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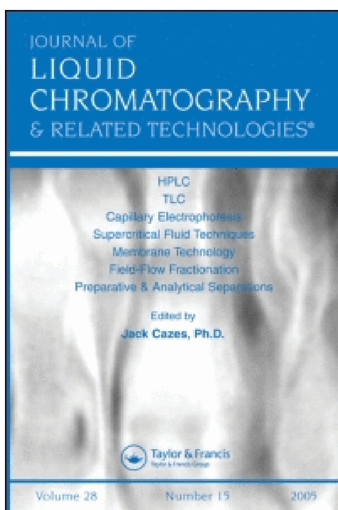

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY FOR CISATRACURIUM AND ITS METABOLITES IN HUMAN URINE

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ABSTRACT

An HPLC assay for the analysis of cisatracurium and its metabolites, laudanosine and the cis-monoquaternary alcohol (MQA), in human urine was developed. The assay involved solid-phase extraction followed by HPLC analysis on a strong cation exchange column with fluorescence detection. Linear regression was used to quantitate cisatracurium and its metabolites over the concentration ranges 10 to 2000 ng/mL for cisatracurium and 10 to 1000 ng/mL for the metabolites. The lower limit of quantitation for the assay was set at 10 ng/mL. The accuracy of the assay ranged from 6.6 to 17.0% for cisatracurium, -1.3 to 2.7% for laudanosine, and 0.2 to 3.4% for MQA. The precision of the assay ranged from 4.7 to 14.0% for cisatracurium, 4.6 to 7.7% for laudanosine, and 3.3 to 7.4% for MQA. The assay is specific for cisatracurium and its metabolites and no interfering endogenous peaks were observed in human urine. Cisatracurium, laudanosine and MQA were stable in acidified human urine (-20°C) for at least 9 months.

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Cisatracurium and its metabolites were also stable in acidified stock solutions and in urine after three freeze-thaw cycles. The mean recoveries for cisatracurium, laudanosine, MQA, and the internal standard from human urine were 104.4, 91.9, 91.9 and 93.4%, respectively.

INTRODUCTION

Cisatracurium, (1*R*, 2*R*)-2,2'-[pentamethylenebis(oxycarbonylethylene)] bis- (1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium) dibenzenesulfonate, (Figure 1), is an investigational intermediate-acting nondepolarizing neuromuscular blocking agent.[1] Cisatracurium is one of ten isomers contained in atracurium and represents approximately 15 percent of the atracurium mixture. Cisatracurium has similar neuromuscular blocking properties to atracurium.[2] However, it is more potent and is significantly weaker as a histamine releaser than atracurium. *In vitro* studies have shown that cisatracurium spontaneously degrades at physiological pH via Hofmann elimination to form laudanosine (Figure 1).[1] In human plasma, cisatracurium is also metabolized by esterases to the monoquaternary alcohol. The other potential product of ester hydrolysis, the monoquaternary acid, has not been detected *in vitro* in human plasma.[1] A high-performance liquid chromatography (HPLC) method was developed to quantitate cisatracurium and two of its metabolites in human plasma.[3,4] The assay was adapted for human urine to support clinical studies designed to investigate the excretion of cisatracurium and the two metabolites. This report describes the accuracy, precision, and specificity of the assay and the stability of cisatracurium and its metabolites in human urine.

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MATERIALS AND METHODS

Chemicals

Cisatracurium and the cis-monoquaternary alcohol (MQA) were synthesized at the Wellcome Foundation, Dartford, Kent, UK. Laudanosine was purchased from Aldrich Chemical Co., (Milwaukee, WI). The internal standard, N-methyl laudanosine, was synthesized at the Wellcome Foundation, Dartford, Kent, UK. High purity sulfuric acid was purchased from GFS Chemicals (Columbus, OH). All solvents used were HPLC grade (Omnisolv, EM Science, Cherry Hill, NJ).

