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RP-HPLC method with fluorescence detection for determination of small quantities of triamcinolone in plasma in presence of endogenous steroids after derivatization with 9-anthroyl nitrile; pharmacokinetic studies☆

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Abstract

A new indirect RP-HPLC method was developed for determination of small, ng/ml, concentrations of triamcinolone (TMC) in human plasma, in presence of endogenous corticosteroids: cortisol (hydrocortisone, F), cortisone (E) and their metabolites, after administration of TMC in a free alcohol form. After solid phase extraction (SPE) in cartridges with octadecyl phase, TMC and prednisolone (I.S.) were derivatized by treatment with 9-anthroyl nitrile (9-AN) in a basic mixture, consisting of triethanolamine and quinuclidine, to receive fluorescent esters at 21-hydroxyl group of the steroid chain. Optimal conditions were also established to purify fluorescent TMC and I.S. derivatives before injection into HPLC column. The fluorescent esters were determined using an isocratic RP-HPLC technique in a C_{18} column. The mobile phase consisted of acetonitrile and 0.3 mM *ortho*-phoshoric acid. The method was validated before using to pharmacokinetic studies. Calibration curve of TMC was linear in the range of 2.5–100.0 ng/ml. Intra- and inter-day measurement precision and accuracy were equal to or lower than 15%. Percent recovery, and limits of detection (LOD) and quantification (LOQ) of TMC were also determined. The method was applied for in vivo conditions after administration of tablets containing TMC to healthy volunteers. Moreover, the method provided potential to determine TMC and, simultaneously, other glucocorticoids: E, F and their metabolites in one analytical run. Column interactions were observed between endogenous metabolites of E. Usefulness of the elaborated method was confirmed in pharmacokinetic studies following administration of a small (4 mg) dose of TMC to human volunteers. The method can provide an alternative to HPLC coupled with RIA in determination of small quantities of TMC.

Keywords: Triamcinolone free alcohol form; Solid phase extraction; Derivatization; Endogenous steroid metabolites; Interaction; Validation; Tablets

1. Introduction

Triamcinolone (TMC, 9α-fluoro-11β,16α,17α,21-tetrahydroxy-1,4-pregnandiene-3,20-dione) is a synthetic glucocorticoid, one of several derivatives of hydrocortisone widely used in medical practice to reduce swelling and to decrease the body's immune responses. TMC is administered orally in small doses, initially 4–16 mg/day for treatment of autoimmune and allergic conditions. In its anti-inflammatory activity, TMC itself is approximately one or two times as potent as prednisolone. Triamcinolone acetonide (TCA), a derivative of TMC, was the first halogenated corticosteroid to be widely used topically and proved to be more effective in treatment of psoriasis than any previously used corticosteroids [1,2]. However, TCA is an adduct of TMC and acetone but it is not a prodrug of TMC. 6-Hydroxytriamcinolone acetonide, but not TMC, is the major metabolite of TCA. After administration of TCA to healthy volunteers, trace amounts of TMC were found in some plasma samples but not in the urine of the volunteers [3,4]. The other derivative of TMC, triamcinolone hexacetonide (TCH) is always applied in treatment. However, the three synthetic glucocorticoids exhibit different solubility in various solvents. Solubility of TMC (1 in 40 in methanol, 1 in 240 in water) is greater than that of TCA (1 in 140 in methanol, almost insoluble in water) and TCH (slightly soluble in methanol, almost insoluble in water) [1]. It causes TMC to be freely absorbed from the GI tract, as compared to TCA and TCH. The absolute bioavailability of the inhaled and oral 2–5 mg formulations of TCA is relatively low,

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about 20%, and indicates incomplete absorption following oral administration, likely to result from the first pass effect [5,6]; however, the influence of lower solubility cannot be excluded. Low solubility of TCH causes it to be absorbed slower than TCA after intra-articular administration. TCH was completely absorbed from the site of injection over a period of 2–3 weeks [7]. However, small concentrations of TCA cause a significant decrease in the number of lymphocytes in circulating blood and significant cortisol suppression [5–7].

Few HPLC methods have been published for determination of TCA [3–6] and none for TMC. The HPLC methods with UV detection were used for pharmacokinetics studies on TCA in plasma after high (40 mg and larger) doses [3,4], but presented limits of quantification (LOQ) were quite variable; i.e. 0.1 μg/ml [3] and 0.6 ng/ml [4]. In order to improve sensitivity in the lower LOQ of TCA the RIA method was used. However, specificity of the method seemed to be rather doubtful [4]. The problem of RIA specificity was solved by combining RIA with HPLC. The HPLC-RIA method was applied in pharmacokinetic studies on TCA after intravenous, oral or inhaled administration of 2–5 mg TCA. Nevertheless, important details of the method were unreported [5,6]. The other way to improve sensitivity of the HPLC methods for determination of some glucocorticoids by HPLC involved pre-column derivatization of the compounds with 9-AN. The fluorescent reagent forms fluorescent esters through the primary hydroxyl group at the 21 position of the carbon chain of E, F, prednisone, prednisolone, 6-β-hydroxycortisol and 6-β-hydroxyprednisolone. However, no HPLC method is available for determination of TMC. Purification of the received fluorescent esters of glucocorticoids from reagents used for derivatization before injection into the column also presented a very important issue [8]. A very sensitive GC–MS method was also published for determination of TCA in human bronchoalveolar lavage fluid, where analytes were converted to acetate derivatives before GC–MS analysis [9].

