# Detecting pM concentrations of prostaglandins in cell culture supernatants by capillary SCX-LC-MS/MS

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Running title:

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Non-standard abbreviations:

| | |
|---|---|
| AcOH | Acetic acid |
| cLC | Capillary LC |
| EIC | Extracted ion chromatogram |

| | |
|---|---|
| EtOH | Ethanol |
| FA | Formic acid |
| FCS | Fetal calf serum |
| hMSC | Human mesenchymal stem cells |
| IL-17 | Interleukin 17 |
| IN | indomethacin |
| MeOH | Methanol |
| MRM | Multiple reaction monitoring |
| NH4FA | Ammonium formate |
| PG | Prostaglandin |
| RP | Reversed phase |
| SCX | Strong cation exchange |
| TNF$\alpha$ | Tumor necrosis factor $\alpha$ |

**Keywords**

human mesenchymal stem cells, LC-MS/MS, on-line SCX-RP, prostaglandins

**Abstract**

A highly sensitive, improved on-line strong cation exchange (SCX) – reversed phase (RP) capillary liquid chromatographic (cLC) method with ion-trap mass spectrometric (IT-MS/MS) detection for the simultaneous determination of prostaglandin (PG) $A_1$, $PGD_2$, $PGE_1$, $PGE_2$, $PGF_{2\alpha}$, 8-iso-(8i)$PGF_{2\alpha}$, 6-keto-(6k)$PGF_{1\alpha}$ and 15-$\Delta^{12,14}$-deoxy-$PGJ_2$ (15d$PGJ_2$) in cell culture supernatants was developed and validated. Pre-treatment of the cell culture supernatants included only dilution and filtration, and the analysis time including all sample preparation steps was 60 min per sample. Peptides/proteins contained in the matrix were removed by the SCX column. Limits of detection in the range of 8-44 pg/mL (25-120 pM) cell culture supernatant were obtained. Excellent linearity ($R^2$>0.99) and satisfactory recoveries and within- and between-day precisions were obtained.

Human mesenchymal stem cells (hMSCs) were stimulated with TNF$\alpha$ or TNF$\alpha$/IL-17, and prostaglandin production was analysed using the developed method. The four prostaglandins, 6k$PGF_{1a}$, $PGF_{1a}$, $PGE_2$ and $PGE_1$ were detected both in non-stimulated and stimulated cells. The amount of PG produced by the cell increased when the cell was stimulated.

## 1. Introduction

Prostaglandins (PGs) are potent biologically active lipid molecules formed by most cells in the body and they have various biological functions [1]. Upon stimulation or cell activation by mechanical trauma, cytokines, growth factors or other inflammatory

stimuli, arachidonic acid is released from membrane lipids. $PGH_2$ is metabolized from arachidonic acid by cyclooxygenase (COX)-1 and COX-2. Prostaglandins are formed by $PGH_2$ metabolizing enzymes and are either released by the cell to activate receptors on other cells or enter the nucleus to activate receptors there [2].

Human mesenchymal stem cells (hMSCs) secrete $PGE_2$ (unpublished manuscript, [3]) and it has been suggested that $PGE_2$ secreted by hMSCs plays an important role in hMSC-mediated immunomodulation [4]. To investigate the molecular mechanism behind the immunomodulating effects of hMSCs, the prostaglandin profile of stimulated hMSCs needs to be characterized, i.e. knowledge of which PGs are produced and released by hMSCs upon stimulation.

Previously, we reported that non-stimulated hMSCs release $PGE_2$, and stimulated hMSCs release $PGE_1$ and $PGE_2$ (unpublished manuscript, [3]). However, we also found evidence for other PGs to be present at lower concentrations. With a method described previously, we were not able to reliable quantify other interesting PGs, such as $PGA_1$, $PGD_2$, $PGF_{2\alpha}$, $6kPGF_{2\alpha}$ and $15dPGJ_2$ in hMSCs. Thus, in the present study we pursued to increase the method's sensitivity.

