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Journal of Pharmaceutical and Biomedical Analysis
16 (1998) 1075–1082

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Validation of an HPLC method for the determination of CP-93,393 in CP-93,393-1 tablets[1]

M.L. Dumont, S.T. Colgan *, R.H. Reed, G.R. Haggan Jr

Pfizer Central Research, Analytical Research and Development, Eastern Point Road, Groton, CT 06340, USA

Received 26 October 1996; received in revised form 14 January 1997

Abstract

A reversed-phase high performance liquid chromatographic (HPLC) assay developed for the CP-93,393-1 drug substance was adapted for use with CP-93,393-1 tablets. Using a novel experimental matrix, validation was performed to obtain linearity, reproducibility and recovery and to meet current regulatory requirements. Deviations in the sample preparation procedure were performed to demonstrate the ruggedness of the assay. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Reversed-phase chromatography; Validation; Ruggedness; Dosage form; CP-93,393

1. Introduction

CP-93,393-1 tablets are currently in clinical trials for the treatment of anxiety and depression. As such, the chromatographic method used to evaluate materials for clinical supplies must be thoroughly validated. Earlier chromatographic methods for CP-93,393-1 tablets utilized two separate isocratic, reversed-phase systems: one for quantitation of impurities and another for quantitation of CP-93,393. These methods were also incompatible with mass spectrometric detection. Compatibility with mass spectrometric detection can be useful when identification of unknown impurities is required. The chromatographic system described here is a single, compound specific, potency and purity-indicating, isocratic, reversed-phase chromatographic system that allows concomitant quantitation of CP-93,393 and all potential impurities from the same injection. This system was adapted from the system used for the CP-93,393-1 drug substance [1]. This system is also compatible with mass spectrometric detection and is more efficient in terms of sample preparation and analysis time. The validation data provided here are consistent with the current USP/NF guidelines for finished pharmaceutical products [2] and the definitions issued by the International Conference on Harmonization (ICH) of requirements for registration for human use [3].

* Corresponding author. Tel: +1 860 4413124; Fax: +1 860 4415423.

[1] Presented at the Analysis and Pharmaceutical Quality Section of the Eleventh Annual American Association of Pharmaceutical Scientists Meeting, October 1996, Seattle, Washington, USA.

PII S0731-7085(97)00056-3

Table 1
Experimental matrix used to validate precision, linearity, accuracy and recovery

Nominal concentration (%)	1	1[a]	2	3	4	5	6
40	$W_{1.40}$	$W^{a}_{1.40}$	$W_{2.40}$	$W_{3.40}$	$W_{4.40}$		
60	$W_{1.60}$	$W^{a}_{1.60}$	$W_{2.60}$	$W_{3.60}$	$W_{4.60}$		
80	$W_{1.80}$	$W^{a}_{1.80}$	$W_{2.80}$	$W_{3.80}$	$W_{4.80}$		
100	$W_{1.100}$	$W^{a}_{1.100}$*	$W_{2.100}$	$W_{3.100}$	$W_{4.100}$	$W_{5.100}$	$W_{6.100}$
100b	$W_{7.100}$						
120	$W_{1.120}$	$W^{a}_{1.120}$	$W_{2.120}$	$W_{3.120}$	$W_{4.120}$		
140	$W_{1.140}$	$W^{a}_{1.140}$	$W_{2.140}$	$W_{3.140}$	$W_{4.140}$		

Nominal concentration, 0.1 mg CP-93,393 ml^{-1}.
The column headings (1–6) represent individual weighings.
[a] Sample solutions prepared without tablet excipients.
[b] This sample contains 1% spikes of Compound 1, Degradant 1 and Degradant 2.

