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# Microdialysis sampling and the clinical determination of topical dermal bioequivalence

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**Abstract**

Our objective was to determine whether the degree of variability associated with dermal microdialysis allows its practical application to determinations of bioequivalence of topically applied agents with a reasonable number of subjects. A statistical review of literature data was conducted to estimate the variances associated with subject-to-subject variability and the probe-to-probe variability within the subjects. In order to successfully utilise dermal microdialysis to establish bioequivalence of topically applied agents, particular care must be applied to study design. Due to the inherent variability between subjects, to maintain subject numbers at reasonable levels, each subject should act as their own control, thus removing the element of subject-to-subject variability from calculations of sample sizes. It is also recommended that measurements are made in duplicate in each subject to reduce the element of variability further. It is then possible to demonstrate, within 80–125% confidence limits and a subject population of approximately 20, that two formulations are bioequivalent.


*Keywords:* Microdialysis; Dermis; Skin; Bioequivalence; Human; Topical

## 1. Introduction

Microdialysis has been extensively reviewed in the literature (Stahl et al., 2002; Benveniste and Hüttemeier, 1990; Elmquist and Sawchuk, 1997; Groth, 1998; Anderson et al., 1998.) but in brief, it is a sampling technique, which can be used to sample endogenous and exogenous solutes in the extracellular space of tissues by means of a dialysis membrane which is permeable to small molecules and water. The microdialysis membrane or probe is implanted in the tissue of interest and perfused, typically with a physiologically relevant media, setting up a concentration gradient along its length. Compounds can diffuse into or out of the probe depending on the direction of the concentration gradient. Data obtained from such studies may be presented in a variety of ways: relative recovery and loss, tissue concentration, AUC and $C_{max}$.

Microdialysis was originally developed in neurosciences in the attempt to directly relate neurochemistry to behaviour. First described in 1966 (Bito et al., 1966), to describe the use of microdialysis to sample extracellular fluid, and since that time the use of microdialysis has escalated. Probes have been adapted, perfusion systems technically improved and along with the coupling of the technique to analytical methodologies, e.g. HPLC, microdialysis has become one of the major tools for bioanalytical sampling. As well as these technical advances, the variety of animal species incorporated has increased, with the progression to the introduction of microdialysis in man in the late 1980s. The extension to clinical pharmacological studies has opened a gateway into obtaining information regarding drug distribution processes to clinically relevant target sites. Microdialysis had been applied to the measurement of free tissue concentrations of endogenous compounds and also to determine the tissue distribution of drug molecules in a large number if human tissues, e.g. bone, lung, heart, brain, skin, neoplastic tissue, soft tissues such as skeletal muscle and blood (Elmquist and Sawchuk, 1997; Müller, 2000; de la Peña et al., 2000).

In the clinical setting, there are currently no sampling techniques which could be used to demonstrate dermal bioequivalence of topically applied agents which are both minimally invasive and which also give an indication of tissue concentrations at a target site in the skin with time. Techniques such as skin stripping, suction blisters, tissue biopsy and dermal imaging techniques such as confocal laser scanning microscopy have all been extensively reviewed in the literature and therefore will

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not be discussed here (Stahl et al., 2002; Pershing et al., 2002; Weigmann et al., 2001; Benfeldt et al., 1999a; Shah et al., 1998). These methods have their place but are restrictive in the amount of information generated and to obtain a concentration/time profile require a large number of subjects or a large number of sampling sites on a particular subject, therefore increasing the invasiveness involved. Concerns have been raised regarding the technique sensitive nature of skin stripping and the associated variability. The assessment of dermal bioequivalence of topical corticosteroids has successfully utilised a vasoconstriction protocol (FDA Guidance for Industry, 1995) however this methodology is restricted to agents demonstrating this specific pharacodynamic endpoint.

Dermal microdialysis has the potential to address the gap in available sampling techniques due to its minimally invasive nature and its ability to generate concentration/time profiles at a target site with good time resolution provided a sufficiently sensitive analytical method is available. Recent publications have commented on the fact that dermal microdialysis is a technique which has utility in a clinical setting (Müller, 2000; Müller et al., 1995; Kreilgaard, 2002) but information is lacking on the manner in which this technique could be applied to a manageable patient population, in particular in the field of dermal bioequivalence testing. The aim of this work is to determine whether the degree of variability associated with dermal microdialysis in particular, allows its practical application to determinations of dermal bioequivalence of topically applied agents with a reasonable number of subjects. This paper summarises a series of analyses undertaken to determine the degree to which such clinical studies must be powered to demonstrate dermal bioequivalence in line with FDA requirements.

