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# Simultaneous determination of triamcinolone acetonide and hydrocortisone in human plasma by high-performance liquid chromatography

S.A. Döppenschmitt[a,*], B. Scheidel[a], F. Harrison[b], J.P. Surmann[c]

[a]*ACC, Analytical Clinical Concepts GmbH, Industriestr. 14–16, 64319 Pfungstadt, Germany*
[b]*Harrison Clinical Research GmbH, Albrechtstr. 43, 80636 München, Germany*
[c]*Humboldt-Universität zu Berlin, Math. Nat. I Fak, Institut für Pharmazie Goethestr. 54, 13086 Berlin, Germany*

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**Abstract**

A validated HPLC method for the simultaneous determination of triamcinolone acetonide and hydrocortisone has been established to monitor the plasma levels of the compounds in healthy volunteers following intramuscular (i.m.) administration of triamcinolone acetonide. Plasma (1.0 ml) was extracted with dichloromethane after addition of the internal standard, fluocortolone. The compounds were separated using a LiChrospher RP 18 column and detected by UV absorbance. Specificity, linearity, as well as the repeatability, intermediate precision and accuracy of the method were established. The limit of quantification was 0.6 ng/ml for triamcinolone acetonide (C.V.=8.7%, R.E.=2.6%, $n$=6) and 2.0 ng/ml for hydrocortisone (C.V.=8.3%, R.E.=2.8%, $n$=6). Data on the stability of triamcinolone acetonide in human plasma are presented. Recovery of the compounds and the internal standard have been studied. The results of quality control samples ($n$=126) determined during routine analysis of volunteer samples are described. Plasma levels of triamcinolone acetonide after i.m. administration of 40 mg of triamcinolone acetonide are presented.

*Keywords:* Triamcinolone acetonide; Hydrocortisone; Corticoids

## 1. Introduction

Triamcinolone acetonide is a potent glucocorticoid, used for anti-inflammatory treatment, e.g. in patients with bronchial asthma or rheumatoid arthritis [1]. Triamcinolone acetonide is an adduct of triamcinolone and acetone, but not a pro-drug of triamcinolone. It has been shown that triamcinolone is not a major metabolite of triamcinolone acetonide [2]. One of the general side-effects of the exogenous supply of glucocorticoids is the suppression of the blood levels of endogenous corticoids such as hydrocortisone. The pharmacokinetics of triamcinolone acetonide have been studied after oral and intranasal inhalation [1,3], intra-articular (i.a.) administration [4] and intravenous (i.v.) administration of triamcinolone acetonide phosphate [2]. Blauert-Cousounis et al. [3] provided some data on pharmacokinetics of triamcinolone acetonide after i.m. administration. Only few HPLC methods for the determination of triamcinolone acetonide in human plasma have been published [5,6]. Rohdewald et al. [7] used triam-

*Corresponding author.

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cinolone acetonide as internal standard for the HPLC determination of different glucocorticoids in human plasma. Some RIA assays have been established, but specificity of these methods seems to be problematic [1,8] or no validation data were reported [3]. For the determination of triamcinolone acetonide in urine and for screening purposes different HPLC and GC methods have been published [9,10].

The limits of quantification for triamcinolone acetonide in human plasma of the HPLC methods were 5 ng/ml [5] and 100 ng/ml [6] respectively. None of these methods took hydrocortisone into consideration.

No data on the stability of triamcinolone acetonide in plasma was provided in the literature mentioned above.

An HPLC method was required for monitoring plasma levels of triamcinolone acetonide and the endogenous steroid hydrocortisone following i.m. administration of triamcinolone acetonide. Therefore it was necessary to improve the limit of quantification for triamcinolone acetonide in comparison to methods in the literature. The validation of the method and the quality assurance during routine analysis was carried out according to published papers [11–18].

## 2. Experimental

### 2.1. Reagents

Triamcinolone acetonide and corticosterone was from Sigma Chemie (Deisenhofen, Germany), hydrocortisone from E. Merck (Darmstadt, Germany) and fluocortolone, used as internal standard, was a gift from Schering (Berlin, Germany). Methanol, tetrahydrofuran, dichloromethane were of HPLC grade (Fisons Scientific Equipment, Loughborough, UK), 0.1 *M* sodium hydroxide was of analytical quality (E. Merck). Nitrogen was of quality 5.0 (Messer-Griesheim, Frankfurt am Main, Germany).

