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# Uncertainty analysis of $^{99m}$Tc-HEPIDA liver clearance determination

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## Abstract

BACKGROUND: The aim of the study was to obtain information on the accuracy and precision of $^{99m}$Tc-HEPIDA hepatic ($Cl_{Hp}$) and plasma ($Cl_{Pl}$) clearances and selection of an appropriate estimator of the measurement uncertainty of a single determination of these quantities.
MATERIAL AND METHODS: In a simulation (Monte Carlo) experiment, it was assumed that the recorded results of plasma and hepatic clearances, as obtained from 185 patients, provided authentic information about $^{99m}$Tc-HEPIDA behaviour in the body over a wide range of the clearances studied. The time-course $^{99m}$Tc-HEPIDA concentration in blood plasma has been described by means of biexponential function with parameter values derived for each patient. For each patient, using these data and urinary excretion data, there had been 5000 simulations performed; in each of the latter, the directly measured numbers have been substituted by simulated ones, obtained by means of varying the real ones, using random generated values. These reflected errors of plasma and radioactive standard pipetting (from 1 to 5%) and stochasticity of counting radioactive decay (1%). The time of blood sampling and urine voiding was also varied, assuming realistic uncertainty. The varied values were then used for computation of the simulated clearances. From the 5000 calculated clearances for each patient, mean-values were calculated, as well as mean standard errors, standard deviations and mean uncertainty of measurements using a widely accepted rule of partial error propagation, and, in addition, a modified rule of the latter. Accuracy of clearance ($Cl_{Pl}$, $Cl_{Hp}$, $Cl_{Ur}$) determination was assessed on the basis of comparison of mean values from simulations with those from directly recorded values. Precision was identified with standard deviation of each of the 5000 simulations. The uncertainty thus obtained was compared with results of calculated traditional and modified uncertainty. There was good agreement between standard deviation of the simulations with results of the modified calculation of total differential. Therefore, a coefficient of variation from simulation computations and a modified means of calculation of the propagated errors was accepted as a measure of uncertainty of a single determination.
RESULTS/CONCLUSIONS: There was a very high correlation between the mean values from simulations and those from direct determinations ($r > 0.98$ in each case). The regression lines practically corresponded to the lines of identity. These correlations were not affected by the assumed range of pipetting uncertainty. In conclusion, the methods of $^{99m}$Tc-HEPIDA clearance determination are satisfactory. Precision of clearance measurements depends substantially upon uncertainty of pipetting. For plasma clearance, the coefficients of variations at $Cl_{Pl} >$ $>$ 350 ml/min and at ab.80 ml/min amounted to 2 and 11% respectively, at pipetting uncertainty of 2%. Similarly, for hepatic clearances of $^{99m}$Tc-HEPIDA of 300 ml/min and 30 ml/min, CV was 2.5 and 25%, respectively (at the same uncertainty of pipetting).
**Key words: plasma clearance, hepatic clearance**

## Introduction

Determination of $^{99m}$Tc-HEPIDA (dimethylacetanilidiminodiacetic-acid) plasma clearance ($Cl_{Pl}$) is an efficacious overall procedure for the evaluation of liver parenchyma damage [1–3]. However, further studies have demonstrated [4] that a fraction of the radiopharmaceutical undergoes elimination from the body via the urinary tract. The substantial and variable share that this pathway takes in total clearance could lead to significant errors in assessment of the functional state of the liver. These observations demanded further investigation into the possibility of determining the specific liver clearance of $^{99m}$Tc-HEPIDA ($Cl_{Hp}$) and to assess its useful potential for evaluation of the liver parenchyma secretory function, as estimated using this compound.

In an earlier study [5] devoted to the theoretical basis for the determination of $Cl_{Hp}$,it could be demonstrated that this quantity is the difference between plasma clearance ($Cl_{Pl}$) and renal clearance ($Cl_{Ur}$) of the substance in question:

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$$Cl_{Hp} = Cl_{Pl} - Cl_{Ur}$$

Determination of both the plasma and renal clearance requires knowledge of the function *C(t)*, adequately describing the decline of $^{99m}$Tc-HEPIDA concentration in plasma after single intravenous administration (bolus). The characteristics of the substances used for clearance studies usually require that they distribute in the body according to the requirements of the open two compartment model. Thus, the general form of the *C(t)* function should be represented by the equation:

$$C(t) = A_1 e^{-b_1 t} + A_2 e^{-b_2 t}$$

To obtain the parameters of this function for an individual, a series of blood samples should be withdrawn at pre-selected points of time after i.v. injection, and the concentration of the radiopharmaceutical in plasma measured. Times of blood samples and respective concentrations for $i = 1,2,..,k$, where $k$ is the number of samples taken, are later used for derivation of *C(t)* function parameters.

The efficacy of $Cl_{Hp}$ of $^{99m}$Tc-HEPIDA when determined by a multisample method (as the difference between the plasma and urinary clearance) for assessment of functional performance of the liver was subsequently demonstrated in several reports [6]. However, for reasonable use of a diagnostic method, a prerequisite are its metrological characteristics, and especially its accuracy and precision.

## Objectives of the study

The principal aim of the study was the assessment of the accuracy and precision of $^{99m}$Tc-HEPIDA liver clearance and an investigation into how several factors, which should be seen as potential sources of errors, affect these characteristics. In addition, the second objective was to select the appropriate estimator of measurement uncertainty of $Cl_{Hp}$. Attaining this aim should enable estimation of the uncertainty [Measurement uncertainty $\Delta x$ – one half of the range $x \pm \Delta x$, in which real value $\zeta$ is contained with high probability. Often as a measure of uncertainty, a standard deviation is used from a given series of measurements (directly or indirectly calculated), as computed according to common rules of error calculations (superposition of errors)] of a single determination of plasma and hepatic clearance of $^{99m}$Tc-HEPIDA. As $Cl_{Hp}$ is a difference of two clearances, $Cl_{Pl}$ and $Cl_{Ur}$, the final objective could be only attained by investigation of respective characteristics of urinary and plasma clearances of $^{99m}$Tc-HEPIDA.