A novel experimental matrix (Table 1) was used to validate the chromatographic system. The multiple weighings used in this approach will identify potential difficulties associated with sample preparation and the results obtained are more indicative of the true reproducibility of the method. Neither the ICH nor USP guidelines stipulate the use of a sample preparation matrix that incorporates multiple weighings for validation of analytical methods. These guidelines state that serial dilutions made from a single stock solution are acceptable, covering a range from 80 to 120% of the test concentration, for determination of linearity. Other articles on the validation of analytical methods have also been published, none of which use a multiple weighing matrix approach [4–10].

2. Experimental

2.1. Materials

HPLC grade methanol and reagent grade glacial acetic acid were purchased from J.T. Baker (Phillipsburg, NJ). Acetonitrile was purchased from Fisher Scientific (Fair Lawn, NJ). Reagent grade ammonium acetate was purchased from Aldrich (Milwaukee, WI). Standards of CP-93,393-1, Degradant 1, Degradant 2, and Compound 1 (the penultimate CP-93,393-1 precursor) were prepared at Pfizer Central Research (Groton, CT) (Fig. 1). The theoretical potency of CP-93,393-1 is 90% (10% represents the hydrochloride counter-ion portion of the CP-93,393-1 salt). CP-93,393 is the free base of CP-93,393-1.

2.2. Equipment

Analyses were performed with an HPLC system consisting of a Waters 510 pump, Waters 717 plus autosampler, a Waters 486 variable wavelength detector and Bioanalytical Systems LC-22C column heater. A Waters Puresil™ C_{18} column (5

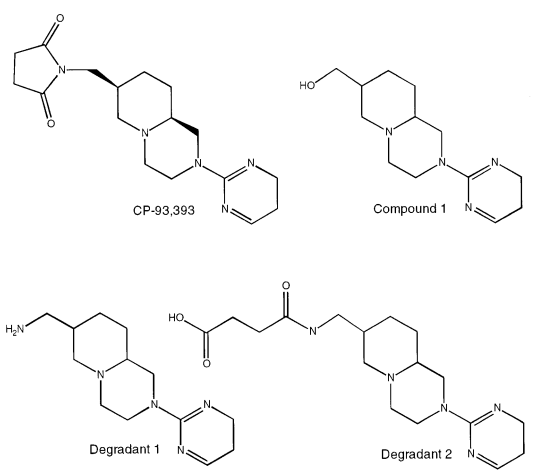

Fig. 1. Structures of CP-93,393, Compound 1, Degradant 1 and Degradant 2.

μ particles, 150 mm × 4.6 mm) (part number WAT044345) was used for all separations. A Brownlee NewGuard™ scrubber column (part number 140–601) containing a Brownlee NewGuard™ C18 insert (part number G18-013) was used between the pump and the injector to increase column longevity. A Benchmark™ Tablet Processing Workstation (TPW), made by Zymark, was used to validate an automated sample preparation procedure for CP-93,393-1 tablets.

2.3. Chromatographic conditions

The following conditions were used for all separations:

Mobile phase:	91/6/3 Buffer*–acetonitrile–methanol (v/v/v) *0.05 M aqueous ammonium acetate, pH adjusted to 4.6 with glacial acetic acid
Flow rate:	2.0 ml min^{-1}
Detection:	UV at 238 nm
Injection:	100 μl
Column temperature:	30°C
Sample concentration:	0.1 mg CP-93,393 ml^{-1}

2.4. Sample preparation

Following the novel sample matrix (Table 1), 26 individual samples were prepared. For each sample, approximately 55 mg CP-93,393-1 was weighed and transferred into a 100 ml volumetric flask. Each sample was dissolved in and diluted to volume with the mobile phase, followed by brief shaking by hand (~ 30 s) or sonication for 20 s to hasten dissolution. The concentration of each stock solution was ~ 0.5 mg CP-93,393 ml^{-1}. Solutions were diluted according to the scheme shown in Table 2.

The appropriate aliquot was transferred to a 25 ml volumetric flask and 290 mg of the tablet excipient mixture was added. The flask was filled to approximately one half its capacity with the mobile phase, stoppered and shaken for 30 min on a reciprocating shaker at 150 oscillations min^{-1}. Each sample was diluted to volume with mobile phase. Solutions were filtered with a Whatman Autovial (catalog no. AV125UAQU) and injected into the HPLC system.

