Pharmacokinetic evaluation of a transdermal anastrozole-in-adhesive formulation

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Background and objective: Anastrozole is a well-established active pharmaceutical ingredient (API) used for the treatment of hormone-sensitive breast cancer (BC) in postmenopausal women. However, treatment with the only available oral formulation is often associated with concentration-dependent serious side effects such as hot flashes, fatigue, muscle and joint pain, nausea, diarrhea, headache, and others. In contrast, a sustained-release system for the local application of anastrozole should minimize these serious adverse drug reactions.

Methods: Anastrozole-in-adhesive transdermal drug delivery systems (TDDS) were developed offering efficient loading, avoidance of inhomogeneity or crystallization of the drug, the desired controlled release kinetics, storage stability, easy handling, mechanical stability, and sufficient stickiness on the skin. In vitro continuous anastrozole release profiles were studied in Franz diffusion cells. In vivo, consecutive drug plasma kinetics from the final anastrozole transdermal system was tested in beagle dogs. For drug analysis, a specific validated liquid chromatography–mass spectrometry method using fragment ion detection was developed and validated.

Results: After efficient drug loading, a linear and sustained 65% drug release from the TDDS over 48 h was obtained. In vivo data showed a favorable anastrozole plasma concentration–time course, avoiding side effect-associated peak concentrations as obtained after oral administration but matching therapeutic plasma levels up to 72 h.

Conclusion: These results provide the basis for establishing the transdermal application of anastrozole with improved pharmacokinetics and drug safety as novel therapeutic approach and promising option to treat human BC by decreasing the high burden of unwanted side effects.

Keywords: anastrozole, breast cancer, transdermal drug delivery system, pharmacokinetics, Franz diffusion cells

Introduction

Among noncommunicable diseases, breast cancer (BC) is the most important reproductive health problem and currently the leading mortality cause in women aged ≥15 years.1 Globally, BC has high incidence rates in all countries,2,3 with 1.7 million new cases per year or 89.7 per 100,000 in Western Europe.4 Although numerous therapy options such as surgery, radiotherapy, chemotherapy, endocrine therapies, and combination of these therapies are available for patients with primary BC, some of these are associated with severe side effects and often cannot prevent cancer-related death due to distance organ metastasis.

A number of targeted drugs have been developed and approved in the last years, including the aromatase inhibitors, which are widely prescribed for the adjuvant treatment of hormone-sensitive advanced metastatic BC in postmenopausal women.5 In comparison to tamoxifen, aromatase inhibitors have shown a significant benefit in reducing BC-related and overall mortalities.6,7 These drugs inhibit the synthesis
of estrogen by reversibly binding and blocking the enzyme aromatase, a key regulator of the metabolic conversion of androgens into estrogens in peripheral tissues.5–10

Anastrozole is the third-generation nonsteroidal aromatase inhibitor approved for the treatment of BC in a variety of both early and advanced disease settings. At present, only an oral immediate release formulation of anastrozole is available on the market. Although the oral preparation of anastrozole provides good therapeutic responses with maximum plasma concentrations within 2 h and long duration of systemic action with its mean elimination half-life ranging from 40 to 50 h, it is often associated with severe systemic adverse events including hot flashes, bone fractures, osteoporosis, arthralgia, myalgia, vaginal dryness, hair loss, skin rash, nausea, emesis, diarrhea, and headache.11,12 Accordingly, patients suffering from nausea and vomiting are not eligible for oral drug delivery. Therefore, transdermal drug delivery systems (TDDS) may be a promising alternative with regard to avoiding side effects due to several advantages: a more even plasma concentration profile of the drug, better patient compliance and therapeutic index, sustained local drug release, and the possibility of quick drug withdrawal, if adverse reactions are observed. This has been shown for a number of drug formulations such as buprenorphine, clonidine, estradiol, fentanyl, ganirelix, lidocaine, methylphenidate, nicotine, nitroglycerine oxybutynin, rivastigmine, rotigotine, scopolamine, selegeline, and testosterone.13,14 Even though drugs with a long half-life seem to be inherently controlled, even in the case of anastrozole, a controlled release system brings major advantages: 1) the sustained release leads to a desired further prolongation of the duration of drug action, 2) the drug levels achieved during the sustained release process are sufficient to ensure therapeutic activity, 3) undesired plasma concentration peaks are avoided, and 4) an easy-to-use system could potentially improve patients’ compliance. Oral therapy with aromatase inhibitors such as anastrozole leads to rather uncontrolled delivery with early high peak levels, more side effects, and poorer compliance. Common side effects such as hot flashes, nausea, and bone damage, related to the depletion of circulating estrogen by aromatase inhibitor treatment in postmenopausal BC patients,15,16 are expected to be decreased with our system. Low molecular weight, high n-octanol/water partition coefficient, and low melting point are ideal physicochemical characteristics to ensure efficient drug penetration through the stratum corneum and should therefore be transferable successfully to anastrozole containing TDDS as well. TDDS are capable of noninvasively and painlessly transporting drugs through the skin into the blood circulation at a fixed rate. Several important advantages of transdermal drug delivery include the avoidance of unwanted plasma peak levels, the prevention of hepatic first pass metabolism, the enhancement of therapeutic efficiency, and the maintenance of steady state plasma levels of the drug.13,17,18 A single layer patch is a widely used design in TDDS, since it is stable and easy to apply, and it is particularly useful for narrow therapeutic index drugs. However, to the best of our knowledge, there has been no research on exploring TDDS for the transdermal delivery of anastrozole.

