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Note

Comparative statistical study of assay methods using mass fragmentography and gas chromatography with nitrogen detection for determination of the tetracyclic antidepressant mianserin in human plasma

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To determine drugs or their metabolites at low levels in biological fluids, sensitive and selective assay methods are required. In general, it is assumed that mass spectrometric methods offer a high degree of specificity and sensitivity. Application of assay methods using mass spectrometric detection can, however, be hampered because not everyone has access to mass spectrometry, the equipment is costly, is susceptible to occasional break-downs, and requires in general more attention from the average laboratory technician for the method to be employed on a routine basis. For the tetracyclic antidepressant drug mianserin (Org GB 94, the lab. code, is used to denote the salt mianserin hydrochloride), a mass fragmentographic method has been described [1,2]. The assay method includes extraction, plasma extract clean-up by liquid chromatography and quantitation by gas chromatography—mass spectrometry (GC—MS). A novel assay method for mianserin [3] using gas chromatography and a nitrogen-sensitive detector (GC—NPD) applies only extraction followed by injection of the raw plasma extract into the GC—NPD system for quantitation. The merits of the simplified GC—NPD assay method with respect to the GC—MS method have been discussed [3].

The present paper deals in detail with the statistical evaluation of data obtained after application of both assay methods to plasma samples from patients receiving mianserin treatment, and to blank plasma samples spiked with mianserin.

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MATERIALS AND METHODS

Assay procedures

Mass fragmentography (GC—MS). The assay method used is in essence the same as described earlier [1,2]. The method has been slightly modified according to the assay procedure for the determination of mianserin in dog plasma [4].

Gas chromatography with nitrogen sensitive detection (GC—NPD). The assay method as described recently [3] has been used.

Samples analyzed for comparison

Spiked samples. Over a period of six months, three artificial preparations A, B and C were made by spiking human plasma free of drug (blank) with approximately 20 ng of mianserin per ml plasma.

Clinical plasma samples. Patients' plasma samples from a clinical mianserin study with 20 mg three times daily or 60 mg nightly [5] have been used.

During mianserin analysis using GC—MS or GC—NPD, each series of measurements consisted of analysis of blank plasma samples, calibration samples, spiked samples, and clinical samples in a random order.

RESULTS AND DISCUSSION

Intra-laboratory variation in the GC—NPD and GC—MS methods

The intra-laboratory variation in both assay methods can be judged from the repeated determinations of the spiked samples of which a number ranging from 1 to 10 was included in each daily series of plasma samples to be analyzed.

In discussing the precision of a method it is important to distinguish between within- and between-day variation. If the same spiked sample is measured repeatedly on each different day using the same method, the logarithm y of the individual observation is assumed to be built up as follows:

$$y = \mu + \alpha_{\text{between days}} + \epsilon_{\text{within day}} \qquad (1)$$

where

$\alpha_{\text{between days}}$ = random error, inherent to all y values that might be observed on the day considered. It is assumed to be randomly drawn from a population of such errors with zero mean and variance σ^2 ($\alpha_{\text{between days}}$). This is the variance responsible for the day-to-day variation.

$\epsilon_{\text{within day}}$ = individual random error, independent from observation to observation and assumed to be randomly drawn from a normally distributed population of individual random errors with zero mean and variance σ^2 ($\epsilon_{\text{within day}}$).

A useful measure for the combined variation of a method is the total variance:

$$\sigma^2_{\text{total}} = \sigma^2(\alpha_{\text{between days}}) + \sigma^2(\epsilon_{\text{within day}})$$

This is the variance in a population of y values, each obtained on a different day. In this population the mean (or expected value) $E(y)$ will be:

$E(y) = E(\mu + \alpha_{\text{between days}} + \epsilon_{\text{within day}}) = \mu$

which shows the meaning of μ in the model eqn. 1.

From each set of observations obtained from one spiked sample on successive days using one method, estimates s^2 ($\alpha_{\text{between days}}$), s^2 ($\epsilon_{\text{within day}}$) and s^2_{total} were calculated from the analysis of variance.

