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Application of single-vial ready-for-use formulation of ^{111}In- or ^{177}Lu-labelled somatostatin analogs

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HIGHLIGHTS

- Optimal quencher combination: ascorbic- and gentisic acid and ethanol.
- Used quencher concentrations had no effect on pharmacokinetics.
- Purging the reaction mixture with N_2 after radiolabelling resulted in 10% higher RCP.
- Quencher mixture stabilize ^{111}In- and ^{177}Lu-labelled SS-analogs during 7 days.
- Enables to store and transport ^{111}In- and ^{177}Lu-labelled SS-analogs in a single-vial.

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ABSTRACT

For the sake of safety it would be desirable to store and transport the ready-for-use liquid formulation (diagnostics and therapeutics) of radiolabelled peptides. The use of ethanol, in combination with a mixture of gentisic- and ascorbic acid, has superior effects on stabilizing radiolabelled somatostatin analogs. As a consequence, ^{111}In- and ^{177}Lu-labelled somatostatin analogs can be stored and transported in a single-vial ready-for-use liquid formulation up to 7 days after radiolabelling.

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1. Introduction

Here we describe the presence of quenchers (de Blois et al., 2012) in a single-vial liquid pharmaceutical formulation of a radiolabelled peptide, in a quantity sufficient to prevent radiolysis of the formulation. Radiolabelled peptides are generally stored and transported in the form of multi-vial kit formulations. Usually the contents of these vials are lyophilized or frozen and should be brought into solution subsequently in a mutual reaction to produce the intended radiolabelled peptide. For the sake of safety it would be desirable to be able to store and transport the ready-for-use liquid formulation of the radiolabelled peptide. Then, a physician could administer the labelled peptide without a radiochemical reaction, simply by diluting the contents of the vial in a radiopharmaceutical liquid that can be administered by injection or by infusion (Filice et al., 2012).

During labeling, storage and transport of the somatostatin analogs (further referred as SS-analogs) the peptide is exposed to radicals produced by *i.e.* ^{177}Lu or ^{111}In as radionuclide. Radiolysis of aqueous solutions produces reactive species (•OH, H•, e^-_{aq}, H_2O_2) that may react with the peptide in the reaction (Garrison, 1987; Jay-Gerin and Ferradini, 2000; Jonah, 1995; Swiatla-Wojcik and Buxton, 2005). Here we describe how to preserve high radiochemical purity – and thus to increase the storage and transport time of radiolabelled SS-analogs – with the use of quenchers. The literature reports successful addition of quenchers such as gentisic acid (Liu and Edwards, 2001), ascorbic acid (Chen et al., 2008; Liu et al., 2001, 2003), methionine (Breeman et al., 2008) and ethanol (Chen et al., 2008; Filice et al., 2012; Fukumura et al., 2004; Schuessler, 1975; Erion et al., 2008) to the reaction mixtures in various combinations and concentrations prior to radiolabelling to prevent radiolysis.

In this study, stability and radiolysis of radiolabelled SS-analogs were monitored by HPLC. HPLC methods were optimized to distinguish between non-labelled and radiolabelled peptides *vs.* the radiolysed peptides. The effect of quenchers on the stability of

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radiolabelled SS-analogs, even under therapeutic conditions was optimized and monitored up to 7 days after radiolabelling.

