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Short communication

Poor reproducibility of in-source collisional atmospheric pressure ionization mass spectra of toxicologically relevant drugs

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Abstract

The purpose of the study was to examine the intra- and interlaboratory reproducibility of mass spectra obtained with liquid chromatography–atmospheric pressure ionization mass spectrometry (LC–API-MS) both in electrospray (ESI) and atmospheric pressure chemical ionization (APCI) modes. Toxicologically relevant drugs of different polarity were selected as test substances: morphine-6-glucuronide, 6-monoacetylmorphine, codeine, lysergic acid diethylamide, methylenedioxymethamphetamine. The study was performed in two laboratories using identical instruments and in one using a slightly different instrument. Basic instrument settings and mobile phase were identical in all laboratories. Mass spectra of drugs were taken at four collision energy voltages and using mobile phase of different composition (four concentration levels of acetonitrile and of ammonium formate buffer). The experiments demonstrated that mass spectra of given drugs, obtained in identical conditions with identical instruments, may show very different degrees of fragmentation. Mass spectra obtained with different instruments differed profoundly not only in the degree of fragmentation, but also different fragments and adducts were observed. Short-term intralaboratory reproducibility of mass spectra was satisfactory. On the other hand, the long-term experiments showed different degrees of fragmentation of APCI-generated mass spectra at nominally identical fragmentation energy. The changes in the composition of the mobile phase (concentration of organic modifier or buffer molarity) did not affect the reproducibility of fragmentation to any relevant degree.The study showed that the interlaboratory exchange and use of mass spectrum library, generated by single-quadrupole LC–API-MS instruments, is hardly feasible at the moment, even under very carefully standardized conditions.

Keywords: Inter-laboratory study; Mass spectrometry; Morphine-6-glucuronide; Monoacetylmorphine; Codeine; Lysergic acid diethylamide; Methylenedioxymethamphetamine; Amphetamines

1. Introduction

The coupling of high-performance liquid chromatography with atmospheric pressure ionization mass spectrometry (LC–API-MS), both in electrospray (ESI) and atmospheric pressure chemical ionization (APCI) modes was a breakthrough for its application to biomedical chromatography and toxicology. Several reviews, published in the last few years, documented very rapid development and a growing application spectrum of these techniques for de-

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termination of toxicologically relevant compounds [1–7]. LC–API-MS became a real alternative for GC–MS, particularly for the dedicated assay of polar drugs and their metabolites and large thermo-labile molecules, which are inaccessible for gas chromatography without derivatization, like e.g. morphine glucuronides, LSD or polar cocaine metabolites [8–14]. On the other hand, GC–MS shows one very important advantage: the electron impact ionization (EI)-generated mass spectra are very reproducible and several databases have been developed for identification purposes [15–17]. These databases are generally commercially available together with GC–MS instruments. Inter- and intralaboratory reproducibility of LC–API-MS mass spectra, which is a prerequisite for establishing a spectrum library, is up to now terra ignota and was not systematically checked. From some anecdotal information one may suspect that API–LC-MS generated spectra are laboratory- or instrument-specific. Also, some authors have reported that mass spectra obtained with ESI or APCI may change in time even when the batch of mobile phase of the same composition was changed [11,18].

The purpose of this study was to examine the reproducibility of API-MS mass spectra (both in ESI and APCI modes) of selected drugs of toxicological importance. The study was organized in order to gain information on the following topics:

- interlaboratory reproducibility of mass spectra obtained under identical conditions with either exactly identical or very similar instruments,
- inter- and intralaboratory reproducibility of molecular fragmentation and its variation by increasing fragmentation energy,
- influence of the controlled variation of the composition of the mobile phase on the reproducibility of mass spectra.

2. Experimental

2.1. Reagents and materials

The solutions of the following drugs (10 ng/μl in acetonitrile (ACN) – 50 m*M* ammonium formate (AMF) buffer, pH 3.0, 25:75) were prepared: morphine-6-glucuronide (M6G), 6-monoacetylmorphine (6-MAM), codeine, lysergic acid diethylamide (LSD) and methylenedioxymethamphetamine (MDMA). These drugs were selected taking into consideration their relevance in toxicology and different polarities.

