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Determination of dihydroergotamine in human plasma by high-performance liquid chromatography with fluorescence detection☆

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Abstract

Dihydroergotamine, a 5-hydroxytryptamine antagonist, is used for the treatment of vascular headaches. A high-performance liquid chromatography assay with fluorescence detection is described for the determination of dihydroergotamine in plasma. The assay was validated over the concentration range 0.1–10 ng/ml plasma and applied to the analysis of plasma samples from subjects treated intramuscularly and intranasally with 2 mg of dihydroergotamine.

Keywords: Dihydroergotamine

1. Introduction

Dihydroergotamine (DHE) is a semisynthetic ergot alkaloid obtained from the natural product, ergotamine, by hydrogenation of the double bond in the lysergic acid moiety.

DHE is used to prevent or treat vascular headaches such as migraine and cluster headaches. Oral administration of this drug leads to incomplete and erratic absorption [1,2]. Drug metabolism studies have shown that DHE is extensively metabolized by the liver [3,4], resulting in very low plasma concentrations [1]. Therefore, the usual route of administration is by injection [5]. In recent years, there has been an interest in the development of alternative routes of administration for DHE [6]. A comparison has been made with DHE nasal spray and subcutaneous sumatriptan for the treatment of migraine [7]. Gallagher [8] has reported that intranasal administration of DHE represents an important new therapeutic option for migraine patients, and a clinical trial has been reported describing the use of DHE suppositories in an outpatient clinical setting [9].

There are reports in the literature describing high-performance liquid chromatography (HPLC) procedures with fluorescence detection for the measurement of DHE in plasma [10,11]. These procedures do not offer the selectivity or sensitivity necessary to describe the bioavailability of the drug. However, Humbert et al. [12] have reported an HPLC assay with fluorescence detection procedure which provided adequate sensitivity for the study of the

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pharmacokinetics of DHE following intramuscular (i.m.) injection to volunteers. The assay used 3 ml of plasma and the assay was validated over the range 0.25–2.5 ng/ml plasma. The disadvantages of this work are that the sample preparation involves the concentration of diethyl ether (15 ml) and requires the use of specialized column switching equipment.

The isocratic HPLC assay with fluorescence detection assay described in this work uses 2 ml of plasma and avoids the use of a step involving the concentration of an organic solvent extract. This sample preparation procedure is analogous to that previously reported for meperidine [13], ambroxol [14], gallopamil [15] and propafenone [16]. The assay is validated over a broader range (0.1–10.0 ng/ml plasma) than previously reported. The procedure has the sensitivity and specificity for pharmacokinetic studies following i.m. and intranasal administration of DHE.

2. Experimental

2.1. Chemicals and solvents

Unless otherwise stated, all solvents and reagents used were HPLC grade. Methyl-*tert*.-butyl ether (MTBE), methanol, water and acetonitrile were obtained from Burdick and Jackson (Muskegon, MI, USA). Trifluoroacetic acid and triethylamine were supplied by Aldrich (Milwaukee, WI, USA), and the purity was at least 99%. The phosphoric acid was supplied by J.T. Baker (Phillipsburg, NJ, USA). Ethanol was obtained from E.M. Sciences (Gibbstown, NJ, USA). Dihydroergotamine mesylate was obtained from the United States Pharmacopoeia (Rockville, MD, USA), and the dihydroergocristine methanesulfonate (DHEC) was obtained from Research Biochemicals International (Natick, MA, USA).

2.2. Standard and internal standard preparation

Stock solutions of dihydroergotamine mesylate and dihydroergocristine mesylate were prepared at 1 mg/ml in methanol. These stock solutions were prepared in amber volumetric flasks and stored at −5°C. Spiking solutions were prepared from the stock solution immediately prior to use by diluting with 10% ethanol in water.

