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ANALYTICAL METHOD VALIDATION AND INSTRUMENT PERFORMANCE VERIFICATION

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The *precision* of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple samples of the same homogeneous sample under prescribed conditions. Precision is usually investigated at three levels: repeatability, intermediate precision, and reproducibility. For simple formulation it is important that precision be determined using authentic homogeneous samples. A justification will be required if a

2.4.4 Robustness

The *robustness* of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in the analytical procedure parameters. The robustness of the analytical procedure provides an indication of its reliability during normal use. The evaluation of robustness should be considered

3.3.7 Robustness

ICH definition: The *robustness* of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal use.

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Precision should be considered at three levels: repeatability, intermediate precision, and reproducibility.

Repeatability. Repeatability expresses the precision under the same operating

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BIOANALYTICAL METHOD VALIDATION

Fabio Garofolo, Ph.D.

8.1.1 Definition of Bioanalytical Method Validation

Bioanalytical method validation is a procedure employed to demonstrate that an analytical method used for quantification of analytes in a biological matrix is reliable and reproducible to achieve its purpose: to quantify the analyte with a degree of accuracy and precision appropriate to the task. Validation data, through

conferences. At the beginning of this document the FDA states very clearly that its guidance for bioanalytical method validation represents its current thinking on this topic and that an alternative approach may be used if such an approach satisfies the requirements of applicable statutes and regulations [4]. This statement allows bioanalytical laboratories to adjust or modify the FDA recommendations, depending on the specific type of bioanalytical method used.

Compliance with the FDA guidance can be considered a minimum requirement to test the performance of a bioanalytical method. Due to the fact that the

- *Accuracy:* the degree of closeness of the determined value to the nominal or known true value under prescribed conditions. This is sometimes termed *trueness*.
- *Analyte:* a specific chemical moiety being measured, which can be intact drug, biomolecule, or its derivative, metabolite, and/or degradation product in a biological matrix.
- *Analytical run (or batch):* a complete set of analytical and study samples with the appropriate number of standards and QCs for their validation. Several runs (or batches) may be completed in one day, or one run (or batch) may take several days to complete.
- *Biological matrix:* a discrete material of biological origin that can be sampled and processed in a reproducible manner. Examples are blood, serum, plasma, urine, feces, saliva, sputum, and various discrete tissues.
- *Stock solutions:* the original solutions prepared directly by weighing the reference standard of the analyte and dissolving it in the appropriate solvent. Usually, stock solutions are prepared at a concentration of 1 mg/mL in methanol and kept refrigerated at -20°C if there are no problems of stability or solubility.
- *Calibration standard:* a biological matrix to which a known amount of analyte has been added or *spiked*. Calibration standards are used to construct calibration curves from which the concentrations of analytes in QCs and in unknown study samples are determined.
- *Internal standard:* test compound(s) (e.g., structurally similar analog, stable labeled compound) added to both calibration standards and samples at known and constant concentration to facilitate quantification of the target analyte(s).
- *Limit of detection (LOD):* the lowest concentration of an analyte that the bioanalytical procedure can reliably differentiate from background noise.
- *Lower limit of quantification (LLOQ):* the lowest amount of an analyte in a sample that can be determined quantitatively with suitable precision and accuracy.
- *Matrix effect:* the direct or indirect alteration or interference in response due to the presence of unintended analytes (for analysis) or other interfering substances in the sample.

- *Method:* a comprehensive description of all procedures used in sample analysis.
- *Precision:* the closeness of agreement (*degree of scatter*) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.
- *Processed sample:* the final extract (prior to instrumental analysis) of a sample that has been subjected to various manipulations (e.g., extraction, dilution, concentration).
- *Quantification range:* the range of concentration, including ULOQ and LLOQ, that can be quantified reliably and reproducibly with accuracy and precision through the use of a concentration–response relationship.
- *Recovery:* the extraction efficiency of an analytical process, reported as a percentage of the known amount of an analyte carried through the sample extraction and processing steps of the method.
- *Reproducibility:* the precision between two laboratories. It also represents precision of the method under the same operating conditions over a short period of time.
- *Sample:* a generic term encompassing controls, blanks, unknowns, and processed samples, as described below:
  - *Blank:* a sample of a biological matrix to which no analytes have been added that is used to assess the specificity of the bioanalytical method.
  - *Quality control sample (QC):* A spiked sample used to monitor the performance of a bioanalytical method and to assess the integrity and validity of the results of the unknown samples analyzed in an individual batch.
  - *Unknown:* a biological sample that is the subject of the analysis.
- *Selectivity:* the ability of the bioanalytical method to measure and differentiate the analytes in the presence of components that may be expected to be present. These could include metabolites, impurities, degradants, or matrix components.
- *Stability:* the chemical stability of an analyte in a given matrix under specific conditions for given time intervals.
- *Standard curve:* the relationship between the experimental response value and the analytical concentration (also called a *calibration curve*).
- *System suitability:* determination of instrument performance (e.g., sensitivity and chromatographic retention) by analysis of a reference standard prior to running the analytical batch.
- *Upper limit of quantification (ULOQ):* the highest amount of an analyte in a sample that can be determined quantitatively with precision and accuracy.
- *Validation*
  - *Full validation:* establishment of all validation parameters to apply to sample analysis for the bioanalytical method for each analyte.
  - *Partial validation:* modification of validated bioanalytical methods that do not necessarily call for full revalidation.

