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Implementation of a single quad MS detector in high-throughput transdermal research of plant extracts

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ABSTRACT

In this study, a new type of single quadrupole mass spectrometric detector was implemented in transdermal research. The local skin pharmacokinetic properties of the plant *N*-alkylamides (NAAs) pellitorine and anacycline, present in an *Anacyclus pyrethrum* extract, and spilanthol, present in a *Spilanthes acmella* extract were investigated. This single quad MS detection method showed great advantages compared to the traditional UV detector. The NAAs could be identified and quantified in the samples with an ultra performance liquid chromatography (UPLC)–single quad MS detection system, even if they were not separated, which is a requirement when using an UV–detector. Another advantage of the UPLC–MS system is that lower limit of detection values could be obtained allowing a more accurate and precise determination of the experimental lag time in the *in vitro* skin permeation experiments. To conclude, this single quad MS detector coupled to UPLC is a useful analytical tool with improved performance compared to high performance liquid chromatography (HPLC)–UV for biomedical-pharmaceutical purposes in transdermal research.

1. Introduction

There are some limitations concerning the use of liquid chromatography–ultraviolet/visible (LC–UV/VIS) for the quantification of chemicals, as there is the possibility for compounds with a very similar structure to co-elute, as well as the relatively high limits of detection (LoD) for most compounds. Therefore, there is a need for a simple technique to overcome these separation problems and to obtain low LoD values. Currently, there is such simple technology available with a single quad MS detector, which is directly compatible with existing LC equipments and software, complying with GLP/GMP requirements. This detector is characterized by easy-to-use smooth lab-integration, increased efficiency and acceptably low cost, providing additional detection possibilities over the traditional UV/VIS detectors. The productivity and information content of the analyses can thus be enhanced. With the single quad MS detector, it is not only possible to make a scan of a selected mass range, but selected ion monitoring (SIM) methods can be used as well, making it possible to look at selected compounds with a known molecular weight. Besides the confirmation of the identity of a compound using the *m*/*z* of the compound, it can thus also be used for the quantification of compounds. Recently, this detector has been implemented in the routine QC analysis of some peptide drugs [1].

In this study, biologically active compounds in plant extracts are quantified. Plant extracts are complex mixtures, containing quite often many bio-active compounds. These plant extracts are frequently used in cosmetics for topical use. Investigation of the transdermal behavior of bio-active compounds is not only important for cosmetics, but also in the development of medicinal compounds to characterize the clinical relevance as well as the toxicological effects of the compounds. A great number of mathematical models are described in the literature to calculate transdermal parameters from *in vitro* and *in vivo* studies. Local skin kinetics of active compounds are investigated *in vitro* using a Franz diffusion cell (FDC) set-up, studying permeation, penetration and partition of the absorbed compounds into the different skin layers. The OECD guideline number 428 is widely accepted to perform skin absorption studies [2–4].

After topical application of a compound, the percutaneous absorption begins, but with a delay of entering the systemic circulation ranging from a few minutes to several hours and even days

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and this time is thus of clinical relevance. The delayed absorption is due to the penetration across the outer layer and main barrier of the skin, *i.e.* the stratum corneum (SC) and the partition into the different viable skin layers. The concept of lag time (t_{lag}) is generally defined as the time it takes for a compound to penetrate the skin. Most therapeutic indications require a drug with a short lag time. The t_{lag} is seldom used alone to describe the transdermal behavior of a penetrant. Besides the lag time, the most relevant and commonly used transdermal parameters are the steady state (SS) flux (J_{ss}) and the permeability coefficient ($K_{p,v}$) [4–9].

However, there exist different interpretations of the term lag time yielding different calculation approaches (Fig. 1). The lag time most often used and calculated in *in vitro* experiments is obtained by the extrapolation to the *x*-axis of the linear part of the slope of the cumulative amount *versus* time plot and is called the "extrapolated" lag time. This approach is derived from a steady state mathematical model, based on Fick's first law of diffusion and used in case of an infinite dose [2,9,10]. However, in most cases, a certain amount of the compound has already penetrated the skin before the extrapolated lag time is reached and this time point is called the "experimental" lag time [11].

Another lag time approach is the "true" lag time, *i.e.* the time point when steady state conditions are reached, taking approximately three times the extrapolated lag time. This true lag time reflects the time it takes for the compound's concentration gradient to become stabilized across the skin [12]. Before this true lag time, the permeation rate across the skin continually increases. It follows that SS-flux and permeability coefficient values can only be calculated after three times the extrapolated lag time at SS conditions [5,13]. After about 1.7 times the extrapolated lag time, there is less than 10% error on the estimation of the actual amount of compound penetrated through the skin at SS [4]. The time it takes to achieve steady state depends on several factors, such as the physical chemical properties of the compound and the permeability of the skin itself, related to the skin thickness on the site of application and the diffusion coefficient [9].

In this study, the *Anacyclus pyrethrum* DC (AP) and the *Spilanthes acmella* (SA) plant extract are used, containing various *N*-alkylamides (NAAs), showing interesting biological activities [14]. The single quad MS detector coupled to UPLC was used for the quantification of pellitorine and anacycline present in the AP extract and spilanthol in the SA extract samples obtained during a FDC experiment.

