ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2013, *58*(7-8), 659-665

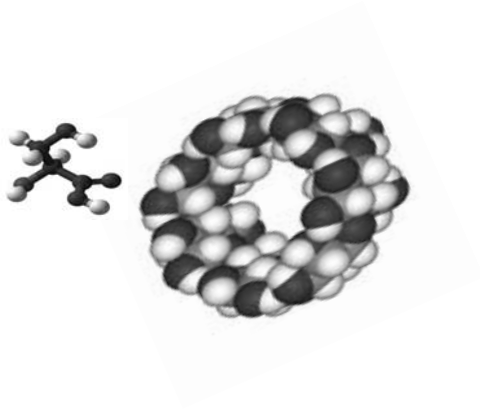
ENANTIOSELECTIVE, POTENTIOMETRIC MEMBRANE ELECTRODES BASED ON CYCLODEXTRINS FOR THE ASSAY OF GLYCERIC ACID IN URINE AND SERUM SAMPLES

Raluca-Ioana STEFAN-VAN STADEN,[a,*] R'afat M. NEJEM[b] and Jacobus F. VAN STADEN[a]

[a] Laboratory of Electrochemistry and PATLAB Bucharest, National Institute of Research for Electrochemistry and Condensed Matter, 202 Splaiul Independenței Str., 060021, Bucharest, Roumania
[b] Department of Chemistry, Al-Aqsa University, Gaza, Palestine

Received December 20, 2012

Hyperoxaluria type 2 (PHII) and D-glyceric academia/aciduria are two inborn inherited metabolic diseases, characterized by excess excretion of L- and D-glyceric acids, respectively. Enantioselective, potentiometric membrane electrodes (EPMEs) based on carbon paste modified with α- and γ-cyclodextrins (CD) (for the assay of L-glyceric acid), and β-cyclodextrin and 2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrin (as chloride salt) (β-CD-derivative) (for the assay of D-glyceric acid) were designed. These electrodes can be reliably used for the analyses of L-glyceric acid in the concentration ranges of 10^{-9}-10^{-7} (α-CD based EPME) and 10^{-5}-10^{-2} mol/L (γ-CD based EPME) and of D-glyceric acid in the concentration ranges of 10^{-5}-10^{-3} (β-CD based EPME) and 10^{-6}-10^{-3} mol/L (β-CD-derivative), with low detection limits. The selectivity of EPMEs was determined over L- or D-glyceric acid, creatine, creatinine, Na^+, K^+, and Ca^{2+}. Recovery tests were applied for the enantioanalysis of each enantiomer in the presence of its antipode as well as for urine and serum samples.

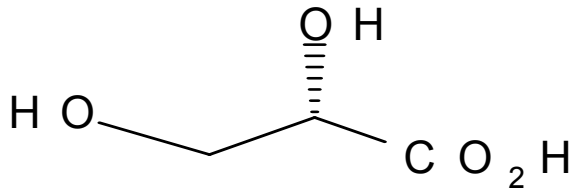


INTRODUCTION

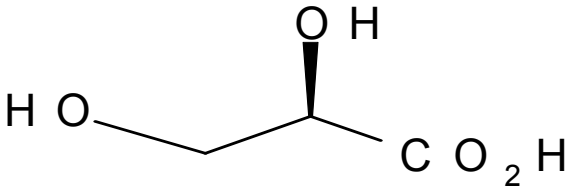
Enantiomer recognition is very important for clinical and biomedical laboratories especially when a fast diagnosis is requested.[1] Different enantioselective methods like electrochemical methods,[2] chromatography,[3] and electrophoresis[4] were applied up to now in biomedical analysis of enantiomers. Electrochemical techniques proved to be very reliable for the assay of enantiomeric purity of chiral compounds.[2] Enantioselective, potentiometric membrane electrodes (EPMEs) based on different chiral selectors such as cyclodextrins,[5-7] maltodextrins,[8] and macrocyclic antibiotics[9,10] were design for the enantioanalysis.

Hyperoxaluria type 2 (PH II) and D-glyceric academia/aciduria are two types of diseases caused by increase production of L- and D-glyceric acids (GAs), respectively.[11-15] PH II is associated with urolithiasis or nephrocalcinosis ended with terminal renal failure,[17] and characterized by high levels of L-glyceric acid. D-glyceric acidemia/aciduria is characterized by increased excretion of D-glyceric acid, delayed psychomotor growth, mental retardation and seizures.[18-20] Accordingly, the enantioanalysis of glyceric acid and its antipode is crucial for early discovery of PH II and D-glyceric acidemia/aciduria.

