ELSEVIER

Journal of Chromatography A, 926 (2001) 199–209

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Tune compounds for electrospray ionisation/in-source collision-induced dissociation with mass spectral library searching

Wolfgang Weinmann[a,*], Mylène Stoertzel[a], Susanne Vogt[a], Jürgen Wendt[b]

[a]*Institute of Legal Medicine, Klinikum der Albert-Ludwigs-Universität Freiburg, Albertstrasse 9, D-79104 Freiburg, Germany*

[b]*Agilent Technologies, Deutschland GmbH, Chemische Analysentechnik, Hewlett-Packard-Strasse 8, D-76337 Waldbronn, Germany*

Abstract

Haloperidol, paracetamol, metronidazole and metamizole have been tested as tune compounds for electrospray ionisation in-source collision-induced dissociation MS (ESI-CID-MS) with two different mass spectrometers (Sciex API 365 and Agilent 1100 MSD SL). The different electrospray sources of API 365 and MSD 1100 SL consist of an orifice with nitrogen curtain gas and a capillary interface, respectively. In-source CID occurs in both interfaces in front of the skimmers, which separate a region with a vacuum of approximately 300 Pa and the high vacuum ($<10^{-3}$ Pa). Comparison of the breakdown curves of selected tune compounds, depending on collision energy (orifice or fragmentor voltage), showed, that very similar fragmentation can be obtained with both instruments, when adjusting the fragmentor voltage of the MSD 1100 SL to higher values than the orifice voltage of the API 365. For three energy levels – low, medium and high – the corresponding voltages were 20, 50 and 80 V for the API 365 and 110, 190, 230 V for the MSD 1100 SL. These voltages resulted in the most similar spectra for haloperidol and paracetamol with both instruments. The comparison of ESI-CID-MS of all tune compounds at three energy levels showed, that – despite variations in relative ion abundances – all significant ions were present in one of the three CID spectra. Therefore, mass spectral library searching of an ESI-CID-MS library set-up with one of the two instruments should be possible with the other instrument after adjusting the CID energies by means of at least two tune compounds such as haloperidol and paracetamol, metronidazole or metamizole.

Keywords: Libraries, MS; Mass spectrometry; Haloperidol; Paracetamol; Metronidazole; Metamizole; Benzoylecgonine

1. Introduction

Fragment ion formation is obtainable by atmospheric pressure ionisation (API) by in-source collision-induced dissociation (CID) and has been observed in thermospray interfaces [1–3], atmospheric pressure chemical ionisation (APCI) [4], electrospray (ESI) or ionspray ionisation [5]. In-source CID can be induced by increasing skimmer, nozzle, cone, capillary and orifice voltages, depending on the construction of the ion source [6–16]. Mass spectral libraries of drugs, pesticides and explosives have been created by several authors using in-source CID mass spectrometry (ESI-CID-MS). To date, the largest library is the library of Marquet and co-workers with more than 1500 compounds [17,18], followed by our own library (430 compounds) [19,20] and the library of Schreiber et al. [21]. These ESI-CID-MS libraries of drugs, pesticides and explosives have been used for the identification of drugs in serum, urine, hair [17–20] and for the identification

*Corresponding author. Tel.: +49-761-2036-854; fax: +49-761-2036-858.

E-mail address: weinmann@sun11.ukl.uni-freiburg.de (W. Weinmann).

0021-9673/01/$ – see front matter
PII: S0021-9673(01)01066-4

of pesticides and explosives in sewage waters [21]. Smaller libraries have been developed to demonstrate the use of library searching, and for testing the reproducibility of ESI-CID-MS [14,22]. Two different concepts for setting-up ESI-CID-MS libraries for general-unknown screening have been reported: one concept uses composite mass spectra which are obtained by averaging two spectra – one spectrum at low and one at high orifice voltage (the same procedure for positive and negative modes) [17,18]; the other concept uses three single mass spectra with different collison energies [19,21]. Glafenine [17] and haloperidol [19] have been used to check instrument performance and the degree of fragmentation by ESI-CID-MS for general-unknown screening and good reproducibility of ESI-CID has been obtained in different laboratories with different API mass spectrometers (Sciex) and ionspray or turbo-ionspray sources [19]. Good reproducibility of ESI-CID-MS and mass spectral library searching has also been found by use of relative ion abundances derived from a mixture of calibration compounds which are usually used for mass calibration with the MSD 1100 system (Agilent) [22]. In contrast, poor reproducibility of ESI-CID-MS spectra with preset source potentials (fixed values) was found in an intra-laboratory assay, although similar or identical instruments (Finnigan SSQ) were used [23]. However, one reason for the poor reproducibility was, that no tune compounds were used for setting-up source potentials prior to running ESI-CID-MS experiments. For quality control in pharmaceutical analysis, an LC–MS performance test kit consisting of aspartame, cortisone, reserpine and dioctylphthalate has recently been developed [24]. This test mixture has been suggested for checking the LC performance and the performance of the mass spectrometer with regard to sensitivity for protonated molecules in the positive mode and deprotoned molecules in the negative mode. However, a higher degree of in-source CID was not taken into consideration when developing this test mixture.

