Estimation Of Repaglinide –Rp Hplc & Uv – Methods

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***Abstract*—Then goal of the current work was to use UV spectrophotometry and High-Performance Liquid Chromatography (HPLC) to develop and evaluate straightforward, accurate, and precise techniques for estimating repaglinide in oral solid dose forms. An oral hypoglycemic medication called repaglinide is frequently recommended to treat type 2 diabetes. For the measurement of repaglinide, a straightforward, quick, cost-effective, accurate, and exact stability indicating RP-HPLC technique has been devised. For the determination of repaglinide, a RP HPLC technique was created. With an isocratic program and a mobile phase consisting of methanol: potassium dihydrogen phosphate (60:40% v/v) at a flow rate of 1.2 ml/min, the separation was accomplished using a Shimadzu C18 column (4.6 x 250 mm, 20µl). The detection was done at 240nm Repaglinide was shown to have a retention time of 5.14 minutes. There was linearity for Repaglinide 100–500 µg/ml. The UV spectrophotometric technique was created using repaglinide at 287 nm in the proper solvent solution. The linearity, accuracy, precision, specialization, and limit of quantification of the method have all been verified. Repaglinide in oral solid dose formulations can be routinely analyzed for quality control using the suggested analytical techniques.**

***Index Terms*—Repaglinide, HPLC Method, UV Method, Validation, second order derivative spectroscopic method.**

I. INTRODUCTION

Repaglinide is an oral hypoglycemic medication that is mainly used to treat Type 2 Diabetes Mellitus (T2DM). It is a member of the class of Meglitinide derivatives. By blocking ATP-dependent potassium channels, it stimulates the release of insulin from pancreatic β-cells. Repaglinide is a commonly recommended medication in diabetes treatment because of its quick start of action and brief duration, which makes it very helpful in reducing postprandial hyperglycemia.

Quality control and quality assurance of pharmaceutical dosage forms require precise, accurate, and validated analytical methods for drug estimation. Among the various analytical techniques, UV spectrophotometry and high-performance liquid chromatography (HPLC) are extensively employed due to their sensitivity, accuracy, and reproducibility.

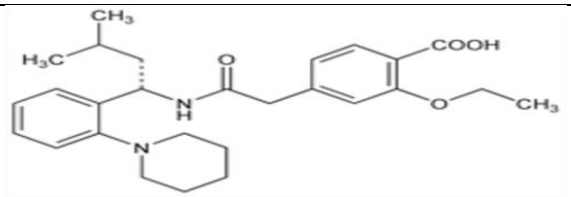
UV Spectrophotometry is an efficient, economical, and swift method commonly employed in standard quality control for the assessment of pharmaceuticals. This technique relies on the principle of ultraviolet light absorption by the drug molecule at a designated wavelength, facilitating quantitative analysis in drug formulations.

HPLC is regarded as a more sophisticated and dependable technique, offering high selectivity, accuracy, and precision. It facilitates the separation of

the drug from its excipients, degradation products, and impurities, thus ensuring precise quantification.

The establishment and verification of these methods are crucial according to the guidelines set forth by the International Conference on Harmonisation (ICH), guaranteeing their appropriateness for standard quality control of repaglinide in oral solid dosage forms (tablets, capsules). Consequently, the assessment of repaglinide through both UV spectrophotometric and HPLC techniques offers a comparative analytical strategy, merging the benefits of simplicity and cost-effectiveness (UV) with the precision and accuracy (HPLC). This research will not only enhance dependable quality control but also aid in regulatory compliance, stability investigations, and pharmaceutical studies related to repaglinide formulations.

DRUG PROFILE:

S.NO	CONTENT	EFFECTIVE
1	Structure	
1	Molecular Formula	C27 H36N2O4
2	Molecular Weight	452.6g/mol-1
3	Chemical Name	2-ethoxy-4-[2-[[(1S)-3-methyl-1-(2-piperidin-1-ylphenyl)butyl]amino]-2-oxoethyl]benzoic acid
4	Melting point	126 to 128 °C
5	Physical Characteristics	Crystal from ethanol : water (2:1) White Crystalline
6	Pharmacodynamic	Repaglinide lowers blood glucose by stimulating the release of insulin from the beta islet cells of the pancreas. It achieves this by closing ATP-dependent potassium channels in the membrane of the beta cells. This depolarizes the beta cells, opening the cells' calcium channels, and the resulting calcium influx induces insulin secretion.
7	INDICATIONS	Absorption: Peak plasma concentrations are observed within 1 hour (range 0.5-1.4 hours. bioavailability is approximately 56%. and plasma insulin for 4-6 hours. 2 mg dose of repaglinide the area under the curve (AUC) is 18.0 - 18.7 (ng/mL/h)^3.
		Distribution: IV administration in 31 L
		Protein- binding More than 98% - albumin- α1-acid glycoprotein)
		Biotranformation: The Repaglinide undergoes rapid metabolism primarily through oxidation and dealkylation processes mediated by the CYP450 enzymes 3A4 and 2C9, resulting in the formation of a dicarboxylic acid derivative known as M2. This compound can further be oxidized to yield an aromatic amine derivative, referred to as M1. Additionally, the carboxylic acid group of repaglinide is subject to glucuronidation, producing an acyl glucuronide metabolite designated as M7. Various other unidentified metabolites have

		also been observed; however, it is important to note that these metabolites do not exhibit significant hypoglycemic activity..
8	ELIMINATION	Excreation:: Nintey percentage excreation The drug is excreted primarily in feces, with less than 2% remaining as unchanged substance, while 8% is eliminated through urine, of which only 0.1% is in its unchanged form.
9	LIFE-Half	One Hours
10	Screening	IV admin- 33-38 L/hour
11	Toxicity	More than LD501 g/kg (mice)

II. MATERIALS AND METHODS

Instrumentation:

The device utilized in the current research was the The Shimadzu UV-1601 is a double beam UV/Visible spectrophotometer designed for precise optical measurements. All measurements were conducted using an electronic balance (Model Shimadzu AUX-220). RP-HPLC was executed on the Shimadzu HPLC system featuring the LC-20AT pump.

Reagents and Chemicals:

The pharmaceutical dosage form used in this study was Repaglinide pure sample were purchase from paris danker pvt Ltd., Chennai.

1.Methnol (HPLC Grade)

2.Distilled Water (HPLC Grade)

3.Potassium Dihydrogen phosphate from Analytical Grade)

ESTIMATION OF REPAGLINIDE BY SUITABLE ANALYTICAL TECHNIQUES:

- CHROMATOGRAPHIC METHOD

1) RP-HPLC

- SPECTROPHOTOMETRIC METHOD

1) UV –Spectrophotometric.
2) Second Derivatives UV-Spectroscopy

METHODS For HPLC – (RP-HPC)

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Step1: Preparation of mobile phase:

Preparation of potassium dihydrogen phosphate buffer.

Dissolve 100g of potassium dihydrogen phosphate in 800ml of distilled water, then adjust the pH to 2.5 using boric acid, and finally, make the total volume up to 1000ml with water.

MOBILE PHASE

Methanol and a 0.1M potassium dihydrogen phosphate buffer were combined in a 60:40 (v/v) ratio and subsequently filtered using a 0.45µ membrane filter.

STOCK SOLUTION

A precisely measured 50mg of Repaglinide was dissolved in methanol and diluted to a final volume of 100ml with the same solvent, resulting in a stock solution with a concentration of 500µg/ml.

LINEARITY OF DETECTOR RESPONSE

A stock solution of repaglinide was diluted with methanol to produce a range of known concentrations: 100, 200, 300, 400, and 500 µg/ml. A consistent volume of 20 µl from each concentration is injected into the system at regular intervals of 10 minutes. The retention time for repaglinide was recorded at 5.14 minutes, which reflects the duration required for the compound to traverse the system.