In the present study, we developed an optimized TDDS for anastrozole delivery. In vitro permeation experiments using Franz diffusion cells were first performed to evaluate the effect of adhesive types, drug loading, and permeation enhancers on anastrozole transport. A long-acting anastrozole-in-adhesive patch of pressure-sensitive adhesives (PSA) was developed, and the in vivo pharmacokinetics of this optimized patch was evaluated in beagle dogs. Plasma anastrozole concentrations were determined by an optimized, sensitive, and accurate liquid chromatography–mass spectrometry (LC–MS) method.

Materials and methods

Materials

Anastrozole (99.8%) and methyl tert-buty ether Chromasolv® (MTBE; ≥99.8%) were obtained from Sigma-Aldrich Co. (St Louis, MO, USA); d12-anastrozole (internal standard [IS]) was purchased from Santa Cruz Biotechnology Inc. (Dallas, TX, USA). All solvents and crystallization inhibitors used for the development of TDDS were at least of analytical grade and obtained from Th. Geyer (Berlin, Germany). 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer was obtained from Thermo Fisher Scientific (Waltham, MA, USA), and silicon matrix BIO-PSA® AC 7-4302 was obtained from Dow Corning (Wiesbaden, Germany). Backing film CoTran™ 9720 was purchased from 3M Company (St Paul, MS, USA). HPLC solvents acetonitrile (HPLC gradient grade) and distilled water (HPLC grade) were obtained from Promochem (Wesel, Germany), formic acid (98%–100%) was obtained from EMD Millipore (Billerica, MA, USA), and ortho-phosphoric acid (85%) was obtained from Th. Geyer.

The 11.28 mm clear glass Franz diffusion cells19 with flat ground and 8 mL of receptor volume were obtained from SES GmbH Analysensysteme (Bechenheim, Germany). Hairless excised mouse skins were obtained from Taconic Biosciences (Cologne, Germany). For visual evaluation of the anastrozole homogeneity in the silicon matrix, a binocular Science MTL 201 (50–800x) polarization microscope (Bresser GmbH,
Rhede, Germany) with a cold light source type IL-FOI-L24 and several color filters was used.

**Preparation of transdermal adhesive patches**

In a first attempt, the direct incorporation of the active pharmaceutical ingredient (API) anastrozole into a silicon matrix BIO-PSA® type 7-4302 was traced but had to discontinue because of disappointing inhomogeneity of the wet mix. Within a second approach, diverse organic solvents were tested to incorporate the API into the PSA matrix in a dissolved form while ensuring compatibility with the adhesive and likewise considering future safety and tolerability aspects. This more promising procedure favoring two eligible solvents provided homogeneity, but this was lost during the drying process; hence modifiers as crystallization inhibitors had to be tested further.

All steps of the pharmaceutical build up from the definition of components up to the final TDDS formulation were performed in a good manufacturing practice-certified pharmaceutical laboratory (Formula GmbH, Berlin, Germany). The principal scheme of matrix preparation procedures and drug loading are shown in Figure 1.

Optimized process parameters were the solvation of 20–30 mg of anastrozole in 10 µL of ethyl acetate (EA), the use of silicon matrix BIO-PSA® type 7-4302, the addition of glycerol as crystallization inhibitor equimolar to anastrozole, the agitation of wet mix for 4 h, the layer film of 120 µm, drying process at room temperature for 24 h, and backing with CoTran™ 9720.

The adhesive layer was examined immediately after drying process visually for completeness and surface structure. The thickness of the prepared TDDS was measured with a micrometer and was calculated by subtracting the combined thickness of the backing membrane and release liner from the thickness of the whole TDDS. The thickness of all TDDS used was 120±10 µm. No anastrozole crystals were found in any formulation by microscopic observation. Content uniformity was studied in 10 samples. The mean recovery of anastrozole from silicon matrix was 105.2% (range 101.8%–112.9%; coefficient of variation 3.14%).