2. Methods and materials

2.1. $^{111}In/^{177}Lu$ labelling of SS-analogs

DOTA-TATE ([DOTA^0,Tyr^3]octreotate), DOTA-NOC ([DOTA^0-Nal^3]octreotide) and DTPA-octreotide ([DTPA^0]-octreotide) were purchased from BioSynthema, (St Louis, MO, USA). $^{111}InCl_3$ was purchased from Covedien (Petten, The Netherlands) and $^{177}LuCl_3$ from IDB Holland (Baarle Nassau, The Netherlands). During optimisation, the typical reaction mixture for radiolabelling consisted of 60 MBq of ^{177}Lu- or $^{111}InCl_3$ in 0.01–0.05 M HCl with 2 nmol peptide dissolved in Milli-*Q* water, sodium acetate as buffer (≤ 2 μL of 2.5 M) and 10 μL of quenchers in a final volume of 0.14 mL (final pH 4–4.5). To inhibit oxidation and radiolysis, quenchers were added in various combinations and concentrations prior to radiolabelling as described in paragraph 2.4. Quenchers included ascorbate (Bufa BV, Uitgeest, The Netherlands), gentisic acid (Tyco Health Care, Petten, The Netherlands), ethanol (Sigma-Aldrich Zwijndrecht, The Netherlands) and methionine (Fluka Biochemika, Switzerland). Radiolabelling of DOTA-TATE and other DOTA-conjugated SS-analogs with ^{111}In or ^{177}Lu requires heating for 15 min at 80 °C (Breeman et al., 2005). DTPA-peptides were incubated for 10 min at room temperature (20–22 °C) (Chavatte et al., 2001). After cooling to room temperature, quality control of DTPA- and DOTA-peptides was performed. Quality control includes incorporation of ^{111}In or ^{177}Lu as measured by ITLC-SG (Bakker et al., 1991) and RCP of radiolabelled DTPA- and DOTA-peptides as measured by HPLC (de Blois et al., 2012). RCP of radiopeptides was determined as function of time post radiolabelling at room temperature at regular time intervals.

In order to avoid false positive quality control results due to colloid formation (Breeman et al., 2007a; Liu and Edwards, 2001), quality control of DOTA-peptides was assessed after the addition of 5 μL 4 mM DTPA post radiolabelling (Breeman et al., 2003a, 2003b).

Any non-incorporated ^{111}In or ^{177}Lu will be rapidly captured by the addition of DTPA. ^{111}In-DTPA, and ^{177}Lu-DTPA after i.v. administration, will be rapidly excreted via the kidneys (Breeman et al., 2004, 2003b).

DOTA-TATE labelling at therapeutical level (patient's dose) was performed under the kit formulation as previously reported (Breeman et al., 2006) in a concentrated form (60 GBq in 3 mL). After QC Lu-DOTA-TATE was diluted with saline (100 mL) for patient infusion. We investigated the storing conditions of a ^{177}Lu-labelled DOTA-TATE and DOTA-NOC patient dose (3.7–7.4 GBq) after dilution with saline (5–100 mL) to maintain high RCP.

2.2. HPLC

HPLC grade methanol and trifluoroacetic acid (TFA) were purchased from Mallinckrodt Baker (Deventer, The Netherlands). All other chemicals were purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands). SS-analogs were analysed with a HPLC system (Breeze, Waters, Etten-Leur, The Netherlands), containing a 1525 binary pump and a UV detector (W2487 Waters Dual λ Absorbance Detector). UV absorption was measured at 278 nm. A Symmetry C_{18} column (5 mm × 4.6 mm × 250 mm, Waters, Etten-Leur, The Netherlands) was used with a gradient profile as described earlier (de Blois et al., 2011), mobile phase 0.1% TFA (A) and methanol (B). Sample injections on the HPLC were < 200 μL). Radioactivity was monitored with a system including a NaI detector, digital multichannel analyzer and dedicated software (MetorX B.V, Goedereede, The Netherlands), connected to the HPLC system.

2.3. Dosimetry within the reaction vial

In order to investigate the influence of dose (Gy) on the radiolysis of SS-analogs, dose in the reaction mixture during the labelling procedure and storage was calculated according to the spherical geometry dosimetry model (Stabin and Konijnenberg, 2000). This model derives the absorbed dose rates in the vials used. The complete emission spectrum of the specific nuclides (^{111}In and ^{177}Lu) was taken into account used in the calculations. Dose was also calculated during storage up to 7 days using different volumes (5, 50 and 100 mL) containing a therapeutical amount (3.7 GBq) of ^{177}Lu-DOTA-TATE.

2.4. Optimizing quencher concentration

To obtain maximum protection of radiolabelled SS-analogs and minor effect in pharmacokinetics, quencher concentration in reaction mixture was investigated time dependently. DOTA-TATE was radiolabelled with 60 MBq ^{111}In or ^{177}Lu in the presence of different concentrations of quenchers and 2 nmol of DOTA-TATE in a final volume of 0.14 mL. Ascorbic acid and gentisic acid were investigated with final concentrations of 1–20 mM, 1–50 mM for methionine and 2–20% (v/v) for ethanol.

