Molecular Weight Test Method Validation and HPLC System Qualification

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Abstract *— The Molecular Weight (Mw) Test Method is performed by a contract laboratory. Performing the method externally affects the product release timeframe and represents additional costs when the results are required to be expedite. It is the intent of the company to validate the Molecular Weight (in-house testing) to avoid the waiting time associated to the samples travel time and to the test processing lead time. Qualifying this test in-house will allow to process results in three days instead of two weeks (current timeframe for contract laboratory to provide the results upon processing). There will not be associated costs to expedite results once the test is qualified at the company laboratory.*

Additionally, the company will be implementing a data acquisition software (Empower) which will allow the automatic processing of the samples as well as the results to avoid data transcription. Empower software is FDA 21 Part 11 compliance fully traceable through an audit trail configuration. This feature will avoid security opportunities (data manipulation).

Key Terms *— Molecular Weight, P_4HB monofilament, HPLC System, Bioresorbable material.*

INTRODUCTION

There are four (4) product families manufactured at an un-named company which contains Poly-4-hydroxybutyrate (P_4HB) monofilament as part of their mesh structure. The Phasix Mesh (Flat mesh) product is knit with P_4HB monofilament to form a surgical mesh. On the other hand, the Phasix ST product combines two market-leading technologies into one product; Phasix Mesh (resorbable monofilament) and a proven HA/CMC PEG hydrogel barrier based (Sepra Technology or ST). The P_4HB is a strong biosynthetic material with remarkable mechanical, biocompatibility and biodegradability properties.

As P_4HB is a bioresorbable material, it is susceptible to degradation over time prior to implant and that is the reason to monitor its Molecular Weight prior to sending the product to the customer. The degradation is established through the Molecular Weight of the P_4HB contained within the structure of the products mentioned at the beginning of this introduction.

The Molecular Weight testing is currently performed at an external laboratory which triggers excessive waiting time and costs associated to the sample analysis. The intent of this project is to qualify the equipment required to execute the test method and to validate the test method (in-house) to avoid excessive hold times and reduce costs.

In general, three primary characteristics of chemical compounds can be used to create HPLC separations. These primary characteristics are:

- Polarity
- Electrical charge
- Molecular size

P_4HB molecular weight is assessed through Size Exclusion Chromatography (SEC) which is a molecular size characteristic. Smaller molecules penetrate more of the pores on their passage through the bed. Larger molecules may only penetrate pores above a certain size, so they spend less time in the bed. The biggest molecules may be totally excluded from pores and pass only between the particles, eluting very quickly in a small volume. Mobile phases are chosen for two reasons; they are good solvents for the analytes, and they may prevent any interactions (based on polarity or charge) between the analytes and the stationary phase surface. In this way, the larger molecules

elute first, while the smaller molecules travel slower (because they move into and out of more of the pores) and elute later, in decreasing order of their size in solution. Hence the simple rule: *Big ones come out first* [1]. Column performance is key for the SEC separation. Colum selection based on packing performance was assessed per Column Handbook recommendations for Size Exclusion Chromatography [2].

PROBLEM STATEMENT

Current Contract Laboratory has a processing period of two weeks approximately. The need of the company to shorten that timeframe is driving the validation of the Molecular Weight (in-house testing) to avoid the waiting time associated to the samples travel time and to the test processing lead time and excessive costs due to expedite results.

METHODOLOGY

The research conducted was outlined through the DMAIC (Define, Measure, Analyze, Improvement and Control) methodology. The DMAIC methodology helped to develop the research of the methods and defined the steps to reach the results. During the Define Phase, a Process Walk was made to build knowledge before moving on to the Measure Phase.

As part of the Define Phase, a Risk Assessment Plan document was developed to assess the impact of having the Molecular Weight Test Method transferred to another location. Additionally, the Risk Plan covered the qualification lifecycle required per the company's standard procedures and policies.

Define

The Voice of the Customer and the Critical to Quality diagram are presented in Figure 1. The In-house Mw Testing was implemented to eliminate the external laboratory cost associated to expedite the Mw results. Additionally, it is the desire of the company to be established the main laboratory to perform Mw test for other branches of the same company that uses the P_4HB monofilament within their product structure.

