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Journal of Pharmaceutical and Biomedical Analysis 37 (2005) 695–701
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Analysis of diltiazem and its related substances by HPLC and HPLC/MS

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Received 5 July 2004; received in revised form 5 November 2004; accepted 18 November 2004
Available online 5 January 2005

Abstract

Diltiazem (DTZ) is an optically active calcium channel blocker having a benzodiazepine structure. The drug used in therapy is (+)-*cis*-diltiazem with configuration (2*S*,3*S*). To describe the analytical profile of DTZ different stationary phases (RP-18, RP-8, monolithic support) were tested. The best separation of DTZ from A, B, E and F was obtained using as stationary phase a RP-8 or a monolithic RP-18. The characterization of impurities was carried out using two analytical systems, HPLC and HPLC/MS.

Keywords: Diltiazem; Impurities; Classical reversed stationary phases; Monolithic support; HPLC; HPLC/MS

1. Introduction

Diltiazem (DTZ), the (2*S*,3*S*)-5-[2-(dimethyl-amino) ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate hydrochloride, is 1 of 10 calcium channel blockers which have been approved for clinical use in the United States. The calcium channel blockers have different chemical structures: DTZ is a benzodiazepine. This drug is generally used in the management of the classical and vasospastic angina pectoris, but also in the treatment of essential hypertension [1–3] and supraventricular tachyarythmias [4]. DTZ is an optically active compound having the asymmetric carbons at the positions 2 and 3. Therefore, it forms the *cis* and *trans* isomers and each isomer exists as two enantiomers, (+) and (−) forms [5,6]. The drug approved by FDA is the (+)-*cis*-diltiazem with configuration (2*S*,3*S*). Together with *cis*-desacetyldiltiazem, the stereoisomers can be present as impurities in the raw material or pharmaceuticals.

All DTZ impurities have (+)-*cis* form, except impurity A which has (+)-*trans* form. The impurities of DTZ are cited in the European Pharmacopoeia [7] as compounds A, B, C, D, E, and F. We did not find on the market the impurities C and D, therefore, in this paper we considered only the impurities A, B, E and F (Fig. 1).

To analyze DTZ, many analytical techniques were used like spectrophotometry [8,9], Gaschromatography [10], HPTLC [11], but primarily HPLC [12–14]. Recently, also HPLC–MS and CE were used to characterize the DTZ metabolites [15–18] or (+)-*cis*-Desacetyl DTZ impurity.

The purpose of this work was just to study a separative method, sensitive and selective, to check the DTZ stability and then its impurity profile. HPLC remains a very important technique to separate drugs from their impurities, specially RP-HPLC. Therefore we tested different columns (RP-8, RP-18 and RP-18 monolithic), with different dimensions, looking at the parameters which influence the resolution of DTZ and related substances. Further, we looking at new silica columns, now on the market, with the aim to reduce analysis time. A relative new column is the monolithic C18 silica column [19,20]. This column differs from classical silica columns, since it consists of a silica rod, instead of particles and possesses a biporous structure of macropores and mesopores with diameters of approximately 2 and 13 μm, respectively. The biporous structure of the stationary phase

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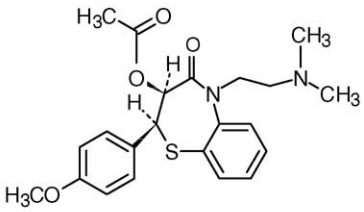
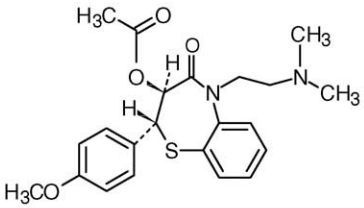
doi:10.1016/j.jpba.2004.11.050



DILTIAZEM

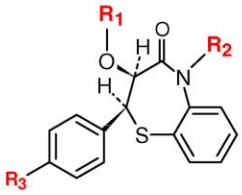
(2*S*,3*S*)-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate



IMPURITY A

A. (2*R*,3*S*)-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate

OTHER IMPURITIES:



B. $R_1 = COCH_3$, $R_2 = H$, $R_3 = OCH_3$

(2*S*,3*S*)-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate

C. $R_1 = COCH_3$, $R_2 = CH_2CH_2N(CH_3)_2$, $R_3 = OH$

(2*S*,3*S*)-5-[2-(dimethylamino)ethyl]-2-(4-hydroxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate

