Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Pharmaceutical and Biomedical Analysis 41 (2006) 725–730
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Comparison of electrospray ionization mass spectrometry and evaporative light scattering detections for the determination of Poloxamer 188 in itraconazole injectable formulation☆

Lakshmy M. Nair*, Jamie Konkel, Mary Thomas, Michael Koberda

Medication Delivery, Baxter Healthcare, Round Lake, IL 60073, USA

Received 4 November 2005; received in revised form 15 December 2005; accepted 21 December 2005
Available online 3 February 2006

Abstract

A high performance liquid chromatography (HPLC) method for Poloxamer 188 using size-exclusion chromatography (SEC) was developed and two different detection mechanisms, evaporative light scattering (ELSD) and electrospray ionization mass spectrometry (ESI-MS), were compared for their quantification capabilities in itraconazole formulation. Both detection techniques coupled with SEC separation were highly effective for the determination of Poloxamer 188, which is difficult to analyze by other common HPLC methods. As expected, ESI-MS detection provided sensitivity and selectivity superior to ELSD. But since the analyte is an excipient in the formulation, high sensitivity was not required and ELSD's simplicity and ruggedness made it more appropriate for routine analysis of this formulation.

Keywords: Poloxamer 188; ELSD; LC–MS; Itraconazole

1. Introduction

Approximately 40% of the drugs listed in United States Pharmacopoeia (USP) and New Chemical Entities (NCE) are practically insoluble in water [1]. This poses a major challenge in the drug delivery industry as water insoluble drug formulations are typically unstable or may lack bioavailability. A model drug that is highly insoluble in water and used in formulation studies described here is itraconazole, a potent antifungal agent. Baxter Healthcare has developed an injectable suspension formulation of itraconazole using their patented NANOEDGE technology [2]. This technology was developed to formulate poorly soluble drug compounds into injectable products. Itraconazole injectable suspension contains drug nanoparticles, and various surfactants, that include Poloxamer 188 added as a surface stabilizer to prevent particle size increase as a result of Ostwald ripening [3].

Poloxamer 188 or *Pluronic F68* is a water soluble, nonionic, triblock copolymeric surfactant consisting of a hydrophobic center chain (block) of polyoxypropylene oxide with two long hydrophilic chains (blocks) of polyoxyethylene oxide (see Fig. 1). The weight average molecular weight of Poloxamer 188 is 8400 Da. It is widely used in pharmaceuticals, biological and cosmetic industries due to its surface-active properties and is approved by the Food and Drug Administration (FDA) as a constituent of various injection products [4]. It is also reported in the National Formulary as a pharmaceutical ingredient.

Even though the use of poloxamers is widely reported, analyses of these compounds are still challenging, especially in pharmaceutical formulations, due to their lack of an ultra-violet (UV) chromophore and poor separation characteristics. The need for an accurate and easy to use analytical tool to measure the concentrations of poloxamers in such matrices still exists. The most common procedure for Poloxamer 188 determination is HPLC with size-exclusion (SEC) separation and refractive index detection (RI) [5,6]. RI detectors have major limitations such as unstable baselines and longer equilibration time. Additionally, this method was found unsuitable for our application due to matrix interferences.

Qualitative and quantitative determination of poloxamers using matrix-assisted laser desorption mass spectrometry (MALDI-MS) and electrospray ionization mass spectrometry

☆ NANOEDGE is a trademark of Baxter Healthcare and PLURONIC is a trademark of BASF Corporation.

* Corresponding author. Tel.: +1 847 270 5942; fax: +1 847 270 5999.
E-mail address: lakshmy_nair@baxter.com (L.M. Nair).

0731-7085/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.12.028

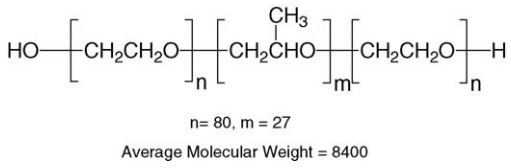

Fig. 1. Structure of Poloxamer 188.

(ESI-MS) was reported by Takats et al. [7]. MALDI-MS was used for the weight average molecular weight determination and ESI-MS was used for concentration determination of Poloxamer 188. The ESI-MS method was complicated, since it required a sample clean-up column. The method also lacked reproducibility.

