AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

RP-HPLC Method Development and Validation for Simultaneous Estimation of Candesartan Cilexetil and Hydrochlorothiazide Tablet Dosage Form

Dhia Eldin A Elmagied Elhag[1*], Omer A. Atiatalla[1], Mohammed B. Mahmoud[2]
1. Faculty of Pharmacy, University of Medical Science and Technology, Khartoum, Sudan P.O Box 12810
2. Blue Nile Pharmaceutical Company, Khartoum, Sudan, P.O Box 1371.

ABSTRACT

A simple, accurate and sensitive RP-HPLC method was developed and validated for the determination of candesartan cilexetil and hydrochlorothiazide in tablet dosage form. The separation of the two drugs was achieved on a Reprospher 100 –C l 8 column (250 x 4.6 mm, 5 µm particle size) with UV detection at 260 nm. The mobile phase consists of two solution, solution (1) orthophosphoric acid (pH 2.2) and the solution (2) which is acetonitrile and purified water in the ratio 55: 45 v/v, respectively. The method was validated in terms of linearity, accuracy, precision, specificity, limit of detection, limit of quantitation and robustness. Linearity was observed in the concentration range 10-70µg/ml for hydrochlorothiazide and 12.8 -89.6µg/ml for candesartan cilexetil. The limit of quantitation was found to be 8.58 and 4.6 µg/ml for hydrochlorothiazide and candesartan cilexetil, respectively whereas the limit of detection, was found to be 2.8 and 1.5 µg /ml for hydrochlorothiazide and candesartan cilexetil, respectively. The % recovery range of candesartan cilexetil was 97.84-101.62% and 100.9-101.53% for hydrochlorothiazide. Variation in HPLC conditions (flow rate, column temperature, mobile phase composition, and wavelength) were used to evaluate the robustness of the method. The method proved to be robust and still produces good results.

Keywords: Candesartan cilexetil, hydrochlorothiazide, Method Development, Validation, RP-HPLC.

*Corresponding Author Email: magied20@gmail.com
Received 09 April 2015, Accepted 21 April 2015

Please cite this article as: Elhag DEAE *et al.*, RP-HPLC Method Development and Validation for Simultaneous Estimation of Candesartan Cilexetil and Hydrochlorothiazide Tablet Dosage Form. American Journal of PharmTech Research 2015.

INTRODUCTION

Candesartan belongs to angiotension II receptor blockers and is used by patients suffering from hypertension[1]. Veeranjaneeyulu D and co-authors reported that the chemical name of candesartan is 2,3-hydroxy-2-butenyl 4-[1-hydroxy-1-methyl]-2-propyl-1-[p(o-1H-tetrazol-5-ylphenyl) benzylimida-2-zole-5-carboxylate,cyclic-2,3-carbonate[2]. The chemical structure is shown in Figure 1.

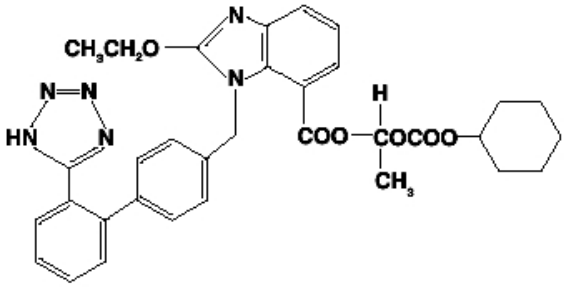


Figure 1: Chemical structure of candesartan cilexetil

Hydrochlorothiazide is diuretic and also used for the treatment of hypertension, it can be formulated on its own or in combination with other drugs e.g. with candesartan[3]. Its chemical name is 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide1,1-dioxide[3-5] and its chemical structure is given in Figure 2.

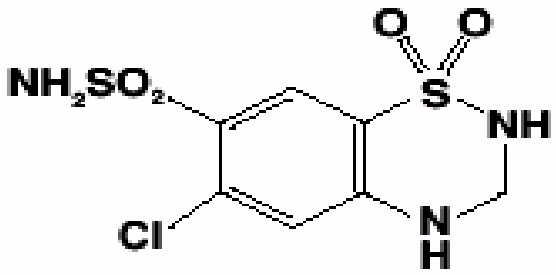


Figure 2: Chemical structure of hydrochlorothiazide

MATERIALS AND METHOD

1. Standard drugs of candesartan cilexetil and hydrochlorothiazide, Ranbaxy Laboratories Limited, India.

2. Zartan tablets (labeled to contain 16 mg of candesartan cilexetil and 12.5 mg of hydrochlorothiazide), Blue Nile Pharmaceuticals, Khartoum, Sudan.

