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Journal of Chromatography B, 698 (1997) 225–233

JOURNAL OF CHROMATOGRAPHY B

Prevalidation statistical design to assess analytical methods Example of a quick liquid chromatographic assay of itraconazole in serum

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Received 20 January 1997; revised 18 April 1997; accepted 21 April 1997

Abstract

(A) An analysis-of-variance spreadsheet program is presented which allows to readily test and/or quantitate in a single run analytical linearity, matrix effect on recovery, repeatability of measurement and of extraction and the ruggedness of these features for up to three sessions. Owing to napierian logarithmic transformation, ANOVA mean squares directly read as relative standard deviations and checking linearity is straightforward. (B) A quick assay for therapeutic drug monitoring of itraconazole and its main metabolite was devised with the help of the program, and subsequently validated according to current quality control recommendations. The assay involves acetonitrile demixing extraction, reversed-phase HPLC and UV detection and shows acceptable performance from 0.06 to 5.0 mg/l (limit of detection about 0.03 mg/l). The prevalidation design fairly predicted precision and accuracy, was more informative about matrix effect and was even more demanding about analytical linearity. © 1997 Elsevier Science B.V.

Keywords: ANOVA; Itraconazole

1. Introduction.

For years our team has been using [1] an analysis-of-variance (ANOVA) design intended to quantitate and test some of the main analytical features of chromatographic drug assays readily and systematically while having the fewest details to keep in mind. These are the linearity of the measurement signal–concentration (response) function, the repeatability of chromatographic measurements, the effect of the biological matrix, the repeatability of analyte recovery from sample preparation (e.g., extraction) and the between-day ruggedness of all these features. In the present work, we will describe the current state of this now called prevalidation design, and examine using a practical example, how well it predicts the results of the currently recommended procedure for bioanalytical method validation [2]. The example provided as an application is a tentative novel assay of itraconazole by liquid chromatography (LC).

Itraconazole (IT) is an "azole" (actually, a triazole) antifungal agent used at a dosage of 100 to 200 mg/day. Current plasma concentrations range from 0.2 to 0.6 mg/l following 100 mg dosage, and up to 1.1 mg/l following 200 mg dosage [3]. Itraconazole pharmacokinetic disposition is sensitive to food intake [4] as well as drugs [5]. Among the

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PII S0378-4347(97)00266-1

over thirty metabolites identified [3], hydroxy-itraconazole (OHIT) has antifungal activity [6]. This requires occasional therapeutic drug monitoring of IT and OHIT, which calls for an easy-to-use assay method.

Itraconazole has been assayed by LC since 1987 [7], but the principal method involves a time-consuming extraction step too long to be used in routine. The method of Allenmark et al. [8] uses column extraction, 2.5 times concentration of the extract (injection volume equivalent to 50 μl of sample) and fluorescence detection; this allows a limit of quantitation (LOQ) of 4 μg/l. The last published method with UV detection [9] uses acetonitrile deproteinisation of 250 μl samples, followed by drying the water–acetonitrile supernatant for 30 min, redissolution and injection of the whole exsiccate.

The aims of the present work are (a) to check the usefulness of the proposed prevalidation design as an aid in optimizing assays before entering the requested validation of the complete procedure; and (b) to validate a quicker IT and OHIT assay method involving acetonitrile deproteinization followed by salt-codemixing of lipophilic analytes and of acetonitrile, and injection of the demixed acetonitrile phase.

2. Experimental

2.1. Apparatus

The chromatograph was composed of a Model AS300 autosampler, a Model SP8810 pump, a Spectra100 variable UV–Vis detector and a Winner-on-Windows integration package, all from Thermo Separation Products (Les Ulis, France). The column was a Lichrocart cartridge (250×4 mm I.D.) filled with LiChrospher RP8 (particle size 5 μm) from Merck (Darmstadt, Germany).

