding author at: Department of Pharmaceutical Chemistry, School of Pharmacy, University of Oslo, P.O. Box 1068 Blindern, 0316 Oslo, Norway.
Tel.: +47 22 85 66 13; fax: +47 22 85 44 02.
E-mail address: leon.reubsaet@farmasi.uio.no (J.L.E. Reubsaet).

1570-0232/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2008.12.023

This was first reported by Barr et al. [7], and has frequently been shown as a successful strategy for the absolute quantification of proteins [4,8–15]. Although the isotope labeled analog of a target molecule fulfills the definition of an ideal IS, the production of large labeled compounds is expensive, if at all possible. To circumvent this challenge a shorter sequence analog to the enzymatic signature peptide product is often used. These isotope labeled internal standards are usually not tryptic substrates, and will therefore not reflect the possible variation in quantification due to incomplete digestion. Hence they are typically added to the samples after the pretreatment and digestion [10,11,13–15]. It is plausible to assume that clinical utilization of a method with an uncompensated source of variation could potentially lead to misinterpretation of the analytical data as addressed by van den Broek et al. [4]. The use of a labeled sequence analog with an internal tryptic cleavage site may improve the analytical quality control, though differences in the digestion kinetics may occur. This paper describes the production of an IS originating from the biomarker, correcting for all the sample preparation steps, including the digestion, as well as its use through an evaluation of the ProGRP (31–98) quantification in serum samples. This relatively inexpensive and uncomplicated labeling approach for the production of the IS involves the use of the *N*-hydroxysuccinimide-based ester acetic acid *N*-hydroxysuccinimide (AA-NHS) [16]. We have previously reported the selection of a suitable signature peptide (NLLGLIEAK) for ProGRP together with the details of the sample preparation [17,18]. The methods suitability for protein quantification in samples which require preparation prior to digestion will be discussed.

2. Experimental

2.1. Chemicals, standards and matrix

Recombinant ProGRP (31–98) were provided by Radiumhospitalet, Rikshospitalet Medical Centre (Oslo, Norway). Tosyl phenylalanyl chloromethyl ketone (TPCK) treated and lyophilized sequencing grade trypsin from bovine pancreas was purchased from Sigma–Aldrich (St. Louis, MO, USA) and human serum from Ullevaal University Hospital (Oslo, Norway). All other chemicals used were of analytical grade.

2.2. Sample preparation and tryptic digestion

All samples were stored at −32 °C and excessive freeze–thaw cycles were avoided.

2.2.1. ProGRP (31–98)

ProGRP (31–98) was cloned from the SCLC cell line NCI-H128 as described elsewhere [3,19]. A 6.9 mg/mL stock solution of ProGRP (31–98) was diluted to various concentrations with 100 mM triethanolamine (TEA) (Fluka, Ulm, Germany) pH 7.3.

2.2.2. Internal standard

The IS was made in-house by specific acetylation of the lysine side chains in ProGRP (31–98) by using AA-NHS as the acetylating reactant (Fig. 1). The acetylation of the ProGRP (31–98) signature peptide NLLGLIEAK, prior to digestion yields the enzymatic peptide product NLLGLIEAKacENR, which was used as a signature peptide for the IS. The production of the IS stock solution was achieved by acetylating 100 μg/mL ProGRP (31–98) in a 20 mM borate reaction buffer pH 9.5. AA-NHS was added in a thousand fold molar excess over primary amino groups in ProGRP (31–98) and the solution was shaken at 1350 rpm for 40 min on a Heidolph® Vibramax vibrating platform shaker (Kelheim, Germany). This solution was diluted to 5 μg/mL with a 100 mM TEA buffer pH 7.3 in order to produce a suitable working solution. The completeness of reaction was tested by digestion of an aliquot of the IS stock solution with the MS set to monitor the mass $[M+2H]^{2+}$ (*m/z* 485.80) for NLLGLIEAK and $[M+2H]^{2+}$ (*m/z* 706.80) and $[M+3H]^{3+}$ (*m/z* 471.50) for NLLGLIEAKacENR. Rest reactivity of excess AA-NHS in the solution was examined by adding 50 μL of the IS working solution to 25 μL of 1 μg/mL neurotensin 1–8 (NT 1–8) in 100 mM TEA and allowing the samples to be shaken for another 40 min. In this experiment the MS was set to monitor the masses of $[M+2H]^{2+}$ (*m/z* 516.10) for NT 1–8 and $[M+2H]^{2+}$ (*m/z* 537.10) for acetylated NT 1–8.

2.2.3. Serum samples

Serum samples were thawed and spiked with different concentrations of recombinant ProGRP (31–98) followed by addition of IS and vortex-mixing for 30 s. The volumes of ProGRP (31–98) and IS added were negligible compared to the total sample volume.

