



The effect of dilution of fusidic acid cream and betamethasone dipropionate cream in complex extemporaneous mixes on formulation performance

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ABSTRACT

The Aron regimen is an unconventional therapy which entails frequent applications of an extemporaneously prepared three component system (a topical antibiotic, a corticosteroid and an emollient), with the intention of decolonising the skin of *S. aureus* whilst treating atopic dermatitis. The impact of heavily diluting these topical medicinal products, to differing extents, on formulation performance is not well understood thus was investigated in this study. Following a single application of a range of compounded Aron mixes (fusidic acid and betamethasone dipropionate diluted to varying extents in an emollient base), significant reductions in the expected drug flux across silicone membrane, *ex vivo* percutaneous absorption and skin retention of both drugs relative to the marketed products were observed. This was attributed to a number of complex formulation effects making such changes difficult to predict in a clinical setting. Further investigations are required to evaluate the impact of frequent applications of the Aron mix to widespread areas on clinical efficacy, antimicrobial resistance and long term side effects.

1. Introduction

In severe cases of atopic dermatitis patients are prescribed a combination of therapies, including emollients, topical corticosteroids (TCSs), topical calcineurin inhibitors, systemic treatments and phototherapy. In cases unresponsive to these treatments, patients may resort to unconventional treatments to manage the skin condition. Once such emerging treatment is the Aron regimen, pioneered by Dr Richard Aron, which combines three components of conventional treatment, an emollient, a topical corticosteroid and a topical antibiotic and uses them in an unconventional way. The Aron regime's aim is to address skin colonisation of *S. aureus*, whilst treating atopic dermatitis (Aron, 2022a). Recent work has reported that *S. aureus* is extensively present in areas of affected and unaffected skin in up to 90% of atopic dermatitis patients (Wollenberg et al., 2018), which may have a causative role in inducing atopic dermatitis flares. The Aron regimen compounds the emollient, TCS and topical antibiotic into a single formulation (the Aron mix), tailored to the patient, which is applied to all affected and unaffected areas of the body frequently (up to six times a day for one to two weeks, tapered thereafter according to response to treatment).

Whilst the success of the Aron regimen has been reported by patients,

carers and Dr Aron (The Daily Telegraph, 2014; The Guardian, 2018; Aron, 2022a), the body of evidence is largely anecdotal. Case series studies (non-blinded, non-controlled) have reported improvements in atopic dermatitis severity following treatment with a compounded antibacterial, steroid, and moisturizer, however, no controlled clinical studies evaluating the effectiveness of the Aron regime have been conducted to date (Lakhani et al., 2017; Rajkumar et al., 2021). Indeed, recent evidence has reported the limited benefit of a topical antibiotic plus a TCS compared to a TCS alone in the treatment of secondary bacterial infection of dermatitis (George et al., 2019; NICE, 2021). Other criticisms have been made of the approach, including that it may promote the development of antibiotic resistance. One of the cornerstones of the Aron regimen is to tailor the degree of dilution of the TCS and antibiotic depending on the patient age, weight and severity of the condition. The rationale behind heavily diluting the TCS and topical antibiotic is to allow uninterrupted therapy (and more frequent applications) thereby preventing the risk of 'steroid rebound' or recolonisation of the skin by *S. aureus*. However, the extent to which drug delivery to the skin is altered does not always correlate with the degree of dilution of a product, thus cannot be readily predicted in a clinical setting (Ryatt et al., 1982; Gibson et al., 1983). This informs the

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recommendation that the extemporaneous dilution of topical products to tailor potency should be avoided (British National Formulary, 2022).

The impact of varying the degree of dilution of an antibiotic and a TCS in an emollient base on the formulation performance is not well understood. Recent work has demonstrated that mixing two topical products can induce complex formulation changes which alters the drug delivery profile relative to the individual marketed products (Beebejaun et al., 2019). These considerations are equally applicable to a further complex extemporaneous mix of a TCS, antibiotic and emollient. Thus, the aim of this work was to evaluate the impact of varying the degree of dilution of a topical antibiotic and TCS in the Aron mix on *in vitro* formulation performance compared to the relative marketed products.

2. Materials and methods

2.1. Materials

Fusidic acid (FA, Ph Eur) and betamethasone dipropionate (BDP; Ph Eur) were acquired from Carbosynth Ltd (Compton, UK). Diprosone cream and Diprobace cream were acquired from the University of Hertfordshire campus pharmacy (Hertfordshire, UK). Fucidin cream was acquired from Bushey Pharmacy (Bushey, UK). Raman grade calcium fluoride slides were acquired from Crystran Ltd (Dorset, UK). Phosphate buffered saline (PBS) tablets, acetonitrile (HPLC grade) and absolute ethanol (99 + %) were acquired from Fisher Scientific (Leicestershire, UK). Sodium chloride (Ph Eur) was acquired from Sigma Aldrich (Dorset, UK). Non-porous, medical grade 0.002" silicone membrane was purchased from Bioplexus (Los Angeles, USA).

2.2. Methods

2.2.1. Raman microscopy of Diprosone cream, Fucidin cream and Aron mix 9

Raman microscopy of crystalline structures in Diprosone cream, Fucidin cream and Aron mix 9 was performed using a Renishaw inVia Raman microscope (Renishaw Plc, UK), calibrated for peak position and intensity using a silicon reference block. Aron mix 9 was prepared 1 h in advance of analysis by weighing appropriate amounts of Fucidin cream, Diprosone cream and Diprobace cream in a 1:2:10 ratio into a glass dish and mixing thoroughly to ensure homogeneity. Samples of Diprosone cream alone, Fucidin cream alone and Aron mix 9 were mounted on Raman grade calcium fluoride slides for spectral analysis. Raman spectra were obtained using the $\times 100$ long working distance magnification lens, a laser excitation wavelength of 785 nm, five accumulations per sample and an acquisition time of 10 s. Three replicate areas were scanned for each analysis and the single, most representative spectrum selected for presentation.

2.2.2. Quantitative analysis of betamethasone dipropionate and fusidic acid

Quantification of betamethasone dipropionate and fusidic acid was achieved using an Agilent 1260 Infinity quaternary pump coupled to an Agilent 1260 multi wavelength UV/Vis detector (Agilent Technologies, UK), set to 210 nm and 240 nm for fusidic acid and betamethasone dipropionate detection, respectively. Chromatographic analysis was performed using a reverse phase Kinetex™ C₁₈ column (5 μ particle size, 250 mm \times 4.6 mm; Phenomenex, UK), a sample injection volume of 40 μ L and a constant flow rate of 1 mL min⁻¹. A tertiary mobile phase system of sodium phosphate buffer (pH 3.1), HPLC grade acetonitrile and water (18.2 M Ω cm⁻¹) was employed in the following ratios, respectively: 65:35:0 from 0 min to 5 min, 30:70:0 from 5 min to 15 min, 0:95:5 from 15 to 16 min, 0:35:65 from 16 min to 22 min, 65:35:0 from 22 min to 24 min. Under these conditions, betamethasone dipropionate and fusidic acid eluted at 13.8 min and 14.4 min, respectively. The HPLC method was fit for purpose with respect to linearity ($r^2 > 0.999$), precision (<2 % RSD), accuracy (<2 %) and sensitivity (BDP limits of

detection and quantification: 0.2 μ g mL⁻¹ and 0.61 μ g mL⁻¹; FA limits of detection and quantification: 0.31 μ g mL⁻¹ and 0.95 μ g mL⁻¹, respectively) in accordance with current ICH guidelines (ICH, 2005).

