ELSEVIER

Journal of Chromatography A, 734 (1996) 303–310

JOURNAL OF CHROMATOGRAPHY A

Feasibility of supercritical fluid chromatography–chemiluminescent nitrogen detection with open tubular columns

H. Shi[a], J.T.B. Strode III[a], L.T. Taylor[a,*], E.M. Fujinari[b]

[a]*Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA 24060, USA*

[b]*Antek Instruments, Inc., 300 Bammel Westfield Road, Houston, TX 77090, USA*

Received 1 August 1995; revised 17 October 1995; accepted 17 October 1995

Abstract

A supercritical fluid chromatography–chemiluminescence nitrogen detection (SFC–CLND) system for open tubular columns is described. The chemistry for the post-restrictor detector is based on the chemiluminescent reaction between ozone generated from oxygen and nitric oxide via high-temperature pyrolysis of nitrogen-containing compounds. The minimum detectable quantity is found to be 60 pg nitrogen of indole by flow injection analysis. Detector linearity was at least three orders of magnitude, and a selectivity of 10^5 was obtained. Several applications of SFC–CLND in the analysis of compounds containing nitrogen are presented.

Keywords: Supercritical fluid chromatography; Detectors, SFC; Chemiluminescence detector; Indoles; Nitrotoluenes; Pyridine; Caffeine; Diphenylamine; Thiocyanates

1. Introduction

In general, chemiluminescence detection is gaining in popularity because it offers many advantages over other optical techniques [1]. The chemiluminescence response together with the low, dark current in the background places these detectors among the most sensitive of analytical instruments. Because of the unique chemistry of each chemiluminescent reaction, the detectors are inherently very selective. These characteristics allow the specific detection of small amounts of desired analytes in complex matrices. The need for extensive sample clean-up and preparation prior to chromatographic analysis is thus eliminated. In addition, good reproducibility and accuracy of the chemiluminescent technique makes it a viable tool for routine analysis.

The analysis of nitrogen-containing compounds is greatly valued in biological, industrial and environmental research endeavors, such as body fluids, food flavors and pesticides. The detection of nitrogen by chemiluminescence became a state of the art technology only after Fontjin and co-workers [2] demonstrated the usefulness of chemiluminescent light resulting from the gas-phase reaction between nitric oxide and ozone. Several years later, a chemiluminescent nitrogen detector for GC (GC–CLND) was developed by Parks et al. [3]. The detection mechanism is illustrated in the following series of reaction:

*Corresponding author.

0021-9673/96/$15.00 © 1996 Elsevier Science B.V. All rights reserved
SSDI 0021-9673(95)01191-9

$$R - N + O_2 \xrightarrow{1000°C} NO + \text{other compounds}$$

$$NO + O_3 \rightarrow NO_2^* + O_2$$

$$NO_2^* \rightarrow NO_2 + h\nu$$

The light emitted in the process is in the wavelength region of 600–900 nm and is detected by a photomultiplier tube.

The first GC detector utilizing nitric oxide/ozone chemiluminescence was the thermal energy analyzer (TEA) developed by Fine et al. [4–6] for nitrosamine determinations. N-Nitroso compounds were analyzed based on a catalytic low-temperature pyrolysis reaction followed by chemiluminescence detection with ozone. Kashihira et al. utilized a chemiluminescent nitrogen oxide analyzer in conjunction with formation of nitrogen monoxide from nitrogen-containing compounds by pyrolysis on a hot platinum catalyst. This system was applied to the GC measurement of atmospheric ammonia and amines [7]. More recently, an improved nitrogen specific GC–CLND system was reported by Fujinari et al. [8]. A wide range of GC-CLND applications was presented, which included pesticide residue, food flavor, pharmaceutical and petroleum light cycle oil samples. The GC-CLND method has proven to be very sensitive and highly selective, enabling detection of as little as 12 pg nitrogen.

The CLND has also been successfully interfaced with high-performance liquid chromatography. Fujinari et al. [9] described a novel HPLC-CLND system in the quantitation of ammonium nitrogen in waste water. The limit of detection of the system was found to be 5 ng nitrogen.

