Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 46 (2008) 449–455
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Quantitative aspects of the analysis of the monoclonal antibody trastuzumab using high-performance liquid chromatography coupled with electrospray mass spectrometry

Carola W.N. Damen [a,*], Hilde Rosing [a], Jan H.M. Schellens [a,b,c], Jos H. Beijnen [a,b]

[a] *Department of Pharmacy & Pharmacology, Slotervaart Hospital/The Netherlands Cancer Institute, Louwesweg 6, 1066 EC Amsterdam, The Netherlands*

[b] *Beta Faculty, Department of Pharmaceutical Sciences, Division of Biomedical Analysis, Utrecht University, P.O. Box 80082, 3508 TB Utrecht, The Netherlands*

[c] *Department of Medical Oncology, Antoni van Leeuwenhoek Hospital/The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands*

Received 14 September 2007; received in revised form 25 October 2007; accepted 3 November 2007
Available online 13 November 2007

Abstract

The analytical possibilities of quantification of the intact monoclonal antibody trastuzumab by high-performance liquid chromatography coupled with electrospray mass spectrometry (HPLC–ESI-MS) were investigated. To clarify the results obtained by LC–MS, complementary experiments were performed using direct UV-spectrophotometry and high-performance liquid chromatography coupled with ultraviolet detection (HPLC–UV). A polystyrene–divinylbenzene (POROS) column was applied with gradient elution using formic acid 0.08% (v/v) in water and formic acid 0.08% (v/v) in acetonitrile as mobile phase for chromatographic analysis. Quantification on LC–MS was performed by using the peak area of the total ion current (TIC) chromatograms of one charge state. Non-linearity and sensitivity loss were the major limitations observed with the LC–MS method, of which the non-linearity is most likely caused by detector saturation. The sensitivity loss during analysis could be reduced by lowering the MS source temperature. This parameter is critical in creating a robust LC–MS system for the quantitative analysis of trastuzumab.
© 2007 Elsevier B.V. All rights reserved.

Keywords: Monoclonal antibodies; Trastuzumab; Reversed phase; Mass spectrometry; Quantification; Sensitivity loss; Robustness

1. Introduction

Monoclonal antibodies (Mabs) encompasses a new group of therapeutic compounds which are used to treat cancer, autoimmune diseases and systemic infections [1]. Trastuzumab (Herceptin®) is a humanized monoclonal immunoglobulin gamma 1 (IgG1) antibody directed against the HER2/neu receptor which is over-expressed in about 25% of all breast cancer patients [2,3]. An IgG monoclonal antibody (Fig. 1) is a tetrameric glycoprotein (approximately 150 kDa) composed of two identical heavy chains and two identical light chains, linked to each other by disulfide bonds. Asparagine at position 297 in the heavy chains is *N*-glycosylated and heterogeneity in this glycosylation profile is common for IgG antibodies produced in mammalian cells [4–12]. The most common glycan structures are shown in Fig. 1 and differ by each other in their glucose or mannose residues.

For qualitative identification of monoclonal antibodies mass spectrometry is the analytical tool of first choice. For the quantification of monoclonal antibodies in biological matrices enzyme-linked immuno sorbent assay (ELISA) is the most widely applied technique. Quantification of trastuzumab in human serum with ELISA methods have been described [13–15]. The aim of our research was to investigate analytical strategies for the quantification of the intact monoclonal antibody trastuzumab with liquid chromatography coupled online with mass spectrometry. The major advantage of mass spectrometry is that a protein specific charge envelope is being generated. Structural changes in the molecule can be detected by the mass spectrometer which is helpful to obtain more insight in

* Corresponding author. Tel.: +31 20 5124073; fax: +31 20 5124753.
E-mail address: carola.damen@slz.nl (C.W.N. Damen).

0731-7085/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2007.11.002

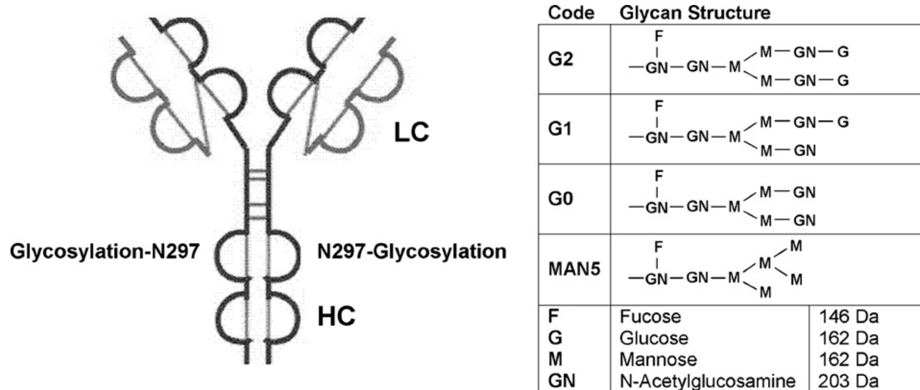

Fig. 1. Structure of monoclonal IgG antibody with the most common glycosylation forms. LC are the light chains, HC are the heavy chains, these are connected by disulfide bonds.

degradation patterns of Mabs or to detect post-translational modifications. ELISA however, generates a result based on antibody binding properties and does not reveal changes in the structure of Mabs. When the structure of the Mab is changed but the molecule is still able to bind to the ELISA plate, the changes are obviously not detected.

