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Determination of Chloramphenicol in Bulk Drug and Pharmaceutical Dosage Forms by HPLC

* P.Suguna, N.V.S.Naidu & B. Sathyanarayana
Department of Chemistry, S.V.University, Tirupati-517502, A.P., India.

ABSTRACT: *A simple, economic, selective, precise, and accurate High Performance liquid Chromatographic method for the analysis of Chloramphenicol in bulk drug and pharmaceutical formulations were developed and validated in the present study. The mobile phase, employed in the present study, consists of a mixture of sodium pentanesulfonate solution, acetonitrile, and glacial acetic acid in the proportion 85:15:1 respectively, the pH of the solutions was maintained at 5.0 ± 0.05 with sodium hydroxide solution . This was found to give a sharp peak of Chloramphenicol at a retention time of 3.551 min. HPLC analysis of Chloramphenicol was carried out at a wavelength of 272 nm with a flow rate of 2.0 ml/min. The linear regression analysis data for the calibration curve showed a good linear relationship with a regression coefficient of 0.999 in the concentration range of 50* ***µg ml^{-1}*** *to 150* ***µg ml^{-1}****. The linear regression equation was y =****38.493****x-****51.484.*** *The developed method was employed with a high degree of precision and accuracy for the analysis of Chloramphenicol. The developed method was validated for accuracy, precision, robustness, detection and quantification limits as per the ICH guidelines. The wide linearity range, accuracy, sensitivity, short retention time and composition of the mobile phase indicated that this method is better for the quantification of Chloramphenicol.*

KEYWORDS: Chloramphenicol. HPLC. Validation

I. INTRODUCTION:

Several analytical methods have been reported for the determination of chloramphenicol in various samples, such as shrimp,[3,8–11] seafood, meat,[7,12–15] eggs,[13] milk,[4,13] honey,[12,13,15] animal feeds,[5] urine, serum[14–16] and pharmaceutical formulations[17–22] based on liquid chromatography (LC),[5,12] liquid chromatography–mass spectrometry (LC-MS),[3,7–11,14,15] gas chromatography (GC), gas chromatography–mass spectrometry (GC-MS),[3,12,14] capillary zone electrophoresis,[16,17] enzyme-linked immunosorbent assay (ELISA),[3,13] spectrophotometry,[18,19] and chemiluminescence.[20–22] LC-MS is a common method that is used to determine chloramphenicol, because of its high sensitivity and low limit of detection. However, it needs expensive apparatus and reagents and is time-consuming. A sensitive, rapid and cheap method for analysis is still needed. Electrochemical methods are widely used in many applications because they are simple and involve no more reagents for derivatization and low cost. Several methods have been developed for the determination of chloramphenicol using electrochemical detection, such as voltammetry at electrochemically activated carbon fiber microelectrodes[4] and capillary-zone electrophoresis with amperometric detection at a carbon disk electrode[17] and a carbon fiber micro-disk array electrode.[16] Boron-doped diamond thin film (BDD) electrodes have many advantages for electroanalytical applications, due to their unique characteristics, which include a very low background current,[23,24] a wide electrochemical potential window in aqueous solutions,[25,26] a long-term stability of response,[27–30] a slight adsorption of polar organic molecules[28] and low sensitivity to dissolved oxygen.[31] Because of these attractive properties, BDD electrodes have been successfully used for the determination of various compounds, such as tiopronin,[30] acetaminophen,[32] D-penicillamine,[33] captopril,[34] lincomycin,[35] sulfonamides,[36] malachite green and leucomalachite green.[37] Sensitive voltammetric determination of chloramphenicol by using single-wall carbon nanotube–gold nanoparticle–ionic liquid composite film modified glassy carbon electrodes was published by Fei Xiao, et al 2007, *Department of Chemistry, Wuhan University, Wuhan 430072, PR China* . The empirical formula for Chloramphenicol mesylate is $C_{11}H_{12}Cl_2N_2O_5$ and the molecular weight is 323.13. It has the following structure.

