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# Cation exchange-based post-processing of $^{68}$Ga-eluate: A comparison of three solvent systems for labelling of DOTATOC, NO2AP$^{BP}$ and DATA$^{m}$

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## HIGHLIGHTS

- Comparison of different $^{68}$Ga post-processing methods through the labelling of DOTATOC, NO2AP$^{BP}$ and DATA$^{m}$.
- Comparison in terms of radiochemical yield, reproducibility and radiolysis.
- Ethanol and acetone post-processed $^{68}$Ga facilitated the highest yields and reproducibility.
- Ethanol post-processed $^{68}$Ga resulted in the lowest degree of radiolysis of $^{68}$Ga-DOTATOC.
- Experimenting with different post-processing methods is an important optimisation step.
- Ethanol-post processed $^{68}$Ga is suitable for clinical application.

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## ABSTRACT

Interest in $^{68}$Ga has led to a number of innovations for its provision suitable for clinical application. Several post-processing methods are available to reduce eluate volume and remove metal trace impurities. In this work three cation exchange resin based post-processing methods (acetone, ethanol and NaCl) have been compared, using three model precursors (DOTATOC, NO2AP$^{BP}$ and DATA$^{m}$), in terms of labelling yield and reproducibility. The acetone and ethanol based methods provided greater reproducibility and yields that makes subsequent purification unnecessary.



## 1. Introduction

By virtue of its favourable radiochemical characteristics ($t_{½}$= 67.7 min, $\beta^+$ yield~89%), $^{68}$Ga has emerged as a promising candidate for non-invasive diagnostic imaging within Positron Emission Tomography (PET). Early examples of the $^{68}$Ge/$^{68}$Ga generator date back to the 1960s, but interest faded in the 1970s as the generator setup didn't fulfil the requirements for routine clinical application (Gleason, 1960; Roesch and Riss, 2010; Roesch, 2013). In the 21st century renewed interest in $^{68}$Ga initiated improvements to the availability and purity of this promising positron emitter.

Routine clinical application of $^{68}$Ge/$^{68}$Ga generators has been restricted in part by metal impurities in the generator eluate; in particular, breakthrough of the generator parent nuclide $^{68}$Ge. To circumvent these problems, and ensure that the $^{68}$Ga derived meets (European) Pharmacopoeia standards, different methods of purifying the eluate have been developed. The simplest of these is the fractionation method, whereby the eluate is fractionated and only fractions with the highest content of $^{68}$Ga are collected and used for labelling procedures (Breeman et al., 2005). Metal impurities such as Ge(IV), Fe(III), Ti(IV) and Zn(II) are not removed directly from the solution in this case, but rather lowered based on differing rates of elution from the generator (Šimeček et al., 2013). Another approach uses ion exchange resins (cationic, anionic or mixed mode) to eliminate metal ion impurities eluted from the

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generator (Loktionova et al., 2011; Meyer et al., 2004; Mueller et al., 2011; Schuhmacher and Maier–Borst, 1981; Zhernosekov et al., 2007). The general principle is that the $^{68}$Ga is trapped on the resin from the initial generator eluate, and subsequently eluted separate from the metal impurities – in the process the concentration of the $^{68}$Ga solution is increased. Various combinations of solvent systems and cationic resins to make purification and concentration possible have been developed over the past ten years.

The first reported cationic exchange resin based post-processing system uses mixtures of acetone and hydrochloric acid to remove metal impurities from the resin in a first washing step whilst $^{68}$Ga is retained on the column. In a second step, pure $^{68}$Ga is eluted in a small volume (400 μL) using a solution which is predominantly acetone based (Zhernosekov et al., 2007). This method provides highly reproducible labelling yields, and has been successfully applied to different types of labelling precursors. However, acetone does not have approval for *in vivo* application and therefore must be removed prior to injection of the final formulation.

To address this problem, a protocol has been published recently in which acetone is substituted by ethanol (Eppard et al., 2014). The ratio of ethanol to HCl in the washing and elution solutions was adjusted to obtain similar results in yield and purity of $^{68}$Ga as the acetone method. Provided that the final formulation contains less than 10% ethanol and the radiochemical purity is $>95\%$, further purification is not necessary (Serdons et al., 2009). Therefore, a simple dilution with saline is sufficient to obtain an injectable tracer solution meeting the requirements of pharmaceutical regulations. Of further benefit, the use of ethanol has also been shown to enhance labelling yields and hinder radiolysis (Perez–Malo Cruz and Roesch, 2012). Following a similar principle, other groups have sought to replace the organic component entirely (Martin et al., 2014; Mueller et al., 2012). In one such example $^{68}$Ga is eluted from the cation exchange resin with an acidified 5 M NaCl solution (500 μL 5 M NaCl and 12.5 μL 5.5 M HCl). The absence of any organic solvents means that the final formulation does not require gas chromatography-based quality control for organic solvents.

