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Simultaneous determination of incretin hormones and their truncated forms from human plasma by immunoprecipitation and liquid chromatography–mass spectrometry

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Abstract

The incretins, glucose-dependent insulinotropic peptide ($GIP_{1–42}$) and glucagon-like peptide 1 ($GLP\text{-}1_{7–36}$), are involved in regulation of gastric emptying, glucose homeostasis, body fat regulation and the glucose-induced insulin secretion from the endocrine pancreas. After release in the circulation both peptides are rapidly degraded by the exopeptidase dipeptidyl peptidase IV (DP IV) to the inactive polypeptides $GIP_{3–42}$ and $GLP\text{-}1_{9–36}$. In vivo stabilization of the active incretins by orally available DP IV-inhibitors is now widely accepted as a new therapeutic approach in antidiabetic treatment. In order to demonstrate the pharmacodynamic effect of DP IV-inhibitors, it is necessary to measure the plasma levels of active and inactive forms of GIP and GLP-1. We previously described an immunoprecipitation method as sample preparation and concentration in combination with a LC–MS analysis for determination of active and inactive GIP. We could improve the efficiency and suitability of this method by reduction of the necessary sample volume to 1.0 ml and simultaneous measurement of $GIP_{1–42}$, $GIP_{3–42}$ and $GLP\text{-}1_{7–36}$, $GLP\text{-}1_{9–36}$, without loss of sensitivity. An LOQ of approximately 5 and 11 pmol/l was maintained for GIP and GLP-1, respectively.
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Keywords: Immunoprecipitation; Incretin hormones

1. Introduction

The incretins, glucose-dependent insulinotropic peptide ($GIP_{1–42}$) and glucagon-like peptide 1 ($GLP\text{-}1_{7–36}$), are peptide hormones that are released postprandially by the gut. Both peptides are involved in regulation of gastric emptying, glucose homeostasis and body fat regulation [1–3]. Concerning blood glucose regulation, pleiotropic effects of these hormones were described including stimulation of insulin release, promotion of β-cell growth and sensitization of muscle and liver for insulin action [4–8].

After release into the circulation, both peptides are rapidly inactivated by the exopeptidase dipeptidyl peptidase IV (DP IV) [9,10]. DP IV is highly expressed in various exo- and endothelial cells in kidney, lung, gut and blood vessels as well as on lymphoid cells. It is a highly specific enzyme releasing the N-terminal dipeptide from peptides with proline or alanine in penultimate N-terminal position A [11,12]. The enzymatic release of Tyr-Ala or His-Ala from the active incretins leads to the truncated inactive peptides $GIP_{3–42}$ and $GLP_{9–36}$, respectively [13,14].

Thus, the in vivo stabilization of the active incretins by orally available DP IV-inhibitors is now widely accepted as a new approach in antidiabetic treatment [15–17]. For the demonstration of the pharmacodynamic effect of DP IV-inhibitors, it is necessary to measure the plasma levels of active and inactive forms of GIP and GLP-1. Besides commonly used ELISA, RIA or HPLC based assays [18–24], we recently developed an immunoprecipitation-LC–MS assay for active and inactive GIP which has advantages in selectivity and sensitivity but needs high plasma volumes [25]. Here, we describe the improvement of this assay now suitable for parallel determination of the active and inactive forms of both incretins, allowing the determination of these four important parameters from only 1.0 ml of plasma.

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2. Experimental

2.1. Chemicals and solvents

All standard peptides $GIP_{1–42}$ (M_w = 4983 Da, peptide content: 72.30%, purity: 98%, used correction factor: 0.7085), $GIP_{3–42}$ (M_w = 4749 Da, peptide content: 68.46%, purity: 98%, used correction factor: 0.6709), GLP-$1_{7–36}$:(M_w = 3297 Da, peptide content: 75.45%, purity: 98%, used correction factor: 0.747), GLP-$1_{9–36}$ (M_w = 3090 Da, peptide content: 75.46%, purity: 98%, used correction factor: 0.747), [Arg^{18}, Glu^{21}]$GIP_{1–42}$ and [Glu^9, Arg^{20}]GLP-$1_{7–36}$ were synthesized in our laboratories. The peptide content of the synthetic human $GIP_{1–42}$ and $GIP_{3–42}$ as well as GLP-$1_{7–36}$ and GLP-$1_{9–36}$ was determined by quantitative amino acid analysis (CAT, Tübingen, Germany).

The polyclonal rabbit-anti-C-GIP antibody was produced by Eurogentec (Herstal, Belgium) and the monoclonal mouse-anti-C-GLP antibody by Biogenes (Berlin, Germany). The peptides used for immunization contained the C-terminal part of GIP (30 AA) and GLP (15 AA) and were synthesized in our laboratory using standard methods of peptide chemistry.

