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Automated sample preparation of protein solid dosage forms: Novel application for the tablet processing workstation

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A B S T R A C T

Biological macromolecule solid dosage forms represent the frontier of orally administered pharmaceuticals. Analysis of these drug products poses new challenges compared to traditional small molecule tablets. In this study we demonstrate the first, to our knowledge, automated Tablet Processing Workstation (TPW) sample preparation of large molecule tablets. Tablets containing a modified version of human insulin were tested for content uniformity and the automated method was successfully validated for recovery, carryover and displayed manual method equivalency in repeatability and in-process stability. Per TPW's ability to process one sample sequentially, the total analysis cycle time is, in fact, increased. In lieu, a net gain in scientist productivity is realized by enabling continuous operation reducing analytical scientist labor time by 71% compared to manually conducted sample preparation.

Introduction

Recently, there has been an intensified interest and effort towards oral drug products with biological macromolecules (BM) serving as the active pharmaceutical ingredient (API). Ordinarily employed BMs include peptides, recombinant proteins, antibodies, or larger biological scaffolds [1,2]. In the search for new drug candidates based on these large and complex molecules, the drive for high-throughput screenings has created a demand for automated solutions across the pharmaceutical industry [3]. For this reason, automated sample analysis represents an essential enabler of drug research moving onwards.

With regards to analysis of BM solid dosage forms, associated challenges are different from those of conventional small molecules. Sample extraction challenges with small molecule formulations would commonly consider poor API solubility and recovery issues [4], when disintegrating and dissolving tablet API and excipients with disparate polarity and hydrophobicity [5,6]. In contrast, sample extractions of BM solids necessitate considerations relating to short API sample solution stability, aggregation and adsorption. The sample extraction media chosen are preferably composed of buffer-organic solvent mixtures. Interplay with buffer ions of interest, ionic strength and pH all contribute towards retaining the API in its monomerized ionic form, averting potential aggregation and thus increasing the sample solution stability. API aggregation and particle formation, caused by protein structure perturbation, is often examined through an analytical test known as high molecular weight proteins (HMWP). HMWP can originate from a multiplicity of analytical sources with the most prevalent being i) chemical interactions: API-API or API-excipient by diluent pH, additives or organic solvents [7,8]; or ii) physical interactions: mechanical stress or shock from arduous agitation inducing heat and cavitation [9,10]. Surfactants are often added to mitigate API electrostatic adsorption to charged glass or metal surfaces [11,12]. This surface interaction reduces API recovery.. All aforementioned considerations need to be balanced during extraction media selection and analytical method development.

Analysis of tablets, e.g., content uniformity and purity determinations, may be a heavy hands-on laboratory workload. Besides the large number of samples, methods generally involve repetitive steps such as pipetting, filtering, and mixing, which demand consistency and precision of the analytical scientist to maintain analytical quality. Automated sample preparation can minimize the human workload and the quality deficiency risk and hence increase overall laboratory productivity. The Tablet Processing Workstation (TPW) is an automated sample preparation robot which performs complete analytical extraction of (primarily) solid dosage formulations. The operations carried out include tablet weighing, solvent addition, homogenization, filtering, dilution and ultimately transfer to UHPLC vials. All additions and dilutions are performed under gravimetrical control throughout the method. Importantly, the TPW processes one sample sequentially, as opposed to a manual analysis where multiple samples can be prepared simultaneously. Thus, TPW increases the total analysis cycle time but provides continuous operation. Although several TPW drug product applications have been described in the literature [13–16], no example has been described for any kind of a BM sample nor a BM solid dosage form.

The purpose of this research was to evaluate automated sample preparation of BM solid dosage forms using the TPW. The tablets contained a fatty acid acylated human insulin analogue (OI338) as the API with excipient components. OI338 (M_w ~ 5800 g mol^{-1}) is a long-acting oral basal insulin analogue that has previously been investigated for the treatment of type-2 diabetes [17,18].

