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Stability of the OECD model compound benzoic acid in receptor fluids of Franz diffusion cells

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The stability of benzoic acid, one of the model compounds recommended for skin absorption studies in the OECD guidelines, in Franz diffusion cell receptor fluids was studied. According to the results, addition of a preservative (sodium azide) to the solution is recommended for long-term skin permeation experiments.

The guidance documents for skin absorption studies of the organization for economic co-operation and development (OECD guidelines) propose three model compounds, with a wide range of octanol/water partitioning coefficient, for the demonstration of the performance and reliability of a laboratory setup for *in vitro* transdermal penetration. These proposed model compounds are caffeine (C), benzoic acid (BA) and testosterone (T) with respectively Log P_{ow} values of 0.01, 1.83 and 3.32 (OECD 2004a, OECD 2004b). Although these compounds are very commonly used in *in vitro* percutaneous experiments, assayed with UV, HPLC or radioactivity, no Franz diffusion cell-relevant stability results have been reported to our knowledge.

As we observed some inconsistencies in our BA assay results obtained by HPLC-UV, a stability study was performed in different media. Previous in vitro transdermal BA-studies have used different receptor fluids as shown in the Table (Nielsen 2006; van de Sandt et al. 2004; Ishikawa et al. 2002; Kitagawa et al. 2000; Yoshida et al. 2000), but none of them investigated the stability, nor explicitly used stability-enhancing ingredients like microbial preservatives.

We incubated the compounds at different temperatures for up to 24 h. Stable results were observed for caffeine and testosterone. However, for BA in human skin extract

Table: *In vitro* transdermal experiments with benzoic acid (BA)

Reference	Receptor fluid	Detection method
Nielsen et al. 2006	1% Tween-20 in 0.9% NaCl	Radio-labelled BA
van de Sandt et al. 2004	0.9% NaCl	HPLC-UV and Radio-labelled BA
Ishikawa et al. 2002	PBS	HPLC-UV
Kitagawa et al. 2000	PBS	UV
Yoshida et al. 2000	0.9% NaCl	HPLC-UV

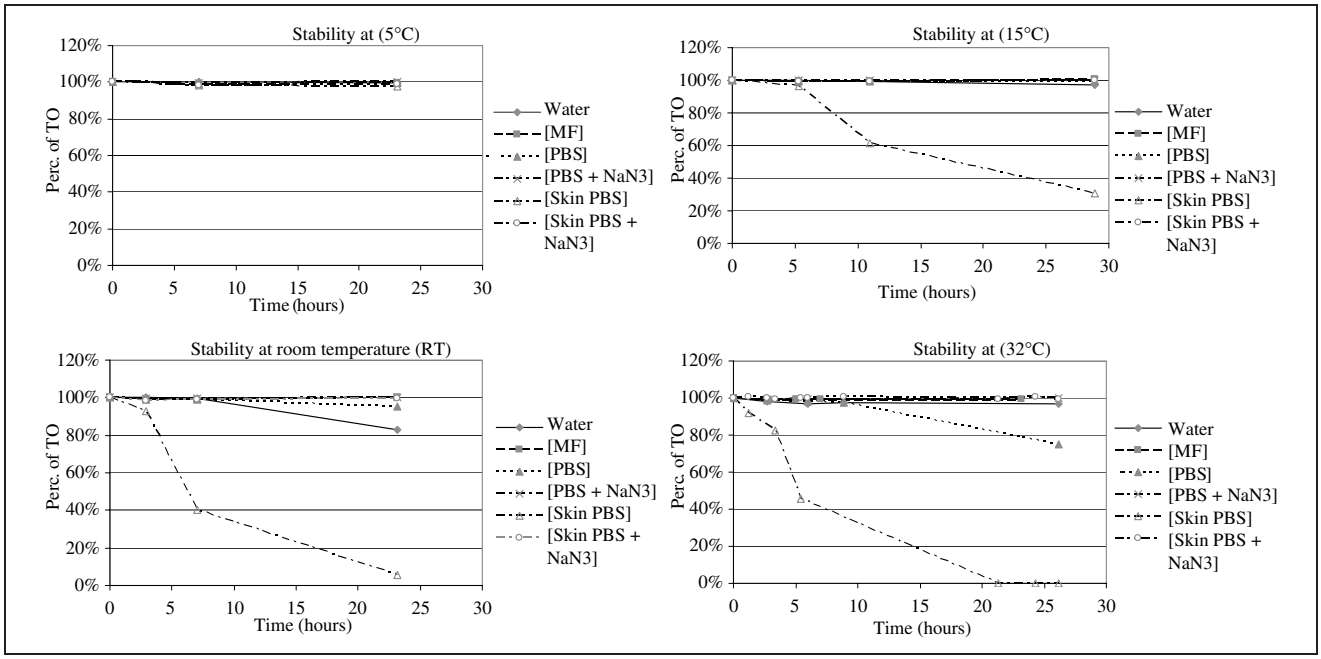

Fig.: Mean assay results of BA for three concentrations. Results for incubation at −20 °C are not shown as they are similar to those of 5 °C

stored at 15 °C or higher temperatures (room temperature or 32 °C), a significant decrease in assay values was observed. When stored at 5 °C or −20 °C, stable assay results were received. While these results gave us information on how to appropriately store the analytical samples for HPLC analysis, it also indicated special precautions for transdermal experiments conducted at 32 °C according to OECD. Adding sodium azide (NaN_3) to the human skin extract prevented effectively the decrease in assay values (Fig.). The same observations were obtained, although to a lesser extent, with BA dissolved in a phosphate buffered saline (PBS) solution and in water.

