

Replacing Glass with Plastic in Analytics: Comparison of UPLC Spectra of Samples Processed in Glass and Eppendorf Conical Tubes 25 mL

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Abstract

Highly sensitive analytical workflows like GC, HPLC or MS are prone to negative influence of leaching and glassware remains a mainstay in most analytical methods. Glassware has however its natural drawbacks and the need to replace it by high-quality, leaching-free plastic consumables is high.

In this Application Note, high-quality polypropylene tubes (Eppendorf Conical Tubes 25 mL) were used to substitute glass vessels in routine UPLC-UV analysis of serum 25OHD2 and 25OHD3 levels. The results indicate that comparable UPLC-UV separation and elution peaks were detected when using Eppendorf Conical Tubes 25 mL as compared to standard borosilicate glass tubes. No interfering peaks from leachables, even at very low noise values, were observed.

Substituting glassware with polypropylene tubes in the described liquid chromatography workflow has saved time, reduced contamination risk (cleaning process) and reduced waste and hazard risk without compromising the chromatography quality.



Introduction

Materials commonly used in medical and life-science lab consumables are typically a blend of base polymers with a broad and heterogeneous range of chemicals added during production. These substances facilitate or accelerate the processability of the polymer, prolong its long-term stability or enhance its performance in the end product in various ways. Increasing scientific evidence indicates however, that substantial part of those processing additives may be released (leach) into samples and significantly affect

experiments. Ultimately, they may pose a likely and largely underestimated source of error in analytical and life-science assay systems [1, 2]. Highly sensitive analytical workflows like GC, HPLC or MS are particularly prone to negative influence of leaching and glassware remains a mainstay as golden standard in most analytical methods. Glassware has however its natural drawbacks (low mechanical stability, cost, safety and cleaning issues) and the need to replace it by comparably leaching-free plastic consumables is high.

In this Application Note high-quality polypropylene tubes (Eppendorf Conical Tubes 25 mL) were used to substitute glass vessels in standard analytical workflow: UPLC-UV measurement and routine analysis of serum 25OHD2 and 25OHD3 levels [3]. No interfering peaks from typical

polypropylene leachables were observed. Substituting glassware with polypropylene tubes allowed substantial time saving, reduced waste and hazard risk without compromising the chromatography quality.

Material and Methods

UPLC analysis of 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 in human serum

For detailed method description refer to: Chin SF, Osman J, Jamal R. Simultaneous determination of 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 in human serum by ultra-performance liquid chromatography: An economical and validated method with bovine serum albumin. *Clinica Chimica Acta*. October 2018; 485: 60-66.

In brief: simultaneous determination of human serum 25-hydroxyvitamin D2 (25OHD2) and 25-hydroxyvitamin D3 (25OHD3) was performed using Ultra Performance Liquid Chromatography (UPLC). Non-human matrix of 4% BSA was used to construct the calibration curve and in quality control samples' preparation to avoid interference of the endogenous 25-hydroxyvitamin D (25OHD) present in the human serum. 25OHD2, 25OHD3 and dodecanophenone (internal standard, IS) were separated on a CORTECS solid-core particle column and monitored by photodiode array detector at wavelength of 265 nm within five min run time.

The relationship between 25OHD concentration and peak area ratio (25OHD:IS) was linear over the range of 12.5 – 200 nM with mean correlation coefficients (r^2) > 0.998.

The limit of detection (LOD) for 25OHD2 and 25OHD3 was 3.00 nM and 3.79 nM, while the lower limit of quantification (LLOQ) was 9.11 nM and 11.48 nM, respectively. High repeatability was obtained for both isomers with intra-day CV% < 5.6% and < 5.3% for inter-day assay. This method was further tested with a commercial lyophilized serum control with an accuracy of 92.87 – 108.31% and applied on 214 human serum samples. In summary, this validated method with BSA can be reliably applied for routine quantification of 25OHD in adults.

Results and Discussion

The results indicate that comparable UPLC-UV separation and elution peaks were detected when using Eppendorf Conical Tubes 25 mL as compared to standard borosilicate glass tubes. No interfering peaks from leachables, even at very low noise values, were observed. Substituting

glassware with polypropylene Tubes in the described liquid chromatography workflow has saved time and reduced waste and hazard risk without compromising the chromatography quality.