2.2. Reagents

Itraconazole (IT), its main metabolite hydroxy-itraconazole (OHIT) and its 2-(3-methylbutyl)-5-methyl analogue R51012 which was used as chromatographic internal standard (I.S.) were kindly supplied by Janssen (Boulogne-Billancourt, France). Other analytical grade reagents and solvents were from Merck–Clevenot (Nogent-sur-Marne, France). From stock solutions of IT, OHIT and I.S., all at 500 mg/l in methanol, working solutions of I.S. at 2 mg/l and of IT and OHIT both at 10.0 mg/l were prepared by proper dilutions in acetonitrile. Five "titrated" solutions were prepared by serial dilutions of the latter working solutions in a 1:3 ratio. Finally, the "spiking extraction solutions" were volume-to-volume mixtures of the I.S. working solution and of one of the five IT–OHIT titrated solutions (to be replaced by pure acetonitrile when assaying unknown samples).

2.3. Preparation of calibration and control samples for chromatography

Water or plasma samples were used for prevalidation, of which only the plasma samples were to enter the validation procedure. To 1 ml of blank sample were successively added 1 ml of spiking extraction solution and an excess (about 0.5 g) of solid potassium chloride. The extraction solution was forcefully pipetted into samples in order to obtain an even protein precipitate, KCl was dissolved by vortex stirring, then the mixture was centrifuged (5 min, 1500 *g*, room temperature) and the supernatant injected.

Owing to the volume-to-volume addition of extraction solutions, the nominal concentrations of the "spiked" samples were those of extraction solutions (half those of titrated solutions) i.e., 0.0617, 0.185, 0.555, 1.67 and 5.0 mg/l.

2.4. Chromatographic analysis and quantitation

A 40 μl volume of the acetonitrile supernatant was injected. The isocratic mobile phase was acetonitrile–water (55:45, v/v) at a flow-rate of 1.5 ml/min; the detection wavelength was 263 nm [7], and the analysis was performed at room temperature. Peak height and area of analytes and I.S. were measured.

2.5. Prevalidation design

As previously described [1], it is a mixed factorial and nested analysis-of-variance design. The three

crossed factors are concentration (fixed, five levels), sample matrix (fixed, two levels) and assay session repetition (random, up to three levels). The four degrees of freedom (DF) of concentration are split further into linear regression, and curvatures of the second and third (sigmoidicity) degrees, common to both matrixes and to all sessions (fourth degree not tested). Of the four DF of concentration–matrix interaction, two are tested: nonparallelism and opposite curvature. Student's *t*-test of regression and *F*-tests of other ANOVA components are performed against the suitable mean squares [10]: the random factor against the residual mean square (MS), and components of fixed factors against the MS of the interaction component between each factor and the random one. The nested intra-cell factor is sample preparation ("extraction", two replicates of the same sample), and two measurements are made from each extract so that residual within-cell variance is the variance of duplicated measurements. In the application presented, the number of sessions was only two, in order to keep the prevalidation design in its minimal configuration: two sessions times five concentrations times two matrixes=twenty samples. Each sample was extracted twice, and the autosampler was programmed for duplicated measurements.

The design computations are programmed under the name Valplan in a spreadsheet (initially Abacus from Psion, UK, readily transferable to other packages)[1]. Measurement data are keyboarded in the same spreadsheet and inputted by file fusion. Data can enter computations as such but are better transformed to napierian logarithms. A Bartlett test of both sets of measurement pairs allows us to judge which one is closer to homoscedasticity. However, since the analysis of regression uses orthogonal coefficients [11], concentration intervals must be equal: concentration levels must follow an arithmetic progression if untransformed data are to be used, or a geometric progression (by serial dilution) if logarithmic transforms are used.

[1] The Valplan program will be made available to interested readers in Works or Lotus 123 format, on request to R.A., above address; Fax: 33 0467 601 182, or e-mail: alric@sc.univ-montp1.fr

2.6. Computational check of the prevalidation design

Sham data were built as follows in order to check the programmed computations against the null hypothesis of the design, namely measurement signal proportional to concentration (analytical linearity), no matrix effect, no session effect and no extraction effect. Concentrations were set at 1, 2, 4, 8, 16. Mean responses were set linearly related at 10, 20, 40, 80, 160 from "water" and half these values from "serum" (mimicking a 50% extraction yield). Mean variation between the three "sessions" was set at −20%, 0 and +20%. "Extraction" variation was calculated as a log-normal increment with standard deviation (S.D.) 5%, and "measurement" variation as a log-normal increment with S.D. 10%. These logarithmic increments were taken in a table of normal random numbers with mean=50 and S.D.= 15, i.e., N(50,15), adjusted to N(0, 0.05) and N(0, 0.10), respectively, and back-transformed to the corresponding arithmetical multiplicator. Completed sham data entered the design as napierian logarithmic transforms.

