Journal of Chromatography B, 910 (2012) 2–13

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Review

Experimental design in chromatography: A tutorial review☆

D. Brynn Hibbert*

School of Chemistry, University of New South Wales, Sydney, NSW 2052, Australia

ARTICLE INFO

Article history:
Received 7 November 2011
Accepted 19 January 2012
Available online 28 January 2012

Keywords:
Experimental design
Design of experiments (DoE)
Response surface methodology
Method validation
Optimisation
Plackett–Burman design
Central composite design
Box–Behnken design
Mixture design
Doehlert design
Factorial design
Fractional factorial design

ABSTRACT

The ability of a chromatographic method to successful separate, identify and quantitate species is determined by many factors, many of which are in the control of the experimenter. When attempting to discover the important factors and then optimise a response by tuning these factors, experimental design (design of experiments, DoE) gives a powerful suite of statistical methodology. Advantages include modelling by empirical functions, not requiring detailed knowledge of the underlying physico-chemical properties of the system, a defined number of experiments to be performed, and available software to accomplish the task. Two uses of DoE in chromatography are for showing lack of significant effects in robustness studies for method validation, and for identifying significant factors and then optimising a response with respect to them in method development. Plackett–Burman designs are widely used in validation studies, and fractional factorial designs and their extensions such as central composite designs are the most popular optimisers. Box–Behnken and Doehlert designs are becoming more used as efficient alternatives. If it is not possible to practically realise values of the factors required by experimental designs, or if there is a constraint on the total number of experiments that can be done, then D-optimal designs can be very powerful. Examples of the use of DoE in chromatography are reviewed. Recommendations are given on how to report DoE studies in the literature.

Contents

1. Introduction

Two decades ago I asked an eminent professor who had just given a major lecture at my university if he used experimental design to optimise the parameters of his syntheses. After a look of total blankness he answered "I have many PhD students. They work

☆ This paper is part of the special issue "Chemometrics in Chromatography" by Pedro Araujo and Bjong Grung (Guest Editors).

* Tel.: +61 2 93854713; fax: +61 2 3856141.
E-mail address: b.hibbert@unsw.edu.au

1570-0232/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2012.01.020

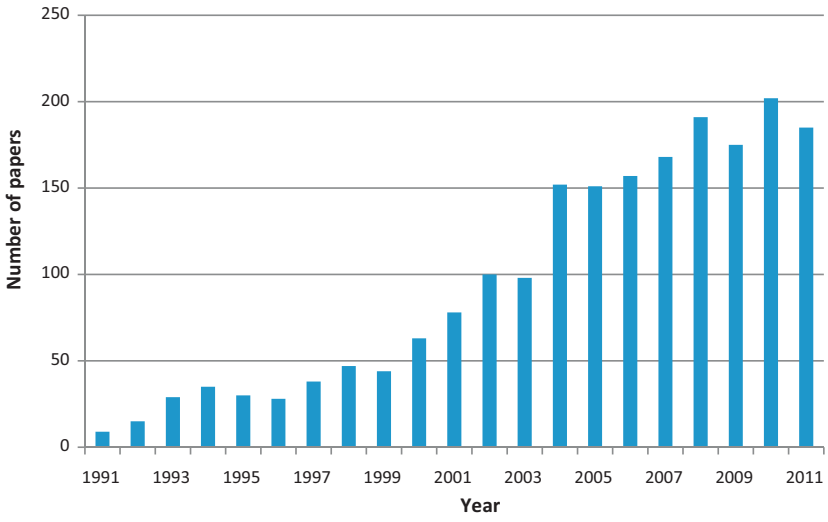


Fig. 1. Numbers of papers referenced by Scopus since 1991 with search term "design of experiments" or "experimental design" and chromatography (search conducted 19.10.2011).

very hard". It is unlikely that a similar response would be given today, and not because PhD students are less diligent than their forebears. The need to maximise the efficiency of scientific discovery, in order to minimise waste and cost, has caused researchers to do smarter experiments that give the most information possible with the fewest experiments. While the classic theories of experimental design have been around since the middle of the twentieth century, and we can find early reviews in the analytical chemical literature, from Deming [1] and Schoenmakers [2], in the late 1980s, adoption of DoE[1] methods in chromatography research have seen increased activity only in the past decade (see Fig. 1).

One of the earliest references is a fully-fledged use of a factorial design to optimise a system that was modelled by an early computer, complete with three-dimensional response surface [3]. Since this time the methodology has remained much the same with factorial, fractional factorial and composite designs being popular.

In this paper after a brief explanation of the approach taken by DoE over other optimisers, the different aspects of a chromatographic separation that have been subjected to experimental design will be reviewed. There is some overlap with other methodology, for example mass spectrometry or sampling and extraction, but this review will mostly be confined to chromatography.

2. Design of experiments

The approach taken by the suite of methods that may be classed as experimental design is that multivariate data can be fitted to an empirical function, usually linear or quadratic with interaction terms, which can be used to provide information about the system (maxima and minima, trends as parameters are changed, etc.). Statistical theory is used to choose values of each factor to generate the data so as to maximise the information about the parameters of the function. Randomisation of the order of experiments ensures, as far as possible, any uncontrolled variables (for example, temperature of the laboratory) contribute to the repeatability variance and do not affect the results in a systematic way. Out of the myriad books on the theory of experimental design I suggest the classic 1976 book by Box, Hunter and Hunter "Statistics for experimenters" which was published in a second edition in 2006 [4], and chapters 21–25 of Massart et al.'s equally classic, in chemistry, "Handbook of Chemometrics and Qualimetrics" [5]. In the context of quality assurance in an analytical chemistry laboratory chapter 3 of Hibbert is also a readable introduction [6].

2.1. Terminology of experimental design

Because the roots of the statistical description of experiments lie in sociological and operations research the terminology is not always obvious to a chemist. The definitions below are given in the style of the International Vocabulary of Metrology (VIM), where definitions of basic concepts in measurement are found [7].

2.1.1. Design of experiments

2.1.1.1. Experimental design. Statistical technique for planning, conducting, analysing, and interpreting data from, experiments.

2.1.1.2. Response. Measured or observed quantity that is the subject of study or optimisation.

Example: chromatographic response factor, retention time, number of theoretical plates.

2.1.1.3. Factor. Quantity that affects a *response*

Notes: (1) Factors are considered as controlled or uncontrolled, depending on whether the levels of the factor can be set in the DoE.

[1] The use of statistical methodology to design experiments and analyse data is termed "design of experiments" or "experimental design" and has the acronym DoE.

Randomisation of the order of experiments might ensure that the effects of uncontrolled factors will contribute to the repeatability variance of the response.

(2) Factors can take discrete or continuous values. Example column temperature, concentration of acetonitrile, stationary phase.

(3) Synonyms are variable, predictor and parameter.

2.1.1.4. Level of a factor. Value of a *factor* that is prescribed in an *experimental design*.

Notes: Designs are named by the number of levels chosen for a factor, e.g. two-level, three-level design.

Examples: temperature: 20, 25, 30 °C; column: C8, C18; gradient time: 1, 3, 5 min.

2.1.1.5. Response surface. Relationship of a *response* to values of one or more *factors*.

Notes: (1) The surface is usually a plot in two or three dimensions of the function that is fitted to the experimental data.

(2) Response surface methodology (RSM) is used to describe the use of experimental designs that give response surfaces from which information about the system is deduced [8].

2.1.1.6. Model. Equation that relates a *response* to *factors*

Notes: (1) A model can be empirical, which is chosen for the mathematical form, or is based on a theoretical understanding of the process that gives the response. Empirical models based on polynomials of the factors are described by the order of the polynomial, e.g. first-order, second-order.

(2) If the equation represents all effects of the system, the difference between the model prediction and the measured response is random variance, usually given the symbol ε.