2.2.3. *In vitro* drug transport studies across silicone membrane: Varying the degree of dilution of Diprosone cream or Fucidin cream in the Aron mix

Aron mixes with the following ratios of Fucidin cream to Diprosone cream to Diprobace cream were prepared as detailed in Section 2.2.1: Aron mix 1 (1:2:20), Aron mix 2 (1:4:18), Aron mix 3 (1:6:16), Aron mix 4 (1:10:12), Aron mix 5 (0.5:2:20.5), Aron mix 6 (3:2:18), Aron mix 7 (5:2:16) and Aron mix 8 (7:2:14). The percent compositions of each product in the Aron formulations investigated are presented in Table 1. Franz cells (Soham Scientific, UK) were mounted with silicone membrane and the receiver chambers filled with a mixture of PBS and ethanol (30 %). Membranes were dosed with 1 g of Diprosone cream alone, Fucidin cream alone or Aron mix 1–8. Six replicate Franz cells were employed for each formulation investigated. Samples (200 μ L) of the receiver fluid were removed periodically up to 26 h and replaced with fresh preheated receiver fluid. Drug quantification in samples was achieved via HPLC UV.

2.2.4. Human skin preparation

Excised human scrotal skin was obtained with informed consent from gender reassignment surgeries following ethical approval from the South London Research Ethics Committee (ethics No. 10/H0807/51). Skin samples were removed from storage (-20 °C) and left to thaw at ambient temperature, the subcutaneous fat was removed using a scalpel and samples were stored at -20 °C until required.

2.2.5. *Ex vivo* finite dose percutaneous absorption studies

Individually calibrated Franz cells (Soham Scientific, UK) with an average surface area of 1 cm² and average receiver volume of 3 mL were assembled with human skin, the receiver chamber was filled with a mixture of PBS and ethanol (20 %) then skin samples were dosed with 10 μ L of Diprosone cream, Fucidin cream or Aron mix 9 using a calibrated positive displacement pipette. Six replicates were performed for each formulation investigated (2 replicates per donor, 3 donors, matched across studies). To ensure contact with the membrane, the product was carefully spread over the surface of the skin with five clockwise, then anticlockwise, motions using the tip of a capillary piston. Aron mix 9 was prepared one hour in advance of dosing in the ratios of 1:2:10 of Fucidin cream, Diprosone cream and Diprobace cream, respectively using the method detailed in Section 2.2.1. Samples (200 μ L) of the receiver fluid were taken at pre-determined intervals up to 24 h and replaced with fresh preheated receiver fluid. Drug quantification was achieved via HPLC UV.

2.2.6. *Ex vivo* finite dose skin distribution studies

Following the 24 h percutaneous absorption study, the residual formulation was collected from the donor chamber and skin surface by three sequential wipes with cotton buds (a dry cotton bud, a cotton bud

Table 1

The percent compositions of Fucidin cream, Diprosone cream and Diprobace cream in the Aron formulations investigated.

	Percent composition of products in the Aron mix		
	Fucidin cream	Diprosone cream	Diprobace cream
Aron mix 1 (1:2:20)	4.35	8.70	86.96
Aron mix 2 (1:4:18)	4.35	17.39	78.26
Aron mix 3 (1:6:16)	4.35	26.09	69.57
Aron mix 4 (1:10:12)	4.35	43.48	52.17
Aron mix 5 (0.5:2:20.5)	2.17	8.70	89.13
Aron mix 6 (3:2:18)	13.04	8.70	78.26
Aron mix 7 (5:2:16)	21.74	8.70	69.57
Aron mix 8 (7:2:14)	30.43	8.70	60.87
Aron mix 9 (1:2:10)	7.69	15.38	76.92

soaked in acetonitrile then a final dry cotton bud) and two tape strips (Scotch Tape strips, 3 M Centre, USA) of the skin surface. The epidermal and dermal layers of skin samples were heat separated by placing the skin in an oven set to 60 °C for 1 min before carefully peeling the epidermis and dermis apart (Kligman and Christophers, 1963). The skin layers were then placed in individual vials and the drug was extracted in aliquots of acetonitrile. Vials containing the samples were sonicated for 10 mins then placed on a roller mixer (Cole-Palmer, UK) for 18 h. To enable maximum recovery of fusidic acid and betamethasone dipropionate from all matrices, a second extraction was conducted for each sample. Drug quantification was achieved via HPLC UV.

2.2.7. Data treatment and statistical analysis

Drug concentration in the receiver fluid was corrected for previous sample removal and profiles constructed to present cumulative amount of drug permeated per unit area ($\mu\text{g cm}^{-2}$) over the exposure period. Linear regression analysis was performed on infinite dose data sets to determine mean drug flux.

Experimental data were expressed as mean ($n = 6$) \pm standard deviation (SD). Statistical analysis was performed using Prism 8.0 (GraphPad, USA). The Shapiro Wilk test was employed to determine the normality of all data sets. Non-parametric analysis for multiple comparisons was performed using Kruskal-Wallis and a Mann-Whitney test applied for post hoc analysis. Parametric analysis for multiple comparisons was performed using analysis of variance (ANOVA) and Tukey's post hoc test. Statistically significant differences were determined at a 95 % confidence interval ($p \leq 0.05$).

3. Results and discussion

3.1. Microscopic evaluation of the Aron mix and marketed preparations

Diprosone cream and Fucidin cream were analysed by Raman microscopy to understand whether drug was present in the formulations at saturated or sub saturated levels (Fig. 1). No potential drug particles were observed for Diprosone cream; thus it is likely that betamethasone dipropionate was formulated at submaximal drug thermodynamic activity in the formulation. Fusidic acid crystals, confirmed by Raman spectroscopy, were present in Fucidin cream (Fig. 2). This indicated that Fucidin cream was formulated as a suspension at maximum stable thermodynamic activity in Fucidin cream. A model Aron mix

formulation employed for the *ex vivo* skin permeation and penetration study was also investigated for the presence of drug crystals (Aron mix 9; 1:2:10). Fusidic acid drug crystals, confirmed by Raman microscopy, were evident in Aron mix 9 following a 13 fold dilution of Fucidin cream in Diprosone cream and Diprosone cream. No drug crystals were attributed to betamethasone dipropionate in Aron mix 9.