Two simple approaches for the determination of Poloxamer 188 in an itraconzole formulation are described in this report. Application of evaporative light scattering (ELS) and electrospray ionization mass spectrometry (ESI-MS) detection in adjunct with size-exclusion separation are described. Both detectors have universal detection properties allowing quantification of compounds that are not suitable with other detection techniques. A comparison study of both methods for Poloxamer 188 quantification is described in this report.

Popularity of the ELS detector has increased considerably since its introduction in the early 80s. ELSD's ability to detect any non-volatile compounds regardless of their structural characteristics is advantageous for compounds that lack UV absorbing groups. It is predominantly used for the determination of surfactants, polymers, carbohydrates and triglycerides in various industries [8–11]. In the pharmaceutical industry, it is used for the determination of drugs, impurities, raw materials and inorganic counter ions [12–16]. Theory and operation of ELS detector is described in various reports [17,18]. In our study, ELS detection was ideal for Poloxamer 188 measurements due to the non-volatile property of the analyte and volatility of the mobile phase.

In parallel with the ELSD method, mass spectrometry (MS) coupled with HPLC was explored for the quantification of Poloxamer 188. Mass spectrometry is known for its dependable, high sensitivity in various sample matrices. Different types of interfaces are used with MS detector, however ESI-MS has been the most commonly technique used since the beginning of 1990s [19]. Theory and applications have been reported previously in numerous papers [20]. In this report, application of an ESI-MS interface connected to a single quadrupole mass analyzer is used for the Poloxamer 188 determinations. This method is not suitable for the molecular weight determination of Poloxamer 188, as it is outside the range of ESI-MS. However, this does not preclude its use as a quantititative tool for monitoring poloxamer concentrations.

The scope of this work was to develop a high performance liquid chromatography method that is compatible with ELSD or ESI-MS to allow for rapid, specific and reproducible determination of Poloxamer 188 in an itraconazole nanosuspension formulation. Both methods were found to be acceptable for use in our formulation studies.

2. Experimental

2.1. Reagents

Acetonitrile (HPLC grade) was purchased from Burdick & Jackson Labs (Muskegon, MI, USA). Distilled water from an in-house source (Baxter Healthcare, Round Lake, IL, USA) was used throughout the analyses. Poloxamer 188 was purchased from BASF corporation (Ludwigshafen, Germany). Both standards and samples were prepared from the same lot of raw material. Ammonium acetate was purchased from Sigma–Aldrich (Milwaukee, WI, USA). Itraconazole suspension was prepared from itraconazle raw material (DSM Pharma, South Haven, MI, USA), and included Poloxamer 188.

2.2. SEC/ELSD chromatographic system

The HPLC system consisted of a Waters 2695 HPLC system (Waters, Milfred, Massachusetts) coupled with an Alltech ELSD 2000 Detector (Alltech Associates, Deerfield, IL, USA). Nitrogen gas (ultra-pure >99%) used to operate the ELSD system was produced using a Nitrogen Generator manufactured by Alltech Associates (Alltech Associates, Deerfield, IL, USA). ELSD was operated in the Impactor "On" mode and the drift tube temperature was set at 40 °C. Nitrogen flow was maintained at 1.9 l/min. The theory and operation of Alltech ELSD 2000 has been discussed in a previously published literature [17]. Poloxamer 188 was chromatographed using a 300 mm × 7.8 mm, Waters ULTRAHYDROGEL, 5 μm, 200 A (pore size) (Waters Corporation, Milfred, MA, USA) size-exclusion column. Mobile phase contained 20% acetonitrile and 80% water, which was operated at a flowrate of 1 ml/min under isocratic conditions. The column temperature was maintained at 40 °C with a column heater. The injection volume was 100 μl. All samples and standards were dissolved in 100% acetonitrile.

2.3. SEC/ESI-MS analysis

The LC–MS system consisted of an Agilent 1100 HPLC system including (binary pump, autosampler, and column oven), and an Agilent single quadrupole MS detector (Agilent, Palo Alto, CA, USA). A Parker Nitrogen Generator (Parker Hannifin Corp., Haverhill, MA, USA) was used as the source for nitrogen gas. Same column described in the ELSD method was also used in this method to separate Poloxamer 188. Mobile phase consisted of 95% 10 mM ammonium acetate and 5% acetonitrile. The flowrate was kept at 1 ml/min and the injection volume was at 20 μl. Samples and standards were prepared in the mobile phase. Table 1 shows the optimized ESI-MS method for the analysis of Poloxamer 188.