Table 2
Dilutions used for sample preparation

Nominal concentration (%)	Desired CP-93,393	
	Concentration (mg ml^{-1})	Dilution
40	0.04	2→25
60	0.06	3→25
80	0.08	4→25
100	0.10	5→25
120	0.12	6→25
140	0.14	7→25

2.5. Parameters for the Zymark Benchmate™ TPW

The following steps were used by the TPW for the automated preparation of CP-93,393-1 tablets: step 1, dispense 100 ml of the mobile phase into the homogenizer; step 2, pour the sample into the homogenizer; step 3, homogenize with 15 pulses, each 10 s long, at 7 K rpm; step 4, soak for 0.5 min; step 5, prewet the transfer path with 8 ml of the homogenate; step 6, transfer 8 ml of the homogenate; step 7, wash the homogenizer for 3 cycles using H_2O; step 8, wash the homogenizer for 1 cycle using the mobile phase; step 9, prewet with 2 ml of the sample and filter 5 ml into the next tube; step 10, end.

The setup parameters for the TPW are shown in Table 3. Tablets were processed starting at rack 1, position 1 until an empty position was encountered. The homogenate was collected in rack 2. Sample solutions were placed in rack 3.

3. Results and discussion

With the exception of sample concentration and injection volume, the chromatographic conditions selected for validation were identical to those

employed to determine the potency and purity of the CP-93,393-1 drug substance. The absolute amount of CP-93,393-1 is constant in the drug substance and tablet assays (10 μg). The sample is injected as a 10 μl aliquot for the drug substance and as a 100 μl aliquot in the tablet assay. Although large injection volumes may decrease chromatographic performance, in this case the effect is insignificant (Table 4). Since the chromatographic performance was essentially equivalent for 10 μl or 100 μl injections and the absolute amount of CP-93,393-1 is the same as in the drug substance assay, the ruggedness data generated for the drug substance assay is applicable here.

The validation data generated for this drug candidate is suitable for a main band chromatographic assay at the third stage of development (phase III, IV candidates). Validation of a method is carried out to demonstrate that it is scientifically sound and that it has been systematically evaluated [11]. Ruggedness, vide infra, confirms that small variations in operating parameters provoke no significant changes in the measured parameter.

Table 3
Setup parameters for the TPW

Start delay
Start-up delay: 0 h
Flow rates
Aspirate: 0.50 ml s^{-1}
Dispense: 1.00 ml s^{-1}
Internal Standard: 0.12 ml s^{-1}
Mix: 1.50 ml s^{-1}
Air push: 0.15 ml s^{-1}
Autowash parameters
Regent volume: 1.00 ml
Sample volume: 0.20 ml
Dispensing
Liquid driven: yes
Gravimetric parameters
Gravimetric on: yes
Weigh tablets 4 places: yes
Weigh probe holdup: yes
Probe holdup volume: 0 ml
Large tablets: no
Filter parameters
Filter flow rate: 0.10 ml s^{-1}
Filtrate confirm: yes
Filtrate density: 1.00 g ml^{-1}
Minimum amount: 0%
Rinse reagent: 1

Table 4
The effect of injection volume on column performance

Injection Volume (μl)	Theoretical plates (USP)	Tailing factor
10	4600	1.7
20	4500	1.7
50	4500	1.7
100[a]	4400	1.7
200	4300	1.7

[a] The specified injection volume was 100 μl at 0.1 mg CP-93,393 ml^{-1}.

3.1. Validation

The validation data (linearity, recovery, precision and system suitability) were obtained using a novel sample matrix (Table 1). The design of this matrix included 26 individual weighings of the drug substance CP-93,393-1. The tablet excipients were added to mimic an actual dosage unit. Since the amount of CP-93,393 present in each sample must be known, actual tablets (which have a range of potencies) could not be used for validation of the method. After appropriate dilutions were made, the weighings in the matrix represented 40–140% of the nominal concentration. Samples were injected in random order and assayed versus an external standard. The samples in column 2 of the matrix were prepared from the same stock solutions as in column 1 without the tablet excipients and used as reference solutions. Excipients were used in all other samples to simulate the tablet formulation.

