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High-performance liquid chromatography–mass spectrometry analysis of diltiazem and 11 of its phase I metabolites in human plasma

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Abstract

The aim of the present work was to develop a high-performance liquid chromatography–mass spectrometry method for analysis of diltiazem (DTZ) and metabolites in human plasma after single dose administration (120 mg). Human plasma samples (1 ml) were cleaned up by a solid phase extraction procedure (C18 cartridges) using codeine as an internal standard. Reconstituted extracts were separated on a reversed-phase C8 column with a linear gradient mobile phase system. The run time per sample analysis was 11 min. Detection was performed using selected ion monitoring following atmospheric pressure chemical ionization. The lower limit of quantification was estimated to be 1 μg/l (in spiked plasma) for all available reference compounds (i.e. DTZ and five metabolites). Validation of the method showed good linearity, precision and accuracy for quantification of these six reference compounds. In addition, tandem MS analyses of human plasma sampled from healthy individuals after peroral intake of 120 mg DTZ revealed that the method enabled detection of six additional metabolites for which reference compounds were not available.

Keywords: Diltiazem; Metabolites; High-performance liquid chromatography; Atmospheric pressure chemical ionization; Mass spectrometry; Pharmacokinetics

1. Introduction

The calcium channel blocker diltiazem (DTZ) is used in treatment of hypertension and angina pectoris. DTZ is subjected to extensive and complex biotransformation involving *N*-demethylation (mono or di), *O*-demethylation and deacetylation (Fig. 1) [1,2]. Several of the metabolites produced through these metabolic pathways might influence the pharmacodynamic response and/or interacting potential in patients treated with DTZ.

The respective hypotensive potencies of the metabolites desacetyl-DTZ (M1), *N*-desmethyl-DTZ (MA) and *N*-desmethyldesacetyl-DTZ (M2)

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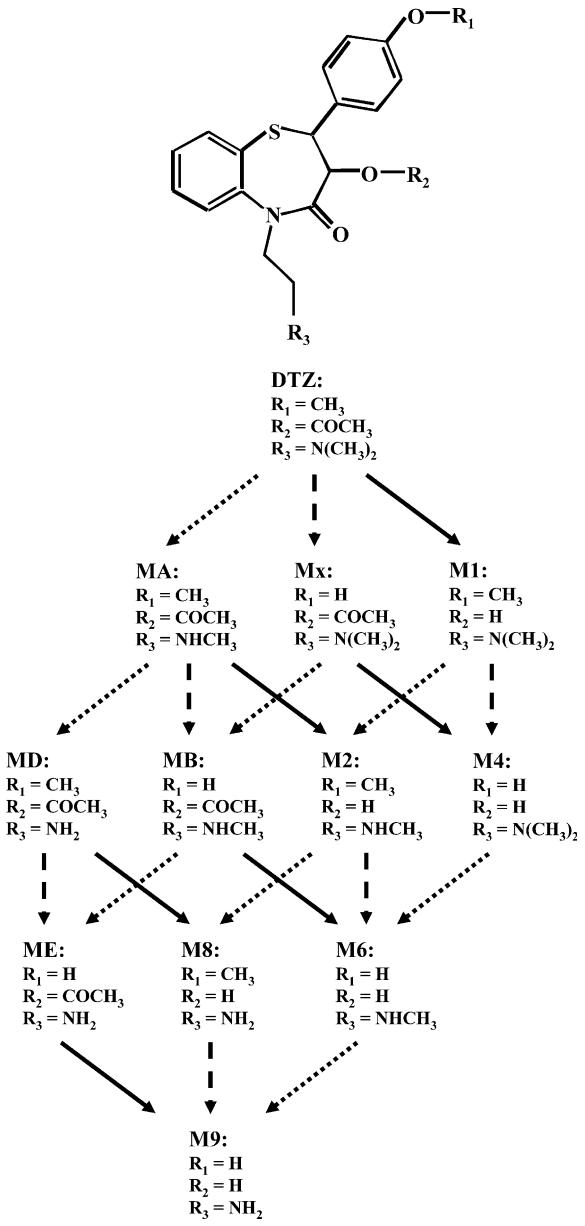


Fig. 1. Chemical structure of DTZ and the metabolites produced through mono/di-*N*-demethylation (dotted line), *O*-demethylation (long dashed line) and deacetylation (solid line). Metabolite abbreviations are adopted from Sugawara et al. [1] and Yeung et al. [2].

have been estimated to be about 1/2–1, 1/3 and 1/3 compared to DTZ in animal studies [3–5]. Combined with the fact that several desacetylated DTZ metabolites, including M1 and M2, have been described to possess considerably stronger ability to reduce thrombocyte aggregation compared to the parent drug in vitro [6], it is likely that metabolites could affect the therapeutic response after administration of DTZ. Moreover, in vitro information [7] indicates that the metabolites MA and/or *N*,*N*-didemethyl-DTZ (MD) might be important in mediating the cytochrome P450-3A4 (CYP3A4) inhibitory action of DTZ treatment, which has been shown to cause a substantial increase in the systemic exposure of many drugs metabolized via CYP3A4 (e.g. buspirone, carbamazepine, cisapride, cyclosporine, lovastatin, midazolam and simvastatin) [8–14].