Quantification of proteins by mass spectrometry is often described [16–18] but, to the best of our knowledge, there are no reports available for proteins as large as monoclonal antibodies. As quadrupole MS detection is usually preferred for quantification purposes due to its sensitivity and large dynamic range [17,18], we selected this type of mass spectrometer coupled with HPLC to set up an analytical method for trastuzumab analysis. For clarification of the results, complementary experiments were executed using UV-spectrophotometry and HPLC–UV.

2. Experimental

2.1. Chemicals

Trastuzumab (Herceptin®) was supplied as a lyophilized powder from Roche (Basel, Switzerland). This pharmaceutical formulation contains additionally L-histidine hydrochloride, L-histidine, α-α-trehalose dihydrate and polysorbate 20. Formic acid was obtained from Merck (Darmstadt, Germany), HPLC grade acetonitrile and ethanol were purchased from Biosolve Ltd. (Amsterdam, The Netherlands). Double distilled water was used throughout the analysis.

Trastuzumab was dissolved in water for injections according to the instruction from the manufacturer resulting in a stock solution of 21 mg/mL. Stock solutions were stored at 2–8 °C and are stable for at least 6 months. Stock solutions were diluted in different concentrations in the range of 1–200 μg/mL in water or 10% (v/v) acetonitrile and used immediately for analysis.

2.2. Instrumentation

HPLC analysis was performed using an 1100 series binary pump with mobile-phase degasser, a column heater and a thermostated autosampler (Agilent Technologies, Santa Clara, CA, USA). A POROS R2/10 2000 Å column (10 mm × 2.1 mm i.d., particle size 10 μm) (Applied Biosystems, Foster City, CA, USA) was heated to 75 °C and used at a flow rate of 0.2 mL/min. Eluent A consisted of 0.08% (v/v) formic acid in water and eluent B of 0.08% (v/v) formic acid in acetonitrile. A linear gradient from 10 to 30% B in 2 min was followed by a linear gradient from 30 to 90% B in 30 s. This mixture was pumped through the column for 3 min before the eluent composition returned to the starting condition. The column was equilibrated for 5.5 min before the next injection. The injection volume was 20 μL and the temperature of the autosampler tray was 7 °C.

For HPLC–MS analysis, the eluent was directed to an API365 triple-quadruple mass spectrometer equipped with a turbo ion spray source (Sciex, Thornhill, ON, Canada). This mass spectrometer can operate in the range *m*/*z* 50–3000. The mass spectrometer was used in the positive ion mode and programmed to scan different masses in de range of *m*/*z* 2400–3000. Nebulizer gas, turbo gas (both compressed air) and curtain gas (N_2) were set at 6 psi, 7.5 mL/min and 8 psi, respectively. The ionspray voltage was kept at 5500 V, with a source temperature of 450 °C. For quantification TIC chromatograms were used and integrated with Analyst software version 1.2 (Sciex). Samples were injected in triplicate.

UV spectrophotometric analysis of the aqueous trastuzumab samples were carried out on a UV-1650PC visible spectrophotometer (Shimadzu, Kyoto, Japan) with UV Probe software version 2.20 (Shimadzu). The UV–vis spectrum of trastuzumab was generated with an aqueous solution of 50 μg/mL in a 1 cm quartz cuvette. Calibration curves were generated by measuring the absorption at 198 and 278 nm.

For HPLC–UV analysis the chromatographic system was directly coupled to a 1100 series programmable wavelength UV detector (Agilent Technologies, Santa Clara, CA, USA) set at a wavelength of 278 nm. Samples were injected in duplicate and chromatograms were processed using Chromeleon Software version 6.5 (Dionex Corporation, Sunnyvale, CA, USA).

