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Research Article

SIMULTANEOUS DETERMINATION OF TELMISARTAN, AMLODIPINE BESYLATE AND HYDROCHLOROTHIAZIDE IN A COMBINED POLY PILL DOSAGE FORM BY STABILITY-INDICATING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A simple, precise and rapid stability-indicating High-performance liquid chromatography (HPLC) method is developed for the simultaneous quantitative determination of Telmisartan, Amlodipine besylate and Hydrochlorothiazide from their innovative poly pill combination drug product in the presence of degradation products. It involves a 150 mm x 4.6 mm, 5 µm C-8 column. The separation is achieved on a simple Isocratic method. The mobile phase contains a mixture of sodium perchlorate buffer pH 2.4 (0.05M): acetonitrile in the ratio 60:40 *v/v*. The flow rate is 1.0 mL min−1 and the column temperature is maintained at 25°C. The detector wavelength is 271 nm for Hydrochlorothiazide and Telmisartan and 237 nm for Amlodipine. The retention times of Telmisartan, Amlodipine and Hydrochlorothiazide are 4.8 minutes, 3.8 minutes and 2.4 minutes respectively. The total runtime for the separation of the three active compounds and their degradation products is 8 minutes. The described method is validated with respect to system suitability, specificity, linearity, precision and accuracy. The precision of the assay method is evaluated by carrying out six independent assays of Telmisartan, Amlodipine and HCTZ (0.032 mg mL−1 of Telmisartan, 0.004 mg mL−1 of Amlodipine, 0.01 mg mL−1 of HCTZ). The accuracy of the method is evaluated in triplicate at three concentration levels,i.e. 50%, 100% and 150% of target test concentration (0.64 mg mL−1 of Telmisartan, 0.08 mg mL−1 of Amlodopine, 0.2 mg mL−1 of HCTZ). The described method is linear over the range, 16 to 48 µg mL−1 for Telmisartan, 2 to 6 µg mL−1 Amlodipine and 5 to 15 µg mL−1 for HCTZ.

INTRODUCTION

Cardiovascular diseases (CVDs) are the disorders of heart and blood vessels and primarily include coronary heart disease, hypertension, cerebrovascular disease, peripheral artery disease, rheumatic heart disease, congenital heart disease and heart failure. CVDs are the major cause of death in developed countries and also are rapidly emerging as a main cause of death in the developing world. An estimated 17.5 million people died from CVDs till 2005, representing almost 30% of all the global deaths. It is projected that almost 20 million people will die from CVDs by 2015. The major risk factors involved in CVDs are high low density lipoprotein (LDL) cholesterol, raised blood pressure, increased serum homocysteine level and platelet aggregation, which are primarily caused by unhealthy diet, physical inactivity and tobacco use. A novel polypill formulation is developed using drugs Telmisartan, Amlodipine besylate and Hydrochlorothiazide for CVDs.

Telmisartan (T) is an angiotensin II receptor (type AT1) antagonist used in the management of hypertension. Telmisartan prevents the constriction (narrowing) of blood

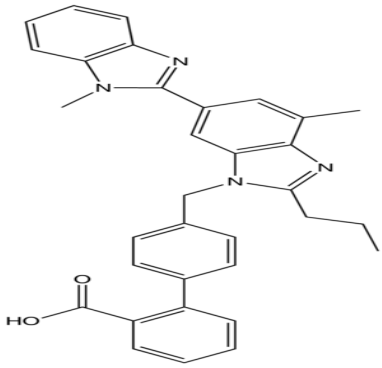
vessels (veins and arteries). Telmisartan is a non-peptide molecule and chemically described as potassium 4'-[(1,7'-dimethyl-2'-propyl-1*H*,3'*H*-2,5'-bibenzimidazol-3'-yl)-ethyl]biphenyl-2-carboxylate (Fig. 1).

Hydrochlorothiazide (H) is a thiazide diuretic (water pill) that decreases the amount of fluid in the body by increasing the amount of salt and water lost in the urine. Hydrochlorothiazide is used to lower blood pressure and to decrease edema (swelling), it is chemically described as 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide (Fig. 1).

