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HPLC evaluation of diclofenac in transdermal therapeutic preparations

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Abstract

High-performance liquid chromatography (HPLC) was selected for analytical evaluation of sodium diclofenac in original transdermal therapeutic preparations containing adjuvant substances (capsaicin, hyoscyamine). After isolation from laminated adhesive patches, diclofenac was analysed on columns with reversed phase, using the mobile phase ethanol and phosphate buffer (pH 6.5) with an addition of tetrabutylammonium iodide and detection at 284 nm. Not only the total amount of diclofenac in the patch was evaluated, but HPLC methodology was also employed to select a suitable acceptor medium for permeation experiments. In patches manufactured in the tested series, HPLC was also employed to examine the release of diclofenac and its in vitro permeation through the human skin.

Keywords: HPLC; Diclofenac sodium salt; Transdermal preparation; Release study; Permeation study

1. Introduction

Sodium salt of diclofenac was another drug tested for transdermal administration by means of new adhesive patches of the laminated type (Doležal et al., 1988) developed at Charles University in Prague, Faculty of Pharmacy in Hradec Králové in co-operation with the firm Chemopharma, Ústí nad Labem. The technology of this new transdermal therapeutic system (TTS) was harmonised in such a way as to suit, as much as possible, the modern trends in these preparations as to their therapeutical application and GMP principles. The methods for the evaluation of newly prepared transdermal patches are extended in the present paper by HPLC analysis of diclofenac.

Diclofenac, like other non-steroidal antiphlogistic agents, can be administered also by external dermal application, i.e. in the form of creams or

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hydrogels with mainly local therapeutic effect. The administration by transdermal route with the use of TTS is studied from the viewpoint of a possible increase in drug penetration through the skin, in order to make the therapy of inflammatory and degenerative rheumatic diseases more effective (Katzung, 1995). In the preparation of new adhesive preparations, the active ingredient was combined with capsaicin (a hyperemic effect) and hyoscyamine (a peripherally dilatational effect), thus achieving an increase in an otherwise low capability of diclofenac (Obata et al., 1993) to penetrate the skin barrier.

In diclofenac determination, HPLC is employed to monitor it in biological material — most frequently in plasma (Lansdorp et al., 1990; Brunner and Luders, 1991; Miller, 1993; Avgerinos et al., 1993; Mohamed et al., 1994; Giagoudakis and Markantonis, 1998), less frequently in blood serum (Moncrieff, 1992), urine (Brunner and Luders, 1991; Mohamed et al., 1994) or body fluids (Kuhlmann et al., 1998). In the analysis of pharmaceutical preparations containing diclofenac, spectral methods (Agrawal and Shivramchandra, 1991; Fabre et al., 1993; Kamath and Shivram, 1993) are often employed, HPLC being used for example in its evaluation in tablets (Beaulieu et al., 1990; Kubala et al., 1993) and in solutions, including injections (Huichang et al., 1992; Szasz et al., 1993).

The purpose of the present study was to elaborate a HPLC analysis of diclofenac in a newly prepared adhesive patch, to document its usability for transdermal patches produced in the tested series in the release testing of batch-to-batch reproducibility and in permeation comparative studies. An important precondition was to evade the interference of adjuvant additives contained in the TTS.

2. Materials and methods

2.1. Chemicals, materials

Diclofenac sodium salt and tetrabutylammonium iodide were purchased from Sigma (St. Louis, MO), methanol HPLC, polyethylene glycol 300 (PEG 300) and propylene glycol from Merck (Darmstadt, Germany). Capsaicin, hyoscyamine, phenylanthranilic acid (IS), potassium dihydrogenphosphate, disodium phosphate, citric acid and octanol were purchased from Lachema (Brno, Czech Republic). Methanol was HPLC grade, ethanol was UV grade and all other chemicals were analytical grade. Water was redistillated.

