SCREENING FOR DRUGS IN SERUM AND URINE BY LC/ESI/CID-MS AND MS/MS WITH LIBRARY SEARCHING*

Wolfgang WEINMANN[1], Natalie LEHMANN[1], Michaela RENZ[1],
Alexander WIEDEMANN[1], Michal SVOBODA[2]

[1]*Institute of Legal Medicine, University of Freiburg, Freiburg, Germany*

[2]*Perkin-Elmer Biosystems, Langen, Germany*

ABSTRACT: A mass spectra library using in-source collision induced dissociation (ESI/CID) as well as a MS/MS library with product ion spectra of ca. 500 drugs have been developed with a triple-quadrupole ionspray mass spectrometer. For the ESI/CID MS library single quadrupole mode and for the MS/MS library triple-quadrupole mode were used. The libraries have been used for general-unknown screening and for identification of drugs and metabolites in serum and urine.

KEY WORDS: LC/MS; Mass spectra library; MS/MS.

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INTRODUCTION

Recently, in-source collision induced dissociation (ESI/CID) of drugs and chemicals using a Perkin-Elmer/SCIEX ionspray source has been investigated with the aim of achieving high reproducibility for setting-up mass spectra libraries with different degrees of fragmentation [8]. For this purpose reference compounds were found for the tuning of the orifice voltage, which produces CID of molecular ions when being increased. ESI/CID of haloperidol, which was used as a test compound, was reproducible in four different laboratories with ionspray and turboionspray sources.

The development of mass spectra libraries was also reported by other authors, who used ionspray ionisation with two orifice voltages for CID of drugs [5], pesticides and explosives [6]. Furthermore, ESI/CID has been found to be reproducible with different electrospray instruments using tuning compounds [4], whereas Bogusz et al. found significant interlaboratory variances in degrees of fragmentation [1].

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* LC/MS – liquid chromatography/mass spectrometry; ESI – electrospray ionisation; CID – collision induced dissociation; GC/MS – gas chromatography/mass spectrometry; MS/MS – tandem mass spectrometry; HPLC-DAD – high performance liquid chromatography/diode array detector.

In contrast to ESI/CID, where all ions which are produced in the ionsource are fragmented simultaneously, in MS/MS-mode only selected ions are fragmented. This has the advantage, that coeluting compounds with different molecular ions do not interfere and that pure product ion spectra are obtained by MS/MS. However, due to the necessity of precursor ion selection, the precursor ions have to be detected in a preliminary LC/MS analysis using single-quadrupole mode, before they can be identified by an LC/MS/MS experiment.

The aim of this work was to create mass spectra libraries for general-unknown screening of drugs and metabolite-identification in forensic cases using both procedures, ESI/CID and MS/MS. Forensic applications of these libraries to drug and metabolite detection and identification in urine and serum samples are shown.

MATERIAL AND METHODS

Substances, reagents and analytical instruments

The following instrumentation was used: a PE/SCIEX API 365 triple-quadrupole mass spectrometer with turboionspray-source (PE/SCIEX, Perkin Elmer, Langen, Germany), Apple Macintosh G3 Power PC, MassChrom 1.0 and Multiview 1.3 software (PE/SCIEX). The following HPLC system (Shimadzu, Duisburg, Germany) was used: pump LC10AD, low pressure gradient mixer, diode array detector (DAD) SPDM10A, interface module CBM10A and 486/100 DOS PC, column RP-C8-select B, 2 mm i.d. x 125 mm, 5 µm particle size (Merck, Darmstadt/FRG). This HPLC/DAD system was coupled without split to a PE/SCIEX API 365 using a TurboIonSpray™ source. Deionized water (< 0.1 µs from cartridge-deionizer, Memtech, Moorenweis, Germany), gradient grade acetonitrile, 25% aqueous ammonia and formic acid (analytical grade, Merck) were used as HPLC solvents or for dissolving drug standards. For HPLC, the following gradient was used with solvent A (5 mm ammonium formate/0.1% formic acid, pH 3) and solvent B (acetonitrile/0.1% formic acid): 0 min: B 10%; 0 – 6.6 min: B linear from 10% to 30%; 6.6 – 26.6 min: B linear from 30% to 70%, 26.6 – 33,3 min: B linear from 70% to 90%, 4 min at 90%.

