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Analysis of Methotrexate from Formulations and Skin Specimens by HPLC

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ABSTRACT

A method for quantitative analysis of methotrexate (MTX) from formulated gels/creams and from tissue/solution samples after percutaneous permeation studies is described. High performance liquid chromatography (HPLC) with a MicroBondapak C_{18}, reverse-phase column was used. A calibration curve of doses versus peak areas for MTX showed linearity over the range of 0.010-14.056 μg with a very high correlation coefficient (r) of 0.99998. Replicate analysis of μg of MTX showed that the peak area was 9627424±22111 counts (mean ± SD; one count is 1/8 μV-sec, 1μV is 2×10^{-6}a.u.) with a relative standard deviation of 0.23%. The resolution factor between MTX and folic acid was 33.35 when the applied doses of both were 1 μg each. Results demonstrated that the photolytic products of MTX, samples from gel and samples extracted from hairless guinea pig skin after percutaneous permeation and epidermis/dermis separation by microwave technique, could be well detected by this method. Preliminary results are satisfactory and meet the requirements of U.S. Pharmacopeia XXII[1]. This report decribes novel approaches for determination of methotrexate content in the formulations and skin specimens. These approaches include the separation of epidermis and dermis by microwaving, the pretreatment procedures employed before analysis, and the use of guard column during HPLC analysis.

Key words : Methotrexate, Percutaneous Absorption, *In Vitro*, *In Vivo*.

INTRODUCTION

Methotrexate (MTX) is a folate antagonist with a wide spectrum of antitumor and immunosuppressant activity. MTX also is a valuable adjunct in the treatment of skin and connective-tissue disorders, non-malignant diseases such as cutaneous sarcoidosis, lymphomatoid papulosis, vasculitis, etc. Currently, MTX is administrated orally for severe cases of psoriatic patient and rheumatoid arthritis[2,3]. Severe adverse effects experienced with MTX include leucopenia, thrombocytopenia, gastro-intestinal effects, bone-marrow depression, liver damage, kidney damage, osteoporosis, pulmonary reac-

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tions (including interstitial pneumonitis), etc. Fatalities have occurred[4]. Topical administration for the treatment of skin and connective-tissue diseases probably is the best application route to minimize the potential therapeutic risks. Since MTX is poorly absorbed by the skin, topical application to date has not been successful.

For several years, we have made great efforts to develop its patented and proprietary Conrex Permeation Excipients, a series of macrocyclic permeation promoters[5-9]. The objective of this study is to develop an analytical method for the determination of MTX in the samples from products and skin tissue samples, both *in vitro* and *in vivo,* from experimental animals.

MATERIALS AND METHODS

Methotrexate (MTX) and folic acid were obtained from Sigma Chemical Company (St. Louis, MO. USA). MTX USP standard was obtained from USP. CPE 215, a pharmaceutical grade macrocyclic lactone, was provided by Conrex Pharmaceutical Corp (PA, USA). MTX preparation for calibration was prepared by dissolving 10.0 mg of MTX (>98% pure) in 100 ml of pH 6.0 buffer solution - acetonitrile mixture (90 : 10) in a volumetric flask[1,10]. This mixture was also used as the mobile phase in HPLC analysis. The pH 6.0 buffer solution was prepared by mixing 370 ml of 0.1 M citric acid solution with 630 ml of 0.2 M dibasic sodium phosphate solution. The final concentration of MTX was about 100μg/ml. This solution was filtered through a 0.22-μm Nylon membrane filter (MSI, Westboro, MA) before using.

For determining the resolution factor, MTX - folic acid mixture, about 0.1 mg per ml each, was also prepared by dissolving both MTX and folic acid in pH 6.0 buffer solution-acetonitrile mixture (90 : 10).

The stability of a 1% MTX gel was surveyed. For the purpose of performing MTX stability tests in acidic or alkaline conditions, MTX was prepared with low and high pH buffer solution. These two buffer solutions were also prepared by mixing different volumes of 0.2 M dibasic sodium phosphate and 0.1 M citric acid to adjust the pH to 3.0 or 8.3. For testing the stability of MTX at various temperature, MTX gel (1%) in protective amber vials was stored at three different temperatures, i.e., at 45°C in an incubator, room temperature (25 ± 1°C) and in a refrigerator (0-4°C) up to four weeks. MTX content was determined periodically by HPLC.

At given time intervals, gel samples were accurately weighed and dispensed in about 100 times the volume of pH 6.0 buffer solution-acetonitrile (90 : 10)in amber volumetric flasks. To remove CPE 215 and other liposoluble ingredients, methylene chloride was used to extract the gel samples three times. To remove the particulate, the above sample was further centrifuged by a centrifuge at 3400 rpm for 30 min followed by filtration with a 0.22 μm Nylon filter. Finally, MTX content was determined by HPLC.