In cLC-MS, higher sensitivity can be achieved either by reducing the inner diameter of the column [5, 6] or by injecting larger sample volumes [7, 8] or a combination of both. Reducing the column inner diameter from 0.3 mm (cLC) to 0.1 mm (nanoLC) however, requires a nano-ESI-interface.

Therefore, we chose to increase the injection volume 10 times, from 50 µL to 500 µL by simply scaling up our existing on-line two-dimensional strong cation exchange (SCX) – reversed phase (RP) capillary liquid chromatographic (cLC) method with ion-trap mass spectrometric (IT-MS/MS) detection.

Due to the complexity of the matrix, an on-line SCX peptide/protein removal step was included prior to reversed phase chromatography for the simultaneous determination of $PGA_1$, $PGD_2$, $PGE_1$, $PGE_2$, $PGF_{2\alpha}$, $8iPGF_{2\alpha}$, $6kPG_{1\alpha}$ and $15dPGJ_2$ (Figure 1). These eight prostaglandins, for which standards were available, were also included in the present method.

## 2. Materials and methods

### *2.1. Cell culture and cell stimulation*

hMSC-TERT20 (a cell line from bone marrow-derived human mesenchymal stem cells transduced with a gene encoding telomerase reverse transcriptase) [9] were grown in RPMI-1640 without phenol red (PAA Laboratories, Pasching, Austria) supplemented with 10% fetal calf serum (FCS, PAA), but without phenol red. Cells were maintained in a humidified incubator at 37°C and 5% $CO_2$ (AGA, Oslo, Norway) and detached when near confluent by incubating with 0.25% trypsin (PAA). The seeding density was 8500 cells/$cm^2$.

hMSC-TERT20 cells were seeded at a density of 1.6 x $10^5$ cells/$cm^2$ and stimulated with either tumor necrosis factor α (TNF$\alpha$), TNF$\alpha$/interleukin-17 (IL-17) or were incubated for 24h in the presence of 100 µM indomethacin (IN) (Sigma, St. Louis, MO, USA) before harvesting the supernatants which were diluted and injected.

*2.2. Chromatographic system*

A schematic presentation of the system is given in figure 2. Valve 1 (V1) contained the sample loop which was made from poly-etheretherketone (PEEK) and was used to inject sample volumes of 500 µL. A HotSep micro pump (pump 1) from G&T Septech (Oslo, Norway) was used to deliver the SCX mobile phase containing 70:30 (v/v) 1 mM ammonium formate (pH 3.0, prepared by mixing equimolar amounts of formic acid (FA, Fluka, Buchs, Switzerland) and ammonia (Merck, Darmstadt, Germany) – EtOH (Arcus, Oslo, Norway) at a flow rate of 50 µL/min. On injection, the positively charged components of the matrix were trapped on a 1 (i.d.) x 50 mm, 5 µm polysulfoethyl aspartamide (PolyLC, Columbia, USA) strong cation exchange (SCX) column. Under these conditions, the non-charged prostaglandins passed through the SCX column without retention and were diluted with water containing 1% MeOH (BDH Hipersolv, Poole, UK) – 0.1% FA in grade 1 water (Milli Q ultrapure water purification system, Millipore, Bedford, MA, USA), which was delivered by a Hitachi L-7110 isocratic LC pump from Merck (dilution pump), prior to trapping on the reversed phase trap column. The flow rate used for the dilution was 100 µL/min, and the dilution pump and the outlet of the SCX column were connected through a MicroTee T-piece with MicroTight tubing sleeves from Upchurch Scientific. The diluted eluate from the SCX column entered the reversed phase system through a piece of 100 µm i.d. fused-silica tubing at a combined flow rate of 150 µL/min. The prostaglandins were trapped on a 1 (i.d.) x 5 mm Tracy trap column packed with 5 µm Kromasil $C_{18}$ (G&T Septech) connected to the second ten-port valve (V2). When trapping was complete, V2 was switched and the prostaglandins were backflushed onto a 0.3 (i.d.) x 100 mm, 3.5 µm Kromasil $C_{18}$ (Eka-Nobel, Bohus, Sweden)