* Corresponding author: iustinavanstaden@yahoo.com



L(-)-glyceric acid



D(+)-glyceric acid

Fig. 1 – L- and D-glyceric acids.

Up to now, colorimetry,[21] capillary electrophoresis,[22] polarimetry,[23] capillary gas chromatography,[24-27] liquid chromatography,[28] and high performance liquid chromatography[29] methods were proposed for the determination of L- and D-glyceric acids.

In this paper, four EPMEs based on carbon paste impregnated with cyclodextrins (α-, β-, γ-, and 2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrin (β-cyclodextrin-derivative) are proposed for the enantioanalysis of L- and D-glyceric acids in biological samples. Cyclodextrins (CD) are oligosaccharides which consist of different glucose units linked to each other through α(1,4)-glucosidic bonds. Only three types of cyclodextrins and their derivatives were used as chiral selectors, termed as α-, β- and γ-CDs, those with six, seven and eight glucose units, respectively. CDs have the shape of a truncated cone and a relative hydrophobic cavity able to host analytes by means of inclusion-complexation.

EXPERIMENTAL

1. EPMEs design

Graphite powder and paraffin oil were mixed in a ratio 1:4 (w/v) followed by the addition of solution containing $1x10^{-3}$mol/L of α-, β-, γ-, or β-CD-derivative (100μL chiral selector solution to 100 mg carbon paste). A plain carbon paste was prepared by thoroughly mixing 100 mg of graphite powder with 40μL paraffin oil. The plain carbon paste was filled into a plastic pipette peak leaving a space of 3-4 mm into the top to be filled with the carbon paste that contains the chiral selector. The diameter of enantioselective, potentiometric membrane electrode was 3 mm. Electric contact was obtained by inserting Ag/AgCl wire into the carbon paste. The internal electrolyte solution of EPMEs was 0.1mol L^{-1} KCl. All the EPMEs tips were gently rubbed on fine abrasive paper to produce a flat surface. The surface of the electrodes was wetted with de-ionized water and then polished with an alumina paper (polished strips 30144-011, Orion) before use for the analysis. When not in use, the EPMEs designed for L- and D-GA electrodes were immersed in a 10^{-3}mol/L solution of L- or D-GA, respectively.

2. Materials and reagents

The cyclodextrins (α-, β-, γ-, and 2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrin (as chloride salt) (β-CD-derivative)) were supplied by Wacher-Chemie GmbH (Germany). Graphite powder (1-2μm, synthetic) was purchased from Aldrich (USA). Paraffin oil was purchased from Fluka (Switzerland). L- and D-GA were supplied by Sigma-Aldrich (USA). Phosphate buffer (pH = 3.5) was supplied by Merck (Germany). De-ionized water from a Modulab system (Continental Water Systems, San Antonio, TX, USA) was used for all reagents and solutions preparation.

Stock solutions (0.1mol/L) of L- and D-GA were prepared by dissolving the required amount of substance in de-ionized water and buffered (1:1 (v/v) de-ionized water: buffer) with phosphate buffer (pH 3.5). Stock solutions were stored at 4°C. Standard solutions of L- and D-GA ($1x10^{-10}$ – $1x10^{-2}$ mol/L) were prepared from the stocks solutions of L- and D-GAs by serial dilutions. All solutions were buffered with phosphate buffer (pH 3.5). 10^{-3} mol/L solutions of each cyclodextrins (α-, β-, γ-, and β-CD-derivative) were prepared.

3. Apparatus

The direct potentiometric measurements were recorded using a Metrohm 663 VA stand (Metrohm, Herisau, Switzerland) connected to a PGSTAT 20 and a software version 4.9 (Eco Chemie, Utretch, The Netherlands). An Ag/AgCl (0.1 mol/l KCl) electrode was used as reference electrode in the cell.

4. Recommended procedure

The direct potentiometric method was used for the potential determination of each standard solution (10^{-10}-10^{-2} mol/L, pH 3.50) of L- and D-GA. Calibration graphs were obtained by plotting E(mV) versus pL-GA and pD-GA, respectively. The unknown concentrations of L- and D-GA were determined in serum and urine samples by interpolation of the potential measured, into the calibration graphs.