3.2. Linearity

The linearity of this method was demonstrated using the first column of samples (with excipients) in the matrix. The peak area was plotted against concentration. The equation of the line was $y =$

Table 5
Recovery from excipients

Weighing	Nominal concentration (%)					
	40	60	80	100*	120	140
$W_{1,X}$	98	100	99	100	102	100
$W_{2,X}$	100	100	103	101	99	102
$W_{3,X}$	103	101	99	99	99	100
$W_{4,X}$	100	102	102	98	98	99
$W_{5,X}$				99		
$W_{6,X}$				99		
Average recovery	100	101	101	99	100	100
R.S.D. (%)	2.0	1.0	2.0	1.0	1.8	1.3

X, Nominal concentration (%).

$(1.87 \times 10^8)x - 327740$. The correlation coefficient was $(r^2) = 0.9993$, the *y*-intercept was 1.8% of the response at the nominal concentration and the residuals were within 2% of the area response for all six concentrations.

3.3. Recovery

Recovery from excipients was determined using the nominal concentration as the reference. The results which are summarized in Table 5 are within the following range: average recovery $= 100 \pm 2$; the relative standard deviation (R.S.D.) was $\leq 2\%$. For assays developed for the pharmaceutical industry, response factors for replicate standards that are within 2% are considered equivalent and within the experimental error of the technique [12].

3.4. System suitability, selectivity and precision

System suitability data were collected during validation. Table 6 summarizes the retention times, theoretical plates, tailing factors and resolution from six injections of a solution containing 0.1 mg CP-93,393 ml^{-1} and 1% spikes of Degradant 1, Degradant 2 and Compound 1. The R.S.D. of 6 injections of a solution containing 0.1 mg CP-93,393 ml^{-1} and tablet excipients was 0.06%. The R.S.D. for Degradant 1, Degradant 2 and Compound 1 were 0.3, 0.3 and 0.4%, respectively. The % R.S.D. of these peak areas are indicative of a well-behaved and stable system. An example of a typical chromatogram containing Degradant 1, Degradant 2 and Compound 1 is show in Fig. 2a. A typical chromatogram of an actual CP-93,393-1 tablet is shown in Fig. 2b.

Table 6
System suitability, selectivity and precision of injection

	Retention time (min)	Plates tangent[a]	Plates Foley [13]	Tailing factor	Resolution
Degradant 1					
Mean ($n = 6$)	2.5	1300	960	1.4	3.6
Range	2.5–2.6	1100–1400	830–1200		
R.S.D. (%)	0.24	8.2	16		
Compound 1					
Mean ($n = 6$)	3.7	1600	1100	1.5	4.7
Range	3.7–3.7	1500–1800	970–1500		
R.S.D. (%)	0.16	7.9	16		
Degradant 2					
Mean ($n = 6$)	5.8	2000	1200	1.4	5.4
Range	5.8–5.8	1700–2400	1100–1600		
R.S.D. (%)	0.13	15	16		
CP-93,393					
Mean ($n = 6$)	10.2	1400	1200	1.6	
Range	10.2–10.3	1200–1700	960–1600		
R.S.D. (%)	0.26	14	20		

[a] USP method.