Recently, it was shown that DTZ is equally effective as ß-blockers and thiazide diuretics in preventing cardiovascular events among hypertensive patients (the NORDIL study) [15]. The results of this long-term study brought DTZ into the category of first-line agents in treatment of hypertension. Thus, it is of interest to further investigate pharmacokinetic aspects of this drug. Since several metabolites of DTZ have pharmacological properties, it is important to perform a thorough metabolite monitoring in pharmacokinetic studies with the parent compound. Many high-performance liquid chromatography (HPLC) methods have been developed for analysis of DTZ and its metabolites in human plasma [16–27], but these are all based on ultraviolet (UV) detection. Due to higher sensitivity and specificity compared to UV, mass spectrometry (MS) has the potential of both lower detection limits and to detect additional metabolites. The purpose of the present work was therefore to establish a HPLC–MS method to enable plasma analysis of DTZ and as many as possible of the metabolites indicated in Fig. 1 after peroral administration of a single therapeutic dose of DTZ (120 mg).

2. Experimental

2.1. Materials

(+)-*cis*-DTZ and codeine (internal standard, IS) were obtained from Sigma Chemical (St. Louis, MO, USA). Reference compounds of the

following five metabolites of DTZ were provided by Tanabe Seiyaku (Osaka, Japan): (+)-*cis*-*N*-desmethyl-DTZ (MA), (+)-*cis*-desacetyl-DTZ (M1), (+)-*cis*-*N*-desmethyldesacetyl-DTZ (M2), (+)-*cis*-*O*-desmethyl-desacetyl-DTZ (M4) and (+)-*cis*-*N*,*O*-didesmethyldesacetyl-DTZ (M6). Unfortunately, we were not able to obtain the other metabolites indicated in Fig. 1. Methanol, acetonitrile, ammonia (25%), formic acid and ammonium acetate were supplied by Merck (Darmstadt, Germany). Deionised water was produced by Easypure UV (Barnstead, IO, USA).

2.2. Apparatus

The HPLC equipment consisted of an AS3000 autosampler and a P4000 quaternary pump coupled to a LCQ^{DUO} ion trap MS detector (all components from Finnigan, Austin, TX, USA). HPLC and MS were interfaced with an atmospheric pressure chemical ionization (APCI) inlet. The software XCALIBUR version 1.2 (Finnigan) was applied for system operation and data acquisition. An Eppendorf Centrifuge 5415 (Eppendorf Gerätebau, Hamburg, Germany) was used to centrifuge extracted plasma samples.

2.3. Standard samples

Validation of the method was performed using DTZ and the five metabolites present as reference compounds (MA, M1, M2, M4 and M6). Standard concentrations used in the preparation of standard samples were 0,5 and 5 mg/l for the analytes and 1 mg/l for the IS (all dissolved in methanol). The analytes were added to drug free pooled human plasma (from three healthy volunteers) to obtain final concentrations of 1, 3, 6, 10, 15, 45, 90, 150 and 225 μg/l. Calibration curves were constructed in two concentration ranges (1–15 and 15–225 μg/l). DTZ, M1, MA and M2 were included in both concentration ranges, whereas M4 and M6 were added only from 1 to 15 μg/l. Standard samples with concentrations of 1, 15 and 225 μg/l for DTZ, M1, MA and M2, and 1, 6 and 15 μg/l for M4 and M6, were used to evaluate intra- and inter-assay precision and accuracy. The IS concentration was 15 μg/l in all samples.

The extraction recoveries were determined at 1 and 15 μg/l (M4 and M6) or 1 and 225 μg/l (DTZ, MA, M1 and M2), and calculated by comparing the analyte/IS peak area ratios of samples spiked prior to solid phase extraction (SPE) with those obtained from samples spiked after SPE.

