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Development and validation of an LC–MS/MS method for the determination of adapalene in pharmaceutical forms for skin application

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DETAILS ABOUT ADAPALENE

In contrast to natural retinoids, adapalene (Fig. S-1) is chemically and photochemically stable.[1] The clinical application of retinoids is limited by various side effects, such as skin irritation and teratogenicity.[2–4] The efficacy of adapalene was similar or better than that of the natural retinoid tretinoin, but the adapalene induced less local side effects after topical application.[5] Adapalene is a selective agonist of retinoic acid receptors RAR-β and RAR-γ.[6] This drug modulates the cellular keratinization and shows anti-inflammatory activity due to inhibition of the lipooxygenase activity and neutrophil chemotaxis. After entering the *stratum corneum*, the adapalene was retained in the epidermis and hair fol-

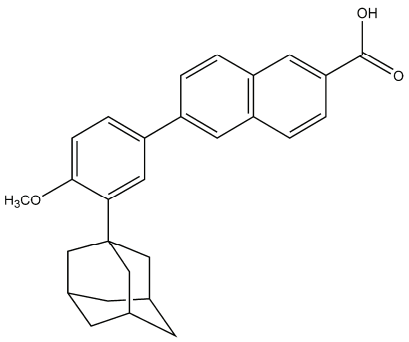

Fig. 1. The structure of adapalene.

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licles, which are the target areas. Absorption through the skin is very low and only traces of adapalene were found in the plasma of acne patients after chronic topical application of this drug in controlled trials.[7] This pharmacokinetic study was performed on male volunteers who were treated with radiolabeled adapalene (in the form of 0.1 % topical gel).

DETAILS ABOUT CHEMICALS AND APPARATUS USED

Adapalene (min. 99.4 %) was kindly donated by the Agency for Medicinal Products and Medical Devices, Bosnia and Herzegovina. Acetonitrile was purchased from Sigma–Aldrich (Steinheim, Germany), formic acid from JT Baker (Phillipsburg, NJ, USA), trifluoroacetic acid from Fisher Chemical (Loughborough, UK) and ammonium acetate from Analytika (Prague, Czech Republic). Pharmaceutical excipients (surfactants and solvents) used in the formulation studies were Plantacare 2000 UP (decyl glucoside, P2000; BASF, Ludwigshafen, Germany), Plantacare 810 UP (caprylic/capric glucoside, P810; BASF, Ludwigshafen, Germany), Emanon EV-E (glycereth-7-caprylate/caprate, EM EV-E; Kao Chemicals, Barbera del Valles, Spain), Capryol 90 (propylene glycol monocaprylate, C90; Gattefosse, Lyon, France), propylene glycol (Kemig, Zagreb, Croatia), ethanol, 96 vol. % (Ada vrenje, Belgrade, Serbia), isopropanol (Lach-Ner, Neratovice, Czech Republic), Polisorbate 80 (polyoxyethylene (20) sorbitan monooleate, P80; Comcen, Belgrade, Serbia), transcutol P (diethylene glycol monoethyl ether, TrP; Gattefosse, Lyon, France) and HPLC grade water (TKA water purification system, Niederelbert, Germany). These solvents and surfactants were used to test the specificity of the LC–MS/MS method presented herein. Commercially available Sona® 0.1 % gel and Sona® 0.1 % cream (Belupo, Koprivnica, Croatia), as well as adapalene (0.1 %) microemulsion APG-1 were used for the method validation. APG-1 was developed in our laboratory and contains C90 (oil phase), P2000 (surfactant), propylene glycol (cosurfactant) and water. The concentration of adapalene in these formulations is 1 mg g^{-1}. Microemulsion APG-1 placebo was prepared in our laboratory. Sona® 0.1 % gel and Sona® 0.1 % cream placebos were prepared by mixing components of gel and cream, according to the composition stated by the manufacturer.

The liquid chromatography analysis was performed on an ultra-high performance liquid chromatography (UHPLC) chromatograph ACELLA (Thermo Fisher Scientific Inc., Madison, WI, USA), coupled to a triple quadrupole mass spectrometer TSQ Quantum Access MAX (Thermo Fisher Scientific Inc., Madison, WI, USA) with heated electrospray ionization (HESI) interface.

