ELSEVIER

Journal of Chromatography A, 729 (1996) 355-361

JOURNAL OF CHROMATOGRAPHY A

# Application of high-performance liquid chromatography with diode-array detection and on-line post-column photochemical derivatization to the determination of analgesics

A.M. Di Pietra, R. Gatti, V. Andrisano, V. Cavrini*

*Department of Pharmaceutical Sciences, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy*

**Abstract**

HPLC analyses of pharmaceutical dosage forms containing analgesics and related compounds (acetylsalicyclic acid, paracetamol, propyphenazone, caffeine and chlorpheniramine) were performed on $C_{18}$ and cyano columns under reversed-phase conditions. The performance of the methods was enhanced by introducing postcolumn on-line photochemical derivatization in combination with a diode-array detection. The column effluents were subjected on-line to UV irradiation (254 nm) and the characteristic photo-induced spectral modifications were useful for the unambiguous identification of the various analgesic compounds. The proposed HPLC methods were successfully applied to the analysis of commercially available analgesic dosage forms.

*Keywords:* Pharmaceutical analysis; Derivatization, LC; Drugs; Acetylsalicylic acid; Paracetamol; Propyphenazone; Caffeine; Chlorpheniramine

## 1. Introduction

Analgesics are widely used drugs, not only as pain relievers but also in several diseases (arthritis, rheumatism) [1]. Their determination in biological fluids (overdose monitoring) and in pharmaceutical dosage forms (quality control) remains of great interest. Among the various analytical techniques, high-performance liquid chromatography (HPLC) constitutes the most popular chromatographic method for separating mixtures of analgesic drugs and related compounds [2–4]; in particular, HPLC methods have been reported for acetylsalicyclic acid (ASA) [5–7], propyphenazone [5,8,9], paracetamol [6–10], caffeine [6–10] and chlorpheniramine [10,11].

For reliable quality control of analgesic products, a selective and versatile detection system is desirable to aid in the positive identification of the column effluents.

The present study was aimed at developing chromatographic conditions suitable for the resolution of the principal analgesic mixtures and at providing information-rich detection to achieve unambiguous identification of the various components. To this end, diode-array detection (DAD) was used in combination with post-column on-line photochemical derivatization. Using a photoreactor arranged on-line between the column and the DAD instrument, the column effluent was subjected to UV irradiation (254

* Corresponding author.

0021-9673/96/$15.00 © 1996 Elsevier Science B.V. All rights reserved
*SSDI* 0021-9673(95)01037-8

nm) and photo-induced chromophore alterations resulted in modified UV spectral properties of the analytes. Thus, the known efficacy of the DAD in confirming the peak identity [12] was enhanced, enabling two different UV spectra (photoreactor on and off) to be obtained for each analyte. The HPLC method was successfully applied to the analysis of commercially available analgesic dosage forms (tablets).

## 2. Experimental

### 2.1. Materials

Acetylsalicyclic acid, caffeine and resorcinol were obtained from Carlo Erba (Milan, Italy), paracetamol from Fluka (Buchs, Switzerland) and propyphenazone from Bracco (Milan, Italy). Chlorpheniramine was kindly supplied by Shering (Milan, Italy). For chromatographic separations, acetonitrile and tetrahydrofuran (THF) of HPLC grade (Mallinckrodt, St. Louis, MO, USA) and doubly distilled, deionized water were used.

Triethylammonium (TEA) phosphate (pH 3.0) solution was prepared by adding phosphoric acid to aqueous triethylamine solution (0.1–0.05 *M*) to adjust the pH to the desired value.

Solid-phase extractions were performed on strong cation-exchange (SCX) Bond Elut cartridges (500 mg) (Varian, Harbor City, CA, USA) using a Baker-10SPE system connected to a water aspirator. The SPE column was conditioned by rinsing with 6 ml of methanol and 3 ml of 0.01 *M* phosphate buffer (pH 4.5) in succession.

### 2.2. Apparatus

The HPLC system consisted of a Varian Model 5020 chromatograph and an HP 1040A diode-array detector connected to a HP 79994A workstation. A Beam Boost Model C6808 photoreactor (ICT, Frankfurt, Germany) was arranged on-line between the analytical column and the detector. The eluate was irradiated on-line in capillary PTFE tubing (10 m $\times$ 0.3 mm I.D.) in a crocheted geometry with an 8-W low-pressure mercury lamp with the main spectral emission at 254 nm. Manual injection were effected using a Rheodyne Model 7125 injector with a 20-$\mu$l sample loop.