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Materials and methods

Reagents and materials

Tablets for testing contained OI338 and excipient components and were prepared according to standard pharmaceutical techniques. Placebo tablets for recovery and accuracy testing were of identical excipient composition and were produced internally. Analytical reference material OI338 for standards was produced by Novo Nordisk. Analytical grade KH_2PO_4, $(NH_4)_2HPO_4$, NaH_2PO_4, NaCl, Titrisol ampoules 1N NaOH and HCl used for pH adjustments, HPLC grade acetonitrile, methanol and 2-propanol were all purchased from Merck (Darmstadt, Germany). Analytical grade KCl was purchased from AppliChem (Darmstadt, Germany). HPLC grade H_3PO_4 solution (49.5-50.5%) was purchased from Sigma-Aldrich (St. Louis, MO, US), HPLC grade trifluoroacetic acid was purchased from Fisher Scientific (Loughborough, UK). Biotechnology grade Brij-35 was purchased from G-Bioscience (St. Louis, MO, US). Deionized water (18 MΩ) was prepared in the laboratory with a Millipore-Q system (Darmstadt, Germany) for all aqueous solutions. When required, media preparations were pH controlled using Meterlab PHM220 pH meter by Radiometer Analytical (Lyon, France). Two-point calibrations were conducted before media measurement by using reference solutions pH 1.679, 4.005 and 7.000 from Hach (Düsseldorf, Germany).

Standard preparation

Two standard solution levels were prepared by diluting the OI338 analytical reference material with media to obtain the OI338 standard concentration of 0.12 mg/mL and 0.5 mg/mL, respectively. The media consisted of a mixture of 50 mM KH_2PO4, 380 mM KCl, 0.3% (w/v) Brij-35 as surfactant, pH was adjusted to 7.4 (± 0.05) and the aqueous buffer (ionic strength: 0.5 M) was mixed with acetonitrile 20% (v/v) to achieve the final media composition. These were used for quantification of the API by means of two-point standard calibration curve, including three injections of each standard level. The relative standard deviation (RSD) of the OI338 standard injections were all below 2.0% for all test sets.

Manual sample preparation

Ten tablets containing OI338 were each transferred to separate 25 mL volumetric flasks. 25 mL of the same media as described above was added to each flask.

Sample solutions were mixed by magnetic stirring for 120 min and afterwards diluted to volume mark. Samples were then filtered into UHPLC vials using syringe filter 25 mm, 0.2 µm nylon membrane (2 mL filter sample prewet volume), purchased from Pall (Basel, Switzerland). All ten samples were used to analyze for content. Two of the ten were chosen at random for additional analyses for purity and aggregation.

TPW sample preparation

Ten tablets of the same tablet batch were prepared on TPW III (manufactured by SOTAX AG, Aesch Switzerland), using the identical media as in the manual preparation. The tablets were placed in separate test tubes of the workstation, followed by automated preparation steps. Sample and media were transferred to the vessel. The volume of 25 mL media was dispensed gravimetrically by solvent density input. Homogenization included 7 times 25 s pulses of 2000 rpm and 7 times 5 s pulses of 4000 rpm. The sample was filtered through syringe filter 25 mm, 0.2 µm nylon membrane (2 mL filter sample prewet volume), identical as for the manual method, before transferred into UHPLC vials. Acquired samples were analyzed offline for content, while two out of these ten samples were randomly chosen and additionally analyzed for purity and HMWP.

TPW method validation

For recovery testing, six placebo tablets were utilized and spiked during homogenization process with OI338 standard solutions to achieve 0.16, 0.20 and 0.24 mg/mL, corresponding to 80%, 100% and 120% of the API target concentration, respectively. All three levels were repeated in two replicates. For carryover examination, three sequential tablet sample preparations were run on TPW, followed by a blank sample tube. This was repeated three times as blanks were collected into vials using the same TPW sample preparation method described above and analyzed for API content.

UHPLC content uniformity method

Manual and TPW samples were analyzed for OI338 content (total protein content) using Waters Acquity UPLC Classic system (Milford, MA, US), equipped with a binary pump and mixer volume 385 µL, autosampler, temperature-controlled column compartment and PDA detector. Column used was a Waters Acquity BEH C18, 50 × 2.1 mm, 1.7 µm. Eluent A: 0.1% (v/v) H_3PO_4 in water and eluent B: 0.1% (v/v) H_3PO_4 in acetonitrile. Elution gradient can be found in Appendix in Table A.1. Flow rate was 0.8 mL/min, injection volume 4 µL and run time 12 min. Column temperature was 50 °C and detection at wavelength of 215 nm. Autosampler temperature was set to 7 °C throughout the experiment. The method was employed for content uniformity analysis, recovery test and carryover test.

