Toxicological detection of dihydropyridine calcium channel blockers and their metabolites in urine by GC-MS after extractive alkylation

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Abstract

Overdose of dihydropyridine calcium channel blockers ("calcium antagonists") may lead to severe cardiovascular disorders like shock. For diagnosis of an intoxication, a GC-MS procedure was developed for the detection of dihydropyridine calcium channel blockers and their metabolites in urine within a general screening procedure for acidic drugs after extractive alkylation. The acidic metabolites were extracted and methylated in one step and analyzed by GC-MS. Mass chromatography with the ions *m/z* 139, 210, 238, 286, 297, 298, 310, 312, 313 and 318 indicated the possible presence of dihydropyridine calcium channel blockers and/or their metabolites. The identity of positive signals in the reconstructed mass chromatograms was confirmed by comparison of the full mass spectra underlying the peaks with the reference spectra recorded during these studies. We were able to detect amlodipine, felodipine, isradipine, nifedipine, nilvadipine, nimodipine, nisoldipine and nitrendipine in urine of patients treated with therapeutic doses of the drugs. Nicardipine could up to now only be detected in rat urine, since human samples were not yet available.

1. Introduction

Calcium channel blockers of the dihydropyridine type are vasodilators widely used as antihypertensives. In overdose case, they may lead to severe cardiovascular disorders like hypotension and shock. For diagnosis of such an intoxication, a GC-MS screening procedure is necessary for the detection of dihydropyridine calcium channel blockers and their metabolites in urine. Since the parent compounds are excreted only in minor amounts and since the urinary metabolites are acidic, they should be detected within our new screening procedure for acidics [1]. This procedure is a suitable supplement to our well known systematic toxicological analysis (STA) procedure for basic and neutral compounds [2]. Acidic compounds can be extracted and derivatized in one step by extractive alkylation [3-5]. In the following, a screening procedure using

extractive methylation is presented for the detection of dihydropyridine calcium channel blockers in urine.

2. Materials and Methods

2.1 Chemicals

The reference drug samples were kindly supplied by the manufacturers. Diethylether was obtained from Asid Bonz (Böblingen, Germany), methanol, methyl iodide for synthesis, toluene were obtained from Merck (Darmstadt, Germany) and tetrahexylammonium hydrogensulfate from Fluka GmbH (Neu-Ulm, Germany). The columns (Isolute Diol, non end-capped, 500 mg/10 mL) for solid-phase extraction (SPE) were obtained from ICT (Bad Homburg, Germany).

2.2 Phase-transfer reagent

The buffer solution was prepared by mixing 70 mL of 1 mol/L aqueous Na_2HPO_4 with 38 mL of 2 mol/L aqueous NaOH. The solution of 0.02 mol/L tetrahexylammonium (THA) hydrogensulfate was prepared by dissolving 0.9 g THA hydrogensulfate in 100 mL buffer.

2.3 Methyl iodide solution

A 1 mol/L solution of methyl iodide was prepared by dissolving 7 g of methyl iodide in 50 mL of toluene.

2.4 Urine samples

The investigations were performed on urine from in-patients treated with therapeutic dosages of calcium channel blockers. When suitable samples from man were not available, urine from rats (Wistar, Ch. River, Sulzfleck, Germany) was used. 10-20 mg/kg body mass of the corresponding calcium channel blocker were administered to female wistar rats in aqueous suspension by gastric intubation. Urine was collected separated from the faeces.

2.5 Extractive alkylation procedure

The extractive alkylation procedure was performed in modification to the method for the determination of diuretics of Lisi et al. [4]. A 2 mL portion of urine was alkalized with 2 mL of buffer (1 mol/L Na_2HPO_4/2 mol/L NaOH) containing 0.02 mol/L THA hydrogensulfate to obtain a pH of 11.5 - 12.0. After addition of 6 mL 1 mol/L methyl iodide in toluene, the centrifuge tubes were tightly closed and shaked in a water bath at 50°C for 30 min. The two phases were separated by centrifugation at 1500 g for 3 min. SPE columns (diol phase, 10 mL/500 mg) conditioned first with 5 mL of methanol and then 5 mL of toluene were used to

remove the coextracted THA salts from the organic phase. The toluene phase was rinsed through the column under mild vacuum at a flow rate of 3 mL/min. The THA salts were adsorbed on the diol sorbent. The analytes, which were also adsorbed at the column, were selectively eluated with 3 mL of diethylether. The combined eluate was carefully evaporated at 60°C to dryness. (vaccum 300-500 mbar). The residue was dissolved in 50 µL of ethylacetate and a 1 µL aliquot of this extract was injected into the GC-MS.