Laminated adhesive diclofenac containing patches (including capsaicin and hyoscyamine) or only with capsaicin and hyoscyamine were obtained from Chemopharma (Ústí nad Labem, Czech Republic).

Excised human skin was from obtained from the Tissue Bank (Faculty Hospital, Hradec Králové, Czech Republic).

2.2. Apparatus and chromatographic conditions

The HPLC system consisted of a solvent delivery system SP 8700 (Spectra Physics, Santa Clara, CA), a detector Spectra 100 (Thermo Separation Products, Santa Clara, CA) and a SP 4100 integrator from Spectra Physics. The samples were applied using 3 and 20 μl loops (Rheodyne, Cotati) to an analytical glass column Separon SGX C-18 (150×3.3 mm I.D., 7 μm) (Tessek, Prague). The mobile phase was methanol — phosphate buffer pH 6.5 (110:100) with an addition of tetrabutylammonium iodide (5 mM/l) at a flow rate of 0.5 ml/min. Detection was performed at 284 nm.

2.3. Preparation of buffers

2.3.1. Preparation of phosphate buffer pH 6.5

Some 350 ml 6.6 mM potassium dihydrogen phosphate solution was adjusted with an addition of (≈ 150 ml) 6.6 mM disodium phosphate solution to the final pH 6.5.

2.3.2. Preparation of McIlvain buffer pH 5.5

A total of 228 ml 0.2 M disodium phosphate solution was adjusted with an addition of (≈ 172 ml) 0.1 M citric acid to the final pH 5.5.

2.4. Standard solutions

The standard solution of diclofenac sodium salt (1 mg/ml) in methanol (diluted to 0.1 or 0.01 mg/ml) was prepared for quantification of diclofenac in the patch, for the elution and dissolution study. Standard solutions of diclofenac (1 mg/ml) in the acceptor medium (diluted to 0.1 mg/ml or 5 μg/ml) were prepared for the release and permeation study. Standard solution of phenylanthranilin acid (IS) of a concentration of 1 mg/ml in ethanol was further diluted according to requirements (0.15 mg/ml for the release study, 0.5 μg/ml for the permeation study).

2.5. Extraction of diclofenac from transdermal patches

The protective anti-adhesive paper was removed from the patch and the base layer was portioned out by cross cuts ≈ 1 mm from each other. A sample patch, made up in such a way, was introduced into a 100-ml conical flask with a ground-glass joint, 40 ml of methanol was added and vigorously agitated in a shaking apparatus for 15 min and exposed in an ultrasound bath for 15 min. Methanolic extract was poured into a 100-ml volumetric flask and extraction was repeated twice with 20 ml of methanol. To combined methanolic extracts, 3 ml of IS solution (1 mg/ml) were added and the volumetric flask was made up to the mark with methanol. A total of 3 μl of the sample was injected onto the column.

2.6. Elution study

After removal of the protective anti-adhesive paper, the patch was covered with 10.0 ml of the acceptor medium and tempered at 37°C for 72 h. The acceptor medium (0.1 ml) was pipetted into a 10-ml volumetric flask and, after making-up with methanol, 3 μl of the sample was injected onto the column.

2.7. Study of solubility

To 10.0 ml of the heated medium under study, an excess of diclofenac sodium salt was added. The developed suspension was shaken at intervals and tempered for 72 h at 37°C and then allowed to settle at the same temperature. Using a pre-tempered pipette, 100 μl of the liquid was withdrawn from clear supernatant and placed into a 10-ml volumetric flask and, after making-up with methanol, 3 μl was injected onto the column.

2.8. Release and permeation study

Modified diffusion cells according to Franz were used for the release and permeation study of diclofenac from the patches of an area or 10.0 cm^2. A PEG 300 solution in water (1:4) tempered to 37°C was used as the acceptor medium. From the acceptor medium, samples of a volume of 0.6 ml (with subsequent replacement of the withdrawn volume with fresh medium) were withdrawn at ten predetermined time intervals (from 1 to 24 or 48 h).

