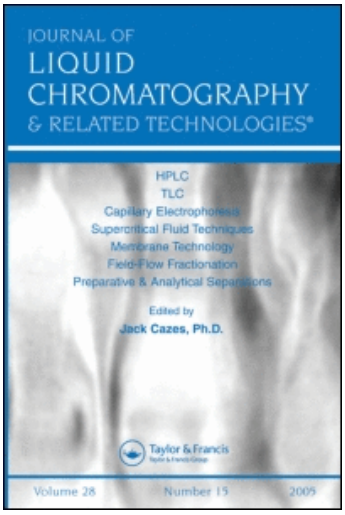

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Akgöl Yesilada[a]; Nesrin Gökhan[a]; Birsen Tozkoparan[a]; Mevlut Ertan[a]; Hassan Y. Aboul-Enein[a]
[a] Faculty of Pharmacy Department of Pharmaceutical Chemistry, Hacettepe University, Ankara, Turkey

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METHOD VALIDATION IN PHARMACEUTICAL ANALYSIS: FROM A GENERAL APPROACH TO CAPILLARY ELECTROPHORESIS

Akgül Yesilada,[1] Nesrin Gökhan,[1] Birsen Tozkoparan,[1]
Mevlut Ertan,[1] Hassan Y. Aboul-Enein[2,*]

[1] Hacettepe University
Faculty of Pharmacy
Department of Pharmaceutical Chemistry
06100 Ankara, Turkey

[2] Bioanalytical and Drug Development Laboratory
Biological and Medical Research Department (MBC-03)
King Faisal Specialist Hospital and Research Centre
P. O. Box 3354
Riyadh, Saudi Arabia

ABSTRACT

This paper gives a rapid review on analytical method validation with special emphasis on capillary electrophoresis. The primary validation parameters such as accuracy, precision, specificity, linearity, and sensitivity are defined; the evaluation procedures are outlined. The recent reports covering the applications on the subject are summarized and discussed.

INTRODUCTION

Validation is an important issue in pharmaceutical analysis and widely required in industrial product development and registration. Simply, it is a tool

used to justify the analytical method used, in other words, to show that the method accomplishes what is claimed or intended. The importance of the subject and detailed explanations on performance criteria had been reported and discussed before by several authors specialized on the subject.[1-5] As deduced from these reports, validation is required for development of a new analytical method, analytical methods submitted as a part of new drug applications (NDA) or abbreviated new drug applications (ANDA), development of a new analytical method, bioequivalance and bioavailability studies, and for the analysis of pharmaceutical samples, and can be performed in three steps as:

1. Identification of appropriate validation parameters.
2. Design of experiments for parameter evaluation.
3. Determination of acceptance criteria.

The contributors to the validation issue cover agencies from various parts of the world, which creates different approaches in application. The Third International Conference on Harmonization (ICH3) has published a text for the use of applicants to bring a solution to this argument. [6]

In the last decade capillary electrophoresis has developed to a promising method in pharmaceutical science especially in areas such as formulation analysis, impurity testing, and pharmaceutical biotechnology. The method may be considered as "new" with respect to application areas, for which the validation phenomena is widely discussed. Recently, several satisfactory reports have been presented to the literature on this subject.[7-10] In this paper, we aimed to review the validated capillary electrophoresis application studies of the past three years in the light of the ICH Guidelines and present a comparative overview. For this purpose, the definitions and assessment procedures of the validation parameters as given in ICH Guidelines were summarized and the selected validated capillary electrophoresis studies were classified according to ICH types of procedures and the knowledge covering the validation experiments were tabulated for the ease of the reader.

Types of Analytical Procedures to be Validated According To ICH3

a. Identification tests.

b. Chiral or achiral impurity tests (Quantitative measurements of content of impurities and limit tests).

c. Main components assay (quantitative measure of active moiety in samples of drug substances or other selected components in the drug product).

Definitions and Assessment Procedures of the Validation Parameters

Accuracy is defined as "the closeness of agreement between the value, which is accepted either as a conventional value, or an accepted reference value and the value, found." Procedures for the assessment of accuracy can be outlined as follows:

Spiked placebo method (if drug product components, samples of impurities (degradation products etc.) are available.

