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Note

Determination of ciramadol and dezocine, two new analgesics, by high-performance liquid chromatography using electrochemical detection

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Ciramadol and dezocine are currently under investigation as synthetic opioid analgesics of the agonist—antagonist type [1—5]. Gas chromatography (GC) utilizing derivatization and electron-capture detection has been the principal method of analysis [6, 7]. This method is sensitive but requires a number of extraction steps, sample derivatization with pentafluorobenzoic acid, followed by extensive sample clean-up before quantitation by GC. A high-performance liquid chromatographic (HPLC) method using UV detection has been recently reported [8], using a modification of the GC method without the derivatization and sample clean-up, but the sensitivity is diminished since the whole extract must be injected to gain the detection limits needed for pharmacokinetic studies [9].

Electrochemical detection takes advantage of the ability of a compound to undergo an oxidation and/or reduction reaction. Not all compounds are electrochemically active, but for those that are this may be a sensitive and selective method of detection for HPLC [10—13]. Utilizing electrochemical detection for the analysis of ciramadol and dezocine allows for a further simplification of the HPLC extraction, and, as the sensitivity is greater, only a small amount of the extract is injected.

EXPERIMENTAL

Instrumentation

A Waters liquid chromatograph [14, 15] was used consisting of an M6000

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dual-piston pump, WISP automatic sampler and data module. The detector was an ESA Coulochem electrochemical detector Model 5100A (Environmental Sciences Assoc., Bedford, MA, U.S.A.). Models 5010 and 5020 were used for the analytical and guard cell, respectively. The column was 12 cm × 3.9 mm I.D., stainless steel, packed with μBondapak C_{18} (particle size 10 μm), with a mobile phase flow-rate of 0.8—1.0 ml/min, operated at room temperature. Settings for the detector were: detector 1, +0.58 μA; detector 2, +0.84 μA; gain, ×10×10. The guard cell was set at a potential of +0.90 μA. Mobile phase for ciramadol was distilled water—methanol—acetonitrile—butanol—phosphoric acid (750:90:10:10:1). The dezocine mobile phase was distilled water—methanol—butanol—phosphoric acid (650:289:10:1). Distilled water was first filtered through a 0.45-μm filter; the solvent was then mixed and filtered through a Nylon 66 0.2-μm filter. The filtration through the 0.2-μm filter is necessary to degas and remove particulates from the solvent that could obstruct the electrodes. This procedure resulted in a background current of between 0.3 and 1.0 A.

Reagents

Ciramadol, dezocine and their internal standards were kindly supplied by Wyeth Labs. (Radnor, PA, U.S.A.). Stock solutions of ciramadol and dezocine (Fig. 1) were made by weighing enough of the hydrochloride salt to equal 10 mg of free base, which was then dissolved in 100 ml of distilled water to make a 100 μg/ml solution. Working solutions of 1 μg/ml for each compound were made by appropriate dilution in 100 ml of distilled water. Stock and working solutions for the internal standard for ciramadol (Wy 15623) and for dezocine (Wy 17288) were prepared the same way. All solutions were stored at 4°C in glass-stoppered bottles and were stable for one year.

A 5 *M* ammonium hydroxide (Fisher Scientific) buffer was made by diluting 333 ml of concentrated ammonium hydroxide to 1 l with distilled water. Methanol and acetonitrile were of HPLC grade (Fisher Scientific), while the ethyl acetate was of pesticide grade (Fisher Scientific).

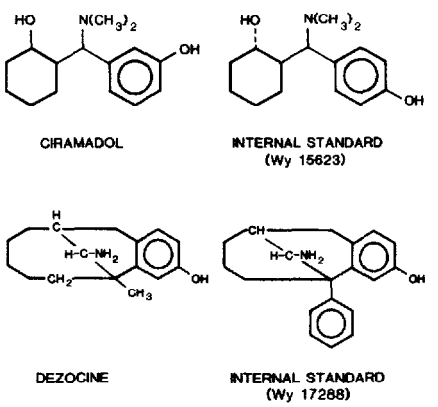

Fig. 1. Chemical structures of ciramadol and its structurally related internal standard (Wy 15623) (top) and dezocine and its structurally related internal standard (Wy 17288) (bottom).

