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Short communication

Liquid chromatographic determination of γ-aminobutyric acid in cerebrospinal fluid using 2-hydroxynaphthaldehyde as derivatizing reagent

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Abstract

gamma Aminobutyric acid (GABA) was determined by precolumn derivatization with 2-hydroxynaphthaldehyde and elution was made using Phenomenex C_{18}, 5 μm column with methanol: water (62:38 v/v) and UV detection at 330 nm. In a mixture containing glycine, l-lysine and tyramine GABA separated completely. A number of amines and amino acids tested did not affect the response of GABA. A linear calibration curve was obtained for GABA in the range of 1.2–28.0 μg/ml with detection limit of 2.8 ng/injection (5 μl). The method was used for the determination of GABA in cerebral spinal fluid (CSF) samples and gave results of 19.0 to 22.4 μg/m1 with coefficient of variation 2.4%

Keywords: Derivatization; LC; γ-Aminobutyric acid

1. Introduction

gamma Aminobutyric acid (GABA) is an important inhibitory neurotransmitter in the central nervous system and essential for brain metabolism and function. A number of analytical methods have been reported for the determination of GABA in biological samples which include magnetic resonance spectrometry [1–4], electrochemical sensor [5–7], fluorimetric [8,9], spectrophotometric [10,11], capillary electrophoresis [12–14], radio receptor assay [15], gas [16–20] and liquid chromatography [21–24]. The high-performance liquid chromatographic (HPLC) methods are more reported with pre and post column derivatization using analytical and micro-bore columns [21–36]. A number of different reagents have been proposed for precolumn derivatization, which include 2,4,6-trinitrobenzenesulphonic acid [25], dansyl chloride [26,31], orthophthalaldehyde [21,22,27–30,32,36], dabsyl chloride [37], 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate [24], 9-fluorenylmethyl chloroformate [38] and phenylthiocarbonyl derivatives [39].

A selective analytical procedure for the determination of GABA from biological fluid using inexpensive reagent could be of value. 2-Hydroxynaphthaldehyde (HN) has a structure somewhat resembling the commonly used 2,3-naphthalene dicarboxaldehyde and has been used for the spectrophotometric, fluorimetric and HPLC determination of hydrazine [40–42], HPLC determination of putrescine, cadaverine [43] and amino acids histidine,

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methionine and tryptophan [44]. The present work examines the use of HN for the selective HPLC determination of GABA from human cerebral spinal fluid.

2. Experimental

2.1. Spectrophotometric studies

To a solution (1 ml) containing gamma amino-butyric acid (GABA) (17.9–89.5 μg), glycine (60–300 μg), tyramine (17.3–86.5 μg), or lysine (14.6–73.0 μg) separately was added 0.6 ml borax buffer pH 8, 2 ml of derivatizing reagent HN (0.3% w/v in methanol) and the solution was heated on a water bath at 80 °C for 10 min. The contents were allowed to cool and the volume was adjusted to 10 ml with methanol. The absorption spectra were recorded within 600–300 nm against reagent blank.

2.2. HPLC Studies

To the solution (1 ml) containing GABA (5.6–140 μg) separately or GABA (5.6–140 μg), glycine (30–750 μg), tyramine (39–975 μg), lysine (58–1460 μg) in a mixture was added 0.6 ml borax buffer pH 8 and 1 ml of derivatizing reagent HN (0.3% w/v in methanol). The solution was heated on a water bath at 80 °C for 10 min and was allowed to cool. The final volume was adjusted to 5 ml with methanol. The solution (5 μl) was injected on Phenomenex C_{18}, 5 μm (150×4.6 mm I.D.) and eluted with methanol: water (62:38 v/v) with a flow-rate 1 ml/min. The detection UV was at 330 nm.