MTX creams and gels for permeation studies were prepared using 0.2% MTX with 4% CPE 215, with or without co-enhancers A and B, other excipients and pH 4.5 buffer solution. This buffer solution was prepared by mixing 0.2 M dibasic sodium phosphate and 0.1 M citric acid. The excipients for preparing the creams were cetyl esters, cetostearyl alcohol, petrolatum, glyceryl monostearate, PEG-40 stearate, methylparaben and propylparaben. The excipients for preparing the gels were guam gum, ethanol, polysorbate 80, and sorbitan monooleate. The control cream was prepared using the same active ingredient and excipients of the test cream except to delete CPE 215. All samples, except those for the light exposure test, were routinely stored in amber glass vials in order to protect from light.

I. *In Vitro Percutaneous Permeation Study*

Franz diffusion cells[11] with a permeation surface area of 0.636 cm^2 were used throughout this study. This system includes amber Frank diffusion cells (FDC-400, Crown Glass Co.

Somerville, Nj), cell drive units and a heating bath/circulator. Freshly excised abdominal skin from female, 7-week old hairless guinea pig (IAF Ha Ho, Charles River Laboratories Inc., New Jersey) was cut into pieces and sandwiched between the two compartments of the permeation cell. The two cell halves were assembled together with a clamp and placed on the drive unit with stratum corneum facing the donor chamber. The subcutaneous surface was bathed from below by 5 ml of 2% Polysorbate 80 in 0.9% sodium chloride solution. In the receiving chamber, the solution was maintained at 37°C with constant stirring at 600 rpm. About 100 mg of 0.2% MTX, or 200 μg MTX, was applied onto the hairless guinea skin in the donor chamber. The permeation study lasted for 24-72 h.

Sample Collection and handling :

(I). Receiving chamber solution : The solution in the receiving chamber was collected at regular intervals of 8-16 h during permeation, in which 1.0 ml of receptor chamber solution was taken and replaced by an equal volume of 2% Polysorbate 80-0.9% sodium choloride solution each time. The receiving chamber solution was then filtered by a 0.45 μm Nylon syringe filter (MAGNA, Cameo 3N, Fisher Scientific) and directly assayed by HPLC. At the end of permeation study, the amount of MTX determined in the receiving chamber solution was regarded as the amount of MTX permeating through the whole layer of skin.

(II). Skin : At the end of each experiment, the skin surface was washed briefly by using 1 ml of 2% Polysorbate 80-0.9% sodium chloride solution three times. The surface of skin was carefully inspected and grease, if any, over the surface was carefully removed using cotton swabs. Then, the skin was removed from the permeation cells and placed on a weighing disc in a microwave oven (GoldStar, Seoul, Korea, Model No. MA-400). An exposure of 8 seconds at medium low setting was sufficient to allow a separation of the epidermis from the dermis. This microwave techniuqe has been successfully and routinely used in our skin uptake studies from topical dosage froms in both *in vitro* and in *vivo* experiments.

(III). Extraction of MTX from skin : After adding one ml of 0.9% sodium chloride to the epidermis or two ml of 0.9% sodium chloride to the dermis in test tubes, the tissue was minced by a high speed Tissue-Tearor (Model 985-370, Biospec Products, Inc., Dremel, Racine, Wl). Two ml of methanol was added into each tube. This mixture was shaken mechanically for 20 min, and then centrifuged at 3400 rpm for 30 min. The supernatant was filtered by a 0.45 μm Nylon syringe filter and assayed by HPLC.

II. *In Vivo Percutaneous Permeation Study*

Female, 7-week old hairless guinea pigs were also used in the *in vivo* portion of this study. Animal were anaesthetized with a rapid-acting anesthetic Ketamine chloride (20-40 mg/kg, i.m). and the abdominal skin was cleaned with gauze soaked with saline. A 10 cm^2 surface area for the application of gel/cream was marked on the abdominal skin surface. About 50 mg of the MTX creams (0.2%) was applied twice a day at intervals of 8 h for four days on the same area. The total dose was 400 mg of gel/cream, or 800 μg of MTX per animal. An occlusive dressing, including a piece of sterile gauze coated with Saran wrap, was used to cover the skin surface of the applied area and further fixed externally by a medical bandage (Johnson & Johnson). At the end of experiment, the animals were euthanized by carbon dioxide asphyxiation and the skin was excised from the animal prior to microwave treatment.

Skin Excision and Separation :

The skin area for gel/cream application was excised immediately after carefully washed with gauze soaked in 0.9% sodium chloride three ti-

mes to remove any preparation remaining on the skin surface. A microwave technique was used for the separation of epidermis from dermis[12]. The skin sample was laid out flat with the epithelium side upward on a weighing disc and placed in a microwave oven (GoldStar, Model No. MA-400). An exposure of 8 seconds at medium low setting was sufficient to allow a separation of the epidermis from the dermis.

III. *Assay*

Samples were assayed by high performance liquid chromatography[1,2,13]. A Hewlett-Packard HPLC apparatus - HP 1050 Series was used. This apparatus includes a pumping unit, an autosampler, a variable wavelength (visible and ultraviolet) detector and a HP 3396 series II integrator. The operating conditions were as follows :

HPLC Column : MicroBondapak-C_{18}, 10 μ m, 300 mm $\times$ 3.9 mm, stainless steel column with Guard-Pak Holder and Guard Pak™ Pre-Column Inserts as guard column (Waters, Division of Milipore Corp. MA).