Chromatographic separations were carried out, at ambient temperature on three different stationary phases: (a) a 5-$\mu$m Spherisorb-CN (Phase Separations, Deeside, UK) column (150 $\times$ 4.6 mm I.D.) using acetonitrile–TEA phosphate (0.1 *M*; pH 3.0) (5:95, v/v) as mobile phase at a flow-rate of 1 ml/min; (b) a 5-$\mu$m Hypersil ODS (HLPC Technology, Macclesfield, UK) column (150 $\times$ 4.6 mm I.D.) using THF–TEA phosphate (0.05 *M*; pH 3.0) with various contents of organic modifier as mobile phase at a flow-rate of 1 ml/min; (c) 5-$\mu$m Hypersil ODS (Phenomenex, Torrace, CA, USA) column (150 $\times$ 3.2 mm I.D.) (mid-bore) using THF–TEA phosphate (0.05 *M*; pH 3.0) (12:88, v/v) as mobile phase at a flow-rate of 0.4–0.6 ml/min.

### 2.3. Calibration graphs

Appropriate drug standard solutions, containing a fixed (0.1 mg/ml) concentration of resorcinol (internal standard, I.S.), were subjected to HPLC analyses and the peak-height ratios (drug to internal standard) were plotted against the corresponding drug concentration to obtain the calibration graphs. The drug standard solutions were as follows.

*Chlorpheniramine*

Standard solutions (10.0–50.0 $\mu$g/ml) were prepared in acetonitrile–0.1 *M* TEA phosphate (pH 3.0) (40:60, v/v).

*Paracetamol–caffeine*

Mixed standard solutions containing paracetamol (0.20–1.0 mg/ml) and caffeine (8.0–40.0 $\mu$g/ml) were prepared in acetonitrile–0.01 *M* phosphate buffer (pH 4.5) (25:75, v/v).

*Acetylsalicylic acid and caffeine*

Mixed standard solutions containing acetylsalicylic acid (200–1000 $\mu$g/ml) and caffeine

(50–100 μg/ml) were prepared in acetonitrile–0.01 *M* phosphate buffer (pH 4.5) (25:75, v/v).

*Acetylsalicylic acid*
Drug standard solutions (15–30 μg/ml) were prepared in methanol–0.05 *M* TEA phosphate (pH 3.0) (40:60, v/v).

### 2.4. Sample preparation

The sample handling was carried out according to the nature and composition of the commercial dosage forms.

*ASA–paracetamol–caffeine (tablets)*
A powdered sample, equivalent to about 68, 51 and 8 mg of the three drugs, respectively, was treated with 80 ml of methanol under sonication for 10 min and then diluted to 100.0 ml with the same solvent. After centrifugation, a 5.00-ml aliquot was transferred into a 20.0-ml volumetric flask, 1.00 ml of resorcinol solution (I.S.) (2.0 mg/ml) and 2.00 ml of methanol were added and the solution was diluted to volume with 0.05 *M* TEA phosphate (pH 3.0).

*Propyphenazone–paracetamol–caffeine (tablets)*
The same procedure as described for ASA–paracetamol–caffeine was followed.

*Paracetamol–caffeine–chlorpheniramine (tablets)*
A powdered sample, equivalent to about 88, 5.5 and 0.45 mg of the three drugs, respectively, was treated with 25 ml of acetonitrile–0.01 *M* phosphate buffer solution (pH 4.5) under sonication for 10 min at ambient temperature and then diluted to 50.0 ml with 0.01 *M* phosphate buffer solution (pH 4.5). After centrifugation, a 10.0-ml aliquot was applied to a previously conditioned SPE cartridge with aspiration. The cartridge was washed with 0.01 *M* phosphate buffer (pH 4.5) ($4 \times 3$ ml) and the retained chlorpheniramine was then eluted with 4 ml of acetonitrile–TEA phosphate (0.1 *M*, pH 8.0) (4:6, v/v).

*ASA–caffeine–chlorpheniramine (coated tablets)*
The same procedure as described for ASA–paracetamol–caffeine was followed.

### 2.5. Assay procedure

The sample solutions were subjected to the described HPLC analyses and the drug content in each sample was determined by comparison with an appropriate drug standard solution.

## 3. Results and discussion

### 3.1. Chromatography

The first objective of this study was the development of a practical HPLC method of general application to the resolution of analgesics and related compounds contained in commercial dosage forms.