3.2. The impact of the degree of dilution of Diprosone cream or Fucidin cream in the Aron mix on drug transport across silicone membrane

The Aron formulations are tailored to suit the patient's age, weight and the severity of the condition, thus varying ratios of Fucidin cream and Diprosone cream diluted in a Diprosone cream base are extemporaneously prepared and dispensed. To investigate the impact on betamethasone dipropionate and fusidic acid transport across silicone membrane when Diprosone cream was diluted to varying extents, a series of formulations were prepared with a fixed concentration of Fucidin cream (4.35 % w/w) and varying concentrations of Diprosone cream (8.70–43.48 % w/w) in a Diprosone cream base (Aron mix 1–4). Silicone membrane is commonly used as a surrogate model membrane for skin to understand the effect of drug concentration or saturation on drug delivery from a topical formulation (Walters, 2002; Edwards et al., 2017). The effect of product dilution on drug thermodynamic activities in the Aron formulation, which influences drug delivery to the skin would be expected to be identified with silicone membrane, drug transport studies. Full details of the Aron mix compositions are presented in Table 1. The drug transport profiles for betamethasone dipropionate and fusidic acid from Diprosone cream, Fucidin cream and Aron mix 1–4 are presented in Fig. 3.

Betamethasone dipropionate flux across silicone was greatest when delivered from Diprosone cream alone and a general trend of decreasing flux with decreasing concentrations of Diprosone cream in the Aron mix was observed for betamethasone dipropionate (Fig. 4). The reduction in drug flux ranged from 2.6 fold – 8.8 fold, when compared to Diprosone cream alone (Table 2; $p < 0.05$). The summary of product characteristics (SPC) for Diprosone cream lists Diprosone cream as the base vehicle for the TCS and suggests that control of the dosage regimen can be achieved by diluting Diprosone cream with Diprosone cream (MSD, 2021). Microscopic evaluations indicated sub saturation of betamethasone dipropionate in Diprosone cream. Thus, it is expected that employing Diprosone cream as a main diluent would have resulted in a decrease in

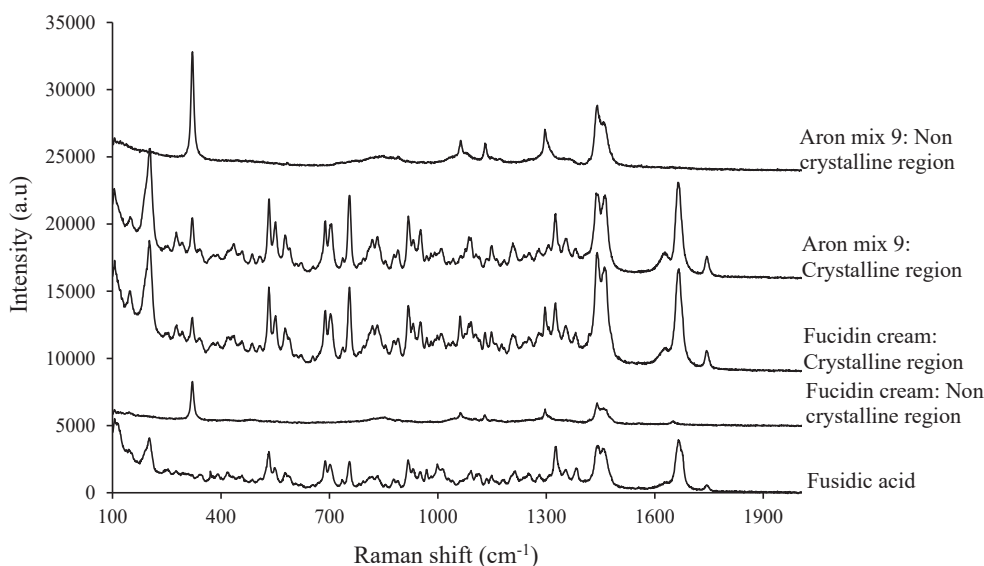


Fig. 1. Raman spectra obtained from fusidic acid, crystalline and non-crystalline regions of Fucidin cream and Aron mix 9. Aron mix 9 contained Fucidin cream, Diprosone cream and Diprosone cream in a 1:2:10 ratio, prepared 1 h before analysis. Spectra were obtained at x100 magnification, a laser excitation wavelength of 785 nm, five accumulations per sample and an acquisition time of 10 s.

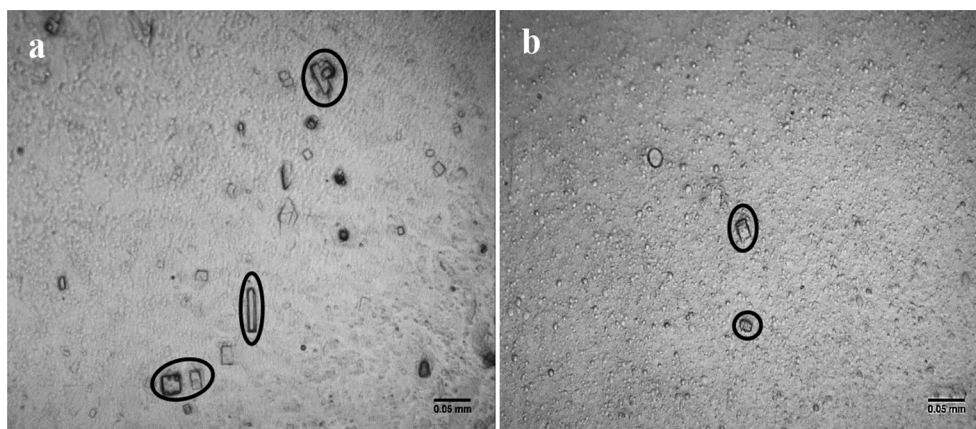


Fig. 2. Representative light microscope images (x 20 magnification) of (a) Fucidin cream and (b) Aron mix 9. Aron mix 9 contained Fucidin cream, Diprosone cream and Diprobase cream in a 1:2:10 ratio, prepared 1 h before analysis. Crystalline structures, circled, were evident in (a) and (b) and attributed to fusidic acid.