**Backing films**

Backing films, such as CoTran™ 9720 as a good adhesive matt and transparent film, Scotchpak 9737 (3M Drug Delivery Systems, St. Paul, MN, USA) as another transparent film, and Scotchpak 9723 as a shiny skin-colored film, were tested for their potency of stickiness in six human volunteers aged between 25 and 58 years (two females and four males), who

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**Figure 1** Scheme of steps of the pharmaceutical TDDS build up.

**Abbreviations:** API, active pharmaceutical ingredient; TDDS, transdermal drug delivery systems.
gave written informed consent. Moreover, stickiness of oval final placebo-TDDS, placed on internal side of the upper arm, was found to be sufficient in 120 µm silicon matrix over up to a maximum of 10 days and CoTran™ 9720 was identified as best backing film with only minimal dermal irritations.

Measurement of drug content and release in patch
For the pharmaceutical analysis of anastrozole concentration in HEPES buffer (acceptor medium) during Franz diffusion cell experiments as well as for the verification of anastrozole content uniformity in the TDDS, a validated HPLC method with UV detection at 205 nm was used. In brief, technical hardware was a Dionex chromatographic system (HPG-3200SD HPLC pump, ASI-100 autosampler and PDA-100UV detector connected to a TCC-100 column oven and Chromeleon Software; Dionex, Idstein, Germany). Chromatographic separation was achieved using a SunFire C18 Reversed-Phase Column (5 µm, 4.6×250 mm ID; Waters, Eschborn, Germany) at a flow rate of 1.0 mL/min and a temperature of 20°C, using a linear gradient elution program from 10% acetonitrile, 90% water, and 0.1% phosphoric acid (v/v/v) to 90% acetonitrile, 10% water, and 0.1% phosphoric acid (v/v/v) at the end of the analytical run. Validation parameters of the external standard method were linearity of calibration function with R=0.995, overall RSD lower than 7.5%, and mean accuracy 102.4%. No additional peaks were detected. When the TDDS were tested in Franz diffusion cells, cumulative permeation of anastrozole from the TDDS into the HEPES acceptor medium was measured by HPLC compared with API permeation from saturated anastrozole solution.

Temperature and stability analyses on patch preparation
The storage stability of anastrozole-containing TDDS in various concentrations was analyzed. To this end, the patches of 60, 90, and 120 µm heights were produced containing 1, 2.5, or 5% anastrozole, prior to drying either at 40°C for 4 h or at room temperature for 24 h. After fixing the backings, the TDDS were assessed upon storage over 24 h up to 1 month at room temperature with or without exposure to light. Subsequent polarization microscopy analyses of the anastrozole-TDDS upon longer-term storage were prepared to validate the homogeneity of the drug in matrix.

In vitro skin permeation study
Skin permeation experiments of anastrozole, directly applied as a freshly prepared saturated aqueous solution or released from the TDDS, were performed using the hairless mouse skin. Before application, the tailored mouse skin was equilibrated in isotonic saline solution for at least 30 min at room temperature. During equilibration time, the Franz diffusion cells were assembled, filled with 8 mL of freshly prepared HEPES buffer, and preheated to 32°C. One hundred fifty microliters of aqueous anastrozole solution, directly applied onto the mouse skin, served as positive control to confirm the ability of the anastrozole to permeate through the skin. Sample collection was performed at predefined time points. Five hundred microliters of sample was taken and replaced by fresh medium. Each sample was analyzed by HPLC. At the end of the permeation experiment, the mouse skins as well as the patches were analyzed for anastrozole residues by extraction with tetrahydrofuran (THF)–water solution (4:1).

In vivo study
Animals
Male healthy beagle dogs weighing 13–14 kg and aged about 8 years were involved in pharmacokinetic studies in vivo. The animals were fed once a day and had free access to water before dosing. Animal studies were performed in accordance with the ethical guidelines for investigation in laboratory animals and in accordance with the guidelines of the German law regarding animal welfare and were approved by the local authorities (Landesdirektion Sachsen).

TDDS application and blood sampling
In vivo studies were carried out in two male healthy beagle dogs. Patches were affixed on the skin of the thoracic-abdominal region. Application areas were shaved and disinfected, and drug patches were affixed and protected from self-manipulation by a textile cover and a neck collar. The 2.8% anastrozole TDDS (10×4 cm², wiper height 120 µm) containing glycerol as crystallization inhibitor was administered, and the animals were monitored for tolerance and overall well-being for 72 h. At predefined time points, ie, 0, 2, 8, 12, 24, 48, and 72 h after TDDS application, 4 mL of whole blood samples was taken by venous puncture. Samples were centrifuged at 1,500×g for 5 min, and plasma was stored at −70°C until analysis.