Supercritical fluid chromatography (SFC) has now become a widely accepted analytical method and is used in various industries. The multidetector compatibility is a major advantage of SFC over both GC and HPLC. A variety of liquid-like and gas-like detectors have been successfully coupled to SFC, such as ultraviolet-visible [10], flame ionization [11], mass spectrometry, Fourier transform infrared spectrometry [12] and sulfur chemiluminescence detection [13–15]. This report describes for the first time the direct interfacing of the CLND to open tubular SFC employing 100% CO_2 as the mobile phase. Results from the performance evaluation of the SFC-CLND system are discussed, and the utility of the system is illustrated by the analysis of several food-related matrices.

2. Experimental

2.1. Instrumentation

A Model 705D CLND nitrogen-specific detector from Antek Instruments (Houston, TX, USA) was interfaced to either a Hewlett-Packard supercritical fluid chromatograph (for flow injection analysis) or a Dionex Lee Scientific (Salt Lake City, UT, USA) series 600 supercritical fluid chromatograph (for chromatographic separation) via a 50 μm I.D. frit restrictor. A SB-cyanopropyl capillary column (20 m$\times$100 μm I.D., 0.25 μm film thickness) obtained from Dionex was used to achieve chromatographic separation. Time-split injection with a helium-actuated Valco (Houston, TX, USA) injector (500 nl rotor) was employed for sample introduction. Data were recorded by a Hewlett-Packard 3310 integrator (Avondale, PA, USA).

2.2. Reagents

Allyl cyanide, allylisothiocyanate, 2,3-dimethylindole, 2,6-dinitrotoluene, diphenylamine, indole, 4-nitrotoluene, 2-phenylindole, phenyl ethyl isothiocyanate and pyridine were purchased from Aldrich (Milwaukee, WI, USA). 2-Butyl isothiocyanate was purchased from Lancaster Synthesis (Windham, NH, USA). 2,5-Lutidine was purchased from Chem Service (West Chester, PA, USA). Caffeine was purchased from Sigma (St. Louis, MO, USA). p-Nitroaniline was purchased from Fisher Scientific (Pittsburgh, PA, USA). Dimethoate was purchased from Accu Standard (New Haven, CT, USA). All chemicals were used without further purification. Horseradish oil standard and hot mustard extract were received from commercial sources. HPLC grade solvents (EM Science, Gibbstown, NJ, USA) were used for preparing standard solutions. Grade 4.3 oxygen (Airco, Murray Hill, NJ, USA) was used as both pyrolysis and ozone-generator gas. SFC-grade CO_2 was obtained from Air Products and Chemical (Allentown, PA, USA).

2.3. Chromatographic conditions

All analyses were performed with pressure programming. In all cases, the oven temperature was held constant throughout the run. Integrator conditions: 1 V input, attenuation 7. CLND conditions: pyrolysis temperature 1050°C, PMT voltage 750 V, range ×50, detector output 1 V. Additional chromatographic conditions are given in the figure legends.

3. Results and discussion

3.1. Detector sensitivity optimization

The CLND was used with capillary SFC employing 100% CO_2 as the mobile phase without modifier. The simple interface between the SFC and the CLND is accomplished by using a conventional length (25 cm) frit restrictor which is threaded through the bottom of the pyrolysis furnace. A schematic diagram of the SFC-CLND is given in Fig. 1. When a sample is chromatographed on a column, the eluting compounds under supercritical fluid conditions are decompressed through the restrictor and then introduced into the pyrolysis furnace where they mix with oxygen and undergo high-temperature oxidation. The responsive nitrogen compounds are converted into nitric oxide. The nitric oxide is then drawn into the reaction chamber and reacted with ozone. This results in the formation of nitrogen dioxide in the excited state (NO_2^*) and the chemiluminescence from the NO_2^* is detected by a photomultiplier tube (PMT).

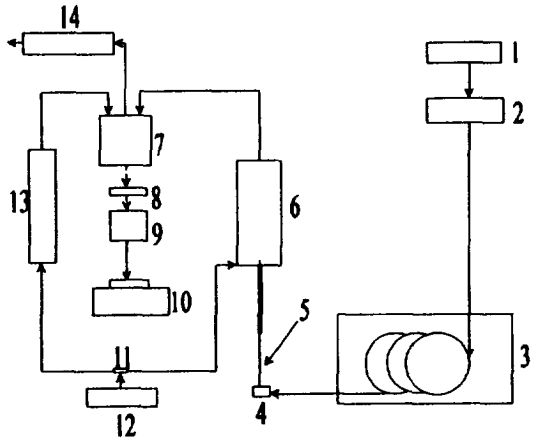

Fig. 1. Schematic drawing of the SFC-CLDN system. (1) CO_2 tank; (2) CO_2 pump; (3) SFC oven; (4) butt connector; (5) frit restrictor; (6) pyrolysis furnace; (7) reaction chamber; (8) filter; (9) PMT tube; (10) integrator; (11) flow meter; (12) oxygen tank; (13) ozone generator; (14) scrubber.