3. Results and discussion

3.1. HPLC development

Reversed phase chromatography for intact proteins can separate species with minor structural differences [19]. For this separation a powerful ion-pairing agent is needed. Trifluoroacetic acid (TFA) is often used for this purpose. TFA, however, forms very strong ion pairs with the protein which do not desintegrate in the electrospray ionization (ESI) interface. This prevents the ionization of the protein, therefore leading to signal suppression in ESI-MS [20]. For the analysis of intact antibodies or their reduced and alkylated chains, perfusion chromatography using polystyrene–divinylbenzene (POROS) columns is often described [7,8,21–23]. POROS R1 and R2 columns are reversed phase columns which can be used without TFA as ion pairing agent. Instead, acetic acid, propionic acid or formic acid can be used [8,20]. A disadvantage of perfusion chromatography is the high optimal flow rate of 0.5–3 mL/min which makes this technique less compatible with mass spectrometry. Recently an LC method coupled directly to an electrospray mass spectrometer for the characterization of intact monoclonal antibodies using a POROS column was developed [19]. This platform was operated at a flow rate of 0.45 mL/min and used formic acid as ion pairing agent. We have used this system as a starting point to develop a chromatographic system for trastuzumab.

For an IgG1 antibody Le and Bondarenko [19] used a gradient of formic acid in water with formic acid in acetonitrile–ethanol 80:20 (v/v). In our hands, this gradient composition resulted in extremely high noise levels in combination with MS. By direct infusion of the separate components of the eluent in the MS, it became clear that the high chemical noise was caused by the presence of ethanol. When ethanol was omitted from the eluent, the noise level was reduced by a factor of 4.2 and the excellent peak shape and peak height of the analyte remained unchanged. Trastuzumab eluted 0.3 min later compared to the retention time in the system with ethanol. A typical chromatogram is depicted in Fig. 2. The capacity factor (k') was 3.1, the peak asymmetry (A_s) was 2.6 and the peak width at 10% height was 12 s.

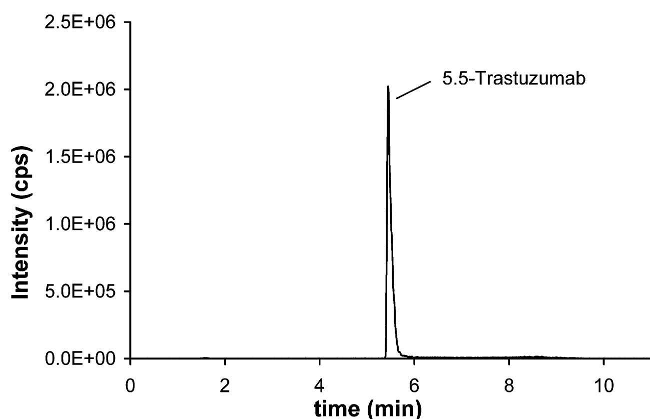

Fig. 2. Representative TIC chromatogram of a full spectrum scan across the range of m/z 2741–2754 after injection of trastuzumab (1000 ng on-column).

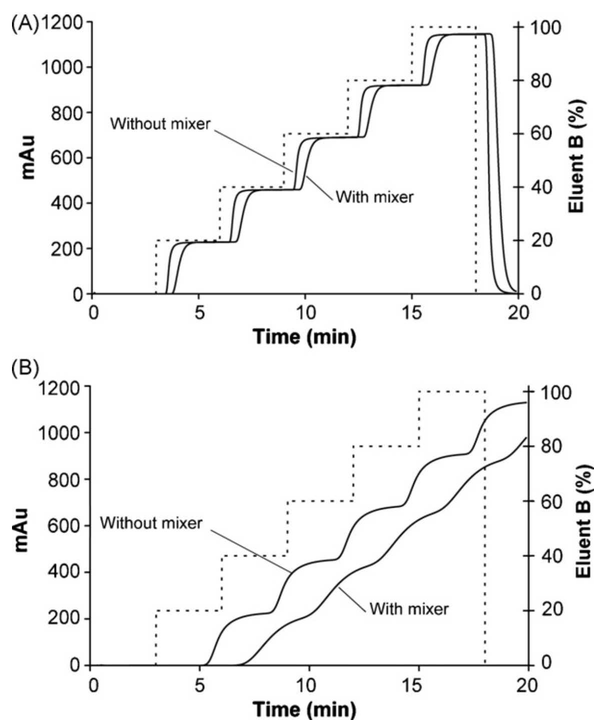

Fig. 3. Gradient performance with and without solvent mixer at a flow rate of 1 mL/min (A) and 0.2 mL/min (B). Dotted line indicates the gradient settings.

