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Matrix Effects and Internal Standards for Prednisolone and Prednisone

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Abstract

The present study was to determine whether the stable isotopically labelled internal standards (SIL-IS) of prednisolone (PL) and prednisone (PN) compensated for urinary matrix effects. In order to assess any such matrix effects, post column infusion of solutions of PL, PN and their SIL-IS were used concurrently with injections of extracted urines samples of varying specific gravities (SG).
Observation of signal intensities for transitions of each analyte were monitored and ratios of the intensities were plotted over the entire run time to determine matrix effects. A constant ratio over the total run time would indicate no matrix effects, while shifts in the ratio either up or down would indicate analyte suppression or enhancement. Results indicated that samples with low SG showed negligible matrix effects, while those samples with higher SG were observed to have marked matrix effects that were not compensated for by the use of SIL-IS.

Introduction

Urine as a matrix consists of many components other than the analytes of interest which can affect the results of an analysis. Due to component similarities in behaviour during extraction and analysis, suppression or enhancement is a known effect for electrospray ionisation.
Controls can be incorporated into analytical procedures to account for matrix effects. The best practice generally involves using a SIL-IS of the analyte itself [1].The analyte and IS should be as similar as possible given that matrix effects are related to chemical structure. Analyte and IS pairs that are not similar may be differently affected by the matrix, causing variability in the response ratio [2]. A SIL-IS provides the highest correlation possible to the analyte co-eluting with it and it is thereby, in theory, expected to compensate for matrix effects.

Experimental

Samples of varying specific gravities (SG) were extracted using a liquid-liquid extraction (LLE) method. The procedure involved adding 1.5 mL phosphate buffer (0.2 M) and 40 µL β-glucuronidase enzyme to 2.5 mL of urine. Samples were incubated at 50°C for 30 minutes, cooled to room temperature and adjusted to pH 9.6 with carbonate buffer. 5 mL tertiary-butyl methyl ether (TBME) was added to samples that were then mixed for 20 minutes. The organic layer was evaporated to dryness and reconstituted with 200 µL of 10% methanol.
The LC-MS/MS system employed was a Waters Acquity Ultra Performance LC coupled to an AB Sciex Q-Trap® 5500 equipped with two different columns; Waters Acquity UPLC BEH C18 (100 mm x 1 mm, 1.7 µm) (BEH) and a Supelco Ascentis® Express F5 HPLC column (100 mm x 2.1 mm, 2.7 µm) (ASC). Mobile phases were 0.2% formic acid in water (A) and 0.2% formic acid in 90% acetonitrile (B).
Standards used to assess matrix effects were prednisolone (PL) and prednisone (PN) obtained from Sigma-Aldrich (Steinheim, Germany) whilst prednisolone-D8 (PL-D8), prednisone-D4 (PN-D4) were from Toronto Research Chemicals (Ontario, Cananda). Infused standards were made to a concentration of 1 µg/ mL.
To assess matrix effects of the method and to determine which column performed better, post-column infusion of solutions containing PL and its SIL-IS or PN and its SIL-IS were continuously infused with injections of urines samples with varying SG.

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Results and Discussion

The signal intensity for each analyte and SIL-IS was monitored and a ratio of the intensities was taken to produce plots showing the behaviour of the ratio throughout the run. Where no matrix effect is observed the ratio will remain constant, whilst the presence of either peaks or valleys represents enhancement or suppression of the analyte respectively.

In samples with low specific gravity (SG), negligible matrix effects were observed for the PL/PL-D8 ratio, despite both signals being individually affected (Figure 1). In samples with high SG, matrix effects were seen to be significantly increased, with two valleys and two peaks observed (Figure 2). The latter of the peaks occurred at the same chromatographic retention time (RT) as PL (7.5 min, marked with arrow Fig. 1 and 2), indicating that quantification would be over estimated.

These observations demonstrate that although the SIL-IS may correct for minor matrix effects experienced in low SG urine extracts, samples with higher SG are more prone to have matrix effects which are not compensated by the use of a SIL-IS. In general it was determined through screening urine samples that circa 20% had significant matrix effects which would adversely effect quantification results even with the use of a SIL-IS.

For PN, ESI- was found to provide the best results for both columns, with ESI+ generally showing more matrix effects. Despite this, 40% of the samples showed the presence of a peak immediately followed by a smaller valley on both columns. Supporting the evidence that although matrix effects are minimised in ESI-, they do occur. These peaks elute at the same RT as PN on the ASC column, and on either side of the PN RT on the BEH column with ≤ 0.4 minutes difference which could lead to erroneous quantification.