To avoid overload of the column and signal suppression in the MS, serum samples were pretreated by precipitation with cold acetonitrile [18]. Equal volumes of sample and cold acetonitrile (−32 °C) were mixed with simultaneous vibration at 1500 rpm using the Heidolph® Vibramax 100. The samples were centrifuged at 10,000 rpm and the supernatant was decanted to another tube and evaporated to dryness at 50 °C under a stream of nitrogen. The samples were reconstituted in 100 mM TEA, pH 7.3 using vibration at 1500 rpm for 30 min prior to tryptic digestion.

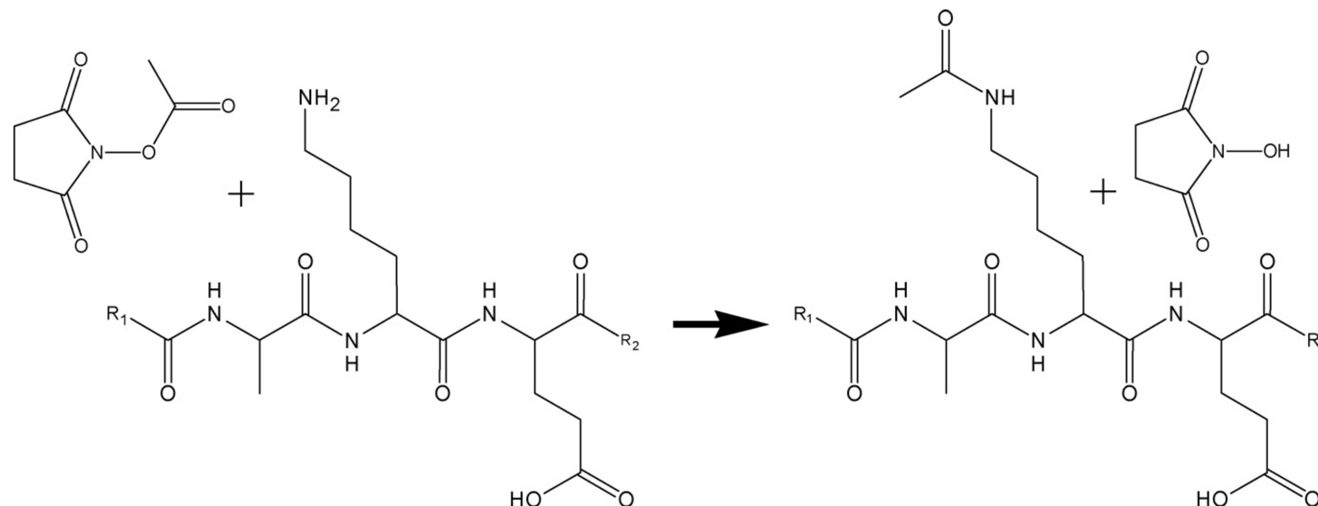

Fig. 1. Specific acetylation reaction of acetic acid *N*-hydroxysuccinimide with the primary amino group of lycine.

As smaller resuspension volumes tended to yield inferior detection limits, the total sample volumes were chosen to be 1 mL after preliminary testing of the reconstitution step (data not shown).

2.2.4. Digest conditions

The enzymatic digestion was obtained using TPCK treated and lyophilized sequencing grade bovine trypsin dissolved in 100 mM TEA buffer pH 7.3. The total protein concentration of the sample was estimated to 4 mg/mL and the enzyme:protein ratio (w/w) in the samples was 1:25. Digestion was carried out at 37 °C for 24 h to ensure complete enzymatic digestion of the proteins, followed by LC–MS and liquid chromatography with tandem mass spectrometric detection (LC–MS/MS) analysis.

2.3. LC–MS

2.3.1. System setup and mass spectrometric conditions

Two different LC–MS and LC–MS/MS systems were used during this study. A single quadrupole was used for evaluation of completeness of reaction and rest reactivity in the production of the IS, whereas a triple quadrupole was utilized for the verification of peptide identity, quantification of ProGRP (31–98) in the samples and evaluation of the method.

2.3.1.1. System setup single quadrupole. The chromatographic system consisted of a Shimadzu SIL-10ADvp auto injector, two Shimadzu LC-10ADvp gradient pumps, a Shimadzu DGU-14A degasser, a Shimadzu SCL-10Avp system controller and a Shimadzu LCMS-2010A single-quadrupole MS detector. Data acquisition and processing were carried out using Shimadzu LCMS Solution software Version 2.04-H3 (all Bergman, Lillestrøm, Norway). The interface used was electrospray ionization (ESI) operated in the positive ionization mode followed by selective-ion-monitoring (SIM) for the masses of interest. The MS operating conditions were as follows: drying gas between 10 and 20 L/min, nebulizer gas 1.5 L/min, CDL temperature 200 °C, block temperature 200 °C and probe voltage: +4.5 kV.