For the sake of quantification, 0.4 ml of the solution from the withdrawn sample was pipetted into a test-tube, 0.1 ml of IS solution was added and after stirring 3 μl (in the release experiment), or 20 μl (in the permeation experiment) were injected onto the column. In the liberation study, direct release of diclofenac from the patch to the medium, i.e. without the use of any additional membranes, was examined. In the permeation study, the passage of diclofenac from the patch through the excised human skin into the acceptor medium was investigated. The patch under study was placed on a ≈ 300 μm thick skin graft of a suitable size (2 or 10 cm^2) and after standardized securing of its adhesion to the skin (a pressure of 10 g/cm^2 for 15 s) the concentration of diclofenac in the acceptor medium was determined at the pertinent time intervals. The number of parallel experiments was $n = 6$.

2.9. Calibration curves

Quantification of diclofenac was based on the calibration curves constructed as the dependence of the ratios of the areas (y) of peaks (analyzed substances to the area of the peak of the internal standard) on concentration (x). For the determination of diclofenac in the patch, five concentra-

tions (20, 30, 40, 50 and 60 μg/ml) of diclofenac (and 30 μg/ml of IS) in methanol were prepared. For the quantification of diclofenac in the liberation study, the calibration curve was constructed from the concentrations of 10, 25, 50, 100 and 200 μg of diclofenac per mililitre (and 30 μg/ml of IS) in the acceptor medium and in the permeation study 0.05, 0.125, 0.25, 0.5, 1.0 and 3.0 μg/ml of diclofenac (and 0.1 μg/ml of IS) were added to the acceptor medium.

3. Results and discussion

A ion-pair HPLC methodology was worked out, making it possible to perform selective analytical evaluation of diclofenac in the final transdermal preparation. The method was applied in the control-analytical evaluation of the test series of TTS, including the selection of the acceptor medium for release and permeation in vitro experiments.

The elaborated analytical method was also successfully used in the laboratory development of TTS, when on the basis of release results the composition of the preparation was being modified until the optimised kinetics of diclofenac release was obtained. The found chromatographic conditions were further applied in the study of diclofenac permeation through the excised skin.

3.1. Quantification of diclofenac in transdermal patches

The HPLC conditions for the evaluation of diclofenac had to be selected in such a way that the peak of diclofenac should not interfere either with the possible peaks of adjuvant additives (capsaicin, hyoscyamine) in TTS, or the residues extracted from the styrene–butadiene–styrene adhesive matrix of the transdermal patch (Fig. 1). Under the described chromatographic conditions, selected with regard to these requirements, the retention time of diclofenac was 5.7 min and it was quantified by the internal standard method. Out of a number of substances, the selection of the internal standard was limited to phenylanthranilic acid and ethylparabene, out of which acid with a retention time of 4.5 min was preferred.

Concentration of diclofenac was determined from the calibration curve $y = 0.0158x + 0.0962$ ($r = 0.9999$). The precision and reproducibility was verified by analysing five samples of a known diclofenac concentration in methanol. The found results of concentrations, including relative standard deviations (RSD) are shown in Table 1; the values of RSD are no larger than 2.5%.

In the manufactured patches, the total amount of diclofenac was determined after its extraction into methanol. Diclofenac concentration was determined from the calibration curve and its total amount in the patch (400.17 ± 0.23 μg/cm^2) was calculated.

