
Quality Control Validation for Exogenous Natural Surfactant Labeled with ^{99m}Tc

Gabriela P. Calmanovici, PhD¹; María J. Salgueiro, Bioch, Pharm¹; Natalia M. Leonardi, Bioch¹; Cinthia G. Goldman Bioch, Pharm¹; Jorge O. Nicolini, Bioch²; Jose R. Boccio, PhD³; Ricardo A. Caro, PhD²; Patricia D. Zubata, Pharm⁴; and Marcela B. Zubillaga, PhD¹

¹Radioisotopes Laboratory, School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina;

²Laboratorios Bacon S.A.I.C., Buenos Aires, Argentina; ³Physics Department, School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina; and ⁴Quality Control Department, School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina

Objective: Exogenous natural surfactant (ENS) labeled with ^{99m}Tc shows an elevated lung specificity allowing the acquisition of high-quality images for ventilation scintigraphy.

Methods: The methods for ^{99m}Tc -ENS quality control (physical properties, pH determination, radiochemical studies, and biologic studies) were evaluated and validated.

Results: The physical properties of the nonradioactive precursor and of the radiopharmaceutical were analyzed as general descriptors of the product. The pH of the radiopharmaceutical was determined by using pH test papers, a method described and validated in the United States Pharmacopeia. Chromatographic studies performed using the acetone/Whatman-1 paper system were validated as a method to evaluate the radiochemical purity of the ^{99m}Tc -ENS. Biodistribution studies on rats after intratracheal administration were validated as a method to estimate the radiopharmaceutical biodistribution in humans.

Conclusion: The proposed method for ^{99m}Tc -ENS quality control studies and stability studies was evaluated and validated following international standards.

Key Words: diagnostic radiopharmaceutical; ventilation; quality control; ^{99m}Tc -ENS; validation

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Exogenous natural surfactant (ENS) labeled with ^{99m}Tc is under study as a specific aerosol scintiscanning agent (1–3). The lung distribution pattern obtained after the administration of ^{99m}Tc -ENS is more homogeneous than the pattern obtained after the administration of diethylenetriaminepentaacetic acid labeled with ^{99m}Tc , the radiopharma-

ceutical most commonly used for this study in our country, Argentina (2,3). The preparation of radiopharmaceuticals should include testing of quality assurance parameters (4) to preclude any undesirable effect on patients. Quality control for ^{99m}Tc -ENS involves the determination of physical properties, pH, radiochemical purity (using acetone/Whatman-1 paper chromatography), and biodistribution in rats (using tracheal instillation) (5). This report describes the evaluation, selection, and validation of quality control methods for ^{99m}Tc -ENS to ensure that these methods perform at the level required by their intended application (6–8).

MATERIALS AND METHODS

Radiolabeling Procedure

The nonradioactive precursor was a freeze-dried formulation of ENS provided by Bacon Laboratories. To obtain the ^{99m}Tc -ENS, we added to the nonradioactive precursor a solution of pertechnetate eluted from a ^{99}Mo - ^{99m}Tc generator (Radiofarma) with an activity of 18,500 MBq. The final volume was 3 mL, and the final activity concentration was 0.37 GBq/mL. The content of each vial was shaken vigorously for 60 s and then allowed to stand at room temperature for at least 10 min before the analysis.

Physical Properties and pH

The physical characteristics before and after reconstitution of the formulation were examined. The sample pH was measured using pH test papers (pH 1–10; Merck), a method that has been described and validated in the United States Pharmacopeia (9,10).

Chromatographic Studies

In previous works, we demonstrated that ^{99m}Tc -ENS remains at the origin and that free pertechnetate travels with the solvent front in this chromatographic system (5). In this work, chromatographic studies were validated as a method

For correspondence or reprints contact: Gabriela P. Calmanovici, PhD, Radioisotopes Laboratory, School of Pharmacy and Biochemistry, University of Buenos Aires, Junín 956, Piso Bajo, 1113 Buenos Aires, Argentina.

E-mail: gcalmano@ffyba.uba.ar

that evaluates the radiochemical purity of $^{99m}\text{Tc-ENS}$. For its determination, an aliquot (15 μL) of $^{99m}\text{Tc-ENS}$ was spotted on Whatman-1 paper (RJM Sales, Inc.) and was developed with acetone as the mobile phase (Merck), with the solvent migrating 10 cm (1,5).

