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Original Article

VALIDATED CHIRAL RP-UFLC METHOD FOR THE QUANTIFICATION OF CHLORTHALIDONE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: Development and validation of new, simple and reliable enantioselective reverse phase ultra-fast liquid chromatography (RP-UFLC) method for quantification of chlorthalidone in bulk and pharmaceutical dosage form.

Methods: In the present study, the isocratic RP-UFLC method was developed on Phenomenex® Lux cellulose 4 column (250×4.6 mm, 5µ) and di-sodium hydrogen phosphate buffer (pH 3.6): methanol (40:60 v/v) as mobile phase. Elute was monitored at 240 nm with a flow rate of 1 ml/min.

Results: The described method provided linear correlation (R^2=0.999) between the range of 2-10 µg/ml. Chlorthalidone enantiomers showed good resolution with a retention time (t_R) of 5.75 min and 7.46 min respectively. The precision of the method revealed that relative standard deviation is within the acceptable limit. The percentage recovery of each chlorthalidone enantiomers was found to be 99.98% and 100.09% respectively. The method was validated in accordance with ICH harmonized tripartite guidelines, validation of analytical procedures: text and methodology Q2 (R1).

Conclusion: An economical, accurate, sensitive and precise RP-UFLC method was developed and fully validated for quality control analysis of chlorthalidone in pharmaceutical dosage form.

Keywords: Chlorthalidone, Enantioselective, RP-UFLC

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INTRODUCTION

Chemically chlorthalidone is (RS) 2-chloro-5-(1-hydroxy-3oxo-2, 3-dihydro-1H-isoindol-1-yl) benzene-1-sulfonamide [fig.1]. Chlorthalidone is an oral diuretic drug widely used for the treatment of hypertension. It is described as a thiazide-like diuretic because it acts similarly to the thiazides. While comparing with other medications of the thiazide class, chlorthalidone has the longest duration of action [1]. Chlorthalidone is practically insoluble in water, but it is soluble in methanol. Its molecular formula and the molecular weight is $C_{14}H_{11}ClN_2O_4S$ and 338.766 g/mol respectively. It was also more effective than amlodipine in reducing congestive heart failure (CHF) in the antihypertensive and lipid-lowering treatments to prevent heart attach trial (ALLHAT) [2]. Furthermore, chlorthalidone prevents reabsorption of sodium and chloride by inhibiting the Na^+/Cl^- symporter in the distal convoluted tubule i.e.; it increases the rate of excretion of $Na^+$and Cl from the body [3].

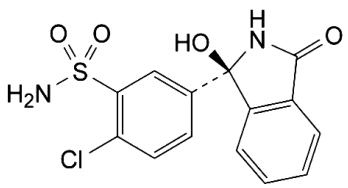

Fig. 1: Chemical structure of chlorthalidone [4]

The literature survey reveals numerous non-stereoselective analytical methods that have been developed for chlorthalidone in their bulk and pharmaceutical formulations. There were reports of ultraviolet spectroscopy [4], supercritical fluid chromatography [5], gas chromatography [6], reverse phase high-performance liquid chromatography (RP-HPLC)[7], Stability indicating RP-HPLC method [8], simultaneous estimations by RP-HPLC in pharmaceutical formulation [9, 10] and biological samples [11, 12]. Also stereoselective UFLC [13] method was developed for the same drug by using kromosil TBB (0, 0'-bis (4-tertbutylbenzoyl)-N, N'-diallyl-Ltartardiamide) chiral column but the reported work was based on normal phase method (makes the method more expensive) and the method is less sensitive. The chief objective of the current work was to develop a simple, sensitive, stereoselective and economic method for quantitative determination of chlorthalidone in bulk and pharmaceutical dosage form.

MATERIALS AND METHODS

Solvents and chemicals

Reference standard of racemic chlorthalidone was obtained from Hetero drugs Pvt. Ltd., Hyderabad, India and was used to develop the RP-UFLC method. HPLC grade methanol was obtained as of Merck specialities Pvt. Ltd., Mumbai, India. Other chemicals and reagents were of analytical grade. Millipore purification system (Direct-Q, Bangalore, India) was used to produce water for the RP-UFLC method. Tablet CTD-12.5, IPCA Laboratories Ltd, India containing 12.5 mg of chlorthalidone IP was procured from the local pharmacy, Mysuru, Karnataka, India.

