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Research paper

Usage of radiopharmaceuticals in the development of pharmaceutical drug delivery systems: validation of [^{99m}Tc]DTPA and [^{99m}Tc]ECD

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Abstract

Tablets containing drugs of different lipophilicity, ranitidine and cinarizine, and placebo were prepared and their in vitro behaviour was studied by dissolution and disintegration tests. [^{99m}Tc]Diethylenetriamine-pentaacetic acid ([^{99m}Tc]DTPA) and [^{99m}Tc]ethyl cysteinate dimer ([^{99m}Tc]ECD) were used as tracers of the process. Both of them were added to tablets during wet granulation. Dissolution and disintegration profiles were assessed at different pH values (1, 4 and 7). Radioactivity was evaluated in filtered samples and scintigraphic studies were carried out in gamma camera. Stability in dissolution media was confirmed for both tracers under these conditions. Dissolution and disintegration velocity constants were calculated. [^{99m}Tc]DTPA proved to be an appropriate tracer for polar drugs such as ranitidine. Nevertheless, it was not a suitable tracer for lipophilic active drugs such as cinarizine. On the other hand, the most lipophilic tracer, [^{99m}Tc]ECD, exhibited the opposite behaviour. Scintigraphic studies of the disintegration process did not show significant differences between placebos and tablets containing active drugs. As disintegration is a physical process it does not discriminate between chemical differences in tablet formulations. Both methods complement each other because the dissolution process can be followed when a suitable radiotracer is chosen according to the physicochemical characteristics of the active drug.

Keywords: Radiopharmaceuticals; Validation; Drug delivery; [^{99m}Tc]ECD; [^{99m}Tc]DTPA; Pharmaceutical development; Tablet formulation

1. Introduction

During the last 20 years, very important improvements have been achieved in pharmaceutical dosage formulation using radiopharmaceuticals as tracers [1–3], but only a few systematic studies have been performed to validate the methodology. Radiopharmaceuticals in nuclear medicine were primarily developed for diagnosis purposes and most of them were administered parenterally. In order to optimise the usage of radiopharmaceuticals in the development of pharmaceutical drug delivery systems, the behaviour of the tracers administered by routes other than the normal must be validated in different dissolution media and in the presence of pharmaceutical excipients [4,5]. Our group has performed stability and in vitro studies of different radiopharmaceuticals with the aim of creating a database of tracers with known physicochemical properties to be used as model drugs in pharmaceutical dosage development [6–9].

The objective of this study is to examine the way radiotracers model the release of drugs from tablet formulations and to validate the feasibility and limitations of two radiotracers with different physicochemical characteristics through basic in vitro studies. The possibility of developing a database of the physicochemical behaviour of radiotracers would allow selection of the most appropriate drug models when using scintigraphic techniques in the development of pharmaceutical dosage forms [10–14].

The characterised radiopharmaceuticals were [^{99m}Tc]diethylenetriamine-pentaacetic acid ([^{99m}Tc]DTPA) and [^{99m}Tc]ethyl cysteinate dimer ([^{99m}Tc]ECD) [15] which were incorporated into tablets during wet granulation. Tablets with drugs of different lipophilicity, ranitidine and cinarizine, and placebo were prepared as previously

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reported by our group [16]. Dissolution and disintegration profiles were assessed at different pH values (1, 4 and 7) [17–19].

2. Materials and methods

2.1. Radiopharmaceuticals labelling

[^{99m}Tc]DTPA and [^{99m}Tc]ECD were obtained by labelling commercial kits with [^{99m}Tc]pertechnetate obtained from an Elumatic III generator (Schering CIS)®. Radiochemical purity tests were performed by chromatographic systems using Whatman No. 1/propanone and Whatman No. 1/NaCl 0.9% for [^{99m}Tc]DTPA complex. [^{99m}Tc]ECD radiochemical purity was studied by chromatography in Whatman No. 1/methanol 85% and HPLC (Shimadzu LC-10 AS) using a Partisphere C18 (Whatman) Column as stationary phase and the mobile phase: phosphate buffer 0.0125 M pH 2.5 (**A**) and anhydride ethanol (**B**). Solvents program was at time zero 100% **A**, and at time = 10 min 70% **A** and 30% **B**, flow rate was set at 2 ml/min.

