Journal of Chromatography B, 877 (2009) 1678–1682

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Simultaneous determination of tryptophan and kynurenine in plasma samples of children patients with Kawasaki disease by high-performance liquid chromatography with programmed wavelength ultraviolet detection

Xiaoqing Zhang[a], Yun He[b], Min Ding[a,*]

[a] Key Laboratory of Laboratory Medical Diagnostics, Ministry of Education, Chongqing Medical University, Chongqing 400016, China
[b] Division of Immunology, Children's Hospital, Chongqing Medical University, Chongqing 400014, China

ARTICLE INFO

Article history:
Received 27 June 2008
Accepted 3 April 2009
Available online 9 April 2009

Keywords:
Tryptophan
Kynurenine
Indoleamine 2,3-dioxygenase
HPLC
Kawasaki disease

ABSTRACT

A simple, fast, sensitive and specific high-performance liquid chromatography (HPLC) method is developed for simultaneous determination of kynurenine (Kyn) and tryptophan (Trp) with ultraviolet (UV) detection setting programmed wavelength. The separation was carried out on an Agilent Hypersil ODS column (125 mm × 4.0 mm, 5 μm) in less than 6 min and the eluate was monitored by the programmed wavelength detection setting at 360 nm from 0 min to 4 min for Kyn, and at 278 nm from 4 min to 6 min for Trp in a single run with UV detector. The linearities of the method were from 0.20 μmol/L to 21.2 μmol/L for Kyn and 2.25–678.0 μmol/L for Trp, and the detection limits were 0.028 μmol/L for Kyn and 0.053 μmol/L for Trp, respectively. Satisfactory precisions and recoveries were obtained by this method. The assay was employed to analyze plasma samples of children patients with Kawasaki disease (KD). The result showed great difference between Kawasaki disease and control group.

1. Introduction

L-Tryptophan (Trp) is an essential amino acid that is required for the biosynthesis of proteins and is important in nitrogen balance and the maintenance of muscle mass and body weight in humans [1–4]. The degradation of tryptophan takes place via two biochemical pathways: biosynthesis of the neurotransmitter serotonin and the kynurenine (Kyn) pathway [5,6]. Trp is the substrate for the first step in both of these pathways. In the former pathway, Trp is transformed to 5-hydroxytryptamine (serotonin) by tryptophan 5-hydroxylase enzyme. In the Kyn pathway which is the major catabolic route for Trp, the indole ring of Trp can be opened by the enzymes tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO), yielding N-formylkynurenine which is then converted to Kyn [6–11]. The reactions catalyzed by both of these enzymes constitute the rate-limiting step in the Kyn metabolic pathway [2,4,6]. Many studies showed that, like TDO, IDO is also a heme-containing enzyme, despite the fact that it shares no sequence homology with TDO [12]. TDO is present mostly in liver, and it is not only found in mammals, but also in mosquitoes and bacteria whereas IDO only exists in mammals and is ubiquitously distributed in tissues including the brain, lung, spleen, placenta and blood [1,12].

The expression of IDO is induced by IFN-γ and is closely linked to a wide spectrum of immune-related pathophysiologic conditions, in contrast to TDO which is inducible by glucocorticoid hormones and is regulated by the availability of the physiological substrate, Trp [12]. Inflammatory cytokines, produced as a consequence of parasite, bacterial or viral infections, increase IDO activity and expression thus causing a reduction in Trp and an increase in Kyn metabolites in blood and brain [10]. IDO has recently been linked with immunoregulation [5,13]. T-cell proliferation can be inhibited by IDO activity and immune response may be suppressed when Trp is diminished due to activated IDO, which occurs not only during infectious diseases but also in cancer and autoimmune diseases [14–16]. IDO activity is characterized by the kynurenine to tryptophan ratio (Kyn/Trp) which correlates with concentrations of immune activation markers such as neopterin [5,7,16]. The Kyn/Trp ratio is therefore considered as a more reliable marker for IDO-induced Trp catabolism than serum Trp or Kyn concentration alone [5].