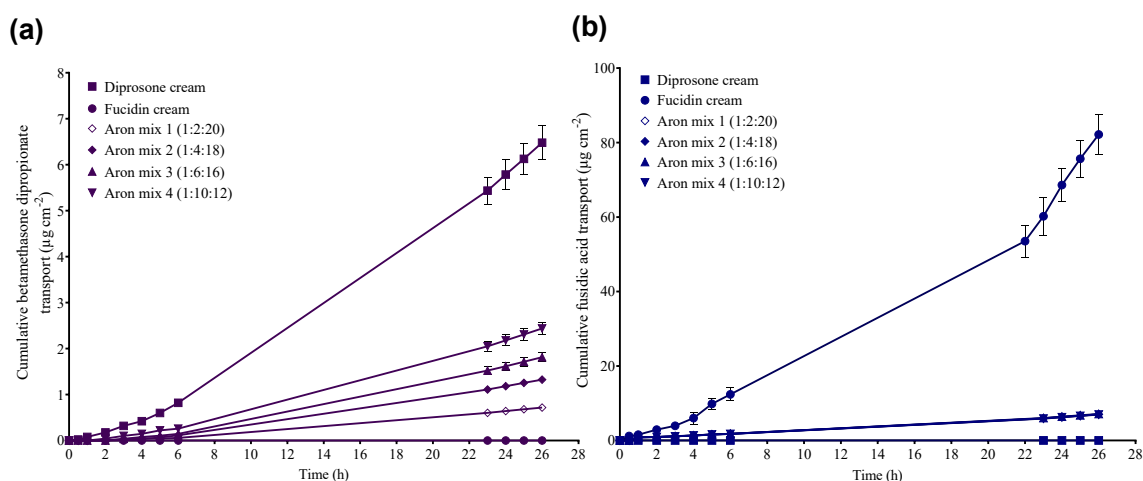


Fig. 3. Cumulative (a) betamethasone dipropionate (BDP) transport and (b) fusidic acid (FA) transport across silicone membrane following the application of an infinite dose of Diprosone cream alone (■), Fucidin cream alone (●) or mixes of Fucidin cream, Diprosone cream and Diprobase cream in the following ratios: Aron mix 1 (1:2:20; ◇), Aron mix 2 (1:4:18; ◆), Aron mix 3 (1:6:16; ▲) or Aron mix 4 (1:10:12; ▼). Data are shown as the mean of six replicates (\pm SD).

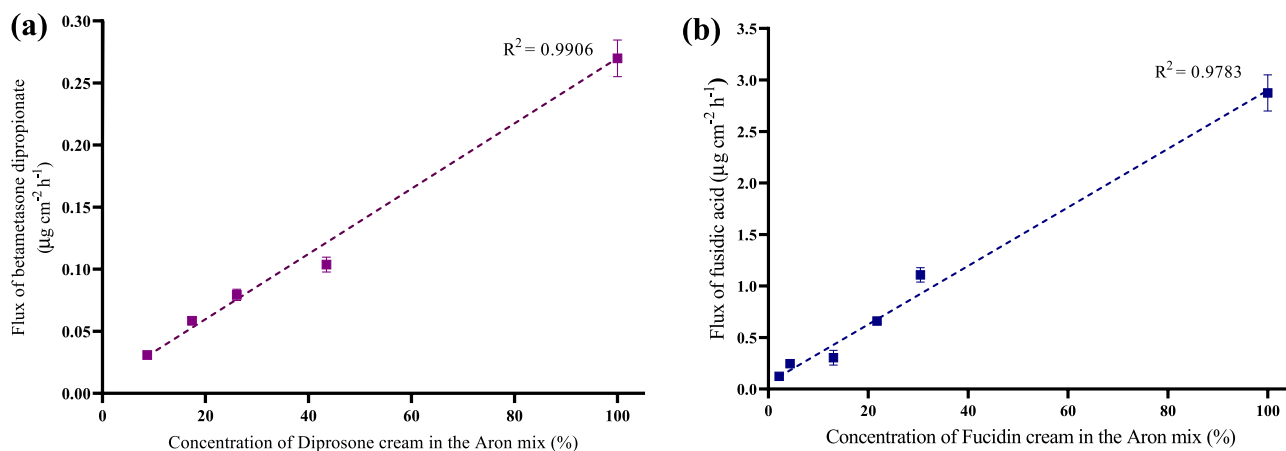


Fig. 4. The correlation between the concentration of (a) Diprosone cream in the Aron mix and betamethasone dipropionate flux across silicone membrane or (b) Fucidin cream the Aron mix and fusidic acid flux across silicone membrane. Data points show the mean of six replicates (\pm SD).

drug thermodynamic activity proportional to the degree of dilution in the Aron mix. It is, however, important to appreciate that the Aron mix entails a complex dilution of Diprosone cream, by Fucidin cream and Diprobase cream, with the potential dilution effect of Fucidin cream on

Diprosone cream being unreported, to date. Thus, the thermodynamic activity of betamethasone dipropionate is not altered by the diluent alone, but also impacted by the excipients and API present in Fucidin cream. On evaluation, a roughly proportional relationship was observed

Table 2

Betamethasone dipropionate (BDP) and fusidic acid (FA) flux from Diprosone cream alone, Fusidic cream alone or an Aron mix with varying proportions of Diprosone cream to Diprobace cream and fixed proportions of Fucidin cream. Data are shown as mean \pm SD (n = 6). * Denotes a significant difference when J4-24 h for BDP and FA in the Aron mixes were compared respectively to Diprosone cream and Fucidin cream ($p < 0.05$).

Formulation	J _{4-24h} for BDP ($\mu\text{g cm}^{-2}\text{h}^{-1}$)	J _{4-24h} for FA ($\mu\text{g cm}^{-2}\text{h}^{-1}$)
Diprosone cream	2.70E-01 \pm 1.48E-02	0.00 \pm 0.00*
Fucidin cream	0.00 \pm 0.00*	2.87 \pm 1.75E-01
Aron mix 1 (1:2:20)	3.09E-02 \pm 1.54E-03*	2.50E-01 \pm 1.72E-02*
Aron mix 2 (1:4:18)	5.85E-02 \pm 2.91E-03*	2.49E-01 \pm 1.58E-02*
Aron mix 3 (1:6:16)	7.94E-02 \pm 4.57E-03*	2.53E-01 \pm 1.84E-02*
Aron mix 4 (1:10:12)	1.04E-01 \pm 5.95E-03*	2.47E-01 \pm 1.52E-02*

between the degree of dilution of Diprosone cream in Aron mix 1–4 and the decrease in betamethasone dipropionate flux across silicone membrane with a 2.3–11.5 fold dilution of Diprosone cream resulting in a 2.6–8.8 fold decrease in betamethasone dipropionate flux, compared to Diprosone cream alone. As Diprobace cream is listed as a compatible base in which to dilute Diprosone cream, the margin of difference observed is likely to be attributable to the formulation effects of Fucidin cream where excipients such as glycerol or Polysorbate-60 (Tween 60) or indeed fusidic acid may alter the solubility of betamethasone dipropionate in the Aron mix, thus alter drug thermodynamic activity and flux to unpredictable extents.

An additional concern is whether dilution of Fucidin cream affects fusidic acid thermodynamic activity and delivery when formulated in the Aron mix. Aron mix 1–4 contained matched concentrations of Fucidin cream (4.35 %) diluted by 23 fold in varying proportions of Diprosone cream and Diprobace cream. Overall, fusidic acid flux from Aron mix 1–4 was significantly reduced by approximately 11.5 fold compared to Fucidin cream alone (Table 2; $p < 0.05$), thus the reduction in drug flux and decrease in drug thermodynamic activity was not proportional to the degree of dilution of the product. Microscopy investigations indicated that fusidic acid is formulated as a suspension in Fucidin cream and thus when diluted with a mix of Diprosone cream and Diprobace cream, a high drug thermodynamic activity may have been maintained if the solid particles of fusidic acid dissolved into the base upon dilution. Additionally, use of a diluent (Diprobace cream) dissimilar to the base of Fucidin cream may have contributed to a change in

drug thermodynamic activity to an unpredictable extent. The change in proportions of Diprobace cream to Diprosone cream appeared to have a negligible effect on fusidic acid flux across silicone membrane with fusidic acid flux decreasing by approximately 11.5 fold following the applications of Aron mix 1–4, compared to Fucidin cream alone. This observation was somewhat unsurprising given the similarity in the excipient lists of Diprosone cream and Diprobace cream.