2.3. Instrumentation and chromatographic conditions

The chromatography system comprised a refrigerated Waters autosampler (Model 717 plus) maintained at 4–12°C with Waters 510 pumps and the Waters 470 dual monochromator fluorescence detector. Chromatography was carried out using a Zorbax Rx-C8 (5 μm, 15 cm×4.6 mm) with the corresponding Zorbax Rx-C8 guard column (5 μm, 2 cm×4.6 mm) at 35°C using the Waters Temperature Control Module. The mobile phase was water–acetonitrile–triethylamine–trifluoroacetic acid (70:30:0.1:0.1) and the flow-rate was 1.2 ml/min. Routinely, 40-μl aliquots of plasma sample extract (total 90 μl) were injected into the chromatography system and the area of the DHE, and the internal standard chromatography (DHEC) peaks were recorded. The column efficiency was routinely monitored using the peak width at half-height method, and the system was maintained at >30 000 plates per meter by changing the guard column. The excitation wavelength was 280 nm at bandwidth 18 nm, and the emission wavelength was 350 nm at bandwidth 18 nm.

2.4. Sample preparation procedure

An aliquot (100 μl) of the DHEC internal standard solution (100 ng/ml) was added to each 2-ml aliquot of plasma in a glass culture tube (with a PTFE-lined screw cap). The samples were extracted with 6 ml MTBE using the rotatory extractor (Glass-Col Apparatus, Terre Haute, IN, USA) for 30 min at a setting of 5. After centrifugation for 5 min at 500 *g*, the samples were transferred to the deep freeze at −70°C for at least 20 min. Immediately upon removing the tubes from the freezer, the organic layer (top layer) was decanted into silanized 15-ml conical tubes. The organic solvent was back-extracted with 100 μl of 0.033 *M* phosphoric acid on the rotary extractor for 30 min at a setting of 5. After centrifugation for 5 min at 500 *g*, the organic layer was removed by aspiration with a pasteur pipette.

Care was taken to rinse the Pasteur pipette with methanol between samples. A 100-μl Gilson pipette was inserted into the aqueous acid phase in the conical tube, and a 90-μl aliquot was transferred to the 100-μl autosampler vials. Care was taken to avoid contamination of the acid aqueous extract with MTBE.

2.5. Validation procedures

Early development work involved the investigation of the sensitivity and precision by assay of replicate plasma samples spiked with DHE. The replicate plasma spikes were stored at −70°C to ensure stability, and samples were removed periodically for development work Plasma samples from six different volunteers were processed by the sample preparation procedure, and chromatography showed the specificity of the method. The plasma samples were obtained from Biological Specialty (Colmar, PA, USA) and additionally from predose samples obtained from clinical studies. Sets of plasma calibration standards at the concentrations 0.0, 0.1, 0.25, 0.5, 1.5, 5 and 10 ng/ml plasma were prepared. Simultaneously, replicate concentrations of DHE in plasma at low (0.1 ng/ml), medium (1.0 ng/ml), and high concentrations (10.0 ng/ml) were prepared. These spiked plasma samples were stored at −70°C. On each of three days, one set of calibration standards was extracted and analyzed simultaneously with one set of six replicate samples at the low concentration, six replicates at the medium concentrations, and six replicates at the high concentration. The plasma standard data (peak area ratio versus concentration) was subjected to a variance-stabilized linear regression procedure [17] in order to obtain the slope and intercept of the calibration line. The plasma concentrations of the DHE in the replicate spikes were calculated from the slope and intercept of the calibration line and the HPLC response variable data (peak area ratios of the drug to the internal standard) of the replicate samples.

The assay between-day accuracy and precision of the assay was monitored during the analysis of clinical samples by the simultaneous assay of quality control samples prepared at three different DHE plasma concentrations (0.2, 2.5 and 7.5 ng/ml). All clinical samples were stored at −70°C prior to assay.

