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Evaluation of the Degradation Profile and Stability of the MAG3 Lyophilized Reagent

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1. Introduction

99mTc-MAG3 (Mercaptoacetyltriglycine) is a radiopharmaceutical that has received significant attention in the field of medical imaging, particularly for its application in assessing renal function, in patients with impaired renal function [1]. Since its initial use, MAG3 LR has suffered from instability problems, and although changes in formulation and labeling procedures have evolved, problems with radiochemical purity persist [2]. The presence of several electron-donor in its molecule such as N, O and S, as showed in the Figure 1, makes S-benzoyl-mercaptoacetyl-triglycine a very reactive reagent that can suffer parallel reactions or degradation, generating nonradioactive impurities [3].

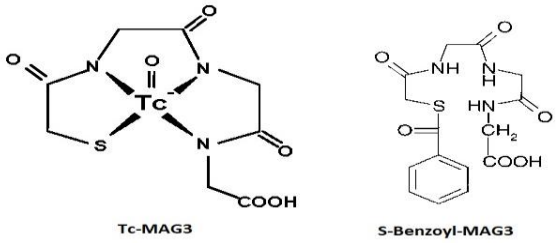

Figure 1. Molecular structure of the compound 99mTc-MAG3 and S-Benzoyl-MAG3 [4].

Nonradioactive impurities can interfere with sensitive radiopharmaceutical preparations, causing to suboptimal quality and leading to preparations being affected and potentially causing unknown radiation doses [5]. High-performance liquid chromatography (HPLC) is an equipment used in the field of radiopharmaceuticals, particularly for the detection of radiochemical impurities of more complex radiopharmaceuticals because is more specific than other methods such as solid-phase extraction (SPE) and thin-layer chromatography (TLC), which can lead to underestimation or overestimation of radiochemical purity (RCP) [6]. When used in final product analysis, HPLC analysis allows determine the active pharmaceutical ingredient (API) content, the stability of the formulation and the degradation profile of the tested product in order to avoid use if any impurity is observed. Forced degradation studies play a pivotal role in the pharmaceutical industry to observe the degradation profile and ensure the quality of radiopharmaceutical products. These studies subject drugs to extreme conditions, aiding in the identification of potential degradation products and pathways [7]. By understanding degradation mechanisms, it is possible to develop robust drug formulations and packaging, ultimately ensuring the safety and efficacy of medications for patient use. To understand the correlation between the degradation of the API and the increase in degradation products in forced degradation studies, the

mass balance help in elucidating the metabolic fate of a drug and is a key in optimizing the design of clinical trials and ensuring compliance with regulatory guidelines [8-9].
In Brazil, there is no mention of MAG3 in the list of radiopharmaceuticals currently registered in ANVISA (National Health Surveillance Agency) [9].
The present work aimed to develop a stability indicative HPLC method capable of identifying and quantifying possible impurities and products degradation profile of the MAG3 LR developed in IPEN.

2. Methodology

The composition MAG3 LR developed by IPEN was described by Silva [10]. Samples for HPLC analysis were prepared adding 3 mL of purified water to 1 mg MAG3 RL.
Calibration curves of S-benzoyl-MAG3 in 1.0 – 40.0 $\mu g.mL^{-1}$ concentration range were obtained by using a HPLC-PDA/UV Shimadzu Prominence LC 20-AT. Analysis were performed using a XTerratm C18 (5.0 µm, 4.0 mm X 150 mm, Waters) column, with 1 $mL.min^{-1}$ flow rate, 10 µL of injection sample volume and 240 nm as wavelenght for quantification purpose at room temperature. The gradient program was composed by a mixture of acetonitrile (phase A) (0 to 5 min: 0%, 5 min to 22.5 min: 50%, 22.5 min to 32.5 min: 50%, 32.5 min to 37.5 min: 0%, 40 min: 0%) and ammonium acetate 1mM (phase B). The analytical method was validated in accordance with RDC 166 requirements [11]. Samples for degradation products and impurities evaluation were subjected to the experimental conditions in solid form. The exposure time and experimental conditions are presented in Table I.

Table I: Exposure time and experimental conditions for degradation products and impurities evaluation.

Condition of degradation	Stress conditions	Exposure time*
Thermal	70°C/ 20% Relative Umidity	21 days
Thermal and humidity	70°C/ 75% Relative Umidity	21 days
Photolitic	Xenon 200 watt/m^2 e 1,2x10^6 lux-hour	44 hours (2x ICH)
Hidrolytic	HCl 0,1M, 25°C	24 hours
	NaOH 0,1M, 25°C	24 hours
Oxydative	FeCl3 1 mM, 30°C	24 hours

** Exposure time in which a degradation rate of at least 10% was reached and/or the maximum exposure time was reached, according to the literature [7].*

Mass balance of degradation study was calculated considering the degradation product peak area, whether these are greater or less than the reference value. The Equation 1 presents the formula to calculate the mass balance percentage.

$$Mass\ balance = \frac{(T_D + PD_D)}{(T_i + PD_i)}\ x\ 100\% \quad (1)$$

where Ti is the content in percentage of API as no degraded sample; TD is the content in percentage of API in degraded sample, PDD is the sum of degradation products in percentage in the sample after degradation exposure and PDi is the sum of observed peaks in percentage in not degraded sample.

