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Application of response surface methodology as mathematical and statistical tools in natural product research

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ABSTRACT

Response Surface Methodology (RSM) models combined with experimental designs became popular in recent years. Their advantages to analyze the interactive effects among different factors and the efficiency considerations encouraged more researchers to conduct natural product research employed with these models. A review about basic principles of factorial designs, central composite designs, Box–Behnken designs, and Doehlert designs which utilized along with the RSM and their applications in natural product research is presented. The objective of this review was to report various experimental design techniques and their applications in the field of natural product research. Furthermore, the characteristic of each design is also presented and the trends of RSM applications in natural product research are also discussed in this paper.

INTRODUCTION

Natural products can be described as products achieved from various natural sources such as plants, microbes, and animals. An entire organism, a part of an organism, an extract from an organism or part of an organism and an exudate, and pure compound are categorized as natural products (Sarker and Nahar, 2007). Secondary metabolites as non-essential small biomolecules from natural products were studied during thousands of years due to their various functions, depending on the origin organism with several variations of the origin, organism's habitat, and organism's activity (Bernardini *et al.*, 2018). Secondary metabolites are classified into five types, namely, alkaloids, fatty acid-derived substances and polyketides, terpenoids and steroids, non-ribosomal polypeptides, and enzyme cofactors (Thirumurugan *et al.*, 2018). Further, the

rapid development of genomics research leads to metabolomics studies which become a powerful tool for discovering and producing secondary metabolite (Breitling *et al.*, 2013).

In the last four decades, the interest for discovering and developing new drugs from natural products has increased significantly (Li and Lou, 2018; Newman and Cragg, 2016). Natural products played important roles to solve several health problems as anti-inflammatory agent (Mahdi, 2010), analgesic agent (Soares-Bezerra *et al.*, 2013), anticancer agent (Handayani and Aminah, 2017; Puspita *et al.*, 2019; Yuliani *et al.*, 2016), antibiotics (Moloney, 2016), anti-angiogenic agent (Aghamohammadi and Hosseinimehr, 2014), antibiofilm (Hamzah *et al.*, 2018), antimalarial drugs (Mojab, 2012), antileukemic agent (Newman *et al.*, 2000), etc. Sources of natural product in drug discovery varied from fungi, plants, marine environment, marine algae, marine sponges, and other marine sources (Dias *et al.*, 2012). Nature is not only providing a new source of drugs but also providing sources for foods (Parodi *et al.*, 2018). There are many different nutrients such as carbohydrates, fats, proteins, minerals, and vitamins that can be achieved by eating

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good meals from natural products (Abdel-Rahman *et al.*, 2011; Burgess and Glasauer, 2004).

Natural products research can become promising in the field of drug discovery and essential future foods development, but there still remain several challenges to be faced by the academic and industrial researcher (Hunter-Cevera *et al.*, 2014). Questions related to active compounds, action mechanism, pharmacology-toxicology aspects, and drugs interaction were frequently asked to ensure the pharmacological effects as well as clinical results (Shan *et al.*, 2007). Hence, it is important to perform standardization and optimization process to evaluate active compounds content (Yuliani *et al.*, 2016), extraction process (Yuliani *et al.*, 2018), computational screening and design (Riswanto *et al.*, 2017), formulation composition (Benali *et al.*, 2014), analytical method validation (Choi *et al.*, 2018), laboratory conditions (Jeong *et al.*, 2017), and instrument settings (Siregar *et al.*, 2017).

Currently, integration of technology and mathematical techniques can be employed to overcome several scientific problems both for education and research (Luneeva and Zakirova, 2017). Since analytical instrument could produce a lot of data and many factors might affect the complexity of experimental results, experimental design as one of chemometrics method can be applied and become the best choice for performing data optimization (Miller and Miller, 2010). Experimental design is commonly used to explain the process of identifying, designing, and evaluating experimental factors statistically (Miller and Miller, 2010; Vera-Candioti *et al.*, 2014). Table 1 exhibits list of the most commonly used design of experiments for optimization study. Response surface methodology (RSM), a special type of experimental design, can be described as a collection of both mathematical and statistical techniques for designing, developing, improving, optimizing, and formulating of product designs where several variables potentially affect characteristics of products designs (Myers *et al.*, 2009). This method improved the traditional optimization which has been carried out by evaluating the effect of one-variable-at-a-time. RSM has the capability to analyze the interactive effects among the variables and minimize ineffective time and reagent used for a research project (Bezerra *et al.*, 2008).

