PEER REVIEWED

Small-Scale Batch Production for Clinical Gamma Scintigraphy Studies of Pharmaceuticals

Evaluation of pharmaceutical dosage forms using clinical gamma scintigraphy in early phase clinical development can provide critical information on drug delivery to the site of action/absorption and enables visualisation and quantitation of regional transit of drug and dosage forms.

Radiolabelling of dosage forms is accomplished by the association of a short lived gamma emitting radioisotope *e.g.* Technetium-99m (^{99m}Tc), with a carefully selected component of the formulation.

It is essential that the inclusion of the radioisotope does not appreciably alter the key pharmaceutical properties of the product, and that it acts as an accurate surrogate for the chosen formulation component. This must be demonstrated by performing *in vitro* assessments, typically standard pharmacopoeial test methods, with additional analysis to quantify the radiolabel.

Following successful radiolabelling method development and process validation, a data package is generated including Master Batch Records (MBR) and information for the Investigational Medicinal Product Dossier (IMPD) which forms part of the Clinical Trial Authorisation (CTA) application. A multi-disciplinary team with appropriate experience and expertise is essential in order to perform such studies.

Introduction

Pharmaceutical gamma scintigraphy is a technique which has been adapted from clinical diagnostic applications to enable the in vivo fate of pharmaceutical dosage forms to be accurately determined in patient populations, or healthy volunteers. It is the gold standard for deposition, retention and transit studies and has been used in many 100's of clinical trials since the 1970's. Its application to pharmaceutical products is dependent upon the physicochemical association of a radiolabel with the active pharmaceutical ingredient (API) or some other carefully selected excipient in the product (Taylor *et al.*, 2018, Strugala *et al.*, 2012).

Pharmaceutical gamma scintigraphy has applications in defining delivery to and residence of drug delivery systems at the site of action/absorption. It also enables visualisation and quantitation of regional transit of drug and dosage forms. PK analyses may also form part of the study protocols to provide further insight into formulation performance. Thus a greater understanding of the fate and variability of delivery systems administered by the oral, pulmonary, nasal, rectal and vaginal routes may be determined. Pharmaceutical gamma scintigraphy is performed using fully quantifiable and trial-validated procedures and has conventionally been applied to assess inhalation systems since it is the only method that allows an absolute measure of the deposition of drug within the lungs (Newman *et al.*, 2012).

The gamma emitting radioisotopes used for this application have short physical half-lives i.e. in the order of hours, and characteristic photon energies in the range of 100–300 keV, *e.g.* Technetium-99m (^{99m}Tc, photo peak 140 keV, physical half-life approx. 6h) and Indium – 111 (^{111}In, photo peaks at 173 keV and 245 keV, physical half-life 2.8 days). For clinical diagnostic applications, the physicochemical properties of the radioisotopes are often modified using commercially available 'kits' containing appropriate ligands which form functional complexes. For example, stable hydrophilic forms of ^{99m}Tc such as ^{99m}Tc-diethylene-triamine-pentaacetate (Technescan, PL 12288/0011) are used for renal scintigraphy, and lipophilic forms such as ^{99m}Tc-exametazime (Ceretec, PL 00221/0126) are used for brain scintigraphy or radiolabelling leucocytes. These same kits and others, can be utilised to modify the properties of ^{99m}Tc or ^{111}In to optimise association with a particular dosage form or API.

The characteristic photon energies and short physical half-lives of these isotopes results in low radiation exposure for diagnostic procedures. Adhering to the principle of as low as reasonably practicable, research studies are usually conducted with administered radioactivity at much lower amounts than the diagnostic reference levels (ARSAC Notes for guidance, 2021) recommended for similar clinical procedures. This can be achieved as imaging protocols for research studies are optimised for highly selective and specific study end-points. The availability of a dedicated research gamma camera is essential to this process.

