

C O N T E N T S

	Page
Zusammenfassung	i
Summary	iv
List of Tables	xi
List of Figures	xii
List of Abbreviations	xiv
1 Introduction	1
2 General Background	4
2.1 Historical Aspects	4
2.2 Biochemistry and Metabolism of Homocysteine	5
2.2.1 The Remethylation Pathway	6
2.2.2 The Transsulfuration Pathway	7
2.3 Classification of Hyperhomocysteinemia	8
2.4 Determinants of Homocysteine	8
2.4.1 Genetics	8
2.4.2 Vitamin Deficiency	9
2.4.3 Age and Gender	10
2.4.4 Lifestyle	10
2.4.5 Medication	12
2.4.6 Pathological Determinants	13
2.5 Homocysteine and Cardiovascular Disease	17
2.5.1 Background and Retrospective Studies	17
2.5.2 Prospective Studies	18
2.5.3 Interaction between Homocysteine and the Usual Risk Factors for Cardiovascular Disease	18
2.6 Homocysteine and Renal Disease	20
2.6.1 Hyperhomocysteinemia in Renal Disease Patients	20
2.6.2 Relation to Atherosclerosis	21

3	Development of a Gas Chromatographic-Mass Spectrometric Method for the Determination of Total Homocysteine and Related Amino Acids by Stable Isotope Dilution	23
3.1	Methods for Determination of Total Homocysteine	23
3.1.1	High-Performance Liquid Chromatography (HPLC)	23
3.1.2	Capillary Electrophoresis	24
3.1.3	Enzyme Immunoassay	25
3.1.4	Gas Chromatography - Mass Spectrometry	25
3.2	Gas Chromatography and Mass Spectrometry Techniques	26
3.2.1	Gas Chromatography	26
3.2.2	Mass Spectrometry	28
3.2.3	Isotope Dilution Method	29
3.3	Experimental	30
3.3.1	Chemicals	30
3.3.2	Sample Collection	31
3.3.3	Sample Preparation for GC-MS	31
3.3.4	Derivatization	31
3.4	GC-MS Measurement	31
3.4.1	Peak Identification	32
3.4.2	Selected Ion Monitoring	34
3.4.3	Quantification	34
3.5	Analytical Performance	36
3.5.1	Specificity	36
3.5.2	Linearity	37
3.5.3	Precision	37
3.5.4	Analytical Recovery	37
3.5.5	Between-Day Variation	37
3.5.6	Limit of Detection and Quantitation	38
4	Comparison of GC-MS Method with the Abbott IMx Homocysteine Assay and an Evaluated HPLC-Method for the Determination of Total Homocysteine	39
4.1	Material and Method	39
4.1.1	Sample Collection	39

4.1.2	GC-MS Determination	40
4.1.3	HPLC Determination	40
4.1.4	IMx Homocysteine Assay	40
4.1.5	Statistical Methods	
4.2	Results	41
4.2.1	Comparison of the GC-MS Measurement with HPLC and IMx Technique in ESRD Patients	41
4.2.2	Comparison of the GC-MS Measurement with IMx in Healthy Subjects	42
5	Application of the GC-MS Method on Clinical Investigations	45
5.1	Total Plasma Homocysteine and Related Amino Acids in ESRD Patients	45
5.1.1	Background	45
5.1.2	Methods	46
5.1.2.1	Sample Collection	46
5.1.2.2	Determination of Total Homocysteine, Methionine, Cysteine and Cystathionine	46
5.1.2.3	Determination of Vitamins	46
5.1.2.4	Statistical Analysis	46
5.1.3	Results	47
5.2	Effects of Leucovorin (N ⁵ -formyltetrahydrofolate) versus Folic Acid on Plasma Total Homocysteine and its Related Amino Acids in ESRD Patients	50
5.2.1	Background	50
5.2.2	Methods and Patients	51
5.2.2.1	Design and Patients	51
5.2.2.2	Biochemical Assay	52
5.2.2.3	Statistical Analysis	52
5.2.3	Results	52
5.2.3.1	Effect of Folic acid and Leucovorin on tHcy and Metabolites	53
5.2.3.2	Relative Response of Folate and Vitamin B ₁₂	55

6	Discussion	57
6.1	Analysis of Plasma Total Homocysteine and Related Amino Acids by GC-MS	57
6.2	Comparison of the New GC-MS Methods with Other Methods	60
6.3	Homocysteine Metabolism in ESRD Patients	62
6.4	Effect of Leucovorin and Folic Acid on Total Plasma Homocysteine in ESRD Patients	64
7	Conclusion	68
8	References	69
	Appendix	89
	List of Publications and Presentations	97
	Acknowledgement	98
	Curriculum Vitae	100