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Review Article

Analytical Techniques for Reverse Engineering of Reference Products for the Development of Generic Oral Solid Dosage Forms

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ABSTRACT

Economical and speedy surrogates are cruces for successful generic product development. With value-driven drug development being key for generic pharmaceutical companies, pacing up innovator product characterization is an effective way to compete with heightened costs and pressures for bioequivalent surrogates. Generic product manufacturers characterize the reference listed drug by reverse engineering techniques that serve as the basis for submission of the abbreviated new drug application. Reverse engineering is a systematic deformulation technique that is classified into three segments: (i) Characterization of small (non-complex) APIs- by determining morphology (including particle size distribution, solid-state and, crystal habit) (ii) Categorisation and analysis of complex peptides, polymeric compounds (APIs), (iii) Assessment of excipients by Q1/Q2 evaluation. As of today, there is no prescribed step-by-step methodology for the process of reverse engineering. This review summarizes the essential analytical processes for the successful deformulation and characterization of the reference listed drug product.

Introduction

The successful metamorphosis of a new drug substance into a commercial drug product is efficiently allied with pharmaceutical formulation development. Of all the new drug products in the preclinical development stage, only 10% successfully reached the market. With escalating costs and mounting pressure for new drug development, innovator pharmaceutical companies face significant challenges in accelerating effective formulation selection. These include navigating complex regulatory requirements, managing the high failure rates in clinical trials, optimizing drug delivery systems, and ensuring scalability from lab to large-scale production all while maintaining safety, efficacy, and cost-efficiency.[1] Generic products can be game changers since they are provident and speedy surrogates to the operose and expensive innovator products. To obtain abbreviated new drug application (ANDA) clearance, the applicant must identify a reference-listed drug (RLD), which is a previously approved pharmaceutical product. An RLD is a single approved drug product that is used to determine the bioequivalence of new generic versions. A generic product manufacturer refers to this product.[2] When two pharmaceutical medicines have the same dosage

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form, safety, strength, mode of administration, quality, performance characteristics, and intended purpose, they are said to be bioequivalent. The FDA-selected medication product known as a "reference standard" is what an applicant requesting approval of ANDA must employ in the *in-vivo* bioequivalence research, that is necessary for ANDA approval. This forms the foundation for submitting ANDA for the generic drug product's approval. Characterization and reverse engineering (RE) of RLD or the innovator are crucial steps in the creation of generic drugs and the submission of ANDA.[3] Characterization of innovator products can serve as both a "support tool" for regulatory submissions and a "developmental tool" for generics. In the process of oral solid generic product development, excipients play a key role in product performance. As a development tool, the innovator product characterization helps understand the effect of excipients like diluents or lubricants in oral solid dosage forms (OSDFs), and categorizes critical and non-critical excipients depending on API characteristics. With the decoding or deformulation of the innovator, the number of experimental trials to optimize the formulation is also reduced and it helps to ensure bioequivalence and also applies for biowaivers thereby. Deformulation is reverse engineering through separation, identification and quantification of individual components or ingredients in a drug product.