The paper presents a highly sensitive, specific, and validated HPLC method for determination of TMC in human plasma. Fluorescent derivatives of TMC, endogenous glucocorticoids and their metabolites, were determined simultaneously in one analytical run. Purification of the fluorescent 9-AN esters of TMC from reaction mixture was performed using cartridges with C_{18} phase. The method was used in pharmacokinetic studies on healthy volunteers after administration of a single tablet, containing 4 mg of TMC in a free alcohol form. Pharmacokinetic data on TMC provided by the method confirmed its analytical usefulness.

2. Experimental

2.1. Materials

Triamcinolone (Farmabios, Italy) and prednisolone (Henan Lihua, China) were obtained from PFC Polfa Pabianice, Poland. Cortisol, tetrahydrocortisone (THE), tetrahydrocortisol (3α,17α,21-trihydroxy-5β-pregnane-11,20-dion) (THF) originated from Research Plus Inc. (Bayonne, NJ, USA). Cortisone, 5α-pregnane-3α,17α,21-triol-11,20-dione (alloTHE), 5α-pregnane-3α,11β,17α,21-tetrol-20-one (alloTHF), 5α-pregnane-3β,11β,17α,21-tetrol-20-one (3βalloTHF), 3βtetrahydrocortisol (5β-pregnane-3β,11β,17α,21-tetrol-20-one) (3βTHF), quinuclidine, 99% triethylamine were purchased from Sigma–Aldrich Chemie, Steinheim, Germany. Eighty-five percent *ortho*-phosphoric acid, acetone (P.O.Ch, Gliwice, Poland) were used. Methanol, acetonitrile (J.T. Baker, Deventer, The Netherland), *n*-hexane, dichloromethane (Merck, Darmstadt, Germany) were HPLC grade. Adequate amounts of dried molecular sieves with 4 Å pores were added to acetonitrile to remove moisture. Demineralised water (0.1 μS/cm) was always used (Seradest USF 1900, USF Seral, Germany). 9-Anthroyl nitrile was purchased from SynChem Laborgemeinschaft OHG (Kassel, Germany). Human plasma was obtained from healthy volunteers in the Regional Blood Bank and Haemotherapy Center in Poznań.

2.2. Preparation of reactants for derivatization

Solutions of 12.5% triethylamine (TEA), 0.5% quinuclidine (Q) and 0.2 mg/ml 9-AN were prepared in anhydrous acetonitrile. The solutions of TEA and Q were mixed at the ratio of 4:1 to receive a final mixture containing 10% TEA and 0.1% Q.

2.3. Apparatus and HPLC conditions

TMC, after extraction from human plasma and pre-column derivatization, was determined in a high performance liquid chromatograph HP 1100 (Hewlett Packard, Vienna, Austria). Chromatographic separation of the analyte, I.S. and endogenous substances, all previously converted to 9-AN derivatives, was accomplished in the LiChrospher RP-18 column (125 mm × 4.6 mm, d.p. 5 μm) which was protected by a guard column (both from Merck, Darmstadt, Germany). The mobile phase consisted of acetonitrile and 0.3 mM *ortho*-phoshoric acid, pH 4.6 (470:530, v/v), filtered through a 0.45 μm cellulose membrane filter (Sartorius, Goettingen, Germany). Before application to the HPLC, the mobile phase was de-aerated using an ultrasonic bath (UM-4 Unitra, Olsztyn, Poland) and a degasser (model G1322A) and pumped by a quaternary pump (model G 1322A) at the flow rate of 2 ml/min. Fluorescence of TMC derivatives was measured at 460 nm emission wavelength with the excitation at 360 nm wavelength, using FL detector at PMT gain equal 18. Extracted and derivatized samples of analytes were injected into the analytical column using an autosampler (model 1314A). The cartridges for solid phase extraction (SPE), of 3 ml capacity with octadecyl phase chemically bound to silica gel (500 mg) (Bakerbond SPE^{TM}, J.T. Baker, Deventer, The Netherlands), were applied for isolation and purification of analytes from serum after derivatization procedure. HP 1100 chromatograph was equipped with ChemStation, used for instrument control, data acquisition and data analysis. The system was controlled by Windows Software.