2.3.1.2. System setup triple quadrupole. This chromatographic system consisted of a Waters in-line degasser AF, Waters 2795 Liquid Chromatograph, Waters 600 LCD controller, Waters W600E gradient pump and a Waters Quattro micro tandem MS detector. System control and data acquisition was performed with MassLynx® version 4.0 SP4 (all Waters Corp., Milford, MA, USA). The interface was ESI operated in the positive ionization mode. The MS was set to monitor the precursor-fragment ion transition of the ProGRP-specific digest peptide NLLGLIEAK $[M+2H]^{2+}$ (*m*/*z* 486.01) to the y_7 ion $[M+H]^{+}$ (*m*/*z* 743.75), and of the IS digest product peptide NLLGLIEAKacENR $[M+2H]^{2+}$ (*m*/*z* 706.75) to the y_6 ion $[M+H]^{+}$ (*m*/*z* 788.70). The MS/MS spectra were acquired at varying collision energies in order to determine the optimal intensities for both product ions. Different dwell time durations were also tested to find the most favorable settings, producing the highest peak intensity for the y_7-fragment of NLLGLIEAK. The optimized fragmentation conditions for the selected reaction monitoring (SRM) of NLLGLIEAK were with a cone voltage of 25 V and collision energy at 14 eV, whereas the cone voltage was 30 V and the collision energy 20 eV for NLLGLIEAKacENR. The dwell time was 600 ms for both scans, with an inter-scan delay of 100 ms.

2.3.2. Columns

On all systems the following columns were used; on-line sample clean-up and enrichment was performed on a LiChrospher RP-8 Alkyl-Diol Silica (C8-ADS) restricted access material (RAM) column (Merck, Darmstadt, Germany). The average pore size is 60 Å, particle diameter is 25 μm and the column dimensions are 25 mm × 4 mm id. This on-line coupled column was used in all the cases when the complex serum samples were analyzed. The analytical separation was carried out on a BioBasic-C8 (Teknolab AS, Kolbotn, Norway) column with average pore size 300 Å, particle diameter 5 μm and column dimensions 50 mm × 1 mm id. The overall system configuration is presented in Fig. 2.

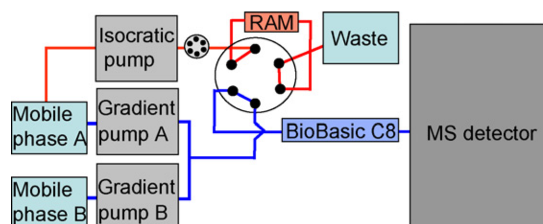

Fig. 2. Schematic diagram for the online sample preparation system setup.

2.3.3. Chromatographic conditions

Analysis of the samples took place in two steps: the samples were initially isocratically loaded on the RAM column (step 1), before they were transferred to the analytical column where gradient elution was carried out (step 2).

Step 1—loading: after sample injection the C8-ADS RAM column was flushed using isocratic conditions (20 mM formic acid and acetonitrile (95:5, v/v) at 0.2 mL/min) to ensure desalting and sample clean-up.

Step 2—transfer and separation: transfer of the retained fraction was performed after 11 min by rotating the six-port, two-position switching valve (see Fig. 2). Mobile phases used here consisted of A: 20 mM formic acid and acetonitrile (95:5, v/v) and B: 20 mM formic acid and acetonitrile (5:95, v/v). The sample was transferred to the analytical column by backflushing with 5% mobile phase B at 50 μL/min. Simultaneously a linear gradient was started and the concentration of mobile phase B rose to 100% at 43 min and this concentration was maintained for 1 min. The reversal to the initial conditions was obtained in 0.4 min and the switching valve was rotated for separate regeneration of the columns. The RAM column was regenerated using mobile phase A, while the analytical column was regenerated with 5% mobile phase B. Regeneration was achieved at 0.2 mL/min for at least 5 column volumes.

2.4. Evaluation of the method

Linearity, LOD, LOQ, intra- and inter-run precision and accuracy was established for the evaluation of the method performance. A calibration curve was generated for ProGRP (31–98) in both 100 mM TEA buffer and in serum samples. The linearity, LOD and LOQ were calculated for these experiments individually.

Injection volumes of 100 μL sample with concentrations of 5, 10, 20, 50 and 100 ng/mL were used to produce the calibration curve in serum samples whereas for buffered samples 0.1, 0.25, 0.5, 1, 2, 4, and 8 ng/mL were used to produce the calibration curves in buffered samples.

This was done in order to evaluate the effect of sample loss and the potential signal loss due to sample preparation and potential ion suppression in the system.

3. Results and discussion

3.1. Production and testing of the internal standard

As the IS is a modified form of the target biomarker, the acetylation reaction has to be complete. An incomplete reaction leads to presence of unreacted ProGRP (31–98) in the IS mixture. This

would cause an uncertainty in the testing results, even with the addition of a standardized amount of IS to the samples. Therefore an aliquot from the IS stock solution was digested as described earlier, and analyzed on the single quadrupole system to evaluate the completeness of the reaction. The ProGRP signature peptide NLLGLIEAK and the IS signature peptide NLLGLIEAKacENR were used for the quantification of unreacted ProGRP (31–98) relative to the acetylated IS. Assuming similarities in ionization of the two tryptic signature peptides the IS: ProGRP (31–98) ratio was at least 1250:1 indicating that more than 99.9% of ProGRP (31–98) was acetylated.