Reversed-phase ($C_{18}$) chromatography was primarily chosen and a simple binary mixture of THF–0.05 *M* TEA phosphate (pH 3.0) (12:88, v/v) was found to provide a complete separation of caffeine, paracetamol, resorcinol (internal standard), acetylsalicyclic acid and propyphenazone (Fig. 1). The organic solvent content can be adjusted according to the composition of the commercial formulation; e.g., caffeine, paracetamol and ASA (with resorcinol as I.S.) were completely separated in 8 min using 20% THF. In order to reduce the cost of both the initial purchase and the waste disposal of the organic HPLC solvents, a "mid-bore" (3.2 mm I.D.) RP-18 column was also used at a flow-rate of 0.4–0.6 ml/min. Using a reduced injection volume (5 μl), the transfer of the HPLC method from the 4.6 to the 3.2 mm I.D. column was successful (Fig. 2).

When multi-ingredient preparations containing chlorpheniramine maleate, a potent antihistamine with moderate sedative effects, were analysed, the described $C_{18}$ reversed-phase mode proved to be inadequate (high retention and peak tailing). Good separations were obtained

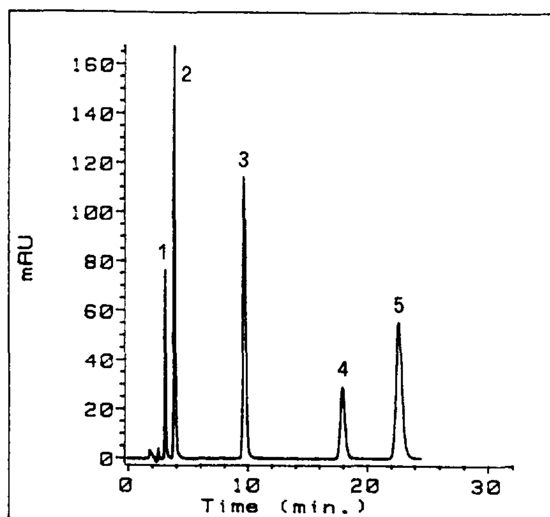


Fig. 1. HPLC separation using a 5-μm Hypersil ODS (HPLC Technology) column (150 × 4.6 mm I.D.) with THF–TEA phosphate (0.05 *M*; pH 3.0) (12:88, v/v) as mobile phase at a flow-rate of 1 ml/min and UV detection at 275 nm. Peaks: 1 = caffeine; 2 = paracetamol; 3 = resorcinol (internal standard); 4 = acetylsalicylic acid; 5 = propyphenazone.

on a nitrile column under reversed-phase conditions; using the binary mixture acetonitrile–0.1 *M* TEA (pH 3.0) (5:95, v/v), paracetamol, ASA, caffeine and chlorpheniramine were completely separated.

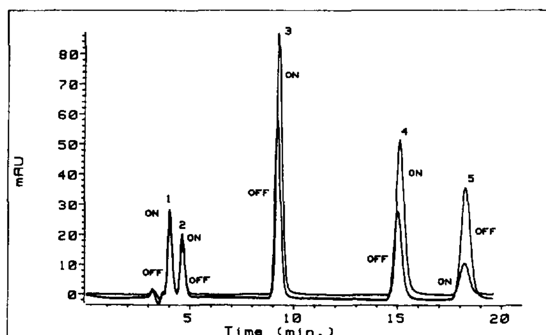


Fig. 2. HPLC separation using a 5-μm Hypersil ODS (Phenomenex) column (150 × 3.2 mm I.D.) with the same mobile phase as in Fig. 1 at a flow-rate of 0.6 ml/min. OFF = photoreactor switched off; ON = photoreactor switched on. Peaks: 1 = caffeine; 2 = paracetamol; 3 = resorcinol; 4 = acetylsalicylic acid; 5 = propyphenazone.

### *3.2. Detection*

The use of DAD in HPLC analyses of pharmaceuticals has gained wide popularity in recent years [13]. The rapid acquisition of spectra (absorption and derivative modes) allows the qualitative work in quality control and stability testing to be improved. Recently, we have successfully used a combination of DAD with postcolumn on-line photochemical derivatization to enhance the selectivity in the HPLC analysis of antimycotics [14] and cosmetics [15]. In the present work, this approach was extended to the determination of analgesics and related compounds. The column effluent was subjected to UV irradiation (254 nm) within a crocheted capillary PTFE tube (10 m × 0.3 mm I.D.) arranged on-line between the column and the detector. On UV irradiation, most of the examined analytes underwent selective structural modifications, resulting in alterated spectral properties (Fig. 3). In particular, propyphenazone and chlorpheniramine were shown to be highly photosensitive, giving significantly different UV spectra with and without UV irradiation. As a consequence, the chromatograms obtained at 275 nm with the reactor switched on and off display significant changes in the peak intensity (Fig. 2).