To investigate the impact of diluting Fucidin cream to varying extents on betamethasone dipropionate and fusidic acid transport, a series of formulations with a fixed concentration of Diprosone cream (8.70 % w/w) and varying concentrations of Fucidin cream (2.17–30.43 % w/w) in a Diprobace cream base (Aron mix 5–8) were investigated. The drug transport profiles are presented in Fig. 5. Following the application of all formulations to silicone membrane, fusidic acid flux from Aron mix 1 and 5–8 was significantly reduced when compared to fusidic acid flux from Fucidin cream alone ($p < 0.05$; Table 3). A trend of decreasing fusidic acid flux with decreasing concentrations of Fucidin cream in the Aron mix was evident (Fig. 2), however as seen above this relationship was not proportional. A 3.3 – 46.1 fold dilution of Fucidin cream resulted in a 2.6–23.3 fold decrease in fusidic acid flux, compared to Fucidin alone ($p < 0.05$). This further confirmed that the decrease in fusidic acid flux cannot be fully explained by simple dilution of the suspension formulation. Instead it is likely that the fusidic acid particles dissolved on dilution to maintain a high drug thermodynamic activity,

Table 3

Betamethasone dipropionate (BDP) and fusidic acid (FA) flux from Diprosone cream alone, Fusidic cream alone or an Aron mix with varying proportions of Fucidin cream to Diprobace cream and fixed proportions of Diprosone cream. Data are shown as mean \pm SD (n = 6). * Denotes a significant difference when J4-24 h for BDP and FA in the Aron mixes were compared respectively to Diprosone cream and Fucidin cream ($p < 0.05$).

Formulation	J _{4-24h} for BDP ($\mu\text{g cm}^{-2}\text{h}^{-1}$)	J _{4-24h} for FA ($\mu\text{g cm}^{-2}\text{h}^{-1}$)
Diprosone cream	2.70E-01 \pm 1.48E-02	0.00 \pm 0.00*
Fucidin cream	0.00 \pm 0.00*	2.87 \pm 1.75E-01
Aron mix 5 (0.5:2:20.5)	5.64E-02 \pm 3.11E-03*	1.23E-01 \pm 2.79E-02*
Aron mix 1 (1:2:20)	5.99E-02 \pm 8.18E-03*	2.45E-01 \pm 3.38E-02*
Aron mix 6 (3:2:18)	6.22E-02 \pm 5.51E-03*	3.04E-01 \pm 7.13E-02*
Aron mix 7 (5:2:16)	5.21E-02 \pm 4.07E-03*	6.59E-01 \pm 3.01E-02*
Aron mix 8 (7:2:14)	5.96E-02 \pm 4.08E-03*	1.11 \pm 7.09E-02*

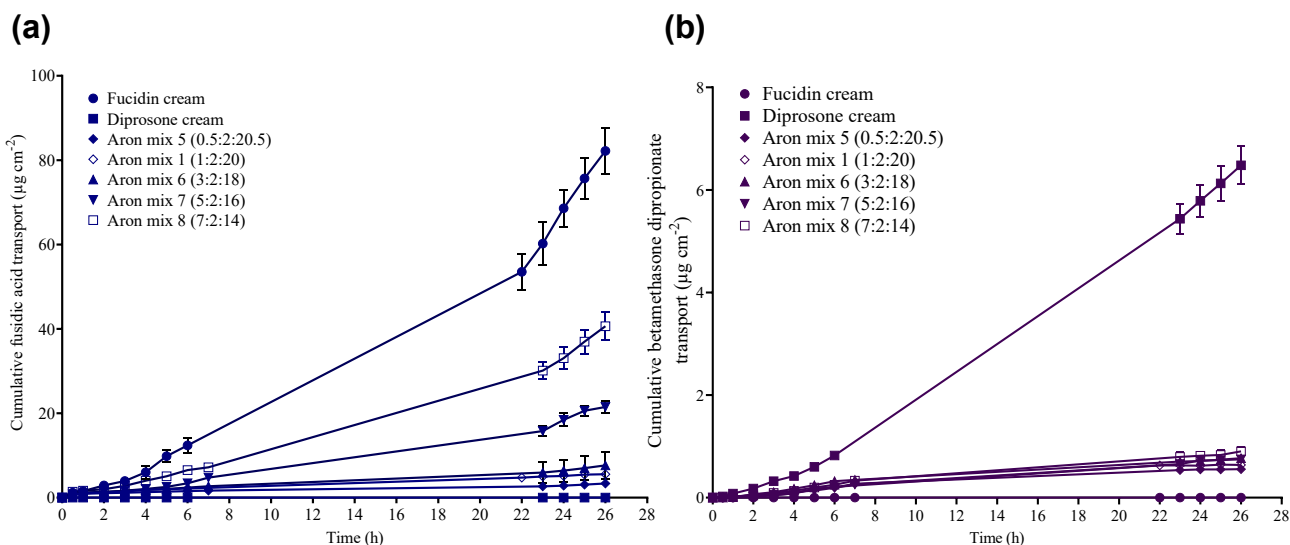


Fig. 5. Cumulative (a) fusidic acid transport (FA) and (b) betamethasone dipropionate (BDP) transport across silicone membrane following the application of an infinite dose of Fucidin cream alone (●), Diprosone cream alone (■) or mixes of Fucidin cream, Diprosone cream and Diprobace cream in the following ratios: Aron mix 5 (0.5:2:20.5; ◇), Aron mix 1 (1:2:20; ◆), Aron mix 6 (3:2:18; ▲), Aron mix 7 (5:2:16; ▼) or Aron mix 8 (7:2:14; □). Data are shown as the mean of six replicates (\pm SD).

which would produce this effect. Moreover the dilution of the topical antibiotic with a base dissimilar to the product (varying ratios of Diprosone cream and Diprosone cream) may have introduced excipients with solubilising or antisolvent effects also contributing to this.

Betamethasone dipropionate flux from Aron mix 1 and 5–8 was significantly decreased by 4.3 – 5.2 fold when compared to betamethasone dipropionate flux from Diprosone cream alone ($p < 0.05$; Table 3). Though betamethasone dipropionate flux was similar when comparing the diluted formulations ($p > 0.05$), a trend of the total amount permeated at 26 h (Q₂₆) decreasing for betamethasone dipropionate with decreasing concentrations of Fucidin cream was observed (and increasing concentrations of Diprosone cream), despite the concentration of Diprosone cream in the mixtures remaining the same (Fig. 5b). The trend was exemplified by Aron mix 5, the formulation with the highest proportion of Diprosone cream (89.13 %), where the 11.5 fold dilution of Diprosone cream resulted in a proportionate 11.6 fold decrease in Q₂₆, compared to Diprosone cream alone ($p < 0.05$). Comparatively, the same degree of dilution of Diprosone cream, but with a different base in Aron mix 8 (60.87 % Diprosone cream) resulted in a less proportional 7.1 fold decrease in Q₂₆ compared to Diprosone cream alone ($p < 0.05$). This trend was likely to be attributable to the increasing proportion of Diprosone cream in the Aron mix, the base vehicle of Diprosone cream, as the proportion of Fucidin cream decreased, decreasing betamethasone dipropionate thermodynamic activity in the formulation to a greater extent than Fucidin cream.