Ultrapure analytical water was obtained by a PURELAP Plus System (USF). Methanol (gradient grade) and formic acid (90%) were obtained from VWR International (Darmstadt, Germany). All other chemicals used were commercially available and of analytical grade.

2.2. Pretreatment of plasma used for preparation of standards

Human citrate plasma (4% sodium citrate solution, approximately 85 ml in 750 ml plasma; Baxter Plasmazentrum, Halle, Germany) used for preparation of standards samples was centrifuged by 4500 × *g* for 10 min at 10 °C (Beckman Allegra™ 21 R, Munich, Germany). The plasma was found to contain significant endogenous amounts of $GIP_{3–42}$ and to a minor degree of GLP-$1_{9–36}$, which interfere with the correct quantification of standard samples. To degrade these interfering peptides, plasma (25 ml) was incubated with 250 μl of a trypsin and 250 μl of a chymotrypsin solution (prepared by dissolving 2 mg of each protein in 10 ml 0.01% TFA). Trypsin and chymotrypsin were purchased from VWR International (Darmstadt, Germany). The protease treated plasma was kept in a water bath at 37 °C for at least 12 h before preparation of standards. After this, proteolysis was stopped by addition of the conservation solution described under 2.3 [25,26].

2.3. Stabilization of test plasma by protease inhibitors

To stabilize the analytes in the plasma samples, we used 100 μl of a conservation solution per 1 ml plasma. We have modified the inhibitor cocktail described by *Pietsch* by addition of our specific DP IV-inhibitor P32/98 to protect the active peptides to DP IV-catalyzed N-terminal degradation [25,26].

2.4. Immunoprecipitation

The procedures of the immunoprecipitation assay are illustrated in Fig. 1.

Magnetic beads (20 μl suspension containing approximately 6.7 × 10^8 beads/ml) with covalently bound anti-IgG-antibodies (Dynabeads® M-280; sheep anti-rabbit IgG for GIP-determination and Dynabeads® M-280 or sheep anti-mouse IgG for GLP-determination, (Dynal, Hamburg, Germany) were washed three times with 1.0 ml buffer (PBS/Tween, pH 7.8, Sigma, Taufkirchen, Germany). For separating the beads from solution a Magnetic Separation Stand (MagneSphere®, Promega, Mannheim, Germany) was employed. Thereafter, the beads were incubated with the polyclonal anti-C-GIP antibodies (20 μl, SA 8682, content 0.1 mg/ml in PBS-buffer, Eurogentec, Herstal, Belgium) or monoclonal anti-C-GLP antibodies (20 μl, 27-64-1, content 0.22 mg/ml in PBS-buffer, Biogenes, Berlin, Germany) in PBS/Tween buffer overnight at 7 °C for binding. After washing (three times with 1 ml PBS/Tween buffer) and merging of the antibody loaded beads, 1.0 ml plasma sample (EDTA- or citrate plasma) stabilized by the inhibitor mixture and containing an appropriate amount (approximately 400 pmol/l) of internal standard was added to the magnetic beads and incubated for 4 h at room temperature under gentle shaking. The plasma was removed carefully and the precipitated peptides were washed three-times with 1.0 ml of PBS/Tween buffer and once with 250 μl PBS-buffer. After that, the precipitated peptides were eluted with 50 μl of 50% methanol containing 0.5% formic acid for 15 min under vortexing. The supernatant containing the peptides was transferred into an autosampler vial for LC–MS analysis.

2.5. HPLC–MS equipment and conditions

The HPLC system consisted of a series 200 microgradient pump (Perkin-Elmer, Überlingen, Germany) equipped with a series 200 autosampler (Perkin-Elmer). Separation was performed using a YMC-Pack ODS AQ (150 mm × 1 mm i.d.) column, particle size 3 μm 200 Å (YMC, Schermbeck, Germany) protected by a guard column YMC-Pack ODS-AQ (10 mm × 2 mm i.d.). Methanol/water (90/10, v/v) containing 0.1% formic acid (A) and methanol/water (10/90, v/v) with 0.1% formic acid (B) were used as eluents. The gradient elution was performed as follows:

0–10 min	100% B with 40 μl/min
10–15 min	100% B to 30% B with 40 μl/min
15–30 min	100% A with 20 μl/min
30–35 min	100% B with 40 μl/min.