To determine the concentrations of Kyn and Trp may help to estimate IDO activity. Several protocols [2,3,5,17] have been proposed to determine Kyn and Trp in biological fluids, among which HPLC-based analyses are the commonly used methods for the determination of Trp and its metabolites, including high-performance liquid chromatography (HPLC) methods with UV detection for Kyn and fluorimetric detection for Trp [8,10,18–20], HPLC with coulometric detection [6], HPLC with electrochemical array detection [21]. Recently, Yamada et al. reported the simultaneous mea-

* Corresponding author. Tel.: +86 23 68485217; fax: +86 23 68485005.
E-mail address: dingmin@cqmu.edu.cn (M. Ding).

1570-0232/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2009.04.013

surement of Trp and Kyn by HPLC–MS/MS [22] both intra- and extracellularly. It took about 12 min to separate the two components. Marklová's group [23] screened the defects in tryptophan metabolism by HPLC. When the simultaneous detection of Kyn and Trp was performed at 254 nm, the two components could not separate effectively from the plasma endogenesis especially when the concentration was very low within 11 min. The limits of detection could not satisfy the clinical plasma samples. Wang and Tang [24] reported simultaneous determination of Kyn and Trp by HPLC with UV detection at 225 nm. As it can be seen in this article, Kyn and Trp could be both detected with a fixed wavelength in 9 min which was the fastest speed for separating the two components which have been reported. However, the inclined baseline and the unsymmetrical peak of Kyn were not beneficial for the exact quantification due to the absorbance of solvent before 3 min at 225 nm. In addition, Kawai's group [25] reported simultaneous measurement of Kyn and Trp in plasma by column switching-HPLC method. The retention time for Kyn and Trp was 21 min and 35 min, respectively. Furthermore, it needs two columns and a high-pressure switching hexagonal valve which made the instrument complex. However, most of these methods are time consuming, employing multi-step detection or multi-detectors. The purpose of this study is to develop a rapid and specific HPLC protocol with UV detection setting programmed wavelength for simultaneous measurement of Kyn and Trp in plasma in a single run. HPLC with programmed wavelength ultraviolet detection was designed to eliminate the interferences of endogenesis and quantify the concentrations of Kyn and Trp simultaneously in clinical plasma samples.

Kawasaki disease (KD) is an acute self-limited vasculitis that occurs predominantly in infants and young children [26]. Although it is first described in Japan in 1967, the immunopathological mechanisms involved in the pathogenesis of KD remains still unclear [27,28]. This paper aims to find some relationship between KD and the Kyn/Trp ratio. The Trp and Kyn in plasma of children patients with KD were analyzed by the developed assay, and then Kyn/Trp ratio was calculated to estimate the activity of IDO. The result showed great difference between KD and the control group which will perhaps provide a potential diagnostic index for clinicians.

2. Experiments

2.1. Chemicals and reagents

Trp and Kyn were purchased from Sigma Chemical (St. Louis, MO, USA). Other chemicals were of analytical reagent grade and obtained from Shanghai Company of Chemical Reagent (Shanghai, China). Chromatographic grade acetonitrile was purchased from Tedia (Fairfield, OH, USA). Chromatographic grade water was obtained from Millipore pure water purification system (Millipore, Milford, MA, USA). The mobile phase was prepared daily and filtered through a 0.22 μm Millipore filter (Millipore, Milford, MA, USA).

Plasma of healthy children and children with KD were obtained from Children's Hospital of Chongqing Medical University (Chongqing, China). The plasma of healthy children was from the children volunteers who came for health checkup. The clinical diagnosis of Kawasaki disease requires a history of fever of 5 days duration, plus 4 of the following 5 associated physical findings: polymorphic exanthema; changes in peripheral extremities, in acute phase: erythema and/or indurative edema of the palms and soles and in convalescent phase: desquamation from finger tips; bilateral non-exsudative conjunctival injection; changes in the oropharynx: injected or fissured lips, strawberry tongue, injected pharynx; acute non-suppurative cervical lymphadenopathy (>1.5 cm in diameter) [29,30]. All plasma samples were stored at −20 °C until analyzed. The sample collection and use for this study have been approved by the ethic committees of Children's Hospital of Chongqing Medical University.

