Automation and Optimization of Sample Preparation of Levothyroxine Sodium Tablets for Drug-Content Analysis by Online HPLC

Application Note

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Abstract

A manual sample preparation workflow for the analysis of drug-content in Levothyroxine Sodium Tablets (0.025 mg) was automated and the extraction times were optimized using the accroma® samplePrep system. The goal was to reduce extraction and overall sample preparation hands-on time without compromising recovery. Levothyroxine drug contents were determined using online HPLC (interfaced to accroma®) to calculate recoveries. 20 Levothyroxine Sodium tablets were prepared manually via ultrasound & intermitted shaking and compared to automated sample preparation workflows of different milling- and extraction times. Grinding and extraction were performed by vertical shaking in the accroma®. The extraction times could be reduced from 30 min manually to 3 min on the accroma ® system while reaching 100% recovery. This corresponds to a reduction of 90% in extraction time. Since shorter milling and extraction times were not tested, the extraction time could be potentially shortened even more. The overall hands-on time could be reduced from 30 min (manually) to 5 min with the accroma® without counting documentation time.

Introduction

The key attributes in the quality control of Oral Solid Dosage Forms (OSDs) are identity, strength, and purity. [1] Active Pharmaceutical Ingredients (API), degradants, excipients and impurities are identified and quantified with chemical analysis assays which require sample preparation. This process is often done manually, which is highly inefficient, expensive, and prone to errors. In chromatography, sample preparation requires 61% of lab technicians time, accounts for a minimum of 30% of all errors and adds up to more than 50% of the total lab costs. [2] As documentation is done manually, full traceability is difficult to achieve. Therefore, the automation of sample preparation workflows is a prerequisite for lean laboratory programs.

Experimental

Chemicals and reagents

- Levothyroxine Sodium Tablets (0.025mg) from batch N22HA18001
- Working standard Levothyroxine (purity 90.3%)
- Water, HPLC grade
- Acetonitrile, HPLC grade
- Methanol, HPLC grade
- Orthophosphoric acid 85%
- Phosphate buffer solution (pH 6.5)
- Extraction solvent (diluent)
  - Methanol (600 parts)
  - Water (400 parts)
  - Orthophosphoric acid 85% (0.5 parts)

Instrumentation

accroma® samplePrep system

- Analytical balance Mettler-Toledo WMS 404C-L/11
- Shaker module
- Liquid- & Filtration module
- Agilent Online analysis interface
- accroLab software

Agilent 1260 Infinity II HPLC (400 bar)

- 1260 Infinity II Quat Pump VL (G7111A)
- 1260 Infinity II Vialsampler (G7129A) with Integrated Column Compartment and External Tray
- 1260 Infinity II Variable Wavelength Detector (G1314F)
- Chromatographic Data System (CDS): Empower 3

Procedure

Chromatography

Parameter	Value
Mobile Phase A	Buffer/Acetonitrile 70:30
Mobile Phase B	Buffer/Acetonitrile 50:50
Flow Rate	1.5 ml/min
Injection Volume	50 µl
Column Temperature	40◦C
UV Wavelength	225 nm
Gradient	**Time** **Min** **%A** **%B** 0.01 95 5 4.40 80 20 4.50 10 90 5.60 10 90 5.70 95 5 8.00 95 5
Column	Zorbax Extend C18, (150 x 4.6) mm, 5µm

Duplicates were performed for every sample and working standard was injected between the experiments.

Standard preparation

26.2mg of working standard (Purity: 90.3%) was accurately weighed and transferred into a 100ml volumetric flask. Methanol was added to the mark and sonication was performed until complete dissolution. 1ml of the solution was then transferred into a 50ml volumetric flask and the volume was filled to the mark with diluent.

