Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 798 (2003) 49–54
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Liquid chromatographic determination of ceterizine hydrochloride and paracetamol in human plasma and pharmaceutical formulations

Basavaraj S. Nagaralli, Jaldappa Seetharamappa*, Babu G. Gowda, Mahaveer B. Melwanki

Department of Chemistry, Karnatak University, Dharwad 580 003, India

Received 22 April 2003; received in revised form 26 August 2003; accepted 26 August 2003

Abstract

An accurate, simple, reproducible and sensitive HPLC method for the determination of ceterizine hydrochloride (CTZH) and paracetamol (PARA) has been developed and validated. The separation of CTZH, PARA and Nimesulide (the internal standard) was achieved on a CLC C_{18} (5 μ, 25 cm × 4.6 mm i.d) column using UV detection at 230 nm. The mobile phase was consisted of acetonitrile–water (55:45 v/v). The linear ranges of detection for CTZH and PARA were found to be 0.715–55 μg/ml ($r^2 = 0.9985$) and 0.55–39 μg/ml ($r^2 = 0.9957$) respectively. Intra- and inter-day assay relative standard deviations were less than 1%. The method has been applied successfully to the determination of binary combination of CTZH and PARA in human plasma and pharmaceutical preparations. There was no interference from drugs commonly administered with CTZH and PARA. The method has been shown to be linear, reproducible, specific, and rugged.

Keywords: Ceterizine hydrochloride; Paracetamol

1. Introduction

Ceterizine, a piperazine derivative and carboxylated metabolite of hydroxyzine used in the treatment of perennial and seasonal allergitic rhinitis and also for chronic uticaria. Paracetamol (PARA) has analgesic and antipyretic activity and is used for the treatment of pain such as headache, toothache, rheumalism and neuralgia. CTZH combined with PARA is frequently used as anti-allergic and antipyretic, and for the treatment of severe allergies and cold. CTZH is official in European Pharmacopoeia [1], while PARA is official in United States Pharmacopoeia [2]. Many HPLC methods [3–26] have been reported for the determination of CTZH [3–12] and PARA [13–26] in biological fluids or in pharmaceutical formulations. But, none of these methods demonstrate the simultaneous quantification of these two drugs in combination in human plasma and pharmaceutical preparations. Moreover, the reported methods are not satisfactory for routine quality assurance for one or the other reason. Some of these methods have low sensitivity [3,7,9,13–19,21–23] or works out only at higher concentrations of the drugs [7,10,14,16–19,21–23] or need more time for analysis [11,17]. Hence, it was felt necessary to develop a simple and sensitive HPLC method, which does not suffer from the above limitations for the determination of binary combination of CTZH and PARA in human plasma and pharmaceutical formulations.

2. Experimental

2.1. Chemicals

CTZH and PARA were obtained from Dr. Reddy's Laboratory, India and Core Healthcare Ltd., India. Acetonitrile (s.d Fine-Chem, Ltd., India) and water (Rankem Ltd., India) used were of HPLC grade. Nimesulide (internal standard) was obtained from Dr. Reddy's Laboratory, India.

2.2. Apparatus

All HPLC measurements were made on a Shimadzu Corporation system (Analytical Instruments division, Kyoto,

* Corresponding author.
E-mail addresses: jseetharam@yahoo.com, j_seetharam@rediffmail.com (J. Seetharamappa).

1570-0232/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2003.08.048

Japan) consisting of a LC10AT solvent pump, SPD10AVP detector and a data station with win chrome software version 3.1. The separation was performed on a CLC C_{18} column (5 μ, 25 cm × 4.6 mm i.d). A CLC ODS (4 cm × 4.6 mm i.d.) was used as a guard column to protect analytical column. A mixture of acetonitrile–water (55:45, v/v) was used as a mobile phase at a rate of 0.8 ml/min. Hamilton 702 μR injector with a 25 μl loop was used for the injection of the samples. Detection was done at 230 nm. The mobile phase was filtered through 0.45 μ Millipore membrane filter and degassed. The separation was carried out at room temperature.

2.3. Stock solutions

CTZH (100 mg), PARA (100 mg) and NIM (100 mg) were accurately weighed separately into different 100 ml calibrated flasks and dissolved in the mobile phase and filled up to volume with the mobile phase. The solutions were diluted as and when required.