With the introduction of concepts of Q1/Q2 equivalence to RLD or innovator products, biowaivers can be accepted. The idea of Q1, Q2, and Q3 equivalence is crucial in the reverse engineering process. Q1 equivalence is a qualitative equivalence that can be established when the formulation has the same components (API and excipients) as the innovator product. Q2 equivalence is quantitative equivalence, which is achieved when the formulation has the same composition (± 5%) as the innovator product. Quantitative characterization of all or critical/ performance modifying excipients is carried out during Q2 equivalence determination. The formulation should include identical components at the same concentration and maintain the same microstructure, with the components displaying equivalent physical and chemical properties. This concept is referred to as Q3 similarity, which serves as a parameter for determining equivalency based on characterization. Q1, Q2, and Q3 represent three essential quality standards that evaluate the degree of similarity between a generic drug formulation and its RLD. The RLD includes the composition, structure, and functional performance of the generic product to be compared, and each standard addresses one aspect of comparability to ensure that the generic product fulfills the composition, structure, and functional performance of the RLD. Q1 equivalency is the term used to describe qualitative comparability. According to the compendia, the active and inactive components in the generic formulation must match the ones in the RLD. This is verified by looking up the innovator product's formula in the literature or on the product label to ensure the same qualitative composition. Quantitative comparability is the main focus of Q2 equivalence, which requires that the generic product have similar excipients but that their proportions be almost comparable (to within ±5% of a generic product) in order to qualify. This necessitates complete analytical separation, identification, and quantification of the 'key excipients' (i.e., excipients with a significant impact upon the formulation's performance) for their replication such that the effects of the RLD are achieved. Q3 equivalency demands a microstructural equivalence, which implies that ingredients in the formulation and their physical and chemical properties should be analogous to those observed in the development formulation. Comprehensive characterization for the API and each excipient is performed to ensure that the factors that drive particle size, crystallinity, and polymorphic form are all maintained to ensure bioavailability and drug release properties. These comparability evaluations are crucial depending on the generic product in order to ensure that it performs similarly to the RLD both *in-vitro* and *in-vivo*. After the comparability of Q1, Q2 and Q3 criteria by the formulation, it can pass *in-vitro* b

ioequivalence (BE) studies. The studies analyze the generic drug's release and absorption profiles that are most similar to those of the RLD. Bioequivalence *in-vitro* is demonstrated successfully and the ANDA referencing drug is submitted. It is a complex, demanding process to arrive at Q1 – Q3 comparability. In many cases, the development of the generic depends on the deformulation or the reverse engineering of the RLD. However, this method faces challenges like characterizing of complex or proprietary excipients and the excipient quality may have an effect on the final formulation. If the generic product fails to satisfy the Q1-Q3 comparability standards, bioequivalence studies incorporating further clinical endpoints might be necessary, resulting in extended development timelines and increased costs. Incomparability studies concentrate on (a) the precise identification of excipients, (b) the solubility and dissolution characteristics of the active pharmaceutical ingredient (API), and (c) the reproduction of the RLD physical attributes. Excipient variability creates major risks surrounding excipients that could affect drug release, poorly soluble API solubility issues that could affect absorption or interaction between excipients and the API, which can alter bioavailability. We believe that managing these factors well will lead to a safe and effective generic product, which is bioequivalent to RLD.[4]

In this article, key processes of reverse engineering and also the pivotal characterization parameters affecting the process of product development are discussed, specifically for OSDFs. Reverse engineering aids to answer questions which include, but are not limited to: (i) How can an obsolete component be replaced in a product, (ii) How

Fig. 1: Summary of step-by-step characterization of the reverse engineering process

can a high in-demand product be recreated, (iii) Explore alternatives to banned excipient(s), (iv) How to enhance product performance in comparison with the innovator product, (v) Identify components causing batch-to-batch variance.[5] RE primarily involves decoding the innovator drug product and has been systematically deformulated into three segments as in Fig 1:[6–9] (i) Characterization of small (non-complex) APIs- by determining morphology (particle size, particle size distribution, solid-form, shape and crystal habit) (ii) Categorisation and analysis of complex APIs (peptides, polymeric compounds), (iii) Assessment of excipients by Q1/Q2 evaluation. As of today, there is no prescribed step-by-step methodology for the process of reverse engineering. This review summarizes essential analytical processes for each deformulation segment as categorized above.

Categorization of the Process of Reverse Engineering

An active strategy for solid-state characterization of the API in the RLD will aid in minimizing the risks associated with the development process.

