Hydrophilic and lipophilic radiopharmaceuticals as tracers in pharmaceutical development: In vitro – In vivo studies
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Abstract

Background: Scintigraphic studies have been performed to assess the release, both in vitro and in vivo, of radiotracers from tablet formulations. Four different tracers with differing physicochemical characteristics have been evaluated to assess their suitability as models for drug delivery.

Methods: In-vitro disintegration and dissolution studies have been performed at pH 1, 4 and 7. In-vivo studies have been performed by scintigraphic imaging in healthy volunteers. Two hydrophilic tracers, (99mTc-DTPA) and (99mTc-MDP), and two lipophilic tracers, (99mTc-ECD) and (99mTc-MIBI), were used as drug models.

Results: Dissolution and disintegration profiles, differed depending on the drug model chosen. In vitro dissolution velocity constants indicated a probable retention of the radiotracer in the formulation. In vivo disintegration velocity constants showed important variability for each radiopharmaceutical. Pearson statistical test showed no correlation between in vitro drug release, and in vivo behaviour, for 99mTc-DTPA, 99mTc-ECD and 99mTc-MIBI. High correlation coefficients were found for 99mTc-MDP not only for in vitro dissolution and disintegration studies but also for in vivo scintigraphic studies.

Conclusion: Scintigraphic studies have made a significant contribution to the development of drug delivery systems. It is essential, however, to choose the appropriate radiotracers as models of drug behaviour. This study has demonstrated significant differences in release patterns, depending on the model chosen. It is likely that each formulation would require the development of a specific model, rather than being able to use a generic drug model on the basis of its physicochemical characteristics.

Background

Development of new drug formulations requires the performance of extensive studies, both in the laboratory, and in vivo, in animals and in volunteers. In vitro studies can be very expensive but costs are even higher when in vivo stages are reached. Methodology that can generate relevant information but shorten the preformulation phases means important savings in economic, human and time terms [1-9]. Gamma scintigraphy provides rapid, complementary information that often cannot be obtained by other methodologies. It has been successfully used during development stages of feasibility studies and in
determining specific parameters of the final product. The information obtained by scintigraphy gives support during investigation and development, and also complements the development of registration dossiers and marketing publicity. The incorporation of a radiopharmaceutical into a drug formulation allows determination of the biodistribution kinetics and the release sites [10-14]. It is very important to choose the proper radionuclide, often $^{99m}$Tc (technetium), as this has optimal characteristics of half-life and energy, allowing images to obtain with high efficiency and low doses [15,16].

Many studies to validate the methodology mentioned above have already been undertaken [17,18], but, in general within these studies, radiopharmaceuticals are only rarely used as drug surrogates. Radiopharmaceuticals were primarily developed for diagnostic purposes in nuclear medicine, most of them being intended for intravenous administration. In order to optimise the usage of radiopharmaceuticals in the development of pharmaceutical drug-delivery systems, the behaviour of these tracers following administration by other routes must be validated. Stability and in vitro studies of different radiopharmaceuticals have been previously reported by our group [17], with the aim of creating a database of radiotracers or model drugs with known physicochemical properties to be used as in pharmaceutical dosage development, particularly of tablet formulations.

Provided that the tablet formulation can be radiolabelled with a suitable gamma emitter without altering its characteristics, imaging with a gamma camera can be used to monitor in vivo transit and dispersion of the tablet, and give some indication of deposition and absorption of the drug. The aim of this work is to assess in vivo and in vitro behaviour of a tablet formulation using scintigraphic studies, to examine the way in which radiopharmaceuticals model the release of drugs, using four different tracers with different physicochemical characteristics. Based on this assessment, the correlation between in-vitro and in-vivo behaviour could be investigated.

The characterised radiopharmaceuticals were $^{99m}$Tc-diethylenetriamine-pentaacetic acid ($^{99m}$Tc-DTPA), $^{99m}$Tc-ethyl cysteinate dimer ($^{99m}$Tc-ECD), $^{99m}$Tc-methylene diphosphonate ($^{99m}$Tc-MDP) and $^{99m}$Tc-sestamibi ($^{99m}$Tc-MIBI) [17]. They were incorporated into the tablets during wet granulation. Disintegration profiles were assessed in vitro in dissolution vessels and in vivo by scintigraphic imaging in healthy volunteers. In vitro dissolution studies were performed for tablets containing the above-mentioned radiotracers.