It is necessary to choose an appropriate experimental design before applying RSM. This present review paper discusses various experimental designs with different basic principles and applications in natural product research.

METHODS

Relevant information in the field of natural product research, herbal chemistry, computational statistics, and RSM applications were investigated and assembled from several sources such as Google Scholar, Elsevier, Science Direct, Pubmed, SciFinder, Scopus, and Web of Science. Supporting references sourced from journal articles, books, theses, and scientific reports were accessed using facilities provided by Gadjah Mada University, Indonesia. The literature search was carried out between March and June 2019. The keywords used in the literature search included "natural product," "response surface methodology," "experimental design," "factorial design," "Box–Behnken design (BBD)," "central composite design (CCD)," and "Doehlert design".

FACTORIAL DESIGN

The term factorial design is defined as an experimental design in which combinations of levels of factors were set as the runs (Hibbert, 2012). Factorial design was categorized as full factorial design and fractional factorial design which both of them with two levels for each factor (k) were commonly used in process of screening design due to their efficiency and economical consideration (Vera-Candioti *et al.*, 2014). It is possible to combine every factor at the designed level using full factorial design as L^k combinations, where k represents number of factors and L represents designed levels. On the other hand, the fractional factorial design only evaluates a specific subset of a full design. Fractional factorial design enables the evaluation of a relatively large number of factor in small number of runs or experiments. This method was designed by fractioning a full factorial design of L^k combinations into L^{k-p} combinations, where p represents the number of independent design generators. It should be noted that the fractional factorial design can reduce the number of runs or experiments but it does not possible to estimate all major and

Table 1. Design of experiments for common optimization study.

Design	Factor levels	Number of experiments	RSM	Characteristics
Factorial design (FD)				
Two-level full factorial (2-FFD)	$2 \leq k \leq 5$	2^k or 2^{k+l} (considering replicates)	No	Commonly used for screening study.
Two-level fractional factorial	> 4	2^{k-p}	No	Commonly used for screening study with a smaller number of runs.
Three-level full factorial (3-FFD)	3	3^k	Yes	Requires a large number of runs.
Central composite design (CCD)	5	$2^k+2k+Cp$	Yes	• All factors are studied in five levels $(-\alpha, -1, 0, +1, +\alpha)$.
				• α-values can be calculated using the formula of $\alpha=2^{(k-p)/4}$
Box–Behnken design (BBD)	3	$2k(k-1)+Cp$	Yes	• All factors should be adjusted in three levels $(-1, 0, +1)$.
				• No factorial or extreme points.
Doehlert design (DD)	Depend on each factor	k^2+k+Cp	Yes	Factor levels are different for each factor.

k = number of factors; p = number of independent generators; Cp = the replicate number of the central point; α = star point location determinator.

interaction effect separately (Brereton, 2007; Hibbert, 2012). However, only full factorial designs at three levels can be applied in conjunction with the RSM by running 3^k experiments. Figure 1 illustrates the model of factorial design at various levels.

Table 2 presents some studies employed by the factorial design. Portilla-Rivera *et al.* (2009) performed a fractional factorial design in order to study the relationship between the type and the ratio of hemicellulosic sugars (Portilla-Rivera *et al.*, 2009). An incomplete/fractional 3^3 factorial design has been developed to study the effect of glucose, xylose, and arabinose on the production of surface-active compounds by *Lactobacillus pentosus*. This study resulted in the optimum ratio of xylose/glucose was 1.5–3.5 for producing biosurfactants achieved from the trimming vine shoots or grape marc. Fractional factorial designs were also useful for extraction optimization both for *Salvia mirzayanii* (Khajeh and Ghanbari, 2011) and *Artemisia annua* (Khalil *et al.*, 2011). Khajeh and Ghanbari (2011) developed a two-level fractional factorial design (2^{4-1}) based on an analysis of variance to evaluate the significance of microwave power, temperature, time, and type before performing a BBD for optimizing the extraction condition of *S. mirzayanii* oil. Another study by Khalil *et al.* (2011) developed a three-level fractional factorial design (3^{k-1}) for extraction of artemisinin. The optimum condition was achieved by sample immersion in chloroform for 2 minutes with the ratio of sample/solvent of 0.5/3 g/ml.