3. Acetonitrile HPLC grade, Scharlau Chemicals, Spain.

4. Nylon syringe membrane filters, PALL Life Sciences, India.

5. Orthophosphoricacid (14.8 M) HPLC grade, Scharlau Chemicals, Spain

6. De-ionized water.

Instrumentation

HPLC Shimadzu LC-2OAB system comprising A - LC-2OAB Prominence model Solvent Delivery pump, the instrument was equipped with auto sampler, and a variable wavelength UV/vis detector (Shimadzu SPD20A Prominence). A CMB-20A System Controller with class LC-20 HPLC software LC Solution version 1.2. Online degasser (DGU-20A3 Prominence), a CTO -20A Prominence Column oven. Reprospher ODS Hypersil-C18, (250mmx4.6mm i.d. 5 µm) column. Shimadzu UV- 160 double beam spectrophotometer (Shimadzu, Japan).

Chromatographic Conditions

Isocratic elution mode was used. The employed flow rate was 1.0 ml/min, column temperature: 40° C, injection volume, 5µl and detection wavelength was 260nm.

Preparation of the solutions for mobile phase

Solution 1

Exactly 1ml of orthophosphoric acid (14.8 M) was transferred into 2000ml flak and was completed to mark (pH, 2.2). The solution was filtered, sonicated for 10 minutes.

Solution 2

Solution 2 was a mixture of acetonitrile and purified water taken in the ratio (55: 45 v/v), respectively. The mixture was degassed for 10 minutes.

Preparation of mobile phase

Solution (1) and solution (2) were mixed in ratio of (20:80 v/v), respectively.

Preparation of Diluents

Diluents-1: a mixture of water and acetonitrile in the ratio of (20: 80 v/v) respectively.

Diluents-2: a mixture of water and acetonitrile in the ratio of (50: 50 v/v).

Each diluent was sonicated for 10 minutes before use.

Preparation of Standard stock solution of candesartan cilexetil

Exactly 64 mg of candesartan cilexetil reference standard material were transferred into 50ml volumetric flask, 35ml of diluent-1was added. The solution was sonicated for 10 minutes and completed to the mark with diluent-1.

Preparation of Standard stock solution for hydrochlorothiazide

An accurately weighed 50 mg of hydrochlorothiazide standard were transferred into 50-ml volumetric flask. 35ml of diluent-1 were added, the solution was sonicated and completed to the mark with diluents-1.

Standard Solution

Exactly 5ml of Standard stock solution for hydrochlorothiazide and 5ml of standard stock solution of candesartan cilexetil were pipetted into a l00ml volumetric flask, the mixture was up to the mark with diluent-2 and was well mixed.

Preparation of sample

The average weight of 20 tablets was determined. 10 tablets were transferred into 500ml volumetric flask and 100ml of diluent-1 were added, the solution was sonicated for 10 minutes, then 200ml of diluent-1 were added and the solution was sonicated again for 30 minute with intermittent shaking and finally was made up to the volume with diluent-1. A portion of the solution was filtered. 10ml of the filtrate were transferred into 50 ml volumetric flask and were completed to mark with diluents-2.