2.4. Standard working solutions

Standard working solutions were prepared individually in mobile phase for CTZH and PARA. Aliquots from each working solutions were combined and diluted with mobile phase to yield a solution with final concentrations of 200 and 250 μg/ml of CTZH and PARA. Studies on the stability of analytes in standard working solution showed that there was no decomposition product in the chromatogram and difference in area-ratios during the analytical procedure and even after storing for 2 days at +4 °C.

2.5. Plasma sample preparation

Ten healthy volunteers, aged 20–35 years, were selected after obtaining their written consent. The Departmental ethical committee clearance was obtained on the proposed human plasma experimental procedures adopted in this research. After an overnight fast, at 08.00, before breakfast, 10 volunteers were given Cheston cold® tablet (labelled to contain PARA, 500 mg and CTZH, 5 mg). Human blood samples were collected after an hour in dry and evacuated tubes. The samples were handled at room temperature and were centrifuged at 1000 rpm for 10 min for separation of plasma within 60 min of collection. The plasma samples were spiked with internal standard and were extracted with ether. The ethereal layer was evaporated to dryness on a water bath under gentle stream of nitrogen gas at 40 °C. The residue was dissolved in the mobile phase and 20 μl solution was injected on to the column.

2.6. Pharmaceutical preparation

Twenty tablets of the selected drugs were finely powdered. An amount equivalent to 25 mg of the drug was weighed accurately and transferred into a 100 ml beaker. Using a mechanical stirrer, the powder was completely disintegrated in mobile phase for CTZH and PARA. The solution was filtered and the filtrate was made up to 50 ml with the mobile phase.

3. Procedure

3.1. Chromatographic conditions

LC analysis was performed by isocratic elution with flow rate of 0.8 ml/min. The mobile phase of acetonitrile and water (55:45, v/v) was used throughout. All solvents were filtered through a 0.45 μ Millipore membranes filter before use and degassed in an ultrasonic bath. Volumes of 20 μl each, prepared and sample solutions (pharmaceutical or plasma samples) were injected into the column. Quantification was effected by measuring at 230 nm and the chromatographic run time was 7 min.

Through out the study, the suitability of the chromatographic system was monitored by calculating the capacity factor (k^1), the resolution (R), the selectivity (α) and the peak asymmetry (T).

3.2. Establishment of calibration

Working standard solutions of CTZH (1.5–55 μg/ml) and PARA (2.0–39 μg/ml) containing fixed concentration (10 μg/ml) of internal standard were prepared in the mobile phase.

Triplicate 20 μl injections were made for each standard solution to see the reproducibility of the detector response at each concentration level. The peak area ratio of standard to internal standard was plotted against the concentration of the drug to obtain the calibration graph. The results were subjected to regression analysis to calculate calibration equation and correlation coefficients. A typical chromatogram obtained was shown in Fig. 1.

3.3. Analysis of plasma samples

The plasma sample obtained as described in plasma sample preparation was taken and 20 μl solution was injected into the chromatographic column and the chromatogram was recorded (Fig. 2). At the above said chromatographic conditions, well resolved peaks for standard and internal standards were observed. The retention times of PARA, CTZH and NIM were observed to be at 2.39, 3.50 and 5.88 min, respectively.

3.4. Analysis of tablet

An aliquot of the drug obtained by following the procedure described for analysis of pharmaceutical preparations was taken and analysed. The chromatogram at 230 nm showed a complete resolution of all the peaks.

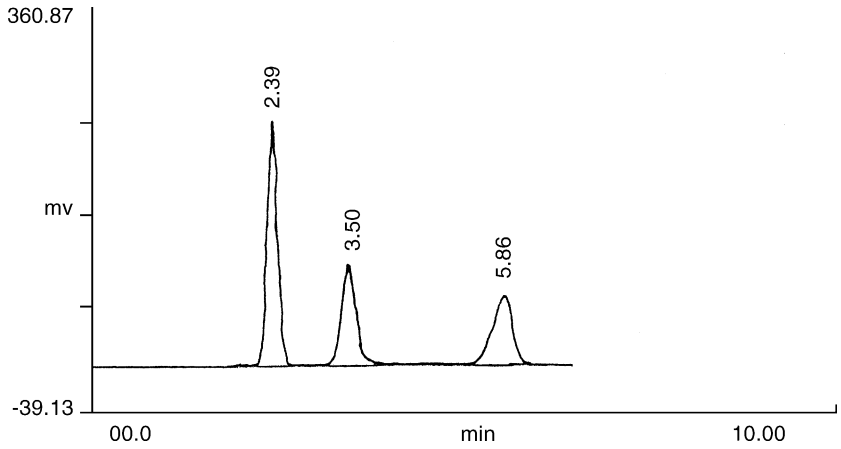

Fig. 1. Chromatogram of CTZH, PARA and NIM in pure form.