2.7. Itraconazole assay validation procedure

The validation design was intended to match current recommendations [12,13] concerning the acceptability of control samples. In order to have enough control measurements available for the standard validation procedure, each serum extract was replicated three times instead of twice and two more samples were extracted and measured at the lowest, the highest and the median concentrations out of the available five. Fig. 1 is a scheme of the spiked serum extracts measured in the prevalidation and validation procedures. Computations for validation were made according to two usual calibration patterns: either one point chosen in the upper part of the concentration range, or unweighted regression of all five points.

3. Results

Table 1 is the spreadsheet output of the computational check of the prevalidation design. Napierian

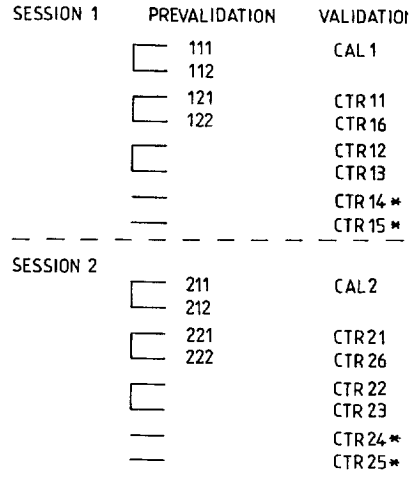

Fig. 1. Pattern of samples measured for each concentration, which were partly used concurrently for prevalidation and for validation calculations. Prevalidation samples from both matrixes bear three digit numbers for session, extract and measure (two measures from each extract). Validation samples were only serum samples, some of which were not used for prevalidation. CAL: calibration samples. CTR: control samples. The control samples with * were measured only at the lowest, medium and highest concentrations.

logarithmic standard deviation, readable as relative standard deviation (R.S.D.) (coefficient of variation, see Section 4.1) of HPLC measurement and of the extraction component, amounted to respectively, 9.9 and 4.6%; however the between-extracts ANOVA component was not significantly higher than between-measures ($F=1.427$ for 30 and 60 DF, P about 0.1). Estimates of measurement R.S.D. range from 6.3 to 14%. The 0.995 regression coefficient (slope) did not significantly differ from one ($t_8=0.33$).

Fig. 2 shows chromatograms obtained from water and serum samples at the lowest and the median concentrations.

Table 2 shows the main results of the prevalidation computations for IT and OHIT when using several current measurement variables: peak area, peak height and either with internal standardization (relative height and area). Overall measurement R.S.D. of IT ranged from more than 20% to about 5% when measuring peak area and from 12% to 1% when measuring height, irrespective of internal standardization. On the contrary, between-session R.S.D. amounted to less than 5% when measuring area to more than 30% when measuring height, with an advantage to internal standardization. The effect of plasma components on extraction was low (about 5% retention) and extraction added virtually nothing to measurement variance. The regression slope was significantly greater than 1 by 5% when using peak height. Measurements of OHIT gave similar results, with a rather higher within-session precision.

Fig. 3 shows the bilogarithmic response function of the assay: linear regression versus concentration of relative peak height measures of itraconazole from water and from serum, topped with the graph of residuals.

Table 3 shows the recovery estimates of IT, OHIT and I.S. from water and from serum using peak height measurement. Recovery ranged around 130% with an overall S.D. of about 9%.

Table 4 presents the assessment of accuracy and precision of IT mg/l determinations when using relative peak height of six control samples at every concentration level. Bias estimate exceeded 3% only at the lowest point, and was often not significant. Five-points calibration did not increase quality by comparison to one-point (highest point). Calibrating the second session with the mean calibration relation of both sessions did not decrease bias.

LOD and LOQ. The width of the detector noise when chromatographing serum extracts was 40 μV (Fig. 4). Peak heights of the 5 mg/l samples of IT and ME were respectively, 18 000 and 9000 μV, thus LODs taken as twice the noise were estimated 0.02 and 0.04 mg/l, and LOQs as twice the LOD [13] were estimated 0.04 and 0.08 mg/l, respectively.