2.4. Standard solutions for calibration curve of TMC in human plasma

Stock solutions of TMC of 100 μg/ml and prednisolone (I.S.) of 500 μg/ml were prepared by dissolving their appro-

priate amounts in anhydrous acetonitrile, previously treated with the 4 Å pore molecular sieves to remove moisture. The standard solutions of 2.00; 1.00; 0.50; 0.30; 0.20; 0.15; 0.10 and 0.05 μg/ml TMC and 2.00 μg/ml I.S. were prepared in the same anhydrous solvent. The standard solutions were stable for a month when refrigerated. The 50 μl volume of the samples of TMC and I.S. were transferred by automatic pipette (Eppendorf, Hamburg, Germany) to glass screw vials, each containing 1.0 ml of blank plasma. The resulting concentrations of TMC were 100.0; 50.0; 25.0; 15.0; 10.0; 7.5; 5.0 and 2.5 ng/ml and 100.0 ng/ml of I.S.

2.5. Procedure for solid phase extraction of plasma samples

Cartridges with C_{18} phase were applied for extraction of TMC from human plasma. Before use, each SPE cartridge was conditioned by means of 5 ml methanol, 3 ml demineralised water, 3 ml acetonitrile and 3 ml demineralised water (in the given order). The cartridges were reused several times. Then, plasma samples containing TMC and I.S were acidified using 0.2 ml 0.1 mol l^{-1} *ortho*-phosphoric acid and then shaken and inserted into conditioned SPE columns. Cartridges with absorbed plasma were rinsed using 3 ml of demineralised water. Subsequently, the analytes were eluted by 1 ml of acetonitrile under low vacuum (2–5 mmHg) using a water pump. Acetonitrile was evaporated to dryness at a gentle stream of nitrogen at 40 °C.

2.6. Derivatization procedure

The evaporated residue of plasma extract was dissolved in 50 μl of anhydrous acetonitrile and 100 μl of the 10% TEA and 0.1% Q solution, and some molecular sieves were added. Then, 100 μl of derivatizing reagent, 9-AN, was added to the vortexed mixture. The mixture was left to react for 30 min at the temperature of 22 ± 1 °C. Then, 0.8 ml of demineralised water was added to the mixture and the contents were vortexed. The mixture was transferred to cartridges with C_{18}, previously activated with 2 ml of acetonitrile and 2 ml of water. The absorbed analytes were rinsed with 2 ml of water and allowed to dry by air flushing for 10 min. Subsequently, the 9-AN derivatives of TMC, I.S. and endogenous steroids were purified using of 2 ml mixture of *n*-hexane and dichloromethane at the ratio 1:1 (v/v) and eluted using 1 ml of acetone. The obtained eluates were dried in a stream of nitrogen at 60 ± 1 °C. The resulting residue was reconstituted in 50 μl of acetonitrile and 50 μl of mobile phase and 30 μl was injected into the analytical column. The volunteers' plasma samples were processed in the same manner, except that each 1 ml plasma sample was spiked with 50 μl I.S.

2.7. Validation parameters

2.7.1. Linearity

Linearity of the calibration curve was estimated for the peak area of TMC after derivatization with 9-AN as related to I.S., as a function of the analyte concentration in plasma samples, covering the range of 2.5–100.0 ng/ml. The equations of calibration curves were used to calculate unknown TMC concentrations in human plasma. A correlation coefficient was calculated to confirm linearity of the calibration curve.

2.7.2. Limit of detection (LOD) and limit of quantitation (LOQ)

LOD for TMC was determined as S/N of 6:1. LOQ was defined as the lowest concentration of TMC of the calibration curve at which the coefficient of variation, CV, did not exceed 15.0% of its nominal value.

2.7.3. Precision and accuracy

Precision and accuracy of determination of TMC concentrations in human plasma by the method was evaluated for concentrations within the calibration curve range. Intra-day precision of the elaborated method was calculated for five samples each of the 50.0 and 10.0 ng/ml TMC concentrations in human plasma. Inter-day precision and accuracy were estimated for all concentrations within the calibration curve range. The precision was expressed as percent of coefficient of variation, %CV = (S.D./mean concentration) × 100. Accuracy was estimated for the same range of TMC concentrations as for evaluation of precision of the method, and was expressed by the percent difference between the determined mean concentration and the nominal concentration: %error = [(mean concentration − nominal concentration)/nominal concentration] × 100.

2.7.4. Recovery

The recovery was calculated for 50.0 and 10.0 ng/ml TMC concentrations. The first series consisted of five 1.0 ml blank plasma samples spiked each with 50.0 μl of 1.0 μg/ml or 0.2 μg/ml and 50 μl of 2.0 μg/ml I.S. The samples were extracted according to the above procedure. Then, five blank plasma samples of the second series were supplemented with I.S. only, and extracted. TMC was added to a dry residue after extraction of the plasma samples containing I.S.-spiked. In the next step, the samples were derivatized. The recoveries were calculated as the ratio of peak area for each extracted or non-extracted TMC to peak area of extracted I.S. using the formula:

$$\text{Recovery}\,(\%) = \frac{P_{\text{TMCextr}}/\text{I.S.}}{P_{\text{TMCnon-extr.}}/\text{I.S.}} \times 100\%$$

where $P_{\text{TMCextr./I.S.}}$ and $P_{\text{TMCnon-extr./I.S.}}$ are peak areas of extracted or non-extracted TMC, respectively, to peak area of extracted I.S.