D. $R_1 = COCH_3$, $R_2 = CH_2CH_2NHCH_3$, $R_3 = OCH_3$

(2*S*,3*S*)-2-(4-methoxyphenyl)-5-[2-(methylamino)ethyl]-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate

E. $R_1 = R_2 = H$, $R_3 = OCH_3$

(2*S*,3*S*)-3-hydroxy-2-(4-methoxyphenyl)-2,3-dihydro-1,5-benzothiazepin-4(5 *H*)-one

F. $R_1 = H$, $R_2 = CH_2CH_2N(CH_3)_2$, $R_3 = OCH_3$

(2*S*,3*S*)-5-[2-(dimethylamino)ethyl]-3-hydroxy-2-(4-methoxyphenyl)-2,3-dihydro-1,5-benzothiazepin-4(5 *H*)one

Fig. 1. Chemical structures of DTZ and impurities.

provide a porosity greater than 80% allowing chromatography with a much lower back pressure than on conventional columns. Monolithic silica columns are more and more commonly used and several applications already were published [21–23]. Until now, the monolithic columns were not used to determine DTZ and its impurity. Therefore, we used a RP-18 monolithic support with the aim to reduce the retention time (t_R), the retention factor (k) and to improve the resolution (Rs) of analytes.

2. Experimental

2.1. Reagents and chemicals

(+)-*cis* Dialtiazem, dimethylamine (DA), dimethyloctylamine (DO) were obtained from Sigma Chemical (St. Louis, MO, USA).

The impurities B, E and F were from Ethypharm (78550 Houdan, France).The impurity A was from European Pharmacopoeia laboratories (EDQM, European Pharm., Council of Europe BP904F67029 – Strasbourg, France).

All other chemicals and solvents, water included, were of analytical or HPLC grade and were obtained from VWR International (Milano, Italia).

2.2. Apparatuses

2.2.1. HPLC

The analyses were performed using a Merck-Hitachi Chromatograph, Series L-7000, equipped with a Merck-Hitachi Series L-7450 photodiode array detector. The chromatograph was controlled by a Flyer Pentium computer, interface D-7000. The data were evaluated by Merck-Hitachi HPLC System Manager Software (version 4.1). The detector conditions during the analyses were: acquisition rate of spec-

Table 1
Chromatographic phases tested to analyze DTZ and impurities A, B, E and F

Stationary phase	Mobile phase	Peaks separated
RP-18 Lichrosphere 100 Merck (12.5 cm length, 5 μm)	Na_2HPO_4 pH 8.5 + 0.2% DA/ACN (60/40)	Diltiazem, impurities B, E. Coelution of impurities A and F. Analysis time 7.6 min (Fig. 2a)
RP-18 Lichrosphere 100 Merck (12.5 cm length, 5 μm)	ACN/EtOH/KH_2PO_4 pH 4.5 + DO 25/5/70	Separation of diltiazem and impurities A, B, E, F. Decreasing of the sensitivity of impurity E. Analysis time 29.4 min (Fig. 2c)
RP-18 Lichrospher 100 Merck (12.5 cm length, 5 μm)	Ammonium acetate pH 6.58 + 0.2% DA/ACN (60/40)	Separation of E, B, DTZ. Coelution of F + A (Fig. 2b)
RP-8 Lichrospher 100 (25 cm, 5 μm)	Ammonium acetate pH 6.58 + 0.2% DA/ACN (60/40)	Separation on line of DTZ and all impurities. Analysis time 27.5 (Fig. 3)
RP-18 Chromolith Merck 10 cm	Ammonium acetate + 0.2% DA pH 6.58/ACN (60/40)	Separation on line of DTZ and all impurities. Analysis time 8.2 min (Fig. 4)

DA: Diethylamine; DO: *N*,*N* dimethyloctylamine.

tra 1600 ms, spectral bandwidth for each channel 4, wavelength 240 nm and reference bandwidth 50.

The solutions were injected via a Rheodyne Model 7725 valve using a 20 μL sample loop.