## Material and methods

### Selection of values (data) assumed to represent reality

To select such data, archive results were taken of 185 determinations of respective clearances in patients referred for investigations on medical indications. The plasma and hepatic clearance were determined using $^{99m}$Tc-HEPIDA obtained from ex promptu kits, manufactured by OBRI "POLATOM".

The set of data for each patient included: the activity administered ($A_p$), parameters of biexponential function fitted to plasma concentrations of the radiopharmaceuticals in the time intervals from 0–90-min p. injection, the results of plasma and hepatic clearance: ($Cl_{Pl}$ and $Cl_{Hp}$, resp.). The latter covered ranges from 64 to 371 and from 16 to 306 ml/min, respectively. The urinary clearance $Cl_{Ur}$ was calculated assuming that voiding was complete and took place at a time $Y = 95$ min post injection of $^{99m}$Tc-HEPIDA.

These values from patients' records and derived results were taken as real, and they served as a reference system. It was also assumed that underlying physiological processes did not vary in the course of determination.

### Errors of activity measurements

Random values of normal distribution were added to real values of $A_p$, $A_{Ur}$ and concentrations (activity) of the radiopharmaceutical in plasma at given moments in time. These latter values corresponded to fluctuations of counts with relative standard deviation of 1 percent. In addition, random pipetting errors were also added, characterized by relative standard deviations $\frac{\Delta p}{p}$ of 1, 2, 3, 4 and 5 percent.

To $A_p$ and $A_{Ur}$ three random values were added (representing three activity measurements of each) and from the sums obtained means were calculated $\overline{A}_p$ and $\overline{A}_{Ur}$ with corresponding standard deviations $S_{A_p}$ and $S_{A_{Ur}}$.

### Errors of time measurements characterising blood sampling and voiding of urine

To the established ideal blood sampling times $T_i$ ($i = 1,...,9$) and the voiding time *Y,* random values were added of rectangular distribution ($-\Delta^*$, $\Delta^*$) (* — wild character). Half of $\Delta T$ range assumed values of 0 s, 2.5 s, 5 s, 10 s and 20 s, and $\Delta Y$ range assumed values of zero and 3 min.

### Fitting a function to plasma concentrations of $^{99m}$Tc-HEPIDA

Values of blood sampling times and plasma concentrations of the radiopharmaceutical were used for derivation of parameters of biexponential function *C(t)*. The function parameters were calculated by an iterative least square method, as reported earlier [7–9].

### Calculation of clearances

Mean values of $\overline{A}_p$ and $\overline{A}_{Ur}$ and obtained parameters of the *C(t)* function were applied for calculation of $Cl_{Pl}$ and $Cl_{Ur}$ according to the formulas:

$$Cl_{Pl} = \frac{A_p}{\int_0^\infty C(t)dt} = \frac{A_p}{\frac{A_1}{b_1} + \frac{A_2}{b_2}}$$

and

$$Cl_{Ur} = \frac{A_{Ur}(Y)}{\int_0^Y C(t)dt} = \frac{A_p}{\frac{A_1}{b_1}\left(1 - e^{-b_1 Y}\right) + \frac{A_2}{b_2}\left(1 - e^{b_2 Y}\right)}$$

and afterwards for calculation of $Cl_{Hp}$ as their difference.

For each real clearance there were $n = 5000$ random simulations performed.

### Data processing

For each simulation of individual clearance the following were computed:

1. Error of the $i^{th}$ result, i.e. $\varepsilon_i$ (for $i = 1,...., n$) as the difference between the result of simulation and real value.

2. A typical superpositional uncertainty of the $i^{th}$ result ($i$ = 1,..., $n$) calculated acc. to the rule of superposition of errors as given by the formula:

$$(\Delta_S Cl_i)^2 = \sum_{j=1}^{5}\left(\frac{\partial Cl}{\partial x_j}\Delta x_j\right)^2$$

In this formula, $x$ denotes a quantity necessary for calculation of a clearance: $A_p$, $A_{Ur}$ and $A_1$, $b_1$, $A_2$, $b_2$, and $\Delta x$ – their respective uncertainties; the sum is 5 squares of partial differentials.

3. A modified superpositional uncertainty $\Delta_m Cl_i$. The modification of uncertainty calculation is related to quotient $\frac{A_i}{b_i}$, where $i$ = 1,2, and depended upon substitution of the sum $\left[\left(\frac{S_{A_i}}{A_i}\right)^2+\left(\frac{S_{b_i}}{b_i}\right)^2\right]$ by a square of difference $\left[\left(\frac{S_{A_i}}{A_i}-\frac{S_{b_i}}{b_i}\right)^2\right]$ for $i$ = 1,2 (see Appendix). The minus mark "–" informs us that errors $S_A$ and $S_b$ compensate for each other (explanations and justification therein).

After $n$ = 5000 simulations of clearance calculations of given real excretion processes (for each patient), further computations followed, namely:

4. Mean clearance values for each clearance: $Cl_{Pl}$, $Cl_{Ur}$, *and* $Cl_{Hp}$.
5. Mean standard error for each of the clearance determinations, i.e.

$$\bar{\varepsilon} = \sqrt{\frac{\sum_{i=1}^{n}\varepsilon_i^2}{n}}$$

6. Standard deviation for each clearance determination (each patient): $S_{ClPl}$, $S_{ClUr}$, $S_{ClHp}$.
7. Mean superpositional uncertainty of the three clearances, as calculated by a modified procedure (see Appendix).

### *Statistical analysis*

Analysis, in this investigation, was concentrated upon the question of how the accuracy and precision of clearance determination are affected by: relative uncertainty of pipetting $\frac{\Delta p}{p}$ [%] i.e. 1,2,3,4 and 5 per cent, measurement of blood sampling time $\Delta T$ of 2,5 through 20 seconds, and uncertainty of measuring the urine voiding time $\Delta Y$ = 0 and 3 minutes.

