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Research Paper

# Combined strategies for enhancing the transdermal absorption of midazolam through human skin

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## Abstract

**Objectives** Midazolam administration by intravenous or intramuscular injection produces pain and stress. For this reason, alternative methods of administration have been proposed. The transdermal administration of midazolam could improve patient comfort, which is especially important for children in the pre-operative period. We aimed to assess the effect of iontophoresis and chemical percutaneous enhancers applied individually and together, to determine if a synergistic effect is achieved when both enhancement techniques are simultaneously employed.

**Methods** This work reports the characterization of the passive diffusion of midazolam hydrochloride through human skin *in vitro* and evaluates the effect of iontophoresis application and chemical percutaneous enhancers on said diffusion when employed both individually and in combination.

**Key findings** Percutaneous absorption assays demonstrated that the physical technique of iontophoresis, when applied alone, moderately increased midazolam hydrochloride permeation flux through human skin, producing a similar effect to that obtained with *R*-(+)-limonene chemical enhancer. Among the strategies assayed, it was observed that Azone produced the most pronounced enhancement effect when applied separately. The combination of pre-treatment with Azone and iontophoresis exhibited a higher capacity for enhancing the transdermal flux of midazolam through human skin than Azone alone.

**Conclusions** In conclusion, when applied individually, Azone exhibited the greatest enhancement effect on the transdermal diffusion of midazolam of the various strategies assayed. The combination of Azone and iontophoresis produce the highest transdermal steady-state flux of midazolam but no synergic effect was achieved when the two enhancement strategies were applied in combination, showing that although selecting the best conditions for iontophoresis application, it is less effective for augmenting the transdermal delivery of midazolam than the chemical enhancer Azone.

**Keywords** chemical percutaneous enhancers; iontophoresis; midazolam; transdermal absorption

## Introduction

Midazolam (Figure 1) is a short-acting, hypnotic–sedative benzodiazepine with anxiolytic and amnesic properties.[1] The pharmacological effects of the drug appear to result from reversible interactions with the gamma-amino butyric acid (GABA) neurotransmitter in the central nervous system (CNS), the major inhibitory neurotransmitter in the brain.[2]

Midazolam is usually administered by the oral and parenteral routes, and less frequently by the intranasal or rectal routes.[3,4] Generally, oral administration is indicated for the short-term treatment of moderately severe insomnia in patients who do not react adequately to other hypnotics, and can be used to produce sedation before surgical interventions. Midazolam parenteral administration is indicated for pre-operative sedation and for the induction of general anaesthesia, and is used in children, particularly in non-ventilated infants, as respiratory depression is associated much less frequently than with other benzodiazepines.[5]

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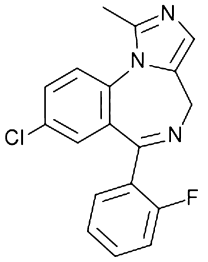


**Figure 1** Structure of midazolam hydrochloride.

Midazolam administration by intravenous or intramuscular injection produces pain and stress. For this reason, alternative methods of administration, such as the oral, rectal, intranasal and even sublingual (whose effect has been demonstrated in studies of paediatric patients) routes have been proposed to avoid the unpleasant nature of injections, especially relevant in the case of children.[3]

However, there are problems associated with these alternative routes; rectal administration is associated with pharmacokinetic disadvantages, and nasal, oral or sublingual formulations are not well accepted by children due to their acid pH and bitter taste. Transdermal administration may thus represent an alternative method of administration of midazolam that avoids the disadvantages of the oral/gastrointestinal route, the traumas of parenteral administration and the rejection of nasal, rectal or sublingual administration among children. Transdermal administration of midazolam could improve patient comfort, which is especially important for children in the pre-operative period.

