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Drug stability and product performance characteristics of a dapivirine-releasing vaginal ring under simulated real-world conditions

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Abstract

In two recent Phase III clinical trials, use of a 25 mg dapivirine vaginal ring significantly reduced HIV acquisition rates. *Post hoc* analysis from one of the trials indicated higher rates of protection among women over the age of 21 years when compared to younger women, most likely due to reduced adherence in the latter group. There is currently no information available on how release of dapivirine from the ring is affected by either its intermittent removal from the vagina or women's cleaning of the ring before re-insertion. Here, *in vitro* drug stability and product performance characteristics of the dapivirine ring were assessed under simulated conditions of real-world use. The impact of systematic deviations from the 28-day continuous use protocol upon *in vitro* release performance, was investigated. Also, the effect of ring exposure to a range of common household chemicals – including bath salts, bleach, detergent and personal lubricants – was examined through measurement of dapivirine content and stability. Dapivirine *in vitro* release under intermittent schedules was similar to that obtained under the normal continuous schedule ignoring the periods of interruption. Ring exposure to various household chemicals had no discernible impact on dapivirine assay value, degradation or stability.

Keywords: HIV microbicide; Antiretroviral; Silicone elastomer vaginal ring; Intermittent use protocol

Abbreviations: DPV – dapivirine, IPA – isopropanol, IPA/water – 1:1 mixture of isopropanol and water, IPM – International Partnership for Microbicides, MTN – microbicides trial network, NOR – 19-norethindrone, RT – room temperature, SVF – simulated vaginal fluid, SVF/Tween –

- 41 simulated vaginal fluid with 0.2% Tween 80, rpm – revolutions per minute, RRT – relative
- 42 retention time, TFA – trifluoroacetic acid.

1. Introduction

Antiretroviral-releasing vaginal rings are a promising female-controlled biomedical strategy for reducing rates of HIV acquisition (Malcolm et al., 2016, 2014; Spence et al., 2015; Thurman et al., 2013). Among the advantages offered by this technology are the ability for the user to easily insert/remove rings without the need for medical intervention and the ability to offer long-acting administration of the antiretroviral agent(s) over many weeks or months (Boyd et al., 2016; Clark et al., 2014; Malcolm et al., 2016).

The dapivirine (DPV) releasing vaginal ring (Ring-004) is the most clinically advanced microbicide ring formulation (Malcolm et al., 2016). This matrix-type design comprises 25 mg micronised DPV homogeneously dispersed throughout a platinum-catalysed silicone elastomer ring. The ring has an outer diameter of 56 mm and an internal diameter of 7.7 mm. In 2016, the results from two Phase III clinical trials were reported in which the DPV ring was tested for prevention of HIV-1 acquisition. A 27% and 31% reduction in seroconversions was measured in the Aspire Study (MTN-020) and The Ring Study (IPM 027), respectively, compared to a non-medicated control ring (Baeten et al., 2016; Nel et al., 2016b). In the ASPIRE study, the level of protection afforded by the ring correlated strongly with participant age. For women aged 25 or over the incidence reduction was 61%. However, among women aged under 25, the protective efficacy was just 10%. In a post hoc exploratory analysis of age-categorized subgroups, the lack of protection among younger women was correlated with lower adherence (Baeten et al., 2016). A similar, though not statistically significant, reduction in efficacy was observed in The Ring Study – 37% vs. 15% for women over and under 21, respectively (Nel et al., 2016b). These correlations between adherence and efficacy mirror results in previous microbicide clinical

66 studies involving tenofovir tablets and vaginal gels in which no protective effect was observed
67 (Marrazzo et al., 2015; Van Damme et al., 2012). Interestingly, subsequent re-analysis of data
68 from the VOICE study showed that a protective effect with a vaginally administered tenofovir
69 gel could be detected among adherent participants (Dai et al., 2016). Although adherence to
70 study medication was deemed to be a major contributing factor to the lack of effect observed, it
71 was not the only factor, particularly for tenofovir-containing products (Kashuba et al., 2015;
72 McKinnon et al., 2018). Interim data from two open-label extension studies of the 25 mg DPV
73 ring – HOPE (MTN-025) and DREAM (IPM 032) – indicate that with a higher adherence rate,
74 the level of protection afforded by the ring rises to approximately 50% (Baeten et al., 2018; Nel
75 et al., 2018).

