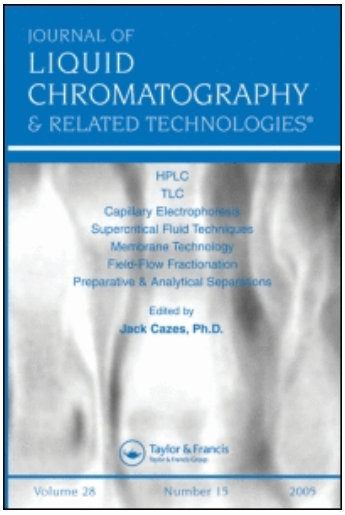


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# ANALYSIS OF PHARMACEUTICAL RESIDUES IN BOVINE LIVER BY HPLC

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# ANALYSIS OF PHARMACEUTICAL RESIDUES IN BOVINE LIVER BY HPLC

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## ABSTRACT

A reversed-phase high performance liquid chromatographic method has been developed for the simultaneous analysis of a group of pharmaceuticals including corticoids (dexamethasone and betamethasone acetate) and major tranquillizers (chlorpromazine, acetopromazine, propionyl promazine, xylazine, and haloperidol) in bovine livers using U.V detection. Next to a fractionated liquid-liquid extraction, the total separation was carried out using a Kromasil $C_{18}$ column and a mixture of methanol:water (80:20, v/v) as mobile phase. All the compounds were detected at 240 nm. The detection limits ranged from 21 $ng \cdot mL^{-1}$ to 298 $ng \cdot mL^{-1}$.

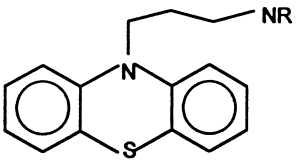
## INTRODUCTION

Corticoids belong to a group of hormones produced by the suprarrenal cortex, although this term is also used to describe all their metabolites that are removed by urine. Depending on its biological action, corticoids have been clasified in glucocorticoids, mineralocorticoids, and gonadocorticoids. Both, dexa-

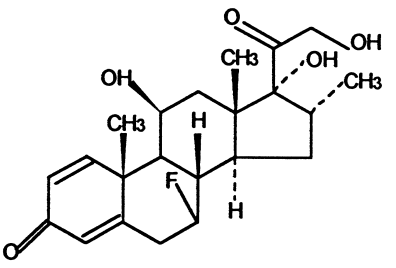
methasone and betamethasone acetate, are included in the group of the glucocorticoids. Among other roles, this group favour the glucidic metabolism, is the cause of osteoporosis, acts like an inhibitor of inflammatories and alergic processes, and finally, diminsh the antibodies formation when they are admistrated in high doses.

This paper proposes a method for the simultaneous analysis of dexamethasona and betamethasone acetate in bovine livers, as well as a group of antipsychotic medicaments that are possible to be administrated to fighting bulls. This group of pharmaceuticals are called "major tranquillizers," because they are able to create an indifferent psychomotive state and, are very effective products in exciting situations. These compounds are hydrophobic substances with affinity for the neuronal membranes where they are able to deactivate the enzymes found there. They present an aesthesic and antiarrhythmic effect, and stabilize the mitochondrial and lisosomic membranes, which is an explanation of its anti-inflammatiory attributes.

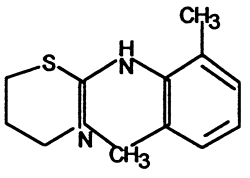
The presence of haloperidol, xylazine, acetopromazine, propionylpromazine, and chlorpromazine were investigated. All the promazines are included in the fenotiazines family. This group was synthesized as an anti-histamine and its sedative action was, for a long time, considered like a collateral effect. This tranquillizer function is caused because they are able to inhibit the dopaminic receivers of S.C.N. Therefore, they decrease the motive activity and the aggressiveness in animals.[1]