4. Discussion

4.1. Computational check

The consistency of calculations programmed in the prevalidation design can be judged from Table 1. Sham data for check afforded analytical linearity, added systematic matrix and session components and involved fixed R.S.D.s for extraction effect and for measurement variability.

Simulated matrix and session components appeared highly significant as expected, and the overall ratio of responses in "serum" over "water" was

Table 1
Computational check of the prevalidation program, presented as the current spreadsheet layout

SS ln	ANOVA component	DF	MS ln	S.D. ln	*F*-tests	
					vs. ia	vs. Intra-cell
136.44694	Total	119	1.1466129			
4.204343	Between-sessions	2	2.1021715	1.45		187
16.659012	Between-matrices	1	16.659012	4.08	1620	
114.22954	Between-concentrations	4	28.557385			
135.43341	Between-cells	29	4.6701175			
1.013534	Within-cells	90	0.0112615	0.106		
0.591552	Between-measures	60	0.0098592	0.0993		
0.421982	Between-extracts	30	0.0140661			1.427
	Extraction component:		0.0021034	0.0459		
114.20966	Linear regression	1	0.9952228	=Slope	0.333	=*t* slope
0.015735	Curvature	1			0.663	
0.002103	Sigmoidicity	1			0.089	
0.005058	Nonparellelism	1			0.367	
0.012985	Opposite curv.	1			0.943	
0.0200475	ia CCN*MAT	4	0.0050119			0.364
0.1897539	ia CCN*SES	8	0.0237192			2.106
0.0205681	ia MAT*SES	2	0.0102840			1.043
0.1101418	ia triple	8	0.0137677			0.979
R.S.D. mes	C1	C2	C3	C4	C5	
(water)	0.111	0.066	0.117	0.127	0.119	
(serum)	0.071	0.070	0.066	0.140	0.063	
Slope water	Slope serum			Response serum/water		
0.9886	1.002			0.475		

SS ln, MS ln: sums of squares, mean squares of napierian logarithmic transforms.
S.D. ln: standard deviations, readable as R.S.D.s (coefficients of variation) of antecedent arithmetical data (see Section 4.1).
DF: degrees of freedom.
ia: factor interaction component.
R.S.D. mes: R.S.D. of measurement pairs.
Cn: nth of the five concentrations tested.

0.475 (expected: 0.50). The slope of the linear regression common to matrixes and sessions was 0.995 (expected: 1.0). No component of regression analysis other than the linear term was significant as expected. In the intra-cell analysis, the logarithmic S.D. of measurements was 0.099 (expected: 0.10) and the logarithmic S.D. of extraction was 0.046 (expected: 0.050).

A noteworthy feature of the logarithmic transformation of measurement data is that constant R.S.D. (i.e., S.D. proportional to measure) results in constant logarithmic S.D.. When using napierian logarithms, logarithmic S.D. is expected to remain numerically close to arithmetical R.S.D. (within 10% up to R.S.D.=20%). When R.S.D.s of the 40 pairs of "measurement" data were calculated as usual, the mean ratio R.S.D./logarithmic S.D. was 0.995 (range: 0.992–0.998), which confirms that napierian logarithmic S.D.s actually read as R.S.D.s (coefficients of variation) of analytical values. Moreover, since analytical precision is usually closer to constant R.S.D. than to constant S.D., logarithms bring ANOVA and unweighted regression closer to homoscedasticity than do calculations from arithmetic data.

Another benefit of the logarithmic transformation of both measurement data and concentrations, is that it makes the assessment of analytical linearity straightforward. Only when measurements are proportional to concentrations the bilogarithmic slope will have unity value and logarithmic curvature will