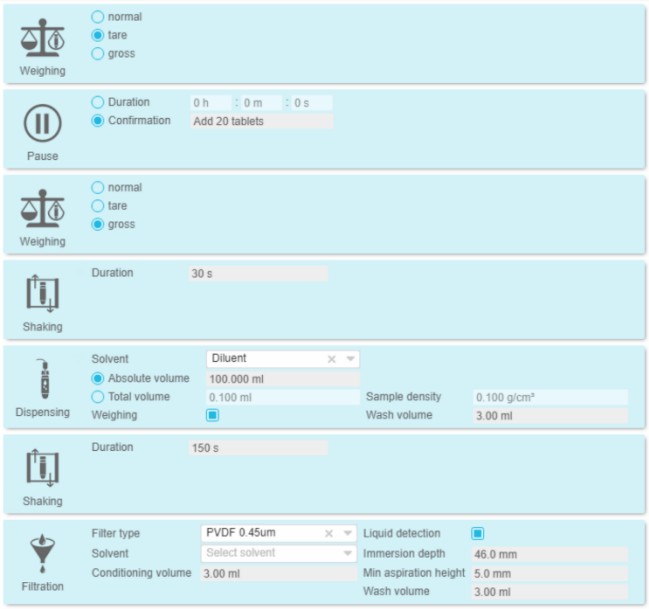
Manual sample preparation method

20 Levothyroxine Sodium tablets (0.025mg) were randomly selected. The tablets were accurately weighed and transferred into a 100ml volumetric flask and 100ml of diluent was added to reach a concentration of 5.0 µ g/ml. The flask was sonicated for 30 minutes with intermittent shaking every 5 minutes. The suspension was then filtered through a 0.45µ PVDF syringe filter discarding the first 3ml of filtrate.

Automated samplePrep method

Compared to the extraction in the manual sample preparation, the samples were first ground. The extraction was performed by vertical shaking of the accroTubes.

Two steel balls (22mm) were added to an accroTube. The closed accroTube was placed into the accroma® system. After starting the workflow, the accroTube was tared automatically. Subsequently, 20 randomly selected Levothyroxine Sodium tablets (0.025 mg) were loaded into the accroTube, placed back into the accroma® system and the workflow was continued.



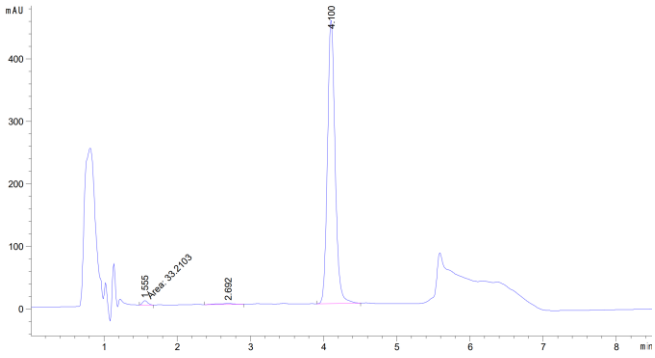
Workflow 4 on the accroma® samplePrep system. Download the workflow under: https://expert.accroma.com/

Four workflow experiments with different milling & extraction times were performed.

Experiment	Milling	Extraction	Total
Workflow 1	5 min	10 min	15 min
Workflow 2	1 min	5 min	6 min
Workflow 3	1 min	4 min	5 min
Workflow 4	0.5 min	2.5 min	3 min

Results and discussion

The acquired chromatograms of the sample solutions showed peaks between 3.0 and 4.5 minutes. No non-expected peaks were identified comparing the working standard and the samples. Therefore, it was assumed that no degradation was taking place during the milling and extraction process.



Chromatogram of sample solution prepared by workflow 4

A full comparison of the measured concentrations and the calculated recoveries of manual and automated sample preparations are shown in the following table:

Experiment	Concentration	Recovery
Working standard	4.70 µg/ml	-
Manual	4.77 µg/ml	100%
Workflow 1	4.84 µg/ml	102%
Workflow 2	4.89 µg/ml	103%
Workflow 3	4.95 µg/ml	104%
Workflow 4	4.83 µg/ml	100%

Whereas the recoveries were slightly higher for the workflows 1-3, the recovery of workflow 4 was measured to be 100%. Possible reasons for obtaining higher recoveries than 100% could be:

- The recovery of manual sample preparation is lower than 100%
- Non uniformity of content of tablets
- Variety of HPLC analysis

The root cause of the higher recoveries was not investigated further.

Conclusion

Extraction times by the accroma® samplePrep system were shown to be significantly shorter than by the manual method of ultrasonication.

Parameter	Manual	accroma	Improvement
Recovery	100%	100%	-
Extraction time	30 min	3 min	90% reduction
Hands-on time	30 min	5 min	83% reduction

The accroma® system proved to be suitable for the automated sample preparation of 20 Levothyroxine tablets (0.025 mg) for assay test. The extraction time could be reduced by 90%. The overall sample preparation hands-on time could be reduced by 83% as the accroma® system can automate sample weighing, pipetting, extraction, filtration and transferring the vial into the HPLC. The system crosschecks every added volume gravimetrically which increases traceability and accuracy. It documents every possible parameter and step of the process. In conclusion, lab efficiency and compliance are significantly improved by using the accroma® samplePrep system.

References

[1] Görög S., Drug Safety, drug quality, drug analysis **2008**, Journal of Pharmaceutical and Biomedical Analysis, Vol. 48, Issue 2.

[2] Majors R. e., Sample preparation – Fundamentals for chromatography, p. 4 (**2013**)

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Printed in Switzerland, Juli 2021
7944-5001EN

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