The aim of our work was to find a way for the comparison of ESI-CID-MS between instruments of two different manufacturers with different interface technologies, i.e., the turbo-ionspray source from Sciex and the electrospray source from Agilent. Tuning compounds were selected from our ESI-CID mass spectral library of drugs [19], which had been set-up with a Sciex mass spectrometer (API 365 in single-quadrupole mode) using three in-source CID energies (20, 50 and 80 V orifice voltages). For every compound, three spectra are available in this library. For setting-up the library, haloperidol had been used for checking the performance of in-source CID. One prerequisite for the selection of the tuning compounds for this work was, that mainly protonated or deprotonated molecules were obtained at low CID energy, and that several fragment ions were obtained at medium and high CID energy. The second prerequisite was the characteristic change of the abundances of molecular ions and fragment ions due to the increase of collision energy – which can be expressed as ‘‘breakdown curves’’. A third prerequisite was, that pure compounds – not complex mixtures with different concentrations of compounds – should be used, preferably those, which are readily available and inexpensive. If similar behaviour was observed for several different compounds on two different instruments, these compounds should then be chosen for the selection of ‘‘corresponding’’ CID energies (i.e., orifice and fragmentor voltages). The ESI-CID-MS library set-up with one instrument should then be tested with spectra acquired with the other instrument.

2. Experimental

Haloperidol, metamizole (dipyrone), 4-acetaminophen (paracetamol) and metronidazole were purchased from Sigma (Deissenhofen, Germany). Deionised water (<0.1 μS from a cartridge deioniser, Memtech, Moorenweis, Germany), gradient-grade acetonitrile and methanol, 25% aqueous ammonia and formic acid (analytical grade, Merck, Darmstadt, Germany) were used for dissolving drug standards. Haloperidol, metronidazole and paracetamol were dissolved (10 μg/ml) in HPLC solvents A–B (1:1, v/v), with A being 1 m*M* ammonium formate–0.1% formic acid (pH 3.1) and B being acetonitrile. These HPLC solvents have recently been used for the acquisition of mass spectra for the ESI-CID-MS library with the API 365 system with gradient-LC conditions using a reversed-phase C_8 column [19]. For negative mode ionisation, metamizole was dissolved in methanol–water (1:1, v/v) at a concentration of 50 μg/ml.

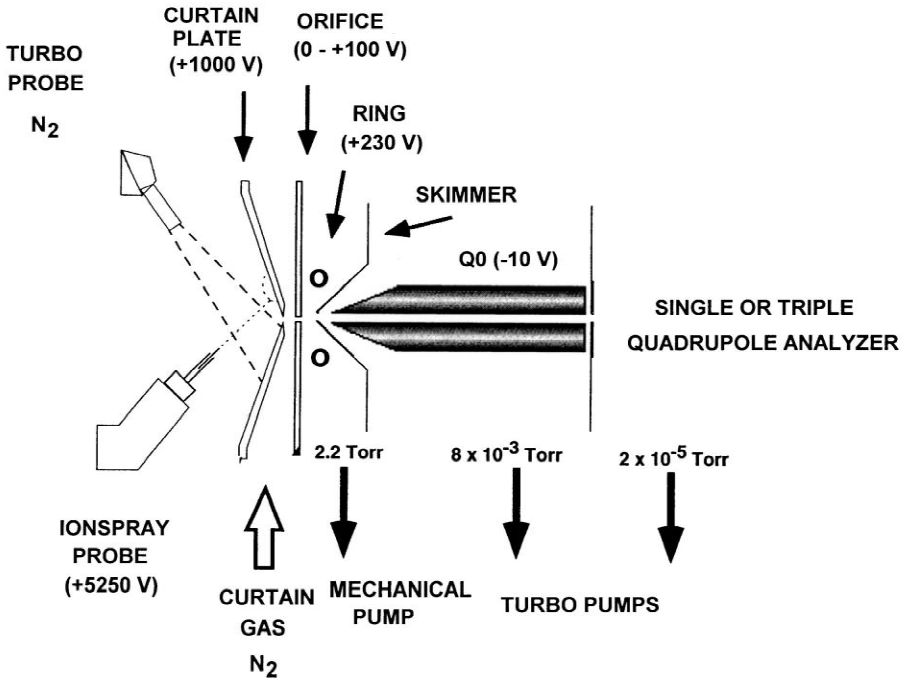

Fig. 1. Turbo-ionspray source of Sciex instruments.