UHPLC purity method

Manual and TPW samples were analyzed for OI338 purity using Waters Acquity UPLC Classic system, equipped with a binary pump and mixer volume 385 µL, autosampler, temperature-controlled column compartment and PDA detector. Column used was a Waters Acquity UPLC Peptide CSH, 130 Å, 1.7 µm, 2.1 × 150 mm. Eluent A: A mixture of 100 mM $(NH_4)_2HPO_4$, pH 3.1 (ionic strength: 0.09 M) and acetonitrile 9:1 (v/v) and eluent B: 80% acetonitrile in water (v/v), used a gradient that can be found in Appendix Table A.2. Flow rate was 0.3 mL/min, injection volume 5 µL and run time 52 min. Column temperature was 50 °C and detection at wavelength of 214 nm. Autosampler temperature was set to 10 °C throughout the experiment. Purity was calculated by subtracting area of total impurities from the OI338 main peak area in percent.

HPLC high molecular weight protein (HMWP) method

Manual and TPW samples were analyzed for OI338 HMWP using a Waters Alliance HPLC 2695 system, equipped with a quaternary pump, autosampler, temperature-controlled column compartment and UV detector. Column used was a Waters Insulin HMWP SEC, 7.8 × 300 mm. Isocratic elution with eluent containing a mixture of 500 mM NaCl, 10 mM NaH_2PO_4, 5 mM H_3PO_4 (ionic strength: 0.6 M) and 50% (v/v) 2-propanol. Flow rate was 0.5 mL/min, injection volume 40 µL and run time 40 min. Column temperature was 50 °C and detection at wavelength of 215 nm. Autosampler temperature was set to 5 °C throughout the experiment. HMWP was calculated by dividing the HMWP peak area with the total peak area, expressed in percent.

Data analysis

Waters Empower software was used to quantitatively analyze chromatograms. Dotmatics GraphPad Prism (Boston, MA, US) software was employed for statistical data analysis, where Student T-test was performed on obtained data sets.

Results and discussion

To compare manual and TPW method for preparation of OI338 tablets, five different tests were performed: content uniformity, recovery, carryover, API purity and aggregation (HMWP) analysis. TPW results were compared to established and validated manual extraction, employing internally validated chromatography methods.

It is important to emphasize that this work was conducted in an early non-clinical research environment, and therefore it is not required that the generated results fulfil the United States Pharmacopeia (USP) and European Pharmacopoeia (Ph. Eur.) drug product criteria.

Method equivalence - content uniformity and repeatability

The TPW analytical method is evaluated by comparing content uniformity results from manual and TPW sample preparation. In an early research setting and non-GMP (good manufacturing processes) laboratories tablet content and percent label claims (% LC) might diverge from the expected amount for several reasons, e.g., unknown purity of novel API materials, suboptimal formulation processes attributed to uncharacterized API, or undesired API and excipient powder properties.

The manual sample preparation method is an internally validated analytical method. Thus, for equivalence, the TPW method parameters were kept unchanged, such as extraction media, sample concentrations and filter type. The only altered parameter is the tablet disintegration mechanism whereby homogenization is employed in TPW versus magnetic stirring in the manual method. Ten tablets prepared by each method, twenty in total of the same tablet batch, were analyzed for content uniformity. As presented in Table 1, manual samples resulted in mean of 84.1% LC OI338. The TPW sample results yielded a comparable mean value, 83.0% LC OI338. Student t-test shows non-significant difference between the means ($p = 0.97$), confirming that the manual and automated methods are indistinguishable from each other.

When analyzing ten tablets prepared by a manual method, the percentage of RSD was 3.3%, while the result for ten tablets prepared on TPW was 3.4% (Table 1). Typically, an automated method is anticipated to improve analysis repeatability and generate lower RSD by eliminating human variability. This was not observed for our result set. It is acknowledged that formulation batches possess an intrinsic variability originated from tablet production, further tests are required to conclude on potential repeatability improvement for the automated method. The TPW contains long tubing systems (polypropylene) and valves (stainless steel) which the sample is transferred through, subjecting the BM to a large surface area. This could give rise to loss due to non-specific adsorption, and thereby affect recovery and repeatability [21]. In light of these considerations, we deemed the equivalent repeatability achieved for the TPW versus manual samples to be acceptable.

