



# Quality criterion to optimize separations in capillary electrophoresis: Application to the analysis of harmala alkaloids



Marcos Tascon<sup>a,b</sup>, Fernando Benavente<sup>b</sup>, Cecilia B. Castells<sup>a</sup>, Leonardo G. Gagliardi<sup>a,\*</sup>

<sup>a</sup> Laboratorio de Investigación y Desarrollo de Métodos Analíticos, LIDMA and División Química Analítica Facultad de Ciencias Exactas, UNLP-CONICET, Calle 47 esq. 115, La Plata B1900AJL, Argentina

<sup>b</sup> Departament de Química Analítica, Facultat de Química, Universitat de Barcelona, Barcelona, Spain

## ARTICLE INFO

### Article history:

Received 30 March 2016

Received in revised form 7 July 2016

Accepted 13 July 2016

Available online 14 July 2016

### Keywords:

Capillary electrophoresis

Optimization

Quality criteria

Harmala alkaloids

## ABSTRACT

In capillary electrophoresis (CE), resolution ( $R_s$ ) and selectivity ( $\alpha$ ) are criteria often used in practice to optimize separations. Nevertheless, when these and other proposed parameters are considered as an elementary criterion for optimization by mathematical maximization, certain issues and inconsistencies appear. In the present work we analyzed the *pros* and *cons* of using these parameters as elementary criteria for mathematical optimization of capillary electrophoretic separations. We characterized the requirements of an ideal criterion to qualify separations within the framework of mathematical optimizations and, accordingly, propose: -1- a new elementary criterion ( $t'$ ) and -2- a method to extend this elementary criterion to compose a global function that simultaneously qualifies many different aspects, also called multicriteria optimization function (MCOF).

In order to demonstrate this new concept, we employed a group of six alkaloids with closely related structures (harmine, harmaline, harmol, harmalol, harmame and norharmame). On the basis of this system, we present a critical comparison between the new optimization criterion  $t'$  and the former elementary criteria. Finally, aimed at validating the proposed methods, we composed an MCOF in which the capillary-electrophoretic separation of the six model compounds is mathematically optimized as a function of pH as the unique variable. Experimental results subsequently confirmed the accuracy of the model.

© 2016 Published by Elsevier B.V.

## 1. Introduction

The development of an analytical separation method typically requires an optimization of the conditions [1–4]. First, the aspects of the analysis that will be optimized must be defined and these aspects constitute, conceptually, the *desirability* or the *optimization quality* and/or *qualification criterion* (QC). The definition of the QC should be designed according to the manner of optimization—i.e., a *maximization*, *minimization*, or a definition of *acceptability limits*. The QCs depend on *operational variables* (OVs), which can be discrete, such as the chemical nature of the background electrolyte (BGE) or type of additive, or continuous such as pH, temperature, or the concentration of components in the separation medium [5]. A given QC can be a very simple concept, with a clear and understandable physical meaning—such as selectivity ( $\alpha$ ), resolution ( $R_s$ ), analysis time ( $t_{an}$ ), and limit of detection (LOD)—or can also involve

a complex function [6–8]. Furthermore, in certain instances, more than one aspect can be optimized simultaneously (i.e., *multiobjective or multicriterion optimizations*) [5,9]. This situation obtains when the aim is to optimize the separation of several compounds, i.e. the optimization is based on many  $\alpha$  or  $R_s$  between all the pairs of compounds. Once all the QCs and OVs have been defined, a number of exploratory experiments are required to acquire real information about the system, in order to fit mathematical equations to the real values, to then interpolate or extrapolate those discrete observations, and finally to find the OV leading to the best possible QC. In one case, mathematical expressions used to fit the experimental results can be empirical and thus require a significant number of experiments to accurately establish the real dependencies of the QCs on the OVs. In the opposite case, the equations can have a sound basis in models derived from solid theories. Those equations require a reduced number of exploratory experiments for obtaining satisfying expressions describing the behaviors of the QC over wide ranges of the OVs so as to enable even extrapolations.

In chromatography, the separation between pairs of compounds is adequately characterized by  $R_s$ . The use of the  $R_s$  as the QC has been implemented in many optimization software programs

\* Corresponding author.

E-mail addresses: [leogagliardi@quimica.unlp.edu.ar](mailto:leogagliardi@quimica.unlp.edu.ar), [leogagliardi@fcnym.unlp.edu.ar](mailto:leogagliardi@fcnym.unlp.edu.ar) (L.G. Gagliardi).

[10–13]. Nevertheless, in CE, the number of phenomena affecting the peak widths and peak shapes are greater than in chromatography, thus requiring a large number of exploratory experiments in order to predict  $R_s$  [8–10]. Alternatively, other QCs could be used to describe the separation between analytes. In general, QCs based on mobilities offer the advantage that the dependence of that parameter on the different OV's becomes more predictable. As a result, the number of required experiments can be reduced significantly yielding favorable consequences in terms of optimization time, reduction of chemical waste, and operational costs [6,14,15]. This is the case when the OV to optimize is pH, a variable affecting directly the ionization of weak acids or bases [16]. The dependencies of effective mobilities on pH are well known, and expressions relating those two parameters can be easily deduced [2,3,17–19]. Different parameters have been proposed as QC for optimizing CE separations using pH as the OV. Giddings postulated an early definition of selectivity ( $p$ ), including a difference of velocities ( $\Delta U$ ) between the pair of ions under consideration and their average velocity ( $U$ ) [20]:

$$p = \frac{\Delta U}{U} \quad (1)$$

Later, Gebauer and Boček defined an analogous expression, but based on mobilities ( $\mu$ ) instead of velocities [16],