The chromatographic separations were carried out using:

- a 5 μm RP-18 Lichrospher 100 Merck column (12.5 cm × 4.6 mm;
- a 5 μm RP-8 Lichrospher 100 Merck column (25 cm × 4.6 mm);
- a 5 μm RP-18 Chromolith Performance Merck column (10 cm × 4.6 mm).

All four columns were obtained from VWR International (Milano, Italia).

2.2.2. HPLC/MS

The HPLC apparatus was a Waters chromatograph 1525 (Waters, Milford, USA). HPLC column (equipped with a guard column, Phenomenex C_{18} $L = 4$, 2 mm i.d.) 250 mm × 2.1 mm Nucleosil C_{18}, 5 μm, (Shandon, England) was slurry packed in our lab.

MS and MS/MS analyses were performed on a Q-TOF mass spectrometer (Micromass, Manchester, UK) equipped with a Z-spray source interface in positive mode. Instrument operation, data acquisition and analysis were performed using MassLynx 4.0 software (Micromass) on a windows NT server.

2.3. Procedures

2.3.1. HPLC

The best analytical conditions to separate DTZ and impurities A, B, E, and F were summarized in Table 1.

2.3.2. HPLC/MS

The analysis was performed in a Nucleosil C18 column (250 mm × 2.1 mm, 5 μm), equipped with a guarde column (Phenomenex C18, 4 mm length, 2 mm i.d., 5 μm). The column was slurry packed in our lab under isocratic elution (80/20) of methanol–ammonium formate (6 mM; pH 6.5) mixture at a flow rate of 150 μL/min under isocratic elution. The injector was a Rheodyne 8125 with a sample loop of 5 μL.

MS and MS/MS analyses, performed using the cone voltage, extraction voltage, microchannel plate (MCP) detector voltage and collision energy, were optimised for each sample. The best values were 30 V for cone voltage, 3300 V for capillary voltage, 1.5 V for extraction voltage and 20 eV for collision energy in MS–MS experiments. Nitrogen was used as a nebulizing gas and as curtain gas and argon as a collisional gas. Desolvation temperature was 350 °C and source temperature, 120 °C.

3. Results and discussion

The possible impurities of DTZ are compounds very similar to the drug (Fig. 1). To obtain a good resolution among DTZ and impurities A, B, E and F we tested different stationary phases considering:

- the features of stationary phase: RP-18, RP-8 and RP-18 monolithic;
- the column dimension (10, 12.5 and 25 cm).

Considering that DTZ and impurities are basic compounds we tested three mobile phases:

1. Na_2HPO_4 (10 mM, pH 8.5), containing 0.2% of diethylamine (DA), and acetonitrile (60/40).
2. ACN/EtOH/KH_2PO_4 (pH 4.5), containing 0.01% *N*,*N*-dimethyloctylamine (DO) (25/5/70). This 2° mixture is the mobile phase used in the European Pharmacopoeia [7] to analyze DTZ related substances.
3. Ammonium acetate buffer (10 mM, pH 6.58) containing 0.2% of DA and acetonitrile (60/40).

The chromatograms of Fig. 2 show the influence of the mobile phase on the resolution of DTZ from the impurities. In fact the chromatograms of Fig. 2a and b, obtained with the same stationary phase, are isocratically eluted with mobile phase 1° or 3°. The mobile phase 1° resolved DTZ, B and E