    - *Cross-validation:* comparison of validation parameters of two bioanalytical methods.
- *Working solutions:* solutions prepared from the stock solution through dilution in the appropriate solvent at the concentration requested for spiking the biological matrix.

8.2.6 Dilutions

The ability to dilute samples originally above the upper limit of the standard curve should be demonstrated by accuracy and precision parameters in the validation.

the internal standard should be assessed over the anticipated run time for the batch size in validation samples by determining concentrations on the basis of original calibration standards. Reinjection reproducibility should be evaluated to determine if an analytical run could be reanalyzed in the case of instrument failure.

Limit of Detection

2000 Conference: the lowest concentration of an analyte that a bioanalytical procedure can reliably differentiate from background noise.

1990 Conference: the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels.

Lower Limit of Quantification

2000 Conference: the lowest amount of an analyte in a sample that can be determined quantitatively with suitable precision and accuracy.

Comment:

Upper Limit of Quantification

2000 Conference: the highest amount of an analyte in a sample that can be determined quantitatively with precision and accuracy.

Comment:

Accuracy

2000 Conference: the degree of closeness of the determined value to the nominal or known true value under prescribed conditions. This is sometimes termed *trueness*.

Comments:

8.3.2 Selectivity/Specificity

Selectivity is the ability of the bioanalytical method to measure and differentiate the analytes in the presence of components that may be expected to be present. *Specificity* is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. In general, analytical methods are selective, and only in some cases also specific (e.g., an LC-MS/MS

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An analysis of the SFSTP guide on validation of chromatographic bioanalytical methods: progresses and limitations

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Abstract

The Société Française des Sciences et Techniques Pharmaceutiques (SFSTP) published in 1997 a guide on the validation of chromatographic bio-analytical methods, which introduces new concepts in three different areas: stages of the validation, test of acceptability of a method and design of experiments to perform. In 'stages of validation', the SFSTP guide requires two phases to validate a method. The first phase, called 'prevalidation', is intended to (1) identify

1. Introduction

Before using an analytical method for quantitative determinations of drugs and their metabolites, an applicant laboratory must first demonstrate that the envisaged method fulfils a number of performance criteria. Since the publications of the 'Washington Conference' [1] and the ICH Guidelines on Validation of Analytical Methods Q2A and Q2B [2,3], which list the performance criteria to reach from a regulatory point of view, many laboratories have started to redesign their processes by involving analysts and statisticians, in order to define strategies that will allow the fulfilment of the regulatory requirements, while

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tions are now available [5,6] that provide the analyst, on the one hand, with a better understanding on the way to proceed and on the other hand, real data for qualifying his own computations that he could perform using a commercial spreadsheet.

other words, questions about the bias, precision and robustness must conduct the actions of the analyst developing a new method and no more focus its efforts only on some performance criteria, such as minimal resolution or maximal retention (migration) time in the case of chromatographic or electrophoretic methods. The ability of an analy-

as stability and robustness.