Standard addition method (if drug product components, samples of impurities are not available).

During acquisition of precision, linearity or specificity data using an appropriate experimental design.

The accuracy of the method should be assessed by a minimum of nine determinations over a minimum of three concentration levels within the prescribed range. The mean results should be reported either as percent recovery or by plotting the recovered amount versus theoretical value.

Precision is "closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions" and may be performed at three levels:

Repeatability (intra-assay precision) is determined under the same operating conditions over a short interval of time. It is assessed by a minimum of nine determinations over the prescribed range for the procedure; e.g. at three concentrations, three replicates each or by a minimum of six determinations at 100% of the test concentration.

Intermediate precision is the establishment of the effect of variations on precision such as different days, analysts, equipment, reagents, etc. Required data for the evaluation of results are the standard deviation, relative standard deviation (coefficient of variation), and confidence intervals.

Reproducibility expresses the precision between laboratories.

Specificity is "the ability of a method to measure only what it is intended to measure" or "the ability to assess unequivocally the analyte in the presence of components which may be expected to be present." The interfering components may be inactive excipients, degradation products, synthetic impurities and precursors, or biological material.

Assessment may be performed by the analysis of a placebo (the sample matrix) without the analyte. There should be no interfering responses. If impurities or matrix material are not available, standard addition methods may be used. For this purpose samples are prepared in the matrix and standards without matrix at equivalent concentrations and analyzed or samples and standards are fortified with equivalent levels of analyte and analyzed. The sample and standards must be in agreement. In addition to that peak re-analysis may be performed by another chromatographic technique or peak purity testing may be carried out by using more informative detectors such as mass spectrometric detector, multiple wavelength uv detector, or diode array detector.

Linearity is "the procedures' ability to obtain test results which are proportional to the concentration of the analyte in the sample within a given range." It should be established across the range of analytical procedure. For this purpose the response is plotted against the analyte concentration. Visual evaluation should establish linearity. If there is linearity, test results are evaluated by an appropriate statistical method such as regression analysis, calculation of correlation coefficient, y-intercept, slope, residual sum of squares. A minimum of five concentrations are used and may be demonstrated on synthetic mixtures.

Range is "the interval between the upper and lower concentration of the analyte (including. these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity."

Sensitivity is "the ability of the method to measure decreasingly small amounts of analyte." Measures of sensitivity are detection limit LD (or limit of detection LOD) and quantitation limit LQ (or limit of quantitation-LOQ).

Detection limit is defined as "the lowest amount of analyte in the sample which can be detected" and may not necessarily be quantitated as exact value.

Quantitation limit is "the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy."

Determination of these parameters can be done by various techniques such as visual evaluation, signal-to-noise ratio, standard deviation of the response, and the slope, and standard deviation of the blank, calibration curve.

Robustness shows the reliability of an analyte with respect to deliberate variations in method parameters. Typical variations are stability of analytical solutions, different equipment, and different analyses.

Capillary Electrophoresis in Pharmaceutical Analysis: The Reflections of Previous Contributions to Recent Applications

Capillary electrophoresis has emerged as an alternative method for HPLC in pharmaceutical analysis, by its practical applicability and low cost. The problems faced during application had been lack of precision and low sensitivity compared to HPLC; partially due to instrumentation and nature of the method. Since validation is the measure of the reliability of a method and especially important in quantitative analysis, the problems faced during method development in capillary electrophoresis extended to validation process and started a capillary electrophoresis focused discussion.

Apart from this, the definitions of validation terminology and evaluation of the defined parameters have been a subject of worldwide discussion amongst the analysts. The terms whose definitions especially discussed are specificity selectivity and reproducibility repeatability.

In the ICH3 text, the term specificity is preferred for selectivity. Repeatability, reproducibility, and intermediate precision are designated as level of precision and discriminated clearly by definition. Sensitivity is represented by the terms limit of detection and limit of quantitation.