2.3. Determination of GABA from CSF sample

Equal volumes of cerebral spinal fluid (CSF) (0.8 ml) and methanol (0.8 ml) were added and centrifuged at 12000 rpm for 10 min. The supernatant liquid (0.7 ml) was transferred to a 5 ml volumetric flask and was treated as in Section 2.2. The amount of GABA from CSF was evaluated from the calibration curve.

Cerebral spinal fluid (CSF) sample (1.0 ml) was treated as in 2.3 and the supernatant liquid (1.0 ml) was divided into two equal parts. To one part (0.5 ml), the procedure was adopted as in 2.2 and to the other part (0.5 ml) was added 56 μg and it was again treated as in 2.2. The increase in the response was used to calculate the percentage recovery of GABA from the calibration curve.

2.4. Equipments and reagents

Spectrophotometric studies were carried out on a Hitachi 220 spectrophotometer (Hitachi (Pvt) Ltd, Tokyo, Japan). HPLC studies were carried out on a Hitachi 655A liquid chromatograph connected to a variable wavelength UV monitor. A Rheodyne 7125 injector and Hitachi 2500 Chromato-injector (Hitachi (Pvt) Ltd, Tokyo, Japan). Phenomenex C_{18}, 5 μm (150×4.6 mm I.D.) (Torrance, CA, USA) were used throughout the study.

GABA, glycine, l-lysine, tyramine, methanol (E. Merck, Darmstadt, Germany), HN (Fluka, Switzerland) and double distilled water from all glass was used. GABA, glycine, l-lysine or tyramine containing 1 mg/ml was prepared by dissolving an appropriate amount in water and an equal volume of methanol was added. The final volume was adjusted with methanol: water (1:1 v/v).

Buffer solutions at unit intervals between pH 1 and 9 were prepared from the following: hydrochloric acid (0.1 *M*) and potassium chloride (1 *M*) (pH 1 and 2); acetic acid (1 *M*) and sodium acetate (1 *M*) (pH 3–7), borax (1 *M*) and hydrochloric acid (0.1 *M*) for pH 8, sodium bicarbonate (1 *M*) and sodium carbonate (saturated) (pH 9–10).

2.4.1. CSF samples

CSF samples were collected from the neurosurgical ward at Liaquat Medical University Hospital, Jamshoro, during ventrico-peritonial shunt operations on patients suffering from hydrocephalus due to tuberculous meningitis and septic meningitis (Table 1). A part of the CSF sample was obtained from the collected CSF during required operational procedure.

3. Results and discussion

GABA, glycine, tyramine and l-lysine containing primary amino group reacted with HN to form Schiff

Table 1
Samples used in study

Patient	Disease	Age years	Sex	Amount μg/ml
1	T.B. meningitis	28	Male	21.8
2	Septic meningitis	10	Female	18.5

bases (Fig. 1). The derivatives formed were examined spectrophotometrically to characterise them and establish the optimal conditions for their formation. The effects of pH, derivatizing reagent concentration and heating time on the derivatization were examined in aqueous–methanolic solution. The absorbance was measured at 417 nm and 415 nm for glycine and GABA derivatives respectively and the condition which indicated the maximum absorbance was considered as optimal. Different buffer solutions in the pH range of 2–10 at unit intervals were examined. A similar response was observed within pH 6 to 9 with a maximum at 8, which was selected. Heating time at 80 °C was varied between 5 and 25 min and a heating time of 10 min proved optimal. The methanolic solution of derivatizing reagent HN containing 1.5 to 7.5 mg was added and 6 mg (2 ml 0.3% w/v) of HN was selected for a final volume of 10 ml. GABA derivative at the optimised condition indicates molar absorptivity of 30 000 l mol^{-1} cm^{-1} at 415 nm. The l-lysine, glycine, and tyramine indicated molar absorptivities of 18 066, 24 330 and 18 814 l $mole^{-1}$ cm^{-1} at 417 nm. The solution stability of the derivative was observed >12 h.