The objectives of the present article are precisely to identify and explain the progress permitted by the SFSTP guide, point out some of the limitations and suggest ways to overcome them.

and the analyst himself that the method can indeed be used for its intended purpose. The validation phase can absolutely not be envisaged as a mean to estimate the performance of the method. If nothing or very little is known about the bias, the

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Themed Issue: Bioanalytical Method Validation and Implementation: Best Practices for Chromatographic and Ligand Binding Assays
Guest Editors - Mario L. Rocci Jr., Vinod P. Shah, Mark J. Rose, Jeffrey M. Sailstad

Key Elements of Bioanalytical Method Validation for Small Molecules

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Bioanalytical methods are used for the quantitation of drugs and their metabolites in biological matrices. In today's drug development environment, highly sensitive and selective methods are required to quantify drugs in matrices such as blood, plasma, serum, or urine. Chromatographic methods (high-performance liquid chromatography [HPLC] or gas chromatography [GC]) have been widely used for the bioanalysis of small molecules, with liquid chromatography coupled to triple quadrupole mass spectrometry (LC/MS/MS) being the single most commonly used technology. for validation parameters are obtained. The essential parameters required according to the FDA Guidance[1] are selectivity, sensitivity, accuracy, precision, reproducibility, and stability. While obtaining these parameters, other parameters are also determined during validation (eg, extraction efficiency, calibration range and response function [linear or nonlinear], positional differences within an analytical run, and dilution integrity for analyzing above limit of quantitation [ALQ] samples). These validation parameters are described below in detail and are summarized in Table 1.

evaluated at room temperature for at least 6 hours. If the stock solutions are kept refrigerated or frozen over a period of time, the stability over that period should be evaluated by comparing the response of the aged stock solution to that of a freshly prepared stock solution. Stock solution stability should be performed at one concentration in at least duplicate.

stability should be determined at several time points (eg, 1, 3, 6, 9, and 12 months) depending on the length of stability required. If possible, it is recommended that some stored in vivo samples are analyzed to assess the long-term stability of incurred samples at storage temperature. Upon obtaining the long-term stability data, the validation report can be amended to include the stability results or a separate report can be

minimum of 2 concentrations (eg, low and high QC concentrations) are frozen overnight, at normal storage temperature (eg, −20°C or −70°C) and thawed unassisted at room temperature. When completely thawed, the samples are frozen again at the same temperature for 12 to 24 hours and thawed. This freeze-thaw cycle

The relationship between the detector response and concentration should be demonstrated to be well defined and reproducible. A calibration curve should consist of a blank sample (matrix sample processed without the IS), a zero standard (matrix sample processed with internal standard), and 6 to 8

nonzero standards. The number of standards can be increased for a complex curve or a curve covering a very large range. The simplest relationship that provides acceptable back-calculated concentrations for the standards should be used to fit the calibration curve. If a weighting factor is used, it should be defined during validation. The concentrations of calibration standards are back-calculated, and the residuals (difference between the back-calculated concentration of the calibration standard and its nominal concentration) determined. The residuals should be no more than ±15% at all concentrations except at the LLOQ level, where they can be up to ±20% of the nominal value. To accept an analytical run, at least 75% of the calibration standards should meet the stated acceptance criteria. Calibration standards not meeting the acceptance criteria should be eliminated from the calibration curve calculations. No extrapolation from the calibration curves is allowed, therefore the range of the calibration curve will be truncated if the end points on the calibration curve are eliminated.

Positional Differences

During a chromatographic analysis, samples are injected in sequence over several hours. Therefore, it is important to determine if the sample position in the chromatographic run sequence has an influence on the observed response (eg, if there is response change over the course of the run or any carryover is observed from previous samples). An evaluation of the situation should be done during the validation of the method and monitored during sample analysis. Procedures

http://www.pharmaresearchlibrary.com/wp-content/uploads/2013/03/Method-Validation-in-Pharmaceutical-Analysis.pdf

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Precision should be obtained preferably using authentic samples. As parameters, the standard deviation, the relative standard deviation (coefficient of variation) and the confidence interval should be calculated for each level of precision.

Repeatability expresses the analytical variability under the same operating conditions over a short interval of time (within-assay, intra-assay). At least nine

Intermediate precision includes the influence of additional random effects within laboratories, according to the intended use of the procedure, for example, different days, analysts or equipment, etc.

Reproducibility, i.e., the precision between laboratories (collaborative or interlaboratory studies), is not required for submission, but can be taken into account for standardisation of analytical procedures.

2.1.2.3 Intermediate Precision and Reproducibility

Intermediate precision includes the influence of additional random effects according to the intended use of the procedure in the same laboratory and can be regarded as an (initial) estimate for the long-term variability. Relevant factors, such as operator, instrument, and days should be varied. Intermediate precision is obtained from

There has been some controversial discussion about the terminology for this validation characteristic. In contrast to the ICH, most other analytical organisations define this as *selectivity*, whereas specificity is regarded in an absolute sense, as the "*ultimate degree of selectivity*" (IUPAC) [68]. Despite this controversy, there is a broad agreement that specificity/selectivity is the critical basis of each analytical procedure. Without a sufficient selectivity, the other performance parameters are meaningless.