RESULTS AND DISCUSSION

Method development

Method development process involves many trials for producing ideal chromatograms in terms of peak symmetry, number of theoretical plates, minimal or noise- free and tailing factor. K. Balamuralikrishna and Syamasundar pointed that different Spectroscopic and analytical methods had been introduced for the identification quantification of candesartan and hydrochlorothiazide in bulk form or as pharmaceutical products[3]. Narendra and co workers presented a chromatographic method for the separation and quantification of candesartan and hydrochlorothiazide by the use of a isocratic RP-HPLC[6]. Patel Jibgesh *et al* developed a rapid and sensitive method based on UV/vis spectrometry to analyze both drugs formulated as tablets[7]. S.S Qutab and others introduced a rapid chromatographic method with Uv detection at λ 271nm for the separation and quantification of candesartan cilextel and hydrochlorothiazide. They employed a phenyl-2 column for the separation and potassium dihydrogen phosphate, methanol and triethyamine mixture as a mobile phase. The method proved to be linear, sensitive and accurate[8]. Candesartan and its related substances were separated successfully by Gunda Srinivas and his colleagues by using a simple chromatographic method[9]. They used a C18 column for separartion and the two mobile phases and a gradient elution mode. They reported that the mobile phase was 0.01 triethylamine (pH2.2), trifloroacetic acid, while the other mobile phase was 0.1% trifloroacetic acid and acetonitrile. Excellent results were reported by the authors. Chandrul Kaushal K. and B.Srivastava[10] reviewed the different factors and the chromatographic experimental conditions (e.g. column type, column temperature stationary phase, mobile phase, mode of elution, flow rate, pH, and the sample properties) that must be

investigated in order to introduce a valid chromatographic method that fits the purpose of the analysis as indicated in the ICH recommendations[11].

Determination of appropriate UV wavelength

The suitable wavelength for the determination of candesartan cilexetil and hydrochlorothiazide in diluents is identified by scanning over the range 200-400 nm. The spectrum indicated that 260 nm is a suitable detection wavelength for the determination of the two drugs.

Validation of Analytical Method

According to J. Ermer and J.H McB Miller[12], method validation process is performed to ensure that the proposed method of analysis can provide excellent performance characteristics such as accuracy, linearity, range, specificity, robustness of detection and limit of quantitation.

System Suitability

It has been emphasized in the protocol of the International Conference on Harmonization (ICH) guidelines[11] that system suitability represents one of the basic requirements and integral part of any method that is to be introduced for routine analysis. The parameters to be determined in this test include the number of theoretical plates, tailing factor, relative standard deviation. Accordingly and in the present study, the relative standard deviation for candesartan cilexetil and hydrochlorothiazide peaks area obtained from six replicate injections are shown in Table1.

Table 1: The measured system suitability parameters

No	Hydrochlorothiazide	Candesartan cilexetil
1	303534	251482
2	301722	250461
3	302749	250368
4	305738	250935
5	309829	251991
6	301281	251991
Average	304142.2	250373
SD	3201.282	674.738
RSD%	1.052561	0.26889
Tailing Factor	1.5	1.14
No. of theoretical Plate	5985.686	17805.28

Linearity

The linearity of the method was investigated by measuring five solutions in the range of 12.8—89.6µg/ml for candesartan cilexetil and 10-70µg/ml for hydrochlorothiazide. Each solution was prepared in triplicate and the average values were used to plot the calibration curves. Figure 3 represents the chromatogram of the two drugs (89.6 µg/ ml candesartan cilexetil and 70 µg /ml for hydrochlorothiazide).