Stability studies for [^{99m}Tc]DTPA with all the excipients of the tablet formulation have been previously reported by our group [16]. Similar studies were performed for [^{99m}Tc]ECD with each one of the excipients: starch, lactose, PVP K30, magnesium stearate and Ac-Di-Sol. Each component was wetted with the radiotracer and dried at 70 °C during 10 min. The same procedure was performed with the granulate used for preparing tablets. [^{99m}Tc]ECD was incubated with each one of the dissolution media (hydrochloric acid pH 1, 4 and water pH 7) at 37 °C during 120 min to establish the stability of the radiotracer during dissolution in vitro studies.

Identical conditions of incubation for those tablets containing active drugs cinarizine and ranitidine were performed. Chemical stability was determined by UV spectrophotometry (Shimadzu-Bausch & Lomb Spectronic 210 UV) at 251 and 314 nm for cinarizine and ranitidine, respectively.

2.2. Partition coefficient

Partition coefficients water/octanol (w/o) were performed for both radiopharmaceuticals. Two millilitres of saline solution and octanol were put into a centrifuge tube. A known amount of [^{99m}Tc]DTPA or [^{99m}Tc]ECD activity in 0.5 ml was added. Then the mixture was shaken in a Vortex mixer during 1 min. Phases were separated by centrifugation. Aliquots of 0.5 ml from each phase were measured in a solid-state scintigraphic counter Ortec-Maestro MCB1. Volume corrections were done and the activity percentage was determined in each phase.

2.3. Tablet preparation

Tablets were prepared by wet granulation according to our previously reported method. The tablets were weighed (target weight 400 mg) and activity measured in a dose calibrator Capintec CRC 25 (target activity 18.5 MBq).

2.4. Dissolution studies

Tablet dissolution was performed in a Vankel USP Type II dissolution apparatus. The same dissolution were evaluated as the stability studies of the radiotracers, 900 ml of filtered and shaken solution, were incubated at 37 °C, at a spindle speed of 50 rev./min. Samples of 2 ml were obtained during the process at 1, 2, 3, 5, 10, 15 and 30 min. They were filtered through cellulose acetate membranes (0.22 μm). Each sample was assessed for radioactivity content, and drug concentration was determined by UV spectrophotometry at 251 nm for cinarizine and 314 nm for ranitidine. Corrections for volume, decay and efficiency were performed. Curves of log% non-dissolved vs. time were plotted.

2.5. Disintegration studies

Disintegration profiles were recorded simultaneously to the dissolution process. Vessels were placed in front of a gamma camera detector Dyna (Picker 4/15) of round field with low energies collimator and high resolution with a 20% window centred in 140 keV (^{99m}Tc photo peak) and NaCl (Tl) detector.

Serial images were registered during 30 min at 1 frame/min with a matrix of 128 × 128 without acquisition zoom. Three regions of interest were delimited: one on the tablet, another one on the dissolution medium and the third one on the external field to determine the background.

2.6. Data processing

First-order kinetics is the most common dissolution profile in 'non-sink' methods. When the drug in solid state dissolves, the dissolution medium increases its concentration, so dissolution velocity depends on the concentration of the drug in the dissolution medium. The dissolution velocity constant of the drug is k_d, which corresponds to the slope of the linear regression:

$$\log[1 - Q_t/Q_\infty] = k_d t/2.303 \quad (1)$$

Considering A, the amount of drug or activity of the tracer present in the solid formulation and Q, the amount of drug or activity of the tracer dissolved in the dissolution medium. The amount of drug that is dissolved at time= t is Q_t and the remaining drug in the solid form is:

$$A_t = Q_\infty - Q_t \quad (2)$$

$$Q_\infty - Q_t = Q_\infty \, e^{-k\,dt} \quad (3)$$

$$e^{-k\,dt} = 1 - Q_t/Q_\infty \quad (4)$$

It can be considered that at infinite time t_∞, $A_0 = Q_\infty$ so

$$A_t = Q_\infty \, e^{-k\,dt} \quad (5)$$

the logarithm of this expression is the one used above to calculate the slope [20].