2.4. Biological samples

Due to lack of reference compounds, validation data could not be obtained for the metabolites *O*-desmethyl-DTZ (Mx), *N*,*O*-didesmethyl-DTZ (MB), *N*,*N*-didesmethyl-DTZ (MD), *N*,*N*,*O*-tridesmethyl-DTZ (ME), *N*,*N*-didesmethyldesacetyl-DTZ (M8) and *N*,*N*,*O*-tridesmethyl-desacetyl-DTZ (M9). However, the ability to detect these metabolites was examined by analysis of human plasma after intake of DTZ (biological samples). The biological samples were obtained from healthy individuals recruited to a study where the aim was to investigate pharmacokinetics of DTZ and metabolites in relation to *CYP2D6* genotype. A detailed description of the study protocol has been published elsewhere [28], but briefly 120 mg DTZ was given as a peroral single dose to healthy individuals who had given their written informed consent. Plasma samples were drawn from 0 to 24 h after drug intake.

2.5. Sample preparation

The human plasma samples (1 ml) were spiked with IS, mixed with 1 ml 0.1 M ammonium acetate and vortexed prior to SPE. C18 (100 mg) cartridges (Varian, Harbor City, CA, USA) mounted on a vacuum manifold device (Supelco, Bellafonte, PA) were used in the procedure. The SPE cartridges were preconditioned with acetonitrile and 0.1 M ammonium acetate (1 ml each) before sample application. After application of the diluted samples, the SPE cartridges were washed with 1 ml water and 1 ml acetonitrile/water (30/70, v/v). Elution of the analytes was performed with 0.75 ml of a methanol/0.1 M ammonium acetate mixture (95/5, v/v) into Eppendorf vials (Treff AG, Degersheim, Switzerland). The extracts were centrifuged for 10 min at 14 000 rpm (63 g) in order to precipitate SPE cartridge fines and 0,5 ml of the

supernatant was evaporated to dryness under a stream of nitrogen. The residues were resolved in 110 μl of the buffer component of the mobile phase (i.e. 10 mM ammonia, titrated to pH 2.75 with formic acid). Aliquots of 100 μl were injected onto the analytical column.

2.6. Chromatographic conditions

An Inertsil C8 column (50 μm, 50 × 3 mm; Varian) with an internal pre-column of similar material (50 μm, 10 × 2 mm) was used for chromatographic separation. The analytes were eluted by a linear gradient mobile phase system. Solution A consisted of a 20/80 (v/v) mixture of methanol and 10 mM ammonia (titrated to pH 2.75 with formic acid), whereas solution B had the same components in a ratio of 85/15 (v/v). The mobile phase changed from 100% A to 100% B within 6 min after sample injection. From 6 to 7 min of the run the mobile phase was 100% B. Then, the mobile phase was reversed to 100% A over 30 s. The analytical column was finally re-equilibrated for three and a half-minutes before injection of another sample. The flow rate was 0.4 ml/min during the whole run.

2.7. Mass spectrometry conditions

Tuning of the MS detector and optimization of the APCI operating conditions were performed by injection of DTZ (1 mg/l in methanol) through a T connector. A vaporizer temperature of 450 °C, a heated transfer capillary temperature of 150 °C, a coronary discharge intensity of 5 μA and a sheath gas flow of 60 units (arbitrary software unit) were obtained as optimal APCI conditions. Several compounds co-eluted under the applied chromatographic conditions. The co-eluting compounds do not have equal mono isotopic masses, and the detector was operated in the selected ion monitoring (SIM) mode for quantification of DTZ and the metabolites. Tandem MS detection was not chosen for quantification, as the co-eluting compounds had identical main MS/MS fragments. However, tandem MS was used to identify metabolites that were present in biological samples, but for which reference compounds were not available.

An attempt was made to detect all the metabolites indicated in Fig. 1 in biological samples, and the following molecular ions $[M+H]^+$ were therefore selected for monitoring: 415 for DTZ, 401 for MA and Mx, 387 for MD and MB, 373 for M1 and ME, 359 for M2 and M4, 345 for M6 and M8, 331 for M9 and 300 for the IS. The chromatographic peaks representing IS, DTZ, MA, M1, M2, M4 and M6 were identified by separate injection of reference compounds. In order to identify the possible chromatographic peaks of Mx, MB, MD, ME, M8 and M9, their respective parent ions were selected individually for fragmentation by helium gas collision in the ion trap during chromatography of extracted biological samples. The relative collision energy was 25% and fragments were detected in the full scan mode from m/z 110 to 425. The MS/MS fragmentation spectra obtained by analysis of biological extracts were then compared with those provided by tandem MS analysis of reference compounds.