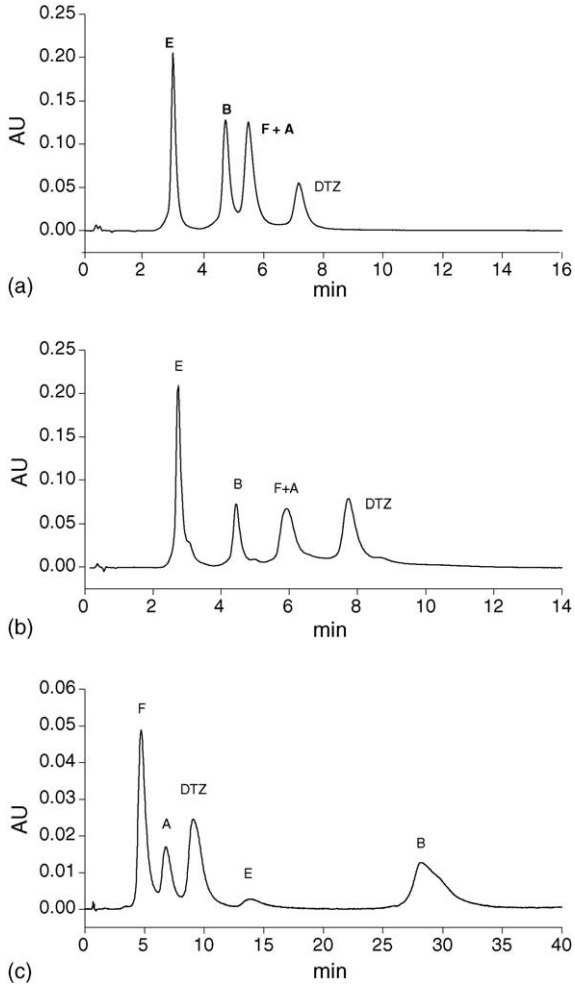


Fig. 2. Separation of DTZ from the impurities using different analytical conditions (see Table 1).

but didn't resolve the impurities A and F. The mobile phase 3° showed a better resolution of all peaks, but also with this eluents A and B were not resolved. The addition of 5% of EtOH and 0.01% DO to the mobile phase allowed the resolution of

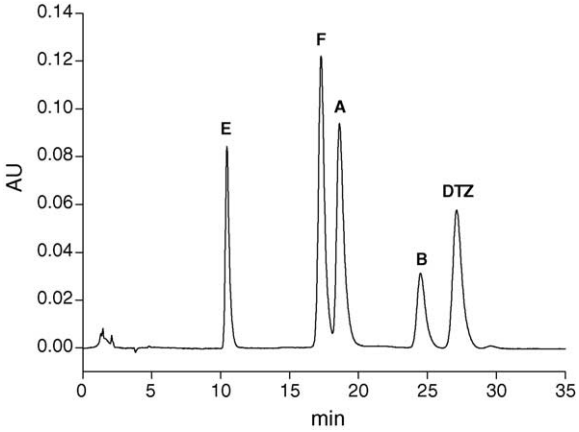


Fig. 3. Separation of DTZ and impurities A, B, F, E mixture using a RP-8 stationary phase eluted with 10 mM ammonium acetate with 0.2% DA/ACN (60/40).

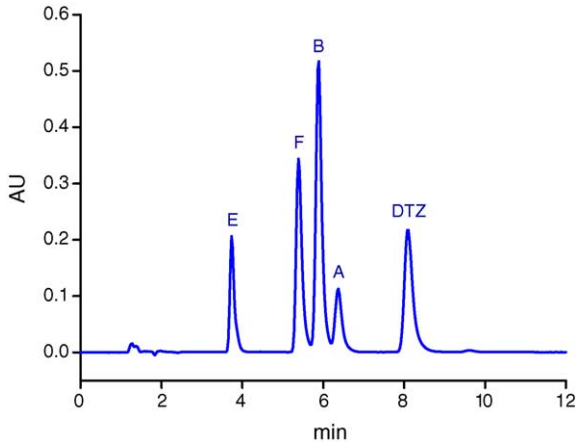


Fig. 4. Separation of diltiazem and impurities A, B, F, E mixture using a Chromolit RP-18, (10 cm length, 5 μm) stationary phase eluted with 10 mM ammonium acetate with 0.2% DA/ACN (60/40). Flow rate 1.5 ml/min.