RESULTS AND DISCUSSION

1. EPMEs response characteristics

The truncated cone shape of cyclodextrins and its relative hydrophobic cavity able to host analytes by means of inclusion-complexation is the reason of the interactions between enantiomers and chiral selectors. Weak bonds between substituent groups on the asymmetric center of analytes and secondary and/or primary hydroxyl groups of the CD are also responsible for chiral recognition.[4] From the proposed EPMEs, only those based on α- and γ-CD were working for the assay of L-GA, while β- and β-derivative-CD based EPMEs were working only for the assay of D-GA. The calibration equations obtained for L- and D-GA are:

E(mV) = 59.00 pL-GA + 139.00 (α-CD based EPME)
E(mV) = 52.80 pL-GA - 64.30 (γ-CD based EPME)
E(mV) = 58.00 pD-GA + 100.33 (β-CD based EPME)
E(mV) = 59.00 pD-GA - 160.50 (β-derivative-CD based EPME)

where E (mV) is the cell potential, pL-GA = -log[L-GA] and pD-GA = -log[D-GA]. All response characteristics of the electrodes are shown in Table 1. EPMEs displayed a good stability and reproducibility over the tests performed for 2 months, when they were used daily for measurements.

The response times were less than 1 min and 1min for the EPMEs designed for the assay of L-GA, respectively based on α- and γ-CD in the concentration ranges (10^{-9}-10^{-7} and 10^{-5}-10^{-2} mol/L). The response times for the determination of D-GA were > 1min when EPME based on β-CD was used in the concentration range 10^{-5}-10^{-3} mol/L and < 1min when EPME based on β-CD-derivative was used in the concentration range 10^{-6}-10^{-3} mol/L.

2. Selectivity of the EPMEs

The selectivity of the electrodes has been investigated using the mixed solutions method proposed by Ren,[30] and it was checked against L- or D-GA, creatine, creatinine, Na^+, K^+, and Ca^{2+}. The ratio between the concentrations of the main analyte and interfering ion was 1:10. The potentiometric selectivity coefficients, K_{sel}^{pot} (Table 2), proved that L(D)-GA, creatine and creatinine do not interfere in the determination of L- and D-GA, and that the proposed EPMEs are enantioselective. Also, inorganic cations such a Na^+, K^+, and Ca^{2+} do not interfere in the analysis of L- and D-GA, as the values of K_{sel}^{pot} obtained were lower than 10^{-3}.

Table 1

Response characteristics of EPMEs used for the determinations of L- and D-glyceric acids

Analyte	EPME based on	EPMEs response characteristics			
		Slope [mv/p(C)]	Intercept, E^o [mV]	Linear conc. range [mol/L]	Detection limit [mol/L]
L-GA	α-CD	59.00	139.00	10^{-9}-10^{-7}	1.48×10^{-11}
	γ-CD	52.80	-64.30	10^{-5}-10^{-2}	1.00×10^{-6}
D-GA	β-CD	58.00	-100.33	10^{-5}-10^{-3}	1.00×10^{-6}
	β-CD-derivative	59.00	-160.50	10^{-6}-10^{-3}	1.00×10^{-7}

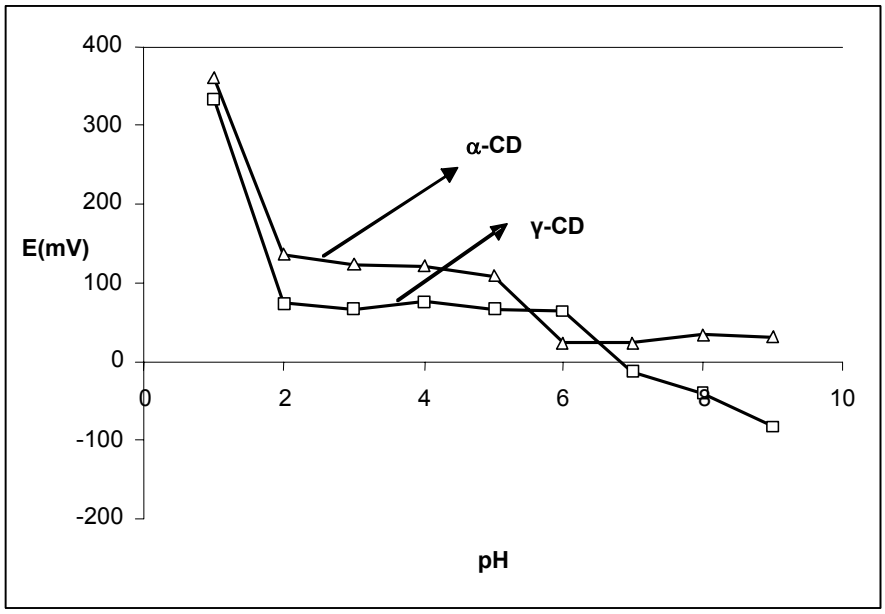
[a]All measurements were made at room temperature; all values are the average of ten determinations.

