RESEARCH ARTICLE

INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

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SIMULTANEOUS ESTIMATION OF OLMESARTAN MEDOXOMIL AND HYDROCHLOROTHIAZIDE BY VALIDATED REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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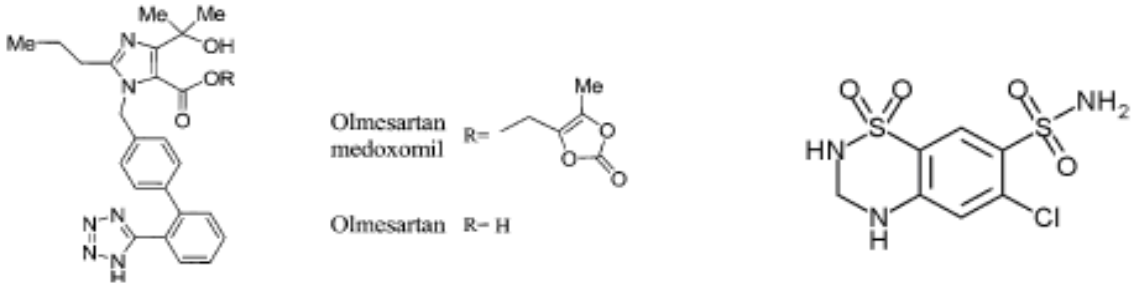
Accepted Date: 28/12/2011 **Publish Date: 27/02/2012**

Abstract: Olmesartan medoxomil belongs to a group of angiotensin II receptor antagonist, in combination with Hydrochlorothiazide it act as a selective and very effective antihypertensive agent, which has been launched in Indian market recently. The ultimate goal of this work was to develop and validate a rapid, specific reversed- phase HPLC method for assaying Olmesartan medoxomil and Hydrochlorothiazide simultaneously. The assay involved an isocratic elution of these two components on Inertsil-phenyl column (25cm X 4.6mm, 5µm) using a mobile phase composition of Buffer: Acetonitirile (480:520) and pH adjusted to 3.0 with dilute orthophosphoric acid. The flow rate was 1.0 mL/min and the analytes monitored at 257nm. Separation was completed within 10 min. Calibration curves were linear with coefficient correlation between 0.99 - 1.0 over a concentration range of 24 to 74 µg/mL of Olmesartan medoxomil and 15 to 47 µg/mL for Hydrochlorothiazide respectively. The developed method was successfully applied to estimate the amount of both the component simultaneously.

Key words: Olmesartan medoxomil, Hydrochlorothiazide, RP-HPLC

INTRODUCTION

Olmy-H fixed dose combination tablet contains Olmesartan medoxomil and Hydrochlorothiazide as Antihypertensive agent[1].



Molecular Structure I - Olmesartan medoximil & II – Hydrochlorothiazide

Olmesartan medoxomil is a prodrug, which, after ingestion, liberates the only active metabolite, Olmesartan. It is a competitive and selective AII type 1 receptor antagonist that is used alone or with other Antihypertensive agents to treat hypertension[2, 3]. The hydrolysis of olmesartan medoxomil occurs readily by the action of esterases, which are present abundantly in the gastrointestinal tract, liver and plasma. Olmesartan blocks the vasoconstrictor effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in vascular smooth muscle[4, 5]. The Hydrochlorothiazide is a thiazide diuretic. Thiazides affect the renal tubular mechanisms of electrolyte reabsorption, directly increasing excretion of sodium and chloride. Indirectly, the diuretic action of hydrochlorothiazide reduces plasma volume, with consequent increases in plasma renin activity, increases in aldosterone secretion. The renin-aldosterone link is mediated by angiotensin II, so co-administration of an angiotensin II receptor antagonist tends to reverse the potassium loss associated with these diuretics. Fixed dose combination of both these component in one tablet is 20 milligrams of olmesartan/12.5 milligrams of hydrochlorothiazide once daily[6, 7].

Literature survey did not reveal any reported method for the analysis of Olmesartan medoxomil neither in combination with any other drug nor alone. Even it is not official in IP, BP, and USP. But various analytical methods for quantitative determination of

Hydrochlorothiazide[8] in pharmaceutical formulations has been reported in literature like Fluorimetric[9], phosphometric[10], UV spectrophotometry[11, 12], electrochemical detection and high-performance liquid chromatography (HPLC)[13, 14, 15]. The assay for API and tablet form of HCTZ has been described in USP[16]. The non-availability of UV and HPLC method until now for the simultaneous analysis of these components made it worthwhile objective to pursue the present research work[17, 18]. Therefore, in the proposed work, a successful attempt has been made to develop analytical method with due consideration of accuracy, sensitivity, rapidity, economy and simplicity.

