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Note

Gas chromatographic determination of carisoprodol in human plasma

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The carbamate drug carisoprodol (N-isopropyl-2-methyl-2-propyl-1,3-propanediol dicarbamate or SOMA®) is widely prescribed as a muscle relaxant in acute painful musculoskeletal conditions. It has been in use for more than twenty years, but only a few quantitative procedures for the determination of the drug in biological fluids have been published [1—8].

One of the earliest methods in use was the colorimetric procedure of Hoffman and Ludwig [1], originally developed for the determination of meprobamate in plasma or urine, but subsequently adapted for the assay of carisoprodol by Kato et al. [9]. However, it lacked specificity since other carbamates or amides were found to interfere. The first gas chromatographic (GC) procedure for the determination of carisoprodol as well as other dicarbamates in plasma and urine of man, dog, rabbit and monkey was published by Douglas et al. [2]. This procedure used a 1.2-m column packed with 3.8% UC-W98 at 180°C and allowed determination of carisoprodol in the range 1—10 μg/ml. Maes and co-workers [3, 4] have described two GC procedures intended for the quantitation of the drug in cases of overdose, but they required frequent column reconditioning [3] or used a relatively complex clean-up procedure. Methods for forensic application have been published by Van der Kleijn et al. [5], who adapted the method of Douglas et al. [2], and by Adams et al. [6] or Brandslund et al. [7]. A GC assay for carisoprodol in serum was also devel-

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oped by Stern and Caron [8] as part of a screening procedure for a wide variety of drugs.

None of the above methods fully demonstrated specificity, linearity, accuracy, precision and limit of detection of carisoprodol in the given biological matrix as presently required by the Food and Drug Administration [10] to show validity of analytical methods used in clinical studies.

At Wallace Labs. (WL) a new GC method was developed for quantitating carisoprodol in human plasma which would be suitable for drug level monitoring. Data obtained from the application of this procedure at both WL and Harris Labs. (HL) is presented showing independent experience with this procedure. More than 1000 clinical specimens have been analyzed using this method in the course of several drug bioavailability studies.

EXPERIMENTAL

Chemicals

Carisoprodol and the internal standard, tybamate (N-butyl-2-methyl-2-propyl-1,3-propanediol dicarbamate), were provided by Wallace Labs. Glass-distilled chloroform and methanol were purchased from commercial vendors. Fresh human plasma was obtained from local sources.

Instrumentation

Sample analysis at WL was performed on a Hewlett-Packard Model 5710A gas chromatograph equipped with a Model 7671A autosampler and Model 18789A nitrogen—phosphorus detector which was interfaced with an HP3354B laboratory automation system.

Sample analyses performed at HL utilized a Sigma III gas chromatograph and nitrogen—phosphorus detector with a Model AS-100 autosampler (Perkin-Elmer, Norwalk, CT, U.S.A.). An HP3390 integrator was used for data collection and analysis.

Chromatographic conditions

Both laboratories used glass columns, 2 m × 6 mm and 2 mm I.D., packed with GP 3% SP2100 DB on 100—120 mesh Supelcoport and silanized glass wool (Applied Science, State College, PA, U.S.A.) with programming as follows: oven 180°C (isothermal), injector 200°C (HL used 300°C), detector 300°C. WL used helium as carrier gas at 60 ml/min and detector gases were oxygen and hydrogen at flow-rates of 60 and 3.4 ml/min, respectively. HL used nitrogen as carrier gas at a flow-rate of 30 ml/min and detector gases were air and hydrogen at 100 and 4 ml/min. The column conditioning was conducted at 210°C at a carrier gas flow-rate of 5 ml/min for 1—2 h followed by overnight at 190°C at 60 ml/min. Approximate retention times were 5.1 and 10.6 min for carisoprodol and tybamate, respectively. Both carisoprodol and tybamate were well resolved from phenacetin, caffeine, acetaminophen and codeine phosphate, which were a few potential drugs that might interfere owing to their presence as co-constituents in the various carisoprodol (SOMA) products or as with meprobamate which is a metabolite of carisoprodol biotransformation in man.