Using optimised imaging protocols, cross-over studies can be conducted in healthy volunteers or patient groups whilst ensuring the radiation dose is minimised. For example, a three-way cross-over study to evaluate lung deposition can be performed with a radiation exposure equivalent to 3–4 months' background radiation, i.e. approx. 0.75 mSv. The average annual UK background radiation exposure is approximately 2.7 mSv (Public Health England) but as with other parts of the world, there are large region to region variations in natural background radiation.

Radiolabelling Pharmaceuticals

Manufacture of the radiolabelled investigational medicinal product (IMP) must be performed according to the principles of Good Manufacturing Practice (GMP) under a Manufacturing and Import Authorisation for IMP (MIA (IMP)). Holders of manufacturing authorisations require the services of a Qualified Person (QP) who is responsible for certifying that the IMP is manufactured and tested in accordance with the terms of the Clinical Trial Authorisation (CTA) and GMP. The method development data and the validation data will be incorporated into the Investigational Medicinal Product Dossier (IMPD) to be submitted as part of the CTA application to the Medicines and Healthcare products Regulatory Agency (MHRA) in the UK.

The key to success is that the formulation characteristics should not be perturbed by the addition of the radiolabel and that the radiolabel must be an accurate surrogate for the desired formulation component. In order to achieve this goal, it is essential that the investigators have a thorough understanding of the formulations under

test, since it is often necessary to be able to reverse engineer the product to ensure the radiolabelling is successful.

Extensive *in vitro* testing is required to demonstrate that the radiolabelling process has not altered the properties of the original, i.e. reference product, and also to show that the radiolabel is an accurate surrogate for the API or selected formulation component. The initial stage of this process involves methods transfer of key analytical techniques from the Sponsor company to the contract research organisation (CRO). Once the radiolabelling method is finalised, master batch records (MBR) describing the manufacturing steps, including in process control measures and release tests are documented and signed off by the Sponsor.

This article describes the key steps in the radiolabelling method development and validation processes in order to successfully manufacture small scale GMP batches of radiolabelled products.

While the focus is on inhalation dosage forms the principles for radiolabelling method development and validation are applicable to other pharmaceutical preparations such as oral dosage forms i.e. tablets, capsules etc.

Development Phase

The target for radiolabel association should be identified, this is usually the API but could under certain circumstances be another component of the formulation that will provide critical information regarding the fate of the dosage form. For example, in the case of a liposomal formulation of the antibiotic amikacin, the physical form of the radiolabel was selected so that it associated with the lipid bilayer and provided information on the fate of the drug carrier (Weers *et al.*, 2009). To investigate delivery from a novel electronic inhaler, a hydrophilic form of the radiolabel i.e. ^{99m}Tc-DTPA was dissolved in the aqueous phase enabling both the delivered dose and also the deposition pattern within the lungs to be quantified (Nikander *et al.*, 2007).

The key formulation performance characteristics of the test product i.e. baseline performance, must be evaluated by the CRO. Formal methods transfer i.e. analytical and product test methods should be performed at this stage. The objective of these experiments is to demonstrate successful methods transfer and to establish the key performance characteristics of the product, *e.g.* aerodynamic particle size distribution (APSD) for an inhaled product or drug release characteristics for oral dosage forms. To fully characterise the reference product, it is necessary to evaluate samples from a number of batches in order to understand batch to batch variability.

The pharmaceutical qualities so determined must remained unchanged following radiolabelling and are used to define the key characteristics that the radiolabel must possess in order to function as a surrogate.

The challenges of radiolabelling formulations for inhalation devices range in terms of complexity from the addition of a suitable chemical form of ^{99m}Tc into a jet-nebuliser formulation through to the production of small batches of radiolabelled API for use in a dry powder inhaler (DPI). These challenges are overcome by application of our knowledge which has been gleaned by performing radiolabelling studies with a wide array of different formulation and device types. Ultimately, every formulation/device combination is unique and requires a bespoke set of methods applicable for small batch radiolabelling.

