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Short communication

Determination of eltanolone in human plasma by high-performance liquid chromatography

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Abstract

A high-performance liquid chromatographic method for the determination of eltanolone in plasma has been developed. Plasma samples containing eltanolone were diluted with acetonitrile to precipitate plasma proteins, and derivatized with 2,4-dinitrophenylhydrazine before direct injection onto a C_{18} column. The mobile phase was acetonitrile–water (70:30, v/v) containing 0.1% trifluoroacetic acid and detection was by UV absorbance at 367 nm. The quantitation limit was 0.020 μg/ml. The method has proven to be rapid, precise and sensitive in the range of concentrations found during and following intravenous anaesthesia. © 1997 Elsevier Science B.V.

Keywords: Eltanolone

1. Introduction

Eltanolone (Careltan, 5β-pregnan-3α-ol-20-one, pregnanolone) (Fig. 1) is an intravenous anaesthetic agent currently undergoing clinical trials [1–3]. Published assays for eltanolone (pregnanolone) in plasma use GC–MS, requiring complex derivatization and expensive equipment [4–6]. A simple method of determining eltanolone by HPLC would be advantageous to laboratories without access to GC–MS equipment. Due to the low molar absorptivity of eltanolone, detection by UV absorbance lacks the sensitivity to measure anaesthetic concentrations and it was considered necessary to derivatize the drug to improve detection sensitivity. Carbonyl compounds react with 2,4-dinitrophenylhydrazine (2,4-DNP) [7] to give characteristically yellow compounds that absorb strongly at the high UV end of the spectrum. The use of 2,4-DNP to derivatize some carbonyl-containing steroids has been described [8,9] but eltanolone was not among the steroids included in that work.

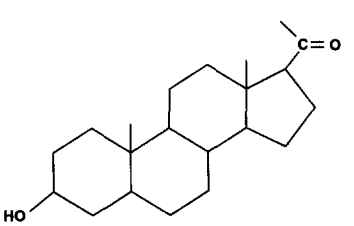

Fig. 1. Structural formula of eltanolone.

In this paper, we describe a simple, low-cost method for the determination of anaesthetic and post anaesthetic concentrations of eltanolone using a simple derivatization with 2,4-DNP and absorbance detection.

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2. Experimental

2.1. Reagents and solvents

Methanol and acetonitrile were HPLC grade from Waters (Lane Cove, Australia). Sulphuric acid (98%) was analytical grade from Ajax Chemicals (Auburn, Australia). Pregnanolone, trifluoroacetic acid and 2,4-dinitrophenylhydrazine (2,4-DNP) were all obtained from Sigma (St. Louis, MO, USA).

2.2. Chromatography

The HPLC system consisted of a WISP 712 autosampler, a Model 510 pump and a Model 481 absorbance detector all from Waters (Milford, MA, USA) interfaced to a PC running Maxima 820 software from Dynamic Solutions (Waters). The column was a 150 mm×3.9 mm Novapak C_{18}, (4 μm, Waters). The mobile phase consisted of acetonitrile–water (70:30, v/v) containing 0.1% trifluoroacetic acid, running at 2.0 ml/min. The column effluent was monitored at 367 nm. Under these conditions the capacity factor (k') of the 2,4-DNP derivative of pregnanolone was 10.2 with a retention time of 6.2 min.

2.3. Preparation of 2,4-DNP solution

2,4-DNP (2.0 g) was suspended in 100 ml of methanol and concentrated sulphuric acid (4.0 ml) was added dropwise over 10 min. The solution was allowed to stir for a further 10 min and then filtered [7].

2.4. Sample preparation

Plasma (1 ml) and acetonitrile (2 ml) were mixed in a sealable polypropylene centrifuge tube and centrifuged for 10 min at 2000 *g*. An aliquot (1 ml) of the centrifuged solution was placed into a second sealable polypropylene tube containing the derivatizing agent, 2% (w/v) 2,4-DNP (100 μl). The reaction mixture was vortexed for 10 s and then allowed to stand for 1 h before being injected onto the column (100 μl for concentrations greater than 5 μg/ml and 200 μl for concentrations less than 5 μg/ml).

2.5. Standards

Stock aqueous solutions of eltanolone (400, 40, 4 and 0.4) μg/ml were stored at 4°C. Drug-free human plasma (950 μl) was spiked with stock solution (50 μl) to produce eltanolone concentrations of 20, 2, 0.2 and 0.02 μg/ml for use as standards.

