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Reconsidering astatine-211 analytics: Retention effects in reversed-phase HPLC

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ABSTRACT

Astatine-211 is a short-lived alpha-emitting radionuclide with high potential for cancer treatment by targeted alpha therapy (TAT). Reversed-phase high-performance liquid chromatography (RP-HPLC) is central to the development and quality control of ^{211}At-labeled radiopharmaceuticals. However, accurate analysis remains challenging due to the ultra-trace levels of astatine and its complex, unpredictable chemical behaviour. In this study, we systematically evaluated the recovery of representative inorganic astatine formulations under different redox conditions using various chromatographic conditions, which is a prerequisite for reliable quantification. Examination of four different columns with distinct stationary phase chemistry demonstrated that significant discrepancies can arise when radiochemical conversion is assessed solely based on eluted activity, as is common standard for RP-HPLC analysis. Among the chromatographic conditions examined, the highest and most consistent astatine recoveries (88−98%) were achieved using a basic mobile phase containing 0.4% triethylamine in combination with a base-tolerant stationary phase, likely due to a shift in astatine speciation under alkaline conditions, reduced secondary interactions with the stationary phase, and/or potentially beneficial ion pairing effects. In contrast, widely used standard solvent systems based on acetonitrile/water with or without 0.1% trifluoroacetic acid resulted in unsatisfactory and highly variable recoveries (7−71% or 30−79%, respectively) across all columns investigated. These findings highlight the necessity of optimized chromatographic conditions and suggest that inadequate recovery of free astatine could lead to substantial misestimation of the radiochemical conversion and purity of radiopharmaceutical formulations if not explicitly accounted for by auxiliary quantification strategies.

1. Introduction

Targeted alpha therapy (TAT) is an emerging cancer treatment modality based on selective delivery of alpha-emitting radionuclides to malignant cells via tumor-targeting vectors. The high linear energy transfer (LET) of alpha particles confers a potent cytotoxic effect, with a relative biological effectiveness (RBE) significantly exceeding that of beta-emitters. Coupled with their short tissue range (typically no more than a few cell diameters), this allows for localized tumor ablation with minimal damage to surrounding healthy tissues.

Among the various alpha-emitters currently under investigation, astatine-211 has garnered considerable attention due to its favorable physical and chemical properties. As a halogen and the rarest naturally occurring element, astatine-211 is unique in its chemical behavior. It also exhibits a simple decay scheme with the emission of a single alpha particle, enabling straightforward dosimetry in contrast to radiometals like actinium-225, lead-212, or bismuth-213, which are part of complex decay chains [1]. Its half-life of 7.2 h provides a practical balance between synthetic accessibility, (pre)clinical workflows, and waste as well as patient management. In addition, the ability of astatine to form

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covalent bonds with carbon atoms allows for direct radiolabeling with minimal perturbation of pharmacophore structure, a significant advantage over radiometal-based chelation strategies [2].

With growing clinical interest, there has been a parallel increase in efforts to develop stable, tumor-specific ^{211}At-labeled radiopharmaceuticals for TAT. Due to the trace amounts of these compounds used for therapeutic applications and the short half-life of most clinical radionuclides, analytical methods must be both highly sensitive and rapid. Consequently, reversed-phase high-performance liquid chromatography (RP-HPLC) has become the standard technique for method development and quality control. However, for certain radionuclides such as fluorine-18 and gallium-68, non-specific adsorption to RP-HPLC columns has been observed and can significantly skew interpretation if not properly addressed [3–5].