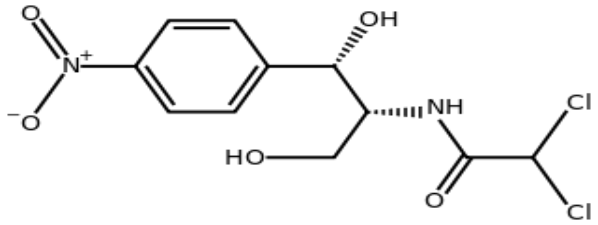


Figure1.

The HPLC method describe here is simple, sensitive, and reproducible for Chloramphenicol determination in formulations with low background interference. An attempt has been made to develop and validate to ensure their accuracy, precision and other analytical method validation parameters as mentioned in various gradients. One method reported for the HPLC determination for developed based on the use of a C-18 column with a suitable mobile phase, without the use of any internal standard. For pharmaceutical formulation the proposed method is suitable for their analysis with virtually no interference of the usual additives present in pharmaceutical formulations.

Experimental Instrumentation

HPLC Analytical column Nucleosil – C-18, 100mm x 4.6mm x 5µm Column

Table – 1.1: Chromatographic conditions of Chloramphenicol

Stationary phase	Mobile phase	Flow rate (ml/min)	Run time (min)	Column Temp (0^{c})	Volume of injection loop(µl)	Detection wavelength (nm)	Retention time (min)
Nucleosil – C18,100mmx4.6mmx5µm Column	sodium pentanesulfonate, acetonitrile, and glacial acetic acid 85:15:1	2.0	10	25	20	272	3.551

Analytical Methodology

1. Preparation of Mobile phase

2.1 g of Sodium pentanesulfonate was weighed accurately and transferred into one liter volumentric flask and dissolved in doubly distilled water and made up to the 1000ml with the water. For isocratic system, a solution of mixture of sodium pentanesulfonate solution, acetonitrile, and glacial acetic acid in the proportion 85:15:1 respectively was prepared and filtered through 0.2 µm Nylon membrane filter paper and degased prior to use.

2. Chromatographic conditions

Separation was performed on Nucleosil - C18, 100mm x 4.6mm x 5µm Column. Methanol was used as a diluent and mobile phase consists of mixture of Sodium pentanesulfonate solution, acetonitrile, and glacial acetic acid in the proportion 85:15:1 respectively. Injection volume of 20 µl was used. Mobile phase was filtered before use through 0.5 µm Nylon membrane filter paper and degassed with helium purge for 20 min. The components of the mobile phase were pumped from solvent reservoir to the column at flow rate 2 ml/min and wavelength was set to 272 nm. The column temperature was set at 25^{o}C.

3. Preparation of Chloramphenicol Standard Solution: (pure sample)

About 100mg of **Chloramphenicol** working standard was weighed accurately and transferred into 100 ml volumetric flask and 10 ml of diluents was added and sonicated to dissolve. The solution in the flask was made up to the mark with diluents.Dilute to volume with diluent. i.e. 1000 µg/ml (Stock solution A)

From the above stock solution A 10 ml of the solution was pipette out into 100 ml volumetric flask and the volume was made up to the mark with methanol to obtained the final concentration of 100 µg/ml (Stock solution B) From the stock solution B ranging from 5-15 ml were transferred into a series of 10 ml volumetric flasks to provide final concentration range of 50-150 µg/ml and each flask was made up to the mark with diluent.