### 2.2. Instrumentation

The chromatographic system from Jasco (Gross-Umstadt, Germany) consisted of a Pu-980 pump, an 851-AS autosampler and a programmable UV-975 UV–Vis absorbance detector. The separation was performed by a LiChrospher RP 18, 5 $\mu$m, 10×4 mm I.D. (Knauer, Berlin, Germany) precolumn and a LiChrospher RP 18, 5 $\mu$m, 250×4 mm I.D. (Knauer) analytical column. Data recording was carried out by Chromcard Software (Fisons Instruments, Rodano, Italy).

### 2.3. Preparation of hydrocortisone-free human plasma

For preparation of hydrocortisone-free human plasma, 250 ml of pooled human plasma were shaken for 40 h with pharmaceutical-grade active carbon (Caesar and Lorentz, Hilden, Germany) for the absorption of hydrocortisone. Subsequently the plasma was separated by filtration using a glass sinter filter (porosity 10–16 $\mu$m).

### 2.4. Preparation of calibration standards and quality control samples

To 1 ml of hydrocortisone-free plasma 100 $\mu$l of a methanolic solution of triamcinolone acetonide and hydrocortisone (resulting in the plasma concentrations given in Table 2) and 50 $\mu$l of a methanolic solution of the internal standard (50 ng/50 $\mu$l) were added.

### 2.5. Preparation of plasma samples of volunteers

To 1.0 ml of the individual plasma sample 100 $\mu$l of methanol and 50 $\mu$l of a methanolic solution of the internal standard (50 ng/50 $\mu$l) were added.

### 2.6. Extraction procedure

After addition of 0.5 ml of 0.1 *M* sodium hydroxide and 2 ml dichloromethane the samples prepared as described above were shaken for 10 min and afterwards centrifuged (2500 *g*) for 10 min. A 1.9-ml volume of the organic layer was transferred in a glass tube and evaporated under a stream of nitrogen at 45°C. The residue was reconstituted in 50 $\mu$l of methanol. A 17-$\mu$l aliquot of this sample was injected onto the HPLC system.

### 2.7. Chromatographic conditions

The mobile phase was methanol–water–tetrahydrofuran (1100:1000:25, v/v). Mobile phase was degassed by sonication for 20 min prior to use. The flow-rate was 1.0 ml/min and the temperature was ambient. The eluate was monitored by UV absorbance at 252 nm (0.005 aufs).

A mixture of corticosterone and triamcinolone acetonide in methanol was used as a test mixture to check the suitability of the system. Slight variations of the content of tetrahydrofuran were used for adjusting chromatographic resolution.

### 2.8. Data evaluation and calculations

All calculations were done on an IBM-type personal computer using Excel 4.0 software (Microsoft, USA).

### 2.9. Defining assay characteristics

#### 2.9.1. Specificity

To demonstrate the specificity of the method, blank plasma was analysed. As recommended [12] plasma samples of six different individuals and in addition three pooled ($n=5$) plasma samples were examined during validation. For each individual volunteer a plasma sample taken prior to the application of the drug was analysed [12].

#### 2.9.2. Stability

Plasma samples spiked at three concentrations in the high, medium and low range of the standard curve were analysed immediately after preparation and after two, four and twelve weeks of storage at −20°C. In addition samples where analysed after three freeze–thaw cycles. Degradation was expressed as percentage loss of content after storage or freeze–thaw cycles compared to the initial content of the sample.

#### 2.9.3. Standard curve and linearity

The standard curve was determined on each day of three-day validation ($n=3$ at each concentration). The variance over the range of the standard curve was examined using Cochran's test [19], in order to choose the correct algorithm for linear regression (weighted or un-weighted). For calculation of the standard curves substance-to-internal standard peak-area ratios were used. For the evaluation of linearity, a plot of concentration versus signal and the relative error (R.E.) of the interpolated concentration of the standards were taken into consideration.

$$\text{R.E.} = \frac{(\text{interpolated concentration of standard} - \text{nominal concentration})}{\text{nominal concentration}} \times 100\%$$

#### 2.9.4. Precision and accuracy

The repeatability (intra-assay precision), the intermediate (inter-day) precision and the accuracy were calculated from data obtained during the three-day validation. Three concentrations were chosen from the high, medium and low range of the standard curve. Plasma samples spiked at these three concentrations were analysed each day of three-day validation ($n=6$ at each concentration). Precision was expressed as the coefficient of variation (C.V.) of the interpolated concentrations. All standard deviations of results obtained from standard curves were calculated considering the rule of error propagation [21]. Accuracy was expressed as the mean relative error (R.E.) of the interpolated concentration of the samples. According to international conference reports for bioanalytical methods precision (C.V.)$\leq$ 15% and accuracy (R.E.)$\leq \pm 15\%$ are acceptable [11,12].

