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Quantitative determination and pharmacokinetics of a new antipsychotic (CGS 13429A) in human plasma using capillary gas chromatography/mass spectrometry

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Abstract: A quantitative analytical method is described for the determination of a new antipsychotic (CGS 13429A) in human plasma. The method relies on capillary gas chromatography/mass spectrometry in the positive chemical ionization mode, utilizing ammonia reagent gas. The limit of quantification (LOQ) was 0.1 ng ml^{-1} and the method was validated over a concentration range of 0.1–50 ng ml^{-1}. The method was used to measure CGS 13429A plasma concentrations following the administration of single oral ascending doses ranging from 0.1 to 10 mg in healthy male volunteers. The drug was rapidly absorbed (Tmax ranged from 1.1 to 3.7 h) and showed a mean terminal elimination half-life of 8.1 h, which was independent of dose. Area-under-the-curve (AUC) along with Cmax values were proportional to the administered dose.

Keywords: *CGS 13429A; capillary gas chromatography/mass spectrometry; log–log regression with* y*-intercept; positive chemical ionization; selected ion monitoring.*

Introduction

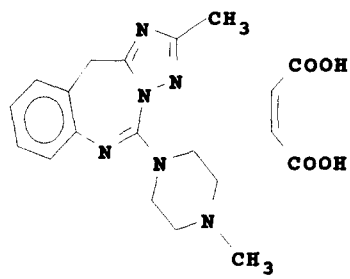
CGS 13429A, 2-methyl-5-(4-methyl-1-piperazinyl)-11H-[1,2,4]triazolo[1,5-c][1,3]-benzodiazepine 2-butenoic acid (Fig. 1) [1], has been shown preclinically (unpublished data) to be a potential antipsychotic agent with relatively low potential to cause extrapyramidal side effects. The results of these animal studies along with the sensitivity requirements anticipated for human bioavailability and pharmacokinetic studies, required the development of an analytical method with a lower limit of quantification than the existing LC method [2].

The present paper describes the development and validation of a gas chromatography/mass spectrometry (GC/MS) analytical method for quantifying CGS 13429A levels in human plasma. The method was used to measure CGS 13429A plasma concentrations following the administration of single oral ascending doses ranging from 0.2 to 10 mg in healthy male volunteers.

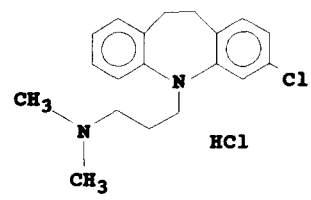
Experimental

Chemicals and reagents

CGS 13429A and the internal standard (clomipramine hydrochloride, Fig. 1) were obtained from Ciba-Geigy Corporation (Summit, NJ, USA). Anhydrous sodium carbonate, LC grade heptane, and reagent grade sulphuric acid were obtained from Fisher



CGS 13429A



Clomipramine Hydrochloride

Figure 1
Structures of CGS 13429A and the internal standard (clomipramine hydrochloride).

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Scientific (Fair Lawn, NJ, USA). Reagent grade 3-methyl-1-butanol was obtained from Mallinckrodt, Inc. (Paris, KN, USA). High purity (99.98%) solvent grade dichloromethane was supplied by Baxter Diagnostics Inc. (McGraw Park, IL, USA). All water was obtained from a Milli-Q water system (Millipore Corporation, Bedford, MA, USA).

Standard solutions

Internal standard solutions. Clomipramine hydrochloride (30 mg), the internal standard (IS), was dissolved in 100 ml of water to produce a 300-μg ml^{-1} stock solution. This solution was serially diluted with water to give two IS spiking solutions (2 μg ml^{-1} and 200 ng ml^{-1}). Spiking solutions were stored at 4°C prior to use (for up to 3 months).

