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A simple and efficient high-performance liquid chromatographic assay for etomidate in plasma

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Abstract

The development and validation of an effective and simplified LC assay for the quantitation of etomidate in beagle plasma is described. The methodology employs a rapid and simple protein precipitation procedure in combination with previously reported chromatographic conditions. Using a 0.3 ml aliquot of plasma, the assay is linear in the concentration range of 50 to 5000 ng/ml, with an extraction efficiency between 97 to 104% and accuracy between 98 and 105%. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Etomidate is a potent non-barbiturate hypnotic agent used in the induction of anesthesia [1] (Fig. 1). Administration of etomidate results in a rapid onset of hypnosis and a rapid recovery while having minimal adverse respiratory and cardiovascular effects [2]. These properties make etomidate an ideal drug for induction of anesthesia in hemodynamically compromised patients and outpatients [3].

Due to the potency of etomidate, sensitive analytical techniques are required for analysis of etomidate concentrations in plasma. After intravenous (IV) administration to humans, typical peak concentrations are between 1000 and 3000 ng/ml, with anesthesia obtained with plasma concentrations between 230 and 240 ng/ml [4]. Several high-performance liquid chromatographic and gas chromatographic methods for the analysis of etomidate in biological samples have been reported, however, each of these methodologies requires either large sample volumes (1–4 ml plasma) or a complex sample preparation. The HPLC methods of Le Moing [5] and Avram [6] provide acceptable accuracy and precision in the determination of etomidate concentration, however, both methodologies involve lengthy multiple extraction procedures from plasma samples. The HPLC method of Ellis and Beck [7] is a single extraction procedure with pentane, but this results in low extraction efficiency and, as a consequence, a 2-ml plasma sample is necessary. The volume of

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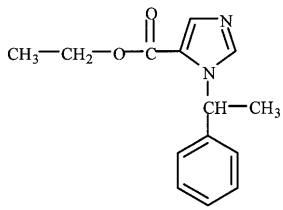

Fig. 1. Chemical structure of etomidate.

blood required for each time point would preclude multiple sampling in small laboratory animals and would limit the number of time points that could be collected from a dog.

The reported gas chromatography methods involve coupling the GC to a variety of detectors that may not be readily available, such as an alkali flame ionization detector [8], a nitrogen–phosphorous detector [9] or a mass spectrometer [10]. Typically, the sensitivity of these assays are high (1–10 ng/ml), however, the plasma sample volumes required range between 1 and 4 ml, volumes which are generally not suitable for laboratory animal studies.

The purpose of this paper is to describe a simple and rapid technique for the extraction of etomidate from beagle plasma samples, which at a later time point will be utilized for a pharmacokinetic evaluation of etomidate in beagles. The quantitation of etomidate concentrations in plasma utilized an HPLC system based on previously reported chromatographic conditions [5].

2. Experimental

2.1. Materials

The pharmacologically active isomer of etomidate (R-(+)-ethyl 1-(α-methylbenzyl)imidazole-5-carboxylate) was employed for the development of this analysis method. Acetonitrile and methanol (Fisher Chemicals, NJ) were LC grade. Analytical grade sodium phosphate (Sigma, MO) and ammonium sulfate (Fisher Chemicals) were used. Water was obtained from a Waterpro PS water purification system (Labconco, MO).

2.2. Chromatographic system

The chromatography was based on a slightly modified method of Le Moing and Levron [5]. The HPLC system was a modular system consisting of a Shimadzu SCL-10A system controller, SIL-10A autoinjector, an LC-10AT pump and an SPD-10AV UV detector set at 242 nm. A Phenomenex LUNA C18 bonded-phase column (5 μm particle size, 150 × 4.6 mm i.d., Phenomenex, CA) and a SecurityGuard C18 guard cartridge system (4 × 3.0 mm i.d., Phenomenex) were used in conjunction with Class-VP Shimadzu (Shimadzu Scientific Instruments, MD) software for data acquisition and analysis. The mobile phase consisted of 25:25:50 (v/v) acetonitrile–methanol–water containing 25 mM phosphate buffer (pH 8.1). The flow rate was 1.5 ml/min and all separations were performed with ambient column and sample temperatures.