3. Results and discussion

3.1. Comparison of ELSD versus ESI-MS for poloxamer188 determination

In the earlier stages of method developments, it is a common practice to use LC–MS in adjunct with ELSD to gather

Table 1
Optimized conditions for Poloxamer 188 determination by ESI-MS

Separation parameters	
Column	Waters ULTRAHYDROGEL, 300 mm × 7.8 mm
Mobile phase	10 mM ammonium acetate/acetonitrile (95/5)
Flow rate	1.0 ml/min
Column temperature	40 °C
Injection volume	20 μl
ESI-MS parameters	
Ionization mode	ESI-MS
Polarity	Positive
Drying gas	12.0 l/min
Nebulizer pressure	50 psig
Vcap (positive/negative)	5000 V
Fragmentor voltage	300 V
SIM ion	976.4

multiple information. For example in combinatorial chemistry, LC–MS and ELSD are connected in sequence so the former can be used to acquire structural information and the latter is used for quantification purpose. This is possible due to the similarities of both detection mechanisms in terms of operating requirements. Most methods are interchangeable with slight or no modification to the mobile phase conditions. Similarities, advantages and disadvantages of both techniques are summarized in Table 2.

3.2. Analysis of Poloxamer 188

3.2.1. SEC/ELSD system

For the determination of Poloxamer 188 in the itraconazole suspension formulation, initially the HPLC/ELSD method was developed. Separation is based on size-exclusion, which is also referred to as gel permeation chromatography (GPC), where retention of molecules in solution is based on their molecular sizes. This is widely used for the separation of polymers, surfactants and proteins. The Waters Ultrahydrogel column used in our experiments is an aqueous GPC column, compatible with up to 20% organic solvent. Fig. 2 shows typical chromatograms of Poloxamer 188 in standard (raw material dissolved in acetonitrile) and in itraconazole suspension sample preparation.

3.2.2. SEC/ESI-MS

Same size-exclusion column was used with the ESI-MS system except slight modification to mobile phase was made in order to protonate the sample analyte. In this case, the mobile phase was modified to 10 mM ammonium acetate (95%) and acetonitrile (5%). Sample volume was changed to 20 μl to accommodate the high sensitivity of mass spectrometer detection.

Analysis of Poloxamer 188 by ESI-MS required two steps. First step is the isolation of a tracking ion, which represents Poloxamer 188 and the second step is the quantification by selected ion monitoring (SIM). In the first step, a stock solution of Poloxamer 188 (conc. = 1 mg/ml) was injected by flow injection analysis (FIA). Mobile phase flow rate was kept at 0.2 ml/min with an injection volume of 20 μl. The MS was set to full scan analysis. The resolution between the peaks was not adequate to determine the molecular weight of Poloxamer 188, however the mass spectrum was useful to provide the most intense peak with m/z of 976.4. This peak represents poloxamer molecule since the number of charges associated with the peak is calculated to be approximately in the range of 8–10. This was determined based on the theoretical molecular weight calculation (8624) from the formula in Fig. 1 and the m/z value of 976.4. This ion was used as a tracking ion for poloxamer quantification and applied in the SIM mode. Table 1 describes the final operating conditions in the SIM mode. Illustration of selected ion and its corresponding chromatogram in itraconazole formulation sample is shown in Fig. 3.

3.3. Specificity

Injections of matrix solution (containing all formulation components except Poloxamer 188), mobile phase and sample diluent were performed on the ELSD system to evaluate specificity of the method. Chromatograms of these samples showed no peaks with signal to noise ratio greater than 3 (S/N > 3) at the retention time of the Poloxamer 188 peak, confirming the specificity of the method. During the course of the analysis, a growing peak was observed at approximately 9.5 min. Injections of individual components in the formulation matrix confirmed that this peak is due to column build-up from glycerin in the sample. Throughout the analysis, blank solutions were injected in between sample injections to eliminate the interference from glycerin build-up.