In general, the availability of UV spectra under different conditions (lamp on and off) for each analyte using the same mobile phase increases the information useful for the unambiguous identification of the mixture components. According to this approach, the chromatographic retention data (capacity factor or retention index), whose intra- and inter-laboratory reproducibility may be variously affected, are completed with specific information on the analyte structure and photoreactivity. For this application to analgesics, the combination of DAD with on-line photoderivatization appears simpler and more feasible than a previously reported HPLC–FTIR method [6].

### *3.3. Analysis of pharmaceutical dosage forms*

Using a $C_{18}$ column (conditions as in Fig. 1), linear relationships between the peak-height

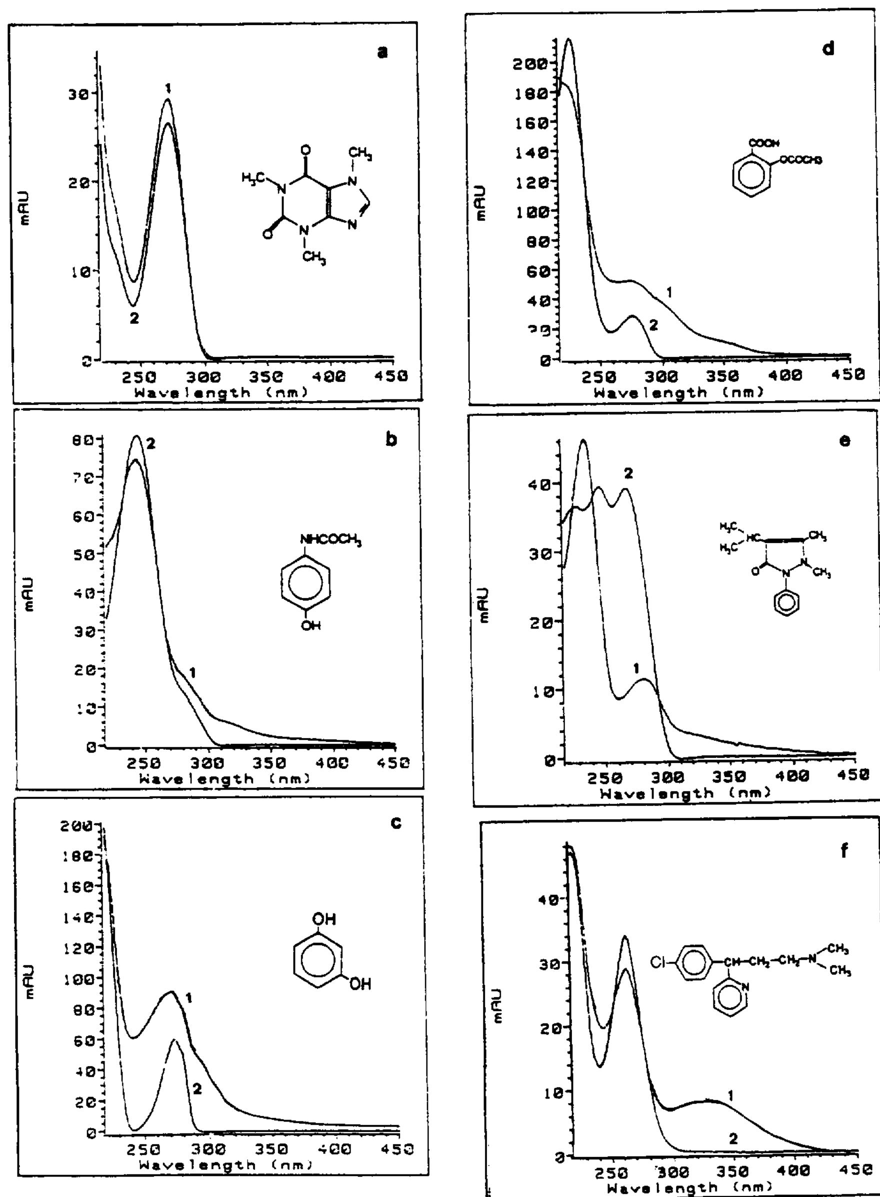


Fig. 3. UV spectra of analgesics and related compounds with on-line photoreactor switched (1) on and (2) off under the same chromatographic conditions as in Fig. 2. UV spectra were taken with an HP 1040 photodiode-array detector. (a) Caffeine; (b) paracetamol; (c) resorcinol; (d) acetylsalicylic acid; (e) propyphenazone; (f) chlorpheniramine.