4. Results and discussion

4.1. Method development

The mobile phase was chosen after several trials with methanol, acetonitrile, isopropyl alcohol, triethylamine, water and buffer solutions in various proportions and at different pH values. A mobile phase consisting of acetonitrile and water (55:45, v/v) was selected to achieve maximum separation and sensitivity. The effects of flow rates in the range of 0.5 to 1.5 ml/min were examined. A flow rate of 0.8 ml/min gave an optimal signal to noise ratio with a reasonable separation time. Using reverse phase C_{18} column, the retention times of PARA, CTZH and NIM were observed to be 2.39, 3.50 and 5.88 min, respectively. The total time of analysis was less than 7 min.

The solution containing PARA, CTZH and NIM exhibited maximum absorption at 230 nm and hence, this wavelength was chosen for the analysis.

4.2. Linearity

The calibration curves were liner in the studied range. The calibration curve equation is $y = bx + c$, where y represents the ratio of CTZH/PARA peak height to NIM peak height and x represents the ratio of CTZH/PARA concentration to that of NIM. The mean equation of the calibration curve ($n = 9$) obtained was $y = 406375.69x + 652478.55$ for PARA and $y = 236462.97x$–12736.44 for CTZH. Excellent linearity was obtained for both drugs between peak area ratios and concentrations of 1.5–55 μg/ml with $r^2 = 0.9985$ and 2.0–39 μg/ml with $r^2 = 0.9957$ for CTZH and PARA, respectively.

4.3. Limits of detection and quantification

Limits of detection (LOD) were established at a signal-to-noise ratio (S/N) of 3 while limits of quantification (LOQ) were established at a signal-to-noise ratio (S/N) of 9. The LOD and LOQ were experimentally verified by

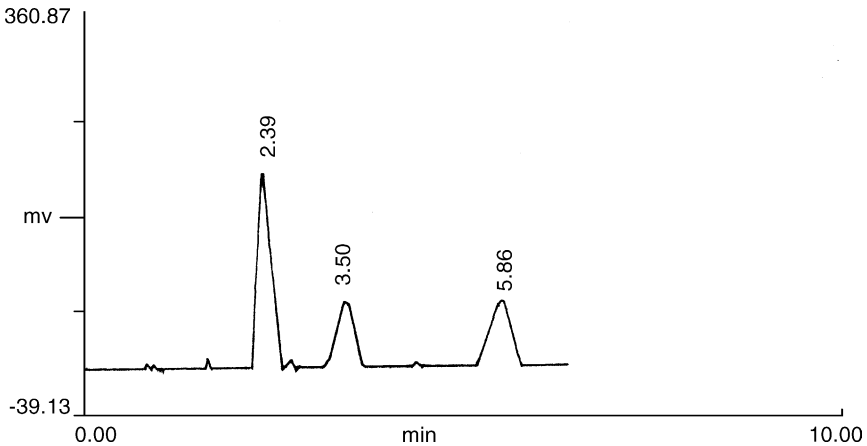

Fig. 2. Chromatogram of plasma sample containing CTZH, PARA and NIM.

Table 1
Precision of the developed method at the LOQ level ($n = 9$)

Compound	λ (nm)	Peak area ($n = 9$, mean)	R.S.D. (%)
PARA	230	171924	2.41
CTZH	230	89567.25	2.28

R.S.D.(%) = ((S.D./mean) × 100).

nine injections of CTZH and PARA at the LOD and LOQ concentrations. The limit of detection was calculated to be 0.248 and 0.208 μg/ml and limit of quantification was calculated to be 0.550 and 0.715 μg/ml for CTZH and PARA, respectively.

4.4. Suitability of the method

The chromatographic parameters such as resolution, selectivity and peak asymmetry were evaluated for the selected drugs. The calculated resolution values R between each peak-pair were found to be 3.44 and 6.78 while the selectivity values (α) were observed to be 1.81 and 1.95 for PARA and NIM, respectively. The capacity factors (k^1) were found to be 1.34, 2.43 and 4.76 while the peak asymmetry (T) values were observed to be 1.10, 1.27 and 1.25 for PARA, CTZH and NIM, respectively.