analytical column by a linear solvent gradient from 35 to 55 % B in 10 min followed by a step to 100 % B which was held for 10 min to elute the most hydrophobic prostaglandins and to wash the column. Mobile phase reservoir A contained 0.1% AcOH (Merck), while mobile phase reservoir B contained ACN (Rathburn, Walkerburn, UK) – MeOH – AcOH (95:5:0.1, v/v). The mobile phase gradient was delivered by an Agilent 1100 capillary gradient pump (Palo Alto, CA, USA) with an incorporated on-line vacuum degasser (pump 2) at a flow rate of 5 µL/min.

Simultaneously with the reversed phase separation, 1 M ammonium formate (pH 3.4) – EtOH (90:10, v/v) was used to wash (backflush) the SCX column and 1 mM ammonium formate (pH 3.0) – EtOH (70:30, v/v) was used for equilibration (20 min) before the next sample was injected. An equilibration time of 10 min was applied between each solvent gradient in the reversed phase separation.

Pump 1 was used to initiate sample injection and pump 2 was started manually at the same time. Thirty minutes after injection, V1 was switched back to load position by pump 1 and V2 was switched manually to start position (backflushing trap column) and the data acquisition was started.

Both the SCX and the RP analytical column were slurry-packed in glass-lined stainless steel housing using a downward high pressure liquid slurry method developed in-house.

### *2.3. Mass spectrometric detection*

The prostaglandins were detected by an Esquire 3000+ iontrap – MS instrument (Bruker Daltonics, Bremen, Germany) equipped with a low-flow nebulizer. The IT-MS instrument was operated in the negative mode with optimum conditions as described

before [3]. Data were acquired using the Esquire Control v 3.5 by Bruker Daltonics in the MRM mode. Helium (99.9999%) to operate the MS was purchased from AGA (Oslo, Norway).

Segmented MS detection was performed since the MS/MS cycle time is reduced if only one precursor ion is to be isolated/fragmented, and higher sensitivity is achieved. To increase the sensitivity additionally, the sum of several fragments was chosen for quantification as in [3].

### *2.4. Standard solutions and sample preparation*

1 mg/mL stock solutions of $PGA_1$, $PGD_2$, $PGE_1$, $PGE_2$, $PGF_{2\alpha}$, $8iPGF_{2\alpha}$, $6kPGF_{1\alpha}$, $15dPGJ_2$ and the internal standards $PGD_2$-$d_4$ and $PGF_{2\alpha}$ -$d_4$ (all from Cayman Europe, Talinn, Estonia) were made by dissolving 1 mg prostaglandin in 1 mL EtOH.

Standard solutions and spiking solutions were prepared by appropriate dilution of the stock solutions with 30% EtOH containing 1% FA.

For validation of the method, 240 µL of cell culture supernatant (hMSC-TERT + IN) were spiked with 10 µL of a solution containing both prostaglandins and internal standards. For determination of prostaglandins released by non-stimulated or stimulated cells, 240 µL of cell culture supernatant were added 10 µL of a solution containing the internal standards.

The samples were diluted 4 times by adding 750 µL 30% EtOH containing 1% FA and allowed to rest for 15 min before filtering through an Acrodisc 0.2 µm nylon syringe filter (Pall Life Sciences, Ann Arbour, MI, USA), and 500 µL of this solution were injected onto the chromatographic system.

The concentrations of PGs in the spiked samples (hMSC-TERT + IN) used for

calibration and validation of the method are shown in Table 1. The calibration curve was made using either the internal standard method, where the ratio of the areas were plotted against the concentration of analyte in the calibration solutions (spiked samples (hMSC-TERT + IN)), or the external standard method.

Gelöscht: Table 1

Eingefügt: Table 1

All spiked solutions were stable for at least 21 days when kept at -21°C in a freezer.