Another challenge was that the presence of excess AA-NHS in the IS mixture could lead to acetylation of endogenous ProGRP in the patient samples, thus resulting in false ProGRP estimates. The IS solution was diluted in an ammonium-based buffer in order to inhibit such an effect, as AA-NHS reacts with the ammonium groups in the solution, and hence the acetylation reactivity is removed. The effect of this quenching was tested by monitoring the acetylation of the model peptide NT 1–8. This peptide contains a lysine amino acid which is rather easily acetylated with AA-NHS. Addition of NT 1–8 to a diluted AA-NHS-solution did not lead to the formation of the acetylated NT 1–8 form, thus proving that the reactivity of the diluted solution could be disregarded (Fig. 3). In addition the vast amounts of free amino groups in the complex serum samples also reduce the probability of acetylation of endogenous ProGRP.

3.2. Verification of the internal standard digest product NLLGLIEAKacENR and the ProGRP specific signature peptide NLLGLIEAK

The presence of a peak for both the doubly charged and the less intense peak of the triply charged peptide NLLGLIEAKacENR (*m*/*z* 706.75 and 471.5) was used as an early identity indication of the peptide. As for the ProGRP specific signature peptide NLLGLIEAK, the identity has been verified on a different instrumentation setup, as earlier reported [17]. However, in order to confirm the identities with the described setup, an MS/MS fragmentation analysis was performed on *m*/*z* 486.01 for NLLGLIEAK and *m*/*z* 706.75 for NLLGLIEAKacENR, which are the most intense charge states of the precursor ions. As shown in Fig. 4, a mass spectrum from *m*/*z* 100 to *m*/*z* 1000 was acquired for both the peptides, and several of b- and y-fragment ions were detected. The presence of fragment ions b_2–b_9 and the complementary y-ions y_1–y_7 for the fragmentation of NLLGLIEAKacENR, and the ions b_2–b_6, b_8 and y_1–y_7 for NLLGLIEAK confirmed the identity of the peptides.

3.3. Selection of SRM masses for NLLGLIEAK and NLLGLIEAKacENR

In the optimization of the fragmentation conditions for NLLGLIEAKacENR, the single charged product ion y_6 (*m*/*z* 788.70) yielded the most intense signal in the spectrum (data not shown),