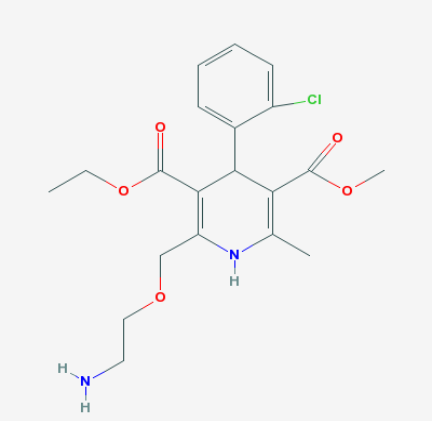
Amlodipine besylate (A) is in a class of drugs called beta-blockers. Beta-blockers affect the heart and circulatory system (arteries and veins). Amlodipine besylate is used to lower blood pressure, lower heart rate, reduce chest pain (angina), and to reduce the risk of recurrent heart attacks. It is chemically described as 3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4- dihydropyridine-3,5-dicarboxylate benzenesulfonate (Fig. 1).

The ever-increasing need for speed and efficient use of time in the pharmaceutical and other fields, there is demand for the development of fast and high throughput analytical procedures. The rapid quantitative determination of combination drugs with big difference in label claims (40 mg for Telmisartan and 5 mg for Amlodipine) with shorter run times is a challenge. For HPLC based assays, the processes of reducing analysis time while adequately resolving analytes from degradation products is often accomplished with column with small particles. The theoretical advantages for small particles are to get well resolved peaks with high theoretical plates over small concentration. Present drug stability test guidance Q1A (R2) issued by international conference on harmonization (ICH) [1] suggest that stress studies should be carried out on a drug product to establish its inherent stability characteristics, leading to identification of degradation products and hence supporting the suitability of the proposed analytical procedures. It also requires that analytical test procedures for stability samples should be stability indicating and they should be fully validated. Accordingly, the aim of the present study was to establish inherent stability of Telmisartan, Amlodipine besylate, and Hydrochlorothiazide through stress studies under a variety of ICH recommended test conditions[1–3] and to develop a rapid stability-indicating reverse phase assay method[4–6].

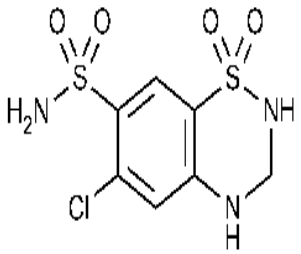
Literature survey reveals that a variety of spectrophotometric and chromatographic methods including UV, colorimetric determination, ratio derivative, and a stabilityindicating HPLC methods have been reported for determination Telmisartan, Amlodipine and HCTZ either single or in combination with other drugs[7–12]. Whereas no liquid chromatography method has been reported for simultaneous quantitative determination of Telmisartan, Amlodipine and HCTZ in the combined dosage form.Hence a rapid simple reproducible High performance liquid chromatography method was developed for simultaneous quantitative determination of Telmisartan, Amlodipine and HCTZ in polypill pharmaceutical dosage forms in the presence of degradation products.



Telmisartan



Amlodipine



Hydrochlorothaizide

Fig. 1: Structure of Amlodipine besylate, Hydrochlorothiazide and Telmisartan

EXPERIMENTAL

Chemicals and reagents

Standards and tablets (40 mg of Telmisartan, 5 mg of Amlodipine besylate, 12.5 mg of Hydrochlorothiazide) were supplied by Aurabindo laboratories limited, Hyderabad, India. The HPLC grade acetonitrile and methanol, analytical grade sodium per chlorate and disodium hydrogen phosphate were purchased from Merck, Darmstadt, Germany. Water was prepared by using Millipore MilliQ Plus water purification system.

Instrumentation

The present work was carried out on Water's HPLC equipped with UV-Visible and Diode Array detectors with pair of 10 mm matched quartz cells. Glassware's used were of 'A' grade and were soaked overnight in a mixture of chromic acid and sulphuric acid, rinsed thoroughly with double distilled water and dried in hot air oven.

Chromatographic conditions

The chromatographic column used was X-Terra RP 8, 150 mm x 4.6 mm i.d with 5 µm particles. The mobile phase contains a mixture of sodium perchlorate buffer pH 2.4(0.05M): acetonitrile (60:40, *v/v*). The flow rate was 1.0 mL min−1 and column temperature was maintained at 25°C. The detection wavelength was 271 nm for Hydrochlorothiazide and Telmisartan, and 237 nm for Amlodipine besylate. The diluent contains a mixture of sodium perchlorate buffer pH 3.0(0.05M): methanol in the ratio 90:10, v / v.