4.5. Precision

The precision (Table 1) of the method (within-day variations of replicate determinations) was checked by injecting nine times of PARA and CTZH at the LOQ level. The precision of the method, expressed as the relative standard deviations (R.S.D., %) at the LOQ level, were 2.41 and 2.28% for PARA and CTZH, respectively.

4.6. Accuracy

A standard working solution containing PARA and CTZH, to give final concentrations respectively 20 and 30 μg/ml was prepared. The prepared mixture of standard was injected nine times as a test sample. From the respective area counts, the concentrations of the PARA and CTZH were calculated using the detector responses. The accuracy, defined in terms of % deviation of the calculated concentrations from the actual concentrations is listed in Table 2.

Table 2
Accuracy of the developed method

Drug	Spiked concentration (μg/ml)	Measured concentration (μg/ml; mean ± S.D.)	R.S.D. (%)	Deviation (%)
PARA	20	19.351 ± 0.229	1.18	3.24
CTZH	30	31.05 ± 0.371	1.19	3.51

R.S.D.(%) = ((spiked concentration − mean measured concentration) × 100)/spiked concentration.

4.7. Ruggedness

The ruggedness of the HPLC method was evaluated by carrying out the analysis of the standard working solution, the same chromatographic system and the same column on different days. Small differences in area ratios and good constancy in retention times were observed after 48 h time period. The R.S.D. value of less than 0.419% for areas was observed (Table 3). The R.S.D. values for nine determinations were found to be 0.2540 and 0.3344% (for intra-day analysis), and 0.365 and 0.279% (for inter-day analysis) for CTZH and PARA, respectively. The low R.S.D. values indicated the ruggedness of the method. The comparable detector responses obtained on different days indicated that the method is capable of producing results with high precision on different days.

Similarly, injecting the standard working solution into a different HPLC unit tested the ruggedness of the method. The high degree of reproducibility of the detector response and retention times indicate that the method is fairly rugged.

4.8. Specificity of the method

Specificity of the chromatographic analysis was confirmed by the fact that the drugs such as ibuprofen, chlorpheniramine maleate, amoxycillin, cloxacillin, pseudoephedrin hydrochloride, cefadroxil, methdilazine hydrochloride, diclofenac sodium, and ciproflaxacin did not interfere in the determination, as evident from their retention times which are different from those of CTZH and PARA.

Table 3
Day to day variability according to area

	25th September 2002		27th September 2002	
	PARA	CTZH	PARA	CTZH
Area	29991142.8	16267707.6	30007643.8	16464718.4
S.D.	99418.1	63135.9	98659.3	69012.4
R.S.D. (%)	0.3314	0.388	0.328	0.419

Mean values of nine determinations.

Table 4
Intra-day (1 representative day) and inter-day precision and accuracy for PARA and CTZH in human plasma samples

Drug	Plasma conc. (μg/ml)	Intra-day ($n = 9$)			Inter-day ($n = 9$)		
		Conc. measured	R.S.D. (%)	Bias (%)	Conc. measured	R.S.D. (%)	Bias (%)
PARA	5	4.71 ± 0.10	2.17	−5.8	5.28 ± 0.10	1.85	5.6
	15	15.85 ± 0.30	1.90	5.66	14.07 ± 0.33	2.34	−6.2
	30	28.13 ± 0.66	2.35	−6.23	31.95 ± 0.69	2.16	6.5
CTZH	5	4.67 ± 0.12	2.57	−6.6	4.76 ± 0.08	1.68	−4.8
	15	14.11 ± 0.31	2.20	−5.93	16.23 ± 0.43	2.64	8.2
	30	32.06 ± 0.73	2.27	6.86	27.59 ± 0.85	3.08	−8.03

Table 5
Analysis of CTZH and PARA in pharmaceutical preparation

CTZH/PARA in spiked tablet (mg/tablet)	CTZH/PARA found (mg/tablet)	R.S.D. (%)	Recovery (%)
PARA (500)	499.89	0.58	100.13
CTZH (5)	4.96	0.63	99.68

4.9. Analysis of plasma samples

The proposed method was applied to the determination of CTZH and PARA in plasma samples for validation. The results obtained in intra-day and inter-day precision and accuracy at three different concentrations in plasma are summarised in Table 4. The largest values of % bias were 6.23 and 6.86 in intra-day and 6.5 and 8.03 in inter-day precision, for PARA and CTZH, respectively. Low values of relative standard deviation indicated high precision of the proposed method.

4.10. Analysis of pharmaceutical preparation

The proposed method was successfully applied to the analysis of PARA and CTZH in Cheston cold® tablet (labelled to contain PARA, 500 mg and CTZH, 5 mg as active substances) and the results were shown in Table 5. The low values of relative standard deviation indicated high precision of the method.