Same experiments were repeated on the ESI-MS system to demonstrate specificity. Results showed no interfering peaks at the retention time of Poloxamer 188. This method did not

Table 2
Comparison of ELSD vs. ESI-MS

Data element	LC/ELSD	LC/ESI-MS
Specificity/selectivity	Separation is necessary to achieve specificity/selectivity	Specificity/selectivity can be achieved from mass analyzer and from the separation
Quantification	Separation is necessary for reliable quantification	No separation is required
Calibration curves	Multi-point calibration is recommended	Multi-point calibration is recommended
Buffers	Compatible with only volatile buffers	Compatible with only volatile buffers
Chemical interferences	Separation is required to isolate analyte from interferences	Internal standards are used in most cases. Baseline separation can be used to isolate the analyte
Ease and cost of operation	Easy and less expensive to operate	Requires special skills and training LC–MS systems are expensive

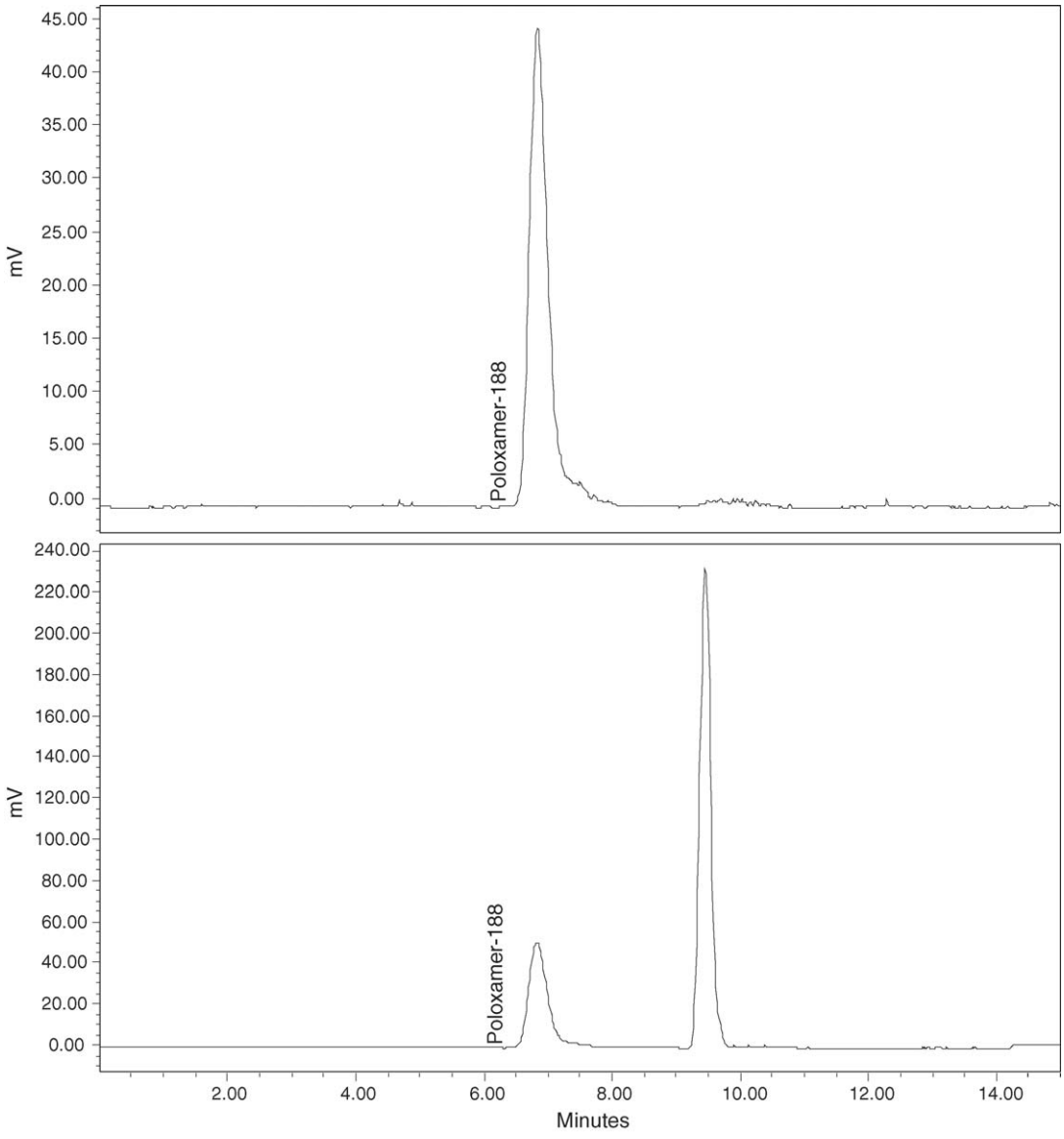

Fig. 2. Poloxamer 188 chromatograms in standard and sample solutions by SEC/ELSD.

require additional injections of blank solutions to clean up the columns, as glycerin did not interfere with the peak of interest.