2.5. Radiolabelled SS-analogs in the presence of quenchers mixtures

Applied quencher concentrations after optimization in reaction vial were: 3.5 mM for ascorbic acid and gentisic acid, 10 mM for methionine and 7–10% (v/v) for ethanol. Single quenchers and combinations of those quenchers were applied and RCP's were measured by HPLC up to 7 days after radiolabelling. RCP measurements were stopped when RCP decreased below 50%.

2.6. Purging labelling mixture with nitrogen (N_2) or oxygen (O_2)

To investigate any influence of O_2 on the formation of radicals, which would lower the RCP, the reaction mixture after radiolabelling was purged for 1 h with N_2 or O_2 (100 mL/min). We hypothesized that N_2 would decrease the O_2 concentration and thereby positively influence RCP. In contrast, purging with O_2 would increase the oxygen concentration and thereby influence RCP negatively. RCP was measured by HPLC up to 7 days after radiolabelling.

2.7. Maintaining RCP and biological activity (receptor affinity)

Autoradiography with stabilized ^{111}In-DOTA-TATE (Fig. 3A) was performed as previously reported (de Jong et al., 2001; de Visser et al., 2007; Hofland et al., 1999) on rat brain sections up to 7 days after radiolabelling. Internalisation with stabilized ^{111}In-DOTA-TATE was performed using CA20948 somatostatin receptor expressing cells as described previously (Bernard et al., 2000), also up to 7 days after radiolabelling.

3. Results

3.1. Dosimetry within the reaction vial

Dose (Gy) within reaction mixture during labelling procedure

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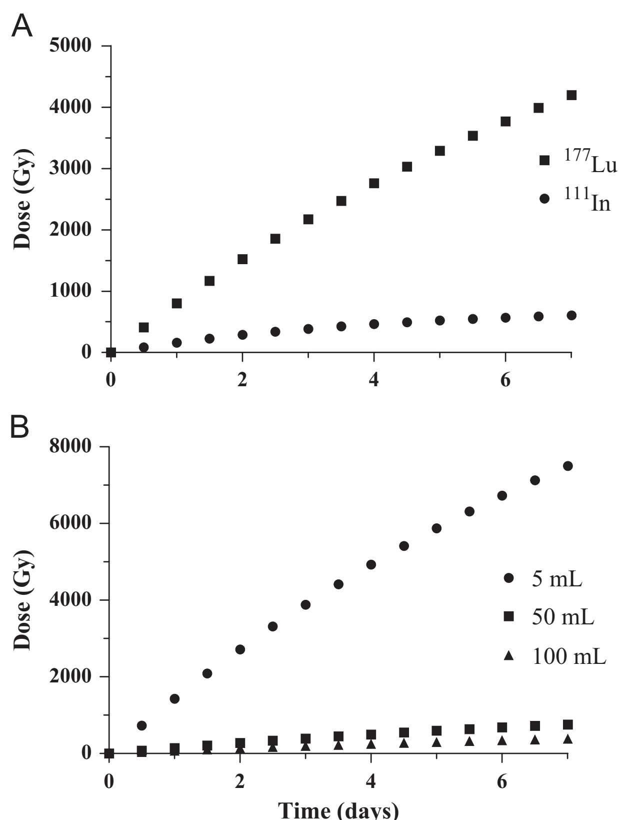

Fig. 1. Dose calculations spherical geometry dosimetry model (Stabin and Konijnenberg, 2000). *X*-axis shows time (days); *Y*-axis dose (Gy). (A) Doses for ^{111}In- and ^{177}Lu-containing vials within standardised reaction mixture up to 7 days of storage. Reaction mixture containing 60 MBq of ^{177}Lu- or $^{111}InCl_3$ labelled SS-analog in a final volume of 0.14 mL. In comparison to ^{111}In, Therapeutic nuclide ^{177}Lu resulted in a 5 times higher dose (Gy). (B) Dose calculations using different volumes (5, 50 and 100 mL) were performed up to 7 days of storage for vials containing 3.7 GBq of ^{177}Lu.

Fig. 1a shows the differences is dose with a factor of 5. Fig. 1b shows the calculated dose during storage using different volumes (5, 50 and 100 mL) of saline containing a therapeutical amount (3.7 GBq) of ^{177}Lu. Maximum dose was obtained (7.5 kGy) in a volume of 5 mL saline.