Methods

Radiopharmaceuticals labelling and control

The radiopharmaceuticals $^{99m}$Tc-DTPA, $^{99m}$Tc-ECD, $^{99m}$Tc-MDP and $^{99m}$Tc-MIBI were obtained by labelling commercial kits with $^{99m}$TcO$_4^-$ from a $^{99m}$Mo/$^{99m}$Tc generator (Technonuclear). Radiochemical purity testing of the $^{99m}$Tc-DTPA complex was performed by chromatographic analysis on Whatman N°1 paper with propanone and sodium chloride solution (0.9%) as developing solvents. $^{99m}$Tc-ECD radiochemical purity was studied by chromatography on Whatman N°1 paper with a methanol/water (85/15) solvent and by HPLC (Shimadzu LC-10 AS) using a Partisphere C18 (Whatman) column as the stationary phase. The mobile phases were phosphate buffer 0.0125 M pH 2.5 (A), and ethanol 100% (B). Solvent program was, at time = 0 min, 100 % A, and at time = 10 min, 70% A, 30% B. The flow rate was set at 2 mL min$^{-1}$.

The radiochemical purity of $^{99m}$Tc-MDP was determined by chromatography on Whatman N°1 paper using propanone and sodium chloride 0.9 % as developing solvents.

The $^{99m}$Tc-MIBI complex was assessed by HPLC (Shimadzu LC-10 AS) using a Partisphere C18 (Whatman) column 12.5 cm as stationary phase. The mobile phases were methanol (A), and ammonium sulphate 0.05 M (B). Solvents program was at time = 0 min, 20 % A, and at time = 5 min, 95% A. The flow rate was set at 2 mL min$^{-1}$.

Tablet preparation

Tablets were prepared by wet granulation using lactose and starch as diluents, PVP K30 (ISP Corp) as granulating agent, Ac-Di-Sol (FMC Biopolymer) as disintegrant and magnesium stearate as lubricant. The excipients were passed through a No. 16 sieve (16 meshes per centimetre) and mixed by a geometric method. After compression, tablets were assessed for weight (target weight 400 mg) and radioactivity content (target activity 18.5 MBq). Activity was measured in an ionisation chamber dose calibrator (Capintec CRC 25).

Tracer stability was verified as in a previous communication [18] both during tablet preparation and in dissolution and disintegration studies.

In vitro studies

Dissolution tests were carried out in a Vankel USP Type II apparatus, 900 mL of dissolution medium was used at three different pH values comprising pH 1 (0.1 M hydrochloric acid), pH 4 (0.016 M acetic acid/sodium acetate) and pH 7 (water). Temperature was maintained at 37 °C, and a rotation speed of 50 r.p.m. used. Samples of 2 mL were withdrawn through cellulose acetate membrane
filters (0.22 \text{\textmu m}) at 1, 2, 3, 5, 10, 15 and 30 minutes and assessed for radioactivity in a solid scintillation counter (Ortec-Maestro MCB1). Corrections were applied for volume, decay and counting efficiency. Curves of log % non-dissolved vs. time were plotted.

Disintegration profiles were recorded concurrently with the dissolution procedure by placing the vessels in front of a circular field of view gamma camera (Dyna (Pyker 4/15) equipped with a low energy, high resolution collimator, operating with a 20\% window centred on 140 Kev. Serial images were recorded at 1 frame/min with a matrix of 128 \times 128 without acquisition zoom. Three regions of interest were delineated, one on the tablet, one on the dissolution medium and the third on the external field to determine the background [19]. Net activity was quantified by subtracting mean background pixel counts from each pixel in the selected region of interest. No attenuation correction was necessary.

In vivo studies
Studies were performed in four healthy volunteers (2 men and 2 women) mean weight 60 Kg, mean age 38 years. They were non smokers and were not receiving any medication. Written informed consent was obtained from all subjects. They were not allowed to consume alcohol during the study period or in the preceding 24 hours. The protocol was in accordance with the Declaration of Helsinki guidelines for ethics in research and with resolutions XXIX and XXXV of the World Medical Assembly.

 Volunteers fasted for 6 hours prior to the study. Tablets were administered with 200 mL of still mineral water. Volunteers stood erect during the acquisition process in front of the detector. Urine samples were collected at 30 and 120 minutes post ingestion [22,23].

Serial images were recorded during the 30 minute period after administration at 2 frames / min using a rectangular field view gamma camera (Sophy). Later static images were recorded 2 hours after tablet intake.

Three regions of interest were delineated, one on the stomach, one on the intestines and a third on a region outside the body perimeter to determine the background. Net activity was quantified by subtracting mean background pixel counts from each pixel in the selected region of interest. Gamma ray attenuation was quantified by calculating the geometric means of count rates in paired anterior and posterior planar views.