Mobile Phase : pH 6.0 Buffer Slution-Acetonitrile, 90 : 10.

Flow Rate : 1.2 ml/min.

Detection wavelength : 302 nm.

Sample Inject Volume : 5 or 15 μl per injection for calibration and 20-40 μl for receiving chamber solution and for skin samples.

RESULTS AND DISCUSSION

Calibration

Different doses of MTX from 0.01 −14.056 μg in 1 − 14 μl of pH 6.0 buffer solution-acetonitrile (90 : 10) were examined. The retention time of MTX peak in 26 determinations was 7.480 $\pm$ 0.145 min (mean $\pm$ SD). Peask areas were measured and expressed as counts (one count is 1/8 μV-sec, 1 μV is 2 $\times$ 10^{-6} a. u.). A calibration curve for MTX showed linearity over the range of 0.010 − 14.056 μg with a very high correlation coefficient (r) of 0.99998, n = 26 (Figure. 1). The linear regression equation was:

$$y = 8047 + 9606305x$$

where y is the peak area of MTX in counts, and x the dose in micrograms.

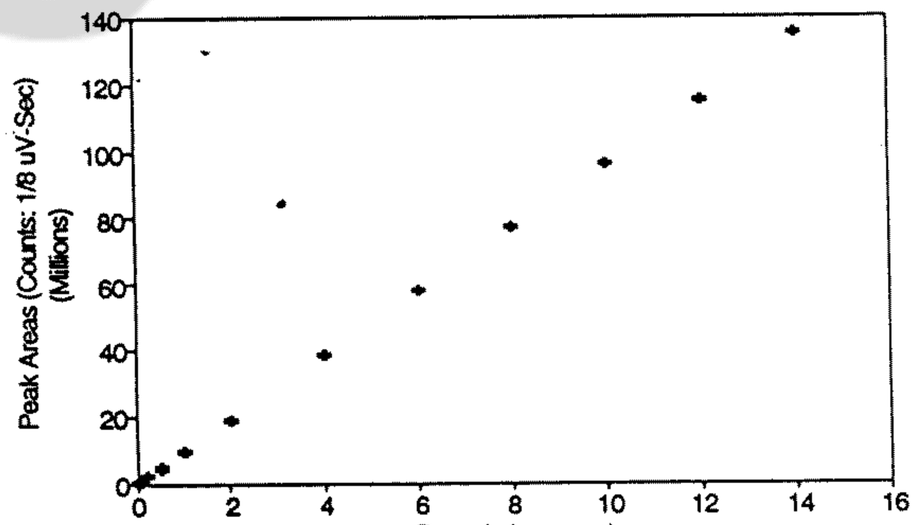

Figure 1. Calibration. MTX doses versus peak areas in 26 determinations. Peak areas were expressed as counts (One count is 1/8 μV-sec, 1 μV is 2 x 10^{-6} a.u.) The linear regression equation is: y = 8047 + 9606305x. Where y is the peak area of MTX, and x the dose of MTX in μg. r = 0.99998, n = 26.

Precision

With seven replicate injections of 1 μg in 10 μl, the peak area was 9627424 $\pm$ 22111 counts (mean $\pm$ SD) with a relative standard deviation of 0.23%. This result meets the requirement of U. S. Pharmacopeia XXII "not more than 2.5%" in six replicate injections[1].

Accuracy

Assay samples were accurately prepared by dissolved MTX (Singma, Lot No. 118F0443) in pH 6.0 buffer solution-acetonitrile. The final concentration of MTX was 75.0 μg/ml or 50.0 μg/ml. The peak areas of six replicate injections (10 μl each, i.e., 0.75 μg or 0.50 μg of MTX per injection) were 7280186 $\pm$ 25229 and 4896904 $\pm$ 15960 counts, respectively. Comparing these mean values with the mean value of the six replicate determinations (10 μl each, i.e., 1.0 μg of MTX per injection) of MTX standard solution in a oncentration of 100 μg/ml (9793679 $\pm$ 18949 counts), the recoveries were 74.34 μg/

ml and 50.00 μg/ml with recovery rates of 99.1140% and 100.0013%, respectively.

Resolution

It is to be expected that its analog folice acid might interfere with the analysis. In this study, the retention time of folic acid was 3.299 ± 0.027 min (mean ± SD). The resolution factor R_s was calculated by equation as following:

$$R_s = \frac{t_2 - t_1}{1/2(w_2 - w_1)}$$

where, t_1 and t_2 are the retention times and, w_1 and w_2 the peak width of the compound 1 and 2, respectively[1].

The chromatogram obtained from this mixed solution showed the MTX could be well separated from folic acid (Figure. 2). In this study, the doses of both MTX and folic acid were about 1 μg each in 10 μl. The resolution factor between these two compounds, calculated from the mean of eight injections, was 33.35. This result also meets the requirement of U. S. Pharmacopeia XXII "not less than 8.0" in 6 replicate injections[1].

