# The development and validation of HPLC methods for the detection of drug and detergent traces on laboratory glassware in a pharmaceutical laboratory

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*"The LORD is my shepherd, I shall not want"*

*Psalm 23:1*

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# ABSTRACT

Pharmaceutical contract testing laboratories carry a responsibility to ensure that medicine made available for consumption by patients is of the approved quality for their intended health use. Glassware is an essential tool in testing of pharmaceutical products. Glassware used in most pharmaceutical contract testing laboratories is non-dedicated hence proper glassware cleaning procedures are essential. Contract testing laboratories need to perform glassware cleaning validation studies to verify that glassware used in the testing of medicines is adequately cleaned from one product to the next and to ensure that the cleaning procedures themselves do not contribute any unwanted residues to the glassware.

The aim of this study was to develop and validate HPLC methods for the detection of drug and detergent residues recovered from glassware in a pharmaceutical contract testing laboratory. The objectives of the study were to:

i. Develop and validate an HPLC method to detect selected glassware cleaning detergents;
ii. Investigate the efficacy of the current in-house glassware cleaning protocol (manual and automatic cleaning);
iii. Investigate the efficacy of cleaning detergents on glassware exposed to drugs;
iv. Develop an efficient glassware cleaning protocol;
v. Validate a glassware cleaning protocol for a pharmaceutical laboratory.

Cleaned laboratory volumetric flasks of varying sizes were randomly used as samples. Glassware washed with the automatic laboratory glass-washer and manually washed glassware was subjected to the rinsing and swabbing sampling procedures. A standard addition and recovery procedure was also employed to prove that the cleaning procedure works and that the glassware is indeed clean after being hand washed or automatically washed with the glassware washer.

The HPLC method was validated on an LC Agilent® 1100 DAD series system using a μBondapak $C_{18}$ (300 mm x 3.9 mm, 10 μm). Acetonitrile: buffer containing 0.02 M hexanesulfonic acid sodium salt with the pH adjusted to 3.0 with phosphoric acid in the ratio 25:75 was used as mobile phase with the flow rate set at 1.0ml/min. UV detection set at 220 nm and the injection volume at 25 μl.

The regression line plot obtained was linear over a concentration range from 5000 μg/ml to 15 000 μg/ml for Ekon D concentrate® and a concentration range from 9700 μg/ml to 39 000 μg/ml for LaboClean FT concentrate®. The correlation coefficient of 0.993 was obtained for Ekon D concentrate® and 0.999 for LaboClean FT concentrate®. The detection limit and quantitation limit were1568 μg/ml and 5228 μg/ml for Ekon D concentrate®, and 917 μg/ml and 3059 μg/ml for LaboClean FT concentrate®. The relative standard deviation (%RSD) obtained for both detergents were below 7.0%. The mean recovery of the method was 99.5%.

In the results obtained detergent traces were recovered from approximately 16% of the total sampled hand washed glassware and in 13% of the hand washed glassware, drug contaminants were also recovered. From the machine washed sampled glassware 10% was contaminated with drug residues and none of the sampled machine washed glassware flasks were contaminated with soap residues.

The HPLC method developed for the detection of detergent and drug traces recovered from laboratory glassware was a success. The automated glassware cleaning procedure was more efficient in the cleaning of laboratory glassware when compared to the manual cleaning procedure. Observation shows that the current in-house glassware cleaning protocol is efficient; however, the SOP is not followed properly. The developed HPLC method was proved to meet all the performance expectations and acceptance criteria for cleaning validation purposes. The aim of this study to develop and validate the HPLC method for the detection of drug and detergent traces recovered from laboratory glassware for a pharmaceutical contract testing laboratory was met.

# UITTREKSEL

Farmaseutiese toetslaboratoriums dra 'n verantwoordelikheid deur toe te sien dat die medisyne wat aan pasiënte beskikbaar gestel word vir 'n bepaalde siektetoestand van aanvaarbare kwaliteit is. Glasware is onontbeerlike toerusting tydens die toets van farmaseutiese produkte. In die meeste laboratoriums word glasware nie toegewys aan 'n bepaalde analitiese proses nie en dit is dus noodsaaklik dat die skoonmaakproses van hoogstaande gehalte is. Dit is dus nodig dat kontraklaboratoriums die skoonmaakprosesse wat gebruik om glasware mee te was sal valideer om sodoende te verseker dat die produk waarvoor dit gebruik was, behoorlik verwyder is. Verder is dit ook noodsaaklik dat vasgestel word dat die skoonmaakmiddels nie bydra tot onnodige residue op die glas nie.

Die doelstellings vir hierdie studie was om Hoë Druk Vloeistof Chromatografie (HPLC) metodes te ontwikkel en te valideer waarmee geneesmiddel- en skoonmaakmiddelresidue wat moontlik in glasware agtergelaat kon word, te kan analiseer. Die doelwitte van die studie was dus om:

i. 'n HPLC metode te ontwikkel en te valideer waarmee sekere skoonmaakmiddels op glasware geanaliseer kon word:
ii. Die effektiwiteit van die skoonmaakproses in die kontraklaboratorium (handwas en outomaties) vas te stel;
iii. Die effektiwiteit van die skoonmaakmiddels om geneesmiddels van glasware te verwyder;
iv. 'n Effektiewe skoonmaakproses te ontwikkel indien nodig, en
v. Die validering van die skoonmaak protokol.