There are several operating variables which are critical to the detector response. These parameters include (a) restrictor position and (b) pyrolysis oxygen flow-rate. First, we optimized the CLND sensitivity by proper insertion of the restrictor to the detector interface. The restrictor positions and the corresponding indole signal responses of the CLND are shown in Fig. 2. The result indicates that the

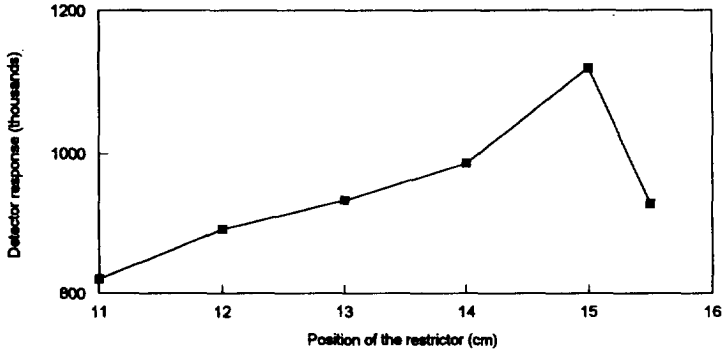

Fig. 2. Effect of restrictor position on CLND response. Conditions: pressure program 100 atm (hold 1 min); ramp to 180 atm at 20 atm/min; hold 3 min; biphenyl (3 m×50 μm I.D., 0.25 μm film thickness) column; time-split injection 0.3 s; sample, indole in methanol.

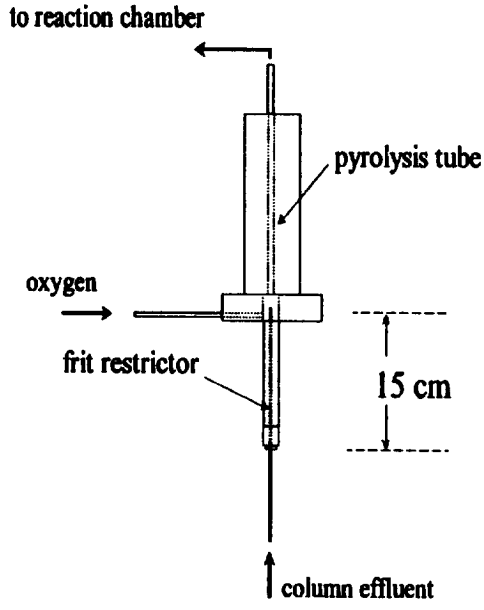

Fig. 3. The detailed interface of the SFC-CLND system.

optimum position for the restrictor is 15 cm into the tube adjoining the detector base of the pyrolysis furnace and is elucidated in Fig. 3. At the optimum position, the restrictor tip is placed above the oxygen inlet to the pyrolysis chamber; therefore, the column effluent is mixed with the pyrolysis oxygen and effectively swept into the furnace. The efficient pyrolysis of the sample and the resulting nitric oxide gas provide the means for a maximum sensitivity.

The CLND was further optimized by maintaining a constant oxygen flow-rate through the ozone generator at 8 ml/min and varying the pyrolysis oxygen flow from 20 to 65 ml/min. The detector response (peak area) in this case was obtained by injecting 2,6-dinitrotoluene in methanol. As shown in Fig. 4, a significant drop in the detector response was observed when the pyrolysis oxygen flow-rate is either below 30 ml/min or above 55 ml/min. A possible explanation for this is that (a) low pyro-oxygen flow-rate into the furnace results in incomplete oxidation of nitrogen to nitric oxide and (b) incomplete oxidation is also obtained from the short residence time of the analyte in the pyrolysis chamber when the pyro-oxygen flow-rate is set too high. The best CLND response was achieved at a flow-rate between 35 and 50 ml/min. The pyrolysis oxygen flow-rate of 40 ml/min was selected and used in the experiment.