Plasma sample preparation
Plasma samples were extracted by liquid/liquid extraction. Aliquots of 1 mL plasma were added each in a 4.5 mL polypropylene tube. Thereafter, 50 µL of IS solution (1 µg/mL) was added to each sample, vortexed (30 s), subsequently mixed with 2 mL of MTBE, and placed on a longitudinal shaker for 5 min. After centrifugation (3,000×g, 4 min),
the upper organic phase was collected and the lower phase was re-extracted with 2 mL of MTBE. Combined organic phases were evaporated to dryness at 55°C under a gentle stream of nitrogen. The obtained residue was reconstituted in 200 µL of mobile phase and transferred to an autosampler vial. Twenty-five microliters were injected into the LC–MS system for analysis.

**LC–MS analysis**

For the determination of the expected low anastrozole concentrations in complex biological samples such as plasma, a specific optimized LC–MS method was developed and validated.

HPLC analysis was performed on a Spectra P4000 system (TSP, San Jose, CA, USA) with an AS3000 autosampler and an SCM1000 degasser. The chromatographic separation of anastrozole and deuterated anastrozole (IS) was achieved on an Atlantis RP C18 column (150x2.1 mm, 3 µm; Waters Corp., Milford, MA, USA) fitted with a precolumn (10x2.1 mm) and a PEEK precolumn filter under isocratic conditions. Acetonitrile/0.1% formic acid (80:20, v/v) was used as mobile phase at a flow rate of 0.2 mL/min, and the column temperature was maintained at room temperature (22°C±2°C).

Single-quadrupole mass spectrometric detection was performed on an SSQ 7000 mass spectrometer (Finnigan MAT, San Jose, CA, USA) equipped with electrospray ionization (ESI) technique. Quantification was performed using the selected ion monitoring (SIM) mode. Fragment ions from anastrozole and the IS generated by collision-induced dissociation (CID) exhibited favorable sensitivity in positive ion detection mode and were therefore used for quantification. Optimized parameters were as follows: capillary voltage, 4.5 kV; sheath/auxiliary gas: nitrogen; sheath gas pressure: 40 psi; auxiliary gas pressure: 10 psi; and ion transfer capillary temperature: 220°C. All data were acquired and processed using the Xcalibur 1.3 software and SSQ Tune program Version 1.1 (Thermo Fisher Scientific).

**Data and statistical analyses**

Standard descriptive statistics using the Sigmaplot 13.0 (Systat Software Inc., San Jose, CA, USA) was performed. Drug concentration data are given as numeric mean and SD or percentage values if not otherwise indicated.

**Results and discussion**

**Effect of solvents on patch preparation**

In the development of optimized TDDS for anastrozole, the following critical points had to be assessed: 1) chemical and physical stabilities of anastrozole incorporated into the polymer matrix with or without additives and 2) the ability of the API incorporated into the matrix to diffuse out the polymer and penetrate the stratum corneum with or without additives.

Because of its simple setup, initially, a monolithic drug-in-adhesive system composed of a polymer matrix with direct incorporation of drug substance and, if necessary, of additives and a protective layer (backing) was favored. For long-term storage, this system was covered by a peel off film (release liner). Considering the requirement of a low-irritating and inert system, the well-established silicon matrix BIO-PSA® type 7-4302 was selected. In the first approach, we directly incorporated anastrozole into the polymer matrix, prior to analyzing homogeneity under a Bresser incident light polarization microscope. Upon direct incorporation of anastrozole into the polymer matrix, however, powder aggregates of different sizes were observed rather than a fine suspension (Figure 2A). Since this would preclude an acceptable drug delivery profile, the addition of organic solvents to the anastrozole solution was necessary. More specifically, defined amounts (20–30 mg) of anastrozole were dissolved in 10 µL of eight different solvents (EA, dimethyl sulfoxide [DMSO], dioxane, THF, ethanol [EtOH], xylene, chloroform, and dichloromethane). The selection of suitable solvents for the TDDS preparation was based on the solubility of anastrozole in the solvents, their compatibility with the adhesive, the absence of skin irritating effects and toxicity, and the boiling points of the solvents.

Crystallization of the API in the silicon matrix after drying was absent only in the case of EtOH (Figure 2B) or DMSO (refer compilation in Figure 2, lower right panel) but persistently observed when using EA, dioxane, THF, or xylene (Figure 2C). The patches showed less crystallized API when dried at room temperature as compared to higher temperature (40°C; data not shown). Based on these data, EtOH was initially selected for further studies. Subsequent polarization microscopy analyses of the EtOH-based anastrozole-TDDS upon longer-term storage, however, revealed sporadic crystal cluster formation across the whole liner area. More specifically, after fixing the backings, the TDDS were assessed upon storage over 24 h up to 1 month at room temperature (Figure 3B) with or without exposure to light. Widespread crystallization was observed, correlating with the applied amount of anastrozole on the release liner, rendering this solvent system as inappropriate.