The derivative obeyed the Beer's law within 1.79–8.95 μg/ml GABA, 6.0–30.0 μg/ml glycine, 1.46–7.3 μg/ml lysine and 1.73–8.65 μg/ml tyramine. The spectrophotometric study enabled examination of HPLC for selective determination of GABA from the biological fluids. The GABA-HN derivative easily eluted from Phenomenex C_{18}, 5 μm (150×4.6 mm I.D.) with methanol or methanol–water and separated completely from the derivatizing reagent HN. Glycine, l-lysine and tyramine also react with HN to form the derivatives. Attempts were therefore made to separate them from GABA-HN. Complete separation was obtained when eluted with methanol: water (62:38 v/v) with a flow-rate of 1 ml/min and UV detection at 330 nm (Fig. 2). The separation was

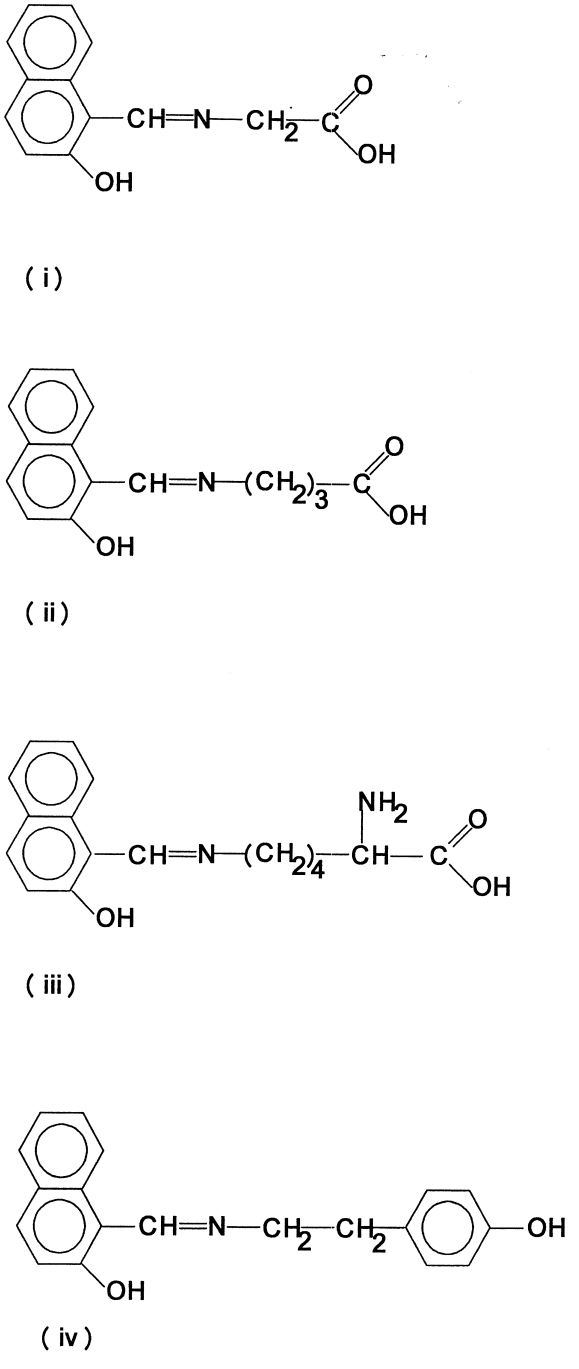

Fig. 1. Structural diagrams: (I) glycine-HN, (II) GABA-HN, (III) lysine-HN and (IV) tyramine-HN.