#### 2.9.5. Limit of quantification (LOQ)

The limit of quantification was determined by repeated analysis of spiked plasma samples ($n=6$). Precision and accuracy of the determination at the limit of quantification were calculated as described above (Section 2.9.4). Following international recommendations a precision (C.V.)$\leq$20% and accuracy (R.E.)$\leq \pm 20\%$ is acceptable at the limit of quantification [11,12].

#### 2.9.6. Recovery

For triamcinolone acetonide and hydrocortisone, recovery was determined at two concentrations. The recovery of the internal standard was determined at the concentration used in this method (50 ng/ml). Therefore the absolute recovery (AR) was calculated using peak areas of extracted plasma samples ($n=6$

at each concentration) and directly injected solutions of the same concentrations:

$$\text{absolute recovery (AR)} = \frac{\text{peak area}_{\text{extracted analyte}}}{\text{peak area}_{\text{solution of analyte}}} \times 100\%$$

### 2.10. Volunteer samples

During routine analysis, each analytical run consisted of plasma samples of volunteers, samples for the calculation of the standard curves ($n = 1$ at each concentration) and quality control samples in the high, medium and low range of the standard curve ($n = 2$ at each concentration).

To test the integrity of an individual run, a limit of not more than ±20% relative error for two of the six quality control samples (not both at the same concentration) has been applied [12].

For further analysis of the quality control samples of each analytical run, the mean of the two results was calculated at each concentration (high, medium, low) and plotted against the day of analysis (mean-plot) [22]. In addition, the range of the two results obtained in each run was calculated and plotted against the day of analysis (range-plot) [22].

## 3. Results

### 3.1. Specificity

Chromatograms of a blank individual volunteer sample, a pooled plasma spiked with the compounds and a plasma sample from a volunteer after the administration of triamcinolone acetonide are shown in Fig. 1. Chromatograms demonstrate that the compounds of interest could be detected separately from endogenous compounds.

### 3.2. Stability

Storage of triamcinolone acetonide in human plasma at −20°C over a period of 12 weeks resulted

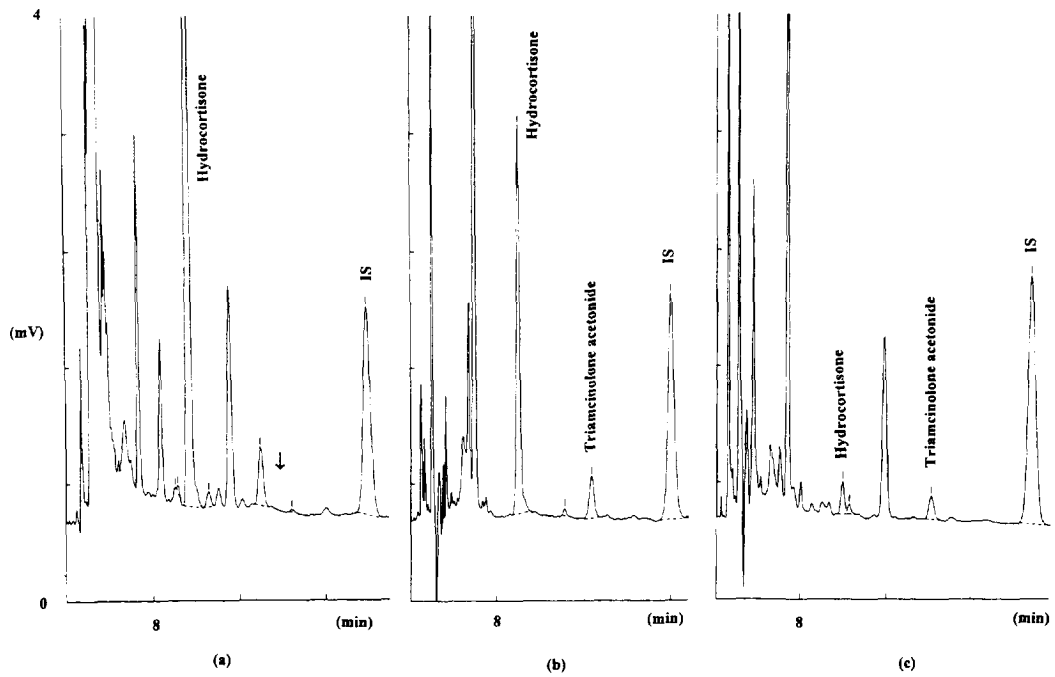


Fig. 1. Chromatograms of (a) blank individual volunteer sample, (b) spiked standard (10.0 ng/ml triamcinolone acetonide, 50.0 ng/ml hydrocortisone), (c) individual volunteer sample (48 h after i.m. administration of triamcinolone acetonide).

in a mean degradation of 2.5%. Three freeze–thaw cycles caused a mean degradation of 4.4%.