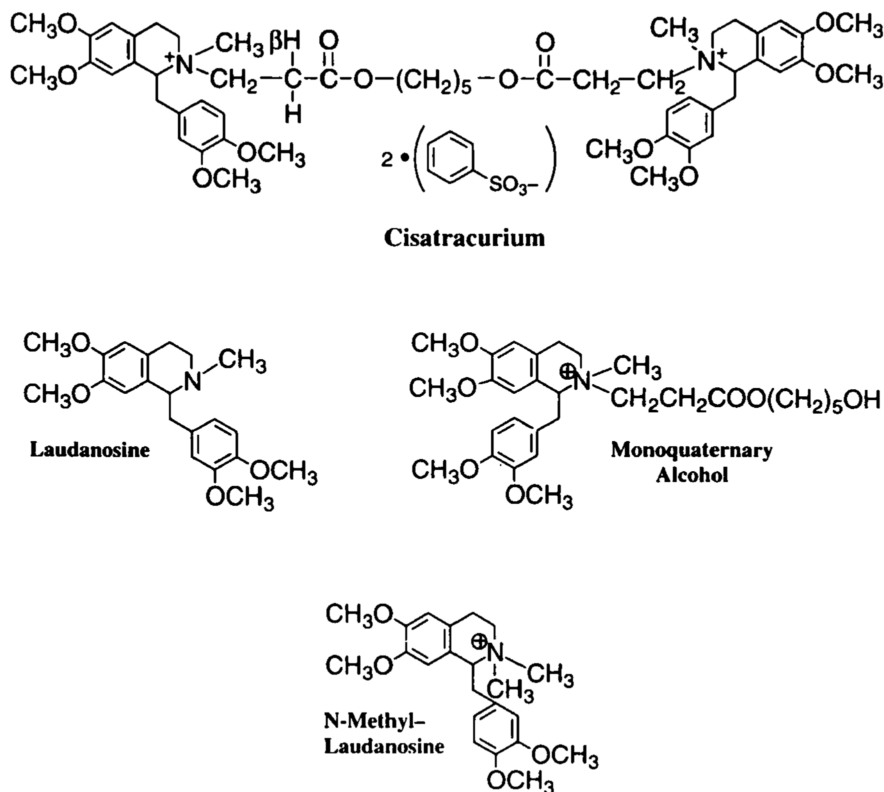

Figure 1. HPLC chromatograms of cisatracurium, its metabolites and internal standard.

Instrumentation

The HPLC system used consisted of either a Hewlett Packard model 1090 with autosampler and column heater (Hewlett Packard, Avondale, PA) or a Waters Model 600 multisolvent delivery system with a model 712 WISP injector (Waters Associates., Milford, MA) and a Systek thin foil heating system (Systek, Minneapolis, MN). A Hewlett Packard model 1046A fluorescence detector was used with both systems. The fluorescence excitation and emission wavelengths were optimized at 230nm for excitation and 315nm for emission. A Spherisorb® strong cation exchange (SCX) HPLC column (250 mm x 4.6 mm, Phase Separations, Norwalk,CT) was used for the analyses. The column was maintained at 50°C. The HPLC system was programmed to deliver acetonitrile:7 mM sodium sulfate in 0.5 mM sulfuric

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acid (6:4,v:v) for 18 min, followed by acetonitrile:56 mM sodium sulfate in 0.5 mM sulfuric acid (6:4,v:v) for 11 min, returning to the initial condition to re-equilibrate for 5.5 min. The flow rate was 2 mL/min and the column temperature was 50°C. Data were collected and analyzed with VG Multichrom software (VG data systems, Altrincham, Cheshire, UK) on a VAX 6000-320 computer (Digital Equipment Corp., Maynard, MA).

Preparation of Calibration Standards and Control Samples

Urine samples containing cisatracurium must be acidified to prevent the degradation by Hofmann elimination and ester hydrolysis.[2] Calibration standards and quality control samples were prepared by adding aliquots of the standard solutions to acidified control urine. For every 1 mL of urine, 4 mLs of 0.05M citrate buffer (pH 3.05) were added. Calibration standards ranged from 10 to 2000 ng/mL for cisatracurium and 10 to 1000 ng/mL for laudanosine and MQA. Urine quality control samples spiked at five concentrations (50, 500, 1000, 5000, and 10,000 ng/mL for cisatracurium and 50, 250, 500, 2500, and 5000 ng/mL for laudanosine and MQA) were prepared by adding aliquots of standard solutions (prepared separately from the calibration standards) to acidified control urine. The control samples were divided into 3.5 mL portions and stored in polypropylene tubes at -20°C. Prior to analysis, the two highest concentrations were diluted 1:10 with citrate buffer to fit the calibration curve range.

