

# Development and Validation of Analytical Methods for Pharmaceuticals

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## Abstract

Analytical methods development and validation play important roles in the discovery, development, and manufacture of pharmaceuticals. The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity, potency, and performance of drug products. This review gives information regarding various stages involved in development and validation of analytical methods like LC, HPLC, MS.

**Keywords:** High performance liquid chromatography (HPLC); Liquid-liquid extraction (LLE); UV detector; Mass Spectrometry; NMR; limit of detection (LOD); Limit of quantitation (LOQ)

## Introduction

### Analytical method development

Analytical chemistry deals with methods for identification, separation, and quantification of the chemical components of natural and artificial materials [1]. The choice of analytical methodology is based on many considerations, such as: chemical properties of the analyte and its concentration [2], sample matrix, the speed and cost of the analysis, type of measurements i.e., quantitative or qualitative and the number of samples. A qualitative method yields information of the chemical identity of the species in the sample. A quantitative method provides numerical information regarding the relative amounts of one or more of the analytes in the sample.

The steps of method development and method validation depend upon the type of method being developed. However, the following steps are common to most types of projects:

- Method development plan definition
- Background information gathering
- Laboratory method development, it includes various stages namely sample preparation, specific analytical method, detection and data processing
- Generation of test procedure

A well-developed method should be easy to validate. A method should be developed with the goal to rapidly test preclinical samples, formulation prototypes, and commercial samples. There are five common types of analytical methods, each with its own set of validation requirements:

- Identification tests
- Potency assays
- Quantitative tests for impurities
- Limit test for the control of impurities
- Specific tests

The first four tests are universal tests, but the specific tests such as particle-size analysis and X ray diffraction are used to control specific properties of the active pharmaceutical ingredient (API) or the drug product [3,4].

The most widely used methods for quantitative determination of drugs and metabolites in biological matrices such as blood, serum, plasma, or urine includes Gas chromatography (GC), High-performance liquid chromatography (HPLC) [5,6], Thin layer chromatography (TLC), combined GC and LC mass spectrometric (MS) procedures such as LC-MS [7,8], LC-MS-MS [9,10], GC-MS [11,12], and GC-MS-MS, techniques like NMR is used for structure identification.

Chromatography in different forms is the leading analytical method for separation of components in a mixture. The chromatographic procedure for the separation of substances is based on differences in rates of migration through the column arising from different partition of the compounds between a stationary phase (column packing) and a mobile phase transported through the system [13]. Chromatographic methods can be classified according to the physical state of the mobile phase into the following basic categories: gas chromatography (GC), supercritical fluid chromatography (SFC) and liquid chromatography (LC). The technique was originally developed by the Russian botanist M.S. Tswett in 1903 [14,15].

Today TLC is rapidly becoming a routine analytical technique due to its advantages of low operating costs, high sample throughput and the need for minimum sample preparation. The major advantage of TLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC thus reducing the analysis time and cost per analysis [16,17]. An enhanced form of thin layer chromatography (TLC) is called as High performance thin layer chromatography (HPTLC) [18,19]. A number of enhancements can be made to the basic method of thin layer chromatography to automate the different steps, to increase the resolution achieved and to allow more accurate quantitative measurements.

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**Received** October 15, 2011; **Accepted** December 02, 2011; **Published** December 06, 2011

**Citation:** Nishant T, Arun Kumar, Sathish Kumar D, Vijaya Shanti B (2011) Development and Validation of Analytical Methods for Pharmaceuticals. J Anal Bioanal Techniques 2:127. doi:10.4172/2155-9872.1000127

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Liquid chromatography can be categorized on the basis of the mechanism of interaction of the solute with the stationary phase as: adsorption chromatography (liquid-solid chromatography), partition chromatography (liquid-liquid chromatography), ion-exchange chromatography (IEC), size exclusion chromatography (SEC) and affinity chromatography.

Early work in liquid chromatography was based on highly polar stationary phases, and nonpolar solvents served as mobile phases, this type of chromatography is now referred to normal-phase liquid chromatography (NPLC) [20]. Chromatography on bare silica is an example of normal-phase chromatography. In reversed-phase high performance liquid chromatography (RP-HPLC), the stationary phase is nonpolar [21,22], often a hydrocarbon, and the mobile phase is relatively polar [23]. In RP-HPLC, the most polar component is eluted first, because it is relatively most soluble in the mobile phase.

The definite break-through for liquid chromatography of low molecular weight compounds was the introduction of chemically modified small diameter particles (3 to 10 $\mu$ m) e.g., octadecyl groups bound to silica in the late 1960s. The new technique became rapidly a powerful separation technique and is today called high performance liquid chromatography (HPLC).

HPLC-UV diode-array detection (DAD) [24,25] and HPLC-MS techniques take advantage of chromatography as a separation method and DAD or MS as identification and quantification methods. The HPLC equipment consists of a high-pressure solvent delivery system, a sample auto injector, a separation column, a detector (UV or DAD) a computer to control the system and display results.

