Piramal
Pharma Solutions

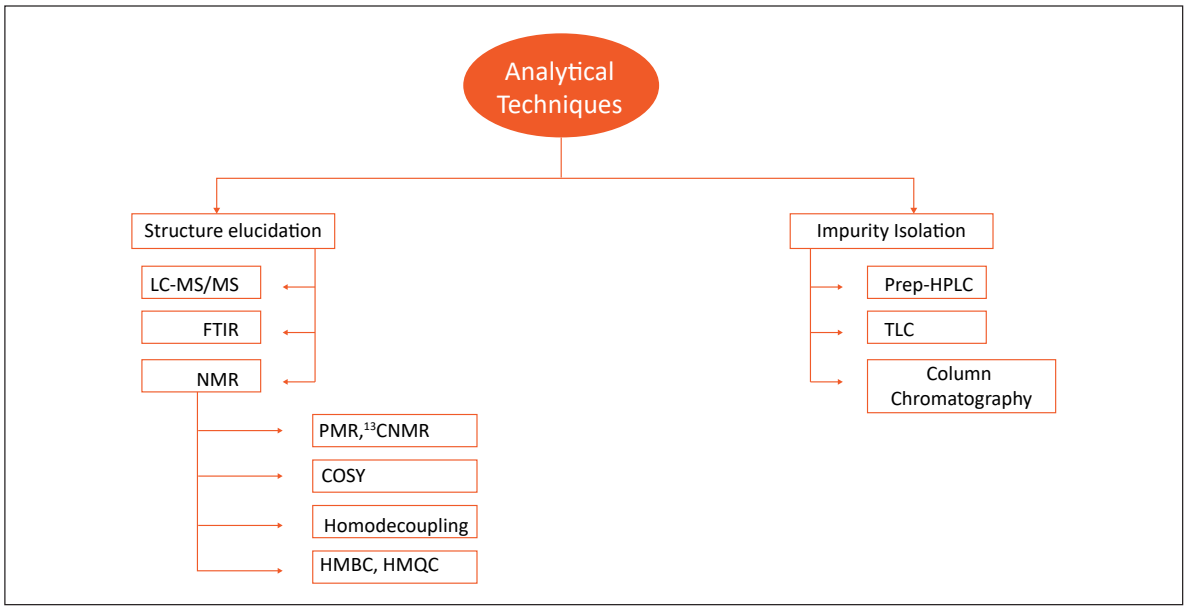
IMPURITY PROFILING AT PIRAMAL

ABSTRACT

The stringent regulatory norms and the efforts for the control of impurities in pharmaceutical products have laid down the need for impurity profiling. In the journey, beginning from synthesis of the crude drug till the expiry of the dosage form in the market, the control of impurities at every step is imperative.

The impurities may occur during the chemical synthesis of the API: byproducts or side-reactions, impurities from the chemicals used for the synthesis, etc. referred as process impurities. During the formulation development, degradation products (DP) may be formed due to stability issues because of API-excipient incompatibilities, or environment (pH, moisture, free radical, oxygen light) etc. Various regulatory bodies such as ICH, USFDA etc., have specified the thresholds for impurities in APIs and drug products. Impurities may cause the drug to become sub potent or more adversely may have toxic effects beyond a threshold.

We, at Piramal Pharma carry out the impurity profiling with various analytical techniques. The impurities are monitored and investigated at every stage of synthesis, formulation development: excipient compatibility, stability studies, stress degradation studies, etc. The efforts are thus made to identify these impurities or degradation products by isolation and characterization techniques if beyond identification threshold or if it very close to the threshold. The analytical department at the Piramal pharma utilizes various techniques for this purpose; a flow diagram of the same is given in figure-1.



The techniques such as prep-HPLC, TLC, column chromatography are used for the isolation of the impurities. The spectrometric methods like LC-MS, GC-MS, NMR and FTIR are used for the structure elucidation. The LC-MS/MS technique which forms to be a bastion for impurity profiling is extensively used and explored at the Piramal Pharma, for the identification and characterization of the impurities which proves to be fruitful when impurities are present even in trace quantities. The key advantage of LC-MS/MS is that the isolation of impurities or DPs is not required, and it provides a tool for online identification. The Quadrapole iontrap mass detector provides the advantage of identification of the pathways of formation of particular impurity by their fragmentation patterns which are very helpful in approaching towards the chemical structure of the impurity.