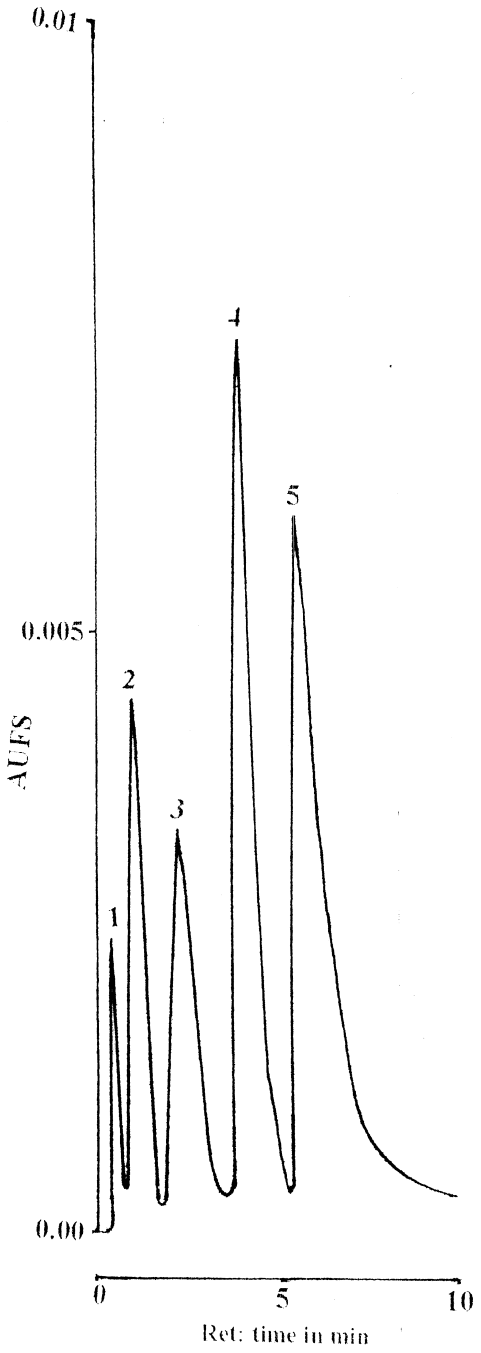

Fig. 2. HPLC separation as HN derivatives of (1) glycine, (2) GABA, (3) l-lysine, (4) tyramine, (5) HN. Column Phenomenex C_{18}, 5 μm (150×4.6 mm I.D.) elution with methanol: water (62:38 v/v) with flow-rate of 1 ml/min and UV detection at 330 nm.

reproducible in terms of retention time and peak height with C.V. 1.6% and 2.4% respectively (n=5). The reproducibility of the peak height on the repeated formation of the derivative with 11.3 μg/ml GABA was examined and inter- and intra-day variation was observed with a coefficient of variation (C.V.) of ±3.5% (n=8). The linear calibration curve for GABA was obtained by recording average peak height (n=3) against concentration with (1.12–28 μg/ml) corresponding to 5.6–140 ng/injection (5 μl) with a coefficient of correlation (r) = 0.998. Test solutions of GABA separately and in the presence of glycine, lysine and tyramine were analysed and the relative error was found within ±0 to 2.5% The detection limit measured as three times the background noise was 2.8 ng/injection (5 μl). Similarly the linear calibration curve for simultaneous determinations for glycine, lysine and tyramine were observed within 6.0–150 μg/ml, 11.8–292 μg/ml and 39–195 μg/ml respectively. The coefficient of correlation (r) for glycine, lysine and tyramine with n=5 points calibration was calculated as 0.9985, 0.999, 0.994 respectively. The detection limits observed were 3.0 μg/ml glycine, 5.8 μg/ml lysine, 1.7 μg/ml tyramine, corresponding to 15 ng, 33.6 ng and 8.5 ng/injection (5 μl) respectively.

The effects of the compounds l-aspargine, histamine, dl-valine, l-arginine, dl-tryptophan, β-alanine, glutamine, phenylalanine, histidine, l-serine, leucine, isoleucine at twice the concentration of GABA (11.2 μg/ml) were examined, following the analytical procedure. The compounds separated completely and did not affect the elution and determination of GABA.