### 3.3. Standard curve and linearity

During the three-day validation, it was shown that the variance over the range of the standard curve was not homogeneous (heteroscedastic data). Thus standard curves had to be calculated using weighted linear regression algorithms [20]. Table 1 shows the characteristics of the standard curves during three-day validation. Table 2 gives the interpolated concentrations of the calibration standards and the relative errors (day one of the three-day validation) to demonstrate linearity. The use of hydrocortisone-free human plasma for preparation of the standards had no influence on slope and intercept of the calibration curves of triamcinolone acetonide in comparison with pooled human plasma.

### 3.4. Precision and accuracy

The values obtained during three-day validation for repeatability, intermediate precision and accuracy are summarised in Table 3.

Intermediate precision ranged from 3.3 to 6.1% for triamcinolone acetonide (concentration 50.0 to 2.0 ng/ml, $n=18$ for each) and from 3.9 to 4.8% for hydrocortisone (concentration 200.0 to 10.0 ng/ml, $n=18$ for each). The accuracy was determined as −9.3 to 3.8% for triamcinolone acetonide (concentration 50.0 to 2.0 ng/ml, $n=6$ for each) and as −4.3 to 3.3% for hydrocortisone respectively (concentration 200 to 10 ng/ml, $n=6$ for each).

### 3.5. Limit of quantification

The limits of quantification, determined by repeated analysis of spiked plasma samples, were 0.6 ng/ml for triamcinolone acetonide and 2.0 ng/ml for hydrocortisone. For triamcinolone acetonide the precision (C.V.) at the lower limit of quantification was determined as 8.7% ($n=6$) and for hydrocortisone as 8.3% ($n=6$). The mean R.E. was 2.6% for triamcinolone acetonide and 2.8% for hydrocortisone.

### 3.6. Recovery

In Table 4 the absolute recoveries of triamcinolone acetonide, hydrocortisone and fluocortolone are listed. The C.V. ranged from 2.3 to 5.4%.

### 3.7. Volunteer samples

Plasma samples of volunteers (756), quality control samples (126) and calibration standards (189) in 21 analytical runs have been analysed over a period of eight weeks.

The results of the quality control samples for triamcinolone acetonide, analysed with each analytical run are shown in Fig. 2. The mean relative error was 0.6% (ranging from −10.5 to 12.8%), −1.7% (−11.3 to 13.3%) and 0.6% (−10.9 to 11.2%) for the high, medium and low concentration range for triamcinolone acetonide as well as 1.1% (−9.3 to 12.0%), 3.6% (−9.8 to 14.3%) and −2.4% (−17.5 to 23.6%) for hydrocortisone. For triamcinolone acetonide 94% of the QC samples ($n=126$)

Table 1
Characteristics of standard curves during three-day validation

| Day | Slope (mean±S.D.) [(ng/ml)$^{-1}$] | Intercept (mean±S.D.) | Coefficient of correlation |
|---|---|---|---|
| *Triamcinolone acetonide* | | | |
| 1 | 0.0152±0.00022 | −0.001731±0.000336 | 0.9971 |
| 2 | 0.0154±0.00015 | −0.000557±0.000224 | 0.9986 |
| 3 | 0.0146±0.00019 | −0.000746±0.000266 | 0.9970 |
| *Hydrocortisone* | | | |
| 1 | 0.0186±0.00028 | 0.001404±0.001819 | 0.9971 |
| 2 | 0.0189±0.00070 | −0.008737±0.003677 | 0.9995 |
| 3 | 0.0183±0.00084 | 0.002096±0.004980 | 0.9989 |