2.2. Instrumentation and chromatographic conditions

The HPLC system was an Agilent (Palo Alto, CA, USA) LC system equipped with vacuum degasser (G1322A), quatpump (G1311A), manual-injector (G1328) and variable wavelength detector (VWD, G1314A). The data were acquired and processed with Agilent Chemstation software. An Agilent Hypersil ODS column (125 mm × 4.0 mm, 5 μm) was used for the analysis at 25 °C. The chromatographic separation was carried out using the mobile phase consisting of 15 mmol/L acetate buffer (pH 4.0) and acetonitrile (95:5, v/v) at a flow rate of 0.8 mL/min. The eluate was monitored by the programmed wavelength detection setting at 360 nm from 0 min to 4 min for Kyn and at 278 nm from 4 min to 6 min for Trp.

2.3. Standard solution and sample preparation

The stock solutions of Kyn and Trp were dissolved in methanol at the concentrations of 5.00 mmol/L and 20.0 mmol/L, respectively and stored at −20 °C. A series of mixed standard working solutions with different concentrations were obtained by further dilution of each standard stock solution with the mobile phase immediately before use. For protein precipitation, 100 μL of the plasma sample was mixed with 13 μL 40% perchloric acid, followed by vortex-mixing for 1 min and centrifuged at 15 000 g for 10 min at 4 °C. Ultimately 20 μL of the supernate was injected into the HPLC system for analysis.

2.4. Method validation

2.4.1. Calibration curve and linearity

The calibration samples were prepared by adding appropriate amount of standard working solutions to both physiological solution and the healthy mixed children plasma. The calibration samples were performed protein precipitation as described above. Nine-point calibration curves were constructed in triplication by adding nine series of 10 μL of mixed standard working solutions of Kyn and Trp into 90 μL of the physiological solution. The calibration curves were established by plotting the peak area of Kyn or Trp, versus the concentrations of Kyn of Trp in the calibration samples. The regression parameters of slope and correlation coefficient were calculated by linear least square regression. And the calibration samples were also added to the healthy mixed children plasma to establish the calibration curve. Since Kyn and Trp are endogenous in plasma, for preparing the calibration curves with mixed healthy plasma, the peak areas of Kyn and Trp in the mixed healthy plasma was subtracted from the peak areas after adding the calibration standards into the mixed healthy plasma in chromatography, which represented the peak areas of Kyn and Trp in calibration standards. The limit of quantification (LOQ) was calculated by $10\sigma/S$ where σ represents the standard deviation of the determination and S represents the slope of the calibration curve.

2.4.2. Accuracy and precision

The accuracy and precision of this method were determined by adding defined amounts of Kyn and Trp into mixed healthy plasma and each was divided into 9 and stored at −20 °C. The accuracy was determined by analyzing three plasma samples adding standard working solutions of Kyn and Trp at the low, middle and high concentrations. The inter-day variation was assessed by measuring these aliquots on 5 consecutive days, using one freshly thawed sample every day, while the intra-day variation was assessed by consecutive determinations of 5 samples in a day.

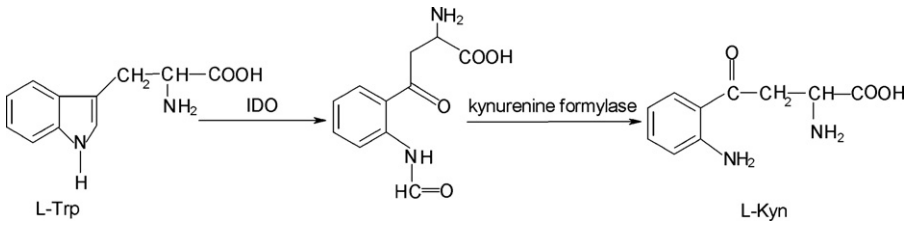

Fig. 1. The catabolism of Trp to Kyn.