The method was used for the analysis of GABA from CSF fluid of three patients. The amount of GABA found was 21.8 and 18.5 μg/ml from patients suffering from tuberculosis meningitis and septic meningitis respectively (Fig. 3). However GABA from a child of 40 days suffering from hydrocephalous was below the determination limits. The determination was checked by spiking the CSF of a patient with 56 μg of GABA. The average percentage recovery was 95% with C.V. 2.5% (n=3). Loscher and Schmidt [45] have reported the concentration of GABA in a CSF as 117±10 and

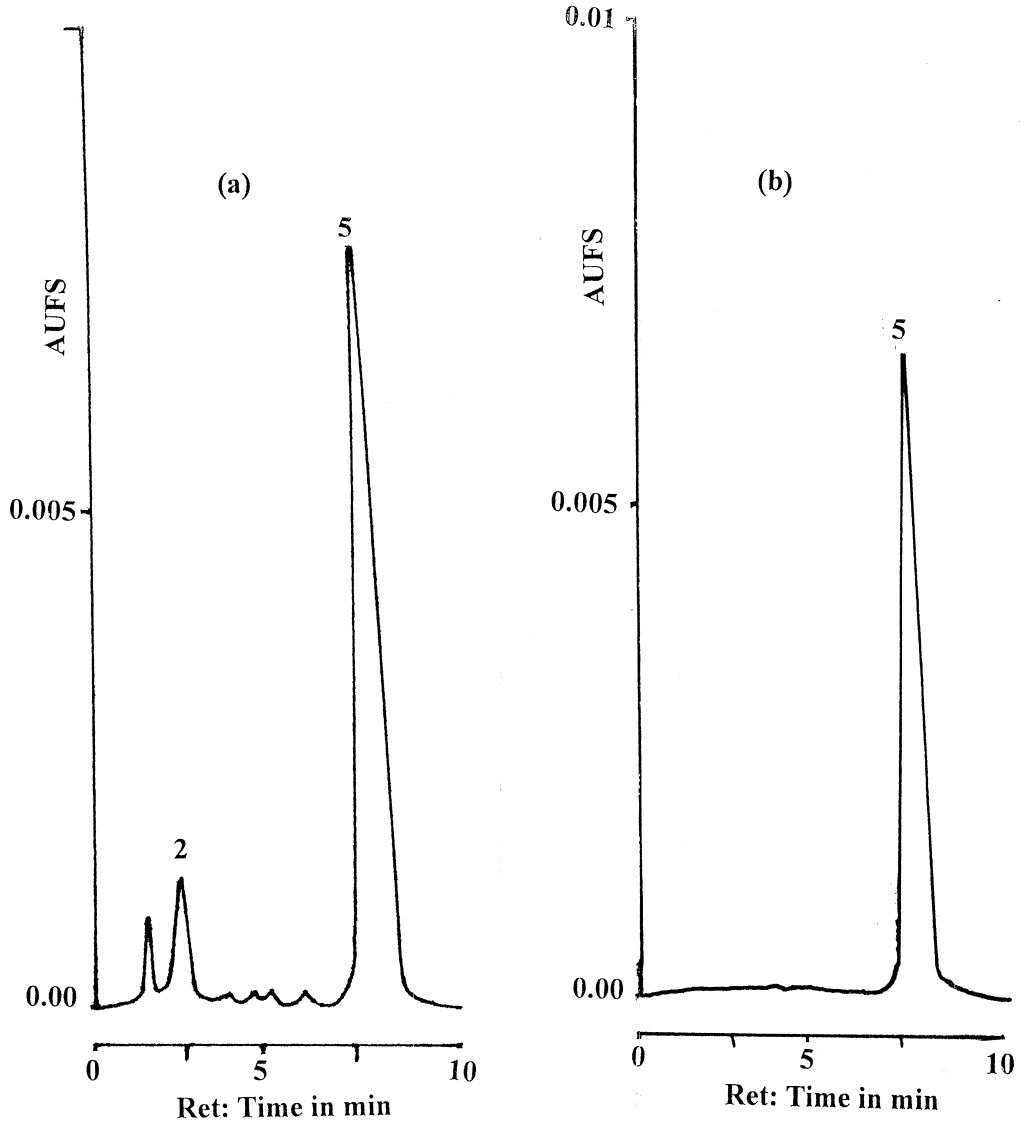

Fig. 3. HPLC determination of (a) GABA in CSF of patient suffering from meningitis; (2) GABA-HN, (5) HN. (b) Elution of reagent HN. Conditions are as Fig. 2.

127±7.4 pmol/ml in normal and patients with neurological disorders. The reported values by Loscher and Schmidt correspond to results for healthy subjects [46]. The high values of GABA in the present work observed in the meningitis patients may be because of higher inflow of GABA in CSF.

4. Conclusion

A simple analytical procedure has been proposed for the selective determination of GABA from CSF samples by isocratic elution with methanol–water. The detection limit observed is 2.8 ng/injection.

Glycine, l-lysine and tyramine could also be determined simultaneously.

