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Assay of acyclovir in human skin layers by high-performance liquid chromatography

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Abstract

This paper describes an assay procedure for acyclovir quantification in human skin after in vitro transdermal transport experiments. The procedure employs warm distilled water for acyclovir (ACV) extraction and high-performance liquid chromatography (HPLC) as analytical method. The procedure has good reproducibility, sensitivity and specificity, resulting in a reliable method for biopharmaceutical studies of ACV distribution in skin tissue. The chromatographic conditions set up, using distilled water as mobile phase, makes the analytical procedure simple and easy to perform. © 1997 Elsevier Science B.V.

Keywords: Acyclovir; Human skin; HPLC; Reversed-phase chromatography

1. Introduction

The low efficacy of dermatological formulations of acyclovir (ACV, Fig. 1), a synthetic analogue of 2′-deoxiguanosine approved over 10 years ago for the treatment of herpes virus infections [1] has been attributed to its poor percutaneous penetration [2].

Recently, the possibility of enhancing ACV transport across the skin using iontophoresis with the aim of delivering greater amounts of acyclovir to the target site of *Herpes simplex* infections, i.e. the basal epidermis, has been studied [3]. Since iontophoresis can promote the accumulation of drugs within the skin [4–6], knowledge of the ACV concentration gradient in skin layers after iontophoresis enables a more efficacious therapeutical treatment to be established.

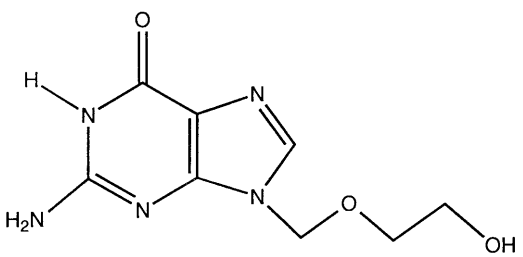

Fig. 1. Chemical structure of Acyclovir ((9-[2-hydroxyethoxy]-methyl) guanine).

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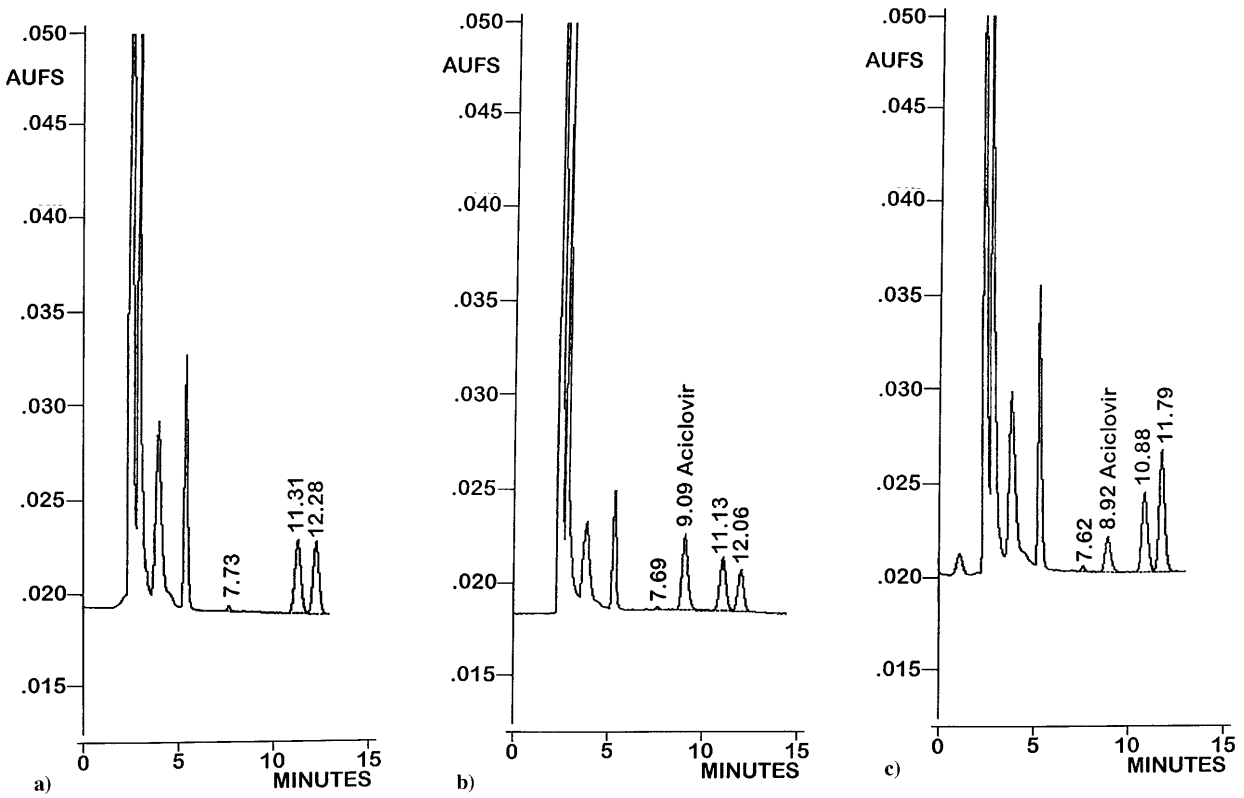