3.3. Percutaneous absorption and skin retention of betamethasone dipropionate and fusidic acid from a model Aron mix

To confirm whether the observed effects of Aron mixes on the drug transport of betamethasone dipropionate and fusidic acid across silicone membrane resulted in significant differences in drug delivery to the skin, the percutaneous absorption and distribution of both drugs was evaluated in human skin following application of Aron mix 9, Fucidin cream and Diprosone cream. The use of human skin can provide understanding of additional formulation effects such as the impact of mixing the formulation on the performance of any chemical penetration enhancers present in the formulation (Walters, 2002). In this study human scrotal skin was used. The scrotum may be affected by atopic dermatitis and is histologically similar to skin from other body regions, although it is usually more permeable to drugs (Smith et al., 1961). The tissue presents a barrier to drug penetration and although drug absorption may be relatively high, the insight gleaned from its use would be expected to be relevant for other body sites and thus it was selected as a suitable skin model for this study (Caserta et al., 2019). Whilst Diprosone cream does not contain any notable potential penetration enhancers, Fucidin cream is formulated with a proportion of glycerol, a hygroscopic excipient which can increase the water holding capacity of the stratum corneum (Batt et al., 1988). Under conditions of low humidity, such as that created in stratum corneum affected by dry skin conditions, glycerol has been shown to interact with lipid bilayers to increase skin permeability of metronidazole (Björklund et al., 2013).

The recovery of betamethasone dipropionate and fusidic acid from the skin surface (residual formulation), epidermis, dermis and receiver fluid following the application of Diprosone cream alone, Fucidin cream alone and the Aron mix 9 is presented in Table 4. The absolute recovery of betamethasone dipropionate and fusidic acid ranged 94–108 % of the applied dose for all experiments conducted, falling within the OECD defined acceptable criteria (OECD, 2019). Betamethasone dipropionate was not detected in the receiver fluid following the application of Aron mix 9 and very low levels of the drug were recovered from the dermis. For clarity the total drug delivery (total drug content in the epidermis, dermis and receiver fluid) was used for statistical analysis as an indication of the change in total betamethasone dipropionate and fusidic acid delivery from Aron mix 9 when compared to Diprosone cream or Fucidin cream alone (Table 4).

Table 4

The distribution of betamethasone dipropionate (BDP) and fusidic acid (FA) in the residual formulation, skin layers, receiver fluid and total absorbed (sum of epidermis, dermis and receiver fluid) following the application of Diprosone cream alone, Fucidin cream alone or Aron mix 9. Data are shown as mean \pm SD ($n = 6$). * denotes a significant difference when total BDP or FA delivery from Aron mix 9 was compared to total drug delivery from Diprosone cream or Fucidin cream, respectively.

	Residual formulation ($\mu\text{g cm}^{-2}$)	Epidermis ($\mu\text{g cm}^{-2}$)	Dermis ($\mu\text{g cm}^{-2}$)	Receiver fluid (Q ₂₄ ; $\mu\text{g cm}^{-2}$)	Total delivery ($\mu\text{g cm}^{-2}$)
Diprosone cream - BDP	3.80 \pm 0.92	0.08 \pm 0.15	0.53 \pm 0.39	0.58 \pm 0.34	1.19 \pm 0.83
Aron mix 9 (1:2:10) - BDP	0.55 \pm 0.13	0.00 \pm 0.00	0.19 \pm 0.04	0.00 \pm 0.00	0.20 \pm 0.04*
Fucidin cream - FA	172.35 \pm 49.81	1.90 \pm 1.22	7.91 \pm 4.31	16.24 \pm 4.18	26.05 \pm 6.94
Aron mix 9 (1:2:10) - FA	8.61 \pm 3.73	0.40 \pm 0.03	1.81 \pm 1.49	2.62 \pm 0.56	4.83 \pm 1.84*

Total betamethasone dipropionate delivery to the skin significantly decreased by 6 fold following the application of Aron mix 9 when compared to Diprosone cream alone ($p < 0.05$). This decrease was roughly proportional to the 6.5 fold dilution of Diprosone cream in the Aron mix. Comparatively, total fusidic acid delivery to the skin from Aron mix 9 significantly decreased by 5.4 fold compared to the application of Fucidin cream alone ($p < 0.05$), disproportionate to the 13 fold dilution of Fucidin cream in the Aron mix. This trend was consistent with the findings of the silicone membrane drug transport studies and suggests that whilst Diprosone cream was a suitable diluent for Diprosone cream to accomplish the intended reduction in betamethasone dipropionate absorption, the dilution effect on fusidic acid permeation was not predictable.

The percutaneous absorption profiles of betamethasone dipropionate and fusidic acid when Diprosone cream and Fucidin cream were applied alone or in Aron mix 9 are presented in Fig. 6. Betamethasone dipropionate permeation was not detectable over 0–10 h, following the application of Diprosone cream alone to human skin. Following the application of Aron mix 9, betamethasone dipropionate was not

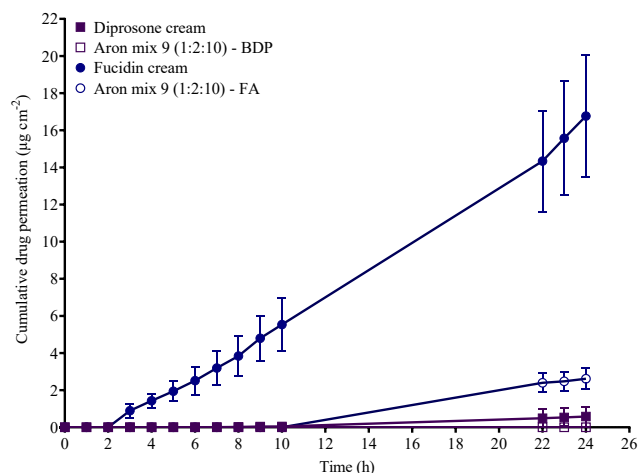


Fig. 6. The cumulative amount of betamethasone dipropionate (BDP) and fusidic acid (FA) permeated across human skin from Diprosone cream alone (■; BDP), Fucidin cream alone (●; FA) and Aron mix 9 (□ denotes BDP and ○ denotes FA). Aron mix 9 contained Fucidin cream, Diprosone cream and Diprosone cream mixed in a 1:2:10 ratio. Data are shown as mean \pm SD ($n = 6$).