In this study ten analytical works performed by validated capillary electrophoresis techniques were examined with respect to validation parameters. Four of these articles were chiral impurity testing while six were achiral impurity testing and main peak assay in formulated drug substances, which shows an increase in validated applications of capillary electrophoresis in pharmaceutical formulations.

In Tables 1 and 2, the articles are tabulated according to the active compound, mode of CE used, and each validation parameter performed is included with a brief description of the evaluation method.

Linearity was conventionally applied in all the work; it is not included in the tables. Accuracy tests were carried out in all the studies either by percent recovery[11,12,15-20] or by plotting theoretical versus practical values.[13-14]

Precision, one of the most discussed parameters of capillary electrophoresis, was evaluated as reproducibility in two of the studies to demonstrate the closeness of agreement between successive runs.[11,12] It is interesting to note that these articles were published in 1995 before the publication of ICH3 text. In contrast, in the other studies, the researchers preferred using the term repeatability and intermediate precision which shows that ICH3 Guidelines brought about the harmonization expected for this term.

Table 1

Studies Classified as Chiral Impurity Tests

Name of Active Cpd.	Mode of CE	Accuracy	Precision	Specificity	Sensitivity	Robustness	References
Triaprofenic acid (pharmaceutical formulation)	CZE-CD	Recovery as mean ±SD, STD% n=3 from tablet.	Reproducibility RSD%, n=8 at one conc. level, for peak area and resolution; used internal std.		LOD by S/N=3 method.	----	11
BMS-180431-09 new chlosterol-lowering drug (drug substance)	CD-MEKC	Recovery as percent.	Reproducibility, RSD% for repeated injections.	Comparison with LC.	LOD 3xSD/slope.	---	12
Ropivocaine (drug substance)	CZE-CD	Theoretical versus practical plot, r^2=0.995, range: 0-0.5%.	Repeatability at two conc. levels; RSD=5.6%, 3.0%, n=6.	By mixing the test solution with possible impurities representative electropherogram.	LOD= 0.05% by dilution, LOQ=0.01%, RSD=15%, n=6.	Tested by variations in conc., pH of BGE; conc of CD, analysis temp.	13

Ropivocaine (injection solution)	CZE-CD	Theoretical versus practical plot, r^2=0.999, range: 0-3%.	Repeatability at five different conc. levels; RSD=6.6-0.6%, n=6 intermediate precision, between assays.	Mixing samples matrix with analogues, representative electropherogram.	LOD=0.05%, by dilution, LOQ=0.1% by dilution, RSD=15%, n=6.	Tested by variations in and BGE conc., pH and temp.	14

Table 2

Studies Classified as Archiral Impurity Tests and Main Peak Assays

Name of Active Cpd.	Mode of CE	Accuracy	Precision	Specificity	Sensitivity	Robustness	References
Cefotoxime (drug substance)	MEKC	Recovery as percent.	Reproducibility (between days) repeatability (within day) for migration time and response factor. RSD=0.04-1.88%, n=10.	Peak purity assessment with photo-diodide array system.	LOD = 0.02% of main peak.	---	15
Oxytetracycline (ointment)	Non aqueous	By standard addition method at 3 conc. levels, as percent recovery, 96.7-98.7%.	Repeatability (overall) at three conc. levels, by standard addition, RSD=2.8-4.3%, n=6; for migration time within and between days.	(Selectivity) by looking for interfering impurities from the ointment matrix and degradation products.	---	---	16