Examples: (1) Krier et al. reported an optimisation of an HPLC method for the analysis of the drug sulindac [9]. The response was the logarithm of the retention factor (k), and was optimised as a function of the duration of the initial isocratic step (P), the mass fraction of acetonitrile at the beginning and end of the gradient (c_l and c_u), and the gradient (g).

$$\log(k) = \beta_0 + \beta_1 P + \beta_2 c_l + \beta_3 c_l^2 + \beta_4 c_u + \beta_5 c_u^2 + \beta_6 g + \beta_7 g^2 + \beta_8 c_l P + \beta_9 c_l P + \beta_{10} g P + \beta_{11} c_u P + \beta_{12} c_l g + \beta_{13} c_u g + \varepsilon$$

This is a second-order model in g, c_l and c_u and first-order in P.

(2) Theoretical and empirical models of aspects of the mixed-mode separation of anionic and cationic pharmaceutically related compounds was reported by Zakaria et al. [10]. Peak width was modelled by a peak compression approach which has a theoretical equation, while analyte retention and methanol content were modelled by linear and quadratic functions.

2.1.1.7. Effect. Coefficient of a term in a model.

Notes: The main effect is the coefficient of the term in the first order of a factor. Interaction effects are coefficients of products of linear terms, e.g. two-way interaction, three-way interaction.

Example: In the example of Krier (see definition of *model*) β_1, β_2, β_4, and β_6 are main effects, β_3, β_5, and β_7 are second order effects, and β_8 to β_{13} are two-way interaction effects.

2.1.1.8. Factorial design. Experimental design in which the runs are combinations of levels of *factors*.

Notes: (1) full factorial designs have every possible combination of factors at the designated levels. There are L^k combinations of k factors at L levels.

(2) Fractional factorial designs are a specific subset of a full design.

Other experimental designs include Plackett–Burman, central composite, Box–Behnken, Doehlert, D-optimal, G-optimal, and mixture designs. These will be described in the course of the review.

2.2. Why experimental design?

2.2.1. Optimisation strategies

In any modelling and optimisation exercise there is an initial decision to be made between empirical approaches or ones that use scientific knowledge about the system under study. For example Zakaria et al. modelled the electrokinetic chromatographic separation of mixtures of organic anions and cations using mixed pseudo-stationary phases using the complex equilibria that pertained in the system [11]. Calculated mobility coefficients were fitted to observations with equilibrium constants as factors. The availability of credible theoretical models should always be considered.

However in the case where such theoretical modelling is not feasible or is overkill in a system that simply requires optimising, DoE has the advantage of providing recipes of experiments that are independent of the system itself. An example of a modelled system that has generated software to predict retention times of ions in ion chromatography is given by Madden et al. [12]. Even when a model is available that is clearly not quadratic, DoE can still give a good optimum with careful choice of design points. DoE also has the advantage over iterative estimators such as Simplex that the number of experiments is determined before any work is done. Assuming the factors and factor levels are chosen appropriately, then the outcome is assured with a known effort. This advantage is particularly useful in modern systems with fully programmable systems with autosamplers in which a run can be set up in the evening and results are available in the morning. It is the opinion of the author (and one reviewer) that DoE should usually be preferred over Simplex.

2.2.2. DoE versus change one at a time

It is often argued that DoE is superior to the traditional change-one-at-a-time approach, and this is usually so when a response is being optimised. If the factors in the design are correlated, that is if the change in response to a change in a factor level depends on the level of another factor, then it is unlikely that the optimum will be discovered and more experiments than necessary will have been done (see Fig. 2).

It can be seen from Fig. 2(a) that for a response surface that indicates there are interactions between the factors (independent factors lead to a circular response surface, or ellipse oriented along the axes), the change-one-at-a-time approach does not even reach the true optimum. However the real value of experimental design is seen in Fig. 2(b) in which the space of the factors is clearly covered by the design (here a central composite design). Information about the response surface will be much greater from the design than the simple approach in Fig. 2(a).

2.3. Kinds of problems informed by experimental designs

There are two kinds of chemical problems that need experimental design for their solution. The first is to discover which factors may significantly affect the response of an experiment, and the second to find factor values that optimise the response.

2.3.1. Screening and robustness studies

The object is to perform a minimum number of experiments on a maximum number of factors. In the terminology of DoE what is needed is a main effects model with a highly fractionated design. The minimum number of levels is two, and apart from fractional

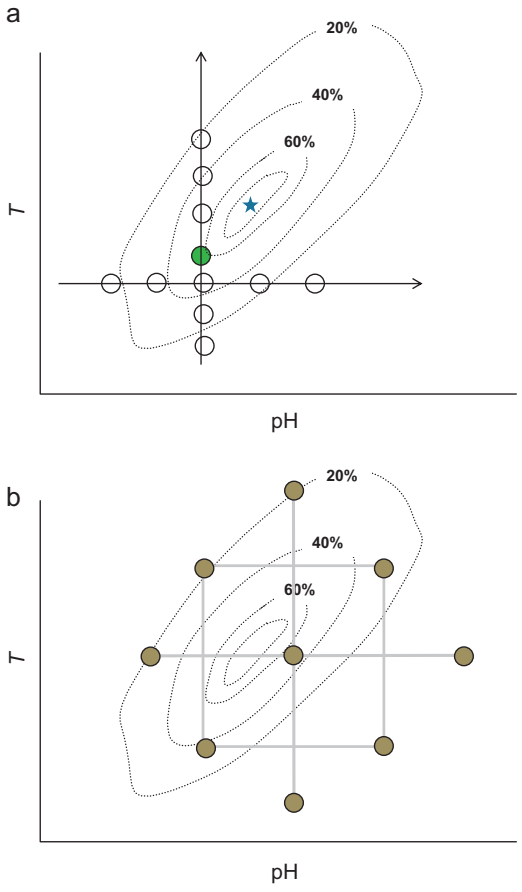


Fig. 2. (a) Change one at a time optimisation (open points) with best experiment (closed point) and true optimum (star) on a hypothetical response surface that shows correlation between factors. The filled point shows the optimum. (b) A central composite design.

two-level designs, possibly the most used designs are due to Plackett and Burman. For $4n$ experiments, the main effects of up to $4n-1$ factors can be estimated $2n$ times. Such a screening design is used in two scenarios. First, these designs are done as a prelude to an optimisation, to make sure that factors being investigated do indeed significantly contribute to the response. Many factorial and fractional factorial designs can be embedded within the subsequent optimisation, thus further saving on runs to be performed. Secondly, during method validation, ruggedness (different normal conditions) or robustness (small changes introduced deliberately) studies are typically done with Plackett–Burman designs. Because the expected outcome is no significant change of the response, allowing the claim of a rugged/robust method, many factors can be screened without concerns about interacting and non-linear effects. A main effects model will suffice.

A main effects model looks like

$$R = \beta_0 + \sum_{i=1}^{i=k} \beta_i x_i + \varepsilon \quad (1)$$

And with interactions

$$R = \beta_0 + \sum_{i-1}^{i=k} \beta_i x_i + \sum_{i=1}^{i=k} \sum_{j-i}^{j-k-1} \beta_{ij} x_i x_j + \varepsilon \quad (2)$$

2.3.2. Choice of response

Chromatography is full of trade-offs and an optimum separation depends on the wider problem with considerations such as time, cost, required measurement uncertainty, that is the use to which the analytical information is to be put. An advantage of DoE is that multiple responses can be measured (resolution, time, throughput) and models developed that arrive at the desired overall optimum without extra experiments.