The following instrumentation was used: an API 365 triple-quadrupole mass spectrometer with turbo-ionspray source (Applied Biosystems/Sciex, Langen, Germany), an Apple Macintosh G3 Power computer, MassChrom 1.1.1 and Multiview 1.4 software. For orifice voltage ramping experiments, solutions of tune compounds were continuously infused by means of a syringe pump (Harvard, Quebec, Canada) with a microliter syringe (maximal volume 1 ml, Hamilton, Bonaduz, Switzerland) at a flow-rate of 20 μl/min while ramping the orifice voltage in 5 V steps. Prior to the orifice-ramping experiments, ring voltage (API 365: 230 V) was optimised using the protonated molecule of haloperidol (MH^+, m/z 376). Nebuliser-gas flow-rate was 1 l/min, curtain-gas flow-rate was 2 l/min, spray needle voltages were +5 kV and −4 kV, respectively. With the MSD 1100 SL single-quadrupole mass spectrometer (Agilent, Waldbronn, Germany), flow injections (FIA) of 5 μl of the tune compound solutions were analysed with a flow-rate of 200 μl/min, each at a different fragmentor voltage between 0 and 400 V in 10 V steps. Ramping of the fragmentor voltage during infusion was not possible with the MSD 1100 data acquisition software; therefore, breakdown curves were created using Microsoft Excel by plotting ion abundances against fragmentor voltage. With both instruments, data acquisition of molecular ions and fragment ions

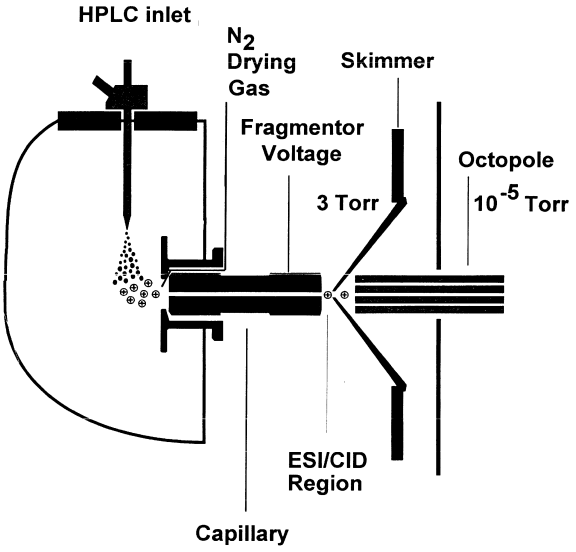

Fig. 2. Turbo-ionspray source of Agilent MSD 1100 SL.

was performed in the selected ion monitoring mode for orifice ramping (dwell-time 200 ms) or by the scan mode with a scan range of 50 to 550 u, dwell time 0.2 ms and step size 0.1 u. Details of spectra acquisition with the MSD 1100 SL were: capillary voltage: 4000 V, drying gas temperature: 350°C, drying gas flow 10 l/min, nebuliser pressure: $3.5 \cdot 10^5$ Pa. For metamizole, the polarity was switched to the negative mode. All other parameters were optimised by the instrument's autotune procedure. Quadrupole resolution was set to obtain a peak width at half height of 0.6 ± 0.1 u in the whole mass range by the manufacturers' calibration procedures.

3. Results and discussion

Ion sources of the API 365 and MSD 1100 SL are shown in Figs. 1 and 2 (1 Torr = 133 Pa). Structures of the tune compounds and ''breakdown curves'', which are obtained by plotting the ion abundances of molecular ions and fragment ions against the increas-