In recent years, the skin has gained importance as a medium for the systemic application of drugs. However, human skin is a remarkably efficient barrier that presents difficulties for transdermal delivery. Few drugs have the characteristics required to permeate sufficiently through the skin to achieve a therapeutic concentration in the blood. For this reason, different methodologies have been investigated and developed to overcome the barrier-like properties of the skin and thus enhance the transdermal absorption of drugs.[6]

Iontophoresis is an enhancement technique that increments transdermal drug transport through the application of a low-level electric current ($\leq$0.5 mA/cm$^2$).[7] The mechanisms by which iontophoresis enhances molecular transport across the skin are: (1) electrorepulsion, in which a charged ion is repelled from an electrode with the same charge;[8–13] (2) electroosmosis, which is the convective flow of solvent that occurs through a charged pore in response to the preferential passage of counter ions when the electric field is applied;[9–14] and (3) current-induced increase in skin permeability. The first two mechanisms are the principal mechanisms involved in drug transport. Then, under the influence of an iontophoretic current, the total transport ($J_t$) of a compound across the skin is the sum of $J_p$ (passive flux), $J_{er}$ (electrorepulsive contribution) and $J_{eo}$ (electroosmotic flux). $J_{er}$ is the main transport mechanism for small charged molecules. It is well accepted that the contribution of $J_{eo}$ becomes greater respective to the $J_{er}$ as the molecular weight increases.[9,10]

Skin has an isoelectric point of 4–4.5. Under physiological conditions, the carboxylic acid groups are ionized and human skin has a net negative charge. In these conditions the electroosmotic flow is from anode (+) to cathode (–).[15–19] This phenomenon has been used to increase the anodic delivery of positively charged drugs and it is especially important for large cations and uncharged polar molecules.[17,19] These molecules have low transport number due to the competition from smaller and more mobile ions comprising the background electrolyte or receptor solution (e.g. physiological buffer).[14] The iontophoretic drug transport across the skin is directly dependent on current density.[14,20,21]

Recently, to enhance transdermal drug transport as much as possible, iontophoresis has been evaluated separately and in combination with strategies based on other mechanisms for incrementing drug transport, such as chemical penetration enhancers.[22,23] The use of chemical percutaneous enhancers is another method of incrementing the absorption of drugs through skin. These enhancers augment the permeation of a drug by increasing its coefficient of diffusion (by disrupting the barrier) into the outermost layer of skin, the stratum corneum, or by improving the partitioning between the formulation and the stratum corneum (perhaps by altering the solvent nature of the skin membrane and subsequently improving access to the tissue). On the other hand, enhancers appear to act by increasing the solubility of the drug in the formulation.[24,25]

Midazolam is a highly lipophilic drug (log P = 2.68) and its molecular weight is 325.76 (362.23 as hydrochloride). Midazolam hydrochloride is a weak acid with a $pK_a$ value of 6.15, so the percentage of the ionised form is dependent on the pH. Its solubility is <0.1 mg/ml at neutral pH and this increases considerably in acidic media.[26] The parenteral solution of midazolam used clinically is buffered to an acidic pH approximately of 3. The oral and sublingual dose of midazolam is 0.5–0.75 mg/kg,[27] intranasal administration requires 0.1–0.35 mg/kg[4,28] and rectal dosage is 0.25–0.35 mg/kg.[29] Parenteral injection requires 0.04–0.15 mg/kg.[30,31]

Midazolam percutaneous absorption through rat skin has been described and the results demonstrate that pre-treatment of rat skin with terpenes and other chemical enhancers produces a significant increment of the transdermal permeation of midazolam with respect to controls.[32] Specifically, *R*-(+)-limonene has been reported to be the greatest enhancer among those evaluated.[32] Azone has also been assessed and has shown a moderate capacity for increasing the transdermal flux of midazolam through rat skin.[32]

The development of a transdermal therapeutic system for the administration of midazolam could improve the patient comfort in pre-operative sedation and induction of general anaesthesia, particularly in children. Considering that transdermal absorption of midazolam has not been characterized across human skin and previous authors report its capability of diffusion across animal skin model,[32] this work reports the characterization of the in-vitro transdermal absorption of midazolam hydrochloride through human skin and assesses the effect of iontophoresis and chemical percutaneous enhancers (Azone and *R*-(+)-limonene), applied individually and together, to determine whether a synergistic effect is achieved when both enhancement techniques are simultaneously employed.

