HPLC-MS TECHNIQUE IN RADIOPHARMACEUTICAL RESEARCH (THE QUALITY CONTROL OF ^{18}F-2-FLUORODEOXYGLUCOSE AS AN EXAMPLE)

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In the nuclear medicine, the radiopharmaceuticals labeled by ^{99m}Tc and many others radionuclides having lifetimes of several hours are in common practice. At present, the more short-lived positron emitting radionuclides of half times from several to hundred minutes (^{11}C , ^{13}N, ^{15}O, and ^{18}F) are used for positron annihilation functional imaging (PET).

Application of radiopharmaceuticals puts increasing demands on their quality control, considering the short half-times, high specific activities (autoradiolytical effects), and general quality (chemical purity, apyrogenity and sterility) of pharmaca [see e.g. http://news.pharmaceuticalonline.com]. Mostly, the radioanalytical control consists of application of several separation and instrumental analytical techniques. In this paper, perspective of the hyphenated HPLC and MS techniques is demonstrated on the example of one of the most spread radiopharmaceutical, 2-[^{18}F]fluoro-2-deoxy-D-glucose (further: FDG).

Labeled FDG [HAMACHER, COENEN and STÖCKLIN, *J. Nucl. Med.* 27, 235 (1986), HAMACHER, NEBELING and BLESSING, *Appl. Radiat. Isot.*, 41, 49 (1990)] is purified by successive column technique using the anion exchange resin, silica-C18 and alumina cartridges. According to pharmacopoeia, following parameters should be under control:

Parameter	**Prescribed value**	**Assessed by**
Volume activity	0.1-2.0 GBq/mL	activity and weight measurement
Molar activity	> 55 MBq/mg FDG	radiometric and refractive index detectors
Radionuclidic purity	> 99.9 % ^{18}F	gamma-spectrometric assay of 511 keV peak of ^{18}F
Radiochemical purity	> 92 % 2-[^{18}F]FDG < 5 % $^{18}F^-$	anion exchange (usually Dionex™) HPLC assay of FDG and F^- (>95:1)
Chemical purity	< 100 μg/mL acetonitril, methanol or acetone, <1000 μg/mL ethanol, acetate	GC
Osmolality	0.3 Osmol/kg (0.15 M NaCl)	conductometry
pH	5.5 - 7.5	potentiometry
Catalysers (Kryptofix®,TBA etc.)	< 50 μg/mL	UV-VIS assay

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Pyrogens - bacterial endotoxins	<17.5 EU/mL (≅ 1.75 μg/mL)	luminescent limulus test (LAL)

The quality control can be performed via radio-HPLC and gas chromatography in 10 minutes. These parameters are controlled at the end of synthesis and at permissible expiration date 8 hours they may change. As follows from the data above, in the applied FDG solutions, FDG concentration is 2-35 mg/mL (0.01-0.2 mol/L), [^{19}F]FDG : ^{18}F[FDG] ratio is above 1: 66, and the dose rate in the radioactive solution is of the order 0.1-0.3 MGy/hr.

Objective of a new method of FDG analysis development is to replace existing tests by a more complex assay that will be also flexible enough to suit for quality control of other fluorine-18 labeled radiopharmaceuticals (L-DOPA etc.). The technique should follow not only the present state of pharmacopoeia, but also emerging demands on pharmacodynamics data at larger storage periods (the assay of autoradiolysis and chemical decomposition products, and metabolites). Analytical performance should proceed with an unaltered 1-5 μL sample, and it would be profitable if it could also provide micropreparative separation and its on-line control.

It was the liquid chromatography combined with mass-spectrometry (LC-MS) which was presented, like an experimental attempt, as a new quality control tool for PET-radiopharmaceuticals [FRANSSEN, LUURTSEMA, MEDEMA, VISSER, VAALBURG, JERONISMUS-SHALINGH, and BRUINS, *Appl.Rad. Isotopes* 45, 937 (1994)]. In principle, a modern LC-MS equipment enables aprior analysis of FDG, both the elemental and isotopic one. Isobaric interferences can be eliminated by HPLC. The pyrogens, lipopolysaccharides with a m.w. of about 10 kDa should be detected by either a direct MS or ion fragmentation procedures. If microsensors available, further parameters to be established in the main stream (fractionated eluate), both osmolality and pH. Electron spray ionization at mass spectrometric analysis can give evidence of pyrogens - bacterial endotoxins, lipopolysaccharides with a m.w. of about 10 kD on a level of picograms.
Electrospray ionization operates by the process of the emission of ions ("ion evaporation") from a droplet into the gas phase. (Fig.1).