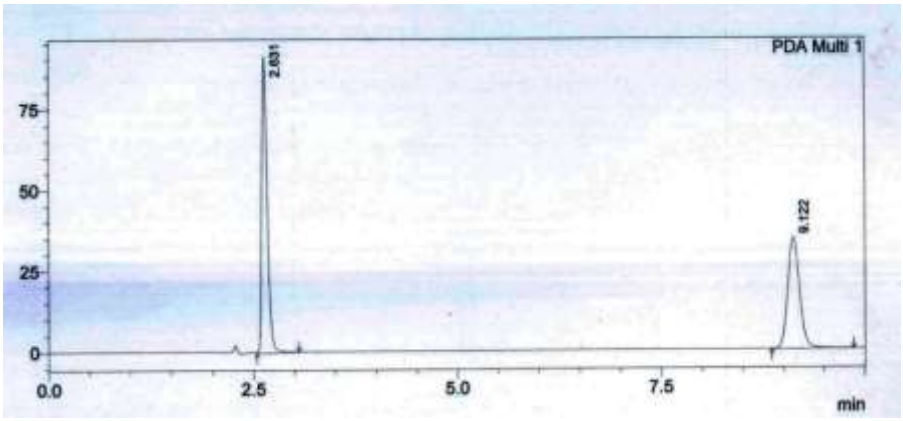
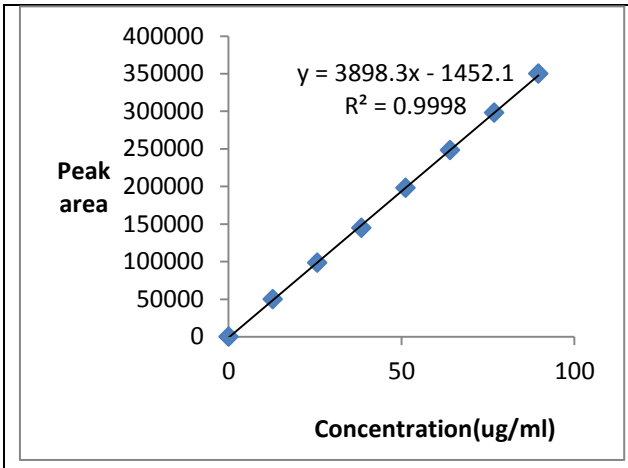
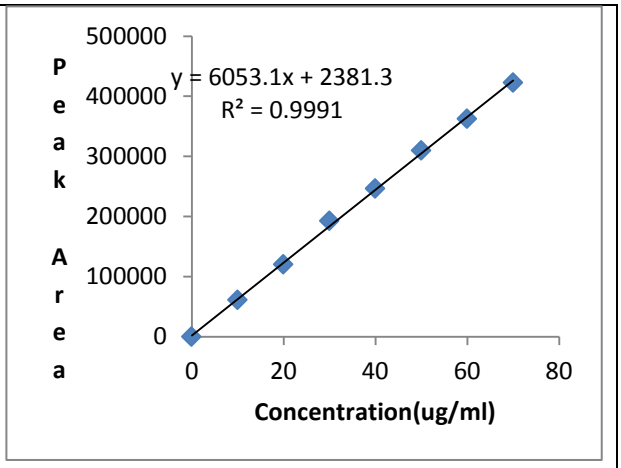


Figure 3: Chromatogram of hydrochlorothiazide 70µg/ml , candesartan cilexetil 89.6µg/ml

The chromatogram shows well resolved, symmetric peaks for both drugs. The calibration curves obtained are shown in Figure 4a and 4b.



(a) Calibration curve of candesartan cilexetil



(b) Calibration curve of hydrochlorothiazide

Figure 4: Chromatogram of candesartan cilexetil and hydrochlorothiazide.

The graphical plots shown in Figure 4 indicate that a very good correlation exists between the peak area and concentration of the drugs. Linear regression least square fit data are shown in the plots.

Accuracy

The accuracy was evaluated by adding known amount of candesartan cilexetil and hydrochlorothiazide standard drug at three different levels (80%, 100%, 120% level) to the tablet powder which were subjected to assay test. Accordingly, 10, 12.5, and 15 mg of hydrochlorothiazide reference drug; and 12.8, 16, and 19.2 mg of candesartan cilexetil standard drug were added along with each 12.5/16 mg Zartan Plus (candesartan cilexetil and hydrochlorothiazide tablet). In this test, each solution was analyzed in triplicate The results (Table 2 and 3) were expressed as the percentage of candesartan cilexetil and hydrochlorothiazide reference standard recovered from the sample.

Table 2: The results of accuracy study of hydrochlorothiazide

Parameters	**Hydrochlorothiazide**		
Tablet amount (mg)	12.5	12.5	12.5
Level of addition (%)	80	100	120
Amount added (mg)	10.23	12.5	14.9
Amount recovered(mg)	10.08	12.62	14.93
Average% recovery	101.53	100.9	100.22
4RSD	±0.9	±3.1	±1.3

Table 3: The results of accuracy study of Candesartan Cilexetil

Parameters	**Candesartan Cilexetil**		
Tablet amount (mg)	16	16	16
Level of addition (%)	80	100	120
Amount added (mg)	12.73	16.1	19.23
Amount recovered(mg)	12.94	16.28	18.82
Average% recovery	101.62	101.15	97.84
4RSD	±2.5	±0.52	±1.3

From the above tables it is clear that high recovery percentage was obtained by the use of this method.

Specificity

Placebo sample solutions were prepared in triplicate by taking a weight of placebo approximately equivalent to its weight in the tablet. Chromatograms of placebo solutions have shown no peaks at the retention time of candesartan cilexetil and hydrochlorothiazide peaks. This indicates that the excipients used in the formulation do not interfere in estimation of candesartan cilexetil and hydrochlorothiazide in tablets.