Following these recent publications and discussions researchers in the field we saw the need for a direct comparison of the three main solvent systems for post-processing of the $^{68}$Ge/$^{68}$Ga generator eluate. The three main post-processing methods permit complete removal of $^{68}$Ge-breakthrough, but to the best of our knowledge a study comparing the three has not yet been carried out or reported (Zhernosekov et al., 2007; Eppard et al., 2014; Mueller et al., 2012). Specifically, we were interested in how the different methods would influence subsequent labelling procedures, yields and reproducibility. With most sites opting to install a single post-processing method, such a comparison is of special interest for established and prospective setups. To conduct this comparison three model precursors were selected (Fig. 1), namely DOTATOC: which is used for detection of neuroendocrine tumours, NO2AP$^{BP}$: which is suitable for detection of bone metastases, and DATA$^{m}$: a recently reported chelator based on the 6-amino-diazepine scaffold (Parker et al., 2013; Parker and Waldron, 2013; Waldron et al., 2013).

## 2. Materials and methods

### 2.1. General

Chemicals were purchased from Sigma-Aldrich® or Merck® and used without further purification. NO2AP$^{BP}$ and DATA$^{m}$ were synthesised according to literature procedures (Holub et al., 2014; Waldron et al., 2013). DOTATOC was purchased from ABX (Radeberg, Germany). For all post-processings a $^{68}$Ge/$^{68}$Ga generator ($TiO_2$-based matrix, Cyclotron Co. Obninsk, Russia) was used, and eluted with 0.1 M HCl (5 or 10 mL). Solutions for eluting the generator and performing post-processing (N1-6 and NaCl) were prepared according to literature (Eppard et al., 2014; Mueller et al., 2012; Zhernosekov et al., 2007). (N1: 80% Acetone, 0.15 M HCl, 1000 μL; N2: 97.56% Acetone, 0.05 M HCl, 400 μL; N4: 80% EtOH, 0.15 M HCl, 1000 μL; N5: 90% EtOH, 0.9 M HCl, 1000 μL; N6: 5 M NaCl, 5.5 M HCl, 512.5 μL). Radio-TLC was performed with silica TLC-plates (silica 60 $F_{254}$, $5 \times 4.5$ cm, Merck) and analysed using a flatbed-imaging scanner (Instant Imager, Canberra Packard).

Radio-TLC plates relating to evaluations of DATA$^{m}$ and DOTATOC were developed in 0.1 M citrate buffer (pH 4). NO2AP$^{BP}$ labelling kinetics were analysed using analytical HPLC (LiChrospher 100 RP-18, 1 mL/min, acetonitrile and water each containing 0.1% TFA were used as mobile phase). Analytical HPLC (LiChrospher 100-RP-18EC, 0.8 mL/min) of $^{68}$Ga-DOTATOC for radiolysis monitoring was based on a gradient using water (0.1% TFA) and acetonitrile. Detection was carried out with a UV (Hitachi L-7400) and a radioactivity (Gamma Raytest) detection system. Solvents were obtained as HPLC grade and degassed by ultra-sonication for 15–20 min before use. pH measurements were conducted using a calibrated pH metre (Mettler-Toledo, SevenEasy pH, Switzerland). All experiments were performed in triplicate.

### 2.2. $^{68}$Ge/$^{68}$Ga-generator elution procedures (Table 1)

Acetone-based method: The generator was eluted with 5 mL of 0.1 M HCl. $^{68}$Ga is trapped on the cation exchange resin, whilst metal cation contaminants are retained to a lesser extent. Residual metal impurities were eluted from the resin with solution N1, whilst $^{68}$Ga is retained. The purified $^{68}$Ga was eluted with solution N2 and used for radiolabelling without further modification. The resin was reconditioned with 4 M HCl and water.

Ethanol-based method: The generator was eluted with 5 mL of 0.1 M HCl. $^{68}$Ga trapped on the cation exchange resin is washed with solution N4 to remove residual metal impurities. The purified $^{68}$Ga was eluted with solution N5, and used for radiolabelling without further modification. Reconditioning of the resin was performed as per the acetone method.