Using this HPLC system, substantial carry-over was experienced. When a blank sample was injected after a trastuzumab injection (2000 ng on-column), a peak appeared of 35% of the height of the original trastuzumab injection. Experiments proved that the carry over originated from the column material. This has been described earlier for POROS material [22] and is ascribed to the interior structure of the pores in the stationary phase trapping proteins [20]. The carry-over was reduced substantially to 8% by increasing the period when 90% 0.08% v/v formic acid in acetonitrile was flushed over the column from 1 s to 3 min and was reduced to 1.9% by removing the solvent mixer from the HPLC system. The volume of a standard mixer in the system is 420 μL, which is apparently not suitable for the low flow rates used with online mass spectrometry. Fig. 3 shows an LC–UV experiment without analytical column performed at a flow rate of 0.2 and 1.0 mL/min with and without solvent mixer. Eluent A was isopropanol–water 10:90 v/v, eluent B was 0.5% acetone in eluent A. Absorbance was measured at 265 nm. In Fig. 3 the gradient is shown with the dotted line. Every 3 min the percentage of eluent B was increased by 20%. Fig. 3A shows the absorbance with a solvent flow of 1.0 mL/min. It can be clearly seen that the eluent composition follows the programmed gradient almost immediately both with and without solvent mixer. On the other hand when the solvent flow is reduced to 0.2 mL/min (Fig. 3B) solvents A and B are not adequately mixed. With a solvent mixer the delay time is approximately 7 min before the eluent composition is the same as the programmed. With a steep gradient and an isocratic hold for 3 min as with our trastuzumab method the amount of eluent B remained too low to elute all trastuzumab from the HPLC column. The mixing of solvent A and B at a flow rate of 0.2 mL/min also takes place without a

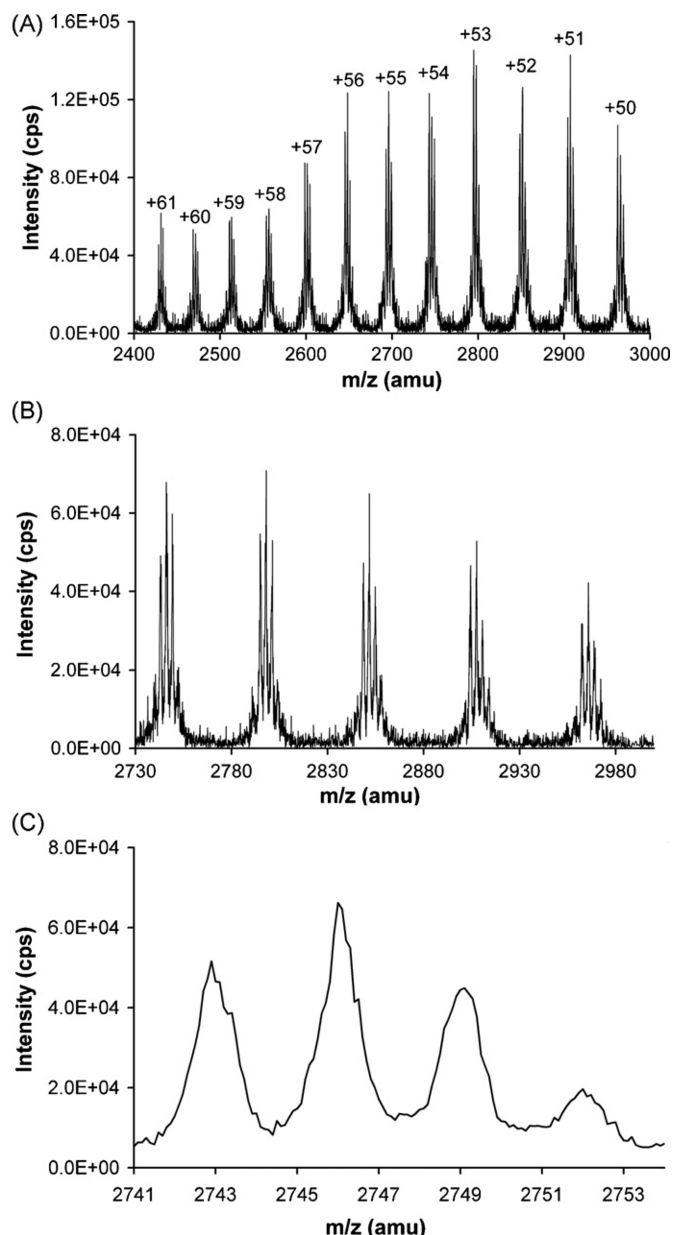

Fig. 4. Positive ESI mass spectrum scan from *m/z* 2400 to 3000 (A) featuring multiple charged ions from 50+ to 61+. Spectrum scans across the range from *m/z* 2730 to 3000 (B) and *m/z* 2741 to 2754 (charge state +54, C), 4 distinctive peaks in each charge state were observed.

solvent mixer. Thus, our final system consisted of a gradient of 0.08% v/v formic acid in water with 0.08% v/v formic acid in acetonitrile at a flow rate of 0.2 mL/min without a solvent mixer in the system.