Table 2

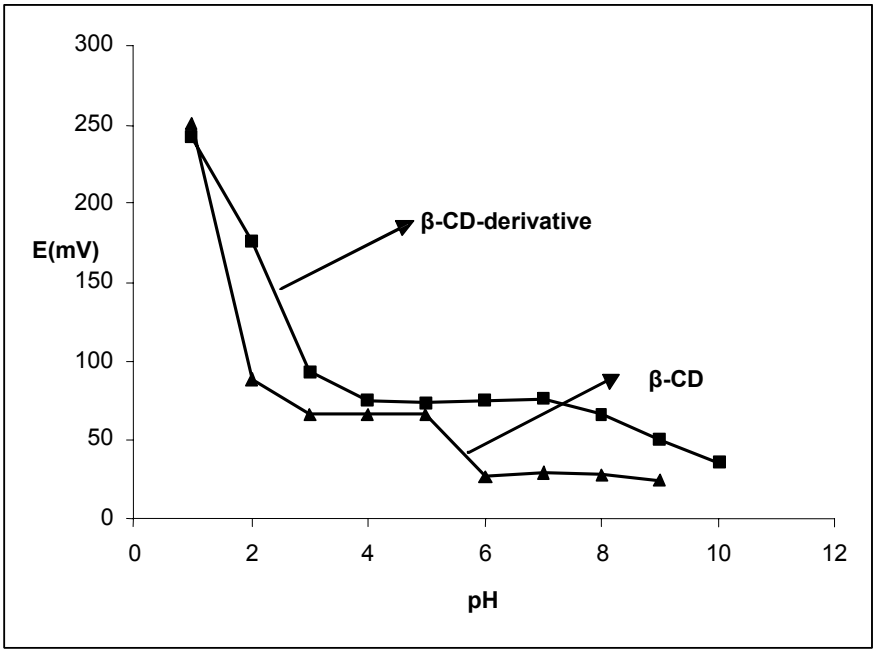
Selectivity coefficients for EPMEs

Interfering species (j)	K_{sel}^{pot} of EPME based on			
	α-CD	γ-CD	β-CD	β-CD-derivative
L-glyceric acid	-	-	3.89×10^{-3}	3.98×10^{-3}
D-glyceric acid	3.98×10^{-3}	4.46×10^{-3}	-	-
Creatine	3.83×10^{-3}	4.27×10^{-3}	4.05×10^{-3}	7.51×10^{-3}
Creatinine	7.51×10^{-3}	8.35×10^{-3}	7.63×10^{-3}	3.83×10^{-3}

All measurements were made at room temperature; all values are the average of ten determinations.



a



b

Fig. 2 – Influence of pH variation on the response of EPMEs based on a. α- and γ-cyclodextrins, respectively, for the assay of L-glyceric acid, ($C_{L\text{-}GA}$ is 10^{-8} and 10^{-4} mol/L, respectively), and b. β-cyclodextrin and 2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrin (as chloride salt) (β-CD-derivative), for the assay of D-glyceric acid ($C_{D\text{-}GA}$ is 10^{-4} mol/L, respectively).

3. Effect of pH on the response of the EPMEs

The influence of pH on the response of the proposed EPMEs was checked by measuring the potentials of the potentiometric cells at pHs between 1 and 10. Solutions of pHs between 1 and 10 of L- and D-GA were prepared by adding different volumes of HCl (0.1 mol/L) or NaOH solutions (0.1 mol/L) to their standard solutions.

Plots showing the variation of E (mV) with pH values are shown in Fig. 2a and b. For L-GA, the responses of EPMEs are pH-independent in the pH ranges 2.0-5.0 (α-CD based EPME) and 2.0-6.0 (γ-CD based EPME), while for D-GA the responses of EPMEs are not depending on pH in the ranges 3.0-5.0 (β-CD based EPME) and 4.0-7.0 (β-CD-derivative based EPME).