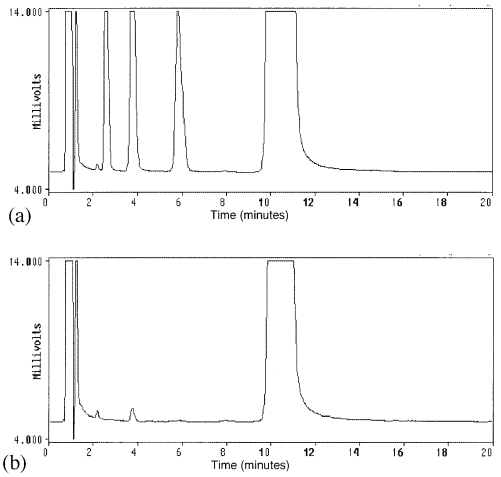

Fig. 2. a, A typical chromatogram of a sample extract containing CP-93,393-1 and 1% spikes of Degradant 1, Degradant 2 and Compound 1. b, A typical chromatogram of a sample extract of a CP-93,393-1 tablet.

The inter-day and intra-day precision of this method can be estimated using drug substance stability data. The sample preparation matrix for the drug substance is similar to the drug product matrix and identical chromatographic conditions were employed. The drug substance data also were generated by a different analyst using a different chromatographic system.

3.5. Stability

Solution stability of CP-93,393 was investigated. Two solutions of CP-93,393 were prepared at a concentration of 0.1 mg ml^{-1} in mobile phase. One solution contained tablet excipients, the other contained no excipients. The solutions were evaluated versus freshly prepared external standards for a period of two weeks using the chromatographic system described here. At the end of two weeks the solutions indicated 101 and 102% recovery from solution or excipients, respectively.

As a compliance issue, the stability of the mobile phase was also investigated. For this study, a batch of the mobile phase was prepared, sealed and stored at ambient conditions on a laboratory bench for 3 months. The mobile phase was then used to evaluate three standard solutions. Two contained the drug substance alone and the other contained the drug substance and 0.2% Degradant 1, 0.2% Compound 1 and 0.5% Degradant 2. The system suitability for the three standards indicated acceptable resolution, peak shape, retention times and theoretical plates. Based on the acceptable results, the mobile phase may be assigned a 3 month expiration.

3.6. Filter validation

Whatman Autovial filters (catalog no. AV125UAQU) were used to remove insoluble excipients from the sample preparation. Three sample solutions were prepared, each contained 0.1 mg CP-93,393 ml^{-1} and the appropriate excipients. For each solution, the filtrate was collected after 0, 2, 4, 6 or 8 ml of filtrate had passed through the filter and assayed. Quantitative (101–103%) recovery of CP-93,393 from all aliquots indicated that these filters were suitable for this analysis. A similar study indicated that Gelman Acrodiscs (part no. 4497) were equivalent to the Whatman Autovial filters.

3.7. Variation of sample preparation parameters

The procedure for extracting CP-93,393 from CP-93,393-1 tablets involved the following steps: (1) cutting the tablet into quarters, (2) transferring the pieces of the tablet to an appropriate size volumetric flask such that the final concentration was 0.1 mg CP-93,393 ml^{-1}, (3) adding one half of the flask volume of the mobile phase (e.g. 50 ml mobile phase to a 100 ml flask) and stoppering, (4) shaking the solution for 30 min on a reciprocal shaker, (5) dilution to volume with the mobile phase, and (6) filtration. The amount of extraction solvent and shaking time were varied to determine if small changes would effect assay results.

Twelve samples were prepared containing 0.1 mg CP-93,393 ml^{-1} and 290 mg excipients. Three samples were shaken on a reciprocating shaker for 10, 20, 25 or 30 min. The sample preparation was then completed as described above and all samples were assayed. These results, which are sum-

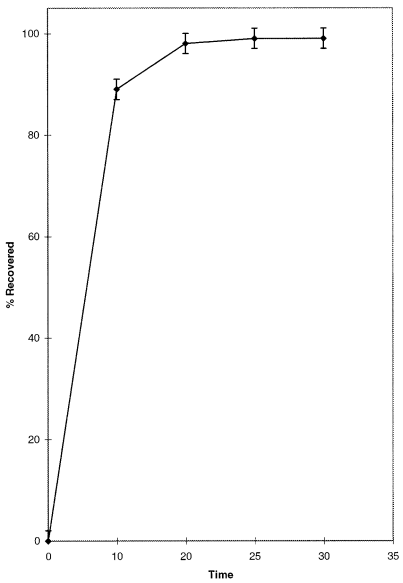

Fig. 3. The effects of shaking time during sample preparation vs. recovery of CP-93,393.

marized in Fig. 3, indicated that quantitative extraction occurred with shaking times of 20 min or longer. To ensure complete extraction, 30 min of shaking is specified in the assay procedure.