Disintegration data is processed in a similar way to that described above, A being the net activity present in the region of interest on the tablet, Q, the net activity present in the region of interest of the vessel and k_d, the first-order disintegration velocity constant.

3. Results

3.1. Labelling process

Tracers [^{99m}Tc]DTPA and [^{99m}Tc]ECD were prepared with radiochemical purity higher than 99% [21].

3.2. Stability studies

No physicochemical change of the radiotracer was observed after 120 min incubation of [^{99m}Tc]DTPA with the dissolution media at pH 1, 4 and 7 and after the process of wetting and drying the components of the formulation.

The w/o coefficient for [^{99m}Tc]DTPA was 0.95 in the saline phase.

[^{99m}Tc]ECD during the granulation process showed no changes in radiochemical purity. When it was incubated in the dissolution media at pH 4 and 7 it did not show any physicochemical change during 120 min, but it showed the presence of hydrolysis products after 60 min at pH 1, lowering the radiochemical purity to 70%.

The w/o coefficient for [^{99m}Tc]ECD was 0.23 in the saline phase.

Stability studies for ranitidine did not show detectable variations in the UV spectrum during 120 min in the dissolution mediums. Cinarizine did not show changes during the time of study at pH 1 and 4 but it decomposed immediately at pH = 7.

3.3. Dissolution studies with [^{99m}Tc]DTPA

Dissolution profiles in all cases reached relatively low levels, between 46 and 60% for [^{99m}Tc]DTPA in placebo, cinarizine and ranitidine tablets (Figs. 1a, 2a, and 3a). When the tracer [^{99m}Tc]DTPA constants were determined from tablets containing ranitidine, values were almost of the same order as that of placebo at pH 1 and 4, but lower at pH 7 (Table 1). Also, dissolution of the tracer was lower at this pH, reaching a maximum value of 25% in 30 min.

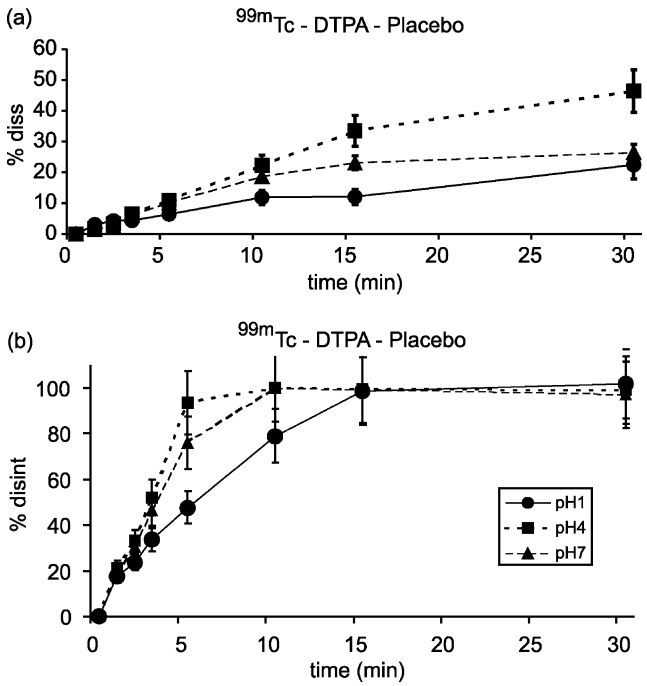

Fig. 1. (a) Dissolution profile of [^{99m}Tc]DTPA in placebo tablets. (b) Disintegration profiles determined by scintigraphy in placebo tablets.

