Asian Journal of Chemistry Vol. 22, No. 9 (2010), 6983-6990

Quantitative Analysis of Glyoxal, Methyl glyoxal and Dimethyl glyoxal from Foods, Beverages and Wines Using HPLC and 4-Nitro-1,2-Phenylenediamine as Derivatizing Reagent

K.P. MAHAR, M.Y. KHUHAWAR*, T.G. KAZI†, K. ABBASI and A.H. CHANNER
High Tech Central Resource Laboratory, University of Sindh, Jamshoro, Pakistan
Fax: (92)(222)771372; E-mail: khalidamahar@yahoo.com; mykhuhawar@usindh.edu.pk

An analytical procedure has been developed for precolumn derivatization, separation and determination of glyoxal, methyl glyoxal and dimethyl glyoxal by HPLC using 4-nitro-1,2-phenylenediamine as a reagent. HPLC elution was from the column Zorbex C-18 and detection was by photodiode arry at 255 nm. The isocratic elution and separation was obtained with methonal-water-acetonitrile (42:56:2 v/v/v) with a flow rate 0.9 mL/min. The linearity of the calibration curves were obtained with 0.2-1.0 µg/mL with limit of detection (LOD) within 41-75 ng/mL for each of the compound. The method was used for the simultaneous determination of glyoxal, methyl glyoxal and dimethyl glyoxal from food items (juices, tea, coffee and yoghurt), beers and wines. The results were obtained with relative standard deviation (RSD) within 0.6-2.5 %.

Key Words: HPLC, Glyoxal, Methyl glyoxal, Dimethyl glyoxal, 4-Nitro-1,2-phenylenediamine, Food analysis.

INTRODUCTION

Glyoxal and methyl glyoxal are biological active small organic molecules and are present in biological fluids. Their concentrations are reported to increase in diabetic patients[1-3]. Glyoxal and methyl glyoxal are potent glycating agents formed by the degradation of glycated proteins, glycolytic intermediates and lipid peroxidation[4]. Glycation reactions occur endogenously in all tissues and body fluids under physiological conditions and also during thermal processing of food where heating increase the rate of glycation processes[5].

Methyl glyoxal is found from food stuffs such as bread, soya sauce, instant tea and roasted coffee and glyoxal is also derived from sugar fragmentation and has been isolated in many food stuffs such as roasted coffee and coca[6]. The toxicological profile of food derived glyoxal and methyl glyoxal is under scrutiny as possible glycotoxins[7-9]. Dimethyl glyoxal is an important α-dicarbonyl compound known as dairy flavour and is usually present in food product obtained by fermentation

†National Center of Excellence in Analytical Chemistry, University of Sindh, Jamshoro, Pakistan.

processes such as wine, brandy, yoghurt, cheese, vinegar or butter[10]. It has a characteristic odour and taste that can affect the organoleptic quality of foods. For instance the concentration of dimethyl glyoxal has to be controlled by the brewing industry during the production of beer, due to its unpleasant butter like taste[11].

Various analytical procedures have been developed for the determination of α-dicarbonyl compounds. Chemical derivatization with 2,4-dinitrophenylhydrazine or 4-amino-5-hydrazino-3-mercapto-1,2,4-triazole has been used for spectrophotometric determinations[12]. More procedures are reported using high performance liquid chromatography (HPLC), gas chromatography (GC) and capillary electrophoresis (CE). HPLC methods for the determination of glyoxal, methyl glyoxal and dimethyl glyoxal primarily involve 1,2-diaminobenzene or 1,2-diamino-4,5-dimethoxy benzene as derivatizing reagent. The resulting quinoxaline compounds formed are monitored using spectrophotometric or spectrofluorimetric detection[13-17]. Recently stilbendiamine has been used for HPLC, capillary electrophoresis (CE) and GC determination of glyoxal and methyl glyoxal[3,18,19]. Rosario *et al.*[20] analyzed methyl glyoxal in water and biological matrix by capillary zone electrophoresis with diode array detection and using 1,2-diamino benzene as derivatizing reagent. The GC determinations of glyoxal, methyl glyoxal and dimethyl glyoxal are based on precolumn derivatization with 0-(2,3,4,5,6-pentaflourobenzyl) hydroxylamine[2,21], cystamine[22], *o*-phenylenediamine[23], 4,5-dichloro-1,2-phenylenediamine or 1,2-propylenediamine[23]. 4-Nitro-1, 2-phenylenediamine (NPD) has same functional group as 1,2-phenylenediamine and has been examined for HPLC determination of glyoxal, methyl glyoxal, dimethyl glyoxal from foods, beverages beers and wines using photodiode array detection. The derivatization and separation conditions are optimized for repeatable and reproducible determinations.