Preparation of solutions and standards

At WL stock methanol solutions of carisoprodol and tybamate (1 mg/ml) were freshly prepared weekly. Appropriate amounts of drug were placed in glass tubes, dried under nitrogen and reconstituted with plasma to give standards containing 0.2, 0.5, 1.0, 5.0 and 20 μg/ml. Three separate stock solutions were used to give triplicate standard curves. In addition three 20-ml batches of carisoprodol-fortified plasma were prepared (20, 5 and 1 μg/ml). From each batch four 3-ml aliquots were placed into 15-ml borosilicate tubes, sealed with Parafilm® and frozen (−5 to −15°C) pending stability analysis at weekly intervals for approximately one month. The remaining plasma was used for day 0 analysis.

The method validation procedure conducted at HL included additional standards to those above, namely 0.05, 0.1, 2, 4 and 8 μg/ml, but not 20 μg/ml.

Extraction procedure

A 1.0-ml plasma aliquot was placed into a 15-ml glass tube with a PTFE-lined screw cap. After addition of 5.0 ml of chloroform, the sample was extracted on a rotary mixer (Model R0250, Kraft Apparatus, Mineola, NY, U.S.A.) for 20 min (speed setting 4) followed by centrifugation for 10 min at approx. 1000 *g*. The aqueous portion was aspirated off, and 4.0 ml of the organic phase were transferred to a 5.0-ml glass tube and dried under nitrogen (40°C water bath) until approx. 0.5 ml remained. This was vortex-mixed and then allowed to evaporate completely. Internal standard solution (100 μl) containing 100 μg/ml tybamate in methanol was used to reconstitute the dried residue, which was transferred to a glass microvial for GC assay. Quantitation of carisoprodol in plasma samples was determined from a standard curve of peak-area ratio (WL) or peak-height ratio (HL) of drug and internal standard.

At HL tybamate internal standard solution was evaporated to dryness under nitrogen and plasma added to it. This was followed by extraction with 6 ml chloroform, centrifugation and evaporation of most of the lower organic phase under nitrogen. The residue was reconstituted in 0.5 ml methanol before injection into the gas chromatograph.

RESULTS AND DISCUSSION

Fig. 1 shows typical chromatograms of extracted blank plasma containing internal standard (A), plasma spiked with carisoprodol (B) and plasma from a subject receiving a single tablet containing 350 mg of carisoprodol (C). Carisoprodol is well separated from the internal standard, tybamate, and was resolved from other possible interferences as listed in Table I. Although not shown, chromatograms of extracted blank plasmas from many sources showed no interferences in the regions of drug and internal standard.

The selected internal standard, tybamate (an N-butyl-substituted dicarbamate), is a close analogue of carisoprodol with similar extraction characteristics. The statistical validation of the assay is listed in Tables II and III. The internal standard was added to the plasma extract (WL) or to plasma before extraction (HL) in order to estimate the variability introduced by the extraction procedure.