Thus, another solvent was chosen in order to avoid unwanted crystal formation over time and the production process was modified by adding crystallization inhibitors.
Figure 2 Effects of incorporation of anastrozole without and with prior solvation into the TDDS polymer matrix.

Notes: Microscopic pictures of anastrozole aggregate formation in TDDS without solvent (A). Absence of crystallization (B) when using EtOH as solvent (the faint structures seen are air bubbles, and green color is filter) but crystal formation upon drying of the TDDS loaded with anastrozole dissolved in EA (C). Table: lower right: compilation of results, demonstrating the absence of crystal formation after drying only when using EtOH or DMSO as solvent. Microscopic pictures from freshly prepared TDDS (A, B) and after drying process (C) before considering crystallization inhibitors.

Abbreviations: DMSO, dimethyl sulfoxide; EA, ethyl acetate; EtOH, ethanol; TDDS, transdermal drug delivery systems; THF, tetrahydrofuran.

| Solvent | Crystallization |
|---------|-----------------|
| EA      | ✓               |
| DMSO    | –               |
| Dioxane | ✓               |
| THF     | ✓               |
| EtOH    | –               |
| Xylene  | ✓               |

Figure 3 Cumulative permeation of anastrozole through the hairless excised mouse skin to HEPES acceptor medium (pH 7.3) in Franz diffusion cells within 48 h.

Notes: (A) Mean value curve (bold) and single experiments (n=4) from freshly manufactured silicone-based, ethanol-solubilized anastrozole TDDS. (B) Microscopic picture of anastrozole crystal clusters formed in these TDDS after drying and storage at room temperature for 24 h up to 1 month. Scale bar, 1.11 µm.

Abbreviation: TDDS, transdermal drug delivery systems.
Poly-ethylene glycol (PEG) 400 in concentration 1 or 2.5% and, based on previous studies, glycerol were explored at ratio equimolar to anastrozole. EA was considered as the most suitable solvent due to its favorable evaporation features, good API solubility, and compatibility with the adhesive that is already dissolved itself in EA. Alternatively, EtOH was tested as well due to its low toxicity and intrinsic enhancer properties. More specifically, anastrozole and the respective crystallization inhibitor (glycerol and PEG 400) were weighed and each diluted in a few microliters of the selected organic solvent (EA and EtOH). Only then, the silicon matrix was weighed and added to this stock solution. The mixture was then applied to the release liner in the desired layer heights of 60, 90, and 120 µm as described earlier. The TDDS were dried at room temperature (~22°C) for 24 and 48 h and studied with regard to crystal formation. Absence of crystal formation was, indeed, observed in all of the above combinations, lasting over 6 months of storage at room temperature (data not shown). However, subsequent tests revealed poor TDDS stickiness on the skin when using EtOH. Since this problem was not observed in the case of EA, this solvent was used for further experiments.

**Drug release and content study**

When initially the EtOH-based TDDS were tested in Franz diffusion cells under similar conditions vs saturated aqueous anastrozole solution, a cumulative permeation of anastrozole from the TDDS into the HEPES acceptor medium was found (Figure 3A) reaching 91.5 and 83.0% compared to the saturated anastrozole solution at 24 and 48 h, which indicated a rather fast anastrozole release from the TDDS. After 48 h, the upper plateau was almost reached and was found to be strongly dependent on the initial anastrozole dosage loaded (data not shown). Adversely, EtOH-based TDDS were found to stick only poorly to the skin and were therefore discontinued; hence, no permeation data were obtained.

**In vitro skin permeation study**

The release profile of EA-based anastrozole TDDS was examined by monitoring the drug permeation from 1% to 2.5% anastrozole patches, containing equimolar ratios of either PEG 400 (Figure 4A) or glycerol (Figure 4B) and sticking on the hairless excised mouse skin in Franz diffusion cells. While in the case of the 1% anastrozole loading, no major differences were observed between PEG 400 and glycerol up to 6 h (Table 1), the 2.5% glycerol containing anastrozole-TDDS showed significantly higher anastrozole permeation from 24 to 48 h (compare open circles in Figure 4A and B). Already after 24 h, a maximum flux of about 50 µg/cm² was determined, with still no steady state being reached (Figure 4B).