3. Analytical applications

The suitability of EPMEs was investigated for the recovery of L- and D-GA in the solutions containing the antipode of the enantiomer assayed. Solutions containing L:D or D:L of glyceric acid were prepared in different ratios (2:1 to 1:99.99) to check the recovery for L- and D-GA, respectively. The recovery tests demonstrated the suitability of the enantioselective, potentiometric membrane electrodes for the enantioanalysis of L- and D-GA (Table 3). No significant differences in the recovery values were recorded for the ratios between L:D or D:L enantiomers varying from 1:9 to 1:99.99.

Healthy volunteers donated serum and urine samples. These samples were stored at -20°C. Serum and urine samples were spiked with L- and D-GA. These samples were used for the recovery of L-GA and D-GA in the real matrices and to show the suitability of the EPMEs for the enantioanalysis of L- and D-GA in serum and urine samples. The results obtained for the analysis of L– and D-GA in serum and urine samples (Tables 4 and 5) were compared with those obtained using a chromatographic method of analysis.[22] The results obtained using the proposed EPMEs are in concordance with those obtained using the chromatographic method[22] showing the suitability of the proposed EPMEs for diagnosis of hyperoxaluria type 2 (PHII) and D-glyceric academia/aciduria.

Table 3

The recovery results obtained for the analysis of L- and D-glyceric acids in the presence of their antipode

L:D (mol/mol)	% L-GA, Recovery	
	EPMEs based on	
	α-CD	γ-CD
2:1	99.88±0.01	99.38±0.01
1:1	99.60±0.03	99.69±0.01
1:2	99.71±0.02	99.94±0.02
1:4	99.65±0.01	99.68±0.02
1:9	99.73±0.01	99.52±0.01
D:L (mol/mol)	% D-GA, Recovery	
	EPMEs based on	
	β-CD	β-derivative-CD
2:1	99.17±0.04	99.99±0.01
1:1	99.52±0.01	99.97±0.01
1:2	99.78±0.01	99.98±0.01
1:4	99.17±0.02	99.98±0.02
1:9	99.99±0.03	99.96±0.01

All measurements were made at room temperature; all values are the average of ten determinations.

Table 4

Recovery of L-glyceric acid in serum and urine samples

Type of sample	Sample no.	% L-GA, Recovery		
		Chromatographic method[22]	EPMEs based on	
			α-CD	γ-CD
Serum	1	99.36	99.44±0.01	99.09±0.04
	2	99.68	98.65±0.02	99.72±0.02
	3	99.39	98.37±0.03	99.03±0.03

Table 4 (continued)

Urine	4	99.75	99.76±0.02	99.73±0.03
	5	99.57	99.54±0.02	99.59±0.01
	6	99.60	99.52±0.01	99.69±0.01
	7	99.57	99.60±0.03	99.50±0.02
	8	99.80	99.81±0.01	99.72±0.01
	9	99.63	99.62±0.01	99.65±0.04

All measurements were made at room temperature; all values are the average of ten determinations.

Table 5

Recovery of D-glyceric acid in serum and urine samples

Type of sample	Sample no.	% D-GA, Recovery		
		Chromatographic method[22]	EPMEs based on	
			β-CD	β-CD-derivative
Serum	1	99.44	99.08±0.02	99.55±0.01
	2	99.79	99.27±0.01	99.98±0.02
	3	99.51	99.36±0.01	99.87±0.02
Urine	4	99.47	99.57±0.02	99.33±0.02
	5	99.68	99.75±0.01	99.63±0.01
	6	99.87	99.99±0.01	99.52±0.02
	7	99.76	99.93±0.02	99.66±0.01
	8	99.78	99.91±0.02	99.59±0.02
	9	99.72	99.89±0.01	99.67±0.01

All measurements were made at room temperature; all values are the average of ten determinations.

CONCLUSIONS

The proposed method proved to be reliable for the enantioanalysis of L- and D-glyceric acids in biological fluids (serum and urine). The serum and urine samples did only need to be buffered before analysis.

The enantioselective, potentiometric membranes electrodes based on cyclodextrins (α- β-, γ-cyclodextrins and 2-hydroxy-3-trimethylammonio-propyl-β-cyclodextrin (as chloride salt)) have excellent features in biomedical enantioanalysis of L- and D-glyceric acids. Miniaturization of these electrodes will make possible *in vivo* diagnosis of hyperoxaluria type 2 (PHII) and D-glyceric academia/aciduria.