4. **Preparation of Test Solutions :(Formulation)**

Twenty tablets containing **Chloramphenicol** were weighed and finely powered. An accurately weighed portion of the powder equivalent to 100 mg of **Chloramphenicol** was transferred into a 100 ml volumetric flask. About 10 ml of diluent was added and shaken for 20 minutes by manually and further sonicated for 30 minutes and diluted up to the mark with diluent. This solution was centrifuged at 8000 rpm for 10 minutes and decanted the supernatant solution into another test tube (. i.e. 1000 µg/ml) and transferred 10 ml of supernatant solution into another 100 ml volumetric flask and made up the volume with diluent (100 µg/ml). Further transfer 5-15 ml of solution was transferred into another 10 ml volumetric flask and made up the volume with diluent. The solution was filtered through 0.45 µm Nylon membrane filter paper. (50-150 µg/ml)

5. Assay procedure:

The column was equilibrated for at least 30 minutes with mobile phase flowing through the system with a flow rate of 1.0 ml/min. Detector was set at a wavelength of 251 nm. Five sets of the Drug solutions were prepared in diluent containing **Chloramphenicol** at a concentration range of 50 - 150 µg/ml. Then 20 µl of each standard and sample solution were injected for five times separately. The retention time for **Chloramphenicol** was found to be 3.551 min (Fig -3.15). The peak areas of the drug concentrations were calculated.

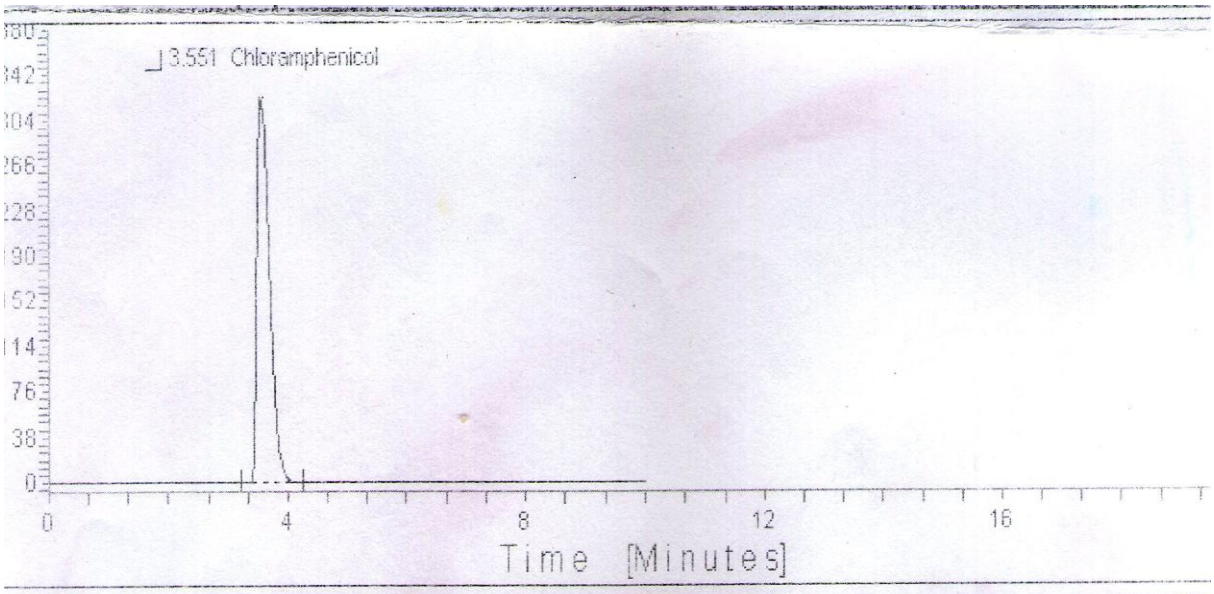
6. System Suitability Solution:

Chloramphenicol standard working solution was used as system suitability solution.

7. Procedure:

Equal volumes of blank and five replicate injections of system suitability solution (Chloramphenicol standard working solution) were separately injected into C-18 column. Then two injections of test solution were injected and chromatograms were recorded. Any peak due to blank in the test solution was disregarded. % RSD of five replicate injections of system suitability solution (Chloramphenicol standard working solution) was calculated. Tailing factor and theoretical plates of the peak in the chromatogram obtained with 5th injection of system suitability solution (Chloramphenicol standard working solution)were checked.