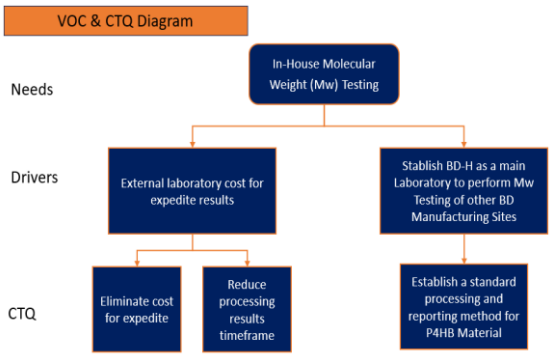

Figure 1
VOC & CTQ Diagram

The Performance and Financial Metrics were defined during this step and are summarized in Table 1. There were two metrics associated to performance and two metrics associated to financial measurements. For the performance measure, the metrics were divided between the equipment and software qualification; while for the financial measure, the metrics were drive by the cost reduction and the revenue.

Table 1
Validation Activities Schedule

Performance Measures		
Metric	Baseline	Goal (If Applicable)
Equipment and Software Qualification	There is no data acquisition software (full 21-part 11 compliance) and a separation system (HPLC) available at BD-H	Qualify and released for production (software and equipment) within six months. IQ, OQ and PQ for software and IQ, OQ and PQ for equipment.
Test Method Qualification	Full transfer of method characteristics (no leverage of an already qualified characteristic)	Complete Test Method Qualification within a one-month period including laboratory to laboratory correlation assessment.
Financial Measures		
Cost Reduction	Contract Laboratory doubles the base rate when results are expedited. External Laboratory Base Cost = $700 per lot External Laboratory Cost for expedite results = $1.4k	N/A
Revenue	BD-Humacao will served as the main laboratory for the Molecular Weight testing for Delran (mesh supplier). Equipment acquired will be paid-off in 0.8 months. Savings are expected at $500k annually.	N/A

The equipment and software qualification consisted of the execution of an Installation Qualification, an Operational Qualification, and a Performance Qualification. The Installation Qualification was focused on assuring that the equipment was properly installed/connected while for the software part, the Installation Qualification assured that the data acquisition was properly installed per the company requirement. The

Operational Qualification for the equipment was focused on the equipment capacity to run within established high and low parameters and calibration within those parameters, while the Operational Qualification for software was focused on the security aspects of the data acquisition system per company's software policies. The Performance Qualification was focused on challenging the proposed controls (Work Instructions) for the equipment operation and for the data acquisition software operation.

The Financial metrics were based on the reduction of the laboratory test base cost as well as the elimination of the cost associated to expedite the results. Additionally, the revenue measurement was included in the Financial metrics since the company expects to perform the implicated testing to other branches within the same company.

The Define phase served to identify the preliminary opportunities related to the project implementation as well as the equipment and service cost associated to the project implementation.

The equipment and service cost associated to the project were requested through an executive summary which is part of an Authorization for Expenditure request. The request included equipment and consumable quotes, as well as the financial analysis required to establish the baseline for the payback period. Details of the equipment and service cost are presented in Table 2.

Table 2

Equipment and Service Cost

Equipment and Service Cost	
Description	**Cost**
Equipment	$96,483
Consumable Materials for Validation	$6,354
External Testing for Validation (Including Materials)	$9,000
Services	$13,850
Freight Charges (6.0% of Total Equipment Cost)	$5,789
Sub Total	$119,733
Contingency 10% of Sub Total	$13,147.61
Grand Total	$144,624

Measure

A Procees Flow Chart of the process was developed under the Measre Phase. This process flow allows the company to identify the key process bottlenecks within the testing at the external laboratory: the travel time of the sample, the sample receiving and preparation, the sample analysis, and the results processing timeframe. The total processing time, taking in consideration the bottlenecks presented sums approximately two weeks. Figure 2 summarizes the current state process flow chart.