3.2. Detector performance

The SFC-CLND system was evaluated by determining linear dynamic range (LDR), response factors, selectivity and minimum detectable quantity. Calibration curve was constructed from standard solutions of indole in methanol ranging from 2.96 to 850 ppm of nitrogen. Linear least-square analysis resulted in a correlation coefficient higher than 0.999. The LDR of three orders of magnitude was

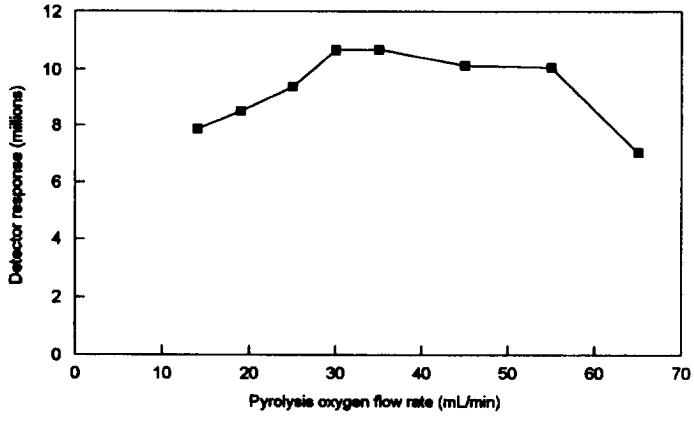

Fig. 4. Effect of pyrolysis oxygen flow-rate on CLND response. Conditions: (flow injection analysis) 60°C transfer line; CO_2 pressure, 300 atm; sample, 2,6-dinitrotoluene in methanol.

Table 1
CLND minimum detectable quantity (MDQ) comparison for SFC, GC and HPLC

Chromatographic system	MDQ (pg nitrogen)	Analyte	Reference
GC	12	Nitric oxide	[8]
SFC	60 (FIA)	Indole	
HPLC	5000	Ammonium ion	[9]

obtained. Minimum detectable quantity was determined by a signal ($S/N=3$) (Fig. 10) resulting from a flow injection of 1.40 ppm nitrogen of indole solution via a 5-μl sample loop with a split ratio of 1/116.5 (CLND/UV), and was found to be 60 pg nitrogen at the detector. The minimum detectable quantity for SFC by CLND is compared to that by GC and HPLC (Table 1). As reported previously, the chemiluminescent nitrogen detector is molar responsive [16,17], and responds to the nitrogen mass or content of each nitrogenous compound. Peak areas per unit mass of nitrogen for some selected compounds were used to calculate relative response factors. Table 2 lists the response factors for those selected nitrogen compounds relative to the response from indole (arbitrary units of the detector response per unit mass of nitrogen). As expected the result showed equimolar nitrogen response for the detector. The advantage of this detector for quantifying nitrogen-containing compounds in a complex sample is that a single calibration curve may be used, provided that the proper chromatographic conditions are used. Selectivity (N/C) of five orders of magnitude using SFC-CLND was determined by comparing the indole to methanol response. In most cases, pressure programming is needed to achieve the necessary SFC separations. Therefore, preliminary linear pressure programming experiments (without sample injection) from 100–400 atm were conducted at various ramp rates (10, 15, 20, 25, 30, 35 and 40 atm/min) in order to study the effects of pressure changes on the detector. The CLND showed a stable baseline for every ramp as depicted in Fig. 5, indicating the feasibility for using pressure programming to obtain SFC separations.

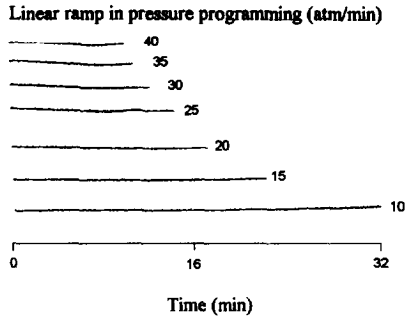

Fig. 5. The CLND baseline stability is shown during linear pressure programming with SF-CO_2. Conditions: pressure program 100 atm (hold 1 min), ramp to 400 atm (hold 1 min) at different ramps; cyano (10 m×100 μm I.D., 0.25 μm film thickness) column; without sample injection.