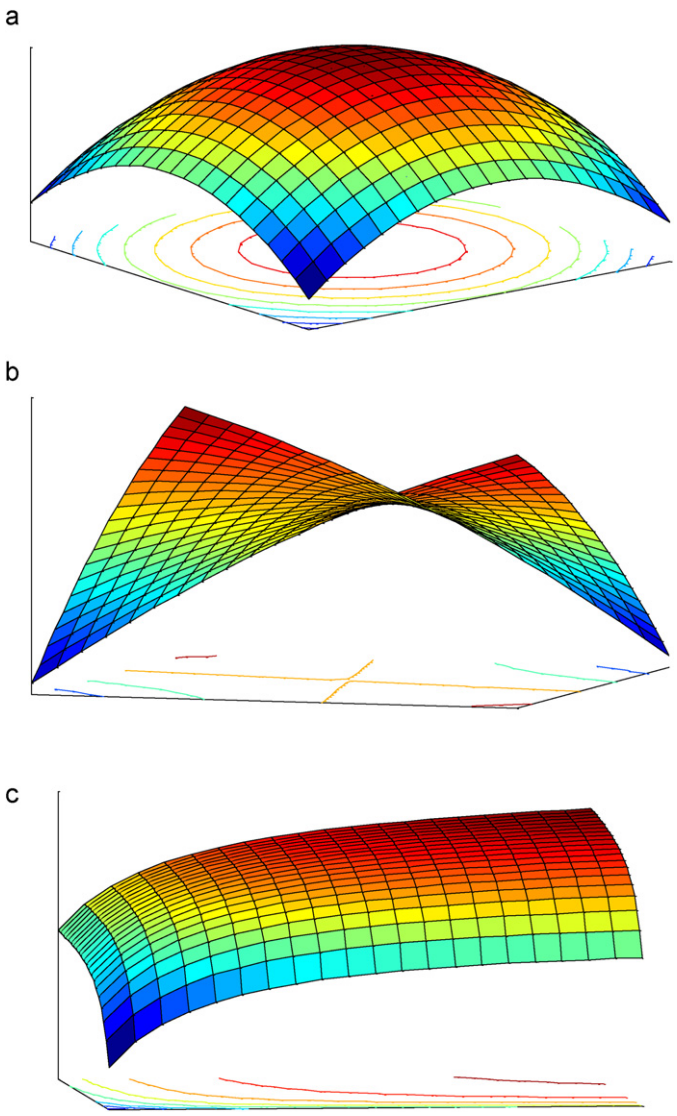


Fig. 3. Response surfaces as functions of two factors. (a) Having a maximum response, (b) having no maximum in the space of the factors and (c) having a plateau.

2.3.3. Optimising responses

Within the span of the values of factors in an experiment there will be better response values and not so good ones. Optimisation is the process of discovering where the best values lie. There is not always a nice single maximum of a function that can be discovered (see Fig. 3). Often the response plateaus and there is an area of response surface with approximately the same value (Fig. 3c). Sometimes the function shows a saddle with maximum values at the edges (Fig. 3b).

For chromatographic separations it is important to have an acceptable response that meets minimum criteria and so the aim is often to locate that region (e.g. high pH, lower temperature) rather than find the absolute optimum. This makes DoE very powerful when the polynomial function does not fit the data perfectly, but does describe the response sufficiently to locate an acceptable region.

Table 1
Factor levels for the 8-experiment, 7-factor Plackett–Burman design. '+' represents one level and '−' the second.

Factor	A	B	C	D	E	F	G
Expt.							
1	+	+	+	−	−	+	−
2	−	+	+	+	−	−	+
3	+	−	+	+	+	−	−
4	−	+	−	+	+	+	−
5	−	−	+	−	+	+	+
6	+	−	−	+	−	+	+
7	+	+	−	−	+	−	+
8	−	−	−	−	−	−	−

In order to have the possibility of a maximum (or minimum) quadratic terms are needed

$$R = \beta_0 + \sum_{i-1}^{i=k} \beta_i x_i + \sum_{i=1}^{i=k} \sum_{j-i}^{j-k-1} \beta_{ij} x_i x_j + \sum_{i=1}^{i=k} \beta_{ii} x_i^2 + \varepsilon \quad (3)$$

2.4. Choosing factors and factor levels

Some thought must be given before starting a design as to which factors will be chosen. Factors to be studied may be obvious from the nature of the system. Ruggedness tests in method validation will have had the factors for study prescribed in the protocol [13]. If it is known that a factor has a great effect of the separation (perhaps column temperature) there is no point in discovering this information in a screening design. It can be included immediately in the factors for optimisation. Discrete valued factors such as column type might be studied separately, rather than as part of a design. Thus mobile phase composition, flow rates and gradients might be optimised for a C8 column and then for a C18 column.

The choice of factor levels in a design is most important, possibly more so than the design itself. Obtaining information using only a small number of factor levels is a strength of DoE, but also a potential weakness. Each level must be appropriate and lead to useful information. Values too close together do not allow sufficient variation in the response to be observed (for example points up on the plateau of Fig. 3(c)). However points that are at the extremes of a reasonable range will give poor responses that might not differ from each other (for example extremes in Fig. 3(a)). Not all combinations of factor levels may be practical. Solubility limitations can lead to combinations of solvent compositions that will not allow high loading of a salt. For example Guo et al. in optimising acetonitrile mass fraction, ammonium acetate concentration and column temperature in the hydrophilic chromatography separation of organic acids report that two of the experiments (85% acetonitrile and 40 mM ammonium acetate concentration) could not be performed [14]. Although the authors proceeded with the rest of the design, it would have been better to choose other values, or go to a design that can accommodate inaccessible areas of factor space (such as a D-optimal design).

2.5. Coded factor levels

Experimental designs are often written in terms of coded variables. For example a design that requires only two values of a variable (so-called 'two-level' design) factor values are usually given as a series of +1 and −1 indicating whether one value (+1) or the other value (−1) is to be chosen. (See Table 1 for an example of this.) There are mathematical reasons for this practice, but also a practical use is that designs can be written independently of the particular factors under study. For designs where there are more than two levels, the values indicate the relative magnitude of the levels. For example in a circumscribed two-factor central composite design (see Section 2.7.2) the five coded levels are $-\sqrt{2}$, −1, 0, +1, $+\sqrt{2}$. Suppose the factor were temperature and the range to be studied was decided to be 50–100 °C then the required design points would be: 50, 57, 75, 93, 100. Note that this can only be done for continuous variables that can be set a predefined values.

2.6. Replication and randomisation

Measurements of responses are contaminated by random variability, and this feeds through to estimates of coefficients of models (effects) and locations of optima. When deciding on the significance of otherwise of effects, the values must be compared with the repeatability variance of the measurement. This assumes that the experiments in the design are performed in a short period of time on the same instrument by the same operator, that is under repeatability conditions of measurement (VIM 2.20 [7]). The repeatability variance might already be known from quality control measurements, but usually experiments to estimate the random variance are part of the design. Every experiment may be duplicated, but typically multiple experiments are performed at the centre of the design. How many are deemed appropriate tends to vary. Reviewing published studies we find 12 design points plus 4 centre, and in the same study 18 plus 3 [15], 19 and 3 [16], 15 and 2 [14], 10 and 3 [17], 54 and 4 [9], 8 and 5 [18], 27 and 2 [19], 38 and 12 [20], 17 and 3 [21], 12 and 3, 31 and 7, and 20 and 6 [22]. Having established the repeatability variance, estimates of effects can be compared with this value by Student's *t*-tests at the appropriate degrees of freedom. For designs that require specific numbers of factors (for example Plackett–Burman, $4n - 1$, i.e. 3, 7, 11 factors), the number can be made up using so-called dummy factors. These are factors that cannot possibly have an effect on the response, for example rotating clockwise before commencing the run as one level and rotating anti-clockwise as another level, or singing progressively different verses of your national anthem at your experiment. If the assumption that the activity cannot change the response, the variance of the measured values of the main effects of these dummy factors must be an estimate of the random variance.

Because the factor values are changed in a systematic way, it is important that the order of experiments is randomised. This negates any spurious systematic effect that would be manifested with the non-random order in time of the experiments, and ensures that the estimates of repeatability variance properly reflect the random aspects of the process.

2.7. Kinds of experimental design

Although there are many different kinds of design, they can be distinguished by the model that is derived (linear or quadratic, with or without interactions), constraints on factor levels, and the purpose of the study (screening, optimisation). Many designs (orthogonal designs) vary the factors independently of each other, which eliminates correlations among the factors.