The on-site release procedures for radioactive products vary depending upon the complexity of the radiolabelling methodology. In some instances, the process may involve incorporation of the radiolabel into a 'finished' product i.e. one that has been through certification and batch release by the original manufacturer, *e.g.* a nebuliser preparation. In contrast, for a DPI the radiolabel must first be exclusively surface associated with the API prior to blending with the carrier, followed by device/capsule filling. The DPI example offers the greatest challenges since the product is being formulated from its constituent components and this has to be produced, characterised and released on the day that it is to be administered. In all scenarios detailed In-Process Control (IPC) checks and Release/Quality Control tests must be performed.

Initial experiments can be conducted in the absence of the radiolabel this is often referred to as 'cold' labelling. This strategy can provide an insight into method feasibility without unnecessary radiation exposure to operators. The mass of radiolabel associated with the dosage forms is very small, in the order of nanograms or less, and so its omission does not diminish the value of cold radiolabelling experiments. During these experiments possible effects of the radiolabelling procedures on formulation performance must be assessed. For example; the effect of varied volumes of the vehicle to facilitate radiolabel incorporation into a nebuliser preparation, or the impact of batch sizes for small scale powder blending for DPIs may be evaluated. It is critical that these procedures do not change the performance of the test product relative to that of the standard reference product. Once this has been established subsequent experiments with the inclusion of radioactivity may be performed in the knowledge that the process itself does not impact product performance. The objective of the next phase of experiments is to demonstrate that the radiolabel acts as an accurate surrogate for the selected formulation component i.e. usually the API.

At this stage draft Master Batch Records (MBR) will be prepared to document the key steps of the method to ensure consistent processing at the next stage of development.

Preliminary Radiolabelling Phase

Once the baseline performance characteristics of the test formulations are established draft acceptance criteria for radiolabel characteristics can be defined. Using the methodology from the 'cold' labelling studies preliminary experiments incorporating low levels of radioactivity are performed. The *in vitro* tests *e.g.* dissolution/disintegration tests for oral dosage forms, APSD for inhalers, are repeated following the inclusion of the radiolabel, with additional analysis, using a gamma counter or a gamma camera, as appropriate for the formulation/delivery system, to quantify the tracer.

The objectives of these tests are two-fold i.e. to show consistent performance with the control, unlabelled formulation, and to demonstrate a good correlation between the radiolabel and API (or chosen excipient) in order to confirm that the radiotracer is a suitable surrogate for the selected formulation component.

For inhalation products, the International Society for Aerosols in Medicine (ISAM) Regulatory Affairs Networking Group (Devadason *et al.*, 2012) published guidance to advocate the standardisation of radiolabelled product criteria in terms of minimal changes to key characteristics of the

reference product *e.g.* total emitted dose, APSD, and ensuring acceptable correlations between radiolabel and API recovered from impaction tests and delivered dose assessments.

Successful outcomes at this stage enables progression to the Validation Phase. However, prior to this step the Master Batch Records (MBR) must be finalised so as to accurately document the manufacturing steps including appropriate IPC checks (Table 1) and Release Tests (Table 2) to ensure the quality and consistency of the final product.

An example of the APSD release test data for DPI batches manufactured during the course of a clinical study is shown in Figure 1. The plot shows the relative recovery of the radiolabelled API and the radiolabel (^{99m}Tc) from inertial impaction tests using a Next Generation Impactor (NGI). The histogram also shows the recovery of API for the reference unlabelled product.

At this stage stability issues should be considered. Generally, because of the short physical half-life of the radioisotopes the dosage forms are administered within hours of completion of the radiolabelling process. However, physical stability should be demonstrated over a period relative to the anticipated clinical dosing procedures. If it is assumed that it will take 4 h to dose all subjects/patients on each dosing day, then the radiolabelled product should be demonstrated to perform as expected over a similar or slightly extended period of time i.e. include a contingency allowance.