Small Molecule API Characterization

When an API is characterized to the microstructure level, wherein the arrangement of matter is analyzed optimally, Q3 sameness can be established. Key aspects essential for the deformulation of the solid form of the active pharmaceutical ingredient (API) in the reference listed drug (RLD) can be classified into molecular, particle, and bulk level analysis. Polymorphs hydrates/solvates, salt form, amorphous and desolvated solvates are categorized under the molecular level of characterization. Polymorphs can be discovered by using different recrystallizing solvents (polar/ non-polar solvents), varying agitation, temperature and pH. Suppose the polymorphs do exhibit different physical properties, in that case, they can be differentiated for identity and purity based on physicochemical stability, solubility profile (intrinsic dissolution rate, equilibrium solubility), crystal habit, calorimetric behavior and %RH profile. Differential scanning calorimetry (DSC), infrared radiation (IR), modulate DSC, X-ray powder diffraction (XRPD), and solid state nuclear magnetic resonance spectroscopy (SSNMR) have all been used to differentiate between a single polymorphic form (quantitative control) and a mixture of polymorphic forms (qualitative control), for example shown in Table 1.[10,11] Depending on the sensitivity, selectivity and resolution of the analytical techniques, tools can be selected to carry out solid-state characterization. For instance, SSNMR provides an effective technique for analyzing and comparing the physical forms of drug substances, both pure and formulated, across the pharmaceutical processing and manufacturing stages in order to analyze mixtures of solid forms in both the pure API and the formulation.[12,13] Primary characterization parameters of hydrates (solvates) are essentially the same as that of polymorphs, in addition to the recrystallization solvent system selection. Solvent-water systems must also be employed to augment the chances of hydrate formation, which can be monitored by moisture uptake studies (%RH and Dynamic Vapor Sorption (DVS). Thermogravimetric analysis (TGA) has been successfully employed to characterize and differentiate between three hydrated forms of fenoprofen, wherein the binding of water molecules to fenoprofen salt forms was successfully determined.[14] Another important consideration is the formation of hydrates/solvates after wet granulation or milling/compression processes of the bulk drug substance, wherein an anhydrous form of the drug may get converted to the hydrate form. Subsequent drying may prove to be inadequate to convert the hydrate form back to the anhydrous state. Therefore, extensive studies are recommended to alternatively try to convert the drug substance to a new crystal form to circumvent issues of conversion of anhydrous to hydrate forms. Solvates that undergo desolvation prior to analysis are termed desolvated solvate, which exhibits lesser orderliness than the solvate forms. Analytical methods need to be employed to distinguish desolvated forms from true anhydrate, which can be carried out by single X-ray structure determination. XRPD and SSNMR methods can perform a comparison of solvated and desolvated forms. Analysis of the solvent of recrystallization by varying the vapor pressure to determine the vapor pressure isotherm can be studied since the recrystallized drug substance shows a plateau in the isotherm as the vapor pressure is reduced. Amorphous forms due to their property of higher solubility than crystalline forms, are of significant interest owing to enhanced bioavailability. These amorphous forms can be produced by freeze drying, spray drying, or by milling. Altered relaxation times on the SSNMR and broad lines on the IR, along with microscopy, can be effectively employed to detect amorphous forms. XRPD

Table 1: Use of different analytical tools

Analytical tool	*Ability to differentiate crystal forms*	*Application example for prednisolone*
IR spectroscopy	Insufficient to differentiate polymorphic forms	Similar spectra for form A and form B, stronger H bonding in form A
DSC	Inadequate for discerning crystal forms	Form A melts at 182°C, Form B shows small endothermic transition and exothermic peak between 173–177°C, thermal phase transformation detected at ~180°C
XRDP	Most efficient analytical tool	Markedly different patterns for form A and form B crystals. Form A peak at 2θ = 8.7°, Form B peak at 2θ = 9.2°
SSNMR	Successful in characterizing crystal forms	Forms A and B differentiated by resonances at a 120 ppm range

has also been successfully employed to detect amorphous forms since they exhibit broad bumps between 2 and 20°C 2θ.[11] Glass transition temperature (T_g) values can also be positively evaluated to assess amorphous forms. When the temperature exceeds T_g, the amorphous forms tend to crystallize, leading to a decrease in stability in the more reactive amorphous solid. Therefore, before choosing the amorphous form of the drug, a temperature above the glass transition temperature of the drug substance must be established in order to evaluate the stability of the amorphous forms.[15]