## 3. Results and discussion

### *3.1. Method Optimalization*

Prostanoids are released by stem cells upon stimulation; however their concentration is highly variable [3]. With our previously developed method concentrations in the range of 0.4-2.2 ng/mL cell culture supernatant could be determined [3]. However, other prostaglandins possibly present at lower concentrations could not be reliably quantified. As mentioned in the introduction, one possibility for detecting lower concentrations is to increase the injection volume. In this case it was increased 10 times from 50 µL to 500 µL by simply scaling up the existing method.

Due to high back-pressure on the SCX column the loading flow rate was limited to 50 µL/min, thus the loading time had to be 30 min (3x loop volume). All prostaglandins eluted from the SCX column within the loading time. In order to accomplish sufficient dilution (3x) of the SCX column eluate for trapping on the RP column, the dilution pump had to deliver a flow of 100 µL/min. Possible breakthrough of the prostaglandins on the trap column at high flow rate used (150 µL/min) over an extended time (30 min) was checked, but no breakthrough was observed.

However, during repeatability testing, the signal of spiked sample was found to be much lower than the signal of the standards (diluted with 30% EtOH and 1% FA),

This was anticipated to be due to signal suppressing from components contained in the matrix.

To investigate whether this was the case or not, two blank samples were injected (Figure 3), one using an injection volume of 50 µL and one using an injection volume of 500 µL. An interference isobaric to 8iPGF$_{2\alpha}$, PGF$_{2\alpha}$ and PGE$_1$ eluted in a narrow band after 6.5 min. It was clearly separated from the PGs when an injection volume of 50 µL was used, while it was much broader, covering several minutes and overlapping with the eluting PGs when an injection volume of 500 µL was used. Thus, because of the 10 times higher amount of matrix injected onto the system, severe signal suppression was observed.

Gelöscht: Figure 3

Eingefügt: Figure 3

The interference was identified to be phenol red which has a molecular mass of 354.38 and interferes with PGE$_1$, PGF$_{2\alpha}$ and 8iPGF$_{2\alpha}$ having the same molecular mass. Thus, the RPMI 1640 cell culture medium was modified. Phenol red has no function other than pH control and it could thus be excluded from the medium.

Previously, 30mM ammonium formate was used as modifier in the reversed phase separation,, in order to reduce interferences by phenol red [3], however, high salt concentrations (> 10mM) should be avoided when MS is used as detection because of ionization suppression. Wu *et.al.* described, that small amounts of weak carboxylic acids, such as AcOH, can be used to increase the negative-ion ESI response [10]. Thus, 0.1% AcOH was chosen as modifier in the reversed phase separation of the present system.

### *3.2. Method validation*

Culture supernatants from hMSC-TERT cultivated in the presence of 100 µM indomethacin as a cyclooxgenase inhibitor were used as matrix, and spiked matrix samples were used to validate the developed method on 4 consecutive days.

With the developed method, a mass limit of detection (mLOD) of 1 ($PGA_1$), 2 ($PGE_1$, $PGE_2$, $15dPGJ_2$), 3 ($PGF_{2\alpha}$, $8iPGF_{2\alpha}$), 3.5 ($PGD_2$) and 5.5 ($6kPGF_{1\alpha}$) pg (on-column) was obtained respectively (Table 2) injecting 500 µL obtained from a 240 µL sample diluted to 1 mL. The mLOD and the mass limit of quantification (mLOQ) were determined as the amount of analyte giving a signal to noise ratio of 3 and 10, respectively. The mLOD is given in pg on column, and to calculate the method concentration limit of detection (cLOD), the mLOD is divided by the injection volume and multiplied with 4 to take the dilution into account. Hence 2 pg $PGE_2$ corresponds to a concentration of 16 pg/mL cell culture supernatant or 45 pM. Thus, using this method we were able to improve the limit of detection by a factor of 5 [3]. Other publications report similar [11-13] (or possibly lower [14]) LODs using triple quadrupole instruments.