Full factorial designs were also used for several studies with similar optimization purpose such as evaluating the extraction process or taking part in screening models of optimization. Setyawan *et al.* (2018) optimized the extraction condition of *Camellia sinensis* L. using a full two-level and two-factor factorial design. The optimum condition was achieved with the water temperature of 95°C and two-times brewing number. Other studies developed full two-level and three-factor factorial designs for evaluating both the preparation process and extraction condition for natural products such as Ginger rhizome (Shah and Garg, 2014), *Lippia sidoides* (Lima *et al.*, 2015), and a fermented product from soybean called tempeh (Yuliani *et al.*, 2018). As reported, the fractional and full factorial designs were widely used in optimization and design screening studies because of their economic advantages and efficiency. Nevertheless, the RSM model was only applicable to be performed along with full three or more level factorial designs due to their ability to generate all possible combinations from a data set of factors in order to investigate the effects of all factors and interaction (Sen, 2016).

RSM is suitable for fitting a quadratic surface in order to optimize process parameters. Further, the RSM strongly supported the experimental studies to determine the optimum process (Behera *et al.*, 2018). The full three-level factorial design with RSM has been performed by de Aragão *et al.* (2005) and Saccani *et al.* (2005) to optimize chromatographic separation.
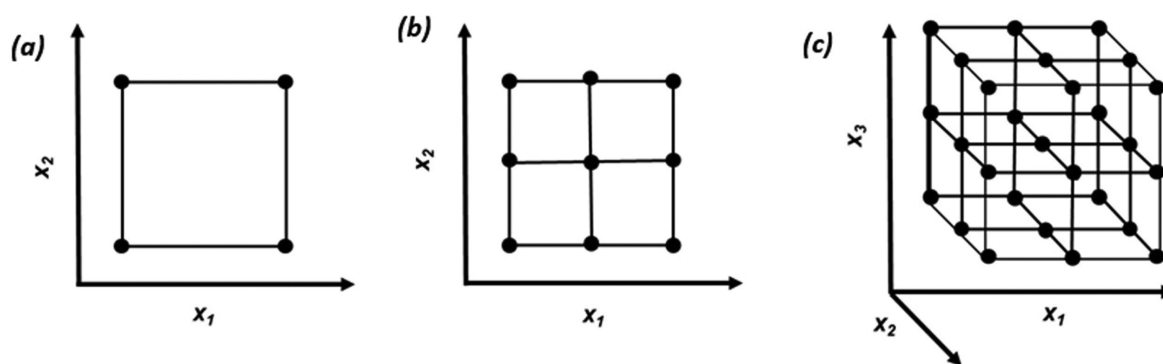

Figure 1. Illustration of factorial design models in various levels: (a) two-levels, two factors (2^2) design; (b) three-levels, two factors (3^2) design; and (c) three-levels, three factors (3^3) design.

Table 2. Reports of factorial design application in natural product research.

Samples	Objective of study	Types of factorial design	References
L. pentosus	Testing the relationship between the type and the ratio of hemicellulosic sugars for producing biosurfactants	Fractional FD	(Portilla-Rivera *et al.*, 2009)
S. mirzayanii	Evaluating the effect of different parameters on the microwave-assisted extraction	Fractional FD	(Khajeh and Ghanbari, 2011)
A. annua	Optimizing extraction process	Fractional FD	(Khalil *et al.*, 2011)
Ginger rhizome	Determining the optimum extraction conditions	Full 2^3 FD	(Shah and Garg, 2014)
L. sidoides	Evaluating the influences of variables of preparation	Full 2^3 FD	(Lima *et al.*, 2015)
Tempeh (a fermented product from soybean)	Evaluating factors of the extraction process	Full 2^3 FD	(Yuliani *et al.*, 2018)
C. sinensis L.	Optimizing the extraction condition	Full 2^2 FD	(Setyawan *et al.*, 2018)
Tea, coffee, and human urine	Improving the chromatographic resolution of three methylxanthines (caffeine, theobromine, and theophylline)	Full 3^2 FD	(de Aragão *et al.*, 2005)
Fresh and dry-cured pork products	Optimizing the chromatographic conditions	Full 3^2 FD	(Saccani *et al.*, 2005)
Berberis species	Optimizing the berberine loaded liposome for oral administration.	Full 3^2 FD	(Sailor *et al.*, 2015)