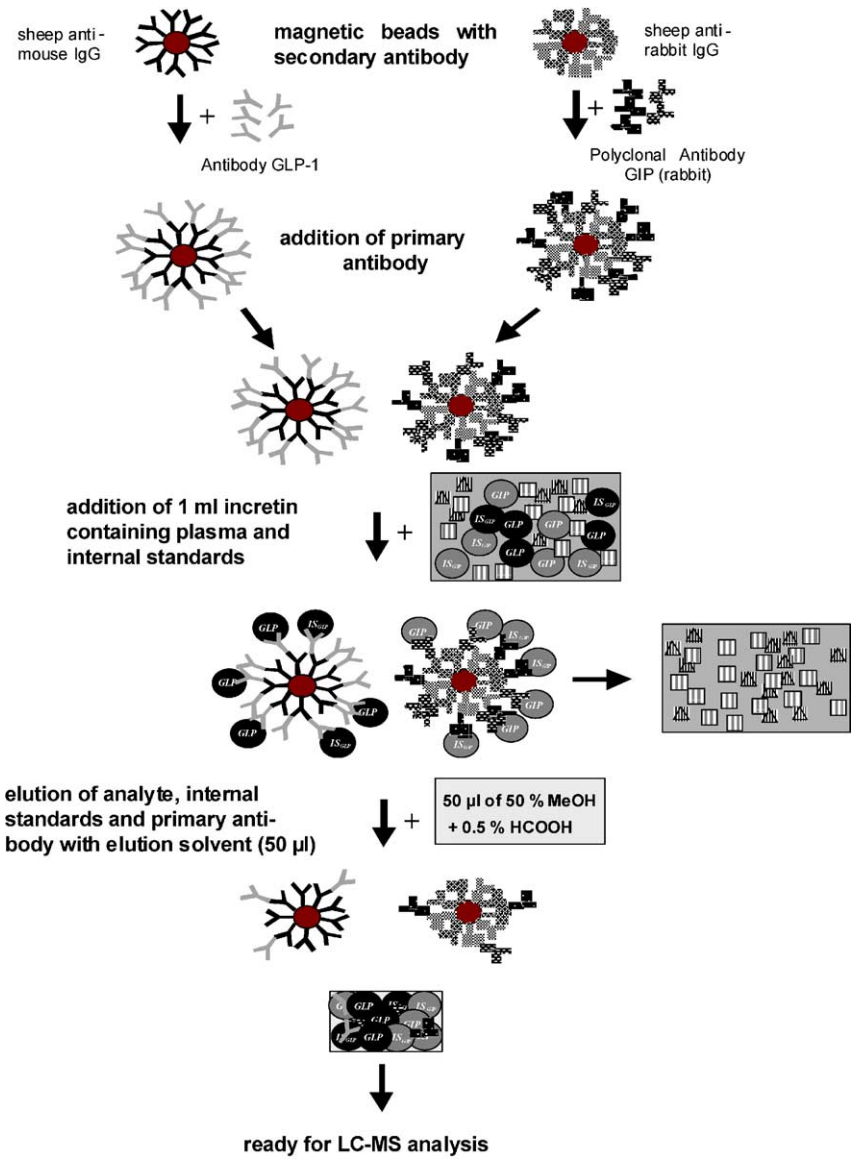

Fig. 1. Principle of the new immunoprecipitation method.

Forty microlitre samples were injected. Autosampler and column were kept at room temperature.

The HPLC was coupled to a single quadrupole mass spectrometer (Applied Biosystems, API 150 EX, equipped with an ionspray (API) source (Darmstadt, Germany). All samples were analyzed in the positive ion mode. The spray voltage was set to 5000 V. The optimization of the mass spectrometric settings was carried out by continuous flow injection (20 μl/min) of a standard solution (all 10 μg/ml, without content correction) of all analytes using a microliter syringe pump (Harvard Apparatus, Holliston, MA, USA).

Sequence modified $GIP_{1–42}$ and $GLP\text{-}1_{7–36}$ with two amino acid replacements (His to Arg in position 18 and Asp to Glu in position 21 for $GIP_{1–42}$ and Asp to Glu in position 9 and Lys to Arg in position 20 for $GLP\text{-}1_{7–36}$) were applied as internal standards. For LC–MS quantification in the selected ion monitoring mode the mass traces of following ions of the six analyzed peptides were used:

$GIP_{1–42}$: $[M + 5H]^{5+}$, $m/z = 997.9$
$GIP_{3–42}$: $[M + 5H]^{5+}$, $m/z = 950.9$
$GIP_{1–42}$ as internal standard $[M + 5H]^{5+}$, $m/z = 1004.4$
$GLP\text{-}1_{7–36}$: $[M + 4H]^{4+}$, $m/z = 825.4, 826.2$ as well as $[M + 3H]^{3+}$, $m/z = 1100.2$
$GLP\text{-}1_{9–36}$: $[M + 4H]^{4+}$, $m/z = 773.4$ as well as $[M + 3H]^{3+}$, $m/z = 1030.8$
$GLP\text{-}1_{7–36}$ as internal standard $[M+4H]^{4+}$, $m/z = 836.2$

2.6. Preparation of standards

One μl of a $GIP_{1–42}$ stock solution (1 mg/ml = 0.2 mmol/l) and 1 μl of a $GIP_{3–42}$ stock solution (1 mg/ml =