2.7.5. Specificity

Specificity is described as a potential ability of a method to discriminate the analyte among all potential interfering exogenous (i.e. drugs, derivatizes reactants) and endogenous (i.e. glucocorticoids) compounds in blood. It should confirm that the signal measured is caused exclusively by the analyte.

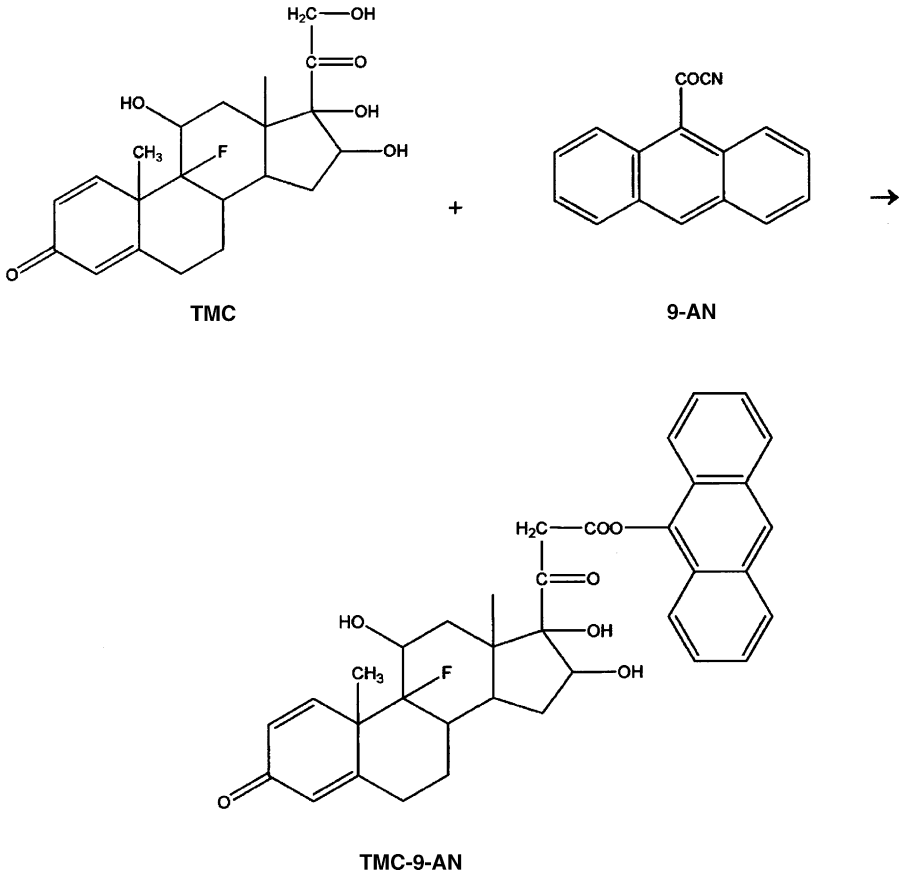

Fig. 1. Scheme of derivatization of triamcinolone with 9-anthroyl nitrile.

2.8. Application of the method in vivo

2.8.1. Pharmacokinetic analysis

The applicability of the elaborated method in pharmacokinetic studies on TMC was estimated in vivo. The studies were approved by the Human Investigation Ethical Committee at the University of Medical Sciences in Poznań. A single tablet with 4 mg of TMC was administered to each of three healthy volunteers. Blood samples (4–5 ml) were obtained (in serum gel tubes S/4.7 ml, Sarstedt, Monovette, Germany) at the following times: immediately before administration and at 0.5, 1.0, 1.33, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0 and 10.0 h following administration. Within 30 min following blood withdrawal, the samples were frozen in plastic vials at −27 °C until analysed. The plasma TMC concentrations were used to calculate pharmacokinetic parameters. All pharmacokinetic parameters were calculated