Analysis of three methylxanthines such as caffeine, theobromine, and theophylline in the matrix of tea, coffee, and human urine was done by validated RP-HPLC/UV (de Aragão *et al.*, 2005). Another study by Saccani *et al.* (2005) conducted a three-level factorial design and RSM in the optimization of a cation-exchange column and UV detection at 262 nm followed by validated IC-MS determination for quantitative analysis of the niacin level in fresh and dry-cured pork products (Saccani *et al.*, 2005). These two studies presented proposed methods with good chromatographic separation from complex matrices of natural products employed by three-level factorial design and RSM. Application of the three-level full factorial design was also developed by Sailor *et al.* (2015) in the optimization of berberine loaded liposome.

CENTRAL COMPOSITE DESIGN

The two-level designs were useful to be applied in the screening study. However, the two-level designs lack information about maxima or any non-linear relationships since its application lead only on linear models (Hibbert, 2012). Performing a full factorial design with the level more than two will affect the effectiveness of the design itself due to the greater number of experiments that should be done. Hence, it is important to develop a design which allows greater level numbers without running every combination experiments. Presented by Box and Wilson (1951), the CCD becomes solution to overcome these problems. This design consists of three parts, namely, a full factorial or fractional factorial design, axial or star points as additional design,

and central point with all factors set to 0. Figure 2 illustrates the CCD. It can be seen that the factorial and star points lie equidistant from the central point. It allows the design to cover the factor space near the central point with more points than at the peripheral area of the factorial design.

The CCD has been widely applied in the field of natural product research. Table 3 presents some reports of CCD application in natural product research. These models were commonly used in several optimization purposes such as formulation, product enhancement, and separation both for extraction and chromatographic condition. Benali *et al.* (2014) developed natural food jelly from *Phoenix dactylifera* L. fruit syrup and orange albedo powder in lemon juice suspension. The effect of cooking temperature, cooking time, Brix, and cooling stage temperature has been evaluated to achieve the optimum hardness and stickiness of resulted product (Benali *et al.*, 2014). Other related study has been conducted by Kaur *et al.* (2018) which optimized blending process of radish (*Raphanus sativus*) juice production. The CCD provided 20 formulation runs with various series concentration of herbal extract, sugarcane juice, and salt (Kaur *et al.*, 2018).

In the aim of product enhancement, the CCD was employed as one of mathematical and statistical strategy to increase secondary metabolites production and compounds related. Farombi *et al.* (2018) optimized preparation conditions of hydroxyapatite from catfish bones using the CCD under the RSM (Farombi *et al.*, 2018). This study claimed to be important since there was only limited study of hydroxyapatite extraction which can be potentially developed as long-term contaminants containment (Alpat *et al.*, 2008; Barakat *et al.*, 2009; Sobczak *et al.*, 2009). Other studies related to product enhancement were dominated by biotechnological studies. *Haemophilus influenzae, Streptomonospora arabica, Streptomyces rimosus,* and *Myroides gitamensis* were investigated to become natural sources of biomass (Momen *et al.*, 2016), antifungal agent (Managamuri *et al.*, 2017), cholesterol oxidase enzyme (Srivastava *et al.*, 2018), and L-asparaginase enzyme (Talluri *et al.*, 2019), respectively.

In the field of separation science from natural products, the CCD was applied for optimization of both extraction condition and performance parameters of the chromatographic method. This design was developed in the extraction of *Lotus plumule* (Xiong *et al.*, 2016), *Curcuma zedoaria* leaves (Azahar *et al.*, 2017), and
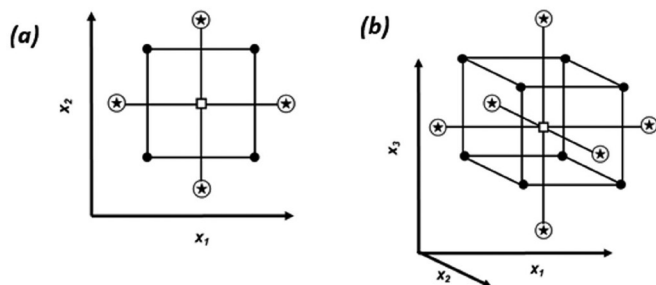

Figure 2. Illustration of central composite designs for (a) two factors and (b) three factors optimization. Every design consists of factorial points (●), star points (⊗), and central points (□).