METHOD FOR DETERMINING THE AMOUNT OF REPAGLINIDE IN TABLETS

Twenty tablets were weighed and powdered, followed by the precise weighing of a quantity equivalent to 10mg of Repaglinide. This was subsequently dissolved in menthol within a 50ml standard flask, sonicated, and filtered through Whatman filter paper. Finally, the solution was adjusted to the mark with methanol. A 20µl aliquot of the prepared solution was injected in triplicate into the chromatographic system at 10-minute intervals to ensure complete elution. The chromatograms were recorded, and the peak area of the sample was utilized to determine the amount of Repaglinide. The quantity of Repaglinide in each tablet is calculated using the following.

$$\text{Amount of drug in each tablet} = \frac{\text{Peak area of sample} \times \text{Concentration of std} \times \text{Dilution factor} \times \text{Average weight}}{\text{Peak area of std} \times \text{Weight taken}}$$

ANALYTICAL METHOD VALIDATION

RECOVERY STUDIES:

These evaluate the precision and specificity of an analytical technique by determining the percentage recovery of a known amount of standard drug incorporated into a pre-analyzed sample. The equation for percentage recovery, along with a sample data table, is included.

$$\%\text{Recovery} = \frac{(\text{Amount of drug in sample After addition of standard}) - (\text{Amount of drug in sample before addition of std})}{\text{Amount of standard added}} \times 100$$

STABILITY OF SAMPLE SOLUTION:

The sample solution remained stable for a minimum of 24 hours, as injections conducted after this duration exhibited no significant alterations in retention time, peak area, or any other pertinent parameters.

SYSTEM STABILITY PARAMETERS:

These assessments are essential for liquid chromatographic systems to guarantee the consistency and suitability of the system for analysis, taking into account the equipment, analytical procedures, and samples. System suitability pertains to a collection of tests and standards employed to confirm that an analytical system, especially in chromatography, is operating properly and yielding dependable results.

UV VISIBLE SPECTROPHOTOMETRY

Establishment for optimum levels of various Parameters:

- Absorption
- Beers Concentration
- Estimation of Analytical Dosage form

PREPARATION OF STANDARD STOCK SOLUTION:

A standard stock solution of the analyte was created by dissolving a sufficient quantity of the drug in methanol, which was then adjusted to the desired volume with methanol. These solutions were utilized for the determination of various parameters.

ABSORPTION MAXIMUM: Were standard stock solution for appropriately diluted in methanol to achieve a concentration of 20 µg/ml. The solution was scanned in the UV region ranging from 200 to 400 nm, using methanol as a blank. It was observed that Repaglinide displayed a pronounced maximum absorption at approximately 287 nm.

BEER'S CONCENTRATION:

The standard solution of Repaglinide was appropriately diluted to produce varying concentrations of 20, 30, 40, 50, 60, 70, and 80 µg/ml. The absorbance was measured at approximately 287 nm and plotted against the drug concentration.

CALIBRATION GRAPH:

A graph depicting absorbance versus concentration was created, revealing that linearity exists within the range of 20 to 80 µg/ml.

DETERMINATION OF REPAGLINIDE IN ORAL SOLID DOSAGE FORM:

The mean weight of 20 tablets was assessed and subsequently powdered. An aliquot of powder corresponding to 2mg of Repaglinide was precisely weighed and placed into a 50ml standard flask. Methanol was introduced to dissolve the powder, and the volume was adjusted with methanol before filtering, resulting in a concentration of 40 µg/ml. The absorbance of this solution was recorded at 287nm, using methanol as a blank. The quantity of Repaglinide in each tablet is determined using the formula provided.

$$\text{Amount of drug in each tablet} = \frac{\text{ABSTest} \times \text{Concentration of std} \times \text{Dilution factor} \times \text{Average weight}}{\text{ABSTest} \times \text{Weight taken}}$$

RECOVERY STUDIES:

In order to evaluate the accuracy, precision, and reproducibility of the proposed method, recovery experiments were conducted by introducing a required amount of standard drug into a pre-analyzed sample, followed by reanalyzing the contents using the proposed method. The formula for calculating recovery of percentage was also provided.

$$\%\text{Recovery} = \frac{(\text{Quantity of drug in sample After addition of standard}) - (\text{Amount of drug in sample before addition of std})}{\text{Quantity of Std. added}} \times 100$$

SECOND ODER DERIVATIVE UV SPECTROSCOPY:

PREPARATION OF STOCK SOLUTION:

A standard stock solution of Repaglinide was created by dissolving a sufficient amount of Repaglinide RS in methanol and adjusting the volume with methanol.

LINEARITY AND CALIBRATION CURVE:

A stock solution is appropriately diluted with methanol to create a series of concentrations at 20, 30, 40, 50, 60, 70, and 80 µg/ml of Repaglinide. The aforementioned solutions were scanned within the wavelength range of 200-400 nm, and the resulting spectra were derivatized to obtain second-order derivative spectra. The amplitude was measured in millimeters. A calibration curve was established by plotting amplitude against concentration.

DETERMINATION OF REPAGLINIDE IN TABLET FORMULATION:

Twenty tablets were precisely weighed and ground into a powder. An amount corresponding to 10mg of Repaglinide was accurately measured, placed into a beaker, and dissolved in methanol. The resulting mixture was sonicated for 10 minutes, then filtered through Whatman filter paper No.1 into a 25 ml standard flask, and the volume was adjusted with methanol. Suitable aliquots were analyzed by scanning within the wavelength range of 200-400nm, and the resulting spectrum when derivative to obtain second-order derivative spectrum. The amplitude was scaled in nm. The quantity of Repaglinide in each tablet was determined using the calibration curve and applying the following formula.

$$\text{Amount of drug in each tablet} = \frac{\text{Concentration of std} \times \text{Dilution factor} \times \text{Average weight}}{\text{Weight taken}}$$

RECOVERY STUDIES:

In order to evaluate the correctness, exactness, and consistency of the suggested technique, retrieval experiments were conducted by introducing a known quantity of a standard drug into a pre-analyzed sample formulation, and the total amount was assessed using the proposed method. The formulas for calculating percentage recovery are included.

$$\%\text{Recovery} = \frac{(\text{Amount of drug in sample After addition of standard}) - (\text{Amount of drug in sample before addition of std})}{\text{Amount of standard added}} \times 100$$

VALIDATION FOR REPAGLINIDE BY ANALYTICAL METHOD

Validation of an analytical method is the process by which it is established, by laboratory studies.

- ✓ RP-HPLC
- ✓ UV-SPECTROPHOTOMETRIC
- ✓ UV- SPECTROPHOTOMETRIC SECOND DERIVATIVE

ACCURACY:

The precision of an analytical technique refers to how closely the test results obtained by the method align with the true value. This is frequently expressed as the percentage of recovery achieved by the assay of known quantities of the analyte.

PRECISION

Where accuracy of an analytical method pertains to the degree to which the results produced by the technique correspond with the actual amount. This is often represented as the recovery of percentage attained through the assay of specified amounts of the analyte.

LINEARITY:

A linearity of an analytical method refers to its capacity to yield test results that are either directly proportional or can be adjusted to be proportional through a clearly defined statistical transformation relative to the concentration of the analyte in a sample within a specified range. This is typically represented by the around the slope of the regression line, which is computed based on a recognized mathematical relationship.

RUGGEDNES:

Ruggedness refers to the consistency of test results when conducted under standard, anticipated operational conditions across different laboratories and among various analysts.