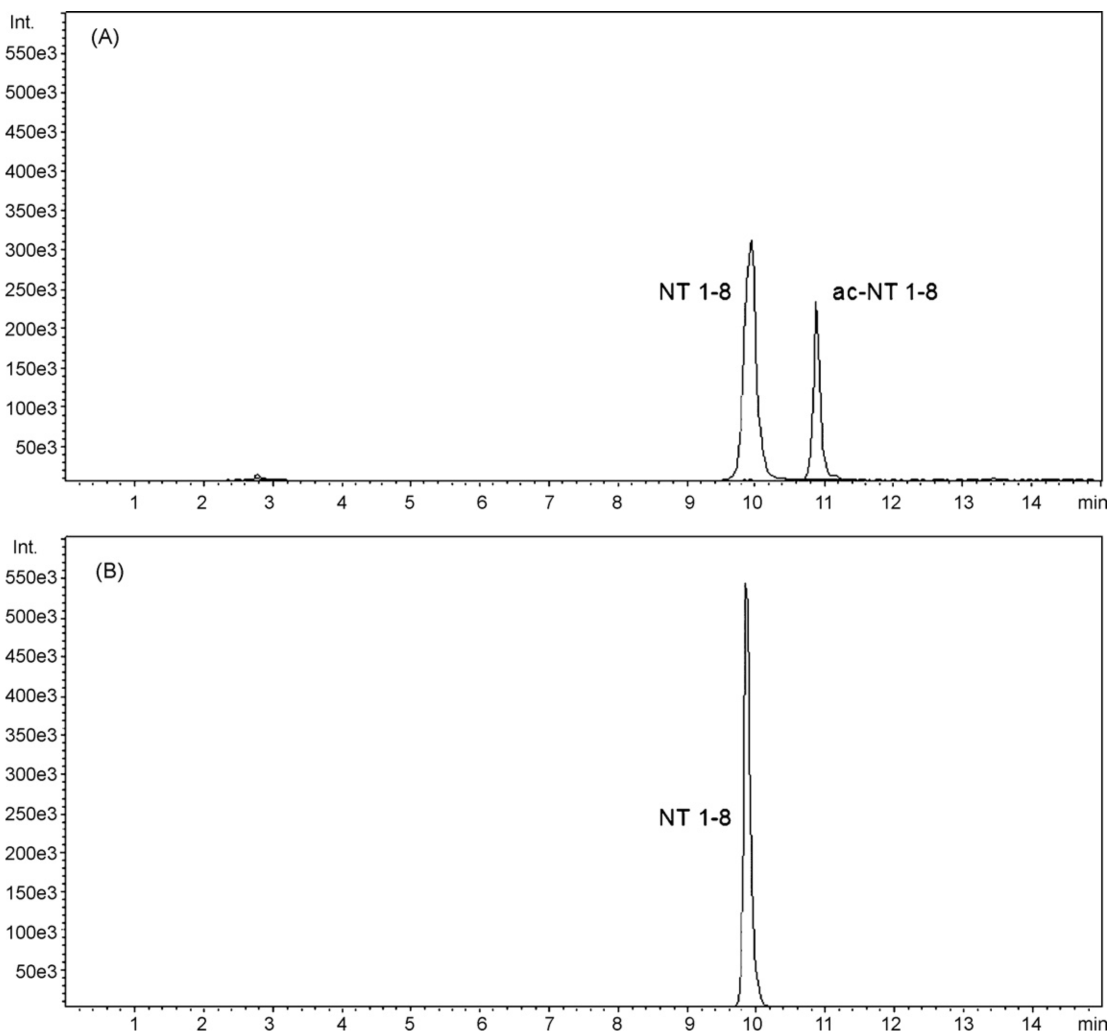

Fig. 3. Selected ion monitoring of *m*/*z* 516.10 and *m*/*z* 537.10 for NT 1–8 and acetylated NT 1–8 respectively. Injection of 20 μL of A: 333 ng/mL NT 1–8 partially acetylated with acetic acid *N*-hydroxysuccinimide and B: 25 μL 1 μg/mL NT 1–8 added 50 μL 100 μg/mL IS batch as described in Section 2.