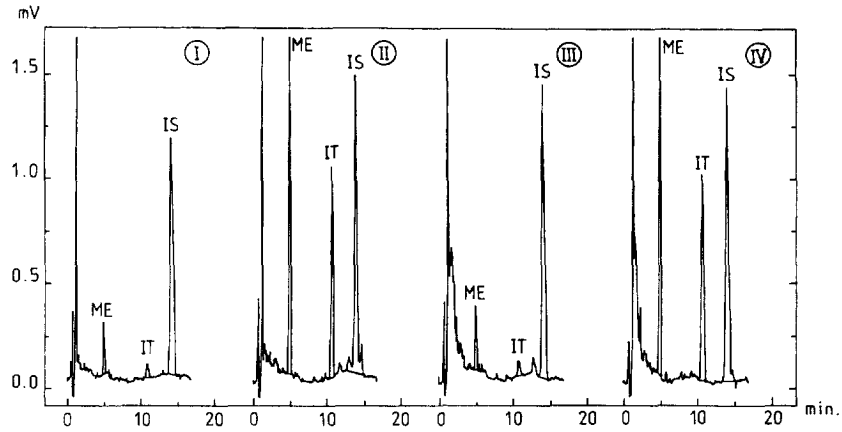

Fig. 2. Chromatograms of the first-coming sample of session 1 (111 of Fig. 1) corresponding to: I, water extract 0.062 mg/l; II, water 0.556; III, serum 0.062; and IV serum, 0.556. ME: hydroxy metabolite; IT: itraconazole; I.S.: internal standard.

be non significant. If the arithmetical response function is linear with positive intercept, the logarithmic slope will be significantly less than one with an upward-bulging curvature, and the opposite if it is linear with negative intercept. Arithmetical curvature results in the logarithmic slope being different from one with or without curvature. Assessing the statistical significance of an extrapolated intercept is intricate and precarious, whereas testing the curvature of bilogarithmic regression is merely a matter of an F-test.

4.2. Application to the itraconazole assay

The prevalidation design can help choosing the best quantitation method: peak area or height, inter-

Table 2
Main prevalidation characteristics of the HPLC assay of itraconazole and metabolite (OH-itraconazole)

	Itraconazole				Metabolite	
	Area	Area/I.S.	Height	Height/I.S.	Height	Height/I.S.
Mes R.S.D. %, overall	10.07	11.6	5.93	6.16	1.02	1.73
Highest level	0.5	4.0	0.4	1.35	0.33	1.4
Lowest level	22.6	26.2	11.6	12.0	1.1	1.4
Session-R.S.D. %	4.3	0.5	30.7	22.7	31.4	23.4
Matrix: ser/wat%	95	89.5	95	95	99	97
P (serum≠water)		>0.5	0.3	>0.5	$F<1$	>0.5
Extraction R.S.D. %	3.11	0	0	0	3.03	0.65
Regression slope	1.02	1.01	1.05	1.04	0.93	0.92
P (slope≠1)	>0.2	>0.2	0.005	0.001	<0.001	<0.001
Curvature P	$F<1$	$F<1$	0.4	0.1	0.05	0.01
Sigmoidicity P	$F<1$	$F<1$	$F<1$	$F<1$	$F<1$	0.35
Nonparallelism P	0.5	0.2	0.2	0.2	0.35	>0.5

Height (area)/I.S.: peak height (area) relative to the internal standard peak height (area).
ser/wat%: percent ratio of measures from serum and from water samples.
P: significance level of F-tests; P (slope≠1): probability of slope being different from 1 (t-test).

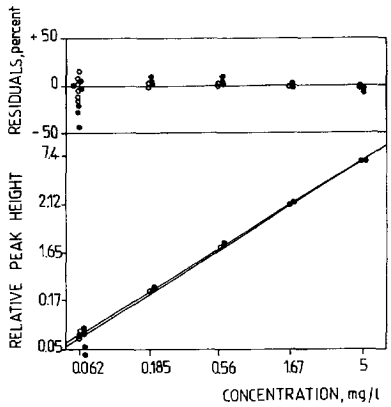

Fig. 3. Bilogarithmic response function: linear regression of itraconazole relative peak height vs. concentration from prevalidation samples. ○: Water samples; ●: serum samples.

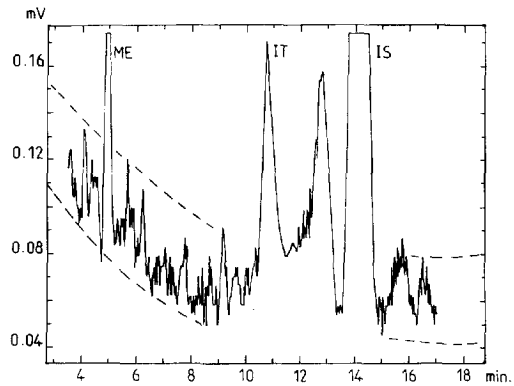

Fig. 4. Enlargement of chromatogram III from Fig. 2, used to estimate baseline noise.