$$p = \frac{\Delta \mu}{\bar{\mu}} \quad (2)$$

Both expressions can be interconverted by taking into account the electric field,  $E$  ( $U = \mu \cdot E$ ). Selectivity between a pair of compounds ( $\alpha_{ij}$ ) is nowadays [6] defined as the ratio of their effective mobilities ( $\mu_{eff}$ ):

$$\alpha_{ij} = \frac{\mu_{eff,i}}{\mu_{eff,j}} \quad (3)$$

where  $\mu_{eff,i} > \mu_{eff,j}$ . This definition is algebraically analogous to the one used in chromatography. A relevant difference exists, however, when  $\alpha$  is used as a QC for separation optimization in chromatography vs. CE, principally related to the domains of the retention factors ( $k_{ij}$ ) and  $\mu_{eff}$ . The  $k_{ij}$  values can range from virtually zero—i.e., the void volume marker—up to infinity for any compound fully retained in the stationary phase. Moreover, based on physical fundamentals,  $k_{ij}$  is often modeled as a logarithm, under which the QC–OV relationships are usually linear or, at least, smooth for mathematical optimization by maximization or minimization. In contrast,  $\mu_{eff}$  values can range from certain discrete positive numbers—i.e., the  $\mu_{eff}$  of the hydrogen ion—to other discrete negative numbers—i.e., the  $\mu_{eff}$  of the hydroxyl ion. Furthermore, many analytes, such as those used in this study, are uncharged within significant zones of the OV domains—e.g., at isoelectric points or pHs more than 2 units away from the  $pK_a$  values. Since inversions in the migration order and reversal of the migration direction are frequent, those changes necessitate a redefinition of  $\alpha$  over the OV scales, resulting in discontinuities and nonderivable points—all of which features constitute drawbacks to mathematical optimizations. These explanations based on the selectivity definition exemplify the importance of a proper definition of QC in determining the success of mathematical optimization. With respect to CE the following considerations must be taken into account:

-1- QCs must be mathematically monotonous over the whole range even when the order of migration between analytes changes. For instance, this issue is relevant to any QCs involving a mobility difference that is positive by definition, or involving a quotient such as  $\alpha_{ij}$ , which must be  $>1$  by definition.

-2- QC must likewise be monotonous when the mobility of one analyte changes its sign. In general, this situation occurs with QCs

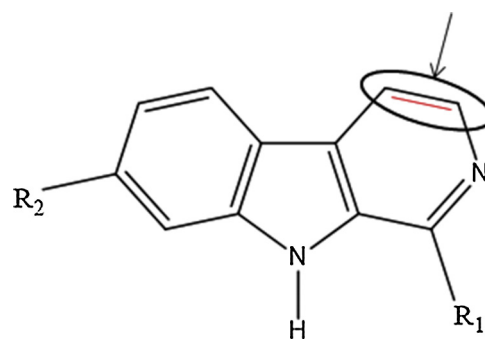


Fig. 1. Basic  $\beta$ -carboline structure of the harmala alkaloids (HALks). Table 1 lists the substituents R1 and R2 and the presence of unsaturation.

given as a multiplication or quotient, such as  $\alpha_{ij}$  as a function of pH around an isoelectric point.

-3- QC must give the same weight to the separations over the entire OV domain and specifically must not approach to infinite when the mobility of one analyte approaches zero. For example, an extreme situation is the co-migration of the slower analyte with all the neutral substances, always present in real samples. The presence of neutral species requires that CE takes into consideration the mobility of all those uncharged species (along with the EOF markers and solvent peaks) as an additional peak from which the analyte peaks should be separated.

Some of these optimization issues have been identified in the early 80's. In an attempt to overcome those problems, Jorgenson and Lukacs included the EOF in the denominator of their definition of selectivity ( $p'$ ) [5]:

$$p'_{ij} = \frac{\mu_i - \mu_j}{\mu_{EOF} + \mu_j} \quad (4)$$

where  $\mu_i > \mu_j$ . Eq. (4), although, is an incomplete solution because the denominator can still become zero. Furthermore, the inclusion of  $\mu_{EOF}$  confers on  $p'$  a dependence on a property having a known variability that depends on the material of the capillary wall and changes with the physical and chemical properties of the BGE [21–24].

In 1994, B.K. Clark et al. proposed the use of the difference between the effective mobilities of the analytes being considered,  $d_{ij}$  [25]:

$$d_{i,j} = \mu_{eff(i)} - \mu_{eff(j)} = \Delta \mu_{i,j} \quad (5)$$

where  $\mu_{eff,i} > \mu_{eff,j}$ . This definition overcomes the mathematical drawback of ratios becoming infinite when denominators approach zero, although,  $d_{ij}$  still retains the shortcomings of changes in migration order and also can give false optimum values that fail to become separated from neutral species.

In this work, we propose an alternative criterion to qualify the separations in CE. Our aim was to develop the simplest mathematical function that would overcome all the aforementioned difficulties. We also describe how to compose a multicriterion function based on an elementary QC that allow a true mathematical optimization in the separation of more than two compounds. The theoretical prediction based on this novel criterion was tested by optimizing the separation of six harmala alkaloids (HALks) (harmine, harmaline, harmol, harmalol, harmane and norharmane) as a function of pH. These compounds share a  $\beta$ -carboline structure (see Fig. 1) and differ only in either one functional group or an unsaturation (Table 1). This similarity in the charge/mass ratio between those analytes makes their separation a relevant analytical challenge [26–29].

Download English Version:

<https://daneshyari.com/en/article/1199996>

Download Persian Version:

<https://daneshyari.com/article/1199996>

[Daneshyari.com](https://daneshyari.com)