3. Results and discussion

3.1. Sample preparation procedure

Previously, the work of Humbert et al. [12] has allowed the study of the pharmacokinetics of dihydroergotamine following i.m. injection. The procedure involves addition of ammonium hydroxide to 3 ml of a plasma sample followed by centrifugation. The supernatant is applied to an Extralut column and after 15 min the drug is extracted from the column with 15 ml of diethyl ether. The ether extract is concentrated to dryness with nitrogen prior to reconstitution in mobile phase and HPLC assay by a column switching procedure with fluorimetric detection. The diethyl ether concentration step described is time-consuming and, in order to avoid decomposition of an air-sensitive drug such as DHE, has to be done most carefully using a gentle stream of nitrogen.

The method researched and developed in this laboratory requires less plasma sample (2 ml). The sample is extracted with organic solvent and, following centrifugation, the organic solvent layer is separated from the aqueous layer by freeze pouring into conical centrifuge tubes. Back extraction of the organic solvent with a small volume of acid, followed by centrifugation and aspiration of the organic layer, provides an aqueous acid extract suitable for chromatography. This approach does not require a step involving the concentration of the solvent to dryness with nitrogen.

Early experiments compared the extraction of the drug with MTBE–hexane (1:1); MTBE–hexane (3:1) and MTBE (100%). MTBE was selected since it gave a higher recovery than the mixtures of MTBE and hexane. Following extraction of the drug into MTBE, the solvent is back-extracted with a small volume of phosphoric acid (100 μl, pH 1). Phosphoric acid was preferred to hydrochloric and sulfuric acids, because it was found that the extract obtained with phosphoric acid had better stability properties.

Chromatography is carried out by isocratic LC and the assay is validated over a broader concentration range (0.1–10 ng/ml plasma) than previously reported. This sample preparation procedure was analogous to that previously reported for gallopamil [15]. This procedure partitions and concentrates the ana-

lytes into the acid layer, while providing a highly purified extract for chromatography. The sample preparation procedure allows calibration standards plus quality control samples and up to 80 clinical samples to be prepared simultaneously within one day and analyzed unattended by HPLC.

3.2. Dihydroergotamine chromatography

The mobile phase was established by altering the ratio of acetonitrile to the aqueous component until a suitable retention time of drug and internal standard was obtained at 1.2 ml/min. The retention time of DHE was around 9 min and DHEC was around 19 min. The chromatographic system provided >30 000 plates per meter, and excellent resolution from the plasma matrix components was obtained. The specificity of the assay was demonstrated by the assay of blank plasma samples from six different subjects. A representative chromatogram is shown in Fig. 1. Fig. 1A shows the HPLC chromatogram of plasma from a subject predose. Fig. 1B shows the HPLC analysis of a plasma calibration standard spiked at the concentration of 0.1 ng/ml DHE. Fig. 1C shows the HPLC analysis of a plasma sample (1.1 ng/ml) obtained from a subject 2 h after intramuscular injection of 2 mg DHE.

3.3. Intra-day assay accuracy and precision

The intra-day accuracy and precision information for this assay was determined by the analysis of six replicate plasma samples at three different concentrations. The intra-day accuracy and precision results are shown in Table 1. The accuracy of the assay ranged from 94.8–119%; the precision range was 2.2–9.6%. Subsequently, when the method was applied to the analysis of plasma samples obtained from subjects treated with DHE, between-run accuracy and precision information was obtained from the quality control samples.

3.4. Between-run assay accuracy and precision

The between-run assay accuracy and precision information from these quality control samples at