76
77 The contraceptive field has a long history of assessing user acceptability and adherence, and the
78 user issues previously reported for contraceptive vaginal rings are likely to be encountered again
79 with microbicide rings (Dieben et al., 2002; Novák et al., 2003; Stifani et al., 2018; Woodsong
80 and Holt, 2015). Studies examining women’s perceptions and motivations for ring use and
81 reasons for ring removal have been reported (MacQueen et al., 2014; Montgomery et al., 2012;
82 Nel et al., 2016a; Van Der Straten et al., 2012). Many factors are known to impact adherence to
83 microbicide rings, including ring removal for menses, sex or washing and the feelings and
84 opinions of sexual partners (Montgomery et al., 2017). In clinical studies, increased familiarity
85 and experience with vaginal ring use among study participants, coupled with counselling and
86 support from clinical staff, can significantly increase positive clinical outcomes (Montgomery et
87 al., 2017).

88

There are a number of ways in which women's use of a ring may deviate from so-called 'perfect use'. For example, the ring may be removed or be expelled periodically. Consequently, the length of time the ring is located outside of the vagina will impact the concentrations of dapivirine in the vaginal fluid and hence product efficacy after removal. The performance of the ring under conditions of imperfect use is unknown. As well as not wearing the ring for the intended duration, it may also be washed or exposed to other cleaning fluids in the course of normal use. Exposure of rings to other household or personal hygiene products could potentially affect their *in vivo* performance. In studies examining acceptability of a tenofovir disoproxil fumarate ring among women from New York, users were concerned about the ring potentially absorbing blood and getting dirty if it remained inserted during menses (Watnick et al., 2018). In a previous study, between 4 and 18% of women reported removing the ring either during menses or for periodic cleaning (Montgomery et al., 2012). Application of a variety of different substances to the vagina for general health, cleaning or sexual function has also been reported in different populations (Gafos et al., 2010; Low et al., 2011; Martin Hilber et al., 2010; Pines et al., 2018). Further, previous clinical studies have observed ring discolouration following use (Spence et al., 2016). To the best of our knowledge, the impact of ring exposure to wash products or other vaginally-administered products has not been reported.

Here, we have examined: (i) the impact of periodic removal and storage of the dapivirine-releasing vaginal ring (Ring-004) during *in vitro* release testing; (ii) ring performance following exposure to various products common in Sub-Saharan African households, including two bath salts, an oral pill containing natural products that was suspected to cause ring discolouration, three vaginal lubricants, a liquid household detergent and a household bleach; and (iii) the

impact of exposure on the DPV content of the ring and the potential for undesirable breakdown product formation.