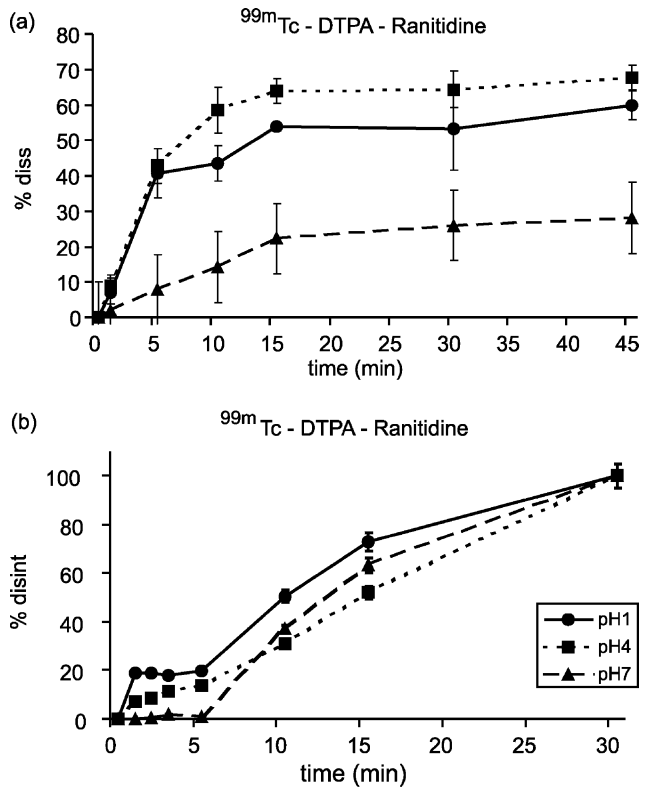

Fig. 2. (a) Dissolution profile of [^{99m}Tc]DTPA in ranitidine tablets. (b) Disintegration profiles determined by scintigraphy in ranitidine tablets.

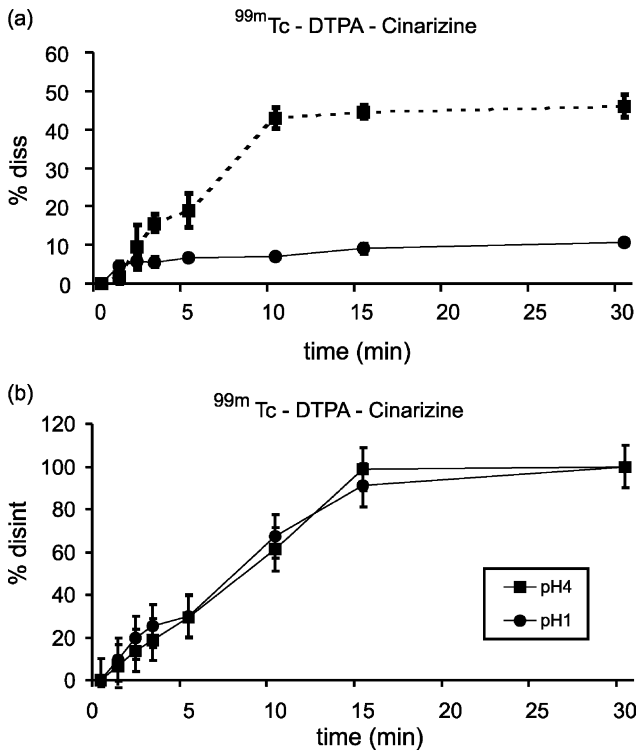

Fig. 3. (a) Dissolution profile of [^{99m}Tc]DTPA in cinarizine tablets. (b) Disintegration profiles determined by scintigraphy in cinarizine tablets.

