Development and validation of ^{99m}Tc ethylene dicysteine and EC determination method by HPLC.

Almeida, E. V.; Fukumori, N. T. O.; Mengatti, J.; Silva, C. P. G.; Matsuda, M. M. N.
Radiopharmacy Center - IPEN – CNEN/SP
Av. Professor Lineu Prestes 2242 - 05508-000 - São Paulo – SP - Brazil

1. Introduction

Technetium-99m labelled L,L-ethylene dicysteine (^{99m}Tc-EC) is a radiopharmaceutical commonly used for the radioisotopic evaluation of the renal function. It is generally prepared by addition of pertechnetate ($^{99m}TcO^-_4$) to the commercially available lyophilized kit containing EC (ethylene dicysteine) reagent, at basic conditions [1]. EC reagent is synthesized via L-thiazolidine-4-carboxylic acid and subsequent dimerization of the intermediate radical in Na/NH_3 medium [2]; and the main impurities are N-methyl-L-cysteine and L-cysteine [3]. Since there is no method for determining the radiochemical and chemical purities of the radiopharmaceutical and its precursor reagent, this work reports an method for ^{99m}Tc-EC and EC lyophilized kit analysis and the HPLC validation analytical parameters, such as linearity and selectivity.

2. Materials and methods

The HPLC system (LC 20AT prominence) (Shimadzu, Japan) was composed by two pumps, autosampler (SIL 20A), system controller (CBM 20A), and diode array (SPD M20A) and radiometric detector (Bioscan). The analysis was performed with a Shim-Pack VP-ODS column (150 mm x 4.6 mm i.d., 5 µm particle size). All solutions were previously filtered, a 20 µL aliquot sample was injected and isocratically eluted in 50 mmol L^{-1} phosphate buffer (pH 2.5) and ethanol (94:6;v/v) mobile phase, at 0.4 mL min^{-1} flow rate. ^{99m}Tc ($Na^{99m}TcO_4$) and EC lyophilized kit were from IPEN-CNEN/SP (Brazil), ethylene dicysteine reagent was acquired from ABX (Germany), and other reagents were from Merck (Germany). Water was purified in a MilliQ® system from Millipore (France).

^{99m}Tc-EC was prepared by adding 1mL $Na^{99m}TcO_4$ in 1.0 mCi mL^{-1}, measured with a radioactive dose calibrator (Capintec). A 500 ppm EC stock standard solution was prepared by dissolving EC-ABX reagent in basic medium (pH 12.00) and 25, 50 and 200 ppm working solutions were prepared by appropriate dilutions. Each standard solution was analyzed in triplicate and EC calibration curves were obtained in the same day (intra-day) and in consecutive days (inter-day).

3. Results and discussion

Figure 1 shows the peak profile and its respective retention time in RP-HPLC chromatogram of (a) ^{99m}Tc-EC, (b) 500 ppm EC standard solution, (c) sample of EC lyophilized kit (IPEN-CNEN/SP) and (d) UV spectrum of 500 ppm EC. By this method, a fast determination of the radiopharmaceutical is possible, which is important for quality control routine analysis, with ^{99m}Tc- EC retention time of 4.9 minutes. $^{99m}TcO^-_4$ was not observed as radiochemical impurity during 4 hour labeling. EC-ABX reagent and EC lyophilized kit produced at IPEN-CNEN/SP did not present the main reported synthesis impurities at these analysis

conditions. The three sample chromatograms show symmetrical peaks; the baseline was stable during the analysis. The method was linear in the range of 0-500 ppm. The calibration curve equation was Abs (λ=225nm) = 0.1408 [EC] + 2.228 (n=5), with good correlation coefficient (r = 0.999).

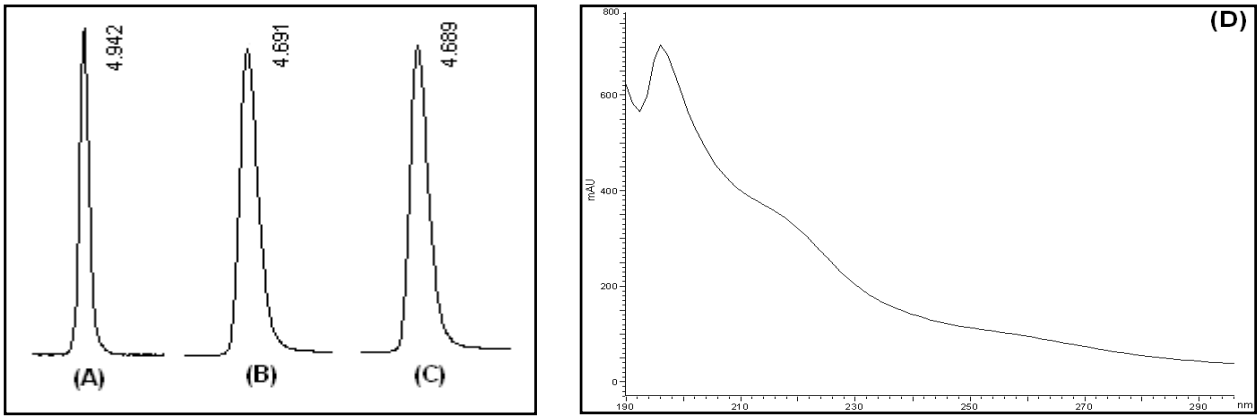

Figure 1 – Peak profile from RP-HPLC chromatogram of (A) ^{99m}Tc- EC, (B) 500 ppm EC standard solution and (C) sample of EC lyophilized kit (IPEN-CNEN/SP). (D) UV spectrum of 500 ppm EC standard solution.

The intraday and interday precision (SD(standard derivation)/calculated x 100) and accuracy (calculated/nominal value x 100) of 4 EC standard solutions are presented in the Table I.

Table I - Intra and Inter-day precision and accuracy values of EC standard solution analysis.

Nominal value (ppm)	Intra-day Average ± SD*	Precision (%) (Intra-day)	Accuracy (%) (Intra-day)	Inter-day Average ± SD	Precision (%) (Inter-day)	Accuracy (%) (Inter-day)
25	26.09 ± 0.31	1.18	104.36	27.04 ± 0.43	1.59	108.16
50	55.71 ± 1.61	2.88	111.42	57.04 ± 1.99	3.49	114.08
200	211.89± 2.92	1.38	105.94	212.00 ± 3.04	1.43	106.00
500	513.00± 6.87	1.34	102.60	515.97 ± 5.82	1.13	103.18

*standard derivation

Repeatability (intra-day) and intermediate precision (inter-day) expressed as RSD (relative standard deviation in percentage), were lower than 3.5%, and the mean recovery percentage was 96,98%. The LD (detection limit) and LQ (quantification limit) were 5.32 ppm and 17.74 ppm, respectively.

4. Conclusion

This work developed a new, selective, precise and accurate method for quantification of ^{99m}Tc-EC and EC in commercial lyophilized kit using HPLC with radioactivity and UV detectors.

References

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