3. Results and Discussion

Selectivity test showed that there was no interference of individual components of MAG3 LR on S-benzoyl-MAG3 determination by peak-purity evaluation. The individual components were added to standard solution in the same concentration as the developed formulation and the chromatogram is presented in Figure 2.

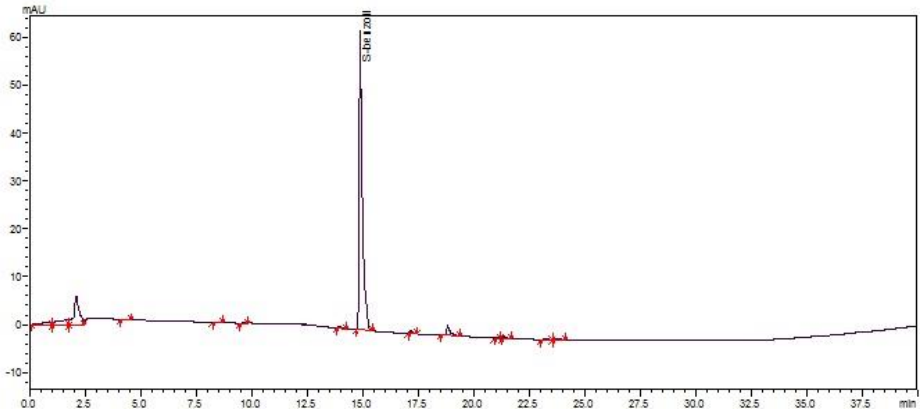

Figure 2. S-benzoyl-MAG3 chromatogram in selectivity test.

S-benzoyl-MAG3 showed a retention time of 14.9 minutes ± 0.5 and a content of 100.5%.
The linear equation for analytical curve for S-benzoyl-MAG3 in 1.0 – 40.0 µg.mL^{-1} concentration range is A = 201.94 [API S-benzoyl-MAG3] + 9300.3, with a determination coefficient (r) of 0.999.
The control sample used as refence showed one unknow peak at 18.85 minutes, wich represents a relative retention time (RTT) of 1.26 minute. The unknow peak showed a content of 3.3%, which was considered on mass balance calculation. The most drastic degradation condition for LR MAG3 was alkaline hydrolysis with 0.1M NaOH. The content measured after exposure time was 0.2%, representing a total variation of 100.3%, which can be considered a total degradation. Chromatogram of alkaline hydrolysis condition is showed in Figure 3. Mass balance of degradation study is showed in the Table II.

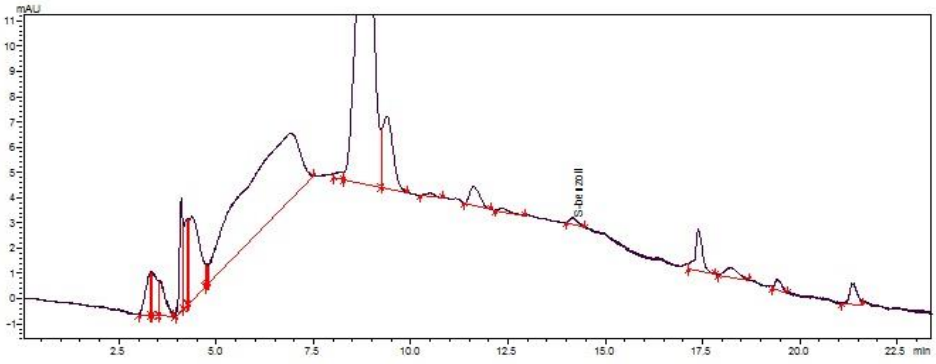

Figure 3. Chromatogram of LR MAG3 in the alkaline hydrolysis condition.

Table II: Mass balance procedure of degradation study.

Condition of degradation	Content (%)	Content variation (%)	Total impurities (%)	Content + Impurities (%)	Mass balance (%)
Control sample	100.50	-	3.30	103.80	-
Thermal (70°C/ 20% UR)	92.20	8.30	4.11	96.31	92.78
Thermal and humidity	102.50	- 2.00	6.26	108.76	104.78
Acid hydrolysis	94.60	5.90	1.36	95.96	92.45
Alkaline hydrolysis	0.20	100.30	49.45	49.65	47.83
Photolysis protected	99.00	1.50	3.23	102.23	98.49
Photolysis exposed	94.60	5.90	4.05	98.65	95.04
Oxidation ($FeCl_3$)	90.20	10.30	18.10	108.30	104.33

4. Conclusions

The developed analytical method is suitable for detecting the resulting impurities of degradation study. The chromatographic profile of degradation conditions for the formulation MAG3 LR demonstrated that the IPEN product matrix has little influence on product degradation. The peak of S-benzoyl-MAG3 reduced 10% and the sum of the recovery values of the unknown products generated a mass balance value close to 100% - an indicator that demonstrates that the method is capable of quantifying impurities of degradation in compliance with the limits proposed by legislation [12].

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