Biodistribution Studies

Biodistribution studies of radiopharmaceuticals have been validated as a methodology that estimates the biodistribution of the radiopharmaceuticals in humans (11). The experiments performed on animals adhered to ethical standards and were conducted according to local animal care regulations. Each of 70 Sprague–Dawley rats (weight range, 250–500 g) was anesthetized with 300 mg of chloral hydrate AR (Mallinckrodt) per kilogram of body weight and received an intratracheal injection of $^{99m}\text{Tc-ENS}$ (0.3 mL). Thirty minutes after injection, each rat was sacrificed using a lethal dose (800 mg/kg) of chloral hydrate, and organs of interest (lungs, gastrointestinal system, heart, blood, liver, spleen, and kidneys) were excised, blotted dry, and measured for radioactivity (1,5).

Measurements

The activity of the radiopharmaceutical was measured in an ionization chamber (RADX model 255 Remote; RADX Corp.). Samples for the radiochemical purity and biodistribution studies were measured in a monochannel γ -spectrometer with a 5×5 cm NaI(Tl) standard well crystal, which was previously set to optimal electronic conditions. All counting measurements were performed with a relative error of less than 1%.

Validation of Methods

Specificity was evaluated by intentional degradation studies of the nonradioactive precursor, including acid hydrolysis (HCl, 5N; 30 min), base hydrolysis (NaOH, 5N; 30 min), oxidation (H_2O_2 , 30 g/L; 30 min), heat treatment (50°C, 20 h), and exposure to artificial light (20 h). After the degradation treatments, the samples were allowed to cool to room temperature, were neutralized when required, and then were labeled with ^{99m}Tc . For comparison, an untreated sample was labeled with ^{99m}Tc (control sample). To evaluate precision, we studied repeatability (instrumental precision) and intermediate precision (method precision). Repeatability was evaluated by the same operator performing 10 determinations on the same day and on the same equipment. Intermediate precision was evaluated by 2 operators performing 10 determinations on different days, different weeks, and different equipment within the same laboratory, with the results compared by the Student *t* test ($P < 0.05$) (12). The results for specificity, repeatability, and intermediate precision were expressed as percentage of radioactivity at the origin (chromatographic studies) and activity concentration in the lungs (biodistribution studies). Accuracy was assessed using the addition-matrix method, by adding 10% of the radiopharmaceutical to each sample ($n = 3$) at 80%, 100%, and 120% of the defined activity concen-

tration. The results were expressed as the percentage of recovery, where the added activity was the spotted activity in the chromatogram (chromatographic studies) and the injected activity by tracheal instillation (biodistribution studies). Linearity was studied by analyzing, 3 times, each of 5 radiopharmaceutical suspensions of activity concentration ranging from 0.28 to 0.56 GBq/mL. The results were expressed as the equation obtained for the linear regression and its correlation coefficient (r^2) with $P < 0.05$ (13). Robustness for chromatographic studies was evaluated by the variation of cube saturation time (0.5–6 h), time for drying of the chromatographic strips (15 min–1 h), and distance to which the solvent migrated (8–12 cm). Robustness for biodistribution studies was evaluated by the variations of age (3–6 mo) and sex of the animals, time of measurement after dissection (15 min–2 h), and injected volume (0.2–0.4 mL) (6–8).

RESULTS

Physical Properties and pH

The nonradioactive precursor of the radiopharmaceutical was a white powder. $^{99m}\text{Tc-ENS}$ was a white suspension with a pH of 4.0–6.0.