Determination of the limit of detection and limit of quantitation

The limit of detection and the limit of quantification of the drug were calculated using the following equations.

Limit of detection = 3.3SD/S.

SD = the standard deviation of the response, (S) = the slope of the calibration curve

Limit of quantitation = 10SD/S.

Based on the above equations, the limit of detection and limit of quantitation for candesartan were found to be 1.5 µg/ml and 4.6 µg/ml, respectively. For hydrochlorothiazide, the limit of detection and limit of quantitation for can were found to be 2.8 µg/ml and 8.58 µg/ml, respectively.

In the rest of the tables, and for simplicity and convenience, hydrochlorothiazide will be denoted as (A) candesartan cilexetil will be denoted as (B).

Precision

The intra-day and inter-day precisions of the proposed methods were determined by estimating the corresponding responses 5 times on the same day and on 3 different days, for 3 different concentrations of 40, 50 and 60 µg/ml hydrochlorothiazide and 51.2, 64 and 76.8µg/ml, for candesartan cilexetil. Method repeatability (intra-day precision) was evaluated and the results are shown in Table 4.

Table 4: Intra-day precision test results summary

	A:B Ratio		**A:B Ratio**		**A:B Ratio**	
	40µg/ml:51.2µg/ml		**50:64 µg/ml**		**60:76.8µg/ml**	
#	**A**	**B**	**A**	**B**	**A**	**B**
Time1	246354	197591.7	309737.7	248180.3	362036.3	297650.3
Time2	248512.3	197000.3	316520.3	252312	367903.7	297340.7
Time3	243620	195006.7	311495	249135	363221.7	293494.7
Time4	243823	195201.7	311452	249262.7	362827.3	293009.3
Time5	244075	194707	312989.3	249917	363296.3	293098.3
Average	245007.6	195901048	313114.2	249761.4	363857.1	294918.7
SD	2343.884	1302.0517	2380.605	1554.89	2316.733	2361.946
RSD%	0.956658	0.6646462	0.760299	0.62255	0.636715	0.800881

The intermediate precision (inter day precision) was performed in days as described and the results are shown in Table 5.

Table 5: Inter-day test results summary

A:B Ratio	**40µg/ml/51.2µg/ml**		**50/64 µg/ml**		**60/76.8µ/ml**	
#	**A**	**B**	**A**	**B**	**A**	**B**
DAY1	246345	197591.7	309737.7	248180.3	362036.3	297650.3
DAY2	249195.3	194933.3	302173	240693.3	360779.3	294718.7
DAY3	243871.3	192258	305233.7	245244	355303.3	297006.3
Average	246470.5	194927.7	305714.8	244705.9	359373	296458.4
SD	2664.219	2666.854	3805.229	3772.397	3580.035	1540.678
RSD%	1.080948	1.368125	1.244699	1.541605	0.996189	0.519694

Method repeatability (intra-day precision) and the intermediate precision (inter day precision) tests showed that the mean %RSD was found to be less than 2.0%.

Robustness

The robustness of the method was evaluated after changing the following parameters: Flow rate, column temperature, mobile phase composition and detection wavelength. The results are shown in Table 6.

Table 6: Results of Robustness test method

parameter	Variation	Ret. time in minutes		Tailing factor		%RSD for 3 injections of standard	
		A	B	A	B	A	B
Flow rate	0.75 ml min^{-1}	3.5	12.53	1.44	1.14	0.61	0.15
	1.00 ml min^{-1}	2.64	9.61	1.48	1.14	0.11	0.34
	1.50 ml min^{-1}	1.75	6.52	1.55	1.16	1.39	1.39
Colum temperature	35° C	2.67	10.59	1.49	1.19	1.05	0.18
	40 ° C	2.63	9.69	1.56	1.2	1.65	0.11
	45 ° C	2.61	9.24	1.58	1.21	1.03	0.03
Mobile phase composition (v/v)	75: 25	6.52	14.02	0.94	0.82	0.69	0.04
	80:20	2.64	9.61	1.48	1.14	0.61	0.32
	70:30	1.4	3.67	1.4	1.30	0.49	0.23
Wave length	265 nm	2.63	8.74	1.5	1.16	0.22	0.12
	260 nm	2.63	8.74	1.51	1.16	0.33	0.34
	255 nm	2.63	8.74	1.53	1.16	1.07	0.14
Average				1.46	1.15	0.77	0.28

In all the deliberately varied chromatographic conditions (flow rate, column temperature and ratio of acetonitrile in mobile phase), the tailing factor and the % RSD for the candesartan cilexetil and hydrochlorothiazide peak areas for five replicate injections of standard were found to be within the acceptable limits, illustrating the robustness of the method.