3.2. Optimizing quencher concentration

Quencher concentrations in reaction mixture were investigated time dependently by measuring RCP during storage time. Each quencher was investigated separately for its minimal concentration with maximum quenching effect (Fig. 2). Quencher mixtures were optimized to achieve maximum protection using these minimal concentrations. Under the experimental conditions the optimal quencher concentrations were 3.5 mM for ascorbic and gentisic acid, 10 mM for methionine and 10% (v/v) for ethanol. These concentrations had no effect on pharmacokinetics, so complete incorporations (> 99%) within described incubation time.

3.3. Radiolabelled SS-analogs in the presence of quencher mixtures

Reaction mixture (60 MBq ^{111}In or ^{177}Lu, 2 nmol DOTA-TATE in 0.14 mL) containing ascorbic and gentisic acid (3.5 mM) and extra addition of ethanol clearly stabilized ^{111}In-DOTA-TATE. Addition of ethanol 10% (v/v) stabilize ^{111}In-DOTA-TATE during 7 days; higher concentrations (> 10% (v/v)) have no additional effect (Fig. 3A and B). We also investigated the influence of ethanol on the RCP of solutions of OctreoScan® ([^{111}In-DPTA^0]octreotide) during storage.

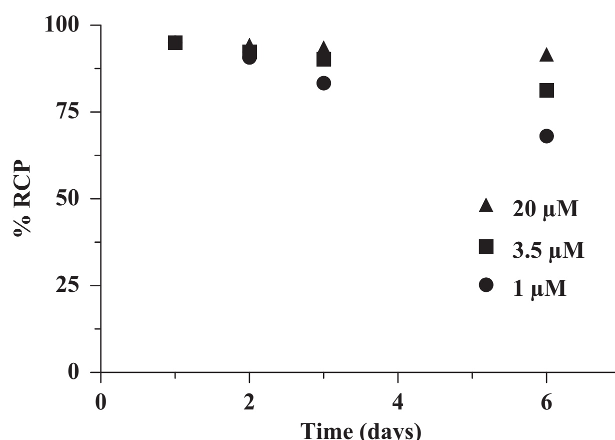

Fig. 2. ^{111}In-DOTA-TATE in the presence of three different concentrations of ascorbic acid. *X*-axis shows time (days); *Y*-axis RCP (%). Labelling was performed with 60 MBq ^{111}In and 2 nmol of DOTA-TATE in a final volume of 0.14 mL. This figure shows the effect of quencher concentration over time. Under same conditions, ^{177}Lu-DOTA-TATE showed similar results.

available kit formulation (OctreoScan®) after reaction of their ingredients; this commercial kit contains a mixture of gentisic- and ascorbic acid as quenchers. Addition of ethanol to the gentisic- and ascorbic acid containing OctreoScan® kit (see instructions for use) even improve stability of radiolabelled peptide (Fig. 4A and B).

The optimal quencher mixtures as described above was tested within a broad range of specific activity of ^{111}In-DOTA-TATE (50–190 MBq/nmol, Fig. 5A) and activity concentration (0.14–1.4 GBq/mL, Fig. 5B). The results were similar to those for RCP (Figs. 3 and 4).

Fig. 6 shows the optimized storing conditions of ^{177}Lu-DOTA-TATE and ^{177}Lu-DOTA-NOC. Comparable results were obtained with ^{177}Lu-DOTA-TATE at a therapeutical level (3.6–7.4 GBq, see Fig. 7) and maintained high RCP after dilution in saline (5–100 mL).

3.4. Purging labelling mixture with N_2 and O_2

Purging the reaction mixture with N_2 after radiolabelling resulted in ± 10% higher RCP after 5 days of storage. Purging with O_2 resulted in RCP values obtained with traditional storage (data not shown).

3.5. Maintaining RCP and biological activity (receptor affinity)

In order to prove the biological activity of the radiolabelled SS-analogs, we studied internalisation and autoradiography during the 7 days of RCP monitoring (Fig. 4). Results showed a constant and specific binding and internalisation of SS-analogs (data not shown) (Breeman et al., 2007b).