Table 1
Tablet content uniformity and repeatability results of OI338 for TPW and manual sample preparation as percentage of label claim (% LC) and relative standard deviation (RSD). N = 10 for each preparation method, N = 20 in total.

Sample	TPW OI338 (% LC)	Manual OI338 (% LC)
Replicate 1	80.1	88.1
Replicate 2	80.4	81.3
Replicate 3	82.5	84.3
Replicate 4	78.2	85.5
Replicate 5	85.0	81.9
Replicate 6	81.7	87.3
Replicate 7	85.9	83.7
Replicate 8	84.7	86.8
Replicate 9	86.2	80.5
Replicate 10	85.1	81.5
Mean	83	84.1
RSD (%)	3.4	3.3

Table 2
Recovery test of TPW method as percentage recovered OI338 of added amount (%) and relative standard deviation (RSD), OI338 added to placebo sample matrix (tablet). Three concentration levels: 80%, 100% and 120% of target API sample concentration performed in duplicate, N = 6 in total.

Sample	80% level OI338 recovery (%)	100% level OI338 recovery (%)	120% level OI338 recovery (%)
Replicate 1	101.8	100.9	100.6
Replicate 2	104.0	102.8	100.3
Mean	102.9	101.9	100.5
RSD (%)	1.5	1.3	0.2

Table 3
Purity and High Molecular Weight Protein (HMWP) of OI338 TPW and manual samples, N=2 in total.

	OI338 Purity (%)		OI338 HMWP (%)	
Sample	TPW	Manual	TPW	Manual
Replicate 1	94.6	94.2	1.0	1.0
Replicate 2	94.7	94.4	1.1	1.0
Mean	94.6	94.3	1.0	1.0

TPW method validation - recovery/accuracy

To validate the TPW method, a recovery test was performed using three API sample concentration levels: 80%, 100% and 120% of target OI338 concentration 0.2 mg/mL. Mean values deviated less than 3% from the target at all three levels (Table 2), which is an acceptable result for investigations performed in an early drug development setting [19]. Furthermore, the observed RSD values of below 2% demonstrated confidence of the recovery results, in accordance to internal guidelines. The obtained recovery results imply a method accuracy range between 80-120% of the OI338 concentration for the solid dosage form used in these studies.

TPW method validation – carryover

To verify the TPW automated cleaning procedure of the vessel and transfer paths between individual sample preparations, OI338 carryover was tested as part of the method validation. Blank samples prepared using TPW preceded by sequential tablet sample preparations showed no carryover peaks of OI338 (Fig. 1), LOQ = 0.1%, N=3, indicating sufficient cleaning between the TPW sample replicates.

API stability – purity and high molecular weight protein (HMWP)

TPW homogenization could, in principle, cause protein degradation and aggregation by exposing the sample solution to high shear forces [20], generated heat and oxygen [10]. Therefore, purity and HMWP tests of the TPW samples were compared to those prepared manually by magnetic stirring. Results presented in Table 3. Verify that no additional OI338 degradation occurs upon homogenization at 2000 and 4000 rpm as revealed by comparable OI338 purity for both methods. In addition, the impurity peak landscape was identical when overlaying and comparing the TPW and manual purity chromatograms (Fig. 2).

HMWP analysis of OI338 protein stability by means of size exclusion chromatography was conducted to evaluate potential protein aggregation during homogenization. The results in Table 3. demonstrate identical HMWP amounts measured in samples generated by TPW and manually. The chromatogram overlay (Fig. 3) confirms that the monomerized structure of OI338 is retained during tablet homogenization as part of the automated workflow, compared to magnetic stirring.