2.7.1. Factorial designs

The workhorse of DoE, factorial designs identify experiments at every combination of factor levels. There are L^k combinations of L levels of k factors. In full factorial designs (see Fig. 4) every experiment is performed, while for fractional factorial designs a specific subset is performed that allows calculation of certain coefficients of the model. Two-level designs are chosen for screening factors and can give main and interaction effects, but not higher orders. Fractionation leads to designs that give main effects only with fewer runs. Calculation of effects in two level designs is easy and can be performed in a spread sheet. If the two levels are coded +1 and −1, then the column of +1 and −1 under each factor (see for example the columns in Table 1) is multiplied by the response for

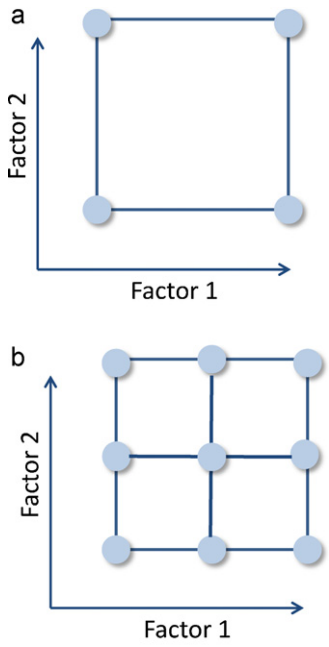


Fig. 4. Full-factorial designs. (a) Two-factor, two-level design and (b) two-factor three-level design. Each point represents the factor values for one experiment (run).

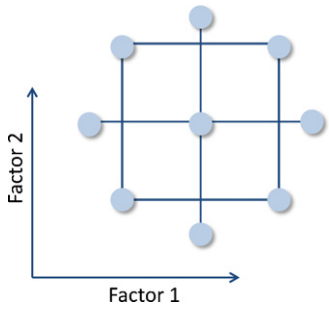


Fig. 5. Circumscribed central composite design for two factors. Each point represents the factor values for one experiment (run).

each experiment. The product is summed and divided by half the number of experiments. This is the main effect for the factor. For an interaction effect a column is created that is the product of the level codes and the procedure outlined above is applied to this column.

2.7.2. Plackett–Burman designs

Plackett and Burman published their paper in 1946 [23], and these have become particularly popular for robustness tests in

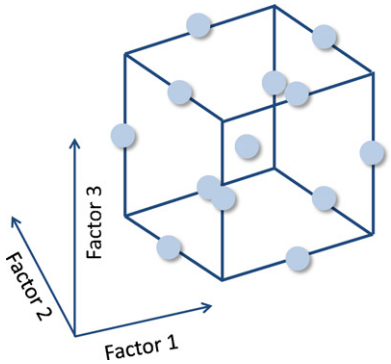


Fig. 6. Box–Behnken design for three factors. Each point represents the factor values for one experiment (run).

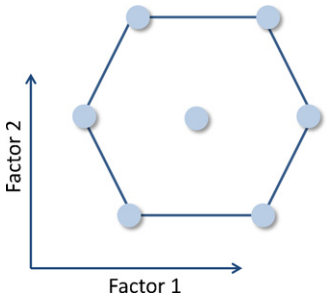


Fig. 7. Dohlert design for two factors. Each point represents the factor values for one experiment (run).

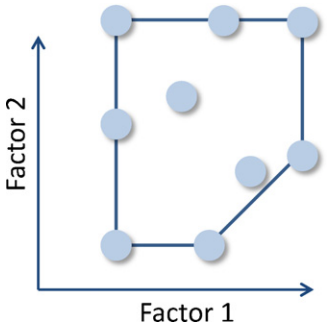


Fig. 8. Example of a D-optimal design for two factors and 9 runs, where the lower right triangle of the design is not accessible (i.e. experiments cannot be performed with these combinations of factor levels).

method validation because one of the runs requires the base level of each factor. A Plackett–Burman design requires $4n$ experiments to be performed to investigate a maximum of $4n - 1$ factors at two levels. For example there are eight experiments in the seven-factor design in Table 1, with the two levels designated '+' and '−'. When deciding the levels, if '−' is allocated to the base level of the factor, then '+' is this base plus a small change that is being investigated as part of the robustness study. Note that the change can be an increase or a decrease. The main effect that is obtained from the

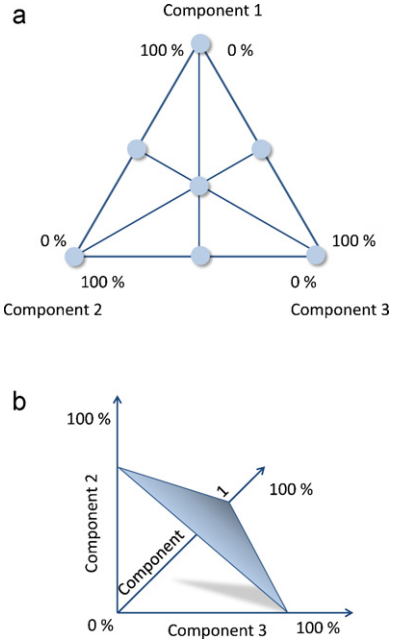


Fig. 9. (a) Mixture design for three components whose value sum to 100% and (b) the plane of the design in three-dimensional factor space.

Table 2
Coded factor levels for the first experiment in a Plackett–Burman experimental design. The design gives the main effects of $4n-1$ factors in $4n$ experiments. See Section 2.7.2 for details of generating the design.

n	Coded factor levels for the first experiment
2	+++−−+−
3	++−+++−−−+−
4	++++−−−−−−+++−+
5	+−++−−−−+−+−++++−−+

analysis of the DoE is an estimate of the change in response as the factor goes from the '−' level to the '+' level. The incorporation of dummy variables is discussed in Section 2.6, and how to calculate the effect in Section 2.7.1. If one of the rows of coded factor levels of a Plackett–Burman design is known, the remaining rows can be generated by cycling the end code to the beginning of the row and moving the rest of the codes one place to the right. Thus in Table 1, the first row is +++−−+− and the second row now starts with the last '−' with the other codes moved along: −+++−−+. The sequence ends with all '−'. (If the process is repeated for the eighth row the first row is regenerated.) Starting sequences for Plackett–Burman designs are shown in Table 2).

2.7.3. Central composite designs

Two-level designs can only lead to linear models of responses and so cannot give information about maxima or any non-linear relationships. However a drawback of full factorial designs at levels greater than two, is the great number of experiments that must be done. Designs that allow greater numbers of levels without performing experiments at every combination of factor levels cover the factor space near the centre with more points than at the periphery. One such design is the central composite design, so named because it combines a two-level factorial design with a star design and centre points. The star and factorial points can lie equidistant from the centre (circumscribed design, see Fig. 5), or the star points can lie within the space of the factorial design (inscribed design) or they can lie on the faces of the factorial design points (faced). Central composite designs require $L^k + Lk + n_c$ where n_c are the number of replicate centre points chosen.

Table 3
Software used for experimental design.

Software	Company and reference	Comments	Used by
Design-Expert	Stat-Ease Inc., http://www.statease.com/	DoE software	[37–41]
Fusion Pro	S-Matrix Corporation, http://www.smatrix.com/	DoE software	[14,42,43]
JMP	SAS Institute Inc., http://www.sas.com/	General statistical software. See book on DoE in SAS [44]	[19,20,45–49]
Matlab	The Mathworks Inc., http://www.mathworks.com.au	General mathematical and computing software. Statistics Toolbox contains DoE routines.	[8,34,36,50–53]
MINITAB	Minitab Inc., http://www.minitab.com	General statistical software	[52,54–59]
Modde	Umetrics, http://www.umetrics.com/modde	DoE software	[15,60,61]
Nemrod-W	LPRAI, Marseille, France http://www.nemrodw.com/html-US/design-of-experiments.html	Windows OS only. Optimal designs.	[17,62,63]
Origin	Microcal Software, http://www.originlab.com/	General data analysis and graphing software	[64]
R	Revolution Analytics http://www.revolutionanalytics.com/	Open source general software	[9,65,66]
SPSS	IBM, http://www-01.ibm.com/software/analytics/spss/	General statistical software	[38,67]
Statgraphics	Statpoint Technologies, http://www.statgraphics.com/	General statistical software	[68,69]
STATISTICA	StatSoft, http://www.statsoft.com	General statistical software	[16,70]
Unscrambler	CAMO AS, http://www.camo.com/	Chemometric and DoE software	[71,72]
Virtual Column	ACROSS and the University of Tasmania, http://www.virtualcolumn.com	Chromatographic modelling software	[12]

Table 4
Reports of the use of DoE in method validation (robustness studies) of chromatographic techniques.