Drugs in salt form have the potential to revolutionize stability and bioavailability. For example, ranitidine hydrochloride has better absorption properties than the free base of ranitidine. Likewise, telmisartan formulated as a sodium salt form exhibited enhanced solubility as compared to telmisartan.[16] For the process of characterization of salt forms, step-by-step salt form characterization, along with analysis techniques for each step, has been tabularised in Table 2.[17]

Particle size and particle size distribution (PSD) are included in particle-level characterization. PSD may have an impact on the rate of dissolution and bioavailability of APIs with dissolution-limited bioavailability. Since it changes during the manufacturing process, such as (i) API solubilization in the processing solvent during wet granulation, (ii) particle size reduction by milling, sieving, or mixing, and (iii) fragmentation during compression, particle size is a crucial parameter. Particle sizing methods such as light scattering/obscuration are ineffective at distinguishing between the formulation's excipients and API. Microscopy can successfully distinguish API from the excipients based on features like particle shape and birefringence patterns. Thereby, particle size and PSD can be analysed by diverse techniques, categorized as per the straplines under Fig. 2.[19–26] To effectively characterize particles in specific size ranges, analytical techniques can be selected based on Fig. 3.[19,27,28] Work carried out by Shete *et al.* on the characterization of atorvastatin calcium (ATC) samples gives detailed information on the application of these analytical techniques for the process of solid-state analysis. ATC is marketed in the amorphous and crystalline state. Karl Fisher titrimetry, XRPD, DSC, TGA, and hot stage and scanning electron microscopy (HSM and SEM) were used to characterize six samples of crystalline and amorphous ATC, respectively. Samples ATC 7 through ATC 12 were amorphous, while samples ATC 1 through ATC 6 were crystalline. Crystalline and amorphous solids were distinguished and their purities were assessed using XRPD. All crystalline and amorphous

Table 2: Step-by-step characterization of salt form

Step of characterization	*Inference*	*Method of analysis*
Identify pK_a and corresponding ionizable groups	Gives an idea about the feasibility of salt form	NMR, high-performance liquid chromatography (HPLC)
Assessment of amorphous or crystalline forms	Amorphous forms are difficult to stabilize as compared to crystalline forms.	Optical-polarized microscopy, single-crystal PXRD, DSC, intrinsic dissolution rate, Raman spec
Hygroscopicity profile assessment	Helps determine if the salt form will retain properties in humid conditions of pharmaceutical operations	Desiccator method [18] Dynamic vapor sorption
Solubility assessment followed by physicochemical stability, polymorphic stability and excipient compatibility	Useful especially in combination formulations	Isothermal microcalorimetry, thermogravimetry, IR/MS, DSC, PXRD
Assessment of polymorphic forms of stable salt forms	Salt forms with a lesser number of polymorphic forms is preferred so that performance is predictable	SSNMR, Raman spec, DSC, intrinsic dissolution rate, hot stage microscopy, optical polarized microscopy