3.2. Selection of the acceptor medium

Five liquid media of different polarities — octanol, water, a mixture of water and propylene glycol (3:2), a mixture of water and polyethylene glycol 300 (4:1) and a mixture of phosphate buffer of pH 5.5 and polyethylene glycol 300 (4:1) were

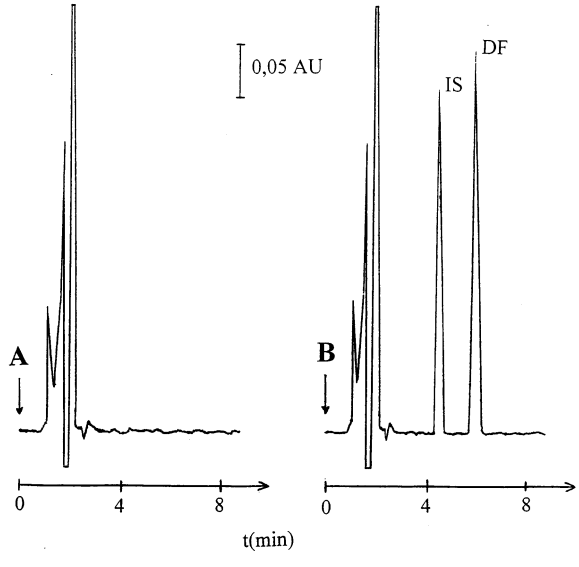

Fig. 1. A chromatographic record of an extract from the patch without diclofenac (A), only with adjuvant additives and from the patch with diclofenac (B) and adjuvant additives (DF, diclofenac; IS, internal standard).

Table 1
Precision and accuracy of diclofenac evaluation in methanol

Added (μg/ml)	Within-day ($n = 6$)		Day-to-day ($n = 11$)	
	Found ± S.D. (μg/ml)	RSD (%)	Found ± S.D. (μg/ml)	RSD (%)
20.0	20.3 ± 0.4	2.0	20.5 ± 0.5	2.4
30.0	30.3 ± 0.5	1.8	30.7 ± 0.6	1.9
40.0	40.5 ± 0.7	1.8	40.0 ± 0.7	1.8
50.0	50.5 ± 0.9	1.7	50.9 ± 0.8	1.6
60.0	60.9 ± 1.0	1.6	60.5 ± 1.0	1.6

tested to select a suitable medium for the release study of diclofenac from a transdermal patch.

It aimed to find such an acceptor medium which would ensure sufficient solubility of diclofenac from the viewpoint of the needs of the release and permeation experiments. In particular, it was necessary to find such a medium in which diclofenac is well soluble (ensuring sink conditions) but which would not simultaneously extract other components of tested patches in an undesirable manner, or which would not elute components of the excised skin in the course of the in vitro permeation study.

The results of both the elution and dissolution studies are shown in Table 2. It follows from the found values that the highest concentration of diclofenac was found in the case of elution with octanol. This liquid, however, due to its relatively high lipophilicity partially dissolved also the adhesive layer of the patch (which was also evident macroscopically in the appearance of the tested patches) and thus, increased the found values of the content of the drug. From the viewpoint of saturation, concentrations of diclofenac, mixtures of water and propylene glycol or polyethylene glycol, or a mixture of buffer pH 5.5 and polyethylene glycol were most advantageous.

On the basis of a comparison of the results of the elution and dissolution studies, as the first step two systems were selected as acceptor media for permeation experiments: a mixture water–polyethylene glycol and a mixture of the phosphate buffer pH 5.5–polyethylene glycol. The found values of the release coefficient k in the case of the release of diclofenac into an unbuffered acceptor medium were somewhat higher ($k = 7.9$ $\mu g/cm^2/h^{1/2}$) than in the case of the medium buffered with to pH 5.5 ($k = 6.1$ $\mu g/cm^2/h^{1/2}$) (see Fig. 2). This finding resulted in the selection of the unbuffered mixture water/PEG as the acceptor phase for subsequent release and permeation experiments.

3.3. Release of diclofenac from transdermal patches

The kinetics of release of the active ingredient belongs to the characteristics of the functionality of the patch with respect to therapeutic effect. Time dependence of release of diclofenac, both from the patch direct into the acceptor medium (study without a membrane) and from the patch through the excised skin into the acceptor medium (permeation study), was investigated.

