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A validated liquid chromatographic method for the antiallergy agent CI-922 in dog and rat plasma

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Abstract: A sensitive, specific and rapid liquid chromatographic procedure to selectively monitor CI-922 in dog and rat plasma was developed and validated. Plasma samples were acidified and extracted with ethyl ether. The organic layer was evaporated to dryness, reconstituted with mobile phase, and the analytes were separated and quantified on an octadecylsilane stationary phase (λ = 340 nm). The procedure was linear from 0.05 to 3.0 $\mu g\ ml^{-1}$ with a detection limit of 0.02 $\mu g\ ml^{-1}$. The accuracy of the method had relative errors of 7.6, 4.2 and 5.4% for dog plasma controls containing 0.5, 1.50 and 2.5 μg CI-922 ml^{-1}, respectively. The corresponding precision was 5.0, 4.0 and 4.8% (RSD). Similarly, relative standard deviations less than 4.6% and relative errors of less than 1.4% were obtained for rat plasma controls. The method is suitable for pharmacology, toxicology and pharmacokinetic studies of CI-922.

Keywords: *Reversed-phase chromatography; UV detection; CI-922/antiallergy; dog, rat plasma.*

Introduction

CI-922 (I) (3,7-dimethoxy-4-phenyl-N-1*H*-tetrazol-5-yl,4*H*-furo[3,2-b]indole-2-carboxamide) (Fig. 1) is a new antiallergenic agent. It is slightly soluble in water (590 $\mu g\ ml^{-1}$), has a pKa value of 5.4, and shows good octanol–water partitioning (log P = 1.12). Pharmacological studies indicate that CI-922 is an orally effective mediator release inhibitor [1–3]. A preliminary report on the metabolic disposition of CI-922 in laboratory

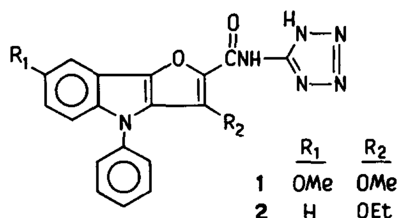

Figure 1
Structure of CI-922 (**I**) and internal standard (**II**).

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animals has recently been published [4]. Currently, CI-922 is being tested in Phase 1 clinical trials. This report describes a procedure for the analysis of CI-922 in dog and rat plasma samples. The method is sufficiently selective, precise and accurate to be applied to pharmacology, toxicology and pharmacokinetic studies.

Experimental

Reagents

CI-922 (**I**) and the internal standard (PD 112,479) (**II**) (3-ethoxy-4-phenyl-N-1*H*-tetrazol-5-yl-4*H*-furo[3,2,6]indole-2-carboxamide) were synthesized at Warner-Lambert/Parke-Davis Pharmaceutical Research (Ann Arbor, MI, USA) [1]. HPLC-grade acetonitrile, glacial acetic acid were obtained from E. M. Industries (Gibbstown, NJ, USA) and J. T. Baker (Phillipsburg, NJ, USA), respectively. Reagent grade hydrochloric acid (MCB, Cincinnati, OH, USA), ethyl ether (E. M. Industries, Gibbstown, NJ, USA) and (0.1 M) sodium hydroxide (Acculute®, Anachemia Chemicals, Champlain, NY, USA) were used as received.

Liquid chromatography

Instrumentation consisted of a Waters 590 solvent delivery system, a Waters 710B intelligent sample processor and a Waters 440 fixed wavelength detector (Milford, MA, USA). Peak height ratios were measured using a Hewlett–Packard Model 3390A Integrator (Avondale, PA, USA).

Separation was achieved using a 150 × 4 mm i.d. stainless steel column packed with 5-μm Biosil-ODS (Bio Rad Laboratories, Richmond, CA, USA). The mobile phase consisted of acetonitrile–water–glacial acetic acid (50:50:1, v/v/v) and was delivered at a flow rate of 1 ml min^{-1}. The eluent was monitored at 340 nm.

The specificity of the method was assessed by obtaining multiple UV wavelength scans (Hewlett–Packard Model 1040A, Avondale, PA, USA) of the CI-922 peak.

Standard solutions

Stock solutions (100 μg ml^{-1}) of CI-922 and the internal standard (PD 112,479) were prepared in 1 mM NaOH. Standard solutions containing 0.2, 0.8, 2.0, 4.0, 6.4, 8.0, 10.0 and 12.0 μg ml^{-1} of CI-922 were prepared by diluting the stock solution with water. The internal standard solution was diluted with water to a final concentration of 5 μg ml^{-1}.

Control pools

Dog plasma control pools were prepared by diluting the CI-922 stock solution to 5 μg ml^{-1} with control dog plasma. This solution was further diluted with control dog plasma to yield control pools containing 0.5, 1.5 and 2.5 μg CI-922 ml^{-1} of plasma. Rat plasma control pools were prepared in an identical manner.