2.3. Extraction procedure

A 300 μl sample of plasma in a 1.5 ml microcentrifuge tube was vortexed for 30 s with 100 μl of saturated ammonium sulfate solution and 300 μl of acetonitrile. To allow complete precipitation of plasma proteins, the sample was left for 30 min at room temperature. No hydrolysis of etomidate in beagle plasma was observed over this 30 min period, however, for plasma samples collected from other species, the addition of fluoride to inhibit esterase activity may be prudent [12]. Previous experience in our laboratory with ACN precipitation techniques have shown that the extraction efficiency for highly protein bound compounds can be improved if the plasma and ACN mixture are left for a short period of time, rather than immediate centrifugation. After protein precipitation, the mixture was centrifuged at 10 000 × *g* for 5 min and 50 μl of the organic layer was injected onto the LC column.

2.4. Standard solutions

A stock solution of etomidate (1 mg/ml) in methanol was prepared and stored at 4°C. Ellis and Beck [7] reported that this stock solution is

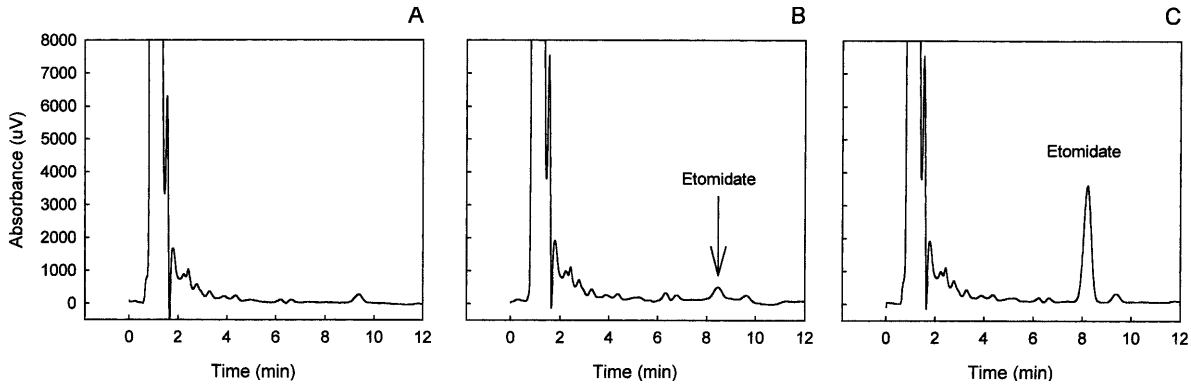

Fig. 2. Representative chromatograms of extracted blank plasma (panel A) and an extracted plasma sample spiked with etomidate to a concentration of 50 ng/ml (panel B) and 500 ng/ml (panel C).

stable for >12 months. Working solutions (0.75–75 μg/ml) in 60:40 (v/v) acetonitrile–water were prepared by serial dilution of the stock solution. Working solutions were prepared on a weekly basis.

2.5. Etomidate quantitation

Aliquots of blank plasma (300 μl) were spiked with 20 μl of standard etomidate solutions to provide final plasma concentrations ranging between 20 and 5000 ng/ml. These spiked plasma samples were used to generate standard curves by plotting the peak area against the concentration. Unknown sample concentrations were calculated from the standard equation $y = mx + c$, as determined by the linear regression of the standard curve. Linearity was confirmed by calculating the correlation statistics of the regression line. The limit of detection (LOD) was defined as the lowest etomidate concentration in plasma with a signal-to-noise ratio >3. The limit of quantitation (LOQ) was defined as the lowest concentration of etomidate in plasma at which the signal-to-noise ratio was $\geqq 10$.

2.6. Assay recovery, precision and accuracy

Recovery of etomidate was calculated by the comparison of the peak area of the etomidate peaks recovered from spiked plasma samples with the peak area of injected standard solvent solutions. Within-day precision ($n = 5$) and the accuracy ($n = 3$) of the assay were determined by replicate analyses of spiked plasma samples at three concentrations (50, 500 and 5000 ng/ml). The accuracy of the assay is expressed as ((observed concentration)/(expected concentration) $\times$ 100). The assay precision for each concentration is expressed as ((peak area S.D.)/(mean peak area) $\times$ 100). Between-day precision was determined by analysis of spiked plasma samples ($n =$ 5) at these concentrations on three different days.