## Materials and Methods

### Materials

Midazolam hydrochloride (Figure 1; 8-chloro- 6-(2-fluorophenyl)- 1-methyl-4*H*-imidazo[1,5-a] [1,4]benzodiazepine hydrochloride) was obtained from a solution for parenteral administration (Midazolam Rovi, 1 mg/ml; Laboratorios Farmaceuticos Rovi S.A., Spain). The excipients of this pharmaceutical form are sulfuric acid, sodium hydroxide and water. Azone (1-dodecyl-azacycloheptan-2-one) was obtained from Netqem (Durham, USA) and *R*-(+)-limonene was purchased from Fluka Chemie (Buchs, Switzerland). HEPES (*N*-[2-hydroxyethyl]piperazine-*N*-[2-ethanesulfonic acid]) was obtained from Sigma-Aldrich Co. (St Louis, USA). NaCl, NaOH, HCl and ethanol (absolute) were purchased from Mallinckrodt Baker B.V. (Deventer, Holland). All the compounds were of analytical grade. Ultrapure water used to prepare the solutions was obtained using a Barnstead NANOpure system (Barnstead International, Boston, USA).

Silver chloride (99%) and 1-mm silver and platinum wire (both 99.9%), used for the manufacture of the Ag/AgCl electrodes employed in the iontophoresis assays, were purchased from Sigma-Aldrich Co. (St Louis, USA).

### Permeation experiments

Permeation experiments were performed on Caucasian abdominal skin samples obtained from three randomly assigned female donors, aged 38–48 years, who had undergone cosmetic surgical procedures. Informed consent was previously obtained from the patients and their identity was masked to the researchers to guarantee their anonymity.

After its extraction, human skin tissue was maintained at 4°C within 2 h until it was stored at −80°C. One hour before use, skin samples were prepared to a thickness of 500 $\mu$m using an Aesculap-Wagner dermatome C. GA 176 (B. Braun Surgical S.A., Barcelona, Spain).

Transdermal experiments using midazolam hydrochloride were performed at room temperature employing standard iontophoresis diffusion cells. The cells employed were a modified Franz type, flat flange joint cells (SCSIE, Servicios Centrales de Soporte a la Investigación, Universitat de València, Valencia, Spain). These cells have an upper donor compartment divided into two 2-ml compartments separated by a glass wall and a 7.75-ml lower receptor compartment. Skin was placed horizontally between donor and receptor compartments with the stratum corneum facing the donor compartment. The diffusion area was 0.9 $cm^2$.

Several donor and receptor solutions, adjusted to different pH values, were employed. The donor anodal solution consisted of 1.2 ml of 1 mg/ml midazolam hydrochloride adjusted to pH 5.5 or pH 5 with NaOH (1 M) and HCl (1 M). The donor cathodal compartment was filled with 1.2 ml of saline (NaCl 150 mM–HEPES 20 mM), also buffered to pH 5.5 or pH 5 with HCl (1 M). The receptor compartment was filled with saline buffer at pH 7.4 or pH 5. Passive diffusion and iontophoresis (0.5 $mA/cm^2$) experiments were carried out for all these conditions.

Subsequent experiments were performed employing different solutions at pH 5 in all donor and receptor compartments. In this case, human skin was pre-treated for 12 h with different solutions. Controls were pre-treated with 600 $\mu$l of saline buffered solution at pH 5 (NaCl 150 mM–HEPES 20 mM). The vehicle used, ethanol, was also assayed by applying 600 $\mu$l of ethanol. Finally, 600 $\mu$l of the solutions tested (5% concentration (w/w) of the chemical enhancer tested in ethanol) were applied onto human skin. After 12 h, the pre-treatment solution was replaced by 1.2 ml of 1 mg/ml midazolam hydrochloride solution (adjusted to pH 5 with NaOH 1 M), placed in one of the donor compartments. The remaining two compartments (donor and receptor) were filled with the same saline buffer (NaCl 150 mM–HEPES 20 mM) at pH 5.