RANGE:

A range for determination method refers to in-between defined by the higher and lower levels of analysis, which are estimated with precision, accuracy & linearity when utilizing the method for a specified range (expressed in percent or parts per million) derived from the analytical method.

SENSITIVITY

Were sensitivity of the analytical method or instrument refers to the ratio of the change in response relative to the change in the quantity of concentration that is measured.

LIMIT OF DETECTION

The limit of detection refers to the minimum concentration of an analyte in a sample that can be identified, although it does not imply the ability to quantify it, under the specified experimental conditions.

LIMIT OF QUANTIFICATION

The limit of quantification refers to the low quantification of an analyte in a sample that can be accurately and precisely scaled under specified experimental conditions. This limit is represented as the concentration of the analyte (for example, percentage or parts per million) within the sample.

RESULTS & DISCUSSION FOR REPAGLINIDE BY ANALYTICAL METHOD

RP-HPLC

A system suitability test was conducted on a representative chromatogram to evaluate various parameters, including column efficiency, resolution, and peak tailing. The linearity of Repaglinide was determined by plotting a graph of the peak area of standard solutions against concentration; it were found to be linear within the range of 100 to 500 mcg/ml. The retention time was recorded at 5.14 minutes. A wavelength of 240 nm was selected based on the UV scanning of Repaglinide. The mobile phase, consisting of a buffer solution, potassium dihydrogen phosphate, and methanol mixed in a ratio of 40:60 (V/V), was deemed ideal for the analysis of Repaglinide, with the concentration of Repaglinide in the oral dosage form falling within acceptable limits. The precision of the method was assessed by performing five injections of the standard, with very low relative standard deviation (RSD) values indicating good precision. The reproducibility and reliability of the method were evaluated through recovery studies, which yielded favorable results.

UV SPECTROSCOPY METHOD

The assessment of Repaglinide in oral solid dosage forms using the UV spectrophotometric method is straightforward, precise, and swift. The percentage deviation and the coefficient of variation are acceptably low, and the recovery rate is nearly indicative of the accuracy and reproducibility of the suggested approaching.

SECOND DERIVATIVE SPECTROSCOPY

Then suggested method of second derivative spectroscopy has proven to be straightforward and precise for estimating Repaglinide in solid oral dosage forms without any interference. The method's reliability and reproducibility were confirmed by an almost complete recovery rate of nearly 100%. Additionally, this approach does not require extensive

calculations, making it a rapid process.

SUMMARY:

- The contemporary instrumental techniques offer:
- Outstanding separation capabilities enable precise measurement of extremely low concentrations of substances within intricate mixtures:
- Analysts are constantly seeking a swift and precise method for analyzing drugs that can be integrated into routine analysis; this current study includes:

1) Simple
2) Precise

Rapid and accurate methods of estimation of Repaglinide in the Oral solid dosage from(Tablet)

1) Reverse Phase HPLC method
2) UV spectrophotometric method

Derivative spectrophotometric method

III. CONCLUSION

RP-HPLC:

The estimation of Repaglinide was performed using Reverse Phase HPLC; the mobile phase for the elution buffer solution consisted of potassium dihydrogen phosphate and methanol in a ratio of (40:60). The elution process was isocratic at ambient temperatures. The column utilized was a hypersil C18 ODS column, with a detection wavelength set at 240nm. The linearity and application of Repaglinide were determined to be within the range of 100-500 mcg/ml. The retention time was recorded at 5.14 minutes, and the sample was diluted to achieve a concentration within this specified range. The results were calculated by comparing the peak area of the sample to that of the standard. The outcomes obtained through this method were consistent with the validation parameters outlined in USP XXIII. The low RSD values achieved indicated the reproducibility of the method.

UV-DERIVATIVE:

Where Derivative Spectroscopic method requires an instrument equipped with a recording facility. This technique removes any spectral interferences from standards of Repaglinide concentrations of 20, 30, 40, 50, 60, 70, and 80 mcg/ml, which were scanned, and the absorption spectrum was derivatized to obtain second-order derivative spectra. The two wavelengths chosen for sampling were 287 nm. The amplitude of the drug on scaling at 287 nm, and a calibration curve was created by plotting concentration against amplitude. The sample formulation was dissolved and appropriately diluted within the linearity range, and their second derivative spectrum was recorded. The amplitudes at 287 nm were measured and compared with the amplitude obtained from the standard. From the calibration curve, the concentration of Repaglinide was determined. Recovery studies were conducted to verify the accuracy of the method.

TABLES FOR ESTIMATION OF REPAGLINIDE BY UV , UV-DERIVATIVE &
RP-HPLC METHOD

PARAMETERS FOR RP-HPLC METHOD

Table:1 The following chromatographic condition were adopted in the proposed method

S.I	DESCRIPTION	EFFECT
1	INSTRUMENT NAME	Shimadzu HPLC System
2	COLUMN	C18, ODS,Hypersil(250 ×4.6 mm)
3	Detector	UV -240 nm
4	Pump	LC-10 ATV
5	Inj. Volume	20µl
6	Mobile Phase	Methanol:Potassium dihydrogen phosphate adjusted to (Ph 2.5) (60:40) v/v
7	Mode of Operation	Isocratic
8	Temperature	Ambient
9	Flow Rate	1.2 ml/min
10	Solvent	Methanol

TABLE-2 LINEARITY OF DETECTOR RESPONSE FOR REPAGLINIDE BY RP-HPLC METHOD

S.I	Concentration of Repaglinide (mcg/ml)	Area of Peak
1	100	19999250
2	200	39433858
3	300	59074432
4	400	78953169
5	500	98887393

TABLE: 3 STATISTICAL PARAMTERS FOR RP-HPLC METHOD

Estimation For Repaglinide by RP-HPLC Method

Formulation	Label Claim (Mg/tab)	Amount Estimated Mg/tab	Label claim(%)	Percentage Deviation	SD	RSD	CV	SE
TABLET	2	1.9912	99.56	-(0.44)	0.7444	0.007444	0.7434	0.43982
	2	1.9999	99.99	-(0.01)				
	2	2.0203	101.01	+(1.01)				

SD – Standard Deviation
RSD – Relative Standard Deviation
CV – Coefficient Variation
SE – Standard Error

Table: 4 – Estimation for Repaglinide Recovery Studies by RP-HPLC Method

S.I	Quantity of drug added mg	Quantity of drug recovery mg	Percentage Recovery (%)
1	2	1.999	99.99
2	1	0.9894	98.94

Table: 5 Calibration of Repaglinide By UV-Spectrometry method

S.I	Concentration in (mcg/ml)	Absorbance (Ab)
1	20	0.174
2	30	0.264
3	40	0.350
4	50	0.438
5	60	0.525
6	70	0.613
7	80	0.701

Table: 6 Estimation for Repaglinide by UV Method

Formulation	Lable-Claim Mg/tab	Estimated Amount Mg/tab	Percentage Label-claim(%)	Percentage Deviation	SD	RSD	CV	SE

	2	1.9970	99.85	0.15				
	2	2.0062	100.31	0.31				
	2	1.9958	99.79	0.21				
TABLET					0.2845	0.002845	0.2845	0.1642

SD – Standard Deviation
RSD – Relative Standard Deviation
CV – Coefficient Variation
SE – Standard Error

Table: 7 Estimation for Repaglinide Recovery Studies by UV Method

S. I	Quantity of drug increase (mg)	Amount of drug recovery mg	(%) Recovery
1	2	1.9792	98.96
2	1	0.9970	99.70

Table: 8 Estimation of Linearity for Repaglinide by UV – Method (Second Derivative)

S. I	Quantity in (mcg/ml)	(Ab)
1	20	11.00
2	30	18.88
3	40	25.50
4	50	34.00
5	60	42.25
6	70	50.25
7	80	57.50

SD – Standard- Deviation
RSD – Relative Standard Deviation
CV – Coefficient Variation
SE – Standard- Error