CGS 13429A solutions. CGS 13429A (0.5 g) was dissolved in 100 ml of water to provide a 5-mg ml^{-1} stock solution. This solution was serially diluted with water to give an intermediate solution (1.0 μg m^{-1}). Spiking solutions of 1, 20, 100, 200 and 500 ng ml^{-1} CGS 13429A (all containing 200 ng ml^{-1} IS) were obtained by mixing aliquots of the 1.0-μg ml^{-1} intermediate solution with 10 ml of the 2-μg ml^{-1} IS spiking solution and diluting to 100 ml with water. Spiking solutions were stored at 4°C prior to use (for up to 3 months).

Calibration curve samples (prepared daily in duplicate). To 1-ml portions of drug-free control human plasma were added 0.1-ml aliquots of the standard spiking solutions, to give the 0.1, 2, 10, 20 and 50-ng ml^{-1} CGS 13429A standards. IS concentration was 20 ng ml^{-1} in all samples.

Quality control (QC) samples. Samples of known CGS 13429A concentrations were prepared at five concentration levels (0.1, 2, 10, 20 and 50 ng ml^{-1}). All QC samples were prepared in advance and stored frozen (−20°C) prior to analysis. Aliquots (1 ml) of these samples were extracted along with each standard curve and the concentrations were calculated based on the regression equations of the standard curves.

Extraction

A 1-ml plasma sample was placed in a tube and 100 μl of IS (200 ng ml^{-1}) was added (excluding calibration curve samples, which already contained IS). One millilitre of 2 M sodium carbonate was added, followed by 4 ml of 1% 3-methyl-1-butanol (v/v) in *n*-heptane. The tube was capped and mechanically vortexed at moderate speed for 1 min, followed by centrifugation at 2000*g* for 30 min. The aqueous layer was frozen in a dry-ice/ethanol bath, and the heptane layer was transferred to a tube containing 400 μl of 0.1 N sulphuric acid. The tube was capped and mechanically vortexed at moderate speed for 1 min, followed by centrifugation at 2000*g* for 10 min. After discarding the organic phase, 0.2 ml of 1 N sodium hydroxide and 1.4 ml of *n*-heptane were added. The tube was capped and mechanically vortexed at moderate speed for 1 min, followed by centrifugation at 2000*g* for 10 min. The aqueous layer was frozen and the heptane layer was transferred to a 5-ml reaction vial, and completely evaporated under a stream of nitrogen at 40°C. Ethanol (10 μl) was added and the solution was transferred to a 0.7-ml crimp-sealed autoinjector vial.

GC/MS analysis

The GC/MS system consisted of a Delsi-Nermag R10-10C equipped with a Varian Vista 6000 gas chromatograph and a CTC-A200S autoinjector (LEAP Technologies, Chapel Hill, NC, USA). The associated computer was a Micro PDP 11/73 (Digital Equipment Corp., Maynard, MA, USA). SIDAR™ software (Version 3.0 supplied by Nermag) was used for data acquisition and QUANTI™ was used for quantification (Version 6.3, also supplied by Nermag).

A 30 m × 0.32 mm Heliflex RSL-150 bonded capillary column (Alltech Corp., Deerfield, IL, USA) was used for the analysis. The carrier gas was helium with an inlet pressure of 20 psi. The injector was used in the splitless mode at 290°C. The injector purge was opened after 0.25 min at a split ratio of 50:1 and closed after 4.75 min. The column temperature was programmed for a 1 min hold at 100°C, followed by a temperature increase of 40°C min^{-1} to 320°C. There was a 2.5-min hold at the end of the temperature gradient.

The mass spectrometer was operated in the positive ion chemical ionization (CI) mode with ammonia reagent gas. The ammonia flow rate was 6 ml-atm min^{-1} resulting in an indicated source pressure of 0.2 Torr. The GC/MS interface was maintained at 290°C and the ion source temperature at 250°C. Selected ion

monitoring was employed using the $[M + H]^+$ ions at *m/z* 297 and 315 for CGS 13429A and IS, respectively.