In contrast, iodine isotopes, often regarded as chemical homologs of astatine, are typically not affected by retention effects, making their chromatographic behavior straightforward [6,7]. Yet, despite their apparent similarity, astatine's chemical properties and chromatographic behavior differ markedly [8]. The element exists in multiple oxidation states and exhibits a complex and poorly understood aqueous speciation that depends on pH and redox potential [9]. In particular, astatine can exist as neutral species (At^0), in reduced forms (e.g., At^-), or as oxidized species (e.g., At^+, AtO^+ and higher oxo-species). The relative distribution of these forms is governed by the chemical environment and can likely shift dynamically under typical radiochemical conditions. In addition, these species are expected to exhibit markedly different chromatographic behaviour in RP-HPLC, with more hydrophobic or neutral forms showing increased retention, while ionic species may interact with residual silanol groups or elute with reduced retention depending on their charge and polarity. Consequently, the complex and variable speciation of astatine is likely a key factor contributing to retention effects and variability in recovery. Its presence in a true carrier-free form further complicates matters, as it is only present in ultra-trace quantities several orders of magnitude lower than those of no-carrier-added (n.c.a.) radionuclides. This extreme dilution can enhance the impact of interactions with stationary phases and contributes to the perceived unpredictability of its chemical and chromatographic behavior, which has been described as chameleon-like and enigmatic [10,11]. In contrast to astatine, recovery of radioactive analytes is well recognized as a critical parameter for established radionuclides such as carbon-11, fluorine-18, or technetium-99m, and has been extensively addressed in the analytical literature as well as in regulatory frameworks, including pharmacopoeial monographs (e.g., European Pharmacopoeia) [12]. For these radionuclides, non-specific adsorption and recovery losses are routinely considered during method development and validation, particularly in the context of radiopharmaceutical quality control [3,13,14]. However, despite the growing popularity of astatine, only a few methods have been proposed to account for retention effects, such as using sodium sulfide column washes to elute and quantify retained free astatine [15], or the use of dual-flow cell radiation detectors for recovery quantification [16]. While the application of these approaches has provided clear evidence for radiation dose-dependent retention of astatine on HPLC columns [17], they are rarely implemented systematically, and a comprehensive evaluation of the chromatographic parameters governing astatine retention and recovery is still lacking. Specifically, the influence of stationary phase chemistry, mobile phase composition, and redox conditions on quantitative recovery has not been systematically investigated. Upon establishing workflows in our recently established astatine laboratory, we also observed low astatine recoveries during activity balancing by post-column injection, a technique routinely employed in our lab to correct for retention effects [5]. These observations prompted the present study, which systematically investigates chromatographic parameters governing retention of astatine under RP-HPLC conditions using complementary online and offline quantification methods. By identifying parameters that minimize retention and improve quantification reliability, this work provides practical guidance for the development and quality control of ^{211}At-labeled radiopharmaceuticals.

2. Materials and methods

2.1. General

All HPLC solvents were of HPLC gradient grade and were purchased from Merck KGaA (Darmstadt, Germany) or Sigma-Aldrich (Taufkirchen, Germany). Ultrapure water (resistivity $>$ 15 MΩ•cm) was freshly prepared using an ELGA Purelab classic system (ELGA LabWater, Celle, Germany). All organic solvents, reagents and HPLC solvent modifiers were of analytical reagent grade or higher and were purchased from Merck KGaA or Sigma-Aldrich. All reagents were used as received without further purification unless otherwise stated. Radioactivity was measured with a Comecer TALETE HC dose calibrator (COMECER S.p.A., Castel Bolognese, Italy) that was cross-calibrated against an EG&G Ortec GEM-20190 high-purity germanium detector (EG&G Ortec, Oak Ridge, TN, USA).

2.2. Astatine-211 production and purification

Astatine-211 was produced via the ^{209}Bi(α,2 n)^{211}At nuclear reaction by bombarding bismuth targets (99.99% purity; Strategic Elements, Deggendorf, Germany) with ≈ 29.2 MeV alpha particles at a current of 50 μAe using an IBA cyclone 30XP cyclotron (IBA International, Louvain-La-Neuve, Belgium) with an IBA external high current target station for 6° slanting angle targets. Targets were produced in-house by melting bismuth layers (30–50 mg/cm^2 thickness) onto aluminum alloy backings. Following irradiation, the target material was mechanically removed from the backings and astatine-211 was isolated according to established procedures for dry distillation of astatine (for examples see [18–20]) with some adjustments. Briefly, the irradiated bismuth was placed in a quartz tube oven preheated to 750 °C and distilled using dry synthetic air as carrier gas. The volatile astatine-211 was collected in a polymer tube at −40 °C and eluted with chloroform (50−200 μL). The chloroform was then evaporated to dryness at ambient pressure under a stream of argon.