EXPERIMENTAL

The chemicals glyoxal and dimethyl glyoxal (Across, New Jersey, USA), methyl glyoxal (Fluka, Switzerland) and 4-nitro-1,2-phenylenediamine (NPD) (Fluka, Switzerland), methanol (RDH, Germany), acetonitrile (Fisher Scientific, Leicestershire, UK) were used. The solution of glyoxal, methyl glyoxal and dimethyl glyoxal containing 0.1 mL each was diluted to 50 mL with methanol. 4-Nitro-1,2-phenylenediamine was recrystallized from *n*-heptane before use. Acetic acid (RDH, Germany), hydrochloric acid (37 %), potassium chloride, sodium acetate, ammonium acetate, boric acid, sodium tatraborate, sodium bicarbonate, sodium carbonate, ammonium chloride and ammonia (25 %) (E. Merck, Germany) were used.

The buffer solutions within pH 1-10 at unit interval were prepared as: hydrochloric acid (0.1 M) and potassium chloride (1 M) (pH 1-2), acetic acid (1 M) and sodium acetate (1 M) (pH 3-6), ammonium acetate (1 M) (pH 7), boric acid (1 M) and sodium tetraborate (1 M) (pH 8), sodium bicarbonate (1 M) and sodium carbonate (saturated) (pH 9), ammonium chloride (1 M) and ammonia solution (1 M) (pH 10).

pH measurements were made with an Orion 420 A pH meter (Orion Research Inc., Boston, USA) with combined glass electrode and internal reference electrode. Spectrophotometric studies were carried out with a double beam Hitachi 220 spectrophotometer (Hitachi (Pvt.) Ltd. Tokyo, Japan) with dual 1 cm silica cuvettes. High performance liquid chromatography (HPLC) was carried out with Agilent model 1100 network HPLC system (Agilent Technology Inc., USA) 1100 series with diode array detection system. The computer with Chemstation software controlled the HPLC. The column Zorbax 300 SB-C-18 (4.6 mm × 150 mm i.d.) (Agilent Technology Inc. USA) was used throughout the study.

Analytical procedures

Spectrophotometric procedure: The aqueous solution (0.1-1.0 mL) containing (5-100 μg) glyoxal, methyl glyoxal or dimethyl glyoxal was transferred to 10 mL volumetric flask separately. Each of the solution was added 4-nitro-1,2-phenylene-diamine (NPD) solution (1.5 mL, 1 % w/v in methanol), acetic acid-sodium acetate buffer pH 3 and contents were warmed at 70 ºC for 20 min and cooled at room temperature. The volume was adjusted to the mark with methanol and absorption spectra were recorded against reagent blank within 500-200 nm. The reagent blank was prepared following the same procedure without the addition of α-diketone.

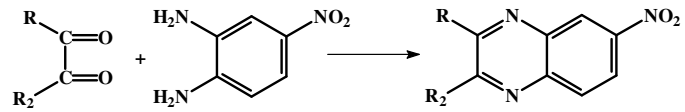
HPLC procedure: The aqueous solution (1-2 mL) containing glyoxal, methyl glyoxal and dimethyl glyoxal (1-20 μg) of each was treated as given in spectrophotometric procedure and volume was adjusted to 10 mL with methanol. The solution (20 μL) was injected on the column Zorbax C-18 (4.6 mm × 150 mm i.d.) and eluted with methanol-water-acetonitrile (42:56:2 v/v/v) with a flow rate 0.9 mL/min. The detection was observed at 255 nm.

Analyses of foods, beverages, beers and wines: Apple juice, lemon juice and orange juice (Nestle Pakistan Ltd., Karachi) was filtered through Watmann filter paper 42 and juice (5 mL) from the each was treated as HPLC procedure. Green tea (Tapal 0.53 g), instant tea, instant coffee decaffeinated (Moccona Dovwe Egberts Joure-Holland), Instant coffee 1 g was added boiling water (100 mL) and was allowed at 80 ºC for 20-25 min. The solution was filtered and (5 mL) was processed as HPLC procedure. Yoghurt (Nestle Pakistan, Ltd., Lahore) (2 g) was added methanol (4 mL) and contents were centrifuged at 3000 g for 0.5 h. The supernatant layer was collected and treated as analytical HPLC procedure. Sample (5 mL) from each of the 5 samples of beers and wines W1 = whisky (Four Aces whisky, W2 = superior whisky (Quetta Distillery, Ltd., (Pvt.) Quetta, Pakistan), W3 = drygin (Club London, drygin (export quality), W4 = beer (Murree beer brewed from barely, malt, hops and water (Murree Brewery Co. Ltd., Rawalpindi, Pakistan) and W5 = beer (Murrees classic lager brewed from malt hops), Murree, Brewery Co. Ltd., Rawalpindi, Pakistan) was processed as HPLC procedure. The quantitation was made by linear regression equation $Y = ax + b$. The blank determination was also recorded for each of the sample. The blank sample was prepared following same procedure but the addition of derivatization reagent 4-nitro-1,2-phenylenediamine was omitted.