METHOD OPTIMIZATION

The chromatography analysis was performed using a Zorbax Eclipse XDB-C18 column (150 mm×4.6 mm, 5 μm particle size) from Agilent Technologies (Palo Alto, CA, USA). Three different mobile phases were tested: acetonitrile/0.1 % trifluoroacetic acid, acetonitrile/0.1 % formic acid and acetonitrile/20 mM ammonium acetate. The percentage of acetonitrile ranged from 60 to 90 %. The flow rate was set to 0.5 or 0.8 mL min^{-1}. The column temperature was 25 °C and injection volume was 10 μL. The optimization of the ion source and MS/MS settings was performed by the automatic optimization function of the MS software TSQ EZ Tune version 2.3.0.1206 SPI, with syringe-pump infusion of adapalene standard solution. Nitrogen was used as a carrier gas, whereas argon was used as a collision gas. The capillary temperature was adjusted to 300 °C, while the vaporizer temperature was set to 400 °C. The following parameters were varied during the optimization of the ion source:

spray voltage, sheath gas pressure, auxiliary gas pressure, ion sweep gas pressure, tube lens offset and skimmer offset. The collision pressure and collision energy were optimized during the MS/MS analysis.

METHOD SPECIFICITY PROOF

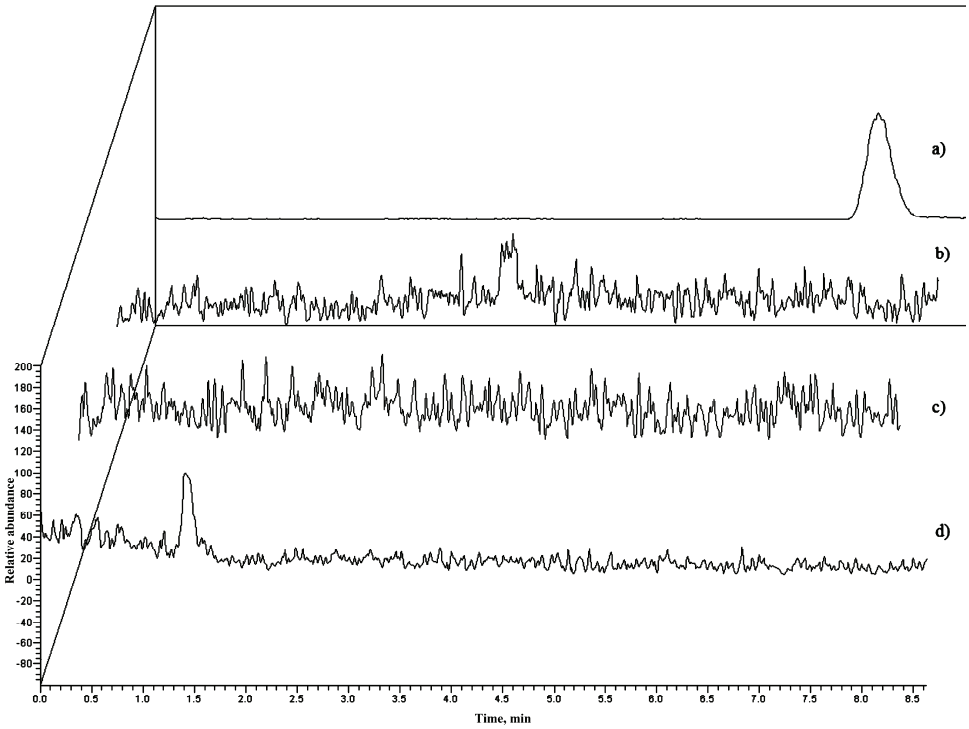

Fig. S-2. LC–MS/MS chromatograms of: a) adapalene standard (400 ng mL^{-1}), b) microemulsion APG-1 placebo, c) p810 and d) Sona® 0.1 % cream placebo.

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