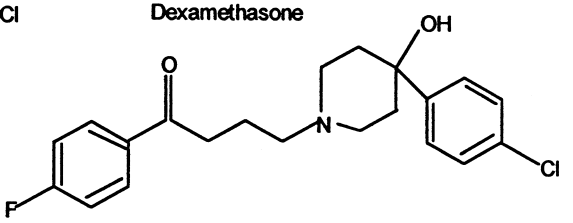

Promazine, R=$C_2H_6$, $C_3H_9$, HCl




Dexamethasone




Xylazine




Haloperidol

Analytical methods previously developed for the determination of this kind of compound in different organic matrices, are usually based in chromatographic techniques with different detection methods: U.V. spectrometry,[2-3] spectrofluotimetry,[4-6] CG-MS previous purification with "on line" immunoaffinity HPLC,[7-8] and capillary electrophoresis.[9] Several samples are investigated, such as tears,[8] humane plasma,[9-10] sheep plasma,[11] urine or feaces,[12] pharmaceuticals,[13] or bovine and pig kidney,[14] among others. The aim of this work is the simultaneous analysis of a total of 7 of these compounds in bovine visceras.

## EXPERIMENTAL

### Reagents

Dexamethasone, betamethasone acetate, chlorpromazine hydrochloride, propionyl promazine hydrochloride, acetopromazine, xylazine, and haloperidol, all of them, from Sigma Chemical Co. (U.S.A.)

All the reagents were of analytical reagent grade. Methanol was HPLC grade (Scharlau, Spain). Stocks solutions of all compounds were prepared in methanol at a concentration of 1000 $\mu g \cdot mL^{-1}$. These solutions were stored at 4°C and protected from light.

### Apparatus

Grinder (Moulinex 1,2,3); vortex-mixer (Rotabit Selecta); centrifuge Universal 16 (Genesys Instrumentation, S,L); sample concentrator Techne DRIBLOCK DB 20, equipped with temperature control and $N_2$ flow (Genesys Instrumentation, S.L).

The HPLC system consisted of a Jasco PU-1580 pumping system equipped with a Rheodyne Model 7125 injector with a 20 µL loop. The detector was a Perkin Elmer 785A UV-vis detector, and the chromatograms were monitored by a computer (Chromatography Interface "Hercule lite Interface." Software: Borwin, from JMBS Software for scientists).

### Chromatographic Conditions

The column was a 150 x 4.0 mm i.d. stainless steel prepacked reversed-phase column containing 5 µm $C_{18}$ particles (Kromasil, Spain). The mobile phase was methanol-water (80:20, v/v). These solutions were filtered through a Millipore Durapore filter (0.45 µm pore size) and deaerated by stirring under

vacuum for 10 min. The flow-rate was 1.0 mL·min$^{-1}$ and the UV detector was operated was at 240 nm.

## Sample Preparation

Samples were kept frozen untill they were analysed. They were ground, then mixed, and later they were subjected to an extraction and purification process as follows:

### Extraction Procedure

About 5.0 g of a sample of liver was ground and homogeneized. The first extraction was carried out in water:acetonitrile (10 : 40, v/v) using the vortex-mixer for 1 h at 2500 r.p.m. Then, the phases were separated by centrifugation (3000 r.p.m. during 15 min) saving the extract, and later, all the extraction process was repeated with 20 mL of acetonitrile. After the centrifugation, both extracts were mixed and evaporated until obtaining a volume of 10 mL.

In a parallel line to the sample, the extraction process was applied to a blank of reactives, and to another two samples, spiked with 10 and 20 μg of the studied compounds.

### Purification Procedure

The purification process was achieved by means of a fractionated liquid-liquid extraction procedure as follows:

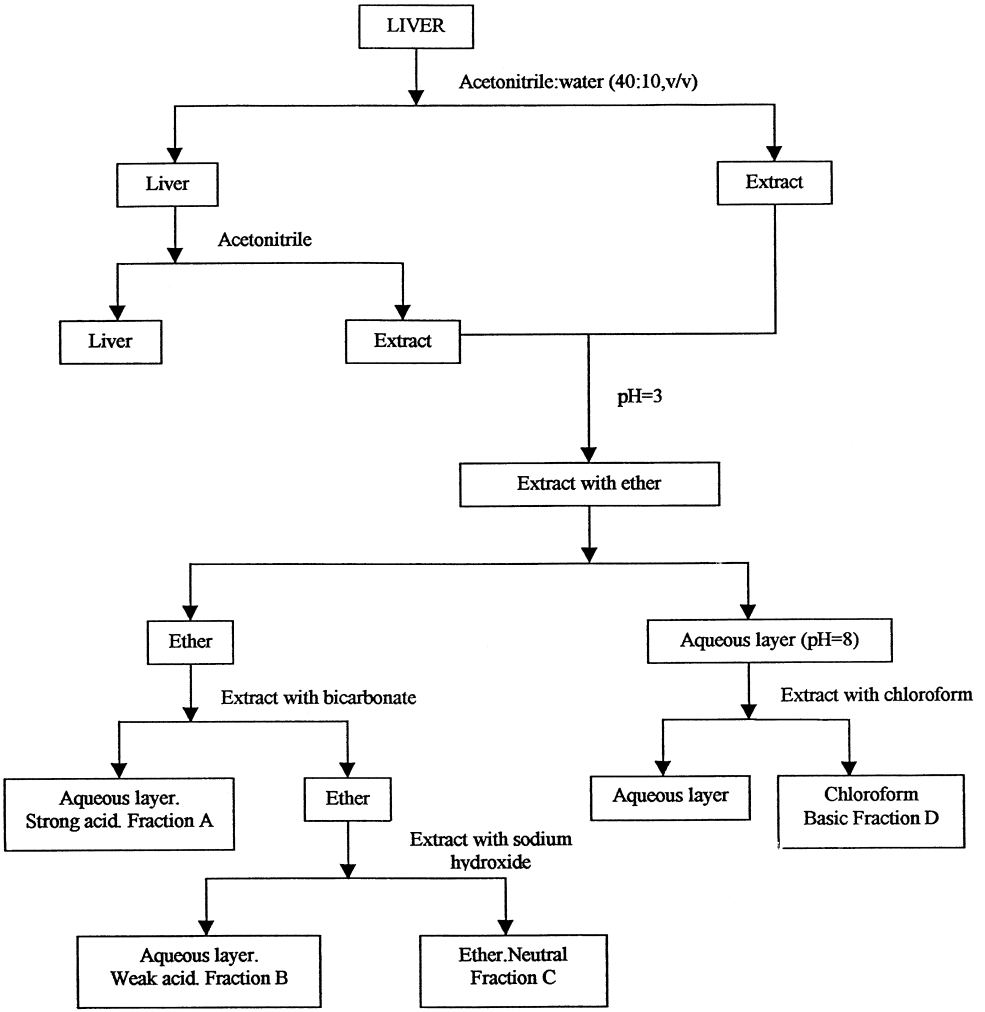
The acetonitrile phase was adjusted to pH = 3.0 using H3PO4 (c). This solution was extracted twice with 30.0 mL of diethyl ether. The organic phases were combined and washed with 5.0 of ultrapure water, adding the washing to the sample, and retaining the aqueous solution for later extraction. The ethereal phase is now extracted with 5.0 mL of saturated sodium bicarbonate solution, discarding the aqueous phase (Fraction A). Then, the ethereal solution is extracted with 5.0 mL of 0.5 M NaOH in order to eliminate the possible presence of acid susbstances like barbiturates (Fraction B).

The ethereal solution is washed with water, discarding the washing, then dried with anhydrous sodium sulphate and evaporated to dryness (Fraction C). The residue is dissolved in 2.0 mL of methanol and reserved for the analysis of corticoids.

The pH value of the aqueous solution, obtained after the first extraction, is adjusted to pH=8.0 with a diluted ammonia solution. Later, it was extracted two

times with 10.0 mL portions of chloroform. The combined extracts are washed with water and evaporated to dryness. The residue is dissolved into 2.0 mL of methanol. This fraction is used for the investigation of neuroleptics (Fraction D).