Determination of glyoxal, methyl glyoxal and dimethyl glyoxal by standard addition: 5 mL of each apple juice, orange juice (Nestle Pakistan Ltd., Karachi) and superior whisky (Quetta Distillary Ltd., (Pvt.) Quetta, Pakistan) was transferred to 10 mL volumetric flask in duplicate. A sample of each was added glyoxal (0.3 μg), methyl glyoxal (0.6 μg) and dimethyl glyoxal (0.4 μg) and both of samples were treated as analytical HPLC procedure. The quantitation was made by linear calibration curve and an increase in the response with added standards.

RESULTS AND DISCUSSION

The reagent 4-nitro-1,2-phenlenediamine (NPD) reacts with α-diketone glyoxal, methyl glyoxal and dimethyl glyoxal to form nitroquinoxalines (Fig. 1). The formation of the derivative was checked and confirmed on spectrophotometer, by measuring the absorbance of the solutions against reagent blank. The absorbance of solutions also increased with an increase in the concentration of glyoxal, methyl glyoxal and dimethyl glyoxal and obeyed the Beers law over the concentration range 1-10 μg/mL. The derivatives once formed were highly stable and did not show any change in absorbance up to 24 h.



Glyoxal = R_1=R_2=H, Methyl glyoxal = R_1=HR_2=CH_3, Dimethyl glyoxal = R_1R_2=CH_3

Fig. 1. Reaction of α-diketones with 4-nitro-1,2-phenlenediamine

Optimization of derivatization and separation: Glyoxal, methyl glyoxal and dimethyl glyoxal formed stable derivatives with 4-nitro-1,2-phenlenediamine and the effect of reaction condition in terms of pH, amount of reagent 4-nitro-1,2-phenlenediamine added per analysis, warming time and temperature on the derivatization was examined. Each of the compounds after derivatization was injected on Zorbex C-18 column and eluted with a mixture of methanol-water. The derivatized analyte easily separated from the derivatizing reagent. The effect of variables on the derivatization, elution and separation was examined. A variable was changed at a time following the HPLC procedure. Constant volume (20 μL) with same concentration of the analyte was injected and the condition which gave maximum response was considered optimum. The pH was varied between 1-10 at unit interval and reaction was observed in acidic medium with maximum in acetate buffer at pH 3 and was selected. The reagent solution 4-nitro-1,2-phenlenediamine (1 % w/v in methanol) was varied from 0.5-4.0 mL at an interval of 0.5 mL. A similar response was observed with the addition of 1 mL and above and addition of 1.5 mL was selected. The warming time at 70 ºC was varied from 10-30 min at an interval of 5 min. Maximum response was observed with a warming time of 15 min and above and 20 min was selected.

For the simultaneous determinations of glyoxal, methyl glyoxal and dimethyl glyoxal HPLC separation from the column Zorbax C-18 was examined. Isocratic elution was carried out with different mixtures of methanol-water and a complete separation was obtained with mixture of methanol-water-acetonitrile (42:56:2 v/v/v) with a flow of 0.9 mL/min (Fig. 2A). Peak identification was made on the basis of comparing the retention time with that of standard and by spiking each of the α-diketone with standards in sequence. The derivatives absorb within UV region due to π-π* transition within quinoxaline rings and wavelength at peak maximum was examined with the diode array detector and wavelength of the UV detector was fixed at 255 nm. The repeatability of the separation (precision) in terms of retention time and peak height was examined (n = 6) and relative standard deviations (RSD) were observed within 0.15-2.00 and 1.5-2.9 %, respectively.