3. Results and discussion

3.1. Chromatography and MS identification

The retention time of the IS was ∼1.8 min, whereas DTZ and its metabolites were eluted from 4.5 to 6.0 min after sample injection (Fig. 2). The chromatographic peaks of IS, DTZ, MA, M1, M2, M4 and M6 detected by SIM were confirmed by separate injections of reference compounds, whereas the peaks that possibly represented Mx, MB, MD, ME M8 and M9 in biological samples were identified by tandem MS experiments. Li et al. have described the fragmentation pattern of the core structure of the metabolites Mx and MB based on HPLC–MS/MS (triple quadrupole) experiments with synthesized compounds and human urine extract [29]. The daughter ions that might be produced through the bond cleavages sketched by these authors are presented in Table 1 for DTZ and its metabolites. All the 60 daughter ions indicated in Table 1, as well as the molecular ions, were present in the MS/MS spectra obtained from reference compounds and extracts of biological samples (Table 2). This provided strong

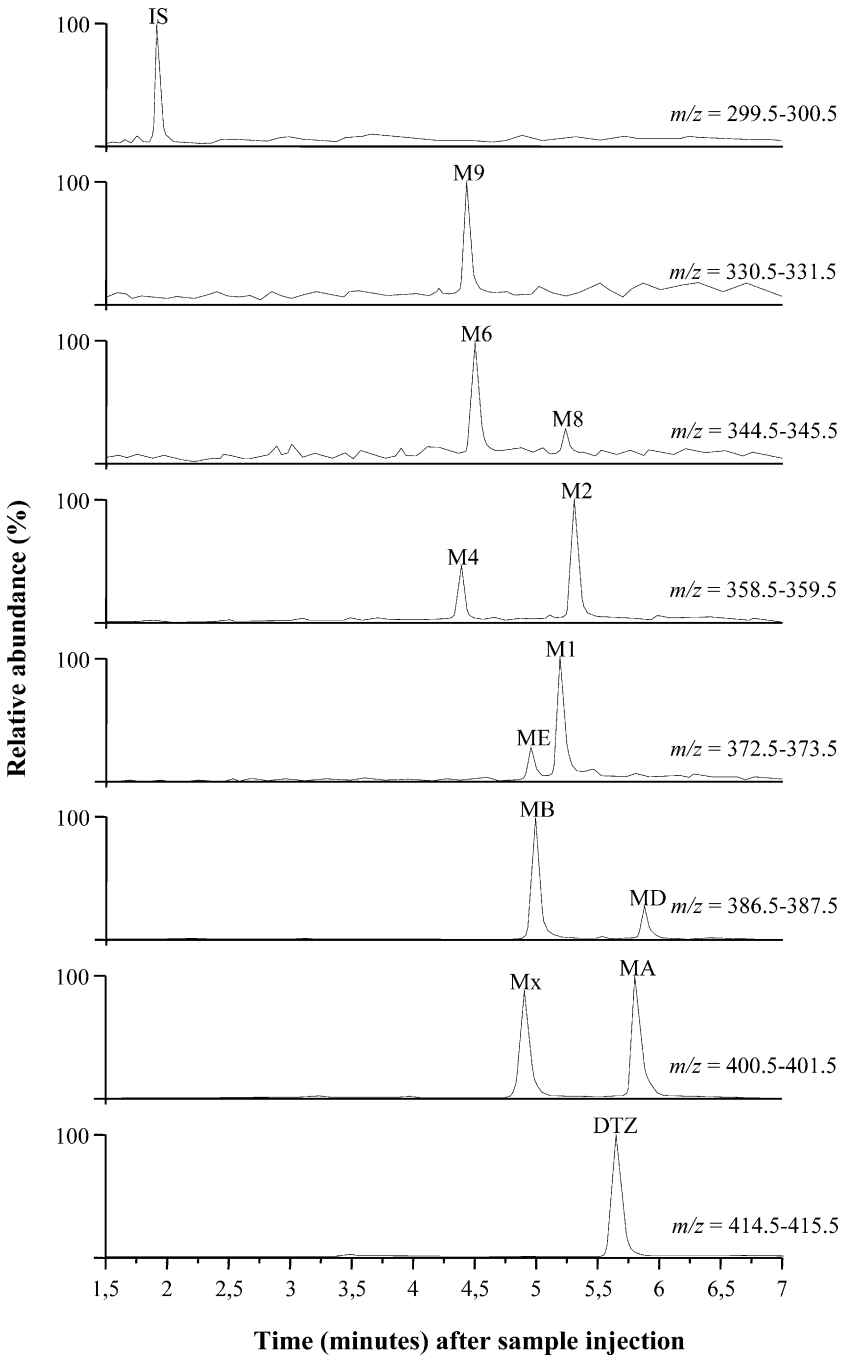
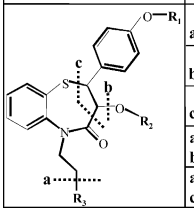


Fig. 2. A representative APCI-single-MS chromatogram obtained by SIM of an extracted plasma sample drawn from a healthy volunteer (genotyped as a homozygous extensive CYP2D6 metabolizer) 4 h after peroral intake of 120 mg DTZ. Measured plasma concentrations (μg/l) of available reference compounds were 72.7 (DTZ), 33.4 (MA), 3.3 (M1), 3.4 (M2), 2.6 (M4) and 3.3 (M6). For metabolite abbreviations and structures, see Fig. 1.