### *Assessment of accuracy*

Comparison, by means of regression analysis of mean values of the simulated clearances with real measured values, enabled assessment of the accuracy of the multisampling methods ($Cl_{Pl}$, $Cl_{Ur}$, $Cl_{Hp}$). As tools of evaluation, the coefficient of determination $R^2$ and standard error of estimation (SEE) were used. To evaluate the concordance of the regression line with the line of identity between real and simulated means (of 5000 runs), the distance between the lines at two points was measured: at the smallest and largest values of the clearances: for $Cl_{Pl}$, $Cl_{Hp}$ and $Cl_{Ur}$ at 64 and 370 ml/min, 16 and 305 ml/min and 5 and 153 ml/min, respectively. These parameters of $R^2$, SEE, and distance ($D_{Cl}$) have been defined as parameters of agreement. The dimension of the latter — with the exception of $R^2$ — is [ml/min].

### *Evaluation of precision*

As a measure of precision, a coefficient of variation (a quotient of standard deviation and mean, in percent) was accepted. However, standard deviation was also used for comparison of standard deviation with mean standard error and for analysis of changes of both these quantities.

### *Selection of the uncertainty estimator of a single determination of a clearance*

To select a proper measure for estimation of the uncertainty of a single determination of a clearance in a given patient, comparison was advocated by a mean value of superpositional uncertainties: typically $\Delta_s Cl$ and in modified version $\Delta_m Cl$ (see Appendix). This comparison enabled selection of a formula for which results were close to those of standard deviation. As a measure of agreement, a quotient was accepted of mean uncertainty over standard deviation: the closer the value of the quotient to unity (but not less than 1), the more concordant the two quantities compared.

## Results

In this study, the graphs and tables display results applying to the hepatic clearance of $^{99m}$Tc-HEPIDA. This latter procedure is the most complex procedure of the three determinations of clearances of this radiopharmaceutical, and errors which apply to $Cl_{Hp}$ assume the highest values due to superposition of partial errors of $Cl_{Pl}$ and $Cl_{Ur}$. However, the character of analysed parameters for these two quantities is very similar to that for $Cl_{Hp}$.

The results presented below, unless indicated to the contrary, have been obtained for conditions most often prevailing during routine clinical work, namely:

— relative uncertainty of pipetting — $\frac{\Delta p}{p}$ = 2%;
— uncertainty of the blood sampling time up to $\Delta T$ = 2.5 s;
— urine voiding time $Y$ = 95 min post injection;
— uncertainty of urine voiding time up to $\Delta Y$ = 3 min.

### *Accuracy*

Figure 1 presents the correlation between real measurements and means from the simulation exercise. As can be seen, the corre-

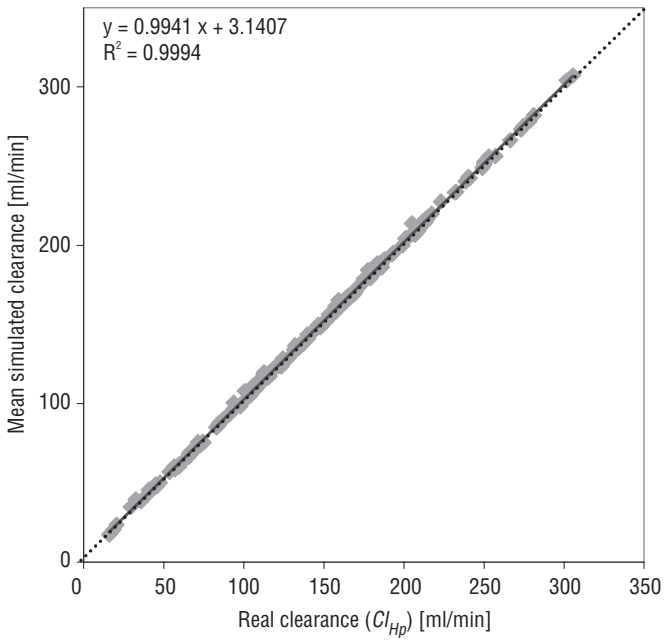


**Figure 1.** Correlation of real (determined) and mean values of simulated hepatic clearances of $^{99m}$Tc-HEPIDA (multisample determination). Continuous line — line of regression, dotted line — line of identity.

**Table 1. Values of the parameters of agreement for multisample determination of $Cl_{Hp}$**

| Parameter of agreement | Values of the parameters *vs.* uncertainty of pipetting 1% | 2% | 3% | 4% | 5% |
|---|---|---|---|---|---|
| $R^2$ | 0.9995 | 0.9994 | 0.9992 | 0.9990 | 0.9988 |
| SEE [ml/min] | 1.35 | 1.54 | 1.72 | 1.92 | 2.14 |
| $D_{16}$ [ml/min] | 2.67 | 3.05 | 3.23 | 3.20 | 3.19 |
| $D_{305}$ [ml/min] | 1.06 | 1.35 | 1.49 | 1.43 | 1.04 |

**Table 2. Values of parameters of agreement for correlations between real values of $Cl_{Hp}$ and mean values from simulations in individuals at varied uncertainty of blood sampling time ($\Delta T$)**

| Parameter of agreement | Values of the parameters *vs.* uncertainty of $\Delta T$ [s] 0 | 2.5 | 5 | 10 | 20 |
|---|---|---|---|---|---|
| $R^2$ | 0.9992 | 0.9994 | 0.9994 | 0.9994 | 0.9994 |
| SEE [ml/min] | 1.53 | 1.53 | 1.34 | 1.35 | 1.32 |
| $D_{16}$ [ml/min] | 3.015 | 3.03 | 3.02 | 3.03 | 3.07 |
| $D_{305}$ [ml/min] | 1.37 | 1.36 | 1.34 | 1.34 | 1.32 |

**Table 3. Values of parameters of agreement for correlation between real values of $Cl_{Hp}$ with mean values from simulations in individuals at varied uncertainty of urinary bladder emptying times ($\Delta Y$) [min]**

| Parameters of agreement | Parameters at two uncertainties of DY [min] 0 | 3 |
|---|---|---|
| $R^2$ | 0.9994 | 0.9994 |
| SEE [ml/min] | 1.52 | 1.53 |
| $D_{16}$ [ml/min] | 3.02 | 3.04 |
| $D_{305}$ [ml/min] | 2.097 | 1.32 |

lation is very tight and the regression line is positioned very close to the line of identity. This indicates that, in general, the clearances are being measured accurately. There are only few points whose distances from the regression line are apparent, but not by more than 2.5 ml/min. Similarly, tight correlations were obtained for $Cl_{Pl}$ and $Cl_{Ur}$, and also at different assumed relative uncertainties of pipetting.