For loop-injection experiments the mobile phase consisted of acetonitrile and AMF buffer. The composition of the mobile phase varied according to experiments described below. The mobile phase for column experiments consisted of ACN and 50 m*M* AMF buffer in the following proportions: 5:95 for M6G, 10:90 for codeine and LSD. Column experiments were performed in two laboratories: in Aachen, using a Superspher 4 μm RP-18 column, 125×3 mm, (E. Merck) and in Teddington on LiChrospher 5 μm RP-18 column, 125×4 mm, (E. Merck). HPLC-grade acetonitrile of the same batch was used as a mobile phase constituent in each laboratory.

2.2. Atmospheric pressure ionization mass spectrometry

In the laboratories of the Institute of Forensic Medicine in Aachen, Germany (IFM) and in the Laboratory of Government Chemist in Teddington, UK (LGC) identical SSQ 7000 instruments from Finnigan MAT (San Jose, CA, USA) were used. In the laboratory in the FBI Academy in Quantico, USA, (FBI) a TSQ 700 instrument, also from Finnigan MAT, was used. The latter instrument was used in single quadrupole mode on the first quadrupole. The following common instrument settings were applied in all laboratories: for APCI, heated vaporizer temperature 450°C, heated capillary temperature 190°C, corona current 5 μA; for ESI, spray voltage 5 kV, heated capillary 250°C. Sheath gas pressure ranged from 45 to 70 p.s.i., the auxiliary gas from 20 to 45 ml/min and the multiplier voltage from 1350 to 1500 V (11 p.s.i.=6894.76 Pa). Mass spectra were taken in full scan mode from m/z 50 to m/z 500, the scan time was 0.5 s.

2.3. Influence of octapole offset voltage on mass spectra

In these experiments, 50 ng/5 μl aliquots of drugs were individually injected into the LC–MS, equipped with APCI or ESI sources. Loop injections

were applied, the mobile phase consisted of ACN–AMF buffer, pH 3.0 (5:95). The buffer concentration was 50 m*M* for APCI and 10 m*M* for ESI. The octapole offset voltage (OOV) was set at 10, 20, 30 and 40 V and the mass spectra were taken in the range from m/z 50 to 500. In these series of experiments 120 mass spectra were obtained. The experiments were repeated after 8 months in one laboratory (IFM) in order to check the long-term reproducibility of fragmentation.

2.4. Influence of organic modifier concentration on mass spectra

The influence of ACN concentration of the mobile phase on the mass spectra of examined drugs was checked using four mobile phases, consisting of a mixture of ACN and AMF buffer, pH 3.0. The buffer concentration was 50 m*M* for APCI and 10 m*M* for ESI. ACN concentrations in the mobile phase were: 1%, 5%, 10% and 50%. In these series of experiments loop injections of drugs (50 ng each) and column injections were used. Both APCI and ESI sources were used. The OOV was set at 10 V and 40 V for each drug. In the IFM laboratory column injections of LSD, 6-MAM and codeine were also carried out. In these experiments 240 mass spectra from loop injections and 48 spectra from column injections were generated.

2.5. Influence of buffer molarity on mass spectra

These experiments were performed using four mobile phases, consisting of ACN and AMF buffer, pH 3.0. The concentration of ACN was always 5%. The buffer molarities were: 5, 10, 25, 50 m*M*. Mass spectra of drugs were taken with the APCI and ESI sources, using an octapole offset voltage of 10 and 40 V. Loop injections of drugs (50 ng each) were carried out. In the LGC laboratory also column injections of drugs at 10 m*M* of AFM buffer were performed. Two hundred and forty mass spectra from loop injections and 5 mass spectra from column injections were obtained. The mobile phases in all experiments were prepared using the same protocol in order to eliminate artifactual differences in the composition.

3. Results and discussion

The large amounts of experimental data gathered in the study cannot be presented as a whole in the paper. Nevertheless, all data concerning fragmentation (15 tables) as well as detailed fragmentation proposals for each drug involved are available from the authors on request.