Fig. 2.

The analytical methods reported for ACV determination in biological matrices included radioimmunoassay [7] and high-performance liquid chromatography (HPLC) [8–10]. Considering the complexity and heterogeneity of skin tissue and the physico-chemical properties of the drug, any determination of the ACV gradient in the skin requires the development of a specific and reliable method. The dosage of ACV in thick skin layers, such as the whole stratum corneum, viable epidermis and dermis, was presented by Parry et al. [11]. The present study describes an assay procedure for ACV quantification in successive thin slices of human skin, obtained after in vitro transport experiments. The procedure employs warm water for ACV extraction and HPLC as analytical method.

2. Experimental

2.1. Materials

Acyclovir (mol. wt. 225.2) was a gift from Lisapharma, Erba (CO), Italy.

Abdominal human skin tissue from caucasian donors (8 individuals, both sex, range of age = 60–88 years old) was obtained from elective abdominoplasty surgery (Istituto di Clinica Chirurgica Generale e Terapia Chirurgica, Faculty of Medicine, University of Parma, I) and used within 3 h of surgery for in vitro transport experiments.

2.2. Apparatus and chromatographic conditions

For the analysis of ACV, an isocratic HPLC system (LC 250 pump, Perkin Elmer, Norwalk,

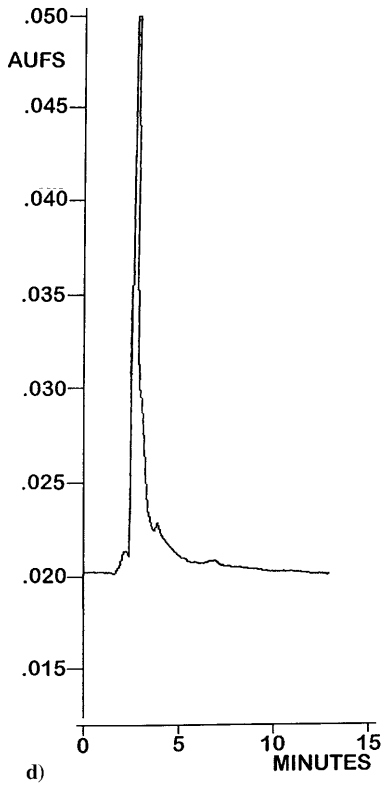

Fig. 2. Representative chromatograms obtained by extraction from (a) blank skin slice; (b) skin slice spiked with ACV; (c) slice of skin submitted to ACV iontophoresis and (d) second extraction of a slice of skin submitted to ACV iontophoresis (column Vydac; eluent: distilled water 1.2 ml min^{-1}; UV detection 254 nm; loop 50 μl).

Connecticut, USA) equipped with a 5-μm C18 250 × 4.6 mm column from Vydac (Hesperia, CA, USA) was used. The eluent was distilled water at flow rate of 1.2 ml min^{-1}. A Rheodyne valve with a loop of 50 μl and a spectrophotometric detector working at 254 nm (UV/VIS Spectrophotometric detector LC290, Perkin Elmer) were employed. The area of chromatographic peaks was recorded and elaborated by PENelson (Perkin Elmer) 1020 software. The analyses were performed at room temperature.

Calibration curves were obtained in the ACV range 0.15–15.0 μg ml^{-1}. The limit of quantification (LOQ) was determined from the standard deviation of the baseline noise (SN) and is defined as the drug concentration resulting in a peak height of 10 times SN [12]. LOQ was further confirmed analyzing an ACV solution having the computed concentration.

The effectiveness of the operating system proposed for ACV HPLC determination was ascertained according to the indications of USP XXIII.