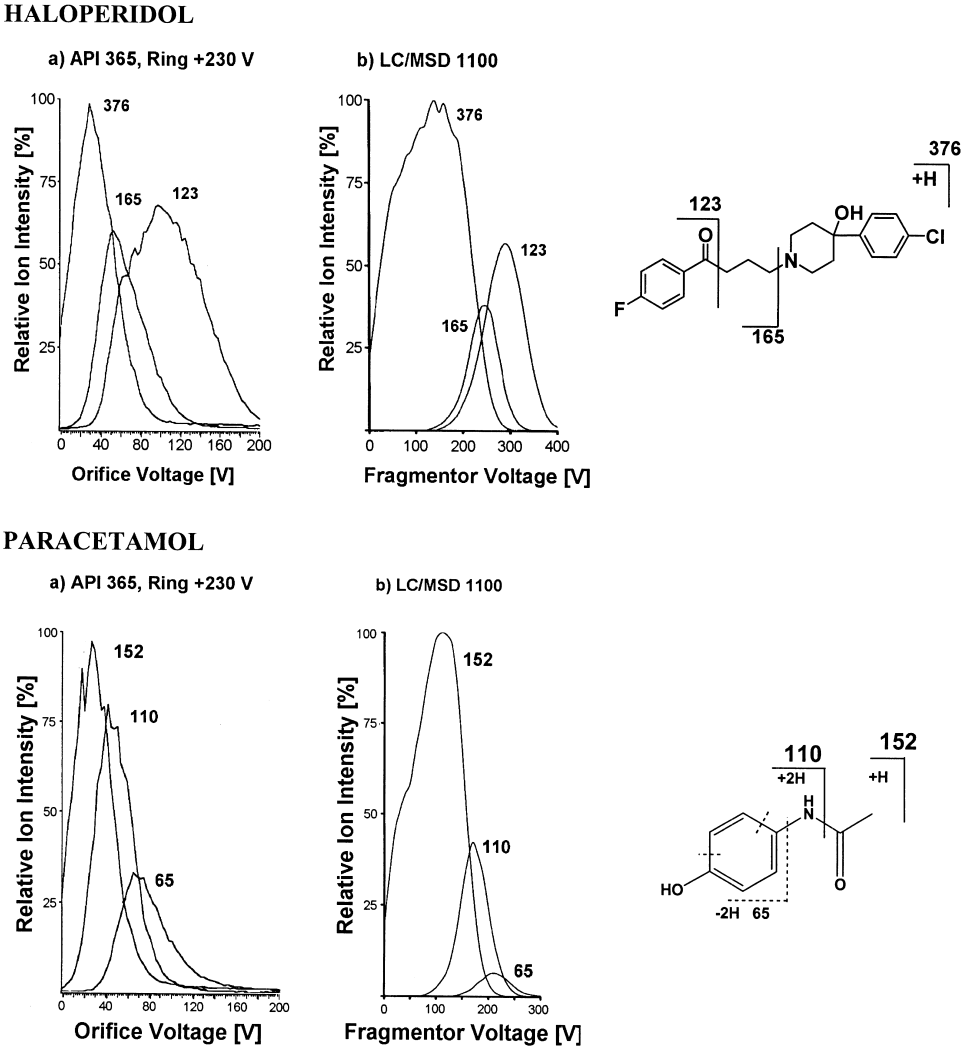

Fig. 3. Comparison of breakdown curves of tune compounds obtained with API 365 and MSD 1100.

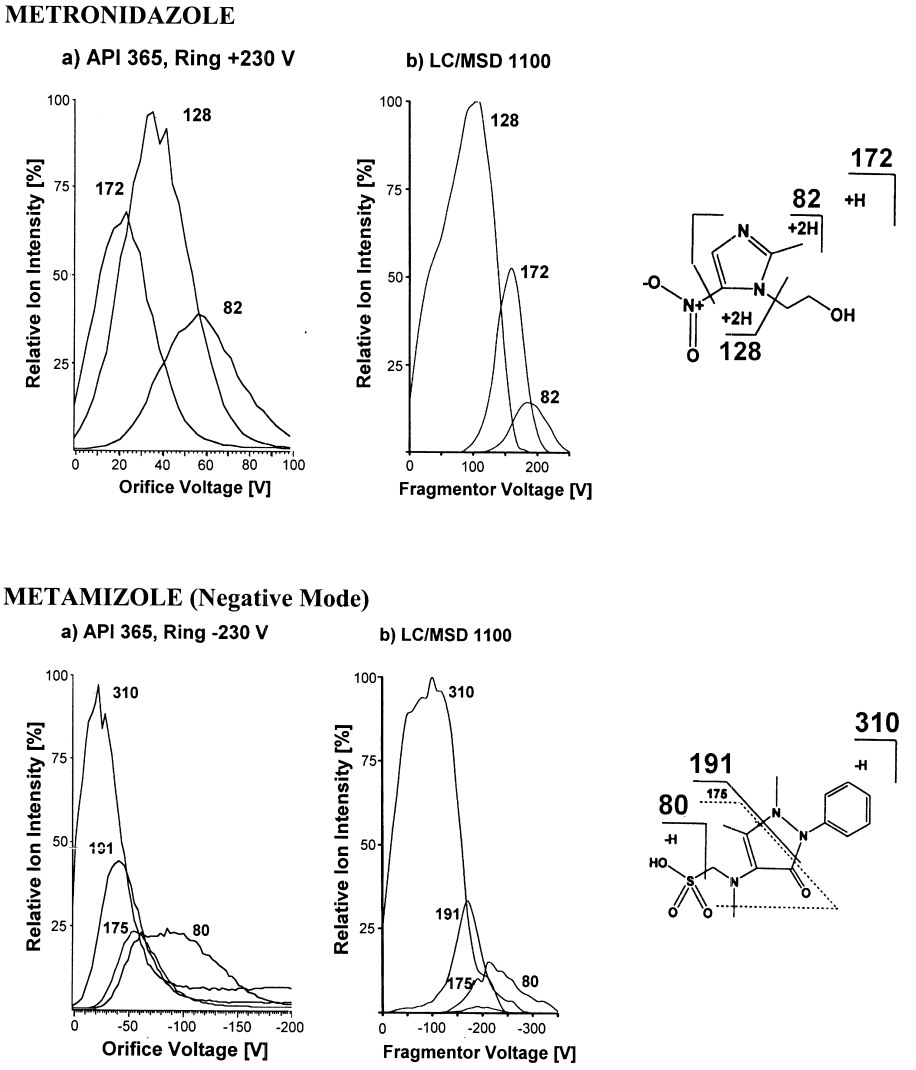

Fig. 3. (*continued*)

ing CID voltage, i.e., orifice or fragmentor voltage, are shown in Fig. 3. In-source CID is influenced by the orifice voltage (API 365, see Fig. 1) and by the fragmentor voltage (MSD 1100) at the exit of the capillary (see Fig. 2). Peak maxima of the breakdown curves were regarded (Fig. 3), and the fragmentor voltage (MSD 1100) was plotted against the orifice voltage of the curves' maxima obtained with the API 365 for all characteristic ions of each compound (Fig. 4). In all cases, the fragmentor voltages in the breakdown curve maxima were higher than the orifice voltages (API 365). While in Fig. 4 the three curves of paracetamol, metronidazole and metamizole almost merged together, for haloperidol relatively high fragmentor voltages were observed at the curves' maxima of the fragment ions (m/z 165 and 123). Therefore, a compromise had to be found for the selection of ''corresponding'' fragmentor voltages – which should result in the most similar CID mass spectra for all compounds. With regard to haloperidol and the other three compounds, the following corresponding fragmentor