2.6 Apparatus

The samples were shaken with a multifix shaker S 300 (Schwinherr, Schwäbisch Gmünd, Germany). A Vac-Master V-10 (ICT) was used for solid phase extraction.

The extracts were analysed using a Hewlett Packard (HP, Waldbronn, Germany) Series 5890 gas chromatograph combined with an HP MSD Series 5970 mass spectrometer and an HP Series 59970C DOS workstation. The GC conditions were as follows: splitless injection mode; column, HP-1 capillary (12 m x 0.2 mm I.D., cross-linked methylsilicone, 0.33 µm film thickness); column temperature, programmed from 100°C to 310°C at 30°C/min, initial time 3 min, final time 8 min; injection port temperature, 280°C; carrier gas, helium; flow rate, 1 mL/min. The MS conditions were as follows: full scan mode; ionisation energy, 70 eV; ion source temperature, 220°C; capillary direct interface heated at 260°C.

2.7 GC-MS procedure

During the gas chromatographic separation full EI mass spectra were recorded and stored. Mass chromatography with typical ions was used to screen for the presence of calcium channel blockers and their metabolites. Positive peaks were identified by computerized comparison of mass spectra underlying the peaks with reference spectra recorded during this study.

3. Results and Discussion

3.1 Sample preparation

Urine samples were available from patients treated with therapeutic doses of amlodipine, felodipine, isradipine, nifedipine, nilvadipine, nimodipine, nisoldipine or nitrendipine. Since human urine samples were not yet available for nicardipine urine samples of rats were used. The parent compounds are excreted only in minor amounts. Therefore the screening should focus on the main metabolites which are acidic compounds. As we have seen, cleavage of conjugates was not necessary for toxicological analysis of these drugs. However, derivatization

was indispensable for sensitive detection of them. Extractive alkylation allowed the extraction and derivatization in one step.

Removal of the coextracted THA salts from the organic phase was essential to avoid decrease of the column separation power caused by pyrolysis of the THA in the GC injection port. We have tested several SPE columns with polar sorbents with different eluents for separation of THA. The diol phase yielded the best reproducibility and recovery under the described conditions. It is a further advantage, that such SPE columns can easy be handled and that they can be reused after washing with methanol.

3.2 GC-MS analysis

For detection of the calcium channel blockers and their metabolites, mass chromatography with the diagnostic ions *m/z* 139, 210, 238, 286, 297, 298, 310, 312, 313 and 318 was used. Five typical fragment ions and their relative abundances of the mass spectra and the gas chromatographic retention indices [6] used for the identification are given in table 1. The full mass spectra will be published elsewere [7-9].

Table 1. Typical fragment ions and their relative abundances of the mass spectra and the gas chromatographic retention indices (RI) used for the identification of the calcium channel blockers and their main metabolites after methylation

Drug or methylated Metabolite					M^+	**RI**
Amlodipine	72_{80}	208_{15}	325_{100}	347_{10}	436_{10}	2815
-M (desamino-COOH-desethyl-)	222_{50}	280_{20}	312_{100}	364_{10}	423_{5}	2800
-M (dehydro-desamino-COOH-)	260_{95}	318_{60}	347_{100}	362_{15}	435_{5}	2640
-M (dehydro-desethylamino-desethyl-)-H_2O	139_{10}	250_{10}	267_{10}	282_{100}	317_{5}	2300
-M (dehydro-desethylamino-COOH-)	139_{10}	224_{10}	296_{85}	356_{100}	391_{5}	2430
Felodipine	210_{30}	238_{100}	310_{10}	324_{10}	383_{5}	2670
-M (dehydro-)	173_{45}	286_{30}	318_{90}	346_{100}	381_{0}	2280
-M (dehydro-desethyl-)	173_{10}	286_{10}	300_{15}	332_{100}	367_{0}	2235
-M (dehydro-desethyl-HO-)-H_2O	164_{10}	284_{10}	301_{15}	316_{100}	351_{0}	2505
-M (dehydro-desmethyl-HO-)-H_2O	164_{10}	267_{10}	302_{100}	330_{90}	365_{0}	2560
Isradipine	178_{10}	210_{100}	252_{95}	284_{20}	371_{10}	2630
-M (dehydro-)	221_{40}	251_{45}	295_{100}	327_{95}	369_{30}	2360
-M (despropyl-)	192_{10}	224_{100}	254_{15}	284_{25}	343_{15}	2610
-M (dehydro-despropyl-)	222_{10}	264_{55}	294_{45}	309_{100}	341_{70}	2270
-M (dehydro-desmethyl-OH-)-H_2O	237_{65}	267_{60}	294_{50}	311_{100}	353_{15}	2635
Nicardipine	91_{100}	147_{60}	210_{20}	315_{10}	479_{5}	3770
-M (desamino-COOH-)	165_{10}	282_{100}	315_{10}	373_{10}	404_{5}	2950
-M (dehydro-desamino-COOH-)	139_{10}	281_{20}	312_{100}	371_{15}	402_{5}	2650
-M (dehydro-desbenzylethyl-methyl-amino-) *	139_{15}	297_{90}	313_{75}	327_{100}	344_{30}	2300

