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Sample preparation optimization for assay of active pharmaceutical ingredients in a transdermal drug delivery system using experimental designs

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Abstract

A simple but very effective sample preparation method is discussed for a matrix or drug-in-adhesive type of transdermal drug delivery system (TDS). The method is a one-step extraction using a methanol/water solvent system. Because of the unique design and physical property of the delivery system, special considerations were taken in selection of sample solvent, sample container and extraction enhancement device. The main focus of the article is on method optimization using experimental designs. A Plackett–Burman design was used to screen multiple method factors including extraction solvent strength, extraction solvent volume, shaking speed of a reciprocating shaker, and shaking time. Later, two of the factors were studied in more details using a 4×5 general factorial design. From the experimental results, the so-called main effects plots and interaction plots were generated using a statistical software. The plots are helpful in choosing the method conditions.

Keywords: Transdermal drug delivery system; Plackett–Burman design; General factorial design; Sample preparation

1. Introduction

Recently, there has been resurgence in development of transdermal delivery systems (TDS or transdermal patch) for therapeutic use because of its better safety profile, better bioavailability, and better patient compliance. TDS can be divided into two categories: the active and passive transdermal systems. The active TDS uses active assisting means, including ultrasound (Sonoporation), laser, iontophoresis and electroporation, to push the drug through the skin. The passive TDS allows the active pharmaceutical ingredient (API) to defuse through the skin layers to achieve drug delivery. [1–3] This paper discusses a particular type of TDS, the so-called drug-in-adhesive matrix (DIAM) system in the context of sample preparation considerations.

The importance of sample preparation has received active discussions in the literature [4–7]. The sample preparation procedure is a pivotal part of an analytical method for quantitative analysis of different products, including pharmaceutical products [8]. The development of a sample preparation method involves selection of suitable reagents, materials and apparatus (sample solvent, container, extraction enhancement devices, filtration devices, etc.), and selection/optimization of method factors (organic solvent concentration, pH, temperature, extraction time, energy level, etc.). The initial selection of sample preparation reagents and materials is based on knowledge of the formulation design and physical properties of the API and the intended purpose of the method. The sample preparation procedure will impact the method's accuracy, repeatability and laboratory-to-laboratory reproducibility as well as its simplicity, safety, and time and cost-effectiveness.

Development of sample preparation method for TDS, particularly the DIAM type of TDS, has proved a challenge due to its unique physical properties. A DIAM system is composed of three layers: the backing, which is usually a piece of flexible polymer; the adhesive layer, which also contains

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the API; and the protective release liner, which is removed before the delivery system is used. The fact that TDS is not designed to release the API(s) in aqueous media makes the sample preparation for a DIAM system difficult. The conventional procedures designed for the common dosage forms such as tablets or capsules will not work. The tackiness of the system makes the sample preparation even more difficult because it will readily attach to the container or fold up on itself potentially resulting in poor recovery.

In this article, we report a simple but effective sample preparation method for the DIAM system. The procedure is a one-step extraction using methanol/water as sample solvent and utilizes a reciprocating shaker to provide agitation. We also demonstrate the use of factorial experimental designs to optimize four method factors including sample solvent strength, sample solvent volume, shaking speed, and shaking time. Compared with one-factor-at-a-time experiments, a factorial experiment is more efficient in multi-factor optimization. More importantly, when the multiple independent variables of a method will generate a maximum point (an optimized condition), the one-factor-at-a-time experiments can easily miss the optima, whereas the factorial experiments will give a combination near the maximum [8]. In this study, we report a two-step optimization process. First, a 10-experiment set Plackett–Burman design was used to screen the four operating factors. This type of design is called the fractional factorial design [9], and has been used elsewhere in method development and validation [10–16]. Plackett–Burman designs are often used to screen a number of factors using a relatively small number of experiments to identify the factors that have the greatest effect on the response variables. In the second step, a 4×5 general factorial design was used to allow for a more detailed examination of two chosen factors.

2. Experimental

2.1. Chemicals and reagents

HPLC-grade methanol was purchased from EM Science (An affiliate of Merck KGaA, Darmstadt, Germany). HPLC-grade equivalent water was obtained from an in-house Millipore Milli-Q-Gradient ultrapure water system (Millipore, USA). This study also involves a proprietary Johnson & Johnson Pharmaceutical Research & Development (J&JPRD) compound, which is identified as API_J&J, and a proprietary transdermal product, which is identified as DIAM_J&J.

2.2. Apparatus

A 4-oz straight-sided round, wide-mouth glass jar (70 mm height $\times$ 50 mm i.d.) with 0.030 mm PTFE disc-lined cap was used as the container for sample preparation. A reciprocating shaker (Model HS501, IKA Works, USA) with a stroke length of 3 cm was used to provide agitation in sample preparation.