Table 1, shows assembled values of agreement parameters of respective regression lines with those of identity, for multisampling methods at different relative uncertainties of pipetting. It seems clear that the latter does not significantly affect the general accuracy of $Cl_{Hp}$ determination. The same applies to $Cl_{Pl}$ and $Cl_{Ur}$.

Table 2 shows values of agreement parameters for lines of regression with lines of identity for $Cl_{Hp}$, when varying the uncertainty of the blood sampling times $\Delta T$. The same applies to $Cl_{Pl}$ and $Cl_{Ur}$. Again, these data indicate that inaccuracy of blood sampling time, of the order studied, does not adversely influence the general accuracy of determination of the three $^{99m}$Tc-HEPIDA clearances.

Table 3 shows the assembled parameters of agreement of regression lines with lines of identity for $Cl_{Hp}$ determination at two values of uncertainty of urine voiding time $\Delta Y$. Again, this uncertainty — at the level studied — does not significantly affect the uncertainty of $Cl_{Hp}$ determination.

## *Precision*

Figure 2 presents the relationships between absolute values of $Cl_{Hp}$ and its mean square error and standard deviations of all clearance determinations in individual patients. From the graph, it follows that standard deviations of $Cl_{Hp}$ are in very good agreement with the corresponding values of mean square errors. For some clearance determinations, however, values of mean square errors assume values somewhat larger than the corresponding standard deviations (by ab. 2.5 ml/min). This could be due, perhaps, to a systematic error of that magnitude.

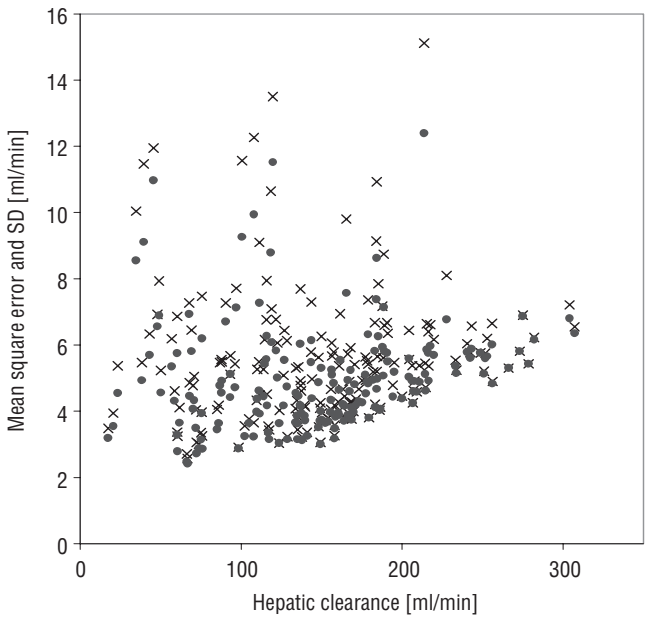


**Figure 2.** Relationship between mean square error (×××) and standard deviation (•••) of simulated hepatic clearance ($Cl_{Hp}$) and value of that clearance.

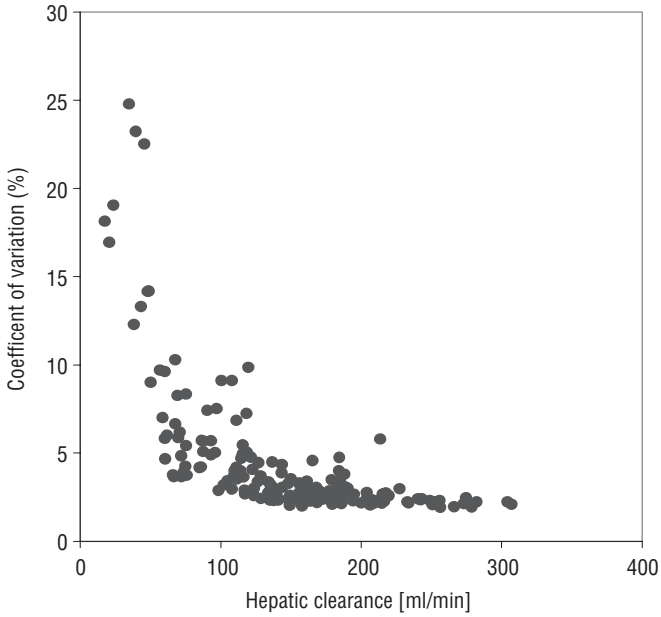


**Figure 3.** Coefficient of variation *vs.* absolute value of hepatic clearance.

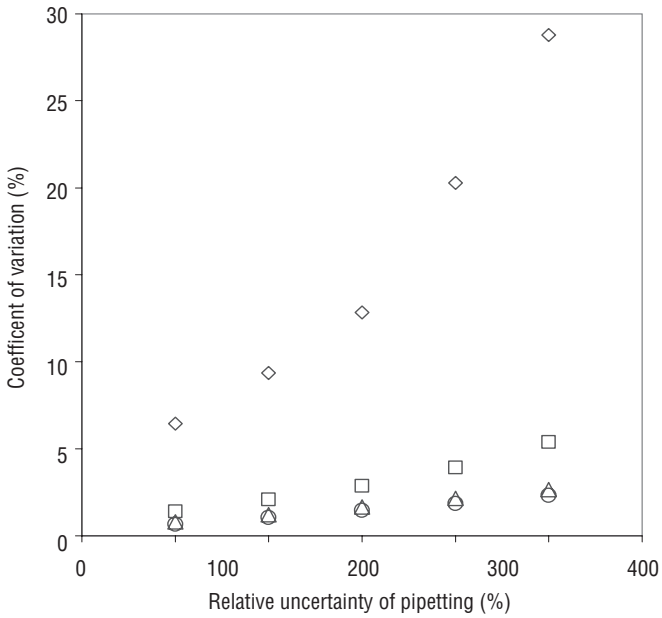


**Figure 4.** Coefficient of variation of simulated hepatic clearance ($Cl_{Hp}$) *vs.* relative uncertainty of pipetting and magnitude of the clearance: ◇◇◇◇ — 17 ml/min, □□□□ — 111 ml/min, ΔΔΔΔ — 214 ml/min, ○○○○ — 307 ml/min.