Finally, transdermal diffusion experiments were carried out to investigate the effect of the combination of chemical enhancers and iontophoresis. In these experiments, skin pre-treatment was performed as previously described with Azone or *R*-(+)-limonene. Following this, and using the pH 5 solutions employed in the iontophoresis experiments described above, a constant current density was applied (0.50 $mA/cm^2$).

In all cases, simultaneously ,passive diffusion experiments were performed as controls.

The sampling protocol was identical in all experiments. One-millilitre samples were withdrawn manually from the receptor chamber hourly over 8 h. The volume of sample removed was replaced with the same volume of buffer. The amount of drug contained in each sample was quantified to determine the cumulative amount of midazolam hydrochloride in the receptor compartment at each time point. Midazolam hydrochloride was quantified in all the samples collected by means of an HPLC method.

### HPLC analysis

The apparatus used for HPLC analysis was a Waters system equipped with a quaternary pump and a diode-array detector (Waters 996 Photodiode Array Detector, Barcelona, Spain). Computerized data acquisition and treatment were performed with Millenium Chromatography Software. Separation was carried out at ambient temperature on a 250 × 4.0 mm, reverse-phase column packed with 5 $\mu$m C18 silica particles (Kromasil $C_{18}$) (Análisis Vínicos, Tomelloso, Spain). The mobile phase was an ammonium chloride–methanol–acetonitrile (40 : 20 : 40, v/v/v) mixture. The pH of the mobile phase was adjusted to pH 5.5 with HCl (0.05 M). The mobile phase was filtered through a 0.45-$\mu$m pore-size nylon membrane. Absorbance was measured at 254 nm. The flow rate of the mobile phase was maintained at 1 ml/min. Samples of 50 $\mu$l were injected.

Validation of the analytical method was carried out. Calibration curves were obtained by the least-square linear regression analysis of the response obtained as a function of the concentration of midazolam hydrochloride. The specificity of the method was investigated by analysing ten blank samples. The linearity of the curves was tested by statistical comparison among the slopes and the intercepts of calibration curves with zero and the correlation coefficients with 1. Accuracy of the method was defined as the relative error from the assay of known concentration solutions. To be acceptable, measures should be within ± 10% for all concentrations.[33,34] The precision of the method was tested as within-day and between-day reproducibility of the assay and was expressed as the residual standard deviation (RSD) of replicate measurements.

To be acceptable, the RSD should be lower than 10% at all concentrations analysed.[33,34] The limit of detection (LOD) was determined as the sample concentration resulting in a peak area of three-times the noise level. LOD was determined by the analysis of the peak baseline noise in ten blank samples.[34,35] The limit of quantification (LOQ) was defined as the lowest concentration, which can be determined with an accuracy and precision below 20%.[33,34]

### Data analysis

By means of HPLC analysis, midazolam in samples was determined and plots of the accumulated amount of midazolam ($\mu$g/cm$^2$) against time (h) were constructed. The transdermal steady-state flux (*Jss*) in all experimental conditions assayed (iontophoresis application and chemical percutaneous enhancers) was estimated from the slope of the linear region of the plot (steady-state portion).

Variation of the steady-state flux of midazolam as a function of the assayed condition was assessed using the one-way analysis of variance. Post-hoc multiple comparison tests were performed by means of the Scheffé test since data were homoscedastic.

Permeation-enhancing activity, expressed as the enhancement ratio of steady-state flux ($ER_{flux}$), was calculated as the ratio between the flux value obtained with the strategies applied (chemical compounds, iontophoresis or both) and that observed with the respective passive diffusion control:

$$ER_{flux} = Jss \text{ with enhancer} / Jss \text{ without enhancer} \quad (1)$$

where *Jss* is midazolam transdermal steady-state flux.