Table 2
Concentrations of the standard curves and interpolated concentrations as well as relative error (R.E.) of the interpolated concentrations for triamcinolone acetonide and hydrocortisone, (day 1 of the three-day validation)

| Triamcinolone acetonide | | | Hydrocortisone | | |
|---|---|---|---|---|---|
| Concentration added (ng/ml) | Concentration interpolated (ng/ml) | R.E. (%) | Concentration added (ng/ml) | Concentration interpolated (ng/ml) | R.E. (%) |
| 100 | 102.6 | 2.6 | 250 | 242.1 | −3.2 |
| | 104.0 | 4.0 | | 248.5 | −0.6 |
| | 102.7 | 2.7 | | 241.9 | −3.2 |
| 50 | 51.3 | 2.7 | 200 | 197.5 | −1.3 |
| | 50.7 | 1.4 | | 197.2 | −1.4 |
| | 51.3 | 2.5 | | 197.5 | −1.3 |
| 20 | 19.6 | −2.0 | 100 | 99.3 | −0.7 |
| | 20.9 | 4.6 | | 99.5 | −0.5 |
| | 20.1 | 0.3 | | 98.0 | −2.0 |
| 10 | 9.8 | −1.9 | 50 | 51.0 | 1.9 |
| | 9.7 | −2.9 | | 50.0 | 0.1 |
| | 9.8 | −2.0 | | 50.6 | 1.2 |
| 5 | 4.8 | −5.0 | 20 | 20.4 | 1.8 |
| | 4.9 | −2.8 | | 21.2 | 6.1 |
| | 4.7 | −6.6 | | 20.5 | 2.4 |
| 2 | 1.9 | −7.0 | 10 | 10.0 | 0.3 |
| | 2.2 | 10.5 | | 10.3 | 2.7 |
| | 2.0 | 0.5 | | 9.5 | −4.8 |
| 1 | 1.0 | −1.0 | 5 | 5.2 | 4.1 |
| | 1.0 | 0.0 | | 4.9 | −1.2 |
| | 1.0 | −1.0 | | 5.1 | 2.1 |
| 0.8 | 0.83 | 3.8 | 2 | 2.0 | 2.2 |
| | 0.74 | −7.5 | | 1.9 | −4.7 |
| | 0.78 | −2.5 | | 2.0 | −0.1 |
| 0.6 | 0.64 | 6.7 | | | |
| | 0.60 | 0.0 | | | |
| | 0.62 | 4.1 | | | |

were in the range of ±10% of the nominal value, no values were outside ±20%. For hydrocortisone 87% were in the range of ±10% ($n=126$) and only 1 sample deviated more than 20% from the nominal value.

No analytical run had to be omitted considering the requirements mentioned above.

## 4. Discussion

An efficient method for the simultaneous determination of triamcinolone acetonide and hydrocortisone in plasma of healthy volunteers ($n=18$) following the i.m. administration of triamcinolone acetonide is described.

Reversed-phase chromatographic systems for the separation of triamcinolone acetonide are described in the literature [5,6], but the limits of quantification were not sufficient for the issue of this study. On the other hand RIA methods used for pharmacokinetic measurements in the literature [1,8] seemed to lack specificity referring to exogenous and especially even to endogenous steroids. Blauert-Cousounis et al. [3] did not provide any data on the performance of the RIA used. Thus we used reversed-phase HPLC for the determination of triamcinolone acetonide.

Liquid–liquid extraction was used for sample preparation. Various organic solvents were evaluated and dichloromethane was found to result in best recovery.

Table 3
Results of the three-day validation for triamcinolone acetonide and hydrocortisone: mean, standard deviation (S.D.) and coefficients of variation (C.V.) for documentation of repeatability and intermediate precision as well as the relative error (R.E.) for documentation of accuracy

| Concentration added (ng/ml) | Day | Repeatability ($n=6$ at each day) | | Accuracy R.E. (%) | Intermediate precision ($n=18$) C.V. (%) |
|---|---|---|---|---|---|
| | | Concentration interpolated (mean±S.D.) (ng/ml) | C.V. (%) | | |
| *Triamcinolone acetonide* | | | | | |
| 50 | 1 | 51.9±1.4 | 2.6 | 3.8 | 3.3 |
| | 2 | 49.6±1.1 | 2.3 | −0.8 | |
| | 3 | 51.6±2.0 | 4.0 | 3.2 | |
| 10 | 1 | 9.71±0.35 | 3.6 | −2.9 | 4.0 |
| | 2 | 9.54±0.34 | 3.5 | −4.6 | |
| | 3 | 10.33±0.58 | 5.6 | 3.3 | |
| 2 | 1 | 1.81±0.11 | 6.1 | −9.3 | 6.1 |
| | 2 | 1.90±0.13 | 6.7 | −5.0 | |
| | 3 | 1.83±0.16 | 8.6 | −8.4 | |
| *Hydrocortisone* | | | | | |
| 200 | 1 | 204.2±6.1 | 3.0 | 2.1 | 3.9 |
| | 2 | 191.4±3.4 | 1.8 | −4.3 | |
| | 3 | 197.0±8.6 | 4.4 | −1.5 | |
| 50 | 1 | 50.4±3.5 | 7.0 | 0.7 | 4.2 |
| | 2 | 49.9±0.8 | 1.7 | −0.2 | |
| | 3 | 51.6±2.2 | 4.2 | 3.3 | |
| 10 | 1 | 9.92±0.48 | 4.8 | −0.8 | 4.8 |
| | 2 | 9.66±0.21 | 2.1 | −3.4 | |
| | 3 | 10.25±0.15 | 1.5 | 2.5 | |