The dissolution profile of the tracer at pH 4 for the cinarizine tablets reached 40% at 30 min but less than 20% for pH 1 (Fig. 3a). Dissolution velocity constants of the tracer from tablets containing cinarizine were significantly reduced at pH 1 and at pH 4 when compared with the same constants measured in placebo tablets (Table 1) [22].

3.4. Dissolution studies with [^{99m}Tc]ECD

Tracer dissolution in placebo tablets was very low at all pH range. Dissolution profiles reached 39% in 30 min at pH 7, 24.5% at pH 4 and 21% at pH 1 (Fig. 4a).

Dissolution percentages were about 4% at the three pH for ranitidine tablets (Fig. 5a).

Table 1
Dissolution velocity constant values

	pH 1	pH 4	pH 7
[^{99m}Tc]DTPA-ranitidine	0.096	0.093	0.079
[^{99m}Tc]DTPA-cinarizine	0.049	0.030	Decomposes
Placebo-DTPA	0.110	0.080	0.110
Ranitidine-DTPA UV	0.108	0.100	0.060
Cinarizine-DTPA UV	0.025	0.078	Decomposes
[^{99m}Tc]ECD-ranitidine	0.025	0.027	0.037
[^{99m}Tc]ECD-cinarizine	0.080	0.097	Decomposes
Placebo-ECD	0.041	0.050	0.054
Ranitidine-ECD UV	0.096	0.103	0.071
Cinarizine-ECD UV	0.031	0.076	Decomposes

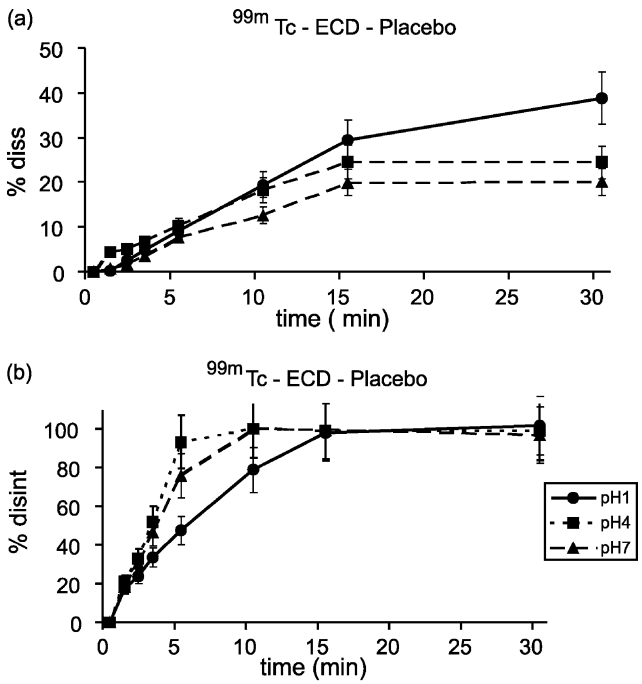

Fig. 4. (a) Dissolution profile of [^{99m}Tc]ECD in placebo tablets. (b) Disintegration profiles determined by scintigraphy in placebo tablets.

Cinarizine tablets reached 27% for pH 1 and 24% for pH 4 (Fig. 6a).

The dissolution of the tracer reached lower values in ranitidine tablets than in placebos. Also the tracer exhibited lower dissolution velocity constants in ranitidine tablets than placebos.