Sample preparation

To a 1.0 ml plasma sample in a 13 × 100 mm screw cap culture tube was added 0.25 ml internal standard solution, 0.25 ml water (or 0.25 ml CI-922 standard), 0.2 ml 1 M HCl and 5 ml anhydrous ethyl ether. Sample aliquots of less than 1 ml were diluted with control plasma. After horizontally shaking for 10 min and centrifuging at 2000 rpm for 5 min, a 4 ml aliquot of the ether phase was evaporated to dryness under nitrogen. The residue was reconstituted in 0.25 ml of mobile phase and a 0.05 ml aliquot was analysed.

Assay validation

The assay was validated over the concentration range (0.05–3.0 $\mu g\ ml^{-1}$ of CI-922 ml^{-1}) in plasma by assaying eight calibration standards and three control pools in triplicate on three consecutive days. The best-fit straight line was determined by least squares linear regression analysis using a weighting factor of 1/concentration squared [5]. CI-922 concentrations in the seeded control pools and samples were calculated using the regression parameters.

Extraction efficiency of CI-922

The extraction efficiency of ^{14}C-CI-922 was determined at concentrations of 0.5 and 2.5 $\mu g\ ml^{-1}$ using a Packard Tri-Carb scintillation counter (Packard Instrument Company, Model 4530, Downers Grove, IL, USA). The extraction efficiency of the internal standard (1.25 $\mu g\ ml^{-1}$) was assessed by comparing peak heights of extracted versus aqueous standards using liquid chromatography. All data from plasma extractions were normalised by 1.25 in order to compensate for sample procedural steps.

Results and Discussion

The procedure for the analysis of CI-922 in dog or rat plasma involves a single step acidic extraction into ethyl ether. The organic phase was evaporated and reconstituted with mobile phase. The validity of the liquid chromatographic assay was established through a confirmatory study of calibration curve reproducibility, extraction efficiency, specificity, and assay accuracy/precision.

Extraction efficiency

The extraction efficiency of CI-922 added to dog plasma at concentrations of 0.5 and 2.5 $\mu g\ ml^{-1}$ were 96.2 ($\pm$1.3)% and 97.0 ($\pm$2.4)% whilst the extracting efficiencies from rat plasma were 99.5 ($\pm$3.0)% and 95.0 ($\pm$1.6)% at identical concentrations. The extraction efficiency of the internal standard was 96.5 ($\pm$2.4)%.

Chromatography

The retention times for CI-922 and the internal standard were 6.3 and 8.4 min, respectively. Figure 2 illustrates typical chromatograms for control dog and rat plasma, and plasma from a dog and rat dosed with CI-922. No endogenous plasma components or metabolites were observed near the retention times corresponding to CI-922 or internal standard. Figure 3 illustrates LC-multiple wavelength scans for a calibration standard and dog and rat plasma samples containing CI-922. Scans were obtained at the upslope, apex and down slope of the CI-922 peak. The three UV spectra from each sample are all superimposable, indicating that no interfering peaks, such as a metabolite, are present in the samples, which confirms analyte identity. In addition, all spectra obtained on the upslope, apex and downslope are superimposable, which implies peak homogeneity.

Linearity, accuracy and precision of the calibration curves

The assay was validated by analysing eight CI-922 standards in triplicate over three consecutive days. Peak height ratios were proportional to CI-922 concentration over the range 0.05–3.0 $\mu g\ ml^{-1}$. The best-fit line was determined daily by least squares regression analysis using a weighting factor of 1/concentration squared [5]. The results of a typical regression were: peak height ratio = 0.865 $\times$ CI-922 concentration + 0.004 (r =

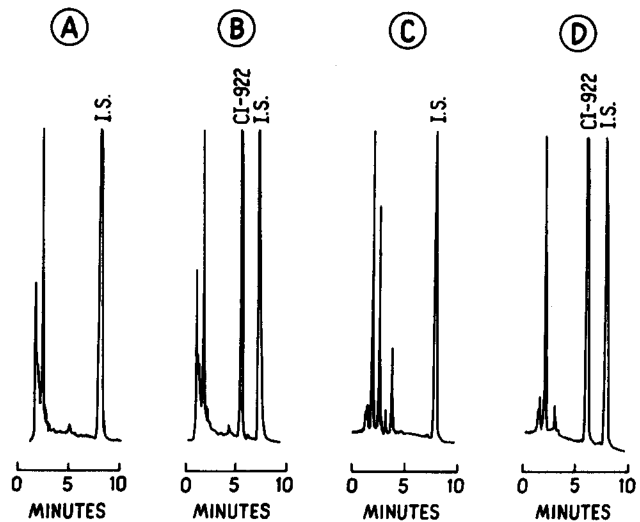

Figure 2
Typical chromatograms for CI-922 in plasma. (A) Control dog plasma containing internal standard. (B) Dog plasma sample containing 1.4 μg ml^{-1}. (C) Control rat plasma containing internal standard. (D) Rat plasma sample containing 1.4 μg ml^{-1}.