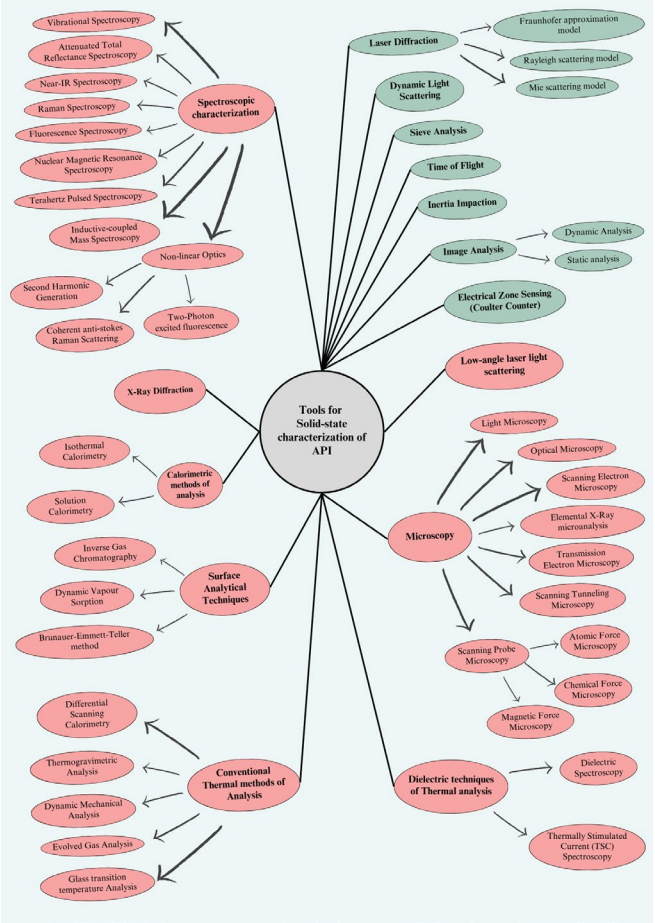

Fig. 2: Analytical tools employed for particle size, crystal habit characterization (in Pink) and for particle size distribution (in Green)

samples were determined to be pure, with the exception of ATC 1, which was discovered to contain other polymorphic crystals and poor crystallinity. ATC 2 through ATC 6 were determined to belong to polymorphic form category-I of ATC by DSC, while TGA assisted in identifying hydrate forms of crystalline ATC. In order to compare the test samples for amorphous ATC, in situ generated samples were used. DSC showed extra endotherm peaks for ATC 12 and ATC 13 above the glass transition temperature Tg, which made it easier to identify and separate test samples with residual crystalline or amorphous content. [29]

API localization and distribution help assess the effect of excipients on the distribution of API in the formulation. In modified-release formulations, combinations of excipients and their distribution alter drug release. Hence, to assess where and how the API is distributed, mapping can be carried out, wherein a peak specific to the API can be picked. XRPD, energy dispersive X-ray (EDX), and infrared and Raman spectroscopy can all be used for mapping. A combination of SEM and EDX can also be used for an API that contains elements that EDX can detect. By the process of mapping, the presence of chloride was ascertained in the API and not in the excipient. Since chloride was present in the API, particle size and dissolution data of two tablets were compared, which helped justify a slower dissolution rate. When the spectral peak of the API differs significantly from the excipient, it is possible to identify the presence of API in the formulation sample. Hence by element and spectral mapping, API distribution can be carried out.[30,31]

Complex API Characterization

Complex API can be categorized as peptides, polymeric drug substances, macromolecular complex, low molecular weight heparin, natural/synthetic polymers, naturally derived complex mixtures, synthetic complex mixtures such as iron-carbohydrate complexes, synthetic nucleotides, etc. Since this review focuses on reverse engineering of only oral solid dosage forms, characterization is limited to drugs that are formulated as OSDFs. Polymeric drug substances are conjugates in which a polymeric moiety is physically encapsulated in a polymeric matrix and covalently bonded to a pharmacologic agent. Polymers work by altering the API's pharmacokinetic characteristics, improving delivery to the site of action, or delaying clearance to lengthen the duration of action. For targeted and localized binding and retention at the binding site, the polymers' inherent qualities—such as avidity and multiple binding sites—are used.[32] One of the earliest synthetic polymers to be extensively utilized as a clinical sequestrant was sodium polystyrene sulfonate (Kayexalate®).[33] Sevelamer hydrochloride (Renagel®), colesevalam hydrochloride (Cholestagel®), patiromer (Veltassa®), cholestyramine (Questran®), colestipol (Colestid®) are examples of other commercially marketed sequestrants.[34] To establish sameness between the test API and the RLD for colesevalam hydrochloride and Sevelamer hydrochloride, the following three criteria have been established: (i) Equivalence to the synthetic manufacturing route, (ii) Polymeric structure characterization- chemical structure and molecular formula determination, (iii) Physicochemical characterization (Table 3).[6,7,35–38]

Characterization of polymeric drug substances requires specific analytical techniques to be employed since methods like NMR and IR do not provide detailed