Calculations

Calibration curves were obtained by plotting the peak area ratios of CGS 13429A to IS against the concentrations of CGS 13429A in the standards. Calibration curve parameters were calculated using log–log regression with *y*-correction ($Y = AX^B + C$). The selection of the mathematical model best suited to fit the calibration curve data set was achieved by analysing the standard curve results, as well as comparing the accuracy and precision of the QC samples using different mathematical models.

The overall accuracy and precision of the method were determined by analyzing QC samples spiked at five different concentrations (0.1, 2, 10, 20 and 50 ng ml^{-1}) in replicate (n = 4) on each of three consecutive analysis days.

Human study

The analytical method was used to measure plasma concentrations of CGS 13429A after single oral administration of the drug in ascending doses (0.1, 0.25, 0.5, 1, 2, 4, 6, 8 and 10 mg) to six healthy male volunteers.

The study protocol was approved internally by Ciba-Geigy Corporation, and approved by the Biomedical Sciences Review Committee (Institutional Review Board) at Ohio State University. The clinical phase of the study was performed at the Clinical Research Unit of the Department of Pharmacology at Ohio State University (College of Medicine) under the direction of Dr Nicholas Gerber. The study was conducted in compliance with FDA regulations concerning institutional review (21 CFR56) and informed consent of human subjects (21 CFR50). Participants in the study signified their consent to participate in writing.

Blood samples (10 ml) were obtained by repeated venipuncture. A heparin lock was inserted into the vein in some cases; the blood sample was obtained only after withdrawing and discarding the contents of the heparin lock. The samples were collected in Venoject® (sodium heparin) tubes.

Following an overnight fast, the volunteers were dosed at 8:00 am, and were given a standardized meal 2.5 h after dosing. Blood samples were collected prior to dosing (0 h), and at 0.5, 1, 2, 4, 8, 12 and 24 h after dosing. After at least 1 week, the next higher dose was administered. The blood samples were centrifuged for 10 min and the separated plasma was transferred to plastic tubes and stored at −20°C until analysed. The QC samples were stored along with the blood samples for no longer than 4 months.

Results and Discussion

Method devlopment

Positive CI (ammonia) GC/MS spectra of CGS 13429A and IS are shown in Fig. 2. For both compounds, the $[M + H]^+$ ions were utilized for selected ion monitoring (SIM) analyses.

Typical selected ion chromatograms for control human plasma and human plasma spiked with CGS 13429A at 0.1 ng ml^{-1}, the

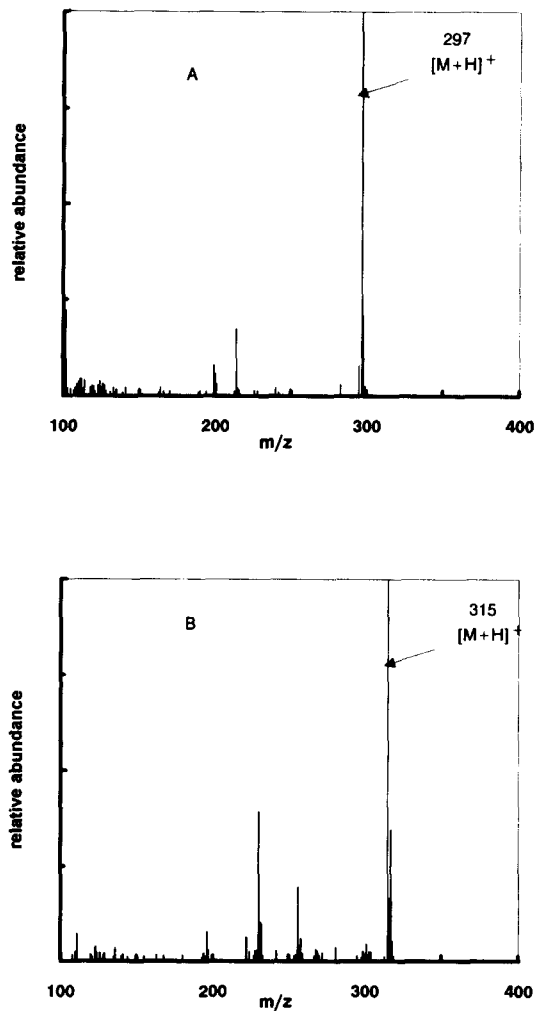

Figure 2
Positive CI (ammonia) GC/MS spectra of (A) CGS 13429A and (B) clomipramine hydrochloride (IS).