2. Materials and methods

2.1 Materials

Matrix-type, silicone elastomer vaginal rings nominally containing 25 mg dapivirine (Ring-004, mean measured DPV content per ring 24.4 ± 0.15 mg) were manufactured at QPharma (Malmö, Sweden) and supplied by International Partnership for Microbicides (IPM). Potassium dihydrogen orthophosphate, potassium hydroxide, trifluoroacetic acid (TFA) and urea (AnalaR, analytical reagent grade) were purchased from VWR International Ltd. (Dublin, Ireland). 19-Norethindrone (NOR) was purchased from LGM Pharma (Nashville, USA). HPLC-grade 2-propanol (IPA), methanol, acetone, acetonitrile, phosphoric acid (85% w/w in water), Tween 80, sodium chloride, calcium hydroxide, bovine serum albumin, lactic acid, acetic acid and glucose were all purchased from Sigma-Aldrich (Gillingham, UK). A Millipore Direct-Q 3 UV Ultrapure Water System (Watford, UK) was used to obtain HPLC-grade water. Simulated vaginal fluid (SVF), pH 4.2 with 0.2% w/v Tween 80 (SVF/Tween) release media, was prepared according to a previously described method followed by the addition of the Tween 80 component (Owen and Katz, 1999). Revive Bath Salts, Sols Bath Salts, and DeWitt's K&B Pills were provided by IPM (Table 1). The DeWitt's K&B Pills (a traditional African product) were reported to have been taken by some participants in Phase III clinical trials; the blue colour of the pills matches that observed for a small number of rings following clinical use (IPM, unpublished data) and is known to cause blue/green urine discolouration. The vaginal lubricants Replens MD, SuperSlik and Sliquid were purchased from Amazon.co.uk (Table 1). The detergent (Fairy Liquid, a

Procter & Gamble UK product) and bleach (Domestos, a Unilever UK product) were purchased from a local supermarket (Table 1).

Table 1. Vaginal lubricants, detergent, bleach and bath salts products tested

	Product name	Ingredients
A	Super Slik water-based lubricant	water, glycerine BP, monopropylene glycol USP, hydroxyethyl cellulose, sodium methylparaben, sodium ethylparaben, sodium propylparaben
B	Replens MD Vaginal Moisturiser	purified water Ph.Eur, glycerin, mineral oil, polycarbophil, carbomer homopolymer Type B, hydrogenated palm oil glyceride, sorbic acid, sodium hydroxide
C	Sliquid Silver Luxury Silicone Lubricant	cyclopentasiloxane, dimethicone, dimethiconol
D	Fairy Liquid washing up liquid	water, sodium laureth sulphate, alcohol denatured, lauramine oxide, C9-11 pareth-8, sodium chloride, 1,3-cyclohexanedimethanamine, polypropylene glycols, dimethyl aminoethyl methacrylate/hydroxypropylacrylate copolymer citrate, parfum, geraniol, limonene, colourant
E	Domestos bleach	sodium hypochlorite, C12-C18 alkyl dimethylamine oxide cationic surfactants, non-ionic surfactants, sodium hydroxide, soap, perfume
F	Revive Bath Salts	sodium sesquicarbonate, sodium bicarbonate, sodium lauryl sulphate, fragrance, propylene glycol, panax ginseng seed oil, eucalyptus globulus leaf oil, amycl cinnamal, citral, citronellol, coumarin, geraniol, butylphenyl methylpropional, limonene, linalool, CI 42090
G	Sols Bath Salt (<i>Itshe Abelungu</i>)	not available; pink coloured viscous liquids and salts (Cocks and Moller, 2002)
H	DeWitt's K&B (Kidney and Baldder) Pills	dry Buchu (<i>Agathosma</i>) extract, Bearberry extract, magnesium stearate, paraffin wax, talc

2.2 *In vitro* release testing of rings

In vitro release testing of the 25 mg DPV rings was performed according to the schedule and conditions summarised in Table 2. On Day 0, rings were weighed and placed into individually

144 labelled 250 mL glass flasks containing 200 mL of either 1:1 mixture of IPA and water
145 (IPA/water) or SVF/Tween. Flasks were sealed and placed in a temperature-controlled orbital
146 shaking incubator (37°C, 60 rpm, 25 mm orbital throw). On Day 1, each flask was removed from
147 the incubator after 24 h $\pm$ 15 min, gently shaken for 10 s, and then a 1–2 mL sample of the
148 release medium taken for HPLC analysis. Rings scheduled to be placed on storage were removed
149 from the release medium, rinsed with distilled water and blotted dry, before being placed in
150 individually labelled polyethylene sample bags and stored in the dark at either ambient
151 temperature or 4°C. Otherwise, the release medium was replaced with 100 mL of fresh medium
152 before the flasks were returned to the incubator. Sampling and complete replacement of the
153 release medium continued daily (except for weekends) for those rings scheduled to be on release.
154 Each Friday, the volume of release medium used was increased to 200 mL, and no sampling or
155 release medium replacement occurred during weekends.