Figure-2: Chromatogram of Chloramphenicol



Result-A Table

Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
	3.551	4640.338	324.091	100	100	0.233
otal		**4640.338**	**324.091**	**100**	**100**	

Figure -3: Linearity of Chloramphenicol standard

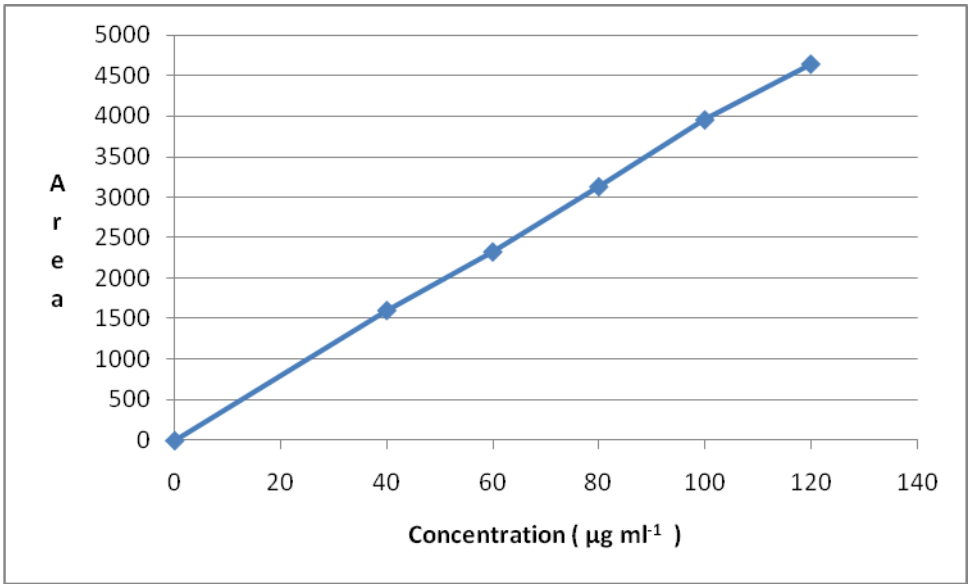


Table -1.2: Performance calculations, detection characteristics precision and acuracy of the proposed method for Chloramphenicol

Parameter	HPLC Method
Wavelength (nm)	272
Retention time (t) min	3.551
Linearity range (µg ml^{-1})	40-120
LOD	1.4039
LOQ	4.6798
Regression equation (y=bc+a)	
Slope (b)	38.493
Intercept (a)	51.484
Standard deviation (SD)	18.0145
Correlation coefficient(r^2)	0.9995
Relative Standard deviation (%RSD)	0.5753
Intermediate Precision (%RSD)	0.34
Range of errors	
Confidence limits with 0.05 level	15.7901
Confidence limits with 0.01 level	20.7517

RSD of 5 independent determinations

Table – 1.3: System suitability - Selectivity

Sr. No.	Area of Chloramphenicol	Tailing factor	Theoretical plates
1	3303.16	1.12	4214
2	3318.64		
Mean	**3310.90**		
Standard Deviation (±)	**10.95**		
(%) Relative Standard Deviation	**0.33**		

Table -1.4: System suitability - Linearity of standard

Sr. No.	Area of Chloramphenicol	Tailing factor	Theoretical plates
1	3045.54	1.09	4025
2	3030.64		
3	2965.65		
4	2946.30		
5	3065.97		
Mean	3010.82		
Standard Deviation (±)	52.06		
(%) Relative Standard Deviation	1.73		

Table -1.5: Results of linearity of standard

Linearity Level	Sample Concentration (in µg ml^{-1})	Average Area (n = 2)	Correlation Coefficient
Level – 1	40	1604.40	**0.999**
Level – 2	60	2326.62	
Level – 3	80	3130.05	
Level – 4	100	3953.47	
Level – 5	120	4640.34	