3.2. LC–MS

Fig. 4 shows a full spectrum scan across the range from *m/z* 2400 to 3000, showing multiple charged ions from 50+ to 61+ as is common for proteins when using mass spectrometry [24,25]. When a smaller scan is performed from *m/z* 2730 to 3000 (Fig. 4B) and from *m/z* 2741 to 2754 (charge state 54+, Fig. 4C) it can be seen that each charge state consists of four distinctive peaks. When the spectrum is deconvoluted the masses of the four clustered peaks are 148,066, 148,228, 148,390 and 148,552 Da with mass differences of 162 Da. This correlates with the difference of one hexose residue (Fig. 1). For quantification the area of the TIC chromatogram of the scan *m/z* 2741–2754, with charge state 54+ was used because this charge state has the highest signal to noise ratio. Furthermore, the area of the TIC chromatogram of a single sugar moiety in this charge state (*m/z* 2745.2–2746.7) was tested. The LLQ of our method with both scans is approximately 1 μg/mL with 20 μL injection on column. With the scan range from *m/z* 2741 to 2754 the signal to noise ratio is 10, as with the narrow range from *m/z* 2745.2 to 2746.7 the signal to noise ratio is 7. Chromatograms of the blank and LLQ samples are shown in Fig. 5. As the scan range *m/z* 2741–2754 has higher signal to noise ratio then the narrower scan range, this scan range was used for further experiments.

The quantitative results of LC–MS analysis of aqueous trastuzumab solutions by using the peak area of the TIC chromatograms of the scan range from *m/z* 2741 to 2754 are shown in Fig. 6A. In the low concentration range, no linear relationship exists. The second part from 5 to 15 μg/mL seems to be linear with correlation coefficients better than 0.986. In the higher concentration range the curves are flattening. Furthermore the MS signal decreased when calibration samples were injected sequentially as can be seen in Fig. 6A.

To investigate whether HPLC–UV is a useful technique for clarifying these results, experiments using UV-spectrophotometry were set up first. The UV spectrum of trastuzumab was recorded. The spectrum shows a distinct maximum at 198 nm and a less abundant maximum at 278 nm. The absorption of diluted aqueous trastuzumab samples were measured at both wavelengths. At 198 nm the LLQ is 1 μg/mL and a linear correlation is found up to 35 μg/mL. Above this concentration maximal absorption is reached. At 278 nm the LLQ is 6 μg/mL and a linear correlation is found over the entire concentration range up to 200 μg/mL. The non-linearity in the low concentration range as seen with LC–MS is not observed with UV-spectrophotometry at 198 nm. As linear relationships are found with UV spectrophotometry over the entire tested concentration range, UV detection in combination with high-performance liquid chromatography can be used for further clarification of the non-linearity as observed with LC–MS.

As 198 nm is not a specific wavelength because eluent components like formic acid and acetonitrile absorb at this wavelength as well and because the maximum measurable concentration is 35 μg/mL, quantitative analysis using LC–UV was performed at 278 nm. The LLQ of HPLC in combination with UV detection at 278 nm is 19 μg/mL with linear calibration curves from 19 to 200 μg/mL and regression coefficients of 0.996 or better. The calibration standards were injected sequentially and no loss in sensitivity was observed.

Employing LC–MS, non-linearity in the low concentration range (<5 μg/mL) was observed, in contrast with UV-spectrophotometry using a wavelength of 198 nm. LC–UV at 278 nm has an LLQ of 19 μg/mL. Thus, this technique does