Table 3. Reports of central composite design application in natural product research.

Samples	Objective of Study	References
P. dactylifera L.)	Optimizing formula of natural food jelly from natural ingredients	(Benali *et al.*, 2014)
Ericaceae plant materials	Establishing optimal separation conditions	(Owczarek *et al.*, 2016)
L. plumule	Optimizing MAE conditions	(Xiong *et al.*, 2016)
Haemophilus influenzae	Determining the optimum cultivation conditions for growth and biomass production	(Momen *et al.*, 2016)
C. zedoaria leaves	Maximizing the extraction yield of total phenolics and flavonoids	(Azahar *et al.*, 2017)
S. arabica VSM25	Studying the impact of five variables on the production of antifungal metabolites	(Managamuri *et al.*, 2017)
Mango seed kernel oil	Optimizing temperature, time, and amount of n-hexane	(Mas'ud *et al.*, 2017)
S. rimosus	Predicting the combination of yeast extract, dextrose, starch and ammonium carbonate at various concentrations	(Srivastava *et al.*, 2018)
Nutritional beverage	Optimizing component in the development of nutritional beverages	(Kaur *et al.*, 2018)
Catfish bones	Optimizing preparation conditions for producing hydroxyapatite	(Farombi *et al.*, 2018)
M. gitamensis BSH-3	Optimizing of process parameters of L-asparaginase enzyme production	(Talluri *et al.*, 2019)

mango seed kernel oil (Mas'ud *et al.*, 2017). Owczarek *et al.* (2016) conducted the standardization of Ericaceae medicinal plants using ultra-high performance liquid chromatography-photodiode array for determining oleanolic and ursolic acids. The CCD played an important role in separation conditions optimization. Temperature and flowrate were established as factors. On the other hand, separation time, resolution, and total response were marked as responses. This study declared that the developed method was successfully applied to evaluate the content of oleanolic and ursolic acids in Ericaceae plant samples.

BOX–BEHNKEN DESIGN

In several cases of natural product research, it is a common possibility to find problems which should be solved using mathematical model or design using three levels or more and three factors or more. Box and Behnken (1960) presented a particular class of three-level fractional factorial design with the specification for studying quantitative variables and now this finding was well known as the BBD. This design was more efficient and economical than other three-level designs due to its ability to allow points selection from the three-level factorial arrangement (Bezerra *et al.*, 2008). The characteristics for the BBD are that there are no factorial or extreme points. This design requires fewer points than the CCD as it was promoted by Ferreira *et al.* (2007) for the optimization of analytical methods. Figure 3 illustrates the model of the BBD.
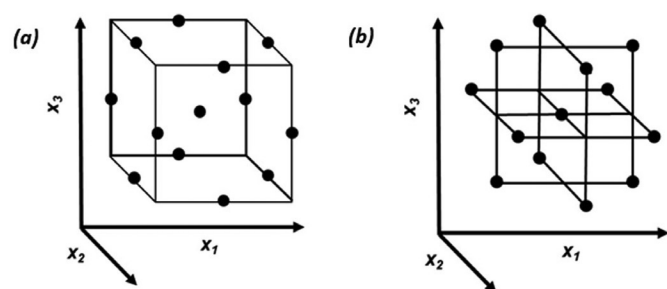

Figure 3. Illustration of the two graphical forms for the three factors BBD: (a) the cube for BBD and (b) three interlocking 2^2 factorial design.

Table 4 presents some reports of BBD application in natural product research. Similar to CCD, the BBD can be applied for optimization study including extraction process, product enhancement, and chromatographic separation. These designs were widely used to optimize several parameters of extraction such as extraction time, extraction temperature, extraction number, solid to liquid ratio, solvent selection, and solvent concentration (Anuar *et al.*, 2013; Jeong *et al.*, 2017; Sousa *et al.*, 2014; Yoo *et al.*, 2018). For specific extraction technique, namely, decoction process, parameters including decocting temperature, total decocting time, and water-to-medicinal sample ratio were evaluated (Ha *et al.*, 2018; Zhang *et al.*, 2017). The BBD can be conducted for other special technique, namely, microwave-assisted extraction to evaluate the effect of microwave power, temperature, and solvent type (Khajeh and Ghanbari, 2011).