Validation Phase

The final stage of the radiolabelling development process is to progress from the low levels of radioactivity used during the preliminary experiments to the levels that will be required for clinical imaging. The validation phase is performed according to the final MBR and using the same batches of product, excipients and/or devices that will be used on the clinical dosing days. Several validation batches should be prepared to demonstrate the robustness of the process. Most clinical study designs will involve a manufacturing campaign spanning a period of weeks or months depending on the subject population; specific patient groups typically take longer to recruit than healthy volunteers. Thus multiple batches will be manufactured and it is essential that the process is robust so that the chance of batch failure is minimised.

Stage of Preparation/ Material	IPC Tests	Method	Batch Limit
Prep of ^{99m}Tc	^{99m}Tc recovery	Dose Calibrator	Not less than or equal to xxx MBq at reference time
Radiolabelling Test powder with ^{99m}Tc	Radioactivity of ^{99m}Tc for addition to Test powder	Dose Calibrator	Not less than or equal to xxx MBq/mL at intended time of first dose.
	Radioactivity of ^{99m}Tc on radiolabelled Test powder	Dose Calibrator	Specific Activity: xx.x – xx.x MBq/mg at intended time of first dose
Radiolabelled Test powder blended with lactose	Blend Uniformity (Dose Calibrator measurement is non-destructive and the same samples will be used for HPLC analysis).	Dose Calibrator	xx.x – xx.x MBq per dose RSD < x%
		HPLC	x blend uniformity samples for HPLC xxx – xxx µg per dose RSD < x%
	Identification	HPLC	Retention time of the main peak from the sample solution is consistent with the retention time of Test drug substance from the standard solution.
Capsule filling with ^{99m}Tc radiolabelled Test powder	Description of filled capsules	Visual Assessment	Sealed, intact, free from visible powder on outside, free from visible defects
	Capsule fill weight (of the radiolabelled product)	Gravimetric determination	xx.x – xx.x mg per capsule

Table 1: Example In-Process Controls for the Manufacture of a Radiolabelled Dry Powder Inhaler Product.

Test Item	Method	Acceptance Criteria
Description	Visual Assessment of capsules	Sealed, intact, free from visible powder on outside, free from visible defects
Capsule Radioactivity Content	Dose Calibrator	xx.x – xx.x MBq
APSD at release	Gamma Scintigraphy	Performance Criteria MMAD (µm), GSD and FPF (%) to comply with release criteria for reference product.
APSD at release	HPLC	Performance Criteria MMAD (µm), GSD, FPF (%), FPD (µg or mg) to comply with release criteria for reference product.

Table 2: Example Release Tests for ^{99m} Tc radiolabelled Capsules for a Dry Powder Inhaler Formulation.

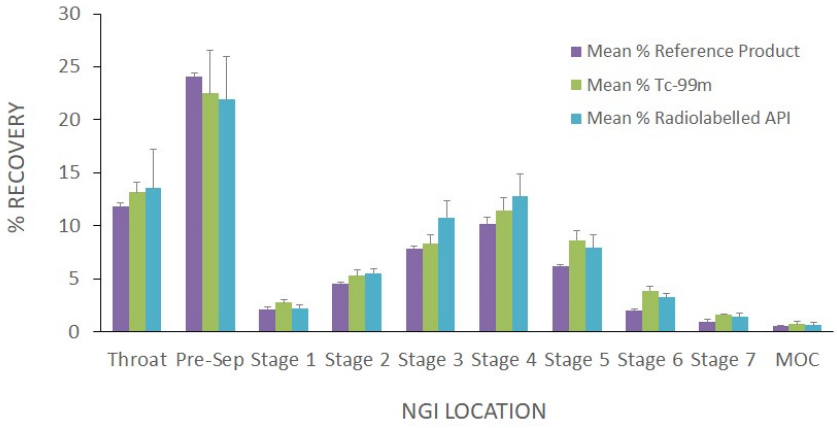


Figure 1: Validation Data Showing Mean % Recovery of ^{99m}Tc, radiolabelled API and API from Reference Product. ^{99m}Tc recovery was measured using a gamma camera, API was quantified by validated HPLC-UV assay.

