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Comparison of quadrupole and (quadrupole) ion-trap mass spectrometers for the analysis of benzodiazepines

D. Borrey, E. Meyer, W. Lambert, A.P. De Leenheer*

Laboratorium voor Toxicologie, Universiteit Gent, Harelbekestraat 72, B-9000 Ghent, Belgium

Abstract

GC–MS with on-column injection is evaluated for the analysis of benzodiazepines. A standard mixture of five benzodiazepines (ketazolam, flunitrazepam, flurazepam, alprazolam and triazolam) was used to compare the applicability of different types of mass spectrometers. Using an ion-trap mass spectrometer excessive peak tailing was observed on the late-eluting peaks. Injections on four other ion-trap systems, both with internal or external ionisation, yielded similar results. However, all peak shapes became acceptable when injections were performed on a quadrupole system. The results obtained on three other quadrupole mass spectrometers confirmed this observation. These data strongly suggest that a quadrupole instrument is more suitable than an ion-trap one for the analysis of some benzodiazepines.

Keywords: Mass spectrometry; Benzodiazepines

1. Introduction

So-called ''benchtop'' mass spectrometers are used in most laboratories for GC–MS analysis. Two types of benchtop instruments can be distinguished, i.e., ion-trap MS (IT-MS) instruments and quadrupole MS (Q-MS) instruments. Although there are many differences between ion-trap and quadrupole mass analyzers, the major one lies in the mass analysis stage. In IT-MS, ions are stored until the trap is full. By varying the radio frequency (RF) voltage applied to the ring electrode, ions are ejected out of the endcap and are detected by the electron multiplier. In Q-MS, ions are continuously formed, accelerated into the quadrupole and mass analyzed. Consequently, both types of mass spectrometers have specific advantages. With IT-MS higher sensitivity is obtained in full scan, whereas ion ratio stability is better for Q-MS [1,2].

Recently, the development of a GC–MS method for benzodiazepine analysis was started in our laboratory. Benzodiazepines are widely prescribed psychoactive drugs used as anxiolytics, sedative hypnotics, anticonvulsants and muscle relaxants with short, intermediate, or long duration of action. These compounds have a high boiling point and a part of their structure in common (Fig. 1). In the literature many GC methods are described, but for toxicological purposes MS detection is preferred as additional spectral information is yielded [3–7]. Therefore, the chromatographic conditions initially optimized on GC with nitrogen–phosphorus detection (NPD) were transferred to IT-MS. Excessive peak tailing was observed on the late-eluting peaks. However, all peak shapes became acceptable when similar injections were performed by Q-MS. As no literature data were found describing this phenomenon, addi-

*Corresponding author.

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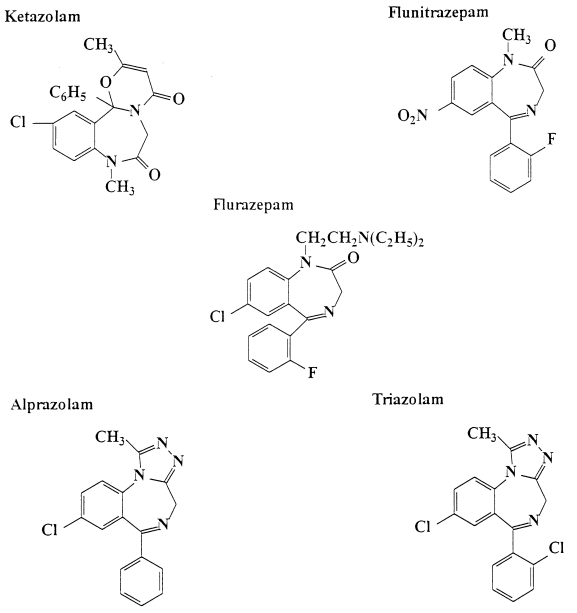

Fig. 1. Structures of the five benzodiazepines used: ketazolam, flunitrazepam, flurazepam, alprazolam and triazolam.

tional experiments were carried out to confirm or reject the hypothesis that the tailing could be attributed to the type of MS detector used.