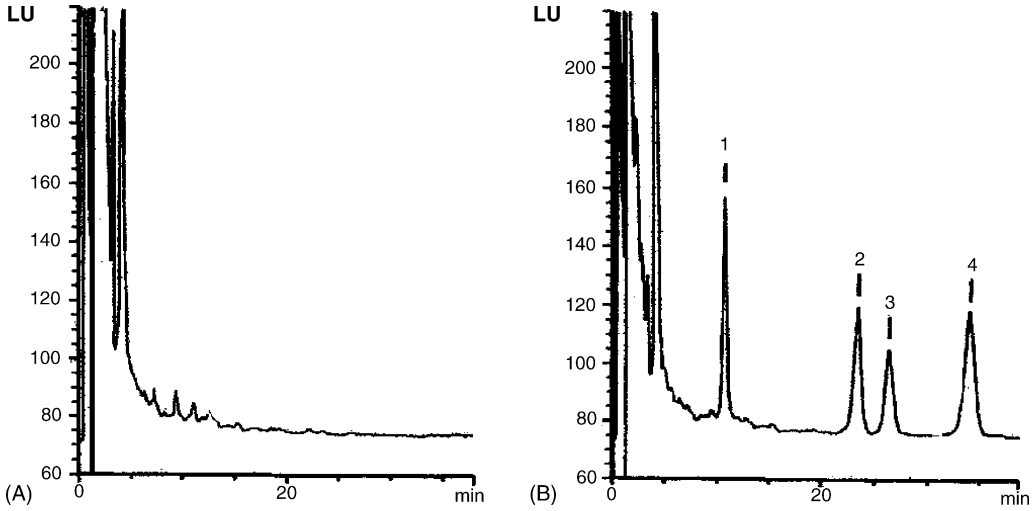

Fig. 2. Chromatograms obtained after extraction of water samples: (A) blank sample; (B) water samples spiked with: TMC (1), I.S. (2), F (3) and E (4). The concentration of each analyte was 100 ng/ml.

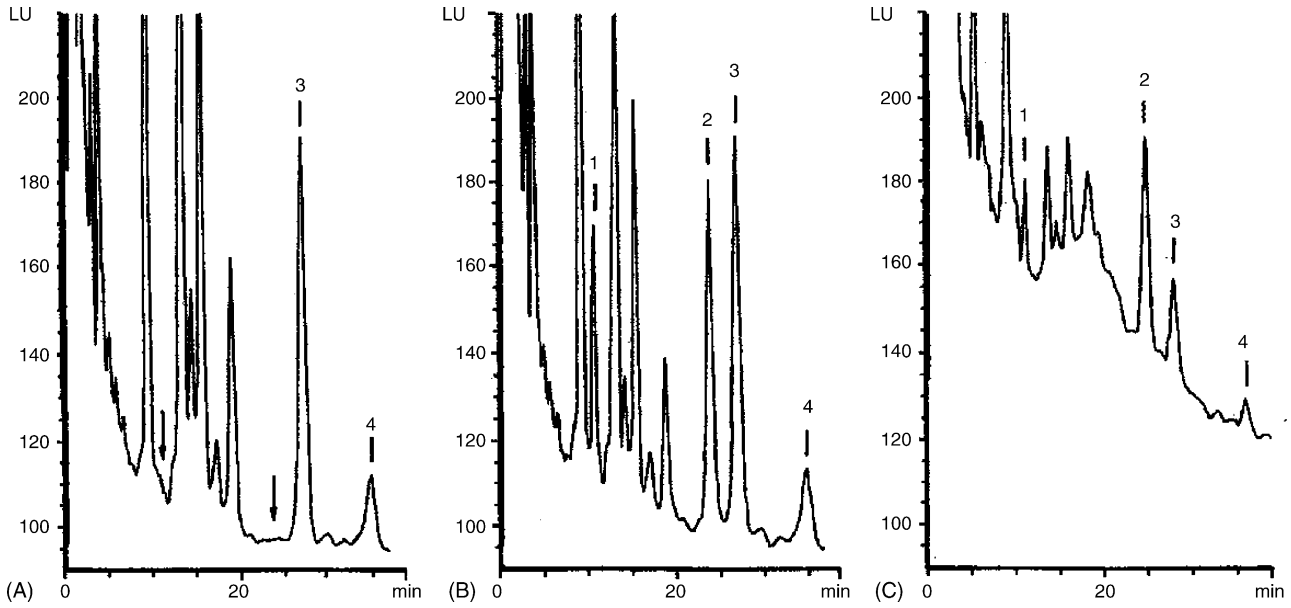

Fig. 3. Chromatograms obtained after extraction of human plasma samples: (A) blank plasma; (B) plasma spiked with the concentration of 2 μg/ml of TMC (1) and I.S. (2), (final concentration of each analyte in plasma was 100 ng/ml), (3) and (4) represent endogenous glucocorticoids, F and E, respectively. Chromatogram (C) corresponds to a sample of a healthy volunteer 1 h after administration of a single 4 mg TMC tablet.

using an open one-compartment model. Area under the curve ($AUC_{0\rightarrow\infty}$) was estimated by the trapezoidal rule with extrapolation to infinity using the ratio C_n/k_{el}, where C_n was the last measurable concentration. The (k_{el}) represented the total first-order elimination rate constant, which was estimated from the terminal linear segment of the log plasma concentration/time data. The elimination half-life ($t_{0.5}$) was calculated from $\ln 2/k_{el}$; t_{max} was estimated from TMC concentration/time curve and C_{max} was read at t_{max}. Clearance of the drug from plasma (Cl) was calculated by dividing the dose (D) of TMC by $AUC_{0\rightarrow\infty}$ (assuming complete bioavailability; $F=1$). Volume of distribution (V_d) was estimated from $D/AUC_{0\rightarrow\infty}$ k_{el} and mean residence time (MRT) from AUMC/AUC, where AUMC is area under the first moment curve. The Topfit 2.0 software package was used for the calculations [10].