2.3. Sample preparation

The purified and dried astatine-211 was reconstituted in one of three solutions to represent different chemical forms that could be present in radiopharmaceutical formulations: (i) 1 mg/mL *N*-chlorosuccinimide in methanol (electrophilic ^{211}At), (ii) 100 mg/mL ascorbic acid in acetonitrile (MeCN)/water (nucleophilic ^{211}At) and (iii) pure MeCN/water (native ^{211}At). These formulations were chosen to approximate oxidizing, reducing, and non-modified conditions commonly encountered in astatine radiochemistry, rather than to represent single, well-defined chemical species. The activity concentration was adjusted to 3 MBq/mL and the samples were incubated for at least 20 min before being injected into the HPLC system. All experiments were performed in triplicate using freshly prepared samples from independently purified astatine-211 batches.

2.4. HPLC

The HPLC system consisted of an AZURA P6.1 L gradient pump (Knauer, Berlin, Germany), two Rheodyne 7725i high pressure injection valves (Rheodyne LLC, Rohnert Park, CA, USA) placed in front and behind the chromatographic column, and an Ortec 3 ″NaI(Tl) well-type scintillation detector combined with an Ortec 925-SCINT single channel analyzer (Advanced Measurement Technology Inc., Oak Ridge, TN, USA). A coil of polypropylene tubing (0.8 mm I.D., ca. 20 cm length) was inserted as flow cell. Samples were applied by full-loop injections using 20 μL sample loops. Data acquisition was performed with Knauer

ClarityChrome software. HPLC columns were used as provided by the manufacturer and their performance (separation efficiency and peak symmetry) was examined prior to use: Phenomenex Luna C18(2), 5 µm, 100 Å, 250 × 4.6 mm (Phenomenex Ltd. Deutschland, Aschaffenburg, Germany; Part No 00G4252-E0); Waters XBridge BEH C18, 5 µm, 130 Å, 250 × 4.6 mm (Waters GmbH, Eschborn, Germany; Part No 186003117); Phenomenex PolymerX, 5 µm, 100 Å, 250 × 4.6 mm (Phenomenex Ltd. Deutschland; Part No 00G-4326-E0); Merck Chromolith Performance RP-18 endcapped, 100 × 4.6 mm (Merck KGaA; Part No 1.02129.0001). Solvent modifiers (TFA, ascorbic acid, triethylammonium acetate, triethylamine, sodium sulfite, and sodium thiosulfate) were individually added to solvent A (aqueous) and B (organic). Due to limited solubility and to prevent precipitation of modifiers during gradient elution, solvent B contained 20% water for experiments with ascorbic acid and 30% water for experiments with Na_2SO_3 or $Na_2S_2O_3$. For all solvent systems, the following gradient was used: 0–1 min: 5% B, 1–6 min: 5–100% B, 6–15 min: 100% B with a flow rate of 1 mL/min.

2.5. Quantification of astatine recovery

Recovery was quantified using both online and offline approaches. Online detection employed a post-column injection method (Fig. 1A), wherein an equal-volume aliquot of the same sample was injected downstream of the separation column (bypassing the stationary phase) via a secondary injection valve. This allowed direct measurement of the total detector response (i.e., total activity injected), independent of potential retention effects [5]. First, the sample was injected via the primary injection valve (=pre-column injection) and the effluent was collected for 12 min in a 20 mL scintillation vial for offline quantification (=effluent A, see below). Immediately afterwards, the post-column injection was performed via the secondary injection valve, and recovery was calculated as the ratio of the combined peak integrals of the pre-column injection to the peak integral of the post-column injection (both decay-corrected to the respective time of injection). For offline quantification, the column was then removed from the flow path and another equal-volume sample was injected via the primary valve and eluted using the same gradient conditions. The effluent was again collected for 12 min into an identical 20 mL scintillation vial (=effluent B). Activity in the two effluent fractions (A and B) was measured with a low-level gamma counter (HIDEX AMG Automatic Gamma Counter, model 425–601, Turku, Finland), and recovery was calculated as the ratio of the decay-corrected count rate for effluent A (collected with column) to that for effluent B (collected without a column). All calculations were performed using the respective instrument software and/or Microsoft Excel 365 (Microsoft Corp., Redmond, WA, USA).