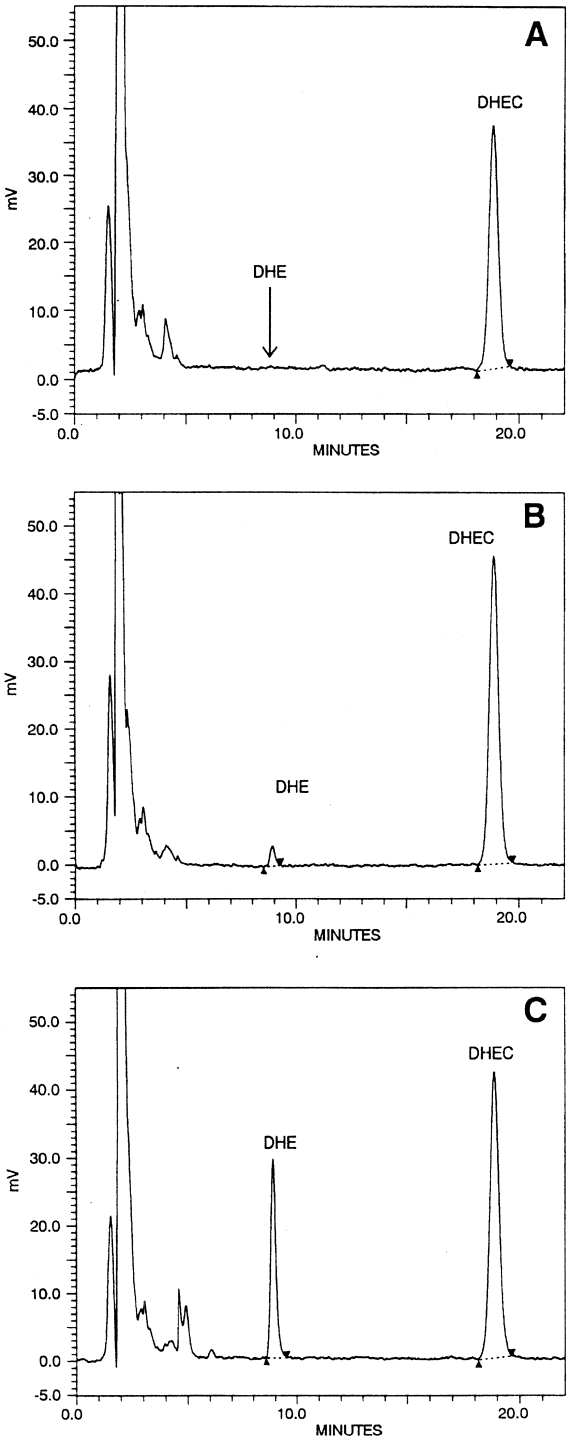

Fig. 1. Representative chromatograms of plasma extracts. (A) Blank plasma. (B) Plasma calibration standard containing 0.1 ng/ml DHE. (C) Plasma extract from a subject 0.5 h after i.m. administration of 2 mg of DHE; plasma contained 1.35 ng/ml and internal standard (dihydroergocristine).

Table 1
The within-day accuracy and precision of the assay of dihydroergotamine in plasma

	Determined concentrations	High (ng/ml)	Medium (ng/ml)	Low (ng/ml)
	Spiked value	10.0	1.0	0.1
Day 1	Mean found (n=6)	10.7	1.0	0.1
	Standard deviation	0.304	0.024	0.007
	Accuracy (%)	106.9	102.1	98.8
	Precision (RSD, %)	2.8	2.3	6.6
Day 2	Mean found (n=6)	9.5	1.0	0.1
	Standard deviation	0.210	0.030	0.005
	Accuracy (%)	94.8	98.9	119.0
	Precision (RSD, %)	2.2	2.6	4.3
Day 3	Mean found (n=6)	11.1	1.08	0.106
	Standard deviation	0.270	0.030	0.010
	Accuracy (%)	111.5	108.0	105.9
	Precision (RSD, %)	2.4	2.4	9.6
Summary of 3 days	Mean found (n=6)	10.4	1.0	0.1
	Standard deviation	0.263	0.025	0.008
	Accuracy (%)	104.4	103.0	107.9
	Precision (RSD, %)	2.5	2.4	7.0

Table 2
The between-run accuracy and precision of the assay of dihydroergotamine in plasma

	High QC (ng/ml)	Medium QC (ng/ml)	Low QC (ng/ml)
Spiked value	7.50	2.50	0.20
Day 1	7.18	2.69	0.17
	8.09	2.95	
Day 2	8.06	2.63	0.18
	8.16	2.82	0.18
Day 3	7.74	2.56	0.18
	7.5	2.5	0.18
Day 4	7.43	2.49	0.18
	7.92	2.53	0.17
Day 5	7.48	2.52	0.19
	8.12	2.51	0.18
Day 6	8.27	2.66	0.17
	8.45	2.73	0.19
Mean	7.87	2.63	0.18
Standard deviation	0.394	0.145	0.007
Accuracy (%)	104.9	105.3	89.5
Precision (RSD, %)	5.0	5.5	3.9