Ultra performance liquid chromatography (UPLC) is a recent technique in liquid chromatography, which enables significant reductions in separation time, solvent consumption and analysis time as compared to the conventional HPLC [26,27].

### Sample preparation

The purpose of sample preparation is to create a processed sample that leads to better analytical results compared with the initial sample. The prepared sample should be an aliquot relatively free of interferences that is compatible with the HPLC method and that will not damage the column [28]. The main sample preparation techniques are liquid-liquid extraction (LLE) [29,30] and solid-phase extraction (SPE) [31]. In these methods the analyte of interest was separated from sample matrix, so that as few potentially interfering species as possible are carried through to the analytical separation stage.

### Detection

After the chromatographic separation, the analyte of interest is detected by using suitable detectors. Some commercial detectors used in LC are: ultraviolet (UV) detectors [32], fluorescence detectors, electrochemical detectors, refractive index (RI) detectors and mass spectrometry (MS) detectors. The choice of detector depends on the sample and the purpose of the analysis.

The UV detectors are the most common HPLC detectors since they are robust, cheap, easy to handle, and since many solutes absorb light in this frequency range [33,34]. The ordinary UV detector measures the absorbance at one single wavelength at the time. A diode-array detector (DAD) can measure several wavelengths at the same time, and since no parts are moved to change wavelength or to scan, there are no mechanical errors or drift with time.

DAD detectors have been proposed for various applications, such as preliminary identification of a steroidal glycoside in seed [35], peptide mapping [36], assay of sulfamethazine in animal tissues [37], or identification of pesticides in human biological fluids [38].

HPLC with a mass spectrometer detector (LC-MS) [39,40] showed superior sensitivity and selectivity compared to HPLC-UV methods [41].

**Mass Spectrometry:** Mass spectrometry (MS) is a widely used detection technique that provides quantitative and qualitative information about the components in a mixture [42]. In qualitative analysis it is very important to determine the molecular weight of unknown compound and MS is capable of that. MS is also more sensitive than an UV detector for quantification. An MS detector consists of three main parts: the ionization source where the ions are generated, the mass analyzer, which separates the ions according to their mass-to-charge ratio ( $m/z$ ), and the electron multiplier (detector). There are several types of ion sources, which utilize different ionization techniques for creating charged species. Three popular ionization techniques are: electrospray ionization (ESI) [43], atmospheric pressure chemical ionization (APCI) and matrix-assisted laser desorption (MALDI). Electrospray is the most widely used ionization technique when performing LC-MS [44-47].

**NMR:** Nuclear magnetic resonance (NMR) spectroscopy is a very powerful tool to determine the structure of compounds [48,49]. This nondestructive spectroscopic analysis can reveal the number of atoms and their connectivity's, and thus the conformations of the molecules.

Near infrared (NIR) spectroscopy is a quick, non-destructive method that is amenable for spot analysis application. In the last two decades, it has been increasingly used in pharmaceutical analysis [50].

### Method Validation

“Validation of an analytical method is the process by which it is established by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical application - “[51]

The methods were validated according to International Conference on Harmonization (ICH) guidelines for validation of analytical procedures [52,53]. Validation is required for any new or amended method to ensure that it is capable of giving reproducible and reliable results, when used by different operators employing the same equipment in the same or different laboratories [54]. The type of validation program required depends entirely on the particular method and its proposed applications.

Typical analytical parameters used in assay validation include:

- Accuracy
- Precision
- Specificity
- Detection Limit
- Quantitation Limit [55]
- Linearity
- Range
- Robustness

**Accuracy:** Accuracy is a measure of closeness between the measured and real value [56].

**Precision:** Precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained

from multiple sampling of the same homogeneous sample under the prescribed conditions of repeatability [57], intermediate precision reproducibility.

**Specificity:** Specificity is the ability to measure the desired analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc [58].

**Detection limit:** The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Can be determined

- Visually
- Signal to Noise ratio [59]
- Standard Deviation of the Response and the Slope [60]

**Quantitation limit:** The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

**Linearity:** The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample. Test results should be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method of least squares.

**Range:** The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

**Robustness:** The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters [61] and provides an indication of its reliability during normal usage.

Only specificity is needed for an identification test. However, the full range of specificity, accuracy, linearity, range, limit of detection (LOD) [62], limit of quantitation (LOQ) [63], precision, and robustness testing is needed for more-complex methods such as quantitative impurity methods.

## Conclusion

Recent development in pharmaceutical and biotechnological field generates a cumulative demand for analytical methods. Rapid and accurate quantification of the substrate and drug product is important in the process development. Improvements in analytical instrumentation leads to development of new techniques like isocratic and gradient RP-HPLC, which evolved as the primary techniques for the analysis of nonvolatile APIs and impurities. These analytical methods are critical elements of pharmaceutical development so it is very important to develop efficient and accurately validated analytical methods to develop safe and effective drugs.

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