Raw data retrieval from the literature for inclusion in these analyses proved challenging. The parameter chosen for analysis was the AUC of the concentration/time profiles and/or $C_{max}$ from as many subjects as possible. The greatest amount of data available to us was that generated by Dr. W. Keene (Keene, 2002). The primary analysis has been conducted using this data and the degree of variability from this study is then compared to available literature.

## 2. Materials and methods

### 2.1. Statistical analysis

The technique of restricted maximum likelihood (REML) as implemented in the SAS V8.02 procedure MIXED was used to estimate the variances associated with subject-to-subject variability and the probe-to-probe variability within the subjects.

Sample size calculations were performed using NQuery Adviser V4.0.

Graphics have been produced using SPLUS 2000 Release 3, StatGaphics Plus V5 and Microsoft Excel 2000.

### 2.2. In vivo study design for primary data set

The data set used in this analysis (Tables 1 and 2) is from an in vivo study on eight human volunteers (four males, four females aged 19–24 years) (Keene, 2002). The design of the trial required each subject to have a total of six microdialysis probes inserted, which were divided into three pairs (probe 1 and probe 2 for each pair) according to the perfusate to be used:

Ringers perfusate—application site unoccluded.
Ringers & Noradrenaline (5 μg/mL) perfusate—application site unoccluded.
Ringers & Noradrenaline (5 μg/mL) perfusate—application site occluded.

Noradrenaline was included in the perfusate to decrease dermal blood flow, hence allowing higher local tissue concentrations to be maintained. Occluding the application site has the effect of further boosting local tissue concentrations. Two kilodalton probes were used, perfused at a rate of 0.4 mL/h. The probes were positioned to a depth of 0.4–0.6 mm (verified using ultrasound) in the ventral forearm of the volunteers.

The topically applied vehicle contained methyl salicylate at saturation in a 50% propylene glycol/50% water vehicle. 0.1 mL was pipetted into a drug well (Comfeel® Plus Ulcer Dressing, Coloplast Ltd.) secured over the application site. Where wells were occluded a sheet of the dressing material was applied over the well immediately following vehicle application.

## 3. Results and discussion

### 3.1. Overview

The concentration of methyl salicylate and its metabolite salicylic acid was measured by HPLC hourly over a 5-h period (Tables 1 and 2). While methyl salicylate is a recognised rubefacient (Cross et al., 1999) it is the final tissue concentrations that are of interest in this analysis rather than the means by which they were achieved.

The purpose of the statistical analysis detailed below is to use the variation observed in this group of subjects to predict the numbers of subjects required in future two group comparative studies. The data show that there is quite a large variation in the concentration profiles across subjects within each of the perfusates. In some cases variability in the profiles can be seen for the two probes within a subject/perfusate combination, in particular for methyl salicylate.

The main response of interest in terms of measuring dermal delivery is the area under the concentration curve (AUC) and this has been calculated for each curve using trapezoidal integration. It is usual for such areas to be transformed by taking logarithms and there is some evidence that this transformation would be beneficial with these data in that the variability of the subjects, as can be seen in Figs. 1 and 2 where areas are plotted on a log-scale. An analysis of the data to estimate the components of variance that can be attributed to variation between subjects and to variation between the probes within each subject has been conducted. The estimates, pooled across perfusates, are shown in Table 3. It can be seen from this data that the estimates of variance components are similar across the two compounds, particularly when we bear in mind the uncertainty in the individual estimates