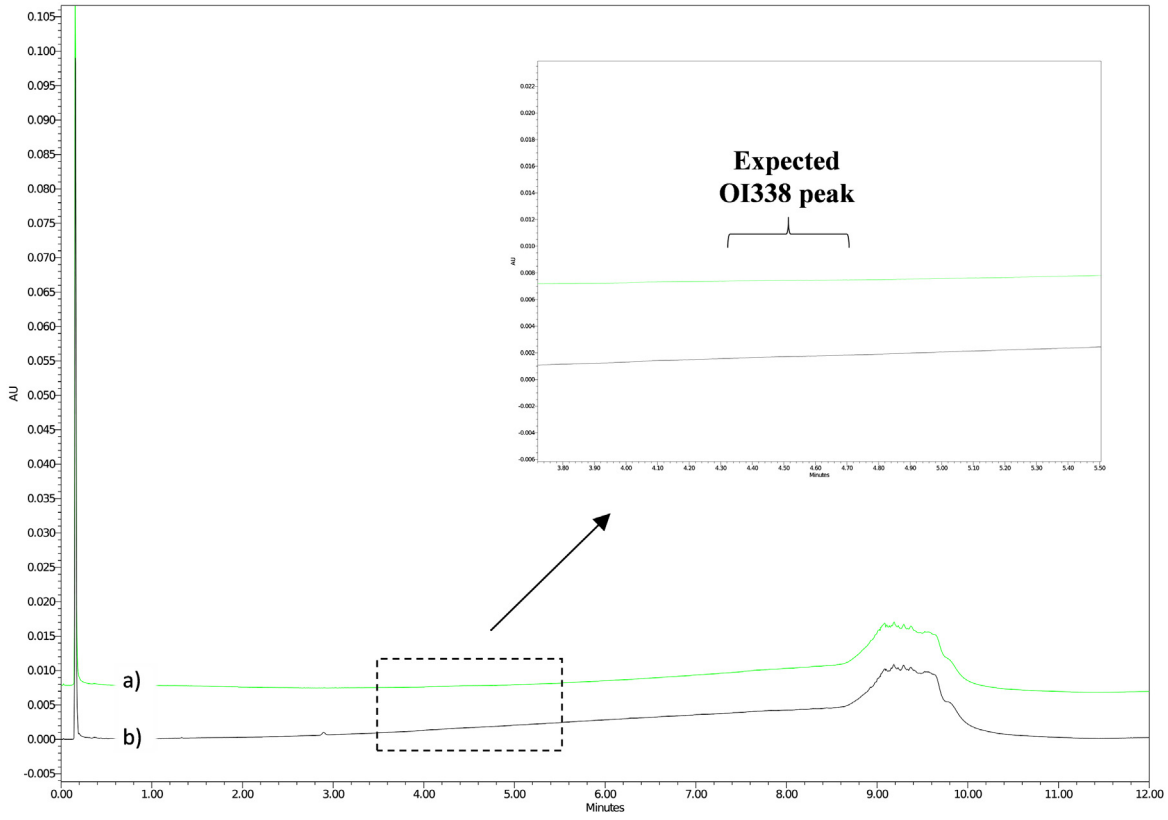

Fig. 1. Carryover chromatograms of OI338 content uniformity analysis of TPW carryover sample and extraction media; a) TPW carryover sample, b) Extraction media (blank). The OI338 peak is anticipated to elute at 4.5 min.