## Results and Discussion

The aim of this work, in addition to characterizing the transdermal permeation of midazolam through human skin, was to evaluate the effect of different enhancing strategies on said permeation. The enhancing strategies selected to be assayed were iontophoresis and two chemical percutaneous enhancers (Azone and *R*-(+)-limonene). Previous reports have demonstrated that iontophoresis is an efficient enhancement technique,[9,10,13,14,20] and that a synergistic effect can be achieved when it is combined with chemical enhancers.[22,23]

To perform the diffusion experiments needed to achieve our objectives, a specific and sensitive HPLC method to determine midazolam hydrochloride was developed and validated. This method enabled us to determine midazolam hydrochloride in samples obtained from in-vitro transdermal permeation experiments. Assay performance of the validated method was assessed by all the following criteria: specificity, linearity, precision, accuracy, LOD, LOQ and applicability to in-vitro transdermal diffusion studies. Ten blank samples were analysed to investigate the specificity of the method. There was no interference found at the retention time of midazolam hydrochloride. The method exhibited linearity between the response and the corresponding concentration of sumatriptan succinate over the 0.1–50 $\mu$g/ml range of concentrations assayed. The results of the least-square linear regression analysis showed a correlation coefficient $\geq 0.999$. The slope of the calibration curve was statistically different from zero, and the intercept was not statistically different from zero.

The results of accuracy and the between-day and within-day precision determined are showed in Table 1 and were within acceptable limits (<10%)[33,34] in all cases. The limit of detection (LOD) and the limit of quantification (LOQ) of midazolam were 0.012 $\mu$g/ml and 0.100 $\mu$g/ml, respectively.

The applicability and specificity of this method has been demonstrated by the study of in-vitro transdermal diffusion of midazolam hydrochloride across human skin.

It is known that iontophoresis is highly efficient in enhancing the permeation of charged molecules, especially cations, and that the efficiency of iontophoretic transport is highly dependent on the physico-chemical properties of the drug (molecular weight, etc.).[14–19] Midazolam hydrochloride is a weak acid with a $pK_a$ value of 6.15, so the percentage of the ionised form is dependent on the pH. To determine the pH for optimum midazolam iontophoretic transdermal absorption, iontophoresis was applied at a current density of 0.5 mA/cm$^2$ and iontophoretic transdermal transport through human skin was investigated at different pH conditions. Passive diffusion experiments (controls) were performed at the same pH values. The first conditions assayed consisted of donor anodal and cathodal solutions, both adjusted to pH 5.5 and a receptor solution adjusted to pH 7.4. In subsequent experimental conditions donor and receptor solutions were both adjusted to pH 5. The pH of the receiver was lowered to pH 5 to ensure the maintenance of sink conditions, since midazolam solubility is higher at pH 5 than at pH 7.4.

**Table 1** Accuracy and between- and within-day variability of the HPLC method for determining midazolam

| Real concn ($\mu$M) | Between-day variability ($n = 10$) | | Within-day variability ($n = 5$) | | Accuracy |
|---|---|---|---|---|---|
| | Concn found ($\mu$M) | RSD (%) | Concn found ($\mu$M) | RSD (%) | (%) |
| 0.1 | 0.103 | 6.197 | 0.106 | 9.793 | 5.973 |
| 0.5 | 0.539 | 8.266 | 0.526 | 8.330 | 4.856 |
| 1 | 1.028 | 9.128 | 1.041 | 9.844 | 3.962 |
| 5 | 5.032 | 5.889 | 4.910 | 5.617 | 1.842 |
| 10 | 10.657 | 9.646 | 10.400 | 9.145 | 3.843 |
| 50 | 51.009 | 3.907 | 50.551 | 1.664 | 1.089 |

RSD, residual standard deviation.

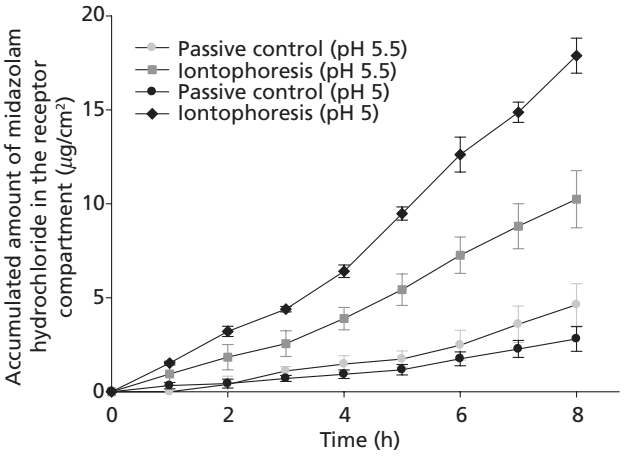


**Figure 2** Amount of midazolam hydrochloride accumulated in the receptor compartment ($\mu g/cm^2$) during the transdermal diffusion experiments across human skin. Error bars show standard deviation.