Table 1
Summary of dialysate methyl salicylate concentrations (μg/mL) for each probe and at each time point

| Subject | Perfusate | Pre-dose | | 0–1 h | | 1–2 h | | 2–3 h | | 3–4 h | | 4–5 h | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Probe 1 | Probe 2 | Probe 1 | Probe 2 | Probe 1 | Probe 2 | Probe 1 | Probe 2 | Probe 1 | Probe 2 | Probe 1 | Probe 2 |
| 1 | Ringers | ND | ND | 0.82 | 1.13 | 0.22 | 0.44 | ND | ND | ND | ND | ND | ND |
| | Ringers + NA | ND | ND | 1.54 | 1.04 | 0.85 | 0.53 | 0.47 | 0.30 | 0.30 | 0.17 | 0.21 | 0.16 |
| | Ringers + NA occluded | ND | ND | 2.01 | 1.55 | 2.19 | 1.33 | 1.27 | 0.96 | 0.89 | 0.83 | 0.80 | 0.58 |
| 2 | Ringers | ND | ND | 0.63 | – | 0.49 | – | 0.45 | – | 0.45 | – | 0.52 | – |
| | Ringers + NA | ND | ND | 0.21 | – | 0.17 | – | 0.25 | – | 1.24 | – | 0.32 | – |
| | Ringers + NA occluded | ND | ND | 0.30 | 0.97 | 0.29 | 0.72 | 0.29 | 0.78 | 0.66 | 0.90 | 0.13 | 0.73 |
| 3 | Ringers | ND | ND | 0.22 | – | 0.21 | – | ND | – | ND | – | ND | – |
| | Ringers + NA | ND | ND | 0.36 | – | 0.19 | – | ND | – | ND | – | ND | – |
| | Ringers + NA occluded | ND | ND | 0.17 | 0.13 | 0.50 | 0.31 | 0.38 | 0.34 | 0.25 | 0.36 | 0.25 | 0.33 |
| 4 | Ringers | ND | ND | 0.63 | 0.54 | 0.47 | 0.12 | 0.15 | ND | ND | ND | ND | ND |
| | Ringers + NA | ND | ND | 0.96 | 1.27 | 0.50 | 0.50 | 0.17 | 0.16 | 0.11 | 0.11 | 0.10 | ND |
| | Ringers + NA occluded | ND | ND | 0.91 | 0.38 | 0.96 | 0.55 | 0.68 | 0.29 | 0.57 | 0.21 | 0.46 | 0.25 |
| 5 | Ringers | ND | ND | 0.63 | – | ND | – | ND | – | ND | – | ND | – |
| | Ringers + NA | ND | ND | 0.97 | – | 0.22 | – | 0.16 | – | ND | – | ND | – |
| | Ringers + NA occluded | ND | ND | 1.94 | 1.00 | 0.91 | 0.73 | 0.50 | 0.49 | 0.61 | 0.33 | ND | ND |
| 6 | Ringers | ND | ND | 0.39 | 1.04 | 0.35 | 0.41 | ND | ND | 0.17 | ND | ND | ND |
| | Ringers + NA | ND | ND | 0.50 | 2.95 | 0.48 | 0.87 | 0.28 | 0.23 | 0.20 | 0.23 | ND | ND |
| | Ringers + NA occluded | ND | ND | 1.13 | 1.12 | 1.15 | 1.40 | 0.50 | 0.69 | 0.48 | 0.47 | ND | 0.42 |
| 7 | Ringers | ND | ND | 0.30 | 0.30 | 0.10 | 0.09 | ND | ND | ND | ND | ND | ND |
| | Ringers + NA | ND | ND | 0.50 | 1.60 | 0.20 | 0.30 | 0.08 | 0.10 | ND | 0.07 | ND | ND |
| | Ringers + NA occluded | ND | ND | 1.00 | 0.30 | 0.60 | 0.30 | 0.40 | 0.20 | 0.20 | 0.10 | 0.10 | 0.08 |
| 8 | Ringers | ND | ND | 1.70 | 1.93 | 0.27 | 0.43 | 0.14 | 0.24 | 0.17 | 0.13 | 0.12 | ND |
| | Ringers + NA | ND | ND | 1.65 | 0.47 | 0.53 | 0.18 | 0.27 | 0.05 | 0.17 | ND | ND | ND |
| | Ringers + NA occluded | ND | ND | 0.65 | 0.48 | 0.64 | 0.34 | 0.34 | 0.21 | 0.21 | 0.16 | 0.09 | 0.05 |

NA, Noradrenaline; ND, none detected; (–) not available due to analytical problems.

as shown by the confidence limits (the width of the confidence intervals reflects the fact that there are relatively few subjects in this study). However, the between probe variation is somewhat higher in the methyl salicylate group.