Table: 9 Estimation for Repaglinide by UV Method (Second Derivative)

Formulation	Label-Claim (Mg/tab)	Estimated Amount (Mg/tab)	Percentage Label - claim(%)	Percentage Deviation	SD	RSD	CV	SE
	2	1.988	99.40	0.6				
	2	1.974	98.70	- -1.30				

TABLET	2	1.9847	99.23	0.77	0.3651	0.003683	0.3683	0.21075

Table :10 Estimation for Repaglinide Recovery Studies by UV Method –(Second Order)

S. I	Estimation of drug added mg	Estimation of drug recovery mg	(%) Recovery
1	2	1.9792	99.77
2	1	0.9970	99.04

Table 11 Estimation for Repaglinide of System suitability parameters for RP-HPLC

S. I	Parameter	Repaglinide
1	No.of Theoretical	6955
2	Tailing factor	1.09

Table: 12: Estimation for Repaglinide of System suitability parameters for RP-HPLC & UV, Second Derivative

S.I	Parameters	RP-HPLC	UV -METHOD	Second derivative
1	Linearity	100-500 mcg/ml	20-80 mcg /ml	20-80 mcg /ml
2	Accuracy	99.99	99.85	99.40
4	Precision	0.007444	0.002845	0.003683
5	Specialty	No interference from additives	No interference from additives	No interference from additives
6	Limit Of Quantification (LOQ)	100mcg/ml	20mcg/ml	20mcg/ml

Table: 13 Results for Estimation of Repaglinide of three methods:

Method	API	Label Claim(mg/tab)	Estimation Found (mg/tablet)	% Label claim	% Deviation	S.D	R.S.D	CV	S.E
RP-HPLC	Repaglinide	2.00mg	1.9999	99.99	-0.01	0.7444	0.0007444	0.7434	0.43982
UV-method	Repaglinide	2.00mg	2.0062	100.31	0.31	0.2845	0.002845	0.2845	0.1642
Second Derivative	Repaglinide	2.00mg	1.9880	99.40	-0.6	0.3651	0.003683	0.3683	0.21079