2.3. Drug extraction from skin samples

The quantitative determination of ACV at different depths from skin surface was performed on horizontal slices of the skin sample [13,14]. At the end of the in vitro transport experiment, a portion of the skin sample, rinsed 3 times with saline solution, was punch biopsed (0.6 cm^2), included in an embedding medium for tissues (O.C.T. Compound, Tissue-Tek, Miles, Elkhart, IN, USA), frozen in liquid N_2 and mounted on a cryostat (Miles, Elkhart, IN, USA). Horizontal slices 20 μm in thickness were cut at −28°C, and sequentially collected in Eppendorf microtubes. The skin slices, having a volume of 1.2 10^{-3} cm^3, were

Table 1
Recovery of acyclovir from skin slices

Amount added (ng)[a]	Amount extracted (ng)	*n*	Recovery (%)	RSD[b] (%)
49.6 ± 0.6	46.0 ± 1.0	7	92.8	2.3
89.6 ± 1.7	88.9 ± 3.2	9	99.2	3.6
214.0 ± 2.3	212.2 ± 8.3	6	99.2	3.9

[a]Determined by direct injection of spiked solution (mean ± SD).
[b]Relative standard deviation.

stored at −20°C until analysis. From each skin slice, ACV was extracted with 200 μl of distilled water at 60°C for 15 min; during this time the tubes were vortexed twice for 10 s. After cooling, 200 μl of 1 N $HClO_4$ were added to precipitate residual proteins and the mixture was centrifuged at 5000 G for 10 min. The supernatant was filtered through a 0.45-μm nylon filter (Lida, Kenosha, USA) and analyzed via HPLC.

For the validation of ACV extraction from the skin, a different series of 20 μm slices of blank skin (which had not previously been in contact with ACV) was cut and used in specificity and recovery determinations. Some of the blank skin slices were submitted to the assay procedure and the retention time of endogenous compounds was compared with that of ACV. Then, different known amounts of ACV solution (5, 10 and 20 μl of ACV 10 μg ml^{-1}) were directly added to blank skin slices, which, after 3 h of contact, were submitted to the above described extraction and analysis. The extraction recovery was determined by computing the ratio of the amount of ACV extracted from spiked skin to the amount of ACV added. The skin slices were from different donors and taken from different depths.

3. Results and discussion

The suitability of the system was checked according to USP XXIII. Specific data were collected from replicated injections of standard solution. The retention time of ACV peak was 9 min and the efficiency of the column, expressed as the number of theoretical plates, was at about 9000. The tailing factor, T, calculated as the ratio of the distance from the leading edge to the tailing edge of the peak, divided by twice the distance from the peak maximum to the leading edge of the peak, was 1.1. The reproducibility, S_R, expressed as relative standard deviation, was found within-day variation ranging between 0.23 and 1.1%.

Initial efforts were directed towards the development of a reliable procedure for the extraction of ACV from skin slices, considering its poor water and oil solubility and the complexity of the biological matrix. This was successfully obtained soaking the sample in distilled water at 60°C for 15 min. In these conditions the solubility of ACV increases to 9 mg ml^{-1} (more than five times the value at room temperature). The stability of standard solutions of ACV in the same conditions as the assay procedure, was also tested and no degradation was observed.

The efficiency of separation of ACV peak from other peaks deriving from the skin, determined as resolution factor, R, was 3.18 ± 0.27 for the peak preceding ACV and 3.39 ± 0.04 for the peak following ACV. Fig. 2 illustrates typical chromatograms obtained from human skin slices (Fig. 2a unspiked (blank sample) and Fig. 2b spiked with ACV). Despite the complexity of the biological matrix, a good resolution was obtained using distilled water as eluent. Satisfactory recoveries were obtained from all samples tested. The results, given in Table 1, made use of an internal standard unnecessary, avoiding the risk of its co-elution with endogenous compounds.

The limit of quantification was calculated as 8 ng ml^{-1} (0.4 ng injected) and allowed the dosage