Abdella *et al.* (2018) evaluated variables for genistein production by deglycosylation process involving commercial β-glucosidase enzyme. Concentration of enzyme, agitation rate, reaction time, and pH has been stated as factors of experiments with 25 runs. This study has successfully obtained the optimal combination of the major reaction affecting factors to enhance genistein production (Abdella *et al.*, 2018).

Studies on chromatography also reported the application of the BBDs with HPLC and high performance thin layer chromatography (HPTLC). Prabaningdyah *et al.* (2017) developed an HPLC method to determine curcumin and desmethoxy curcumin in Curcuma syrup formulation employed by the BBD with RSM. This method was successfully optimized and validated according to the International Conference on Harmonization (ICH) guidelines. Hence, this method could be applied for routine analysis of curcuminoids content in syrup formulation. The BBD was not only useful to be applied along with HPLC method but also with other chromatographic technique, namely, HPTLC. Gohel *et al.* (2018) conducted BBD-assisted optimization for quantitative analysis of quercetin, kaempferol, and keto-β-boswellic acid. Three HPTLC conditions including volume of *n*-hexane, solvent front, and chamber saturation time have been chosen as experimental factors to evaluate five responses related to optimum separation. This study resulted that the proposed

Table 4. Reports of BBD application in natural product research.

Samples	Objective of Study	References
S. mirzayanii	Optimizing conditions for extraction	(Khajeh and Ghanbari, 2011)
Melastoma malabathricum Fruit	Optimizing of variables for obtaining anthocyanins pigment from the extraction process	(Anuar *et al.*, 2013)
Yukmijihwang-tang (an herbal formula)	Optimizing extraction process	(Kim *et al.*, 2014)
Stryphnodendron adstringens (Mart.) Coville bark extracts	Optimizing extraction parameter	(Sousa *et al.*, 2014)
Ephedra intermedia, Rheum palmatum, and *Lithospermum erythrorhizon*	Optimizing the experimental conditions in the laboratory to extract (−)-ephedrine and (+)-pseudoephedrine	(Jeong *et al.*, 2017)
Curcuma syrup	Optimizing HPLC condition	(Prabaningdyah *et al.*, 2017)
Huang-Qi-Liu-Yi Tang (an herbal formula)	Optimizing the decoction conditions	(Zhang *et al.*, 2017)
Soy flour	Evaluating variables for genistein production	(Abdella *et al.*, 2018)
S. oleracea and *B. serrate*	Optimizing of the HPTLC conditions	(Gohel *et al.*, 2018)
Eungyo-san (EGS) (an herbal formula)	Investigating optimal conditions for "Decocting later"	(Ha *et al.*, 2018)
M. officinalis L.	Evaluating the effects of three independent variables on *M. officinalis* L. extraction	(Yoo *et al.*, 2018)

method has met the requirements for validated HPTLC method for the quality control testing of *Spinacia oleracea* and *Boswellia serrata*.

DOEHLERT DESIGN

Doehlert (1970) developed an alternative experimental design which has several advantages such as few experimental points, high efficiency, and economically effective. Different from central composite and BBDs, these designs are not rotatable due to their number of estimation for varied factors. Nevertheless, Doehlert designs have different numbers of levels for different factors and allow to fill the provided factor space uniformly according to its possibility (Hibbert, 2012). Belonging to a second-order experimental design, Doehlert designs describe different characteristics for different levels: 1) a circular domain for two variables; 2) spherical domain for three variables; and 3) hyperspherical domain for four and more variables, which accents the uniformity of the studied variables in the experimental domain (Bezerra *et al.*, 2008). Figure 4 illustrates the model of the Doehlert design.

Table 5 presents some reports of Doehlert design application in natural product research. Although commonly applied in chromatographic optimization (Araujo and Janagap,

Figure 4. Illustration of the model of the Doehlert design for the optimization of (a) two variables and (b) three variables.

Table 5. Reports of Doehlert design application in natural product research.