MATERIAL AND METHODS

Chemicals and Materials: MSN and Ipca Laboratories supplied Olmesartan medoxomil and Hydrochlorothiazide respectively. Acetonitrile (HPLC grade) and Sodium dihydrogen orthophosphate were purchased from Spectrochem and E-Merck Limited respectively. In-house purified water (USPgrade) was used throughout the study.

Instrumentation:

The chromatographic separations were performed using Shimadzu LC 2010C integrated system equipped with quaternary gradient pump, 2010C UV-VIS detector, 2010C Column Oven and 2010C programmable auto sampler controlled by CLASS-VP software. The Inertsil-Phenyl (250X4.6 mm), 5µm was used as a stationary phase. The system suitability result displayed in Table 1 was evaluated throughout the study. Electrolab TDT-08L autosampler dissolution apparatus were used for comparative dissolution study.

HPLC condition:

Detector	257 nm for assay
Injection volume	20 µl
Flow rate	1.0 ml/min
Temperature	30˚ C
Run time	10 min
Mobile phase	Buffer: Acetonitrile: Methanol (480:520)
Diluent	Acetonitrile: Methanol (1:1)

Buffer preparation:

Weighs 2.9g of sodium dihydrogen orthophosphate in to 1000 ml of Milli Q water and adjust pH 3.0 with orthophosphoric acid. Filtered it through 0.45 µ HVLP nylon filter.

For Dissolution:

Standard preparation:

Standard stock solutions were prepared in diluent and further for second dilution, dilute it with dissolution media and then dilute 5.0 ml of this to 10.0ml with buffer

solution pH 1.2 to make final concentration Olmesartan medoxomil 11 µg and Hydrochlorothiazide 7 µg respectively.

Sample preparation:

Place one tablet each in six different vessels and operates the instrument as mentioned above. Withdraw about 10 ml of the sample solution, filter and dilute 5.0 ml of this to make the final concentration. The samples withdrawn above were analyzed on HPLC.

RESULTS

The detection wavelength of 257 nm was chosen in order to achieve a good sensitivity for quantitative determination of Olmesartan medoxomil and Hydrochlorothiazide in tablet dosage. The mobile phase consisting of Buffer: Acetonitirile (480:520) (pH 3.0) with orthophosphoric acid helped to produce well resolved chromatogram at ambient temperature using a flow rate of 1.0 ml/min and a runtime of 10 min, Hydrochlorothiazide elutes at first and then Olmesartan medoxomil shown in the chromatogram, Figure 1 and 2 which illustrate the separation of both active ingredients. The isocratic program throughout HPLC method was adopted to analyze both components in a short single run time. The proposed method was simple and economic, which did not require extraction or separation of the analyte.

The specification of the dissolution method was set as per considering the solubility, permeability, dissolution and pharmacokinetics of the drug substance. A model-independent method was used for the comparison of in vitro dissolution profiles. In this study f_1 (difference factor) and f_2 (similarity factor) was calculated. The use of these factors was also recommended for dissolution profile comparison in the FDA's guides for industry.

Linearity:

The plot of peak area responses against concentration was shown in Figure 3 and 4. It can be seen that plot is linear over the concentration range of 24 to 74 µg/ml and 15 to 47 µg/ml for Olmesartan medoxomil and Hydrochlorothiazide respectively with a correlation coefficient (r^2) 0.9999. The results of linearity, limit of detection and limit of quantification were presented in Table 2.

Specificity:

There was no interference from sample placebo and peak purity of Olmesartan medoxomil and Hydrochlorothiazide was 0.99930 and 0.99892 respectively. It showed that developed analytical method was specific for its intended purpose.

Standard and sample solution stability:

Standard and sample solution stability was evaluated at room temperature for 24 hr. The relative standard deviation was found below 2.0%. It showed that both standard and sample solution was stable up to 24 hr at room temperature.

Method precision:

The relative standard deviation for six replicate injections was less than 1.0 %, which met the acceptance criteria established for the method. The results obtained were presented in Table 3.