2.4.3. Recovery

To estimate the recovery of the method, 90 μL of the mixed healthy plasma was spiked with 10 μL of mixed standard working solution of Kyn and Trp at low, middle and high concentrations. And 90 μL of the mixed healthy plasma was spiked with 10 μL of the mobile phase was used as the blank sample to estimate the endogenous concentration. Sample recovery is expressed as [(found concentration − endogenous concentration)/spiked concentration] × 100%.

2.5. Clinical application

40 children patients with KD and 20 healthy children, less than 15 years old, were analyzed using the developed assay and the Kyn/Trp ratio was calculated. The data were presented as means ± S.D. and analyzed by one-way analysis of variance (ANOVA) using SPSS13.0 statistical software. p values less than 0.05 were considered as statistically significant.

3. Results and discussion

3.1. Detection condition

The proposed HPLC method for simultaneous determination of Kyn and Trp in human plasma is based on the programmed wavelength detection.

Trp which contains an indole ring can produce natural fluorescence, whereas Kyn cannot emit fluorescence itself owing to its intrinsic characteristic (Fig. 1). The samples must be derivatized with fluorescent agents by simultaneous determination of Kyn and Trp by fluorescence detection. Most simultaneous determination of Kyn and Trp was based on the fluorescence determination of Trp and the ultraviolet determination of Kyn. Thus the HPLC system needs to equip with both the fluorescence detector and the ultraviolet detector.

This study aims to develop a simple method for simultaneously rapid determination of Kyn and Trp in a single run with a single detector. The concentration of Kyn is much lower than Trp in plasma, so the most appropriate condition should first be considered for Kyn. There are three absorbance peaks in ultraviolet spectrogram of Kyn at 225 nm, 258 nm and 360 nm, respectively. Most plasma endogenesis, such as kynurenic acid, 3-hydroxykynurenine, 5-hydroxytrptophan and N-formylkynurenine have absorptions at 225 nm or 258 nm which will affect the quantification of Kyn. The obvious interference of endogenous components or solvent peaks near the peak of analyte at 225 nm or 258 nm greatly decreases the accuracy of the assay. Although the absorption coefficient of Kyn at 360 nm is lower than 258 nm, there is little absorbance of endogenous components at 360 nm and the lowest limit of quantification can satisfy the analysis in clinical plasma samples. Accordingly the determination is performed at 360 nm for Kyn. However, there is no evident absorption for Trp at 360 nm which makes simultaneous determination of the two components difficult. The maximum absorption wavelength of Trp emerges at 278 nm. So programmed wavelength ultraviolet detection was designed in this study and 360 nm was selected to eliminate the interference peaks of other endogenesis and quantify Kyn from 0 min to 4 min and quantification of Trp at 278 nm from 4 min to 6 min. The baseline was adjusted to zero before sample injection. The wavelength changed from 360 nm to 278 nm at 4 min which would make the baseline elevated. To make the chromatogram looks friendly, the baseline back to zero was selected at 4 min. Thus simultaneous determination of Kyn and Trp could be realized in a single run, while most methods reported needs to detection of Kyn and Trp, respectively which made the determination complicated. The retention times of Kyn and Trp are 3.3 min and 5.0 min, respectively. It took only 5 min to separate the two components in plasma samples, which was more rapid than other methods reported previously. The resolution is more

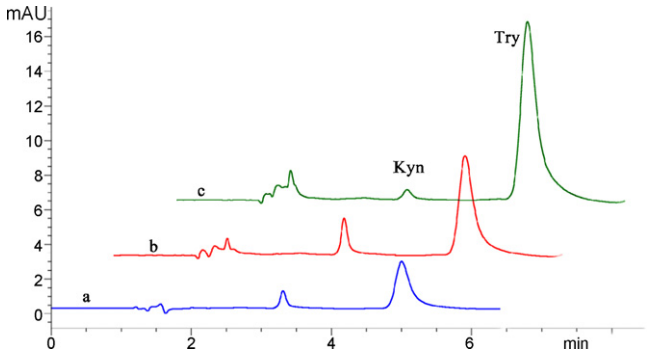

Fig. 2. HPLC chromatograms of Kyn and Trp by programmed wavelength detection. 360 nm was set from 0 min to 4 min and 278 nm was set from 4 min to 6 min. (a) Mixture of standards. (b) Plasma of a child patient with KD. (c) Plasma of a healthy child.