3. Results and discussion

3.1. Derivatization, HPLC, SPE and conditions for resolution of TMC

Preliminary studies on determination of TMC levels by HPLC with UV detection, after administration of the small (4 mg) dose in tablet formulation, failed. Similarly, UV detection following the 2–5 mg dose of TCA [4,11] was not suitable. The UV detector proved to be insufficiently sensitive for that purpose. However, UV detection was possible following the 40–80 mg dose. Pharmacokinetic studies after administration of the small, 2–5 mg, formulations of TCA were performed applying a much more sensitive method of RIA coupled with HPLC, where LOQ was 0.1 ng/ml [5,6], but details of

Table 1
Precision and accuracy data on standard solutions of calibration curves[a] for analysis of TMC in human plasma

Nominal concentration of analyte (ng/ml)	TMC		
	Mean assayed value (ng/ml)	Accuracy (error %)	Precision (CV %)
Intra-day ($n=5$)			Repeatability
50.00	53.21	6.4	15.0
10.00	9.17	8.3	14.3
Inter-day ($n=5$)[a]			Reproducibility
100.00	100.32	0.3	15.0
50.00	49.60	0.8	13.8
25.00	22.02	11.9	14.8
15.00	15.09	0.6	11.4
10.00	1.10	14.3	14.3
7.50	2.24	8.1	13.6
5.00	5.11	10.2	14.9
2.50	10.64	8.10	2.6

Linear equation: $y=(P_{TMC})/(P_{I.S.})=0.0047\times+0.0141$; $r=0.9992$; ($P_{TMC(I.S.)}$ − peak area of TMC or I.S.).

[a] Five calibration curves were prepared in a period of 4 weeks.

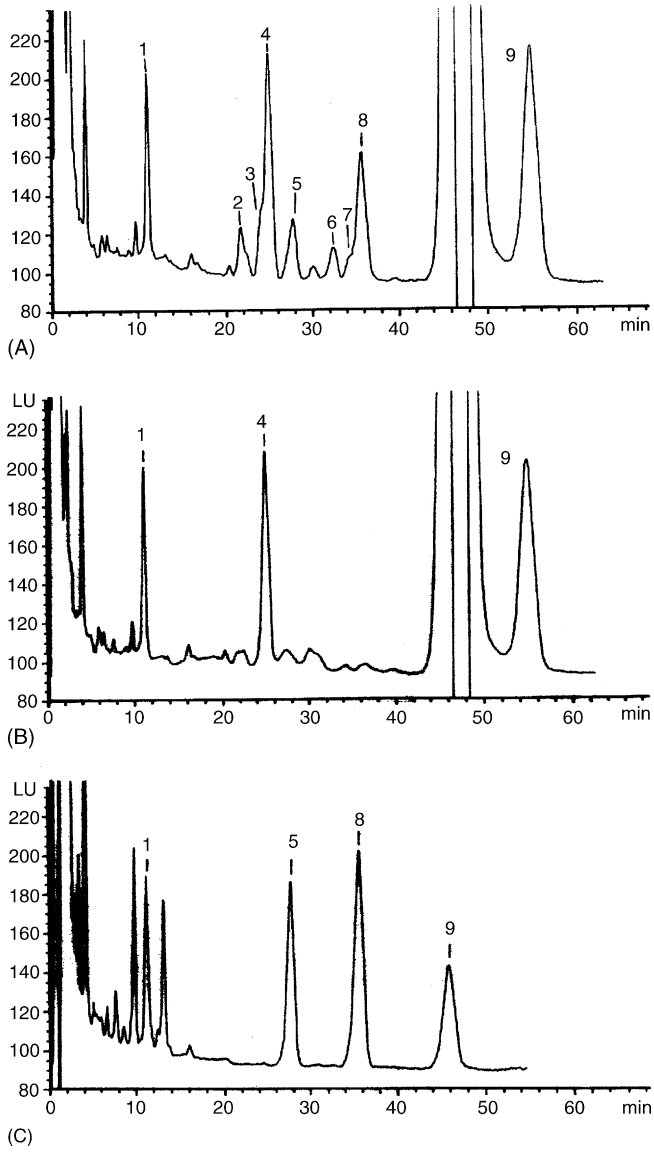

Fig. 4. Influence of alloTHE on split of THE. (A) Chromatogram of TMC (1), endogenous glucocorticoids F (5), E (8) and their metabolites: 3βalloTHF (2), 3βTHF (3), alloTHE (4), THF (6), alloTHF (7) and split THE (9) obtained after extraction of water samples supplemented with the above compounds. (B) Split of THE in presence of alloTHE. (C) Chromatogram with regular peak of THE in absence of alloTHE.