detected in the receiver fluid over the entire experimental period. In comparison, fusidic acid was detectable in the receiver fluid at early timepoints following the application of Fucidin cream alone and its permeation profile was typical of an infinite dose study as would be expected from a suspension formulation, where the drug particles can dissolve in the formulation base to replace drug that had permeated the skin. This keeps the fusidic acid thermodynamic activity/saturation high in the formulation on the skin surface as drug is absorbed. Following the application of Aron mix 9, fusidic acid permeation was not detectable over 0–10 h, and considerably lower than that from Fucidin cream after 24 h (Fig. 6). The low drug permeation rates from Aron mix 9 were consistent with the observed decrease in drug delivery to the epidermis and dermis. The premise for heavily diluting the TCS and antibiotic in the Aron mix is to allow an increase in the frequency of product application even to unaffected areas, up to six times daily (Aron, 2022b). Dilution (reduced drug concentration) of a topical formulation does not necessarily correlate with the extent of drug delivery to the skin and whilst the overall decrease in betamethasone dipropionate delivery to the skin was proportional to the degree of dilution of Diprosone cream, this was not the case for Fucidin cream. For Fucidin cream, the substantial dilution of the suspension formulation with a base dissimilar to the product resulted in disproportionate reductions fusidic acid delivery to the skin, making this difficult to predict in a clinical setting. The fusidic acid delivery from Aron mix 9 was not lowered to the same extent as the dilution factor. This is likely to be because of the fusidic acid particles present in Fucidin cream dissolving in the diluted Aron mix formulation raising the drug thermodynamic activity/saturation above that expected by a simple formulation dilution. However, the Aron mix 9 formulation still contained fusidic acid particles as shown by Raman microscopy (Section 3.1). In theory, higher fusidic acid delivery from Aron mix 9 might therefore be expected, perhaps matching that of the Fucidin cream suspension. It is possible that the dissolution rate of the fusidic acid crystals in the Aron mix 9 formulation may not have been rapid enough to fully maintain the high drug thermodynamic activity in the formulation. The fusidic acid crystals observed in Aron mix 9 could potentially dissolve further in the Aron mix base with time, altering the formulation performance. Consideration of product stability following the preparation of Aron regime formulations was outside the scope of this study. Simultaneous to the decreasing fusidic acid thermodynamic activity in Aron mix 9, mixing Fucidin cream with Diprosone cream and Diprosone cream is likely to have resulted in a decrease in the thermodynamic activity of glycerol in the extemporaneous mix, compared to Fucidin cream alone which may have affected its delivery to the skin. Thus, the extent to which total fusidic acid delivery to the skin was altered is likely to have been influenced by (i) the decrease in drug thermodynamic activity and (ii) the decrease in thermodynamic activity of potential penetration enhancers in Aron mix 9.

S. aureus infections are typically localised to the skin surface and stratum corneum (Arikawa et al., 2002). However, skin colonisation of *S. aureus* has been found to extend beyond the epidermal barrier and into the dermis in lesional sites of atopic dermatitis patients (Nakatsuji et al., 2016). Thus, successful treatment may require delivery of fusidic acid in sufficient concentrations into the skin to decolonise the affected sites and prevent recurrent skin infections. The summary of product characteristics for Fucidin cream advises that fusidic acid concentrations of 0.03–0.12 µg mL⁻¹ (equivalent to 58–232 nM) are sufficient to inhibit nearly all strains of *S. aureus* (Leo Laboratories Ltd, 2019). The recovered drug levels in the epidermis and dermis are presented in Table 5 for comparison with the reported MIC for fusidic acid.

The drug concentration in the epidermis was 3,365 fold and 1,221 fold greater than the MIC for fusidic acid, following the application of Fucidin cream alone and Aron mix 9, respectively. Drug concentration in the dermis was 528 fold and 236 fold greater than the MIC of fusidic acid, following the application of Fucidin cream alone and Aron mix 9, respectively. Care should be taken however before assuming that the drug was delivered to the epidermal and dermal tissue in sufficient

Table 5

Fusidic acid (nM) recovered from human epidermal and dermal skin following the application of Fucidin cream alone and Aron mix 9. Data are shown as the mean of 6 replicates.

	Epidermis	Dermis
Mean drug recovery from Fucidin cream (nM)	781,528	283,576
Ratio compared to MIC ^a	3365.194	528.6072
Mean drug recovery from Aron mix 9 (nM)	122,763	54927.94
Ratio compared to MIC ^a	1221.055	236.5151

^a Ratio was calculated as mean drug recovery/ MIC of 232 nM (equivalent to 0.12 µg mL⁻¹).

concentrations, following a single application of Aron mix 9, for activity against *S. aureus*, as drug binding to keratin or other proteins may affect its antibiotic efficacy. It is also important to consider this finding in the context of the Aron regimen, which involves repeat applications of the product to affected and unaffected areas of the skin up to six times a day during the initial phase of treatment (minimum of two weeks). Clinical recommendations widely caution against the extended use of topical antibiotics in the treatment of clinically infected atopic dermatitis (Bath-Hextall et al., 2010; Eichenfield et al., 2014; Wollenberg et al., 2018) and where offered, treatment should be applied to localised areas only for a maximum of seven days (NICE, 2021). To fully evaluate the role of the Aron regimen in the treatment of severe cases of infected (or uninfected) atopic dermatitis, controlled investigations into the clinical efficacy of combined TCS and antibiotic therapies are required.

4. Conclusions

Compounding a complex dual therapy of a TCS and a topical antibiotic in an emollient base reduced the expected drug transport profiles, percutaneous absorption and skin retention of betamethasone dipropionate and fusidic acid relative to the individual marketed products. For Diprosone cream, the decrease in betamethasone dipropionate transport across silicone membrane and delivery to the skin largely correlated to the degree of dilution of the product in the Aron mix. For Fucidin cream, the impact of dilution did not correlate with the extent of reduction in fusidic acid transport across silicone membrane or skin absorption and was attributed to several formulation effects occurring in the complex extemporaneous mixtures. These effects are difficult to predict in a clinical setting. Preliminary investigations revealed that fusidic acid was delivered to the epidermal and dermal tissue in relatively high concentrations following a single application of a model Aron mix. To evaluate whether these findings correlate with clinical efficacy, further studies are required. In addition, the effect of frequent daily applications of the Aron mix to widespread areas on TCS side effects and antimicrobial resistance should be investigated.