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Extraction Procedure

Calibration standards, spiked control samples, and study samples were processed as follows. Acidified urine samples (1.5 mL) and internal standard (75 μL of a 1 mg/mL solution) were combined in a polypropylene tube. Samples were mixed, allowed to equilibrate for 5 minutes and then centrifuged at 3000 x g for 10 minutes. Phenyl Bond Elut® cartridges were conditioned with acetonitrile and 5 mM sulfuric acid (1 mL of each).

Acidified urine samples (1 mL of the supernatant) were loaded onto the cartridges. Cartridges were washed with 5 mM sulfuric acid and 1:1 methanol:water (1 mL each), and cisatracurium and its metabolites eluted with acetonitrile:80 mM sodium sulfate in 5 mM sulfuric acid (6:4,v:v), (0.6 mL). The eluate volumes were reduced under nitrogen and then transferred to polypropylene vial inserts for HPLC analysis.

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Calculations

The peak height ratios of each compound and internal standard were calculated from the calibration standards and the data fitted to a least-squares linear-regression model. The concentrations of the analytes were calculated from the regression parameter estimates obtained from the calibration curve.

Assay Validation

The appropriate regression model for the assay was determined by analyzing replicate spiked calibration standards (10 to 2000 ng/mL for cisatracurium; 10 to 1000 ng/mL for laudanosine, n=6 at each of 6 concentrations). The concentration-peak height data were fitted to a least-squares linear regression model (LSLR) with four weighting schemes (unweighted, 1/c, $1/c^2$, and log-log transformed). The residuals at each concentration were calculated, and a plot of the Studentized residuals versus concentration was used to determine the appropriate model and weighting.

The accuracy and precision of the method were estimated by assaying spiked urine samples at five concentrations (50, 500, 1000, 5000, and 10000 ng/mL for cisatracurium and 50, 250, 500, 2500, and 5000 ng/mL for laudanosine and MQA).

One-way analysis of variance (ANOVA) was used to partition the total observed variance of the assay into its two components, within-assay variance (random error) and between-assay variance (error associated with differences in day-to-day conditions). Precision was expressed as the coefficient of variation (CV) of the means of these runs. Accuracy was calculated as the percentage difference between the mean calculated concentration and the amount added (% bias).

$$\text{Precision: } CV = \text{Standard deviation/mean} \times 100$$

$$\text{Accuracy: } \%Bias = \frac{\text{Mean measured value - Theoretical value}}{\text{Theoretical value}} \times 100$$

The stability of cisatracurium and its metabolites in acidified human urine stored at -20°C was determined. Urine spiked at five concentrations (50, 500, 1000, 5000, and 10000 ng/mL for cisatracurium and 50, 250, 500, 2500, and 5000 ng/mL for laudanosine and MQA) was assayed on the day of preparation and at various time intervals after storage at -20°C.