2.3. Sample preparation method

Solutions for each DIAM_J&J system were prepared by carefully placing one sample into a 4-oz wide-mouth glass jar, making sure that the adhesive-side faces up and does not attach to the wall of the jar. Subsequently, 25.0 mL of sample solvent (70% methanol, unless otherwise specified) was added to the jar via pipette and the jar capped tightly. Next (immediately after solvent addition) samples were placed on a reciprocating shaker at a frequency of 150 rpm for 3 h (unless otherwise specified). After the shaking was completed, samples from each glass jar were immediately transferred into HPLC vials for sample analysis.

2.4. Computer software

Minitab, the statistical software, was purchased from Minitab Inc. (State College, PA, USA).

2.5. HPLC analysis of samples

A Waters (Milford, MA) Alliance HPLC system equipped with a photodiode array detector was used for the sample analysis. The Waters Millennium32 software was used to acquire, store, and process the chromatographic data and to report results. All chromatographic runs were performed using a Supelco (Bellefonte, PA, USA) Discovery® RP Amide C16 column (4.6 mm $\times$ 250 mm, 5 μm particle size) and water (A) and acetonitrile/methanol (50/50, B) mobile phases. The gradient elution was programmed to start with 45% and end with 68% B in 23 min with no holding time at a flow rate of 1.0 mL/min. UV detection at 220 nm, column temperature of 40 °C, and an injection volume of 25 μL were used in the method.

3. Results and discussion

3.1. Selection of extraction method and solvent system

Two different approaches were considered for sample preparation of the DIAM_J&J system. In one approach the adhesive layer was dissolved in hexane. Then a liquid–liquid extraction step is performed using methanol and water. An aliquot of the aqueous phase was then used for HPLC analysis. One of the major disadvantages of this approach is that the drug delivery system self-folds as soon as it is in contact with hexane, which can cause incomplete recovery of the API. Additional measures had to be taken to prevent this from happening, which had the potential of introducing contaminants. The second approach, which is the topic of this article, was to use an aqueous solvent to extract the API without dissolving the adhesive layer. This approach is based on the fact that the API has very limited solubility in the adhesive phase and the adhesive layer is relatively thin, which will allow the API to diffuse into the extraction solvent in an acceptable time

period with the help of a vigorous agitation. Also, the patch does not self-fold in aqueous solution, which avoids the use of additional devices or measures during the extraction procedure. In preliminary experiments, acetonitrile/water and methanol/water were compared as extraction solvents. It was found that the level of chromatographic interference from the backing material and other excipients was worse when acetonitrile/water was used as extraction solvent. To the contrary, the methanol/water system showed excellent selectivity in suppression of excipient component extraction compared to both acetonitrile and hexane. Therefore, methanol/water was chosen as the sample solvent of choice for the method.

3.2. Selection of container and extraction enhancement device

Due to unique physical property of the sample, selection of sample container warrants some special considerations. A round, straight-sided, wide-mouth glass jar was chosen so that the analyst could transfer the patch easily into the container and lay it flat on bottom of the jar. On the other hand, the internal diameter of the container was chosen, so that the patch would fit nicely in the container without too much extra room. This is important later in the shaking process to ensure the generation of vigorous sweeping waves over the surface of the patch, which makes the extraction complete in a relatively short period of time. A reciprocating shaker was chosen over a sonicator or other extraction enhancement devices for the reason that it will provide the mechanical shaking energy in a consistent and reproducible way, independent of other factors, including shaker brands, number of samples prepared and temperature as long as the shaking speed and stroke length are specified.

3.3. Screening of method factors using Plackett–Burman experimental design

After the sample solvent, container and extraction enhancement device were selected, method factors were chosen for optimization. There are many factors that may affect the sample preparation method. Four of them, including sample solvent strength (methanol, %), sample solvent volume (mL), shaking time (h) and shaking frequency (rpm), were chosen for optimization. Other factors, such as temperature, different brands of shakers, the stroke length of shaker, were not investigated either due to infeasibilities of conducting the experiment or due to unavailability of the required devices. The Plackett–Burman experimental design was used in the preliminary screening study. A 10-experimental set Plackett–Burmann experimental design was used to study these factors (Table 1), using the statistics software Minitab. The purpose of the study was to identify the factor(s) that make the most important contributions to extraction variations. In each experiment, two independently weighed standard solutions and two sample solution prepared from two different lots of the DIAM_J&J product, as well as a sample from a placebo lot, were injected. The results for API_J&J from the two lots of DIAM_J&J product are also presented in Table 1. From these results, the main effects plot (Fig. 1) and interaction plot (Fig. 2) were generated for lot 1 using Minitab. Similarly, main effects plot and interaction plot were observed for lot 2. The main effects plot demonstrates that among the four investigated method factors, the shaking speed shows much less significance compared with the other three, which show very similar behavior. Regarding shaking speed, it was observed that it should be maintained at close to 150 rpm. Above this speed, the shaking becomes too vigorous to maintain sweeping waves over the surface of the patch. Therefore, 150 rpm was chosen for the method.