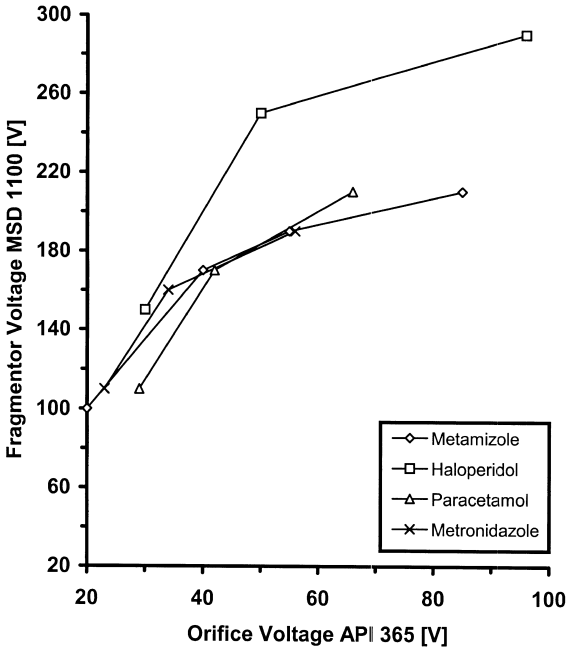

Fig. 4. Differences of fragmentor voltage and orifice voltage in peak maxima of breakdown curves of the tune compounds.

voltages were selected from Fig. 4: 110, 190, 230 V (MSD 1100 SL) corresponding to 20, 50 and 80 V orifice voltages – which recently had been used for setting up the ESI-CID-MS library of drugs with the API 365 [19].

As a result of this selection, the medium and high CID-energy levels (MSD 1100: 190 and 230 V) were not the optimum for haloperidol, because a fragmentor voltage of 230 V (high CID-energy level) yielded spectra similar to the spectra obtained with 50 V orifice at medium CID-energy level (API 365) (see Fig. 5), and a fragmentor voltage of 290 V yielded a spectrum (data not shown) with higher similarity to the 80 V spectrum (API 365).

Relative ion abundances of all substances using these corresponding orifice and fragmentor voltages were calculated and are shown in Tables 1–4. In most cases, the base peak was the same with both instruments at each CID-energy level; the two exceptions were haloperidol at medium and high levels – as was expected from the compromise when selecting fragmentor voltages. However, significant variations of relative ion abundances of the fragment ions were found for all compounds when the two instruments were compared. Maximum differences in relative ion abundances, i.e., the ''worst case'', were 92% for haloperidol at high CID-energy level, 41% for paracetamol at high CID-energy level, 70% for metronidazole at low CID-energy level and 60% for metamizole at medium CID-energy level.

A practical application of the corresponding fragmentor voltages is the library search with spectra obtained with the MSD 1100 and an ESI-CID mass spectral library set-up with the API 365. ESI-CID mass spectra acquired with both instruments using corresponding fragmentor and orifice voltages are shown in Fig. 5 for haloperidol and in Fig. 6 for benzoylecgonine, the major cocaine metabolite. A library search was performed with the API 365 by importing the MSD 1100 data as text files into the Multiview software of the API 365. Fit values ranged from 91 to 54%, when spectra of the same CID-energy levels were regarded. The high CID-energy level spectrum of haloperidol acquired with 230 V fragmentor voltage had higher similarity with the 20 and 50 V (API 365) spectra (fit values: 76 and 95%, respectively) than with the 80 V spectrum (fit

Table 1
Comparison of relative abundances (R.A., % base peak) at three CID-energy levels for haloperidol

		R.A.		
		m/z 376	*m/z* 165	*m/z* 123
Low CID-energy level	20 V API	100	6	1
	110 V MSD	100	0	0
Medium CID-energy level	50 V API	98	100	44
	190 V MSD	100	11	6
High CID-energy level	80 V API	8	59	100
	230 V MSD	100	85	55