Table 2. Summary of the test groups, schedule and conditions for DPV ring *in vitro* release testing, mean residual content values, mean cumulative *in vitro* release and total calculated DPV recovery values (n = 3 rings per study group). Ring codes beginning with ‘P’ and ‘S’ refer to rings released into IPA/water and SVF/Tween media, respectively.

Ring code	Testing days	Release medium*	Storage temperature #□	Mean residual content (mg)	Mean cumulative release (mg)	Mean total DPV recovery (mg)	% Recovery
Control	–	–	–	24.30 ± 0.41	–	–	99.6
PC	1–28	IPA/water	–	11.21 ± 0.18	12.84 ± 0.30	24.05	98.6
P1	1 and 28	IPA/water	RT	21.07 ± 0.19	3.40 ± 0.02	24.47	100.3
P2	1 and 28	IPA/water	4°C	20.79 ± 0.29	3.38 ± 0.02	24.17	99.1
P3	1, 8, 15, 22 & 29	IPA/water	RT	18.89 ± 0.26	5.53 ± 0.03	24.42	100.1
P4	1, 8, 15, 22 & 29	IPA/water	4°C	19.02 ± 0.27	5.57 ± 0.01	24.59	100.8
P5	1–7 and 21–28	IPA/water	RT	14.89 ± 0.40	9.40 ± 0.02	24.28	99.5
P6	1–7 and 21–28	IPA/water	4°C	14.88 ± 0.15	9.39 ± 0.04	24.27	99.5
SC	1–28	SVF/Tween	–	19.23 ± 0.28	4.89 ± 0.10	24.12	98.8
S1	1 and 28	SVF/Tween	RT	23.73 ± 0.33	0.72 ± 0.03	24.45	100.2
S2	1 and 28	SVF/Tween	4°C	24.01 ± 0.34	0.74 ± 0.03	24.76	101.5
S3	1, 8, 15, 22 & 29	SVF/Tween	RT	23.21 ± 0.60	1.62 ± 0.02	24.83	101.8
S4	1, 8, 15, 22 & 29	SVF/Tween	4°C	22.62 ± 0.33	1.57 ± 0.06	24.19	99.2
S5	1–7 and 21–28	SVF/Tween	RT	21.38 ± 0.46	3.08 ± 0.11	24.26	100.3
S6	1–7 and 21–28	SVF/Tween	4°C	21.06 ± 0.19	3.17 ± 0.08	24.23	99.3

* IPA/water – 1:1 mixture of isopropanol and water; SVF/Tween – simulated vaginal fluid with 0.2% w/v Tween 80

#□ RT – room temperature

2.3 Ring exposure to bath salts, detergent, bleach and personal lubricants

DPV rings were exposed to a range of household products (two different bath salts, a pill containing natural extracts, a household liquid detergent and a household bleach) and three different personal lubricants to assess the impact on drug content and stability (Table 1). The range of wash solutions included bath salt brands widely available in South Africa as well as commonly used detergent and bleach products from the UK. Exposure conditions (wash solution, temperature, time) for each test are outlined in Table 3. The personal lubricants were selected to include aqueous-based, mineral oil-based, and silicone oil-based products. Exposure durations (1–4 h), temperatures (37–50°C) and product test volumes were selected to mimic real world conditions. Untreated rings, rings exposed to SVF/Tween only, and rings exposed to water only were used as study controls. Diluted solutions of each personal lubricant product (to mimic product dilution with vaginal fluid) were prepared by mixing 15 g of the lubricant with 5 g of SVF/Tween prior to ring addition. Detergent and bleach products (4 g) were diluted in water (16 g). Bath salt solutions were prepared by addition of 1 g bath salt to 100 mL water with stirring for 5 min. Revive Bath Salts and DeWitt's K&B Pills were not fully dissolved after mixing at either ambient temperature or 40°C for 30 min, but were still used for testing. 20 mL or 20 g of each product solution/dispersion was used to fully cover the ring in the flask.