Validation batch results must comply with all IPC and Release Test specifications, the data generated, along with the preliminary radiolabelling results, will be included in the IMPD. The IMPD contains chemical and pharmaceutical quality information about the Investigational Medicinal Product (IMP). For scintigraphy studies the following information related to the radiolabelling process should be provided:

- Radiolabel
- Other Excipients
- Manufacturing Process Development
- Manufacturing Process Validation
- Analytical Method Validation

- Representative Batch Analysis Data
- Stability Data

This information can be incorporated into the existing IMPD or alternatively submitted as a standalone abbreviated radiolabelling IMPD making reference to appropriate sections of the standard document.

Clinical Phase

Once the radiolabelling method is successfully validated and the full regulatory review and approval process is complete the clinical phase of the study may begin. On a manufacturing day the ^{99m}Tc is eluted from a generator early in the morning and quality control (QC) tests are performed to ensure compliance with appropriate Pharmacopoeial and manufacturer specifications. The radiolabelled product is then manufactured in accordance with the approved MBR. The Production Manager and Quality Assurance Pharmacist perform a QC check of the completed MBR and IPC/Release Test results. In addition to information captured on the MBR, QC checks of all analytical data *e.g.* inertial impaction results, HPLC data relating to content uniformity of DPI blends and associated system suitability results for HPLC performance. Following the QC checks batch certification and release is conducted by the QP.

Dosing and imaging typically commences as soon as possible after the product is released due to the short physical half-lives of the radioisotopes. Figure 2 (B) shows lung deposition in a healthy volunteer following dosing from a DPI product. Figure 2 (A) shows the corresponding ventilation image achieved by the subject inhaling a short-lived radioactive gas i.e. Krypton-81m (physical half-life approx. 13s), which enables the ventilated regions of the lung to be determined and thus drug deposition and distribution to be accurately assessed. Additionally, transmission scans of each subject are routinely acquired in order to measure regional tissue attenuation to ensure accurate quantitation of radioactivity. In the case of GI studies suitably radiolabelled drinks may be administered to outline anatomical features *e.g.* stomach, or colon.