3.1. Influence of octapole offset voltage on mass spectra

3.1.1. Interlaboratory reproducibility of APCI and ESI mass spectra

Tables 1 and 2 show the fragmentation observed in the three participating laboratories for LSD and M6G, respectively. It may be generally stated that very different fragmentation intensities were observed for the same drugs, examined under the same conditions on identical instruments in different laboratories. E.g., for codeine and 6-MAM, no fragmentation was observed in LGC at any voltage in APCI mode, whereas in both other laboratories gradually increasing fragmentation of these drugs began at 20 V. For M6G, no fragmentation was observed in the LGC and in the IFM laboratories in the ESI mode up to 30 V OOV, whereas in the FBI laboratory very profound fragmentation and adduct formation appeared even at 10 V OOV. Figs. 1 and 2 show the differences in the fragmentation intensity of MDMA observed in each laboratory in APCI and ESI. When different instruments were used, not only quantitative but also qualitative differences in mass spectra were observed. However, the adjustment of the OOV may allow more similar spectra to be obtained among the laboratories at different voltage settings. It was also notable in comparing spectra from different laboratories that the intensities and reconstructed ion currents changed more sharply for the FBI instrument than the others. These values and thus spectra changed gradually for the IFM instrument and especially for the LGC instrument.

3.1.2. Short-term and long-term intralaboratory reproducibility of APCI and ESI mass spectra

In all three groups of experiments (influence of octapole voltage, ACN concentration and buffer

Table 1
Interlaboratory and long-term intralaboratory reproducibility of LSD fragmentation. Observed peaks and intensities at indicated values of offset voltages[a]

Laboratory	OOV[b] 10			OOV 20			OOV 30			OOV 40		
	S	M	W	S	M	W	S	M	W	S	M	W
IFM1[c]-APCI	324	304		324	223, 304 281	208	223	208, 304, 324 180, 281		223, 208	180	
IFM2[d]-APCI	324		304	324		281, 304 223	324, 223	281, 304 208, 197	180	223, 208	180, 281	197, 304 324, 209
IFM1-ESI	324	242		324	242		324, 223	242, 281 142		223	208, 142, 324	197, 281 242
IFM2-ESI	324	242		324	242		324	242, 223	281	223	324, 142, 208 224, 242, 208	297
LGC[e]-APCI	324		293, 281	324			324	223		223, 324	181, 208 197, 180	192
LGC-ESI	324		***387, 475***[g]	324			324, 223		281, 208 197	223	208, 324 197, 281	128
FBI[f]-APCI	324		281, 279	324	281, 223	221, 283	223	208, 281, 197	324, 180	223, 208	281, 192, 209	167
FBI-ESI	324		281, 223	324, 223	281	251, 208 197	223, 208	197	281, 180	208, 223 207	180, 192 194, 197	

[a] The ions were listed by decreasing intensity: S = strong intensity (40–100%), M = medium (10–40%), W = weak (4–10%).
[b] OOV = octapole offset voltage.
[c] IFM1 = IFM laboratory, first examination.
[d] IFM2 = IFM laboratory, second examination.
[e] LGC = LGC laboratory.
[f] FBI = FBI laboratory.
[g] ***Bold italics***: adducts.

molarity) one particular set of conditions was applied for each drug, i.e. 5% ACN, 50 m*M* (APCI) or 10 m*M* (ESI) AMF and OOV 10 V or 40 V. Since the experiments were completed in a few days, this made it possible to determine the short-term intralaboratory reproducibility of mass spectra. Generally speaking, the short-term intralaboratory reproducibility of mass spectra was satisfactory in all laboratories. Small differences in ion abundances occurred sporadically and involved smaller fragments.

The long-term reproducibility was tested in one laboratory (IFM), where the series of experiments on the influence of octapole voltage on fragmentation was repeated after 8 months. For all drugs distinct differences were observed between two experiments in APCI mode in the extent of fragmentation. In the first experiment, the fragmentation was obviously more profound at the same OOV values. Moreover, the tendency for adduct formation was observed for 6-MAM (M + 28, M + H + 41, M + 56, M + H + 101) only in the first series of experiments in APCI mode and indicates that spectra are dependent on the system history. Fig. 3 illustrates the differences in mass spectra observed for 6-MAM in two series of the experiments. The comparison of mass spectra obtained in ESI mode showed generally good long-term reproducibility.

