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# Analysis of radiopharmaceutical metabolism

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Nuclear imaging techniques such as PET can assess time-dependent changes of radioactivity concentration or show the regional distribution of a radiotracer within a tissue of interest, but they cannot distinguish between different chemical species. Most radiopharmaceuticals are rapidly metabolized in the mammalian body. Tracer metabolism takes place primarily in the liver but in some cases also in other tissues (kidneys, intestines, lungs, blood cells)[1]. Metabolites released from these organs can reach the tissue of interest. It is generally assumed that the uptake of radioactivity in target tissue (brain, heart, tumour) represents only unchanged radiopharmaceutical. This assumption can be tested in experimental animals by analyzing radioactivity in tissue samples. If the chemical structures of the metabolites are known, such compounds can be labelled themselves and be injected into volunteers to check whether they enter the human brain.

A strong feature of PET is the possibility to quantify transport rates in biochemical pathways. This information has to be extracted from serial measurements of radioactivity in target tissue (the output) and in blood plasma (the input), using a suitable mathematical model of the process under investigation [2]. In order to calculate the input to target tissue accurately, the fraction of plasma radioactivity representing unchanged radiopharmaceutical should be determined in samples of arterial blood at several time intervals after injection. First, parent radiopharmaceutical and radioactive metabolites should be extracted as quantitatively as possible and all protein should be removed from the biological sample. Liquid-liquid solvent extraction is commonly used for protein removal [3], but several other techniques are possible, such as solid-phase extraction (SPE) [4] and internal-surface reversed phase chromatography (ISRP) 1.

Second, the parent radiopharmaceutical should be completely separated from all radioactive metabolites, using an appropriate analytical method. Radio-HPLC is the most widely used technique because it combines speed with resolution. Less commonly employed separation methods are SPE, thin-layer chromatography (TLC), and overpressure-thin layer chromatography (OPTLC).

Finally, the relative amounts of radioactivity in parent and metabolites should be determined. These can be measured: (i) by a sensitive positron detector placed behind the HPLC column [5], (ii) by collecting fractions of eluate and counting radioactivity in the fractions with a gamma counter, (iii) by measuring the regional distribution of radioactivity on a TLC plate with a phosphor storage imaging system [6], (iv) by cutting the TLC plate into thin strips and counting radioactivity within the strips. The amount of radioactivity under the parent peak divided by the sum of all radioactivity within the chromatogram gives the fraction of unmodified parent at the time interval in question. Both radioactivity values should be corrected for background.

Some pitfalls and sources of errors in metabolite analysis[1,7] will be discussed such as:

- Modification of radioactive species in the biological sample during protein removal;
- Decomposition of the radiopharmaceutical during sample spotting (TLC);
- Loss of radioactivity due to incomplete extraction or physical inclusion in a protein pellet;
- Incomplete elution of injected radioactivity from the HPLC column;
- Incomplete separation of radioactive metabolites from the parent peak.
- Due to rapid clearance of radioactivity from plasma, the amount of radioactivity in each sample may soon become too low to be measured with adequate statistical accuracy. This problem is severe for radionuclides with short half-lives, such as $^{13}N$ ($t_{0.5}$ 10 min) and $^{11}C$ ($t_{0.5}$ 20 min).

**References**

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