Streamlining Sample Preparation with Second Generation Enzymes

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Abstract

Analyte recovery and column lifetime was studied for enzymatic hydrolysis of seven different glucuronide conjugates using first and second generation enzymes (DR2102, BG100® and BGTurbo™). Glucuronide-free analyte recovery was 15 and 16 % higher for BGTurbo™ and BG100®, respectively, than for DR2102. Also, BGTurbo™ and BG100® showed significantly lower remaining protein in the samples to be loaded into the column, indirectly improving column lifetime. These new enzymes can significantly improve sample preparation methods used for drug analysis.

Introduction

Even though enzymatic hydrolysis is the optimum approach used for sample preparation in analytical toxicology (Fu et al., 2010), some drawbacks of this technique include long incubation times, poor analyte recovery and short column lifetime due to high concentration of proteins loaded onto the HPLC column. We tested a first generation beta-glucuronidase currently in use in our laboratory (DR2102 from Campbell Scientific) against a beta-glucuronidase also isolated from *Haliotis rufescens* (BG100® from Kura Biotec) and a second generation enzyme (BGTurbo™ also from Kura Biotec), assessing final analyte recovery and total protein concentration loaded onto HPLC column. The aim of this work was to compare hydrolysis efficiency and potential column lifetime of first and second generation beta-glucuronidase enzymes used for analyte quantification.

Method & Materials

Blank urine was fortified with 300 ng of conjugated buprenorphine, codeine, hydromorphone, lorazepam, morphine, oxymorphone and THC-COOH glucuronides. The sample hydrolysis was performed, in triplicate, as follows: 50 µL of fortified urine, 25 µL of buffer, 20 µl of the internal standard drugs and 10 µL of DR2102 and BG100® enzymes or 20 µL of BGTurbo™ enzyme. Incubation was carried out for 60 minutes at 62 °C for samples hydrolyzed with DR2102; 30 minutes at 68 °C for BG100®; and 15 minutes at 50 °C for BGTurbo™.

Samples were then centrifuged at 24,000 rpm to lower protein content, diluted with 400 µL for DR2102 and BG100 or 380 µL for BG Turbo (95% water; 5% methanol) and loaded onto LC column (Phenomenex®) for analyte quantification. Total protein in the samples before and after centrifugation was measured in triplicate by absorption at 280 nm with NanoDrop™ (Thermo Fischer Scientific).

Results & Discussion

Glucuronide-free analyte recovery values are shown in Table 1. Besides reducing the sample incubation time with second generation enzymes, average analyte recovery was also improved by 16 % in half the incubation time for BG100® and 15 % in one fourth of the incubation time for BGTurbo™ (figure 1), when compared to hydrolysis conditions with DR2102. BG100® was the best enzyme for buprenorphine and oxymorphone recovery, while BGTurbo™ was particularly more reactive to codeine than to any other analyte (table 1).

Table 1. Analyte recovery obtained for the beta-glucuronidases tested.

	Analyte recovery (ng/mL)		
Analyte	DR2102	BG100®	BGTurbo™
Buprenorphine	223.1	271.1	224.4
Codeine	159.4	173.8	243.2
Hydromorphone	176.8	196.0	196.2
Lorazepam	214.9	202.5	185.9
Morphine	174.5	190.9	198.1
Oxymorphone	155.8	189.0	169.4
THC-COOH	75.7	146.0	140.6

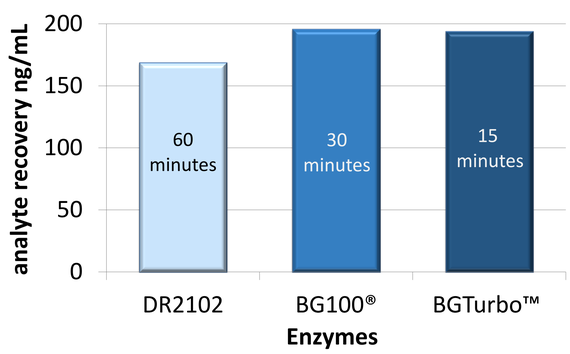

Figure 1. Average analyte recovery obtained for the beta-glucuronidases tested. Incubation time is shown inside the columns.

The amount of protein remaining in the samples after centrifugation was decreased by 61 % for hydrolysis with BG100® and 81 % for BGTurbo™ compared with DR2102 (figure2). Also, the protein concentration in the samples treated with BG100® and BGTurbo™ was lower before the centrifugation than samples treated with DR2101 after centrifugation. This is interesting because it allows the user to skip the centrifugation step (used to lower the protein loaded into the column and prevent column lifetime shortage; Polson et al., 2003) with the new enzymes, improving even more the sample preparation setup.

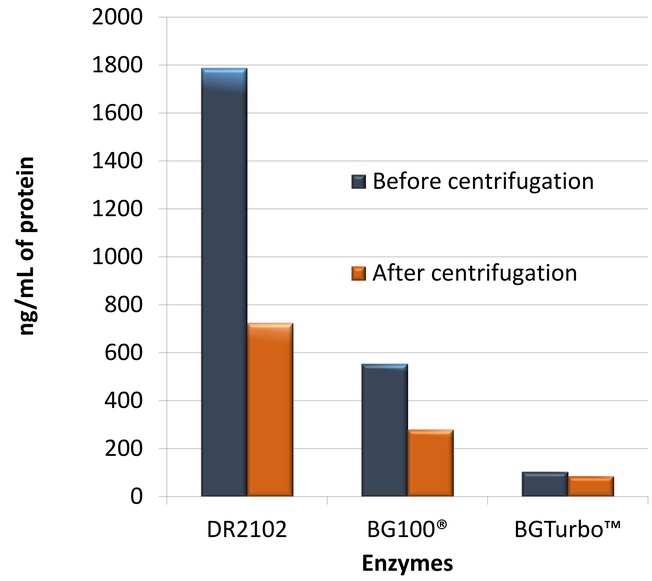

Figure 2. Total protein concentration before and after centrifugation.

Previous results of the work of A. M. Spiekerman, PhD, show that almost a 4 fold increase in column lifetime with samples treated with BG100® compared with DR2102 (>1500 vs. 400 column inyections). This longer column life could be related to the lower protein content present in the BG100® treated samples (figure 2).

Conclusion

The new enzymes BG100® and BGTurbo™ were able to:

- Reduce up to 4 times sample preparation.
- Improve overall analyte recovery by up to 16 %.
- Decrease total protein concentration of samples up to 88 %.

All these improvements will help directly to extend column lifetime, as well as to improve both sample preparation efficiency and precision of the results obtained in analytical toxicology laboratories. A benchmark of authentic urine patient samples will be the next step to quantify the analytical benefits and operational savings that can be derived by second generation beta-glucuronidases.

Reference

- Fu et al., 2010. Journal of Analytical Toxicology, 34:243-351.
- Polson et al,, 2003. Journal of Chromatography 785:263-275.

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