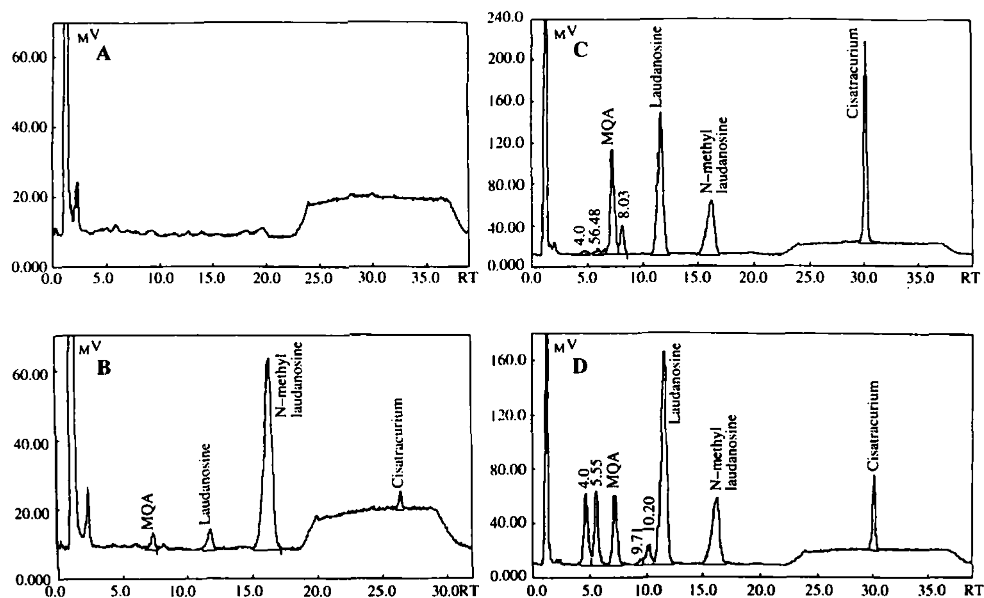

Figure 2. HPLC Chromatograms of Cisatracurium and Its Metabolites. a) Chromatogram of extracted acidified blank human urine. b) Chromatogram of extracted acidified blank urine spiked with Cisatracurium and its metabolites (10 ng/mL of each). c) Chromatogram of extracted QC sample spiked with 500 ng/mL of Cisatracurium and 250 ng/mL of each metabolite. d) Chromatogram of extracted patient urine sample 109-203. Sample collected from 5 t 10 hours after a 7.2 mg dose was received.

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The stability of cisatracurium and its metabolites was also determined in stock solutions stored at 4°C, and in urine samples after three freeze-thaw cycles. The extraction efficiency of the assay was determined by comparing the peak heights for each analyte in extracted standards with those obtained by the injection of unextracted standards.

Biomedical Application

A clinical study was designed to investigate the safety, efficacy and pharmacokinetics of cisatracurium in human volunteers.[5] During the surgical procedure, volunteers received an intravenous bolus dose of 0.1 mg/kg cisatracurium.

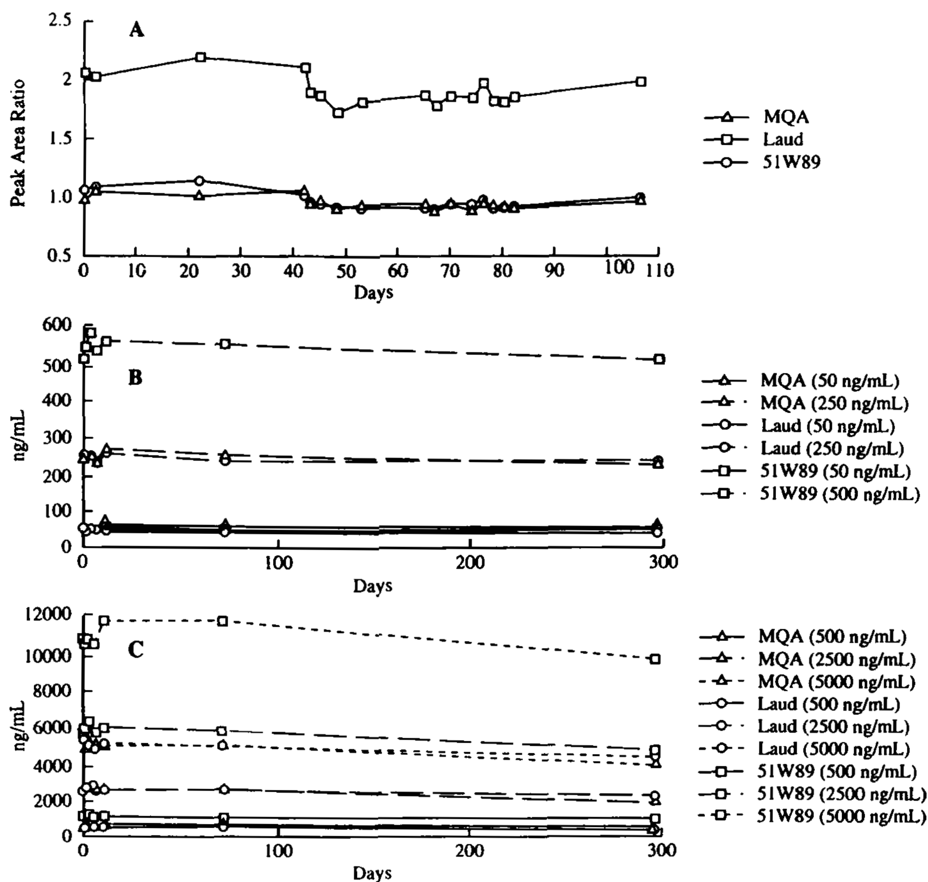

Figure 3. Stability of 51W89, laudanosine, and MQA. a) Stock solutions (2 μL of a 10μL solution). b) Human urine. c) Human urine.