NaCl-based method: Preconditioning of the resin for the NaCl solvent system was performed with 5 M HCl and water. $^{68}$Ga was eluted from the generator and trapped on the resin using 10 mL of 0.1 M HCl. Solution N6 was slowly passed over the resin to elute $^{68}$Ga ready for radiolabelling.

Specific details regarding the type of resin and resulting eluate acidity for each post-processing method are provided in Table 1.

### 2.3. $^{68}$Ga-labelling procedures (Table 2)

Specific details regarding the content of the labelling media, precursor concentrations and varied parameters are provided in Table 2.

The amount of each precursor used for labelling was kept constant for all experiments with a given precursor, and selected from previous radiolabelling experience. Precursor concentrations differed for each solvent system as different volumes of labelling media and eluate were used. A labelling protocol for each combination of precursor and post-processing method is provided. For DOTATOC and NO2A$^{BP}$ the temperature of the labelling reaction was varied, and for DATA$^{m}$ the pH of the reaction was varied. Outside of these variations the pH, precursor amount and temperature were kept constant.

Acetone method: DOTATOC was dissolved in $H_2O$ and preheated in a heater-shaker device. The $^{68}$Ga containing solution

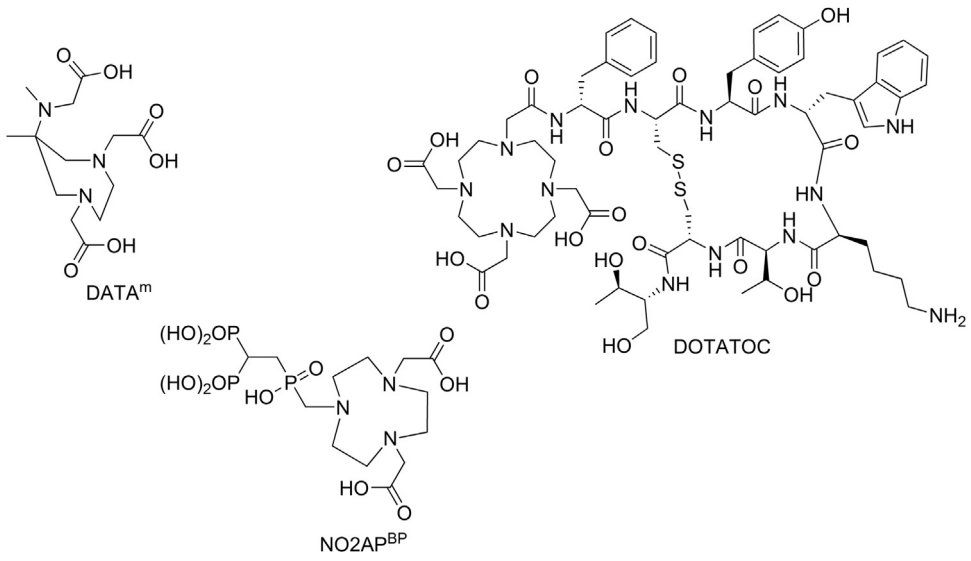


**Fig. 1.** Model precursors (DOTATOC, NO2AP$^{BP}$, DATA$^{m}$) labelled with $^{68}$Ga post-pocessed by each of the three methods (acetone, ethanol and NaCl based).

**Table 1**
Specific details of the different post-processing methods evaluated (Eppard et al., 2014; Mueller et al., 2012; Zhernosekov et al., 2007).

| Method | Resin | Vol. of 0.1 M HCl for elution (mL) | $H^+$ in eluate (mol) | Vol. of purified $^{68}$Ga eluate (μL) |
|---|---|---|---|---|
| Acetone | Bio-Rad AG50WX8-400 | 5 | 2∗10−5 | 400 |
| Ethanol | Bio-Rad AG50WX4 | 5 | 9∗10−4 | 1000 |
| NaCl | Merck Lichrolut SCX | 10 | 7∗10−5 | 512.5 |

(400 μL N2) was added. TLC samples were taken at 1, 3, 5, 10 and 15 min. Labelling of DOTATOC has been carried out in buffer media (0.2 M NaOAc), but was not pursued as the protocol of choice here since it did not offer any discernible advantage in terms of precursor concentration or temperature.

NO2AP$^{BP}$ was dissolved in NaOAc-buffer and preheated in a heater-shaker device and the $^{68}$Ga containing solution (400 μL N2) was added. HPLC samples were taken at 1, 2, 10 and 20 min.

