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Review

Mass spectrometric tools for cell and tissue studies

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ABSTRACT

Mass spectrometry (MS) is a powerful tool for identification and quantitation of organic molecules from various matrices, especially when combined with liquid chromatography (LC). The aim of this review is to present different MS techniques and methods which can be utilized in drug and metabolism studies using cells and tissues. The first part focuses on the use of LC/MS in permeability studies across cell lines as well as in ABC transporter protein experiments. The second part describes the utilization of MS in drug metabolism studies using cell lines. The third part presents a relatively new application area of MS, namely mass spectrometric imaging (MSI) or imaging mass spectrometry (IMS). Several different MSI techniques can be used for characterization of surfaces, in terms of abundance of proteins and peptides but also small molecules, such as drug compounds and their metabolites, at the surface. The final part gives a review of MS based techniques for direct analysis of cell contents.

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1. Introduction

Mass spectrometry (MS) is a powerful tool for identification and quantitation of organic molecules from various matrices, especially when combined with liquid chromatography (LC). The main advantages of MS are sensitivity and selectivity which enable detection, identification, and quantitation of both small molecules and large biomolecules from complex mixtures. Recent development of mass analyzers, for example very sensitive triple quadrupole instruments, high resolution time-of-flight and orbitrap instruments, and their hybrid instruments, has brought capabilities of MS instruments to a new level which enables direct and selective analysis of organic molecules at very low concentration levels. Therefore, it is ideal also for analysis of organic molecules when biological material, such as cell and tissues, are involved. The purpose of this review is to highlight different mass spectrometric techniques and methods which can be utilized in permeability and metabolism studies using cell lines and in a direct analysis of small and biomolecules from cells and tissues.

2. Permeability studies

2.1. Measurements with cell lines

In order to have a desired effect a drug molecule must be able to reach its target protein in the body. Following oral administration, which is the most desired route of drug administration, the drug can be absorbed into systematic circulation via passive and active transport routes across the gastrointestinal epithelium. Therefore, one of the most important properties of a drug candidate is its permeability through biological membranes. Permeability by passive diffusion is determined mainly by physicochemical properties of a drug candidate such as molecular weight, charge, and hydrophobicity (Van Pelt et al., 2003; Smith and van de Waterbeemd, 1999). In order to be able to predict drug absorption in the gastrointestinal track *in vitro* permeability assays have been developed. Most common models to predict drug absorption are various cell culture models (Braun et al., 2000; Hidalgo, 2001). At present, a monolayer of human colon adenocarcinoma cell line, Caco-2, is the most often used system to predict human absorption based on a permeation rate of compounds across the cell monolayer (Press and Di Grandi, 2008). Another commonly used cell lines are Madin-Darby Canine Kidney cells (MDCK) (Cereijido et al., 1978) and 2/4/A. The *in vitro–in vivo* correlation of Caco-2 cell line has been known to be problematic and, thus, caution is needed when conclusions are drawn from Caco-2 cell line experiments. However, because the passive diffusion driven by the concentration gradient is the most important factor in drug absorption, Caco-2 and other cell lines can be considered as valuable *in vitro* prediction tools. From an analytical point of view permeability samples produced by Caco-2 cell assays are fairly simple. In an experimental setup a compound is added to either side of the monolayer and the compound concentration is measured from the opposite side of the monolayer by a suitable analytical technique. Radiolabeled compounds were used in the original Caco-2 monolayer assays but recently LC/MS assays have been the method of choice in permeability studies. The sensitivity and selectivity of LC/MS/MS make it a powerful tool for routine analysis of cell culture samples (Wang et al., 2000). In addition, the utilization of LC/MS has eliminated the need of radiolabeled compounds and permits simultaneous analysis of multiple compounds in so called cassette dosing or n-in-one studies (Markowska et al., 2011). In this approach compounds of interest are administered together under identical conditions. Compounds need to be selected carefully to avoid drug–drug interactions but successful implementation of cassette dosing can substantially increase total throughput. The cassette dosing puts pressure on the analytical method due to the large amount of compounds with varying properties. Problems in mass spectrometric detection can be avoided to some extent by using ultra high resolution chromatography to achieve better separation for the sample components. Higher chromatographic resolution is obtained by using columns packed with sub-2 micrometer particles. The prize of the better chromatographic performance is the need of better quality pumps capable of handling clearly higher operation pressure. The utilization of ultra high performance chromatography instruments allows decreasing the length of analytical run even to less than 1 min, resulting significantly higher total throughput.

The common LC/MS set-up in the quantitative analysis of cell culture samples is liquid chromatography using a reversed-phase C_{18} column combined with a triple–quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source. As well known, ESI is limited to fairly polar analytes and, thus, lipophilic compounds cannot be detected or the signal intensity is very poor. Therefore, other ionization methods have been utilized in order to be able to analyze a larger variety of compounds. Hakala et al. used atmospheric pressure photoionization (APPI) as an interface in LC/MS analysis to increase the number of possible analytes (Hakala et al., 2003). Another commonly used atmospheric pressure ionization technique is atmospheric pressure chemical ionization (APCI) which also can be used to analyze non-polar compounds in permeability studies. The major drawback of APPI and APCI is that they are not suitable for thermolabile compounds. On the other hand, APPI and APCI are less prone to ion suppression caused by salts and interfering endogenous compounds. Most of the mass spectrometer vendors provide ionization sources capable of utilizing more than one ionization technique in one ion source. These multi-mode ionization sources enable the combinations such as ESI and APPI, or ESI and APCI. However, often some compromises are needed in order to operate each ionization mode successfully. As a result, some losses in performance can be observed, for example detection limits can be higher with a multi-mode ion source than with a specific ion source alone.

LC/MS techniques can be combined with on-line sample preparation methods by utilizing column switching technology. Typically solid-phase extraction (SPE) is coupled to LC/MS analysis and it has also been used for permeability studies. For example, Soldner et al. used on-line sample preparation to determine permeability rate of losartan through different cell monolayers (Soldner et al., 2000). The drawback with on-line sample preparation is the prolonged total analysis time. Thus, automated sample preparation performed off-line usually results in higher total throughput.

However, when higher throughputs in quantitation are required a traditional LC/MS/MS analysis can be considered slow. Thus, methods with higher throughputs have been developed. Fung et al. presented a method which utilized multiple sprayers in LC–ESI/MS (Fung et al., 2003). Higher throughput was achieved by using four parallel chromatographic columns which were connected to four sprayers. They were able to reach the total throughput of 800 samples per day. At about the same time Van Pelt et al. described a fully automated method utilizing disposable nanoelectrospray tips for Caco-2 samples increasing throughput even further (Van Pelt et al., 2003). In the method 96 samples were analyzed in 72 min increasing number of samples per day to almost 2000. In their system each sample is analyzed using disposable nanoelectrospray tip, thus minimizing the carry over problems. However, no chromatographic separation is performed, therefore a risk of interfering compounds needs to be taken account. On the other hand, very low sample volumes and solvent consumption are achieved by miniaturizing the sample introduction system. Later, the same technique was used as combined with a fraction