The experiments demonstrated that the long-term reproducibility of APCI-generated mass spectra, obtained with the same instrument in nominally identical conditions, was not satisfactory. Since the ESI-generated spectra were not affected, the possible common causes, like a slightly changed geometry of the ion optics, a different condition of the heated capillary or a different vacuum in skimmer area, are

Table 2
Interlaboratory and long-term intralaboratory reproducibility of M6G fragmentation. Observed peaks and intensities at indicated values of offset voltages[a]

Laboratory	OOV[b] 10			OOV 20			OOV 30			OOV 40		
	S	M	W	S	M	W	S	M	W	S	M	W
IFM1[c]-APCI	286	268		286	227, 177, 270		286			286		
IFM2[d]-APCI	286, 462	248, 284	331, 444	286	270, 462, 248	295, 444	286	462, 248	284, 105	286, 462	105, 270, 209	444
	270, 268	287			268, 284, 287			287, 268	237		219, 191, 193	
IFM1-ESI	462			462			462			462		286
IFM2-ESI	462			462			462			462	324, 142, 208	297
											224, 242, 208	
LGC[e]-APCI	286	268, 150, 115, 173		286			286			462	286	
											197, 180	
LGC-ESI	462			462			462			462	286	
FBI[f]-APCI	355, 429	281, 149, 286	***489***[g]	355, 429	415, 221	299, 341	355, 429	341, 221	149, 295	355, 281, 429	415, 149, 401	341, 221, 239
	503	299, 415, 267		281	149		281	415				
		371, 177									267, ***503***	
FBI-ESI	462, 355	267, 281	299	355, 281	341, 221		355, 281	415, 341, 401		281, 341, 221	239, 401, 181	
	371, 429	211		429, 462	415, 299		221, 429	327, 239		327, 114, 267		

[a] The ions were listed by decreasing intensity: S = strong intensity (40–100%), M = medium (10–40%), W = weak (4–10%).
[b] OOV = octapole offset voltage.
[c] IFM1 = IFM laboratory, first examination.
[d] IFM2 = IFM laboratory, second examination.
[e] LGC = LGC laboratory.
[f] FBI = FBI laboratory.
[g] ***Bold italics***: adducts.

not probable. This leaves the heated vaporizer itself as a possible source of variability in fragmentation and adduct formation.

3.2. Influence of mobile phase composition on mass spectra

Controlled changes in ACN concentration in the range from 1% to 50% did not cause any dramatic variation in mass spectra within each laboratory. At higher concentrations the ACN adduct was more often visible. Also, the changes in the concentration of buffer did not play an important role as a factor in altering the intralaboratory reproducibility of mass spectra. Only in the case of extremely different buffer molarities were some changes in abundances observed. It seems that small changes in the concentration of the organic modifier or in the buffer molarity of the mobile phase (e.g. during preparation of a new batch of mobile phase) cannot alter the reproducibility of mass spectra. Mass spectra obtained from column runs were virtually identical with those recorded during loop injections.

4. Conclusions

It is never simple to present a study showing some drawbacks or disadvantages of a given methodology. The opposite is much more attractive. Nevertheless, the precise recognition of pitfalls of some new technique is a first step of its improvement. Therefore, the entire material gathered in this study is not a mere collection of irreproducible results; it may be regarded as a reference data for further experiments on interlaboratory comparability of API mass spectra.