For each method the estimates $s^2(\alpha_{\text{between days}})$ as well as the estimates $s^2(\epsilon_{\text{within day}})$ were combined over the three preparations, the latter combination following the standard method for pooling mean square estimates of the same variance [6]. For the combination of the three estimates $s^2(\alpha_{\text{between days}})$ the reciprocal of the estimated variance of $s^2(\alpha_{\text{between days}})$ was used as weighting factor. The combined value for s^2_{total} was obtained by addition. Finally, all estimated variances of y (= log observed plasma level) were converted to relative standard deviations (or coefficients of variation) of the observed plasma level itself, denoted as $s_{\text{rel. between days}}$, $s_{\text{rel. within day}}$ and $s_{\text{rel. total}}$ (see Table I).

TABLE I

RESULTS CALCULATED FROM DIFFERENT ARTIFICIAL PREPARATIONS A, B AND C, WHICH WERE INCLUDED IN EACH DAILY SERIES OF PLASMA SAMPLES TO BE DETERMINED

Means are geometric means. d.f. = degrees of freedom.

	A	B	C	Combined
GC—NPD method				
Total No. of observations	37	14	41	
No. of measuring days	8	4	11	
$s_{\text{rel. within day}}$	5.4 %	2.4 %	4.2 %	4.5 % (69 d.f.)
$s_{\text{rel. between days}}$	4.2 %**	6.2 %***	5.6 %***	5.2 %
$s_{\text{rel. total}}$	6.8 %	6.6 %	7.0 %	6.9 %
Weighted mean observed (ng/ml)	18.11	20.80	19.76	
S.D. of this mean (ng/ml)	0.37	0.60	0.34	
GC—MS method				
Total No. of observations	20	10	22	
No. of measuring days	6	3	3	
$s_{\text{rel. within day}}$	4.8 %	3.0 %	7.4 %	6.0 % (40 d.f.)
$s_{\text{rel. between days}}$	4.5 %*	1.8 %//	√—5.4 %//	2.6 %
$s_{\text{rel. total}}$	6.6 %	3.5 %	7.0 %	6.5 %
Weighted mean observed (ng/ml)	18.61	21.15	20.54	
S.D. of this mean (ng/ml)	0.33	0.52	0.42	

//Level of significance $P > 0.05$.
*Level of significance $0.05 > P > 0.01$.
**Level of significance $0.01 > P > 0.001$.
***Level of significance $0.001 > P$.

Clearly the total scatter of the two methods, as applied in our laboratory, is virtually the same, but there is a rather marked difference in the scatter components: the GC—NPD method shows the smallest within-day fluctuations but, in contrast to the GC—MS method, very pronounced day-to-day errors.

Systematic deviation between the results obtained by GC—MS and GC—NPD

Table I also shows the mean and its standard error of each spiked sample per method. Each mean was calculated as a weighted mean of the day means, using the reciprocal of the estimated variance of the day mean as a weighting factor. As can be seen, the difference of any two weighted means pertaining to the same preparation is small in comparison to the standard error of these means. The weighted mean of the three differences is calculated to be 0.50 with a standard error of 0.33, again suggesting no significant difference.

For a better analysis of a possible existing systematic difference between the GC—MS and GC—NPD assay method, 74 human plasma samples from a clinical mianserin study [5] were analyzed by GC—MS (including clean-up by liquid chromatography) as well as by GC—NPD. The mianserin steady state levels encountered during this study were within the range of 6 to 120 ng of mianserin per ml plasma. In Fig. 1, the plasma levels of each sample determined by the two different assay methods are represented by a dot, using logarithmic scales in order to equalize the scatter over the whole range. The straight line minimizes the sum of squares of the perpendicular distances from the points