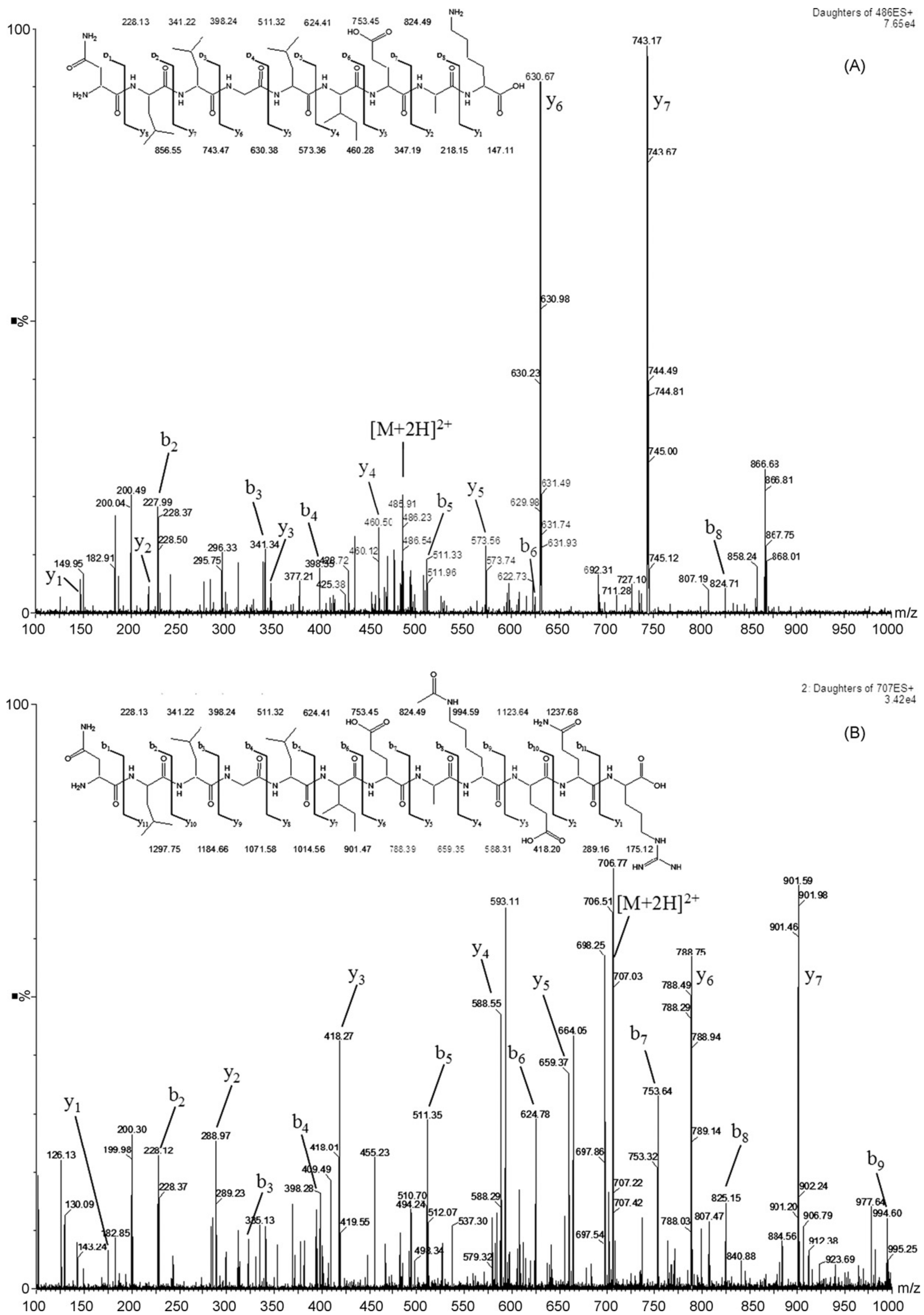

Fig. 4. Triple quadrupole mass spectra of a product ion scan on of the ProGRP signature peptide NLLGLIEAK (A) and the IS NLLGLIEAKacENR (B). The high degree of equivalence between the theoretical and experimentally observed fragment ions verifies the identity of the peptides.

Table 1
Evaluation of the methods performance using linear and polynomial calibration.

	Number of replicates, *n*	Nominal (theoretical) concentration ProGRP (31–98) (ng/mL)	CV (%)	$y = bx + c$		$y = ax^2 + bx + c$	
				Measured concentration ProGRP (31–98) (ng/mL)	Bias (%)	Measured concentration ProGRP (31–98) (ng/mL)	Bias (%)
Intra-run	8	5	25.2	1.4 ± 0.3	−72.8	6.1 ± 1.5	22.0
	7	20	15.7	19.7 ± 3.1	−1.5	17.9 ± 2.8	−10.6
	8	100	12.0	94.6 ± 11.3	−5.4	99.2 ± 11.9	−0.8
Inter-run	5	5	8.5	1.5 ± 0.1	−70.6	7.4 ± 0.6	47.1
	5	20	8.4	24.2 ± 2.0	21.1	21.7 ± 1.8	8.5
	5	100	7.5	99.4 ± 7.5	−0.6	108.8 ± 8.2	8.8

and hence was chosen for the SRM. In the product ion scan for NLLGLIEAK on the triple quadrupole system the most abundant product ion was the single charged y_7-fragment ion (m/z 743.75) and was hence selected for the monitoring of digested ProGRP (31–98) in the SRM.

3.4. Evaluation results

3.4.1. Buffered samples

The calibration curve based on the buffered samples analyzed using the triple quadrupole confirmed an expected high degree of linearity ($r^2 = 0.9835$). The mLOD and mLOQ at 2.5 pg (S/N = 3) and 8.3 pg (S/N = 10) ProGRP (31–98) on column (OC) respectively indicate that, under optimal conditions, endogenous normal levels of ProGRP could be measured. Comparison of these LOD/LOQ values with those obtained from earlier ProGRP experiments on a single quadruple MS [17], show an approximated fivefold improvement of the S/N level using the triple quadrupole MS.

3.4.2. Serum samples

3.4.2.1. mLOD and mLOQ. For ProGRP (31–98) in serum, the calibration curve was generated by spiking serum samples to known concentrations of ProGRP (31–98) and IS prior to digestion. It was decided that the amount of IS to be added should approximately produce the same signal intensity as 100 ng/mL of ProGRP (31–98). In the SRM analysis of a precipitated and digested serum sample, the concentration of IS to generate such a signal was 1 μg/mL. Based on the signal to noise ratio, the mLOD and mLOQ were calculated to be approximately 150 and 500 pg OC in serum samples. These values are comparable with the earlier estimated results obtained with a single quadrupole system [18]. This is in contrast with the findings from the ProGRP analyses in buffered solutions, and limited improvement of using a more sensitive detector is therefore likely to result from signal suppression in the ESI interface of the system.

3.4.2.2. Calibration curves. For serum samples the calibration area was chosen in accordance with the LOQ calculated in the previous experiments. The calibration plots tend to be curved, and the linearity reduction due to the samples at 100 ng/mL is distinct. For this reason, both a linear regression curve ($y = bx + c$) and a quadratic polynomial curve ($y = ax^2 + bx + c$) were adapted to the data set. The effect of addition of IS to the samples was also examined for both the linear and curved trendlines (see Fig. 5).