Using this EA/glycerol formulation, the distribution of anastrozole between the TDDS and the mouse skin was determined after 48 h. HPLC measurements of the API after exhaustive extraction of the TDDS and mouse skin with a THF–water mix revealed that ~1.7 µg (3%) and ~24.3 µg (30%) of the total 72.73 µg anastrozole were still found in the TDDS and mouse skin, respectively. Thus, about 65% of the anastrozole from the TDDS had permeated through the mouse skin. In vitro permeation profiles of the different TDDS formulations are given in Table 2. The kinetic evaluation of the in vitro permeation profiles was performed using

![Figure 4](image-url)
Higuchi, zero-order, and Korsmeyer–Peppas kinetic models. Higuchi kinetic model exhibited linear relationship between drug release vs square root of time showing the best fit with the coefficient of regression of 0.979 and 0.983 for equimolar glycerol containing 1 and 2.5% anastrozole films, respectively (Figure 5), thus supporting the sustained release from final transdermal films.

Optimized LC–MS method for anastrozole detection in biological samples

Using an improved LC–MS method, the best signal-to-noise ratio was seen when analyzing the fragment ions of anastrozole and the deuterated d12-anastrozole at m/z 225 and m/z 237 rather than the whole molecule ions (m/z 294 and 306). Due to the fragmentation pattern, the deuteration remains in the measured ion.21 The major benefit of this approach was improved robustness by increased specificity and sensitivity. Figure 6A shows total ion current and mass traces of detected fragment ions of anastrozole at m/z 225 and m/z 237 and deuterated anastrozole (IS). This figure depicts a blank plasma sample (upper panel) and a blank plasma sample spiked with 5 ng/mL of anastrozole (lower panel). Figure 6B presents the optimized maximum response of fragmentation at mass trace m/z 225 for anastrozole in relation to the CID voltage.

The method was fully validated according to the European Medicines Agency guideline on bioanalytical method validation22 and proved to be selective, linear, accurate, and precise. For the preparation of the calibration curve and the calculation of plasma concentrations, least-squares linear regression analysis with weighting factor (1/concentration) obtained by the IS method using peak height was used. In each analytical batch (daily), a calibration curve as well as quality control standards at low, medium, and high concentration levels was analyzed. A typical calibration curve (calibration range: 2.0–200 ng/mL) function was $y = 0.0103 + 0.0147 \times C$ ($r^2 = 0.9990$) (with $C$= concentration and $y$= relative abundance). The lower limit of quantification (LLOQ; mean $\pm 3\times SD$) and the limit of detection (mean $\pm 6\times SD$) were calculated

### Table 1

| Time (h) | 1% anastrozole PEG 400 TDDS 0.79 cm² (µg/8 mL) | 2.5% anastrozole PEG 400 TDDS 0.79 cm² (µg/8 mL) |
|----------|-----------------------------------------------|-----------------------------------------------|
|          | Cell 1 | Cell 2 | Average | Cell 1 | Cell 2 | Average |
| (a)      |        |        |         |        |        |         |
| 2        | 0.54   | 1.40   | 0.97    | 2.02   | 5.82   | 3.92    |
| 4        | 1.92   | 3.27   | 2.60    | 16.62  | 12.40  | 14.54   |
| 6        | 3.15   | 4.44   | 3.80    | 19.58  | 16.20  | 17.89   |
| 24       | 12.91  | 7.61   | 10.26   | 32.96  | 27.33  | 30.15   |
| 30       | 14.70  | 7.49   | 11.10   | 32.96  | 26.58  | 29.77   |
| 48       | 19.48  | 8.29   | 13.89   | 32.75  | 27.42  | 30.09   |
| (b)      |        |        |         |        |        |         |
| 2        | 0.95   | 0.75   | 0.85    | 3.85   | 1.62   | 2.74    |
| 4        | 2.13   | 1.92   | 2.03    | 19.14  | 5.57   | 12.36   |
| 6        | 3.24   | 2.94   | 3.09    | 26.43  | 9.72   | 18.08   |
| 24       | 12.46  | 14.68  | 13.57   | 58.73  | 39.90  | 49.32   |
| 30       | 14.38  | 16.20  | 15.29   | 59.65  | 43.34  | 51.50   |
| 48       | 17.62  | 19.54  | 18.59   | 62.28  | 56.51  | 59.40   |

**Abbreviations:** PEG, poly-ethylene glycol; TDDS, transdermal drug delivery systems.

### Table 2

| Formulation | Crystallization inhibitor (w/w) | Anastrozole content (%) | Jss (µg/cm²/h) | Lag time (h) | Cp (cm²/h) |
|-------------|---------------------------------|-------------------------|----------------|--------------|------------|
| AC 7-4302   | 1% PEG                           | I                       | 0.30           | 4.20         | 1.15x10⁻⁷  |
| AC 7-4302   | 2.5% PEG                          | 2.5                     | 0.60           | 14.08        | 3.42x10⁻⁸  |
| AC 7-4302   | 1% glycerol                       | I                       | 0.42           | 1.81         | 2.66x10⁻⁷  |
| AC 7-4302   | 2.5% glycerol                     | 2.5                     | 1.31           | 4.80         | 1.00x10⁻⁷  |