3. Results and discussion

3.1. Quantification of astatine recovery

Recovery of astatine-211 from the HPLC system was determined using two complementary quantification approaches: (1) online detection after pre- and post-column injection via an in-line radiation detector, and (2) offline quantification by gamma counting of the entire effluent collected with and without a column (for details see 2.5). Online detection is the standard method for radiopharmaceutical quality control and typically results in sharp, well-defined peaks for ^{211}At-labeled organic compounds. However, it proved unreliable for free astatine species, which often produced broad, tailing signals that lacked clear peak boundaries and suffered from elevated noise levels (Fig. 1B). Notably, these phenomena were not observed for post-column injections bypassing the stationary phase, suggesting that they reflect non-specific retention effects that result in gradual release of radioactivity from the column due to, e.g., interconversion between astatine species with different retention behavior.

To address this, offline detection by gamma counting of the whole effluent (collected with and without column) was implemented, taking special care to minimize self-adsorption artifacts associated with the low-energy X-ray emissions of astatine-211 through standardized sample size and geometry. While impractical for routine use, this method offered improved quantification of activity due to extended acquisition times, eliminated ambiguities in signal integration, and offered clearer insight into whether astatine was actually retained on the column or simply escaped detection during online monitoring. In general, online and offline detection showed consistent trends in recovery and variance, with some notable exceptions (Table 1). For example, nucleophilic and electrophilic astatine samples analyzed on the Merck Chromolith column under neutral conditions occasionally showed online recoveries