Scheme 1 shows all the process described above:




***Scheme 1.*** Extraction and purification procedure.

## RESULTS AND DISCUSSION

The purification procedure carried out, allows the investigation of corticoids and neuroleptics. Chlorpromazine, propionyl promazine, acetopromazine, haloperidol, and xylazine are investigated in fraction D, while fraction C corresponds to the betamethasone acetate and dexamethasone. 20 µL of each fraction of untreated samples, and 20 µL of the treated samples, are injected into the chromatograph.

Good resolution was possible on a Kromasil $C_{18}$ reversed phase column with methanol:water (80:20, v/v) as the mobile phase at a flow rate of 1.0 $mL \cdot min^{-1}$. According to the UV spectra of all the compounds which have been investigated and, in order to obtain better signals, a wavelength of 240 nm was chosen for the simultaneous detection.

When the optimized working conditions are used, it is possible to separate and to indentify, simultaneously, dexamethasone ($t_r$ = 2.37min) and betamethasone acetate ($t_r$ =2.78 min). Figure 1 shows the chromatogram obtained when a mixture of both corticoids are injected into the chromatograph.

Better selectivity and sensibility is obtained in methanol:water (85:15, v/v) solution as mobile phase in the analysis of fraction D. Figure 2 shows a chro-



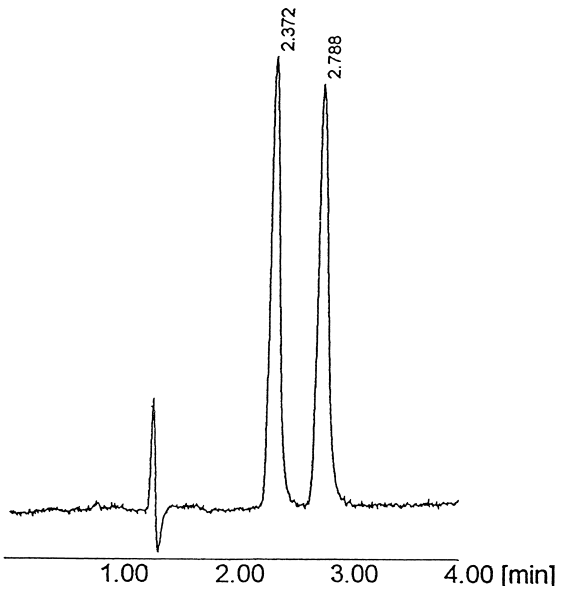



***Figure 1.*** Chromatograms of corticoids: dexamethasone and betamethasone acetate. Amount injected 6 ng. Mobile phase: methanol:water (80:20, v/v). Dexamethasone: $t_r$ = 2.372 min. Betamethasone acetate: $t_r$ = 2.788 min.

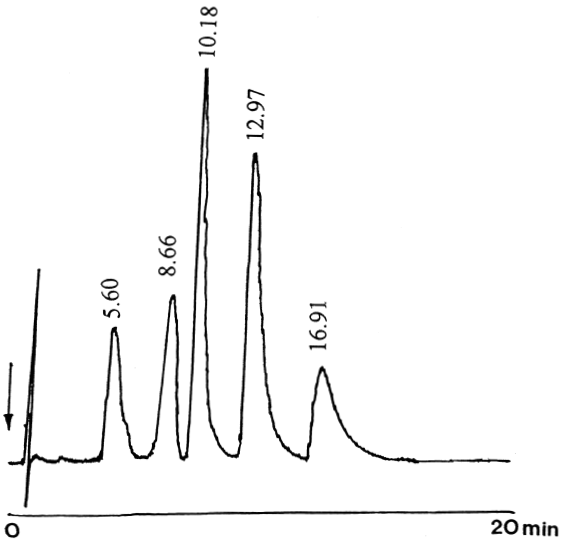



***Figure 2.*** Chromatograms of the tranquillizers investigated. Amount injected 250 ng. Mobile phase: methanol:water (85:15, v/v). Xylacine: $t_r$ = 5.60 min; haloperidol: $t_r$ = 8.66 min; acetopromazine: $t_r$ = 10.18 min; propionylpromazine: $t_r$ = 12.97 min; chlorpromazine $t_r$ = 16.91 min.

matogram obtained from an injection of fraction D. The separation of the whole five substances into the chromatographic system once the corticoids were separated is possible.

