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Screening, library-assisted identification and validated quantification of oral antidiabetics of the sulfonylurea-type in plasma by atmospheric pressure chemical ionization liquid chromatography–mass spectrometry☆

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Abstract

An atmospheric pressure chemical ionization liquid chromatographic–mass spectrometric (APCI–LC–MS) LC–MS assay is presented for fast and reliable screening and identification as well as precise and sensitive quantification of oral antidiabetics of the sulfonylurea-type (OADs) in plasma. It allowed the specific diagnosis of an overdose situation or a Munchausen syndrome caused by ingestion of OADs. After liquid–liquid extraction, the OADs glibenclamide, glibornuride, gliclazide, glimepiride, glipizide, gliquidone, glisoxepide, tolazamide and tolbutamide were separated using fast gradient elution. After screening and identification in the scan mode using our new LC–MS library, the OADs were quantified in the selected-ion mode. The quantification assay was validated according to the criteria established by the *Journal of Chromatography B*. All validation data were inside the required limits. The assay is part of a general LC–MS procedure for fast screening, identification and quantification of different toxicologically relevant compounds in plasma and has proven to be appropriate for OADs.

Keywords: Screening; Quantification; Library-assisted identification; Antidiabetics; Sulfonylurea-type

1. Introduction

Sulfonylurea-type oral antidiabetics (OADs) have been used for more than 50 years in treatment of hyperglycemia in patients with diabetes mellitus type 2. Besides this therapeutic use, sulfonylureas are misused, e.g. by Munchausen patients who want to imitate an illness or by suicidal patients. For differential diagnosis of unclear hypoglycemia, antidiabetics must be screened for to allow differentiation between a surreptitious misuse of sulfonylureas or pathophysiological reasons like insulinoma [1]. Before exploratory surgery or even subtotal pancreatectomy, misuse of hypoglycemic sulfonylurea drugs should analytically be excluded [2]. Micellar electrokinetic capillary chromatography (MECC) was described for detection of sulfonylureas in urine with diode-array or UV detection [3]. However, as the authors have learned afterwards, this procedure was not suitable for screening of sulfonylurea drugs of

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the third generation, since these are excreted in an almost completely metabolized form. Therefore, 2 years later the same working group published a modified MECC procedure for the detection of the metabolites of sulfonylureas of the third generation [4]. Sulfonylureas can sufficiently be separated by reversed-phase liquid chromatography. Diode array detectors were applied for the general screening procedures, which covered some sulfonylureas besides other drugs [5,6]. The use of UV at one wavelength [7] is critical for toxicological analysis due to low specificity. Magni et al. [8] described the identification of four sulfonylureas in serum by electrospray LC–MS. The LC–MS identification procedure of Susanto and Reinauer [9] is of minor specificity because they used only one ion per compound for selected-ion mode (SIM) detection. Concerning quantification, they stated, that their LC–MS method can be used for ''prequantification''. Some sulfonylureas can be quantified by HPLC [10,11].

In the following, a new APCI–LC–MS procedure will be presented for fast screening, reliable identification and fully validated quantification of glibenclamide, glibornuride, gliclazide, glimepiride, glipizide, gliquidone, glisoxepide, tolazamide and tolbutamide in plasma from subtherapeutic to overdose concentrations. This procedure is based on our general extraction procedure which is routinely used in clinical toxicology for both, our general GC–MS [12] and our LC–MS screening, identification and quantification procedure [13–15].

2. Experimental

2.1. Chemicals and reagents

The reference substances of the studied oral antidiabetics were kindly supplied by the following manufacturers: glibenclamide and glimepiride by Aventis (Bad Soden, Germany), glibornuride and tolbutamide by Hoffmann–La Roche (Grenzach-Wyhlen, Germany), gliclazide by Servier (München, Germany), glipizide by Pfizer (Karlsruhe, Germany), gliquidone by Yamanouchi (Heidelberg, Germany), glisoxepide by Bayer (Leverkusen, Germany) and tolazamide by Pharmacia (Erlangen, Germany). Trimipramine-D3 (internal standard, I.S.) was obtained from Promochem (Wesel, Germany). Ammonium formate (analytical grade) was obtained from Fluka (Neu-Ulm, Germany). Acetonitrile (HPLC grade) and all other chemicals (analytical grade) were obtained from E. Merck (Darmstadt, Germany).

2.2. Biosamples

Pooled blank human plasma samples were obtained from a local blood bank. Authentic patient plasma samples had been submitted to our laboratory for toxicological analysis.

2.3. Sample preparation

Plasma (1 ml) was extracted with 5 ml of a mixture of diethyl ether–ethyl acetate (1:1, v/v) after addition of 0.1 ml I.S. solution (0.01 mg/ml trimipramine-D3 in methanol) and 5 ml saturated sodium sulfate solution. After phase separation by centrifugation, the organic phase was transferred to a pear-shaped flask and evaporated to dryness. The aqueous phase was basified with 0.5 ml of 1 mol/l aqueous sodium hydroxide and extracted a second time with 5 ml of the solvent mixture. This organic phase was transferred to the same pear-shaped flask and evaporated. The combined residues were dissolved in 100 μl of methanol. For the described LC–MS procedure, 50 μl of this solution were evaporated and redissolved in 50 μl of acetonitrile. The rest could be used for GC–MS screening and quantification [12].