Accuracy/recovery:

The data presented in Table 4 show excellent recoveries at all levels. The average recoveries for triplicate determinations at 50, 100, and 150% levels were with in the acceptable criteria. Excellent recovery and low relative standard deviation value showed that the method was suitably accurate for potency assay of Olmesartan Medoxomil and Hydrochlorothiazide simultaneously in the drug substances.

Method robustness:

The method was found to be robust, as small but deliberate changes in the method parameters have no detrimental effect on the method performance as shown in Table 5. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was robust.

Method ruggedness:

Ruggedness test was determined between two different analysts, instruments and columns. The value of percentage RSD was below 2.0%, showed ruggedness of developed analytical method. The results of ruggedness were presented in Table 6.

DISCUSSION

Considering the efficiency of HPLC, attempt has been made to develop simple, accurate, precise, rapid and economic method for simultaneous estimation of Olmesartan medoxomil and Hydrochlorothiazide in a tablet dosage form. Thus, method described enables to the quantification of Olmesartan medoxomil and Hydrochlorothiazide. The advantages lie in the simplicity of sample preparation and the low costs of reagents used. It has been found that this method is also applicable for Inertsil $C_{8\ and}$ C_{18} column (250 X 4.6 mm), 5µm. The contribution of another important factor was its LOD. Results from statistical analysis of the experimental results were indicative of satisfactory precision and reproducibility. Hence, this HPLC method can be used for analysis of commercial formulation provides useful information for studies.

ACKNOWLEDGEMENTS

The authors are thankful to Zydus Cadila Healthcare Limited, Ahmedabad, India for providing reference standards and all facilities to complete this research work.

Table 1

System Suitability and System Precision

Compound	Retention time (Mean ± SEM)	n	k'	R	T	α
HCTZ	4.078 ± 0.0020	4448.75	0.4561	-	1.32	-
Olmesartan Medoxomil	7.290 ± 0.0024	3812.24	0.7882	9.18	1.35	1.728

HCTZ = Hydrochlorothiazide, n= Theoretical plates, k'= Capacity Factor, R= Resolution,

T= Asymmetry, α = Selectivity

Table 2

Characteristics of the Analytical Method Derived from the Standard Calibration Curve

Compound	**LOD µg/ml**	**LOQ µg/ml n=(5)**	**Linearity µg/mL**	**Correlation co-efficient σ**	**Residual standard regression S**	**Slope of regression**
HCTZ	0.007	0.021	15-47	099993	7815.145	34502.838
Olmesartan Medoxomil	0.012	0.041	24-74	0.99997	10653.250	46087.139

HCTZ = Hydrochlorothiazide

LOD= Limit of detection

LOQ= Limit of quantification

Table 3

Method Precision

Compound	Concentration µg/ml (n=6)	Retention time Mean ± SEM (n=6)	% Assay Mean ± SEM (n=6)	% RSD of Assay
HCTZ	31.5	4.10 ± 0.0000	99.66 ± 0.272	0.7
Olmesartan Medoxomil	50	7.20 ± 0.0000	96.01 ± 0.195	0.5

HCTZ = Hydrochlorothiazide

Table 4

Method Accuracy for HCTZ

Level	Drug Added (mg)	Drug Recovered (mg)	% Assay (Mean ± SEM) (n=3)	% RSD of Assay (n=3)
50 %	32.00	32.38	101.4 ± 0.057	0.1
100 %	62.83	63.50	101.3 ± 0.120	0.2
150 %	94.00	95.09	101.4 ± 0.152	0.3

HCTZ = Hydrochlorothiazide

Method Accuracy for Olmesartan Medoxomil

Level	Drug Added (mg)	Drug Recovered (mg)	% Assay (Mean ± SEM) (n=3)	% RSD of Assay (n=3)
50 %	50.43	50.46	100.5 ± 0.378	0.7
100 %	100.36	100.55	100.6 ± 0.404	0.7
150 %	150.06	150.82	100.9 ± 0.264	0.5

Table 5

Method Robustness

Compound	**% RSD in Normal and Changed condition (n=5)**		
Temperature	% RSD Normal	% RSD (-5°C)	% RSD (+5°C)
HCTZ	0.08	0.07	0.05
Olmesartan Medoxomil	0.06	0.09	0.04

Compound	% RSD in Normal and Changed condition (n=5)		
Flow Rate	% RSD Normal	% RSD (-10%)	% RSD (+10%)
HCTZ	0.08	0.04	0.06
Olmesartan Medoxomil	0.06	0.10	0.04