The system suitability of the chromatographic system was monitored and tetrahydrofuran as component of the mobile phase was used to control chromatographic performance. In chromatograms of human plasma a peak was observed directly prior to that of triamcinolone acetonide. This peak was not present in plasma samples taken from subjects after administration of glucocorticoids and appeared again with declining plasma levels of the glucocorticoid. The peak may correspond to an endogenous steroid which is suppressed by exogenous glucocorticoids, as known from, e.g., hydrocortisone. The quality of the chromatographic system was determined by the baseline resolution of this endogenous peak and triamcinolone acetonide. Because of its chromatographic behaviour corticosterone was used as model-substance for the endogenous peak.

Stability of triamcinolone acetonide in human

Table 4
Absolute recoveries of triamcinolone acetonide, hydrocortisone and fluocortolone (IS) ($n=6$ for each concentration)

| Compound | Concentration added (ng/ml) | Absolute recovery (mean±S.D.) (%) | C.V. (%) |
|---|---|---|---|
| Triamcinolone acetonide | 50 | 100.1±2.3 | 2.3 |
| | 5 | 98.9±3.3 | 3.4 |
| Hydrocortisone | 200 | 89.5±4.5 | 5.1 |
| | 10 | 93.0±5.0 | 5.4 |
| Fluocortolone | 50 | 98.9±3.3 | 3.3 |