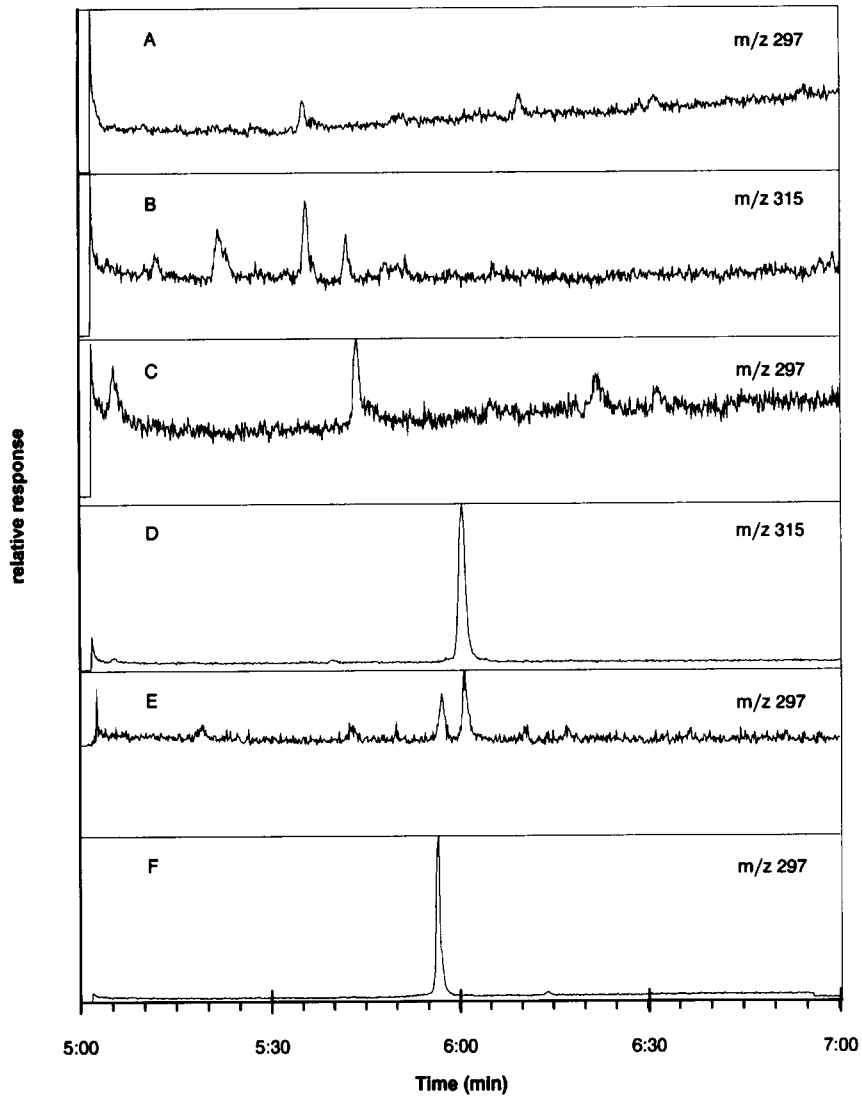

Figure 3
Selected ion chromatograms for control plasma samples spiked with CGS 13429A. (A) and (B) blank plasma; (C) and (D) blank plasma with 20 ng ml^{-1} IS; (E) 0.1 ng ml^{-1} drug and 20 ng ml^{-1} IS; (F) 50 ng ml^{-1} drug and 20 ng ml^{-1} IS.