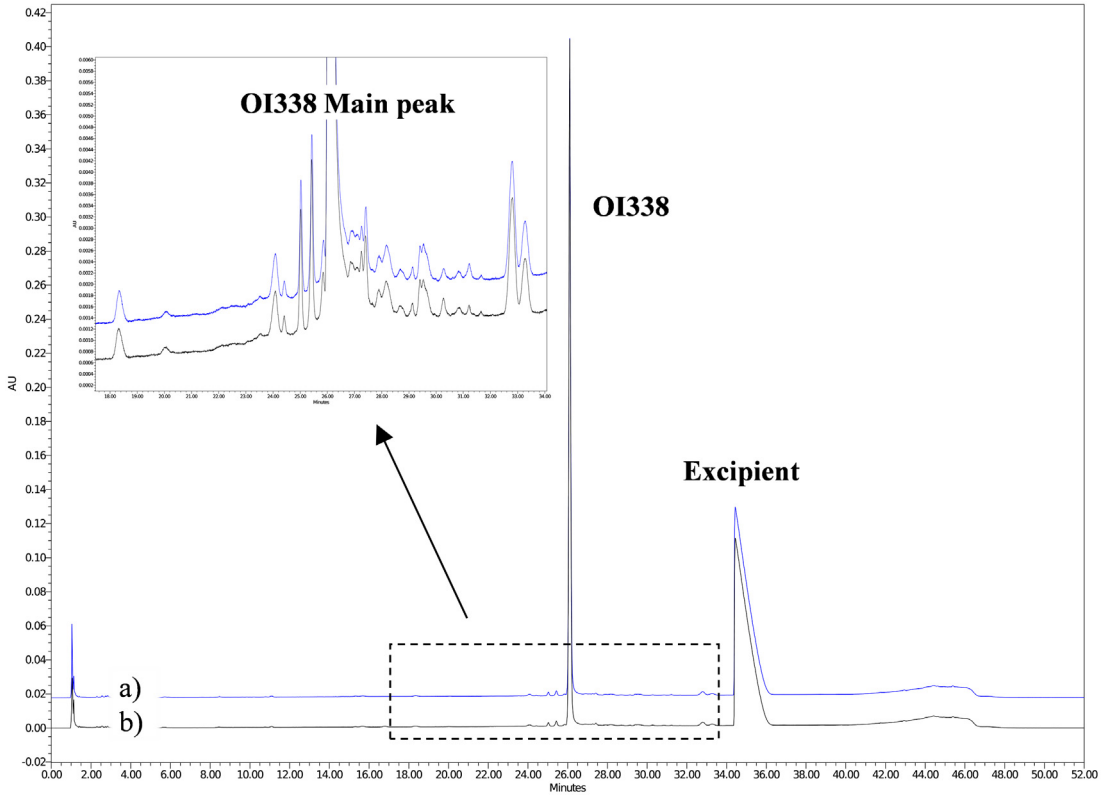

Fig. 2. Purity chromatograms of OI338 TPW and manual samples; a) manual sample, b) TPW sample.

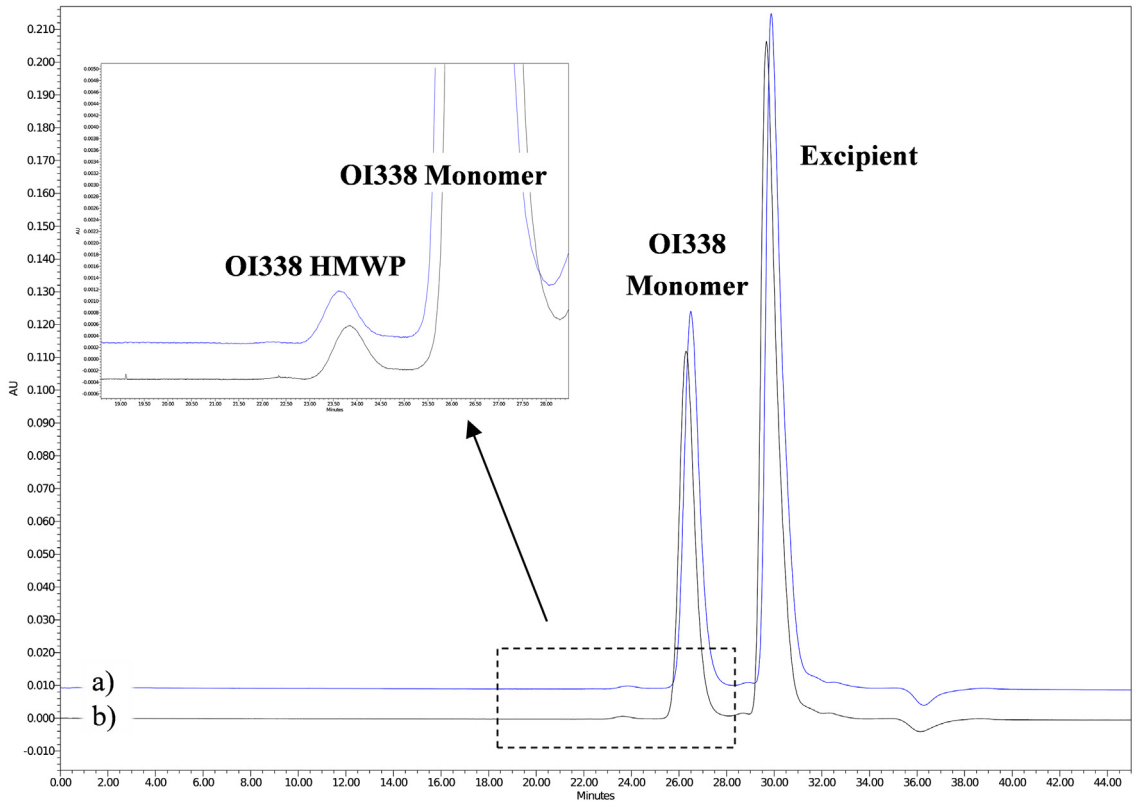

Fig. 3. High Molecular Weight Protein (HMWP) chromatograms of OI338 TPW and manual samples; a) manual sample, b) TPW sample.