Table 1
The intra-day and inter-day precisions of Kyn and Trp in plasma (n = 5).

	Intra-day		Inter-day	
	$\bar{x}$ ± S.D. (μmol/L)	CV (%)	$\bar{x}$ ± S.D. (μmol/L)	CV (%)
Kyn	2.24 ± 0.014	0.61	2.20 ± 0.045	2.04
	4.01 ± 0.092	2.31	4.05 ± 0.237	5.86
	11.2 ± 0.28	2.48	12.3 ± 0.59	4.82
Trp	57.1 ± 0.66	1.15	56.5 ± 0.62	1.09
	113.7 ± 3.79	3.34	121.6 ± 6.95	5.71
	357.1 ± 8.33	2.33	373.1 ± 22.8	6.12

Table 2
The recoveries of Kyn and Trp in plasma (n = 5).

Component	Spiked concentration (μmol/L)	Recovery (%)	CV (%)
Kyn	0.66	112.6 ± 2.07	1.84
	2.65	94.8 ± 3.49	3.68
	10.6	92.0 ± 2.63	2.86
Trp	21.2	118.0 ± 3.10	2.63
	84.8	96.3 ± 4.48	4.65
	339.0	95.9 ± 2.46	2.56

Table 3
Concentrations of Kyn and Trp in children plasma of control group and KD group.

Group	c(Kyn) (μmol/L)	CV (%)	c(Trp) (μmol/L)	CV (%)	c(Kyn)/c(Trp)	CV (%)
Control	1.61 ± 0.31	19.2	44.1 ± 7.52	17.0	0.037 ± 0.007	18.0
KD	2.40 ± 0.89*	37.3	28.5 ± 17.3*	60.7	0.119 ± 0.089*	74.8

* There is significant difference between the KD group and the control group, $p < 0.01$.

than 6.0, indicating that the two components can be separated effectively. No obvious absorbance of other endogenous components in the plasma sample can be seen from the chromatogram (Fig. 2).

3.2. Linearity

The linear equation for Kyn was $Y = 6.09X - 0.82$ ($r = 0.9991$) and the linear equation for Trp was $Y = 6.71X - 7.77$ ($r = 0.9993$) in physiological solution. X means the concentrations of Kyn or Trp in μmol/L. Y means the peak area of Kyn or Trp in the corresponding calibration standards adding to physiological solution. And the LOQ were 0.20 μmol/L for Kyn and 2.25 μmol/L for Trp, respectively. Peak areas for Kyn in physiological solution were correlated over a range of concentrations from 0.20 μmol/L to 21.2 μmol/L and for Trp over a range of concentrations from 2.25 μmol/L to 678.0 μmol/L, respectively. The limit of detection (LOD) that can be reliably detected with an S/N ratio of 3 was found to be 0.028 μmol/L for Kyn and 0.053 μmol/L for Trp, respectively. And the linear equation for Kyn was $Y = 5.94X - 0.78$ ($r = 0.9992$) and the linear equation for Trp was $Y = 6.65X - 17.8$ ($r = 0.9995$) in mixed healthy plasma. X means the concentrations of Kyn or Trp in μmol/L. Y means the peak area of Kyn or Trp in the calibration standards added to mixed healthy plasma subtracting the corresponding peak area of Kyn or Trp in the mixed healthy plasma. And the LOQ were 0.27 μmol/L for Kyn and 3.35 μmol/L for Trp, respectively. Peak areas for Kyn were correlated over a range of concentrations from 0.27 μmol/L to 21.2 μmol/L and for Trp over a range of concentrations from 3.35 μmol/L to 678.0 μmol/L, respectively in mixed healthy plasma. Compared with the calibration of adding the standards in plasma, calibration curve in physiological solution showed that the matrix effect was not evident. So we set the calibration curve in physiological solution in the work.