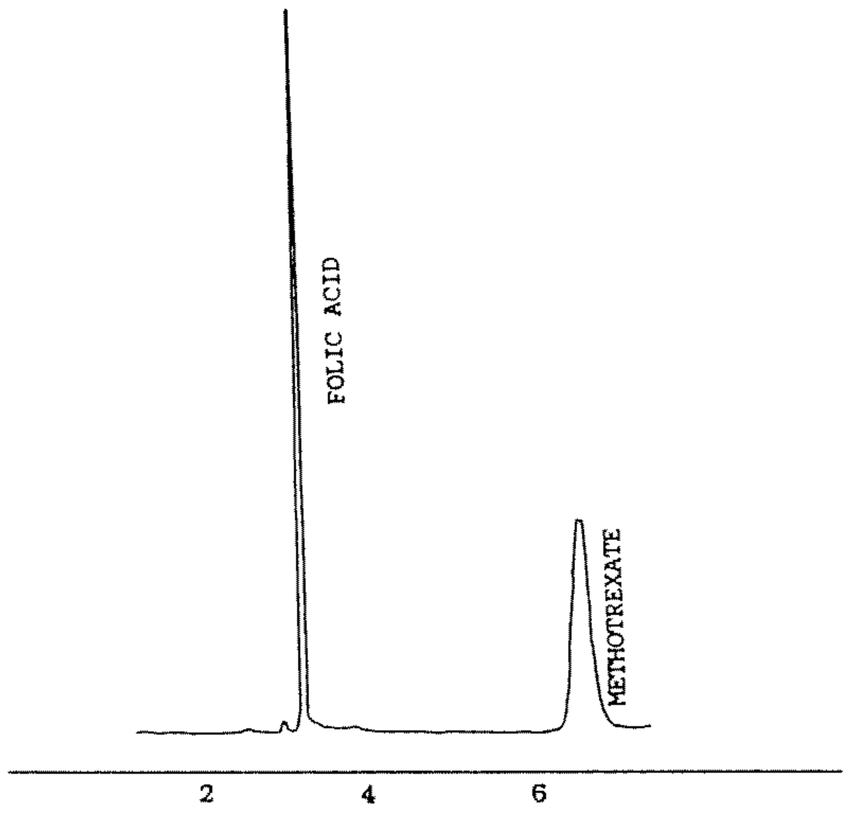

Figure 2. Resolutin between MTX and folic acid. The chromatogram obtained from MTX and folic mixed solution contains about 1 μg of each compound. The resoltution factor between these two compounds, calculated from the mean of eigh injections, was 33.35.

The Stability of MTX under Different Conditions

Short-term stability of MTX stored under different conditions was surveyed. MTX so-