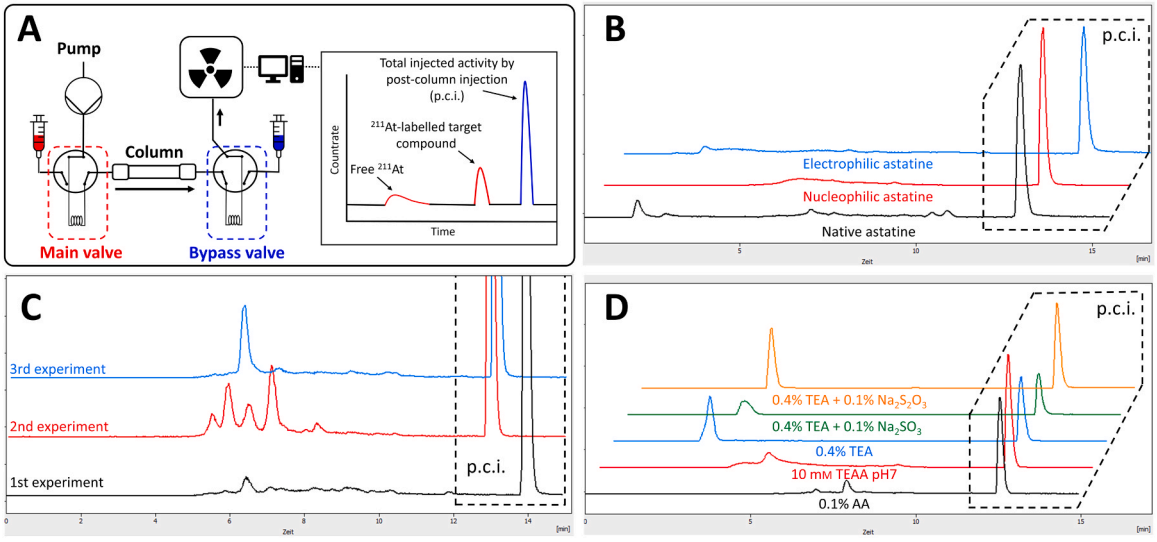

Fig. 1. Quantification of astatine-211 recovery by online detection. (A) Schematic representation of the HPLC setup used for determining astatine recovery via post-column injection. (B) Representative chromatograms of the different astatine formulations obtained with a Waters XBridge column and MeCN/water as the mobile phase. (C) Triplicate chromatograms of electrophilic astatine-211 prepared and analyzed under identical experimental conditions (column: Waters XBridge, solvent: MeCN/water/0.1% TFA). (D) Representative chromatograms of nucleophilic astatine-211 obtained with a Waters XBridge column and different mobile phases designed to improve astatine recovery (see Table 2). All experiments were performed using a gradient of 5–100% organic phase at a flow rate of 1 mL/min (see Section 2.4 for details).

Table 1
Astatine-211 recovery from different HPLC columns using MeCN/water with or without 0.1% trifluoroacetic acid (TFA) as mobile phase.

Column	Astatine recovery [%] [a]							
	Electrophilic At			Nucleophilic At	Native At		*Sample average*	
	neutral	+TFA	neutral	+TFA	neutral	+TFA	*neutral*	*+TFA*
Phenomenex Luna C18(2)	33 ± 10	16 ± 5	40 ± 18	44 ± 24	41 ± 21	8 ± 4	*38 (40)*	*23 (26)*
	(29 ± 9)	(22 ± 9)	(41 ± 4)	(43 ± 19)	(50 ± 11)	(14 ± 5)		
Waters XBridge C18	63 ± 4	37 ± 13	60 ± 12	71 ± 16	43 ± 10	14 ± 4	*55 (46)*	*40 (32)*
	(49 ± 7)	(37 ± 15)	(30 ± 12)	(42 ± 10)	(60 ± 12)	(16 ± 5)		
Phenomenex PolymerX	24 ± 13	9 ± 2	28 ± 12	31 ± 2	39 ± 11	7 ± 14	*30 (33)*	*16 (18)*
	(24 ± 8)	(12 ± 4)	(36 ± 18)	(30 ± 13)	(39 ± 14)	(12 ± 4)		
Merck Chromolith	80 ± 20	18 ± 2	89 ± 26	68 ± 8	68 ± 13	21 ± 1	*79 (69)*	*36 (30)*
	(73 ± 14)	(25 ± 5)	(66 ± 11)	(47 ± 13)	(68 ± 4)	(18 ± 3)		
Column average	*53 (47)*	*19 (24)*	*54 (43)*	*54 (41)*	*47 (54)*	*13 (15)*		

[a] Recovery was quantified by online detection with post-column injection and offline detection (numbers in parenthesis) by gamma counting of the effluents collected with and without column (n = 3 per value).

exceeding 100%, an artifact attributed to integration errors from distorted signals. In contrast, offline values remained within the theoretical maximum of 100%, underscoring the value of offline detection as a reference method.

3.2. Influence of sample composition

Astatine exists in multiple oxidation states, and its chemical form strongly influences chromatographic behavior. Accordingly, three distinct formulations were evaluated, which comprised electrophilic astatine (oxidized with *N*-chlorosuccinimide), nucleophilic astatine (reduced with ascorbic acid) and native astatine (dissolved without redox manipulation to simulate deastatination products). Due to the complex and dynamic aqueous chemistry of astatine, these formulations are not expected to represent single, well-defined chemical species, but rather mixtures of species governed by the prevailing redox conditions. While these do not encompass the full spectrum of astatine species that may result from the numerous electrophilic and nucleophilic strategies used for production of ^{211}At-labeled radiopharmaceuticals [21,22], they can nevertheless be considered representative of the main classes of unreacted or free astatine likely to be encountered in practical formulations.