2.6. Application of method

Following informed consent in accordance with approved institutional protocol, two patients were administered a single i.v. bolus dose of eltanolone 24 mg as part of anaesthesia for surgery. Blood samples were collected into heparinized tubes at 0, 2, 5, 10, 15, 30 min after the eltanolone dose. The samples were centrifuged and the plasma stored at −20°C until analysis.

3. Results and discussion

Typical chromatograms of blank human plasma and human plasma after a single dose of eltanolone are shown in Fig. 2. The within-day and between-day coefficients of variation at concentrations of 20.000 to 0.020 μg/ml eltanolone are presented in Table 1. Standard curves were linear over the range 20.000 to 0.020 μg/ml with r^2 coefficients greater than 0.998

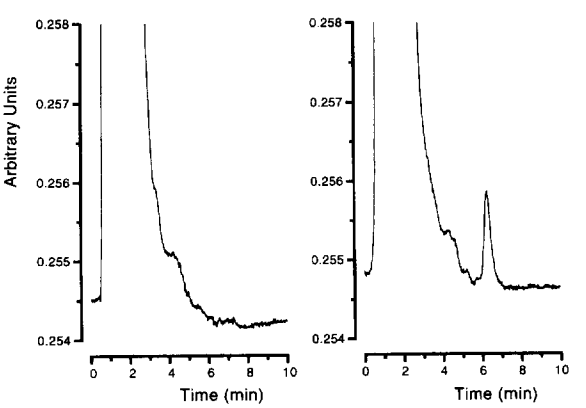

Fig. 2. Chromatograms of 2,4-DNP derivatives of blank plasma and plasma 15 min after 24 mg eltanolone i.v. (0.105 μg/ml). Detection by UV absorbance at 367 nm. Injection volume 200 μl for both chromatograms.

Table 1
Within-day accuracy and precision ($n=6$) along with between-day reproducibility ($n=5$), mean±S.D.

Spiked concentration (μg/ml)	Within-day measured concentration (μg/ml)	C.V.[a] (%)	Between-day measured concentration (μg/ml)	C.V.[a] (%)
20.000	20.040±0.240	1.2	19.920±0.180	0.9
2.000	2.010±0.046	2.3	2.014±0.052	2.6
0.200	0.194±0.010	4.9	0.204±0.014	6.9
0.020	0.022±0.002	9.5	0.019±0.002	10.5

[a] Coefficient of variation.

in all cases. The limit of detection was 0.020 μg/ml (signal-to-noise ratio of 4:1) with an injection volume of 200 μl. This was the effective limit with the 3.9 mm diameter column, as the injection of greater sample volumes resulted in significant deformation of the eltanolone derivative peak. Representative concentration profiles following the administration of a single dose of 24 mg of eltanolone in two adult surgical patients are shown in Fig. 3.

Derivatization of eltanolone with 2,4-DNP increased the molar absorptivity of the parent compound and improved the selectivity of the assay by allowing detection at 367 nm. The derivatization reaction equilibrium was achieved at approximately 30 min at room temperature, with no further change on standing at room temperature up to at least 24 h. Blank plasma treated with 2,4-DNP gave no interfering peaks under the chromatographic conditions used for the determination of eltanolone. Eltanolone is primarily metabolised to sulphate and glucuronide conjugates [4,5], which are too polar to be retained on the column. The only known unconjugated metabolite, 5β-pregnan-3α,20α-diol [10], does not contain a carbonyl group and therefore will not react with 2,4-DNP.

This method, while not as sensitive as the GC–MS methods [4–6], is sufficiently sensitive for the analysis of eltanolone concentrations during and after anaesthesia [2]. The major advantages over the GC methods are the simplicity of the sample handling allowing rapid processing and the requirement for only the simplest and most widely available HPLC equipment.

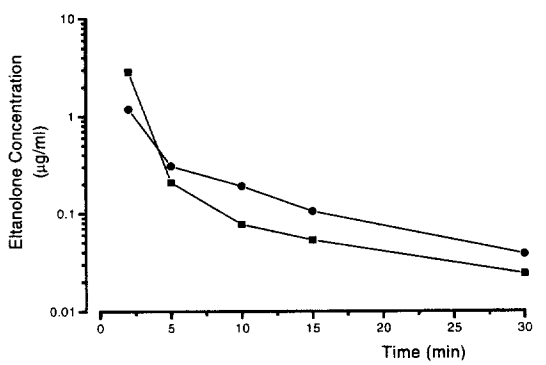

Fig. 3. Representative plasma concentrations in two surgical patients following the i.v. administration of 24 mg of eltanolone.

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