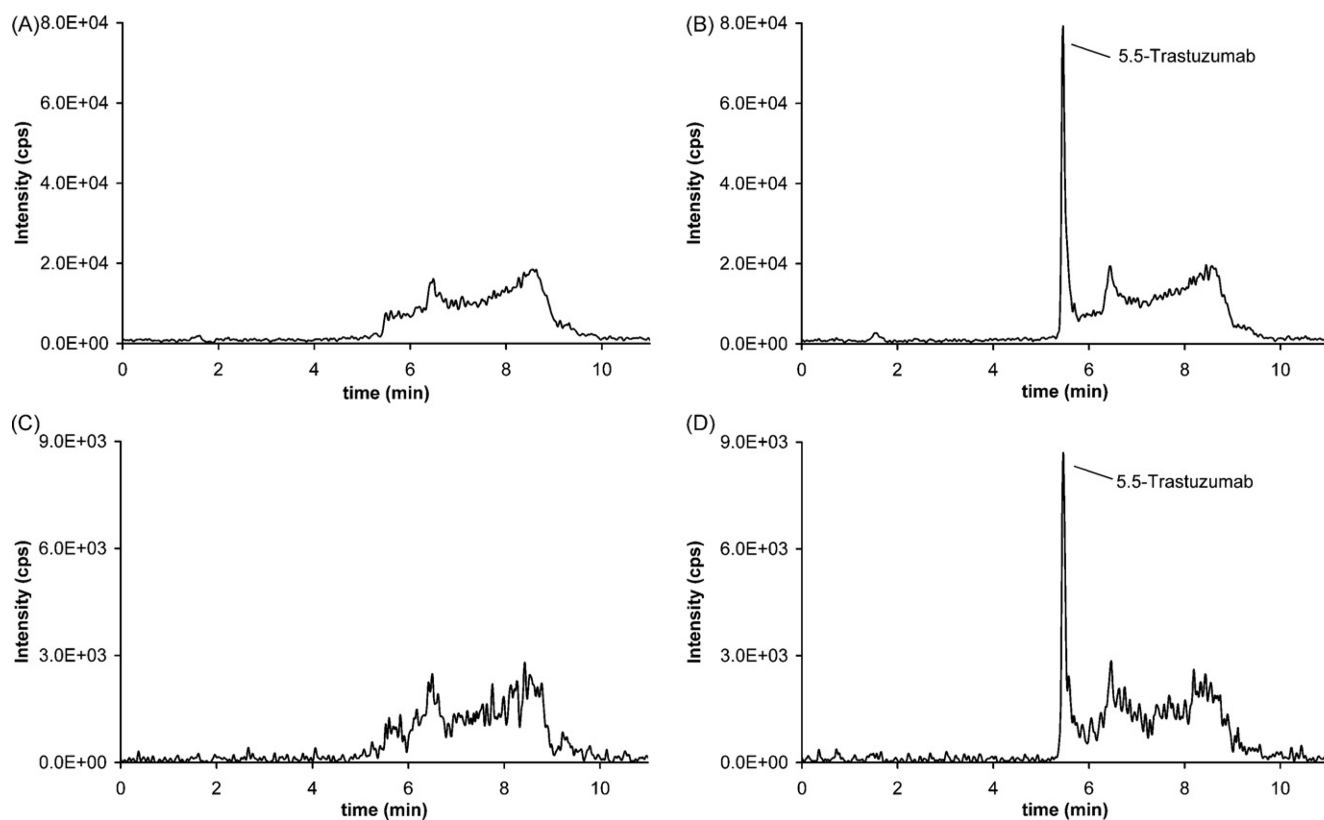

Fig. 5. TIC chromatograms of blank (A) and 1 μg/mL solution (B) in the scan range of *m/z* 2741–2754 and of blank (C) and 1 μg/mL solution (D) in the scan range of *m/z* 2745.2–2746.7.

not explain the non-linearity of LC–MS in the low concentration range. The non-linearity in the low concentration range was found to result from loss of analyte in the system, but could be prevented by using 10% acetonitrile as solvent instead of water for the preparation and dilution of calibration standards. Linearity was now observed in the range of 1–15 μg/mL trastuzumab.

From 15 μg/mL on, no linear relationship exists and the curve is flattening. On LC–UV the calibration curves are linear up to at least 200 μg/mL. Thus, this non-linearity is caused by the mass spectrometer and not by the chromatographic system. The observed non-linearity using LC–MS may occur in the source (sample introduction and ionization processes), in transit from the source region to the detector (absorption), and in the detector (saturation processes) [18]. Quantification is performed on one single charge state. For peptides, linearity of two orders of magnitude has been reported when one single charge state was used for the quantification in selected ion monitoring (SIM) [26,27]. For proteins as large as monoclonal antibodies, as far as we know, no data are present in literature about quantification on one single charge state, therefore it is unknown what dynamic range can be expected.

By sequential injection of the calibration samples on LC–MS the MS signal decreased (see Fig. 6A). When these samples were injected on LC–UV, no signal decrease was observed and the calibration curves superimposed. A robustness test of the LC–MS method was performed by injecting a 15 μg/mL solution in 10% acetonitrile 50 times. After 10 injections the peak area was decreased by 11%. After 30 injections the decrease was 22% and the signal stabilized at this level for the other injections. The same phenomenon was seen by Aronov et al. [28] for the small molecule kinoprene-PTAD (MW 451) while running 200 samples. After 50 injections the sensitivity decreased 85% and then remained stable for the rest of the analysis. The loss of sensitivity was probably due to accumulated residue on the sample cone and hexapoles. When the same behavior occurs for a molecule as large as trastuzumab then this could explain that by injecting the trastuzumab dilution series multiple times on our LC–MS system the sensitivity becomes lower within each series.