2.4. Liquid chromatography–mass spectrometry

2.4.1. Apparatus

The oral antidiabetics were separated, screened for, identified and quantified in plasma using an Agilent Technologies (AT, Waldbronn, Germany) AT 1100 series atmospheric pressure chemical ionisation electrospray (APCI) LC–MSD, SL version and a LC–MSD ChemStation using the A.08.03 software.

2.4.2. HPLC conditions

Gradient elution was achieved on a Merck LiChroCART® column (125×2 mm I.D.) with Superspher® 60 RP Select B as stationary phase and a LiChroCART® 10-2 Superspher® 60 RP Select B guard column. The mobile phase consisted of ammonium formate (0.005 *M*, adjusted to pH 3 with formic acid) (eluent A) and acetonitrile (eluent B). Before use the mobile phases were degassed for 30 min in an ultrasonic bath. During use, the mobile phases were degassed by the integrated AT 1100 series degasser. Until the beginning of the analysis, the HPLC system was flushed with a 60:40 (v/v) mixture of the two eluents. The gradient and the flow-rate were programmed as follows: 0–4 min 40% B (flow: 0.4 ml/min), 4–6 min 90% B (flow: 0.6 ml/min), 6–7 min 90% B (flow: 0.8 ml/min), 7–10 min 40% B (flow: 0.4 ml/min). After 10 min, the HPLC column was reequilibrated and the autosampler began with the next injection.

2.4.3. Electrospray conditions

The following APCI inlet conditions were applied: drying gas (7000 ml/min, 300 °C) and nebulizer pressure (25 p.s.i.) (both nitrogen); capillary voltage, 4000 V; drying gas temperature set at 300 °C, vaporizer temperature set at 400 °C; corona current was 5.0 μA; positive full scan mode for screening and library-assisted identification, mass range *m/z* 50–600, fragmentor voltages 100 and 200 V (cycle time: 0.59 s); positive SIM mode for quantification, fragmentor 100 and 200 V. The HPLC effluent entered the electrospray chamber only in the time window between 0 and 7.0 min.

Tuning of the MSD was performed with the help of the autotune feature of the LC/MSD ChemStation software (rev. A.08.03) using the APCI acetonitrile solution tuning mix supplied with the apparatus.

2.4.4. LC–MS procedure for screening and identification

The presence of OADs was screened for by mass chromatography in the 100 V trace and the 200 V trace of the same run with the following ions (*m/z*): 450, 271, 446, 312, 324, 298 (I.S.), 367, 494, 491 and 528. Positive peaks in each of the two traces were identified by library search comparing the underlying APCI mass spectra with the reference spectra of our new LC–MS library of drugs, poisons, pesticides and their metabolites [15]. This library was created for the NIST98 search algorithm, version 1.7. This algorithm is offered by Agilent Technologies and other mass spectrometer companies.

2.4.5. LC–MS procedure for quantification

For quantification, the following target ions (*m/z*) were used in the SIM mode: time window 0–4.8 min: 450 at 200 V for glisoxepide, 271 at 100 V for tolbutamide, 446 at 100 V for glipizide, 312 at 100 V for tolazamide; time window 4.81–7 min: 324 at 100 V for gliclazide, 298 at 100 V for the I.S. trimipramine-D3, 367 at 200 V for glibornuride, 494 at 100 V for glibenclamide, 491 at 100 V for glimepiride and 528 at 100 V for gliquidone. The peak area ratios of the target ions of the drugs vs. that of the I.S. were compared with the calibration curve in which the peak area ratios of the standards vs. that of the I.S. were plotted versus their concentrations.

2.5. Assay validation for plasma analysis

The LC–MS assay was validated for the quantification of nine oral antidiabetics in plasma according to the criteria established by Lindner and Wainer [16].

2.5.1. Preparation of stock solutions, calibration standards and control samples

Stock solutions of each of the nine OADs in different concentrations (0.01, 0.1, 1.0, and 10.0 g/l) were prepared in methanol by separate weighings. The calibration standards were prepared using pooled blank plasma and spiking solutions prepared from the stock solutions as mixtures of the nine OADs in methanol at concentrations ten times higher than the corresponding calibrations standards (Section 2.5.3.). The quality control samples (concentrations given below) were prepared using pooled blank plasma and independently prepared mixtures of the nine OADs at concentrations ten times higher than the corresponding quality control samples. For recovery studies, standard solutions containing the I.S. (1 mg/l) and the nine OADs (concentrations given

below) were prepared in methanol. All solutions were stored at 4 °C

2.5.2. Peak purity and selectivity

Six different blank plasma samples were analyzed for peaks interfering with the detection of the analytes or the I.S.

2.5.3. Linearity of calibration

Calibration standards with concentrations of 0.01, 0.05, 0.1, 0.15 and 0.2 mg/l of glibenclamide, 0.3, 0.5, 0.7, 0.9 and 1.1 mg/l of glibornuride, 0.5, 1.5, 3.0, 4.5 and 6.0 mg/l of gliclazide, 0.1, 0.75, 1.5, 2.25 and 3.0 mg/l of glimepiride, 0.05, 0.5, 1.0, 1.5 and 2.0 mg/l of glipizide, 0.3, 0.5, 0.7, 0.9 and 1.1 mg/l of gliquidone, 0.05, 0.25, 0.5, 0.75 and 1.0 mg/l of glisoxepide, 30.0, 50.0, 75.0, 100.0 and 120.0 mg/l of tolazamide and 30.0, 50.0, 75.0, 100.0 and 120.0 mg/l tolbutamide were assayed ($n=5$).