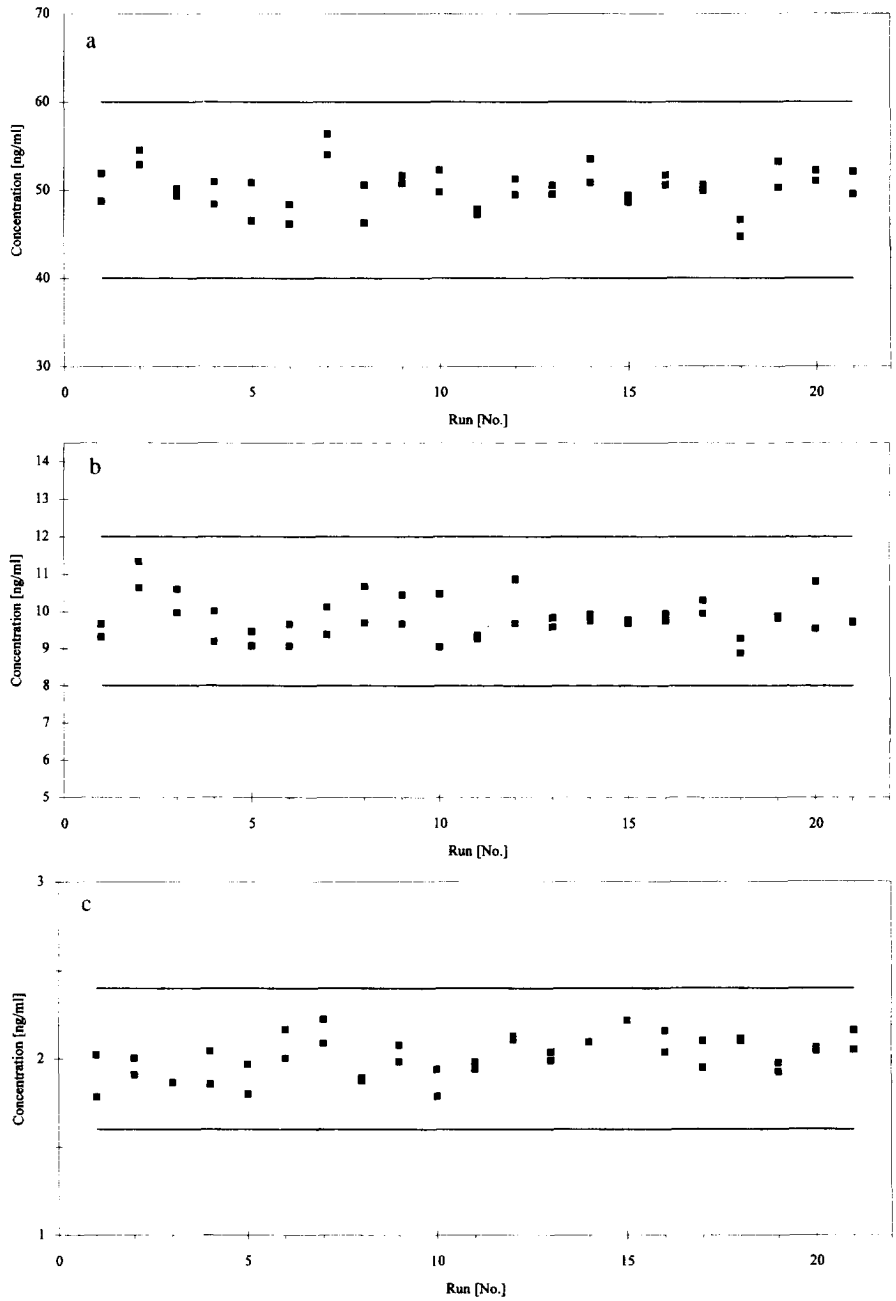


Fig. 2. Individual results of quality control samples (□) in the (a) high (50.0 ng/ml), (b) medium (10.0 ng/ml) and (c) low (2.0 ng/ml) range of the standard curve for triamcinolone acetonide during measurement of volunteer samples, $n=42$ for each concentration (——— = acceptance limit of nominal value, ±20%).

plasma was studied. Plasma samples of triamcinolone acetonide were stable at −20°C for at least three months. Sufficient stability was determined after three thaw–freeze cycles in plasma, too.

The standard curves obtained during three-day validation demonstrate linearity over the ranges of the standard curves. McLean et al. [23] used a variance stabilising technique for the calculation of standard curves to improve precision and accuracy in the low concentration range of the standard curve. This method was applied to the data obtained during three-day validation, but gave no improvement in precision and accuracy compared to the used weighted linear regression method.

In comparison to published HPLC methods [5,6] precision and accuracy of the presented method for the determination of triamcinolone acetonide in plasma was comparable or better. The limit of quantification for triamcinolone acetonide (0.6 ng/ml) could be effectively improved compared to the published HPLC methods, which describe limits of quantification of 5.0 ng/ml [5] and 100.0 ng/ml [6], respectively.

Precision and accuracy at the limits of quantification of the method presented meet the requirements of current recommendations in bioanalytical method validation [12].

For each analytical run individual standard curves and quality control samples were prepared as recommended for routine bioanalytical analysis [12,14,17]. Quality control samples were used to accept or reject every run. Acceptance criteria used were taken from published international conference reports on bioanalytical method validation [12,14].

Fig. 3 presents the plasma concentrations of triamcinolone acetonide of a representative volunteer. In two of the eighteen volunteers plasmal levels of triamcinolone acetonide were ten-to-twenty times higher than in the other sixteen volunteers.

Using this method it was possible to describe the pharmacokinetic properties of triamcinolone acetonide after i.m. administration of 40 mg triamcinolone acetonide in eighteen healthy volunteers and to estimate simultaneously the side-effect of the exogenous glucocorticoid on the plasma levels of the physiological glucocorticoid hydrocortisone.

## References

[1] B.A. Zaborny, P. Lukacsko, I. Barinov-Colligon and J.A. Ziemniak, J. Clin. Pharmacol., 32 (1992) 463.
[2] H. Möllmann, P. Rohdewald, E.W. Schmitt, V. Salomon and H. Derendorf, Eur. J. Clin. Pharmacol., 29 (1985) 85.