Table 1
Corrected concentrations of the used calibration standard samples

Calibration solution	Concentrations of plasma standard samples of $GIP_{1–42}$ (pmol/l)	Concentrations of plasma standard samples of $GIP_{3–42}$ (pmol/l)	Concentrations of plasma standard samples of GLP-$1_{7–36}$ (pmol/l)	Concentrations of plasma standard samples of GLP-$1_{9–36}$ (pmol/l)
I-1	354.25	335.45	373.5	373.5
I-2	177.13	167.73	186.75	186.75
I-3	88.56	83.86	93.38	93.38
I-4	44.28	41.93	46.69	46.69
I-5	22.14	20.97	23.34	23.34
I-6	11.05	10.48	11.67	11.67
I-7	5.53	5.24	5.84	5.84
QC 1	118.1	111.8	124.5	124.5
QC 3	29.52	27.95	31.13	31.13
QC 4	14.76	13.98	15.56	15.56
QC 5	7.38	6.99	7.78	7.78

0.2 mmol/l) were diluted with 38 μl PBS-buffer. Ten μl of this solutions were diluted with 990 μl PBS-buffer to the standard calibration solution I with a final nominal concentration of 50 nmol/l.

One μl of a GLP-$1_{7–36}$ stock solution (1 mg/ml = 0.32 mmol/l) and 1 μl of a GLP-$1_{9–36}$ stock solution (1 mg/ml = 0.32 mmol/l) were diluted with PBS-buffer to a nominal concentration of 50 nmol/l.

Human citrate plasma (trypsin/chymotrypsin treated and shifted with inhibitor cocktail) were spiked with the standard solutions of all analytes to obtain the final concentrations of the plasma standard samples and the used QCs samples (see Table 1).

The calibration curves of all incretins were constructed using ratios of the observed analyte peak area to internal standard versus concentration of analyte. Quadratic regression analysis of the data yielded in slopes, intercept parameters and correlation coefficients (better then 0.999) which were used to determine the concentration of each analyte.

The quality control samples QC 1, QC 3 and QC 5 were used for the determination of the accuracy and QC 4 for the determination of the method precision.

Final molar concentrations were calculated considering peptide content and purity of the peptides. The concentrations used for the determination of all analyzed incretins are shown in Table 1.

3. Results and discussion

3.1. Chromatographic system

Fig. 2 shows typical TIC chromatograms of a standard sample including all analytes as well as a plasma blank sample after immunoprecipitation. SIM-chromatograms of a GIP and GLP calibration sample after immunoprecipitation are shown in Figs. 3 and 4. Under the specified chromatographic conditions all GIP forms ($GIP_{1–42}$, $GIP_{3–42}$ and the internal standard [Arg^{18},Glu^{21}]$GIP_{1–42}$) coelute after approximately 24 min. Investigating plasma GLP-1 forms, a good separation of GLP-$1_{7–36}$ (retention time approximately 27 min) and GLP-$1_{9–36}$ (approximately 28 min) was achieved. The internal standard coelutes with GLP-$1_{7–36}$. All $[M + 5H]^{5+}$ peaks of GIP forms and $[M + 4H]^{4+}$ peaks and corresponding $[M + 3H]^{3+}$ peaks of GLP-1 forms are simultaneously detectable.

Primary antibodies still present in the samples show no interaction with the stationary phase and elute with the dead volume. Therefore, quantification of the incretins was not affected.

3.2. Selectivity

Blank samples (containing mobile phase only) did not show any relevant peaks. The mass trace m/z = 825.4 (base peak of GLP-$1_{7–36}$) showed an unspecified peak after immunoprecipitation, which overlapped with the peak of GLP-$1_{7–36}$ in the HPLC (see Fig. 2 the plasma blank sample). Therefore, this mass trace is unsuitable for the quantitative determination of GLP-$1_{7–36}$ in human plasma samples after immunoprecipitation. That is why, we slightly changed the mass trace for detection of GLP-$1_{7–36}$ to m/z = 826.2 were the interfering peak is not present. Furthermore, a

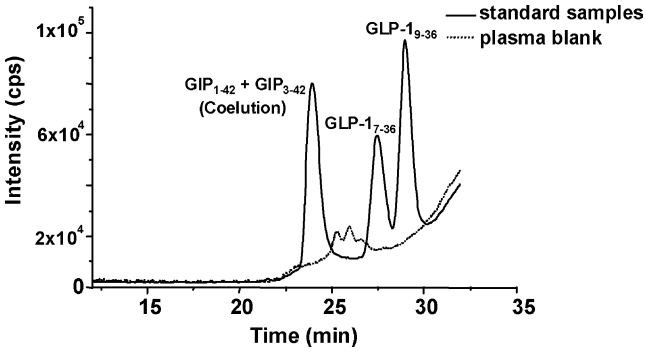

Fig. 2. Representative LC–MS chromatogram of an aqueous standard solution containing all analytes without immunoprecipitation compared to a plasma blank sample after immunoprecipitation (TIC of all selected ions).