We conclude that adding a preservative such as NaN_3 is required to perform reliable long-term *in vitro* skin permeation experiments with BA.

Experimental

Human skin extract was obtained by placing a full-thickness skin piece of approximately 6.5 cm in 50 mL of a 0.1 M phosphate buffered saline (PBS) solution pH 7.4 (Sigma, St Louis, CA, USA) [skin PBS], to which 0.1% m/V sodium azide (Mallinckrodt Baker, Phillipsburg, NJ, USA) was added [skin PBS + NaN_3]. The solutions containing the skin pieces were incubated for 24 h at 37 °C and used immediately.

This gave the same skin exposure/solution ratio as in our experimental settings for *in vitro* transdermal penetration experiments with Franz diffusion cells. The skin used was obtained from the abdominal region of a 45 years old healthy male patient (plastic surgery); the subcutaneous fat was removed and the full-thickness skin was kept in a refrigerator at −20 ± 3 °C until it was used.

BA solutions at three different target concentrations were prepared (i.e. 80 μg/mL, 20 μg/mL and 2 μg/mL) in six different solvents. The chosen solvents were: water (HPLC grade, Fisher Scientific, Leicestershire, UK) [water], 0.1% m/V formic acid in water/acetonitrile 70/30 V/V (i.e. mobile phase of the used HPLC system) [MF], 0.1 M PBS solution at pH 7.4 [PBS], 0.1% m/V sodium azide in the same PBS solution [PBS + NaN_3], skin extract in PBS solution [Skin PBS] and skin extract in 0.1% m/V sodium azide in PBS solution [Skin PBS + NaN_3].

Solutions were incubated for up to 24 h at five different temperatures: −20 ± 3 °C, 5 ± 3 °C, 15 ± 3 °C (i.e. the temperature of the autosampler of the HPLC), room temperature (21 ± 3 °C) and at 32 ± 3 °C (i.e. the most commonly used working temperature for *in vitro* transdermal penetration experiments and recommended by OECD).

Samples taken at different times were assayed by an in-house developed and validated HPLC method using a Waters Alliance 2695 equipped with a Waters 2996 PDA detector and thermostatted autoinjector (all Waters Corporation, Milford, Massachusetts, USA). Analytes were separated on a LiChrospher 100 RP (125 × 4 mm, 5 μm, Merck, Darmstadt) column kept at 30 °C. The mobile phase was a mixture of acetonitrile/water (70/30 V/V) buffered with 0.1% m/V formic acid at a flow rate of 1 ml/min. UV detection was at 229 nm. Injection volume was 25 μL. Under these conditions, BA eluted at approximately 4.5 min.

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Studies of the skin permeation of lipophilic drugs: paclitaxel

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The objective of this investigation was to develop and validate skin permeation methodology for a highly lipophilic drug with respect to sink conditions and intactness of barrier during the course of experiment, using water permeation and trans epidermal water loss as tools.

Skin, being a lipophilic and highly tortuous barrier, severely limits the molecular transport of drugs into the body. However, permeation of lipophilic molecules is less restricted than that of hydrophilic drugs. The ideal characteristics required for efficient transdermal delivery of a drug include (Naik et al. 2000) a) molecular weight < 500 Da; b) log P: 1 to 3 and c) daily dose <10 mg. A practical problem in transdermal delivery research is the development of a suitable permeation methodology for poorly water-soluble molecules. This problem is even more severe during the evaluation of transdermal escape of topical drugs. In such a situation, higher amounts of drug will be localized in the skin without sink conditions, leading to false positive results.

Aqueous solubility of paclitaxel (PCL) is as low as 0.7 μg/ml, but the compound is appreciably soluble in ethanol (EtOH, 36.4 mg/ml, Straubinger 1995; Panchagnula et al. 2004). Due to its poor aqueous solubility, physiological buffers cannot provide necessary sink conditions. Therefore, EtOH was incorporated into phosphate buffered saline (PBS) (pH: 7.4). However, EtOH itself, being a penetration enhancer (Williams and Barry 1992) can alter the skin barrier if it diffuses back into the stratum corneum (SC) from the receptor compartment. In the current study, to evaluate the effect of ethanolic receptor phase on skin barrier function, a series of binary combinations of EtOH and PBS were prepared and their influence on skin barrier function was evaluated, using ^3H_2O permeation and transdermal epidermal weight loss (TEWL) as markers.

Hydro alcoholic solutions have often been used as receptor phase in skin permeation studies of poorly water soluble drugs (Liu et al. 1991; Dayan and Touitou 2000; Ben-Shabat et al. 2005). However, this methodology was not systematically evaluated yet. In the current investigation, skin barrier integrity was evaluated using two complimentary methodologies. Water permeation and TEWL were earlier employed to assess skin barrier alteration upon the