2. Experimental

2.1. Solvents and reagents

Ethyl acetate was of analytical grade purity and obtained from Merck (Darmstadt, Germany). Standards of ketazolam, alprazolam and triazolam were a gift of Upjohn (Kalamazoo, MI, USA). Flunitrazepam was a gift of Hoffmann–La Roche (Basel, Switzerland) and flurazepam was a gift of Madaus–Therabel (Brussels, Belgium). Individual stock solutions with a concentration of 1.0 mg/ml were prepared in ethyl acetate. A standard mixture working solution containing 2.5 μg/ml of each drug was prepared by repeated dilution of the stock solutions with ethyl acetate.

2.2. Chromatographic conditions

The chromatographic system consisted of a Supelco (Bellefonte, PA, USA) intermediate polarity retention gap (3 m×0.53 mm I.D.) coupled to a SGE (Achrom, Zulte, Belgium) BPX5 capillary column (25 m×0.22 mm I.D. and 0.25 μm film thickness). The same temperature conditions (60 to 210°C at 40°C/min, ramped at 30°C/min to 275°C, held for 5 min, finally ramped at 30°C/min to 300°C and held for 10 min) were used throughout all experiments. The transfer line was held at 300°C. The carrier gas was helium at an inlet pressure of 17 p.s.i. (1 p.s.i.=6894.76 Pa)

2.3. Apparatus

The chromatographic conditions mentioned above were initially optimized on a GC–NPD system consisting of a Hewlett–Packard HP 5890 Series II gas chromatograph coupled to a NPD system (Avondale, PA, USA).

Further experiments were mainly carried out on two GC–MS systems, one with an ion-trap (IT-MS, a) and one with a quadrupole (Q-MS, a). Four additional ion-trap (IT-MS, b–e) and three additional quadrupole (Q-MS, b–d) MS detectors were used for the comparison experiments. Although on-column injection was used in-house, splitless injection was also performed for some of the analyses. Slight modifications of the chromatographic conditions (column dimensions and temperature program) were allowed for some of the external GC–MS analyses.

2.3.1. IT-MS

(a) A Series 3400 Varian gas chromatograph (Sunnyvale, CA, USA) in combination with a Finnigan Mat Magnum mass-selective ion-trap detector (San José, CA, USA) with internal ionisation and a self-installed HP on-column capillary inlet at 60°C. Both electron impact (EI) and positive chemical ionisation (PCI) are possible.

(b) A Hewlett–Packard HP 5890 Series II in combination with a Finnigan Mat GCQ with external ionisation and a HP split/splitless capillary inlet at 280°C used in the splitless mode. EI as well as PCI and negative chemical ionisation (NCI) are possible.

(c) A Varian gas chromatograph in combination with a Finnigan Mat ITS 40 mass-selective ion-trap detector with internal ionisation and a Varian septum programmable injector (SPI) used in the on-column mode.

(d) A Series 3400 Varian gas chromatograph in combination with a Finnigan Mat ITS 40 mass-selective ion-trap detector with internal ionisation and a Varian 1093 SPI used in the splitless mode.

(e) A Series 3400 CX Varian gas chromatograph in combination with a Varian Saturn 2000 ITD mass spectrometer with internal ionisation and a Varian 1078 split/splitless injector.

2.3.2. Q-MS

(a) A Hewlett–Packard HP 5890A gas chromatograph in combination with a Hewlett–Packard HP 5970 Series mass-selective detector and a self-installed HP on-column capillary inlet at 60°C.

(b) A Hewlett–Packard HP 5890 Series II gas chromatograph in combination with a Hewlett–Packard HP 5972 mass-selective detector and an Analytical Applications (Brielle, The Netherlands) cold injection system used in the splitless or the on-column mode.

(c) A Hewlett–Packard HP 5890 gas chromatograph in combination with a Hewlett–Packard HP 5972 mass-selective detector and a Hewlett–Packard split/splitless injector used in the splitless mode.