2.7.1.1 International Conference on Harmonisation (ICH)

According to ICH Q2A [1a] *"the robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage"*.

Furthermore, it is stated in ICH Q2B [1b], *"The evaluation of robustness should be considered during the development phase and depends on the type of procedure under*

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Guidance for Industry

U.S. Department of Health and Human Services Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Veterinary Medicine (CVM)
May 2001

Bioanalytical Method Validation

three) will depend on the total number of samples in the run. The results of the QC samples provide the basis of accepting or rejecting the run. At least four of every six QC samples should be within "15% of their respective nominal value. Two of the six QC samples may be outside the "15% of their respective nominal value, but not both at the same concentration.

each run. The results of the QC samples provide the basis of accepting or rejecting the run. At least 67% (four out of six) of the QC samples should be within 15% of their respective nominal (theoretical) values; 33% of the QC samples (not all replicates at the same concentration) can be outside the ±15% of the nominal value. A confidence interval approach yielding comparable accuracy and precision is an appropriate alternative.

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REVIEW PAPER

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Bioanalytical method validation and its implications for forensic and clinical toxicology – A review

cent years. This is also reflected in the increasing requirements of peer-reviewed scientific journals concerning method validation. Therefore, this topic should be extensively discussed on an international level to reach a

A review on validation of bioanalytical methods was published by Karnes et al. in 1991 which was intended to provide guidance for bioanalytical chemists [1]. One

year later, Shah et al. published their report on the conference on "Analytical Methods Validation: Bioavailability, Bioequivalence and Pharmacokinetic Studies" held in Washington in 1990 (Conference Report) [2]. During this conference, consensus was reached on which parameters of bioanalytical methods should be evaluated, and some acceptance criteria were established. In the following years, this report was actually used as guidance by bioanalysts. Despite the fact, however, that some principle questions had been answered during this conference, no specific recommendations on practical issues like experimental designs or statistical evaluation were made. In 1994, Hartmann et al. analysed the Conference Report performing statistical experiments on the established acceptance criteria for accuracy and precision [3]. Based on their results they questioned the suitability of these criteria for practical application. From 1995 to 1997, application issues like experimental designs and statistical methods for bioanalytical method validation were discussed in a number of publications by Dadgar et al. [4, 5], Wieling et al. [6], Bressolle et al. [7] and Causon [8]. An excellent review on validation of bioanalytical chromatographic methods was published by Hartmann et al. in 1998, in which theoretical and practical issues were discussed in detail [9]. In an update of the Washington Conference in 2000, experiences and progress since the first conference were discussed. The results were again published by Shah et al. in a report (Conference Report II) [10], which has also been used as a template for guidelines drawn up by the U.S. Food and Drug Administration (FDA) for their own use [11]. Besides, it should be mentioned that some journals like the Journal of Chromatography B [12] or Clinical Chemistry have established their own criteria for validation. Two other documents that seem to be important in this context have been developed by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and approved by the regulatory agencies of the European Union, the United States of America and Japan. The first, approved in 1994, concentrated on the theoretical background and definitions [13], the second, approved in 1996, on methodology and practical issues [14]. Both can be downloaded from the ICH homepage free of charge (www.ich.org). Finally, in 2001 Vander Heyden et al. published a paper on experimental designs and evaluation of robustness/ruggedness tests [15]. Despite the fact that the three last mentioned publications were not especially focussed on bioanalytical methods, they still contain helpful guidance on some principal questions and definitions in the field of analytical method validation.

Terminology

The first problem encountered when studying literature on method validation are the different sets of terminology employed by different authors. A detailed discussion of this problem can be found in the review of Hartmann et al. [9]. Therein, it was proposed to adhere, in principle, to the terminology established by the ICH [13], except for accuracy, for which the use of a more detailed definition was recommended (cf. Accuracy). However, the ICH terminology lacked a definition for stability, which is an important parameter in bioanalytical method validation. Furthermore, the ICH definition of selectivity did not take into account interferences that might occur in bioanalysis (e.g. from metabolites). For both parameters, however, reasonable definitions were provided by Conference Report II [10].