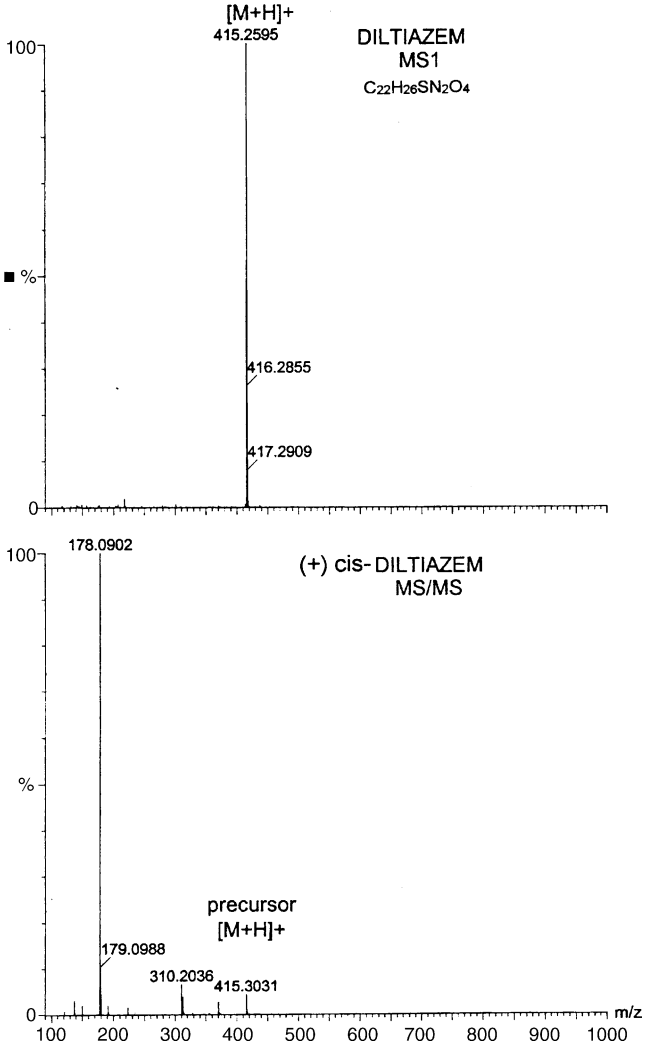


Fig. 5. MS spectrum and MS–MS spectrum of DTZ. MS conditions: 30 V for cone voltage, 3300 V for capillary voltage, 1.5 V for extraction voltage. MS/MS conditions: 20 eV for collision energy. Precursor ion *m*/*z* 415. The spectra were obtained by infusion (10 μL/min) of the standard 10 ng/μL in MeOH–ammonium formate 6 mM.

all impurities (Fig. 2c), but the analysis time increased from 8 to 30 min. Further the sensitivity of impurity E dramatically decreased.

The RP-8 column (25 cm length) gave a very good separation of all considered compounds (Fig. 3), but the best separation of DTZ and impurities was obtained with a monolitic column RP-18 (Fig. 4). As we can see the retention time (t_R), retention factors (k) and resolution (Rs) of analytes are strongly affected by the monolithic stationary phase and basic modifiers DA or DO. Actually the high performance of the monolitic column was emphasized from the reduced analysis time (from 28 min of Fig. 3 to 8.5 min of Fig. 4) and increased sensitivity.

The quantitative analysis was carried out with the monolithic column.

The minimum detectable amount (LOD) of diltiazem was 25 ng and the limit of quantitation (LOQ) was 75 ng. Using the usual stationary phases (RP-18 or RP-8) LOD and LOQ were, respectively, 152 and 456 ng. By appropriate dilution of a DTZ standard watery solution (0.54 mg/mL), the linearity of calibration curve was controlled in the concentration range of 0.0054–0.18 mg/mL ($n = 6$; $r = 0.999$).

To increase the selectivity we used a HPLC/MS system allowing to recognise also the compounds coeluted in the same chromatographic peak (Fig. 2a and b)

Initially acquisition MS parameters were optimised in ion spray mode by direct continuous infusion of the analytes standard solution (10 ng/μL in MeOH–ammonium formate 6 mM) at a flow rate of 10 μL/min in the mass spectrometer by ESI–MS and then by ESI–MS–MS.

The Micromass® Q-Tof micro™ is a high resolution mass spectrometer that enables automated exact mass measurements. The instrument also features a quadrupole mass filter and collision cell for MS/MS analyses. This powerful combination delivers simple exact mass measurement of fragment ions to yield increased confidence in structural elucidation and data bank search results.