2.5.4. Apparatus precision

Quality control samples in the low (0.05 mg/l of glibenclamide, 0.5 mg/l of glibornuride, 2.0 mg/l of gliclazide, 1.0 mg/l of glimepiride, 0.25 mg/l of glipizide, 0.5 mg/l of gliquidone, 0.25 mg/l of glisoxepide, 60.0 mg/l of tolazamide and 60.0 mg/l of tolbutamide, LOW) and high concentration ranges (0.1 mg/l of glibenclamide, 0.7 mg/l of glibornuride, 4.0 mg/l of gliclazide, 2.0 mg/l of glimepiride, 1.0 mg/l of glipizide, 0.7 mg/l of gliquidone, 0.5 mg/l of glisoxepide, 100.0 mg/l of tolazamide and 100.0 mg/l of tolbutamide, HIGH) of the nine analytes were extracted. Each sample was injected five times within a single sequence and during the course of five consecutive sequences alternately (sequence order: LOW/HIGH/LOW/HIGH/LOW/HIGH/LOW/HIGH/LOW/HIGH).

2.5.5. Accuracy and precision

Quality control samples ($n=5$) at two concentrations of each of the nine OADs (concentrations of each particular compound given in Table 1) were assayed against calibration curves to determine the accuracy. The concentrations of the analytes were calculated using a linear regression model. The calculated values at each concentration were averaged and the percentage bias was calculated to estimate accuracy. The intra- and inter-day precision (relative standard deviation) of the method was assessed from the comparison of the analysis of

Table 1
Intra-day ($n=5$) and inter-day ($n=15$, 3 days) precision and accuracy data of the LC–MS assay for OADs

Drug	Spiked concentration (LOW and HIGH) [mg/l]	Mean calculated concentration [mg/l]	Accuracy [%][a]	Precision [%][b]	
				Intra-day	Inter-day
Glibenclamide	0.05	0.05	−0.9	6.8	17.0
	0.1	0.10	1.9	3.4	3.2
Glibornuride	0.5	0.52	3.7	9.1	9.7
	0.7	0.75	6.5	8.1	8.1
Gliclazide	2.0	2.05	2.2	5.8	14.0
	4.0	4.01	0.2	6.2	7.6
Glimepiride	1.0	1.04	4.3	6.0	10.3
	2.0	1.94	−3.0	5.9	12.2
Glipizide	0.25	0.26	4.9	8.0	1.7
	1.0	1.03	3.4	4.6	11.6
Gliquidone	0.5	0.49	−1.5	6.4	12.2
	0.7	0.72	2.1	4.3	11.5
Glisoxepide	0.25	0.24	−5.6	5.6	9.4
	0.5	0.48	−4.3	6.6	10.3
Tolazamide	60.0	61.25	2.1	5.8	5.7
	100.0	101.55	1.6	3.8	4.2
Tolbutamide	60.0	61.65	2.8	5.2	4.1
	100.0	103.86	3.9	4.2	6.5

[a] Accuracy=((mean calculated concentration−actual concentration)/actual concentration)×100.
[b] Precision=(SD/mean)×100.

control samples ($n=5$) on each of 3 days by one-way ANOVA using day as the grouping variable.

2.5.6. Stability

Analyte stability for longterm storage was tested by analyzing spiked samples ($n=3$) before and after storage for 6 months at −20 °C. The samples were analyzed together with a freshly prepared calibration curve.

2.5.7. Limits

For determination of the limit of detection (LOD, signal-to-noise ratio greater than 3:1), quality control samples with 0.002 mg/l of glibenclamide, 0.03 mg/l of glibornuride, 0.05 mg/l of gliclazide, 0.01 mg/l of glimepiride, 0.005 mg/l of glipizide, 0.03 mg/l of gliquidone, 0.005 mg/l of glisoxepide, 3.0 mg/l of tolazamide and 3.0 mg/l of tolbutamide were assayed. The criteria for the limit of quantification (LOQ, signal-to-noise ratio greater than 10:1) were fulfilled by the lowest point of the calibration curve (0.01 mg/l of glibenclamide, 0.3 mg/l of glibornuride, 0.5 mg/l of gliclazide, 0.1 mg/l of glimepiride, 0.05 mg/l of glipizide, 0.3 mg/l of gliquidone, 0.05 mg/l of glisoxepide, 30.0 mg/l of tolazamide and 30.0 mg/l tolbutamide). The noise data from the assay of blank matrices was taken from the selectivity experiments (see Section 2.5.2.).

2.5.8. Recoveries

Absolute analytical recoveries were tested at the LOW (0.05 mg/l of glibenclamide, 0.5 mg/l of glibornuride, 2.0 mg/l of gliclazide, 0.5 mg/l of glimepiride, 0.1 mg/l of glipizide, 0.5 mg/l of gliquidone, 0.1 mg/l of glisoxepide, 60.0 mg/l of tolazamide and 60.0 mg/l of tolbutamide) and HIGH (0.15 mg/l of glibenclamide, 0.7 mg/l of glibornuride, 4.0 mg/l of gliclazide, 2.0 mg/l of glimepiride, 1.5 mg/l of glipizide, 0.7 mg/l of gliquidone, 1.0 mg/l of glisoxepide, 100.0 mg/l of tolazamide and 100.0 mg/l of tolbutamide) concentration levels ($n=5$). Solutions (0.1 ml) containing the nine OADs as a mixture (LOW or HIGH) in methanol was spiked to 1 ml of blank plasma. The samples were extracted according to the procedure described above. The combined organic residues were redissolved in 0.1 ml of I.S. solution (0.01 mg/ml trimipramine-D3 in methanol), 50 μl of this solution were evaporated and redissolved in 50 μl of acetonitrile.

