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Conceptual Paper

Hyphenated Analytical Methods: Role in Pharmaceutical Analysis

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A hyphenated technique is an on-line combination of a separation technique and one or more spectroscopic detection techniques. These techniques have received ever-increasing attention in pharmaceutical analysis over conventional chromatographic or classical spectroscopic techniques for both qualitative and quantitative analysis of unknown compounds in complex and varied matrices with a minimum of preparation.

The hyphenation involves linking liquid chromatography (LC), usually a high performance liquid chromatography (HPLC), gas chromatography (GC), or capillary electrophoresis (CE) to spectroscopic detection techniques such as photodiode array (PDA) UV-Vis absorbance, Fourier-transform infrared (FTIR), nuclear magnetic resonance (NMR), mass spectroscopy (MS), inductively coupled plasma (ICP)- mass spectroscopy. These couplings result in the introduction of any of these modern hyphenated techniques, namely GC or LC-PAD, GC or LC-FTIR, GC or LC-NMR, GC or LC-MS and CE-MS etc. Furthermore, the coupling of separation and detection techniques could entail more than one separation or detection techniques in the hyphenation process. For instance, LC-PDA-FTIR, LCPDA-NMR-MS, LC-PDA-MS, LC-MS-MS, LC-NMR-MS and GC-ICP-MS and the like could be coupled.

Chromatography generates pure or nearly pure chemical compounds through separation of chemical components in a mixture while spectroscopy produces selective information for identification and quantification using library spectra and standards respectively. Chemical compounds that are adequately volatile and stable in high temperature GC conditions can be easily analyzed by GC-MS technique. However, polar compounds, especially those with amino, hydroxyl and thiol groups can also be analyzed by this technique following chemical derivatization. High performance liquid chromatography (HPLC) allows determination of compounds that are thermolabile, very polar or of high molecular weight. The capillary electrophoretic method allows the separation of biomolecules with high performance where HPLC fails and also permits the quantification of small molecules that cannot be separated by gel electrophoresis. Inductively coupled plasma (ICP) uses argon plasma (7000 - 8000 Kelvin) to atomize and ionize elements in chemical compounds.

Ultraviolet and visible detection allows the identification and quantification of chemical organic compounds because of the conjugation system in such compounds The infrared (IR) or FTIR detection allows the identification and quantification of chemical organic compounds because the structures of organic compounds have many vibrational absorption bands that are characteristic of particular functionalities. Nuclear magnetic resonance (NMR) detection permits identification and quantification of unknown compounds by chemical shifts and integrated areas of the signals. The mass spectrometry detection permits the identification and quantification of chemical compounds on the basis of mass-charge ratio spectral data.

In terms of detection techniques, IR and NMR are much less sensitive compared to various other detection techniques for example UV and MS. However, NMR sensitivity is currently being enhanced by pulse field gradients and solvent suppressions methods and the introduction of high-field magnets (800 - 900 MHz).

Pharmaceutical analysts have exploited the properties of these hyphenated analytical methods in the analysis of chemical compounds, some of which might be in trace or ultra trace levels. Such analyses performed are very often in complex and varied sample matrices and they include:

(i) Identification and quantification of pharmaceutical active ingredients including cytotoxic compounds in bulk or finished products, in body tissues or biological fluids (whole blood, plasma, serum, urine, saliva, synovial fluids etc).

(ii) Identification and quantification of volatile organic compounds in pharmaceutical active ingredients or excipients, in air (autoexhausts, soots of interest etc.), in water (phenols, polycyclic aromatic hydrocarbons PAH, polychlorinated biphenyls PCB, pesticides, herbicides etc.) and in soil (pesticides, herbicides etc).

(iii) Identification and quantification of elemental compositions of chemical and pharmaceutical compounds.

(iv) Identification and quantification of pharmaceutical active ingredients in natural products.

(v) Identification and quantification of antibiotics in foods of animal origin.

In conclusion, of all the hyphenated analytical methods, capillary gas chromatography coupled with tandem mass spectrometry (GCMS/MS) seems to be the most effective and efficient technique in terms of resolution, sensitivity, specificity and speed of analysis, as necessary for ultra trace analysis. Finally, it is pertinent to note that hyphenation of at least two techniques would be of importance to a pharmaceutical analyst only when the analytical powers (separation and detection) of both instruments are enhanced.

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