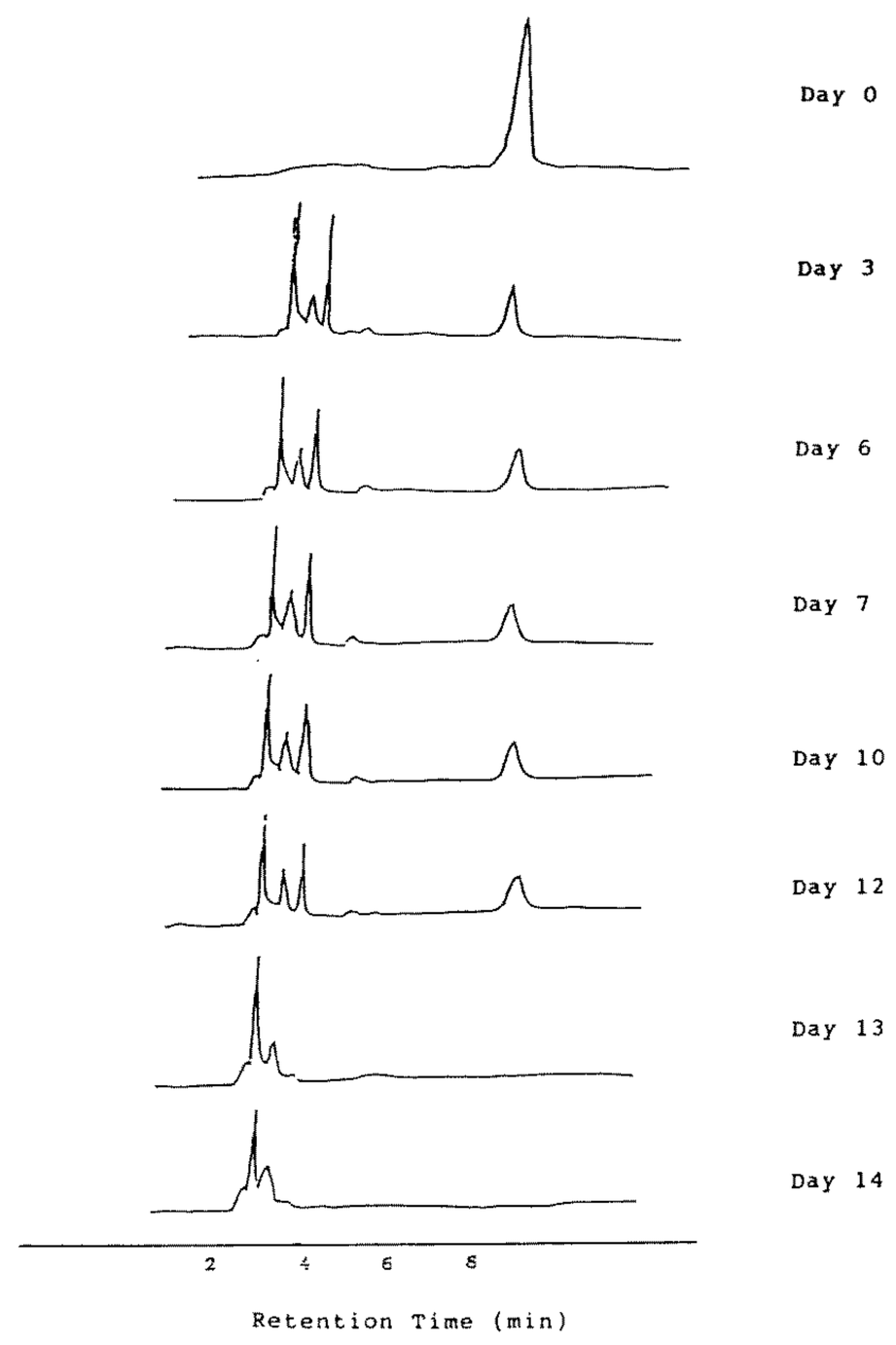

Figure 3. The chromatograms of MTX in low/high pH buffer solution and after exposure to the daylight. Group I & II: MTX was dissolved in pH 3.0 or pH 8.3 buffer solution-acetonitrile and stored in amber vials to protect from light. Results showed no significant decomposition. Group III: MTX was dissolved in pH 6.0 buffer soltuion-acetonitrile, stored in transparent vials and exposed to the daylight. Results showed that the peak area of MTX decreased progressively and almost compppletely disappeared within 12 days.

lution, perpared with pH 6.0 buffer solution-acetonitrile and stored in amber vials to protect from light, could be maintained stable at least for two weeks without significant decomposition. However, the decomposed products could be detected if MTX preparation was stored in transparent vials and exposed to the daylight. The peak area of MTX decreased to 25% after 3 days, 16% after 4 days and almost completely disappeared after 12 days. At the same time, more than 5 new peaks, with retention times of around 2-5 min (Figure. 3) appeared within 3 days. These peaks initially increased and finally decreased progressively. There were no significant change in MTX concentration within a period of two weeks while MTX samples were prepared by pH 3.0 or pH 8.3 buffer solution - acetonitrile (Table. 1 & Figure. 4). These results show that MTX is a light sensitive compound and will eventually be destroyed even by exposing to mild daylight in few days.

Comparing with the MTX reference standard solution, the recovery rates of MTX from fresh prepared gel were 90.94 ± 2.96% by HPLC. The recovery rates of MTX from gel after storage at different temperature for four weeks in amber vials were 91.46 ± 2.49% (mean ± SD) at 45°C, 91.08 ± 2.36% at 25 ± 1°C and 87.94 ± 1.95% at 0-4°C. These results showed that, in the absence of light, there were no significantly change in MTX content in the gel even kept at 45°C for four weeks.

In Vitro Percutaneous Permeation Study

To give confidence in the use of permeation test for pharmaceutical and pre-clinical assessments, experiments were performed *in vito* and *in vitro* to see if applying different formulations of model preparations which contained same concentration of MTX produced various responses. Table 2 and Figure 5 illustrate the amount of MTX in guinea pig skin and receptors after 48 h *in vitro* permeation. Formulation (G-301) containing CPE 215 in the gel increased the amount of MTX residence in the skin (retention, $p < 0.01$) as well as the amount across the skin

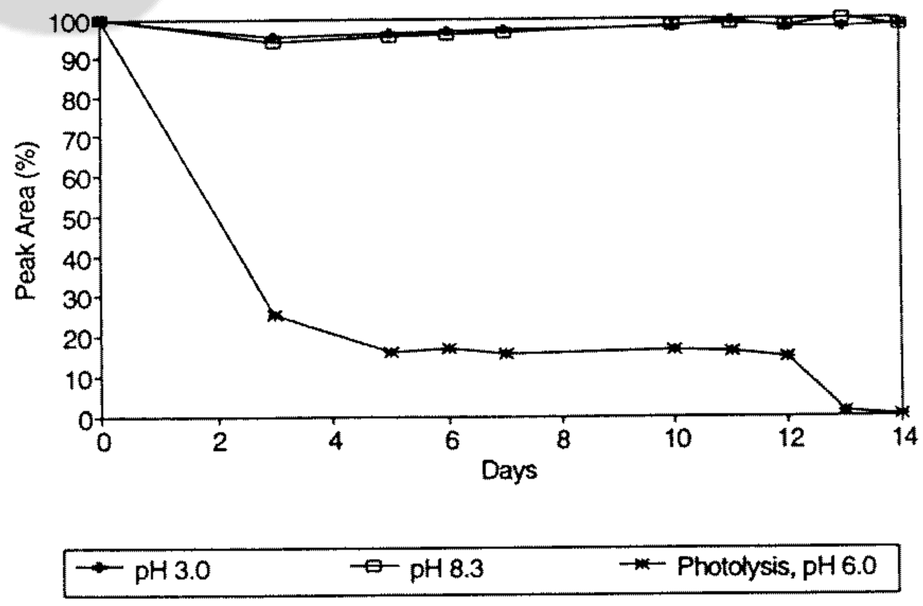

Figure 4. MTX stability in the low/high pH buffer soltuion or after exposure to daylight.

to the receptor (permeation, $p < 0.05$). Formulations containing CPE 215 with 5% propylene glycol or 5% glycerol in the creams did not further increase MTX retention and permeation.