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References

[1] K. Kanamori, B.D. Ross, Adv. Neurochem. 8 (1997) 65.
[2] M. Hajek, M. Dezortova, V. Komarek, Magn. Reson. Mater. Phys. Biol. Med. 7 (1996) 95.
[3] J. Shen, D.C. Shungu, D.L. Rothman, Magn. Reson. Med. 41 (1999) 35.
[4] M. Hajek, M. Burian, M. Dezortova, Magn. Reson. Mater. Phys. Biol. Med. 10 (2000) 6.
[5] O. Niwa, R. Kurita, T. Horiuchi, K. Torimitsu, Anal. Chem. 70 (1998) 89.
[6] F. Mazzei, F. Botre, G. Lorenti, F. Porcelli, Anal. Chim. Acta 328 (1996) 41.
[7] M. Sawai, A. Saito, K. Inoue, Y. Ohta, K. Yoda, J. Adv. Sci. 9 (1997) 93.
[8] S. Uhlhaas, K. Olek, J. Clin. Chem. Clin. Biochem. 22 (1984) 379.
[9] I. Mohner-Pen, I. Bozor, J. Chromatogr. A 798 (1998) 37.
[10] G. Zhang, A.W. Brown, Phytochemistry 44 (1997) 1007.
[11] M.L. Sethi, J. Pharm. Biomed. Anal. 11 (1993) 613.
[12] Y. Takada, M. Yoshida, M. Sakairi, H. Koizumi, Rapid. Commun. Mass Spectrom. 9 (1995) 895.
[13] J. Bergquist, M.J. Vona, C.O. Stiller, W.T.O. Connor, T. Falkenberg, R. Eekman, J. Neurosci. Meth. 65 (1996) 33.
[14] L.S. Kei, F. Zaccardo, M. Little, P. Banks, J. Chromatogr. A 809 (1998) 203.
[15] H. Mousah, P. Jacqmin, M. Lesne, Clin. Chim. Acta 170 (1987) 151.
[16] J.M. Schaaf, A.W. Teelken, F.A.J. Muskiet, G. Nagel, B.G. Wolthers, J. Neurosci. Meth. 13 (1985) 257.
[17] J.T.F. Wong, G.B. Baker, R.T. Coutts, Res. Commun. Chem. Pathol. Pharmacol. 70 (1990) 115.
[18] E.J. Norman, E.L. Wee, H.K. Berry, E.F. Zimmerman, J. Chromatogr. Biomed. Appl. 38 (1985) 21.
[19] P. Cao, M. Moini, J. Chromatogr. A. 710 (1995) 303, (J. Chromatogr. 337).
[20] I.M. Kapetanovic, W.D. Yonekawa, H.J. Kupferberg, J. Chromatogr. Biomed. Appl. 58 (1987) 265, (J. Chromatogr. 414).
[21] Y. Qu, L. Arckens, E. Vandenbussch, S. Geeraerts, F. Vandesande, J. Chromatogr. A. 798 (1998) 19.
[22] J. Kehr, J. Chromatogr. B: Biomed. Sci. Appl. 708 (1998) 49.
[23] D. Ma, J. Zhang, K. Sugahara, T. Ageta, K. Nakayama, H. Kodama, J. Chromatogr. B: Biomed. Sci. Appl. 726 (1999) 285.