Method/analyte	DoE	Factors (levels)	Ref.
Review			[30]
HPLC-UV process-related impurities in pridinol mesylate	Fractional factorial 3^{4-2}	pH (6.3, 6.4, 6.5), fraction of modifier (78, 80, 82%), T (27, 30, 33 °C), flow rate (0.95, 1.00, 1.05 mL/min)	[38]
HPLC-UV (2S,3R)-2-amino-3-octadecanol hydrochloride as anticancer drug	Plackett–Burman with 6 factors and 1 dummy	Mobile phase: x_{ACN}, pH, flow rate, Detector wavelength, Column: T, type (levels not given)	[42]
HPLC-fluorescence amyloid β (Aβ) protein	Plackett–Burman with 6 factors and 1 dummy. 3 centre points added	Mobile phase: x_{ACN} (7, 9%), x_{MeOH}(16.5, 18.5%), flow rate (0.90, 1.1 mL/min) Buffer: $m_{phosphate}$ (15, 25 mM), pH (7.4, 7.6) Column: T (20, 30 °C)	[73]
HPLC-UV immediate-release low-dose tablet formulation	Split-plot robustness design with 16 runs. Points straddle the nominal values.	Mobile phase: x_{ACN} (33,37%), pH (2.8,3.2), flow rate (0.4, 0.6 mL/min) Column: T (35, 45 °C)	[74]
HPLC omeprazole and related compounds in formulation	Paper not available		[75]
HPLC-fluorescence + pre-column derivatisation Pregabalin, gabapentin and vigabatrin in human serum	Plackett–Burman with 6 factors, 12 runs and 2 centre points	Mobile phase: x_{ACN} (7, 9%), x_{MeOH}(16.5, 18.5%), flow rate (0.7, 0.9 mL/min) Buffer: $m_{phosphate}$ (15, 25 mM), pH (7.4, 7.6) Column: T (20, 30 °C) (Note: robustness study on sample preparation also).	[76]
Isocratic HPLC-DAD anti-epileptic drugs	Fractional factorial design, 7 factors plus 3 centre points (11 runs)	Mobile phase: x_{ACN} (19.0, 20.0%), x_{MeOH}(14.0, 15.0%), pH (6.6, 6.8), flow rate (0.8, 1.0 mL/min) Buffer: $m_{phosphate}$ (20, 30 mM), m_{NaCl} (10, 15 mM), Column: T (43,47 °C) (Note: robustness study on sample preparation also).	[77]
HPLC-UV small molecule drug product	Plackett–Burman 7 factors in 8 runs	Mobile phase: x_{ACN} (13, 17%), pH (2.8, 3.2), flow rate 1.3, 1.7 mL/min), Buffer concentration (22, 28 mM) $\lambda_{Detector}$ (276, 280 nm) Column: T (35, 45 °C), type ('symmetry', 'Zorbax')	[78]

Table 5
Reports of the use of DoE in optimising factors in chromatographic techniques ordered by design approach (FF = full factorial, CCD = central composite design, BB = Box–Behnken design).

Method	Analyte	DoE method (factors/levels)	Ref.
(a) Optimisations relying on factorial and central composite designs grouped by chromatographic technique			
Fluid extraction LC–MS	Acrylamide in coffee	CCD (3)	[58]
GC-Ion mobility spectrometry	Exhaled breath condensate	FF (4/2)	[79]
GC × GC–MS	C8 and C9 phenols	FF (5/2), CCD (3)	[80]
GC-FID with SPME	Residual solvents in pharmaceutical products	FF (3/2)	[18]
GC–MS	Estrogenic compounds in environmental samples	PB screen (8) then CCD (3)	[21]
GC–MS	Extraction conditions of polycyclic aromatic hydrocarbons in milk	FF (3/2) CCD (3)	[67]
GC–MS	Drugs in urine	FF (3/2) CCD (3)	[57]
GC–MS	Metabolites	Fractional factorial (6 factors in a 2^4 design)	[34]
GC–MS after membrane extraction	Test compounds of different polarities	FF(2/2 + 1/4) and fractional factorial (4 factors in a 2^3 design)	[63]
GC–MS with SPME	Personal care products	CCD (4)	[50]
GC–MS with SPME	Pesticides	Fractional factorial (7 factors in a 2^4 design with centre points)	[48]
GC–MS with stir-bar SE/thermal desorption	Persistent organic pollutants	FF (3/2) for extraction conditions	[72]
GC–MS with stir-bar SE/thermal desorption	Synthetic musks	CCD (3)	[68]
GC–MS/MS (large volume injection)	PAH in airborne particles	FF (4/2) for injection factors CCD (4)	[22]
GC–MS/MS (large volume injection)	Polyhalogenated compounds	PB design (5) for MS factors CCD (4) for injection factors CCD (3) for extraction factors	[81]
HPLC-amperometric	Compounds in mouse brain tissue	Fractional factorial (5 factors in a 2^4 design)	[82]
HPLC-DAD	Drug design	FF (5/2)	[65]
HPLC-DAD	Cannabinoid drugs	FF (2/3 + 1/5)	[83]
HPLC-DAD	Sulindac	FF (3/3, 1/2)	[9]
HPLC-pulsed amperometric	Polyribosyl-ribitol phosphate in complex combination vaccines	CCD (3)	[37]
HPLC-UV	Tertiary alkaloids from plant	FF (3/3)	[19]
HPLC-UV	Process-related impurities in pridinol mesylate	FF (2/3)	[38]
HPLC-UV	Organic acids	CCD (5)	[46]
HPLC-UV	Malaria drug screening	FF (2/3 + 1/5)	[66]
HPLC-UV	Cephalosporins in plasma and amniotic fluid	FF (3/2)	[33]
HPLC-UV	2-Arylimidazoline derivatives	FF (3/2)	[64]
Hydrophilic interaction LC-UV	Uric acids	CCD (3)	[43]
LC-evaporative light scattering	Cellulose derivatives in pharmaceutical formulations	FF (2/2 + 1/3)	[59]
LC–MS	Estrogenic compounds	FF (4/2)	[84]
LC–MS (semi-preparative)	Cortisones	FF (4/2 + centre 3/1)	[15]
LC–MS after supercritical fluid extraction	Indole alkaloids from plant	FF (4/2)	[61]
LC–MS/MS	12 ionic per- and polyfluorinated alkyl substances (PFAS) in fine airborne particulate matter	CCD (4) optimising extraction parameters	[54]
LC–MS/MS	Siderophores	CCD (5)	[52]
LC–MS/MS after enzyme digestion	Drug–protein adducts	CCD-face centred (3)	[20]
LC–MS/MS with microwave-assisted extraction	Pesticides in air	CCD (3)	[56]
Micellar electrokinetic LC–MS	Enantiomers of binaphthyl derivatives	CCD (4)	[85]
Micellar LC	Test ionic compounds	FF (4/2)	[59]
Micellar LC–MS with SPE	Flavonoids in honey	FF (4/2)	[70]
Molecular imprinted polymers	Sulphonamide residues	FF (3/3)	[39]
Size exclusion chromatography	Molecular size distribution of natural organic matter	CCD (3)	[71]
Size exclusion chromatography	Complex organic mixtures	FF (4)	[86]
Supercritical fluid C	Metoprolol and analogues	CCD (3)	[87]
UHPLC	Acetyl cholinesterase inhibitors	CCD (2)	[40]
(b) Box–Behnken and other optimising designs grouped by chromatographic technique. Design points are shown in two or three-dimensions for non-standard designs			
Review		Response surface designs	[45]
Review		D-Optimal, Doehlert, Mixture designs	[28]
Review		Box–Behnken	[25]
Review		Doehlert, Box–Behnken, CCD	[8]
GC-ECD after single-drop ME	2,4,6-Trichloranisole in wine	BB (2)	[16]
GC–IDMS (headspace)	Benzene in food	Irregular factorial (4 factors, 15 runs)	[88]