Quantitation and validation: Linear calibration curves were obtained by recording average peak height/peak area (n = 4) *versus* concentrations of glyoxal, methyl glyoxal and dimethyl glyoxal within 0.2-1.0 μg/mL with coefficient of determination (r^2) 0.999, 0.998 and 0.999, respectively (Table-1, Fig. 3). The limits of detection (LOD) measured three times the signal to noise ratio (3:1) were obtained glyoxal 45 ng/mL, methyl glyoxal 71 ng/mL and dimethyl glyoxal 75 ng/mL. The limits of quantitation measured as signal to noise ratio (10:1) were with glyoxal 0.14 μg/mL, methyl glyoxal 0.21 μg/mL and dimethyl glyoxal 0.22 μg/mL. The analysis of four test solutions of the mixtures of glyoxal, methyl glyoxal and dimethyl glyoxal indicated relative error within ± 0.1-2.9 %. The derivatization reaction, separation and quantitation was repeatable and reproducible and the variation in the response (peak height/peak area) for each of the α-diketones was examined intra and inter day at the final concentration of 1 μg/mL each. The analysis was carried out by same operator under same condition on the same day (n = 6) and different days (n = 5) and RSDs were observed within 0.4-2.6 and 0.6-3.2 %, respectively.

TABLE-1
HPLC PARAMETER FOR GLYOXAL, METHYL GLYOXAL AND DIMETHYL GLYOXAL USING 4-NITRO-1,2-PHENYLENEDIAMINE AS A DERIVATION REAGENT

Name of compound	Calibration range (μg/mL)	Co-efficient of determination R^2	Least square or regression	Detection limit (LOD) (μg/mL)	Limit of quantitation (LOQ) (μg/mL)
Glyoxal	(0.2-1.0)	0.9991	Y = 5.4843x + 0.0371	0.045	0.135
Methyl glyoxal	(0.2-1.0)	0.9983	Y = 9.9429x + 0.2286	0.071	0.213
Dimethyl glyoxal	(0.2-1.0)	0.9995	Y = 4.9586x + 0.0057	0.075	0.225

Samples analyses: The samples of juices (apples, lemon and orange), tea, coffee, yoghurt, beers and wines were analyzed for the contents of glyoxal, methyl glyoxal

and dimethylgloxal. The samples after derivatization were recorded for analyses. The samples without derivatization were also run on the HPLC and provided background signal.

The results of analysis are summarized in Tables 2 and 3. The results indicated RSD within 0.6-2.5 %. The samples of orange, apple juice and whisky were also analyzed by standard addition and the results agreed with the amounts of glyoxal, methyl glyoxal and dimethyl glyoxal calculated by linear external calibration and indicated recovery of glyoxal 97 %, methyl glyoxal 90 % and dimethyl glyoxal 95 % with RSD of 3.1, 2.6 and 2.8 % (Fig. 2B), respectively. Now comparing the results of analysis with the reported values from foods, beverages and wines with da Silva Ferreira *et al.*[24] work, they reported values ranged from 2.1-29.0 mg/L for glyoxal, 0.4-15.6 mg/L, methyl glyoxal and 0.9-4.4 mg/L, dimethyl glyoxal from the 15 wines analyzed. Esteve *et al.*[25] have reported concentration of dimethyl glyoxal 1.40 ± 0.52 µg/g in yoghurt and 0.1 µg/g in orange juice. Hayashi and Shibamoto[22] reported that one cup of instant coffee (1 g/100 mL) and one cup of brewed coffee (8 g/100 mL) contained 12.6 and 42-78 µg of methyl glyoxal, respectively. They have also reported 0.26 µg/mL methyl glyoxal in apple juice[22]. The reported values are within the range reported in the developed HPLC.

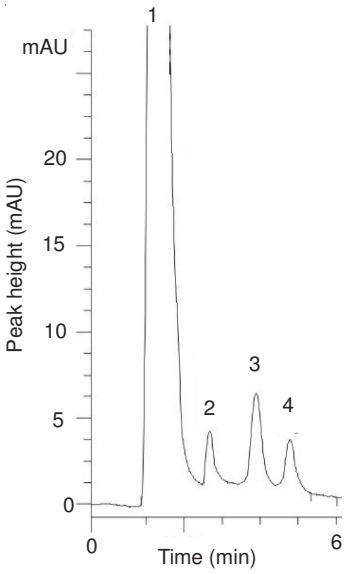


Fig. 2A. HPLC separation of (1) Reagent, (2) glyoxal (3) methylglyoxal (4) dimethylglyoxal Conditions: column Zorbax C-18 (4.6 x 150 mm id) isocratic elution with methanol-water-acetonitrile (46:52:2 v/v/v) with a flow rate of 0.9 mL/min. UV detection by photodiode array at 255 nm