### 3.2. Number of subjects required for a parallel group study

For a study comparing two treatments, if each subject is assigned to one or other of the two treatment groups then the subject-to-subject variability is important in the calculations of sample sizes. We can use these estimates to predict what the variation will be in a future study with any number of repeat probes for each subject. Here the calculations have been made for up to four probes per subject and the results are presented in terms of the standard deviation (S.D.) (Table 4).

### 3.3. Sample sizes required to detect changes in area

Given the variability estimates in Table 4 it is possible to calculate how big a change in AUC between two groups of subjects

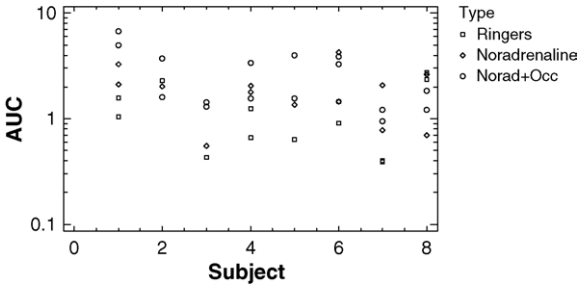


Fig. 1. Methyl salicylate. Plot of AUC against subject showing variation between and within subjects.

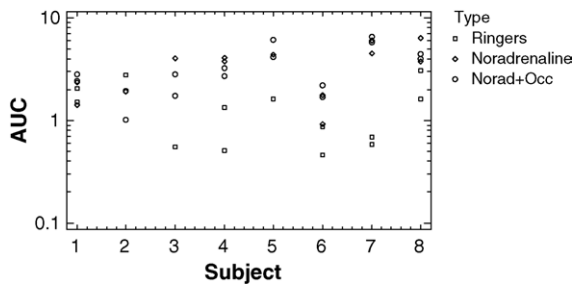


Fig. 2. Salicylic acid. Plot of AUC against subject showing variation between and within subjects.

Table 2
Summary of dialysate salicylic acid concentrations (μg/mL) for each probe and at each time point