Productivity gain

The workflow of manual and automated content uniformity sample preparation of ten OI338 samples is shown in Table 4. Analytical activities in which are identical for both methods have been exempt in the assessment, i.e., media and reference standard preparations, UHPLC analysis and data processing. A scientist conducting the manual sample preparation can simultaneously handle ten samples and spends 70 min in total laboratory time in addition to sample attention during the magnetic stirring. To conduct the automated TPW sample preparation of the identical number of samples, the total scientist time required is 20 min. Hence, the scientist uses approximate a third of the time for manual preparation. This liberates 50 min of labor (a 71% reduction).

It is important to highlight that the total analysis cycle time is, in fact, increased by virtue of the TPW's ability to only process one sample at a time using 50 min per replicate. The cleaning step of the TPW method is the most time-consuming segment of the method (34 min per sample), which shows a great potential for further optimization to obtain a shorter preparation method. Despite the longer analysis cycle time, analyses could be queued up overnight on the TPW for continuous operation, sample vials were stored in a cooled sample manager until the next day. Chemical stability of OI338 in the filtered sample solution has been determined to 48 h in vials kept at 10 ^{0}C (previous internal data from the manual method validation). In this regard, the TPW is equipped to allow for connectivity to online UHPLC for further automation and analysis cycle time reduction. Furthermore, analytical scientists experience a net gain in overall productivity by enabling time for other tasks. Additional indirect time gains correlate to reductions in human-related errors or false sample results, and the use of electronic audit trails facilitating method technology transfer. Finally, and importantly, work safety is enhanced by minimized human exposure to chemicals and potent APIs.

Table 4
Analytical scientist activity workflow and duration of manual and automated TPW content uniformity sample preparation of ten OI338 solid dosage forms. In manual method ten samples are handled simultaneously, the TPW method handles one sample at the time.

Manual sample preparation		TPW automated sample preparation		
Activity	Scientist time (min) N = 10	Activity	TPW time (min) N = 10, [N = 1]	Scientist time (min)
Laboratory setup (glassware, dispense reagent)	20	TPW setup (load reagents, samples and disposables; start software)	NA	20
		Automated sample and reagent handling	60 [6]	NA
Sample disintegration (magnetic stirring 120 min)	NA	Automated sample homogenization	60 [6]	NA
Sample work-up (dilute to mark, filtering, pipette to vial)	30	Automated sample work-up (filtering, vial transfer)	40 [4]	NA
Cleaning	20	Automated cleaning	340 [34]	NA
Total time for 10 samples (min)	70		500 [50]	20[a]

[a] Total scientist time for 1 to 10 TPW samples are approximately identical.NA: not applicable

Conclusions

Over the past years, there has been an evident rise of laboratory automation within the pharmaceutical industry. Sample preparation represents a time consuming, routinely performed laboratory procedure that can readily be superseded by robotics. In this work we have evaluated the use of a TPW automated method for OI338 solid dosage form content uniformity sample preparation. To the best of our knowledge, this is the first reported application of the TPW to biological macromolecule solid dosage forms.

The described analysis, such as content uniformity, recovery demonstrated method equivalency of TPW versus manual sample preparation method. Furthermore, the purity and HMWP results confirmed intact protein structure upon homogenization during the automated workflow, when compared to manual magnetic stirring. Future method optimization should focus on TPW method time reduction by means of further method parameter screening and refinements, such as cleaning. The established automated procedure significantly reduces scientist time spent on sample preparation, consequently enhancing laboratory safety. Overall, the TPW is an applicable and valuable instrument for biological macromolecule analysis in the pharmaceutical research environment and beyond.

Declaration of Competing Interests

IF and VA are both employees of Novo Nordisk. The authors declare no financial interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.slast.2023.02.006.

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