An internal standard method is always used in bioanalysis quantification. However, it is difficult to find a suitable internal standard in this study due to the significant difference of the concentrations of the analytes. The external standard curve was used in this method. 10 μL of mixed standard working solutions were added into 90 μL of physiological solution to analyze the Kyn and Trp. The accuracy of quantity of the sample injection became a most important issue.

3.3. Accuracy and precision

The intra-day and inter-day precision data were given in Table 1, indicating intra-day CV values were less than 4% and inter-day CV values were less than 7% for both Kyn and Trp.

3.4. Extraction recovery

The recoveries were analyzed and the result was shown in Table 2. The mean recoveries of the extraction procedures were in the range of 92.0–118.0%.

3.5. Clinical application

The concentrations of Kyn and Trp in plasma of 40 children patients with KD and 20 healthy children were analyzed using the developed assay and the result was shown in Table 3.

For the etiopathogenesis of KD, there is no agreement even as to whether KD is an infectious disease or an immune-mediated disease [28], saying nothing of any diagnosis index for KD. Diagnosis of KD is made on the basis of clinical criteria and non-specific laboratory findings [31]. Thus it will delay the optimal time for therapy of the disease.

Previous reports show that KD is associated with the activation of cellular immune system [32]. A decrease of Trp with a parallel increase of Kyn indicates an enhanced cytokine-induced degradation of tryptophan. Furthermore, the ratio of Kyn to Trp seems to be a sensitive indicator for interferon-γ induced tryptophan degradation and therefore for an activated immune system [2,3]. The Kyn to Trp ratio of the first product of TDO and IDO versus the concentration of their substrate is an appropriate indicator of tryptophan degradation. Many works have been done to demonstrate concomitant immune system activation in order to substantiate that tryptophan degradation is due to activation of IDO rather than TDO [2,33]. Thus, activated IDO is indicated when Kyn/Trp correlates with an immune activation parameter and endogenous IFN-γ formation [7].

The concentrations of Kyn and Trp in children plasma of healthy controls are 1.61 ± 0.31 μmol/L, 44.1 ± 7.52 μmol/L, respectively and the ratio of Kyn to Trp is 0.037 ± 0.007, which are different from the concentrations of adult healthy controls 1.92 ± 0.58 μmol/L for kynurenine, 73.0 ± 14.9 μmol/L for tryptophan and 0.0269 ± 0.0081 for the ratio by Fuchs and co-workers [2]. Kyn was found to be higher concentrations while the concentration of Trp be lower in KD group ($p < 0.01$) compared with the control group. And Kyn/Trp ratio showed much higher levels in the KD group ($p < 0.01$) than in the control one. A decrease of Trp with a parallel increase of Kyn was seen in KD group and the Kyn/Trp increased, indicating that IDO was activated with concomitant immune system activation. Furthermore, T-cell proliferation and immune response could be inhibited by IDO activity in KD group, which might provide the potential mechanism for clinical study of KD. Larger clinical trials will be necessary to find out more about the potential prognostic expressiveness of tryptophan metabolism in patients suffering from KD.

4. Conclusion

In summary, the present method by HPLC with programmed wavelength detection is well suited for high throughput of samples due to its more simple, rapid and sensitive than other methods of Kyn and Trp simultaneous determination. The simple sample preparation method improves the reproducibility so that an external standard curve method could be used in quantification with satisfactory precision and accuracy. The method meets the requirements for routine analysis of Kyn and Trp in plasma and Kyn/Trp which is of interest in immunological research may provide a useful tool for clinical studies. The concentrations of Kyn and Trp in plasma of children patients with KD were analyzed by this assay and the Kyn/Trp ratio was calculated. The result showed great difference in concentrations of Kyn and Trp between Kawasaki disease and control group which will perhaps provide a potential diagnosis index for clinicians.

Acknowledgments

The authors would like to thank Children's Hospital of Chongqing Medical University kindly for providing plasma samples of children patients with Kawasaki disease as well as healthy children plasma.