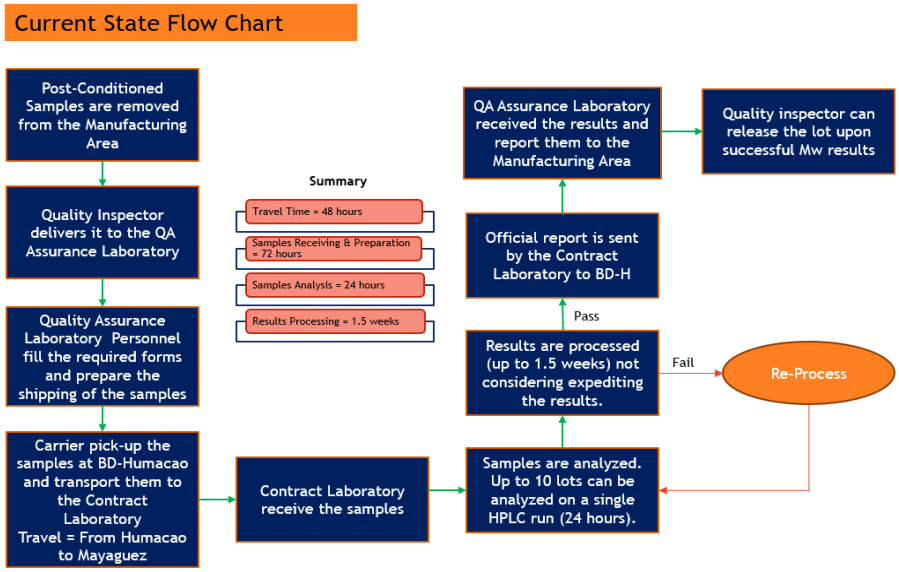

Figure 2

Current State Flow Chart Diagram

The current state of the process reflected the total timeframe for the Test Method (analysis). The Samples required a travel time of approximately 48 hours, a receiving timeframe of 72 hours, an analysis of up to 24 hours and a processing period of 1.5 weeks. The actual analysis process does not fit the company's need of having a processing timeframe that can allow the release of the lot in a timely manner. Usually, the lot is hold for two weeks in the manufacturing area waiting for the results.

Analyze

Three opportunities were identified during the Analyze phase. Opportunities are summarized in Figure 3. The first opportunity was identified when analyzing the sample travel time (approximately 48 hours). The second opportunity was identified during the data acquisition process which is further converted into a report (approximately 1.5 weeks). The third opportunity is focused on the reporting process which usually can take up to 0.5 weeks.

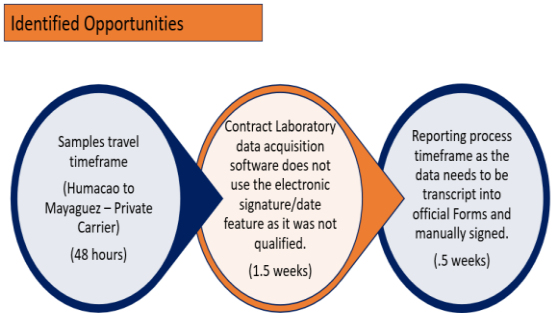

Figure 3
Identified Opportunities

An algorithm to integrate the components being analyzed on a faster way was achieved using the ApexTrack Algorithm. This algorithm does not require the manually integration of each component peak as the algorithm already detects it and provides the molecular weight of the peak upon integration [3].

ApexTrack effectively detects and integrates shouldered peaks, providing more reliable detection of low-level peaks on noisy or sloping baselines. Peak detection using the curvature approach is much more sensitive than the slope criteria used in traditional integration and requires less manual integration and fewer adjustments of integration parameters. This algorithm easily detects even the most subtle peak shoulders with a Detect Shoulders integration event. The addition of the Gaussian Skim integration event replaces vertical drop lines with Gaussian skims, where appropriate.

Three different examples of peaks containing shoulders in a progression are shown n Figure 4. From left to right: optimized traditional integration, ApexTrack integration with default parameters and Detect Shoulders event; ApexTrack integration with default parameters and Detect Shoulders Gaussian Skim events. These examples show that the ApexTrack algorithm simply and effectively integrates shoulders, whether in simple clusters with defined shoulders, or in complex clusters with subtle shoulders. Shoulders are detected whether they are located on the front or the tail of the parent peak.