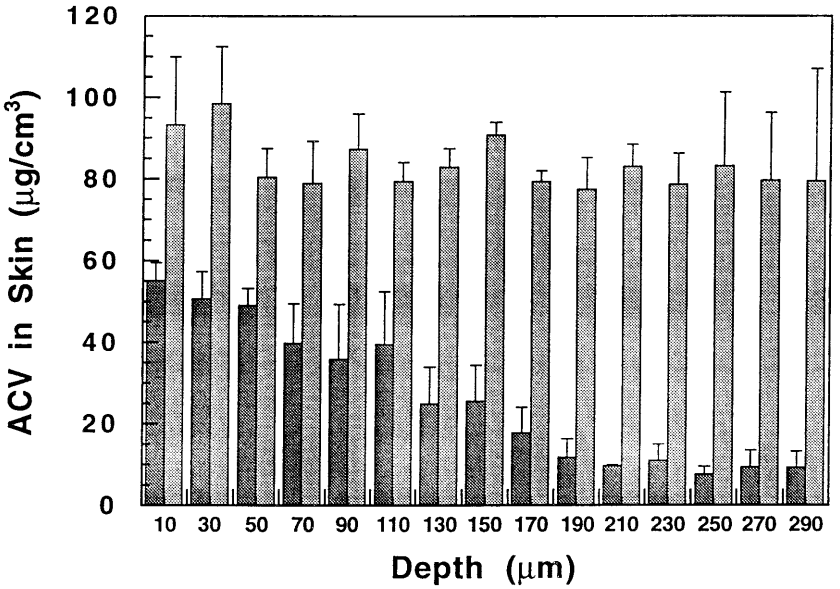

Fig. 3. Distribution of ACV in human skin layers following (■) 7 h of passive diffusion and (▩) after applying 30 min of iontophoresis (0.5 mA.cm^{-2}) followed by passive diffusion for up to 5 h (donor solution: 6.88 mM ACV pH 3.0 buffered solution).

of 3 ng of ACV in each skin slice. In Fig. 2c the first and Fig. 2d the second extraction of ACV from a slice of skin obtained after transdermal iontophoretic experiments are shown. Further extractions of the same material, additionally realized for control purposes, never presented measurable residual amounts of ACV.

In the course of iontophoretic ACV transport investigations, several hundred skin slice samples have already been analyzed using this method [15]. Fig. 3 shows ACV concentrations determined in human skin slices after 7 h of passive diffusion experiment and after applying 30 min of iontophoresis (0.5 mA.cm^{-2}) followed by passive diffusion for up to 5 h. It can be seen that iontophoresis induced high accumulation of ACV in stratum corneum, epidermis and dermis.

In conclusion, this simple assay procedure has good reproducibility, sensitivity and specificity, resulting in a reliable method for biopharmaceutical studies of ACV distribution in skin tissue. The chromatographic conditions set up, using distilled water as mobile phase, make the analytical procedure simple and easy to perform.

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References

[1] G.D. Morse, M.J. Shelton, A.M. O'Donnell, Clin. Pharmacokinet. 24 (1993) 101–123.
[2] J.J. O'Brien, D.M. Campoli-Richards, Drugs 37 (1989) 233–309.
[3] N.M. Volpato, P. Santi, P. Colombo, Pharm. Res. 12 (1995) 1623–1627.
[4] P.G. Green, M. Flanagan, B. Shroot, R.H. Guy, in: K.A. Walters, J. Hadgraft (Eds.), Pharmaceutical skin penetration enhancement, Marcel Dekker, New York, 1993, pp. 311–33.
[5] P. Singh, H.I. Maibach, Crit. Rev. Therap. Drug Carr. System 11 (1994) 161–213.

[6] L.P. Gangarosa, J.M. Hill, Int. J. Pharm. 123 (1995) 159–171.
[7] R.P. Quinn, P. de Miranda, L. Gerald, S.S. Good, Anal. Biochem. 98 (1979) 319–328.
[8] R.L. Smith, D.D. Walker, J. Chromatogr. 343 (1985) 203–207.
[9] J. Salamoun, V. Sprta, T. Sladek, M. Smrz, J. Chromatogr. 420 (1987) 197–202.
[10] K.J. Swart, H.K.L. Hundt, A.M. Groenewald, J. Chromatogr. 663 (1994) 65–69.
[11] G.E. Parry, P. Dunn, V.P. Shah, L.K. Pershing, J. Invest. Dermatol. 98 (1992) 856–863.
[12] G.P. Carr, J.C. Wahlich, J. Pharm. Biomed. Anal. 8 (1990) 613–618.
[13] P.G. Green, B. Shroot, F. Bernerd, W.R. Pilgrim, R.H. Guy, J. Control. Release 20 (1992) 209–218.
[14] A. Jadoul, C. Hanchard, S. Thysman, V. Préat, Int. J. Pharm. 120 (1995) 221–228.
[15] N.M. Volpato, S. Nicoli, C. Laureri, P. Colombo, P. Santi, J. Control. Rel. (1997) submitted.