Compound	% RSD in Normal and Changed condition (n=5)		
pH	% RSD Normal	% RSD (-0.2 unit)	% RSD (+0.2 unit)
HCTZ	0.08	0.06	0.06
Olmesartan Medoxomil	0.06	0.05	0.06

Compound	% RSD in Normal and Changed condition (n=5)		
Mobile phase ratio	% RSD Normal	% RSD (-2%)	% RSD (+2%)
HCTZ	0.08	0.06	0.06
Olmesartan Medoxomil	0.06	0.04	0.06

HCTZ = Hydrochlorothiazide

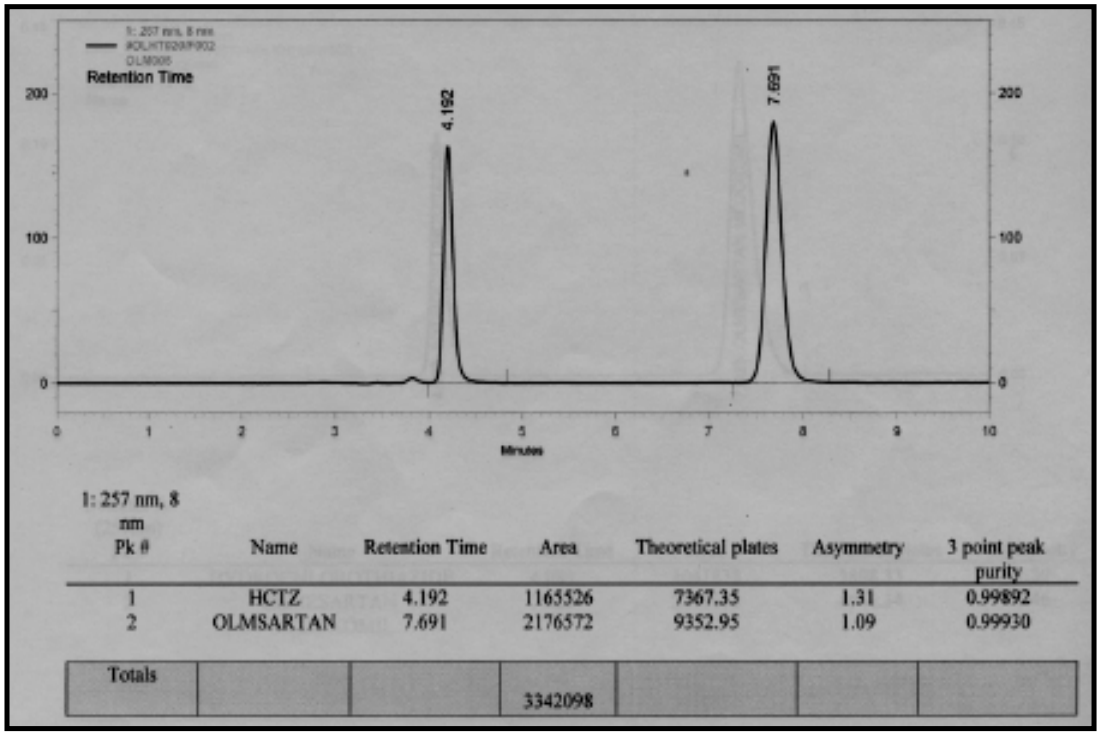
Table 6

Method Ruggedness

Compound	% Assay Mean ± SEM (n=6)	% RSD of Assay (n=6)
Day 1	Analyst-1, Instrument-1 & Column-1	
HCTZ	99.66 ± 0.272	0.7
Olmesartan Medoxomil	96.01 ± 0.195	0.5

Compound	% Assay Mean ± SEM (n=6)	% RSD of Assay (n=6)
Day 2	Analyst-2, Instrument-2 & Column-2	
HCTZ	102.23 ± 0.261	0.6
Olmesartan Medoxomil	95.98 ± 0.214	0.6