Figure 3 shows the chromatograms of the ethereal fraction obtained from a liver sample (5g) where dexamethasone is detected (chromatogram Ia) and a sample where no corticoids are detected (chromatogram Ib), as well as chromatograms obtained from samples spiked with 10 μg ( III) and 20 μg (III) of each corticoid.

A total of 31 samples of bovine livers were analysed following the indicated procedure. Dexamethasone was detected in 15 of them, whereas betamethasone acetate was identified only in 5 of them. Positive results were not detected in the investigation of the tranquillizers studied.

## Calibration Graphs, Sensibility, and Precision

For all the compounds, the precision and accuracy of the method, the linearities of calibration graphs, and detection limits ($x_b+3\sigma_b$) and determination limits ($x_b+10\sigma_b$) were calculated.

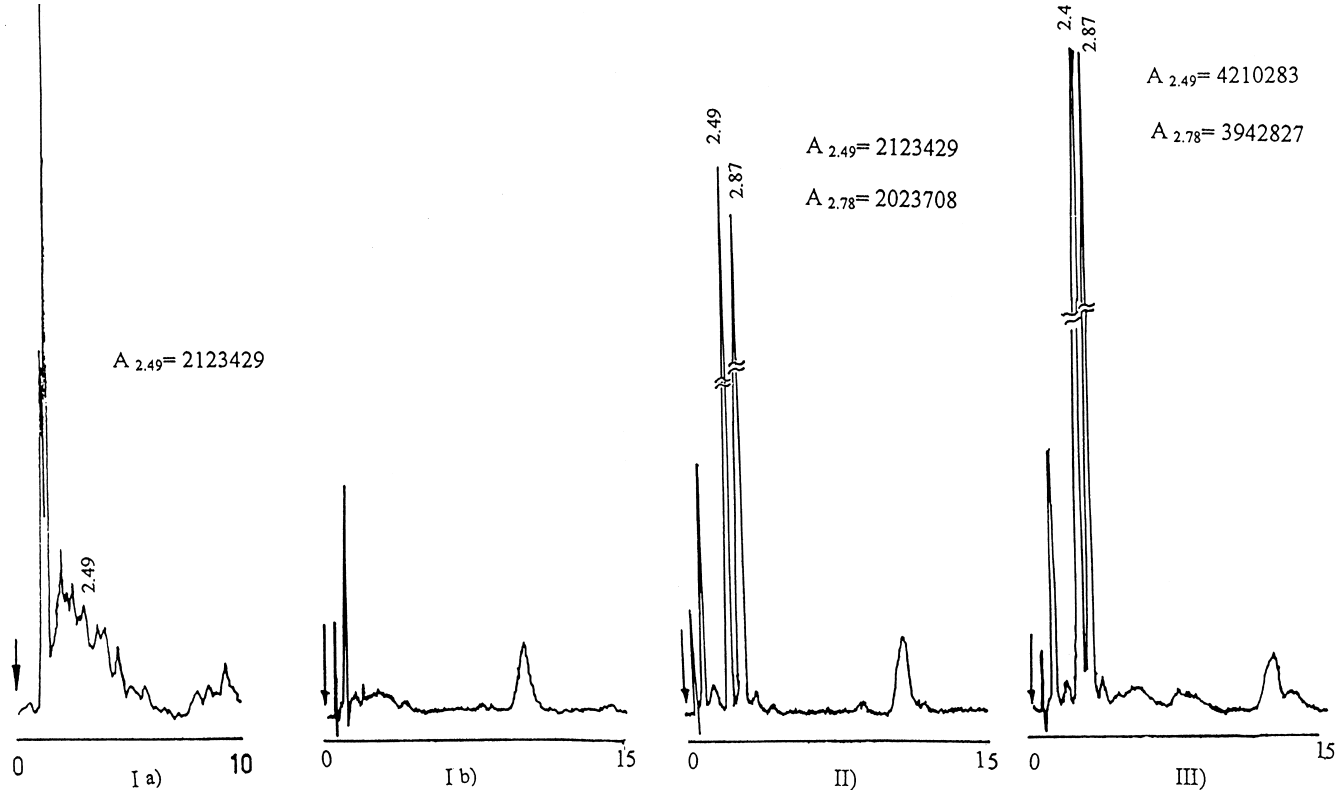



***Figure 3.*** Chromatograms of the ethereal fraction obtained from liver samples. Ia) Sample (5g) where dexamethasone is detected; Ib) Sample (5g), no corticoids are detected; II) Sample (5g), + 10 μg of each corticoid; III) Sample (5g), + 20 μg of each corticoid.

Table 1 shows the results obtained for each one of the compounds studied.

The linearity of the calibration graph is excellent in all cases, as is shown by the correlation coefficients which are very close to unity, however, the sensibility of the method is higher in the case of corticoids.