Extraction procedure

The appropriate amount of internal standard was added to a series of 125 × 16 mm (13 ml) PTFE-lined screw-capped culture tubes. The extraction method for both compounds was the same, with calibration standards used in each run. For the ciramadol analysis, calibration standards consisted of 10, 25, 50, 75, 100, 150 and 200 ng of ciramadol with 100 ng/ml Wy 15623 as internal standard. For dezocine, standards contained 5, 10, 25, 50, 75 and 100 ng of dezocine with 500 ng/ml Wy 17288 as internal standard. Drug-free serum or plasma (0.5 ml) was added to all calibration tubes, while 1.0 ml of unknown patient serum or plasma was added to all the others. The samples were alkalinized with 1 ml of 5 *M* ammonium hydroxide, vortexed briefly to mix, then extracted by agitation on a vortex mixer for 5 min with 5 ml of ethyl acetate. The samples were centrifuged at 400 *g* for 15 min, the organic layer was drawn off to a clean set of conical tubes, and the samples were dried down under conditions of heat (40°C) and mild vacuum. The dried samples were reconstituted with 200 μl of the appropriate mobile phase and transferred to autosampling vials with limited-volume inserts. A 20—50 μl aliquot was injected onto the HPLC system.

Kinetic studies

In a pharmacokinetic study, a healthy male volunteer received a single 30-mg oral dose of ciramadol in the fasting state, followed by plasma sampling during the next 15 h. In another study, a male volunteer received 10 mg of dezocine intravenously, followed by plasma sampling over the next 8 h. Plasma concentrations of ciramadol and dezocine were analyzed as described above, and kinetic variables determined as described previously [16].

RESULTS

Evaluation of the method

Figs. 2 and 3 depict a calibration standard, patient blank and patient sample taken through the above extraction and chromatographed under the conditions given earlier. The method yields two well defined peaks, resolved from each

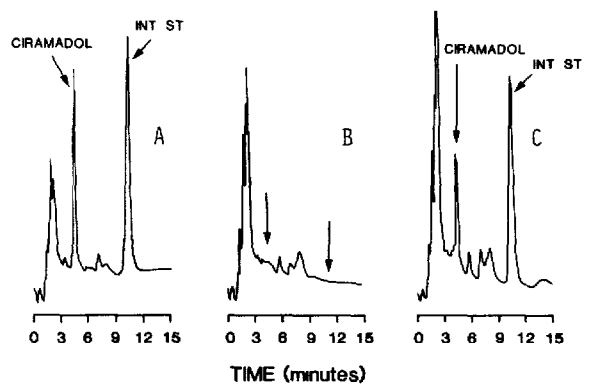

Fig. 2. Chromatograms of (A) a 100 ng/ml calibration standard for ciramadol, (B) extracted patient blank and (C) extracted patient sample, representing 62 ng/ml ciramadol.

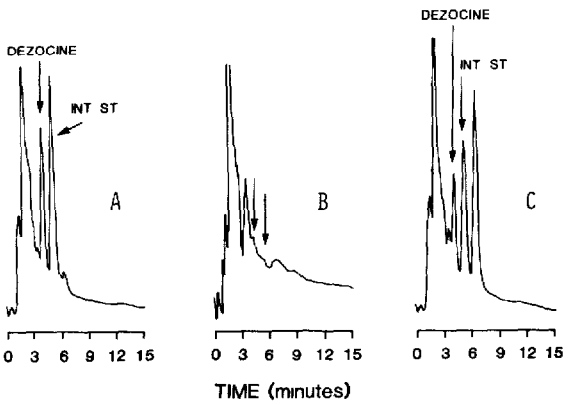

Fig. 3. Chromatograms of (A) a 50 ng/ml calibration standard for dezocine, (B) extracted patient blank and (C) extracted patient sample, representing 50 ng/ml dezocine.

other, with a retention time for ciramadol at 4.1 min, and the internal standard at 9.7 min at a flow-rate of 1.0 ml/min. Retention times for dezocine and its internal standard are 4.1 and 5.4 min at a flow-rate of 0.8 ml/min, respectively. Plasma samples stored in polypropylene tubes may contain a contaminant that elutes close to ciramadol, but this can be controlled by limiting the total number of injections onto the column.

Within-day coefficient of variance for both compounds is given in Table I. Replicate samples (n = 6) of each standard yielded coefficients of variance that ranged from 1.3 to 9.2% for dezocine and 0.7 to 5.8% for ciramadol. Calibration curves are linear, yielding, for ciramadol, a correlation coefficient (r) of 0.9993 with a typical regression equation of $y = 110.31x - 2.6$, where x = peak height ratio and y = concentration. The corresponding relations for dezocine are $r = 0.9999$ and $y = 52.39x - 0.4$. The lower limit of detection of dezocine is 1—2 ng/ml, while the limit for ciramadol is 3 ng/ml. Residue analysis for both compounds indicated a recovery of greater than 95%. Between-day variation for the dezocine 50 ng/ml standard was 8.0%, while the variation for the ciramadol 100 ng/ml standard was 9.7%.