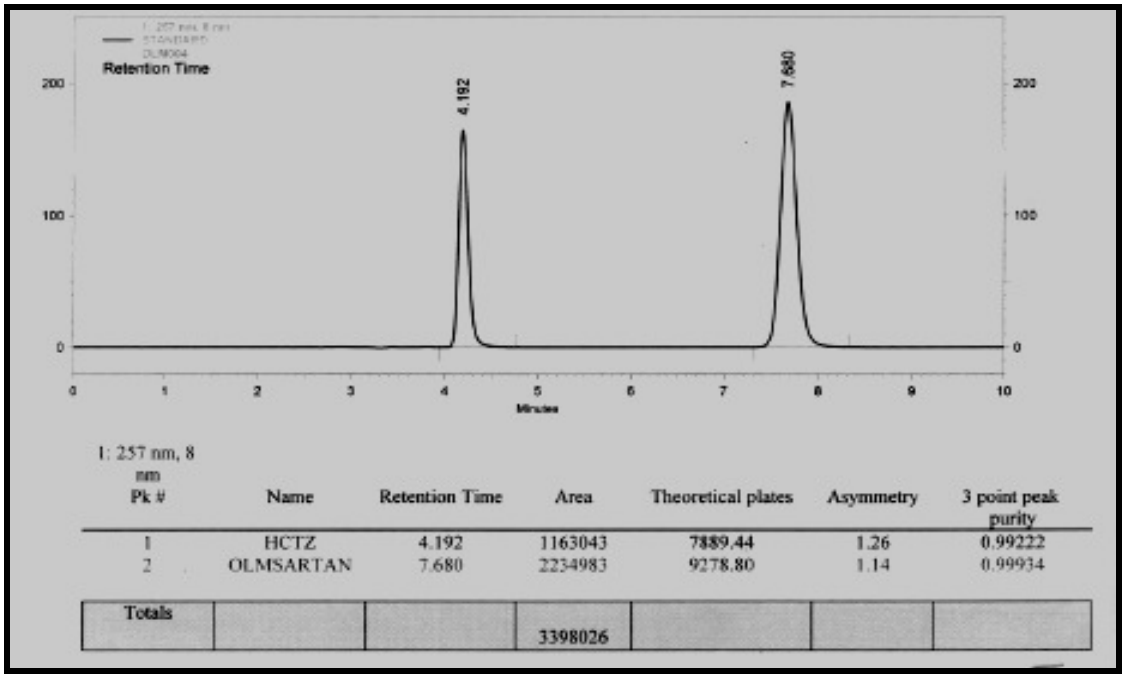
HCTZ = Hydrochlorothiazide



1: 257 nm, 8 nm

Pk #	Name	Retention Time	Area	Theoretical plates	Asymmetry	3 point peak purity
1	HCTZ	4.192	1165526	7367.35	1.31	0.99892
2	OLMSARTAN	7.691	2176572	9352.95	1.09	0.99930
Totals			3342098			

Figure 1 Chromatogram for Test solution.



1: 257 nm, 8 nm

Pk #	Name	Retention Time	Area	Theoretical plates	Asymmetry	3 point peak purity
1	HCTZ	4.192	1163043	7889.44	1.26	0.99222
2	OLMSARTAN	7.680	2234983	9278.80	1.14	0.99934
Totals			3398026			

Figure 2 Chromatogram for Standard solution.