FIGURES FOR ESTIMATION OF REPAGLINIDE BY UV, UV-DERIVATIVE &
RP-HPLC METHOD

RP-HPLC FOR REPAGLINIDE ESTIMATION
Figure:1 –Chromatogram –Related Substance

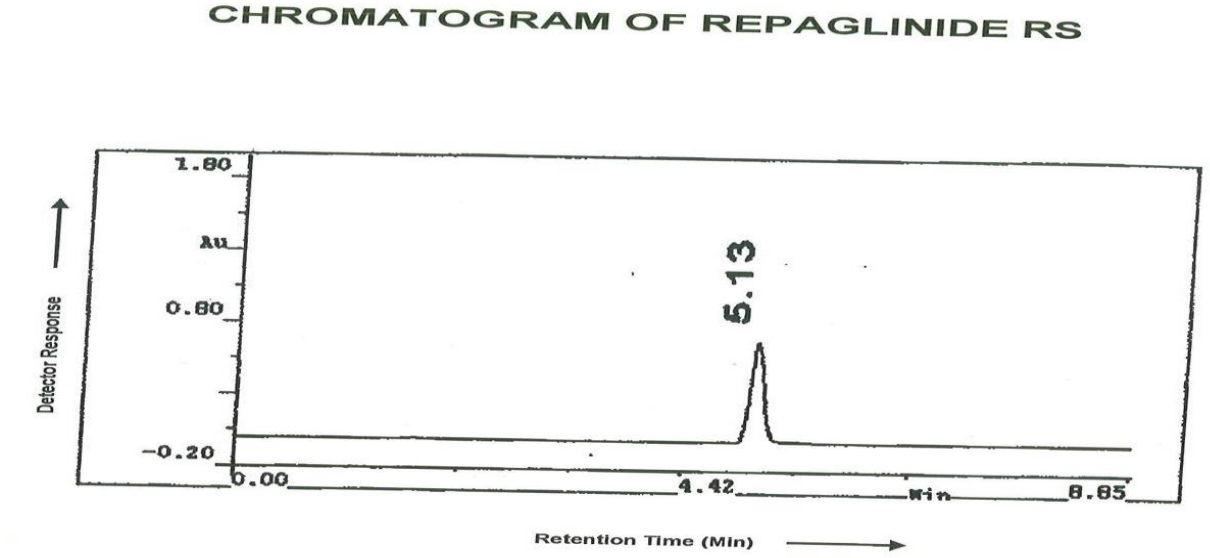


Fig.1 : Chromatogram Of Repaglinide RS