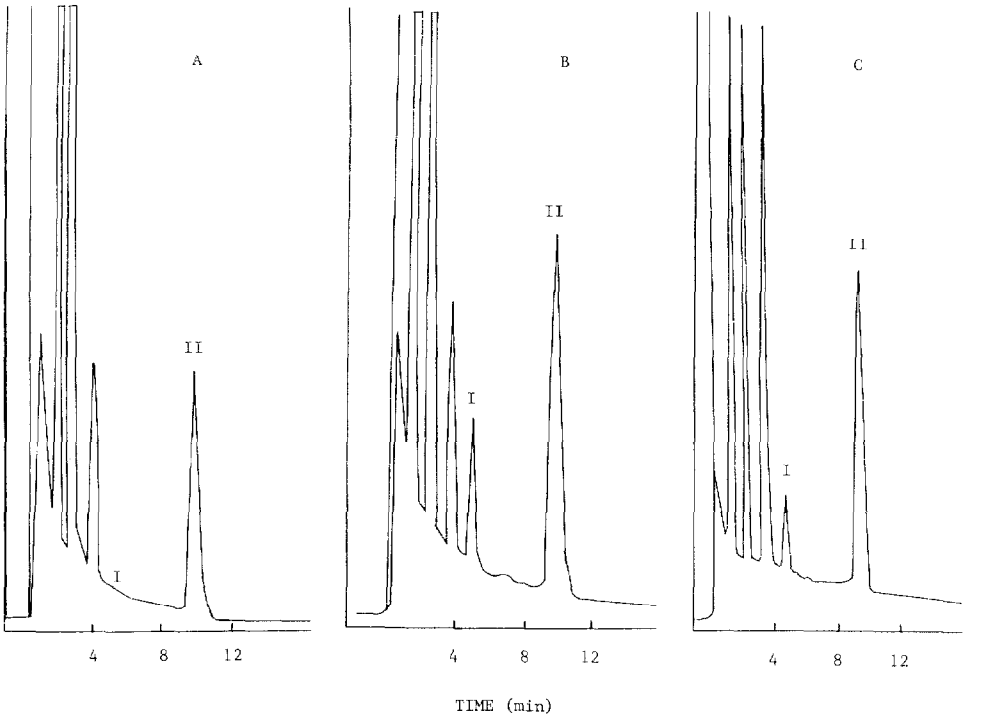

Fig. 1. Chromatograms of (A) 1 ml blank plasma with internal standard, (B) 1 ml blank plasma + 2 μg/ml carisoprodol and (C) plasma extract from a subject that received 350 mg of carisoprodol. Peaks: I = carisoprodol; II = internal standard.

TABLE I

RETENTION TIMES AND RELATIVE RESPONSE FACTORS OF OTHER DRUG SUBSTANCES SUSPECTED TO PRODUCE INTERFERENCE

Compound	Retention time (min)	Relative response factor
Phenacetin	2.62	1.04
Caffeine	2.80	6.44
Meprobamate	2.94	0.04
Carisoprodol	4.51	1.00
Tybamate	9.49	1.04
Codeine phosphate	21.71	0.72
Acetaminophen	N.D.*	—

*N.D. = Not detected.

Linearity of detector response was observed for both curves (0.2—20.0 μg/ml or 0.2—10.0 μg/ml) as evidenced by the correlation coefficients. Whether calculated by peak-area ratios or peak-height ratios, the linear regression lines of the best fit data of carisoprodol response versus concentration gave equations of $y = -0.002 + 1.00x$ ($r = 0.9998$) and $y = -0.015 + 0.999x$ ($r = 0.9994$), respectively. Linearity of response was achieved with standards as high as 40 μg/ml.

TABLE II

PRECISION, ACCURACY AND RECOVERY FOR THE DETERMINATION OF CARISOPRODOL IN HUMAN PLASMA ON THE SAME-DAY RUNS (INTRA-ASSAY VARIABILITY)

For WL, $n = 3$; for HL, $n = 4$.

Stated concentration (μg/ml)	Determined concentration (μg/ml)		Coefficient of variation (%)		Relative mean error (%)		Absolute recovery* (%)
	WL	HL	WL	HL	WL	HL	WL
0.2	0.120	—	38	—	40	—	—
0.5	0.474	0.471	14.1	4.6	5.2	5.8	65.1
1.0	0.969	—	11.3	—	3.1	—	60.1
2.0	—	2.051	—	6.4	—	2.6	—
5.0	5.255	4.738	4.4	5.6	4.5	5.2	82.5
20.0	19.934	—	0.3	—	0.3	—	82.3

*Recovery estimated based on peak areas of directly injected samples without the extraction step. At HL recovery of carisoprodol from plasma was estimated at 85.71—94.90% for concentrations between 0.2 and 8 μg/ml.