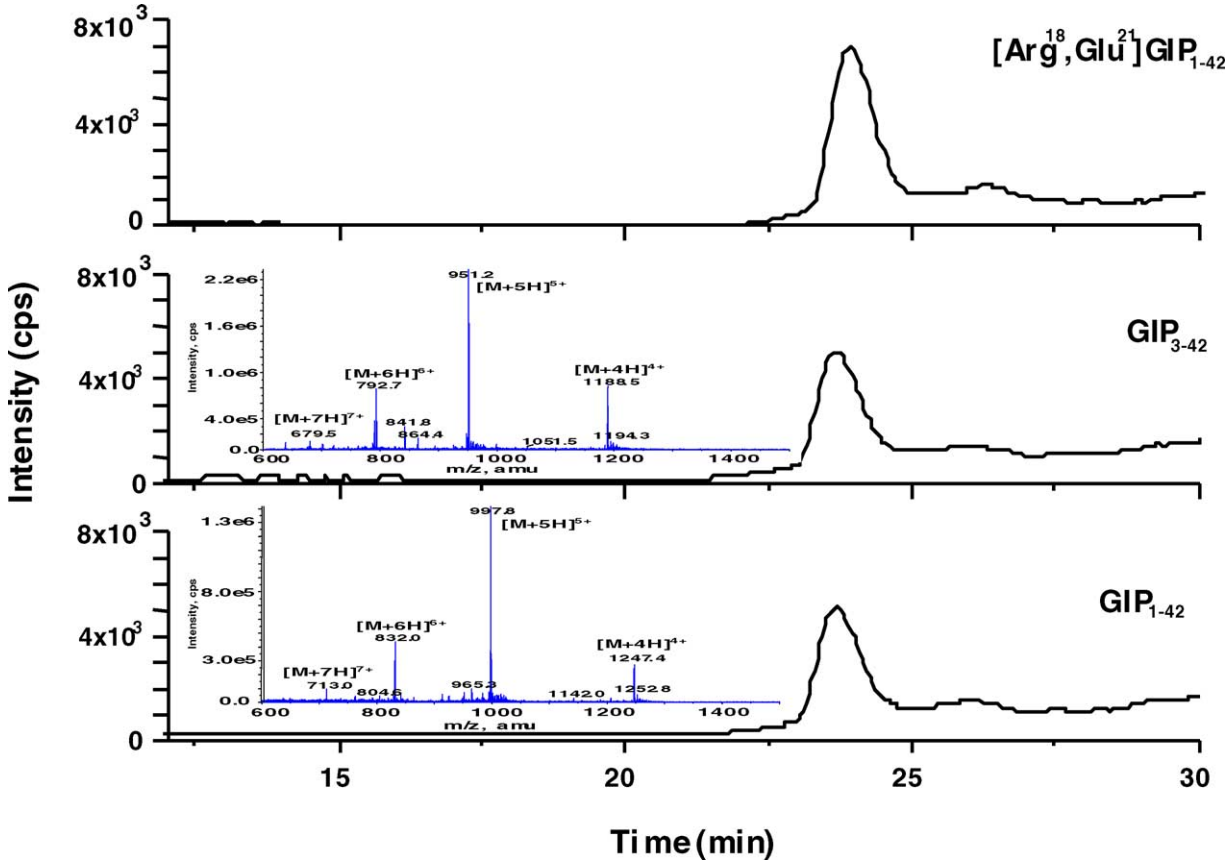

Fig. 3. Representative selected ion monitoring LC–MS chromatogram of $GIP_{1–42}$ (m/z = 997.9), $GIP_{3–42}$ (m/z = 950.9) and internal standard (m/z = 1004.4) of a plasma calibration sample (I-2) after immunoprecipitation under the described conditions. The insets show the respective mass spectra of $GIP_{1–42}$ and $GIP_{3–42}$.

quantification by the usage of the $[M + 3H]^{3+}$ peak (m/z = 1100.2) is possible. At this mass trace, no plasma interference was found but the peak intensity is lower compared to m/z = 826.2.

In case of the mass trace m/z = 773.4 (base peak of GLP-$1_{9–36}$), the increase of the baseline in HPLC causes a poor detection limit (46.69 pmol/l). Therefore, quantification was performed using mass trace $[M + 3H]^{3+}$ m/z = 1030.8.

Small peaks of endogenous $GIP_{3–42}$ and GLP-$1_{9–36}$ were detected in blank samples of human plasma used for preparation of the calibration standards. These blank peaks interfere with the exact quantification of the standards. To remove these endogenous incretins a protease treatment of the plasma as described in the Section 2.2 was performed. By this treatment, the interfering endogenous incretins could be successfully hydrolyzed resulting an improvement of lower limit of quantification. Effects on immunoprecipitation and LC–MS by changes in the plasma matrix were not observed.