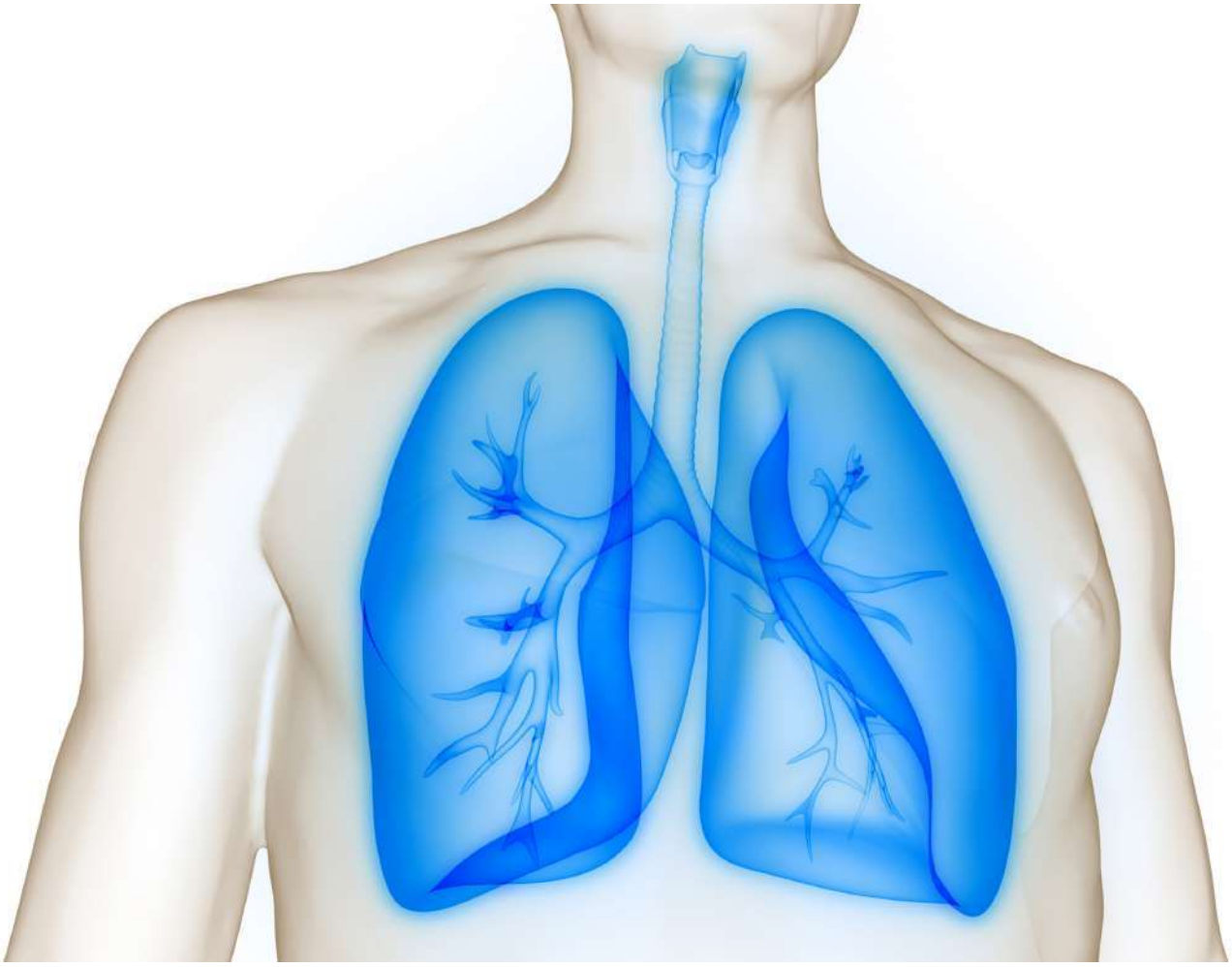
Conclusions

Gamma scintigraphy enables non-invasive determination of the biological fate of pharmaceutical dosage forms administered to healthy volunteers and/or patients.

Effective radiolabelling of the pharmaceutical is achieved following detailed characterisation of the test product, followed by meticulous method development and validation in order to demonstrate a robust, reproducible and accurate process. Successful outcomes are dependent upon the CRO having the necessary technical expertise and experience of dosage form evaluation and development.

Scintigraphy studies can provide information that will facilitate informed decisions to ensure optimal device/formulation selection thereby streamlining the product development process and saving costs in terms of time and finance.

Information from scintigraphy studies can be used to support product licence applications for a range of pharmaceuticals. For example, in the case of anti-reflux agents i.e. alginate containing liquids, tablets and powders, scintigraphy has been used to demonstrate efficacy in terms of the