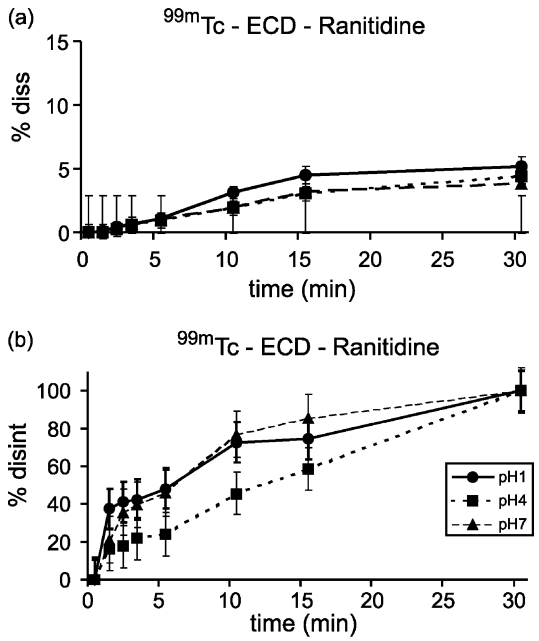

Fig. 5. (a) Dissolution profile of [^{99m}Tc]ECD in ranitidine tablets. (b) Disintegration profiles determined by scintigraphy in ranitidine tablets.

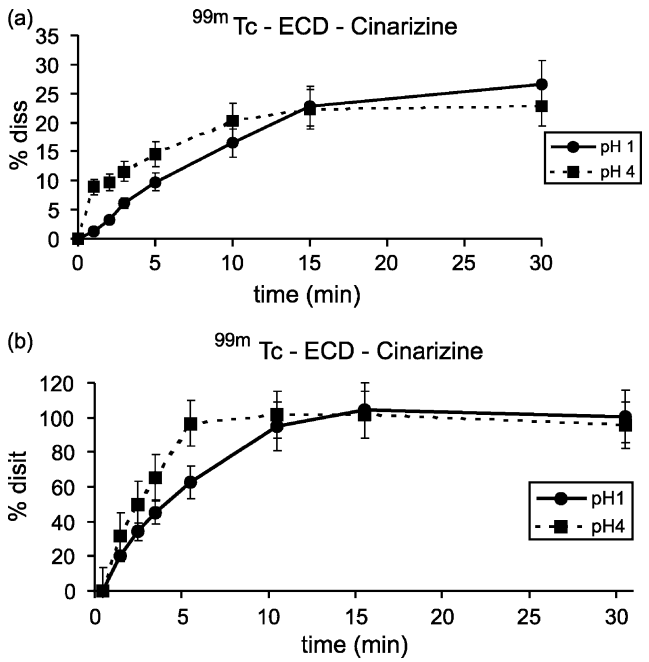

Fig. 6. (a) Dissolution profile of [^{99m}Tc]ECD in cinarizine tablets. (b) Disintegration profiles determined by scintigraphy in cinarizine tablets.

Release of tracer from tablets containing cinarizine demonstrated higher values for dissolution velocity constants (Table 1).

3.5. Drug release

The samples obtained during dissolution studies were assessed not only for radioactivity but also for drug concentration to determine drug release profile. Ranitidine reached 94% at pH 4 and 7 and 24% at pH 1. Cinarizine showed 92% at pH 4, 43% at pH 1 and decomposed at pH 1. These data was the same not only for tablets containing [^{99m}Tc]DTPA as a tracer but also for [^{99m}Tc]ECD. As the radiopharmaceuticals are present in about 10^{-9} M they do not affect the drug delivery profile.

3.6. Scintigraphic studies with [^{99m}Tc]DTPA

Studies for tablets containing [^{99m}Tc]DTPA did not demonstrate significant differences in disintegration velocity constants values for cinarizine or ranitidine tablets when compared with placebo profiles (Figs. 1b, 2b, and 3b).

3.7. Scintigraphic studies with [^{99m}Tc]ECD

Tablets containing [^{99m}Tc]ECD as a tracer demonstrated similar profiles both for placebo and cinarizine (Figs. 4b and 6b), but ranitidine tablets reached lower disintegration velocity constants (Table 2). In all the cases, the constants increased with increasing pH values (Table 2) [22].