(d) A Carlo Erba 9800 Top gas chromatograph (Rodano, Italy) in combination with an Interscience Voyager mass-selective detector (Louvain-la-Neuve, Belgium) and a Carlo Erba on-column injector.

3. Results and discussion

The chromatographic conditions earlier described (Section 2) were initially optimized on a GC–NPD system. On-column injection was chosen because splitless injection was not sensitive enough for our purposes. Much effort was put into optimizing the on-column injection conditions. Critical parameters are the polarity of the retention gap in function of the solvent used and the water content of the solvent [8]. A chromatogram of the standard mixture of the five benzodiazepines (ketazolam, flunitrazepam, flurazepam, alprazolam and triazolam) is presented in Fig. 2. However, when the same conditions were used on the IT-MS (system a; Section 2.3.1) excessive peak tailing was observed on the late-eluting peaks as shown in Fig. 3.

Peak tailing usually is considered to be of chromatographic origin. Therefore, minor modifications were performed in order to solve the problem. The following parameters were changed: the temperature program of the oven and the injector, the type of solvent, the type of retention gap and the injection mode. None of these modifications yielded a significant improvement. These experiments in combination with the GC–NPD observations confirmed that the peak tailing was not a chromatographic problem.

As the only difference between the GC–NPD and the GC–IT-MS was the detector, the literature on the basic ion-trap theory and the parameters that affect the ion-trap operation was consulted [1,2,9–11]. The typical ion-trap experiment can be divided into two steps, ion accumulation and mass analysis. In the first step, ions are stored between two endcap electrodes and a ring electrode until the trap is full. The ions are cooled to the center of the ion-trap by collisions with helium which removes kinetic energy from the ions. An RF voltage applied to the ring electrode causes a rapid change in field polarities. By fluctuating the electric field, ions are alternatively accelerated and decelerated in an oscillating manner, forming a stable trajectory within the trap. Automatic gain control is a gating system which ensures that the trap is filled in an optimum manner. In the second step, ions are ejected out of the endcap at specific mass-to-charge ratio values by varying the RF voltage and are detected by the electron multiplier. The axial modulation voltage is an additional voltage applied to the endcap electrodes which facilitates ion ejection.

The following potentially relevant parameters were varied to improve the peak shape. First, the input of helium into the trap was increased to improve trapping efficiency. For this purpose the column head pressure had to be changed because a direct helium inlet to the trap was not present. Although a positive influence was seen up to a pressure of 27 p.s.i., an acceptable peak shape was never obtained. Moreover, peak resolution was compromised at these high pressure settings. Injections on the GC–NPD were performed with electronic pressure control (EPC). To check if the absence of EPC on the IT-MS was responsible for the tailing, the inlet pressure was raised manually during the run time to imitate the effect of EPC. Again an optimum