REFERENCES

1. H. Y. Aboul-Enein, "The Impact of Stereochemistry on Drug Development and Use", Marcel Dekker, New York, 1997.
2. R.I. Stefan, J.F. van Staden and H.Y. Aboul-Enein, "Electrochemical Sensors in Bioanalysis", Marcel Dekker Inc., NY, USA, 2001.
3. T.J. Ward and A.B. Farris III, *J. Chromatogr. A*, **2001**, *906,* 73-89.
4. S. Fanali, *J. Chromatogr. A*, **1996**, *735*, 77-121.
5. R. I. Stefan, J. F. van Staden and H. Y. Aboul-Enein, *Electroanalysis*, **1999**, *11***,** 1233-1235.
6. R. I. Stefan, J. F. van Staden and H. Y. Aboul-Enein, *Chirality,* **1999**, *11*, 631-634.
7. R. I. Stefan, J. F. van Staden and H. Y. Aboul-Enein, *Anal. Lett.*, **1998**, *31*, 1787-1794.
8. R. I. Stefan, J. F. van Staden and H. Y. Aboul-Enein, *Fresenius' J. Anal. Chem.***, 2002**, *370*, 33-37.
9. A. A. Rat'ko, R. I. Stefan, J. F. van Staden and H. Y. Aboul-Enein, *Talanta,* **2004,** *63*, 515-519.
10. A. A. Rat'ko, R. I. Stefan, J. F. van Staden and H. Y. Aboul-Enein, *Sens. Actuators B*, **2004**, *99*, 539-543.
11. H. E. Williams and L. H. Smith, *N. Engl. J. Med.*, **1968**, 278, 233-238.
12. N. J. Brandt, S. Brandt, K. Rasmussen and F. Schønheyder, *British Med. J.*, **1974**, *4*, 344-347.
13. E. van Schaftingen, J. P. Draye, and F. van Hoof, *Eur. J. Biochem.*, 1989, *186*, 355-359.
14. P. D. Dawkins and F. Dickens, *Biochem. J.*, **1965**, *94*, 353-367.
15. E. van Schaftingen, *FEBS Lett.*, **1989**, *243*, 127-131.

16. H. E. Williams and L. H. Smith, *Science*, **1971**, *171*, 390-391.
17. M. A. Mansell, *Nephro. Dial. Trans.*, **1995**, *10*, 58-60.
18. N. J. Brandt, K. Rasmussen, S. Brandt, S. Kølvraa and F. Schønheyder, *Acta Paediatric Scandinavica*, **1976**, *65*, 17-22.
19. S.K. Wadman, M. Duran, D. Ketting, L. Bruinvis, P.K. de Bree, J.P. Kamerling, J.F.G Vliegenthart, H. Przyrembel, K. Becker, H.J. Bremer, *Clin. Chim. Acta.*, **1976**, *71*, 477-484.
20. M. Duran, F. A. Beemer, L. Bruinvis, D. Ketting, S.K. Wadman, *Pediatr. Res.*, **1987**, *21*, 502-506.
21. G. R. Bartlett, *J. Biol. Chem.*, **1959**, *234*, 469-471.
22. A. Garcia, M. Muros, C. Barbas, *J. Chromatogr. B*, **2001**, *755*, 287-295.
23. M. Fontaine, N. Porchet, C. Largilliere, S. Marrakchi, M. Lhermitte, J. P. Aubert and P. Degand, *Clin Chem.*, **1989**, *35*, 2148-2151.
24. A. Kaunzinger, A. Rechner, T. Beck, A. Mosandl, A. Sewell and H. Bohles, *Enantiomer*, **1996**, *1*, 177-182.
25. J. P. Kamerling, G. J. Gerwig, J. F. G. Vliengenthart, M. Duran and S. K. Wadman, *J. Chromtogr.*, **1977**, *143*, 117-123.
26. D.J. Dietzen, T.R. Wilhite, D.N. Kenagy, D.S. Milliner, C.H. Smith and M. Landt, *Clin. Chem.*, **1997**, *43*, 1315-1320.
27. M. Petrarula, C. Vitale, P. Facchini and M. Marangella, *J. Nephr.*, **1998**, *11*, 23-28.
28. M.S. Rashed, H.Y. Aboul-Enein, M. AlAmoudi, M. Jakob, L.Y. Al-Ahaideb, A. Abbad, S. Shabib and E. Al-Jishi, *Biomed. Chromatogr.*, **2002**, *16*, 191-198.
29. M. Petrarula, M. Marangella, D. Coseddu and F. Linari, *Clin. Chim. Acta*, **1992**, *211*, 143-153.
30. K. Ren, *Fresenius' J. Anal. Chem.*, **1999**, *365*, 389-397.