Validation parameters

There is a general agreement that at least the following validation parameters should be evaluated for quantitative procedures: selectivity, calibration model (linearity), stability, accuracy (bias, precision) and limit of quantification. Additional parameters which might have to be evaluated include limit of detection, recovery, reproducibility and ruggedness (robustness) [2, 4–10, 12].

Selectivity (specificity)

In Conference Report II, selectivity was defined as follows: "Selectivity is the ability of the bioanalytical method to measure unequivocally and to differentiate the analyte(s) in the presence of components, which may be expected to be present". Typically, these might include metabolites, impurities, degradants, matrix components, etc. [10]. This definition is very similar to the one established by the ICH [13], but takes into account the possible presence of metabolites, and thus is more applicable for bioanalytical methods.

There are two points of view on when a method should be regarded as selective. One way to establish method selectivity is to prove the lack of response in blank matrix [1, 2, 4–10, 12, 14]. The requirement established by the Conference Report [2] to analyse at least six different sources of blank matrix has become state of the art. However, this approach has been subject to criticism in the review of Hartmann et al., who stated from statistical considerations, that relatively rare interferenc-

1. Karnes HT, Shiu G, Shah VP (1991) Pharm Res 8:421–426
2. Shah VP, Midha KK, Dighe S, McGilveray IJ, Skelly JP, Yacobi A, Layloff T, Viswanathan CT, Cook CE, McDowall RD, Pittman KA, Spector S (1992) Pharm Res 9:588–592
3. Hartmann C, Massart DL, McDowall RD (1994) J Pharm Biomed Anal 12:1337–1343
4. Dadgar D, Burnett PE (1995) J Pharm Biomed Anal 14:23–31
5. Dadgar D, Burnett PE, Choc MG, Gallicano K, Hooper JW (1995) J Pharm Biomed Anal 13:89–97
6. Wieling J, Hendriks G, Tamminga WJ, Hempenius J, Mensink CK, Oosterhuis B, Jonkman JH (1996) J Chromatogr A 730:381–394
7. Bressolle F, Bromet PM, Audran M (1996) J Chromatogr B 686:3–10
8. Causon R (1997) J Chromatogr B 689:175–180
9. Hartmann C, Smeyers-Verbeke J, Massart DL, McDowall RD (1998) J Pharm Biomed Anal 17:193–218
10. Shah VP, Midha KK, Findlay JW, Hill HM, Hulse JD, McGilveray IJ, McKay G, Miller KJ, Patnaik RN, Powell ML, Tonelli A, Viswanathan CT, Yacobi A (2000) Pharm Res 17:1551–1557
11. U.S. Department of Health and Human Services, Food and Drug Administration (2001) Guidance for industry, bioanalytical method validation http://www.fda.gov/cder/guidance/index.htm
12. Lindner W, Wainer IW (1998) J Chromatogr B 707:1–2
13. International Conference on Harmonization (ICH) (1994) Validation of analytical methods: Definitions and terminology ICH Q2 A
14. International Conference on Harmonization (ICH) (1996) Validation of analytical methods: Methodology ICH Q2 B
16. Penninckx W, Hartmann C, Massart DL, Smeyers-Verbeke J (1996) J Anal At Spectrom 11:237–246
17. International Organization for Standardization (ISO) (1994) Accuracy (trueness and precision) of measurement methods and results: ISO/DIS 5725–1 to 5725–3. ISO, Geneva, Switzerland
18. NCCLS (1999) Evaluation of precision performance of clinical chemistry devices; Approved guideline. NCCLS, Wayne, Pa., USA
19. Toennes SW, Kauert GF (2001) J Anal Toxicol 25:339–343
20. EURACHEM/CITAC (2000) Quantifying uncertainty in analytical measurement http://www.eurachem.bam.de/guides/quam2.pdf

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Determination of loratadine and its active metabolite in human plasma by high-performance liquid chromatography with mass spectrometry detection

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Two stock solutions of loratadine and desloratadine, respectively, with concentration of 2.5 mg/ml were prepared by dissolving appropriate quantities of reference substances (weighed on an Analytical Plus balance from Ohaus, USA) in 10 ml methanol. Two working solutions were then obtained for each

2.6. Validation

As a first step of method validation [19–21], specificity was verified using six different plasma blanks obtained from

healthy human volunteers who had not previously taken any medication.