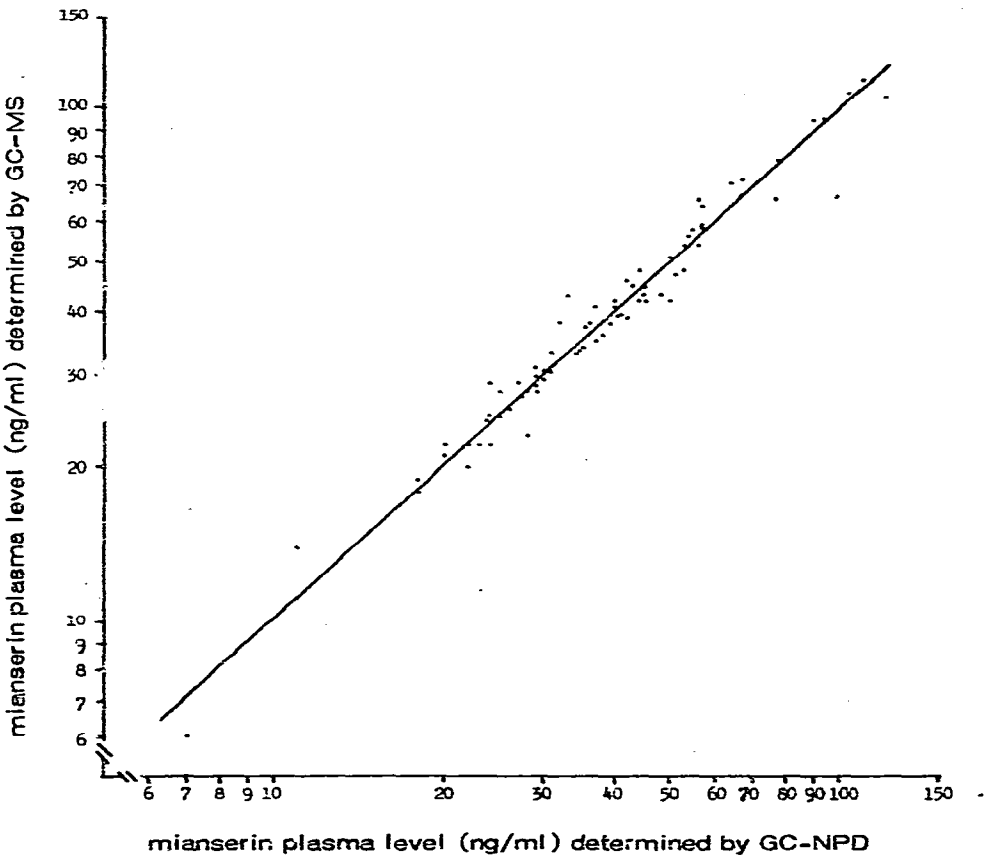

Fig. 1. A plot of the mianserin levels of 74 plasma samples from a clinical study determined by GC—NPD and GC—MS. The straight line minimizes the sum of squared perpendicular distances from the points to the line.

to the line. Its slope (in log units/log unit) is 0.992, very close to 1 and therefore compatible with the assumption that a systematic deviation, if any, from unity in the ratio between the results of analysis applying both assay methods, does not change with the plasma level in the range considered.

Analysis of variance of the observed plasma levels (in log units) reveals that the geometric mean ratio GC—NPD/GC—MS = 0.995 with 95% confidence limits of 0.977—1.014. Therefore, systematic deviation between the two assay methods, if existing at all, can only be very small.

CONCLUSIONS

For the determination of mianserin in human plasma, it is demonstrated that the GC—MS and GC—NPD assay methods are virtually the same with respect to the overall precision. A statistically significant day-to-day variation of the GC—NPD assay method is found. Because at present no explanation for this phenomenon can be given, it is recommended to analyze a particular plasma sample with the GC—NPD assay method on different days whenever possible. Averaging the results will lead to a still better estimate of the actual mianserin plasma level.

By application of both assay methods to plasma samples from depressed patients, no systematic difference between the results in mianserin plasma levels is observed.

Owing to the simplicity of the assay procedure, the GC—NPD method has advantages over the GC—MS assay method for routine mianserin plasma level determinations.

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