[24] H. Liu, M. Sanuda-Pena, J.D. Harvey-White, D. Judith, S. Kalra, S.A. Cohen, J. Chromatogr. A 828 (1998) 383.
[25] T. Yamamoto, C. Nanjoh, I. Nuruma, Neurochem. Int. 7 (1985) 77.
[26] M.A. Desiderio, P. Davalli, A. Penn, J. Chromatogr. Biomed. Appl. 63 (1978) 285, (J. Chromatogr. 419).
[27] T.A. Durkin, G.M. Anderson, D.J. Cohen, J. Chromatogr. Biomed. Appl. 72 (1988) 9, (J. Chromatogr. 428).
[28] S. Murai, H. Saito, H. Nagahama, H. Mujate, Y. Masuda, T.I. toh, J. Chromatogr. Biomed. Appl. 89 (1989) 363, (J. Chromatogr. 497).
[29] H.R. Zielke, J. Chromatogr. 347 (1985) 320.
[30] A.S. Herranz, J. Lerma, R. Martin del Rio, J. Chromatogr. Biomed. Appl. 34 (1984) 139, (J. Chromatogr. 309).
[31] S.L. Pahuja, J. Albert, T.W. Reid, J. Liq. Chromatogr. 7 (1984) 2261.
[32] P.G. Zambonin, A. Guerrieri, T. Rotunno, F. Palmisano, Anal. Chim. Acta 251 (1991) 101.
[33] H.A. Carchon, J. Jacksen, E. Jansen, E. Eggenment, Clin. Chim. Acta 201 (1991) 83.
[34] S. Smith, T. Sharp, J. Chromatogr. B: Biomed. Appl. 652 (1994) 228.
[35] B.W. Boyd, S.R. Witowski, R.T. Kennedy, Anal. Chem. 72 (2000) 865.
[36] H.P. Fitznar, J.M. Lobbes, G. Kattnor, J. Chromatogr. A 832 (1999) 123.
[37] I. Krause, A. Bockhard, H. Neckermann, T. Henle, H. Klostermeyer, J. Chromatogr. A 715 (1995) 67.
[38] Z.J. Shen, Z.G. Wang, X.P. Li, C.H. Hu, S.D. Yang, Shengwn Huaxueyu Shengwu Wuli Jinzhan 25 (1998) 543.
[39] A. Vasanits, L. Molnar-Perl, J. Chromatogr. A. 832 (1999) 109.
[40] J. Manes, P. Campillus, G. Font, H. Marte, P. Prognon, Analyst 112 (1987) 1187.
[41] J. Manes, M.J. Gimeco, J.C. Malto, G. Font, J. Pharma. Biomed. Anal. 6 (1988) 1023.
[42] M.Y. Khuhawar, A.D. Rajper, Pak. J. Sci. Ind. Res. 45 (2002) 371.
[43] M.Y. Khuhawar, M.B. Rind, G.A. Qureshi, J. Chem. Soc. Pak. 18 (1996) 119.
[44] A.M. El-Brashy, S.M. Al-Ghannam, Analyst 122 (1997) 147.
[45] W. Loscher, Schmidt, Drug Monitoring 6 (1984) 227.
[46] V. Rizzo, A. Anesi, L. Montalbetti, G. Bellantoni, R. Trolli, G.V. Melqi d'Eril, J. Chromatogr. A 729 (1996) 181.