Validation of Chromatographic Studies

In specificity studies we observed—after oxidation, acid hydrolysis, and alkaline hydrolysis of the nonradioactive precursor—a heterogeneous distribution of radioactivity in the chromatogram, with a lower percentage of radioactivity at the origin than that in the control sample. We observed no heat-treatment or light-exposure differences from the control sample. In the repeatability studies (instrumental precision), the mean radioactivity at the origin was 99.5% (SD, 0.3%; coefficient of variation [CV], 0.3%). In the intermediate precision studies (method precision), the mean remaining at the origin was not statistically different between the 2 operators (99.5% and 99.0%; SD, 0.3% and 0.6%; CV, 0.4% and 0.6%; $P > 0.05$). The accuracy studies demonstrated nearly 100% recovery (98.0%–99.5%; mean, 98.9%), with a CV of 0.95% (Table 1). The regression curve proved linearity ($r^2 = 0.9999$; $P < 0.05$), with a *y*-intercept of (0.01046 ± 0.00979) and a slope of (7.92900 ± 0.02715) (Fig. 1). The robustness studies showed a lack of influence of the operative variables under study compared with the results obtained without variables (Table 2).

TABLE 1
Accuracy of Chromatographic Studies ($n = 9$)

Added activity (kBq)	Mean recovered activity (kBq)	Mean recovery (%)	SD
2.39	2.37	99.1	0.8
3.01	2.95	98.0	0.7
3.52	3.50	99.5	0.7

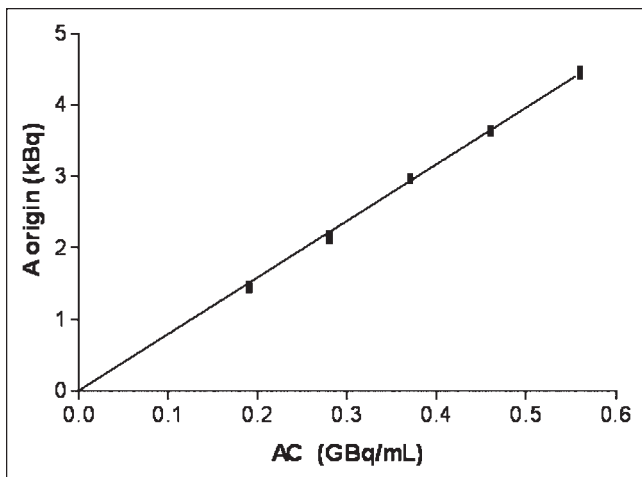


FIGURE 1. Linearity evaluation for chromatographic studies. AC = activity concentration of ^{99m}Tc -ENS; A origin = activity at origin of acetone/Whatman-1 paper chromatography.

TABLE 2
Robustness of Chromatographic Studies

Variable	% origin*
Cube saturation time (h)	
0.5	99.1
1	99.0
Chromatographic-strip drying time (h)	
0.25	99.3
1	99.4
Solvent migration distance (cm)	
8	99.8
12	99.2

*Percentage of radioactivity remaining at origin.

Validation of Biodistribution Studies

Table 3 shows the results for the specificity studies. After oxidation, acid hydrolysis, and alkaline hydrolysis of the nonradioactive precursor, the percentage of radioactivity was heterogeneously distributed in the studied organs, with a lower percentage of activity in the lungs than that obtained for the control sample. For heat treatment and light exposure, the results were similar to those for the control sample and were in accordance with the

TABLE 3
Specificity of Biodistribution Studies

Treatment	Activity (%)						
	Lungs	GIS	Heart	Blood	Liver	Spleen	Kidneys
Acid hydrolysis	33.50	9.64	4.11	26.25	10.23	8.43	7.84
Base hydrolysis	33.81	8.38	2.93	28.87	8.61	8.88	8.52
Oxidation	30.28	7.91	2.13	29.67	9.82	10.66	9.53
Heat treatment	99.78	0.05	0.05	0.00	0.00	0.00	0.11
Light exposure	94.10	5.69	0.00	0.02	0.03	0.00	0.16
Control	98.63	1.25	0.00	0.02	0.03	0.00	0.07

GIS = gastrointestinal system and content.