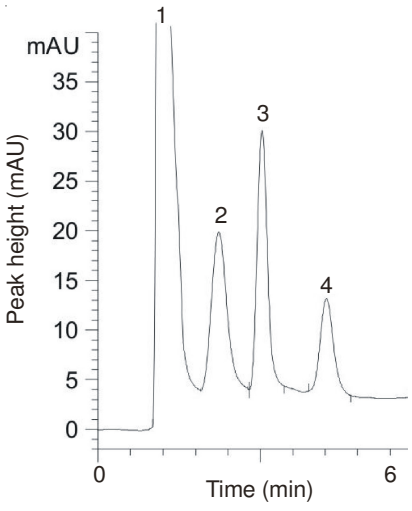


Fig. 2B. HPLC separation of (1) Reagent, (2) glyoxal (3) methylglyoxal (4) dimethylglyoxal after spiking as condition Fig 2A

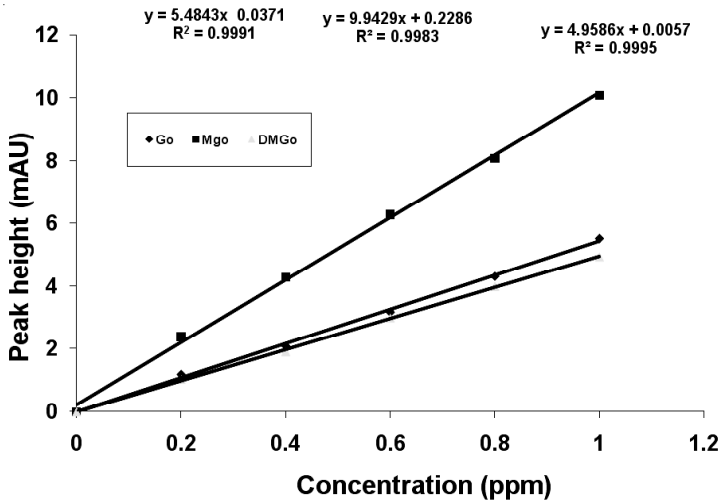


Fig. 3. Calibration of glyoxal, methyl glyoxal and dimethyl glyoxal

TABLE-2
QUANTITATIVE ANALYSIS OF GLYOXAL, METHYL GLYOXAL AND DIMETHYL GLYOXAL IN FOOD PRODUCTS USING 4-NITRO-1,2-PHENYLENEDIAMINE AS A DERIVATION REAGENT

Name of variety	Brand	Glyoxal (RSD) (n = 6) (µg/10 mL)	Methyl glyoxal RSD) (n = 6) (µg/10 mL)	Dimethyl glyoxal (RSD) (n = 6) (µg/10 mL)
Apple juice	Nestle Pakistan Ltd.	0.180 (1.8)	0.235 (0.7)	0.290 (2.0)
Apple juice		0.245 (1.3)	0.126 (1.1)	0.152 (0.9)
Orange juice	Nestle Pakistan Ltd.	0.052 (0.6)	0.056 (1.8)	0.112 (1.1)
Yoghurt	Nestle Pakistan Ltd.	0.306 (2.1)	0.448 (2.5)	0.221 (1.8)
Instant tea		0.195 (0.4)	0.293 (1.3)	0.186 (2.0)
Green tea	Tapal	0.056 (1.6)	0.114 (2.0)	0.145 (1.9)
Instant coffee	Nescafe	0.152 (0.9)	0.104 (0.9)	0.232 (1.1)
Brewed coffee	Nescafe	1.45 (1.8)	0.80 (1.2)	0.694 (1.9)

TABLE-3
QUANTITATIVE ANALYSIS OF GLYOXAL, METHYL GLYOXAL AND DIMETHYL GLYOXAL IN WINE USING 4-NITRO-1,2-PHENYLENEDIAMINE AS A DERIVATION REAGENT

Name of variety	Brand	Glyoxal (RSD) (n = 6) (µg/mL)	Methyl glyoxal (RSD) (n = 6) (µg/mL)	Dimethyl glyoxal (RSD) (n = 6) (µg/mL)
W1	–	1.23 (1.1)	0.0	0.0
W2	–	3.24 (1.6)	1.38 (0.9)	5.56 (2.0)
W3	–	2.21 (1.9)	0.0	0.0
W4	–	3.64 (0.9)	8.07 (1.8)	0.0
W5	–	5.59 (1.3)	0.83 (0.4)	1.0 (0.9)

Conclusion

An analytical procedure has been developed for the determination of glyoxal, methyl glyoxal and dimethyl glyoxal from foods berevages beers and wines by HPLC using 4-nitro-1,2-phenlenediamine as derivatizing reagent. Linear calibrations were obtained with 0.2-2.0 μg/mL and LOD at 41-75 μg/mL. The method was examined for the analysis of food, beverages and wines.

