LIQUID CHROMATOGRAPHY - MASS SPECTROMETRY ANALYSIS OF PHARMACEUTICALS

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The drugs represent mostly non-volatile and thermally labile solutes, often available only in small amounts like it is in case of radiopharmaceuticals. Therefor, the favourable separation techniques for such compounds are HPLC, capillary electrophoresis and also TLC [1]. Liquid chromatography with mass spectrometric detector (LC/MS) is especially powerful for their microanalysis.

Mass spectrometry separating the ions in high vacuum was presumably used as detector for gas chromatography effluent but the on-line coupling with liquid eluant flow 0.1-1 mL/min is far more challenging. New types of ion sources were constructed for simultaneous removal of solvent and ionisation of solutes at atmospheric pressure (API). Ion evaporation from charged droplets (electrospray, ESI) is one of the most convenient technique. Mass spectrum obtained is rather simple and usually a single molecular or pseudomolecular ion (ion associate) of analyte occurs. When further structural information is necessary, like peptide sequencing, a collisional fragmentation and tandem mass analyser (MS/MS) is a requisite. [2,3]

At present, a relatively wide choice of successfully designed commercial equipment is available either for small organic molecules and larger biomolecules (Perkin-Elmer, Agilent, JEOL, Bruker Daltonics, ThermoQuest, Shimadzu). The features of the LC/MS systems are presented.

Pharmacopoeia normally prescribes a number of methods for quality control of pharmaceuticals. A robust LC/MS analysis is potentially able to replace nearly each of them except the test for volatile components performed by GC. Examples of the analyses are conferred.

LC/MS as a new quality control tool for [^{18}F]fluorodeoxyglucose (FDG) radiopharmaceutical, which has became the most spread radiopharmaceutical for positron emission tomography (PET), was proposed. [4] A fast progress in the instrumental field made the task easier. We use the Agilent1100 equipment granted by the IAAE. E.g., in acetonitrile (MeCN) - 0.025% ammonium formate (NH_4HCO_2) solvent (80:20), glucose electrospray ionisation provides dominating lines of FDG.HCO_2^- ions at *m*/*z* 227 suitable for an analytical signal: the limit of FDG quantitation, LOQ = 20 ng was found. [5] Toxic impurity from the FDG synthesis, Kryptofix® 222 provides an intense mass-spectrometric detector (MSD) signal of the positive ion associated with NH_4^+. Expired FDG injection samples contain decomposition products from which at least one is labelled by ^{18}F and characterised by signal of negative ions at *m/z* 207 which does not correspond to FDG fragments but to C_5 decomposition products. [6]

Decomposition of thymidine, the precursor for synthesis of 3-deoxy-3-fluorothymidine (FLT) radiopharmaceutical, goes to thymine and 4,5-thymidineglycol., Without MSD the products are difficult to confirm.

Biomolecules, such as the peptides of molecular weight about 2-5000 can be analysed even by the MSD enabling range of m/z under 1500 due to formation of multicharged ions and mass spectrum deconvolution [7]. E.g. bombesin ($C_{72}S_2N_{25}O_{18}H_{120,}$ Mw=1722) can be analysed by M^{3+} ions formation in acidic eluant and deconvolution in the m/z=500-750 range isotopic peaks.

Identification of endotoxins (Mw about 50-80 kDa) at the level about 1 ppm, proposed for their GC/MS assay in food [8] is another challenge for the pyrogens assay in the injection radiopharmaceuticals.

Last not least, LC/MS may provide also very important figure of specific activity of radiopharmaceuticals by estimation of isotope composition [9] but for practical purposes a shielded equipment housing is required.

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