DATA$^{m}$ was dissolved in NaOAc-buffer and placed in a shaker device. The $^{68}$Ga containing solution (400 μL N2) was added at room temperature (22–24 °C) and the solutions agitated on a shaker device. TLC samples were taken at 1, 2, 10 and 20 min.

Ethanol method: DOTATOC was dissolved in $NH_4OAc$-buffer and preheated in a shaker-heater device. The $^{68}$Ga containing solution (1000 μL N5) was added. TLC samples were taken at 1, 3, 5, 10 and 15 min.

NO2AP$^{BP}$ was dissolved in $NH_4OAc$-buffer and preheated in a heater-shaker device. The $^{68}$Ga containing solution (1000 μL N5) was added. HPLC samples for monitoring were taken at 1, 2, 10 and 20 min.

DATA$^{m}$ was dissolved in $NH_4OAc$-buffer of and placed in a shaker device. The $^{68}$Ga containing solution (1000 μL N5) as added at room temperature (21–24 °C). TLC samples were taken at 1, 2, 10 and 20 min.

NaCl method: DOTATOC was dissolved in 3 mL $H_2O$ containing 300 μL 1 M $NH_4OAc$-buffer and preheated in a heater-shaker device. The $^{68}$Ga containing solution (512.5 μL N6) was added. TLC samples were taken at 1, 3, 5, 10 and 15 min.

NO2AP$^{BP}$ was dissolved in 3 mL water containing 700 μL 1 M $NH_4OAc$-buffer and preheated in a heater-shaker device. The $^{68}$Ga containing solution (512.5 μL N6) was added. The reactions were monitored at 1, 2, 10 and 20 min using HPLC.

DATA$^{m}$ was dissolved in 3 mL water containing 400 μL 1 M $NH_4OAc$-buffer of pH 5 or 6 and placed in a shaker device. The $^{68}$Ga containing solution (512.5 μL N6) was added at room temperature (22–24 °C). TLC samples were taken at 1, 2, 10 and 20 min.

Labelling procedures for examination of radiolysis of $^{68}$Ga-DOTATOC were carried out in a similar manner to those described earlier using the optimum parameters for each post-processing method. Analyses of the radiolabelled compounds were conducted via radio-HPLC at $t=0$, 45 and 90 min.

## 3. Results and discussion

Initially, optimal labelling conditions for each precursor were determined for each of the three post-processing methods. In order to evaluate the versatility of different methods the radiolabelling parameters (temperature, pH, buffer concentration, buffer type) have been varied systematically starting from the optimised conditions. Taking into consideration future clinical application, only buffers approved for *in vivo* application were used for labelling media. While labelling efficiencies for the two compounds NO2AP$^{BP}$ and DATA$^{m}$ for the three post-processing methods reported here present novel data, $^{68}$Ga labelling of DOTATOC can be compared with several publications (Eppard et al., 2014; Mueller et al., 2012; Zhernosekov et al., 2007). A common

**Table 2**
Labelling parameters for the different solvent systems investigated.

| Substance | nmol chelator | Parameter variations | Acetone labelling media | Ethanol labelling media | NaCl labelling media |
|---|---|---|---|---|---|
| DOTATOC | 14 | 80 °C, 95 °C | 4 mL $H_2O$ | 1 mL 1 M $NH_4OAc$ | 3 mL $H_2O$; 300 μL 1 M $NH_4OAc$ |
| NO2AP$^{BP}$ | 11 | 40 °C, 60 °C | 5 mL 0.2 M NaOAc | 5 mL 1 M $NH_4OAc$ | 3 mL $H_2O$; 400 μL 1 M $NH_4OAc$ |
| DATA$^{m}$ | 10 | pH 4, pH 5 | 1 mL 0.2 M NaOAc | 1.1 or 1.5 mL 1 M $NH_4OAc$ | 3 mL $H_2O$; 400 μL 1 M $NH_4OAc$ |

**Table 3**
Radiolabelling results for each of model precursors performed using $^{68}$Ga post-processed by the three different methods.