Instrumentation

A Shimadzu ultra fast liquid chromatography (Prominence LC-20AD) equipped with prominence SIL-20ACHT autosampler, 1260 binary pump VL (35MPa) and Shimadzu prominence SPD-M20A diode array detector were utilized. All another weighing for the analysis was accomplished on Shimadzu electronic analytical balance AY-220. Data collection and analysis were performed using LC solution Software. Quantification of chlorthalidone was achieved using Lux 5µ cellulose 4 column. The mobile phase was composed of Di-sodium hydrogen phosphate (Na_2HPO_4) buffer pH 3.6 and methanol in the ratio 40:60 v/v. The optimized chromatographic conditions are shown in table 1.

Table 1: Chromatographic conditions for the developed method

Parameters	Methods
Stationary phase	Lux cellulose 4 column(250×4.6 mm, 5µ)
Mobile phase	Na_2HPO_4 buffer pH 3.6: methanol (40:60 v/v)
Flow rate (ml/min)	1.0
Elution	Isocratic
Runtime (minutes)	10
Column temperature (°C)	Ambient
Volume of injection loop (µl)	10
Detector	PDA
Detection wavelength (nm)	240
Enantiomer 1 t_R (min)	5.75
Enantiomer 2 t_R (min)	7.46

Preparation of phosphate buffer pH 3.6

0.900 g of anhydrous disodium hydrogen phosphate and 1.298 g of citric acid monohydrate was dissolved inadequate water to make 1000 ml. The pH (3.6) of the resultant solution was adjusted with o-phosphoric acid. Finally, the mobile phase was filtered through a membrane filter (0.45 µ) prior to use.

Preparation of standard solution of racemic chlorthalidone

Chlorthalidone (1000 µg/ml) standard stock solution was prepared by precisely weighing 50 mg in 50 ml volumetric flask, and the volume was made up with methanol. Taking appropriate aliquot from standard stock solution, 10µg/ml was prepared using the same solvent.

Assay

From the fine crushed tablet powder, an accurate quantity of 100 mg of powder was transferred into a 100 ml volumetric flask and diluted with methanol. The solution was filtered through 0.45µm membrane filter to remove undissolved particulates. The resulting stock solution was diluted further to obtain the concentration of 4µg/ml. The mobile phase was equilibrated before 1 hr of the injection of the sample. The flow rate was set at 1.0 ml/min with the wavelength of 240 nm.

Method validation

The developed RP-UFLC method was validated as per ICH guidelines [14].

Linearity and range

The linearity of the method is an ability to get a test result which gives a clear mathematical relationship to the concentration (amount) of an analyte. A standard stock solution of chlorthalidone (1000 µg/ml) was prepared by dissolving precisely 50 mg of chlorthalidone in 50 ml volumetric flask and volume were made up with methanol. The stock solution was diluted further to obtain 2-10µg/ml concentration range and all dilutions were prepared in triplicate. The peak areas, retention times, intercept and slope, were noted.

Precision

System precision and method precision was determined by means of repeatability (intraday) and intermediate precision (inter-day) studies by measuring the peak area and retention time of 3 different sets of each different concentration (2, 4 and 6 µg/ml) of chlorthalidone.

Repeatability

It was performed by repeated injections of 3 different concentrations from single batch under the same experimental conditions on the same day. % RSD for both retention time and peak area was calculated.

Intermediate precision

Intermediate precision of the method was evaluated by performing the same repeated study on the day after that for three different concentrations of chlorthalidone. From the results, % RSD values for mean retention time and mean peak area were calculated.

System suitability tests

Data obtained after giving six replicate injections of a standard solution (6µg/ml) of chlorthalidone were assessed. The peak areas, theoretical plates (N), resolution (Rs), tailing factor (T), capacity factor (k') and asymmetric factor (As) were noted and calculated.

Accuracy

Accuracy assesses the closeness of the experimental value to that of the true value and was measured by standard addition method. Accuracy was implemented at three levels (50%, 100% and 150%) where known amount of standard was added to a pre-analyzed (fixed) sample formulation. The tests were carried out in triplicate. For all concentrations, the % RSD and % recovery were calculated.