From the interaction plot (Fig. 2), the factor shaking speed showed no sign of interaction with the factors “methanol percent” and “sample solvent volume” and some interaction with “shaking time”. On the other hand, interactions were observed between the other three factors. It should be pointed out that the interactions were much more significant at the lower end of methanol concentration, sample solvent volume, or shaking time. In addition to the assay results for API_J&J, the results for the placebo system were also obtained, which showed an increase in interference level due to the unwanted components from excipients after the shaking time exceeds 3.5 h or with a methanol concentration $\geq$75%. An additional study was conducted to confirm that a 3-h extraction time should be used to achieve the best extraction selectivity. Based on the above results, the shaking time was set at 3 h. However, based on the results from the Plackett–Burman design, it was not clear what were the opti-

Table 1
Plackett–Burman design and results

RunOrder	Center point	Blocks	Methanol (%)	Volume (mL)	Shake speed	Shake time (h)	Result (%) lot 1	Result (%) lot 2
1	1	1	55	25	100	4.5	98.1	97.3
2	0	1	65	20	130	3.5	99.9	99.7
3	1	1	75	25	160	2.5	100.1	100.4
4	1	1	55	15	100	2.5	82.7	81.9
5	1	1	75	25	100	2.5	100.6	100.9
6	1	1	75	15	100	4.5	97.9	97.8
7	1	1	55	15	160	2.5	84.1	84.9
8	0	1	65	20	130	3.5	98.4	98.7
9	1	1	75	15	160	4.5	99.4	98.7
10	1	1	55	25	160	4.5	103.2	102.9

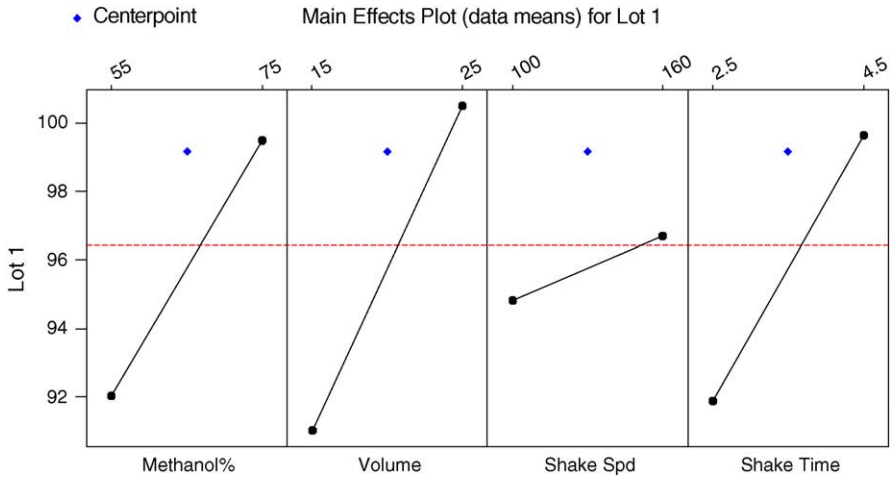

Fig. 1. Main effects plot of lot 1 results from Plackett–Burman design.

mized value for methanol concentration and sample solvent volume.

3.4. Use of general factorial design for method optimization

The final optimization was performed using a 4×5 general factorial design for the remaining two factors, methanol concentration (five levels) and sample solvent volume (four levels). The same samples mentioned above were prepared in the new experiments. The 20-experiment set general factorial design and results are presented in Table 2. Figs. 3 and 4 are the main effects plot and interaction plot, respectively. Table 3 is the two-way analysis of variance for lot 1 results from the general factorial design. If we take into consideration the expanded experimental range for the factor "methanol concentration", actually the main effects plots from both designs are consistent for the two factors studied, with the general factorial design results showing more details. The most significant impact of the operating factors on extraction completeness was observed at low methanol concentration (50–55%). We not only observed low recovery of the active but also interaction between the operating factors. To the contrary, at high methanol concentration ($\geq$70%), we observed good recovery and insignificant interaction between the operating factors. However, at methanol concentration of $\geq$75%, an increased level of interference from the placebo was observed. After taking into consideration all of these factors and lim-