Table 1, continued

Drug or methylated Metabolite					M^+	RI
Nicardipine: -M (dehydro-desbenzylethyl-methyl-amino-HO-)-H_2O	139_{10}	281_{45}	297_{20}	311_{100}	328_{20}	2635
Nifedipine	224_{65}	268_{45}	284_{90}	329_{100}	346_{10}	2595
-M (dehydro-) **	252_{10}	267_{10}	298_{100}	313_{15}	344_{5}	2255
-M (dehydro-COOH-)	195_{10}	254_{10}	342_{100}	357_{10}	388_{5}	2695
-M (dehydro-desmethyl-HO-)-H_2O ***	139_{10}	267_{20}	282_{100}	297_{10}	328_{5}	2490
Nilvadipine	145_{10}	221_{100}	263_{45}	342_{20}	385_{10}	2810
-M (dehydro-)	164_{40}	282_{50}	310_{40}	324_{100}	383_{15}	2565
-M (dehydro-despropyl-)	164_{15}	308_{15}	324_{55}	340_{100}	355_{50}	2520
-M (dehydro-despropyl-HO-)	217_{10}	249_{55}	312_{100}	340_{15}	371_{15}	2705
Nimodipine	196_{55}	254_{80}	296_{100}	331_{10}	418_{10}	2890
-M (dehydro-)	281_{60}	298_{100}	342_{80}	357_{45}	416_{10}	2655
-M (dehydro-desmethyl-COOH-)	281_{60}	298_{100}	340_{75}	371_{20}	430_{10}	2740
-M (dehydro-desethylmethyl-ether)	252_{85}	298_{80}	313_{100}	330_{65}	372_{20}	2390
-M (dehydro-desmethyl-despropyl-)	252_{15}	299_{50}	312_{100}	331_{10}	374_{10}	2665
Nisoldipine	210_{80}	266_{50}	270_{75}	371_{100}	388_{10}	2730
-M (dehydro-)	252_{15}	284_{100}	313_{10}	340_{45}	386_{5}	2450
-M (dehydro-HO-)	253_{20}	284_{100}	313_{45}	356_{80}	402_{5}	2615
-M (dehydro-despropyl-) **	252_{10}	267_{10}	298_{100}	313_{15}	344_{5}	2255
-M (dehydro-despropyl-HO-)-H_2O ***	139_{10}	267_{20}	282_{100}	297_{10}	328_{5}	2490
Nitrendipine	178_{15}	210_{50}	238_{100}	287_{10}	360_{10}	2700
-M (dehydro-)	139_{25}	281_{45}	313_{100}	341_{90}	358_{40}	2370
-M (desethyl-)	192_{10}	224_{100}	287_{10}	315_{10}	346_{10}	2690
-M (dehydro-desethyl-) *	139_{15}	297_{90}	313_{75}	327_{100}	344_{30}	2300
-M (dehydro-desmethyl-HO-)-H_2O	139_{10}	266_{90}	297_{100}	325_{90}	342_{30}	2695
common metabolites: *, **, *						

Generation of the mass chromatograms could be started by clicking on the corresponding pull down menu item which executed the user defined macros. The identity of the peaks in the mass chromatograms was confirmed by computerized comparison of the peaks underlying full mass spectra with reference spectra recorded during the presented study [8-9]. In fig. 1 reconstructed mass chromatograms are shown indicating the presence of dihydropyridine calcium channel blockers and their metabolites in a urine sample from a patient treated with therapeutic doses of nitrendipine.