As controls ($n=5$), a mixture of 0.1 ml of the above mentioned solutions of the nine OADs in methanol (LOW or HIGH) and 0.1 ml of I.S. (0.01 mg/ml trimipramine-D3 in methanol) was evaporated carefully. The residues were then redissolved in 0.1 ml of acetonitrile. Recoveries were calculated by comparing the peak areas of spiked plasma samples and controls.

2.5.9. Proof of applicability

Plasma samples from authentic clinical cases were assayed with the described method.

3. Results and discussion

3.1. Sample preparation

The OADs were isolated by our standard plasma liquid–liquid extraction procedure [12] using trimipramine-D3 as routine I.S. This extraction procedure has proved to be very versatile for GC–MS and LC–MS analysis in clinical toxicology and drug monitoring [13,14,17,18]. The price for the universality was the rather high matrix background in GC–MS runs. This disadvantage could be overcome in LC–MS by using a fast gradient elution separating most of the matrix compounds from the analytes. Recovery values of 65–100% for the different OADs showed that our standard extraction procedure was also suitable for this application.

3.2. LC–MS screening, identification and quantification

The presence of OADs was successfully screened for by mass chromatography with selected ions followed by library search of the underlying APCI mass spectra with our new LC–MS reference library [15]. We preferred the APCI over the ESI mode, because it markedly reduced the well known ion suppression which might lead to false negative results [19]. Moreover, the used LC–MSD showed higher sensitivity when operated in the APCI mode. In Fig. 1, APCI electrospray mass spectra recorded at 100 V and 200 V fragmentor voltage and the

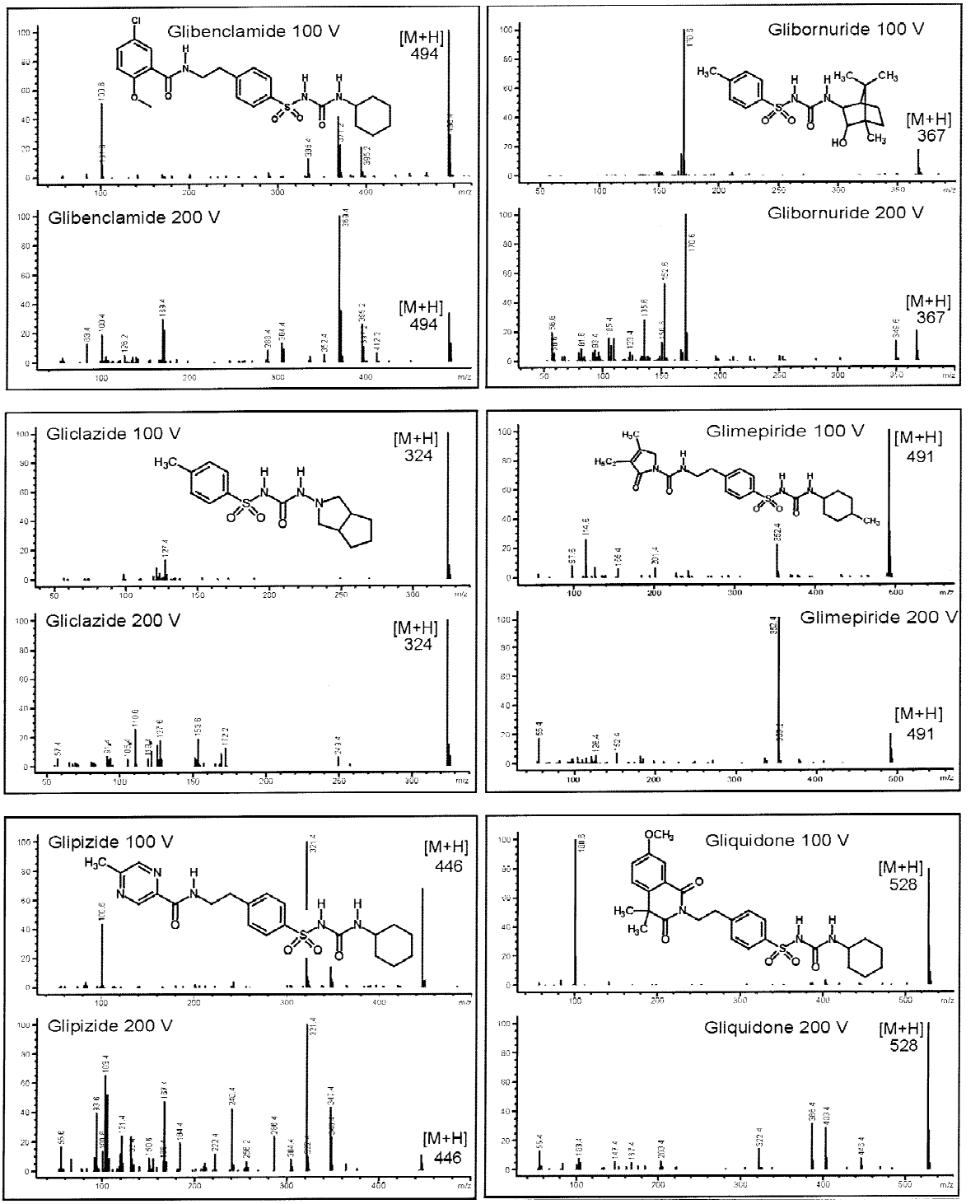

Fig. 1. APCI electrospray mass spectra recorded at 100 and 200 V fragmentor voltage and structures of glibenclamide, glibornuride, gliclazide, glimepiride, glipizide, gliquidone, glisoxepide, tolazamide, tolbutamide and the I.S. trimipramine-D3. The abscissa represents the m/z value [u] and the ordinate the relative abundances of the fragment ions [%].