Scintigraphic studies enabled the determination of tablet transit characteristics within the gastrointestinal tract specifically in the stomach. Curves of Log % non-disintegrated vs. time were plotted [24,25].

Data processing
Disintegration velocity constant of the tablets was $k_d$, which corresponded to the slope of the linear regression in first order kinetics [26]:

$$\log \left[\frac{Q_t}{Q_\infty}\right] = \frac{k_d t}{2.303}$$

Where

- $Q_t$ the amount of activity of the tracer disintegrated at time $t$ present in the region of interest.
- $Q_\infty$ the maximum amount of activity measured in the region of interest.

Disintegration velocity constants in the stomach and the constant of appearance in small intestine were determined in a similar way, considering $Q_t$ the amount of activity of the tracer disintegrated at time $t$ present in the region of interest and $Q_\infty$ the total amount of activity of the region at the end of the study [27].

Results and Discussion
Radiotracers were incorporated during the wet granulation process of tablet preparation and appropriate controls of quality were applied as previously described [17]. All the radiopharmaceuticals were produced with radio-

### Table 1: In vitro dissolution and disintegration constants (p 0.05)

| Radiopharmaceutical | Dissolution velocity Constant (min$^{-1}$) (n = 6) | Disintegration velocity Constant (min$^{-1}$) (n = 6) |
|---------------------|--------------------------------------------------|-----------------------------------------------------|
| $^{99m}$Tc-MIBI     | $(0.05 \pm 0.02)$ all pH range                   | $(0.04 \pm 0.01)$ all pH range                       |
| $^{99m}$Tc-ECD      | $(0.040 \pm 0.005)$ all pH range                 | $(0.05 \pm 0.01)$ (pH 1)                            |
| $^{99m}$Tc-MDP      | $(0.020 \pm 0.005)$ all pH range                 | $(0.09 \pm 0.01)$ (pH4 and 7)                       |
| $^{99m}$Tc-DTPA     | $(0.06 \pm 0.01)$ (pH 1 and 7)                   | $(0.20 \pm 0.04)$ (pH 1 and 7)                      |
|                     | $(0.11 \pm 0.02)$ (pH 4)                         | $(0.040 \pm 0.005)$ (pH 4)                          |
|                     | $(0.011 \pm 0.002)$ (pH 1 and 7)                 | $0.011 \pm 0.002$ (pH1 and 7)                       |
|                     | $0.080 \pm 0.006$ (pH 4)                          | $0.080 \pm 0.006$ (pH 4)                            |
chemical purities higher than 95%, in accordance with manufacturers' specifications.

Disintegration velocity constants in the gastrointestinal tract were determined by in vivo scintigraphic studies, specifically in stomach and small intestine for the different radiopharmaceuticals evaluated as tracers.

**In vitro results**

**99mTc-MIBI**

Dissolution velocity constants for tablets containing this lipophilic tracer showed no significant variation across the whole pH range studied and the values were very similar to disintegration velocity constants determined under the same conditions (Table 1).

The Pearson statistical test was used to quantify correlation within both series of data. Pearson's correlation coefficient \( r \) is always between -1 and 1 indicating that the points are near a negative or a positive slope respectively. The closer the \( r \) value is to -1 or 1 the higher the correlation of the series is. \[27\]

Dissolution and disintegration velocity constants \( (r \) values) were compared using this test. Correlation coefficients are shown in Table 3.

They were not so high indicating a probable retention of the radiotracer in the formulation.

**99mTc-ECD**

This lipophilic radiopharmaceutical demonstrated identical dissolution velocity constants across the whole pH range studied, while disintegration velocity constants increased with pH (Table 1).

Pearson correlation coefficients are shown in Table 3. A high correlation was found.