CRedit authorship contribution statement

M.T. Beebeejaun: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing – original draft. **M.B. Brown:** Conceptualization, Supervision, Writing – review & editing. **V. Hutter:** Conceptualization, Supervision. **L. Kravitz:** Conceptualization, Supervision. **W.J. McAuley:** Conceptualization, Project administration, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- Arikawa, J., Ishibashi, M., Kawashima, M., Takagi, Y., Ichikawa, Y., Imokawa, G., 2002. Decreased levels of sphingosine, a natural antimicrobial agent, may be associated with vulnerability of the stratum corneum from patients with atopic dermatitis to colonization by *Staphylococcus aureus*. *J. Invest. Dermatol.* 119 (2), 433–439.
- Aron, R., 2022a. Aron regimen: About, Dr Aron. Available at: <http://www.draron.com/about> (Accessed: 19 April 2022).
- Aron, R., 2022b. 'Aron regimen: Treatment, prescription and application instructions'. Available at: <http://www.draron.com/treatment-prescription-and-application> (Accessed: 19 April 2022).
- Bath-Hextall, F.J., Birnie, A.J., Ravenscroft, J.C., Williams, H.C., 2010. Interventions to reduce *Staphylococcus aureus* in the management of atopic eczema: an updated Cochrane review. *Br. J. Dermatol.* 163 (1), 12–26.
- Batt, M.D., Davis, W.B., Fairhurst, A., Gerrard, W.A., Ridge, B.D., 1988. Changes in the physical properties of the stratum corneum following treatment with glycerol. *J. Soc. Cosmet. Chem.* 39, 367–381.
- Beebejaun, M.T., Brown, M.B., Hutter, V., Kravitz, L., McAuley, W.J., 2019. Does altering the topical corticosteroid and emollient application regime affect drug delivery?. In: *Skin at Bath Conference [Poster presentation]*. Bath, UK.
- Björklund, S., Engblom, J., Thuresson, K., Sparr, E., 2013. Glycerol and urea can be used to increase skin permeability in reduced hydration conditions. *Eur. J. Pharm. Sci.* 50 (5), 638–645.
- British National Formulary, 2022. BNF 83, Treatment summaries: Topical corticosteroids. Pharmaceutical Press, London. Available at: <https://bnf.nice.org.uk/treatment-summary/topical-corticosteroids.html> (Accessed: 19 April 2022).
- Caserta, F., Brown, M.B., McAuley, W.J., 2019. The use of heat and chemical penetration enhancers to increase the follicular delivery of erythromycin to the skin. *Eur. J. Pharm. Sci.* 132, 55–62.
- Edwards, A., Qi, S., Liu, F., Brown, M.B., McAuley, W.J., 2017. Rationalising polymer selection for supersaturated film forming systems produced by an aerosol spray for the transdermal delivery of methylphenidate. *Eur. J. Pharm. Biopharm.* 114, 164–174.
- Eichenfield, L.F., Tom, W.L., Berger, T.G., Krol, A., Paller, A.S., Schwarzenberger, K., Bergman, J.N., Chamlin, S.L., Cohen, D.E., Cooper, K.D., Cordero, K.M., Davis, D.M., Feldman, S.R., Hanifin, J.M., Margolis, D.J., Silverman, R.A., Simpson, E.L., Williams, H.C., Elmets, C.A., Block, J., Harrod, C.G., Smith Begolka, W., Sidbury, R., 2014. Guidelines of care for the management of atopic dermatitis: section 2. Management and treatment of atopic dermatitis with topical therapies. *J. Am. Acad. Dermatol.* 71 (1), 116–132.
- George, S.M., Karanovic, S., Harrison, D.A., Rani, A., Birnie, A.J., Bath-Hextall, F.J., Ravenscroft, J.C., Williams, H.C., 2019. Interventions to reduce *Staphylococcus aureus* in the management of eczema. *Cochrane Database of Systematic Reviews [Preprint]*, (10).
- Gibson, J.R., Kirsch, J., Darley, C.R., Burke, C.A., 1983. An attempt to evaluate the relative clinical potencies of various diluted and undiluted proprietary corticosteroid preparations. *Br. J. Dermatol.* 109, 114–116.
- ICH, 2005. Q2 (R1): Validation of analytical procedures: Text and methodology. (Proceedings of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use).
- Kligman, A.M., Christophers, E., 1963. Preparation of Isolated Sheets of Human Stratum Corneum. *Arch. Dermatol.* 88 (6), 702–705.
- Lakhani, F., Lee, K., Lio, P.A., 2017. Case Series Study of the Efficacy of Compounded Antibacterial, Steroid, and Moisturizer in Atopic Dermatitis. *Pediatr. Dermatol.* 34 (3), 322–325.
- Leo Laboratories Ltd, 2019. Summary of Product Characteristics: Fucidin cream. Electronic Medicines Compendium.
- MSD, 2021. Summary of Product Characteristics: Diprosone cream. Electronic Medicines Compendium.
- Nakatsuji, T., Chen, T.H., Two, A.M., Chun, K.A., Narala, S., Geha, R.S., Hata, T.R., 2016. *Staphylococcus aureus* Exploits Epidermal Barrier Defects in Atopic Dermatitis to Trigger Cytokine Expression. *J. Invest. Dermatol.* 136 (11), 2192–2200.
- NICE, 2021. NG190: Secondary bacterial infection of eczema and other common skin conditions: antimicrobial prescribing. National Institute for Health and Care Excellence. Last updated 2nd March 2021. Available at: <https://www.nice.org.uk/guidance/ng190/chapter/recommendations#treatment> (Accessed: 19 April 2022).
- OECD, 2019. Guidance notes on dermal absorption: Draft second edition. OECD Environment, Health and Safety Publications, pp. 1–66. Available at: https://www.oecd.org/chemicalsafety/testing/Guidance%20Notes%20Dermal%20Absorption%20156_Oct2019_clean.pdf.
- Rajkumar, J., Jetter, N., Samuels, K., Schwartz, A., 2021. Combined antibiotic, steroid, and moisturizer for atopic dermatitis: A two-year case series of patient-reported outcomes. *Pediatr. Dermatol.* 38 (3), 623–628.
- Ryatt, K.S., Feather, J.W., Mehta, A., Dawson, J.B., Cotterill, J.A., Swallow, R., 1982. The stability and blanching efficacy of betamethasone-17-valerate in emulsifying ointment. *Br. J. Dermatol.* 107 (1), 71–76.
- Smith, J.G., Fischer, R.W., Blank, H., 1961. The epidermal barrier: a comparison between scrotal and abdominal skin. *J. Invest. Dermatol.* 36 (5), 337–343.
- The Daily Telegraph, 2014. Eczema expert defended by parents. Telegraph Media Group, 5 October.
- The Guardian, 2018. Our daughter's eczema was out of control until we found Dr Aron. Alex Lake, 5 August.
- Walters, K.A., 2002. *Dermatological and Transdermal Formulations*. CRC Press.
- Wollenberg, A., Barbarot, S., Bieber, T., Christen-Zaech, S., Deleuran, M., Fink-Wagner, A., Gieler, U., Girolomoni, G., Lau, S., Muraro, A., Czarnecka-Operacz, M., Schäfer, T., Schmid-Grendelmeier, P., Simon, D., Szalai, Z., Szepletowski, J.C., Taïeb, A., Torrelo, A., Werfel, T., Ring, J., the European Dermatology Forum, the E.A. of D., Venereology, the E.A. of A., Clinical Immunology, the E.T.F. on A.D., Airways Diseases Patients' Associations, the E.S. for D., Psychiatry, the E.S. of P.D., Asthma European Network and the European Union of Medical Specialists (2018) 'Consensus-based European guidelines for treatment of atopic eczema (atopic dermatitis) in adults and children: part I'. *J. Eur. Acad. Dermatol. Venereol.* 32(5), 657–682.