Table 2
Relative response factors in CLND

Component	Response factor
4-Nitrotoluene	1.01
2,6-Dinitrotoluene	1.05
2,3-Dimethylindole	1.04
Pyridine	1.01
Caffeine	1.07
Diphenylamine	1.06
Indole	1.00
2-Phenylindole	1.01

3.3. Applications

Capillary SFC-CLND chromatography of a mixture of 2,5-lutidine, 4-nitrotoluene, 2,6-dinitrotoluene, caffeine, indole, dimethoate and *p*-nitroaniline prepared in methanol has been done (Fig. 6). No significant base-line shift was observed by the detector when pressure programming from 100 to 340 atm. Analysis of a hot mustard extract by the capillary SFC-CLND system is demonstrated in Fig. 7. Peaks A and B are allylisothiocyanate and 2-butylisothiocyanate, respectively. These nitrogen-containing compounds are also the "hot" components present in horseradish oil. Peak C is an unknown component in the hot mustard sample.

Horseradish oil, a very high impact oil, is used in low amounts to enhance flavors. The separation of a horseradish oil standard by capillary SFC-CLND is demonstrated in Fig. 8. The peaks A, C, D and E were identified by injecting single component standards. A standard for identifying peak B was un-

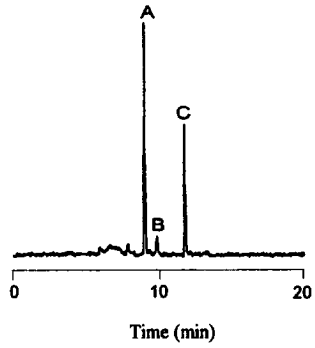

Fig. 7. SFC-CLND separation of a hot mustard extract (0.1 gram of hot mustard powder extracted with a 1-ml water/methanol (30:70) mixture). A=allylisothiocyanate; B=2-butylisothiocyanate; C=unknown nitrogen-containing compound. Chromatographic conditions: pressure program from 80 atm (hold 5 min), ramp to 150 atm at 10 atm/min, then ramp to 200 atm at 15 atm/min; cyano (20 m×100 μm I.D., 0.25 μm film thickness) column; time-split injection 0.2 s.

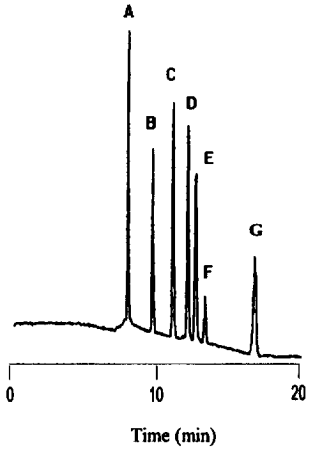

Fig. 6. SFC-CLND separation of a nitrogen-containing mixture. Peaks: A=2,5-lutidine; B=4-nitrotoluene; C=2,6-dinitrotoluene; D=caffeine; E=indole; F=dimethoate; G=*p*-nitroaniline. The concentration of each component in the mixture varies from 50 to 80 ppm. Chromatographic conditions: pressure program from 100 atm (hold 4 min), ramp to 250 atm at 15 atm/min, then ramp to 340 atm at 30 atm/min (hold 3 min); cyano (20 m×100 μm I.D., 0.25 μm film thickness) column; time-split injection 0.2 s; sample in methanol.

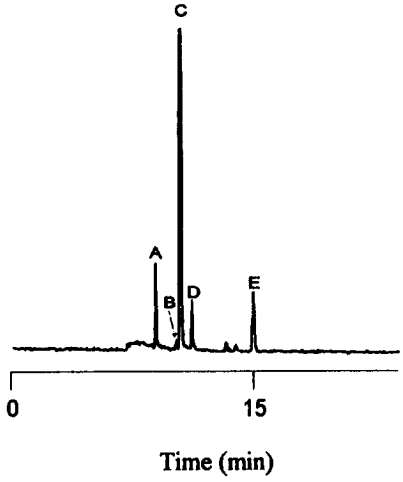

Fig. 8. SFC-CLND separation of a 0.03% (w/w) horseradish oil standard. A=but-3-enonitrile; B=allylthiocyanate; C=allylisothiocyanate; D=2-butylisothiocyanate; E=phenylethylisothiocyanate. Chromatographic conditions: pressure program from 80 atm (hold 5 min), ramp to 150 atm at 10 atm/min, then ramp to 200 atm at 15 atm/min; cyano (20 m×100 μm I.D., 0.25 μm film thickness) column; time-split injection 0.2 s; sample in ethanol.