Table 3
"Extraction recovery": percent ratio of peak height measures from extracted samples and from the corresponding extraction solution (standard deviation, $n=8$)

Matrix	Analyte	Concentration (mg/l)		
		0.0617	0.556	5.0
Water	OHIT	126 (6.5)	125 (0.9)	134 (0.9)
	IT	118 (9)	125 (1.8)	132 (0.6)
	I.S.	134 (6)	128 (1.7)	128 (1.6)
Serum	OHIT	135 (2.6)	123 (1.5)	132 (6.1)
	IT	125 (23)	123 (2.1)	130 (5.3)
	I.S.	160 (3.4)	132 (1.5)	133 (5.7)

nal standardization or not, by successively inputting the corresponding data into the statistical program. In the proposed itraconazole assay, it was clearly shown as reported in Table 2 that peak heights had better within-session repeatability than peak areas. This is not unexpected, since integration error mostly affects the base, i.e., the broadest part, of the peak. Another remark was that the double measurement involved in internal standardization did not increase the measurement variance. In other words, the safeguard against occasional technical defects (e.g., volumetric error) is not payed for with less precision, thus internal standardization is worthwhile and the substance chosen as internal standard may be considered adequate.

Table 4
Accuracy and precision of itraconazole assay determinations from relative peak height, all from six control replicates: %bias±%R.S.D. (*P* or N.S.)

	Concentration (mg/l)		
	0.067	0.555	5.0
1-point calibration (5 mg/l)			
1st session	−13±15 (NS)	−2.6±1.6 (0.015)	1.4±4.4 (NS)
2nd session	−12±9.2 (0.025)	0.4±3.5 (NS)	−0.6±3.0 (NS)
5-point calibration			
1st session	5.0±12.4 (NS)	−1.0±1.6 (NS)	1.2±4.4 (NS)
2nd session	13±7.2 (0.01)	3.0±3.5 (NS)	−0.5±0.3 (NS)
1st+2nd	12±7.4 (0.015)	5.1±3.5 (0.02)	1.9±3.0 (NS)

R.S.D.: relative standard deviation (C.V.) of determinations.
Bias: relative mean deviation from nominal concentration.
P: probability of bias being different from zero (*t*-test).
1st+2nd: second session controls calculated with the mean regression line of the two calibrations.

The prevalidation design proved also useful in assessing the effect of the matrix and its repeatability during sample processing. For example, a tentative previous itraconazole technique that used acetonitrile–trichloroacetic acid deproteinization was shown to add a significant extraction component to the measurement variance and thus was abandoned. Interestingly, in the proposed technique (Table 2), measurements from serum and from water proved almost (95%) equivalent (i.e., there is little analyte retention on serum components). This (a) explains the very tiny contribution of extraction to the measurement error, and (b) allows to safely calibrate by incorporating calibrators to the AcN extraction solution and "extracting" blank serum, or even water, instead of spiking sera, which may considerably simplify routine analysis. Table 3 shows that measures from extracts are higher than measures from acetonitrile extracting solutions: the reason is that the demixed acetonitrile volume is lower than the added volume, whereas more lipophilic analytes are virtually completely extracted, which results in analyte concentration being higher in the demixed phase.

In addition to the estimation of the overall measurement R.S.D. directly from ANOVA, estimates of imprecision are calculated for every measured concentration in each matrix. This allows us to plot error R.S.D. versus concentration (ccn), and to fit it to some simple decreasing function, e.g., measurement R.S.D.$=b+a(1/\text{ccn})$ equivalent to: S.D.$=a+b\cdot$ccn, a model already proposed by Jelliffe [14].

Such an error function is likely to help assess the LODs and LOQs by usual statistics rather than by an arbitrary multiple of the measurement noise [13]. The LOD is the limit below which a measurement cannot be considered different from zero [15]. If the measurement (m) variation is assumed to be gaussian, then LOD is the confidence limit of the difference $m-0$. At the one-tailed alpha risk of 5% and ν degrees of freedom for the S.D. estimate, LOD$=t_\nu\cdot$S.D. with S.D.$=a+b\cdot$LOD. Therefore, LOD$=t_\nu\cdot(a+b\cdot\text{LOD})$ equivalent to LOD$=t_\nu\cdot a/(1-t_\nu\cdot b)$. In the itraconazole application, S.D.(ccn) was estimated from five times two pairs of measurements, whence $\nu=(5\cdot2)-2=8$ DF and $t_\nu=1.86$. When applied to IT and OHIT data, this calculation provided estimates of LOD of respectively, 0.024 mg/l and 0.026 mg/l, to be compared to the estimates given in Section 3 by reference to signal noise.