In order to improve the robustness of the method we investigated whether maintaining 90% eluent B (0.08% v/v HCOOH in acetonitrile) for 4 min after the standard gradient, could clean the sample cone and hexapoles and prevent sensitivity loss during the run. Unfortunately no improvement was observed. However, when we lowered the source temperature from 450 to 375 °C and injected the 15 μg/mL solutions 100 times by using the original gradient no sensitivity loss was noted anymore. The relative standard deviation for the peak area of the TIC chromatograms was 6.9%. At this lower source temperature we injected the trastuzumab dilutions from 1 to 15 μg/mL three times sequentially. Fig. 6B shows that the curves superimposed and that no sensitivity loss was observed. The explanation for this observation remains to be elucidated.

Table 1
Assay performance parameters for quantifying intact trastuzumab

Method	Detection	LLQ	Linear range (μg/mL)
UV-Spectrophotometry	198 nm	1 μg/mL in 1 cm cuvette	1–35
UV-Spectrophotometry	278 nm	6 μg/mL in 1 cm cuvette	6–200
LC–UV	278 nm	380 ng on column	19–200
LC–MS	*m/z* 2741–2754	20 ng on column	1–15
LC–MS	*m/z* 2745.2–2746.7	20 ng on column	1–15

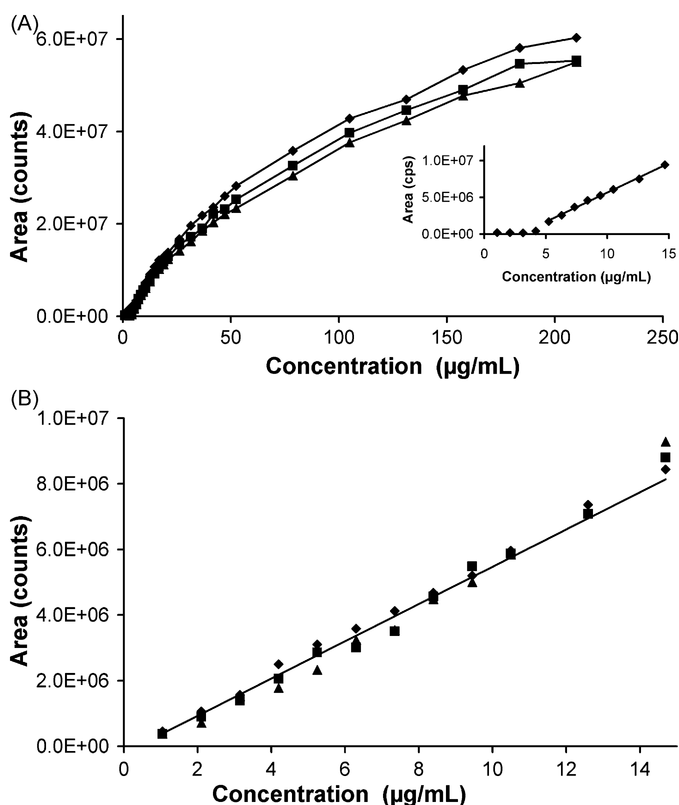

Fig. 6. Calibration curves of trastuzumab (1–200 μg/mL) in water based on peak area measurements in TIC chromatograms of the scan range *m/z* 2741–2754 (A). Calibration standards were injected sequentially in triplicate: (♦) represents the first, (■) the second and (▲) the third series of injections. The inset in (A) shows the range from 1 to 15 μg/mL for the first injection series. (B) The improved system using 10% acetonitrile instead of water and with a MS source temperature of 375 °C.

4. Conclusion

The assay performance parameters of the investigated methods for the monoclonal antibody trastuzumab are summarized in Table 1. The results on UV-spectrophotometry at 278 nm and LC–UV show linear relationships up to at least 200 μg/mL. No sensitivity loss is observed by injecting samples sequentially. Therefore, the loss in sensitivity on LC–MS is due to the mass spectrometric detection and not due to deterioration of samples in de-autosampler. As linear relationships exist on LC–UV, the non-linearity in the LC–MS method is not caused by the liquid chromatographic system, but due to the coupling with mass spectrometry. The adsorption at low concentrations is substantially reduced by using 10% of acetonitrile instead of water as dilution solvent. The source temperature is a critical parameter in creating a robust analytical system on LC–MS. By lowering the source temperature from 450 to 375 °C no sensitivity loss was observed anymore during the run.

Our mass spectrometric method is less sensitive than the ELISA methods reported however, using mass spectrometry quantification is more specific. The mass of the molecule can be confirmed by de-convoluting the protein specific envelope.