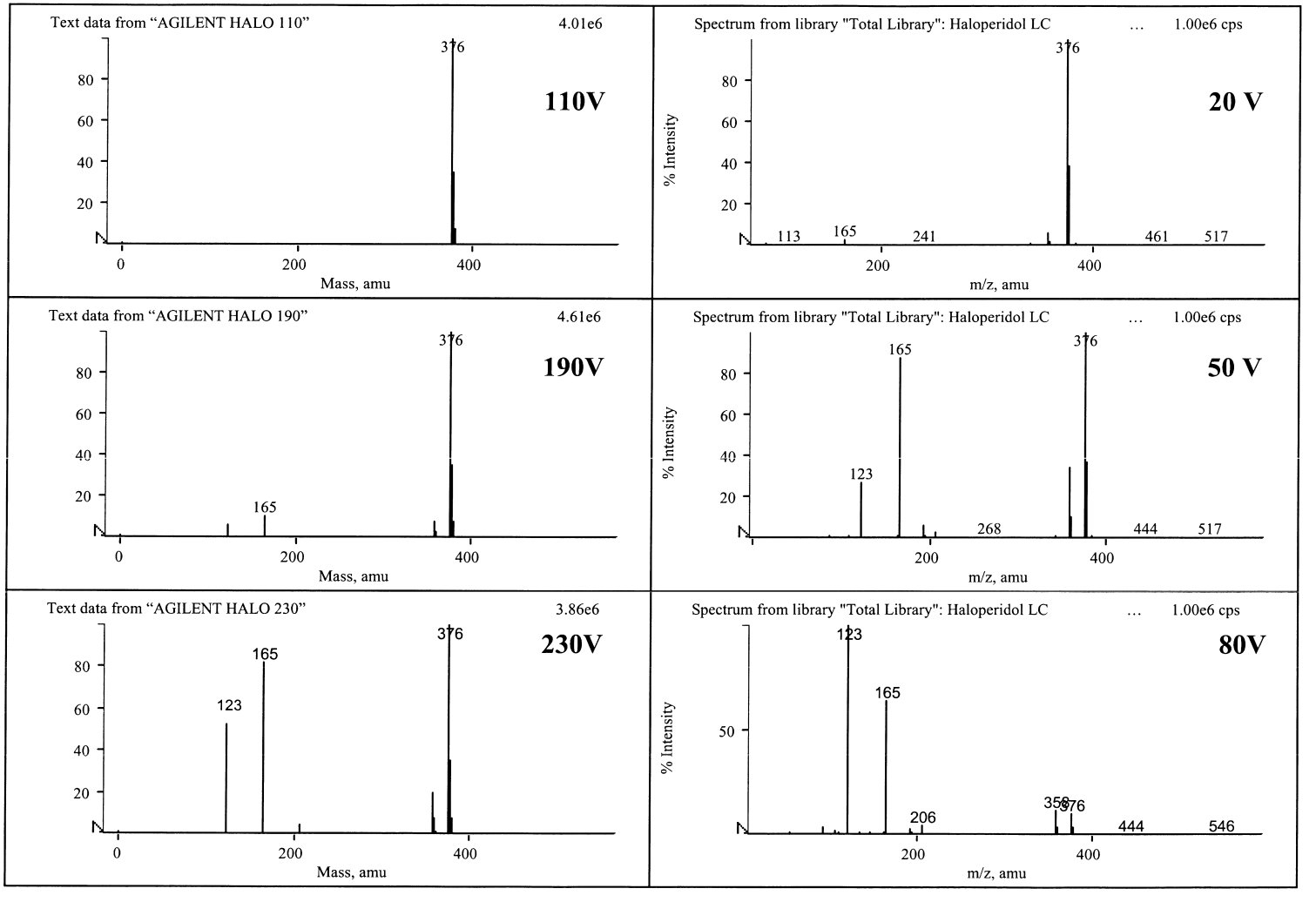

Fig. 5. Haloperidol spectra [API 365 (right) and MSD 1100 (left)]. Fit values of library search were 91% (low CID-energy level), 83% (medium CID-energy level) and 66% (high CID-energy level).

Table 2
Comparison of relative abundances (R.A., % base peak) at three CID-energy levels for paracetamol

		R.A.		
		m/z 152	*m/z* 110	*m/z* 65
Low CID-energy level	20 V API	100	23	0
	110 V MSD	100	4	0
Medium CID-energy level	50 V API	54	100	25
	190 V MSD	24	100	15
High CID-energy level	80 V API	19	57	100
	230 V MSD	0	98	100

Table 3
Comparison of relative abundances (R.A., % base peak) at three CID-energy levels for metronidazole

		R.A.		
		m/z 172	*m/z* 128	*m/z* 82
Low CID-energy level	20 V API	100	76	5
	110 V MSD	100	6	0
Medium CID-energy level	50 V API	14	100	53
	190 V MSD	0	100	99
High CID-energy level	80 V API	1	25	100
	230 V MSD	0	0	100

value: 66%) due to the way the corresponding fragmentor voltages were selected. The lowest fit value for benzoylecgonine (54% at high CID energy) resulted from small fragment ions ($m/z<120$), which were only present in the library entry. In our experience with the API 365, lower fit values are very often obtained in general-unknown screening of serum or urine samples with the high CID-energy level than with the low and medium CID-energy levels due to non-specific background ions generated by LC–MS conditions.

It has to be mentioned, that our preliminary experiments with an MSD 1100 VL – which has a slightly different transport-region geometry com-

Table 4
Comparison of relative abundances (R.A., % base peak) at three CID-energy levels for metamizole

		R.A.			
		m/z 310	*m/z* 191	*m/z* 175	*m/z* 80
Low CID-energy level	20 V API	100	21	1	0
	110 V MSD	100	5	0	0
Medium CID-energy level	50 V API	88	100	68	45
	190 V MSD	54	100	8	41
High CID-energy level	80 V API	48	52	57	100
	230 V MSD	31	46	8	100