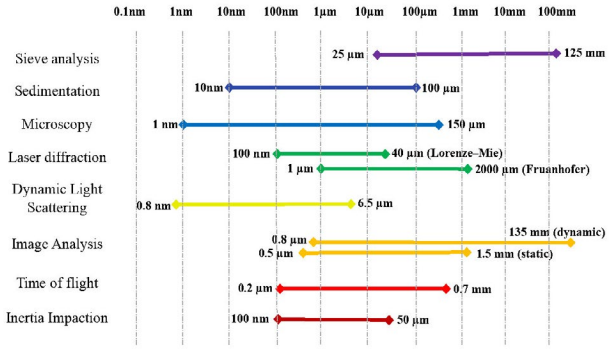

Fig. 3: Comparison of various particle size analysis methods

Table 3: Polymeric API characterizations for API of RLD

Characterizations for establishing sameness between test API and RLD	*Colesevelam hydrochloride*	*Sevelamer hydrochloride*
Determining synthetic manufacturing route	Poly(allylamine hydrochloride) crosslinked with epichlorohydrin and alkylated with 1-bromodecane and (6-bromohexyl)- trimethylammonium bromide	Poly(allylamine hydrochloride) crosslinked with epichlorohydrin
Physicochemical characterization	Bile acid binding by HPLC	Phosphate binding by ion exchange chromatography (IEC)
	Quantify degree of protonation by titration- to determine chloride content	
	Determine total titratable amine	
	Elemental analysis- C, H, N, Cl	
	Determining T_g which helps identify intermediates formed	
	Identification of impurities by gas chromatography (GC) Other tests: Disintegration time, loss on drying, uniformity of mass, swelling index	
Polymeric structure characterization	Determine degree of cross-linking in the polymers and quantify the same in the intermediate by ^{13}C SSNMR spectroscopy	

characterization owing to the cross-linking in the polymers. A few examples of cross-linking agents are toluene diisocyanate, ethylene glycol diacrylate/dimethacrylate, methylene bisacrylamide/bismethacrylamide, and epibromohydrin.[39] For physicochemical characterization, spectroscopic analyses using fourier transformation infrared spectroscopy (FT–IR), Raman spectroscopy, and X-ray diffraction can be performed in addition.

Peptides and polypeptides, being macromolecules, have limited delivery through the oral route due to acidic conditions and, degrading enzymes of the stomach, and lower permeation across the intestine. Linaclotide (Constella®, Linzess®), semaglutide (Rybelsus®), and octreotide acetate (Mycapssa®) are few of the FDA-approved oral peptide formulations. To establish sameness to the RLD, active ingredient sameness can be ascertained by determining structural similarity by identifying the primary peptide sequence, the configuration of chiral centers and defining optical rotation (Table 4.).[40,41]

Excipient Characterization

Assessment of excipients to establish sameness to the RLD can be carried out by: (i) Critical excipient(s) characterization, (ii) Establish Q1/Q2 equivalence by quantifying and qualifying each excipient, (iii) Performing comparative characterizations on critical excipient(s) as per guidance.[9] Q1/Q2 is an inactive ingredient assessment approach used for ANDA submissions, which will also efficiently help characterize excipients. The test formulation is said to be Q1 when it is qualitatively the same as that of the RLD, wherein the grade of each inactive ingredient should be exacted. With the excipient(s) established, critical/performance-modifying excipients can be identified if present and quantitatively characterized for Q2 equivalence.[41] Since the concentration of the excipient or excipients in the test formulation should not vary by more than ±5% of the concentration in the RLD, Q2 establishes quantitative sameness. [42] Separation of the API and individual components of the formulation helps identify and quantify the excipients employed. This helps in the faster development of the optimal prototype formulation without extensive experimentation. Techniques like differential solubility, SEC, filtration on the basis of pore size of membranes, HPLC/high-performance thin layer chromatography (HPTLC), etc., help separate the components. Based on differential solubility, Koradia *et al.*[43] successfully separated API and the excipients. In addition to the API, the innovator product was made with magnesium stearate, hydroxypropyl methylcellulose (HPMC), microcrystalline cellulose (MCC), triacetins,