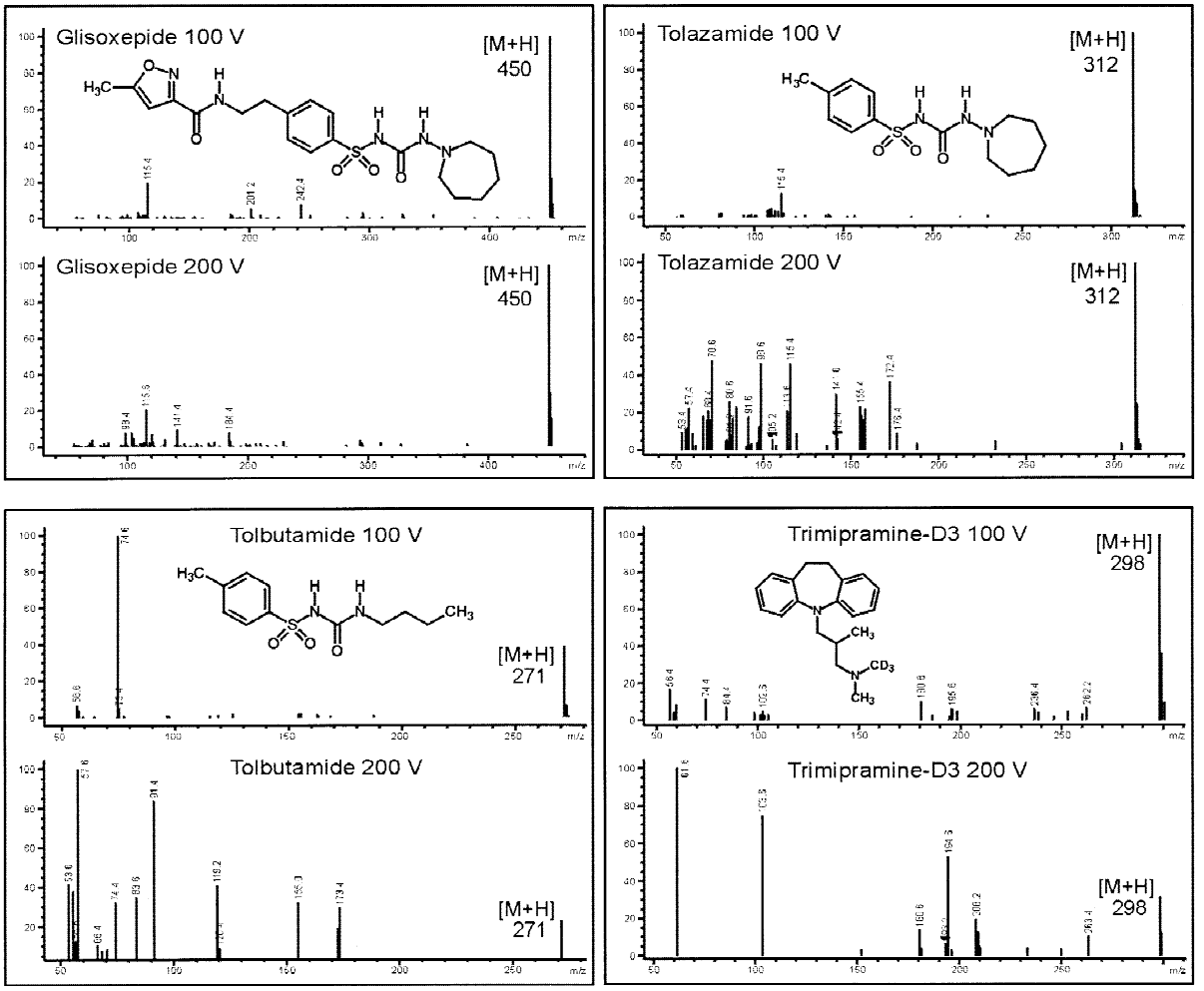

Fig. 1. (*continued*)

structures of glibenclamide, glibornuride, gliclazide, glimepiride, glipizide, gliquidone, glisoxepide, tolazamide, tolbutamide and the I.S. trimipramine-D3 are shown. As can be seen from the spectra, the different OADs resulted in spectra of various significance at different fragmentor voltages. Therefore, we recorded the full scan spectra at 100 and 200 V with a cycle time of 0.59 s. It should be kept in mind that same fragmentor voltage selected in different apparatus may result in different abundances of the formed fragments [20]. Therefore, each user has to select that fragmentor voltage of his specific apparatus which produces mass spectra similar to those shown in Fig. 1. In our experience with three different LC–MSD apparatus, this allowed the successful use of the presented screening procedure and the use of our new LC–MS library.