Sensitivity

The sensitivity of the method can be measured from the limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ can be calculated on the basis of response, i.e., the y-intercept of the regression line and slope of the calibration curve. The equation was given below,

$$LOD = \frac{3.3\sigma}{S}\ LOQ = \frac{10\times\sigma}{S}$$

Where,

σ = standard deviation of response (y-intercept of the regression line)

S = slope of the calibration curve

Robustness

The consequence of the small but deliberate change of the chromatographic conditions was studied to assess the robustness of the method. Robustness can be measured by varying the organic solvent (60±5%), flow rate (1.0±0.1 ml/min), ionic strength of buffer (3.6±0.1) and detection wavelength (240±1). Their effects on tailing factor (T), theoretical plates and % RSD were studied.

RESULTS AND DISCUSSION

Linearity and range

The linearity is a capability of the method to produce test results that are directly proportional to the concentration of an analyte in a given range as shown in fig. 2. Table 2 and 3 specify the regression statistics obtained from the linearity test. Fig. 3 and 4 are the blank and standard chromatogram.

Table 2: Calibration curve of chlorthalidone enantiomer 1 and 2

Concentration(µg/ml)	Enantiomer 1 Peak area	Enantiomer 2 Peak area
2	22042	21533
4	45782	42609
6	69281	66906
8	90729	88221
10	111065	108340

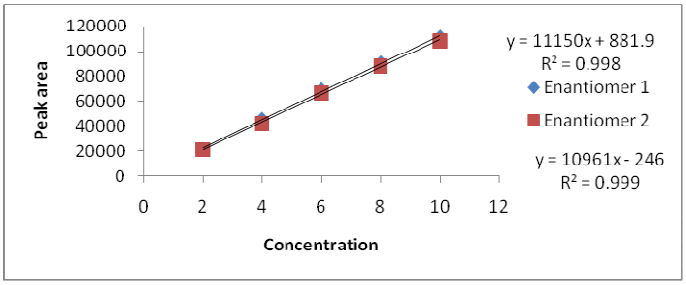

Fig. 2: Calibration curve of chlorthalidone enantiomer 1 and enantiomer 2

Table 3: Linearity data of chlorthalidone

Parameters	Enantiomer 1	Enantiomer 2
Linearity	2-10µg/ml	2-10µg/ml
Regression equation	y=11172x+793.9	y=10961x-246
Slope	11172	10961
Intercept	793.9	246
Correlation coefficient	0.998	0.999
Retention time (t_R)	5.75 min	7.46 min
Resolution	--	2.55
Tailing factor	0.99	1.01
Theoretical plates	3417	3036