Figure 3 shows how the coefficient of variation (a measure of precision) varies with mean values of $Cl_{Hp}$. As would be expected, the magnitude of the coefficient declines with increasing absolute value of the measured clearance (the precision improves). These observations are systematized (by exemplification) in Table 4. Figure 4 shows the relationship between coefficients of variation and the relative uncertainty of pipetting for $Cl_{Hp}$ at absolute values of the latter of 17, 111, 214 and 307 ml/min.

In Figure 5, the relationship between coefficients of variation of $Cl_{Hp}$ and uncertainty of blood sampling time measurements are depicted. Practically, the latter uncertainty — in the range studied — had no influence upon the precision of $Cl_{Hp}$ determination. This also applies to $Cl_{Pl}$ and $Cl_{Ur}$.

As shown in Table 5, it is also demonstrated that uncertainty (at two levels) of bladder voiding time measurement does not affect the precision of $Cl_{Ur}$ and $Cl_{Hp}$ of $^{99m}$Tc-HEPIDA.

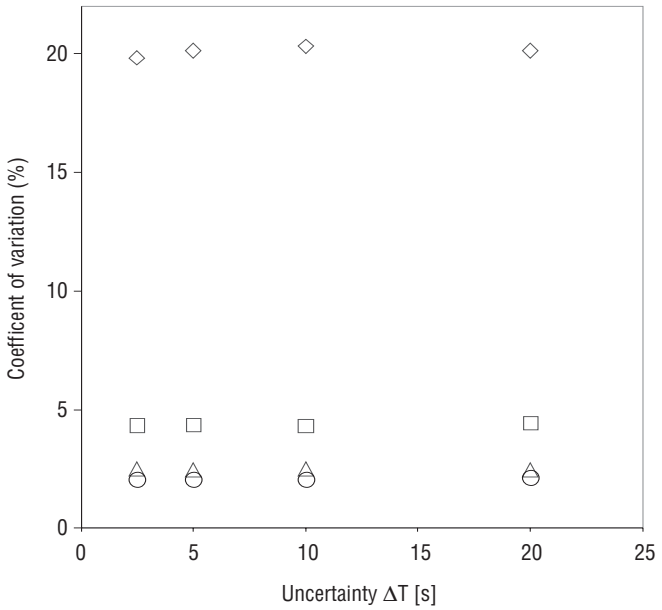


**Figure 5.** Coefficient of variation of simulated hepatic clearance ($Cl_{Hp}$) *vs.* uncertainty of blood sampling time ($\Delta T$) and absolute magnitude of the clearance: ◇◇◇◇ — 17 ml/min, □□□□ — 111 ml/min, ΔΔΔΔ — 214 ml/min, ○○○○ — 307 ml/min.

### *Choice of the uncertainty estimator of single clearance determination*

Figure 6 shows the variation of the: standard deviation, typical superpositional uncertainty $\Delta_s Cl$ and modified superpositional uncertainty $\Delta_m Cl$ (see Appendix) of individual clearance ($Cl_{Hp}$) determinations with their absolute magnitude. It can be clearly seen that values of $\Delta_s$, as typically estimated, greatly exceed the respective standard deviations, on average, by a factor of ~ 2.5.

**Table 4. Selected values of the coefficient of variation for three $^{99m}$Tc-HEPIDA clearances at 3 selected levels of each [ml/min]**

| Level of the clearance | $Cl_{Pl}$ | | $Cl_{Ur}$ | | $Cl_{Hp}$ | |
|---|---|---|---|---|---|---|
| | Level [ml/min] | Coefficient of variation (%) | Level [ml/min] | Coefficient of variation (%) | Level [ml/min] | Coefficient of variation (%) |
| Low | 80 | 11 | 7 | 3 | 30 | 25 |
| Average | 200 | 3 | 80 | 2 | 160 | 4,5 |
| High | 370 | 2 | 150 | 2 | 305 | 2,2 |

**Table 5. Values of coefficient of variation of urinary and hepatic clearances of $^{99m}$Tc-HEPIDA for four values of clearances at two uncertainties of bladder emptying $\Delta Y$**

| Values of coefficient of variation (%) for clearances | | | | | |
|---|---|---|---|---|---|
| Urinary clearance | | | Hepatic clearance | | |
| Values [ml/min] | $\Delta Y$ | | Values [ml/min] | $\Delta Y$ | |
| | 0 | 3 | | 0 | 3 |
| 4.7 | 1.9 | 1.9 | 17 | 17.8 | 18.2 |
| 56 | 1.7 | 1.8 | 110 | 4.1 | 4.2 |
| 108 | 2.1 | 2.2 | 214 | 2.4 | 2.4 |
| 152 | 1.9 | 2.1 | 307 | 2.1 | 2.1 |

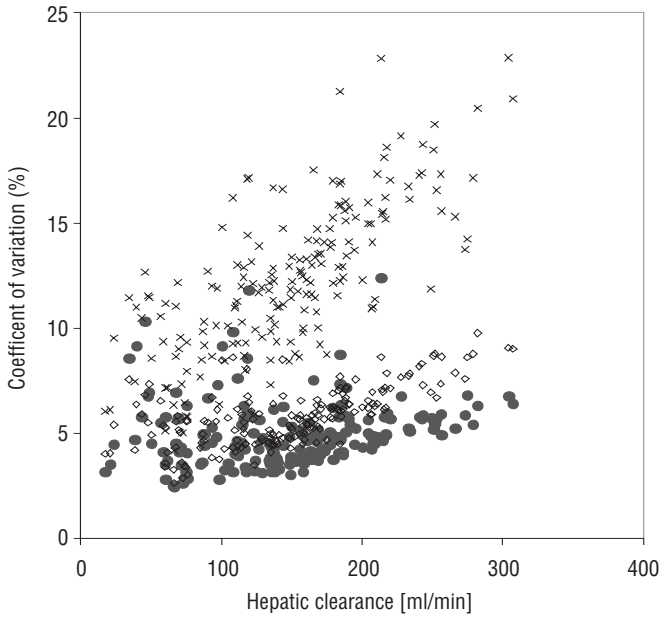


**Figure 6.** Uncertainty of single clearance ($Cl_{Hp}$) determination vs. its magnitude [ml/min] as expressed by: standard deviation (●●●●), total differential — calculated in typical (××××) and in modified way (◇◇◇◇).