Selection of optimal formulations of topically targeted drugs should achieve optimal retention in specific regions of the skin, and not necessarily high permeation which is required for systemically targeted drugs. Drug retention and permeation appear to be inversely related. Knowledge of the drug's local tissue concentration offers a parameter which can be used to make this differentiation and to optimize the formulation further and reevaluate it for clinical efficacy[14].

In Vivo Percutaneous Permeation Study

Since a microwave technique was used for the separation of epidermis from dermis skin samples, a control study was performed. MTX samples were prepared with known amounts of MTX and the MTX content in these samples were assayed before and after the exposure to microwave.

MTX solution containing about 1 mg MTX in each ml of mobile phase was sealed in amberized vials and exposed to microwave for 8 seconds (same for the skin samples). The MTX content of these solutions was assayed triplicately by HPLC. The recovery rates of MTX (com-

Table 1. MTX Stability Test

	I		II		III	
	pH 3.0		pH 8.3		pH 6.0 Photolysis	
T(day)	Area	A/C(%)	Area	A/C(%)	Area	A/C(%)
0	12304476	100.00	10010938	100.00	9540337	100.00
3	11720212	95.25	9412298	94.02	2417649	25.34
5	11840272	96.23	9529133	95.19	1526976	16.01
6	11885701	96.60	9596126	95.86	1593464	16.70
7	11942300	97.06	0643795	96.33	1508223	15.81
10	12019812	97.69	9807558	97.97	1576969	16.53
11	12227288	99.37	9883290	98.72	1538842	16.13
12	12026100	97.74	9826704	98.16	1412681	14.81
13	11974208	97.32	10000461	99.90	83936	0.88
14	12074616	98.13	9821486	98.11	5450	0.06

I. MTX gel was prepared with pH 3.0 buffer soltuion and stored in amber vials. II. MTX gel was prepared with pH 8.3 buffer solution and stored in amber vials. III. MTX gel was prepared by pH 6.0 buffer solution, stored in transparent vials and exposed to daylight. The peak areas were compared with the peak area obtained at day 0 and expressed as percentages.

Table 2. MTX in Skin and Receptor (*in vitro* single application, permeation 2 days)

	Epidermic & dermis $\mu g/cm^2$		Recptor $\mu g/cm^2$	
	Mean	SEM	Mean	SEM
Control Cream	1.989	0.017	0.010	0.010
G-301	10.657**	1.794	2.242*	0.600
C-26(5% PG)	9.575*	1.657	2.644*	0.713
C-27(5% G)	10.491**	1.387	1.510	0.631

Hairless gunea pig abdominal skin *in vitro* permeation for 2 days. 100 mg of the 0.2% MTX cream or 200 μg of MTX was applied onto 0.636 cm^2 surface area of freshly excised skin. 0.2% MTX cream was prepared using the active ingredient MTX, with 4% of CPE 215, propylene gllycol (PG) or glycerol (G), and other excipients. The other excipients were cetyl esters, cetostearyl alcohol, petrolatum, glyceryl monostrearate, PEG-40 stearate, methylparaben and propylparaben. Control cream was prepared with the same active ingredient and excipients except to delete CPE 215 and propylene glycol and glycerol. G-301 was prepared with CPE 215, but without propylene glycol and glycerol in the gel. C-26 and C-27 creams were prepared using the same basic formulation containing CPE 215 incorporated with 5% propylene glycol or 5% glycerol. n = 3 each group. t-test results expressed as differences related to control cream: (*) $p < 0.05$; (**) $P < 0.01$.

pared with the MTX content in the same MTX solution before the microwave treatment) were 100.17 ± 0.71%, 99.93 ± 0.64% and 99.83 ± 0.28%, respectively.