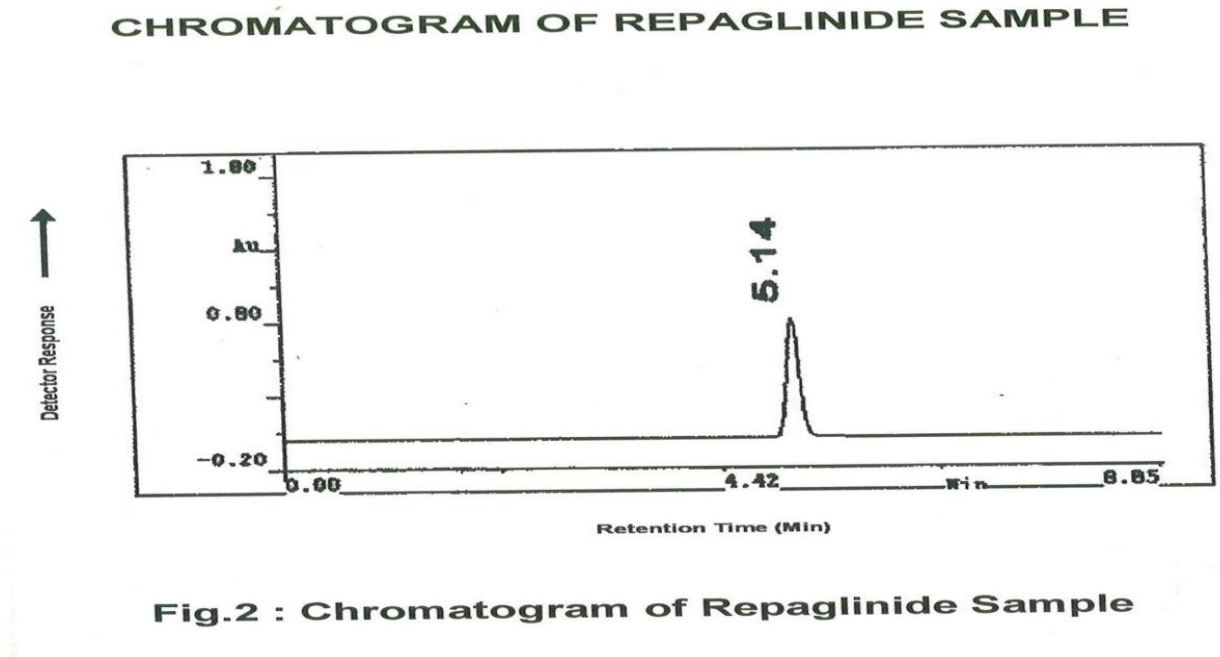


Fig.2 : Chromatogram of Repaglinide Sample

Figure :3 LINEARITY CURVE OF REPAGLINIDE BY RP-HPLC METHOD

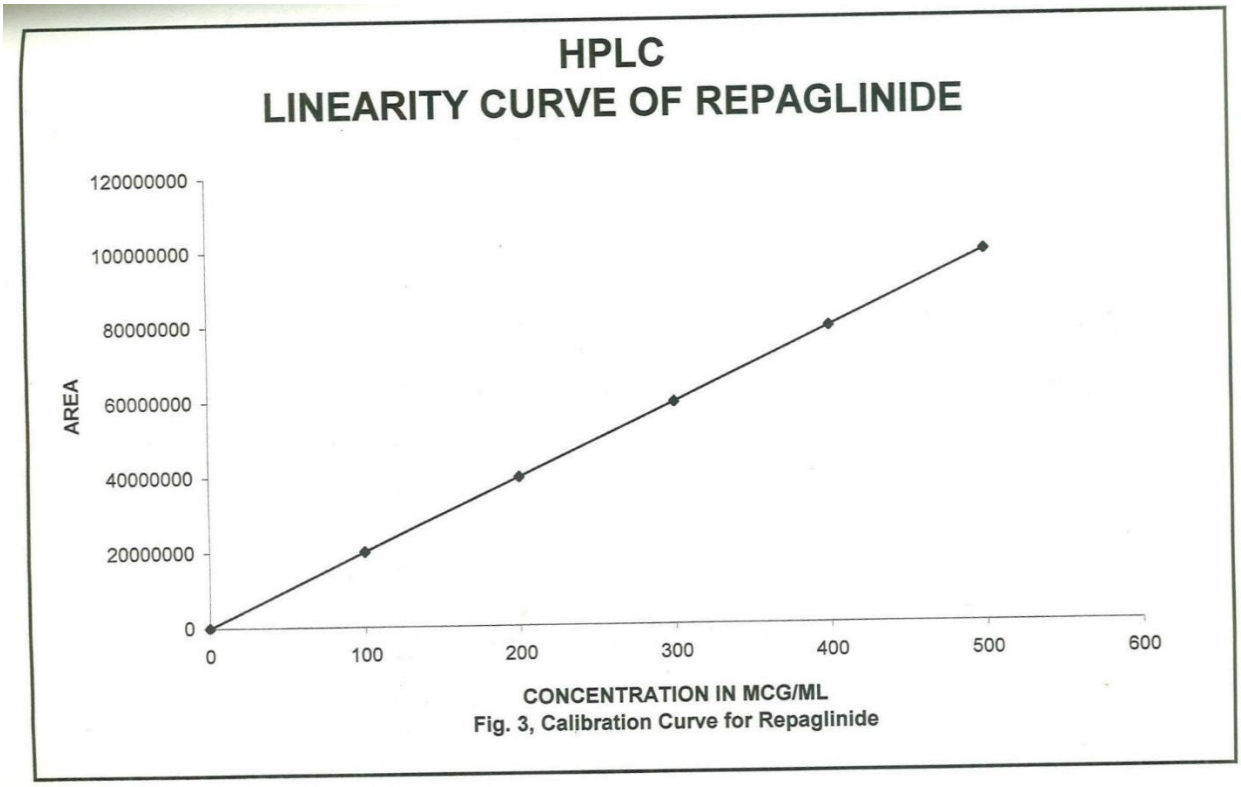


Figure: 4 Recovery Studies For RP-HPLC

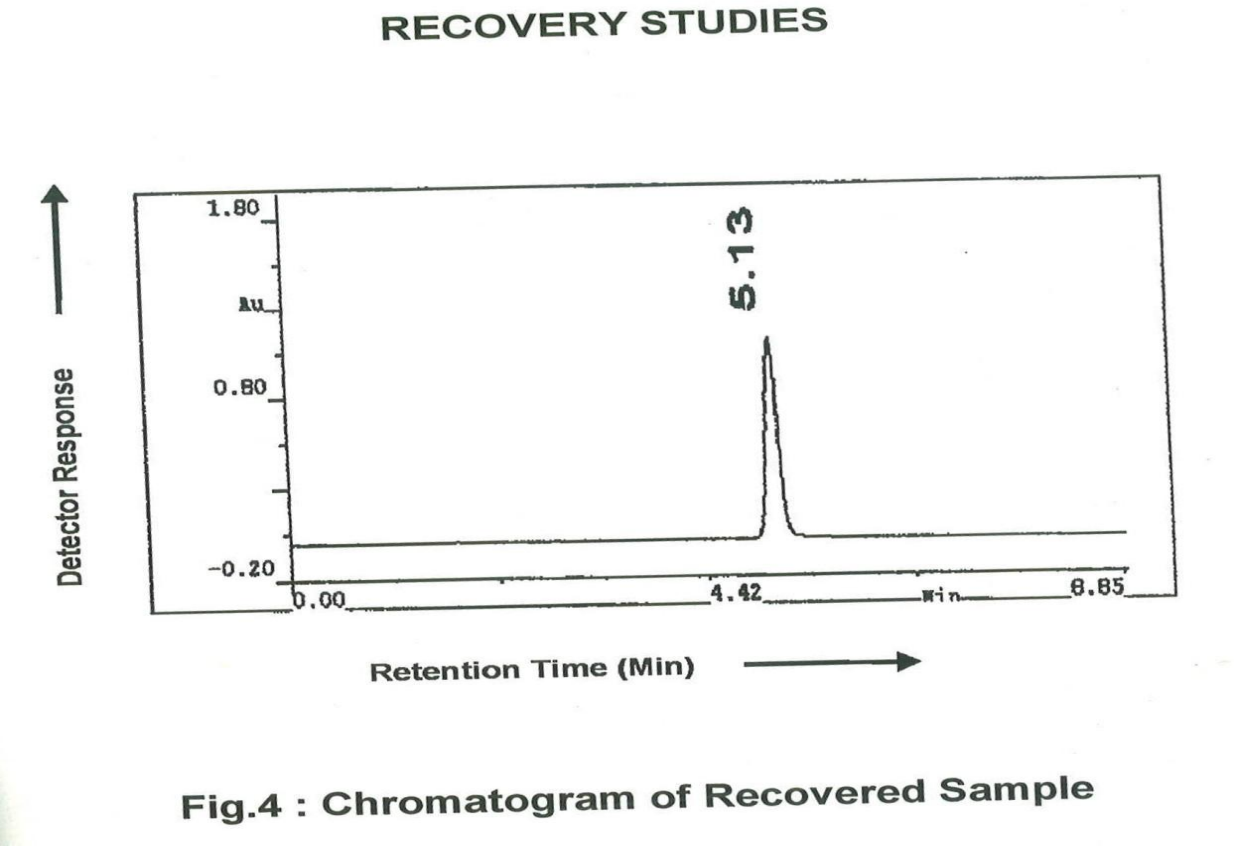