ratios (analyte to internal standard) and the drug concentration (mg/ml) were obtained for each drug; when the nitrile column was used, the internal standard was omitted and linear relationships were obtained between peak area and the corresponding drug concentration ($\mu$g/ml) (Table 1). As shown, good linearity and within-run precision (R.S.D.) were obtained also in the absence of the internal standard.

The sample preparation was carried out by

Table 1
Data for the calibration graphs ($n = 6$)

| Drug | HPLC column | Slope | Intercept | Correlation coefficient | Concentration range | R.S.D. (%)[a] |
|---|---|---|---|---|---|---|
| ASA | $C_{18}$ | 2.8440 | 0.0031 | 0.9985 | 0.15–0.30 mg/ml | 1.15 |
| Caffeine | $C_{18}$ | 30.230 | 0.0182 | 0.9999 | 0.008–0.040 mg/ml | 1.07 |
| Paracetamol | $C_{18}$ | 19.16 | 0.0120 | 0.9999 | 0.050–0.250 mg/ml | 0.96 |
| ASA | Cyano | 546.00 | −0.650 | 0.9986 | 200–1000 $\mu$g/ml | 0.80 |
| Paracetamol | Cyano | 1691.93 | 74.054 | 0.9990 | 200–1000 $\mu$g/ml | 1.20 |
| Caffeine | Cyano | 5243.95 | 6.626 | 0.9996 | 50–100 $\mu$g/ml | 0.70 |
| Chlorpheniramine | Cyano | 546.10 | −0.6496 | 0.9985 | 10–50 $\mu$g/ml | 1.60 |

[a] From replicate analyses ($n = 8$) of a drug standard solution at the lowest concentration within the working range.

simple solvent extraction for tablets B and C, whereas a preliminary solid-phase extraction (SPE) procedure was necessary for the formulations containing low concentrations of chlorpheniramine. The SPE step, using a strong cation-exchange sorbent (propylsulphonate), allowed chlorpheniramine to be concentrated and separated from the other compounds. In fact, chlorpheniramine was quantitatively retained on the SPE column, whereas paracetamol, ASA and caffeine passed through; the selectivity of the SPE step is illustrated in Fig. 4. In the SPE step the chlorpheniramine recovery was essentially quantitative.

The described methods were then applied to the analysis of commercially available analgesic dosage forms. Using DAD in combination with post-column on-line photoderivatization, the identity of the various analytes was easily confirmed. For quantitative assay, direct UV detection at 275 nm, without photochemical derivatization, was used and the data obtained (Table 2) were in good agreement with the claimed contents. The accuracy of the method was ascertained by recovery studies by analysing samples fortified with 30% of the declared content. In every case essentially quantitative recoveries (98–99.4%) were obtained.

The photochemical derivatization in quantitative applications could be useful for the determination of chlorpheniramine in more complex matrices, because the favourable photoinduced spectral alterations (Fig. 3f) allow measurements at higher wavelengths (330 nm) to be carried out.