The values of $\Delta_m$ are very close to SD and exceed the latter by ~ 10 percent only. Similar observations apply to $Cl_{Pl}$ and $Cl_{Ur}$.

## Discussion

Determination of clearances, mostly renal, using proper radiopharmaceuticals, has been known for decades. The theoretical basis, when single intravenous injection is the route of administration, has been given by Sapirstein et al [10]. Regardless of good theoretical foundations of such procedures, very few studies have been devoted to their metrological characteristics and, in particular, to the accuracy and precision of respective methods. Rigorous assessment of the former seems impossible, because there is no standard for the measured quantity and there is no etalon method as a substitute. Attempts to estimate the precision of clearance measurements using urinary markers were based on the principle of repeated measurements (two or three times) in the same individuals. Analysis of these data lead to the conclusion that the multisample method of plasma clearance assumes the position of 'golden standard', and the relative uncertainty of a single measurement has been estimated at 10–15%.

This dilemma may be solved by assuming that:

— the method of determination, as outlined in detail in section, is appropriate and yields real values if properly executed;

— a Monte Carlo simulation, by choosing realistic distribution characteristics of range of variation of the respective parameters, can provide insight into the reproducibility of the procedure, and thereby provide a substitute for absolute assessment of the accuracy. It is this sense in which the term "accuracy" has been used in this study.

Studies of precision and accuracy of urinary plasma clearance determination using $^{99m}$Tc-ethylenedicysteine as the marker were made by us [11]. Analysis was based on results of Monte Carlo simulation of a real clearance study. The results strongly suggest that both accuracy and precision of multisample and single sample methods (in the range of generally normal values of clearance) were approximately 5%.

### *Monte Carlo simulation procedure*

Application of the Monte Carlo simulation procedure requires the creation of a model in the form of a series of assumptions, which form the basis for random variations of the respective parameters. Assumptions in the present study include relative uncertainty of: pipetting of activity measurements, blood sampling time and emptying time of urinary bladder. There factors were selected on the basis of daily laboratory experience.

Two assumptions may be questioned. The first would be related to the completeness of urinary voiding. There could be some fraction of urine retained in the bladder. This could be corrected based on external activity measurements or ultrasound measurements of the volume of urine retained (this was not done in the present study, but basically the correction is possible).

The second assumption that may be questioned is that during the course of the determination the clearance rate is stable in time. Any situations that could conceptually lead to changes of renal function are carefully avoided, but, of course, some variation of physiological processes is probable, and what is eventually measured is a "mean" value of the clearance rate over the course of time during which the sampling is made.

With both these reservations in mind, the Monte Carlo model of clearance simulation may be utilized for uncertainty assessment of the whole procedure.

### Accuracy

Results obtained from simulation based on the model outlined above demonstrate that mean values of 5000 virtual determinations of the same clearance correlate very highly with the real values.

The regression lines, even if their position is very close to the line of identity, do not coincide completely. The regression lines for $Cl_{Pl}$ and $Cl_{Hp}$ lie above, and for $Cl_{Ur}$ below, the respective line of identity. This might be interpreted as showing that the former are systematically overestimated, while the last is somewhat underestimated. Analysis of Tables 1 through 3 indicates that distances between respective lines of identity and regression do not vary significantly with less rigorous performance of measurements, perhaps with exception of pipetting where distances change slightly.

These distances are generally small; for most lax time and pipetting conditions, they do not exceed 3 ml/min for all clearances. One should take note that for $Cl_{Pl}$ and $Cl_{Hp}$ they are noticeable at lower absolute clearance values; however, at these low values (below 65 and 16 ml/min for $Cl_{Pl}$ and $Cl_{Hp}$, respectively), overestimation of their values by 3 ml/min is of no clinical importance for assessment of patients' condition. For higher values of the clearances, these differences are even smaller and appear to be completely negligible.

For renal clearance of $^{99m}$Tc-HEPIDA, a distance of 3 ml/min applies to higher values ($\sim$ 130 ml/min) and seems roughly proportional to the measured values of $Cl_{Ur}$ at lower values. In clinical conditions, correction for these small systematic errors does not appear to serve any purpose.

Summarizing: the accuracy of all clearances investigated in this study appears to be entirely satisfactory.

### Precision

The scatter of the results of measurements, which is responsible for an imprecise determination, results from the interference of incidental errors. As these errors are not known, (and because in the case when there are no known systematic errors, deviations and errors are identical) analysis was concentrated on estimators of errors, i.e. standard deviations. In this study, it was possible to compare mean square errors with their counterparts — standard deviations. As has been shown in Figure 2, that for the overwhelming majority of $Cl_{Hp}$ values, the standard deviations are practically identical (with a few exceptions) to the values of respective mean square errors; there are only a few values of $Cl_{Hp}$ where the differences reach 2.5 ml/min. Similar observations apply to $Cl_{Pl}$ and to $Cl_{Ur}$; in the last cases the differences are still smaller and do not exceed 3 ml/min. This small difference between mean square errors and standard deviations are most likely due to the small systematic errors already mentioned above, which are not incorporated into standard deviations. These small differences are relatively unimportant, and in analysis of coefficient of variation (CV) as a measure of precision, are really of no importance. Therefore, as a measure of precision in this study, a coefficient of variation (SD/mean) was accepted.