Fig.4 : Chromatogram of Recovered Sample

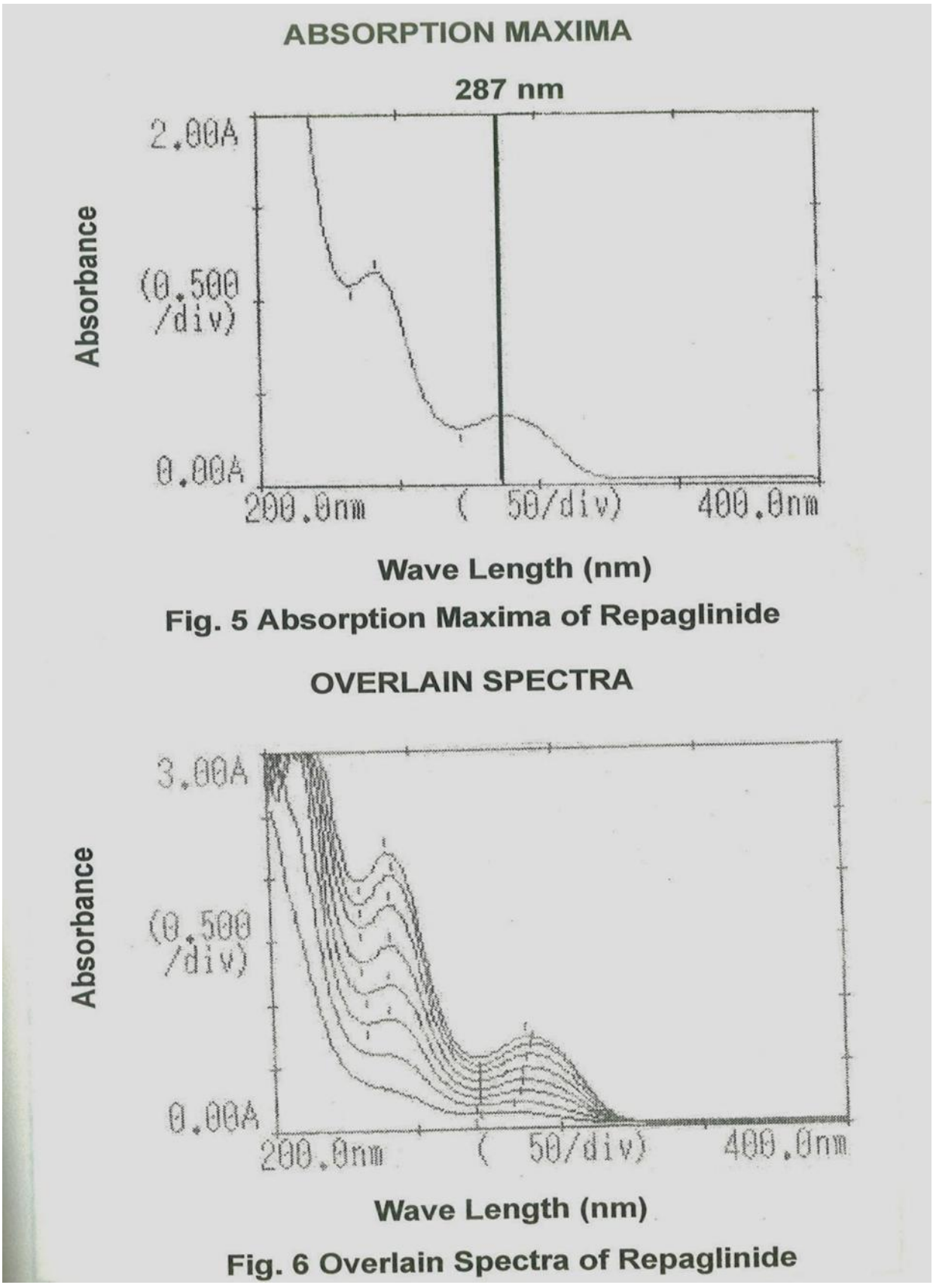


Fig. 5 Absorption Maxima of Repaglinide

Fig. 6 Overlain Spectra of Repaglinide

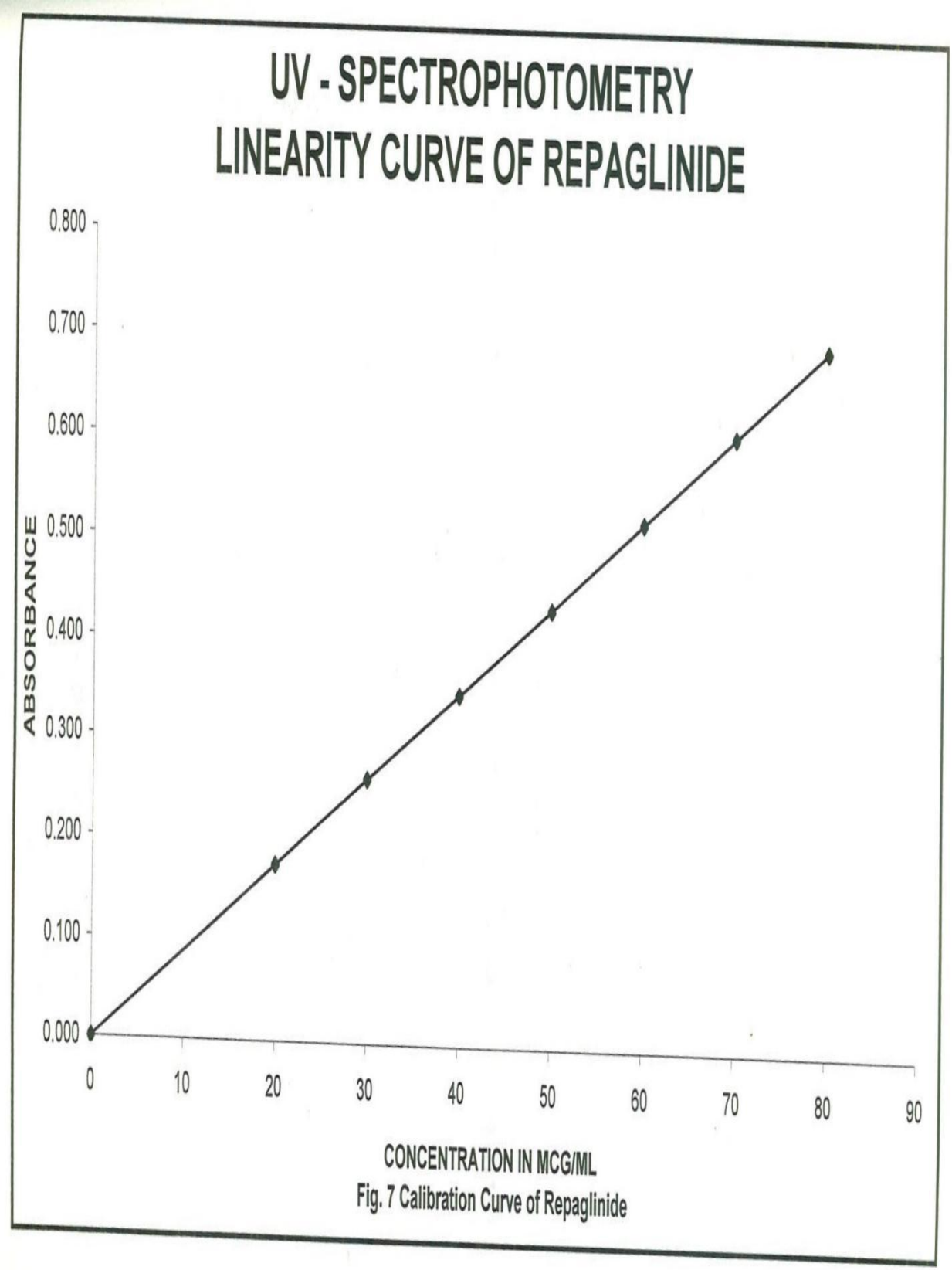
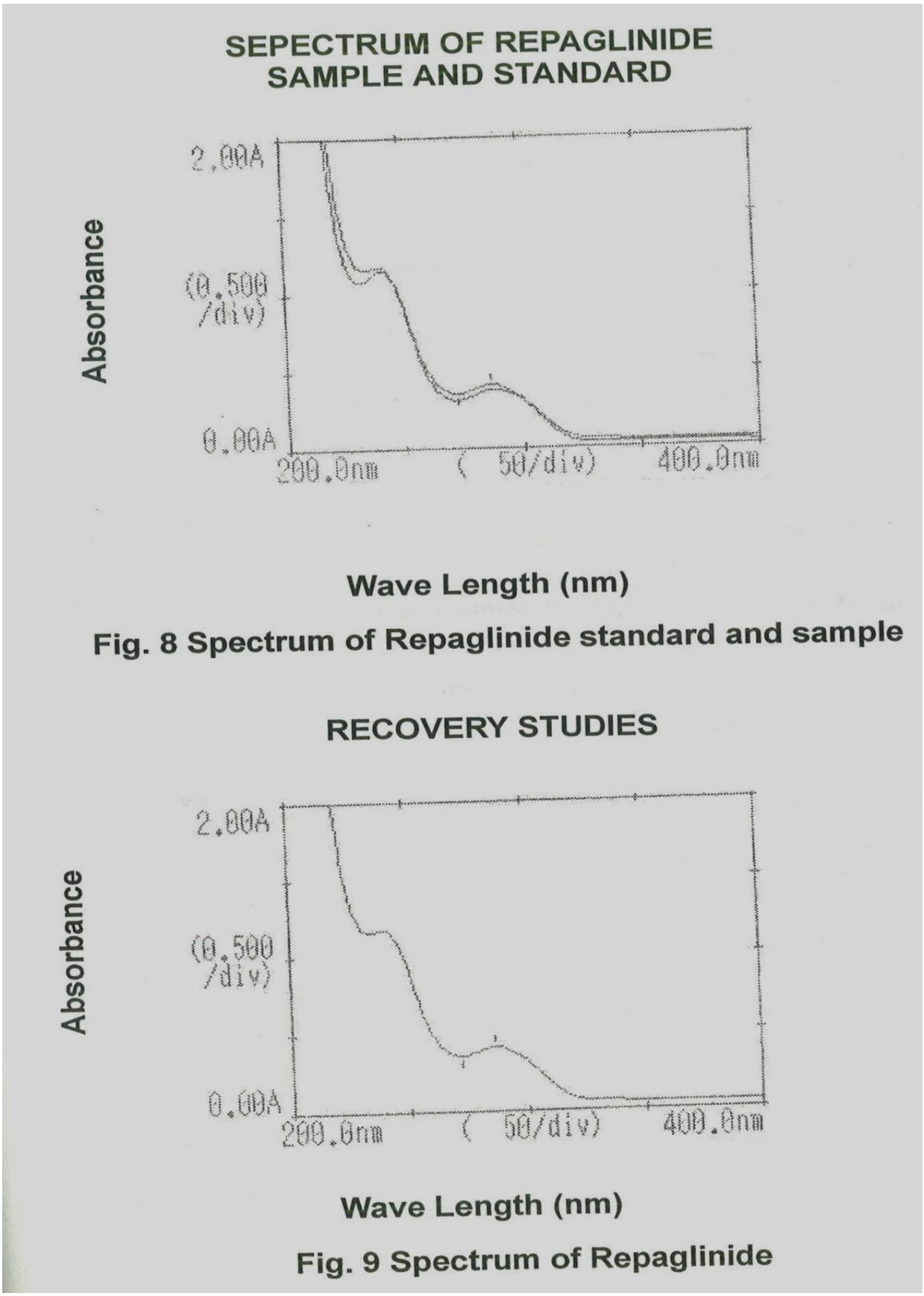