In Fig. 2, smoothed, normalized and merged mass chromatograms (scan mode, 100 V) are shown of a blank plasma sample spiked with I.S. and therapeutic concentrations of OADs. The selected diagnostic ions (m/z) and the spiked concentrations (mg/l) were as follows: 450 and 0.25 (glisoxepide), 271 and 50.0 (tolbutamide), 446 and 0.5 (glipizide), 312 and 50.0 (tolazamide), 324 and 1.5 (gliclazide), 298 and 1.0 (I.S. trimipramine-D3), 367 and 0.5 (glibornuride),

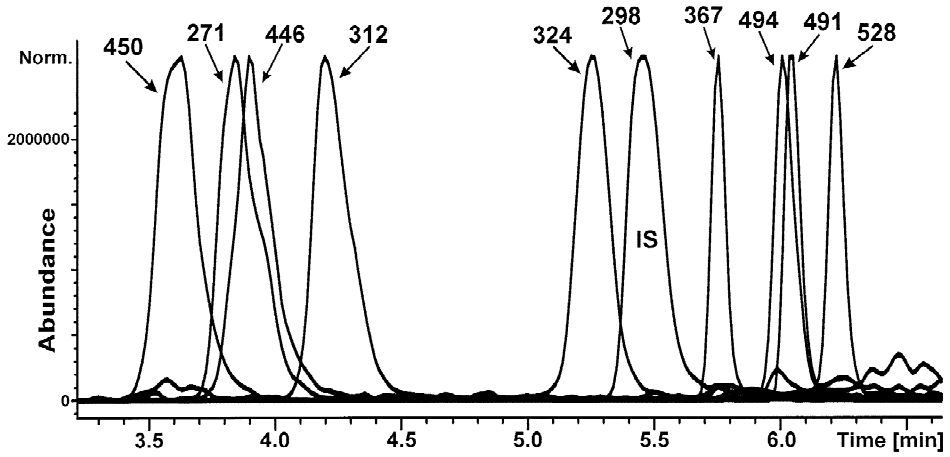

Fig. 2. Smoothed, normalized and merged mass chromatograms (scan mode, 100 V) of a blank plasma sample spiked with I.S. and therapeutic concentrations of OADs. Selected diagnostic ions (m/z, u) and spiked concentrations (mg/l): 450 and 0.25 (glisoxepide), 271 and 50.0 (tolbutamide), 446 and 0.5 (glipizide), 312 and 50.0 (tolazamide), 324 and 1.5 (gliclazide), 298 and 1.0 (I.S. trimipramine-D3), 367 and 0.5 (glibornuride), 494, and 0.05 (glibenclamide), 491 and 0.75 (glimepiride), 528 and 0.5 (gliquidone).

494, and 0.05 (glibenclamide), 491 and 0.75 (glimepiride), 528 and 0.5 (gliquidone). All drugs were sufficiently separated within only 7 min.

For illustration of the screening and identification procedure, smoothed and merged mass chromatograms (scan mode, 100 V) of the ions m/z 450, 271, 446, 312, 324, 298, 367, 494, 491, 528 of an authentic plasma extract indicating the presence of OADs are shown in the upper part of Fig. 3. The mass spectrum underlying the marked peak (lower spectrum), the reference spectrum (upper spectrum), the structure and the hit list found by computer library search [15] are shown in the lower part of Fig. 3.

Quantification of OADs was performed in the SIM mode to improve sensitivity and precision. In Fig. 4, smoothed and merged mass fragmentograms normalized in relation to the abundance of the I.S. (SIM mode, 100 V, ions m/z 450, 271, 446, 312, time window 0–4.8 min; 324, 298, 367, 494, 491, 528, time window 4.81–7 min) are shown of a blank plasma sample extract (top) and of a blank plasma sample spiked with 1.0 mg/l of the I.S. (middle) and of a blank plasma sample spiked with OADs (bottom). The spiked plasma extract was the same extract as used in Fig. 2. The corresponding fragmentograms recorded at 200 V were also free of interfering peaks. As the drugs were first identified in the full scan mode, the use of only the target ion for quantification without qualifiers was acceptable.

3.3. Validation data

The quantification assay was validated according to the criteria recommended by the editors of *Journal of Chromatography B* [16]. The validation data are summarized in Tables 1 and 2. As shown in Fig. 4 (top), no interfering peaks were observed in the blank plasma extract. Interferences with common drugs typically taken in combination were tested and could be excluded due to different retention time and/or mass spectra.

The quantification assay was found to be selective for all the nine tested compounds. The assay was linear from subtherapeutic to overdose concentrations of each compound (data for each compound are shown in Table 2). The low and high level recoveries ranged from 65% for glisoxepide to 100% for glibenclamide (Table 2). The limits of detection with a signal-to-noise ratio of at least 3 in the scan mode screening ranged from 0.002 mg/l for glibenclamide to 0.03 mg/l for tolbutamide. The LOQs corresponded to the lowest concentrations used for the calibration curves with a signal-to-noise ratio of at