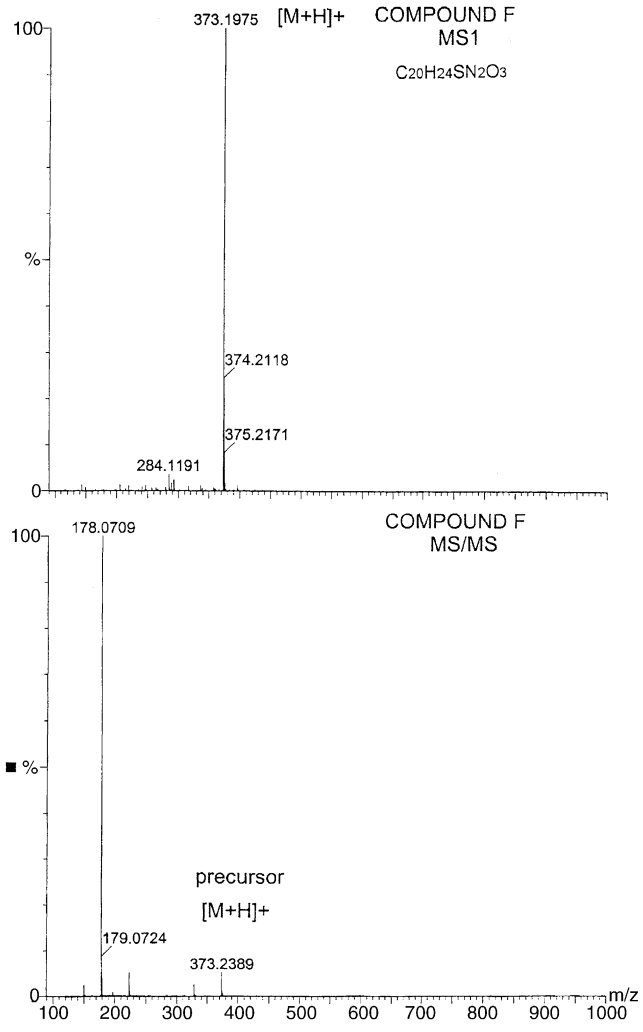


Fig. 6. MS spectrum and MS/MS spectrum of impurity F. Precursor ion m/z 373. Experimental conditions as in Fig. 5. Compound A has the same MS and MS/MS spectrum of DTZ.

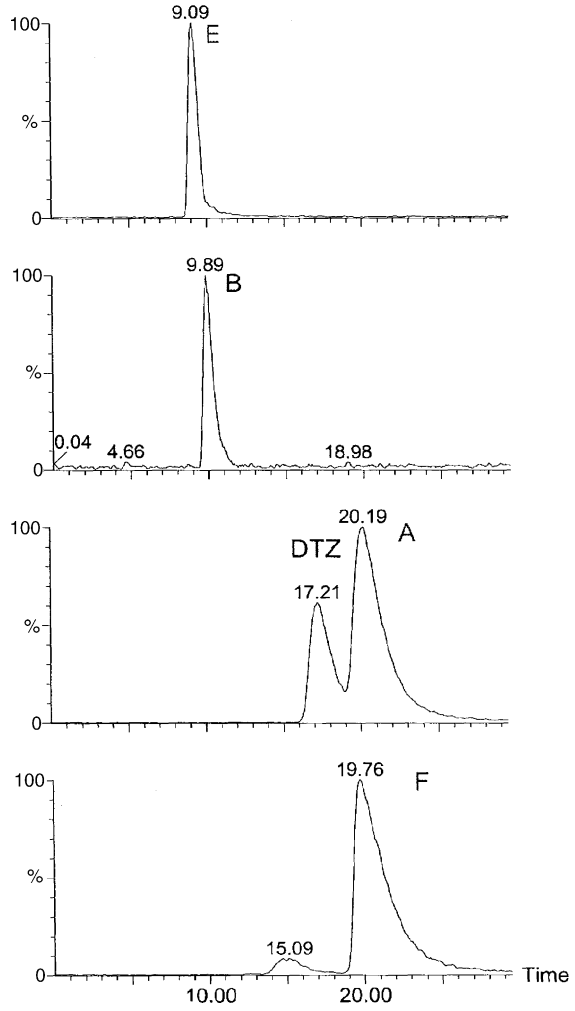


Fig. 7. TIC HPLC–MS/MS of a mixture of DTZ and A, B, E, F (5 ng/μL), injection 5 μL. Flow rate 150 μL/min, mobile phase methanol/ammonium formate (6 mM) 80/20. Column 250 mm × 2.1 mm Nucleosil C18, 5 μ. MS/MS conditions as in Fig. 5. Chosen precursor ions, respectively, (in order of elution), m/z 324, 366, 415, 373, 415.