Concerning directly the assay precision, comparison of Tables 2 and 4 shows that estimates of measurement R.S.D.s as calculated in the prevalidation design fairly predict those that will be made from assay (mg/l) results during the validation procedure.

Considering the itraconazole response function, it appears that area measurement meets the criterion of analytical linearity: logarithmic slope not different from 1 and no significant curvature, whereas peak height or relative peak height give a logarithmic slope significantly higher than 1 and a significant curvature (Fig. 4, Table 2). In other words, whereas (relative) peak height measurement may be preferred because of its better repeatability, it may somewhat distort the Bert–Lambert behavior of peak area. For example, peak width (including tailing) may increase due to column saturation when the amount of analyte increases. This is typically observed with cationic (basic) analytes and silica-based stationary phases and suggests a partially adsorptive mechanism in addition to the phase partition main mechanism of reversed-phase HPLC. Peak flattening results in lowered height/area (H/A), or augmented A/H, ratio. Table 5 shows that the mean H/A ratio of the itraconazole peak lessens continuously as concentration (i.e., peak height) lessens, in water as well as in serum samples. This is opposite to saturation behavior. The ratio A/H may be preferred to H/A for the following reason. If the chromatographic peak is likened to a Gaussian distribution and A and H are measured in coherent units (e.g., mV and mV s), then when A equals 1, H equals $1/\sigma\cdot\sqrt{(2\pi)}$ and A/H equals $\sigma\cdot\sqrt{(2\pi)}$, i.e., 2.5σ, a commonly used dispersion parameter. This simple calculation virtually provides the traditional "width at half-height" which equals 2.36σ of a Gaussian peak. We are currently using A/H estimates to monitor the performance of our columns during time. If computation facilities are available, a more sensible correction of the "height distortion" could be: measuring heights and areas, plotting A/H vs. H during an assay session, fitting this plot e.g., with a polynomial f(H), and finally recalculating the area of every individual peak i as: calculated area=measured height$\cdot$f(H_i).

The lack of linearity of peak height causes more

Table 5
Itraconazole peak height/peak area ratio (s^{-1}), means from eight prevalidation measures

Concentration (mg/l)	0.062	0.185	0.556	1.67	5.0
Water samples					
Mean	0.0271	0.0286	0.0298	0.0306	0.0309
S.D.	0.0072	0.0010	0.0005	0.0004	0.0001
Serum samples					
Mean	0.0258	0.0298	0.0302	0.0307	0.0310
S.D.	0.0064	0.0018	0.0011	0.0006	0.0004

inaccuracy (bias) of mg/l determinations from the lowest IT controls when using current linear calibration. As it can be expected, inaccuracy on lower concentrations is less when calibration uses 5-points over the whole assay range instead of 1-point at the upper part (where precision is better). In fact, inaccuracy is merely more evenly distributed over the calibration range. However, Table 4 shows that the inaccuracy index differs between the two sessions, i.e., shows a random between-session component. In routine daily analysis, it is perhaps good practice using a "moving average regression" over two to five consecutive sessions, in order to lower the random part of these between-session differences. An illustration is given in Table 4 by averaging the regression parameters of the two sessions available.

It was observed by Leskinen et al. [16] that intra-laboratory analytical variance is currently greater than inter-laboratory variance and this unexpected observation was confirmed by Tsanaclis and Wilson [17] after an extensive survey. This was true as well for automated methods supplied with batch calibrators as for chromatographic methods currently using laboratory-made calibrators. The latter authors concluded that improved precision should be sought in more careful day-to-day calibration and standardization procedures. The question remains: how careful these controls, and which analytical parameters to put particularly under control? In the case of chromatographic assays, the above suggestions of restoring linearity and of using a moving average may be liable to improve the results obtained.

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