181 **Table 3.** Experimental conditions and mean DPV recovery values and percentages for rings exposed to bath salts, detergent, bleach and personal
182 lubricant test solutions. n = 3 rings per test solution. Rings were exposed to all test solutions under controlled agitation (60 rpm, except for the 'no
183 wash solution' control).
184

Test solutions	Exposure time (h)	Exposure temperature (°C)	Mean DPV recovered (mg) ± SD	Mean % recovery ± SD
No wash solution	-	-	24.44 ± 0.41	100.2 ± 1.7
SVF/Tween	4	37	24.34 ± 0.37	99.8 ± 1.5
Super Slik Lubricant + SVF/Tween (3:1)	4	37	24.17 ± 0.31	99.1 ± 1.3
Replens MD + SVF/Tween (3:1)	4	37	24.18 ± 0.20	99.1 ± 0.8
Sliquid Silver Luxury Silicone Lubricant + SVF/Tween (3:1)	4	37	24.35 ± 0.17	99.8 ± 0.7
Detergent (Fairy Liquid) + water (1:4)	1	50	25.02 ± 0.27	102.5 ± 1.1
Bleach (Domestos) + water (1:4)	1	50	25.25 ± 0.42	103.5 ± 1.7
Water	3	40	24.81 ± 0.41	101.7 ± 1.7
Revive Bath Salts in water	3	40	24.89 ± 0.30	102.0 ± 1.2
Sols Bath Salts in water	3	40	24.70 ± 0.19	101.2 ± 0.8
DeWitt's K&B Pills in water	3	40	24.69 ± 0.17	101.2 ± 0.7

2.4 Content/residual content assay

Initial and residual DPV content in the control rings and the rings exposed to the various test solutions, respectively, was assessed by solvent extraction and subsequent HPLC analysis. Upon removal from the test solutions, rings were rinsed with deionised water, blotted dry and weighed. Rings were then sectioned into approximately 2 mm pieces and reweighed before being placed into individually labelled 250 mL glass flasks. 5 mL of NOR internal standard solution (2.5 mg/mL) and 95 mL acetone was added to each flask. Flasks were sealed and placed in a temperature-controlled orbital shaking incubator (37°C, 60 rpm, 25 mm orbital throw). After 24 h, the flasks were removed and allowed to cool to ambient temperature for at least 1 h. A 5 mL aliquot of the acetone extraction solution was transferred to a 25 mL volumetric flask and diluted with methanol. Flasks were allowed to stand for a further 20 min at ambient temperature before final dilution to volume with methanol. Samples were transferred to HPLC vials and analysed against standard solutions of known DPV and NOR concentrations.

2.5 Degradation products assay and impurity identification

For degradation product assay and impurity identification, rings were treated and processed using a similar method as for content assay testing. Rings were extracted using 100 mL acetone (without internal standard to avoid interference in subsequent chromatography) in a temperature-controlled (37 °C) orbital shaking incubator. After the flasks were cooled for ~ 60 min, a 5 mL aliquot of the extraction solution was transferred to a boiling tube and evaporated to dryness. A 1:1 mixture of acetonitrile and water (5 mL) was added to each boiling tube and the samples reconstituted by agitation on a whirl mixer for 30 s followed by 5 min sonication. Samples were

then centrifuged at 3000 rpm for 2 min and the resulting solutions filtered through a 0.45 µm filter before being transferred to HPLC vials for analysis.

