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ORIGINAL ARTICLE

# Analytical quality-by-design approach for sample treatment of BSA-containing solutions

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Sample preparation;
Design of experiment (DOE);
Derringer desirability (D)

**Abstract** The sample preparation of samples containing bovine serum albumin (BSA), *e.g.,* as used in transdermal Franz diffusion cell (FDC) solutions, was evaluated using an analytical quality-by-design (QbD) approach. Traditional precipitation of BSA by adding an equal volume of organic solvent, often successfully used with conventional HPLC-PDA, was found insufficiently robust when novel fused-core HPLC and/or UPLC-MS methods were used. In this study, three factors (acetonitrile (%), formic acid (%) and boiling time (min)) were included in the experimental design to determine an optimal and more suitable sample treatment of BSA-containing FDC solutions. Using a QbD and Derringer desirability (*D*) approach, combining BSA loss, dilution factor and variability, we constructed an optimal working space with the edge of failure defined as $D<0.9$. The design space is modelled and is confirmed to have an ACN range of $83\pm3\%$ and FA content of $1\pm0.25\%$.


## 1. Introduction

Within the context of *in vitro* dermal absorption studies, the choice of receptor fluid is a very important factor with the major consideration of the receptor fluid not acting as a rate-limiting step in the permeation process due to the limited solubility of the test compound in the medium [1]. During these Franz diffusion cell (FDC) experiments, usually aqueous-based solutions are used as receiver phase beneath the mounted human or animal skin section membranes [2]. However, it is well recognized that for lipophilic compounds this may cause flux limiting solubility or unstirred layer phenomena which can obscure the true flux of the drug through the skin. In such cases, it is advised to use a solubilising additive in order to guarantee sink conditions throughout the experiment [1–2]. In almost 20% of recent studies, bovine serum albumin (BSA) was used in a

*Abbreviations:* ACN, acetonitrile; BSA, bovine serum albumin; D, Derringer desirability; DF, dilution factor; DOE, design of experiments; FA, formic acid; FDC, Franz diffusion cell; MLR, multiple linear regression; PBS, phosphate buffered saline; QbD, quality-by-design

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concentration up to 5% (m/V), in which it also contributed to the *in vivo* relevance [3–4].

During the FDC studies, samples of receptor fluid are taken at regular time intervals in order to calculate the cumulative concentration of permeated drug and its kinetic permeation parameters. Prior to the chromatographic analysis of the drug, a sample preparation is required to eliminate the BSA protein and liberate the drug before HPLC injection. Beside the many existing techniques for deproteinising samples, the traditional and most simple approach is to add an equal volume of organic solvent, *e.g.* acetonitrile. After centrifugation of the precipitated BSA, an aliquot of the clear supernatant is then injected into the HPLC system [3–10].

While this approach was often successful for traditional HPLC-UV analysis, recent experience within our laboratory has indicated that such a simple sample preparation is insufficient when switching to more advanced fused-core and/or UPLC-MS systems. This has led to bad peak shapes with shifted retention times and increased variability, giving unreliable results, but has also led to increased column back pressure, insufficient column robustness, which can also drastically shorten its life span.

The objective of this work was to determine an optimal and more suitable sample treatment of BSA-containing FDC solutions, aiming at a BSA precipitation of minimally 95%. We applied an analytical quality-by-design (QbD) approach, using a multivariate experimental design to construct a design space.

## 2. Materials and methods

### 2.1. Reagents

HPLC gradient grade acetonitrile (ACN) was supplied by Fisher Scientific (Leicestershire, UK). LC-MS formic acid (FA) was purchased from Fluka (Buchs, Switzerland). Water was purified using an Arium 611 purification system (Sartorius, Göttingen, Germany), resulting in ultrapure water of 18.2 MΩ cm quality. Phosphate buffered saline (PBS) (pH 7.4; 0.01 M) and Bradford reagent came from Sigma (St. Louis, MO, USA). BSA used for the FDC samples was bought from Merck (Darmstadt, Germany), while the 2 mg/mL BSA protein standard stock for analytical quantification came from Thermo Scientific (Waltham, MA, USA).

### 2.2. Experimental design and modelling

In this study, three factors were examined: boiling time (0.25–3 min), formic acid content (0.5–5%) and acetonitrile content (70–90%). The main and first response, *i.e.*, percentage BSA loss, was calculated according to the following formula: BSA loss (%) = (1–([BSA]$_{left}$/[BSA]$_{applied}$)) × 100%, where [BSA]$_{left}$ is the calculated BSA concentration after sample preparation and [BSA]$_{applied}$ is the

**Table 1** Experimental set-up and results.

| Exp. no. | Factors and levels | | | Dilution factor (DF) | Blue colored reaction | BSA loss (%) | RSD (%) |
|---|---|---|---|---|---|---|---|
| | Boiling time (min) | FA (%) | ACN (%) | | | | |
| 1 | 1 | 0.5 | 70 | 4.5 | - | 97.07 | 47.13 |
| 2 | 1.75 | 0.5 | 70 | 4.5 | - | 97.61 | |
| 3 | 1.75 | 2.75 | 70 | 4.5 | + | 37.65 | |
| 4 | 1.75 | 2.75 | 70 | 4.5 | + | 38.96 | |
| 5 | 1.75 | 2.75 | 70 | 4.5 | + | 39.40 | |
| 6 | 1 | 2.75 | 70 | 4.5 | + | 40.82 | |
| 7 | 3 | 2.75 | 70 | 4.5 | + | 41.15 | |
| 8 | 1 | 2.75 | 70 | 4.5 | + | 42.97 | |
| 9 | 0.5 | 2.75 | 70 | 4.5 | + | 45.29 | |
| 10 | 1 | 2.75 | 70 | 4.5 | + | 46.40 | |
| 11 | 0.25 | 2.75 | 70 | 4.5 | + | 46.62 | |
| 12 | 1.75 | 2.75 | 70 | 4.5 | + | 53.71 | |
| 13 | 1.75 | 5 | 70 | 4.5 | + | 11.23 | |
| 14 | 1 | 5 | 70 | 4.5 | + | 38.02 | |
| 15 | 0.25 | 0.5 | 80 | 5.5 | - | 99.04 | 0.15 |
| 16 | 1.75 | 0.5 | 80 | 5.5 | - | 99.12 | |
| 17 | 1 | 2.75 | 80 | 6 | - | 99.13 | |
| 18 | 1.75 | 5 | 80 | 7 | - | 98.78 | |
| 19 | 0.25 | 5 | 80 | 7 | - | 98.88 | |
| 20 | 3 | 0.5 | 90 | 19 | - | 96.72 | 0.50 |
| 21 | 0.5 | 0.5 | 90 | 19 | - | 97.00 | |
| 22 | 1.75 | 2.75 | 90 | 19 | - | 96.91 | |
| 23 | 3 | 5 | 90 | 23.5 | - | 95.83 | |
| 24 | 0.5 | 5 | 90 | 23.5 | - | 96.29 | |

$DF = \frac{V_{total}}{V_{sample}}$ with $V_{total} = V_{H_2O} + V_{FA} + V_{ACN} + V_{sample}$ and $V_{sample}$ = 200µL.