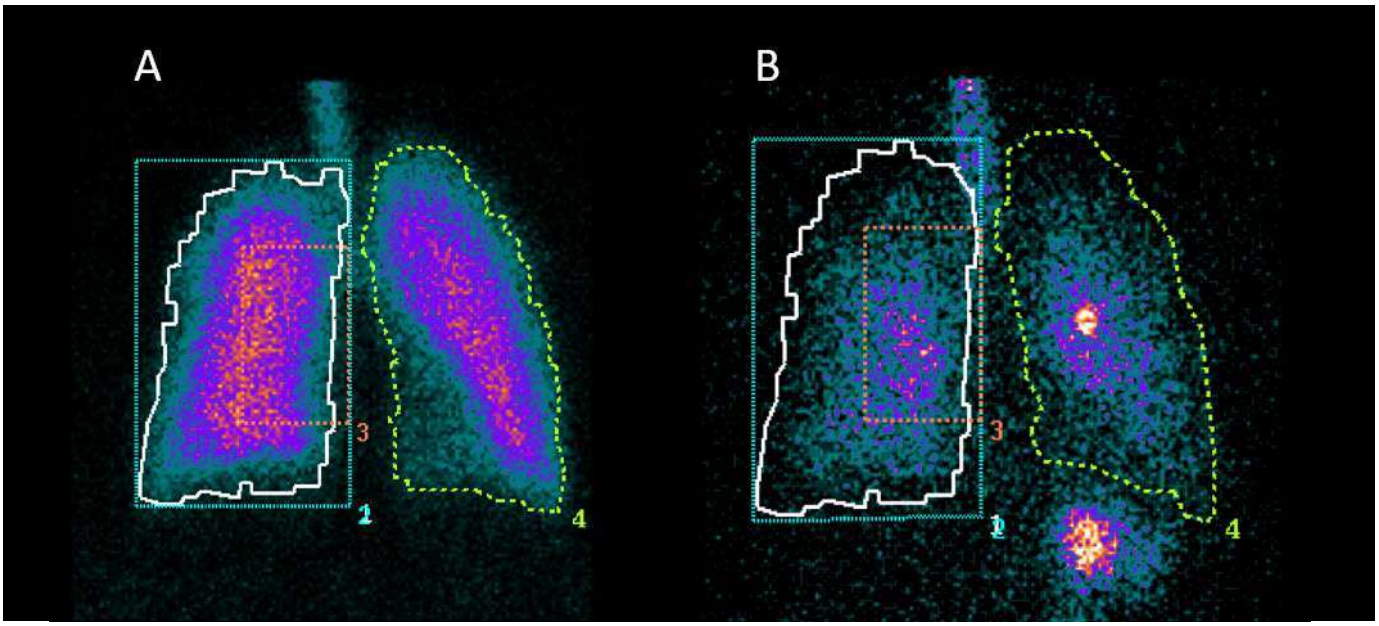
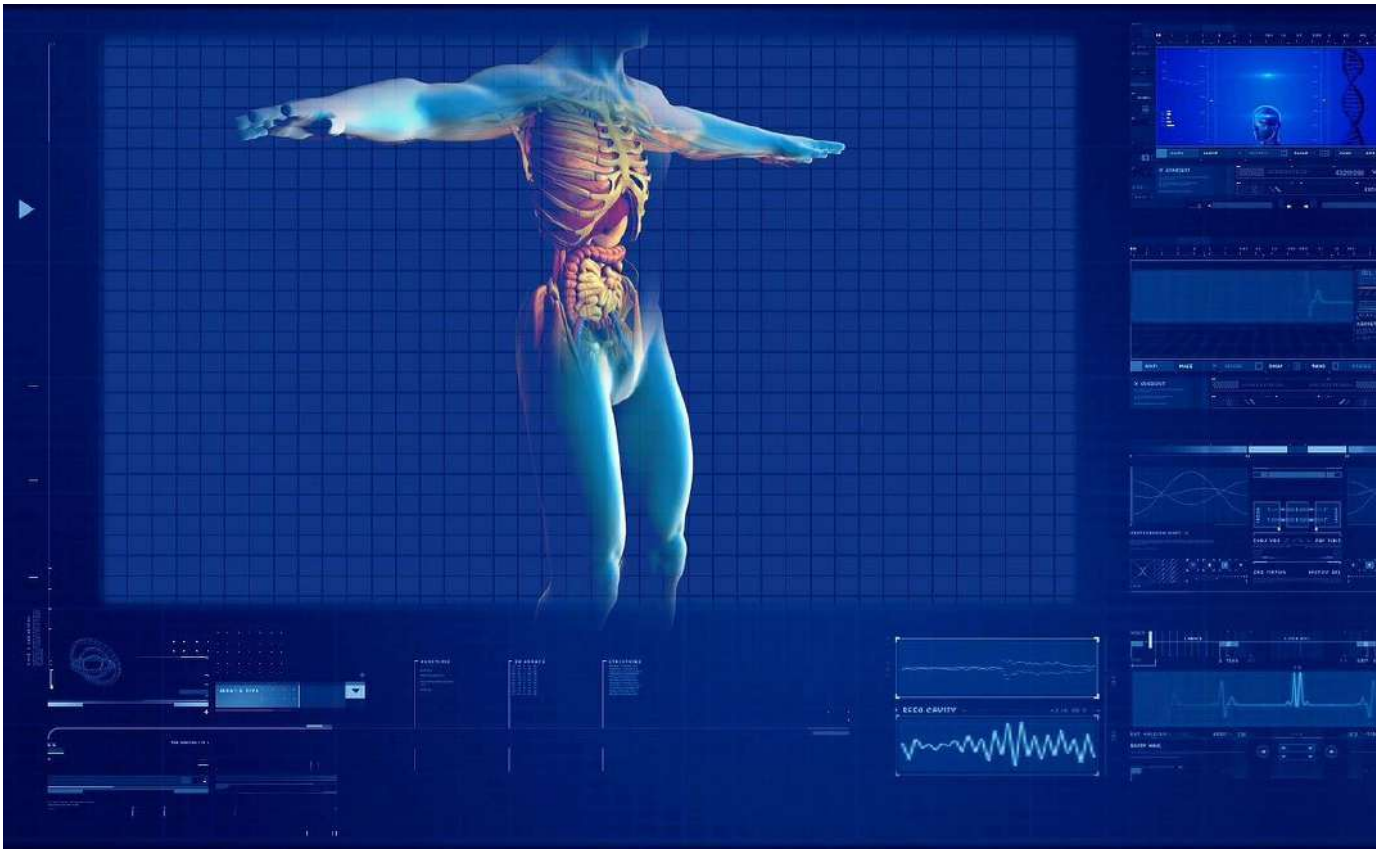