2.6 HPLC methods

A Waters HPLC system (Elstree, UK) consisting of; a 1525 binary HPLC pump, a 717 plus autosampler, an in-line degasser unit, a 1500 series column heater, a 2487 dual wavelength absorbance detector and a 2998 photodiode array detector was used for all analysis.

In vitro release

25 µL of sample was injected on to a Thermo Scientific BDS Hypersil C18 HPLC column (150 x 4.6 mm, 3 µm particle size) fitted with a guard column. The column was held at 45°C and isocratic elution performed using a mobile phase of 45% acetonitrile and 55% phosphate buffer (pH 3.0, 7.7 mM) with a total flow rate of 1.2 mL/min and a run time of 8 min. DPV was detected at 240 nm after approximately 6.1 min.

Content assay

10 µL of sample was injected on to a Kromasil C18 column (150 x 4.6 mm, 5 µm particle size) fitted with a guard column. The column was held at 25°C and isocratic elution performed using a mobile phase of 75% methanol and 25% water. The total flow rate was 0.75 mL/min and the run time was 12 min. DPV was detected at 257 nm after 10.8 min and NOR was detected at the same wavelength after 5.3 min.

Degradation products assay

50 μ L of sample was injected on to a Kromasil C18 column (250 $\times$ 4.6 mm, 5 μ m particle size) fitted with a guard column. The column was held at 25°C and gradient elution performed using a mobile phase of 0.1% v/v TFA in water and 0.1% v/v TFA in acetonitrile mixed according to the schedule in Table 4. The total flow rate was 1.0 mL/min and the run time was 51 min. DPV was detected at 245 nm after 21 min. A resolution solution containing a DPV isomer and a closely related structural analogue were also run to ensure adequate separation.

Table 4. Mobile phase composition for the gradient elution of DPV in the degradation products analysis.

Time (min)	% mobile phase A (0.1% TFA in water)	% mobile phase B (0.1% TFA in acetonitrile)
0.0	70	30
40.0	30	70
40.5	5	95
45.5	5	95
46.0	70	30
51.0	70	30

2.7 Analysis

In vitro release concentrations obtained via HPLC were used to calculate total daily amount of DPV released and cumulative release amount. Residual content values were calculated for each ring and compared to the mean content assay value determined for this batch of rings. To determine when differences between groups were statistically significant, mean values were compared using a one-way analysis of variance. Where appropriate, post hoc comparisons were conducted using Tukey-Kramer multiple comparisons test with a 95% significance level. In the degradation products assay, injections of blank extraction solutions and mobile phase were examined for baseline quality. Chromatograms from replicate samples were overlaid to ensure consistency of peak appearance. A relative retention time (RRT) window of $\pm$ 0.02 either side of

an unknown peak eluting before DPV was used as the basis for assigning a similar peak label to a subsequent chromatogram. For peaks eluting after DPV, an RRT window of ± 0.04 was used. Degradation products providing peak areas greater than 0.1% w/w were recorded and investigated further. Degradation products at peak areas representing between 0.02 and 0.1% w/w were noted but not investigated further. Integrated peaks which gave degradation values less than 0.02% w/w were not considered further.

3. Results and Discussion

3.1 In vitro release testing of rings on intermittent use

In vitro release versus time profiles for 25 mg DPV rings placed on either continuous or intermittent release over 28 days are presented in Figure 1. DPV release into IPA/water (P1–P6, Table 2) are presented in plots A, B and C, and release into SVF/Tween (S1–S6, Table 2) in plots D, E and F. Data for 28-day continuous release of DPV from the ring into IPA/water (group PC) or SVF/Tween (group SC) is also presented in each plot for reference. Plots A and D display daily DPV release from rings tested on Day 1 and Day 28 for IPA/water (group P1 and P2, Table 2) and SVF/Tween (group S1 & S2, Table 2), respectively. Plots B and E show daily release from rings tested on days 1, 7, 14, 21 and 28 into IPA/water (groups P3 and P4, Table 2) and SVF/Tween (groups S3 and S4, Table 2), respectively. Plots C and F display daily release from rings tested on days 1–7 and 21–28 into IPA/water (groups P5 and P6, Table 2) and SVF/Tween (groups S5 and S6, Table 2), respectively. The dashed arrows in each figure correlate the daily release values obtained under the different intermittent release testing schedules with the equivalent daily release values for rings tested under the continuous 28-day release schedule. Where the gradient of the arrow is zero or close to zero, there is no substantial difference