### Determination of Dexamethasone and Betamethasone Acetate

Quantification of dexamethasona and betamethasone acetate was carried out with those samples where the corticoids were identified through the qualitative analysis carried out before. To this end, all the extraction and purification processes was realized with different fractions of each sample that have been spiked with increasing and known amounts of the corticoids under investigation. On the other hand, the selectivity of the method was investigated under working conditions. So, 20 μL solutions of dexamethasone (0.3 $\mu g \cdot mL^{-1}$) and betamethasone acetate (0.3 $\mu g \cdot mL^{-1}$) were injected in the chromatographic system.

After the addition of increasing concentrations of the corresponding corticoid, the chromatograms were recorded. It was considered that a substance caused interference when the signal varied above the error of the method. In the studied concentration range (a ratio 20:1 for each compound), no interference was observed.

***Table 1.*** Experimental Results

| | Calibration curve | Detection limit ($\mu g \cdot ml^{-1}$) | Determination limit ($\mu g \cdot ml^{-1}$) | Er (%) | C.V. (%) |
|---|---|---|---|---|---|
| Dexamethasone | A=77457 C<br>R = 0.9990 | 0.021 | 0.027 | 5.7 | 4.6 |
| Betamethasone acetate | A =36753.2C<br>R=0.99990 | 0.034 | 0.041 | 2.8 | 5.8 |
| Haloperidol | A=12534.9C<br>R=0.9994 | 0.128 | 0.174 | 2.5 | 3.4 |
| Xylazine | A=41370.7C<br>R=0.9997 | 0.142 | 0.239 | 7.8 | 7.0 |
| Clorpromazine | A=-1067.7+10599.0C<br>R=0.996 | 0.298 | 0.387 | 6.7 | 5.7 |
| Propionyl promazine | A=-1167.3+47177.3C<br>R=0.997 | 0.224 | 0.289 | 6.6 | 5.4 |
| Acetyl promazine | A=-667.0+15913.09C<br>R=0.998 | 0.191 | 0.246 | 3.7 | 6.3 |