Table 1
Reproducibility of CGS 13429 A daily calibration curves (0.1–50 ng ml^{-1})*

Analysis day	A	B	C	Correlation coefficient	Mean error†
1	0.02059	0.9887	0.00165	0.9992	7.5
2	0.02003	1.1398	0.00677	0.9969	15.4
3	0.01889	0.9959	0.00774	0.9969	14.1
Mean	0.0198	1.0415	0.00539	0.9977	
SD	0.0009	0.0852	0.00327	0.0013	
RSD (%)	4.4	8.2	60.7		

*Regression model used: $Y = AX^B + C$.
†Mean error — mean of the absolute deviation of each standard from the calibration curve.

Table 2
Inter- and intra-day accuracy and precision data for CGS 13429A quality control samples

	Mean relative recovery (%RSD)			
CGS 13429A added (ng ml^{-1})	Intra-day (n = 4) Run 1	Run 2	Run 3	Inter-day (n = 12) (%RSD, n = 12)
0.1	101.0 (11.7)	104.8 (9.7)	103.5 (5.6)	103.1 (8.6)
1	101.4 (8.2)	109.9 (9.9)	97.4 (5.8)	102.9 (10.6)
5	99.4 (2.8)	109.8 (5.1)	98.1 (5.8)	102.4 (6.4)
20	95.1 (6.9)	93.5 (5.5)	109.3 (5.3)	99.3 (13.0)
50	105.8 (2.4)	109.0 (8.8)	103.1 (11.8)	106.0 (7.4)
Overall mean (SD) recovery = 102.7 (9.4)				

Table 3
Mean pharmacokinetic parameters after single oral ascending doses of CGS 13429A to healthy male volunteers

	T_{max} (h)		C_{max} (ng ml^{-1})		AUC (ng h ml^{-1})		$T_{1/2}$ (h)*	
Dose (mg)	mean	SD	mean	SD	mean	SD	mean	SD
0.1	2.7	2.9	0.7	0.2	4.4	2.9	—	—
0.25	2.7	1.2	1.3	0.4	9.5	3.3	—	—
0.5	2.3	1.5	2.3	0.4	21.0	12.4	9.3	—
1	3.7	2.5	5.8	1.1	55.3	9.1	6.5	1.9
2	1.8	1.3	16.4	5.1	124.6	37.3	6.6	1.5
4	1.5	0.5	33.6	23.0	234.4	47.2	8.6	1.6
6	1.5	0.5	51.9	37.6	336.2	86.3	5.7	1.2
8	1.1	0.5	55.0	23.7	405.6	104.6	9.3	2.6
10	1.4	0.5	77.2	29.9	538.4	88.4	10.0	1.0

* Elimination half-life not reported if the absolute value of the associated correlation coefficient was less than 0.975.