Table 2
Concentration of diclofenac found in tested media in the elution and dissolution studies

Medium	Elution study	Dissolution study
	Concentration of diclofenac (mg/ml)	
Octanol	0.273	52.53
Water	0.031	101.82
Water–propylene glycol (3:2)	0.053	469.30
Water–polyethylene glycol (4:1)	0.055	472.74
Buffer pH 5.5–polyethylene glycol (4:1)	0.057	479.13

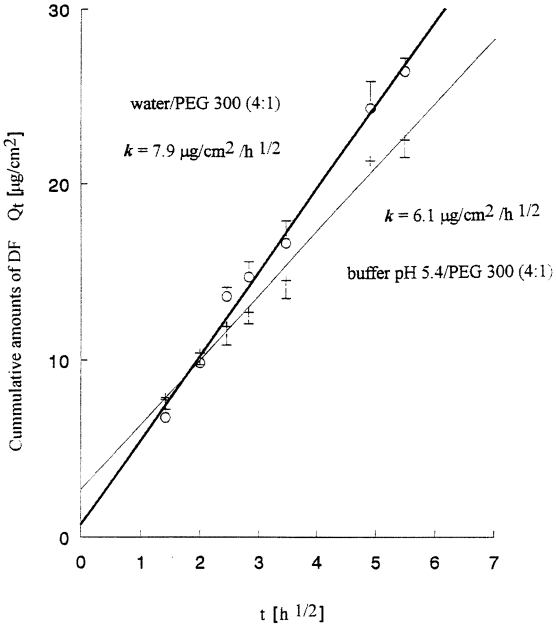

Fig. 2. Release profiles of diclofenac (DF) from the patches into two different acceptor media at 37°C ($n = 6$).

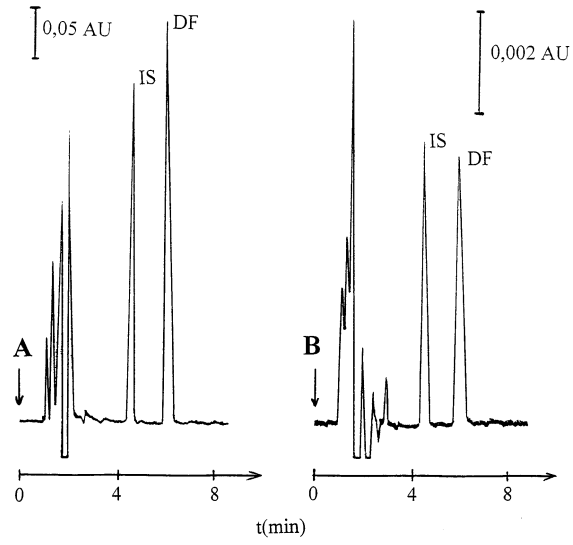

Fig. 3. A chromatographic record of the medium withdrawn after 24 h of diclofenac release from the patch direct (A) to the medium and through the skin (B) into the medium (DF, diclofenac; IS, internal standard).

In the release study, diclofenac concentration was determined from the calibration curve $y = 0.0109x + 0.1250$ ($r = 0.9999$) constructed with the use of an acceptor medium composed of PEG 300/water (1:4). The precision and reproducibility of the analysis was verified by measuring samples of a known diclofenac concentration prepared in this medium, in which medicated patches without diclofenac (only with adjuvant additives) were eluted first and then a defined amount of diclofenac was added to the extract. The found results of concentrations, including RSD are shown in Table 3, the values of RSD not being larger than 3.0%.

Within the framework of the analysis of the liquid acceptor medium from the release study, the residues were eluted in the course of the first 2 min (Fig. 3A) and they did not interfere with the peaks of analysed samples.