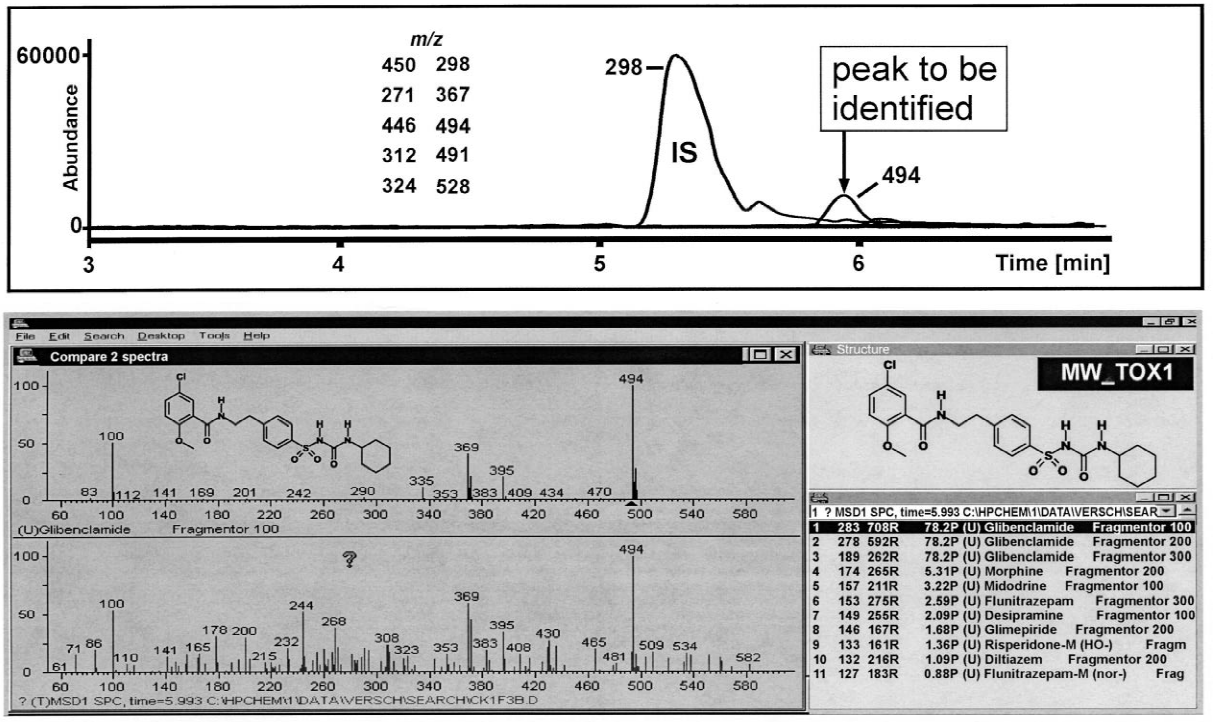

Fig. 3. Smoothed and merged mass chromatograms (scan mode, 100 V) of the ions m/z 450, 271, 446, 312, 324, 298, 367, 494, 491, 528 of an authentic plasma extract indicating OADs (upper part). Mass spectrum underlying the marked peak (lower spectrum), the reference spectrum (upper spectrum), the structure and the hit list found by computer library search [15] (lower part).

least 10. Apparatus precision was examined in the indicated manner ($n=10$; see Section 2.5.4.). Intra- and inter-day precision and accuracy were determined for each compound and the results were within the required limits (Table 1). The analytes in frozen plasma samples were stable for more than 6 months. For demonstration of applicability, authentic plasma samples were analyzed. For example, Fig. 5 shows smoothed, normalized and merged mass fragmentograms (SIM mode, 100 V) with the given ions of an authentic plasma extract indicating 0.15 mg/l of glibenclamide.

However, there is no strong correlation between plasma levels of OADs and blood glucose levels. Therefore, in emergency toxicology, it might be advantageous to confine to a one point calibration at a high therapeutic level. This confinement should be acceptable for a preliminary estimation, because this assay has proved to be linear, accurate and precise. The presented assay is the first procedure for determination of OADs that was fully validated according to the criteria established by the *Journal of Chromatography B* [16].

4. Conclusions

The LC–MS assay presented here allowed very fast and reliable screening and identification as well as precise and sensitive quantification of oral antidiabetics of the sulfonylurea-type in plasma, thus allowing the specific diagnosis of an overdose situation or a Munchausen syndrome caused by ingestion of OADs. The quantification method fulfilled the requirements for a validated assay. The assay has proved to be efficient in authentic cases. In addition, this assay is part of a general LC–MS procedure for