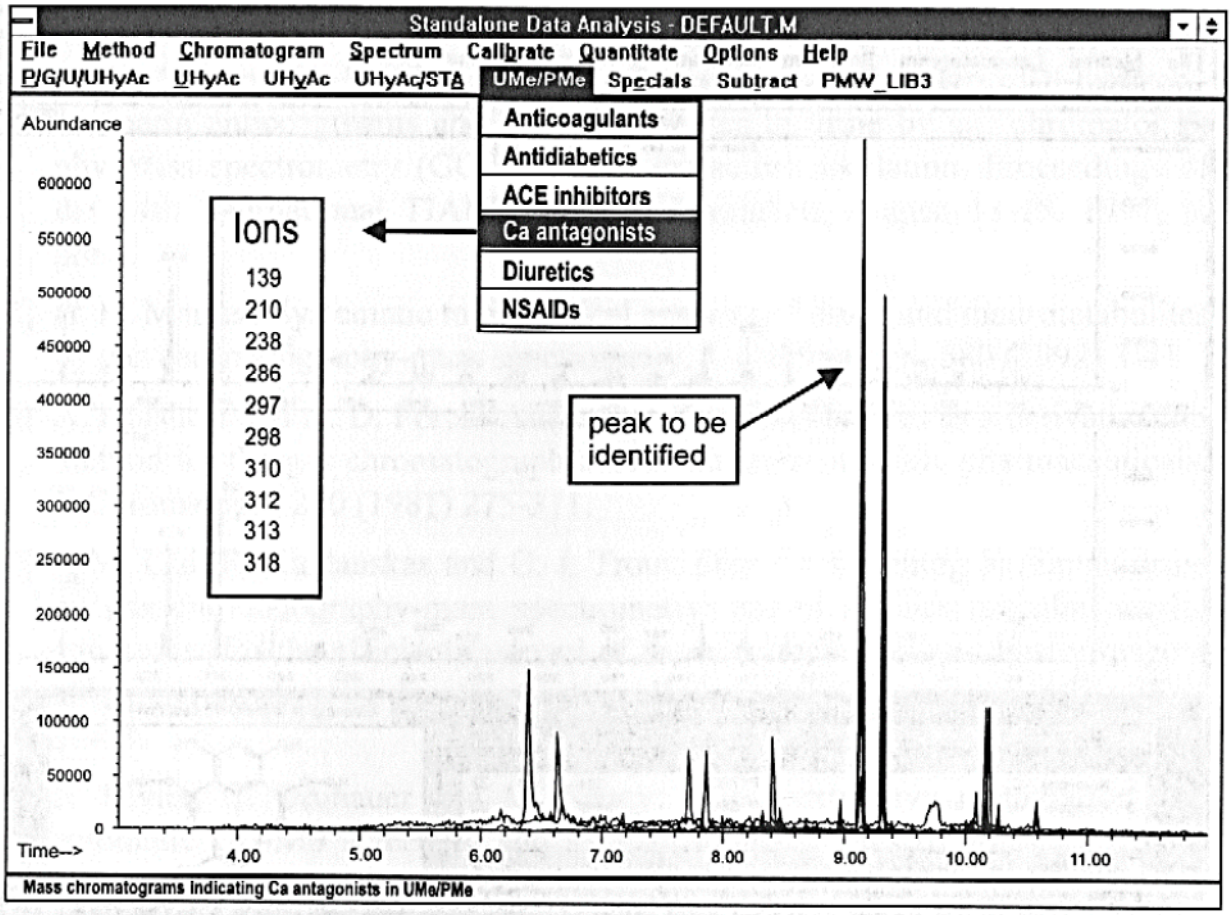

Fig. 1. Mass chromatograms indicating the presence of dihydropyridine calcium channel blockers and their methylated metabolites in urine.

In fig. 2 the mass spectrum underlying the marked peak in fig. 1 and the reference library spectrum of the methylated dehydrodesethyl metabolite of nitrendipine are shown. The drugs and their metabolites were sufficiently separated by GC. Most of the compounds could be differentiated by their mass spectra. However, some of the drugs lead to common metabolites and/or derivatives. Nevertheless, the detection of the parent drugs and/or further unique metabolites or derivatives allowed the differentiation of the drugs. Interferences with other drugs or endogenous biomolecules are improbable since in our experience these compounds have different gas chromatographic and/or mass spectral properties [8-9].