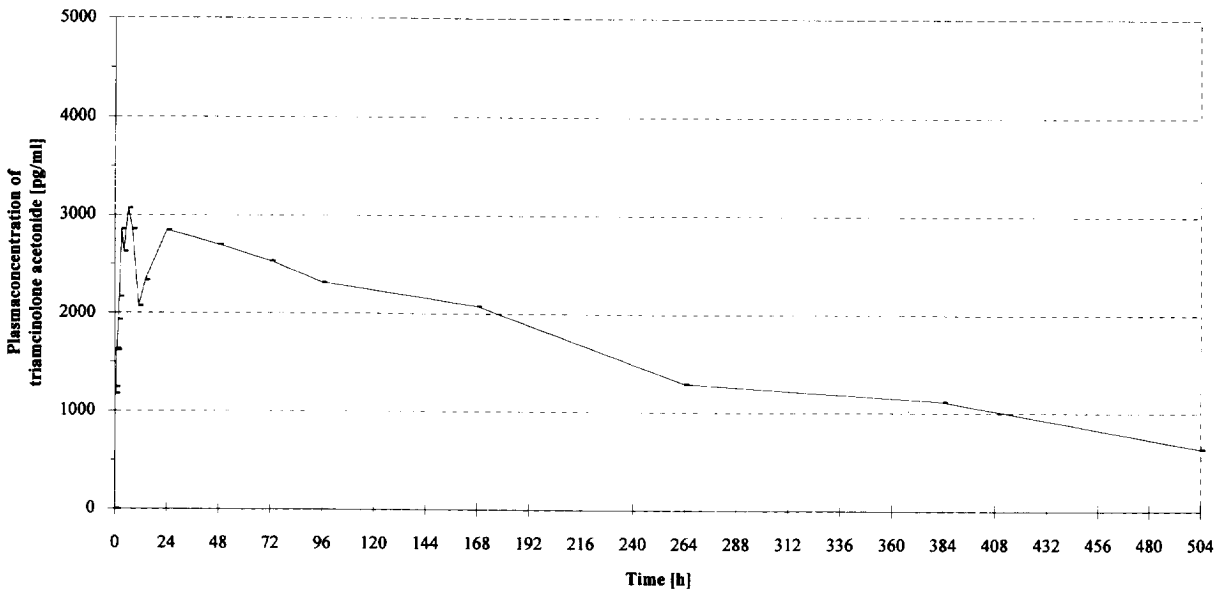


Fig. 3. Plasma concentrations of triamcinolone acetonide after i.m. administration of 40 mg of triamcinolone acetonide to a healthy male volunteer.

[3] S.P. Blauert-Cousounis, J.A. Ziemniak, S.C. McMahon and P.E. Grebow, J. Allergy Clin. Immunol., 83 (1989) 221.
[4] H. Derendorf, H. Möllmann, A. Grüner, D. Haack and G. Gyselby, Clin. Pharmacol. Ther., 39 (1986) 313.
[5] M.E. Tenneson and M.L. Robison, Proceedings of the Symposium on the Analysis of Steroids, Eger, 1981, p. 321.
[6] H. Derendorf, P. Rohdewald, G. Hochhaus and H. Möllmann, J. Pharm. Biomed. Anal., 2 (1986) 197.
[7] P. Rohdewald, J. Rehder, G. Drehsen, G. Hochhaus, H. Derendorf and H. Möllmann, J. Pharm. Biomed. Anal., 6 (1985) 565.
[8] D. Haack and P. Vecsei, Drug Res., 32 II (1982) 832.
[9] R.S. Gardner, M. Walker and D.A. Hollingsbee, J. Pharm. Biomed. Anal., 8 (1990) 8.
[10] A. Santos-Montes, A.I. Gasco-Lopez and R. Izquierdo-Hornillos, J. Chromatogr. B, 620 (1993) 15.
[11] H.T. Karnes, G. Shiu and V.P. Shah, Pharm. Res., 8 (1991) 421.
[12] V.P. Shah, K.K. Midha, S. Dighe, I.J. McGilveray, J.P. Skelly, A. Jacobi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D., McDowall, K.A. Pittmann and S. Spector, J. Pharm. Sci., 81 (1992) 309.
[13] V.P. Shah, Clin. Res. Pract. Drug Reg. Affairs, 5 (1987) 51.
[14] A.C. Cartwright, Drug Inf. J., 25 (1991) 471.
[15] ICH Harmonised Tripartite Guideline, Text on Validation of Analytical Procedures, ICH-Secretary, c/oIFPMA, Geneve, 1994.
[16] CPMP Working Party On Quality Of Medical Products, Note For Guidance, Analytical Validation, Brussels, 1989.
[17] A.G. Causey, H.M. Hills and L.J. Phillips, J. Pharm. Biomed. Anal., 8 (1990) 625.
[18] A.R. Buick, M.V. Doig, S.C. Jeal, G.S. Land, R.D.McDowall, J. Pharm. Biomed. Anal., 8 (1990) 629.
[19] H. Bubert and R. Klockenkämper, Fresenius Z. Anal. Chem., 316 (1983) 186.
[20] J.S. Garden, D.G. Mitchells and W.N. Mills, Anal. Chem., 52 (1990) 2310.
[21] L. Aarons, Analyst, 106 (1981) 1249.
[22] J.R. Lang and S. Bolton, J. Pharm. Biomed. Anal., 9 (1991) 435.
[23] A.M. McLean, D.A. Ruggirello, C. Banfield, M.A. Gonzalez and M. Bialer, J. Pharm. Sci., 79 (1990) 1005.