**99mTc-MDP**

In vitro dissolution velocity constants did not show significant variation across the whole pH range while in vitro disintegration velocity constants increased with pH (Table 1). In vitro correlation coefficients were high at all pH values. (Table 3)

**99mTc-DTPA**

In vitro dissolution and disintegration data showed similar patterns but the values were different (Table 1). In this case at pH 1 and 7 both disintegration and dissolution velocity constants were lower than those at pH 4. The profiles were similar in both series of data and Pearson correlation was very high at all pH ranges studied. (Table 3)

**In vivo results**

**99mTc-MIBI**

No significant variability within volunteers was observed in stomach disintegration velocity constants. Small intestine appearance constant showed similar behaviour but different values and did not represent significant differences within volunteers (Table 2). Two hours after administration there was no significant uptake in any organ except urinary bladder (Figure 1). Pearson Test was used to compare in vitro – in vivo disintegration velocity constants (Table 4) assuming pH 1 for stomach and pH 4 for small intestine no correlation was found. When Pearson test was used for in vivo disintegration velocity constants and in vitro dissolution ones, (Table 5) correlation coefficient was again low for stomach and a bit higher for small intestine, but in neither case was correlation considered to be established.

| Radio pharmaceutical | Stomach disintegration velocity Constant \( \text{(min}^{-1}\text{)} \) \( n = 4 \) | Small intestine appearance Constant \( \text{(min}^{-1}\text{)} \) \( n \geq 4 \) |
|-----------------------|---------------------------------|---------------------------------|
| 99mTc-MIBI            | \((0.016 \pm 0.006)\)           | \((0.13 \pm 0.02)\)             |
| 99mTc-ECD             | From \((0.7 \pm 0.1)\) to \((0.010 \pm 0.002)\) | \((0.02 \pm 0.01)\)           |
| 99mTc-MDP             | \((0.007 \pm 0.002)\)           | \((0.037 \pm 0.006)\)           |
| 99mTc-DTPA            | From \((0.13 \pm 0.03)\) to \((0.07 \pm 0.01)\) | From \((0.004 \pm 0.001)\) to \((0.08 \pm 0.01)\) |

**Table 3: In vitro dissolution – disintegration Pearson correlation coefficient**

| pH | 99mTc MIBI | 99mTc ECD | 99mTc MDP | 99mTc DTPA |
|----|------------|-----------|-----------|------------|
| 1  | 0.75       | 0.99      | 0.68      | 0.99       |
| 4  | 0.77       | 0.78      | 0.90      | 0.99       |
| 7  | 0.50       | 0.94      | 0.68      | 0.99       |

This lipophilic radiopharmaceutical demonstrated identical dissolution velocity constants across the whole pH range studied, while disintegration velocity constants increased with pH (Table 1).

Pearson correlation coefficients are shown in Table 3. A high correlation was found.

In vitro dissolution velocity constants did not show significant variation across the whole pH range while in vitro disintegration velocity constants increased with pH (Table 1). In vitro correlation coefficients were high at all pH values. (Table 3)

In vitro dissolution and disintegration data showed similar patterns but the values were different (Table 1). In this case at pH 1 and 7 both disintegration and dissolution velocity constants were lower than those at pH 4. The profiles were similar in both series of data and Pearson correlation was very high at all pH ranges studied. (Table 3)
In this particular case there was a substantial inter subject variability with coefficient variations during the 30-minute study period ranging from 14 to 95 % (Table 2). Small intestine appearance velocity constants were more homogeneous and late images 2 hours after administration showed liver uptake (Figure 2).

Correlation coefficient was not significant when in vitro disintegration – in vivo appearance velocity constants were compared (Table 4).

When Pearson correlation was quantified between in vivo disintegration velocity constants and in vitro dissolution ones (Table 5) as indicated for \(^{99m}\)Tc-MIBI, there was no correlation at pH 1 (stomach) and it was low at pH 4 (small intestine).

\(^{99m}\)Tc ECD

No significant variations within volunteers were observed in stomach disintegration velocity constants. Small intestine appearance constant showed similar behaviour but different values and did not represent significant differ-

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Table 4: In vitro-In vivo disintegration velocity constants Pearson correlation coefficient.

| pH | \(^{99m}\)Tc MIBI | \(^{99m}\)Tc ECD | \(^{99m}\)Tc MDP | \(^{99m}\)Tc DTPA |
|----|-----------------|----------------|--------------|--------------|
| 1  | 0.29            | 0.44           | 0.29         | 0.8          |
| 4  | 0.21            | 0.29           | 0.08         | 0.12         |

Table 5: In vitro dissolution – in vivo disintegration Pearson correlation coefficient

| pH | \(^{99m}\)Tc MIBI | \(^{99m}\)Tc ECD | \(^{99m}\)Tc MDP | \(^{99m}\)Tc DTPA |
|----|-----------------|----------------|--------------|--------------|
| 1  | 0.13            | 0.08           | 0.68         | 0.07         |
| 4  | 0.51            | 0.69           | 0.59         | 0.12         |
ences within volunteers (Table 2). Two hours after administration there was no significant uptake in any organ except urinary bladder (Figure 3). Pearson Test was used to compare in vitro – in vivo disintegration velocity constants (Table 4). In this case correlation was negligible. When Pearson Test was used for in vivo disintegration velocity constants and in vitro dissolution ones, (Table 5) correlation coefficients had the highest values found in this study.