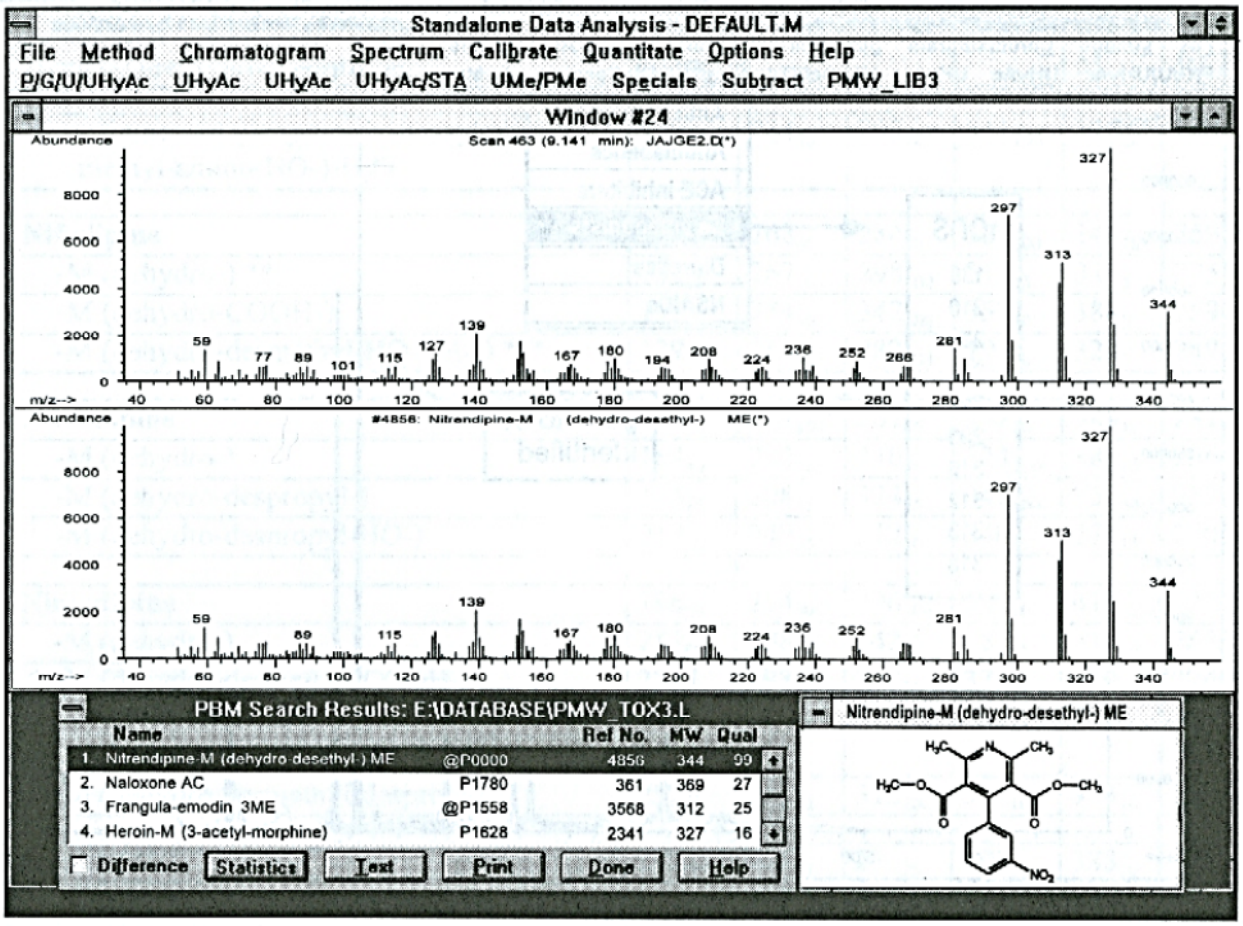

Fig. 2. Mass spectrum underlying the marked peak in fig. 1 (upper part) and the reference library spectrum of methylated dehydrodesethyl nitrendipine (lower part).

The information on the all-over-all recovery (70-90 %) and on the limit of detection (50 ng/mL, *S/N 3*) of the parent compounds was of minor relevance, since the parent compounds were excreted in urine in only minor amounts. Therefore, we studied the detectability of these drugs after therapeutic dosage by determination of their metabolites in urine. Doing so, we were able to prove in humans the intake of amlodipine, felodipine, isradipine, nifedipine, nilvadipine, nimodipine, nisoldipine and nitrendipine. Nicardipine could up to now only be detected in rat urine, since human samples were not yet available.

4. Conclusions

The presented procedure allowed the identification and differentiation of dihydropyridine calcium channel blockers and their metabolites in urine. It is part of a new screening procedure for simultaneous detection of acidic drugs and poisons like coumarin anticoagulants, ACE inhibitors, diuretics, NSAIDs and oral antidiabetics in urine, plasma and gastric contents.

5. References

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