Table 1
The recovery, within-day and between-day precision and accuracy of the assay procedure for etomidate from spiked beagle plasma samples

Etomidate conc. (ng/ml)	Within-day precision (CV%)	Between-day precision (CV%)	Accuracy (%)	Recovery (%)
50	2.2	6.2	97.7	103.8 ± 4.8
500	4.4	4.1	98.6	98.0 ± 2.1
5000	3.1	4.0	104.6	97.4 ± 1.3

Table 2
Comparison of previously published etomidate HPLC assay conditions with current method

Reference	Plasma volume (ml)	Method summary	Reconstitution volume (μl)	Injection volume (μl)	LOQ (ng/ml)	Recovery (%)	Accuracy (%)	Precision (%)
Current method	0.3	Protein precipitation Direct injection	–	50	50	104	98	2.2
5	1.0	Pentane extraction Acid wash Pentane extraction pH basic Pentane extraction x2 Evaporate to dryness	100	50	10	95–98	98	10.6
6	0.5	pH 10 buffer Hexane-ether extraction × 2 Acid wash Hexane–ether extraction pH basic Methylene chloride extraction Evaporated to dryness	100	15–30	20	82	118	13.6
7	2.0	Pentane extraction Evaporate to dryness	200	75	50	60	–	8.3

3. Results and discussion

Previously reported methods for HPLC determination of etomidate concentrations in plasma samples have employed complex and time-consuming methodologies. The method reported here is simple and rapid to perform, while maintaining sensitivity and accuracy.

Fig. 2 shows representative chromatograms for blank plasma (panel A), etomidate in plasma spiked to a concentration of 50 ng/ml (LOQ; panel B) and 500 ng/ml (panel C) using the extraction procedure described. The retention time for etomidate under these conditions was 8.4 min and a 12-min run time was utilized.

The limit of detection for the assay was 20 ng/ml, where the signal-to-noise ratio was $\approx 3{:}1$. The limit of quantitation (50 ng/ml) was identified as the concentration where the CV for five replicate samples was $< 2.5\%$ (Table 1). Other HPLC methods for etomidate have reported lower limits of quantitation using UV detection, however, the variability in the measurements of 10 and 20 ng/ml concentrations were reported as 10.6 and 13.6%, respectively [5,6]. Using our criteria for the determination of LOQ, all of the reported HPLC methods using UV detection would have a similar LOQ and precision at 50 ng/ml. Table 2 lists a comparison of this HPLC method with three previously reported HPLC assays for etomidate.

Across the concentration range studied, the extraction efficiency from plasma was between 97 and 104% and, as a consequence, an internal standard was not required. However, propoxate hydrochloride has been identified as a suitable internal standard compound and is often included in the extraction procedure in the analysis of etomidate [5–7,11]. The assay was linear between the concentration range of 50 and 5000 ng/ml, with correlation coefficients > 0.99.

The accuracy of the assay was determined by injecting three samples at 50, 500 and 5000 ng/ml concentrations and calculating the sample concentration from the equation describing the standard curve. The accuracy of the assay was between 98 and 105% for these concentrations, which is similar to the values reported by Avram et al. [6] and Le Moing and Levron [5].

For analysis of in vivo samples containing etomidate, potassium fluoride [7] or sodium fluoride [3] was added to the blood sample before centrifugation to inhibit esterase activity and to ensure that etomidate was not further metabolize after sample collection. Van Hamme et al. [12] reported that, unlike the situation with rat plasma, hydrolysis of etomidate did not occur in human plasma even in the absence of fluoride. Therefore, blood collection during pharmacokinetic analysis may require the addition of fluoride to the blood sample depending upon the species used for the study.

4. Conclusions

The methods described here for the analysis of etomidate in plasma allows for rapid sample preparation and HPLC analysis of etomidate concentrations between the range of 50 and 5000 ng/ml, with a level of accuracy and sensitivity equal to previously reported more expensive and time-consuming methodologies. The plasma volume required for sample analysis is sufficiently small that etomidate pharmacokinetics in laboratory animals may be studied.

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