In the permeation study, diclofenac concentration was determined from the calibration curve $y = 1.9376x + 0.3331$ ($r = 0.9993$) constructed in the same acceptor medium. The precision and reproducibility of the evaluation was verified by analysing samples of a known diclofenac concen-

Table 3
Precision and accuracy of diclofenac evaluation in an extract coming from the acceptor medium in the release study

Added (μg/ml)	Within-day ($n = 6$)		Day-to-day ($n = 11$)	
	Found ± S.D. (μg/ml)	RSD (%)	Found ± S.D. (μg/ml)	RSD (%)
10.0	10.3 ± 0.3	2.7	10.2 ± 0.3	2.9
50.0	49.8 ± 1.0	2.0	50.5 ± 1.1	2.2
100.0	100.2 ± 1.8	1.8	100.8 ± 1.7	1.7
150.0	152.1 ± 2.2	1.5	151.8 ± 2.4	1.6
200.0	201.7 ± 2.7	1.3	202.4 ± 2.9	1.4

Table 4
Precision and accuracy of diclofenac evaluation in an extract coming from the acceptor medium in the permeation study

Added (μg/ml)	Within-day ($n = 6$)		Day-to-day ($n = 11$)	
	Found ± S.D. (μg/ml)	RSD (%)	Found ± S.D. (μg/ml)	RSD (%)
0.05	0.044 ± 0.002	4.1	0.043 ± 0.002	4.3
0.5	0.511 ± 0.012	2.3	0.050 ± 0.013	2.6
1.0	1.011 ± 0.015	1.5	1.015 ± 0.016	1.6
2.0	2.013 ± 0.028	1.4	2.012 ± 0.029	1.5
3.0	3.030 ± 0.032	1.1	3.025 ± 0.036	1.2

tration prepared in a medium into which patches were first eluted only with adjuvant additives (without diclofenac) and then a defined amount of diclofenac was added to this extract. The found results of concentrations, including RSD, are shown in Table 4; RSD values are not larger than 4.5%.

In the permeation study, the HPLC record also shows the residues extracted from the excised skin, but the peaks of the background of the acceptor medium did not extend a retention time of 3.5 min (Fig. 3B), thus not interfering with the peaks of analysed substances. The amount of diclofenac found in the permeation study shows a gradually increasing character, as seen in Fig. 4. The character of the course of the measured diclofenac permeation curves through the excised skin, as illustrated in Fig. 4, provided a possibility of problem-free numerical expression of average values J of the fluxes of diclofenac under study, so they served as an aid for the optimisation of the patches composition.

4. Conclusion

A method has been worked out for HPLC analysis of diclofenac making possible its evaluation both in the final preparation of the type of laminated adhesive transdermal patches and in the selection of a suitable acceptor medium. A part of the problems under study was also the use of the elaborated analytical method in the release and permeation study of diclofenac from the transdermal patch by the in vitro excised skin. The HPLC analytical method was applied to the control-analytical evaluation of a test series of TTS.

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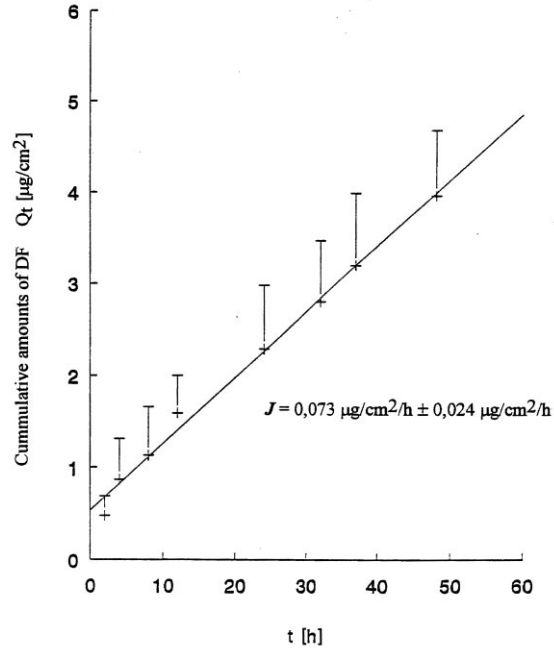

Fig. 4. Cumulative permeation profiles of diclofenac (DF) from six patches of one production bath (at 37°C).

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