Application of the Method to Tablets

The method was used for determination of hydrochlorothiazide and candesartan cilexetil in tablet formulation (Zartan tablets (labeled to contain 16 mg of candesartan cilexetil and 12.5 mg of hydrochlorothiazide). The results obtained showed high recovery percentage and low RSD values. The results of tablet assay are shown in Table 7.

Table 7: percentage recoveries and RSD% of tablets assay

Batch .No	CNHTZ005		CNHTZ006		CNHTZ007	
component	A	B	A	B	A	B
% Assay	100.77	100.75	100.24	99.96	101.13	100.20
% RSD	0.25	0.73	0.28	0.49	0.21	0.15

CONCLUSION

The proposed method for simultaneous determination of candesartan cilexetil and hydrochlorothiazide proved to be accurate, simple and precise. The method was validated according to ICH guidelines in terms of specificity, linearity, accuracy, precision, robustness detection limit and quantification limit and can be used for the analysis of this drug combination and as stability indicating method.

REFERENCES

1. Martindale: The complete drug reference. 33th edition, Pharmaceutical press, Lambeth High Street, London. 2002**:**853.
2. Veeranjaneeyulu D, Aneesha A, Nandakishore Agarwal. Stability Indicating method for the Determination of Candesartan Cilextel and Hydrochlorothiazide in Bulk and Dosage Form. Indian Journal of Research in Pharmacy and Biotechnology, 2013; 1(5):720-724.
3. K. Balamuralikrishna and Syamasundar "Development and Validation of High Performance Liquid Chromatography for the Simultaneous Estimation of Candesartan Cilextel and Hydrochlorothiazide in Combined Dosage Form" Der Pharmacia Chemica, 2013; 2(6):231-237.
4. Y.Sunandama. Single RP-HPLC Method for the Quantification of Candesartan and Hydrochlorothiazie in Formulation. The Experiment,7(4): 428-437.
5. Martindale: The complete drug reference. 33th edition, Pharmaceutical press, Lambeth High Street, London. 2002**,** 907-911.
6. Narendra Devanaboyina, Satyanarayana T, Ganga, Rao B. Simultaneous Determination of Candesartan and Hydrochlorothiazide in Combined Pharmaceutical Dosage form by New RP-HPLC. Res J Pharma Biological and Chemical Sci 2012, 3, 1, 270-278.
7. Patel Jibgesh, Dave J B, Patel C N, and Patel Dhrumil. Q-Analysis Spectrophotometric Method for Estimation of Candesartan Cilextel and Hydrochlorothiazide in Tablet Dosage Form. J. Chem .Pharm. Res, 2010;2(3):10-14.
8. S.S Qutab S.N Razzaq M. Ashfaq, Z.A. Shuja, and I.U. Khan. Simple and Sensitive LC-UV Method for Simultaneous Analysis of Hydrochlorothiazide and Candesartan Cilextel in Pharmaceutical Preparations. Acta Chromatographica, 2007; 19:119-129.
9. Gunda Srinivas, Kakumani Kishore Kumar, Gangaram V. Kanumula, M. Vishnu Priyal,, K Mukkanti. A Stability Indicating UPLC Method for Candesartan in Bulk Drug Samples. American J Analytical Chemistry 2012; 3:704-709.
10. Chandrul Kaushal K. and B. Srivastava. A process of Method Development: A chromatographic Approach. Journal of Chemical and Pharmaceutical Research, 2010; (2)2:519-545.
11. ICH Harmonized Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology, ICH Geneva, 2005:Q2 (R1).

12. J.Ermer and J.H McB Miller,(eds) "Method Validation in Pharmaceutical Analysis: A Guide to Best Practice" WILEY-VCH-Verlag, Gmb, &Co, KGaA Weinham, 2005.