Table -1.6: Results of linearity of sample

Linearity Level	Sample Concentration (in µg ml^{-1})	Average Area (n = 2)	Correlation Coefficient
Level – 1	40	1699.97	**0.999**
Level – 2	60	2457.98	
Level – 3	80	3115.05	
Level – 4	100	3942.70	
Level – 5	120	4648.41	

Table -1.7: Results of Linearity of standard in presence of placebo

Linearity Level	Sample Concentration (in µg ml^{-1})	Placebo added to the standard solution (µg)	Average Area (n=1)	Correlation Coefficient
Level-1	40	137.2	1268.38	0.999
Level-2	60	137.2	2082.58	
Level-3	80	137.2	2676.42	
Level-4	100	137.2	3657.2	
Level-5	120	137.2	4104.67	

Table –1.8: System precision

Sr. No.	Area of Chloramphenicol	Tailing factor	Theoretical plates	Standard Deviation (±)	(%) Relative Standard Deviation
1	3237.79	1.16	4036	**19.25**	**0.59**
2	3266.92				
3	3236.73				
4	3246.17				
5	3240.59				
6	3245.22				
7	3243.92				
8	3274.16				
9	3281.76				
10	3218.77				
Mean	**3249.20**				

Table – 1.9: Results of method precision

Test Solution	% Assay of Chloramphenicol
1	100.84
2	100.02
3	100.84
4	99.24
5	100.09
6	100.67
Mean	**100.28**
Standard Deviation (±)	**0.63**
(%) Relative Standard Deviation	**0.63**

Table -1.10: Results of intermediate precision

Test Solution	% Assay of Chloramphenicol
1	100.50
2	100.64
3	99.96
4	100.62
5	100.93
6	100.26
Mean	100.49
Standard Deviation (±)	0.34
(%) Relative Standard Deviation	0.34

Table -1.11: Results of twelve test solutions of Chloramphenicol in Ocupol-D Eye/Ear Drops (Six of method precision & six of intermediate precision)

Analysis performed during method precision study By Analyst 1 on system 1 and on column 1 on day 1	
Same column	**% Assay of Chloramphenicol**
1	100.84
2	100.02
3	100.84
4	99.24
5	100.09
6	100.67
Analysis performed during intermediate precision study By Analyst 2 on system 2 and on column 2 on day 2	
Column sr. no.	**015337030136 01**
Test Solution	**% Assay of Chloramphenicol**
7	100.50
8	100.64
9	99.96
10	100.62
11	100.93
12	100.26
Mean of twelve samples	**100.38**
Standard Deviation (±)	**0.49**
(%) Relative Standard Deviation	**0.49**

Table-1.12(A): Determination of accuracy of Chloramphenicol

Level of % Recovery	Amount of CPC in formulation (mg)	Amount of Standard CPC added (mg)	Total amount found (mg)	% Recovery
50%	99.92	100	199.84	99.91
	99.87	100	199.74	99.86
	99.95	100	199.90	99.94
100%	99.96	150	249.90	99.95
	99.89	150	249.72	99.88
	99.87	150	249.67	99.86
150%	99.91	200	299.73	99.90
	99.98	200	299.94	99.97
	99.85	200	299.55	99.84

Table-1.12(B): Statistical data for accuracy determination

Level of % Recovery	Total amount found (mean)	Standard deviation	% RSD
50%	199.82	0.0808	0.0404
100%	249.76	0.1209	0.0484
150%	299.74	0.1951	0.0650

Table – 1.13: Robustness with Change in Column Lot

Flow rate →	**Same column**	**Diff column**
Sample	**% Assay**	
Test solution	99.95	100.53
Average assay result from method precision	100.28	100.28
Mean	**100.12**	**100.41**
Standard Deviation (±)	**0.23**	**0.18**
(%) Relative Standard Deviation	**0.23**	**0.18**