Skoon volumetriese flesse in verskillende groottes is ewekansig gekies vir die toetse. Beide glasware wat met die hand gewas is en wat met die outomatiese wasser gewas is, is met gedistilleerde water gespoel en/of smere met katoenstokkies is geneem, om monsters vir analises te bekom. 'n Standaard herwinningsprosedure is ook gebruik om te verseker dat die glasware inderdaad skoon was nadat dit gewas is.

Die HPLC metode is op 'n LC Agilent® 1100 DAD sisteem ontwikkel en valideer. 'n µBondapak $C_{18}$ (300 mm x 3.9 mm, 10 µm) kolom is gebruik. Asetonitriel en 0.02M heksaansulfoonsuur buffer met 'n pH van 3.0 (aangepas met fosforsuur) in 'n verhouding 25:75 is as mobiele fase gebruik. Die vloeisnelheid was 1.0ml/min en UV deteksie by 220nm is gebruik. Die inspuitvolume was 25 µl.

Tydens die validasie van die analitiese metode vir die skoonmaakmiddels is die volgende resultate verkry. Vir Ekon D concentrate® is 'n lyn in die konsentrasiegebied 5000 µg/ml tot 15 000 µg/ml opgestel en vir LaboClean FT concentrate® 9700 µg/ml to 39 000 µg/ml. Die korrelasie koëffisiënt vir Ekon D concentrate® was 0.993 en vir LaboClean FT concentrate® was dit 0.999. Die deteksielimiete was onderskeidelik 1568 µg/ml en 917 µg/ml en die kwanitifeseringslimiete was onderskeidelik 5225 µg/ml en 3059 µg/ml. The persentasie relatiewe standaard afwyking (%RSD) vir beide middels was laer as 7.0%. Die gemiddelde herwinning van die metode was 99.5%.

Die resultate wat verkry is dui daarop dat ongeveer 16% van die totale aantal monsters vir glasware wat met die hand gewas is, steeds seep residue bevat het en 13% daarvan steeds geneesmiddel residue bevat het. Vir glasware wat met die wasser gewas is, was daar 10% wat steeds geneesmiddel residue bevat het en geen daarvan het seep residue bevat nie.

Die HPLC metode wat vir die studie ontwikkel is kon suksesvol gebruik word om die analises van die monsters te doen. Die metode is suksesvol gevalideer en dit het aan al die kriteria voldoen. Die outomatiese wasser is meer effektief om glasware skoon te was. Tydens obeervasie is vasgestel dat die handwas metode ook meer effektief sal wees indien die voorgestelde prosedure noukeuriger gevolg word. Die doelwitte van hierdie studie is dus suksesvol behaal.

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# ABBREVIATIONS

| | |
|---|---|
| **API** | Active pharmaceutical ingredient |
| **cGMP** | Current good manufacturing practices |
| **FDA** | The Food and Drug Administration, USA |
| **GLP** | Good laboratory practices |
| **GMP** | Good manufacturing practices |
| **HPLC** | High performance liquid chromatography |
| **HSA** | Hexane sulphonic acid |
| **LC** | Liquid chromatography |
| **MS** | Mass spectrometry |
| **LOD** | Limit of detection |
| **LOQ** | Limit of quantitation |
| **PDA** | Photodiode array |
| **% RSD** | Percentage relative standard deviation |
| **SOP** | Standard operating procedure |
| **UFLC** | Ultra fast liquid chromatography |
| **UPLC** | Ultra pressure liquid chromatography |
| **USP** | United States Pharmacopoeia |
| **UV/Vis** | Ultraviolet/visible |

# AIM AND OBJECTIVES

## AIM

The aim of the study was to develop and validate HPLC methods for the detection of drug and detergent traces recovered from laboratory glassware in a pharmaceutical contract testing laboratory.

## BACKGROUND

Pharmaceutical contract testing laboratories carry a responsibility to ensure that components, containers and closures, in-process materials, and finished drug products conform to the established standards and specifications (FDA, 2006). Data generated by contract testing laboratory is expected to be meaningful, thorough, timely, unbiased, well documented, and scientifically sound as it ensures quality control and compliance by pharmaceutical manufacturing companies (Ahuja, 2005).

Volumetric glassware forms part of nearly all critical analytical experiments in contract testing laboratories. Contamination of glassware may therefore compromise the reliability of the generated data. Unreliable data violates cGMP regulations that clearly mandate the reliability and accuracy of data generated in contract testing laboratories. Limited information is available regarding the chemical composition of laboratory detergents. This issue is caused by competition amongst the detergent suppliers and the need to protect detergent formulations from being copied. The detergents efficacy on glassware exposed to drugs is a matter based on the user's discretion in cases where user instructions are not specified by the supplier.

Research is not clear in describing protocols relating to laboratory glassware care and maintenance in pharmaceutical environments. This puts contract testing laboratories at a risk of using laboratory glassware that might not be properly cleaned for generating valuable data.

## OBJECTIVES

To achieve the aim of the study the following objectives were pursued:

1. Develop and validate an HPLC method to detect selected glassware cleaning detergents;
2. Investigate the efficacy of the current in-house glassware cleaning protocol;
3. Investigate the efficacy of cleaning detergents on glassware exposed to drugs;
4. Develop an efficient glassware cleaning protocol;
5. Validate a glassware cleaning protocol for a pharmaceutical laboratory.