Equivalency of the GC and HPLC methods was demonstrated by simultaneous analysis by both methods of 29 samples from a pharmacokinetic

TABLE I

REPLICABILITY OF IDENTICAL PLASMA SAMPLES (n = 6)

Ciramadol concentration (ng/ml)		C.V.* (%)	Dezocine concentration (ng/ml)		C.V. (%)
Added	Measured		Added	Measured	
10	10.7	3.8	5	5.2	2.5
25	22.7	5.8	10	9.6	9.2
50	45.8	2.2	25	24.2	3.8
100	94.8	3.9	50	49.7	2.3
150	148.1	2.6	100	100.3	1.3
200	204.6	0.7			

*C.V. = Coefficient of variation for identical samples.

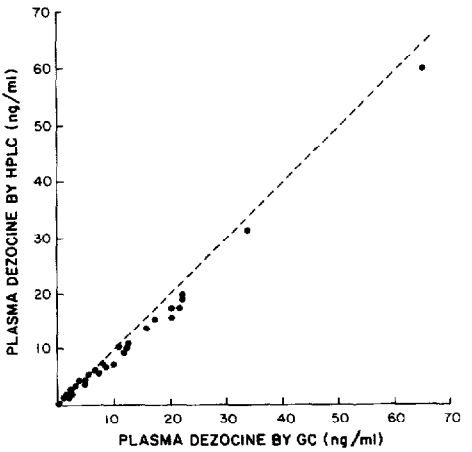

Fig. 4. Methods comparison plot of dezocine plasma concentrations measured by GC and by HPLC. The dashed line is the line of identity ($y = x$). See text for details.

study of dezocine. The GC method, as previously described by Sisenwine and co-workers [6, 7] involves samples extraction, clean-up, derivatization and quantitation by electron-capture gas–liquid chromatography. The methods comparison plot yielded a correlation coefficient of 0.997 and a regression equation of $y = 0.91x - 0.23$, where x and y are the plasma dezocine concentrations simultaneously measured by GC and HPLC, respectively (Fig. 4).

Kinetic studies

Fig. 5 shows plasma concentrations and kinetic variables for the pharmacokinetic studies of dezocine and ciramadol.

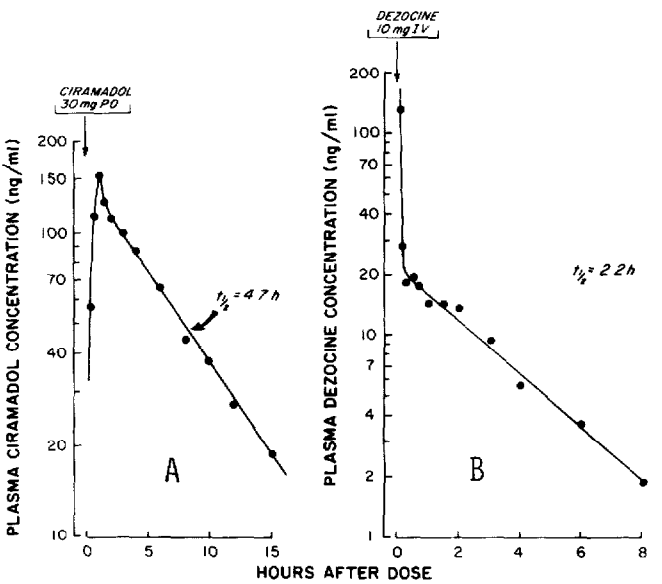

Fig. 5. Representative pharmacokinetic study of (A) ciramadol following a single 30-mg oral dose ($t_{½}$ = 4.7 h); (B) dezocine following a single 10-mg intravenous dose ($t_{½}$ = 2.2 h; V_d = 459 l and clearance = 2.32 l/min).

DISCUSSION

Dezocine and ciramadol can be quantitated in biological samples by several methods. GC with electron-capture detection is selective, but sample preparation is extensive and derivatization is necessary to yield a suitable product [6, 7]. HPLC with UV detection allows for determination of the underivatized compounds, with extraction somewhat simplified. However, sensitivity is considerably decreased [8]. The utilization of the HPLC system coupled with an electrochemical detector allows for a simple one-step sample extraction with no loss of recovery, and sensitivity equivalent to the GC method. Three organic modifiers were needed for the ciramadol mobile phase to optimize peak shape and retention. Attempts to alter proportions or eliminate one resulted in loss of peak shapes or retention. The number of ciramadol injections was monitored, since column aging negatively influenced contaminant and compound resolution. The within- and between-day coefficients of variance for both compounds fall into acceptable ranges. The method is applicable to single-dose pharmacokinetic studies of dezocine and ciramadol in humans.

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