The implementation of the single quad MS detector in transdermal research, overcomes the resolution problems encountered with UV. Moreover, in transdermal research, low concentrations of the compound(s) of interest are often obtained in the FDC experiments. With the MS detector, lower LoD values can be obtained compared to UV. The lower the LoD values, the more accurate transdermal parameters such as the lag time, can be determined.

2. Materials and methods

2.1. Chemicals and reagents

The *A. pyrethrum* root extract came from a local supplier in Sagar (India) and was processed as previously described [14]. The analytical characterisation of the extract was reported earlier by Veryser et al. [15]. Spilanthol, supplied as a 30 w/w% ethanolic *S. acmella* flower extract, was a generous gift of Robertet (Grasse, France).

0.01 M phosphate buffered saline (PBS) was bought from Sigma-Aldrich. Absolute ethanol (EtOH, 99.8% V/V) came from Fisher Scientific (Erembodegem, Belgium), while ULC–MS grade formic acid (FA, 99%), ULC–MS grade acetonitrile (ACN, 99%) and ULC–MS grade methanol (MeOH) were purchased from Biosolve (Valkenswaard, the Netherlands). Ultrapure water (18.2 MΩ cm) was produced using an Arium 611 VF water purification system (Sartorius, Vilvoorde, Belgium).

2.2. Preparations of Franz diffusion dose solution

The *A. pyrethrum* extract was first dissolved in ethanol, vortexed, sonificated for 2 h and centrifuged at 3220 × *g* at room temperature for 15 min. Then, a 30:70H_2O:EtOH (V/V) solution was prepared, centrifuged one more time and the supernatant was filtered using a 0.45 μm Whatman nylon HPLC filter. The experimentally determined concentrations of pellitorine (deca-2E,4E-dienoic acid isobutylamide or $C_{14}H_{25}NO$) and anacycline (tetradeca-2E,4E-diene-8,10-diynoic acid isobutylamide or $C_{18}H_{25}NO$) in the dose solution were 495 μg/ml and 96 μg/ml, respectively. The concentration of pellitorine in the AP extract dose solution was determined using purified pellitorine as reference standard [15].

Spilanthol (deca-2E,6Z,8E-trienoic acid isobutylamide or $C_{14}H_{23}NO$) dose solutions of 1.335 mg/ml and 0.233 mg/ml (experimentally determined) in 30:70H_2O:EtOH (V/V) were prepared with the *S. acmella* extract.

2.3. In vitro *skin permeation study*

The skin penetration of pellitorine, anacycline and spilanthol were evaluated using static Franz diffusion cells (Logan Instruments Corp., New Jersey, USA) with an available diffusion area of 0.64 cm^2. The receptor compartment was filled with 5 ml 0.01 M PBS, free of air bubbles. A randomized blocked design was applied and analyses were done in fivefold (pellitorine and anacycline) and in fourfold (spilanthol). Human skin of two healthy female patients of 62 ± 14 years old (mean ± SD) was obtained after esthetic body contouring surgery, with confidential procedures and informed consent, from the Department of Plastic and Reconstructive Surgery of the University Hospital (Ghent, Belgium). The skin was cleaned with 0.01 M PBS pH 7.4 immediately after the surgical procedure and the subcutaneous fat was removed. Subsequently, the skin was stored at −20 °C for not longer than 6 months. Just before the start of the FDC experiments, the full-thickness skin was thawed, mounted on a template and sliced with an electrical powered dermatome to obtain split-thickness human skin. An actual skin thickness of 492 ± 61.8 μm (mean ± SD, n = 15) for donor 1 and 402 ± 39.0 μm (mean ± SD, n = 15) for donor 2 was experimentally determined with a micrometer (Mitutoyo, Tokyo, Japan) used for pellitorine and anacycline, while for spilanthol, an actual skin thickness of 530 ± 121 μm (mean ± SD, n = 10) and 377 ± 36.2 μm (mean ± SD, n = 10) were experimentally determined from the different patients. After visual inspection for skin damage, the intact skin pieces with the epidermis side upwards were mounted on the FDC between the donor and the receptor chambers ensuring that no air was present under the skin. During the whole experiment, the receptor fluid was mixed continuously using a Teflon coated magnetic stirring bar (400 rpm). The skin impedance was measured to check for skin integrity using an automatic micro-processor controlled Tinsley LCR Impedance Bridge (Croydon, U.K.). Skin pieces displaying an impedance value below 10 kΩ were discarded and replaced by a new skin piece [16]. With a micropipette, 500 μl of the dose solutions were applied on the skin and afterwards covered with parafilm to prevent evaporation of solvents. Due to a water jacket, the receptor compartment was kept at 32 °C. After 1 h, 2 h, 4 h, 8 h, 12 h, 18 h, 21 h and 24 h (pellitorine and anacycline) and after 15 min, 30 min, 45 min, 1 h 30, 2 h, 2 h 30, 3 h, 3 h 30, 4 h, 4 h 30, 5 h, 6 h (spilanthol), 200 μl samples of the receptor fluid were taken from the sample port and were immediately replaced by 200 μl fresh PBS solution. This was taken into account for the calculation of the cumulative permeated concentrations. At the end of