| Precursor | nmol | Parameter | Acetone method | | Ethanol method | | NaCl method | |
|---|---|---|---|---|---|---|---|---|
| | | | Time of 95% (min); σ | Max yield (%) [t (min)] | Time of 95% (min); σ | Max yield (%) [t (min)] | Time of 95% (min); σ | Max yield (%) [t (min)] |
| DOTATOC | 14 | 80 °C | / | 93.9 ± 3.7 [15] | 1; ± 2.2 | 97.6 ± 0.3 [10] | 5; ± 1.1 | 96.4 ± 2.5 (15) |
| | | 95 °C | 10; ± 1.0 | 97.7 ± 0.6 [15] | 3; ± 1.5 | 98.0 ± 0.6 [15] | / | 85.6 ± 4.7 (15) |
| NO2A$^{BP}$ | 11 | 40 °C | / | 94.9 ± 3.5 [20] | 2 ± 0.6 | 98.0 ± 0.6 [20] | / | 85.0 ± 1.0 (20) |
| | | 60 °C | 2; ± 0.1 | 99.3 ± 0.6 [20] | 10; ± 0.2 | 99.3 ± 1.5 [20] | / | 93.7 ± 0.6 (20) |
| DATA$^{m}$ | 10 | pH 4 | 2; ± 0.2 | 99.0 ± 0.2 [20] | / | 93.4 ± 3.3 [20] | / | 19.2 ± 4.2 (20) |
| | | pH 5 | 1; ± 0.5 | 99.3 ± 0.1 [20] | 10; ± 0.3 | 98.5 ± 0.3 [20] | / | 92.9 ± 3.6 [20] |

problem experienced during radiopharmaceutical preparation is radiolysis of the radiolabelled product when larger amounts of $^{68}$Ga are used Eppard et al. (2014) noted that radiolabelling using ethanol post-processed $^{68}$Ga showed a significantly lower degree of radiolysis compared to that when acetone post- processed $^{68}$Ga was used. To examine this is effect DOTATOC was labelled, under optimum conditions, with a greater amount of $^{68}$Ga processed by each of the three post-processing methods.

### 3.1. Radiolabelling of model precursors

The model precursors were labelled with each of the three methods, and one labelling parameter varied for each combination i.e., pH of 4 and 5 for DATA$^{m}$, 80 and 95 °C for DOTATOC, 40 and 60 °C for NO2AP$^{BP}$. An overview of this is provided in Table 3.

Radiolabelling using acetone post-processed $^{68}$Ga: The maximum yield for DOTATOC obtained after 15 min at 80 °C was 94%, which would require a further purification step before *in vivo* application. At 95 °C a maximum yield of 97% was achieved after 15 min with excellent reproducibility. At 60 °C, NO2AP$^{BP}$ showed yields > 95% after 2 min and 99% after 20 min with a high reproducibility. At 40 °C labelling was slower, with yields < 95% after 20 min. The acetone method showed very good labelling characteristics for DATA$^{m}$, with yields of > 95% after 1 (pH 5) and 2 min (pH 4) and a high degree of reproducibility. Even higher yields (≥ 99%) were achieved after 20 min for both pH values.

Radiolabelling using ethanol post-processed $^{68}$Ga: The ethanol method was developed using the acetone method as a starting point, and indeed follows a very similar protocol. However, to account for the greater acidity of the purified $^{68}$Ga eluate a five-fold higher concentration of the buffer is needed to ensure sufficient capacity. DOTATOC labelled to > 95% at 80 °C after 1 min, and showed a quicker and more reproducible labelling performance compared to the acetone method at the same pH, temperature and precursor concentration. DOTATOC labelling at 95 °C resulted in a similar rate of reaction with > 95% after 3 min. Labelling yields ≥ 98% could be achieved with high reproducibility at 80 °C after 10 min and 95 °C after 15 min. NO2AP$^{BP}$ showed labelling yields of > 95% after 2 and 10 min at 40 and 60 °C respectively. At 40 °C a maximum yield of 98% could be obtained after 20 min, which is superior to the result of the acetone method under the same conditions where only 94% were achieved. Labelling performance at 60 °C was slightly slower over 10 min than the acetone method, yet with the same yield of 99% at 20 min. Interestingly, the labelling kinetics of DATA$^{m}$ was more sensitive to pH variations when using $^{68}$Ga post-processed by the ethanol method compared to that of the acetone method. Labelling yields of 93 (pH 4) and > 95% (pH 5) were obtained after 20 and 10 min respectively – significantly slower than that observed at the same pH with the acetone method. Yield reproducibility was in an excellent range and the almost quantitative labelling at pH 5 in accordance with our expectations for this new class of $^{68}$Ga-chelates.