Table 3 and Figure 6 illustrate the amount of MTX in the epidermis and dermis after eight topical applications, 50 mg each, onto the same skin area within 96 hours. The MTX content

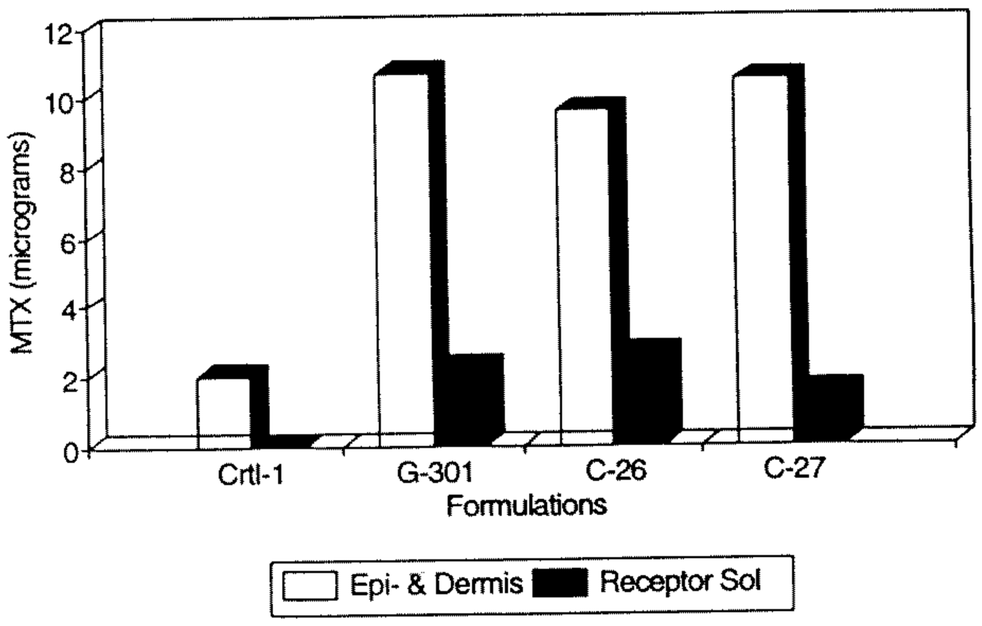

Figure 5. Hairless guinea pig abdominal skin *in vitro* permeation for 2 days. 100 mg of the 0.2% MTX cream or 200 μg of MTX was applied onto 0.636 cm^2 surface area of freshly excised skin. 0.2% MTX creams were prepared using active ingredient MTX, with 4% of CPE 215, 5% propylene glycol, or 5% glycerol, and other excipients. Control cream was prepared with the same active ingredient and excipients except to delete CPE 215, propylene glycol and glycerol. G-301 was prepared with CPE 215, but without propylene glycol and glycerol. C-26 and C-27 creams were prepared using the same basic formulation containing CPE 215 incorporationg of 5% propylene glycol or 5% glycerol.

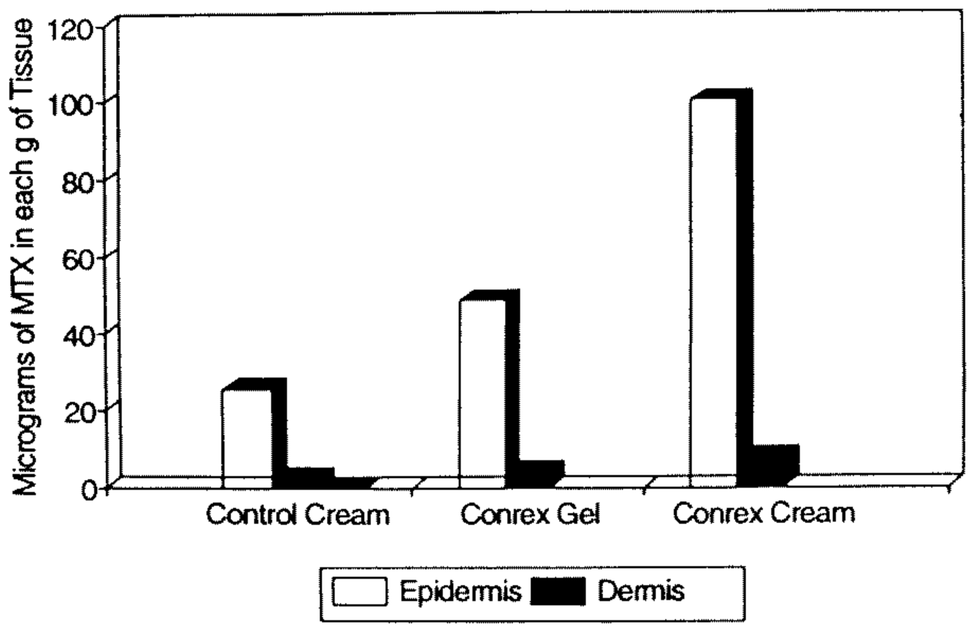

Figure 6. Hairless guinea pig abdominal skin *in vivo* permeation for 4 days. 50 mg of the same 0.02% MTX cream, gel or control cream was applied onto the same 10 cm^2 skin surface for eight times (at 8 a. m. and 4 p.m. each day). The total dose was 400 mg of cream, or 200 μg of MTX.

after topical application of Conrex cream, a formulation incorporated MTX with CPE 215, was about 3-4 times higher in the dermis ($p < 0.01$) and epidermis than the content after application of cream control.