Table 1
Daughter ions (*m/z*) that are possible to derive from the bond cleavages (**a**, **b** and **c**) described by Li et al. [29]

		DTZ: $[M+H]^+ = 415$ $R_1 = CH_3$ $R_2 = COCH_3$ $R_3 = N(CH_3)_2$	MA: $[M+H]^+ = 401$ $R_1 = CH_3$ $R_2 = COCH_3$ $R_3 = NHCH_3$	Mx: $[M+H]^+ = 401$ $R_1 = H$ $R_2 = COCH_3$ $R_3 = N(CH_3)_2$	MB: $[M+H]^+ = 387$ $R_1 = H$ $R_2 = COCH_3$ $R_3 = NHCH_3$	MD: $[M+H]^+ = 387$ $R_1 = CH_3$ $R_2 = COCH_3$ $R_3 = NH_2$	ME: $[M+H]^+ = 373$ $R_1 = H$ $R_2 = COCH_3$ $R_3 = NH_2$	M1: $[M+H]^+ = 373$ $R_1 = CH_3$ $R_2 = H$ $R_3 = N(CH_3)_2$	M2: $[M+H]^+ = 359$ $R_1 = CH_3$ $R_2 = H$ $R_3 = NHCH_3$	M4: $[M+H]^+ = 359$ $R_1 = H$ $R_2 = H$ $R_3 = N(CH_3)_2$	M6: $[M+H]^+ = 345$ $R_1 = H$ $R_2 = H$ $R_3 = NHCH_3$	M8: $[M+H]^+ = 345$ $R_1 = CH_3$ $R_2 = H$ $R_3 = NH_2$	M9: $[M+H]^+ = 331$ $R_1 = H$ $R_2 = H$ $R_3 = NH_2$
		m/z	*m/z*	*m/z*	*m/z*	*m/z*	*m/z*	*m/z*	*m/z*	*m/z*	*m/z*	*m/z*	*m/z*
	a	370	370	356	356	370	356	328	328	314	314	328	314
	b	355	341	341	327	327	313	355	341	341	327	327	313
	c	223	209	223	209	195	195	223	209	223	209	195	195
	a b	310	310	296	296	310	296	310	310	296	296	310	296
	a c	178	178	178	178	178	178	178	178	178	178	178	178

All the indicated daughter ions, as well as the molecular ions ($[M+H]^+$), were present in the MS/MS spectra obtained in our experiments (Table 2).

support for identification of the chromatographic peaks of metabolites in biological samples for which no reference compounds were available. The MS/MS spectra from the four metabolites that together represent the metabolic complexity studied in this work (i.e. deacetylation, *O*-demethylation, mono-*N*-demethylation and di-*N*-demethylation) are presented in Fig. 3.