Gelöscht: Table 2
Eingefügt: Table 2

Theoretically, by increasing the injection volume 10x, a 10x lower detection limit than reported [3] should be obtained. However, when 500 µL of a sample is injected onto the system more matrix components are injected which leads to more severe background and thus more signal suppressing. One possibility to get around this problem is to include an off-line sample preparation step; however, this would lead to a greater loss of sample and analyte, and most probably increasing the analysis time.

The relative recovery of prostaglandins was calculated for three different concentration levels (1, 3 and 5) of spiked samples (Table 1) using

Gelöscht: Table 1
Eingefügt: Table 1

$$\frac{\text{area of spiked sample/area of IS}\,(n=3)}{\text{area of standard solution/area of IS}\,(n=3)}\cdot 100\,\% \ .$$

This represents the total relative method recovery including the dilution and filtration steps. In general recoveries of more than 80% were obtained. However, the relative recoveries for level 1 and 3 varied between 40 and 250% (Table 2) dependent on PG and concentration level. The poor relative recoveries can be explained by a high

Gelöscht: Table 2
Eingefügt: Table 2

variation in internal standard signal. The concentration of the internal standards was quite low, and signal variability at low concentration levels is often observed. This might explain some of the variability of the internal standard signal at level 1 and 3. Low concentrations of internal standards were chosen since low concentrations of analytes were expected and thus to avoid signal suppression of the analyte signal by its corresponding internal standard as described by Liang and co-workers [15]. Due to co-elution of the internal standards with their corresponding analyte, ion-suppression of the internal standards was observed for level 5.

Signal suppression by compounds in the matrix also occurred. Because of this latter signal suppression the internal standard had to be included in calculation of the recovery, since the PG signal was suppressed too. Additionally, PGs and internal standards might have interactions with matrix components retained on the SCX column, and these may result in poorer recoveries. However, these interactions should be similar for PGs and internal standards and hence corrected for by the calibration procedure.

All samples were prepared with spiked matrix for calibration, repeatability testing and analysis of real samples. Thus, a reliable quantification could be made even though the recovery calculated without including internal standards was poor, since the within and between day precisions were satisfactory.

Calibration curves were made using spiked hMSC-TERT + IN with and without internal standards. All calibration curves, except that of 15dPGJ$_2$ and PGE$_1$, showed excellent linearity with $R^2$-values higher than 0.99 (Table 2) both with and without internal standard. Thus, the method can also be used without addition of internal standards.

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**Gelöscht:** Table 2

**Eingefügt:** Table 2

The within- and between-day precisions measured as RSD (Table 3) were determined for three different concentration levels (1, 3 and 5) of spiked samples (hMSC-TERT + IN). The precisions were calculated without using the internal standard, since the RSD was higher when the internal standards were included (data not shown). For level 3 and 5, all prostaglandins showed a within- and between day precision of less than 30% RSD, and some PGs even well below 20%.

Blank matrix was diluted as described above and injected to check mass measurement interferences of the analytes or internal standards. However, no such interference was observed (data not shown) and thus can be ruled out to be responsible for the variation observed.

The variance is the sum of the analyte signal variance and internal standard signal variance when using internal standard for quantification. However, when lower RSD is obtained without internal standard calculation, this points towards that the variation of the internal standard signal is the limiting factor. As discussed above, the internal standards were added in low concentrations due to the low concentrations of analytes expected. Thus, higher variation of the signal is expected, which also was observed at the lowest concentration level of the PGs. However, since the method includes few sample preparation steps and the sample is only filtrated and diluted prior to injection, reliable quantifications were also obtained without internal standards since good linearity and precisions were obtained.