Table 2
Disintegration velocity constants

	pH 1	pH 4	pH 7
[^{99m}Tc]DTPA-ranitidine	0.03	0.05	0.05
[^{99m}Tc]DTPA-cinarizine	0.06	0.06	Decomposes
Placebo-DTPA	0.04	0.07	0.07
[^{99m}Tc]ECD-ranitidine	0.02	0.02	0.05
[^{99m}Tc]ECD-cinarizine	0.04	0.06	Decomposes
Placebo-ECD	0.05	0.065	0.081

4. Discussion

According to the results [^{99m}Tc]DTPA is a suitable model for the dissolution of a polar drug such as ranitidine. This is supported by the fact that the dissolution velocity constants of both drug and tracer are of the same order at pH 7 (Table 1). It should be taken into account that according to USP XXV, the dissolution test for ranitidine should be performed at pH 7. Also disintegration studies showed similar profiles (Figs. 1b and 2b) as confirmed by scintigraphic studies. [^{99m}Tc]DTPA was a suitable tracer in terms of its stability in dissolution media.

Cinarizine tablets showed very different dissolution velocity constants when compared with this radiotracer (Table 1). This might be due to their physicochemical differences, so that [^{99m}Tc]DTPA is not an appropriate tracer for lipophilic active drugs in tablet formulations. Cinarizine shows very low dissolution velocity constants at both pH 1 and 4. Scintigraphic disintegration profiles show that the tracer can be useful as a marker for disintegration but not for dissolution.

The dissolution velocity constants of the most lipophilic tracer [^{99m}Tc]ECD in ranitidine tablets were lower than the same constants for the active drug (Table 1). This could be explained by the physicochemical differences between the lipophilic nature of [^{99m}Tc]ECD and a hydrophilic drug such as ranitidine. Dissolution measurements showed different behaviour for both tracer and ranitidine (Table 1), although the same profiles could be observed for both from the disintegration process (Table 2). So [^{99m}Tc]ECD is not a suitable tracer for in vitro studies of hydrophilic drugs like ranitidine.

Dissolution velocity constants of [^{99m}Tc]ECD in cinarizine tablets and the constants of the active drug were of the same order (Table 1). Similar behaviour could be observed in scintigraphic imaging of the disintegration process (Fig. 6b). Therefore, the radiopharmaceutical [^{99m}Tc]ECD can be used as a tracer not only for dissolution but also for disintegration profiles when in vitro studies are performed

during pharmaceutical formulation development of lipophilic drugs.

5. Conclusions

Dissolution studies demonstrate differences in behaviour between similar formulations containing different drugs, presumably due to the physicochemical nature of the drugs themselves and interactions with the tracer. Scintigraphic studies of the disintegration process did not show significant differences between placebos and tablets containing active drugs. As disintegration is a physical process it does not discriminate between chemical differences in tablet formulations. This method does show differences in the disintegration process with pH changes. It would be an interesting tool to determine the behaviour of different formulations but not for the same formulation with different active drug.

Both methods complement each other because the dissolution process can be followed by choosing a suitable radiotracer, according to the physicochemical characteristics of the active drug. On the other hand, different formulations could be assessed using scintigraphic disintegration profile.

Further studies should be performed to determine a broader range of radiopharmaceuticals of different physicochemical properties that could be used as drug model tracers.