**Abbreviations:** PSA, pressure-sensitive adhesives; Cp, coefficient of permeability; Jss, J (flux in steady state); PEG, poly-ethylene glycol; TDDS, transdermal drug delivery systems.
to be 1.4 and 0.5 ng/mL, respectively. No carry over effect was observed even at the highest 75 ng/mL of anastrozole concentration used for quality control. Recovery rates were measured at low (2.0 ng/mL), medium (10.0 ng/mL), and high (75.0 ng/mL) anastrozole concentrations and calculated at 61.6 ± 3.4, 67.0 ± 8.2, and 52.5 ± 1.6%, respectively. Matrix effects observed were found to be below 5%. The inter-day accuracy was between 89.1 and 109.2%, with a precision of 3.8%–4.3%. Precision data obtained from the results of the intra- and inter-day assays are given in Table 3. All relative SD (RSD) were lower than 10%, thus verifying the precision of the method.

Effect of final TDDS on time-dependent anastrozole plasma levels in beagle dogs

Based on the results from in vitro mouse skin permeation experiments, an optimized 2.8% anastrozole, BIO-PSA® AC 7-4302-based TDDS containing glycerol, was used to study pharmacokinetic profiles in a relevant animal in vivo setting. The data of anastrozole plasma concentrations after the exposure of two dogs to 2.8% anastrozole-loaded TDDS are shown in Figure 7A. As expected, lower initial transfer rates of anastrozole from the experimental patches to the systemic circulation were observed when compared with oral administration in humans (Figure 7B).23,24 After 24 h, the mean anastrozole concentration in dog plasma decreased only slowly to 4.2 and 3.4 ng/mL at 48 and 72 h, respectively. The calculated data on the estimated partial area under the curve (AUC0–72h) and half-life of anastrozole from the TDDS are given in Table 4. While the AUC was found to be comparable with results upon oral administration,24 an almost doubled anastrozole half-life was achieved after patch application.

With the aim of improving the risk–benefit relation of anastrozole in adjuvant treatment of BC in postmenopausal women, we successfully developed and optimized an anastrozole drug-in-adhesive TDDS as ready-to-use formulation. The pharmaceutical development process required several issues to be addressed among others, such as drug inhomogeneity/crystallization, storage stability, favorable release properties, and TDDS stickiness.

Although anastrozole has already been introduced in 1996 for the treatment of hormone-sensitive BC in postmenopausal women and represents the leading aromatase inhibitor, no anastrozole drug formulations other than film tablets for oral administration have been developed so far. While at first glance, this could be indicative of an overall favorable profile of side effects, the opposite is true, including thrombo-embolic events as severe side effects according to a categorization in good clinical practice. Moreover, non-serious side effects impairing the patient’s quality of life are underestimated, although the frequency of unfavorable side effects such as anorexia, cephalgia, nausea,
arthritis, asthenia, and others are categorized as common in the summary of product characteristics. Crew et al reported a 45%–60% prevalence of arthritis, leading to the early termination of this medication in 20% of all aromatase inhibitor-treated women.

Thus, the aim of this work is more than rational. Following TDDS application, a favorable profile of therapeutically relevant systemic drug concentrations over time was shown in beagle dogs. This animal model has been extensively used for in vivo studies of systemic effects after local application of benzoic acid, hydrocortisone, and testosterone and found to be even better suited than the often favored model of porcine skin. According to Monteiro-Riviere et al and Godin and Touitou, the thickness of the stratum corneum (8.6) and epidermis (16.7–27.8 µm) in beagle dog and human (17/47 µm) is not solely predictive for human absorption rates. Indeed, in the order of in vitro determined skin permeability characteristics, the dog as a model is in close proximity to monkey, minipig, and human.

However, drug release profile and amount, as a percentage, of the applied dose may differ in human skin. Other major application challenges of drug-in-adhesive patches relate to the physicochemical properties of the skin, varying within a patient population and thus posing difficulties for the design of a universal formulation with regard to meeting the intended adhesiveness, drug release profile, and drug permeation performance. Notably, despite some variations between the individual animals, an identical time course was observed in our study, with a rather rapid and linear increase in anastrozole plasma concentrations within the first 24 h that was, upon reaching a plateau, followed by a very slow decrease within the next 2 days. Plasma concentrations were comparable to human data after a single oral dose of 1 mg and when considering a typical accumulation factor of 3–4 reflecting therapeutically relevant levels in long-term therapy. This indicates a comparably rapid initial delivery rate and the maintenance of rather stable plasma levels thereafter. While this is an already well-suited profile, it also provides the

