WHAT ARE THE CAUSES OF ERRORS IN CHROMATOGRAPHIC DRUG ASSAYS?

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INTRODUCTION

Measurements of the newer antiepileptic drugs are made almost exclusively using HPLC, GC or LCMS. An exception is topiramate for which a commercial FPIA assay is also available. Analysis of the variability measurements reported by participants in the United Kingdom National External Quality Assessment Scheme (UKNEQAS) for the newer antiepileptic drugs may therefore provide general insights into the origin and cause of errors in chromatographic drug assay techniques

STUDY 1 – ANALYSIS OF WITHIN- AND BETWEEN-LABORATORY ERRORS

A freeze-dried sample prepared by spiking weighed-in mid-therapeutic concentrations of 10 newer antiepileptic drugs into drug-free serum was circulated to UKNEQAS participants on three occasions. Two samples were circulated together for analysis at the same time and the third 2 months later. Measurements reported by participants were screened to reject those >3SD from the consensus mean by the robust method of Healy (Clin Chem 1979;25:675-677). Non-rejected measurements were analysed where a laboratory had reported data for all three samples using the same analytical technique. Three components of variance were extracted 1). within-assay, 2). between-assay intra-laboratory, and 3). inter-laboratory variance from comparison between samples distributed at the same time or 2 months apart.

Components of variance (CV = coefficient of variation)

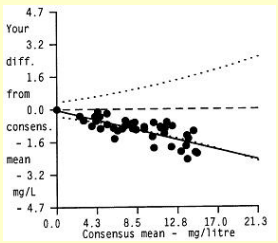
Drug (no. labs)	Within -assay CV%	Between-assay intra-laboratory CV%	Inter-laboratory CV%
Lamotrigine (59)	2.8	5.4	5.9
OH-oxcarbazepine (36)	4.8	3.2	4.1
Pregabalin (9)	1.7	8.0	3.3
Gabapentin (23)	6.8	1.5	5.8
Zonisamide (12)	3.0	7.1	5.0
Felbamate (14)	3.3	8.7	0.0
Levetiracetam (25)	6.9	6.6	0.0
Topiramate (22)	3.9	4.7	8.2
Vigabatrin (14)	4.2	9.4	6.8
Tiagabine (7)	3.8	23.5	18.0
Mean	4.1	7.8	5.7

Taken together, the two sources of within-laboratory variance constitute the greatest measurement errors being 70.5% of the total variance

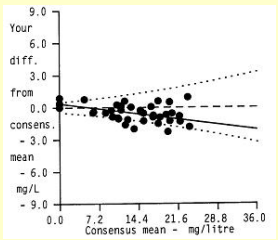
STUDY 2 – ANALYSIS OF WITHIN-LABORATORY ERRORS

Routine monthly UKNEQAS results were assessed after rejection of outliers for the 36 months from August 2003 to July 2006. Data for all laboratories were analysed by weighted linear regression versus the spike value. The residual sums of squares resulting from weighing by the variance of measurements was adjusted to unity in an iterative procedure (Wilson JF et al. Clinica Chimica Acta 1984;143:203-216) in order that residuals from individual laboratories could be assessed by comparison the the Chi Square distribution. Residuals resulting from 4 different types of error were assessed. They were additive errors or bias, slope or proportional errors, errors of curvature (none linearity), and random errors. Each of the 4 tests was performed on a total of 353 laboratory data sets using the accumulated data from the 10 drug compounds.

The 4 error tests are not independent. Notably, there were 66 occurrences where the bias and slope tests were both significant. In 59 of these cases, the intercept did not differ from zero (Figure) and the error was taken to be in slope and the resulting bias disregarded. Seven cases with non-zero intercepts were taken to show both slope and bias errors. One further bias test was ignored that resulted from a non-linearity error.



Slope error – zero intercept



Slope error with bias

Error type	Percent significant tests P <0.05
Slope	30.9% (+ve slopes 12.5%, -ve slopes 18.4%)
Random	19.8%
Bias	13.0%
Curvature	9.9%

DISCUSSION

The biggest source of variance in chromatographic measurements result from within-laboratory factors. The greatest of these derive from proportional errors in slope that typically result from co-eluting interferences with the drug being measured or with the internal standard. The next most frequent error type was in random errors followed by bias errors. There were few errors of curvature.