The permeation profiles obtained based on the amount of midazolam hydrochloride accumulated in the receptor compartment versus time are plotted in Figure 2. As can be observed, iontophoresis led to a major accumulation of the drug in the receptor compartment with respect to both passive diffusion controls (pH 5.5 and pH 5). When iontophoresis was applied under pH 5 conditions the amount of midazolam accumulated in the receptor compartment after 8 h doubled that reached at pH 5.5.

$ER_{flux}$ was calculated respective to the corresponding passive diffusion controls (pH 5.5 or pH 5) depending on the pH conditions of the experiment. Table 2 shows the steady-state flux of midazolam (*Jss*) and the enhancement ratio ($ER_{flux}$) calculated for all the conditions assayed.

Considering passive diffusion, as expected, the higher pH (pH 5.5) tended to produce a midazolam steady-state higher flux ($1.09 \pm 0.38$ $\mu g/cm^2$ h) than the lower pH condition (pH 5) ($0.50 \pm 0.16$ $\mu g/cm^2$ h). Nevertheless, although the lipid nature of the transepidermal pathway favours the permeation of drugs in their molecular stage the fluxes did not prove to be significantly different (analysis of variance; Scheffé, $P > 0.05$). The application of iontophoresis at pH 5.5 and pH 5 produced an increment of the drug flux with respect to the corresponding passive diffusion control. Iontophoresis at pH 5.5 doubled the midazolam flux when compared with that obtained with its passive diffusion control (pH 5.5) but data analysis revealed these differences not to be statistically significant (analysis of variance; Scheffé, $P > 0.05$). On the other hand, iontophoresis applied at a low pH (pH 5), exhibited a great capacity for increasing the transdermal flux of midazolam hydrochloride to a significant extent (analysis of variance; Scheffé, $P < 0.05$) with respect to the passive control and the high pH conditions (both passive and iontophoresis), leading to an enhancement ratio of 7.74.

The results obtained are highly consistent, since midazolam is a lipophilic drug with a $pK_a$ value of 6.15 and is 93% positively charged at pH 5. Iontophoresis is an enhancement technique that specially promotes the transdermal transport of positively charged molecules due to the negative charge of human skin, which functions as a perm-selective membrane to cations.[17] This explains why the iontophoretic transdermal transport of midazolam is more efficient when iontophoresis is applied at pH 5 rather than at pH 5.5, when only 80% of the drug is positively charged. A 13% increase in the percentage of ionization almost doubles the flux of the drug. In this sense, if the pH were lowered, a higher percentage of ionised drug (99%) may be obtained. However, to achieve such high percentages it is necessary to work within pH values that are close to the isoelectric point of human skin (~ 4.5), conditions in which the negative charge of the skin is neutralised and its perm-selective properties for cations disappear.

According to the results of the study of the effect of pH on the transdermal absorption of midazolam, and bearing in mind the arguments discussed above, we selected pH 5 to continue our investigation, since this condition was found to be optimum for the transdermal transport of midazolam hydrochloride under iontophoresis and did not interfere to a significant extent with passive diffusion. Two chemical compounds that are known to be effective percutaneous enhancers were selected and their effect on midazolam transdermal absorption at pH 5 was assessed. Subsequently, iontophoresis was applied in conjunction with these chemical percutaneous enhancers to determine whether a synergistic effect was produced.