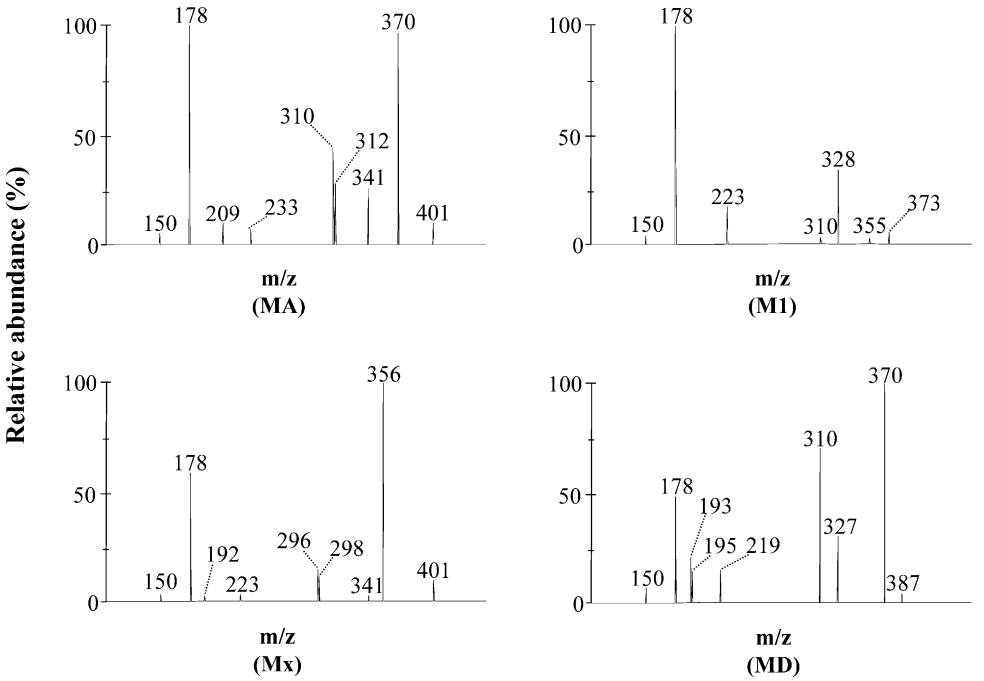


Fig. 3. MS/MS fragmentation spectra of the metabolites MA, M1, Mx and MD. The spectra were obtained from analysis of available reference compounds (MA and M1) or monitoring of human plasma extracts after peroral administration of 120 mg DTZ (Mx and MD). For metabolite abbreviations and structures, see Fig. 1.

Table 2
Fragments (m/z) detected in MS/MS experiments with DTZ and metabolites, relative abundance (%) in parenthesis

DTZ	MA	Mx	MB	MD	ME	M1	M2	M4	M6	M8	M9
178 (100)	*178* (100)	*356* (100)	*356* (100)	*370* (100)	*356* (100)	*178* (100)	*178* (100)	*178* (100)	*178* (100)	*178* (100)	*314* (100)
370 (96)	*370* (94)	*178* (60)	*178* (58)	*310* (72)	*178* (41)	*328* (43)	*328* (40)	*314* (70)	*314* (70)	*327* (96)	*178* (78)
415 (40)	*310* (43)	*296* (17)	*296* (22)	*178* (50)	193 (26)	*223* (18)	*209* (33)	*223* (11)	*209* (14)	*195* (81)	206 (50)
310 (22)	312 (30)	298 (15)	298 (18)	*327* (31)	*296* (22)	*373* (6)	*341* (10)	*359* (5)	*327* (4)	*328* (80)	*195* (37)
312 (17)	*341* (28)	*401* (10)	*327* (12)	193 (20)	*313* (20)	150 (4)	284 (6)	150 (4)	*345* (2)	220 (78)	*313* (23)
355 (7)	*401* (11)	150 (5)	233 (10)	219 (15)	219 (10)	*355* (3)	234 (5)	*341* (2)	*296* (2)	169 (11)	169 (8)
150 (6)	*209* (10)	*223* (4)	*209* (6)	*195* (12)	337 (9)	*310* (3)	150 (3)	*296* (2)	270 (2)	299 (5)	*331* (6)
223 (5)	233 (7)	*341* (3)	*387* (5)	150 (7)	319 (8)		*359* (2)		233 (2)	284 (5)	240 (6)
	150 (5)	192 (2)	150 (4)	*387* (4)	*195* (8)		*310* (2)		220 (2)	152 (5)	*296* (5)
					249 (6)				150 (2)	303 (4)	281 (4)
					267 (5)					281 (4)	267 (3)
					373 (3)					*345* (2)	152 (3)
					150 (3)					*310* (2)	150 (2)
										269 (2)	
										150 (2)	

The MS/MS spectra of DTZ, MA, M1, M2, M4 and M6 were obtained from reference compounds, whereas those of Mx, MB, MD, ME, M8 and M9 were obtained by monitoring of biological samples. The daughter ions indicated in Table 1 and the molecular ions are in cursive. For metabolite abbreviations and structures, see Fig. 1.

3.2. Method validation

Validation of the method was performed using available reference compounds. The average extraction recoveries ($n = 3$) were greater than 80% for all analytes at all concentrations tested. The lower limit of quantification (LLOQ), defined as the spiked plasma concentration that resulted in an analyte-relative-to-noise signal ratio of approximately 10 was estimated to be 1 μg/l for all compounds.

Calibration curves were prepared for all the six available reference compounds in the lower concentration interval (1–15 μg/l, $n = 5$ for each analyte), whereas M4 and M6 were excluded in the higher concentration interval (15–225 μg/l, $n = 5$ for each analyte). Linear regression coefficients (r^2) ranged between 0.993–0.999 and 0.997–0.999 at lower and higher concentrations, respectively. The relative standard deviations (R.S.D.) of the estimated linear slopes were less than 9.3% (lower interval) and 2.6% (higher interval). The origo was included in all 95% confidence intervals that were calculated for the intercepts of the calibrating curves.

Accuracy and precision data are presented in Table 3. For both intra- and inter-assay the average calculated concentrations deviated $< \pm 20\%$ at LLOQ and $< \pm 15\%$ above LLOQ ($n = 5$ for each analyte at each concentration). Similarly, both intra- and inter-assay R.S.D. was $< \pm 20\%$ at LLOQ (except intra-assay of M4; 20.6%) and $< \pm 15\%$ above LLOQ ($n = 5$ for each analyte at each concentration).