the method were not published. The elaborated method with fluorescence detection of TMC derivative was shown to be also adequately sensitive for the 4 mg formulation of TMC, with LOQ equal to 2.5 ng/ml and comparable to the method [8] where six glucocorticoids, but not TMC, were determined. However, a chromatographic column with normal phase was used in the latter method. Before applying samples the column was tested with a normal and a reversed phase. Better resolution of TMC, I.S. and endogenous glucorticoids derivatives was obtained on the reverse phase C_{18} column and, therefore, all determination of TMC were conducted in the column. Furthermore, the mobile phase consisted of acetonitrile and acidified water (pH 4.6), a combination much simpler and more friendly to the environment than multiple organic compounds used by Shibata et al. [8].

Before injection to the column, TMC was derivatized using of a very reactive acyl cyanide, 9-AN, to receive a fluorescent ester (Fig. 1). The derivatization of glucocorticoids with 9-AN occurs through the hydroxy group at the position 21 of the carbon chain. The reaction occured in basic conditions, so that a mixture of 10% triethylamine and 0.1% quinuclidine at a 4:1 ratio was required at room temperature, in anhydrous conditions [8,12]. In order to prolong the life of the analytical column, TMC and endogenous steroid derivatives were purified in C_{18} cartridges, which were more efficient than cartridges with a normal phase. Optimum purification of the analytes from derivatization reagents was achieved by applying 2 ml of the phase consisting of dichloromethane and hexane at a 1:1 ratio (v/v) (Fig. 2).

3.2. Validation parameters

3.2.1. Linearity, LOD and LOQ

Standard curves for TMC proved to be linear in the concentration range of 2.5–100.0 ng/ml and covered the TMC concentrations later determined in the volunteers' samples. Equation of the mean standard curve is presented in Table 1. The established equations of TMC standard curves were used to calculate serum concentrations in volunteers following administration of a 4 mg TMC tablet. LOD for the elaborated technique, at S/N = 6:1, was 1.0 ng/ml. On the other hand, LOQ, defined as the lowest concentration in the standard curve at which CV-defined precision ≤15%, was determined to be 2.5 ng/ml.

Table 2
Validation parameters of standard curves for analysis of F and E

Nominal concentration (ng/ml)	F			E		
	Mean assayed value (ng/ml)	Accuracy (error %)	Precision (CV %)	Mean assayed value (ng/ml)	Accuracy (error %)	Precision (CV %)
100.00	99.88	0.1	11.2	98.9	1.1	3.3
50.00	48.72	2.6	8.8	53.65	7.3	1.8
25.00	23.13	7.5	10.2	22.99	8.0	7.3
15.00	16.16	7.7	5.6	16.42	9.5	14.3
10.00	9.19	8.1	10.2	9.12	8.8	9.9
7.50	6.86	8.5	10.4	8.03	7.1	4.4
5.00	5.69	13.8	5.2	5.47	9.4	16.7

Linear equation: $P_F/P_{I.S} = 0.0086\times \pm 0.011$; $P_E/P_{I.S} = 0.137\times \pm 0.025$; $r = 0.9993$; $r = 0.9982$. The three calibration curves were prepared in a period of 3 weeks.

3.2.2. Precision, accuracy and recovery

The designed technique was characterized by the high precision of estimation of TMC concentrations, both within a day and between days of treatment, as proved by low values of the variability coefficient (CV ≤ 15%). Also the accuracy of estimates fit the range accepted for techniques of drug testing in body fluids and amounted to ≤15% (Table 1). Recovery of TMC from plasma, taking under consideration the multiple step extraction procedure with SPE, was relatively high, ranging between 76 and 83%.

3.2.3. Specificity

The separated peaks of TMC, after extraction from plasma samples and derivatization with 9-AN, are presented on the HPLC chromatogram (Fig. 3). However, other additional peaks, which could originate from endogenous steroids or their metabolites, were also present on the chromatogram. These did not interfere with TMC or I.S. peaks. The two additional peaks with retention times of 26.8 and 34.4 min (Figs. 2 and 3) were identified as F and E, respectively. The potential for F and E determination in presence of TMC or prednisolone is of significance for investigation of disturbances in glucocorticoid excretion caused by TMC therapy [6,13]. Also, metabolites of F (3βalloTHF, 3βTHF, THF), as well as metabolites of E, do not interfere with TMC determinations. Moreover, peaks related to the above endogenous compounds are in many cases completely separated from each other (Fig. 4A). Interaction was observed between alloTHE and THE. In presence of alloTHE, the peak of THE was split to two additional peaks of extensive areas, and the retention time of THE was increased (Fig. 4B). Other analytes did not cause such phenomena (Fig. 4C). Preliminary studies were also performed on quantitative determination of F and E in one analytical run with TMC. Calibration curves for F and E in aqueous medium were linear in the range between 5 and 100 ng/ml of each analyte. The range covered the steroid levels that were expected in human plasma. In the morning, concentrations of F were approximately 80–200 ng/ml, while in the evening, they were in the range of 40–100 ng/ml [14]. The concentrations of F and E standard curves were determined with high precision and accuracy. The correlation coefficient r exceeded 0.99 (Table 2).