D_3-doxepine (internal standard) was obtained from Promochem/Radian (Wesel, Germany). Extraction of urine samples was performed by solid phase extraction (SPE) using mixed mode SPE-cartridges ("chromabond drug", 3 ml/200 mg, Macherey-Magel, Düren, Germany) and an automated SPE-device (RapidTrace™, Zymark Idstein/FRG) [2]. The extraction method was developed in-house and has been described for the extraction of drugs of abuse from serum samples [7]. 1 ml urine was spiked with 100 ng D_3-doxepine and extracted using this method. Enzymatic hydrolysis of serum was performed by adding 70 µl of β-glucuronidase/arylsulfatase (Merck, Darmstadt, Germany) to 1 ml serum and 1 ml phosphate buffer (pH 6) and incubation at 37°C for 5 h. For the extraction of benzodiazepines from serum, a RP-C18 extraction column was used (C18, endcapped,

100 mg, Machery-Nagel, Düren, Germany), rinsed with methanol and water (2 ml each). Serum sample (1 ml serum spiked with 100 ng clobazam, 1 ml 0.1 M Na_2CO_3 added) was applied to a preconditioned (2 ml MeOH, 2 ml water) SPE cartridge, after drying with 0.1 ml MeOH and nitrogen-gas, elution was performed with 0.75 ml MeOH. All evaporated eluates were redissolved in 100 µl HPLC-solvent (A:B, 1:1 (v/v)) and 20 µl were injected into the LC/MS-system in positive ionisation mode (5.2 kV needle voltage). Full scan spectra of reference compounds and sample extracts were aquired in single-quadrupole mode (Q1-scan) with a scan-range of 50–550 amu using a looped experiment with orifice voltage switching (20, 50 and 80 V) between each scan, a dwell-time of 2 ms and a step-size of 0.5 amu. This caused a total scan-time for the looped experiment of 6 s. Product ion spectra for library entries were acquired with MS/MS of the molecular ions of drug standards, using varying collision energies (RO2, 30 to 60 V). For MS/MS of MH^+ 491 in the urine extract RO2-40 V was used.

For the identification of drugs and metabolites by LC-ESI/CID-MS the "Ionspray-CID/MS Library of Drugs 1.0" [5, 8], for the identification of sildenafiloxide using product ion scan (MS/MS), the "Ionspray-MS/MS Library of Drugs 1.0" was used [3].

RESULTS

The diagramms in Figure 1 show the modes of operation used for in-source or ESI/CID (Figure 1a) and for production scanning by MS/MS of molecular ions (Figure 1b). ESI/CID was used for the analysis of a serum extract (Figure 2). Figure 2a shows the LC/ESI-CID/MS analysis of the extract of the native serum, Figure 2b of the serum after treatment with glucuronidase. Using the ESI/CID MS library, signals at t = 13.6 min (in Figure 2a) and t = 16.8 min (Figure 2b) were both identified as lorazepam. Closer look at the 20 V-orifice spectrum (Figure 2a second panel) showed, that it was not lorazepam but its glucuronide (as sodium adduct MH^+ 519; protonated molecular ion MH^+ 497), showing a molecular weight difference of 198 amu compared to lorazepam (MH^+ 321 – see Figure 2b). At 20 V orifice, the glucuronide bond is already partially cleaved by ESI/CID. By enzymatic hydrolysis the glucuronide was hydrolyzed to free lorazepam (see Figure 2b, t = 16.8 min). At that time, there was no library entry present for lorazepam-glucuronide.

In Figure 3a the total ion chromatogram of a urine-extract of a patient, having been prescribed 25 mg sildenafil (Viagra®) is shown. The analysis was run in ESI/CID mode (see Figure 1a). Major ions 491, 449, 461 and 475 were extracted (see Figure 3b). Signal 4 (m/z 475) could be identified as sildenafil, signals 1, 2 and 3 showed very similar UV-spectra (not shown here). MS/MS product ion scan was used for further identification of signal 1 (m/z 491). Due to the loss of 18 amu (491 – > 473), it was supposed to be oxidized in the propyl-sidechain (or alternatively in the ethyloxy-sidechain). Although the library search in the MS/MS library showed pretty good fit results (90%) (Figure 3c),

the reverse fit was much lower (32%) due to less similarity of the unknown than the product ion spectrum of signal 4 (Figure 3d) (fit: 91%; reverse fit: 57%) when compared

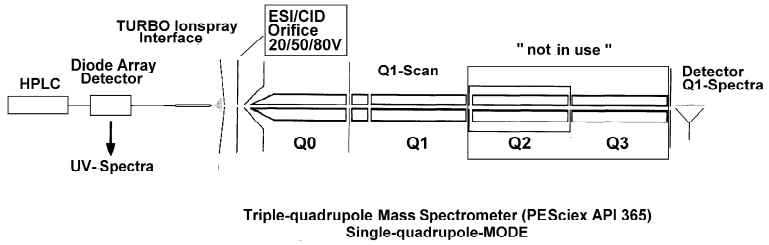

Fig. 1a. Scheme for ESI/CID screening for drugs with Q1-scan (single-quadrupole mode) to obtain molecular ions present at low orifice-voltage (20 V) and to get structural information by CID at higher orifice-voltage (50 or 80V) for identification with the "Ionspray/CID MS Library of Drugs 1.0" [8].