TABLE 4
Accuracy of Biodistribution Studies ($n = 9$)

Added activity (kBq)	Mean recovered activity (kBq)	Mean recovery (%)	SD
4.07	4.02	98.7	0.9
5.26	5.25	99.9	0.8
6.36	6.29	98.9	0.6

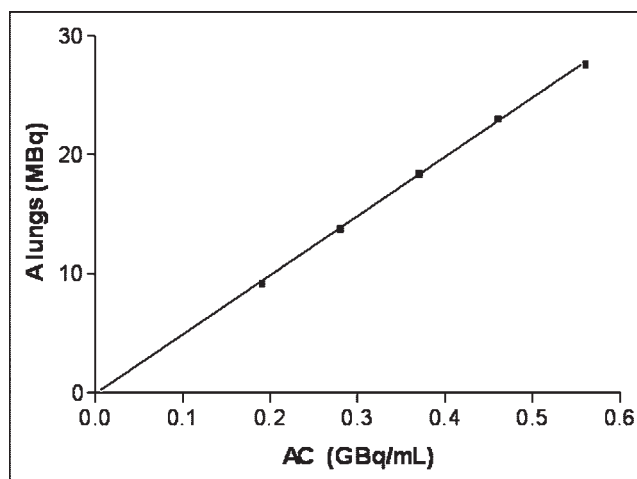


FIGURE 2. Linearity evaluation for biodistribution studies. AC = activity concentration of ^{99m}Tc -ENS; A lungs = activity in lungs in biodistribution studies.

range established for this method (5). The repeatability studies (instrumental precision) found a mean activity of 96.3% in the lungs (SD, 2.3%; CV, 2.4%). The intermediate precision studies (method precision) found mean activities of 97.4% and 95.7% in the lungs (not significantly different between the 2 operators [SD, 2.1% and 2.5%; CV, 2.1% and 2.6%; $P > 0.05$]). The recovery percentages in the accuracy studies were nearly 100% (98.7%–99.9%), with a mean value of 99.2% and a CV of 0.9% (Table 4). For linearity, the regression curve proved linearity ($r^2 = 0.9970$) with a y-intercept of (0.01562 ± 0.47810) and a slope of (49.77000 ± 1.21300) (Fig. 2). Table 5 shows the results for the robustness studies. A comparison of the results obtained with and without the

TABLE 5
Robustness of Biodistribution Studies

Variable	% lungs*
Age of animal (mo)	
3	99.62
6	94.70
Sex of animal	
M	97.71
F	99.36
Time of measurement after dissection (h)	
0.25	99.11
2	98.65
Injected volume (mL)	
0.2	99.25
0.4	98.32

*Percentage of activity in lungs.

operative variables shows that the variables under study lacked influence.

DISCUSSION

As general descriptors of the product, the physical properties of the nonradioactive precursor and of the radiopharmaceutical were analyzed—a useful preliminary evaluation (14). The pH of the radiopharmaceutical was determined by a method described in the United States Pharmacopeia (9,10). Application of this method took into account the fact that radiopharmaceuticals must have an appropriate concentration of hydrogen ions for their stability and integrity (11) and that degradation of many drugs in solution is accelerated or decelerated exponentially as the pH is varied over a specific range (15).

The chromatographic studies were validated as a method to evaluate the radiochemical purity of ^{99m}Tc -ENS. The biodistribution studies were validated as a method to estimate radiopharmaceutical biodistribution in humans according to biodistribution in animals. Both methods were proven to be specific, precise, accurate, linear, and robust. Regarding specificity, after intentional degradation of the nonradioactive precursor of the radiopharmaceutical, we observed a heterogeneous distribution of the radioactivity in the chromatographic studies and in the biodistribution studies, indicating the presence of degradation products. Precision was proven by the low SD shown for both methods in the repeatability studies (instrumental precision) and by the nonsignificant difference between the 2 operators shown in the intermediate precision studies (method precision). Accuracy was proven by the nearly 100% recovery shown for both methods. Linearity was proven by the regression curve for both methods. Regarding robustness, for the variables studied we observed neither a variation in the percentage of radioactivity remaining at the origin (for the chromatographic studies) nor a variation in the percentage of radioactivity in the lungs (for the biodistribution studies) (6–8,16).

CONCLUSION

The proposed method for ^{99m}Tc -ENS quality control and stability studies was evaluated and validated following international standards (6–8). This method involves determining the physical properties of the nonradioactive precursor and of the radiopharmaceutical, determining pH, determining radiochemical purity by acetone/Whatman-1 paper chromatography, and determining biodistribution in rats after intratracheal administration of the radiopharmaceutical. In this way, we can guarantee the quality of the product for its proposed use.

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