HPLC method has been widely used recently for the quantitative determination of methotrexate, methotrexate-monoclonal antibody conjugates and its metabolites[15-21]. The other methods used infrequently for the determination of MTX are fluorescence polarization immunoassay[16], enzyme immunoassay[22] and radioimmunoassay[23]. The chief advantages of HPLC method is simplicity and rapidity in the estimation of MTX. MTX in biological fluid (such as serum, plasm, urine, bile, peritoneal dialysis fluid, etc.), biopsy specimens, and samples from cream, gel and/or other pharmaceutical products were well separated and assayed by HPLC. It is sensitive, up to 0.01 microgram/ml, which is three times below the toxic MTX concentration[16]. Although somethimes this method requires the use of relatively large biological sample volume to obtain precise quantitative results, however, this is rarely necessary in practice. Because of specificity, this method is recommended for therapeutic drug monitoring. The results of this study also indicated that this method is valuable for the determination of MTX from the skin tissue samples, both *in vitro* and *in vivo*, in percutaneous permeation studies.

For *In vitro* and *in vivo* studies, the selection of experimental tissue or animal species to be used is still an area of active research and exploration. Hairless guinea pig skin was used in these studies, because π value, a measure of the degree to which a particular skin can differentiate among compounds of increasing lipophilicities, is very close to that obtained for the human shin[24]. Information is needed to establish the correlation between data on drug retention in skin from *in vitro, in vivo* as well as from clinical

Table 3. MTX Retention in Skin (*in vivo* multiple topical application for 4 days)

	Epidermic μg/g		Dermis μg/g	
	Mean	SEM	Mean	SEM
Control Cream	25.52	10.79	2.32	0.48
Conrex Gel	48.37	4.89	3.86	1.09
Conrex Cream	100.17	33.73	7.43**	0.82

Hairless pig abdominal skin in vivo permeation for 4 days. 50 Mg of the same 0.02% MTX cream, gel or control was applied to the same 10 cm^2 skin surface for eight times (at 8 a.m. and 4 p.m. each day). The total dose was 400 mg of 0.2% MTX cream/gel or 800 μg of MTX. 0.2% MTX cream and gel were prepared using active ingredient MTX, with 4% of CPE 215, and pH 4.5 buffer solution. The excipients for preparing cream were cetyl esters, cetostearyl alcohol, petrolatum, glyceryl monostearate, PEG-40 stearate, methylparaben and propylparaben. The exipients for preparing gel were guam gum, ethanol, polysorbate 80, and sorbitan monooleate. Control cream was prepared with the same active ingredient and excipient except to delete CPE 215. n = 3 each group. t-test results expressed as difference related to the control cream: (**) $p < 0.01$.

studies.

The microwave technique reported here is simpler than the other techniques such as chemical, hot water immersion and hydration, enzyme digestion, adhesive tape stripping and mechanical methods[12]. The adhesive tape stripping method provides mainly stripped stratum corneum, as opposed to epidermis/dermis separation. In contrast, the microwave technique provides a facile separation of epidermis and dermis. Exposure duration is critical. After a series of experiments, in our hands, an exposure of 8 seconds at medium low setting was sufficient to allow a separation of the epidermis from the dermis. If an optimal exposure time was used, the peeling process was easy. This microwave technique has been successfully and routinely used in our skin uptake studies from topical dosage forms in both *in vitro* and in *vivo* experiments.

The problem was encountered during the extraction of MTX from the skin sample using methanol as solvent. It was found that lipid substance was contaminated in the extract. This would cause the blockerage of the flow of eluting solvent during HPLC analysis, leading to the increase in the pressure built up in the column. In order to minimize this problem, we employed pretreatment procedures before injecting into HPLC. This pretreatment column consists of the same silicone material as C_{18} μ Bondapak in the analytical column. We found out that the use of this pretreatment procedure can avoid the problem stated above.

In conclusion, this paper describes novel approaches to analyze methotrexate from both formulations and skin specimens. These novel approaches include the separation of epidermis and dermis by microwaving, the pretreatment procedures employed before analysis, and the HPLC methods for analyzing methotrexate. With view to the future potential applications of topical and transdermal applications of methotrexate for the treatment of psoriasis, rheumatoid arthritis, cutaneous sarcoidosis, and other diseases, this paper provides useful methodology, techniques, and information for those who are interested in methotrexate products. It also provides analytical methods for determining the content of methotrexate in current products.

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應用高效液相色譜分析藥劑和皮膚樣品中的甲氨蝶呤

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摘　要

本文介紹以高效液相色譜分析藥劑和透皮試驗後眞皮及內皮中的甲氨蝶呤。採用MICRO-BODAPAK C_{18}, 20mm × 3.9mm反相柱。藥量（範圍0.010至14.056 ug)和峰面積的標準曲線呈良好的直線關係。相關係數高達0.9999。用1 ug的甲氨蝶呤重覆分析七次，其峰面積爲9627424＋22111點（平均數＋標準差；每點等於1/8毫伏特一秒，每毫伏特爲2×10^{-6}a.u.），其相對標準差爲0.23%。甲氨蝶呤和葉酸的用量各爲1 ug時，其分離系數爲33.54。此法可很好地測出凝膠和透皮試驗後眞皮及內皮中的甲氨蝶呤。初步結果令人滿意，合乎第二十二版美國藥典的各項要求。本文提供新的皮膚分離方法（利用微波爐），皮膚抽取液的前處理，和在甲氨碟呤的高效液相分析中保護柱的使用。