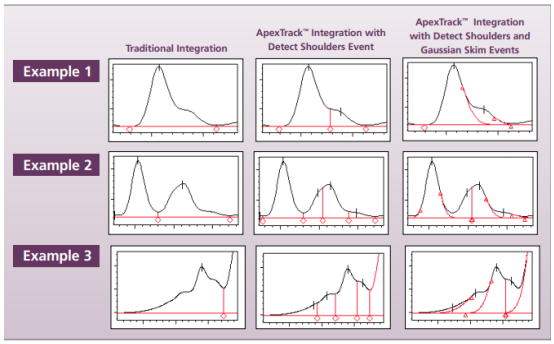

Figure 4
Peaks Containing Shoulders Integrated Using Traditional Integration and ApexTrack Integration [4]

ApexTrack automatically determines the proper peak width and threshold parameters to use for optimal peak detection. These critical parameters are obtained directly from the data. Using automatic parameters can significantly reduce the method development times. The ApexTrack processing parameters that controls peak detection and baseline placement are independent of each other. This is not the case with other integration packages. The algorithm accurately controls both the sensitivity and baseline placement because these parameters do not affect one another. This means that changing the

parameter that affects the peaks' baseline placement does not affect the sensitivity, or the number of peaks detected (and vice-versa) [4]. This reduces the time required to develop the method and the need to manually integrate peaks.

ApexTrack algorithm allowed the peak optimization for the component/sample peak detection by simplifying the processing method and reducing the time required for method development. Refer to Figure 5 for a P_4HB peak detection and integration using ApexTrack algorithm.

Improvement

The process was improved by eliminating the travel time of the samples to the contract laboratory, reducing the processing time or sample analysis and the release of results at the contract laboratory. The improvement of the process reduced the test base cost by 30% (from $700 to $490). Also, the cost for expedite results per lot was eliminated ($1.4k). Equipment acquired will be paid off within a 0.8-month timeframe. The company's laboratory is serving as the main location for Molecular Weight Testing for other manufacturing sites. The in-house testing at the company laboratory sums up a total revenue of approximately $500k annually. The Improved Process Diagram is included in Figure 6.

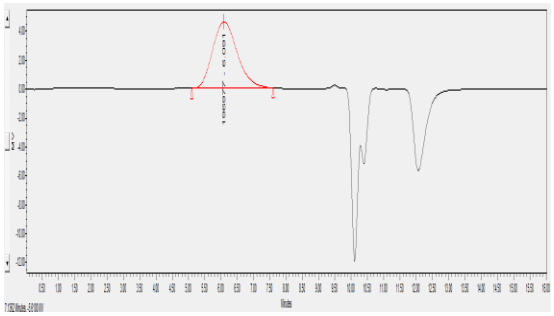

Figure 5
Chromatogram Optimization (Mesh Sample) for Molecular Weight Testing Using ApexTrack