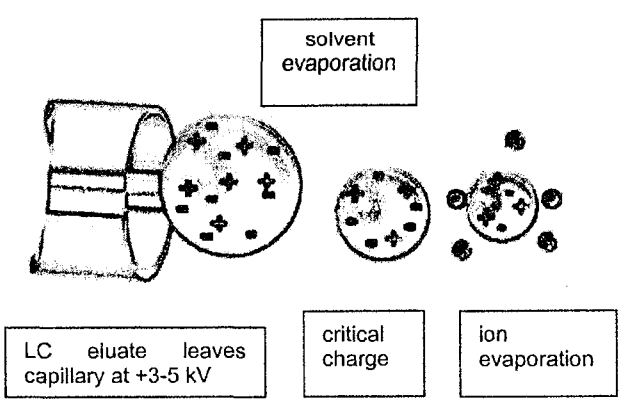

Fig.1 *Electrospray ionization of chromatographic eluate*

A flow of warm nitrogen drying gas helps the evaporation process at atmospheric pressure. Ions are transported into the high vacuum system of the mass spectrometer while lighter solvent and drying gas molecules can be more readily pumped away (Fig.2).

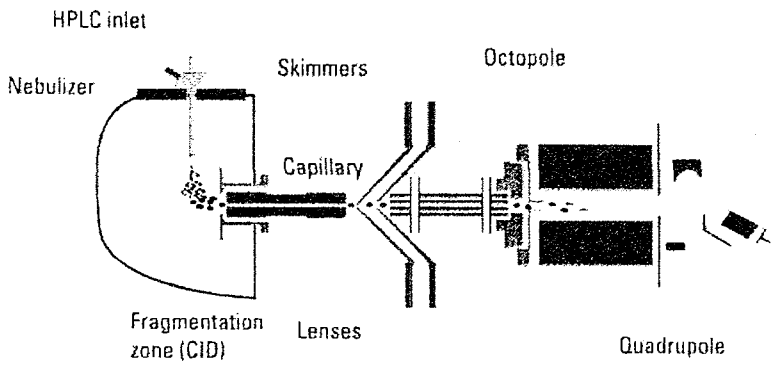

Fig.2. *Agilent 1100 HPLC - MSD interface with orthogonal spray*

For smaller molecules, singly charged ions by the loss (acidic compounds) or gain (basic compounds) of a proton. Formation of either an $[M+H]^+$ or an $[M-H]^-$ ion occurs as a "soft" ionization process, but also some fragmentation may be apparent. Because the presence of added or contaminant ions, such as and Na^+, NH_4^+ or K^+, and Cl^- ions is unavoidable in picoamounts, the adduct formation with the ions is typical for each ESI spectrum. Thus, for FDG we can in e.g. ammonium formate buffer solutions following ions can be expected.

Table 1. The expected molecular ions at ESI of FDG and some saccharides mixture in ammonium formate eluent

ANALYTE	FORMULA	M	NEGATIVE IONS			POSITIVE IONS			
			M-H	MCl	MHCOO	MH	MNH_4	MNa	MK
Glucose	$C_6H_{12}O_6$	**180.1**	179.1	215.0	225.1	181.1	198.1	203.1	219.0
^{18}F-FDG	$C_6H_{11}O_5{}^{18}F$	**181.6**	180.1	216.0	226.1	182.1	199.1	204.1	220.0
^{19}F-FDG	$C_6H_{11}O_5{}^{19}F$	**182.1**	181.1	217.0	227.1	183.1	200.1	205.0	221.0
Sorbitol	$C_6H_{14}O_6$	**182.1**	181.1	217.1	227.1	183.1	200.1	205.1	221.0
^{18}O-Glucose	$C_6H_{12}O_5{}^{18}O$	**182.1**	181.1	217.0	227.1	183.1	200.1	205.1	221.0

When the isobaric components provide similar ionization and fragmentation patterns, e.g. ^{18}O-glucose, which can result from cyclotron targetry materials, an admixture of sorbitol (mannitol) and the FDG carrier (^{19}F-FDG), a high-resolution mass spectrometer should be available, or the chromatographic separation of these components must be ensured for their assay. The same concerns all isobaric epimeric saccharides.

The biomolecules (proteins, lipopolysaccharides etc.) with higher molecular masses up to 200 kDa would produce a series of multiply charged ions which can

give evidence of their presence even on mass-spectrometers of much lower mass scale, and be processed to provide their molecular weight.

In this work, a liquid chromatography/refractive index detector/ radiometric detector/mass spectrometric detector combination (HPLC/RID/RAD/MSD) was used for development of a complex routine technique. Optimization of HPLC/MS analysis was performed investigating the electrospray ionization (ESI) analytical signal of mass spectrometer as a function of various eluent composition [BRUDER, MACÁŠEK, PATAKYOVÁ and BÚRIOVÁ, poster at this Conference]

Some results, illustrating the glucose and FDG ESI-MS, and also composition of FDG after autoradiolysis, which were obtained either by a TOF Mariner (Perkin Elmer) mass-spectrometer and Agilent 1100 HPLC-MS equipment with quadrupole MS detector (Fig.3) are discussed. They give evidence of admixtures and radiolytical formation of deoxyglucose, deoxychloroglucose, erythrose, erytritol, gluconic acid, lactose, raffinose, saccharic acid, sorbitol/[^{19}F]FDG, sorbitol/[^{19}F]FDG, xylitol, and also univalent ions of $C_6H_{10}O_7F.H_2O$ and $C_6H_{12}O_8$ compounds.

The results indicate that the emerging demands on radiopharmaceuticals quality control can be fulfilled in-time only by the radio-HPLC technique developed by the help of a tandem mass-spectrometric detector.