Values of CV, obtained for various values of the clearances at conditions typical for routine analytical work while determining the $Cl_{Hp}$, have been presented in Figure 3; similar variations of CV were seen for $Cl_{Pl}$. As could be anticipated, values of coefficients of variation for both clearances decline with the absolute value of the clearance. Above 200 and 160 ml/min for $Cl_{Pl}$ and $Cl_{Hp}$ respectively, the corresponding coefficients of variation stabilize at 2 and 2.2%. On the other hand, CVs for $Cl_{Ur}$ in the entire range from 4 to 130 ml/min remain at roughly 2–2.5%. So, low coefficients of variation are somewhat astonishing, because this observation may indicate that clearances in healthy individuals, or in those with slight impairment of liver parenchyma, are being determined more precisely than hitherto postulated (usually $\sim$ 10% precision).

The precision becomes worse at low clearance values, and this fact may, to some extent, jeopardize the unambiguous classification of patients with $Cl_{Hp}$ to the order of $\sim$ 80 ml/min. At even lower values of $Cl_{Hp}$ the problem disappears again and diagnosis of liver parenchyma impairment becomes clear.

The results presented above make it clear that coefficients of variation rise with the increasing uncertainty of pipetting, and this observation applies practically to low values of $Cl_{Hp}$ and $Cl_{Pl}$ (Figure 4). When uncertainty of pipetting rises from 1 to 5%, the CV of $Cl_{Pl}$ = 64ml/min and of $Cl_{Hp}$ = 16 ml/min; it increases by a factor of 5 (from 4 to 20%). At normal values of $Cl_{Pl}$ and $Cl_{Hp}$ (360 and 300 ml/min), the coefficients of variation increase marginally (from 2 to 3%).

As shown above, the precision of urinary clearance determination remains practically stable. Other analyzed factors do not affect the precision of determination of this clearance (Table 5).

### Estimator of uncertainty

Another practically relevant issue is the assessment of uncertainty of a single determination. The magnitude of such uncertainty with regard to clearance determination is particularly important when monitoring a patient. A comparison of changes in clearance over time with the uncertainty is required in order to answer the question of whether the former are real.

In situations where performing a series of measurements of physical quantity in standardized conditions is possible, uncertainty of determination is identified with the value of standard deviation.

When determination of a clearance, and particularly if radionuclide-containing substances are applied, such a strategy is, in practice, unacceptable because:

— maintaining absolute constancy of measurement conditions, particularly those of a physiological nature, becomes impossible;
— exposing a patient to another dose of ionizing radiation, however small, requires sound, and difficult to apply in practice, justification.

Thus, another approach should be sought to solve the problem of estimation uncertainty of a single clearance determination.

In the case of plasma and hepatic clearance of $^{99m}$Tc-HEPIDA, we deal with quantities measured indirectly. In such a case, the uncertainty in question should be estimated in assessment of partial quantities and application of the law on superposition of the partial errors. Thus, one should try to compare such an estimate with the respective standard deviation, of course when the comparison is possible. This is the case in the Monte Carlo experiment presented above (Figure 2 and 6).

When the results of three forms of clearances ($Cl_{Pl}$, $Cl_{Hp}$, $Cl_{Ur}$) were utilized, the uncertainty calculated according to the law of error superposition exceeded the respective values of standard deviation by a factor of 2.5. The width of such a range should

provide a 98 percent probability of containing the real value, if the criteria of standard deviation are applied. While looking for explanation of that discrepancy, it became obvious that the excessive width of the classical error superposition uncertainty might be due to the underlying assumption that partial errors are being added "in algebraic fashion" (in fact, their squares are being summed this way). However, at least some of them compensate for each other, as has been demonstrated in the Appendix. These motives led the author of this study to look for another formula which would conform with the condition that uncertainty calculated on the basis of superposition of partial errors would fulfil the condition $\Delta Cl_m \geq SD_{Clm}$ (for $m = Pl, Ur, Hp$). In a more descriptive way, two conditions should be satisfied, namely:

— values of the uncertainty calculated according to a modified formula should be close to values of respective standard deviations;
— however, these values, should not be smaller than the corresponding standard deviations.

From several analyzed possibilities, a formula was selected according to which the uncertainty was calculated, as presented in the methods section.

As follows from Figure 6, the uncertainty of a single clearance determination calculated in a modified way fulfils the above-specified conditions and its values are equal on average to 1.1•SD. These values are much closer to each other than those calculated by traditional principles of error superposition. The other formula led to values smaller than those of standard deviations, and therefore were discarded. In summary, selection of a modified formula to calculate the uncertainty of clearance determination applies to the objectives of this study; full justification for this step is presented in the Appendix. The calculated values of uncertainty slightly exceed the respective coefficients of variation (by a factor 1.1), but this does not, in the author's view, invalidate the method.

## Conclusions

1. Methods of plasma and hepatic $^{99m}$Tc-HEPIDA clearance determinations are characterised by satisfactorily high accuracy.
2. Precision of plasma clearance determination depends primarily on the absolute value of the clearance and varies from 2% at $Cl_{Pl} > 300$ ml/min to 11% at 80 ml/min.
3. Precision of hepatic $^{99m}$Tc-HEPIDA clearance is somewhat inferior to that of $Cl_{Pl}$ and varies from 2.5% at 300 ml/min to 25% at 30 ml/min.
4. A modified formula for estimation of uncertainty of single clearance determination permits assessment of the latter during routine clinical work. The uncertainty thus estimated is in agreement with the standard deviation of individual determinations derived from simulation exercises.

## Appendix

Let us assume that during an experiment for different $x_i$ values of a variable $X$, at $i = 1, 2, ..., k$, for a physical quantity $Y$,the corresponding values of $y_i$ were obtained. In addition, the values $x_i$ were error free, but those of $y_i$ were subject to error, i.e.

$$y_i = \dot{y}_i + \varepsilon_i,$$

where $\dot{y}_i$ is a real true value of the $i^{th}$ result.

Form other considerations, let us assume that it is known that variable $Y$ depends exponentially upon the quantity $X$:

$$Y = Ae^{-bX}$$

and that $A$ and $b$ are greater than zero.