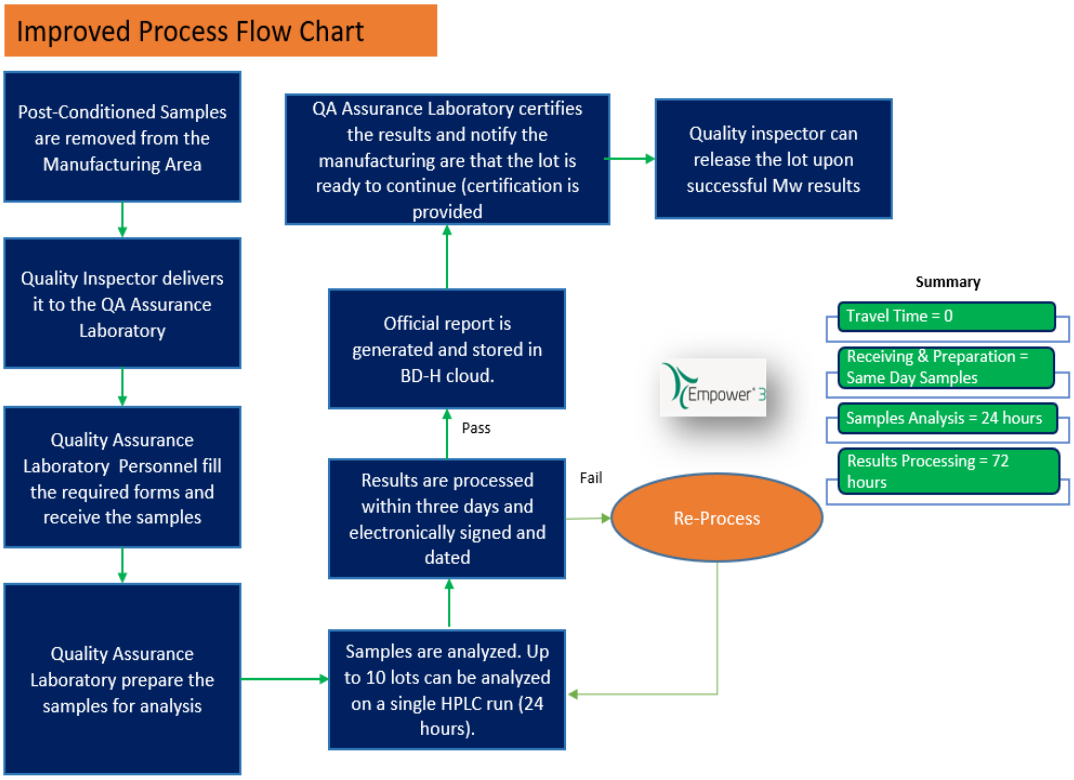

Figure 6
Improved Flow Chart Process

Control

Four controls were implemented during the Control phase. The Test Method for Molecular Weight Testing was validated following the company standard procedure for analytical separations. Once the validation was completed, the Test Method was introduced to the company Quality System as an approved procedure. A Work Instruction to operate the separation system (HPLC) was also created and challenged during the equipment Performance Qualification. Upon the Performance Qualification completion, the HPLC Work Instruction was introduced to the company Quality System as an approved document to operate the HPLC System. A Work Instruction to control the security aspects of the Empower software and its operation was created and challenged during the Performance Qualification. Upon completion, the Empower software Work Instruction was introduced to the company Quality System as an approved document to operate the Empower software. Finally, several laboratory logbooks were designed and introduced within the company Quality System to standardize the documentation or verifications of the laboratory equipment that are used as part of the Molecular Weight Test. The Controls implemented assures the method execution standardization as well as the proper operation of the separation system and the software for the data acquisition system, while the logbooks implementation assures good laboratory practices. Figure 7 presents a description of the Controls implemented.

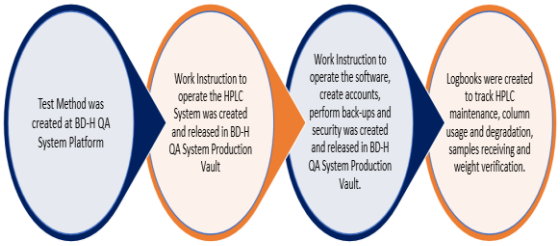

Figure 7
Controls

CONCLUSIONS

The qualification activities for the software were conducted considering the requirements for the qualification of a computerized system capable of performing electronic signature/date. The equipment (separation system) itself was qualified to challenge each function. After qualifying the equipment and the software, a method validation was conducted to validate each required parameter for an analytical method. The processing time was reduced from two weeks to three days. Also, there is no associated cost related to expedite samples results; therefore, lots can be released in a faster way.

FUTURE WORK

This project will allow the establishment of a data trending system for the Molecular Weight data obtained on a lot by lot basis. The Molecular Weight is a critical to quality attribute of the P_4HB material and the trending (data behavior) will allow to determine any opportunities within the process (process stability). Additionally, this qualification allows the validation of future methods requiring the use of a separation system such as the High-Performance Liquid Chromatography separation.

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