4. Discussion

4.1. RCP quantification

Since radiolysed radiopeptides often differ in charge and shape *vs.* structure of intact radiolabelled peptides, radiolysis of radiolabelled peptide can be separated by HPLC and quantified by radiodetection. Typically, RCP of radiolabelled SS-analogs measured by HPLC is expressed as % of radiodetected peak area (µV/s^{-1}) of the intact radiolabelled peptide *vs.* all other radio peaks measured during the same HPLC-analyses.

There are no quality criteria within the field of Nuclear

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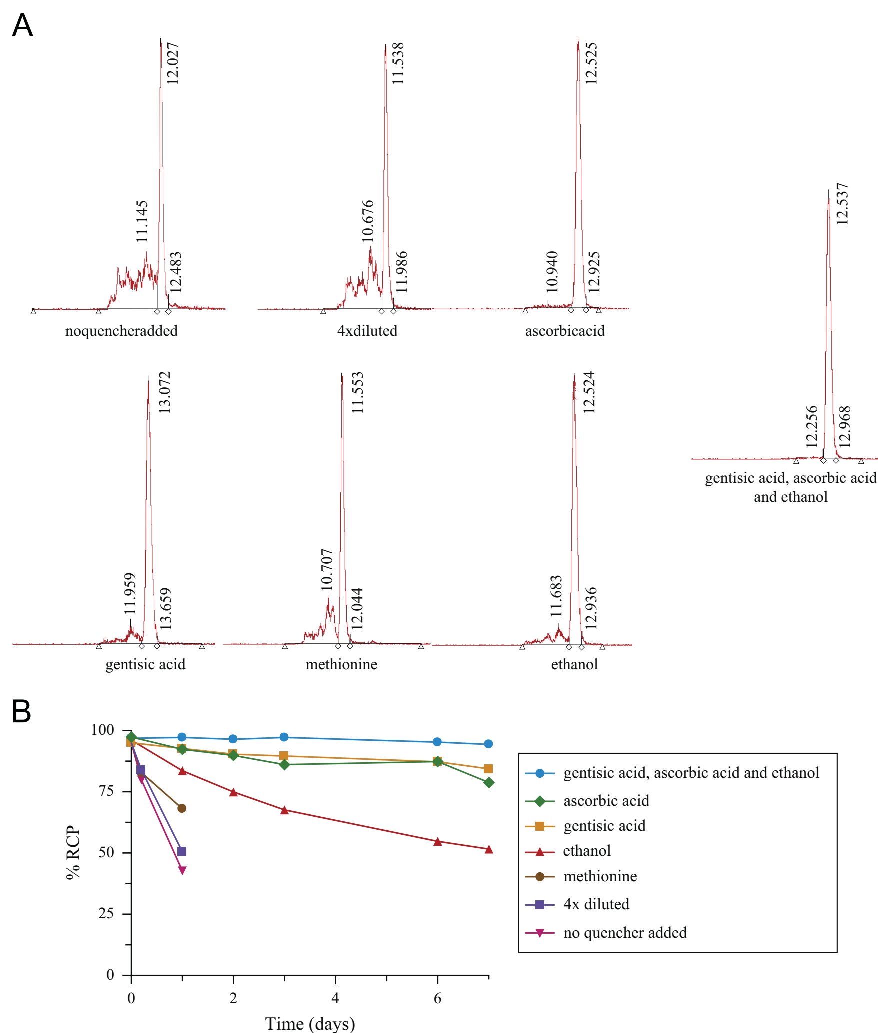

Fig. 3. (A) HPLC-chromatograms of ^{111}In-DOTA-TATE and effect of the difference quenchers and mixtures of quenchers 24 h after radiolabelling. Applied quencher concentrations after optimization (See paragraph *optimizing quencher concentration*) in reaction vial were: 3.5 mM for gentisic acid and ascorbic acid, 10 mM for methionine, and 7–10% (v/v) for ethanol. HPLC-chromatograms 24 h after radiolabelling can be compared in a relative way and show specific patterns of radiolysed peptide in the presence of different quenchers, which indicates that quenchers specifically scavenge different chemical groups and/or formed radicals (B) ^{111}In-DOTA-TATE in the presence of quenchers or combinations of quenchers up to 7 days after radiolabelling. This figure shows the effect of quenchers and combination separately. The maximum error of inter and intra-observer was $<3\%$ and were performed $n \geq 2$.