3.3. Calibration

Calibration curves over the concentration range from of 5.54–354.3 pmol/l for $GIP_{1–42}$ and 5.24–335.5 pmol/l for $GIP_{3–42}$ and as well as 5.84–373.5 pmol/l for GLP-$1_{7–36}$ and 11.67–373.5 pmol/l for GLP-$1_{9–36}$ (see Fig. 5) were set up.

The respective LC–MS data can be fit to a quadratic function of the type:

$$y = Ax^2 + Bx + C, \quad \text{weighted } 1/y$$

Due to the saturation characteristics of the immunoprecipitation, the calibration curves can be described best by a quadratic function [27]. However, over a concentration range of one order of magnitude the curves show linearity. Study samples rarely reached concentrations exceeding the linear range.

The results and statistic parameters of the calibration samples are shown in Table 2.

Concentrations ranging from approximately 5 pmol/l (GLP-$1_{9–36}$ approximately 12 pmol/l) to approximately 335–375 pmol/l can be accurately measured from a 1 ml patient plasma sample.

3.4. Precision and accuracy

Quality control standards (n = 4) containing all incretins were assayed at three concentration levels (for method pre-

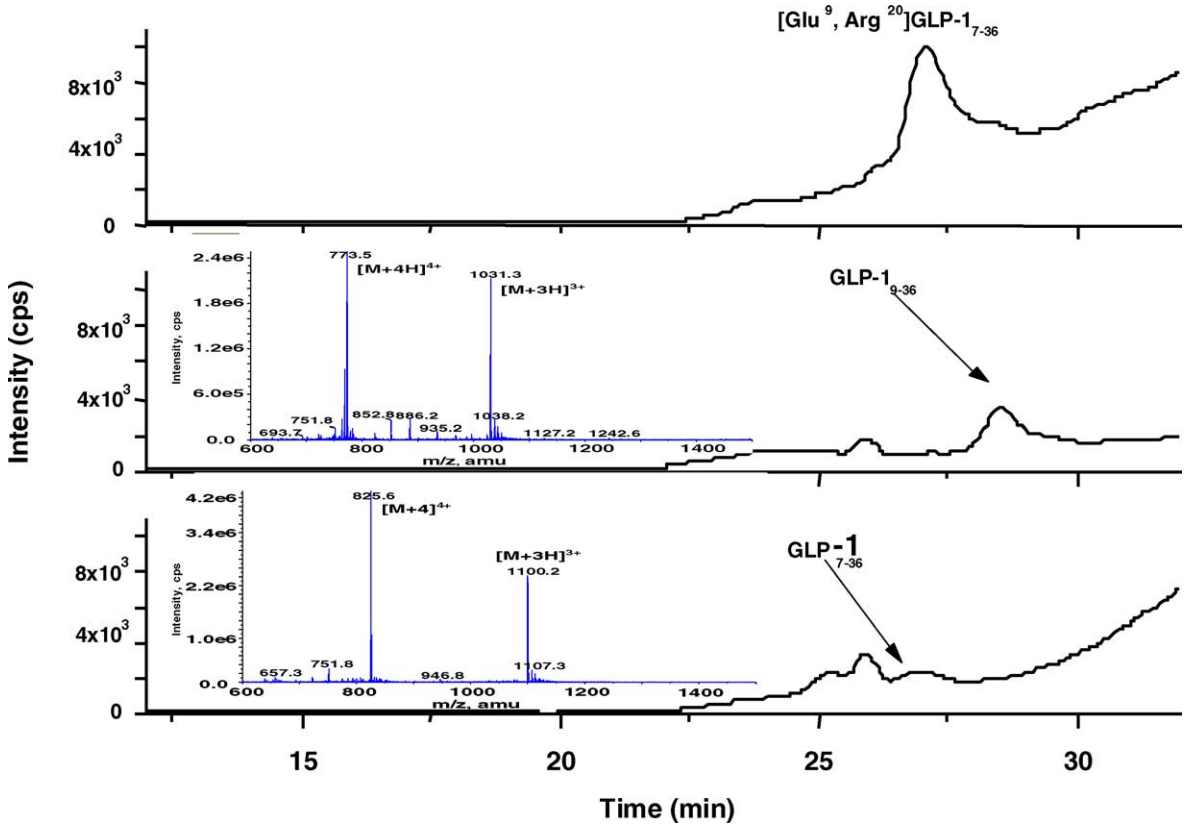

Fig. 4. Representative selected ion monitoring LC–MS chromatogram of GLP-$1_{7–36}$ (m/z = 826.2), GLP-$1_{9–36}$ (m/z = 1030.8) and internal standard (m/z = 836.2) of a plasma calibration sample (I-2) after immunoprecipitation under the described conditions. The insets show the respective mass spectra of GLP-$1_{7–36}$ and GLP-$1_{9–36}$.

cision additionally one QC, n = 7–9) with each calibration curve. Measured concentrations of the quality control standards were calculated daily from each associated calibration curve. The accuracy and precision for the QC1, QC3 and QC5 standards are presented in Table 3.