The addition of IS to the samples improves the coefficient for both the linear and the polynomial calibration curve, although the

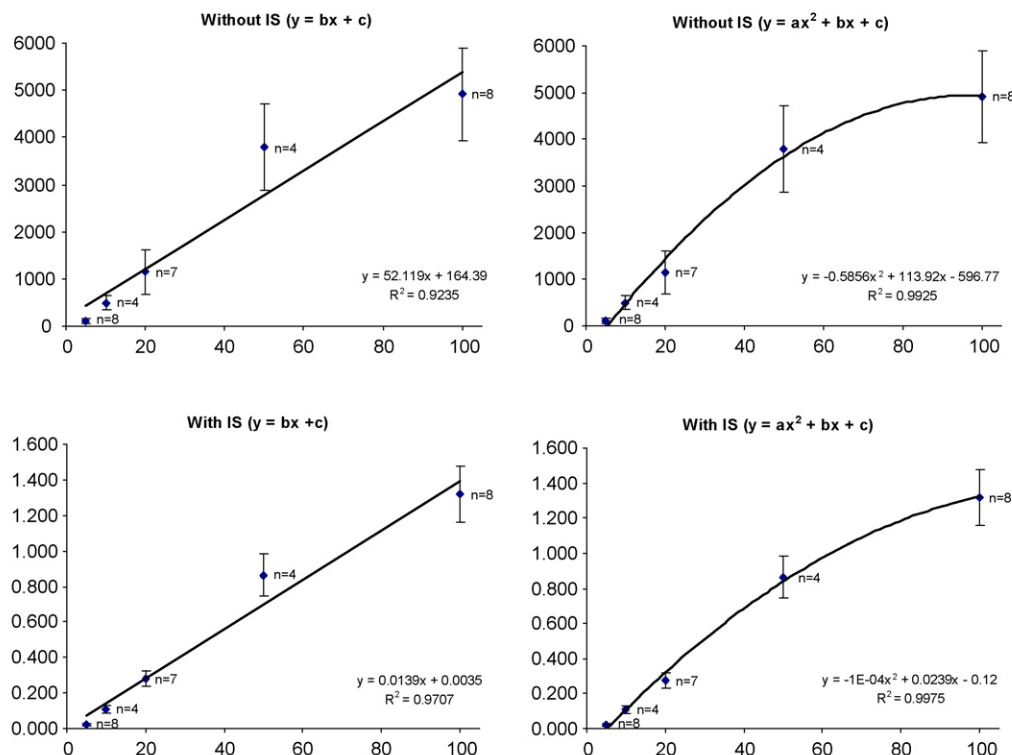

Fig. 5. Comparison of different modeling equations for the calibration curve and the effect of the addition of the IS.

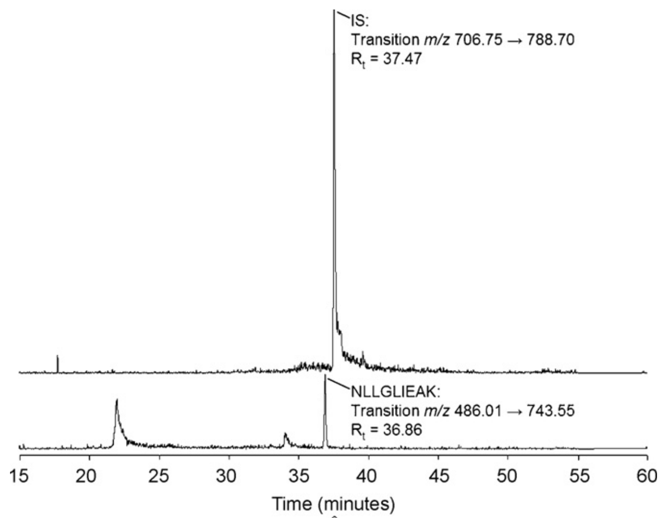

Fig. 6. MRM chromatogram with the transition m/z 486.01 → 743.55 and m/z 706.75 → 788.70 for the injection of 100 μL a realistic sample with its proGRP concentration level to determined at 13.9 ± 1.2 ng/mL (n = 3).

effect is more pronounced for the linear regression, the beneficial effect of the IS addition is observed for both trendlines. From these values the polynomial calibration curve corrected with IS best describes the dataset. The polynomial calibration model is further verified by the calculation of the accuracy (bias) at three concentration levels (see Table 1). Both the intra- and inter-run accuracy was improved by applying the quadratic polynomial calibration model for the calibration curve. The reduced intra-run precision (CV) of the method could indicate the need for improvement in the sample preparation procedure. It is highly plausible that this reduction is due to signal suppression, as indicated in the sensitivity measurements. Although there might be a difference in absolute ionizability between NLLGLIEAK and NLLGLIEAKacENR, the ratio of these ionizablities is constant, allowing absolute quantification.

3.4.3. *Applicability of the method*

Quantification of ProGRP (31–98) with the above described LC–MS/MS method was compared against an established immunoassay routinely performed at Radiumhospitalet, Rikshospitalet Medical Centre. The quadratic polynomial calibration curve obtained with IS was chosen for the quantification of ProGRP (31–98) with the LC–MS/MS method. Two samples with unknown ProGRP (31–98) concentration were measured applying both methods and the results were evaluated. Using the LC–MS/MS method, the ProGRP (31–98) concentration was calculated to 13.9 ± 1.2 ng/mL (n = 3) and 69.9 ± 3.8 ng/mL (n = 3), whereas the immunoassay yielded 8.1 ng/mL (n = 1) and 56.0 ng/mL (n = 1) respectively. Fig. 6 shows the LC–MS/MS chromatogram of the sample with the lowest concentration. The signature peptide NLLGLIEAK produced a peak with intensity significantly above the LOQ. As can be seen from the above values, the LC–MS/MS method yields elevated values compared to the immunoassay. This might be explained as follows: the latter method is more susceptible to be influenced by biomarker degradation both in vivo and in vitro, e.g. as a result of excessive freeze–thaw cycles. When assuming that degradation may affect the immunoreactivity without changing the NLLGLIEAK sequence, higher ProGRP (31–98) concentrations could potentially be observed using LC–MS/MS compared to immunoassay. However, it should be stressed that this must be studied thoroughly before any conclusions can be drawn.