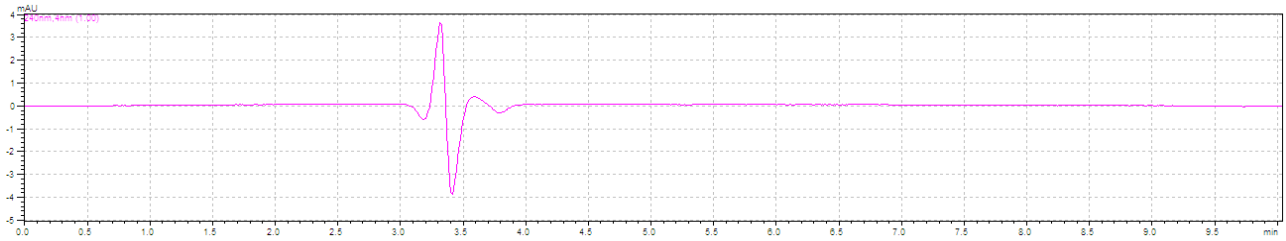

Fig. 3: Blank chromatogram

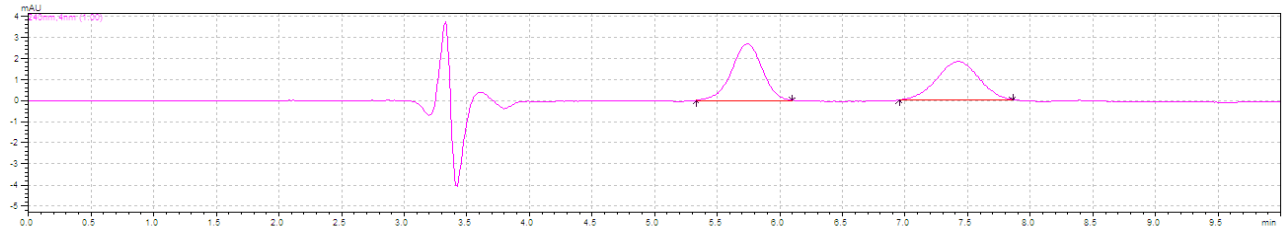

Fig. 4: Standard racemic chlorthalidone chromatogram

Table 4: Assay of chlorthalidone formulation

Parameters	Brand name	Available form	Label claim	Amount found	Assay
Enantiomer 1	CTD 12.5	Tablet	12.5 mg	6.28 mg	50.24%
Enantiomer 2				6.20 mg	49.6%

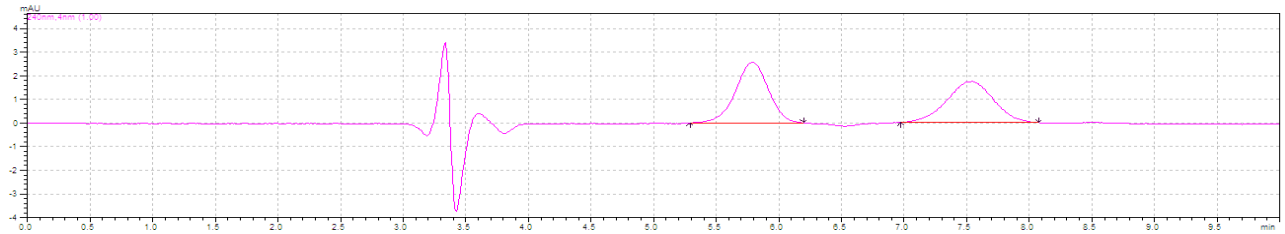

Fig. 5: Formulation of chlorthalidone chromatogram

Assay

Assay determines the potency or content of analyte present in the sample. Assay results are tabulated in table 4 and chromatogram shown in fig. 5.

Precision

System precision

The system precision was carried out to ensure that the analytical system was working properly. The system precision is performed by 6 sample injections and checking the reproducibility in the peak area. Chromatograms were recorded. The results are shown in table 5.

Method precision

Intraday precision

The precision express reliability of the method, where it defines the extent for the individual test results can agree to repeated test result on same operating conditions at a short time period. Repeatability of the method is accepted, as % RSD of chlorthalidone enantiomer 1 and 2 are (0.33 and 0.73) of the retention time (<1%) and % RSD of chlorthalidone enantiomer 1 and 2 are (0.033 and 0.029) of the peak areas of six replicates injection of 2, 4 and 6µg/ml (<2%) which was within the limits.

Intermediate precision

Repeatability method procedure was repeated on the next day. The method passed the test, as both retention time (<1%) and response peak areas (<2%), % RSD obtained were in the limits and was shown in table 6.

System suitability studies

System suitability test was carried out to confirm the resolution and reproducibility of the system on which analysis are to be done. This study data was summarised in table 7. All the values are well within the limit.

Table 5: System precision of chlorthalidone enantiomers

Parameter	Concentration (µg/ml)	Intra-day		Inter-day	
		Enantiomer 1 peak area	Enantiomer 2 peak area	Enantiomer 1 peak area	Enantiomer 2 peak area
Average	4	45774	42608	45761	42613
Mean SD		8.62	5.56	11.93	8.02
Mean %RSD		0.018	0.013	0.026	0.018

(mean±SD) n = Number of determinations, *Average of six determinants, SD=Standard deviation, RSD=Relative standard deviation