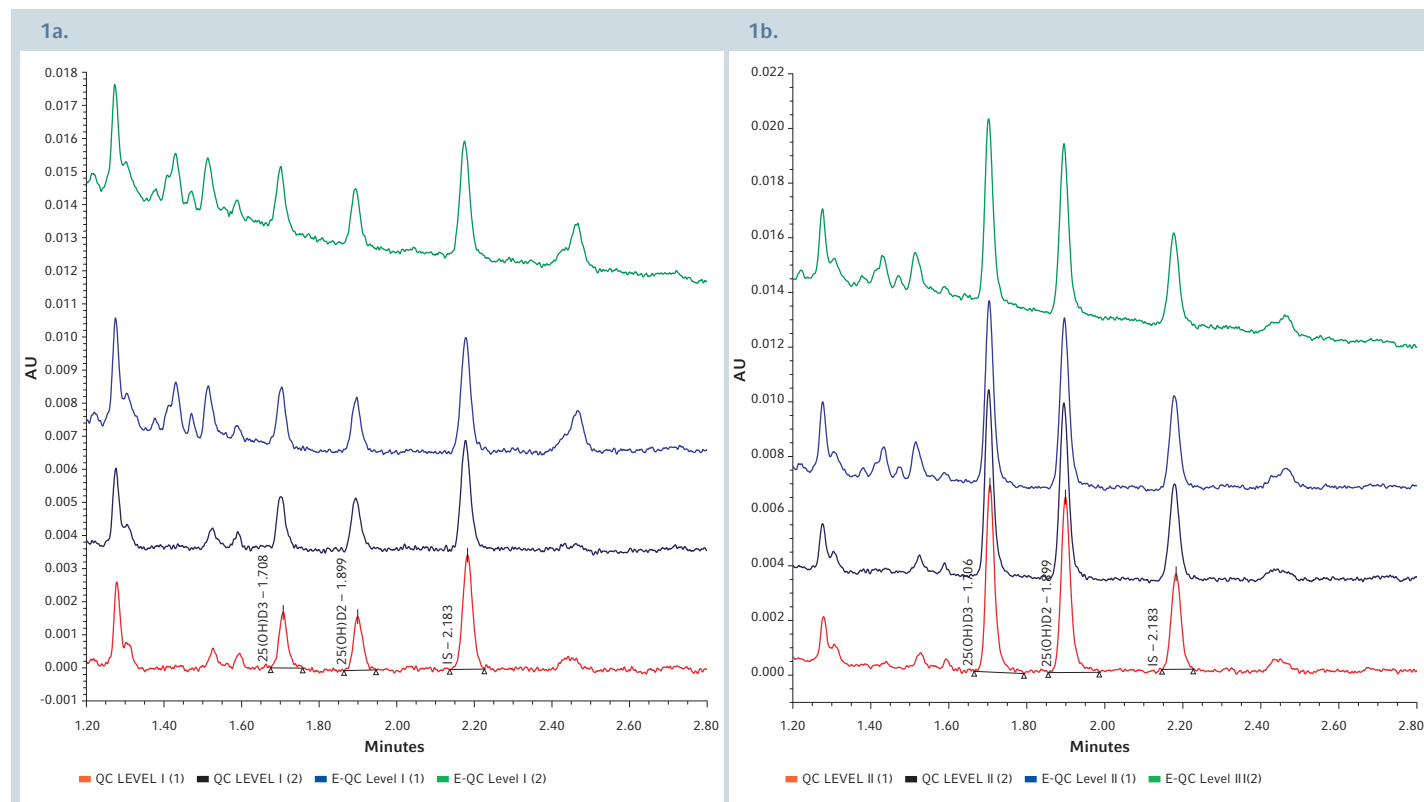


Figure 1: UPLC-UV spectra of 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 in human serum obtained using borosilicate glass (QC, red and black curves) and polypropylene Eppendorf Conical Tubes 25 mL (E-QC blue and green curves).