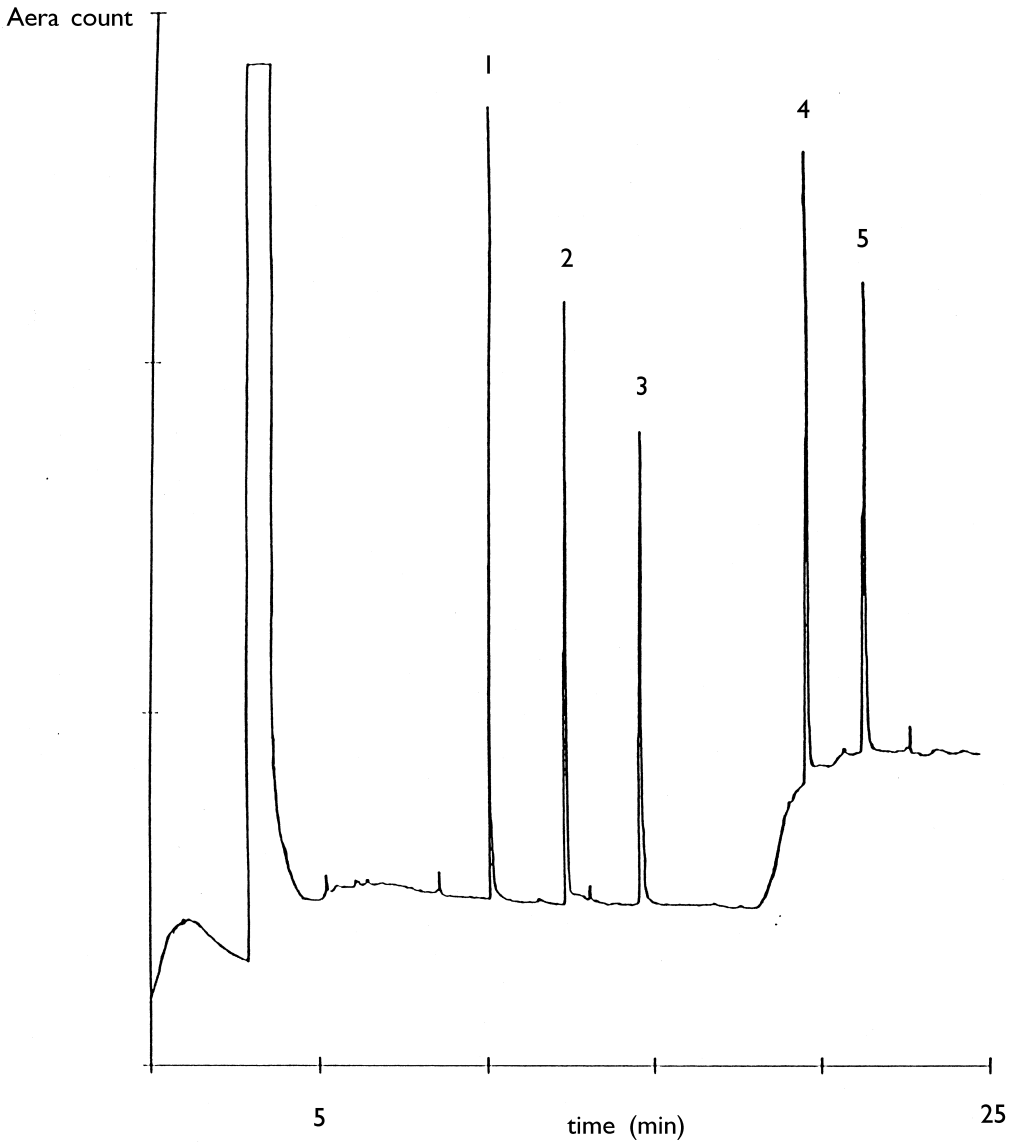

Fig. 2. Representative GC–NPD chromatogram. Peak identification: (1) ketazolam, (2) flunitrazepam, (3) flurazepam, (4) alprazolam, (5) triazolam.

was observed but the amelioration of the peak shape was never satisfactory.

Secondly, the effect of the source temperature was evaluated. Condensation of the vapor effluent from the GC column on the relatively cold source has been described [1]. This condensation is followed by slow revaporisation, ionisation and detection leading to broad chromatographic peaks. Therefore the source was heated to a maximum of 250°C. As peak tailing was not reduced with this parameter setting, the source temperature was set back to 220°C for the other experiments.

In a next step the axial modulation voltage (A/M voltage) was varied to influence the ejection process. The A/M voltage is applied to the endcap electrodes at a fixed frequency and amplitude during the ramp