Radiolabelling using NaCl post-processed $^{68}$Ga: DOTATOC obtained maximum yields of 96% and 86% after 15 min at 80 and 95 °C respectively. These compare poorly with the results obtained using $^{68}$Ga after ethanol post-processing in which yields ≥ 98% were achieved in a shorter reaction time and with greater reproducibility. NO2AP$^{BP}$ could be labelled with maximum yields of 85% and 94% after 20 min at 40 and 60 °C respectively. These labelling efficiencies are significantly lower than those achieved with the acetone (at 60 °C) and ethanol post-processed $^{68}$Ga. Very poor labelling kinetics was observed for DATA$^{m}$ at pH 4 (max. 19%, 20 min) that improved at pH 5 (max. 93%, 20 min), but remained well below that achieved with the other methods. This trend is similar that observed with the ethanol method, but the yields are lower and show a greater difference. The reproducibility at both pH values was also slightly lower compared to the other two methods. Parker and co-workers have shown that the DATA$^{m}$ ligand conformation is sensitive to the chemical environment of the labelling media. It is possible that the NaCl post-processing media promotes a conformation that is not conducive to efficient labelling (Parker and Waldron, 2013).

Although labelling reactions showed a high degree of reproducibility at all temperatures and with all methods, there are several important differences. Table 3 compares labelling efficiencies in two ways: it shows the time needed to achieve the critical yield (95%), and the maximum yield of each labelling reaction (up to a maximum time of 20 min). Because maximum yields for some labelling reactions were only achieved at 15 min or more, Table 4 also compares radiochemical yields for each labelling experiment at 10 min.

All model precursors showed good labelling yields and high reproducibility when labelled with $^{68}$Ga processed by the acetone method. Radiochemical purity was > 95% in all cases with the exception of DOTATOC at 80 °C. The data collected suggest that the eluate permits fairly robust labelling procedures, with variations in different labelling parameters having a relatively small influence on the final yield.

Substituting acetone for ethanol required the use of more acidic solutions to ensure efficient post-processing. As a consequence the buffer concentration was increased from 0.2 M (for acetone

**Table 4**
Labelling yields determined for all experiments at 10 min.

| Precursor | nmol | Parameter | Labelling yield (%) | | |
|---|---|---|---|---|---|
| | | | Acetone method | Ethanol method | NaCl method |
| DOTATOC | 14 | 80 °C | 90.9 ± 4.3 | 97.6 ± 0.3 | 95.5 ± 3.2 |
| | | 95 °C | 97.4 ± 1.0 | 97.4 ± 0.9 | 85.5 ± 3.9 |
| NO2AP$^{BP}$ | 11 | 40 °C | 91.0 ± 4.4 | 97.3 ± 1.2 | 82.7 ± 1.5 |
| | | 60 °C | 98.0 ± 0.1 | 97.0 ± 0.1 | 93.0 ± 1.0 |
| DATA$^{m}$ | 10 | pH 4 | 98.7 ± 0.1 | 90.7 ± 3.5 | 12.6 ± 2.2 |
| | | pH 5 | 98.7 ± 0.1 | 97.9 ± 0.3 | 82.2 ± 7.8 |

method) to 1.0 M to obtain the desired pH of the labelling media. Furthermore, $^{68}$Ga is provided in about twice the volume of the acetone method. In five of six experiments, ligands were labelled to $>98\%$ over 20 min and showed a high degree of reproducibility. The exception in this instance was DATA$^{m}$, which achieved a yield of 93% at pH 4. As reported recently the presence of organic solvents (such as ethanol) in the aqueous labelling media facilitates radiometal-complex formation processes such that higher labelling yields and specific activities can be achieved with less labelling precursor and shorter reaction times (Perez–Malo Cruz and Roesch, 2012). Importantly, these yields would permit clinical application following a simple dilution of the radiopharmaceutical. It is evident that the ethanol method is well suited to clinical application and the labelling of DOTATOC in particular.