Table -1.14: Results for change in flow rate

Flow rate →	**1.8mL/minute**	**2.2 mL/minute**
Sample	**% Assay**	
Test solution	99.96	101.09
Average assay result from method precision	100.28	100.28
Mean	**100.12**	**100.69**
Standard Deviation (±)	**0.23**	**0.57**
(%) Relative Standard Deviation	**0.23**	**0.57**

Table -1.15: Results for change in wavelength

Wavelength →	**270 nm**	**274 nm**
Sample	**% Assay**	
Test solution	100.03	99.95
Average assay result from method precision	100.28	100.28
Mean	100.16	100.12
Standard Deviation (±)	0.18	0.23
(%) Relative Standard Deviation	0.18	0.23

Table – 1.16: Robustness with Change in pH of mobile phase

pH	**4.6**	**5.0**
Sample	%Assay	
Test solution	98.81	98.27
Average assay result from method precision	98.09	98.09
Mean	98.45	98.68
Standard Deviation(±)	0.58	0.84
(%) Relative Standard Deviation	0.56	0.83

Table -1.17: Results for solution stability

% Assay results calculated against the freshly prepared system suitability standard	
Sample	**% Assay of Chloramphenicol**
0^{th} hr	99.94
12^{th} hr	100.12
24 hr	100.66
36 hr	100.58
48 hr	100.02
Mean	100.26
Standard Deviation (±)	0.33
(%) Relative Standard Deviation	0.33

RESULTS AND DISCUSSIONS

The appropriate wavelength in UV region has been selected for the measuring of active ingredient in the proposed method. This method was validated by linear fit curve and all the parameters were calculated.

Parameters Fixation: In developing methods, systematic study of the effects of various parameters was undertaken by varying one parameter at a time controlling all other parameters. The following studies were conducted for this purpose.

a) Mobile phase characteristics

In order to get sharp peaks and baseline separation of the components, carried out number of experiments by varying different components like percentage of organic phase in the mobile phase, total pH of the selected mobile phase and flow rate by changing one at a time and keeping all other parameters constant. The optimum conditions obtained were included in the procedure proposed.

b) Detection Characteristics

To test whether Chloramphenicol had been linearly eluted from the column, different amounts of Chloramphenicol were taken and analyzed by the above mentioned procedures. The peak area ratios of component areas were calculated and the values are graphically represented in Fig –2. The linear fit of the system was illustrated graphically. Least square regression analysis for the method was carried out for the slope, Intercepts and correlation coefficient. The results were presented in Table -1.2.

c) Performance Calculations

To ascertain the system suitability for the proposed method, a number of statistical values have been calculated with the observed readings and the results were recorded in Table-1.2.

d) Method validations

The UV absorption maximum for Chloramphenicol was fixed at 272 nm respectively. As the final detection was made by the UV absorption spectrum, each method was validated by linear fit curve.

e) Precision

The precision of the method was ascertained separately from the peak area ratios obtained by actual determination of a fixed amount of drug. The percent of relative standard deviation was calculated for Chloramphenicol and were presented in Table-1.8, 1.9. 1.10 & 1.11. The precision of the assays was also determined in terms of intra and inter-day variation in the peak areas for a set of drug solution was calculated in terms of coefficient of variation (CV)

f) Accuracy

To determine the accuracy of the proposed methods, different technical grade samples of Chloramphenicol within the linearity limits were taken and analyzed by the proposed methods. The results (percent error) were recorded n Table-1.12.

g) Interference Studies

The effect of wide range of excipients and other additives usually present in the formulations of Chloramphenicol in the determinations under optimum conditions were investigated. The common excipients such as colloidal Silicon dioxide, ethyl cellulose, hydroxyl propyl methyl cellulose, magnesium state, microcrystalline cellulose provide have been added to the sample solutions and injected. They have not disturbed the elution or quantification of drug. In fact many have no absorption at this UV maximum.

h) Analysis of Formulation

To find out the stability of the proposed methods for the assay of formulations containing Chloramphenicol was analyzed by the proposed and reference methods. The proposed method does not differ significantly in precision and accuracy from reference method. The results were recorded in Table-5.42.