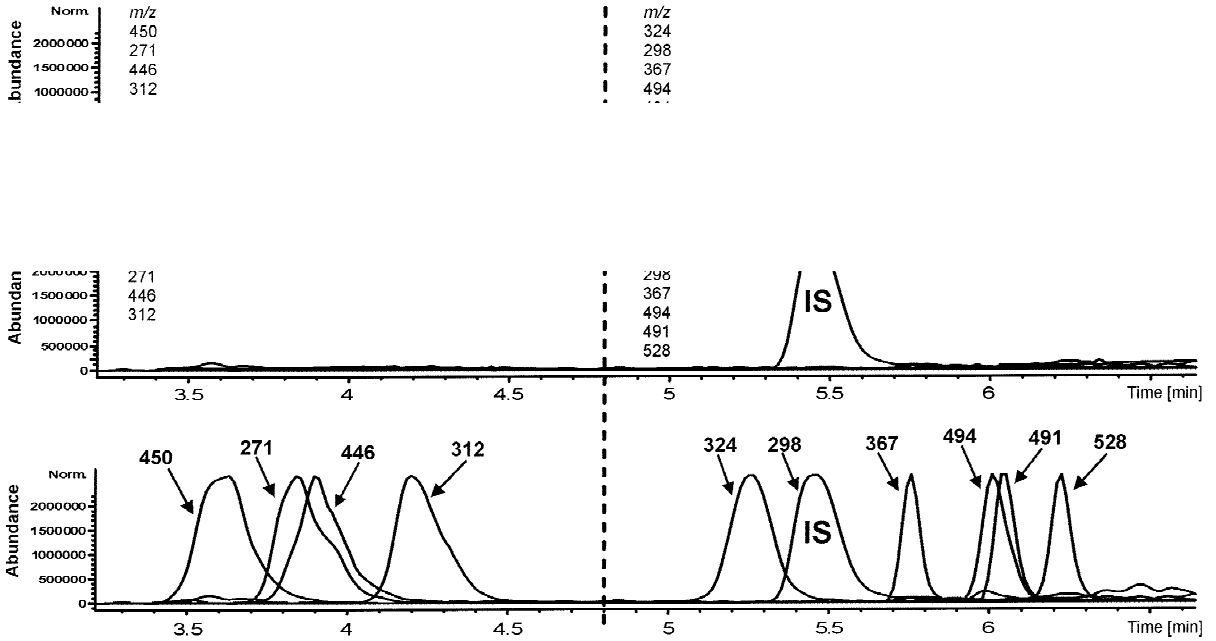

Fig. 4. Smoothed and merged mass fragmentograms normalized in relation to the abundance of the I.S. (SIM mode, 100 V, ions m/z 450, 271, 446, 312, time window 0–4.8 min; 324, 298, 367, 494, 491, 528, time window 4.81–7 min) of a blank plasma sample (top) and of a blank plasma sample spiked with 1.0 mg/l of the I.S. (middle) and of a blank plasma sample spiked with I.S. and OADs (same extract as used in Fig. 2) (bottom).

Table 2
Linearity, LOD and LOQ, apparatus precision and recovery data of the LC–MS assay for OADs

Drug	Linearity concentration range [mg/l]	Coefficient of determination (R^2)	Limits [mg/l]		Apparatus precision		Recovery	
			LOD	LOQ	Concn. [mg/l]	[%]	Concn. [mg/l]	[%]
Glibenclamide	0.01–0.2	0.9941	<0.002	0.01	0.05	4.1	0.05	79.0
					0.1	7.9	0.15	100.1
Glibornuride	0.3–1.1	0.9487	<0.03	0.3	0.5	7.5	0.5	82.4
					0.7	5.0	0.7	68.0
Gliclazide	0.5–6.0	0.9657	<0.05	0.5	2.0	1.8	2.0	69.2
					4.0	7.2	4.0	67.8
Glimepiride	0.1–3.0	0.9871	<0.01	0.1	1.0	9.2	0.5	76.2
					2.0	4.4	2.0	76.4
Glipizide	0.05–2.0	0.9867	<0.005	0.05	0.25	3.3	0.1	74.1
					1.0	3.7	1.5	72.3
Gliquidone	0.3–1.1	0.9862	<0.03	0.3	0.5	6.8	0.5	96.4
					0.7	7.4	0.7	82.6
Glisoxepide	0.05–1.0	0.9911	<0.005	0.05	0.25	3.1	0.1	71.4
					0.5	4.6	1.0	65.9
Tolazamide	30.0–120.0	0.9696	≪3	30.0	60.0	4.2	60.0	76.6
					100.0	3.6	100.0	79.1
Tolbutamide	30.0–120.0	0.9589	≪3	30.0	60.0	6.9	60.0	72.0
					100.0	5.9	100.0	83.4

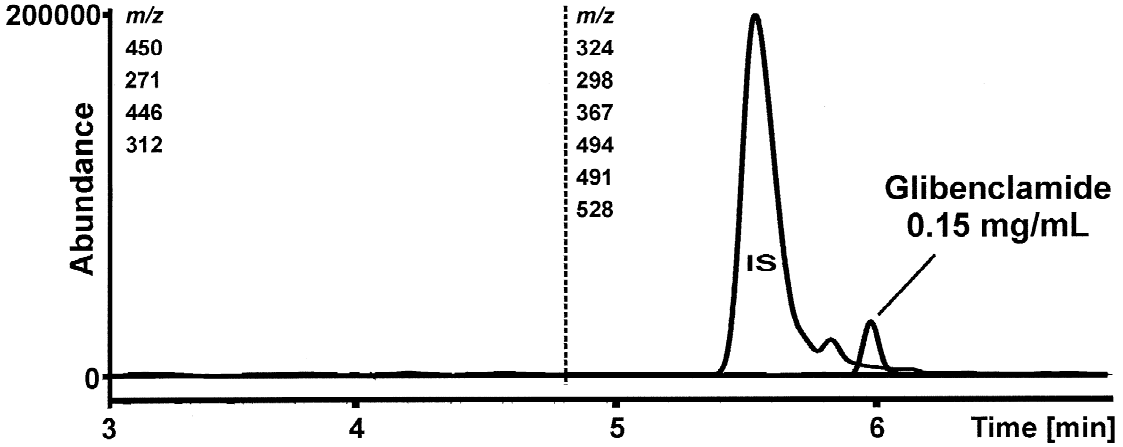

Fig. 5. Smoothed and merged mass fragmentograms (SIM mode, 100 V) with the given ions of an authentic plasma extract (same extract as used in Fig. 3) indicating 0.15 mg/l of glibenclamide.

screening, identification and quantification of different toxicologically relevant compounds in plasma.

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