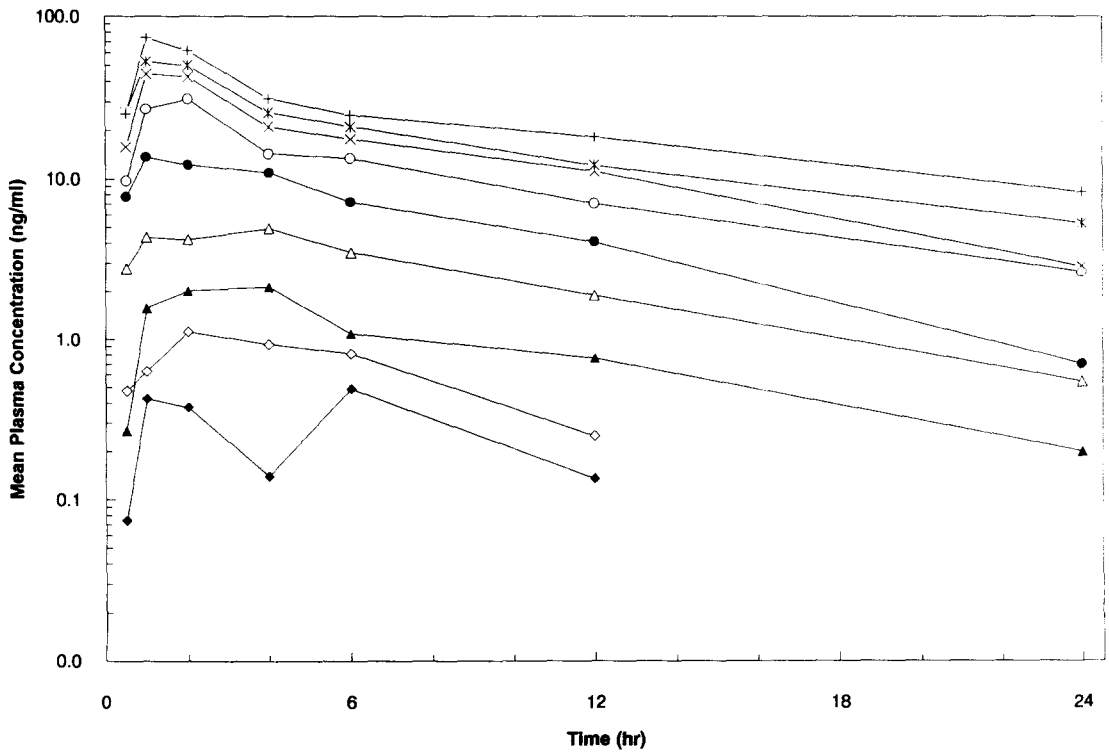

Figure 4
Mean plasma concentration-time profiles for CGS 13429A after single oral doses of 0.1 mg (◆), 0.25 mg (◇), 0.5 mg (▲), 1 mg (△), 2 mg (●), 4 mg (○), 6 mg (×), 8 mg (*) and 10 mg (+) to healthy volunteers.

LOQ and 50 ng ml^{-1}, the upper limit of the validation range are shown in Fig. 3. The retention times of CGS 13429A and IS were 5.9 and 6.0 min, respectively. There were no interfering peaks in any control plasma samples at either the retention time of CGS 13429A or IS. The reproducibility of daily calibration curves was demonstrated by analyses of control human plasma samples spiked in duplicate at six different concentration levels during three separate analytical runs. The mean correlation coefficient for the fit to the regression equation was 0.9977, indicating a good fit to the mathematical model (Table 1). Since parameter *C* (the intercept) is close to zero (Table 1), it would be expected to vary widely from day to day.

The inter- and intra-assay accuracy and precision of the method were evaluated by analyzing QC samples spiked at five different concentrations (0.1, 1, 5, 20 and 50 ng ml^{-1}) in replicate ($n = 4$) on each of three consecutive analysis days (Table 2). The overall mean ± SD relative recovery was found to be 102.7 ± 9.4% indicating good accuracy over this concentration range. The LOQ is defined as the lowest drug concentration where acceptable accuracy (100 ± 15%) and precision (RSD <20%) are obtained. The LOQ for this method is 0.1 ng ml^{-1}.

Clinical pharmacokinetics

The method was successfully applied to the analyses of clinical samples from a rising single-dose safety, tolerability and pharmacokinetic study in healthy male volunteers. The mean plasma concentration-time profiles for all nine doses are illustrated in Fig. 4. Corresponding mean pharmacokinetic parameters and their standard deviations are shown in Table 3. The overall mean terminal elimination half-life was 8.1 h and was independent of dose. Both Cmax and AUC values increased in a dose-proportional manner with increasing doses.

AUC values were obtained using the trapezoidal rule [3]. Elimination half-life values were obtained by calculating the reciprocal of the slope obtained from the linear regression of sample time vs natural logs of the concentrations of the end of the curve [4]. The last three points of the curve were used, then the last four, etc., until the best possible regression correlation coefficient was found.

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