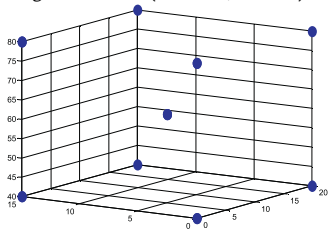
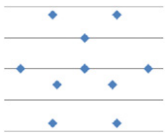
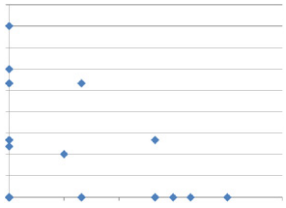
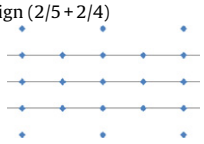
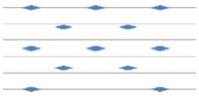
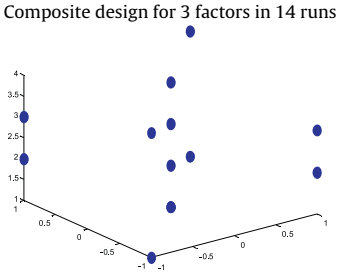
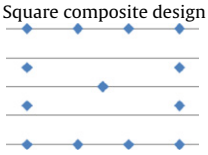
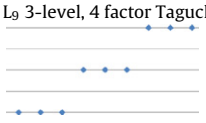
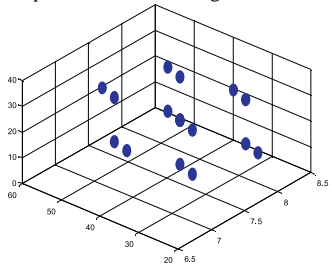


Table 5 (*Continued*)

Method	Analyte	DoE method (factors/levels)	Ref.
GC–MS	Anabolic steroids	Dohlert (2) 	[17]
GC–MS	Metabolites	D-optimal (5 factors, 33 runs) 	[34]
GC–MS SPME	Non-steroidal anti-inflammatories	D-optimal (7/2, 1/4) 21 runs	[62]
GC–ToFMS	Metabonomic compounds	D-optimal (3 factors, 10 runs)	[89]
HPLC-UV	Sulindac	Composite design (2/5 + 2/4)	[3]
HPLC-UV	Synthesis of thiol-modified silica	2 factor design 	[35]
HPLC-UV	Test organic compounds	D-optimal (4/3, 22 runs)	[47]
HPLC-UV	Test ionisable compounds	D-optimal and G-optimal designs on 5 factors	[36]
HPLC-UV	Extracts of tea	Mixture design (optimising extraction solvent and mobile phase)	[90]
Hydrophilic interaction C-DAD	Organic acids	Composite design (3/5) 	[14]
Hydrophilic interaction C-DAD	Organic acids	BB (4)	[41]
LC–MS	Acids	Composite design for 3 factors in 14 runs 	[91]
LC–MS (semi-preparative)	Cortisones	Square composite design (2/5) 	[15]
LC–MS/MS	Protein in liver	orthogonal array L25 (5/3)	[55]
LC–MS/MS	Geniposide and genipin	L_9 3-level, 4 factor Taguchi fractional design 	[92]
LC-UV	Monolithic polymers as stationary phase	D-optimal (5 factors, 17 runs)	[93]
LC-UV–MS	Iodination of obestatin	Draper-Lin cube-star design 	[69]
Mixed mode chromatography	Monoclonal antibody purification processes	BB(5)	[94]
Size exclusion C-MS	Synthetic polymers	D-optimal (5/3, 30 runs)	[51]

2.7.4. Box–Behnken design

A Box–Behnken design [24] has three levels (see Fig. 6) or more and can be applied to problems having three or more factors. There are no factorial or extreme points and the design requires $2k(k-1)+n_c$ points. This is fewer than the central composite design and for three factors the same as the Doehlert design. The use of Box–Behnken designs over central composite was promoted by Ferreira et al. in 2007 [25]. Use of Box–Behnken should be contemplated for systems with greater than two factors where the optimum is known to lie in the middle of the factor ranges (Fig. 7).

2.7.5. Doehlert designs

Doehlert designs, unlike central composite and Box–Behnken are not rotatable, i.e. they can give different qualities of estimates for different factors. However they are very efficient and have different numbers of levels for different factors. Thus factors that are considered more important can be measured at more levels. The design attempts to fill the given factor space as uniformly as possible. A Doehlert design requires k^2+k+n_c points (Fig. 8).

2.7.6. D-optimal designs

So-called optimal designs are becoming more popular and are particularly useful when the factor space is not uniformly accessible, perhaps when combinations of solvent composition and solute concentration are not possible. Another useful aspect is that the number of experiments is specified. These must be the minimum required to calculate the coefficients of the effects model (the number of effects plus a constant term). The D-optimal [26] solution answers the question given a number of design points to choose from a total number, what is the optimal distribution of the points? This can be shown to be when the determinant of $\mathbf{X}\,\mathbf{X}^{\mathrm{T}}$ is maximised, where $\mathbf{X}$ is the matrix of design points and T denotes the transpose. Once a minimum design is analysed, further points can be added to refine knowledge of the system that are guided by the same principle.

2.7.7. Mixture designs

A special kind of design is used when the factors are constrained by having to total some constant value. For example, in chromatography a mobile phase has components that total 100%. Mixture designs address this issue. Three factors that sum to 100%, for example methanol, acetonitrile and water in a mobile phase [27] fix one the components when the other two are chosen. The available space becomes a triangular plane in the three-dimensional factor space (Fig. 9).

2.7.8. Software

Although many designs can be set up in a spread sheet and effects calculated, most researchers will use specialised software. In terms of the basic designs and analysis, any validated software should do the job. Most statistical packages have a DoE component and there are standalone products and add-ins for Microsoft Excel (Microsoft® Office® 2010, Microsoft Corp., USA). Before any software is used there should be some consideration of the most appropriate method and some awareness of the principles that the DoE is based on. If homemade software is used, it must be validated using known data, and this information must be made available for review. Table 3 gives details of software used in the literature referenced by this paper.

3. Review of DoE in chromatography

There have been some very good reviews of experimental design in chromatography. Most notable of the general reviews is that of Dejaegher and Vander Heyden [28]. Other reviews of more focussed scope are on validation and ruggedness and robustness [29,30], capillary electrophoresis [31], Box–Behnken designs [25] and optimisations in GC–MS [8]. Boulanger in a discussion of the French guide from the Société Française des Sciences et Techniques Pharmaceutiques on validation of chromatographic bioanalytical methods advised strongly the use of DoE [32].

The majority of uses of DoE in chromatography can be classed as either method validation robustness studies or optimising method conditions. Typically two, three or four factors are studied including mobile phase composition (not always using a mixture design), gradient parameters (initial and final composition, gradient of flow rate), pH, temperature, injection volume, flow rate. As part of an analysis using chromatography, there are other steps that can be optimised such as extractions, and derivatisation, both which lend themselves to DoE. In Tables 4 and 5 references are made to reports of separations using DoE for method validation robustness testing or optimisation, grouped by chromatographic method and DoE approach.

It is seen that central composite designs are most popular for optimisation, even when D-optimal designs (accessibility of factor space) or Box–Behnken or Doehlert designs (greater efficiency) might be better (Tables 4 and 5).

4. Conclusions

With the greater availability of statistical software and the general ability to batch multiple runs on modern instrumentation, the increasing popularity of design of experiments is not surprising. The present review does not claim to be comprehensive as many papers report the use of DoE without creating keywords or going beyond a brief description. Plackett–Burman designs are the best for robustness studies where a small deviation from method conditions is required and main effects only considered. Plackett–Burman can also be used for screening designs, but has the drawback that it cannot be embedded into an optimising design in the way a two-level factorial design can. The author has not seen this done, but building a D-optimal design on a completed Plackett–Burman design might be an interesting approach. Central composite designs will continue to be popular, but if extremes of the factor space are not critical, then Box–Behnken or Doehlert designs should be considered. As discussed above, the choice of factors and levels is more important that the design, (assuming the design can actually do the intended job).