Preparation of standard solutions

Stock standard solutions of Telmisartan, Amlodipine and HCTZ (0.54 mg mL−1 of Telmisartan, 0.2 mg mL−1 of Amlodipine, 0.5 mg mL−1 of HCTZ) were prepared by dissolving appropriate amounts of the compounds in methanol. Working solutions 0.032 mg mL−1 of Telmisartan, 0.004 mg mL−1 of Amlodipine, 0.01 mg mL−1 of HCTZ were prepared from above stock solution in mobile phase for assay determination.

Preparation of sample solution

Weighed and crushed twenty tablets into a clean and dry mortar-pestle. An equivalent to 20 mg of HCTZ (64 mg of Telmisartan, 8 mg of Amlodipine besylate) was transferred to a 100 mL volumetric flask, added 15 mL of 0.1% orthophosphoric acid and kept on rotary shaker for 20 minutes. Added 50 mL of methanol and sonicated for 20 minutes. Made the volume to 100 mL with methanol (0.64 mg mL−1 of Telmisartan, 0.08 mg mL−1 of Amlodipine, 0.2 mg mL−1 of HCTZ). About 5 mL of supernant solution was taken and diluted to 100 mL with diluent to get working concentrators (0.032 mg mL−1 of Telmisatan, 0.004 mg mL−1 of Amlodiine, 0.01 mg mL−1 of HCTZ). The solution was then filtered through 0.45 µ (Nylon 66- membrane) filter.

RESULTS AND DISCUSSION

Chromatographic Method Development

The method was optimized to separate major degradation products formed under different stress conditions. The main target of the chromatographic method is to get the separation for closely eluting degradation products. The degradation samples were run using different stationary phases like C18, C8, and Mobile phases containing buffers like phosphate and perchlorate with different pH (2.0–7) and using organic modifiers like acetonitrile and methanol in the mobile phase. The isocratic method was working since telmisartan and amlodipine peaks were separated with a proper resolution and also the degradents of hydrochlorothiazide were separating from three actives. Hence the method is optimized with a Isocratic program. As the concentration of amlodipine is very less as compared to telmisartan, so that to improve response, resolution and peak shapes the method was tried at different column temperatures. But the separation, response of peaks and peak shapes were satisfactory in the

adopted chromatographic conditions only. It indicated that the Isocratic method with 10% acetonitrile as organic modifier in mobile phase was successful in separating drugs and all chromatographic degradation products. Some of the trials were summarized as below.

Selection of Diluent (Extracting solvent)

As the tablets are film coated, and all the active drugs are stable in acidic conditions, the 0.1% orthophosphoric acid is added in order to open the film coating and to use the same assay method for quantification of uniformity of dosage units by HPLC.The trials were 0.1N HCl also but the peak shapes are not good in 0.1N HCl. As all the three actives Telmisartan, Amlodipine, & HCTZ are freely soluble in methanol, so it is used as the extracting solvent.

Selection of Stationary Phase

The stationary phases like C8 and C18 were tried but on C18 the peak shape for Telmisartan and the peak symmetry factor for hydrochlorothiazide is more than 1.7, the separation between Amlodipine and Telmisartan is not adequate. On the other hand the peak shapes for all the three components are good and all the three peaks are well separated from each other and their degradents. So that the column with C8 stationary phase is selected.

Selection of mobile phase buffer

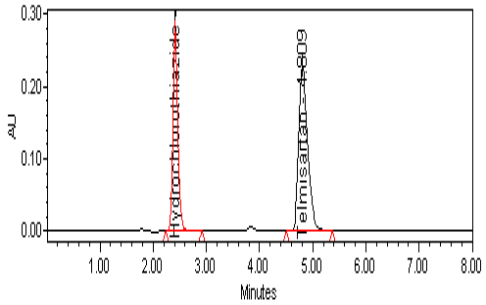
The buffers like Phosphate buffer With Buffer with pH 3.2, pH 7.5 and the perchlorate buffer with pH 2.4 and 3.2 were tried as mobile phase buffer. But with pH 3.2, pH 7.5 phosphate and per chlorate buffer with pH 3.2 the results were not enough good in terms of peak separation and peak shapes. On the other hand the perchlorate buffer with pH 2.4, the results were found satisfactory so that the mobile phase with pH 2.4 perchlorate buffer is finalized.