Table 3 Precision and accuracy data of the LC–MS method for the quantification of anastrozole in beagle dog plasma (mean ± SD, precision, and accuracy [%])

| Nominal concentration (ng/mL) | Intra-day assay | Precision (percentage of CV) | Accuracy (%) |
|------------------------------|----------------|-----------------------------|--------------|
|                              | Mean ± SD      |                             |              |
| 2                            | 2.03±0.26      | 12.98                       | 101.4        |
| 5                            | 4.89±0.47      | 9.70                        | 97.78        |
| 10                           | 10.36±0.30     | 2.90                        | 103.63       |
| 20                           | 19.72±0.45     | 2.27                        | 98.62        |
| 50                           | 48.03±0.96     | 2.01                        | 96.06        |
| 75                           | 72.16±2.56     | 3.55                        | 96.26        |
| 100                          | 96.12±2.48     | 2.58                        | 96.12        |
| 200                          | 206.24±5.61    | 2.72                        | 103.12       |

Abbreviation: LC–MS, liquid chromatography–mass spectrometry.
basis for testing modifications of anastrozole loading within the used matrix patches as well as other matrix compositions for further improving and fine-tuning absorption rates and bioavailability. Committed to the principle of the three Rs, a direct pharmacokinetic control from topical anastrozole (not formulated in TDDS) was not performed, albeit this is an important aspect that might have provided additional information. Additionally, a simple, accurate, and sensitive LC–MS method was developed in this work for the reliable measurement of anastrozole in animal plasma. In contrast to more powerful LC/MS/MS protocols, the current method only requires a single quadrupole MS, with the fragment ion detection considerably improving the signal-to-noise ratio and the sensitivity. While the rapid developments in analytical mass spectrometry techniques require rather cost-intensive hardware, our method provides a way for drug-specific exploiting and optimizing the potency of traditional LC/MS.

**Conclusion**

Based on a BIO-PSA® AC 7-4302 silicone matrix, EA as an anastrozole solvent, and the use of glycerol as a crystallization inhibitor, a homogeneous drug wet mix was produced, capable of building up TDDS as ready-to-use formulation. This anastrozole drug-in-adhesive patch was found to be stable after drying and stored over months. In Franz diffusion cell experiments, a continuous anastrozole release profile, as well as permeation of 65% of TDDS drug loading within 48 h, was found, providing the basis for a proof of functioning study in an experimental TDDS exposure to animals. In vivo data showed a favorable plasma anastrozole concentration–time course following application of the final anastrozole transdermal system to beagle dogs, comparable to plasma concentrations 24–72 h following oral administration in humans. The study attests to the eligibility of the API anastrozole (drug) for transdermal application route and shows the feasibility of anastrozole TDDS in a proof of principle design, which demands expanded confirmatory animal studies, predicting the convenient alternative use of anastrozole to treat BC in women with expected better therapeutic index and patient compliance.

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**Disclosure**

Margarita Voskanian and Christian Brätter are employees of Formula GmbH, Pharmaceutical and Chemical Development Company, Berlin, Germany. The authors report no other conflicts of interest in this work.

![Figure 7](A) Pharmacokinetic profile of anastrozole after application of the optimized (ethyl acetate and glycerol) TDDS formulation containing 2.8% of anastrozole (dotted line: mean of both animals) in dog plasma and (B) mean concentration time course in previously published data from healthy human study following oral administration of 1 mg of anastrozole film tablets. Reprinted from Journal of Chromatography B, vol 850. Mendes GD, Hamamoto D, Ilha J, Pereira AS, de Nucci G Anastrozole, Anastrozole quantification in human plasma by high-performance liquid chromatography coupled to photospray tandem mass spectrometry applied to pharmacokinetic studies, pages 553–559, copyright (2007), with permission from Elsevier.¹⁶

**Note:** Plasma concentration course from the in vivo study (A) compared with published data obtained from human following oral drug application (B) of anastrozole standard dose.

**Abbreviation:** TDDS, transdermal drug delivery systems.

**Table 4** Calculated data on estimated partial (*) AUCl₂₄₋₇₂ to beagle dogs compared with data from Mendes et al²⁴

| Parameter          | Mendes et al (2007) | This study subject 1 | This study subject 2 |
|--------------------|---------------------|----------------------|----------------------|
| AUCl₂₄₋₇₂ (mg/L)   | 536.6               | 715.7*               | 578.8*               |
| T₁/₂ (h)           | 42.5                | 74                   | 86                   |

**Notes:** AUCl₂₄₋₇₂, AUCl from time points 70 to 724 h; T₁/₂, half-life.

**Abbreviations:** AUCl, area under the curve; TDDS, transdermal drug delivery systems.
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