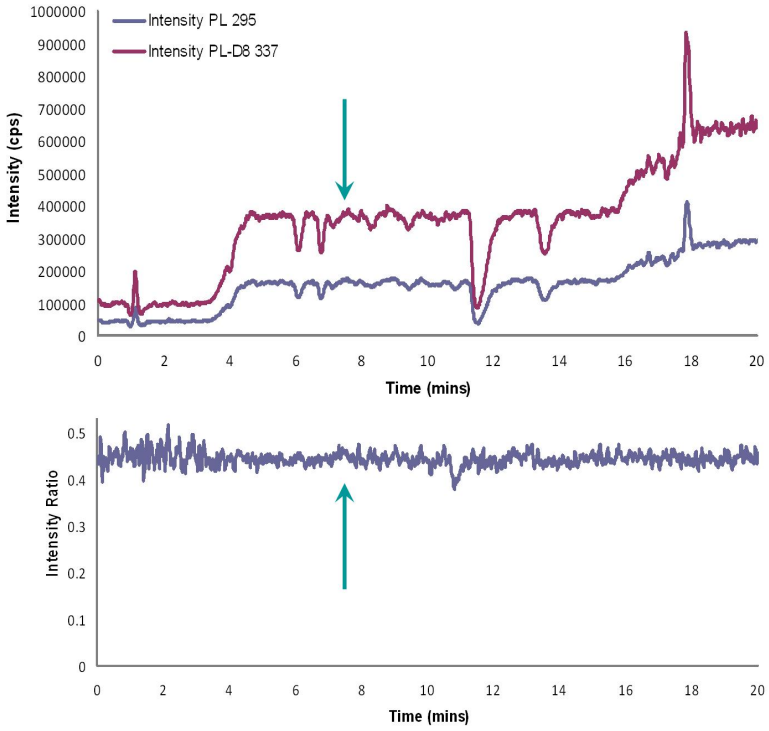

Figure 1. Blank extracted urine sample with a low SG and post column (BEH) infusion of PL and PL-D8. (A) Plot of signal intensities for precursor ion of PL m/z 295 and PL-D8 m/z 337 (B) Response ratio of PL/PL-D8. Arrows indicate retention time of PL for the chromatographic conditions used.

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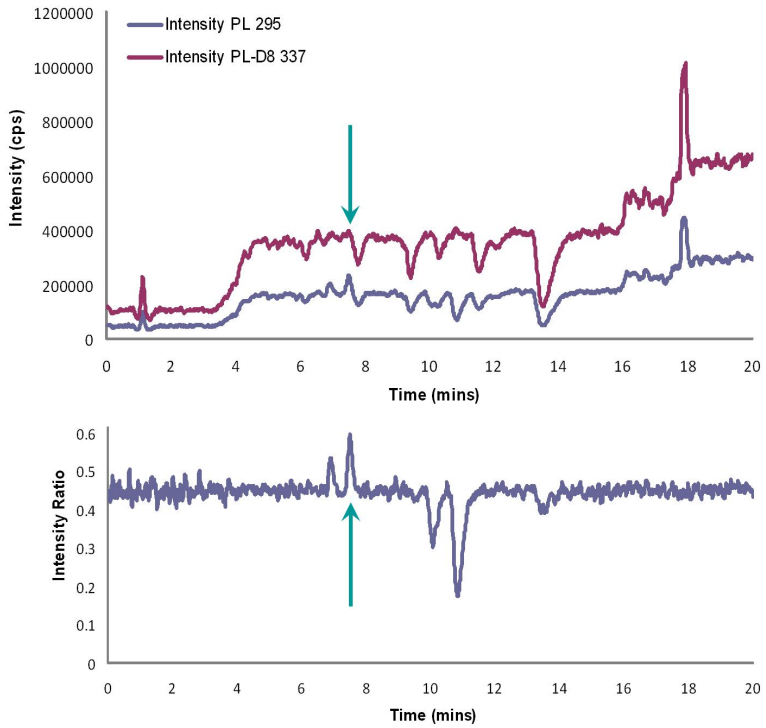

Figure 2. Blank extracted urine sample with a high SG and post column (BEH) infusion of PL and PL-D8. (A) Plot of signal intensities for precursor ion of PL m/z 295 and PL-D8 m/z 337 (B) Response ratio of PL/PL-D8. Arrows indicate retention time of PL for the chromatographic conditions used.

Conclusions

Matrix effects were assessed via post-column infusion of the PL and PN plus their SIL-IS in a selection of urine samples with varying SG. While the assumption that matrix effects are compensated for by using deuterated versions of the compound, the presence of valleys and peaks in post-column infusion experiments demonstrate that the analyte and it's SIL-IS can behave differently. So for PL and PN, their deuterated standards cannot be relied upon to provide accurate quantification in all urine samples.
A possible solution to this problem would be to include a standard addition to one aliquot of the sample in a confirmation process such that the existence of matrix effects can be detected.

References

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