Finalization of Chromatographic conditions

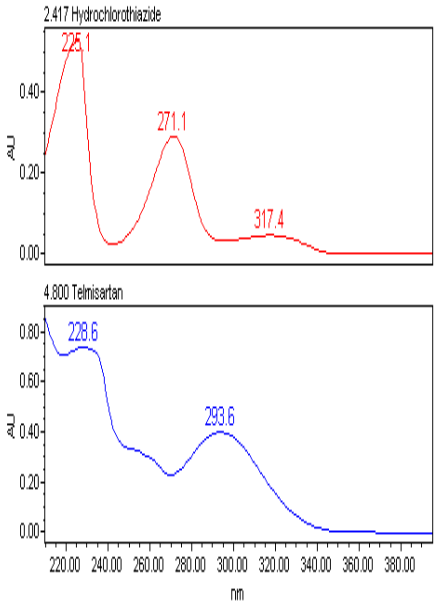
By considering all the experiments the chromatographic conditions are finalized. The chromatograph with finalized chromatographic condition s as follows

(1) Liquid chromatography equipped with PDA detector.Detection Wavelength 271nm for HCTZ and Telmisartan, 237 nm for Amlodipine.
(2) Column: X-Terra RP-8,150mm x 4.6mm,5 µm
(3) Column temperature : 25°C
(4) Flow rate: 1.0 mL per minute
(5) Injection volume:20 µL
(6) Run time: 8 minutes

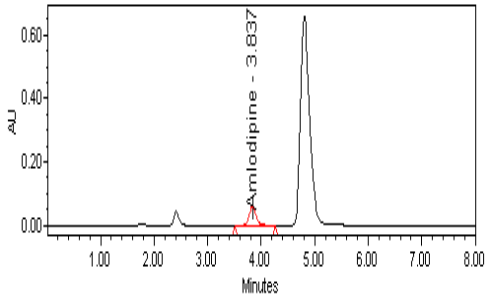
Further the analysis was performed for pharmaceutical dosage forms of both the label claim tablets (40 mg Telmisartan, 5 mg Amlodipine, 12.5 mg HCTZ and 20 mg Telmisartan, 2.5 mg Amlodipine, 6.25 mg HCTZ). The assay (% of active drug content present in the tablets with respect to its label claims) results for drug product, 99.8%, 99.5% of Telmisartan, 99.7%, 98.8% of Amlodipine and 100.1%, 99.8% of HCTZ, respectively. Analysis also performed (n = 3) separately for Telmisartan, Telmisartan and Hydrochlorothiazide tablets and Amlodipine besylate and Hydrochlorothiazide tablets. The assay results for Telmisartan were 99.5%, 99.7%, 99.9%. The assay results for Telmisartan and Hydrochlorothiazide were, 99.7%, 99.5% and 99.9% for Telmisartan and 99.3%, 98.5% and 99.1% for Hydrochlorothiazide. The assay results for Amlodipine besylate and Hydrochlorothiazide were, 99.5%, 99.7% and 98.7% for Amlodipine besylate and 99.8%, 98.5% and 99.3% for Hydrochlorothiazide. It indicated that, adopted HPLC method also can be used separately for assay estimation of Telmisartan tablets, simultaneous estimation of Telmisartan and Hydrochlorothiazide tablets and simultaneous estimation of Amlodipine besylate and Hydrochlorothiazide tablet.



Typical chromatogram for HCTZ and Telmisartan at 271nm



Typical chromatogram for Amlodipine at 237 nm



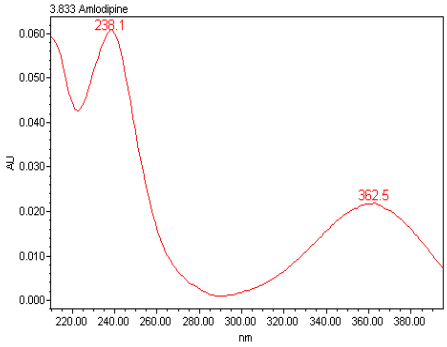


Fig. 2: Typical chromatogram of Telmisartan, Amlodipine besylate and Hydrochlorothiazide at 271 nm and 237 nm from drug product

METHOD VALIDATION

System suitability

In order to find the adequate peak separation (resolution) and repeatability of the proposed method, suitability parameters including retention factor, selectivity and asymmetry factor were investigated and the results were abridged in Table 1.