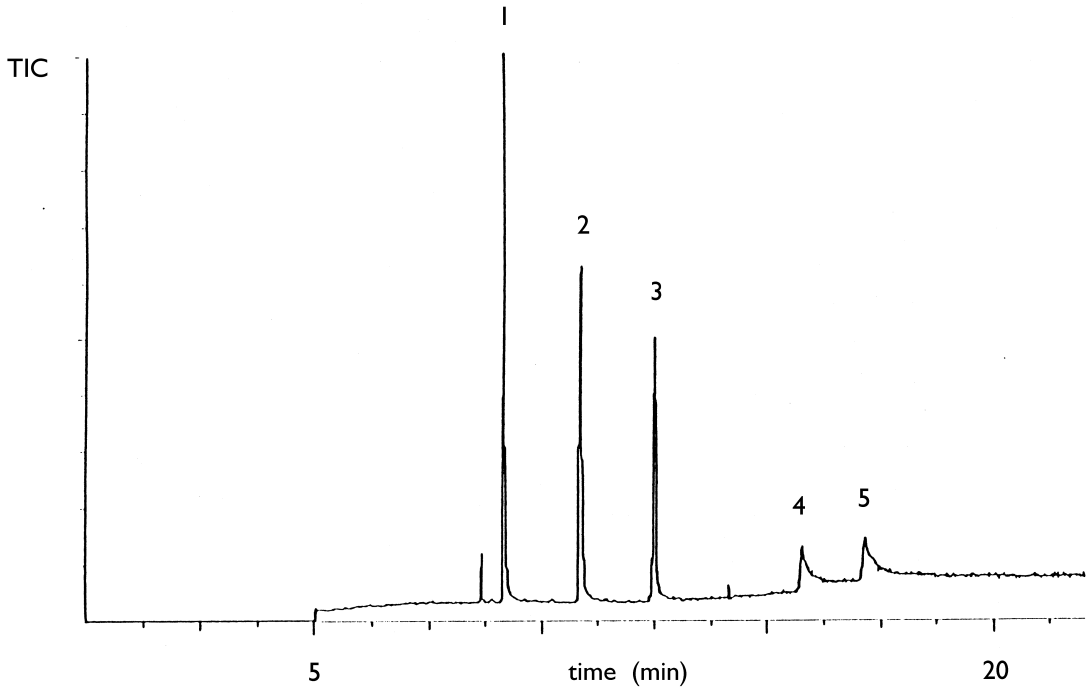

Fig. 3. Representative GC–IT-MS chromatogram. Peak identification: see Fig. 2.

of the RF voltage. By matching the frequency of this supplementary potential to the particular frequency of the ion (the frequency at which trapped ions of a given m/z oscillate), resonance conditions are induced. When an ion comes into resonance with this frequency, it absorbs energy from the applied field and moves away from the center of the trap until it becomes unstable and is ejected [9,11]. The default setting of the A/M voltage is 3.5 V. It was varied stepwise between 1 and 12 V and the best result was obtained at 2 V. This latter setting was maintained for further experiments.

Finally, the filament emission current (FEC) and the electron multiplier voltage were evaluated simultaneously to improve the ionisation and the ejection process, respectively. The FEC is the flow of electrons emitted from the filament. The electrons are accelerated towards the lens assembly which focuses the electrons into the cavity of the ion-trap. The default setting of the FEC is 11 μA. It was increased stepwise to 40 μA and the best result was obtained at 32 μA. The electron multiplier is of the continuous-dynode type and is positioned beneath the bottom endcap electrode. Positive ions ejected from the ion-trap are attracted to the cathode by the negative electron multiplier voltage applied to it. The initial setting of the electron multiplier voltage was −1900 V and was changed to −2100 and −2300 V, respectively. No improvement of the peak shape was observed.

As none of the described parameter variations solved the peak tailing problem, the standard mixture was injected in the splitless mode on a Q-MS system. Chromatograms comparable to the GC–NPD results were immediately obtained (Fig. 4). The hypothesis that the type of mass spectrometer determined the apparent chromatographic peak shape was further investigated. Similar injections were performed on four other IT-MS and three other Q-MS systems. Excessive peak tailing on the late-eluting peaks was systematically observed for all IT-MS and disappeared for all Q-MS systems. Moreover, the tailing was independent of the injection (on-column or splitless) and ionisation mode (external or internal, as well as EI or CI). To quantify the degree of tailing on the late-eluting