The lack of reference compounds did not allow validation of the other metabolites detected in biological samples (i.e. Mx, MB, MD, ME, M8 and M9). However, the satisfactory validation data obtained for quantification of DTZ and the available metabolites supports that the method provides consistent IS-adjusted arbitrary measurements of the additional metabolites detected in biological samples as well. These arbitrary plasma measurements could be used to estimate time to maximum plasma concentration and elimination half-life, as well as pseudo values for maximal plasma concentration and area under plasma concentration vs. time curve. Such data could be

Table 3
Precision and accuracy data obtained for quantification of DTZ and the five metabolites available as reference compounds in spiked plasma samples

	Precision							Accuracy						
	Intra-assay				Inter-assay			Intra-assay				Inter-assay		
μg/l	1	6	15	225	1	6	15	225	1	6	15	225	1	6	15	225
DTZ	12.7	n.t.	4.6	7.6	8.8	n.t.	8.0	2.7	−11.8	n.t.	−0.7	+2.6	−3.3	n.t.	+8.4	+3.7
MA	15.5	n.t.	9.3	6.6	2.4	n.t.	7.2	2.9	+10.7	n.t.	+3.7	+1.0	+12.3	n.t.	+14.1	+2.4
M1	9.7	n.t.	7.8	3.3	8.2	n.t.	7.3	1.7	−17.5	n.t.	−2.4	+0.4	−8.4	n.t.	+4.9	+2.8
M2	11.9	n.t.	7.8	7.5	6.6	n.t.	7.8	4.4	+7.3	n.t.	+3.0	+1.0	+18.9	n.t.	+14.9	+4.1
M4	20.6	13.7	14.4	n.t.	10.1	10.8	3.1	n.t.	+4.6	−2.0	+2.2	n.t.	+2.2	+4.1	+2.1	n.t.
M6	16.5	9.4	11.5	n.t.	8.0	8.1	4.3	n.t.	+9.5	+1.5	+0.8	n.t.	+12.4	+10.8	+7.2	n.t.

Data for precision are the R.S.D. of the measurements (in %, $n = 5$), whereas accuracy refers to the relative deviations of measured compared to nominal concentrations (in %, $n = 5$). For metabolite abbreviations and structures, see Fig. 1. n.t, not tested.

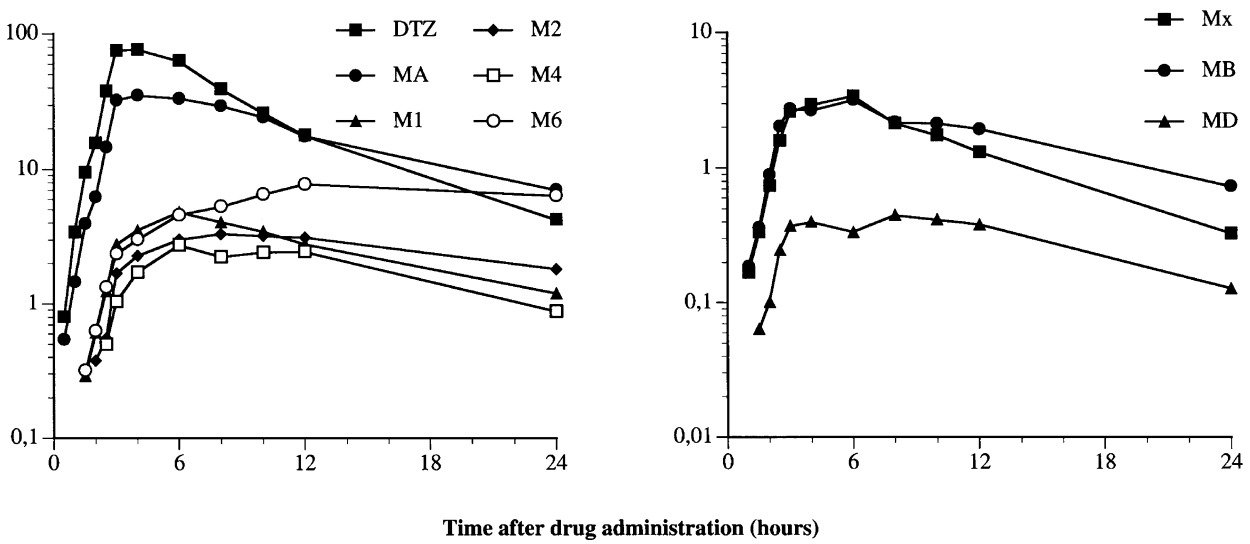


Fig. 4. Plasma-level vs. time profiles for DTZ and eight metabolites in a healthy volunteer (genotyped as a homozygous extensive CYP2D6 metabolizer) after peroral intake of a single 120 mg dose. (A) DTZ and metabolites available as reference compounds (plasma levels in μg/l), and (B) metabolites confirmed by MS/MS analysis (arbitrary plasma levels). For metabolite abbreviations and structures, see Fig. 1.

used for relative comparisons, but it is important to point out that pharmacokinetic results obtained from arbitrary measurements must be adopted on the basis that accuracy, precision and linearity have not been documented.