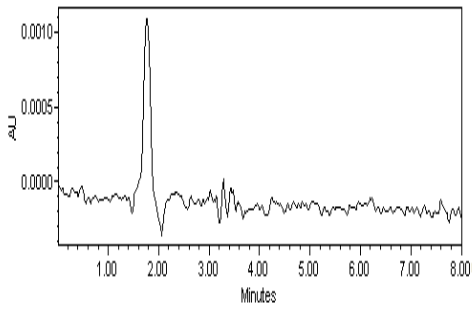
Specificity

The specificity of an analytical method is the ability of the method to determine the analyte response in the presence of additional components such as impurities, degradation products and matrix. The solution of analytical placebo (containing all the excipients except Telmisartan, Amlodipine, & HCTZ) was prepared according to the sample preparation procedure and injected. To identify the interference by these excipients, a mixture of inactive ingredients, standard solutions, and the commercial pharmaceutical preparations including Telmisartan, Amlodipine, and HCTZ were analyzed by the developed method.

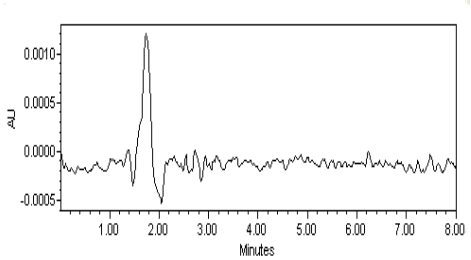
The specificity of the method was also evaluated to ensure there were no interference products resulting from forced degradation.

Placebo Interference

The placebo (excepients present in the tablet) sample were prepared as per the test method and analyzed in the HPLC. The Fig.3 shows there is no peaks at the retention time of the Telmisartan, Amlodipine, & HCTZ in the chromatograph indicates that there is no placebo interference.



Typical chromatogram for placebo at 271 nm



Typical chromatogram for placebo at 237 nm

Fig. 3: Typical chromatogram of Telmisartan, Amlodipine besylate and Hydrochlorothiazide tablets placebo blend at 271 nm and 237 nm from drug product

Forced degradation studies

Stress testing of a drug substance can help to identify the likely degradation products, which can help to establish the degradation pathways and the intrinsic stability of the molecule. All stress decomposition studies were performed at an initial drug concentration 0.64 mg mL−1 of Telmisartan, 0.08 mg mL−1 of Amlodipine, 0.2 mg mL−1 of HCTZ. The degradation conditions are selected on the basis of literature survey[13-17].

Linearity

Linearity solutions were prepared from stock solution at six concentration levels from 50 to 150% of analyte concentrations (16 to 48 µg mL−1 for T, 2 to 6 µg mL−1A and 5 to 15 µg mL−1 for H). The linear regression analysis of T, A, and H were constructed by plotting the peak area of the analytes (y) versus analytes concentration in (x) axis. The calibration curves were linear in the range of 16 to 48 µg mL−1 for T, 2 to 6 µg mL−1A and 5 to 15 µg mL−1 for H with a correlation coefficient of more than 0.999 for all the three drugs. The slope, Y-intercept and correlation coefficient were calculated and summarized in Table 3.

Precision

The precision of the assay method was evaluated by carrying out six independent assays of T, A and H (0.032 mg mL−1 of T, 0.004 mg mL−1 of A, 0.01 mg mL−1 of H) test samples against qualified reference standard. The percentage of RSD of six assay values was calculated. Different analyst from the same laboratory evaluated the intermediate precision of the method. The R.S.D. values of intra- and inter-day studies for T, A, and H confirming good precision of the method. (Table 4).

Accuracy

The accuracy of an analytical method expresses the nearness between the references value and found value. The accuracy of the method was evaluated in triplicate at three concentration levels, i.e. 50%, 100% and 150% of target test concentration (0.64 mg mL−1 of T, 0.08 mg mL−1 of A, 0.2 mg mL−1 of H) in tablets. The results obtained are shown in Table 5.