A slightly reduced labelling performance was observed for all substances using $^{68}$Ga post-processed by the NaCl method. Lower yields and reproducibility also revealed a significantly reduced versatility compared to the other two methods, which necessitates further purification after labelling. An explanation for the lower yields achieved with the NaCl-method might lie in the presence of metal impurities in the reagents used. Using BioXtra grade NaCl (Sigma-Aldrich®) for preparing the N6 solution there are possible contaminations of $\leq 2.5$ nmol of Fe and $\leq 12.5$ nmol of Cu present in each labelling reaction. Considering that the amount of precursor used is comparable it is possible that the metal cation contaminants have a negative influence in the labelling. Such an influence of non-$^{68}$Ga metal ions has been reported by Šimeček et al. (2013), where equimolar amounts (with respect to the precursor) of competing metal ions negatively influenced the labelling characteristics of DOTA- and NOTA-based chelators. Another possible reason for slower labelling kinetics might lie in the high Na(I) concentration of the processed eluate. Although a Na(I) complex of the precursors is not expected to be stable, the cations can bind to the precursor's donor atoms prior to formation of the proper $^{68}$Ga-complex and slow down the labelling process. Furthermore, the three post-processing methods differ in terms of the $^{68}$Ga-species they deliver into the synthesis vial. For the acetone- and the ethanol-based systems this is the $^{68}$Ga-cation as the hexa-aquo species, immediately ready for metal-chelator complex formation. For the NaCl-method the $^{68}$Ga exists as the anionic tetrachloro complex, $[^{68}GaCl_4]^-$. It is possible that the different forms of $^{68}$Ga are playing a role in the differing rates of reaction. Whatever the reason, it is clear that all model precursors showed lower labelling yields with the NaCl method, compared to the acetone- and ethanol-based methods. The disadvantage of the NaCl method is clear when the labelling yields at 10 min are compared, cf. Table 4. The ethanol-method provides yields of $\geq 95\%$, each highly reproducible, for all the precursors. In contrast, the NaCl post-processed $^{68}$Ga resulted in a weaker labelling performance with only one experiment (DOTATOC, 80 °C) reaching the 95% critical yield with 10 min.

### 3.2. Radiochemical purity of the radiolabelled product

An important consideration when samples for *in vivo* studies are being prepared are possible consequences the higher levels of radioactivity may have on the radiochemical purity. High levels of activity are known to cause radiolysis of a prepared radiopharmaceutical, thereby reducing the purity prior to application. To hinder this radiolytic protectants are often added to the radiolabelling media. Following a recent report (Perez–Malo Cruz and Roesch, 2012) which stated that the ethanol used in ethanol post-processing also serves as a radiolysis protectant, we were interested to compare the different methods on this basis. For this experiment DOTATOC (14 nmol) was labelled with $^{68}$Ga (750–900 MBq) post-processed by each of the three methods. No separation from free $^{68}$Ga was performed in order to evaluate the inhibitory characteristics of each solvent system. This explains the presence of free $^{68}$Ga which can be seen as part of each HPLC-chromatogram with a retention time of 3 min. Radio-HPLC evaluations for the grade of radiolytic decomposition of $^{68}$Ga-DOTATOC were carried out at 0, 45 and 90 min post labelling. After 90 min there was no evidence for radiolytic decomposition of $^{68}$Ga-DOTATOC (retention time of 24 min) prepared using $^{68}$Ga post-processed by the ethanol method. In contrast, the $^{68}$Ga-DOTATOC prepared using $^{68}$Ga post-processed by the other methods showed obvious signs of decomposition at early time points (retention time 15–22 min). A comparison of the three experiments is shown in Fig. 2 at 45 min post labelling. It is clear from the HPLC analysis that radiolysis is a considerable problem when acetone, and to a lesser extent when NaCl post-processed $^{68}$Ga is used. These results are in agreement with results published previously (Mueller et al., 2012). This confirms the ability of ethanol as protection agent for radiolysis and underlines the applicability in routine clinical application of this method. An overview of parameters and theoretical specific activities is given in Table 5. HPLC Chromatograms for each experiment are supplied in the Supporting Information.

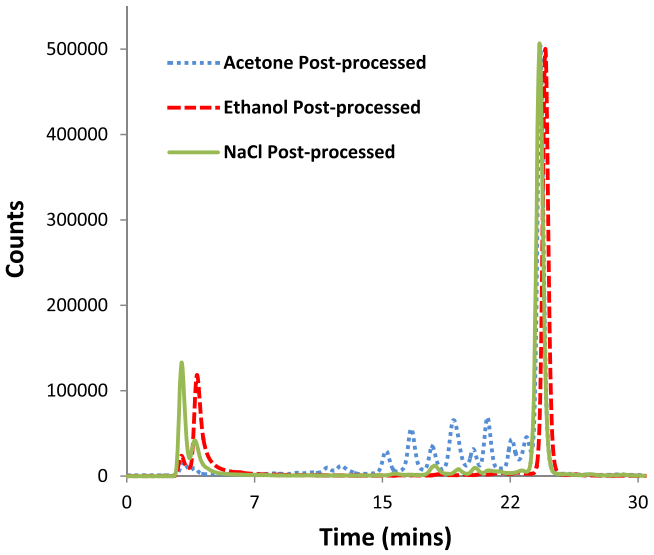


**Fig. 2.** HPLC chromatograms of $^{68}$Ga-DOTATOC ($R_t$=24 min) 45 min after preparation using $^{68}$Ga obtained using each of the three different post-processing methods. Count values have been normalised for clarity. Unreacted $^{68}$Ga from the labelling solution elutes with a retention time of 3–4 min. Radiolytic decomposition is indicated by impurities eluting between 15 and 24 min.