between the daily release value measured upon testing after storage and an equivalent release value measured from rings which were not on storage. It can therefore be concluded that the majority of rings showed no appreciable difference in the amounts of DPV released from rings used intermittently and those used continuously when daily release values were compared based only on the number of days – and not necessarily consecutive days – spent on release (Figure 1A, B, C, D and F). S3 and S4 rings displayed a small, but not statistically significant, difference between the amounts of DPV released for rings with and without interruption of release testing into SVF/Tween (Figure 1E). The daily release values for these rings tested after storage was slightly higher than for rings tested under the continuous regimen (hence the dashed arrow has a negative gradient, Figure 1E). The mean residual content and mean cumulative release values measured for each ring are presented in Table 2, demonstrating complete DPV recovery when cumulative release and residual content values are summated, and indicating that no loss of drug to other surfaces had occurred during the period of ring removal. Ring storage temperature (room temperature vs. 4°C) did not have any substantial impact on the daily release values for any of the rings tested.

The observation that stored rings pick up the expected *in vitro* drug release rate after return to the release medium is interesting. No leaching or loss of drug from the system is possible during storage, as there is no surrounding medium for dissolved drug to partition into. Without release of dissolved drug from the polymer matrix, the remaining solid drug will be unable to dissolve as the system is already saturated. Of course, this observation would likely not hold if the rings were to be stored in a liquid medium following removal from the body. In the event that a ring is removed during use – either by choice or expulsion – the concentrations of DPV already present

in the biological compartments would be expected to decline according to the terminal elimination half-life of DPV in that compartment e.g. 12–14 h in vaginal fluid and 67 h in plasma (Nel et al., 2014).

3.2 Ring exposure to bath salts, detergent, bleach and personal lubricating agents

To assess the impact of exposure to bath salts, liquid detergent, bleach or personal lubricants upon the 25 mg ring, rings were exposed under controlled conditions to test solutions and the DPV content quantified following solvent extraction. Extraction solutions were also assessed for DPV degradation products. The mean residual DPV content values for each ring group following exposure to the various test solutions are presented in Table 3. The lowest amount of DPV measured per ring was 24.17 mg, equivalent to 99.1% of theoretical based on a measured mean value of 24.4 mg DPV per ring for this batch. These data indicate that no significant reduction in DPV content was observed following exposure to any of the personal lubricants or wash solutions tested in this study. Of note, a pink discolouration was visible on the surface of all rings exposed to the Sols bath salt solution (Figure 2A), which rinsing with deionised water did not remove. On sectioning the rings, the discolouration was determined to be largely a surface effect, although given the limited exposure time, substantial migration through the ring would seem unlikely (Figure 2B). The discolouration had no impact on the amount of DPV recovered from this ring (Table 3). The discolouration observed in this study is consistent with ring discolouration previously observed in some used silicone elastomer rings returned from clinical sites (IPM, unpublished results).

319 To determine whether exposure of the ring to any of the wash solutions had the potential to cause
320 DPV degradation, a further set of rings were treated and extracted – this time without addition of
321 an internal standard – and the extraction solutions tested for the presence of significant
322 breakdown products. The results are summarised in Table 5. Rings exposed to personal lubricant
323 solutions were not examined due to concerns about injecting certain formulation components
324 onto the HPLC system.