Solution Stability and Mobile Phase Stability

The solution stability of T, A and H was carried out by leaving the test solution in tightly capped volumetric flask at room temperature for 48 hrs. The same sample solution was assayed for a 24 hours interval up to the study period against freshly prepared standard solution of T, A and H. The mobile phase stability was also carried out by assaying the freshly prepared standard solution for 24 hours interval up to 48 hours. The mobile phase preparation was kept constant during the study period. The percentage RSD of assay of T, A and H was calculated for the study period during mobile phase and solution stability experiments. The % RSD of the assay of T, A, and H during solution stability and mobile phase experiments were within 1% and it indicates that both standard and test preparation and mobile phase were stable for 2 days on bench top at room temperature.

CONCLUSIONS

The established HPLC method proves to be simple, linear, precise, accurate and specific. The total runtime was 8 minutes within which three drugs and their degradation products were separated. The method was validated and shows satisfactory data for all the method validation parameters tested. The Developed method is stability indicating and can be used for simultaneous quantitative determination of the drugs T, A and H in presence of degradation products in stability by the industry. The adopted HPLC method also can be used separately for assay estimation of Telmisartan tablets, simultaneous estimation of Telmisartan and Hydrochlorothiazide tablets and simultaneous estimation of Amlodipine besylate and Hydrochlorothiazide tablets.

Table 1: System suitability parameters for Telmisartan, Amlodipine and HCTZ

System suitability parameters	Telmisartan	Amlodipine	HCTZ
Retention time (min) (Mean ± S.D., n = 5)	4.800 0.0018	3.837 0.0010	2.417 0.001
Repeatability of retention time; R.S.D. % (n = 5)	0.0460	0.0300	0.3342
Repeatability of peak area; R.S.D.% =(S.D. / mean) x 100	0.1501	0.5403	0.0752
Tailing factor (asymmetric factor)	1.2	1.2	1.4
USP plate count	34765	43314	2974

Table 2: Peak purity results of Telmisartan, Amlodipine and HCTZ

Stress condition	Purity angle			Purity threshold			Purity flag		
	T	A	H	T	A	H	T	A	H
Heat stress	0.070	1.118	0.114	0.538	4.580	1.140	No	No	No
Aqueous stress	0.057	0.902	0.131	0.499	3.973	1.384	No	No	No
Acid stress	0.064	1.108	0.108	0.493	4.301	1.125	No	No	No
Base stress	0.065	1.306	0.122	0.536	5.057	1.212	No	No	No
Peroxide stress	0.060	1.727	0.241	0.533	5.776	1.202	No	No	No

Table 3: Linearity

Analyte	Concentration range (µg/ml)	Correlation coefficient	slope	Intercept
Telmisartan	16.024 – 48.073	0.9999	6282.0	-888.486
Amlodipine	2.020 – 6.020	0.9996	10044.1	-14.5193
HCTZ	5.000 – 15.000	0.9998	13499.7	-827.248

Table 4: Intra-day and inter-day Precision results of T, A and H from tablets (n = 6)

	Active name	Prep-1	Prep-2	Prep-3	Prep-4	Prep-5	Prep-6	%RSD	%Mean
Intra-day precision	T	100.2	99.7	100.2	100.4	99.4	100.6	0.45	100.1
	A	99.0	99.3	98.6	99.4	101.2	99.2	0.90	99.5
	H	98.9	98.8	99.3	99.9	101.2	99.2	0.54	99.5
Inter day precision	T	98.0	100.0	100.9	99.0	99.3	100.4	1.04	99.6
	A	99.9	99.4	98.4	99.4	99.8	99.4	0.53	99.4
	H	100.0	100.6	100.6	99.7	100.0	99.3	0.51	100.0

Table 5: Accuracy Results of T, A and H from tablets (n = 6)

Analyte	Recovery level (%)	Actual concentration (µg/ml)	Found concentration (µg/ml)	% Recovery	%RSD
T	50	320	321.86±0.567	100.58	0.564
	100	640	640.51±0.449	100.08	0.449
	960	960	964.51±0.638	100.47	0.635
A	50	40	39.99±0.480	99.97	0.480
	100	80	79.56±0.903	99.45	0.908
	150	120	119.78±0.194	99.82	0.194
H	50	1000	993.2±0.492	99.32	0.495
	100	2000	198.94±0.547	99.47	0.549
	150	3000	298.50±0.297	99.50	0.298

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