5. Conclusions

The data demonstrate that the analytical method we have developed showed acceptable linearity, precision and accuracy over the concentration range. The method described is rapid since preparation of plasma samples prior to chromatography is relatively simple and the total chromatographic run time is about 7 min. The limit of quantification values for PARA and CTZH are observed to be 0.715 and 0.550 μg/ml, respectively. The proposed chromatographic method can be used to analyse a large number of plasma samples from patients each day in clinical and analytical laboratories, since they need same reversed-phase column, mobile phase, detection system and plasma sample preparation. High percentage recovery values show that the compounds are completely extracted from tablet preparations and the results indicate that the method can be used to quantify PARA and CTZH in binary combinations without the interference from other ingredients. In conclusion, the proposed method could be routinely used for the analysis of CTZH and PARA in plasma samples and pharmaceutical preparations.

Acknowledgements

The authors express their sincere thanks to Dr. Reddy's laboratory; India and Core Healthcare Ltd. India, for supplying gift samples of CTZH and PARA. Thanks are also due to the authorities of Karnatak University, Dharwad for providing necessary facilities.

References

[1] European Pharmacopoeia. Part I. Third edition, 1997, p. 578.
[2] The United States Pharmacopoeia, 22nd Revision, USP Convention Inc., Rockville, MD, 2000, p. 17.
[3] S.S. Zarapkar, V.P. Halkar, S.H. Rane, Indian Drugs 35 (1998) 658.
[4] M.V. Suryanarayana, B.P. Reddy, G.L. Krupadanam, V.S. David, C.S.P. Sastry, Indian Drugs 29 (1992) 605.
[5] A.F.M. El-Walily, M.A. Korany, A. El-Gindy, M.F. Bedair, J. Pharm. Biomed. Anal. 17 (1998) 435.
[6] A. Jelenska, A. Ostrowicz, Acta. Pol. Pharm. 57 (2000) 171.
[7] S.O. Choi, S.H. Lee, H.S. Koo, K. Eomjong, C. Hae-Yong, P. Arch. Pharm. Res. 23 (2000) 178.
[8] M.T. Rossel, R. Lefebore, J. Chromatogr. 565 (1991) 504.
[9] C. Yingquan, Q. Yuhua, S. Weidong, Zhongguo. Yiyuan. Yaoxue. Zazhi. 19 (1999) 716.
[10] M.F. Zater, Y.R. Tahboub, N.M. Najib, J. Pharm. Biomed. Anal. 22 (2000) 739.
[11] S.O. Choi, S.H. Lee, H.S. Koo, K. Eomjong, C. Hae-Yong, J. Chromatogr. 744 (2000) 201.
[12] J. Macek, P. Ptacek, J. Klima, J. Chromatogr. 17 (1999) 736.
[13] L. Zhuoyu, H. Shanshan, Fenxi. Ceshi. Xuebao. 17 (1998) 66.
[14] M.J. Akthar, S. Khan, H. Mushtaq, J. Pharm. Biomed. Anal. 12 (1994) 379.
[15] V.M. Shinde, B.S. Desai, N.M. Tendulkar, Indian J. Pharm. Sci. 57 (1995) 35.
[16] B. Raman, D. Patil, Indian Drugs 37 (2000) 437.

[17] A.P. Argekar, S.J. Shah, Indian Drugs 34 (1997) 437.
[18] R.A. Sodhi, J.L. Chawla, R.T. Sane, Indian Drugs 33 (1996) 280.
[19] J.L. Chawla, R.A. Sodhi, R.T. Sane, Indian Drugs 33 (1996) 171.
[20] H. Ismail, Anal. Lett. 27 (1994) 71.
[21] J.L. Chawla, R.A. Sodhi, R.T. Sane, Indian Drugs 34 (1997) 199.
[22] M. Kartal, J. Pharm. Biomed. Anal. 26 (2001) 857.
[23] A.G. Goicoechea, M.J. De Alda, J.L.L. Villa-Jato, J. Liq. Chromatogr. 18 (1995) 325.
[24] M. Gilar, Chem. Listy. 89 (1995) 89.
[25] R. Kalman, F. Katalin, G. Bela, Acta Pharm. Hung. 60 (1990) 156.
[26] A. Esteban, N. Graells, J. Satorre, M. Perez-Mateo, J. Chromatogr. 573 (1992) 121.