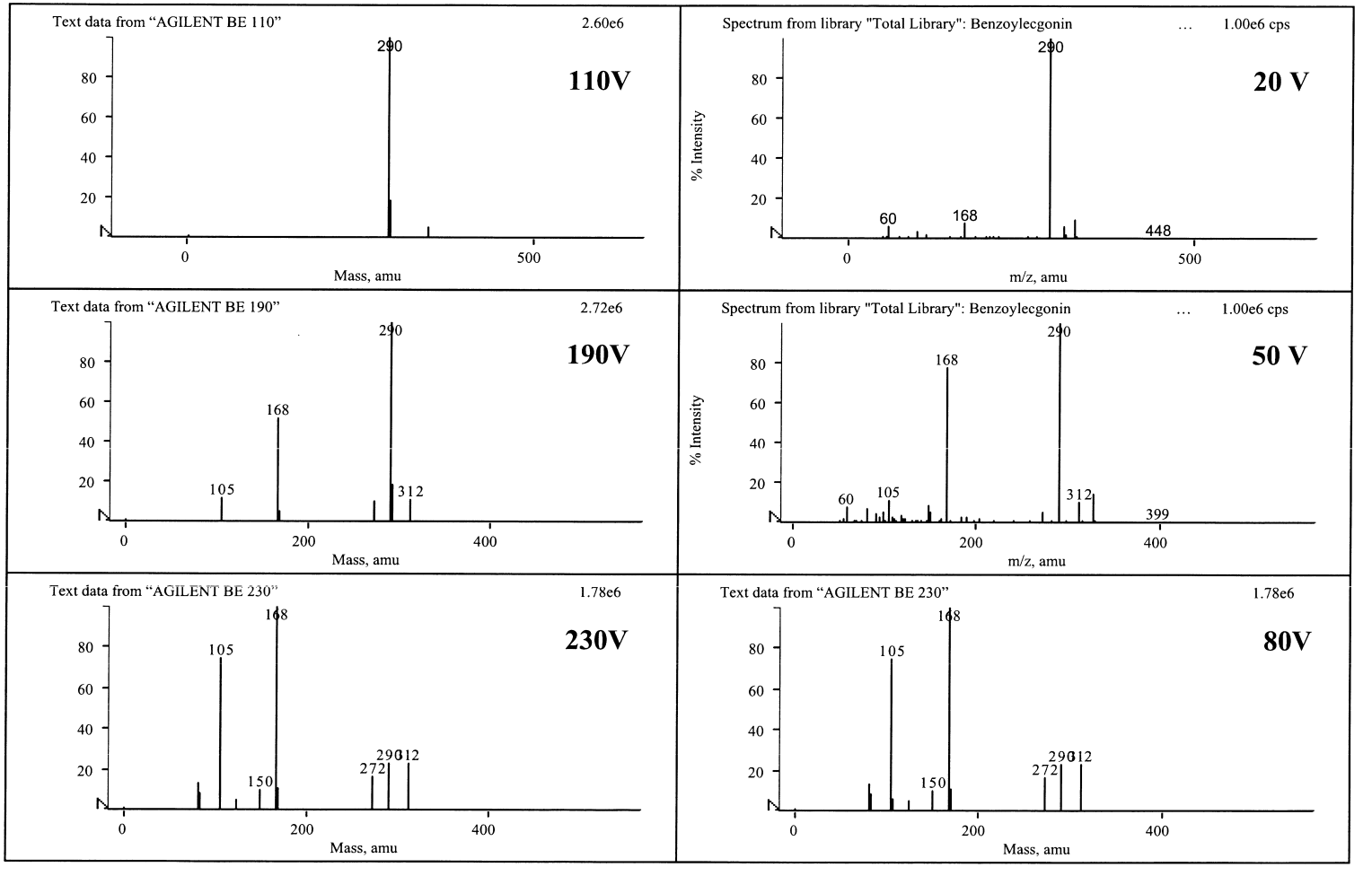

Fig. 6. Benzoylecgonine spectra [API 365 (right) and MSD 1100 (left)]. Fit values of library search were 72% (low CID-energy level), 72% (medium CID-energy level) and 54% (high CID-energy level).

pared to MSD 1100 SL series used in this work – showed similar tendencies for the tune compounds at lower fragmentor voltages; for the MSD 1100 VL, 70, 130 and 170 V, respectively, had been used as corresponding fragmentor voltages [25].

4. Discussion

When comparing two different instruments with the presented tune compounds, differences in the degree of fragmentation were found for haloperidol and the other three compounds (see Fig. 4). This made necessary a compromise for the selection of the corresponding fragmentor voltages, to obtain the most similar fragment ion spectra for many compounds – a prerequisite, which is important for mass spectral library searching in general-unknown screening. The relative ion abundances varied significantly when the two instruments were compared with the corresponding fragmentor and orifice voltages. This leads to the conclusion, that relative ion abundances of one compound are not sufficient for the selection of corresponding fragmentor voltages. However, the example in Fig. 5 showed, that good library search results can be obtained even for haloperidol, for which the fragmentor voltage was not optimal. In our opinion, the selection of corresponding CID energies should be done by at least two tune compounds, e.g., haloperidol and metronidazole or paracetamol, by means of breakdown curves as demonstrated here.