Finally I shall offer some advice about reporting DoE. Compiling this review has made it very clear that many authors believe that writing "design of experiments generated by software X was used to optimise the chromatographic conditions" suffices. This is analogous to stating that "chromatography using an instrument from company X was used to analyse the samples" without mentioning the kind of chromatography, detector, conditions etc. On the other hand, some papers in which DoE was used in optimisation feel the need to rehearse the history of DoE, discussing the choice of one method over another for what is a trivial application. In this author's opinion we should recognise that DoE is a well-known chemometric tool that can be taken as granted, and so the following information should be given either in the text or supporting information without too much more elaboration:

- The name of the design (Plackett–Burman, D-optimal, etc.) with an appropriate reference for the actual variant used.
- The name and details of the software used. If this is homemade then there should be validation information available.
- The factors and their levels (in a table, see for example Table 1 [19]).
- The design, including replicates (in a table, see for example Table 1 [33], Tables 1 and 2 [34]).

- The response variables and their optimised values (predicted and observed, in a suitable table).
- The equation of the response model and calculated values of effects with confidence intervals at stated probability. A confidence interval is preferred to a statement of significance. (see for example Table 2 [35]).
- Response surfaces only if they show some feature of interest. Very colourful, three-dimensional surfaces can be generated by most software, but their use is limited to showing the effect of two factors, and perspective views can be misleading. Graphs showing points in a D-optimal design are useful (see for example Fig. 2 in [36]).

At the end of the day a chromatographer can see if her experimental conditions have been improved by optimisation, and this is, perhaps, all that matters.