i) Ruggedness and Robustness

Ruggedness of the proposed method was determined by carrying out the analysis by two different analysts using similar operational i.e. Robustness with Change in Column Lot, change in flow rate, change in wavelength and change in p^H of the mobile phase . The results were indicated by % CV in Table -1.13, 1.14, 1.15 & 1.16. Robustness of the method was determined by carrying out the analysis at two different wavelengths i.e. at 270 nm and 274 nm and the results were indicated by % CV in Table 1.15.

j) Recovery Studies

Recovery studies were conducted by analyzing each formulation in the first instance for the active ingredient by the proposed methods known amounts of pure drug was then added to each of the previously analyzed formulations and the total amount of the drug was once again determined by the proposed methods

after bringing the active ingredient concentration within the linearity limits. The results were recorded in Table -1.12.

h) Solution Stability

The stability of the solutions under study was established by keeping the solution at room temperature for 48 h. The results indicate no significant change in assay values that indicates stability of drug in the solvent used during analysis. The results were recorded in Table -1.17.

REFERENCES

[1] L. Agüí, A. Guzmán, P. Yáñez-Sedeño, and J. M. Pingarrón, *Anal. Chim. Acta*, **2002**, *461*, 65. S.
[2] B. K. Neuhaus, J. A. Hurlbut, and W. Hammack, *Lab. Inform. Bull*., **2002**, 4290.
[3] A. Pfenning, S. Turnipseed, J. Roybal, C. Burns, M. Madson, J. Storey, and R. Lee, *Laboratory Information Bulletin*, **2002**, 4284.
[4] Impens, W. Reybroeck, J. Vercammen, D. Courtheyn, S. Ooghe, K. De Wasch, W. Smedts, and H. De Brabander, *Anal. Chim. Acta*, **2003**, *483*, 153.
[5] P. Mottier, V. Parisod, E. Gremaud, P. A. Guy, and R. H. Stadler, *J. Chromatogr., A*, **2003**, *994*, 75.
[6] M. Ramos, P. Muñoz, A. Aranda, I. Rodriguez, R. Diaz, and J. Blanca, *J. Chromatogr., B*, **2003**, *791*, 31.
[7] J. Storey, A. Pfenning, S. Turnipseed, G. Nandrea, R. Lee, C. Burns, and M. Madson, *Lab. Inform. Bull*., **2003**, 4306.
[8] P. A. Guya, D. Royerb, P. Mottiera, E. Gremauda, A. Perisseta, and R. H. Stadlerc, *J. Chromatogr., A*, **2004**, *1054*, 365.
[9] D. A. Skoog, D. M. West, F. J. Holler, and S. R. Crouch,"*Fundamentals of Analytical Chemistry*", 8th ed., **2004**, Thomson Learning, Belmont, 694.
[10] H. Y. Shen and H. L. Jiang, *Anal. Chim. Acta*, **2005**, *535*, 33.
[11] G. Scortichini, L. Annunziata, M. N. Haouet, F. Benedetti, I. Krusteva, and R. Galarini, *Anal. Chim. Acta*, **2005**, *535*, 43
[12] P. Viñas, N. Balsalobre, and M. Hernández-Córdoba, *Anal Chim. Acta*, **2006**, *558*, 1
[13] R. J. Shakila, S. A. Vyla, R. S. Kumar, G. Jeyasekaran, and G. I. Jasmine, *Food Microbiology*, **2006**, *23*, 47.
[14] A. Preechaworapun, S. Chuanuwatanakul, Y. Einaga, K. Grudpan, S. Motomizu, and O. Chailapakul, *Talanta*, **2006**, *68*, 1726.
[15] P. Ngamukot, T. Charoenraks, O. Chailapakul, S.Motomizu, and S. Chuanuwatanakul, *Anal. Sci.*, **2006**, *22*, 111.