Table 6: Precision study of chlorthalidone

	Components	Intraday Precision			Interday Precision		
		Theoretical concentration µg/ml			Theoretical concentration µg/ml		
Retention time	Enantiomer 1 Mean RT	5.75	5.74	5.75	5.74	5.737	5.75
	Enantiomer 1 Mean % RSD	0.33	0.28	0.38	0.36	0.31	0.43
	Enantiomer 2 Mean RT	7.48	7.47	7.46	7.46	7.51	7.45
	Enantiomer 2 Mean % RSD	0.76	0.62	0.81	0.87	0.86	0.89
Peak area	Enantiomer 1 Mean Peak area	22047	45774	69281	22042	45761	69285
	Enantiomer 1 Mean % RSD	0.072	0.018	0.0108	0.0806	0.026	0.013
	Enantiomer 2 Mean Peak area	21533	42608	66906	21532	42613	66910
	Enantiomer 2 Mean % RSD	0.062	0.013	0.013	0.0715	0.018	0.016

(mean±SD) n = Number of determinations, *Average of six determinants, RSD=Relative standard deviation

Table 7: System suitability parameters of chlorthalidone

Parameters	Enantiomer 1	Enantiomer 2
Peak area	69282	66905
Theoretical plates (N)	3407	3033
Resolution	--	2.55
Tailing factor (T)	0.99	0.992
Capacity factor	1.4	2.1
Asymmetric factor	1.1	1.1
% RSD	0.38	0.81

n = Number of determinations, *Average of six determinants

Table 8: Recovery data of chlorthalidone

% of recovery	Formulation conc.	Spiked conc.	Total conc.	Conc. Obtained (enantiomer1)	% recovery	% RSD	Conc. Obtained (enantiomer2)	% recovery	% RSD
50	4	2	6	5.97	99.5	0.25	6.03	100.5	0.44
				5.95	99.17		5.99	99.83	
				5.98	99.66		5.98	99.66	
				Mean	99.44		Mean	99.99	
100	4	4	8	7.99	99.87	0.38	7.98	99.75	0.50
				8.03	100.37		8.05	100.62	
				7.97	99.62		7.98	99.75	
				Mean	99.95		Mean	100.04	
150	4	6	10	10.17	101.7	0.31	10.08	100.8	0.55
				10.03	100.3		10.03	100.3	
				9.97	99.7		9.97	99.7	
				Mean	100.56		Mean	100.26	

n = Number of determinations, *Average of three determinants of each concentration.

Accuracy

Recovery data of chlorthalidone enantiomers which was applied at three different levels (50%, 100% and 150%) of enantiomer 1 and enantiomer 2 were found to be 99.8% and 100.09% respectively. Recovery data were summarized in table 8.

Limit of detection and limit of quantification

The LOD of chlorthalidone enantiomer 1 and 2 results were summarized in table 9.

Robustness

Deliberate changes in organic solvent (±%), flow rate (±ml/min), the ionic strength of buffer (±pH) and detection wavelength (±nm) on experimental parameters such as tailing factor *(T*), theoretical plates and resolution of the peak were studied in robustness test.

The obtained results were calculated and summarized in table 10.

Table 9: Limit of detection and limit of quantification of chlorthalidone

Parameter	Enantiomer 1	Enantiomer 2
LOD(µg/ml)	0.086967	0.023078
LOQ(µg/ml)	0.263536	0.069933

Table 10: Robustness of chlorthalidone

Condition		Chlorthalidone	Tailing factor	Theoretical plates	% RSD	Resolution
Optimized condition		Enantiomer 1	0.99	3417	--	--
		Enantiomer 2	1.01	3036	--	2.55
Mobile phase ratio	55:45	Enantiomer 1	0.987	3415	0.87	--
(60:40 v/v)		Enantiomer 2	1.004	3026	0. 98	2.48
	65:35	Enantiomer 1	0.99	3420	0.92	--
		Enantiomer 2	1.007	3029	0. 89	2.51
Flow rate(ml/min)	0.9	Enantiomer 1	0.99	3419	1.002	--
(1.0 ml/min)		Enantiomer 2	1.012	3033	0.96	2.5
	1.1	Enantiomer 1	0.985	3421	0.98	--
		Enantiomer 2	1.004	3037	0.95	2.57
pH of phosphate buffer	3.5	Enantiomer 1	0.979	3409	1.12	--
(3.6)		Enantiomer 2	1.011	3029	0.998	2.54
	3.7	Enantiomer 1	0.985	3418	1.10	--
		Enantiomer 2	1.008	3034	1.002	2.542
Wavelength(nm)	239	Enantiomer 1	0.984	3422	0.78	--
(240)		Enantiomer 2	1.006	3027	0.83	2.53
	241	Enantiomer 1	0.988	3411	0.96	--
		Enantiomer 2	1.01	3038	0.98	2.5

n = Number of determinations, *Average of three determinants.