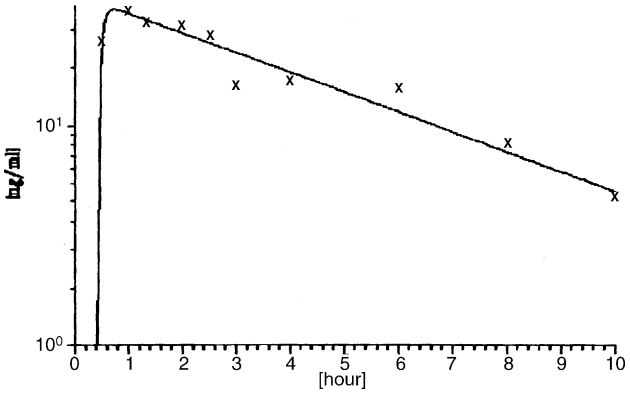

Fig. 5. Semi-logarithmic plot of mean TMC concentrations vs. time after administration of a single 4 mg dose in tablet to healthy volunteers.

3.3. Test in vivo-pharmacokinetic studies

The method was designed for pharmacokinetic and bioavailability studies on TMC in humans after administration of small doses of the drug. The pharmacokinetic profiles were followed for up to 10 h after administration of a single standard tablet to healthy volunteers. The changes in TMC concentration with time followed a one-compartment model. The semi-logarithmic relationship of TMC concentration versus time in the disposition phase was linear (Fig. 5). The pharmacokinetic data showed that TMC was rapidly absorbed from GI tract and reached maximum plasma levels, $C_{max} = 38.4 \pm 14.6$ ng/ml, after the short time of 0.7 h. Values of other pharmacokinetic parameters such as biological half-life ($t_{0.5}$), total clearance (CL) and volume of distribution (V_d) obtained using this method (Table 3) were very similar to literature values on TMC as well as TCA. However, the obtained data were based on RIA results [15], or results of HPLC coupled with the RIA method [5]. Also, the $AUC_{stand.} = 37.17$ ng h/ml for the TMC tablet (AUC standardized to a 1 mg dose) was nearly identical to $AUC_{stand.} = 34.87$ ng h/ml after oral administration of 16 mg TMC [15] or quite similar to TCA data [5], $AUC_{stand.} = 28.85$ ng h/ml. The data confirmed high reproducibility of the elaborated HPLC-FLD method.

Table 3
Pharmacokinetic parameters (mean ± S.D.) of TMC after administration of a single dose (4 mg) in tablet to healthy volunteers

Pharmacokinetic parameters	4 mg TMC tablet[a] HPLC-FLD	Literature data		
		16 mg TMC tablet RIA[15]	2 mg TCA iv RIA-HPLC[5]	5 mg TCA oral solution RIA-HPLC[5]
λ_z (h^{-1})	0.274 ± 0.064	0.261 ± 0.121	–	–
$t_{0.5}$ (h)	2.58 ± 0.44	2.7 ± 1.3; 2–4	2.0 ± 0.7	2.6 ± 1.4
t_{max} (h)	0.64 ± 0.18	1.9 ± 0.52	–	1.0 ± 0.7
C_{max} (ng/ml)	38.4 ± 14.6	94.65 ± 23.05	–	10.5 ± 6.2
$AUC_{0\rightarrow\infty}$ (ng h/ml)	148.7 ± 46.1	557.92 ± 50.16	57.7 ± 14.9	30.4 ± 12.3
Cl (l/h)	28.8 ± 9.2	28.9 ± 2.5	37.3 ± 12.8	–
V_d (l)	110.0 ± 52.3	131.8 ± 56.8	103.2 ± 58.7	–
MRT_{tot} (h)	4.15 ± 0.78	–	2.7 ± 0.8	2.4 ± 1.4

[a] The pharmacokinetic parameters calculated from mean concentrations as means for three volunteers. The parameters were obtained using an open one-compartment model (TopFit 2.0).

4. Conclusion

The elaborated HPLC method with fluorescence detection is specific, adequately accurate and precise and there is no doubt that it can be used for pharmacokinetic and bioavailability studies on triamcinolone, administered in small doses (milligrams). This method also gives the potential for quantitative determination of hydrocortisone and cortisone during a single analytical run. The method can provide an alternative to HPLC with RIA detection for determination of small quantities of TMC.

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