2012). Nevertheless, differences in eluents, gradient, flow, column type and length might result in the detection of different degrees of impurity, and thus variation in RCP.

Therefore, in our opinion, RCP are actually expressed in percentage of arbitrary units (de Blois et al., 2012). Moreover, to enable comparison, HPLC runs were performed under standardized conditions. All HPLC-measurements were corrected for background. Even at low activity, the influence of background on calculated RCP was $<3\%$.

4.2. Addition of ethanol as a quencher

Adding ethanol during or after labelling procedure could have severe side effects in patients with a neuroendocrine carcinoid tumor. Intravenous administration of a ^{177}Lu-labelled SS-analogs mixture containing ethanol could lead to carcinoid syndrome (Adamson et al., 1971). Long et al. (1981) showed that development of a carcinoid syndrome could be blocked by administration of somatostatin. Unfortunately there is no standard maximum tolerated amount of ethanol which can be administered to these automated system for labelling of different SS-analogs, including a C_{18} separation using 2.5 mL of 50% of ethanol, has been applied in many patients world-wide, without reports of carcinoid crisis (Petrik et al., 2011). According to Serdons et al. (2008), radiolabelled peptides can safely be used without removal of the ethanol after appropriate dilution with normal saline to a concentration of ethanol not exceeding 10% and injection volume below 20 mL.

The ready-for-use liquid formulation of the radiolabelled peptide allows physicians to administer labelled peptide without a radiochemical reaction—simply by diluting if necessary.

Users still should be aware that while dilution indeed results in lower dose radiation, however this also decreases quencher concentration and thus negatively influences RCP during further storage. Therefore after dilution radiolabelled peptide should be administered as rapidly as possible to decrease the influence of radiolysis on radiolabelled peptide. To overcome this problem we recommend diluting the liquid formulation with quencher added to maintain a constant

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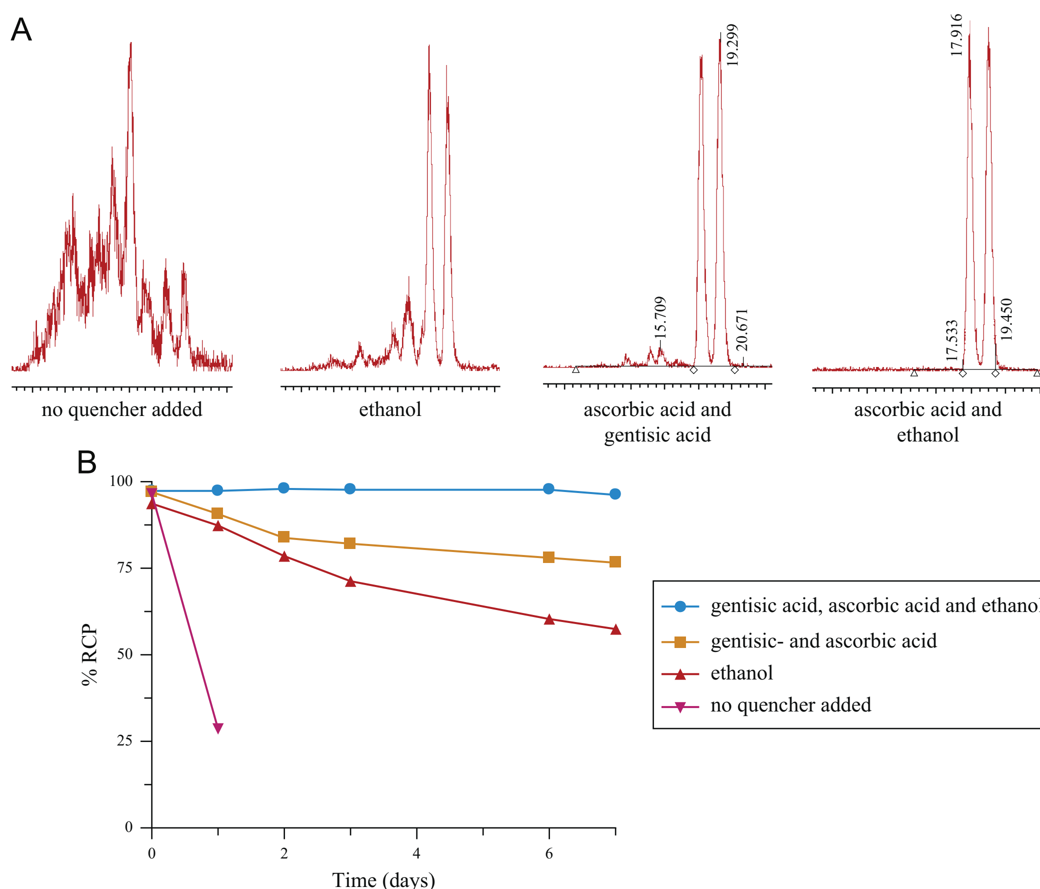