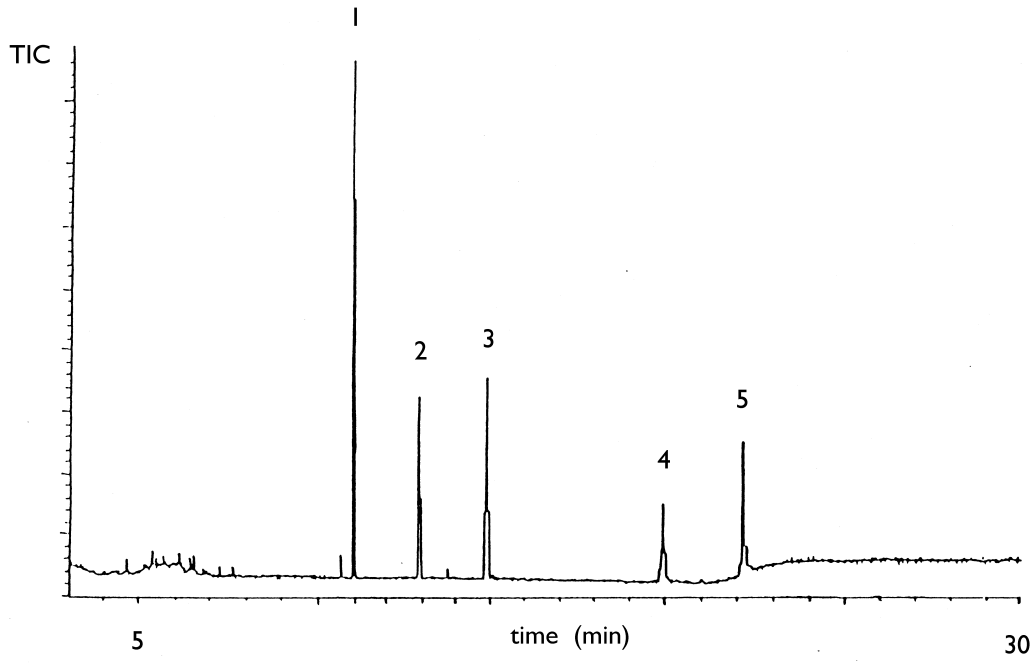

Fig. 4. Representative GC–Q-MS chromatogram. Peak identification: see Fig. 2.

peaks, the tailing factor (T_f) was calculated. The tailing factor is a simple measurement based on comparing the peak width for the front (A) and the back (B) half of the peak at 5% of the peak height. The following formula is used for these calculations: $T_f=(A+B)/2A$. For symmetrical peaks $T_f=1.0$. This value increases with the degree of tailing [12]. Results are summarized in Table 1. The difference between the two systems is also evaluated statistically using a nonparametric permutation test [13]. The two-sided P-value obtained with the StatXact software program is 0.0159, clearly demonstrating the statistically significant difference between the two groups of observations.

4. Conclusions

The experiments described in this manuscript were designed to be a comparison of IT-MS with Q-MS for the analysis of benzodiazepines. In spite of all parameter changes performed with IT-MS the problem of peak tailing persisted, while for Q-MS peak symmetry was always obtained. Thus, our data

Table 1
Comparison between IT-MS and Q-MS for peak shape of some late-eluting benzodiazepines

	IT-MS					Q-MS			
	a	b	c	d	e	a	b	c	d
Injector type	OC	SL	SPI	SL	SL	OC	CIS	SL	OC
Ionisation mode	EI/PCI	EI/NCI	EI	EI	EI	EI	EI	EI	EI
T_f alprazolam	4.5	7.0	5.5	3.6	4.8	1.3	1.1	1.2	1.0
T_f triazolam	4.0	7.8	5.0	3.3	3.0	1.5	1.1	1.2	1.0

Abbreviations: OC, on-column; SL, splitless; SPI, septum programmable injector; CIS, cooled injection system; EI, electron impact ionisation; PCI, positive chemical ionisation; NCI, negative chemical ionisation; $T_f=(A+B)/2A$, tailing factor, see Section 3; a–e: see Section 2.3.

highly suggest that a quadrupole instrument is more suitable than an ion-trap for the analysis of some benzodiazepines.

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