References

[1] S.N. Deming, J.M. Palasota, J. Lee, L. Sun, J. Chromatogr. 485 (1989) 15.
[2] P.J. Schoenmakers, M. Mulholland, Chromatographia 25 (1988) 737.
[3] M.L. Cotton, G.R.B. Down, J. Chromatogr. 259 (1983) 17.
[4] G. Box, W. Hunter, J. Hunter, Statistics for Experimenters, Design, Innovation and Discovery, 2nd ed., John Wiley & Sons Inc., New York, 2006.
[5] D.L. Massart, B.G.M. Vandeginste, J.M.C. Buydens, S. de Jong, P.J. Lewi, J. Smeyers-Verberke, Handbook of Chemometrics and Qualimetrics, 1st ed., Elsevier, Amsterdam, 1997.
[6] D.B. Hibbert, Quality Assurance for the Analytical Chemistry Laboratory, Oxford University Press, New York, 2007.
[7] Joint Committee for Guides in Metrology, JCGM 200, BIPM, Sèvres, 2008, http://www.bipm.org/vim.
[8] L.K. Skartland, S.A. Mjøs, B. Grung, J. Chromatogr. A 1218 (2011) 6823.
[9] F. Krier, M. Brion, B. Debrus, P. Lebrun, A. Driesen, E. Ziemons, B. Evrard, P. Hubert, J. Pharm. Biomed. Anal. 54 (2011) 694.
[10] P. Zakaria, G. Dicinoski, M. Hanna-Brown, P.R. Haddad, J. Chromatogr. A 1217 (2010) 6069.
[11] P. Zakaria, M. Macka, J.S. Fritz, P.R. Haddad, Electrophoresis 23 (2002) 2821.
[12] J.E. Madden, M.J. Shaw, G.W. Dicinoski, N. Avdalovic, P.R. Haddad, Anal. Chem. 74 (2002) 6023.
[13] D.B. Hibbert, in: P.J. Worsfold, A. Townshend, C.F. Poole (Eds.), Encyclopedia of Analytical Science, Elsevier, Oxford, 2005, p. 469.
[14] Y. Guo, S. Srinivasan, S. Gaiki, Chromatographia 66 (2007) 223.
[15] D.C. Cole, N. Pagano, M.F. Kelly, J. Ellingboe, J. Comb. Chem. 6 (2004) 78.
[16] E. Martendal, D. Budziak, E. Carasek, J. Chromatogr. A 1148 (2007) 131.
[17] Y. Hadef, J. Kaloustian, H. Portugal, A. Nicolay, J. Chromatogr. A 1190 (2008) 278.
[18] S. Legrand, J. Dugay, J. Vial, J. Chromatogr. A 999 (2003) 195.
[19] I. Nistor, M. Cao, B. Debrus, P. Lebrun, F. Lecomte, E. Rozet, L. Angenot, M. Frederich, R. Oprean, P. Hubert, J. Pharm. Biomed. Anal. 56 (2011) 30.
[20] L. Switzar, M. Giera, H. Lingeman, H. Irth, W.M.A. Niessen, J. Chromatogr. A 1218 (2011) 1715.
[21] A. Vallejo, L.A. Fernández, M. Olivares, A. Prieto, N. Etxebarria, A. Usobiaga, O. Zuloaga, J. Chromatogr. A 1217 (2010) 8327.
[22] V. Yusà, G. Quintas, O. Pardo, A. Pastor, M. d. l. Guardia, Talanta 69 (2006) 807.
[23] R.L. Plackett, J.P. Burman, Biometrika 33 (1946) 305.
[24] G.E.P. Box, D.W. Behnken, Ann. Math. Stat. 31 (1960) 838.
[25] S.L.C. Ferreira, R.E. Bruns, H.S. Ferreira, G.D. Matos, J.M. David, G.C. Brandão, E.G.P. da Silva, L.A. Portugal, P.S. dos Reis, A.S. Souza, W.N.L. dos Santos, Anal. Chim. Acta 597 (2007) 179.
[26] R.W. Kennard, L.A. Stone, Technometrics 11 (1969) 137.
[27] A.A. de Almeida, I.S. Scarminio, J. Sep. Sci. 30 (2007) 414.
[28] B. Dejaegher, Y. Vander Heyden, J. Pharm. Biomed. Anal. 56 (2011) 141.
[29] C. Hartmann, J. Smeyers-Verbeke, D.L. Massart, R.D. McDowall, J. Pharm. Biomed. Anal. 17 (1998) 193.
[30] G. Sofer, M. Ahnfelt, BioPharm Int. (2007) 20.
[31] G. Hanrahan, R. Montes, F.A. Gomez, Anal. Bioanal. Chem. 390 (2008) 169.
[32] B. Boulanger, P. Chiap, W. Dewe, J. Crommen, P. Hubert, J. Pharm. Biomed. Anal. 32 (2003) 753.
[33] E. Nemutlu, S. Kir, D. Katlan, M.S. Beksaç, Talanta 80 (2009) 117.
[34] A. Jiye, J. Trygg, J. Gullberg, A.I. Johansson, P. Jonsson, H. Antti, S.L. Marklund, T. Moritz, Anal. Chem. 77 (2005) 8086.
[35] R. Dabre, A. Schwämmle, M. Lämmerhofer, W. Lindner, J. Chromatogr. A 1216 (2009) 3473.
[36] J.R. Torres-Lapasió, S. Pous-Torres, J.J. Baeza-Baeza, M.C. García-Álvarez-Coque, Anal. Bioanal. Chem. 400 (2011) 1217.
[37] M. Belfast, R. Lu, R. Capen, J. Zhong, M.A. Nguyen, J. Gimenez, R. Sitrin, R. Mancinelli, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 832 (2006) 208.
[38] R.M. Bianchini, P.M. Castellano, T.S. Kaufman, Anal. Chim. Acta 654 (2009) 141.
[39] M.P. Davies, V. De Biasi, D. Perrett, Anal. Chim. Acta 504 (2004) 7.
[40] G.R. Deshpande, A.K. Roy, N.S. Rao, B.M. Rao, J. Rudraprasad Reddy, Chromatographia 73 (2011) 639.
[41] N. Hatambeygi, G. Abedi, M. Talebi, J. Chromatogr. A 1218 (2011) 5995.
[42] M.W.J. Den Brok, B. Nuijen, E. Miranda, P. Floriano, S. Munt, I. Manzanares, J.H. Beijnen, J. Chromatogr. A 1020 (2003) 251.
[43] N.S. Quiming, N.L. Denola, Y. Saito, A.P. Catabay, K. Jinno, Chromatographia 67 (2008) 507.
[44] A.C. Atkinson, A.N. Donev, R.D. Tobias, Optimum Experimental Designs, SAS Oxford University Press, Oxford, 2007.
[45] A.C. Atkinson, R.D. Tobias, J. Chromatogr. A 1177 (2008) 1.
[46] E. Destandau, J. Vial, A. Jardy, M.C. Hennion, D. Bonnet, P. Lancelin, Anal. Chim. Acta 572 (2006) 102.
[47] K. Le Mapihan, J. Vial, A. Jardy, J. Chromatogr. A 1061 (2004) 149.
[48] E. Passeport, A. Guenne, T. Culhaoglu, S. Moreau, J.M. Bouyé, J. Tournebize, J. Chromatogr. A 1217 (2010) 5317.
[49] L.S. Riter, O. Vitek, K.M. Gooding, B.D. Hodge, R.K. Julian Jr., J. Mass Spectrom. 40 (2005) 565.
[50] G. Basaglia, L. Pasti, M.C. Pietrogrande, Anal. Bioanal. Chem. 399 (2011) 2257.
[51] T. Gruendling, M. Guilhaus, C. Barner-Kowollik, Macromol. Rapid Commun. 30 (2009) 589.
[52] M. Moberg, J. Bergquist, D. Bylund, J. Mass Spectrom. 41 (2006) 1334.
[53] S. Pous-Torres, J.R. Torres-Lapasió, J.J. Baeza-Baeza, M.C. García-Álvarez-Coque, Anal. Bioanal. Chem. 394 (2009) 625.
[54] M.I. Beser, O. Pardo, J. Beltrán, V. Yusà, J. Chromatogr. A 1218 (2011) 4847.
[55] J. Cao, V.M. Covarrubias, R.M. Straubinger, H. Wang, X. Duan, H. Yu, J. Qu, J.G. Blanco, Anal. Chem. 82 (2010) 2680.
[56] C. Coscollà, V. Yusà, M.I. Beser, A. Pastor, J. Chromatogr. A 1216 (2009) 8817.
[57] S. Costa, M. Barroso, A. Castañera, M. Dias, Anal. Bioanal. Chem. 396 (2010) 2533.
[58] O. Pardo, V. Yusà, C. Coscollà, N. León, A. Pastor, Food Addit. Contam. 24 (2007) 663.
[59] J. Rashan Jr., R. Chen, J. Pharm. Biomed. Anal. 44 (2007) 23.
[60] J. Lin, M. Su, X. Wang, Y. Qiu, H. Li, J. Hao, H. Yang, M. Zhou, C. Yan, W. Jia, J. Sep. Sci. 31 (2008) 2831.
[61] A. Verma, K. Hartonen, M.L. Riekkola, Phytochem. Anal. 19 (2008) 52.
[62] D. Arroyo, M.C. Ortiz, L.A. Sarabia, J. Chromatogr. A 1218 (2011) 4487.
[63] C. Vincelet, J.M. Roussel, D. Benanou, Anal. Bioanal. Chem. 396 (2010) 2285.
[64] K. Vučićević, G. Popović, K. Nikolic, I. Vovk, D. Agbaba, J. Liq. Chromatogr. Relat. Technol. 32 (2009) 656.
[65] B. Debrus, P. Lebrun, A. Ceccato, G. Caliaro, E. Rozet, I. Nistor, R. Oprean, F.J. Rupérez, C. Barbas, B. Boulanger, P. Hubert, Anal. Chim. Acta 691 (2011) 33.
[66] B. Debrus, P. Lebrun, J.M. Kindenge, F. Lecomte, A. Ceccato, G. Caliaro, J.M.T. Mbay, B. Boulanger, R.D. Marini, E. Rozet, P. Hubert, J. Chromatogr. A 1218 (2011) 5205.
[67] F. Bianchi, M. Careri, A. Mangia, M. Mattarozzi, M. Musci, J. Chromatogr. A 1196–1197 (2008) 41.
[68] N. Ramírez, R.M. Marcé, F. Borrull, J. Chromatogr. A 1218 (2011) 156.
[69] V. Vergote, B. Baert, E. Vandermeulen, K. Peremans, H. van Bree, G. Slegers, C. Burvenich, B. De Spiegeleer, J. Pharm. Biomed. Anal. 46 (2008) 127.
[70] M.R. Hadjmohammadi, S.S.S.J. Nazari, J. Sep. Sci. 33 (2010) 3144.
[71] R.M.B.O. Duarte, A.C. Duarte, Anal. Chim. Acta 688 (2011) 90.
[72] A. Prieto, O. Zuloaga, A. Usobiaga, N. Etxebarria, L.A. Fernández, J. Chromatogr. A 1174 (2007) 40.
[73] R.N. Rao, P.K. Maurya, D.D. Shinde, S. Khalid, J. Pharm. Biomed. Anal. 55 (2011) 282.
[74] J.S. Space, A.M. Opio, B. Nickerson, H. Jiang, M. Dumont, M. Berry, J. Pharm. Biomed. Anal. 44 (2007) 1064.
[75] H.K. Trivedi, M.C. Patel, Int. J. ChemTech Res. 2 (2010) 1355.
[76] T.A.C. Vermeij, P.M. Edelbroek, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 810 (2004) 297.
[77] T.A.C. Vermeij, P.M. Edelbroek, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 857 (2007) 40.
[78] C. Ye, J. Liu, F. Ren, N. Okafo, J. Pharm. Biomed. Anal. 23 (2000) 581.
[79] M.A. Molina, W. Zhao, S. Sankaran, M. Schivo, N.J. Kenyon, C.E. Davis, Anal. Chim. Acta 628 (2008) 155.
[80] A. Vallejo, M. Olivares, L.A. Fernández, N. Etxebarria, S. Arrasate, E. Anakabe, A. Usobiaga, O. Zuloaga, J. Chromatogr. A 1218 (2011) 3064.
[81] V. Yusà, O. Pardo, A. Pastor, M. De La Guardia, Anal. Chim. Acta 557 (2006) 304.
[82] A.T. Nguyen, T. Aerts, D. Van Dam, P.P. De Deyn, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 878 (2010) 3003.
[83] B. De Backer, B. Debrus, P. Lebrun, L. Theunis, N. Dubois, L. Decock, A. Verstraete, P. Hubert, C. Charlier, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 877 (2009) 4115.
[84] N.C. Maragou, E. Rosenberg, N.S. Thomaidis, M.A. Koupparis, J. Chromatogr. A 1202 (2008) 47.
[85] J. He, S.A. Shamsi, J. Chromatogr. A 1216 (2009) 845.
[86] R.M.B.O. Duarte, A.C. Duarte, J. Chromatogr. A 1217 (2010) 7556.
[87] J. Lundgren, J. Salomonsson, O. Gyllenhaal, E. Johansson, J. Chromatogr. A 1154 (2007) 360.
[88] R.M. Vinci, M. Canfyn, B. De Meulenaer, T. de Schaetzen, I. Van Overmeire, J. De Beer, J. Van Loco, Anal. Chim. Acta 672 (2010) 124.

[89] L. Pan, Y. Qiu, T. Chen, J. Lin, Y. Chi, M. Su, A. Zhao, W. Jia, J. Pharm. Biomed. Anal. 52 (2010) 589.
[90] C.N. Borges, R.E. Bruns, A.A. Almeida, I.S. Scarminio, Anal. Chim. Acta 595 (2007) 28.
[91] C. Seto, K.P. Bateman, B. Gunter, J. Am. Soc. Mass Spectrom. 13 (2002) 2.
[92] S.C. Wang, H.J. Liao, W.C. Lee, C.M. Huang, T.H. Tsai, J. Chromatogr. A 1212 (2008) 68.
[93] J. Urban, F. Svec, J.M.J. Fréchet, J. Chromatogr. A 1217 (2010) 8212.
[94] M. Toueille, A. Uzel, J.F. Depoisier, R. Gantier, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 879 (2011) 836.