As can be observed in Table 2, the vehicle used for pre-treatment with the chemical enhancers (ethanol) did not produce any significant effect on the transdermal flux of midazolam (analysis of variance; Scheffé, $P > 0.05$). Limonene produced a moderate (4.46 fold) but significant increment of the flux of the drug with respect to the control ($P < 0.05$). Limonene was expected to improve the transdermal absorption of midazolam to a major extent, since previous publications have reported its capacity for increasing the absorption of midazolam through rat skin.[32] Azone has proved to be a highly efficient chemical percutaneous enhancer of the diffusion of midazolam through human skin, producing a significantly greater increment of the drug transdermal flux ($6.89 \pm 0.56$ $\mu g/cm^2$ h) (analysis of variance; Scheffé, $P < 0.05$). Of the chemical compounds assayed, the most marked capacity for increasing the transdermal diffusion of midazolam was exhibited by Azone, which produced a midazolam transdermal flux 13.8-fold higher than that obtained with the passive diffusion control.

As shown in Table 2, the combination of either of the chemical percutaneous enhancers, Azone or limonene, with iontophoresis (pH 5) increased the transdermal flux of midazolam to a significant extent when compared with respect to passive diffusion control ($P < 0.05$). The combined application of limonene pre-treatment and iontophoresis does not represent any advantage over the application of limonene alone.

Furthermore, the combined application of Azone skin pre-treatment and iontophoresis produced the best midazolam transdermal flux enhancement, leading to a flux value of $8.94 \pm 1.23$ $\mu g/cm^2$ h ($ER_{flux}$ 17.9), significantly higher than the midazolam transdermal flux obtained with Azone and the rest of the conditions assayed ($P < 0.05$). Nevertheless, no synergistic effect was found when comparing these results

**Table 2** Transdermal steady-state flux (Jss) and enhancement ratio ($ER_{flux}$) of midazolam across human skin, for each condition assayed

| Condition | *n* | Donor/ Receptor pH | *Jss* ($\mu g/cm^2$ h) | $ER_{flux}$ |
|---|---|---|---|---|
| Control | 5 | 5.5/7.4 | 1.09 ± 0.38 | – |
| Iontophoresis | 5 | 5.5/7.4 | 2.23 ± 1.30 | – |
| Control | 5 | 5/5 | 0.50 ± 0.16 | – |
| Iontophoresis | 5 | 5/5 | 3.87 ± 0.87* | 7.74 ± 3.03 |
| Ethanol | 5 | 5/5 | 0.84 ± 0.27 | 1.68 ± 0.76 |
| Limonene | 5 | 5/5 | 2.23 ± 0.49* | 4.46 ± 1.73 |
| Azone | 5 | 5/5 | 6.89 ± 0.56* | 13.8 ± 4.55 |
| Limonene + Iontophoresis | 5 | 5/5 | 3.02 ± 0.73* | 6.04 ± 2.42 |
| Azone + Iontophoresis | 5 | 5/5 | 8.94 ± 1.23* | 17.9 ± 6.23 |

Data are expressed as means ± SD. Experimental conditions, pH of the donor and receptor compartments and number of experiments performed [*n*] are also shown. *$P < 0.05$ with respect to their corresponding pH control (analysis of variance).

with those obtained when each enhancement strategy was applied separately.

Taking into account midazolam plasma clearance (323 ml/min) and the maximum plasma level of the drug required (55.9 ng/ml),[31,35] the necessary entrance of drug is approximately 1080 $\mu$g/h. Considering the results obtained in this in-vitro study this value could be obtained with a transdermal formulation of approximately 11 × 11 cm that would combine the application of Azone and iontophoresis. Obviously, these results need to be verified *in vivo*, with a more realistic formulation.

## Conclusions

In conclusion, when applied individually, Azone exhibits the greatest enhancement effect on the transdermal diffusion of midazolam of the various strategies assayed. The combination of Azone and iontophoresis produced the highest steady-state flux of midazolam but no synergic effect was achieved when the two enhancement strategies were applied in combination, showing that although selecting the best conditions for iontophoresis application, it is less effective for augmenting the transdermal delivery of midazolam than the chemical enhancer Azone.

## Declarations

### Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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