325

326 Exposure of the DPV ring to different bath salts, a pill, and various cleaning solutions (Table 1)
327 produced up to three additional, low-level peaks in the degradation products analysis relative to
328 the control rings (Table 5). However, in all cases, a similar mean sum of degradation products
329 (0.3–0.4% w/w) was observed for untreated control rings. Exposure to detergent or bleach
330 solutions produced no new unknown peaks greater than 0.1% w/w. Untreated rings also
331 produced unknown peaks > 0.1% w/w at the same relative retention time as those observed with
332 the detergent and bleach treatments. This suggests that exposure to the products tested in this
333 study did not significantly impact the impurity profile of the 25 mg DPV ring.

Table 5. Degradation products observed after treatment of the 25 mg DPV ring with bath salts, bleach and detergent solutions. n=3 rings per study group. Unk – unknown peaks

Treatment solution	Average sum of degradation products (%)	Number of unknowns	Unknowns > 0.1 % w/w	Remarks
Untreated	0.4	4	Unk 1 & 2	–
Detergent (Fairy Liquid) + water (1:4)	0.4	6	Unk 1 & 2	Unk 1 & 2 present in untreated ring extracts
Bleach (Domestos) + water (1:4)	0.4	3	Unk 1 & 2	Unk 1 & 2 present in untreated ring extracts
Water	0.3	5	0	–
Revive Bath Salts in water	0.4	6	0	–
Sols Bath Salts in water	0.4	7	0	–
DeWitt's K&B Pills in water	0.3	5	0	–

4. Conclusions

In vitro release testing of the 25 mg DPV matrix-type silicone vaginal ring under various intermittent use schedules produced no substantial impact on the rate of DPV release when compared to rings released continuously for 28 days. Ring storage conditions (ambient temperature or 4°C) had no impact on DPV release rates *in vitro*. Exposure of rings to a range of aqueous solutions derived from bath salts, an oral pill, bleach, detergent and personal lubricants had no significant impact on the amount of DPV present. No new degradation products ($\geq 0.1\%$ w/w) were observed following exposure to detergent, bleach, pill or bath salt treatments. These results indicate that drug release from the 25 mg DPV ring is unaffected by periodic removal and reinsertion of the ring. In addition, the ring is tolerant of exposure to a range of wash or personal lubricant solutions, in terms of both drug content and stability.

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Author Contributions

All authors contributed to the design of experiments and analysis of data. D.J.M and C.F.M conducted the experimental work. The manuscript was drafted by D.J.M, C.F.M and R.K.M with input from other authors. All authors approved submission of the manuscript.

Declaration of Interest

The authors declare no competing financial or personal interest.

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Figure Legends

Fig. 1. *In vitro* daily release versus time profiles for rings releasing into IPA/water (group P rings, plots A, B and C) and SVF/Tween (group S rings, plots D, E and F). Data for control rings PC and SC, representing continuous release over 28 days into IPA/water or SVF/Tween respectively, are presented in each panel for reference. P1, P2 & S1, S2 represent rings released on days 1 and 28 only. P3, P4, S3 and S4 represent rings released on days 1, 7, 14, 21 and 28. Groups P5, P6, S5 and S6 represent rings released on days 1–7 and 21–28. Rings from groups 1, 3 and 5 were stored at room temperature when not on release, and rings from groups 2, 4 and 6 at 4°C. Dashed lines are intended to invite comparisons between daily release values measured after a period of storage with the equivalent timepoint for rings that underwent continuous release testing, e.g. for rings that were released on days 1 and 28 only, the day 28 value is compared with the day 2 value from the continuous release test.

Fig. 2. Discolouration observed upon treatment of 25 mg DPV matrix-type rings with Sols bath salts solution; (A) full ring, (B) ring segments.

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Declaration of Interest

The authors declare no competing financial or personal interest.

Figure 1

Figure 2A

Figure 2B