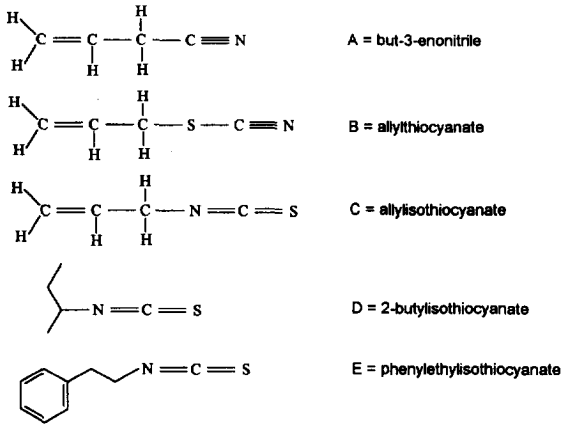

Fig. 9. Structures of the nitrogen-containing components in the horseradish oil of the SFC-CLND chromatogram in Fig. 8.

Fig. 10. Peak resulting from flow injection of 1.40 ppm nitrogen of indole via a 5-μl sample loop with a split ratio of 1/116.5 (CLND/UV). Minimum detectable quantity is 60 pg nitrogen at $S/N=3$.

available; therefore, assignment (B=allylthiocyanate) was made by comparing the SFC-CLND trace to a known GC–CLND chromatogram [18] of a horseradish oil standard. The molecular structures of these compounds are elucidated in Fig. 9.

4. Conclusions

The chemiluminescent nitrogen detector has been successfully interfaced to capillary SFC. In addition to equimolar nitrogen responses, the detector demonstrated high nitrogen selectivity, sensitivity (minimum dectectable quantity 60 pg nitrogen; Fig. 10) and linearity. Pressure programming used in this study showed no perturbation of the detector base line. The SFC-CLND technique provides an alternative approach to current GC and HPLC methods, thus providing new avenues to meet challenges in analytical chemistry.

Acknowledgments

The authors thank Antek Instruments Inc., for the loan of 705D nitrogen specific detector. Additionally, we would like to acknowledge Air Products and Chemicals, Inc., for the donation of carbon dioxide, Dionex, Lee Scientific Division, and Hewlett-Packard Company for the loan of the SFC systems.

References

[1] R.S. Hutte, R.E. Sievers and J.W. Birks, J. Chromatogr. Sci., 24 (1986) 499.
[2] A. Fontijn, A.J. Sabadell and R.J. Ronco, Anal. Chem., 42 (1970) 575.
[3] R.E. Parks and R.L. Manetta, U.S. Patent 4,018,562, 24 October 1975.
[4] D.H. Fine, F. Rufeh, D. Lieb and D.P. Roundbehler, Anal. Chem., 47 (1975) 1188.
[5] D.H. Fine and D.P. Roundbehler, J. Chromatogr., 109 (1975) 271.
[6] D.H. Fine, D. Lieb and F. Rufeh, J. Chromatogr., 107 (1975) 351.

[7] N. Kashihira, K. Makino, K. Kirita and Y. Watanabe, J. Chromatogr., 239 (1982) 617.
[8] L.O. Courthaudon and E.M. Fujinari, LC·GC, 9 (1991) 732.
[9] E.M. Fujinari and L.O. Courthaudon, J. Chromatogr., 592 (1992) 209.
[10] S.M. Fields, K.E. Markides and M.L. Lee, Anal. Chem., 60 (1988) 802.
[11] B.E. Richter, J. High Resolut. Chromatogr. Chromatogr. Commun., 8 (1985) 297.
[12] D.W. Later, D.J. Bornhop, E.D. Lee, J.D. Henion and R.C. Weibolt, LC·GC, 5 (1987) 804.
[13] D.J. Burnhop and B.J. Murphy, Anal. Chem., 61 (1989) 797.
[14] H.-C.K. Chang and L.T. Taylor, J. Chromatogr., 517 (1990) 491.
[15] A.L. Howard and L.T. Taylor, Anal. Chem., 65 (1993) 724.
[16] A.J. Robbat, P.N. Corso, J.P. Doherty and H.M. Wolf, Anal. Chem., 58 (1986) 2078.
[17] E.M. Fujinari and J.D. Manes, in G. Charalambous (Editor), Food Flavors: Generation, Analysis and Process Influence, Elsevier Science, Amsterdam, 1995, pp. 929.
[18] S.M. Benn, K. Myung and E.M. Fujinari, in G. Charalambous (Editor), Food Flavors, Ingredients and Composition. Elsevier Science Publishers, Amsterdam, 1993, pp. 65.