Table 4: Linaclotide characterizations for API of RLD

Characterizations for establishing sameness between test API and RLD	*Linaclotide*
Peptide structure characterization	Primary peptide sequence: cysteinyl-cysteinyl-glutamyl-tyrosyl-cysteinyl-cysteinyl-asparaginyl-prolyl-alanyl--cysteinyl-threonyl-glycyl-cysteinyl-tyrosine Configuration of amino acids: L- configuration of chiral amino acids Characterization of disulfide bonds: Between C_1-C_6, C_2-C_{10}, C_5-C_{13} Identify multimers: By SEC (Size Exclusion Chromatography)
Physicochemical properties	Assess optical rotation, dissolution characteristics comparison

Table 5: Analytical methods for characterization of excipients

Excipient	*Method of analysis*
Hydroxypropyl methylcellulose	HPLC- SEC (Gel Filtration C column) HPLC-ELSD
Microcrystalline cellulose	Rietveld XRD Transmission electron microscopy (TEM) wide angle X-ray scattering (WAXS) NMR
Sodium lauryl sulphate	Reverse-phase liquid chromatography HPTLC
Magnesium stearate	Atomic absorption spectroscopy HPLC ICP-MS
Croscarmellose sodium, Sodium carbonate, Sodium bicarbonate	IEC Complexometric titration
Hydroxypropyl cellulose	SEC ELSD IR
Mannitol	LC-IR ELSD
Povidone	HPLC UV Spec

TiO2, and synthetic red iron oxide. Employing the method of differential solubility, the first step of separation was carried out using methanol since only the API was soluble in the organic solvent. Other excipients, such as MCC and magnesium stearate, were insoluble in water, so in the second separation step, water served as the separating solvent to extract HPMC from the excipient residue. Since there was no weight loss in the residue from the second separation, it was determined at this stage that HPMC was a component of the tablet coating rather than the core. Such techniques employed not only help assess the distribution of the excipients in the innovator product but also facilitate establishing the functionality of excipients. Since the API in this formulation is highly water soluble, it helps establish that magnesium stearate doesn't play a role in enhancing the dissolution of the formulation, thereby making it a non-critical excipient. Following identification, different separation methods can be chosen based on the quantity of interfering substances and their physicochemical characteristics. HPLC can be used in conjunction with evaporative light-scattering detectors (ELSD), ultraviolet-visible light (UV-vis), and/or refractive index detectors. Such hyphenated techniques and others like inductively coupled plasma conjugated with spectroscopic methods like mass spectroscopy (ICP-MS) can be employed based on the properties of all the components of the formulation. Based on the quantities of the excipients, analytical techniques can be selected. For excipients like polymers, which are high molecular weight compounds, gravimetry is best suited. Sophisticated separation methods, like HPTLC/HPLC, are used to quantify excipients, which are employed in small quantities Table 5.[44–60] summarizes analytical techniques for a few critical excipients employed in OSDFs.

In addition to excipient separation and quantification, characterization techniques are used to identify degraded drug distribution using surface-enhanced Raman chemical imaging (SER-CI), identify excipients influencing process parameters, and typify polymorphic forms of the excipients and their resulting effects on the physicochemical properties of the tablet using SSNMR[61-64] When such aspects of components of a formulation can be rationalized. It eases the process of reverse engineering since polymorphic forms of excipients, alternatives to interfering excipients, and excipients affecting critical process parameters of manufacturing can be ascertained.

Conclusion

Innovator product characterization by the process of reverse engineering is an economical and time-saving methodology for generic formulation development. Deformulation of the RLD, which entails a methodical breakdown of the drug product into different elements, including identity, strength, purity, and efficacy, along with identification of the APIs employed, is crucial to understanding its composition once it has been defined. With the API and excipients characterized as per the RLD/innovator product, chances of biofailures are reduced, the number of trials to formulate the test is lessened and biowaivers can be achieved comfortably. A detailed rundown of the process of characterizing each component of a formulation helps seamlessly reverse engineer the product to successfully deliver generic surrogates, for this appropriate analytics acts as an enabler for the development success of a generic product, reducing time to market and associated risks and costs.

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