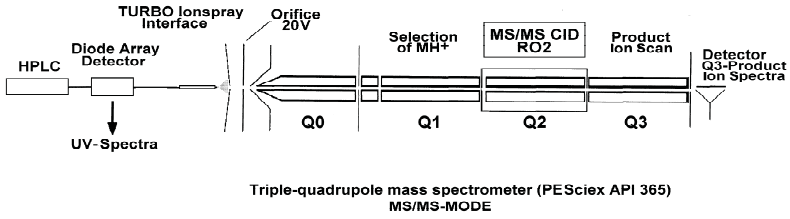

Fig. 1 b. Scheme for the identification of drugs and metabolites by MS/MS-analysis by product ion scanning of MH^+ and subsequent comparison with the "Ionspray/MS/MS Library of Drugs 1.0" [8].

to the MS/MS library entry of sildenafil. The MS/MS library gave a good hint for compound identification, but could not solve the structure of the metabolite in detail.

DISCUSSION

It could be demonstrated that ESI/CID mass spectra libraries can be used for the identification of drugs or metabolites in serum and urine samples by use of low, medium and high orifice-voltage. In adddition the MS/MS-spectra library can be used to get more information about the origin of a molecular ion, even if signals with ESI/CID are overlayed by matrix or other substances. With more knowledge about fragmentation

pathways of small molecules, it will also be possible to identify metabolites with MS/MS and with the help of the MS/MS library.

CONCLUSION

a b

Fig. 2. Total ion chromatogram (TIC) of: a) native serum extract; b) serum extracted after glucuronidase treatment, and ESI/CID mass spectra at three different orifice voltages used for library searching.

ESI/CID and MS/MS spectra libraries are valuable tools for general unknown screening in forensic toxicology with LC/MS or LC/MS/MS with electrospray- or ion-spray-ionisation if CID conditions can be reproduced, e.g. by tuning of CID with reference compounds such as haloperidol [8].

References:

1. Bogusz M. J., Maier R. D., Krüger K. D., Webb K. S., Romeril J., Miller M. L., Poor reproducibility of in-source collisional atmospheric pressure ionization mass spectra of toxicologically relevant drugs, *Journal of Chromatography A* 1999, vol. 844, pp. 409–418.

Fig. 3. TIC of: a) urine extract after consumption of sildenafil using ESI/CID; b) extracted ion chromatograms of major molecular ions; c) comparison of product ion mass spectrum of signal 1 (MH^+ 491) with MS/MS library entry of sildenafil; d) comparison of product ion mass spectrum of signal 4 (MH^+ 475) with MS/MS library entry of sildenafil.

2. Diamond F. X., Vickery W. E., DeKanel J., Extraction of benzoylegonine (cocainemetabolite) and opiates (codeine and morphine) from urine samples using the Zymark RapidTrace™, *Journal of Analytical Toxicology* 1996, vol. 20, pp. 587–591.
3. http:\\www.chemicalsoft.de
4. Keever J., Voyksner R. D., Hough J., Development and evaluation of electrospray CID mass spectra searching for compound identification, Proceedings of the 46th ASMS Conference on Mass Spectrometry and Allied Topics, Orlando, 31 May – 4 June, 1998, p. 86.
5. Marquet P., Vénisse N., Gaulier J. M., Lacassie E., Duchoslav E., Shushan B., Anacleto J., Dupuy J. L., Lachàtre G., "General unknown" screening of xenobiotics in plasma by LC-ES-MS, 37th TIAFT Triennial Meeting, 5–9 September, 1999, Cracow, Poland.
6. Schreiber A., Efer J., Engewald W., Untersuchungen zur Anwendbarkeit von Spektrenbibliotheken für die HPLC/MS, *GIT Spezial Separation* 1999, Bd. 1, S. 10–12.
7. Weinmann W., Svoboda M., Fast screening for drugs of abuse by Solid-Phase-Extraction combinded with Flow-Injection Ionspray – Tandem Mass Spectrometry, *Journal of Analytical Toxicology* 1998, vol. 22, pp. 319–328.
8. Weinmann W., Wiedemann A., Eppinger B., Renz M., Svoboda M., Screening for drugs in serum by Electrospray Ionization/Collision-Induced Dissociation and Library Searching, *Journal of the American Society for Mass Spectrometry* 1999, vol. 10, pp. 1028–1037.