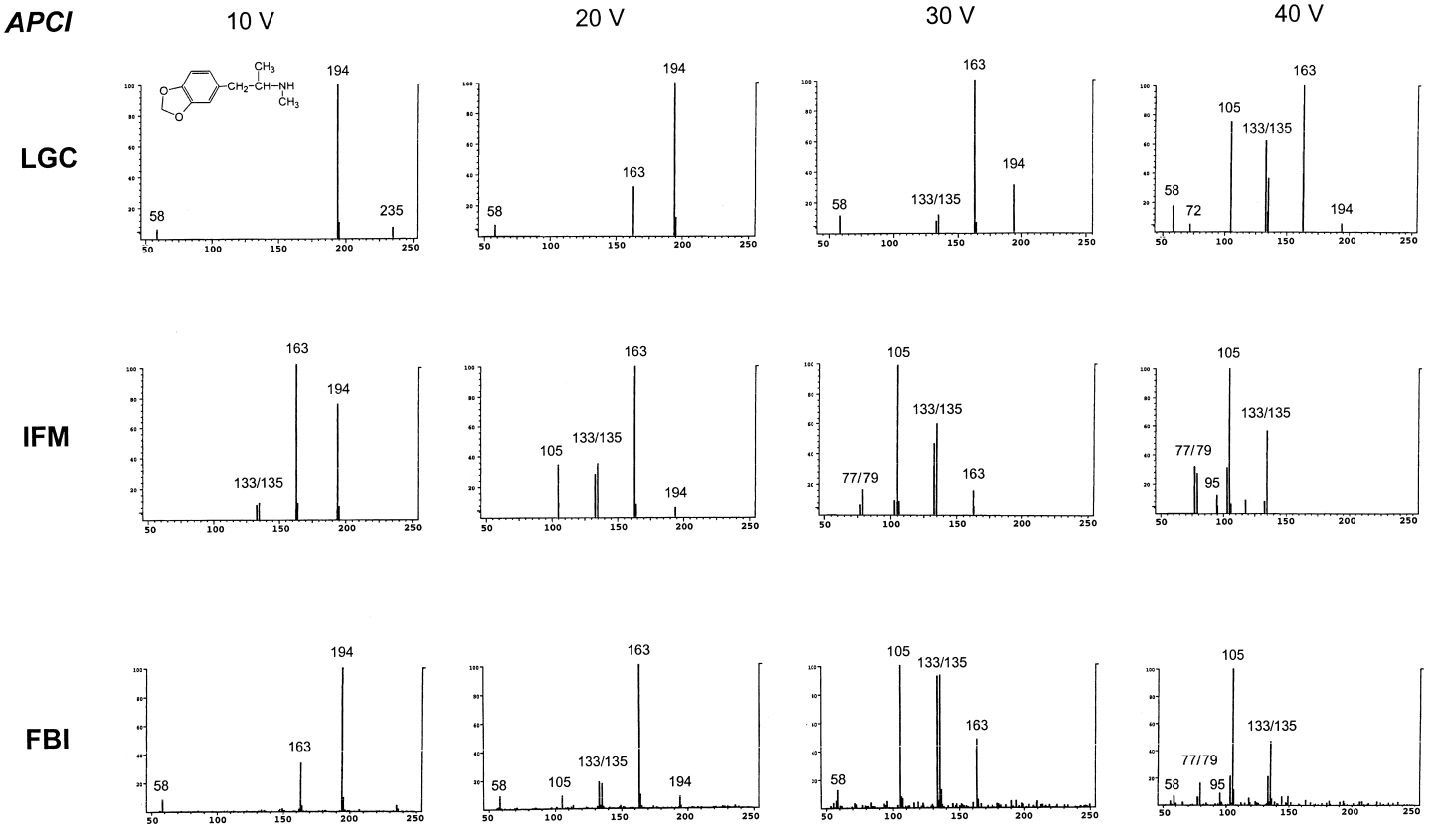

Fig. 1. Mass spectra of MDMA, taken in three participating laboratories in APCI mode at increasing octapole offset voltages (from 10 V to 40 V).