It is obvious that $Y$ is a diminishing function of $X$, and if $X \to \infty$ then $Y$ asymptotically tends to zero.

For individual measurements, their results may be expressed by the formula:

$$y_i = Ae^{-bx_i}$$

for $i = 1, 2,..., k$.

For analysis and interpretation of experimental data $x_i$ and $y_i$, we need estimates of parameters $A$ and $b$. To reach this objective we need to:

a) logarithmically transform both sides of the equation:

$$ln\,(y_i) = ln\,(A) - bx_i$$

and if $z = \ln(y)$ and $a = \ln(A)$ we obtain the simple equation:

$$z_i = a - bx_i$$

b) utilize a classical least square method.

In accordance, the slop may be calculated from the formula:

$$-b = \frac{k\sum_{i=1}^{k} x_i z_i - \sum_{i=1}^{k} x_i \sum_{i=1}^{k} z_i}{k\sum_{i=1}^{k} x_i^2 - \left(\sum_{i=1}^{k} x_i\right)^2}$$

and after changing the sign we obtain:

$$b = \frac{\sum_{i=1}^{k} x_i \sum_{i=1}^{k} z_i - k\sum_{i=1}^{k} x_i z_i}{W}$$

where

$$W = k\sum_{i=1}^{k} x_i^2 - \left(\sum_{i=1}^{k} x_i\right)^2$$

An intercept will then be:

$$a = \bar{z} - (-b)\bar{x} = \bar{z} + b\bar{x}$$

Due to the existence of the errors $y_i$, their logarithms may be expressed as $z_i = \dot{z}_i + \delta_i$. If $x_i$ are error free, then

$$\sum_{i=1}^{k} x_i = g = const$$

$$\sum_{i=1}^{k} x_i^2 = const$$

$W = const$; and

$$\sum_{i=1}^{k} z_i = \sum_{i=1}^{k} (\dot{z}_i + \delta_i) = k\bar{z} + \sum \delta_i$$

where

$$\bar{z} = \frac{\sum_{i=1}^{k} \dot{z}_i}{k}$$

and the slop will assume the form of:

$$b = \frac{k(g\bar{z} - \sum_{i=1}^{k} x_i \dot{z}_i)}{W} + \frac{g\sum_{i=1}^{k} \delta_i + \sum_{i=1}^{k} x_i \delta_i}{W}$$

From this equation, it follows that the error of $b$ will be determined predominantly by

$$A = e^{a} = e^{\bar{z} + (\beta + \varepsilon)\bar{x}} =$$

$$e^{\bar{z} + \beta\bar{x}} e^{\varepsilon\bar{x}} = He^{\varepsilon\bar{x}}$$

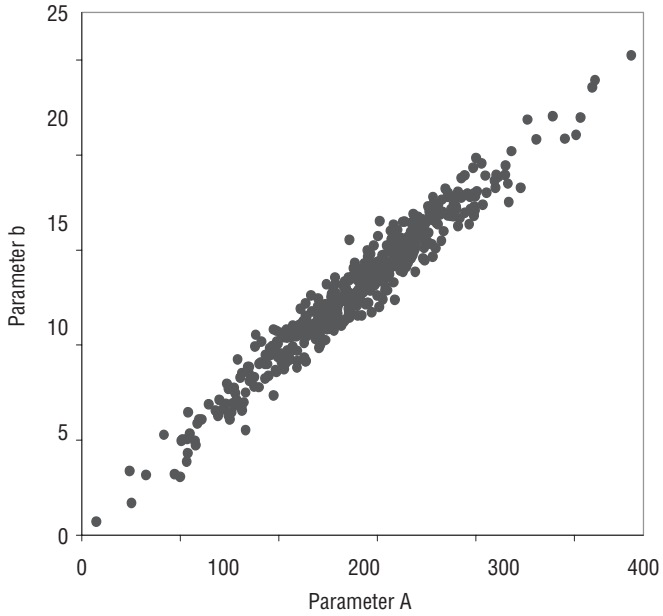


**Figure 7.** Exemplar positive correlation between the errors of exponential slope (*b*) and intercept (*A*) of exponential function $y = Ae^{-bx}$.

the second component of the right side. For simplicity, this formula may be rewritten in the form: $b = \beta + \varepsilon$; $\beta$ corresponds to the first component of the right side and $\varepsilon$ to the second. Parameter *A* could then be presented as:

For small values of $\varepsilon$, the last relationship could be approximated by the linear function

$$A = H + H\varepsilon\bar{x}$$

From the form of the formulas depicting parameters, it follows that, due to the presence of measurement errors, if the value of parameter *b* increases (positive value of $\varepsilon$) then also parameter *A* increases, because for $\varepsilon > 0$, $e^{\varepsilon\bar{x}} > 1$. When in turn $\varepsilon < 0$ then the value of *b* becomes smaller and *A* declines due to the fact that $e^{\varepsilon\bar{x}} < 1$.

The relationship obtained above can be used when calculating an integral (definite or improper) of the experimental function $Ae^{-bx}$ if the quaotient $\frac{A}{b}$ plays a role. Due to parameters errors, the value of the quotient changes but due to the considerations presented above, it follows that if *b* increases then *A* also increases and vice versa. In other words, changes of both parameters have a common direction. Therefore, the value of the quotient – in spite of changes in both parameters — is not sensitive to them because the errors compensate for each other.

Figure 7 presents the relationship of parameters *A* and b obtained from a series of $n = 500$ calculations of $x_i$ and $y_i$ for $i = 1,..,4$, where $x_i$ assumed constant values of 45, 60, 75 and 90, and $y_i$ were obtained by means of a Monte Carlo procedure and had a normal distribution. In the course of such an experiment, mean „standard deviations had the following values: A = 15606 ± 803 (coefficient of variation 0.0515), and b = 0.001559 ± 0.000721 (coefficient of variation 0.0463). The mean value of the quotient and its standard deviation were 1000765 ± 12274 (CV = 0.0123).

The example presented in Figure 7 clearly demonstrated a positive correlation of the errors of both parameters. This confirms the previously presented theoretical considerations and compensations of errors in the quotient of these two parameters.

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