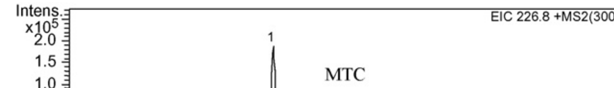
The concentration of analytes was determined automatically by the instrument data system using the internal standard method. Calibration was performed using singlicate calibration standards on five different occasions. The calibration curve model was determined by the least squares analysis. The applied calibration model was $y = c + bx + ax^2$, weight $1/y$ $(1/y)$ quadratic response, where y, area ratio and x, concentration ratio. Distribution of the residuals (%difference of the back-calculated concentration from the nominal concentration) was investigated. The calibration model was accepted, if the residuals were within

long-term stability, in the first validation day, there were injected and analyzed four samples at each of low and high concentrations, and values were calculated against calibration curve of the day. Other two sets with the same plasma concentrations were stored in freezer below −20 °C and analyzed together with calibration samples after 5 months. The values were calculated against calibration curve of the day and the mean values for the stored samples and nominal concentrations were compared. The requirement for stable analytes was that the difference between mean concentrations of the tested samples in various conditions and nominal concentrations had to be in ±15% range.

The within- and between-run precision (expressed as coefficient of variation, CV%) and accuracy (expressed as relative difference between obtained and theoretical concentration, bias%) of the assay procedure were determined by analysis on the same day of five different samples at each of the lower (1.68 ng/ml), medium (10.47 ng/ml) and higher (20.94 ng/ml) levels of the considered concentration range and one different sample of each on five different occasions, respectively. The selected concentration values are relevant in practice taking in account to the ensure that it continued to perform satisfactorily during analysis of volunteer samples. To achieve this objective, a number of QC samples prepared in duplicate at three concentration levels were analyzed in each assay run and the results compared with the corresponding calibration curve. At least 67% (four out of six) of the QC samples should be within 15% of their respective nominal values; 33% of the QC samples (not all replicates at the same concentration) can be outside ±15% of the nominal value.

The stability of the analytes in human plasma was investigated in three ways, in order to characterize each operation during the process of bioequivalence studies: room-temperature stability (RTS), post-preparative stability (PPS) in the autosampler, freeze-thaw stability (FTS) and long-term stability (LTS) below −20 °C. For all stability studies, plasma standards at low (1.68 ng/ml both LOR and DSL) and high concentrations (20.94 ng/ml both LOR and DSL) were used. Four plasma

case of desloratadine, the sum of ions from MS spectrum (*m*/*z* 259, 294, 282) was chosen for quantification because the detection is about 40% more sensitive that the case based only on ion *m*/*z* 259.



of the samples in autosampler before injection. For the freeze-thaw stability, aliquots at the same low and high concentrations were prepared. These samples were subjected to three cycles of freeze-thaw operations in 3 consecutive days. After the third cycle, the samples were analyzed against calibration curve of the day. The mean concentration calculated for the samples subjected to the cycles and the nominal ones were compared. For

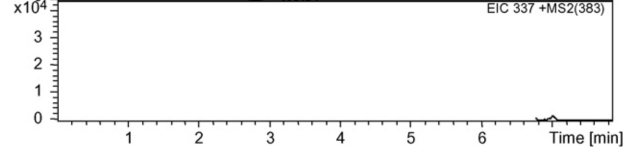


Fig. 2. Chromatograms of a plasma blank containing metoclopramide (MTC, internal standard).

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New validated method for piracetam HPLC determination in human plasma

Augustin Curticapean *, Silvia Imre

system and were injected in time, after the following timetable: 2, 5, 7, 10, 24, 36 and 48 h. For the freeze-thaw stability (FTS), aliquots at the same low (10 μg/ml) and high (100 μg/ml) concentrations were prepared. These samples were subjected to 3 cycles of freeze-thaw operations in three consecutive days. After the third cycle the samples were analyzed against calibration curve of the day. The mean concentration calculated for the samples subjected to the cycles and the nominal ones were compared. For long-term stability (LTS), in the first validation day, there were injected and analyzed four samples at each of low (10 μg/ml) and high (100 μg/ml) concentrations, and values were calculated against calibration curve of the day. Other three sets with the same plasma concentrations were stored in freezer below −20 °C and analyzed together with calibration samples, after two, three and four weeks, each time being used four pairs of replicates for low and high concentrations. The values were calculated against calibration curve of the day and the mean values for the stored samples and nominal concentrations were compared.

The requirement for a stable analyte in the frozen matrix is that the difference between mean concentration of the stored samples and nominal concentration is between ±15%.

The recovery was determined at four levels of concentration as follows: one for the LLQ concentration, and the rest for the QC concentrations. Five replications of each level of con-