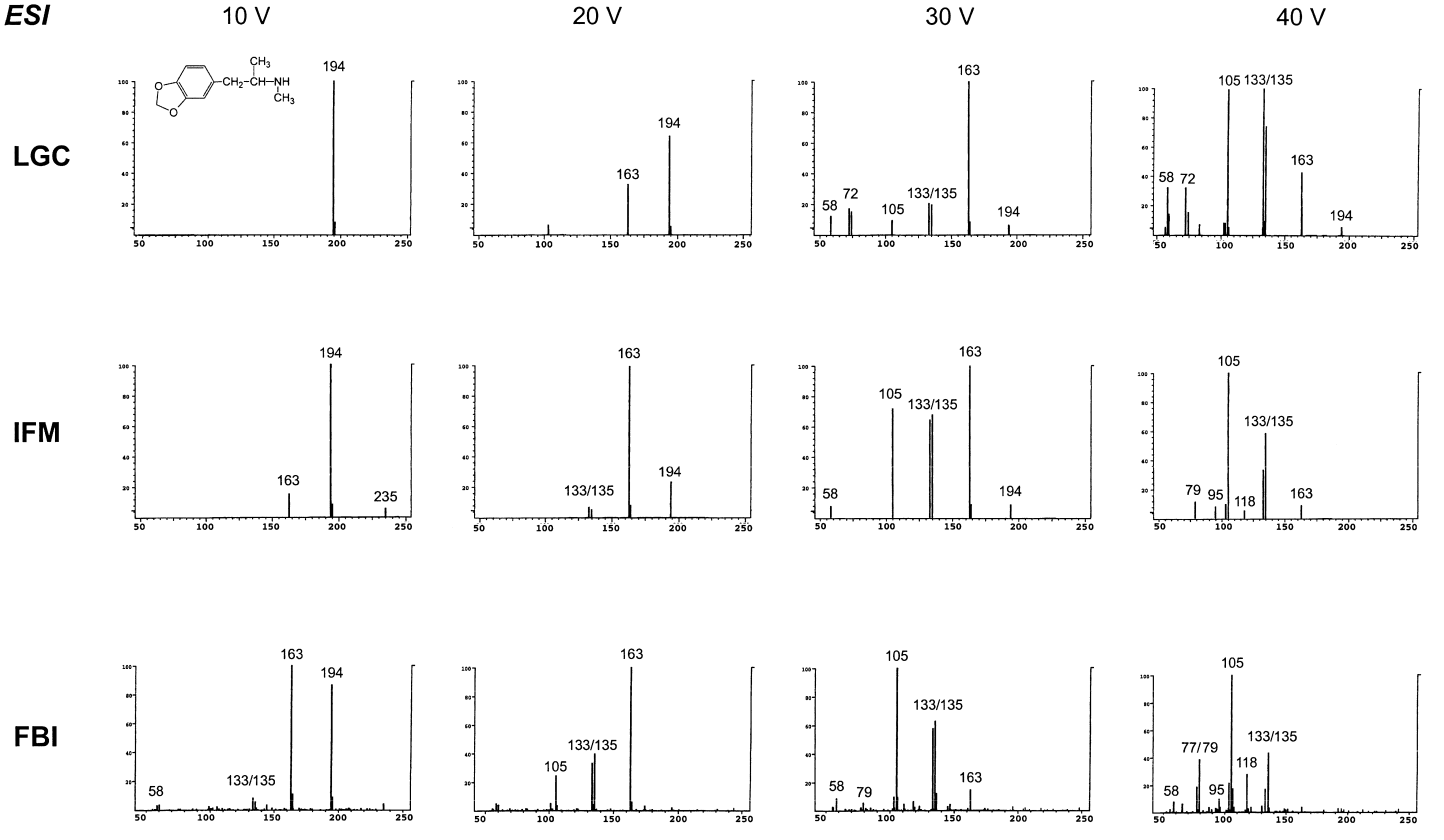

Fig. 2. Mass spectra of MDMA, taken in three participating laboratories in ESI mode at increasing octapole offset voltages (from 10 V to 40 V).