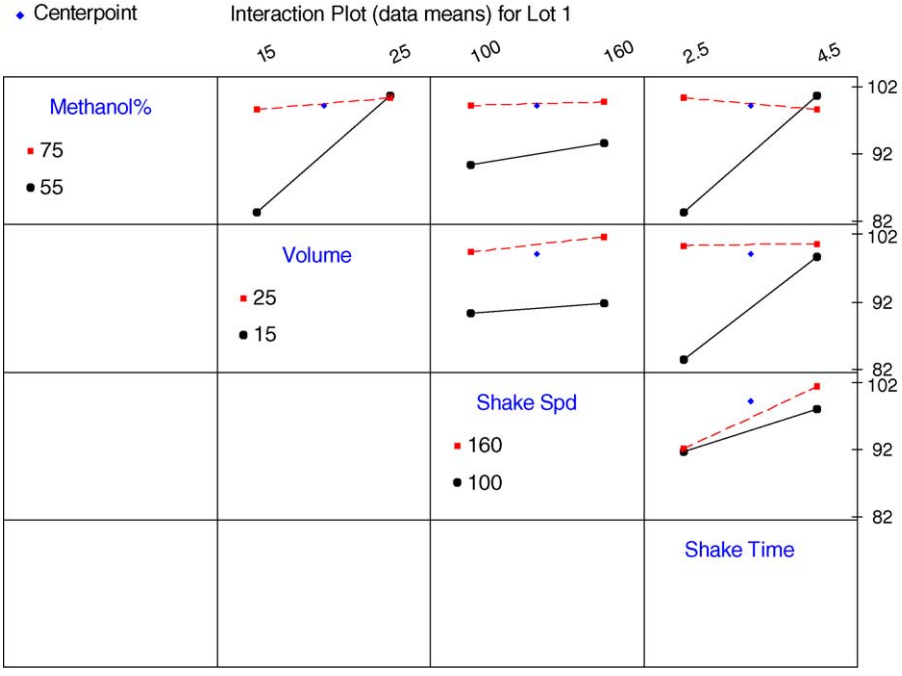

Fig. 2. Interaction plot of lot 1 results from Plackett–Burman design.

Table 2
General factorial design and results

StdOrder	RunOrder	Blocks	Methanol (%)	Volume (mL)	Result (%) lot 1	Result (%) lot 2
1	5	1	50	15	71.1	80.8
2	10	1	50	20	80.3	91.4
3	12	1	50	25	85.4	95.9
4	1	1	50	30	81.3	91.9
5	2	1	55	15	89.9	97.1
6	19	1	55	20	89.3	96.5
7	11	1	55	25	90.2	96.6
8	3	1	55	30	90.9	98.1
9	8	1	60	15	92.3	96.7
10	17	1	60	20	92.7	97.3
11	14	1	60	25	96.3	99.8
12	4	1	60	30	95.5	98.8
13	15	1	70	15	95.3	97.1
14	9	1	70	20	95.7	97.2
15	13	1	70	25	98.4	99.6
16	18	1	70	30	97.0	98.2
17	7	1	75	15	98.8	100.3
18	20	1	75	20	97.8	98.8
19	6	1	75	25	98.5	99.3
20	16	1	75	30	96.7	97.4

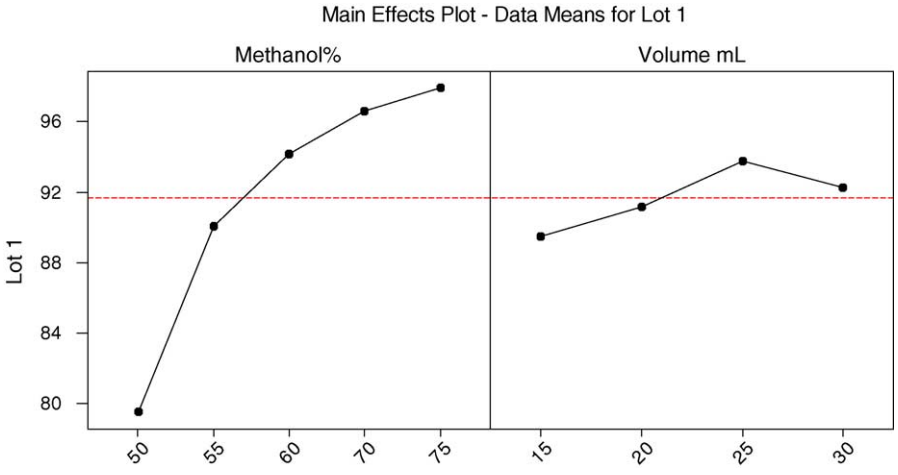

Fig. 3. Main effects plot of lot 1 results from general factorial design.