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Urine samples were collected at 5-hour intervals for 10 hours after cisatracurium administration via an in-dwelling urinary catheter. Urine samples were stored at -20°C until analyzed. Urine was collected into bottles containing sodium citrate buffer, pH 3.1.

At the end of the collection period, the total volume was measured and a 50 mL aliquot removed for the determination of cisatracurium and its metabolites.

Table 1

Recovery of Cisatracurium, Its Metabolites, and the Internal Standard from Human Urine

Compound	Concentration (ng/mL)	% Recovery (mean ± S.D.)
Cisatracurium	1.00.0	108.3 ± 5.3
	1000.0	100.4 ± 2.2
Laudanosine	100.0	92.1 ± 5.9
	250.0	93.3 ± 8.6
	500.0	90.2 ± 8.2
MQA	100.0	92.7 ± 5.0
	250.0	94.7 ± 9.2
	500.0	88.4 ± 10.7
N-methyl-laudanosine	25.0	90.7 ± 1.7
	50.0	96.0 ± 10.2
	100.0	93.4 ± 5.5

(n=3 at each concentration)

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RESULTS AND DISCUSSION

Figures 2a - d show representative chromatograms of blank urine, urine spiked with cisatracurium and its metabolites near the lower limit of quantitation (10ng/mL), urine quality control, and urine from a patient who received cisatracurium. The retention times of cisatracurium, the internal standard, laudanosine and MQA were typically 30, 16, 11.5, and 7.5 min, respectively, and no endogenous interferences were noted.

Residual plots from the calibration curve data fitted to an LSLR model indicated that the variance associated with the response (peak height) was homogeneous throughout the concentration range when the data were weighted by 1/(concentration).[2]

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Table 2

Accuracy and Precision Data for the Assay in Human Urine - Results of ANOVA

Conc. Nominal ng/mL	Conc. Assayed ng/mL	Std. Dev.	Overall CV(%)	Within-Day CV(%)	Between Day CV(%)	Bias (%)
Cisatracurium						
50	53.1	3.6	6.8	5.0	4.6	6.2
500	551.7	10.5	5.1	3.0	4.2	10.3
1000	1091.0	127.9	11.7	11.7	0.0[a]	9.1
5000	5848.4	274.8	4.7	3.7	2.9	17.0
10000	11137.3	1554.4	14.0	14.0	0.0[a]	11.4
Laudanosine						
50	49.9	3.8	7.7	7.7	0.0[a]	-0.2
250	250.9	11.5	4.6	1.8	4.2	0.4
500	493.3	22.8	4.6	4.3	1.6	-1.3
2500	2566.6	134.6	5.3	5.3	0.0[a]	2.7
5000	5026.9	375.2	7.5	7.5	0.0[a]	0.5
MQA						
50	51.7	3.2	6.2	5.9	1.7	3.4
250	255.6	14.1	5.5	5.3	1.5	2.2
500	501.1	16.4	3.3	2.1	2.5	0.2
2500	2581.1	146.3	5.7	5.7	0.0[a]	3.2
5000	5068.6	373.9	7.4	7.4	0.0[a]	1.4

(n=5 assays)
[a]ANOVA estimated this as zero because within-day variability was significantly larger than between-day variability.

Results from the residual plots also indicated the assignment of the upper and lower limits of quantitation which were 2000 and 10 ng/mL for cisatracurium and 1000 and 10 ng/mL for laudanosine and MQA. The concentrations of cisatracurium, laudanosine and MQA in acidified stock solutions stored at 4°C were stable for at least 3 months.