| Subject | Perfusate | Pre-dose | | 0–1 h | | 1–2 h | | 2–3 h | | 3–4 h | | 4–5 h | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Probe 1 | Probe 2 | Probe 1 | Probe 2 | Probe 1 | Probe 2 | Probe 1 | Probe 2 | Probe 1 | Probe 2 | Probe 1 | Probe 2 |
| 1 | Ringers | 0.12 | 0.13 | 0.28 | 0.32 | 0.54 | 0.60 | 0.39 | 0.51 | 0.29 | 0.36 | 0.18 | 0.13 |
| | Ringers + NA | 0.14 | 0.11 | 0.25 | 0.04 | 0.41 | 0.36 | 0.50 | 0.43 | 0.72 | 0.37 | 0.48 | 0.37 |
| | Ringers + NA occluded | 0.12 | 0.13 | 0.37 | 0.32 | 0.83 | 0.53 | 1.02 | 0.84 | 1.23 | 0.70 | 1.31 | 0.94 |
| 2 | Ringers | 0.04 | – | 0.15 | – | 0.48 | – | 0.84 | – | 0.93 | – | 0.61 | – |
| | Ringers + NA | 0.10 | – | 0.20 | – | 0.39 | – | 0.48 | – | 0.50 | – | 0.47 | – |
| | Ringers + NA occluded | 0.09 | 0.06 | 0.21 | 0.14 | 0.47 | 0.22 | 0.44 | 0.27 | 1.17 | 0.46 | 0.99 | 0.47 |
| 3 | Ringers | 0.08 | – | 0.07 | – | 0.15 | – | 0.15 | – | 0.08 | – | 0.09 | – |
| | Ringers + NA | ND | – | 0.52 | – | 1.01 | – | 1.05 | – | 1.16 | – | 0.97 | – |
| | Ringers + NA occluded | 0.03 | 0.04 | 0.13 | 0.12 | 0.47 | 0.65 | 0.73 | 1.29 | 0.91 | 1.46 | 1.11 | 1.55 |
| 4 | Ringers | ND | ND | 0.11 | 0.05 | 0.45 | 0.16 | 0.47 | 0.17 | 0.31 | 0.09 | 0.21 | 0.07 |
| | Ringers + NA | 0.07 | 0.04 | 0.36 | 0.44 | 1.15 | 1.24 | 1.22 | 1.11 | 1.23 | 0.84 | 0.69 | 0.59 |
| | Ringers + NA occluded | 0.05 | 0.04 | 0.25 | 0.20 | 0.98 | 0.70 | 1.46 | 1.06 | 1.59 | 1.14 | 1.59 | 1.07 |
| 5 | Ringers | ND | – | 0.37 | – | 0.54 | – | 0.38 | – | 0.21 | – | ND | – |
| | Ringers + NA | 0.10 | – | 0.65 | – | 1.41 | – | 1.35 | – | 0.09 | – | ND | – |
| | Ringers + NA occluded | 0.05 | ND | 0.44 | 0.44 | 1.49 | 1.74 | 1.33 | 2.88 | 2.26 | 2.32 | – | – |
| 6 | Ringers | ND | 0.04 | 0.07 | 0.30 | 0.20 | 0.28 | 0.11 | 0.13 | 0.09 | 0.14 | 0.06 | 0.07 |
| | Ringers + NA | 0.04 | 0.10 | 0.34 | 0.10 | 0.38 | 0.24 | 0.04 | 0.25 | 0.36 | 0.24 | 0.29 | 0.21 |
| | Ringers + NA occluded | 0.12 | 0.04 | 0.12 | 0.30 | 0.51 | 0.60 | 0.63 | 0.62 | 0.62 | 0.85 | 0.69 | 0.96 |
| 7 | Ringers | ND | ND | 0.19 | 0.20 | 0.23 | 0.24 | 0.14 | 0.13 | 0.06 | 0.03 | ND | 0.06 |
| | Ringers + NA | ND | ND | 0.61 | 1.31 | 1.46 | 2.08 | 1.28 | 1.37 | 0.82 | 0.99 | ND | 0.64 |
| | Ringers + NA occluded | 0.03 | 0.04 | 0.76 | 0.86 | 1.97 | 2.40 | 2.46 | 2.54 | 2.05 | 2.03 | 1.70 | 1.84 |
| 8 | Ringers | ND | ND | 0.36 | 0.62 | 0.45 | 1.02 | 0.53 | 0.82 | 0.19 | 0.55 | 0.13 | 0.33 |
| | Ringers + NA | ND | 0.02 | 0.54 | 0.80 | 1.29 | 1.77 | 1.17 | 1.71 | 0.81 | 1.36 | 0.48 | 0.86 |
| | Ringers + NA occluded | ND | ND | 0.52 | 0.54 | 1.44 | 1.26 | 1.82 | 1.61 | 1.83 | 1.52 | 1.66 | 1.17 |

NA, Noradrenaline; ND, none detected; (–) not available due to analytical problems.

we might reasonably expect to detect using a two-sample *t*-test at the 5% level of significance. The change in AUC is measured in terms of a ratio (this is a consequence of using the log transform of the areas). A reasonable chance of detecting a given ratio is defined here as 80% (the power of the test). How big a ratio that can be detected will depend on the number of subjects in each of the groups, Fig. 3 shows this relationship for the methyl salicylate data. For example, with just five subjects in each group with only one probe we could only reasonably expect to detect areas that were about four times greater in one group than in the other. By increasing the number of probes to 2 this ratio reduces to about three times. Increasing the number of probes per subject further results in smaller and smaller reductions in the ratio. By

Table 3
Estimates of variance components

| Compound | Source of variation | Variance component estimate | Approximately 95% confidence limits |
|---|---|---|---|
| Methyl salicylate | Subjects | 0.20 | 0.09–0.89 |
| | Probes | 0.21 | 0.12–0.46 |
| Salicylic acid | Subjects | 0.26 | 0.13–0.67 |
| | Probes | 0.11 | 0.06–0.23 |

Table 4
Estimates of standard deviation (S.D.)

| Compound | Number of probes per subject | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Methyl salicylate | 0.64 | 0.55 | 0.52 | 0.50 |
| Salicylic acid | 0.60 | 0.56 | 0.55 | 0.54 |