3.4. Accuracy and precision

For both methods, an external standard calibration procedure was used to quantify Poloxamer 188. External standard is seldom used in LC–MS and its use with ELSD is often challenged by its non-linear response over wide concentration ranges [11]. However, in both cases, choosing three calibrations within a relatively small range, the calibration curve was demonstrated to be linear within the range.

For the ELSD method, accuracy was studied through an evaluation of Poloxamer 188 recoveries from 0.1 to 0.3 mg/ml in test articles. Since mass spectrometry is more sensitive than ELSD, the test articles for the ESI-MS method were prepared at concentrations ranging from 0.05 to 0.15 mg/ml.

Table 3 shows comparison of accuracy and precision results from the evaluation of both methods.

Table 3
Accuracy and precision: ELSD vs. ESI-MS

Test articles (%) ($n = 3$)	Mean % recovery ELSD	Mean % recovery ESI-MS	%R.S.D. ELSD	%R.S.D. ESI-MS
50	102.1	99.9	1.2	1.5
100	97.2	101.2	1.5	0.5
150	101.2	98.4	1.1	0.6

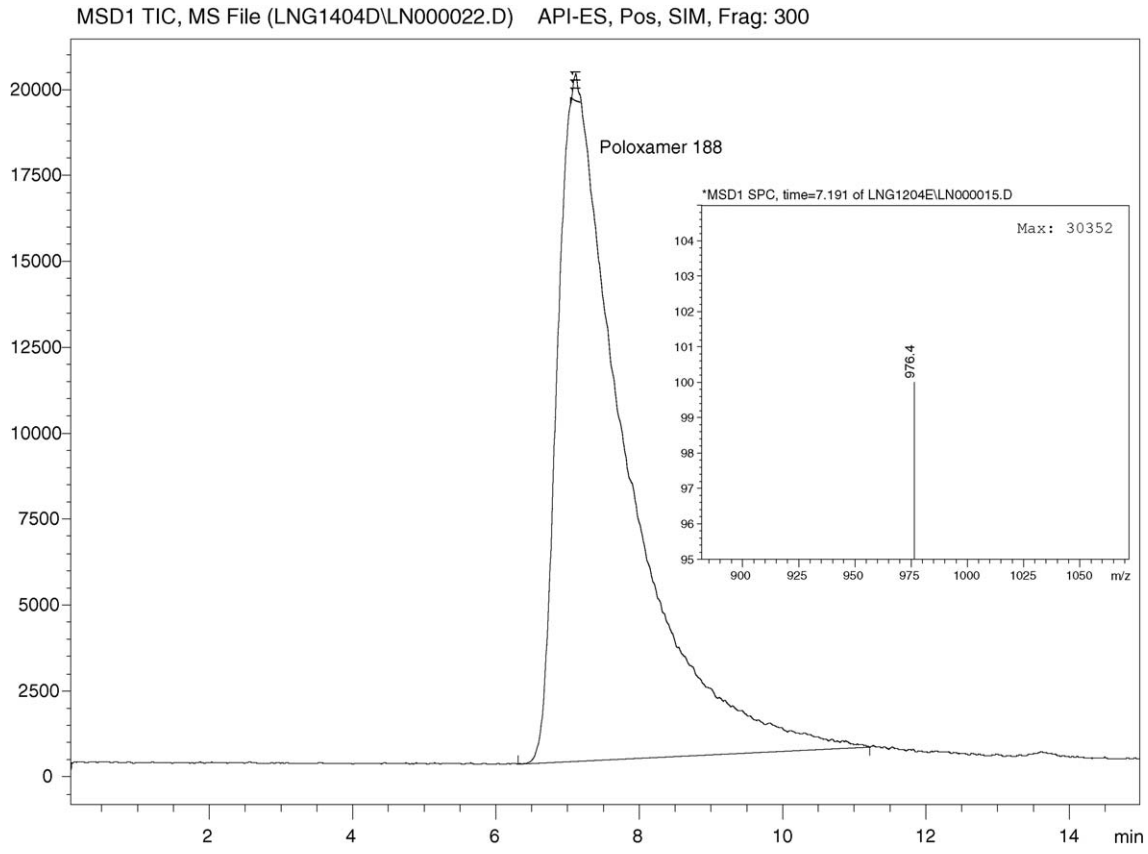

Fig. 3. Chromatogram of Poloxamer 188 and corresponding selected ion with SEC/ESI-MS.