TABLE III

PRECISION AND ACCURACY FOR THE DETERMINATION OF CARISOPRODOL IN HUMAN PLASMA ON DIFFERENT DAYS (INTER-ASSAY VARIABILITY)

Internal standard was added after extraction (WL), $n = 27$, nine different daily calibration curves in triplicate, or before extraction (HL), $n = 10$, ten different calibration curves, single determinations.

Stated concentration (μg/ml)	Determined concentration (μg/ml)		Coefficient of variation (%)		Relative mean error (%)	
	WL	HL	WL	HL	WL	HL
0.2	—	0.175	—	16.1	—	12.5
0.5	0.503	0.547	17.1	8.4	0.6	9.4
1.0	1.021	0.969	11.0	3.1	2.1	3.1
2.0	—	1.989	—	2.2	—	0.6
5.0	5.080	5.058	7.2	2.8	1.6	1.2
10.0	—	10.171	—	2.0	—	1.7
20.0	19.975	—	4.7	—	0.1	—

The precision, accuracy and absolute recovery of drug for the determination of carisoprodol in human plasma was calculated from a "within-one-day" set of concentrations, each run in triplicate. The results are given in Table II. Except for the lowest concentration of 0.2 μg/ml, the precision and accuracy of the assay procedure with the internal standard added after extraction was found to be acceptable.

The reproducibility of the assay performed on different days for both laboratories was determined from nine different daily sets of data (Table III). Whenever the internal standard was added before the extraction step rather than after the extraction process, the coefficient of variation was found to be

smaller. Comparison of the intra- and inter-assay coefficients of variation showed that the assay is reproducible and the day-to-day variability added another 3% to the total assay precision.

The lowest quantifiable concentration (limit of detection or D) was found to be 0.58 μg/ml (WL) and 0.23 μg/ml (HL). This was calculated using the following formula: $D = X_b + fS$ where X_b represents the mean determined values for a series of blank samples, S is the standard deviation of the blank readings and f is a factor associated with the number of replicates as defined elsewhere [11]. The apparent difference here was probably due to a cleaner blank plasma employed by HL. No improvement in sensitivity was achieved by increasing the volume of plasma used.

Freezer storage of carisoprodol-fortified plasmas (1.0, 5.0 and 20.0 μg/ml) showed no apparent deterioration after 9, 13, 22 and 37 days as tested by one-way analysis of variance (ANOVA) calculations, $F_{0.05}$ (4,5) < 5.19, on average values obtained at each concentration level for the time intervals indicated.

Approximately thirty samples, in duplicate, could be manually injected per working day based on 9—10 min run time. With autosampling this estimate has been exceeded since sample preparation is relatively facile.

Typical plasma level data obtained for subjects dosed with a single 350-mg carisoprodol tablet is shown in Table IV. The assay has proven to be reproducible, sensitive, rapid and selective for drug level monitoring of clinical specimens. With minor modifications we have also used this GC procedure for quantifying carisoprodol in rat and dog plasma samples.

TABLE IV

MEAN CONCENTRATION OF CARISOPRODOL IN PLASMA OF EIGHTEEN SUBJECTS ADMINISTERED A SINGLE 350-mg TABLET OF SOMA

Time (h)	Determined concentration of carisoprodol	
	Mean (μg/ml)	Standard error of the mean
0.50	1.51	0.26
0.75	2.01	0.27
1.00	2.07	0.23
1.50	1.65	0.14
2.00	1.57	0.13
3.00	1.11	0.13
4.00	0.66	0.09
6.00	0.24	0.04
8.00	0.08*	0.02

Range for individual subjects 0.05—3.79 μg/ml

*Below limit of detection; estimated value.

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