The inaccuracy was less than 7% and the precision was better than 15% of all quality control concentrations.

Low concentration quality control samples were run in between to ensure method stability. The results are summarized in Table 4.

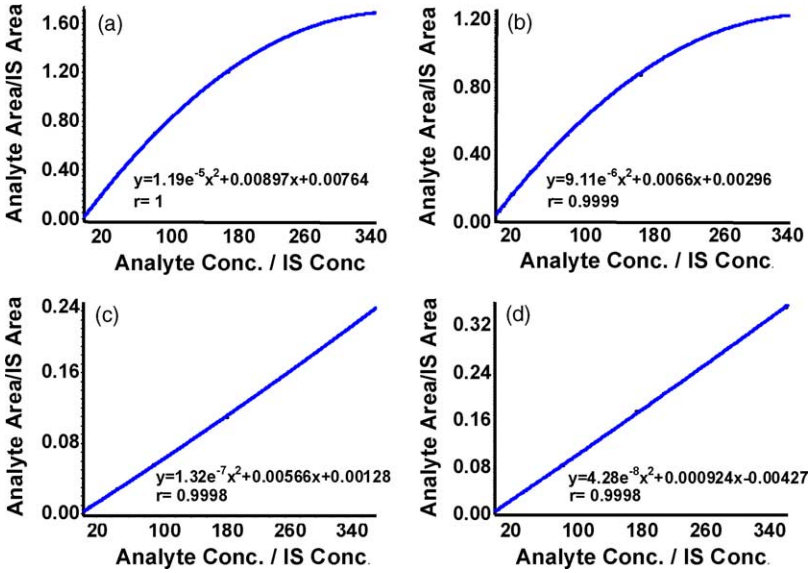

Fig. 5. Calibration curves of $GIP_{1–42}$ (a), $GIP_{3–42}$ (b), GLP-$1_{7–36}$ (c), and GLP-$1_{9–36}$ (d) in the determined range in human plasma after immunoprecipitation.

Table 2
Accuracy (% error) and precision (% CV) of calibration samples for all analytes

$GIP_{1–42}$							
Analyte concentration (pmol/l)	5.54	11.07	22.14	44.28	88.56	177.1	354.3
Calculated concentration (pmol/l) mean ($n = 4$)	5.56	10.96	22.04	45.00	88.06	177.03	355.68
S.D.	0.049	0.091	0.31	0.88	1.33	1.34	6.14
% CV	0.89	0.83	1.46	1.96	1.51	0.76	1.73
% error	0.32	−0.97	−0.44	1.63	−0.57	−0.04	0.39
$GIP_{3–42}$							
Analyte concentration (pmol/l)	5.24	10.48	20.97	41.97	83.86	167.7	335.4
Calculated concentration (pmol/l) mean ($n = 4$)	5.11	10.36	21.22	42.93	83.40	166.50	338.48
S.D.	0.21	0.30	0.45	0.80	0.78	1.51	2.15
% CV	4.09	2.94	2.12	1.87	0.94	0.90	0.63
% error	−2.56	−1.12	1.17	2.28	−0.55	−0.72	0.92
$GLP\text{-}1_{7–36}$							
Analyte concentration (pmol/l)	5.84	11.67	23.34	46.69	93.38	186.80	373.50
Calculated concentration (pmol/l) mean ($n = 4$)	5.54	11.79	23.45	47.82	95.39	181.90	376.18
S.D.	0.31	0.52	0.07	1.06	0.60	0.97	1.42
% CV	5.56	4.37	0.32	2.21	0.63	0.53	0.38
% error	−5.18	1.05	0.47	2.43	2.15	−2.62	0.72
$GLP\text{-}1_{9–36}$							
Analyte concentration (pmol/l)	5.84	11.67	23.34	46.69	93.38	186.80	373.50
Calculated concentration (pmol/l) mean ($n = 4$)	n.a.	11.61	23.68	46.51	93.37	186.90	373.50
S.D.	n.a.	0.042	0.48	1.58	1.34	3.59	1.24
% CV	n.a.	0.36	2.02	3.40	1.44	1.92	0.33
% error	n.a.	−0.51	1.47	−0.38	−0.01	0.08	0.00