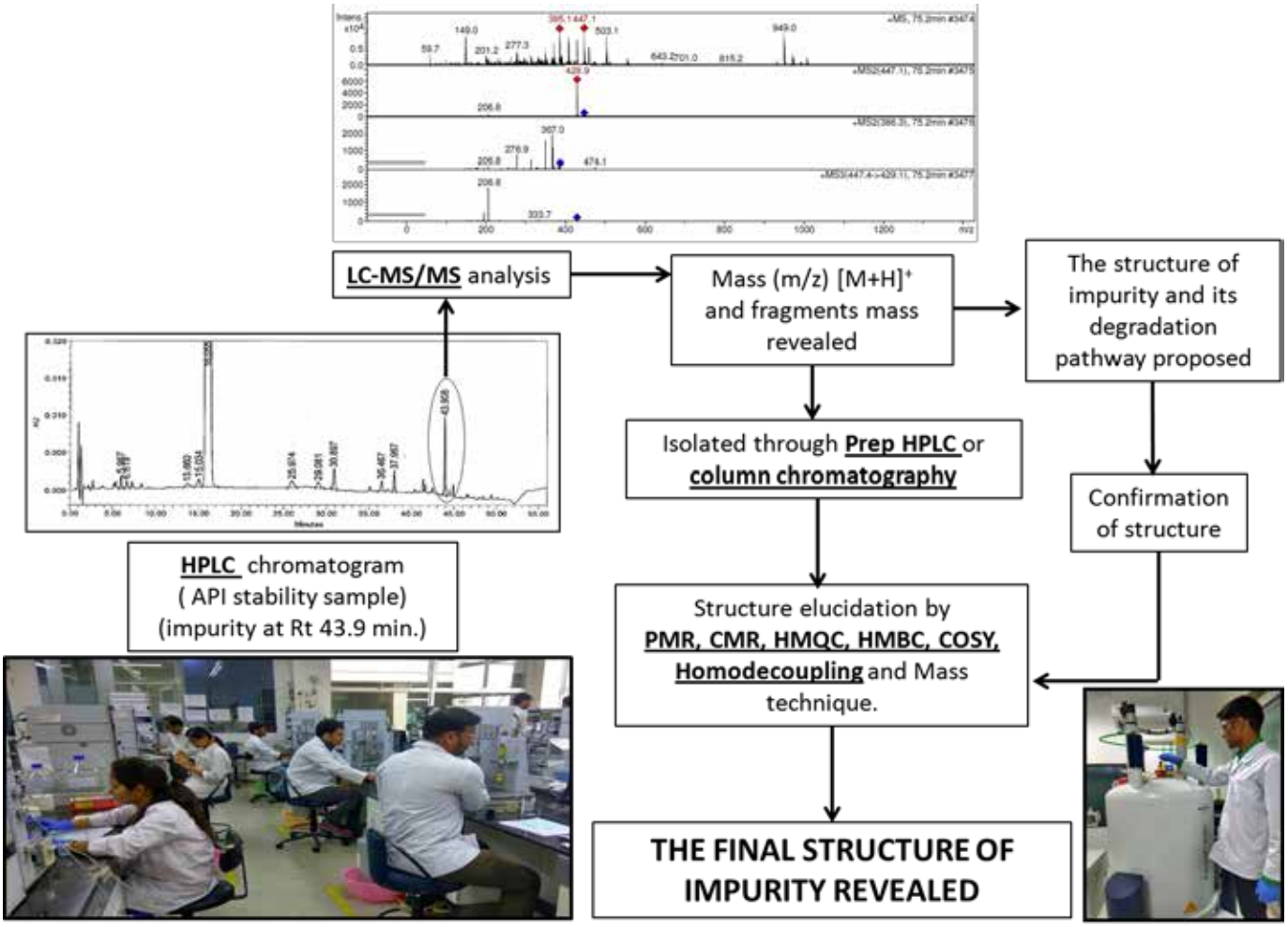
The review of various techniques through which the impurity profiling is carried out at the Piramal premises is briefed out in the following table. Multiple techniques are useful to elucidate the structure based on organic chemistry and instrument data interpretation