Figure 2: A: Anterior images in a healthy subject showing; A: Krypton-81m gas lung ventilation and B: deposition from a 99mTc radiolabelled DPI, radioactivity in the stomach (following oropharyngeal deposition) can be seen below the left lung. Also shown are the regions of interest defining the lung margins (white and green) and the regions used to define the outer (blue) and inner (red) lung regions (right lung only).

formation of alginate rafts floating on the stomach contents (Hampson *et al.*, 2010) as documented in the following summary of product characteristics documents; Gaviscon Double Action Liquid PL 00063/0156, Gaviscon instants oral powder cool mint/Fresh tropical, PL 00063/0173, 0367).

For orally inhaled products (OIP) containing new APIs establishing lung deposition via imaging can enable the selection of optimal device/formulation variables to be taken forward to clinical studies to assess therapeutic efficacy. Evaluating lung distribution can be important if specific airways are to be targeted *e.g.* conducting or peripheral airways. Imaging studies can be combined with PK sampling to provide a greater understanding of the relationship between regional lung deposition, and drug effects and absorption (Taylor *et al.*, 2018). Equipped with this knowledge the development process may be streamlined with associated cost savings.

The value of imaging studies is also recognised in the EMEA guidance (CPMP/EWP/4151/00 Rev. 1, 2009) on the stepwise approach to establishing equivalence between OIP products. Pulmonary deposition from



imaging studies can provide supportive data for the design of PK and/or clinical studies to assess therapeutic efficacy.

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