Table 3

Urinary Excretion Data from a Clinical Study

Patient Group	Cis-atracurium Dose (mg/kg)	N	Mean % of the Dose Recovered Unchanged in Urine[a]	Amount of Dose Recovered in Urine as Cistracurium[a] (ug)	Amount of Dose Recovered in Urine as Landanosine[a] (ug)	Amount of Dose Recovered in Urine as MQA[a] (ug)
End-Stage Liver Disease	0.1	11	11.0 ± 6..9 (5.1 - 27.9)	909.9 ± 460.1 (341.6 - 1719.5)	602.4 ± 280.1 (79.1 - 1245.9)	385.9 ± 232.4 (142.4 - 758.5)
Healthy Adults	0.1	8	14.3 ± 4.0 (6.4 - 18.9)	1302.8 ± 458.8 (398.5 - 1912.0)	503.5 ± 259.7 (61.2 - 803.2)	391.3 ± 138.5 (86.7 - 551.0)

[a] Data presented as mean ± S.D. (range)

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Cisatracurium and its metabolites were also stable in urine frozen at -20°C over a period of at least 9 months, and in acidified urine after three freeze-thaw cycles. No trends in concentration were observed in any of these stability studies (Figure 3).

The recovery results of cisatracurium, laudanosine, MQA and the internal standard from human urine are shown in Table 1. The mean recoveries ranged from 100.4 to 108.3% for cisatracurium, 90.2 to 93.3% for laudanosine, 88.4 to 94.7% for MQA and 90.7 to 96.0% for the internal standard, respectively.

The accuracy of the assay, expressed as % bias, and the within-day and between-day precision data determined by ANOVA are shown in Table 2. The % bias of the assay ranged from 6.2 to 17.0% for cisatracurium, -1.3 to 2.7% for laudanosine, and 0.2 to 3.4% for MQA.

The precision of the assay ranged from 4.7 to 14.0% for cisatracurium, 4.6 to 7.7% for laudanosine, and 3.3 to 7.4% for MQA.

The average % bias over the entire concentration range was 10.0, 0.25, and 1.9 for cisatracurium, laudanosine, and MQA, respectively. The average precision over the entire concentration range was 8.9, 5.9, and 5.6% for cisatracurium, laudanosine, and MQA, respectively

The assay has been used to quantitate urine concentrations of cisatracurium and its metabolites in volunteers after an intravenous bolus dose of cisatracurium. As an example of the utility of the assay, urinary excretion data in healthy adults and end-stage liver disease patients are shown in Table 3. The data indicate that urinary excretion is a minor elimination pathway for cisatracurium in humans.

In summary, a sensitive and selective HPLC assay has been developed and validated for the simultaneous quantitation of cisatracurium and two of its major metabolites in urine. The method has been used to study the pharmacokinetics of cisatracurium and its metabolites in humans.

REFERENCES

1. R. M. Welch, A. R. Brown, R. Dahl "The *In Vitro* Degradation of Cisatracurium, the R,Cis-R',Cis Isomer of Atracurium, in Human and Rat Plasma," Clin. Pharm. & Therap., **58(2)**, 132-142 (1995).

2. R. L. Stiller, D. R. Cook, S. Chakravorti, "*In vitro* Degradation of Atracurium in Human Plasma," Br. J. Anaesth., **57**, 1085-1088 (1985).

3. C. D. James, M. O'Mara, C. Rawls, B. J. Bryant, L. Kanics, G. Ridout, G. Lovell, J. C. Harrelson "A High-Performance Liquid Chromatographic Assay for the Quantitation of Cisatracurium and its Metabolites in Human Plasma," Submitted for internal review; Prepared for Journal of Chromatography.

4. G. Lovell, J. Morgan, "The Analysis of the Geometric Isomers of Atracurium and Principal Metabolites in Biological Fluids Using Solid-Phase Extraction and Gradient Cation Exchange HPLC with Fluorescence Detection," 2nd International Symposium on Pharmaceutical and Biomedical Analysis, 4-7 Apr 1990.

5. M. R. Belmont, R. B. Maehr, W. B. Wastila, J. J. Savarese, "Pharmacodynamics and Pharmacokinetics of Benzylisoquinolinium (Curare-Like) Neuromuscular Blocking Agents," Anes. Clin. of North America, Quarterly Review, **2(2)**, June 1993.

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