Table 3
Accuracy and precision of quality control samples for all analytes

	$GIP_{1–42}$			$GIP_{3–42}$		
Analyte concentration (pmol/l)	7.38	29.52	118.1	6.99	27.95	111.8
Calculated concentration (pmol/l) mean ($n = 4$)	7.57	31.51	117.2	6.51	29.52	111.3
Accuracy (%)	102.54	106.80	99.23	93.12	105.62	99.52
Mean inaccuracy (%)	±2.76	±6.80	±1.12	±6.88	±5.62	±2.93
Precision (% CV)	2.88	2.061	2.06	8.80	1.94	4.17
% error	2.53	6.73	−0.76	−6.88	5.61	−0.47
	$GLP\text{-}1_{7–36}$			$GLP\text{-}1_{9–36}$		
Analyte concentration (pmol/l)	7.78	31.13	124.5	7.78	31.13	124.5
Calculated concentration (pmol/l) mean ($n = 4$)	8.17	32.23	119.13	n.a.	31.02	126.63
Accuracy (%)	105.05	103.52	95.68	n.a.	99.65	101.71
Inaccuracy (%)	±6.65	±7.45	±9.64	n.a.	±4.805	±7.59
Precision (% CV)	6.19	10.46	13.62	n.a.	5.65	8.28
% error	5.03	3.53	−4.31	n.a.	−0.35	1.71

Table 4
Accuracy and precision of a low concentration quality control sample to determine the method precision

	$GIP_{1–42}$	$GIP_{3–42}$	$GLP\text{-}1_{7–36}$	$GLP\text{-}1_{9–36}$
Analyte concentration (pmol/l)	14.76	13.98	15.56	15.56
Calculated concentration (pmol/l) mean	14.49 ($n = 9$)	14.53 ($n = 9$)	15.29 ($n = 7$)	14.23 ($n = 7$)
Accuracy (%)	98.16	103.91	97.19	91.48
Mean inaccuracy (%)	±3.37	±5.16	±12.18	±12.73
Precision (% CV)	3.68	4.89	14.31	13.86
% error	−1.85	3.93	−1.697	−8.53

Table 5
Accuracy and precision of LLOQ samples for all analytes

	$GIP_{1–42}$	$GIP_{3–42}$	GLP-$1_{7–36}$	GLP-$1_{9–36}$
Analyte concentration (pmol/l)	5.54	5.24	5.84	5.84
Calculated concentration (pmol/l) mean	5.64 ($n = 4$)	4.82 ($n = 4$)	5.77 ($n = 5$)	n.a.
Accuracy (%)	101.72	92.02	98.87	n.a.
Mean inaccuracy (%)	±3.83	±7.98	±5.88	n.a.
Precision (% CV)	4.38	6.15	7.26	n.a.
% error	1.73	−7.98	−1.14	n.a.

3.5. Lower limit of quantification

In addition to the calibration standard curves, the lowest concentrations of all analyzed incretins was determined several times. All samples meet the specified criteria (below ±20%) for accuracy and precision, see Table 5.

4. Discussion

Recently, we developed a method for quantitative determination of $GIP_{1–42}$ and $GIP_{3–42}$ in human plasma by immunoprecipitation LC–MS [25]. Using this method, it was possible to detect these gastrointestinal peptide hormones in a concentration range from 5 to 350 pmol/l and to 370 pmol/l, respectively. However, one major drawback of this method is the high plasma volume of 1.9 ml necessary for each determination. Here, we demonstrate that this sample volume can be reduced by improvement of the sample pretreatment and immunoprecipitation procedure. Moreover, a relative reduction of the sample volume was possible by simultaneous detection of multiple parameters in the same sample.

The introduction of several centrifugation steps and a protease pretreatment of the plasma used for standard preparation which depletes endogenous peptide hormones was found to be crucial factor to reach a sensitivity of 5 pmol/l for the GIP peptides with 1.0 ml of plasma.

It could be demonstrated that the additional of determination GLP-$1_{7–36}$ and GLP-$1_{9–36}$ did not influence the determination of the GIP peptides. A prerequisite was that the antibodies (Ab) do not interfere with each other concerning binding to the beads. This could finally be achieved by using anti peptide antibodies from different species, a polyclonal rabbit Ab and a monoclonal mouse Ab for GIP and GLP-1, respectively. Moreover, the above described method has proven useful in combination with the use of biotinylated Ab and streptavidin coupled beads (or primary Ab coupled covalently to the beads) to detect other possibly important peptides in the same plasma sample.

The application of the described method in initial human studies demonstrates that the detection range for the GIP forms is sufficient for the determination of its basal levels and postprandial release [28]. In contrast, the sensitivity of the GLP-1 detection does not allow a safe quantification of basal plasma levels.

Although quantitative immunoprecipitation is not yet a standard technique and further research is needed to optimize its performance (plasma volume, sensitivity), the present results clearly demonstrate its potential. This is especially important for a growing number of microanalytical approaches for bioanalytical applications.

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