## 4. Conclusions

Labelling using acetone post-processed $^{68}$Ga can deliver the necessary radiochemical purities for the three precursors, however further purification steps are required because acetone is not approved for *in vivo* application. In the context of application of the relatively short-lived $^{68}$Ga, these disadvantages become a serious deterrent for their use. The NaCl method overcomes this problem and can be applied for the radiolabelling of each precursor. However, in general it provided lower yields (or longer labelling times) with a lower degree of reproducibility. The NaCl method is also disadvantaged by the fact that changes to the labelling parameters (pH and temperature) had a larger effect on the labelling yield. In contrast, the ethanol method has emerged as one which provides $^{68}$Ga that results in labelling yields $>98\%$ for all three precursors

**Table 5**
Comparison of DOTATOC labelling using high activities of $^{68}$Ga post-processed by the three methods.

| Post-processing method | Volume (mL) | DOTATOC (nmol) | $T$ (°C) | $t$ (s) | pH | Yield (%) | Specific activity[a] (MBq/nmol) | Source[b] |
|---|---|---|---|---|---|---|---|---|
| NaCl | 3.7–3.9 | 28 | 90 | 420 | $3.6 \pm 0.3$ | 99 | 35 | Mueller |
| | 3.8 | 14 | 80 | 900 | $3.9 \pm 0.1$ | 96 | 40 | This work |
| Acetone | 4.4–4.9 | 14 | 98 | 600 | $2.3 \pm 0.1$ | 95 | 68 | Zhernosekov |
| | 4.4 | 14 | 95 | 900 | $2.4 \pm 0.1$ | 97 | 69 | This work |
| Ethanol | 2.0 | 14 | 95 | 300 | 4.0 | 98 | 70 | Eppard |
| | 2.0 | 14 | 95 | 900 | $3.8 \pm 0.4$ | 98 | 70 | This work |

[a] Specific activities shown are theoretical values calculated using a starting activity of 1 GBq.
[b] References as follows: Mueller et al. (2012), Zhernosekov et al. (2007), and Eppard et al. (2014).

with a high degree of reproducibility. Furthermore, since the ethanol does not need to be removed prior to injection, the ethanol method can be incorporated into a kit-type synthesis in which further purification is not needed – as is the case for many $^{99m}$Tc-radiopharmaceuticals. In addition we have shown that the ethanol containing solution showed no sign of radiolysis over 90 min using high activities. In contrast, there was clear evidence for radiolysis when $^{68}$Ga post-processed by the acetone and NaCl methods was used. Radiolysis protecting agents can be added to prevent this, but the advantage of the ethanol system is that this is not necessary.

Typically when labelling of a given precursor is performed for the first time various parameters as temperature, pH and labelling media are optimised. The results gathered here provide support for the idea that there is value to also experimenting with different post-processing techniques during this optimisation phase. The three methods tested use similar setups and can be exchanged with relative ease, making such evaluations a real possibility. In our experience it has been possible to obtain satisfactory labelling yields and reproducibility with all three methods. However, the optimum conditions (in terms of pH, labelling media, temperature, time and precursor amount) for labelling are not transferrable between the different $^{68}$Ga post-processing methods. Therefore, each precursor must be carefully evaluated to determine which combination is the best for a given precursor before routine application. During this optimisation stage the important aspects to consider are the labelling yield and reproducibility. Taking into account the relatively short half-life of $^{68}$Ga, and that clinical application favours simple preparation protocols, the labelling yield is an important consideration. Ideally, the yield should be high enough such that the radiopharmaceutical can be directly applied after sterile filtration, i.e. no further purification is necessary before application. According to European Pharmacopeia legislation, the radiochemical purity of a $^{68}$Ga-DOTATOC preparation for *in vivo* application must be $\geq 91\%$. If this regulation is satisfied then the preparation can be simply diluted and sterile filtered before injection. In our experience the yields obtained using NaCl post-processed $^{68}$Ga are not consistently high enough to avoid purification after radiolabelling. Considering further developments any modular $^{68}$Ga-labelling setup would benefit from introducing the ethanol method for routine clinical preparation of $^{68}$Ga-radiopharmaceuticals.

## Conflict of interest

The authors declare no conflict of interest.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.apradiso.2015.01.023.

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