Once the shaking time was set at 30 min, the amount of extraction solvent was varied. Sample preparation instructions require one half of the final sample volume be used as extraction solvent. Three samples were prepared (with tablet excipients) such that their final concentration was ~ 0.1 mg CP-93,393 ml^{-1}. One sample was extracted with the recommended extraction volume. The other two solutions contained either 50 or 150% of the recommended amount of extraction solvent. Assay results indicated that the amount of extraction solvent has essentially no effect on the amount of drug extracted. Recoveries were as follows: 50% volume, 98% recovered; 100% volume, 100% recovered; 150% volume, 98% recovered.

3.8. Sample preparation using a robotic procedure

An automated method of preparing samples of CP-93,393-1 tablets was developed and validated. This sample preparation used a Benchmate™ Tablet Processing Workstation (TPW). Validation involved using the TPW to prepare 10 tablets from a batch that had previously been prepared manually. The TPW samples were then assayed using the chromatographic system described here. An interval hypothesis approach was used to test for equivalence of analytical results [12]. It was determined that no significant difference existed between the two sets of data at the 95% probability level.

4. Conclusions

The validation data provided here indicated that the chromatographic assay for CP-93,393-1 tablets is a rugged, transferable method and is suitable for regulatory filing. The method satisfies the requirements of linearity, precision and selectivity to quantitate CP-93,393 in a formulated product.

Acknowledgements

The authors wish to acknowledge Andrew Lipczynski of Pfizer, Sandwich, UK for his sample matrix approach to validation of analytical methods.

References

[1] S.T. Colgan, R.H. Reed, M.L. Dumont, G.R. Haggan, J. Chromatogr. (1997) in press.
[2] United States Pharmacopeia XXIII/National Formulary XVIII, United States Pharmacopeial Convention, Inc., Rockville, MD, USA, 1994.
[3] ICH Harmonised Tripartate Guideline Q2A: Validation of Analytical Methods: Definitions and Terminology, Step 4, Final Draft, 26th October, 1994.
[4] D. Gopal, N. Grinberg, T. Dowling, H. Perpall, G. Bicker, P. Tway, J. Liq. Chromatogr. 16 (8) (1993) 1749–1768.
[5] B. Renger, H. Jehle, M. Fischer, W. Funk, J. Planar Chromatogr. 8 (1995) 269–278.
[6] E. Pina, A. Sousa, A. Brojo, J. Liq. Chromatogr. 18 (13) (1995) 2683–2693.

[7] F. Ortiz-Boyer, M. Tena, M. Luque de Castro, M. Valcarcel, J. Pharm. Biomed. Anal. 13 (1995) 1297–1303.
[8] G.P. Carr, J.C. Wahlich, J. Pharm. Biomed. Anal. 8 (1990) 613.
[9] T.D. Wilson, J. Pharm. Biomed. Anal. 8 (1990) 389.
[10] E. Debesis, J. Boehlert, T. Givand, J. Sheridan, Pharm. Tech. September (1982) 120.
[11] S.T. Colgan, G.R. Haggan, R.H. Reed, J. Pharm. Biomed. Anal. 14 (1996) 825.
[12] C. Hartmann, J. Smeyers-Verbeke, W. Penninckx, Y. Vander Heyden, P. Vankeerberghen, D.L. Massart, Anal. Chem. 67 (1995) 4491–4499.
[13] J.P. Foley, J.G. Dorsey, Anal. Chem. 55 (1983) 730–737.