5. Conclusions

These results show, that a library consisting of CID spectra of at least three energy levels – as proposed by Schreiber, Weinmann, and Hough – which have to cover the most important range of CID energies, can be used with different instruments for compound identification. For practical applications, e.g., for drug screening in serum or urine samples, library search parameters should be set in a way, that only minor weight is given to the relative ion abundance to overcome the problem of variations of relative ion abundance; instead, the presence of characteristic ions should be regarded as a criterion for a good library fit value – and last but not least, the instrument operator should be familiar with these problems for the identification of an unknown.

Since the instrument manufacturers Agilent (SL versus VL series) and Sciex (API 365 versus newer instruments API 2000 and API 3000) changed the interface geometry during the development of different instrument series, everybody should acquire breakdown curves of several tune compounds for selecting corresponding CID voltages for subsequent library-searching. In addition, for setting-up ESI-CID-MS libraries, CID conditions should be characterised by breakdown curves of well-defined and readily available tune compounds, too. Thus, the presented tune compounds could become as important for LC–MS with in-source CID as perfluorotributylamine for the tuning of GC–MS instruments.

References

[1] J. Yinon, T.L. Jones, I.D. Betowski, in: 38th ASMS Conference on Mass Spectrometry and Allied Topics, Tucson, AZ, Abstracts, 1990, p. 43.
[2] R.D. Voyksner, T. Pack, T.J. Cornish, in: 38th ASMS Conference on Mass Spectrometry and Allied Topics, Tucson, AZ, Abstracts, 1990, p. 1218.
[3] C.E.F. Heeremans, R.A.M. Van der Hoeven, W.M.A. Niessen, U.R. Tjaden, J. Van der Greef, N.M.M. Nibbering, in: 38th ASMS Conference on Mass Spectrometry and Allied Topics, Tucson, AZ, Abstracts, 1990, p. 1216.
[4] A.P. Bruins, Mass Spectrom. Rev. 10 (1991) 53.
[5] J.A. Loo, H.R. Udseth, R.D. Smith, Rapid Commun. Mass Spectrom. 2 (1988) 207.
[6] V. Katta, S.K. Chowdhury, B.T. Chait, Anal. Chem. 61 (1991) 174.
[7] V. Katta, S.K. Chowdhury, B.T. Chait, in: 38th ASMS Conference on Mass Spectrometry and Allied Topics, Tucson, AZ, Abstracts, 1990, p. 214.
[8] J.D. Henion, J. Conboy, A. Mordehai, G. Hopfgartner, T. Huggins, G. Rule, T. Wachs, in: 40th Conference on Mass Spectrometry and Allied Topics, Washington, DC, Abstracts, 1992, p. 499.
[9] G.J. Van Berkel, S.A. McLuckey, G.L. Glish, Anal. Chem. 63 (1991) 1098.
[10] J.A. Loo, C.G. Edmonds, H.R. Udseth, R.D. Smith, in: 38th ASMS Conference on Mass Spectrometry and Allied Topics, Tucson, AZ, Abstracts, 1990, p. 140.
[11] J.R. Perkins, C.E. Parker, K.B. Tomer, J. Am. Soc. Mass Spectrom. 3 (1992) 139.

[12] D.J. Grindstaff, M.H. Allen, R.D. Voyksner, in: 40th Conference on Mass Spectrometry and Allied Topics, Washington, DC, Abstracts, 1992, p. 37.
[13] T. Wachs, J.D. Henion, in: 40th Conference on Mass Spectrometry and Allied Topics, Washington, DC, Abstracts, 1992, p. 1663.
[14] J. Keever, R.D. Voyksner, J. Hough, in: 46th ASMS Conference on Mass Spectrometry and Allied Topics, Orlando, FL, Abstracts, 1998, p. 86.
[15] R. Straub, R.D. Voyksner, in: 40th Conference on Mass Spectrometry and Allied Topics, Washington, DC, Abstracts, 1992, p. 1879.
[16] J.L. Josephs, Rapid Commun. Mass Spectrom. 9 (1995) 1270.
[17] P. Marquet, N. Venisse, E. Lacassie, G. Lachâtre, Analusis 28 (2001) 925.
[18] P. Marquet, G. Lachâtre, J. Chromatogr. B 733 (1999) 93.
[19] W. Weinmann, A. Wiedemann, B. Eppinger, M. Renz, M. Svoboda, J. Am. Soc. Mass Spectrom. 10 (1999) 1028.
[20] W. Weinmann, N. Lehmann, C. Müller, A. Wiedemann, M. Svoboda, Forensic Sci. Int. 113 (2000) 339.
[21] A. Schreiber, J. Efer, W. Engewald, J. Chromatogr. A 869 (2000) 411.
[22] J.M. Hough, C.A. Haney, R.D. Voyksner, R.D. Bereman, Anal. Chem. 72 (2000) 2265.
[23] M.J. Bogusz, R.-D. Maier, K.D. Krüger, K.S. Webb, J. Romeril, M.L. Miller, J. Chromatogr. A 844 (1999) 409.
[24] L. Tang, W.L. Fitch, M.S. Alexander, J.W. Dolan, Anal. Chem. 72 (2000) 5211.
[25] W. Weinmann, M. Goerner, in: I. Rasanen (Ed.), Proceedings of TIAFT 2000, University of Helsinki, Helsinki, 2000, p. 11.