REFERENCES

1. H. Odani, T. Shinzato, Y. Malsumoto, J. Usami and K. Maeda, *Biol.-Chem. Biophys. Res. Commun.*, **256**, 89 (1999).
2. A. Lapolla, R. Flamini, A.D. Vedova, A. Senesi, R. Reitano, D. Fedele, E. Basso, R. Seraglia and P. Traldi, *Clin. Chem. Lab. Med.*, **41**, 1163 (2003).
3. M.Y. Khuhawar, A.J. Kandhro and F.D. Khand, *Anal. Lett*., **38**, 2205 (2006).
4. N. Ahmed, B. Mirshekar-Syahkal, L. Kennish, N. Karacha-Lias, R. Babaei-Jadidi and P.J. Thornally, *Mol. Food Res.*, **49**, 691 (2005).
5. D.A. Papetti, C. Aceti, B. Sordelli, V. Spini and G. Gazzani, *J. Argic. Food Chem.*, **55**, 8877 (2007).
6. T. Shibamoto, *J. Pharm. Biomed. Anal*., **41**, 12 (2006).
7. N. Shangari, R. Mehta and P.J.O. Brien, *Chem. Biol. Interact*, **165**, 146 (2007).
8. T. Goldberg, W. Cai, P. Melpomemi, V. Dardaine, B.S. Baliga, J. Uribarri and H. Vlassara, *J. Am. Diet Assoc.*, **104**, 1287 (2004).
9. A. Forster, Y. Kuhme and T. Henle, *Ann. N.Y. Acad. Sci.*, **1043**, 474 (2005).
10. M. Yamaguchi, J. Ishida, Z. X. Xuan, M. Nakamura and T. Yashitake, *J. Liq. Chromatogr.*, **17**, 203 (1994).
11. T.W. Wright, *J. Inst. Brew*., **79**, 451 (1973).
12. R.S. Li and G.L. Kenyon, *Anal. Biochem*., **230**, 37 (1995).
13. A.C. Mclellan, S.A. Philips and J.J. Thornally, *Anal. Biochem*., **206**, 17 (1992).
14. I. Nemet, L. Varga-Defterdarovic and Z. Turk, *Chim. Biochem*., **37**, 875 (2004).
15. K. Akira, Y. Matsumoto and T. Hashimoto, *Clin. Chem. Lab. Med.*, **42**, 147 (2004).
16. E.W. Randell, S. Vasdev and V. Gill, *J. Pharmacol. Toxicol. Methods*, **51**, 153 (2005).
17. C. Cordeiro and F.A. Ponces, *Anal. Biochem.*, **234**, 221 (1996).
18. M.A. Mirza, A.J. Kandhro, M.Y. Khuhawar and R. Arain, *Electrophorsis*, **28**, 3940 (2007).
19. A.J. Kandhro, M.A. Mirza and M.Y. Khuhawar, *Chromatogr. Sci.*, **31**, 3037 (2008).
20. P.M.Á. do Rosário, C.A.A. Cordeiro, A.P. Freire and J.M.F. Nogueira, *Electrophoresis*, **26**, 1760 (2005).
21. A. Lapolla, R. Flamini, T. Tonus, D. Fedele, A. Senesi, R. Reitano, E. Marotta, G. Pace, R. Geraglia and P. Traldi, *Rapid Commun. Mass Spectrom.*, **17**, 876 (2003).
22. T. Hayashi and T. Shibamoto, *J. Agric. Food. Chem*., **33**, 1090 (1985).
23. S. Ohmori, M. Kawase, M. Mori and T. Hirota, *J. Chromatogr*., **415**, 221 (1987).
24. A.C. Da Silva Ferreira, S. Reis, C. Rodrigues, C. Oliveira and P. Guedes De Pinho, *J. Food. Sci.*, **72**, 5314 (2007).
25. M.J. Esteve, A. Frigola, M.C. Rodrigo and M. Rodrigo, *Food Addit. Contam.*, **19**, 519 (2002).

(*Received*: 7 January 2010; *Accepted*: 14 June 2010) AJC-8789