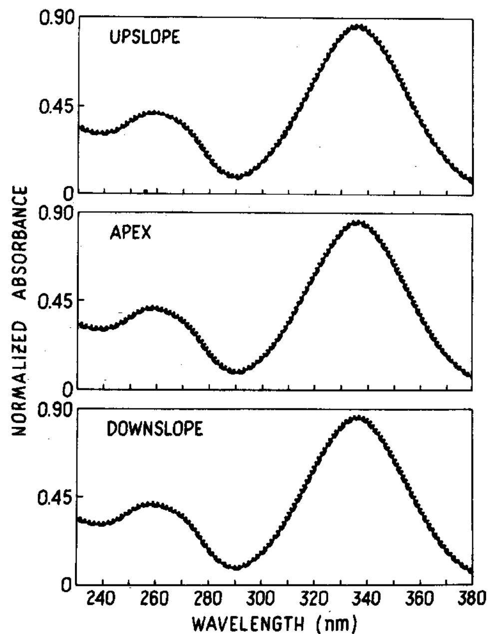

Figure 3
Liquid chromatographic UV scans obtained at the upslope, apex and downslope of the CI-922 peak from a CI-922 standard (. . .), rat plasma sample (– – –) and dog plasma sample (———).

0.999). The mean slope and intercept data from a three-day period are 0.865 (±0.021) and 0.0035 (±0.003), respectively.

The accuracy and precision of the calibration curves was determined from the variation of the standards from the regression line. Precision for the dog calibration standards ranged from 2.6 to 6.6% (RSD) with relative errors of −9.1 to 4.9% (Table 1).

Table 1
Precision and accuracy of CI-922 calibration standards in dog and rat plasma during a three-day period

	Dog plasma					Rat plasma			
Conc. (μg ml^{-1})	N	Conc. found (μg ml^{-1})	RSD (%)	Relative error (%)	Conc. (μg ml^{-1})	N	Conc. found (μg ml^{-1})	RSD (%)	Relative error (%)
0.050	9	0.051	6.00	2.7	0.050	9	0.050	3.11	0
0.200	9	0.182	5.64	−9.1	0.250	9	0.244	1.97	−4.0
0.500	8	0.467	6.59	−6.7	0.500	9	0.485	1.67	−3.0
1.00	9	1.01	2.64	1.4	1.00	9	0.993	1.62	−0.7
1.60	9	1.63	2.86	1.6	1.50	9	1.52	1.78	1.3
2.00	9	2.02	4.77	1.2	2.00	9	1.91	1.78	−4.7
2.50	9	2.60	3.20	3.8	2.50	9	2.55	2.88	1.9
3.00	8	3.15	3.10	4.9	3.00	7	3.08	2.38	2.5

Precision for rat calibration standards ranged from 1.6 to 3.1% with relative errors of −4.7 to 2.5% (Table 1). Based on these results, the method is linear from 0.05 to 3.0 μg ml^{-1}. The detection limit is 0.020 μg ml^{-1}.

Assay precision and accuracy

Assay precision and accuracy were determined by analysing three control pools in triplicate on three consecutive days. Assay precision was ±5% based on RSDs of 5.0, 4.0 and 4.8% for dog plasma controls containing 0.5, 1.5 and 2.5 μg CI-922 ml^{-1}. The accuracy of the method was ±7.6%, with relative errors of 7.6, 4.2 and 5.4% for the same three dog control pools (Table 2). Similarly, an assay precision of ±4.6% with relative errors of ±1.4% were obtained for rat control pools (Table 2). In addition, control samples stored for over 3 months show no evidence of decomposition.

Table 2
Precision and accuracy of the method during a three-day period

	Dog plasma			Rat plasma		
Conc. added (μg ml^{-1})	0.500	1.50	2.50	0.500	1.50	2.50
*N**	9	9	9	9	9	9
Mean conc. found (μg ml^{-1})	0.538	1.56	2.64	0.493	1.50	2.49
RSD (%)	5.0	4.0	4.8	4.6	2.7	3.5
Relative error (%)	7.6	4.2	5.4	−1.4	0	−0.2

*Triplicates of each control pool were assayed over a three-day period.

Conclusion

A precise, accurate and specific liquid chromatographic method has been developed for the selective monitoring of CI-922 in dog and rat plasma samples. Greater than 1000 dog samples and 1200 rat plasma samples have been processed using this method. No endogenous plasma components or metabolites interfere. The method is linear from 0.05 to 3.0 μg ml^{-1} and the detection limit is 0.020 μg ml^{-1}. Maximal plasma concentrations from pharmacological and pharmacokinetic studies do not exceed 2.0 μg ml^{-1}. Samples greater than 3.0 μg ml^{-1} (i.e. toxicological/support studies) should be appropriately diluted with control plasma. Data obtained from this method have allowed precise and accurate pharmacological, toxicological and pharmacokinetic interpretations.

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