$^{99m}$Tc- DTPA

Significant variations within volunteers were observed in stomach disintegration velocity constants and small intestine appearance constants (Table 2). Two hours after administration there was no significant uptake in any organ except urinary bladder (Figure 4). Pearson Test was used to compare in vitro – in vivo disintegration velocity constants (Table 4).

In this case correlation was negligible for pH 4 and higher for pH 1. When Pearson Test was used for in vivo disintegration velocity constants and in vitro dissolution ones, (Table 5) correlation was negligible for the entire gastrointestinal tract.

A good correlation was found between in vitro disintegration and dissolution velocity constants of tablets containing each of the radiopharmaceuticals used as radiotracer.

As scintigraphic studies give information of a physical process, tablets containing the same components, but different tracers, might be expected to have a similar behavior in all cases. As tracers are present in low concentrations (lower than $10^{-9}$ M), it is unlikely that they are responsible for the observed differences in the disintegration constants. It is possible that the tracers are bound to different components within the tablet, which disperse at different rates, which would be a likely explanation. Although the dissolution profiles might be expected to differ depending on the model drug used, the same would not be expected of the disintegration profile. This suggests that the measured disintegration profile can depend on the model chosen.
The radiopharmaceutical $^{99m}$Tc ECD showed absorption in the gastrointestinal tract. This is not a desirable characteristic and makes it an unsuitable tracer for this type of study. It was probably the main factor giving rise to the greater inter individual variability disintegration velocity constants observed when in vivo studies were performed for $^{99m}$Tc-ECD formulations. This fact was not observed with the other radiotracers under the same conditions of study.

Despite the physicochemical differences between $^{99m}$Tc-MIBI and $^{99m}$Tc-DTPA, both tracers presented very low correlation coefficients when in vitro dissolution – in vivo disintegration velocity constants were compared throughout the whole gastrointestinal tract (Table 3).

Only $^{99m}$Tc-MDP showed high correlation coefficients both in vitro between dissolution and disintegration, and between in vitro dissolution and in vivo disintegration.

**Conclusion**

Even though $^{99m}$Tc-ECD, $^{99m}$Tc-MIBI and $^{99m}$Tc-DTPA showed high in vitro correlation between dissolution and disintegration, statistical tests revealed that none of them was an adequate predictor of in vivo performance for this particular tablet formulation, for any region of the gastrointestinal tract.

The hydrophilic radiotracer $^{99m}$Tc-MDP was the only radiopharmaceutical suitable as a drug model for this particular tablet formulation in the prediction of its in vivo behavior.

The method is an interesting tool especially at early stages of pharmaceutical formulation development when different formulations are being chosen, but may not have adequate sensitivity to discriminate between formulation variables.

Careful choice of drug model, together with substantial in vitro validation is essential in order to reduce in vivo studies and make significant savings of human and financial resources.

**Competing interests**

The author(s) declare that they have no competing interests.

**Authors’ contributions**

Mrs. Mariella Terán carried out all the experimental work and together with Dr. Eduardo Savio made substantial contributions to conception, design, analysis and data interpretation. They also gave their final approval of the version to be published. Mrs. Andrea Paolino contributed to data acquisition, analysis and interpretation. Dr. Malcolm Frier was involved in revising it critically for important intellectual content.

**Acknowledgements**

The authors thank TECHI S.A. for supplying radiopharmaceutical kits and Centro de Medicina Nuclear, Hospital de Clínicas for Nuclear Medicine equipment facilities.