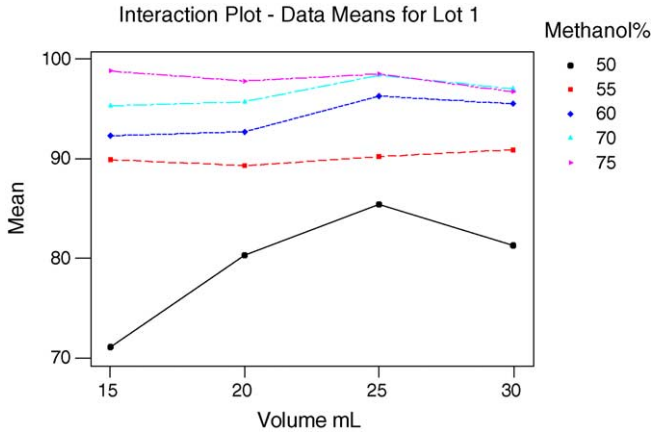

Fig. 4. Interaction plot of lot 1 results from general factorial design.

itations, the following operating conditions were chosen for the method:

- Methanol concentration, 70%
- Shaking time, 3 h
- Shaking speed, 150 rpm
- Sample solvent volume, 25 mL

Table 3
Two-way analysis of variance for lot 1 results from general factorial design

Source	d.f.	Sum of square	Mean square	*F*	*P*
Solvent strength	4	880.76	220.19	32.20	0.000
Solvent volume	3	48.98	16.33	2.39	0.120
Error	12	82.06	6.84		
Total	19	1011.80			

Table 4
Method comparison results (% label)

Sample lot	Method 1		Method 2	
	API	Degradent	API	Degradent
Lot 1				
1	97.8	0.14	96.6	0.15
2	97.6	0.14	98.2	0.13
3	97.2	0.14	97.8	0.13
4	97.4	0.14	96.1	0.13
5	97.2	0.14	98.3	0.12
6	97.7	0.14	97.6	0.12
7	97.2	0.14	97.2	0.10
8	97.3	0.13	98.0	0.10
9	97.7	0.14	97.1	0.10
10	97.1	0.13	96.7	0.10
Mean	97.4	0.14	97.4	0.12
% R.S.D.	0.27	3.1	0.78	14.8
Lot 2				
1	97.9	0.10	98.7	<0.10
2	97.6	<0.10	98.5	<0.10
3	97.2	<0.10	97.0	<0.10
4	97.6	<0.10	96.7	<0.10
5	96.8	<0.10	98.0	<0.10
6	98.5	<0.10	98.2	<0.10
7	97.5	<0.10	97.7	<0.10
8	96.4	<0.10	97.6	<0.10
9	97.3	<0.10	97.8	<0.10
10	97.9	<0.10	97.2	<0.10
Mean	97.5	NA	97.7	NA
% R.S.D.	0.61	NA	0.64	NA

Method 1, this method; Method 2, reference method.

The extraction should be conducted at ambient temperature using a reciprocating shaker with a stroke length of at least 3 cm.

As a side note, the different lots were included in the general factorial design. At lower methanol concentration, lot 1 and lot 2 did show different behaviour in recovery. However, this difference became insignificant when the methanol concentration was increased to 65–75%. Because this sample preparation procedure is for an assay method, sample discrimination in this case is not desired. By choosing the 70% methanol as sample solvent, the sample discrimination effect was minimized.

3.5. Validation of the sample preparation procedure

The sample preparation procedure has been validated by a method comparison study. Two lots of the DIAM_J&J were analyzed using this procedure and a validated reference method. For each lot, 10 samples were prepared and each sample was injected once. The results are presented in Table 4. The reference method uses a two-step procedure for sample preparation. In step one, the adhesive layer of the patch with active is dissolved in hexane. In step two, the active and related impurities are extracted into 50% methanol. Equivalent results were obtained by using both of the sample preparation procedures.

4. Conclusion

It has been demonstrated that experimental design is a very powerful tool in optimization of sample preparation methods. Although in this paper, the study involves a very special sample type, this approach should be applicable to many other sample types. Depending on the complexity of the sample preparation method, either the Plackett–Burman design and/or the general factorial design can be adopted. It should be pointed out that the method developer is ultimately responsible for identifying the relevant method factors for optimization, and sometimes it is not feasible to study some of the factors. Nevertheless, use of experimental designs will give method developer additional assurance that the optimized conditions are obtained.

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