As evident from the high standard deviations, even identically prepared samples of a given formulation exhibited large variations in elution profiles (Table 1). This is exemplified in Fig. 1C, where three nominally identical samples produced markedly different chromatograms, varying not only in peak integrals but also in the number of resolved peaks. This variability underscores the dynamic speciation of astatine and the difficulty in obtaining consistent chromatographic behavior for free astatine-211 in the absence of stabilizing media.

3.3. Stationary and mobile phase effects

To assess whether column chemistry influences retention of astatine-211, like it has been shown for n.c.a [^{18}F]fluoride [3], we also compared four RP-HPLC columns with distinct surface properties. Selection was guided by the need to cover a broad range of surface characteristics known to influence non-specific interactions, particularly for trace-level species with variable speciation like astatine. The Luna C18(2) column, based on fully porous high-purity silica particles with *n*-octadecylsilyl-modification and trimethylsilyl-endcapping, was included as a widely used standard in RP-HPLC method development. In contrast, the Waters XBridge C18 column features ethylene-bridged hybrid organosilicon core particles that offer enhanced pH stability and low silanol activity, both factors that could affect interaction with astatine species. To further explore non-silica-based stationary phases, the Phenomenex PolymerX column was included. This column consists of a polystyrene-divinylbenzene (PSDVB) copolymer, which lacks silanol activity and combines hydrophobic retention with high chemical robustness across a wide pH range. Lastly, the Merck Chromolith Performance RP-18 column was selected due to its monolithic silica structure and demonstrated low retention of [^{18}F]fluoride, which makes it a promising candidate for minimizing non-specific retention of halide-like species.

Initial experiments confirmed varying degrees of astatine retention across all columns. Notably, residual activity from prior injections was frequently observed, indicative of strong or delayed desorption from the stationary phase. This “carry-over” could compromise subsequent runs and complicate interpretation of recovery data. Given the impracticality of waiting multiple half-lives between measurements (~72 h for ten half-lives of astatine-211), column regeneration was attempted using reducing agents according to the work of Guérard and coworkers [15]. Injection of sodium sulfite solution via a relatively small sample loop (20 μL, 10 mg/mL) partially removed retained activity, but residual activity was still detected in subsequent runs. Complete removal was achieved when columns were flushed with at least five column volumes of 0.5% sodium thiosulfate solution. This milder reducing agent (pH 6.6) was preferred over sodium sulfite solution (pH 9.5) for columns sensitive to alkaline conditions (such as Luna C18(2) and particularly Chromolith RP-18) to avoid degradation of the silica matrix and, e.g., exposure of reactive silanol groups.

To accommodate the unknown polarity of astatine species formed under different conditions, all columns were eluted using a broad gradient (5–100% MeCN in water). Under these neutral conditions, substantial differences in apparent recovery (determined by online detection) were observed among columns (Table 1). Averaged across all three sample types, recoveries ranged from 30% for the Phenomenex PolymerX column, to 38% for the Phenomenex Luna C18(2), 55% for the Waters XBridge C18, and 79% for the Merck Chromolith. However, these values were associated with large standard deviations, and high recovery outliers, such as the 89% value for the nucleophilic formulation on the Chromolith column, could not be replicated by offline detection (66%), indicating that tailing and integration artifacts were likely contributing factors.

To explore the effect of mobile phase acidity and ion-pairing, 0.1% trifluoroacetic acid (TFA) was added to both aqueous and organic solvent. This modification, widely used in RP-HPLC, acidifies the mobile phase and introduces an ion-pairing agent that could influence both astatine speciation and retention mechanisms. With TFA-containing mobile phases, retention of native and electrophilic astatine increased markedly for all columns (by a factor of about 3), consistent with the formation of more strongly interacting cationic species. In contrast, nucleophilic astatine showed minimal change in recovery, with values ranging from 30% (Phenomenex PolymerX) to 68% (Merck Chromolith).

Given the limitations of all tested columns when used with standard solvent systems, further optimization of the mobile phase was performed with the Waters Xbridge C18 column, chosen for its superior pH

tolerance (pH 1−12). Initially, MeCN was replaced by methanol, but this led to negligible changes in retention and was not pursued further (Table 2).