**References**

1. Wilson CG, Perkins AC. Gamma Scintigraphy and the study of drug deposition. Advances in pharmaceutical sciences. London Academic Press; 1992.
2. Alan Perkins, Malcolm Frier: Nuclear Medicine in Pharmaceutics. Research. London Taylor and Francis; 1999.
3. Wilson CG: Washington N. Chichester. In Physiological Pharmaceutics: Biological Barriers to Drug Absorption London Ellis Horwood; 1989.
4. Budisantoso N, Frier M, Wilson CG: Lipophilic models for scintigraphic evaluation of drug delivery systems. J Pharmacy and Pharmacology 1997, 49(4):49.
5. Adkin DA, Davis SS, Sparrow RA, Huckle PD, Phillips AJ, Wilding IR: The effects of pharmaceutical excipients on small intestinal transit. Br J Clin Pharmac 1999, 39:381-387.
6. Wood E, Wilson CG, Hardy JG: The spreading of foam and solution enemas. Int J Pharm 1985, 25:191-197.
7. Newman SP, Woodman G, Clarke SW: Deposition of carbencillin aerosols in cystic fibrosis; effects of nebuliser system and breathing pattern. Thorax 1988, 43:318-322.
8. Hak-Kim Chan , Evangelia Daviakas , Stefan Eberl , Michael Robinson , George Bautovich , Iven Young : Deposition of aqueous aerosol of $^{99m}$Tc-DTPA and delivered by novel system (AER) in healthy subjects. Eur J Nud Med 1999, 26:4.
9. Bartlett RJ, Perkins A, Ware FW, Riley A, Robinson PJ: Reproducibility of oesophageal transit studies: several single swallows must be performed. Nucl Med Commun 1987, 8:317-326.
10. Hardy JG: Radiopharmaceuticals: using radioactive compounds in pharmaceuticals and medicine. Radiouclide imaging in drug formulation. London: Ellis Horwood Limited, 1989.
11. Dams TTM, Correnti H: Lessons for medicine and nuclear medicine research. Eur J Nud Med 1999, 26:311-313.
12. Newman SP, Wilding IR: Imaging techniques for assessing drug delivery in man. Pharm Sci Technol Today 1999, 2:181-189.
13. Davies SS, Hardy JG, Newman SP, Wilding IR: Gamma scintigraphy in the evaluation of pharmaceutical dosage forms. Review article. European Journal of Nuclear Medicine 1992, 19:971-986.
14. Perkins AC, Frier M: Nuclear medicine techniques in the evaluation of pharmaceutical formulations. Pharmacy World and Science 1999, 18:97-104.
15. Early Pj, Sodee DB: Principles and Practice of Nuclear Medicine. Second edition. USA. Mosby -Year Book Inc; 1995:94-117.
16. Jarisson S, Berning D, Wei Jia , Dangshe Ma : Coordination compounds in Nuclear Medicine. Chemical Reviews 1993, 3:1137-1156. 93
17. Terán M, Paolino A, Savio E, Gaudiano JP, León AS, Frier M: Centellografia en el desarrollo farmacéutico: Estudios in vitro e in vivo de comprimidos de ranitidina. Acta Farmacéutica Bonaerense 2000, 19(3):217-224.
18. Terán M, Savio E, Paolino A, Frier M: Usage of radiopharmaceuticals in the development of pharmaceutical drug delivery systems: validation of $^{99m}$Tc-DTPA and $^{99m}$Tc-ECD. European Journal of Pharmaceutics and Biopharmaceutics 2004, 57:347-352.
19. Donald Burns H, Raymond Gibson E, Robert Darnall , Peter KS: Nuclear Imaging in Drug Discovery, Development and Approval. Sieg Birkhauser-Boston; 1993.
20. David Ganderton : Advances in Pharmaceutical Sciences. Academic Press London, England; 1992.
21. Wilding IR: Using product visualization techniques to characterize dosage form performance in man. British Pharmaceutical Conference Abstract Book 2001:306.
22. Wilson CG, Mc Jury M, O'Mahoy B, Frier M, Perkins AC: Imaging of oily formulations in the gastrointestinal tract. Advanced Drug Delivery Reviews 1997, 25:91-101.
23. Perkins AC, Mann C, Wilson CG: Three dimensional visualisation of the large bowel: A potential tool for assessing targeted drug delivery and colonic pathology. Eur J Nucl Med 1995, 22:1035-1038.

24. Perkis AC, et al: Oesophageal transit, disintegration and gastric emptying of a film – coated resindronate placebo tablet in gastro – oesophageal reflux disease and normal control subjects. Aliment Pharmacol Ther 2001, 15:115-121.

25. Perkins AC, et al: Oesophageal transit of risedronate cellulose – coated tablet and gelatin capsule formulations. International Journal of Pharmaceutics 1999, 186:169-175.

26. Cid Cárcamo E: Control de calidad biofarmacéutico de medicamentos. Ed Churchill Livingstone Spain; 1993.

27. Donald L: Smith Probability, Statistics and Data Uncertainties in Nuclear Science and Technology. American Nuclear Society Volume 4. LaGrange Park, Illinois, USA: 1991.

Pre-publication history
The pre-publication history for this paper can be accessed here:

http://www.biomedcentral.com/1471-2385/5/5/prepub