3.5. Linearity

For the ELSD method, calibration curves were generated for solutions containing 0.1 through 0.3 mg/ml Poloxamer 188 concentration levels. Peak areas were plotted versus the respective theoretical standard concentrations that provided a regression line of $Y = 1.0071x - 0.0014$. The correlation coefficient (r) for linear least squares was 0.997. The ESI-MS method was linear in the working range of 0.05–0.15 mg/ml. The regression line for this method was $Y = 11177x + 174217$ with a correlation coefficient (r) of 0.999.

3.6. Limit of quantification (LOQ) and limit of detection (LOD)

To determine the LOQ for Poloxamer 188, the lowest concentration sample was analyzed and recovery and precision were calculated using both methods. With the ELSD method the LOQ was calculated to be 25 μg/ml (based on signal-to-noise ratio ≥10), which gave % recovery of 93% with the %R.S.D. of 2% for repeated injections ($n = 3$). Limit of detection (LOD) for this method was estimated to be approximately 8 μg/ml (calculated theoretically).

ESI-MS is clearly more sensitive for Poloxamer 188 determination and required only 20 μl injection volume. For comparison purpose, LOQ and LOD were assessed based on 100 μl injection volume. The quantification limit for Poloxamer 188 (the concentration needed to produce a S/N ratio of 10:1) was determined to be 2 μg/ml and the detection limit (S/N ≥ 3) was calculated to be 0.8 μg/ml respectively.

4. Conclusion

Two different HPLC methods were developed and compared for the quantification of Poloxamer 188 in an itraconazole suspension. Size-exclusion chromatography coupled with ELSD was used in one method while ESI-MS detection was used in the second one. Compared to the previously reported SEC-RI method, both the methods are free of interferences and provided better sensitivity. Investigations of both methods to evaluate precision, accuracy, linearity and specificity gave excellent results. As predicted, ESI-MS method provided higher sensitivity. SEC-ELSD is easy and less expensive to operate than the ESI-MS, and easier to transfer from a research environment to a manufacturing facility.

References

[1] C. Lipinski, Am. Pharm. Rev. 5 (2002) 82–85.
[2] J. Kipp, J. Wong, M. Doty, C. Rebbeck, United States Patent No. US 6607784B2 (19 August 2003).
[3] J. Kipp, Int. J. Pharmaceut. 284 (2004) 109–122.
[4] FDA/Center for Drug Evaluation (CDER) and Research, Website.
[5] B. Erlandsson, B. Wittgren, G. Brinkmalm, J. Pharm. Biomed. Anal. 31 (2003) 845–858.

[6] J.M. Grindel, T. Jaworski, O. Piraner, R.M. Emanuele, M. Balasubramanian, J. Pharm. Sci. 91 (2002) 1936–1947.
[7] Z. Takats, K. Vekey, L. Hegedus, Rapid Commun. Mass Spectrom. 15 (2001) 805–810.
[8] G.R. Bear, J. Chromatogr. 459 (1988) 91–107.
[9] Y. Mengernik, H.C.J. De Man, S.J. Van De, R. Wal, J. Chromatogr. 552 (1991) 593–604.
[10] B.L. Neff, H.J. Spinelli, J. Appl. Polym. Sci. 42 (1991) 595–600.
[11] R. Macrae, J.J. Dick, J. Chromatogr. 210 (1981) 138–145.
[12] W.S. Lettar, J. Liq. Chromatogr. 16 (1993) 225–230.
[13] J.G. Wilkes, J.B. Sutherland, M.I. Churchwell, A.J. Williams, J. Chromatogr. A 695 (1995) 319–323.
[14] M.D. Lantz, D.S. Risley, J.A. Peterson, J. Liq. Chromatogr. & Rel. Technol. 20 (1997) 1409–1415.
[15] D.S. Risley, J.A. Peterson, K.L. Griffiths, S. McCarthy, LC/GC 14 (1995) 1040–1042.
[16] D.S. Risley, K.F. Hostettler, J.A. Peterson, LC/GC 16 (1998) 562–565.
[17] Alltech Associate Inc. ELSD 2000 Product Literature, Bulletin # 424.
[18] A. Kuch, R. Saari-Nordhaus, Am. Lab. (2001) 61–64.
[19] J.B. Fenn, J. Am. Soc. Mass Spectrom. 4 (1993) 524–535.
[20] R.B. Cole (Ed.), Electrospray Ionization Mass Spectrometry: Fundamentals, Instrumentation, and Applications, John Wiley & Sons, New York, 1997.