Data acquisition were performed preliminarily on the standard compounds in full scan, in positive mode (mass range, 50–450 Da). The MS spectra were dominated by the protonated $[M+H]^+$ or $[M+Na]^+$ ion. In some experiments, carried out in negative mode, no detectable $[M-H]^-$ ions were observed. Besides, also changing to more acidic condition (pH < 2) with formic acid, the precursor ion formation $[M+H]^+$ is not increased.

The $[M+H]^+$ ions were chosen as precursor for the MS–MS experiments. MS/MS product ion scans were then recorded (*m/z* 50–430). Figs. 5 and 6 show the mass spectra and the MS–MS spectra of DTZ and compound F. Compound A has the same MS and MS/MS spectra of DTZ. It is interesting to note that these compounds, containing the groups $-CH_2-CH_2-N(CH_3)_2$ can be protonated giving the $[M+H]^+$ ion which is a good precursor for MS/MS experiments. The compounds B and E instead do not contain this group and so in ESI source only a strong $[M+Na]^+$ adduct is formed not useful for MS/MS.

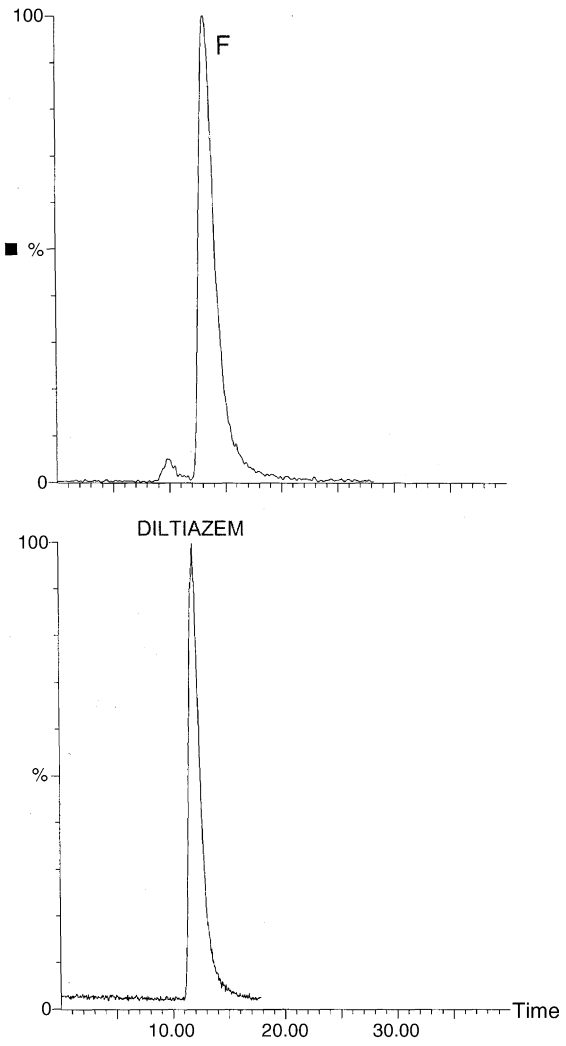


Fig. 8. LC–MS/MS of DTZ and its decomposition product F, obtained after sun days light exposure. Chosen precursor ions, respectively, *m/z* 415, 373.

Finally, the analyses were carried out by HPLC–MS–MS selecting as precursors the "quasi" molecular ion $[M+H]^+$.

As shown in Fig. 7, although compounds A and F are not chromatographically resolved, they can be detected singularly and separated by mass spectrometry, as their structures and spectra are different. DTZ, if exposed to light, after some days (4–5) tends to decompose to compound F (Fig. 8).

4. Conclusion

The quality control of DTZ here proposed shows sensitivity and selectivity. Among the stationary phases tested, the RP-8 and monolithic RP-18 both show a good selectivity, but the monolithic RP-18 column is much more sensitive and rapid. We observed that, as it is also reported in the literature, the (2*S*,3*S*) configuration of DTZ in the solid state was found to be stable.

Acknowledgements

This work was supported by a grant from MIUR 40% (National Research Project 2002–2004) and from Ist. Biochimica Molecolare of Italian C.N.R.

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