References

[1] O.H. Brekke, I. Sandlie, Nat. Rev. Drug Discov. 2 (2003) 52–62.
[2] P. Carter, L. Presta, C.M. Gorman, J.B. Ridgway, D. Henner, W.L. Wong, A.M. Rowland, C. Kotts, M.E. Carver, H.M. Shepard, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 4285–4289.
[3] C.A. Hudis, N. Engl. J. Med. 357 (2007) 39–51.
[4] A. Beck, M.C. Bussat, N. Zorn, V. Robillard, C. Klinguer-Hamour, S. Chenu, L. Goetsch, N. Corvaia, A. Van Dorsselaer, J.F. Haeuw, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 819 (2005) 203–218.
[5] T.M. Dillon, P.V. Bondarenko, D.S. Rehder, G.D. Pipes, G.R. Kleemann, M.S. Ricci, J. Chromatogr. A 1120 (2006) 112–120.
[6] T.M. Dillon, P.V. Bondarenko, R.M. Speed, J. Chromatogr. A 1053 (2004) 299–305.
[7] D.S. Rehder, T.M. Dillon, G.D. Pipes, P.V. Bondarenko, J. Chromatogr. A 1102 (2006) 164–175.
[8] H.Z. Wan, S. Kaneshiro, J. Frenz, J. Cacia, J. Chromatogr. A 913 (2001) 437–446.
[9] L. Huang, S. Biolsi, K.R. Bales, U. Kuchibhotla, Anal. Biochem. 349 (2006) 197–207.
[10] Y. Lyubarskaya, D. Houde, J. Woodard, D. Murphy, R. Mhatre, Anal. Biochem. 348 (2006) 24–39.
[11] G.D. Roberts, W.P. Johnson, S. Burman, K.R. Anumula, S.A. Carr, Anal. Chem. 67 (1995) 3613–3625.
[12] Y. Wang, S.L. Wu, W.S. Hancock, Glycobiology 16 (2006) 514–523.
[13] L. Maple, R. Lathrop, S. Bozich, W. Harman, R. Tacey, M. Kelley, A. Danilkovitch-Miagkova, J. Immunol. Methods 295 (2004) 169–182.
[14] M.D. Pegram, A. Lipton, D.F. Hayes, B.L. Weber, J.M. Baselga, D. Tripathy, D. Baly, S.A. Baughman, T. Twaddell, J.A. Glaspy, D.J. Slamon, J. Clin. Oncol. 16 (1998) 2659–2671.
[15] H.J. Stemmler, M. Schmitt, A. Willems, H. Bernhard, N. Harbeck, V. Heinemann, Anticancer Drugs 18 (2007) 23–28.
[16] B. Domon, R. Aebersold, Science 312 (2006) 212–217.
[17] J. Lill, Mass Spectrom. Rev. 22 (2003) 182–194.
[18] S.E. Ong, M. Mann, Nat. Chem. Biol. 1 (2005) 252–262.
[19] J.C. Le, P.V. Bondarenko, J. Am. Soc. Mass Spectrom. 16 (2005) 307–311.
[20] M.C. Garcia, A.C. Hogenboom, H. Zappey, H. Irth, J. Chromatogr. A 957 (2002) 187–199.
[21] N.B. Afeyan, N.F. Gordon, I. Mazsaroff, L. Varady, S.P. Fulton, Y.B. Yang, F.E. Regnier, J. Chromatogr. 519 (1990) 1–29.
[22] J.E. Battersby, B. Snedecor, C. Chen, K.M. Champion, L. Riddle, M. Vanderlaan, J. Chromatogr. A 927 (2001) 61–76.
[23] J. Bongers, J.J. Cummings, M.B. Ebert, M.M. Federici, L. Gledhill, D. Gulati, G.M. Hilliard, B.H. Jones, K.R. Lee, J. Mozdzanowski, M. Naimoli, S. Burman, J. Pharm. Biomed. Anal. 21 (2000) 1099–1128.

[24] J.B. Fenn, M. Mann, C.K. Meng, S.F. Wong, C.M. Whitehouse, Science 246 (1989) 64–71.
[25] R.D. Smith, J.A. Loo, R.R. Ogorzalek Loo, M. Busman, H.R. Udseth, Mass Spectrom. Rev. 10 (1991) 359–451.
[26] D.C. Delinsky, K.T. Hill, C.A. White, M.G. Bartlett, Rapid Commun. Mass Spectrom. 18 (2004) 293–298.
[27] D.C. Delinsky, K.T. Hill, C.A. White, M.G. Bartlett, Biomed. Chromatogr. 18 (2004) 700–705.
[28] P.A. Aronov, K. Dettmer, J.A. Christiansen, A.J. Cornel, B.D. Hammock, J. Agric. Food Chem. 53 (2005) 3306–3312.