Six water soluble vitamins (pharmaceutical formulation)	CZE	By spiking a placebo with six vitamins at three conc. levels (50,100,150%) percent recovery (98.2-101.3%).	Repeatability and intermediate precision at three conc. levels (50,100,150%), n=6, RSD=4.5-1.1%.	(Selectivity) by examining a synthetic mixture of excipients for interferences; by checking peak homogeneity by photo-diode array detector.	—	—	17
Mitoguazone, 2HCl (drug substance)	CZE	By analysing triplicate samples at four (80,100, 120%) conc. levels, recovery by least squares analysis, (99.1% as average).	As coefficient of variation of nine recovery results, RSD=2.5%.	—	For impurity levels, as 0.1% w/w of the standard.	—	18
BMS-188494-04 as cholesterol lowering drug (formulated in capsules)	MEKC	By spiking placebo capsule contents with active component at nominal conc. and known degradation products at one conc. level, as percent recovery, (99.9-103.7%).	Ten replicate determinations of active component from the same vial, RSD=0.5% (range 0.4-0.8 over three days).	Representative electropherogram of the active component in the presence of degradation products, injection of solutions of placebo capsule blends.	LOD and LOQ for known degradant, 0.05-0.1% determined by dilution of standard solution.	—	19

(continued)

Table 2 (continued)

Studies Classified as Archiral Impurity Tests and Main Peak Assays

Name of Active Cpd.	Mode of CE	Accuracy	Precision	Specificity	Sensitivity	Robustness	References
A range of acidic drugs and excipients	CZE	As percent recovery of the label claim.	Repeatability determined by duplicate analysis of ten calibration and sample solutions, for response factors and assay results.	(Selectivity) by constructing a database for screening a wide range of compounds in comparison with internal standard. Representative separations.	LOD (peak with 3xsignal to noise), LOQ (assessed by ten replicate injections of low level conc.).	For variation parameters such as rinse cycle, buffer conc., temp injection time and voltage; by using experimental designs.	20
Morphine in pharmaceuticals	Aqueous and non aqueous CZE	As percent recovery of the label claim assessed by standard addition method at three conc. levels (25,250, 100%) and by comparison with validated HPLC method	Repeatability determined by injecting six separately prepared samples, n=6.	LOD=0.3 μg/mL for aqueous and 0.2 μg/mL for non aqueous systems.	---	---	21

Naphazoline, HCL and degradation product naphtylacetylethy-lenediamine (NAED) in bulk drug	CZE	Theoretical versus practical plot r^2=0.999, range 0.5-16%.	Repeatability at one conc. level, RSD% for peak area (0.49), peak height (0.053) and migration time (0.08), n=5.	By mixing known amount of naphazoline, HCL with NAED at various levels (0.1-16%). Representative electropherogram.	LOD=0.25 μg/mL for degradation product, LOQ= 0.5μg/mL for degradation product, RSD= 3.99%, n=5.	—	22

On the other hand, ways of optimizing precision include pre-conditioning of the capillary and injection of buffer between injections, buffer replenishment at regular intervals to prevent depletion,[11-20] trimming and cleaning parts of the injection system, [16] use of internal,[7,10,11] or external standard.[8]

As for specificity, it is observed that some authors[16,17,20] still prefer the term selectivity instead of specificity as ICH3 recommended which gives the impression that the usage of this term is in question.

In addition to conventional methods of evaluation, comparison of the results with those obtained by HPLC or applying peak purity testing with photo-diode array detector[15,17,18] were noted as other ways of testing selectivity. For the improvement of selectivity buffer additives such as cyclodextrins and organic modifiers were used. Interestingly one the investigators recommended non-aqueous CE to improve selectivity.[21]

Sensitivity is another important validation parameter to be assessed especially in impurity testing. In the recent studies it is mentioned as LOD or LOQ depending on the purpose of the study. The values obtained were comparable to HPLC. The low injection volume which reduces sensitivity was compensated by using wide capillary bores, long injection times, high sample concentrations, use of water as sample solvent to produce sample stacking (except for non-aqueous CE), low uv wavelengths (195-200 nm),[11,12] and, as a new application, use of bubble capillaries.[10]

Robustness testing shows the reliability of the method with respect to deliberate variations in method parameters such as stability of solutions, buffer concentration, operating voltage, temperature, and capillaries. Researchers from the industry[13,14,20] carry out this parameter. The data obtained by variation of several parameters were evaluated by experimental design.

CONCLUSIONS

ICH3 Guidelines seems to have brought the expected harmonization in the application of validation issue in pharmaceutical analysis. The reflection of this development can be perceived in the increased number of validated capillary electrophoresis studies.

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