Fig. 4. (A) HPLC-chromatograms of OctreoScan® and effect of the difference quenchers and mixtures of quenchers 24 h after radiolabelling. Applied quencher concentrations after optimization as described in paragraph *optimizing quencher concentration*. HPLC-chromatograms 24 h after radiolabelling show specific patterns as well, as also described in Fig. 3A. The two major peaks (17.9–19.2 min) in the HPLC-chromatograms were caused by stereo isomers of the ^{111}In-labelled DTPA (Liu et al., 2001). The two major pre-peaks seen after radiolysis were caused by stereo isomers of ^{111}In-DTPA (see HPLC-chromatogram of gentisic and ascorbic acid). (B) OctreoScan® in the presence of quenchers or combinations of quenchers. *X*-axis shows time (days); *Y*-axis % RCP. These figures show the effect of quenchers and combination up to 7 days after radiolabelling.

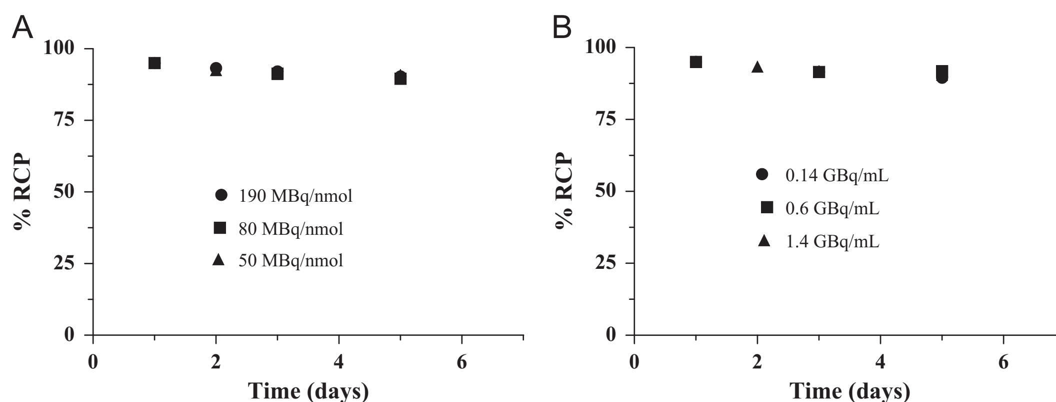

Fig. 5. ^{111}In labelled DOTA-TATE as function of time in the presence of gentisic acid, ascorbic acid and 7% of ethanol (v/v) and the influence of specific activity (50–190 MBq/nmol) (A) or by decrease of the concentration of activity (0.14–1.4 GBq/mL) (B). Labelling was performed in a final volume of 0.14 mL and only the peptide amount (1–4 nmol) or activity (50–190 MBq) was adjusted. *X*-axis shows time (days); *Y*-axis RCP (%). Both figures show the applicability of the quencher mixture within a broad ranch of specific activities, thus independent of the peptide mass or activity concentration (MBq/mL).

Moreover sterility is still a matter of concern, there are microbiological implications for 7-day shelf-life. When the ready-for-use liquid formulation of the radiolabelled peptide will be used over a longer time period sterility should be determined before administration, this could be performed by measuring endotoxins in final

5. Conclusion

Our experiments showed that ethanol, in combination with a mixture of gentisic- and ascorbic acid has a superior effect in stabilizing radiolabelled peptides. This property enables to store

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