Gelöscht: Table 3
Eingefügt: Table 3
Gelöscht: ¶ Generally, the RSDs were higher
Gelöscht: as expected

### *3.3. Application*

hMSC-TERT20 cells were cultured either in the presence of TNFα, in the presence of TNFα and IL-17 or without stimuli, and culture supernatants were analyzed using the

present method. Culture supernatants from cells grown in the presence of 100 µM indomethacin (a cyclooxygenase inhibitor) were used as control samples.

Low concentration of four prostaglandins, 6kPGF$_{1\alpha}$, PGF$_{2\alpha}$, PGE$_2$ and PGE$_1$ were secreted by non-stimulated cells (figure 4-6). When the cells were stimulated with TNF$\alpha$, higher concentrations of the four PGs were secreted (figure 4-6). When a combination of TNF$\alpha$ and IL-17 was used for cell stimulation PGF$_{2\alpha}$, PGE$_1$ and PGE$_2$ concentration increased further.

Thus it can be anticipated that non-stimulated hMSC-TERT produce and secrete 6kPGF$_{1\alpha}$, PGF$_{2\alpha}$, PGE$_2$ and PGE$_1$. Upon stimulation, hMSC-TERT secrete higher concentrations of the four PGs, thus these PGs are likely to be produced upon cell-stimulation.

In our previous method [3], we were not able to detect 6kPGF$_{1\alpha}$ and PGF$_{2\alpha}$, thus by increasing the methods sensitivity more information about which PGs are secreted by hMSC-TERT was gained.

## 4. Concluding remarks

An improved sensitive and fast method using on-line SCX for sample preparation and reversed phase chromatography MS/MS for the simultaneous determination of seven prostaglandins, PGA$_1$, PGD$_2$, PGE$_1$, PGE$_2$, PGF$_{2\alpha}$, 8iPGF$_{2\alpha}$, 6kPGF$_{1\alpha}$ and 15dPGJ$_2$, in cell culture supernatant has been developed. Pre-treatment of the cell culture supernatant included only dilution and filtration prior to on-line SCX-LC-MS/MS analysis. Peptides/proteins contained in the matrix were removed by the SCX column. The method showed 5 times lower limits of detection than presented

previously [3]. Excellent linearity as well as satisfactory recovery and within- and between-day precisions were obtained. Because of a simple sample preparation with few steps, the use of an internal standard was not necessary; however, internal standards were included for method control.

It was shown that non-stimulated hMSC-TERT produce and secrete low concentrations of four PGs, 6kPGF$_{1\alpha}$, PGF$_{2\alpha}$, PGE$_2$ and PGE$_1$ and that the concentration of these PGs increases upon cell stimulation. Both non-stimulated and stimulated cells secreted two additional PGs, 6kPGF$_{1\alpha}$ and PGF$_{2\alpha}$, which were not detected previously [3].

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**Figure 1:** Structures of the prostaglandins investigated.

**Figure 2:** Schematic drawing of the on-line SCX-LC-MS/MS system.

**Figure 3:** EIC (*m/z*=353) of a 50 µL and 500 µL injection of blank TERT (hMSC-TERT + IN) and a 500 µL injection of hMSC-TERT20 + IN spiked with prostaglandins at level 5 (Table 1).

**Formatiert:** Schriftart: 12 pt, Nicht Fett, Komplexe Schriftzeichen-Schriftart: 12 pt

**Gelöscht:** Table 1

**Eingefügt:** Table 1

**Figure 4:** EIC of 6kPGF$_{1\alpha}$ in cell culture supernatant from hMSC-TERT cultivated in the presence of TNF$\alpha$, TNF$\alpha$ /IL-17 or without stimuli (only time window is shown).

**Figure 5:** EIC of PGF$_{2\alpha}$ and PGE$_1$in cell culture supernatant from hMSC-TERT cultivated in the presence of TNF$\alpha$, TNF$\alpha$/IL-17 or without stimuli (only time window is shown).

**Figure 6:** EIC of PGE$_2$ in cell culture supernatant from hMSC-TERT cultivated in the presence of TNF$\alpha$, TNF$\alpha$/IL-17 or without stimuli (only time window is shown).