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References

[1] A. Perkins, M. Frier, Nuclear Medicine in Pharmaceutical Research, Taylor & Francis, London, 1999, pp. 71–112.
[2] H.S. Bean, Advances in Pharmaceutical Sciences, Academic Press, London, 1992, pp. 154–162.
[3] L.G. Martini, J.K. Embleton, R.J. Malcolmson, C.G. Wilson, The use of small volume ocular sprays to improve the bioavailability of topically applied ophthalmic drugs, Eur. J. Biopharm. 44 (2) (2003) 121–126.
[4] H.K. Chan, E. Daviskas, S. Eberl, M. Robinson, G. Bautovich, I. Young, Deposition of aqueous aerosol of [^{99m}Tc]DTPA and delivered by novel system (AER$_x$) in healthy subjects, Eur. J. Nucl. Med. 26 (4) (1999) 320–327.
[5] S.S. Davies, J.G. Hardy, S.P. Newman, I.R. Wilding, Gamma scintigraphy in the evaluation of pharmaceutical dosage forms (Review article), Eur. J. Nucl. Med. 19 (1992) 971–986.
[6] O. Kosch, Magnetic markers in gastroenterology, Bioelect Biomagnet, http://www.berlin.ptbde/8/82/821/gastroe.htm
[7] C.G. Wilson, M. Mc Jury, B. O'Mahoy, M. Frier, A.C. Perkins, Imaging of oily formulations in the gastrointestinal tract, Adv. Drug Deliv. Rev. 25 (1997) 91–101.
[8] C.G. Wilson, M. Bakhashaee, N.N.E. Stevens, An evaluation of gastro-resistant pulsed release delivery system (Pulsincap) in man, J. Drug Deliv. 5 (1997) 201–206.
[9] A.C. Perkins, M. Frier, Nuclear medicine techniques in the evaluation of pharmaceutical formulations, Pharm. World Sci. 18 (1996) 97–104.
[10] T.M. Dams, H. Cortens, Lessons for medicine and nuclear medicine research (Editorial), Eur. J. Nucl. Med. 26 (1999) 311–313.
[11] A.C. Perkins, C. Mann, C.G. Wilson, Three dimensional visualisation of the large bowel: a potential tool for assessing targeted drug delivery and colonic pathology, Eur. J. Nucl. Med. 22 (1995) 1035–1038.
[12] S.P. Newman, I.R. Wilding, Imaging techniques for assessing drug delivery in man, Pharm. Sci. Technol. Today 2 (1999) 181–189.
[13] A.C. Perkins, Oesophageal transit, disintegration and gastric empting of a film-coated resindronate placebo tablet in gastro-oesophageal reflux disease and normal control subjects, Aliment Pharmacol. Ther. 15 (2001) 115–121.
[14] A.C. Perkins, C.G. Wilson, M. Frier, R.M. Vincent, P.E. Blackshaw, R.J. Dansereau, K.D. Juhlin, P.J. Bekker, R.C. Spiller, Esophageal transit of risedronate cellulose-coated tablet and gelatin capsule formulations, Int. J. Pharm. 186 (1999) 169–175.
[15] S. Jurisson, D. Berning, W. Jia, M. Dangshe, Coordination compounds in nuclear medicine, Chem. Rev. 93 (3) (1993) 1137–1156.
[16] M. Terán, A. Paolino, E. Savio, J.P. Gaudiano, A.S. León, M. Frier, Centellografía en el desarrollo farmacéutico: estudios in vitro e in vivo de comprimidos de ranitidina, Acta Farmacéutica Bonaerense 19 (3) (2000) 217–224.
[17] H.D. Burns, E.R. Gibson, R. Dannals, P.K. Siegl, Nuclear Imaging in Drug Discovery, Development and Approval, Birkhäuser, Boston, 1993, pp. 301–318.
[18] C.G. Wilson, Use of imaging techniques to interpret variability in drug delivery, Drug Delivery Supplement, http://www.pharmabrieffing.com/businessbrieffing/pdf/pharmatech2001/reference/reference16.pdf
[19] I.R. Wilding, Using product visualization techniques to characterize dosage form performance in man, British Pharmaceutical Conference, 2001. Abstract Book: 306, 2001.
[20] E. Cid Cárcamo, Control de calidad biofarmacéutico de Medicamentos, Ed. Baigraf, Chile, 1992, pp. 60–112.
[21] ARCAL XV, Manual de protocolos de calidad de radiofármacos, OIEA, Japan, 1999, pp. 39–52.
[22] D.L. Smith, Statistics and Data Uncertainties in Nuclear Science and Technology, vol. 4, American Nuclear Society, LaGrange Park, IL, 1991, pp. 178–230.