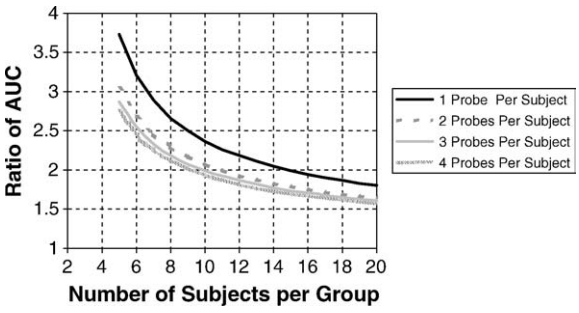


Fig. 3. Relationship between the true ratio of areas that can be detected with 80% power and the number of subjects in each group based on the variance estimates from the methyl salicylate data.

increasing the number of subjects the ratio rapidly decreases and then flattens out. By using 20 subjects per group, each with two probes, you could expect to detect areas in one treatment group that were roughly half as big again as in the second treatment group.

### 3.4. Number of subjects required for a within patient comparison study

If each subject has both treatments applied then the subject-to-subject variability is eliminated from the calculations of sample sizes. However, there could be an important source of variability due to a subject by treatment interaction which would mean that the true underlying difference between the treatments was not the same for each subject. We have no information as to whether this source of variability is likely to be important in a future study as clearly we have no treatment information in the background data and it is assumed that the individual subject reactions to the treatments would not alter the resulting absorption. This would have to be established on a compound-by-compound or treatment-by-treatment basis in any given study. If we assume that the treatment by subject interaction is negligible then we can use the probe variances in Table 4 to estimate the variability we would see from using two probes per treatment on a number of subjects in a paired *t*-test (or the corresponding equivalence test). Because of this assumption the plots presented should be treated as a best case scenario in terms of the numbers of subjects required. By removing the subject-to-subject variability we are able to detect much smaller changes in the areas than was the case for the two-sample *t*-test. For example, with just five subjects in each group we could reasonably expect to detect areas that were about two times greater in one group than in the other.

### 3.5. Sample sizes required to meet bioequivalence criteria

The FDA accepted bioequivalence criterion states that a 90% confidence interval for the ratio of the group means should lie within 80–125% (FDA Guidance for Industry, 1998). A standard requirement is that a sufficient number of subjects should be used in each group so that there is an 80% chance of declaring equivalence when there is no difference between the groups. In a parallel group study, to achieve this with one probe per subject we would need to have 147 subjects per group, with two probes per subject this reduces to 105 subjects per group. This study design therefore would require a very large number of subjects in order to establish the bioequivalence of two topically applied agents. A reasonable number of subjects (e.g. 20 per group), using this design, would only establish bioequivalence if the equivalence limits were set at 56–180%, well outside the current criteria.

If however the study design is altered to a within subject comparison study, each subject having both treatment groups applied to two probes then the subject-to-subject variability is eliminated from the calculations of sample sizes. Assuming the treatment by subject interaction is negligible then we can use the probe variances in Table 4 as estimates of the variability we would see from using two probes on a number of subjects in a paired *t*-test (or the corresponding equivalence test).

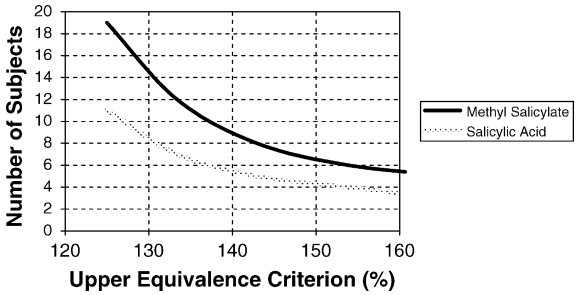


Fig. 4. Relationship between the number of subjects required and the upper equivalence limit.

Fig. 4 shows the total number of subjects required to achieve 80% power for a range of upper equivalence limits (the lower limit is simply the reciprocal of the upper limit). So in this case with a total of 20 subjects there is a reasonable chance of meeting the 80–125% equivalence criterion if the treatments really are the same.