Two levels of detection are shown: **Fig. 1a:** low (Level I) and **Fig. 1b:** high (Level II).

Each acquisition was performed in duplicates (spectra 1 and 2).

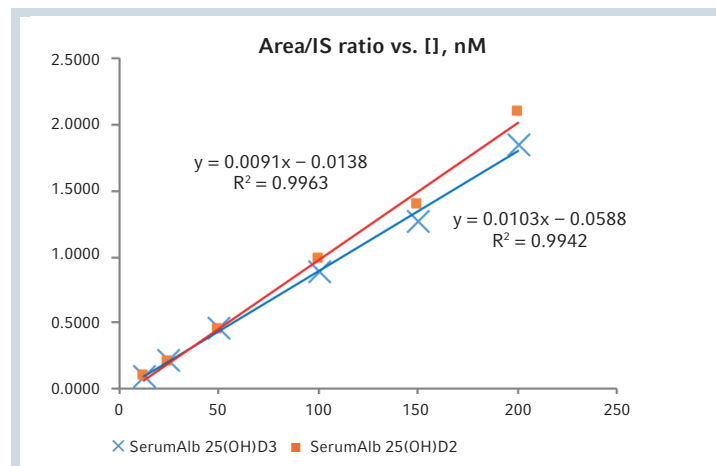


Figure 2: Correlation of peak-area ratios of 25OHD3 and 25OHD2, respectively to Dodecanophenone (IS) with 25OHD concentrations. The six-point calibration curves were linear over the range of 12.5 – 200 nM with mean correlation coefficients (r^2) > 0.994. All QC samples with known concentration were quantified against the calibration curve to assess the validity of the experiment.

Conclusions

In this Application Note the high-quality polypropylene tubes (Eppendorf Conical Tubes 25 mL) were used to substitute glass tubes in standard analytical workflow: routine UPLC-UV analysis of serum 25OHD2 and 25OHD3 levels. Comparable UPLC separation and elution peaks were detected when using Eppendorf Conical Tubes 25 mL as compared to standard borosilicate glass tubes.

No interfering peaks from possible UV-absorbing leachables, even at very low noise values, were observed. Substituting glassware with polypropylene Tubes in the described liquid chromatography workflow has saved time (glassware cleaning), reduced waste and hazard risk without compromising the chromatography quality.

Literature

- [1] Schauer KL, et. al. Mass Spectrometry Contamination from Tinuvin 770, a Common Additive in Laboratory Plastics. Journal of Biomolecular Techniques July 2013; 24(2):57-61
- [2] Grzeskowiak R, Gerke N. Leachables: Minimizing the Influence of Plastic Consumables on the Laboratory Workflows. White Paper 026; www.eppendorf.com
- [3] Chin SF, Osman J, Jamal R. Simultaneous determination of 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 in human serum by ultra-performance liquid chromatography: An economical and validated method with bovine serum albumin. Clinica Chimica Acta October 2018; 485: 60-66

Ordering information

Description	Order no.
Eppendorf Conical Tubes 25 mL with snap cap, Eppendorf Quality™, colorless, 200 tubes, (5 bags x 40 tubes)	0030 118 405
Eppendorf Conical Tubes 25 mL with snap cap, PCR clean, colorless, 200 tubes, (5 bags x 40 tubes)	0030 118 413
Eppendorf Conical Tubes 25 mL with snap cap, sterile, pyrogen-, DNase-, RNase-, human and bacterial DNA-free, colorless, 150 tubes, (6 bags x 25 tubes)	0030 118 421
Eppendorf Conical Tubes 25 mL with screw cap, Eppendorf Quality™, colorless, 200 tubes, (4 bags x 50 tubes)	0030 122 410
Eppendorf Conical Tubes 25 mL with screw cap, PCR clean, colorless, 200 tubes, (4 bags x 50 tubes)	0030 122 429
Eppendorf Conical Tubes 25 mL with screw cap, sterile, pyrogen-, DNase-, RNase-, human and bacterial DNA-free, colorless, 200 tubes, (8 bags x 25 tubes)	0030 122 437

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