Instead, based on the observation that average recovery for the electrophilic formulation was lower under acidic conditions (which should result in the presence of oxidized, cationic species like At^+ or AtO^+), alternative additives aiming to lower the redox potential and stabilize anionic species such as At^-, were evaluated. Addition of ascorbic acid was hampered by low solubility in MeCN and did not improve recovery (Fig. 1D). In contrast, the use of a pH-neutral, ionic solvent containing triethylammonium acetate as organic-soluble buffer resulted in more consistent recoveries between 75% and 91%. Further pH elevation using pure triethylamine (pH 10.5) yielded recoveries of 88−98% across all formulations, with elution occurring as a single, well-defined peak with low retention time (Fig. 1D). This effect is likely attributable to a combination of increased pH, which shifts astatine speciation towards less strongly interacting forms, and suppression of secondary interactions with residual silanol groups on the stationary phase, thereby reducing non-specific adsorption. Furthermore, ion pairing effects between triethylammonium cations and anionic astatine species that are predominantly stable at higher pH could contribute to increased recoveries and improved peak shape. Such conditions markedly enhance quantification reliability by minimizing peak distortion and improving resolution between free astatine species and ^{211}At-labeled compounds. Nevertheless, while they represent a significant improvement compared to default solvent systems, such high-pH mobile phases come with notable trade-offs. Most silica-based columns are not stable under highly alkaline conditions, and analytes with acidic functional groups may suffer from reduced retention.

To further improve robustness, mobile phases combining triethylamine with the reducing agents sodium sulphite or sodium thiosulfate were evaluated. Surprisingly, while these systems maintained single-peak elution of free astatine (albeit with slightly different retention times and peak shapes), they failed to improve reproducibility and actually reduced recovery. This suggests that astatine retention is governed by multiple, potentially interacting factors beyond oxidation state and pH.

Table 2
Astatine recovery from Waters XBridge C18 columns using different mobile phases.

Mobile phase[a]	**Astatine recovery [%]**[b]			
	Electrophilic At	**Nucleophilic At**	**Native At**	***Average***
MeCN/H_2O	63 ± 4 (49 ± 7)	60 ± 12 (30 ± 12)	43 ± 10 (60 ± 12)	*55 (46)*
MeOH/H_2O	53 ± 4 (55 ± 3)	52 ± 5 (49 ± 7)	52 ± 7 (64 ± 7)	*52 (56)*
MeCN/H_2O **+ 0.1% AA**	56 ± 23 (62 ± 21)	47 ± 6 (52 ± 4)	35 ± 2 (46 ± 2)	*46 (53)*
MeCN/H_2O **+ 10 mM TEAA (pH = 7)**	75 ± 6 (71 ± 6)	91 ± 3 (92 ± 2)	82 ± 5 (86 ± 2)	*83 (83)*
MeCN/H_2O **+ 0.4% TEA (pH = 10.5)**	98 ± 9 (79 ± 9)	88 ± 16 (95 ± 8)	98 ± 4 (103 ± 3)	*95 (92)*
MeCN/H_2O + 0.4% TEA **+ 0.1% Na_2SO_3**	84 ± 17 (75 ± 12)	74 ± 5 (74 ± 8)	72 ± 6 (71 ± 7)	*77 (73)*
MeCN/H_2O + 0.4% TEA **+ 0.1% $Na_2S_2O_3$**	78 ± 9 (74 ± 3)	87 ± 7 (84 ± 8)	78 ± 4 (74 ± 10)	*81 (77)*

[a] The respective parameter being examined is indicated in bold.
[b] Recovery was quantified by online detection with post-column injection and offline detection (numbers in parenthesis) by gamma counting of the effluents collected with and without column (n = 3 per value). Abbreviations: AA: ascorbic acid; TEAA: triethylammonium acetate; TEA: Triethylamine