Samples	Objective of Study	References
Medicinal herbs	Evaluating instrumental parameters of the LIBS	(Andrade *et al.*, 2017)
S. mombin L Apple Bagasse Agroindustrial Residues	Achieving the maximum response for the extraction of total phenolic compounds	(Santos-Felix *et al.*, 2018)
Solanaceae family plants	Optimizing extract purification procedure	(Ciechomska *et al.*, 2016)
Belladona extract	Optimizing electrophoretic conditions	(Mateus *et al.*, 1998)
Bovine milk	Optimizing extraction condition for determining of organochlorine pesticides	(Merib *et al.*, 2014)
Almond shells	Optimizing the dioxane organosolv	(Quesada-Medina *et al.*, 2011)
Sugar	Optimizing experimental design methodology	(Pinkowska *et al.*, 2019)
Polygonum cuspidatum	Optimizing the ultrahigh pressure extraction (UPE) process	(Wang and Liu, 2005)

2012), this design was used in other fields of natural product and environmental research. Mateus *et al.* (1998) optimized the micellar electrokinetic capillary chromatography conditions using Doehlert design to determine selected tropane alkaloids. Three factors were evaluated; namely, the buffer pH, the micelle concentration, and the acetonitrile percentage. The most favorable conditions were achieved with 30 mM borate–phosphate buffer (pH = 8.7), micelle concentration of 40 mM sodium dodecyl sulfate, and 16.5% acetonitrile.

The Doehlert designs were also applied in environmental research. Merib *et al.* (2014) conducted the optimization of extraction condition in order to determine organochlorine pesticides residue in bovine milk employed by the Doehlert design (Merib *et al.*, 2014). Other related study implemented Doehlert design in evaluation of instrumental parameters of the laser-induced breakdown spectroscopy (LIBS) for analyzing essential elements (Ca, K, and Mg) from medicinal herbs (Andrade *et al.*, 2017).

In extraction process optimization, Doehlert design was applied for optimizing procedure including solvent used, extraction time, temperature, and agitation speed. Various samples such as *Spondias mombin* L. (Santos-Felix *et al.*, 2018), Solanaceae family plants (Ciechomska *et al.*, 2016), and almond shells have been involved in these studies (Quesada-Medina *et al.*, 2011). More advanced extraction techniques such as hydrothermal extraction, ultrahigh pressure extraction (UPE) were also employed by the Doehlert design (Pinkowska *et al.*, 2019; Wang and Liu, 2005).

CONCLUSION

The RSM designs were widely applied in the field of natural product research due to their advantages compared to the traditional one-variable-at-a-time design. In recent years, there were a large number of publications related to RSM designs and their application. However, this present review focuses on basic principles of factorial designs, CCDs, BBDs, and Doehlert designs which utilized along with the RSM and their applications in natural product research.

Natural product research conducted using CCDs, BBDs, and Doehlert designs were found more frequent in the last decade compared to the full three-level factorial design. The efficiency and economic approach were assumed to be the main consideration for why the popularity of fractional design was increased nowadays. Optimization of the extraction process and analytical method development were found to be the most popular consideration on generating the RSM in the natural product research due to their ability to evaluate the interactive effects among different variables. Although, other considerations such as secondary metabolite production and related compound enhancement as well as environmental assessment encouraged researchers to utilize the RSM models.

Finally, RSM models combined with experimental design can be employed as mathematical and statistical tools in natural product research. This strategy was not only good in the optimization process but also proved to be validated for the analytical method and effective for product enhancement in natural product research.
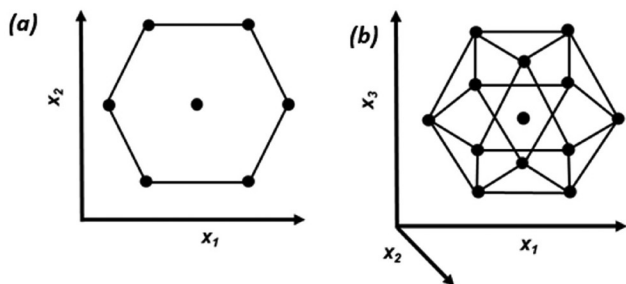
CONFLICT OF INTEREST

All the authors declared there is no conflict of interest.

AUTHORS' CONTRIBUTION

FDOR carried out the literature search, investigation, and initial draft writing. AR reviewed the initial draft and carried out content supervising in the field of chemometrics, analytical chemistry, and computational statistics. SP reviewed the initial draft and carried out content supervising in the field of natural product research and separation science. SM provided the conceptualization of the article, reviewed the initial draft, and carried out content supervising in the field of analytical chemistry and analytical instrumentations.

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