CONCLUSION

A new RP-UFLC method was developed and validated for the separation of chlorthalidone enantiomers in bulk and pharmaceutical dosage form on a Phenomenex® Lux cellulose 4 column (250×4.6 mm, 5µ) using disodium hydrogen phosphate buffer: methanol (40:60 v/v, pH 3.6). The method is fast and cost effective as both the enantiomers were eluted within ten min. The resolution between two peaks was more than 1.5. The method is accurate and precise as the %RSD of both interday and intraday were<2. The percentage recovery of chlorthalidone enantiomers was found to be 99.98% and 100.09% respectively. The LOD and LOQ were within the limit. From the discussion, it can be concluded that the proposed method is specific, robust, precise and accurate. This method utilizes mobile phase which can be effortlessly prepared and cost efficient. Results are in good agreement with label claim which indicates there is no interference of excipients. Therefore the proposed method can be used for routine analysis of chlorthalidone pharmaceutical dosage form.

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CONFLICT OF INTERESTS

No conflict of interest

REFERENCES

1. Oparil S, Cushman WC, Lederle FA. Chlorthalidone versus hydrochlorothiazide in hypertension treatment: do we have the evidence to decide? Am J Kidney Dis 2014;63:387-9.
2. Walters SM, Stonys DB. Determination of chlorthalidone and clonidine hydrochloride in tablets by HPLC. J Chromatogr Sci 1983;21:43-5.
3. Barry LC, Michael EE, Jerome DC. Hydrochlorothiazide versus chlorthalidone evidence supporting their interchangeability. Hypertension 2004;43:4-9.
4. Niraimathi V, Suresh AJ, Kumar IS. UV spectrophotometric methods for the estimation of chlorthalidone in bulk and oral dosage form. Indo Am J Pharm Sci 2013;3:7160-7.
5. Jiri V, Kveta K, Eva T. Rapid supercritical fluid chromatography method for separation of chlorthalidone enantiomers. Anal Lett 2013;46:2860-9.
6. Fleuren HL, Van RJM. Determination of chlorthalidone in plasma, urine and red blood cells by gas chromatography with nitrogen detection. J Chromatogr 1978;152:41-54.
7. Brijesh S, Patel DK, Ghosh SK. A reversed-phase high-performance liquid chromatographic method for determination of chlorthalidone in a pharmaceutical formulation. Int J Pharm Pharm Sci 2009;1:24-9.
8. Naazneen S, Sridevi A. Stability-indicating rp-hplc method for the simultaneous estimation of azilsartan medoxomil and chlorthalidone in solid dosage forms. Int J Pharm Pharm Sci 2014;6:236-43.
9. Kumar GS, Ramya V, Mondal S, Sai PK. Development and validation of a RP-HPLC method for simultaneous estimation of atenolol and chlorthalidone from pharmaceutical formulation. Int Res J Pharm 2012;3:216-9.
10. Rosenberg MJ, Kathryn KL, Thomas ED. Analysis of chlorthalidone in whole blood by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 1986;375:438-43.

11. Mohamed SE, Samia MM, Abdalla AE. Simple and rapid HPLC method for simultaneous determination of atenolol and chlorthalidone in spiked human plasma. Saudi Pharm J 2011;19:43–9.
12. Bijay K, Sharanjit K, Sami M, Harinder JS. First among equals: A comparative study of the effect of hydrochlorothiazide and chlorthalidone on recently diagnosed hypertensives. Asian J Pharm Clin Res 2015;8:195-8.
13. Sirisha T, Gurupadayya B, Bharath KI. Enantiomeric separation and quantitative estimation of chlorthalidone enantiomers by chiral ultra fast liquid chromatography. Int J Res Pharm Chem 2015;5:361-7.
14. International conference on harmonization of technical requirements for registration of pharmaceuticals for human use, ICH harmonized tripartite guideline validation of analytical procedures: Text and Methodology Q2 (R1), Current Step 4 version. London; 2005.

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