**Table 1:** Concentrations of PGs used for calibration and validation of the method. All concentrations are given in pg prostaglandin/mL spiked and diluted hMSC-TERT + IN (1 mL) of which 500 µL were injected.

| | **L1** | **L2** | **L3** | **L4** | **L5** |
|---|---|---|---|---|---|
| **$PGA_1$** | 5 | 10 | 20 | 100 | 200 |
| **$PGD_2$** | 17.5 | 35 | 70 | 350 | 700 |
| **$PGE_1$** | 10 | 20 | 40 | 200 | 400 |
| **$PGE_2$** | 10 | 20 | 40 | 200 | 400 |
| **$PGF_{2\alpha}$** | 15 | 30 | 60 | 300 | 600 |
| **$8iPGF_{2\alpha}$** | 15 | 30 | 60 | 300 | 600 |
| **$6kPG_{1\alpha}$** | 27.5 | 55 | 110 | 550 | 1100 |
| **$15dPGJ_2$** | 10 | 20 | 40 | 200 | 400 |
| **$PGD_2$-$d_4$** | 15 | 15 | 15 | 15 | 15 |
| **$PGF_{2\alpha}$ -$d_4$** | 15 | 15 | 15 | 15 | 15 |

**Table 2:** Recovery [%], linearity, mLOD, cLOD and mLOQ

| | $R^2$ | $R^2$ | mLOD | cLOD | mLOQ | Recovery | | |
|---|---|---|---|---|---|---|---|---|
| **level** | **(with IS)** | **(without IS)** | **[pg]** | **[pM][a]** | **[pg]** | **L1** | **L3** | **L5[b]** |
| **$PGA_1$** | 0.994 | 0.999 | 1 | 25 | 2.5 | 57 | 63 | 15 |
| **$PGD_2$** | 0.997 | 0.993 | 3.5 | 80 | 8.8 | 131 | 61 | 47 |
| **$PGE_1$** | 0.980 | 0.997 | 2 | 45 | 5 | 93 | 142 | 59 |
| **$PGE_2$** | 0.998 | 0.995 | 2 | 45 | 5 | 126 | 168 | 59 |
| **$PGF_{2\alpha}$** | 0.997 | 0.994 | 3 | 65 | 7.5 | 59 | 82 | 56 |
| **$8iPGF_{2\alpha}$** | 0.996 | 0.993 | 3 | 65 | 7.5 | 94 | 101 | 54 |
| **$6kPG_{1\alpha}$** | 0.999 | 0.991 | 5.5 | 120 | 13.8 | 124 | 243 | 86 |
| **$15dPGJ_2$** | 0.987 | 0.984 | 2 | 50 | 5 | 38 | 101 | 11 |

[a]pM in cell culture supernatant

[b]ion suppression of IS at this level

**Table 3:** Within- and between-day RSDs [%] (without internal standard)

| | **Within-day RSD** | | | **Between-day RSD** | | |
|---|---|---|---|---|---|---|
| **Level** | **L1 (n=4)** | **L3 (n=6)** | **L5 (n=5)** | **L1 (n=3)** | **L3 (n=4)** | **L5 (n=4)** |
| **$PGA_1$** | 62 | 33 | 45 | 64 | 36 | 52 |
| **$PGD_2$** | 10 | 15 | 23 | 13 | 12 | 21 |
| **$PGE_1$** | 38 | 13 | 4 | 52 | 10 | 4 |
| **$PGE_2$** | 21 | 10 | 5 | 104 | 5 | 9 |
| **$PGF_{2\alpha}$** | 26 | 9 | 7 | 17 | 19 | 8 |
| **$8iPGF_{2\alpha}$** | 13 | 11 | 23 | 20 | 24 | 9 |
| **$6kPG_{1\alpha}$** | 29 | 24 | 33 | 19 | 26 | 12 |
| **$15dPGJ_2$** | 41 | 42 | 6 | 47 | 19 | 18 |