References

[1] A.M. Myint, Y.K. Kim, R. Verkerk, J. Affect. Disord. 98 (2007) 143.
[2] B. Widner, E.R. Werner, D. Fuchs, Clin. Chem. 43 (1997) 2424.
[3] A. Laich, G. Neurauter, B. Widner, D. Fuchs, Clin. Chem. 48 (2002) 579.
[4] A. Matin, I.M. Streete, I.M. Jamie, J.F. Jamie, Anal. Biochem. 349 (2006) 96.
[5] M. Pertovaara, A. Raitala, H. Uusitalo, Clin. Exp. Immunol. 142 (2005) 155.
[6] B. Maneglier, C.R. Kreuz, P. Cordonnier, Clin. Chem. 50 (2004) 2166.
[7] K. Schröcksnadel, B. Wirleitner, C. Winkler, D. Fuchs, Clin. Chim. Acta 364 (2006) 82.
[8] E. Kwidzinski, J. Bunse, O. Aktas, FASEB J. 19 (2005) 1347.
[9] T. Fukushima, S. Mitsuhashi, M. Tomiya, Clin. Chim. Acta 377 (2007) 174.
[10] A. Cozzi, A.L. Zignego, R. Carpendo, F. Moroni, J. Viral Hepat. 13 (2006) 402.
[11] H. Sugimoto, S.I. Oda, Y. Shiro, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 2611.
[12] D. Batabyal, S.R. Yeh, J. Am. Chem. Soc. 129 (2007) 15690.
[13] S. Lob, A. Konigsrainer, R. Schafer, Blood 111 (2008) 2152.
[14] R.R. Brown, Y. Ozaki, S.P. Datta, Adv. Exp. Med. Biol. 294 (1991) 425.
[15] O. Karola, A. Oliver, S.K. Kwang, J. Virol. 79 (2005) 7768.
[16] K. Schroecksnadela, C. Winkler, L.C. Fuith, D. Fuchsa, Cancer Lett. 223 (2005) 323.
[17] W. Wang, B. Qiu, X.Q. Xu, G.N. Chen, Electrophoresis 25 (2004) 903.
[18] J. Vignau, M.C. Jacquemont, A. Lefort, M. Lhermitte, Biomed. Chromatogr. 18 (2004) 872.
[19] E. Alegre, A.S. López, A. González, Anal. Biochem. 339 (2005) 188.
[20] S.L. Hwang, N.P.Y. Chung, J.K.Y. Chan, C.L.S. Lin, Cell Res. 15 (2005) 167.
[21] A. Vaarmann, A. Kaska, U. Mäeorg, J. Chromatogr. B 769 (2002) 145.
[22] K. Yamadaa, T. Miyazakib, T. Shibatac, J. Chromatogr. B 867 (2008) 57.
[23] E. Marklová, H. Makoviková, I. Krákorová, J. Chromatogr. A 870 (2000) 289.
[24] R. Wang, A.G. Tang, Chin. J. Chromatogr. 24 (2006) 140.
[25] K. Kawai, H. Ishikawa, K. Ohashi, Intern. Congr. Ser. 1304 (2007) 415.
[26] K. Stankovic, P. Miailhes, D. Bessis, J. Infect. 55 (2007) 488.
[27] C. Catalano-Pons, C. Giraud, F. Rozenberg, D. Gendrel, Clin. Microbiol. Infect. 13 (2007) 1220.
[28] K.Y. Lee, J.W. Han, J.S. Lee, Med. Hypotheses 69 (2007) 642.
[29] A.M. Rauch, E.S. Hurwitz, Pediatr. Infect. Dis. 4 (1985) 702.
[30] E.J. Tizard, Curr. Paediatr. 15 (2005) 62.
[31] L.E. Wood, R.M.R. Tulloh, Paediatr. Child Health 18 (2008) 70.
[32] J.C. Burns, M.P. Glodé, Lancet 364 (2004) 533.
[33] D. Fuchs, A.A. Möller, G. Reibnegger, J. Acquir. Immune Defic. Syndr. 3 (1990) 873.