### 3.6. Literature variability

The analyses discussed thus far have been based on results from eight volunteers and one study centre. In order to determine if the degree of variability seen in these studies was representative of studies being conducted at other centres a literature review was conducted. In general very few papers publish raw data in sufficient detail to conduct a full statistical analysis. The papers presented here (Kreilgaard et al., 2001; Müller et al., 1997; Benfeldt et al., 1999b; Tegeder et al., 1999; Hegemann et al., 1995; Müller et al., 1998; Cross et al., 1998) have had their summary statistics converted to an estimate of the coefficient of variation (CV). This measure (also known as the relative standard deviation) simply expresses the standard deviation as a percentage of the mean. For this literature data, it is therefore not possible to look at the relative sizes of the between and within subject variation. In most cases, we have summary statistics based on a single probe per subject and so we are reviewing the sum of these two components of variation. This has therefore been used as the common measure of variation wherever possible and represents the variability observed from a study where only one probe per subject was used.

The studies cover a range of locations, probe types and compounds analysed with details summarised in Table 5. It would be expected that a large range of CV values between the various studies would be seen just by chance, especially given that some of the estimates of mean and standard deviation are based on relatively small numbers of subjects. However, even though it does appear that underlying CV is not the same in every study there is still a measure of agreement between the studies. Therefore, the variability associated with the primary study used to conduct this analysis falls within the variability range seen in the literature and it therefore appears we can reasonably apply the statistical approach used here to the wider use of microdialysis for dermal bioequivalence testing.

Table 5
Summary of analysis of literature data

| Reference | Probe | Tissue site | Number of subjects | Formulation | %CV | |
|---|---|---|---|---|---|---|
| | | | | | AUC | $C_{max}$/tissue concentration |
| Keene (2002) | Gambro, 2 kDa, 30 mm | Dermis | 8 | Saturated methyl salicylate, 50% propylene glycol/50% water (v/v) | 71 | |
| | | | | Salicylic acid—levels measured following application of formulation above | 67 | |
| Kreilgaard et al. (2001) | Gambro GFS + 12, 2 kDa, 30 mm | Dermis | 8 | Microemulsion containing 7.5% (w/w) lidocaine | 93 | |
| | | | | Xylocain 5% (w/w) cream (lidocaine) | 56 | |
| Müller et al. (1997) | CMA 10, 20 kDa, 16 mm | Superficial adipose tissue | 7 | Diclofenac gel (Emugel) | 98 | |
| | | Deep subcutaneous tissue | | | 150 | |
| Benfeldt et al. (1999b) | Gambro GFE 18, 2 kDa, 30 mm | Dermis | 15 | 5% (w/v) Salicylic acid in ethanol | 32 | |
| Tegeder et al. (1999) | CMA 60, 20 kDa, 30 mm | Muscle | 11 | 5% Ibuprofen gel | 142 | 169 |
| | | Dermis | | | 83 | 78 |
| Hegemann et al. (1995) | CMA 10, 20 kDa, 10 mm | Dermis | 9 | Nicotine patch | | 48 |
| Müller et al. (1998) | CMA 10, 20 kDa, 16 mm | Muscle | 12 | 5% Diclofenac foam | | 104 |
| Cross et al. (1998) | CMA 70, 20 kDa | Dermis | 3 | 20% Methylsalicylate | | 42 |
| | | Subcutaneous tissue | | 20% Methylsalicylate | | 75 |
| | | Dermis | | 7% Glycolsalicylate | | 90 |
| Median | | | | | 83 | 78 |

## 4. Conclusion

The development of novel treatments to be applied to the skin may be hindered by the lack of appropriate methods to determine the bioequivalence of various formulations during the development programme. The minimally invasive technique of dermal microdialysis can be utilised in order to establish bioequivalence of topically applied agents in humans by measuring local tissue concentrations with time. The study design must be optimised in order to ensure reasonable subject numbers for a given study. From the studies reviewed in this paper this would require a within subject comparison study where at least two probes are used for both formulations tested and both formulations are applied to each subject so that the subject-to-subject variability is eliminated from the comparison formulations. In this situation it is then possible to demonstrate, with 80% power and a subject population of approximately 20, that two topically applied formulations deliver the same tissue AUC, within 80–125% equivalence limits.

## Acknowledgements

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