3.4. Implications for analysis of ^{211}At-labeled radiopharmaceuticals

While the present study focused on defined model systems to enable systematic evaluation of chromatographic parameters, the findings have direct implications for the analysis of ^{211}At-labeled radiopharmaceuticals, where accurate determination of radiochemical purity is essential. In such systems, the presence of free astatine species may be underestimated due to retention effects, leading to overestimation of radiochemical conversion and purity. The use of basic mobile phases containing triethylamine, as identified in this study, can improve quantification reliability by reducing non-specific retention, although compatibility with specific radiopharmaceuticals and column stability must be considered. In addition, the results highlight the importance of complementary quantification strategies or careful method validation when conventional RP-HPLC conditions are employed. Overall, these findings emphasize the need to adapt analytical protocols to account for astatine-specific retention phenomena in practical applications.

Despite the insights provided by this study, several limitations should be acknowledged. The experiments were conducted using defined model systems to enable systematic evaluation of chromatographic parameters, and therefore do not fully capture the complexity of actual radiopharmaceutical formulations. In addition, astatine speciation was not directly characterized but inferred from the applied redox conditions, reflecting the inherent challenges associated with its ultra-trace chemistry.

Future work should focus on validating the identified chromatographic conditions in complex ^{211}At-labeled radiopharmaceutical samples and under routine quality control conditions. Furthermore, a more detailed investigation of astatine speciation and its direct correlation with chromatographic behavior would contribute to a deeper mechanistic understanding and support the development of more robust analytical methodologies.

4. Conclusion

This study demonstrates that under commonly employed RP-HPLC conditions, free astatine (as generated during electrophilic and nucleophilic radiolabeling protocols) exhibits significant retention on stationary phases. In addition, the fraction of free astatine eluted from the column often produces broad, tailing peaks with poor signal-to-noise ratios, rendering integration unreliable and resulting in high variability of apparent recovery. These effects were most pronounced with TFA-containing mobile phases and occurred across all four column types investigated.

Retention of free astatine on the stationary phase can lead to substantial underestimation of its presence in radiopharmaceutical samples, thereby inflating calculated values for radiochemical conversion and purity. This poses a risk for mischaracterization, especially in formulations intended for preclinical or clinical use. When quantification must be performed using conventional HPLC systems, our findings support the use of a basic mobile phase containing triethylamine in combination with a column offering high pH stability. Under these conditions, reproducible recoveries of 88–98% were achieved, which may be sufficient for applications such as radiolabeling optimization.

However, for actual quality control of radiopharmaceuticals intended for *in vivo* applications, complementary measurements should be employed to confirm accurate quantification. Given the complex and unpredictable speciation of astatine, which is already evident from the limited formulations investigated in this study, retention effects should be routinely considered in the analysis of astatine-211 containing samples.

Disclaimer

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however, those of the author(s) only and do not necessarily reflect those of the aforementioned parties. Neither of the aforementioned parties can be held responsible for them.

CRediT authorship contribution statement

Swen Humpert: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Lucas Mues:** Writing – review & editing, Validation, Investigation, Formal analysis, Data curation. **Bom Pedersen Nadia Renée:** Writing – review & editing, Investigation. **Felix Neumaier:** Writing – review & editing, Writing – original draft, Visualization. **Herth Matthias Manfred:** Writing – review & editing, Funding acquisition. **Bernd Neumaier:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Bernd Neumaier reports financial support was provided by Innovative Health Initiative Joint Undertaking (IHI JU). Matthias Manfred Herth reports financial support was provided by Innovative Health Initiative Joint Undertaking (IHI JU). This project was supported by the Innovative Health Initiative Joint Undertaking (IHI JU) under grant agreement No 101173001 — Accelerate.EU. The JU receives support from the European Union's Horizon Europe research and innovation programme and COCIR, EFPIA, Europa Bío, MedTech Europe, Vaccines Europe, IBA, Tetrakit, and LabLogic. Matthias Herth is the Chief Executive Officer (CEO) of Tetrakit Technologies ApS and Chief Scientific Officer (CSO) of PreTT ApS. These affiliations are disclosed as potential conflicts of interest. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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