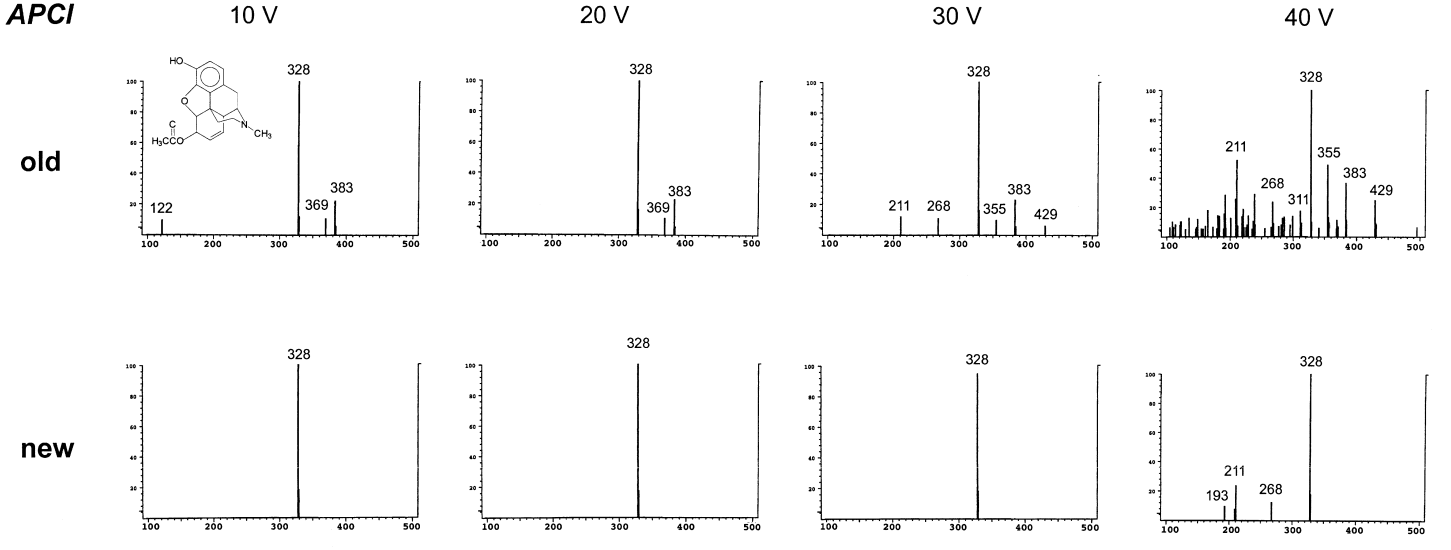

Fig. 3. Comparison of mass spectra of 6-MAM, taken twice in APCI mode in the same laboratory on the same instrument under identical conditions. The time interval between the two experiments (‘old’ and ‘new’) was 8 months.

4.1. Interlaboratory reproducibility

The experiments demonstrated that the mass spectra of given drugs, obtained under identical conditions with identical instruments may show very different degrees of fragmentation, both in ESI and APCI mode. It seems that apparent fragmentation energy, despite nominally identical conditions, is quite different. The comparison of mass spectra obtained with not identical, but similarly constructed instruments (SSQ 7000 and TSQ 700) revealed profound differences not only in the degree of fragmentation, but also in the nature of fragments and adducts which were observed.

In a review of collisional activation by McLuckey, he stated 'it is often difficult to either correct or fully account for instrumental effects when comparing MS/MS spectra obtained under different collisional activation conditions or from different instruments [19].' The same principles apply for collisional activation in OOV fragmentation as MS–MS fragmentation. Molecular ions can undergo collisional activation in two regions on the Finnigan API interface. The first area is in the capillary/skimmer region and ion energy is dependent here on the capillary and tube lens voltages. Variations between the three laboratories for these voltages are tune dependent. The values tuned on the instruments are in turn dependent on the state of cleanliness of the ion optics (and on the tuning compound used) and will vary over time for a given instrument. Therefore they were not controlled in the experiments, but they will affect the fragmentation of the analytes. The second area for collisional activation is the octapole region. Assuming the primary collision gas is nitrogen in both areas and the source ion kinetic energies and the instrumental factors are similar, the only other difference in the laboratories is the collision gas pressure. Due to the design of the instruments the collision regions pressures are not precisely controlled as they would be for MS–MS instruments which use collision cells with pressure gauges. Therefore, the collision activation energies input to the ions will vary among instruments. In order to collect spectra with greater similarity the OOV will need to be varied until a defined standard is obtained.

4.2. Intralaboratory reproducibility

Short-term intralaboratory reproducibility of mass spectra, obtained under identical conditions, was satisfactory. Also, the changes in the composition of the mobile phase constituents (concentration of organic modifier or buffer molarity) did not affect the reproducibility of fragmentation to any relevant degree. On the other hand, the long-term experiments demonstrated that mass spectra, generated in APCI mode, showed different intensities of fragmentation at nominally identical fragmentation energy. Mass spectra obtained in ESI mode, were reproducible and remained so in long-term observation. This makes possible to establish a data base of ESI-generated mass spectra, but only for intralaboratory use.

It must be generally stated that interlaboratory exchange and use of API-generated mass spectra is not possible at the moment, even under carefully standardized conditions. Therefore, LC–API-MS, both in APCI and ESI options, cannot be used as a general identification tool in toxicology, as it is used by GC–EI-MS, at the moment. Nevertheless, LC–API-MS is indispensable as a method of dedicated analysis (particularly in SIM mode). The experiments performed demonstrated that the method is robust and is not sensitive to changes in mobile phase composition, which may occur in everyday use.

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