# Standard line slopes as a measure of a relative matrix effect in quantitative HPLC-MS bioanalysis.

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## Standard line slopes as a measure of a relative matrix effect in quantitative HPLCâ€"MS bioanalysis

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## Abstract

A simple experimental approach for studying and identifying the relative matrix effect (for example â€œplasma-to-plasmaâ€ and/or â€œurine-to-urineâ€) in quantitative analyses by HPLCâ€"MS/MS is described. Using as a database a large number of examples of methods developed in recent years in our laboratories, the relationship between the precision of standard line slopes constructed in five different lots of a biofluid (for example plasma) and the reliability of determination of concentration of an analyte in a particular plasma lot (or subject) was examined. In addition, the precision of standard line slopes was compared when stable isotope-labeled analytes versus analogs were used as internal standards (IS). Also, in some cases, a direct comparison of standard line slopes was made when different HPLCâ€"MS interfaces (APCI versus ESI) were used for the assay of the same compound, using the same IS and the same sample preparation and

chromatographic separation conditions. In selected cases, the precision of standard line slopes in five different lots of a biofluid was compared with precision values determined five times in a single lot. The results of these studies indicated that the variability of standard line slopes in different lots of a biofluid [precision of standard line slopes expressed as coefficient of variation, CV (%)] may serve as a good indicator of a relative matrix effect and, it is suggested, this precision value should not exceed 3–4% for the method to be considered reliable and free from the relative matrix effect liability. Based on the results presented, in order to assess the relative matrix effect in bioanalytical methods, it is recommended to perform assay precision and accuracy determination *in five different lots* of a biofluid, instead of repeat ($n = 5$) analysis in the same, *single* biofluid lot, calculate standard line slopes and precision of these slopes, and to use <3–4% slope precision value as a guide for method applicability to support clinical studies. It was also demonstrated that when stable isotope-labeled analytes were used as internal standards, the precision of standard line slopes in five different lots of a biofluid was ≤2.4% irrespective of the HPLC–MS interface utilized. This clearly indicated that, in all cases studied, the use of stable isotope-labeled IS eliminated relative matrix effect. Also, the utilization of the APCI interface instead of ESI led to the elimination of the relative matrix effect in all cases studied. When the precision of standard line slope values exceeds the 3–4% limit, the method may require improvements (a more efficient chromatography, a more selective extraction, a stable isotope-labeled IS instead of an analog as an IS, and/or a change in the HPLC–MS interface) to eliminate the relative matrix effect and to improve assay selectivity.

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## Keywords

Bioanalysis; Relative matrix effect; Standard line slopes; HPLC; Tandem mass spectroscopy

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