Table-1

Instruments Used for Characterisation		Principle	Importance/Advantage	Disadvantages/ Limitation
LC/MS-MS		The molecular mass of component can be identified along with the molecular mass of the fragments in its structure. The degradation pathway of the impurity can be predicted	LC-MS can be used for non-volatile and thermally fragile molecules. The technique can be used to separate a variety of organic compounds from metabolites to proteins	The molar mass of component (impurity or DP) can be known and its degradation pathway can be predicted from fragmentation pattern. But for the confirmation of structure other techniques like NMR are required
LC-MS mass analysers	Quadrapole analyser	The stability of ion trajectories in oscillating electric fields forms the basis of separation of molecular ions	High selectivity and sensitivity. Increased sensitivity through Single Ion Monitoring mode	Have Low resolution and the exact elemental mass can't be obtained
	Ion Trap analyser	The ions of all m/z get separated by sweeping the RF potential applied between the endcaps of the analyser and electrodes. The basic principle is resonant ejection	More sensitive than quadrapole. Routinely used to carry out tandem experiments. Subsequent fragmentation of daughter ions can be achieved for detailed study.	Ions have to be selected: non-selected ions exit the trap Neutral molecules that do not ionize may give rise to abnormally larger [M+H]+. This complicates the spectrum.
	Time of flight analyser	Separation of ions is based on the kinetic energy of the molecular ions in the flight tube (field free drift region). The ion with lower mass has greater velocity and hence shorter flight time	Mass range is unlimited like quadrapole; they have excellent sensitivity due to lack of resolving slits. Hence most useful for biological molecules	Limited dynamic range hence fast digitizers are used for accurate resolution

PMR		Based on the spin of protons under the influence of electromagnetic energy	Prediction of entire carbon skeleton: position of protons and functional groups	Difficult to predict the structure in case of dimerization or for complex structures. Overlapping of signals in case of complex structures
13CNMR		Prediction of the number of non-equivalent carbons based on the resonance	13CNMR spectra will give a map of the carbon framework	Only the number of carbon atoms in the structure can be predicted hence support of other techniques required. Require more amount of samples
Different NMR techniques	COSY	COSY spectra indicate through-bond coupling	Can be used to gain structural information of molecules for wide range of sizes	Suitable only for abundant nuclei (ideally 100%) viz. 1H, 31P or 19F
	NOESY	A NOESY spectrum based on space correlations via spin-lattice relaxation. It determines the signals that arise from protons that are close to each other (<2.5 A^o) even if they are not bonded.	For structure elucidation of proteins. Important tool for identification of the stereochemistry of molecule in solvent.	Computationally expensive, the results are dependent on the accurate measurements of NOE volumes which makes the technique difficult
	HMQC (Heteronuclear Multiple Quantum Coherence)	Carbon (or other heteroatom) to hydrogen connectivity can be determined	Selective for direct C-H coupling	Carbons, which have no protons attached (quaternary carbons), do not appear in these NMR techniques
	HMBC (Heteronuclear Multiple Bond Coherence)	Carbon (or other heteroatom) to hydrogen connectivity can be determined.	Gives longer range couplings (2-4 bond coupling)	Quaternary carbons cannot be detected. An artifact can occur in HMBC as it is "tuned" to detect the small couplings arising from long-range interactions.
	Homodecoupling	It performs the measurements of 1H-1H coupling constants which results into residual multiplets. Allows simplifying multiplet structures by irradiating a specific 1H resonance.	It is very useful technique to separate the overlapped proton NMR spectra. The resulting spectra consist of single lines suggesting proton-decoupled C-13 spectra at natural abundance without multiplicity.	Low sensitivity due to the spatial selective excitation. Requires a special processing scheme to link the data thus generated.

Piramal team has done impurity chracertisation of multiple products using very sophisticated techniques which present cutting edge technology and very high intellectual captial is utlised to interpret the results. Ability to use multiple techniques and tools gives Piramal advantage to characterise exact structure of impurities, which help in controlling the degradation pathways and/or use for identification of the impurities.



Flow Diagram for Impurity Characterisation having asymmetrical dimer as impurity, which are difficult to characterize and synthesize.

The Piramal Pharma analytical development team is actively involved in the research of the impurity profiling, thus making a bright contribution to the horizons of pharma sector.

Piramal Pharma Solutions is a contract development and manufacturing organization (CDMO), offering end-to-end development and manufacturing solutions across the drug life cycle. We serve our clients through a globally integrated network of facilities in North America, Europe and Asia. This enables us to offer a comprehensive range of services including Drug Discovery Solutions, Process & Pharmaceutical Development services, Clinical Trial Supplies and Commercial supply of APIs and Finished dosage forms. We also offer specialized services like development and manufacture of Highly Potent APIs, Antibody Drug Conjugation and are well versed in technologies such as Bio-catalysis, Route Scouting etc. Our capability as an integrated service provider & experience with various technologies enables us to serve Innovator and Generic companies worldwide.

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