Fig. 7 Calibration Curve of Repaglinide


SEPECTRUM OF REPAGLINIDE
SAMPLE AND STANDARD
Absorbance
2.00A
(0.500
/div)
0.00A
200.0nm
(50/div)
400.0nm
Wave Length (nm)
Fig. 8 Spectrum of Repaglinide standard and sample
RECOVERY STUDIES
Absorbance
2.00A
(0.500
/div)
0.00A
200.0nm
(50/div)
400.0nm
Wave Length (nm)
Fig. 9 Spectrum of Repaglinide

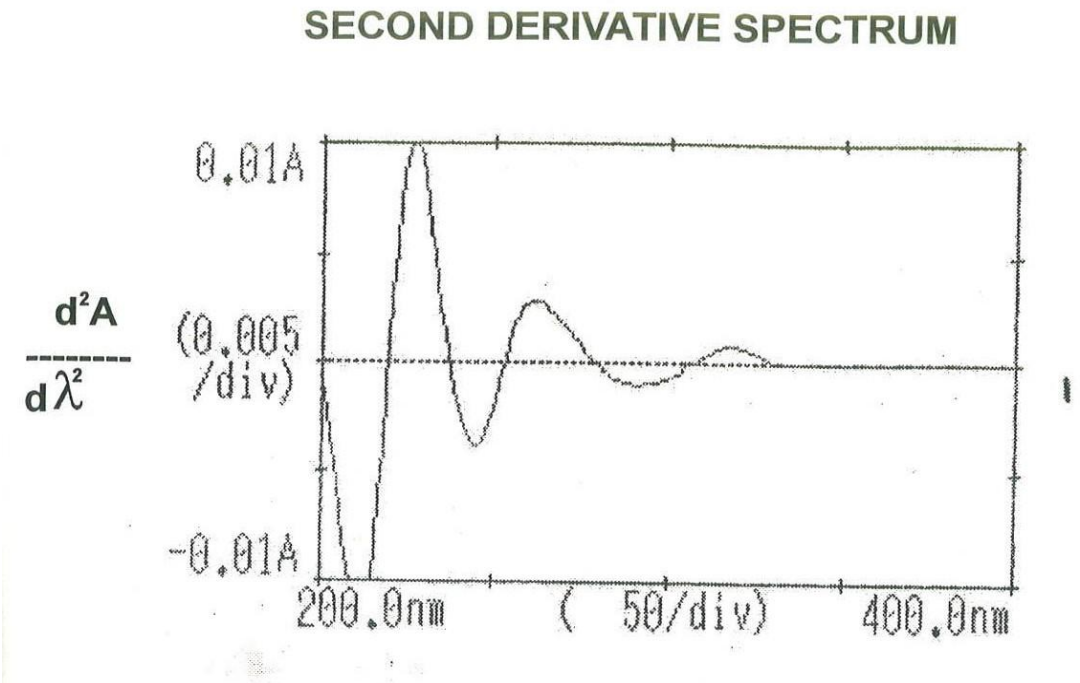


Fig. 10 Second Derivative spectrum of Repaglinide RS

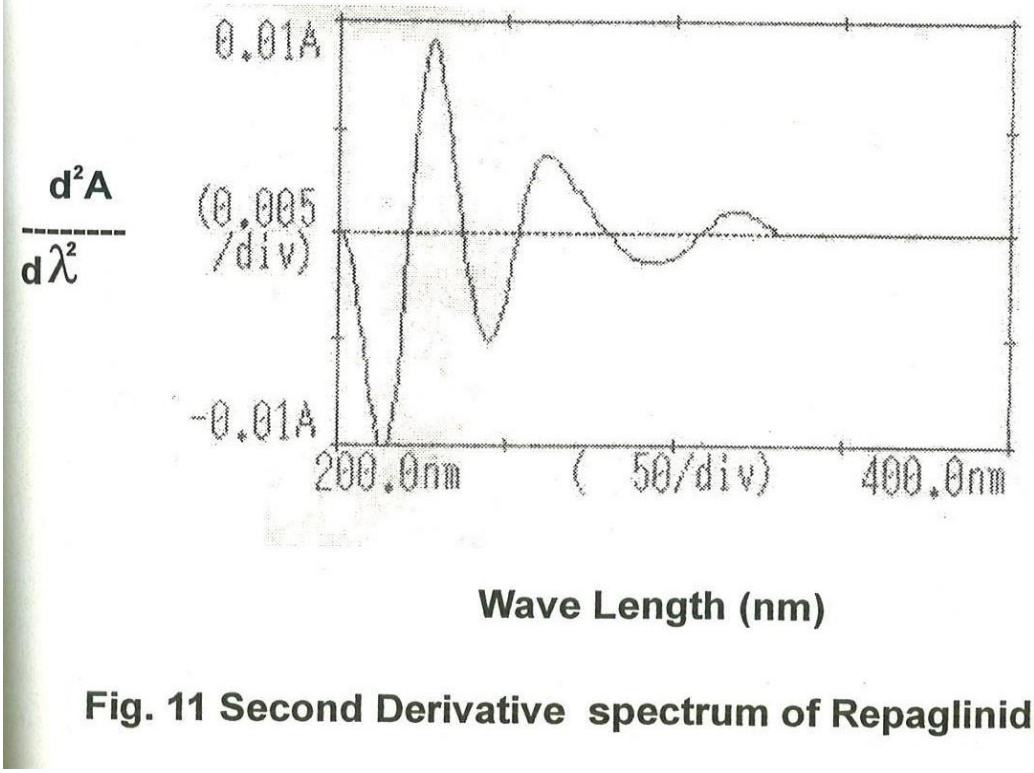


Fig. 11 Second Derivative spectrum of Repaglinide Sample

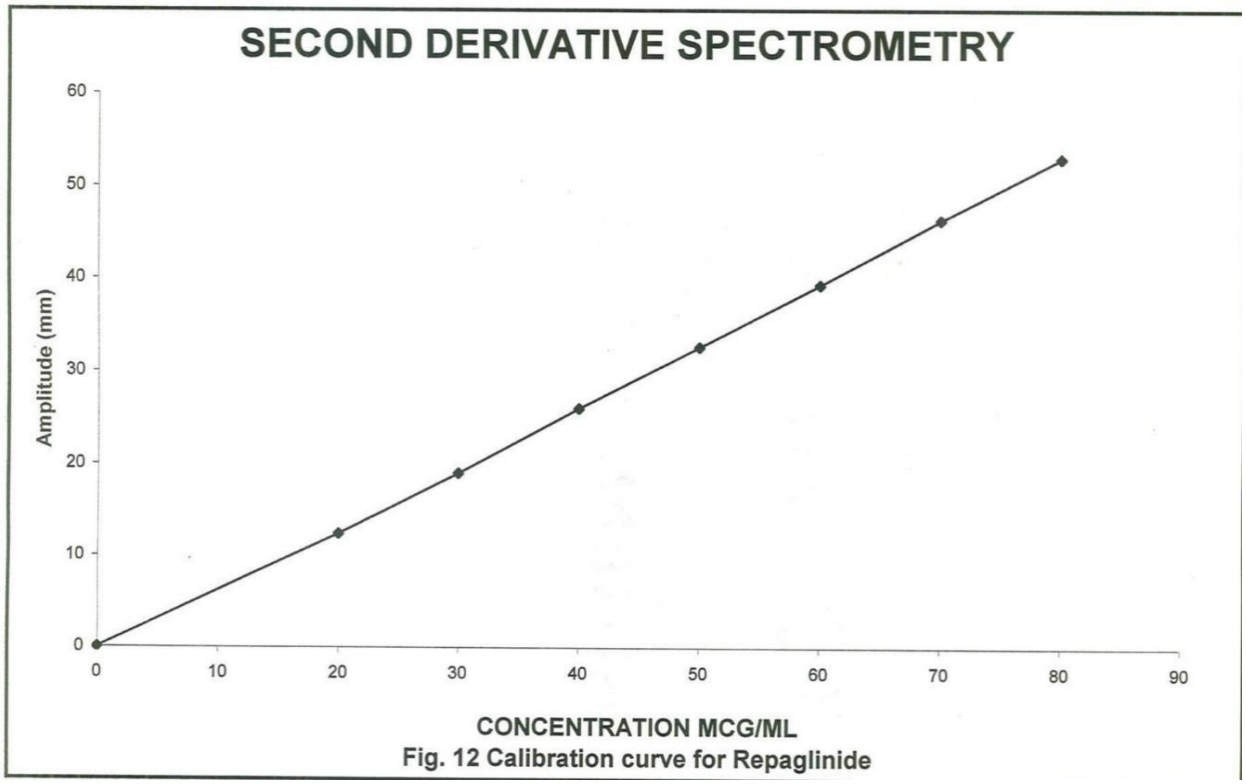


Fig. 12 Calibration curve for Repaglinide

REFERENCE

[1] Jaffery, Bassett J. Mendham J., Denny R.C. Vogels Textbook of Quantitative Chemical Analysis 1989 5ª Edn, 3-13,216.

[2] Beckett A.H., Stenlake J.B., Practical Pharmaceutical Chemistry, Part Two, 1998. 85-170.

[3] Gordeep Chatwal, Sham Anand, Instrumental methods of Chemical Analysis, 2000, 585-614.

[4] Chatwal G.R., Analytical Chromatography, 1995 1ª Edn 85-98.

[5] Martindale, 33rd Edn. The Complete Drug Reference. Edited by W.grell 8220, Page No.1458.

[6] Remington, The Science and Practice of Pharmacy, 1995, Vol-1, 19ª Edn. 534-538.

[7] Sharma B.K., Instrumental Method of Chemical Analysis 1994 14ª Edn. 112, 782-809.

[8] The Unites States Pharmacopoeia, (U.S.P) XXIV Rev., National Formulary. (N.F) XIX, Asian Edn., U.S Pharmacopoeial, Convention Inc., Rockvile M.D., 2000, 1922-2151.

[9] HPLC determination in human plasma by A greshcel eta. Chromatogram 568, 246.

[10] Eur.J. Clinical Pharmacology 193:45:113-16.

[11] Repaglinide for type 2 diabetes mellitus Med Lett Drugs Therapetics 1998 40: 55-6.

[12] Novo Nordisk 2360 (Physician's Desk Reference) drug information of repalinide, consult 2003 PDR Supplements.

[13] Goodman and Gilman's The Pharmacological basis of Therapeutics, 2002 10th Edn.

[14] Ji,HG.Xie.Li. Yan, MJ. Zhang, L. Liu, Xd of China Pharmaceutical university 32 (1): P. 30-33, 2001.

[15] W.Grell of preparation of Repaglinide WO 9300337 (1993 to Thomae). 34.

[16] F.S. Ampudia Blasco of dia betologia 37, 703 (1994).

[17] B.H.R. wolffenbuttel of comparative Clinical evaluation in NIDDM Eur.J Clinical Pharmacol 45, 113 (1993).

[18] CIMS APRIL, (Update 2) Page No: 26, 27 & 43.

[19] Bidstrup TB, Sidelmann UG, Thomsen Ms, Hansen KT BR J Clin Pharmacol 2003 Sep 56: 305-14.

[20] Moses R Diabetes Nutr Metab 2002 Dec 15:33-8.

[21] Gromada J diabetes Nutr Metab 2002 Dec 15: 15-8.

[22] Derosa G, Myugellini A, Ciccarelli L, Crescenzi G, Fogri R Clin Ther 2003 Feb 25 47284.
[23] Thomsen MS, Chassard D, Evcine E, Nielsen KK, Jestrgensen M J Clin Pharmacol 2003 Jan 43: 238.
[24] Matsuda M, Kaku K Nippon rinsho 2002 Sen 60 suppl 9: 555-8.
[25] Moses R, Exp Clin Endocrinol Diabetes 1999 107 Suppl 4: S136-9.
[26] Balfour JA, Faulds D Drugs Aging 1998 Aug 13: 173-80.
[27] Kaku K, Nippon Rinsho 1997-Nov 55 Suppl: 180-5.
[28] Hatorn V Clin Pharmacokinet 2002 41: 471-83.
[29] Quast U, Dtsch Med Wochenschr 2001 May 126: 559-60.