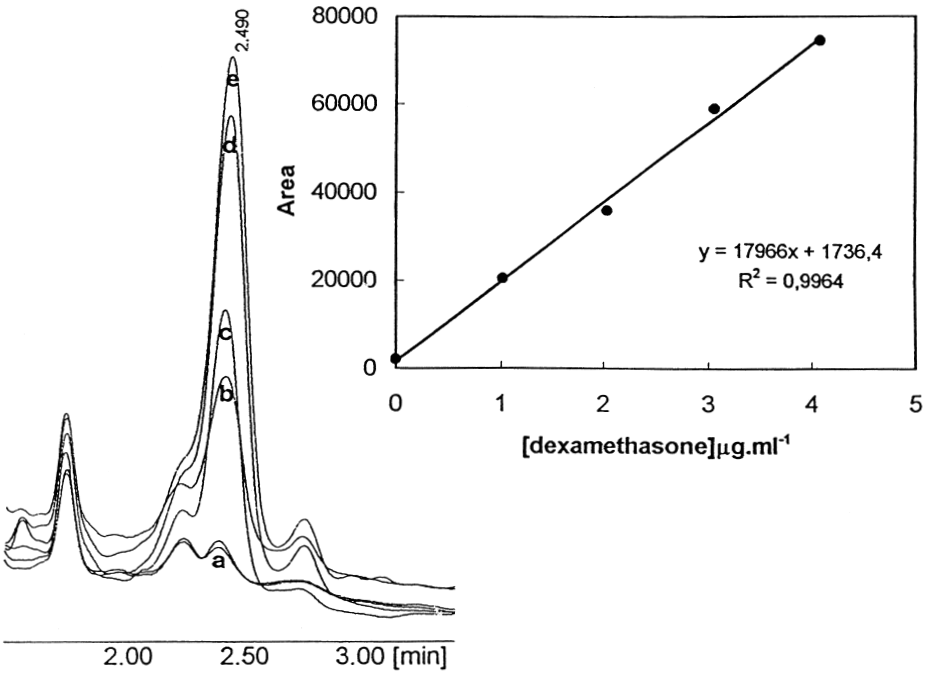



***Figure 4.*** Quantification of dexamethasone. a) Sample, b) sample spiked with 2.04 µg, c) sample spiked with 3.06 µg, d) sample spiked with 4.08 µg, e) sample spiked with 8.16 µg of the corticoid.

Figure 4 shows the chromatograms obtained, under optimal working conditions, from the injection of 20 µL of the ethereal fraction (fraction C) obtained from roughly 5 g of liver, not spiked, and another 4 samples of aproximately 5 g of the same liver that have been spiked with increasing and known amounts of dexamethasone and betamethasone acetate. It is possible to see the characteristic signal of dexamethasone and how this signal intensity increases linearily when the added amount is higher.

All the positive samples were quantified in this way. The results obained for the amounts of dexamethasone found were between 31 and 516 ng per gramme of liver. The precision of the determinations (n=3) is given from a coefficient of percent variation (C.V.%) values between 2.0 and 10.0%. The recovery values for the complete process were calculated between 40 and 80% . These values were calculated for each sample that has been analyzed.

## REFERENCES


1. *Páginas de Farmacología. Psicofarmacología (1)*; Serie Nacional Para la Formación de Postgrado, Primera Serie No. 13; 1991; 396-407.
2. Liu, K.R.; Chen, S.H.; Wu, S.M. J. Chromatogr. **1994**, *676* (2), 455.
3. Dusi, G.; Curatolo, M.; Faggionato, E. J. Liq. Chromatogr. & Rel. Technol. **1996**, *19* (10), 1607.
4. Payne, L.D.; Hicks, M.B; Wehner, T.A. J Agric. Food Chem. **1995**, *43* (5), 1233.
5. Prabhu, S.V.; Wehner, T.A.; Tway, P.C. J. Agric. Food Chem. **1991**, *39* (8), 1468.
6. Bagnati, R.; Ramazza; V.; Fanelli, R. Anal. Biochem. **1996**, *235* (2), 119.
7. Creaser, C.S.; Feely, S.J.; Teale, P. Anal. Comm. **1996**, *33* (1), 5.
8. Baeyens, V.; Varesio, E.; Gurny, R. J. Chromatogr. B, Biomed, Appli. **1997**, *692* (1), 222.
9. Schild, P.N.; Charles, B.G. J. Chromatogr. **1994**, *658* (1), 189.
10. Wu, S.M.; Wu, H.L.; Chen, S.H. Anal. Chim. Acta **1995**, *307* (1), 103.
11. Livinstong, A.; Muge, D.K.; Chambers, J.P. J. Vet. Pharmacol. Therapeut. **1995**, *18* (1), 24.
12. Courtheyn, D.; Vercmmen, J.; Peteghem, C. The Analyst **1994**, *119* (12), 2557.
13. Santos Montes, A.; Gasco Lopez, A.I.; Izquierdo Hornillos, R. Chromatographia **1994**, *39* (9/10), 539.
14. Roybal, J.E. J. AOAC Int. **1993**, *76* (4), 720.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