Stability of DTZ and metabolites in plasma during storage has been investigated previously, and samples should not be stored longer that 8 weeks at −20 °C before analysis [30]. All analyses were therefore performed within 6 weeks of storage (−20 °C) in the present work.

3.3. Application of the method

To our knowledge, this is the first HPLC–MS method that has been described for analysis of DTZ and metabolites in human plasma. Recently, the method was used in a study investigating pharmacokinetics of DTZ and seven of its metabolites in relation to interindividual differences in cytochrome P450-2D6 (CYP2D6) metabolic capacity following administration of a single peroral dose of DTZ (120 mg) to healthy volunteers [28]. Altogether, it was possible to measure DTZ and eight of the metabolites throughout the whole sampling interval (0–24 h) in all 15 study participants (an example is shown in Fig. 4). An interesting finding was that the pharmacodynamically active metabolites M1 and M2, in contrast to the parent drug, were extensively ($\geq$5-fold) accumulated in poor compared to extensive CYP2D6 metabolizers [28]. The ability to detect subsequent metabolites of M1 and M2 lead to the observation that a reduced *O*-demethylation rate of these precursor metabolites was due to their accumulation in poor CYP2D6 metabolizers.

Analysis of the *N*,*N*-didemethylated DTZ derivates (i.e. MD, ME, M8 and M9) were not included in the pharmacokinetic study, but these metabolites could be detected in extracts of plasma sampled in this investigation (Fig. 2). The highest IS-adjusted arbitrary measurements were observed for MD, and this was the only *N*,*N*-didemethylated metabolite that could be regularly detected up to 24 h post-dose. The obtained plasma-level vs. time profiles for MD in individuals with

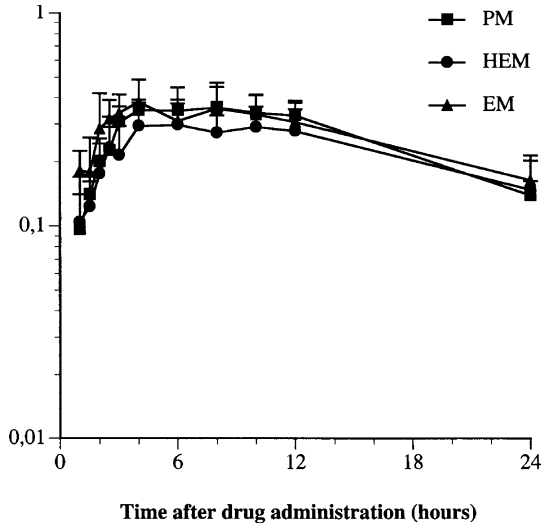


Fig. 5. Plasma-level vs. time profiles for the metabolite *N*,*N*-didesmethyl-diltiazem (MD) in individuals genotyped as either CYP2D6 poor metabolizers (PM), heterozygous extensive metabolizers (HEM) or (homozygous) extensive metabolizers (EM) after single peroral administration of 120 mg DTZ (arbitrary plasma levels, $n = 5$ in each subgroup; mean ± S.D.). For metabolite structure, see Fig. 1.

different CYP2D6 metabolic activity are shown in Fig. 5. No statistical differences in the pharmacokinetics of MD in relation to CYP2D6 activity were observed. The average elimination half-lives ranged from 10 to 13 h (estimated from the last two samples). Former human studies reporting plasma measurements of *N*,*N*-didemethylated DTZ metabolites seem to be absent, as well as investigations outlining their pharmacodynamic activities relative to the parent compound. However, an in vitro study with human liver microsomes showed that MD is about 200-fold more potent as a CYP3A4 inhibitor compared to DTZ itself [7]. Thus, it will be of interest to monitor MD in studies where the influence of DTZ treatment on drugs metabolized by CYP3A4 is explored.

4. Conclusions

The present HPLC–MS method is more sensitive, and enables detection of additional metabolites, compared to previous methods for analysis of DTZ and metabolites in human plasma. Validation of the method was restricted to DTZ and five metabolites due to limited access of reference compounds, but tandem MS analyses confirmed the chromatographic peaks of another six metabolites in biological samples. It has been shown in a clinical study with healthy volunteers that DTZ and eight metabolites could be measured regularly throughout a 24 h sampling interval after peroral administration of a single therapeutic DTZ dose (120 mg). Thus, the method is appropriate for monitoring of DTZ and metabolites in pharmacokinetic studies.

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