4. Concluding remarks

This paper demonstrates the potential of using protein digestion in combination with LC–MS/MS as means of biomarker measurements in serum. As with all multi-step analytical methods, the use of an IS is necessary. The possibility to correct for all pretreatment steps, even the digestion, could be carried out by using the slightly modified ProGRP (31–98) as IS. The acetylation of ProGRP (31–98) is relatively easy to accomplish, and utilization of the product as an IS perform well in the absolute quantification of the SCLC biomarker ProGRP. The LOQ for the method is in a clinically relevant region. Although the LOQ is higher than the reference value for the biomarker, the levels of accuracy, precision and sensitivity indicate the value of further research in this area. One of the major bottlenecks in the use of ESI-MS on biological samples is the risk of signal suppression in the interface due to the complexity of the sample. It is consequently necessary to do further studies in the sample preparation step in order to obtain further improvements in the MS-based quantification of this biomarker. Compared to traditionally immunoassays, the throughput of the LC–MS/MS method is lower. An advantage however, is that in future research several biomarkers can be determined within a single run. These multiplex analyses increases throughput and will be less laborious.

References

[1] M.S. Nordlund, D.J. Warren, K. Nustad, J. Bjerner, E. Paus, Clin. Chem. 54 (2008) 919.
[2] K. Aoyagi, Y. Miyake, K. Urakami, T. Kashiwakuma, A. Hasegawa, T. Kodama, K. Yamaguchi, Clin. Chem. 41 (1995) 537.
[3] M.S. Nordlund, C. Fermer, O. Nilsson, D.J. Warren, E. Paus, Tumor Biol. 28 (2007) 100.
[4] I. van den Broek, R.W. Sparidans, J.H.M. Schellens, J.H. Beijnen, J. Chromatogr. B 854 (2007) 245.
[5] D. Chelius, P.V. Bondarenko, J. Proteome Res. 1 (2002) 317.
[6] V. Kulasingam, C.R. Smith, H. Batruch, A. Buckler, D.A. Jeffery, E.P. Diamandis, J. Proteome Res. 7 (2008) 640.
[7] J.R. Barr, V.L. Maggio, D.G. Patterson, G.R. Cooper, L.O. Henderson, W.E. Turner, S.J. Smith, W.H. Hannon, L.L. Needham, E.J. Sampson, Clin. Chem. 42 (1996) 1676.
[8] D.J. Janecki, K.G. Bemis, T.J. Tegeler, P.C. Sanghani, L. Zhai, T.D. Hurley, W.F. Bosron, M. Wang, Anal. Biochem. 369 (2007) 18.
[9] D.R. Barnidge, M.K. Goodmanson, G.G. Klee, D.C. Muddiman, J. Proteome Res. 3 (2004) 644.
[10] E. Kuhn, J. Wu, J. Karl, H. Liao, W. Zolg, B. Guild, Proteomics 4 (2004) 1175.
[11] S. Kirsch, J. Widart, J. Louette, J.F. Focant, E. De Pauw, J. Chromatogr. A 1153 (2007) 300.
[12] E.Y. Zhen, M.J. Berna, Z. Jin, M.L. Pritt, D.E. Watson, B.L. Ackermann, J.E. Hale, Proteom. Clin. Appl. 1 (2007) 661.
[13] F.G. Zhang, M.J. Bartels, J.C. Brodeur, K.B. Woodburn, Environ. Toxicol. Chem. 23 (2004) 1408.
[14] F.G. Zhang, M.J. Bartels, W.T. Stott, Rapid Commun. Mass Spectrom. 18 (2004) 491.
[15] M. Yanagida, H. Nakayama, F. Yoshizaki, T. Fujimura, K. Takamori, H. Ogawa, K. Iwabuchi, Proteomics 7 (2007) 2398.
[16] E. Miland, M.R. Smyth, C. ÓFágáin, Enzyme Microb. Technol. 19 (1996) 63.
[17] B. Winther, J.L.E. Reubsaet, J. Sep. Sci. 30 (2007) 234.
[18] B. Winther, P. Moi, E. Paus, J.L.E. Reubsaet, J. Sep. Sci. 30 (2007) 2638.
[19] C. Fermér, I. Andersson, K. Nilsson, O. Nilsson, Tumor Biol. 25 (2004) 7.