Table 3
The concentration of back-calculated calibration standards

	Calculated concentrations (ng/ml)					
Standard concentration	10.0	5.0	1.0	0.5	0.25	0.1
Day 1	9.81	5.12	0.991	0.489	0.259	0.099
Day 2	8.77	5.29	0.989	0.529	0.262	0.097
Day 3	10.4	4.93	0.955	0.504	0.255	0.1
Day 4	10.4	4.93	0.955	0.504	0.255	0.1
Day 5	9.85	5.12	0.931	0.548	0.241	0.1
Day 6	10.4	4.93	0.955	0.504	0.255	0.1
Mean	9.92	5.06	0.962	0.513	0.254	0.0992
Standard deviation	0.62	0.147	0.023	0.021	0.007	0.0011
Accuracy (%)	99.2	101.1	96.2	102.6	101.7	99.2
Precision (RSD, %)	6.2	2.9	2.4	4.1	2.9	1.1

three different concentrations (0.2, 2.5 and 7.5 ng/ml) is shown in Table 2. The between-run accuracy ranged from 89.5–105.3% with the precision ranging from 3.9–5.5%. The corresponding between-day calibration standard data is shown Table 3.

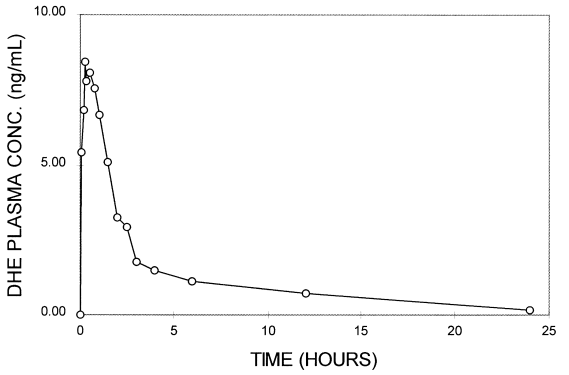

Fig. 2. Representative plasma concentration–time profile for one subject treated i.m. with 2 mg DHE.

3.5. Application of the analytical method to pharmacokinetic studies

A representative plasma concentration–time curve of a subject treated i.m. with 2 mg of DHE is shown in Fig. 2. The utility of the method for monitoring the bioavailability of drug following an alternate route of administration (intranasal) was investigated. The plasma concentration–time profile of one subject treated intranasally with 2 mg DHE together with the corresponding crossover i.m. treatment is presented in Fig. 3.

It was evident that this lower limit of quantitation (0.1 ng/ml) was adequate to routinely monitor the bioavailability of drug following i.m. and intranasal treatment up to 24 h after drug administration.

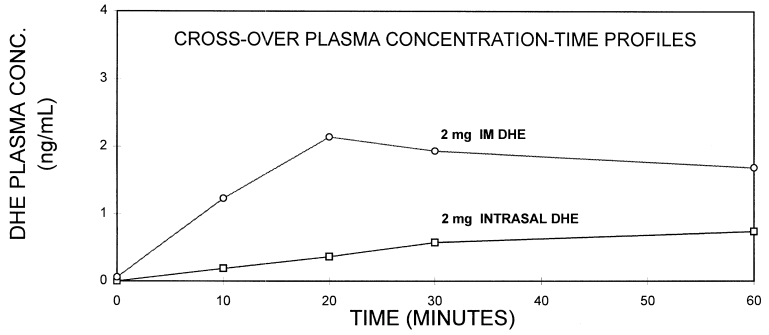

Fig. 3. Plasma concentration–time profiles from one subject treated i.m. and intranasally with 2 mg DHE.

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