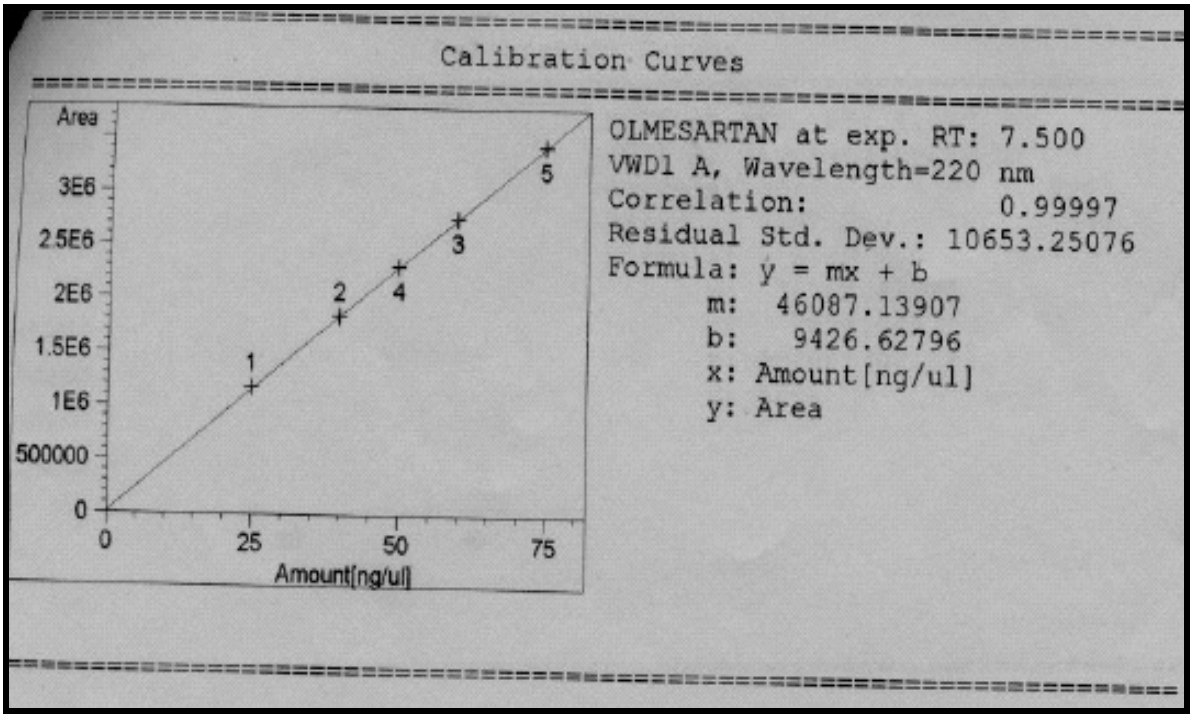


Figure 3 Linear calibration curve for Olmesartan medoxomil.

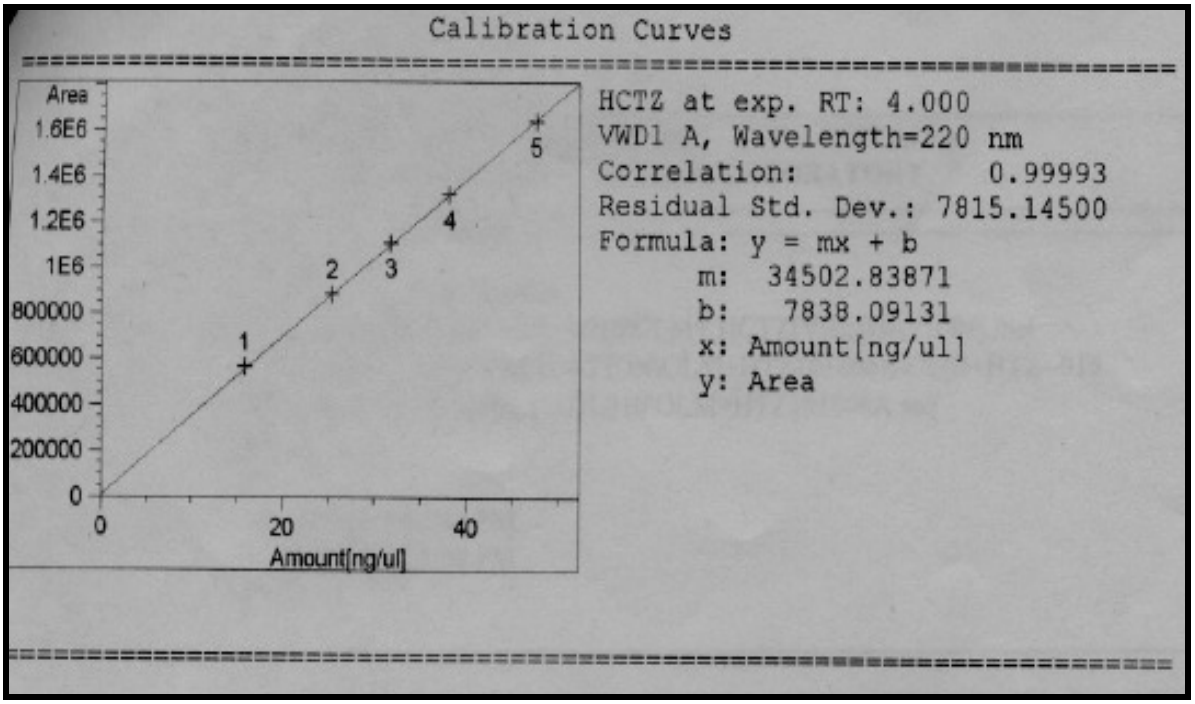


Figure 4 Linear calibration curve for Hydrochlorothiazide.

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