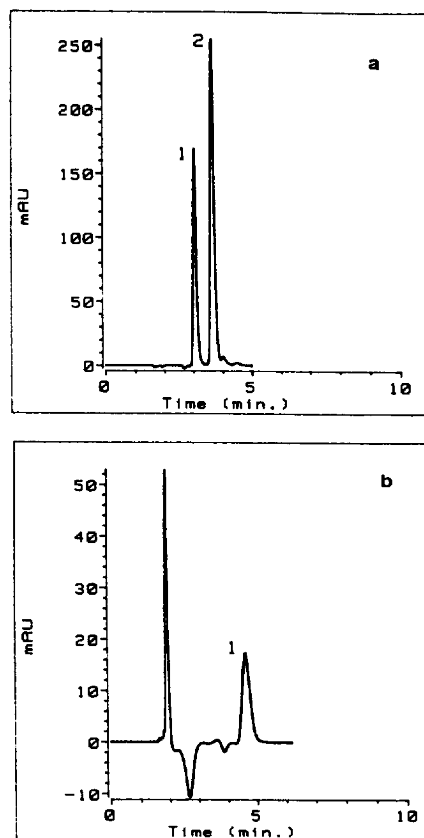


Fig. 4. HPLC of a commercial mixture of ASA–caffeine–chlorpheniramine. Chromatographic conditions: column, 5-$\mu$m Spherisorb-CN (Phase Separations) (150 × 4.6 mm I.D.); mobile phase, acetonitrile–TEA phosphate (0.1 $M$; pH 3.0) (5:95, v/v) at a flow-rate of 1 ml/min; detection, UV at 275 nm. Peaks: (a) 1 = caffeine and 2 = acetylsalicylic acid; (b) 1 = chlorpheniramine after the SPE step.

Table 2
Assay results from the HPLC analyses of commercial analgesic dosage forms (mean values of five analyses, expressed as a percentage of the claimed content)

| Formulation | Components | HPLC column | Found (%) | R.S.D. (%) |
|---|---|---|---|---|
| Coated tablets | ASA | CN | 102.00 | 1.3 |
| | Caffeine | | 97.10 | 1.1 |
| | Chlorpheniramine | | 101.00 | 1.6 |
| Tablets A | Paracetamol | CN | 98.10 | 1.1 |
| | Caffeine | | 101.20 | 1.0 |
| | Chlorpheniramine | | 99.90 | 1.8 |
| Tablets B | Propyphenazone | $C_{18}$ | 97.50 | 1.2 |
| | Paracetamol | | 102.40 | 1.4 |
| | Caffeine | | 99.60 | 0.8 |
| Tablets C | Paracetamol | $C_{18}$ | 99.25 | 1.3 |
| | ASA | | 103.00 | 1.5 |
| | Caffeine | | 99.40 | 1.0 |

Moreover, higher sensitivity could be achieved for ASA, but for this application this was not necessary.

In conclusion, the combination of DAD with postcolumn on-line photoderivatization constitutes a simple and effective approach to enhancing the intrinsic selectivity of an HPLC method and offers the opportunity to achieve further information useful for the reliable quality control of analgesic dosage forms.

## Acknowledgements

This work was supported by MURST (Rome, Italy). Thanks are due to Miss S. De Propris for her valuable technical assistance.

## References

[1] J.E.F. Reynolds (Editor), Martindale, The Extra Pharmacopoeia, Part 1, Pharmaceutical Press, London, 1989, p. 1.
[2] A.C. Moffat (Editor), Clarke's Isolation and Identification of Drugs, Pharmaceutical Press, London, 1986.
[3] M. Bogusz and M. Wu, J. Anal. Toxicol., 15 (1991) 188.
[4] R.K. Gilpin and L.A. Pachla, Anal Chem., 65 (1993) 117R.
[5] G. Santoni, L. Fabbri, P. Gratteri, G. Renzi and S. Pinzauti, Int. J. Pharm., 80 (1992) 263, and references cited therein.
[6] S. Shah and L.T. Taylor, LC·GC Int., 2 (1989) 340.
[7] M.A. Abuirjeie, M.E. Abdel-Hamid and E.S.A. Ibrahim, Anal. Lett., 22 (1989) 365.
[8] H.M. Stevens and R. Gill, J. Chromatogr., 370 (1986) 39.
[9] M.G. Mamolo, L. Vio and V. Maurich, J. Pharm. Biomed. Anal., 3 (1985) 157.
[10] B.R. Thomas, X.G. Fang, P. Shen and S. Ghodbane, J. Pharm. Biomed. Anal., 12 (1994) 85.
[11] T.A. Biemer, J. Chromatogr., 410 (1987) 206.
[12] H.J.P. Sievert and A.C.J.H. Drouen, in L. Huber and S.A. George (Editors), Diode Array Detection in HPLC, Marcel Dekker, New York, 1993, Ch. 4, p. 51.
[13] R.W. Giuffre, in L. Huber and S.A. George (Editors), Diode Array Detection in HPLC, Marcel Dekker, New York, 1993, Ch. 6, p. 163.
[14] A.M. Di Pietra, V. Cavrini, V. Andrisano and R. Gatti, J. Pharm. Biomed. Anal., 10 (1992) 873.
[15] V. Andrisano, R. Gotti, A.M. Di Pietra and V. Cavrini, Chromatographia, 39 (1994) 138.