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Comparison between gas chromatography–atomic emission detection and gas chromatography–mass spectrometry for the assay of propofol

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Abstract

Quantitation by gas chromatography–atomic emission detection (GC–AED) is based on the intensity of the signal measured at a wavelength characteristic of an element, after atomisation by the plasma. This response depends only on the number of atoms of this element present in the molecule under investigation, and is independent of the structure of the molecule. This technique was used for the assay of propofol, and the estimation of its two metabolites, after calibration with standard solutions of pure propofol. The results were compared with those obtained by gas chromatography–mass spectrometry (GC–MS). Propofol was quantified with higher precision and accuracy by GC–AED than by GC–MS which exhibited larger residual values. Concentration assessment for two metabolites showed a better agreement with the theoretical value by GC–AED since the response depends only on the number of carbon atoms in each molecule.

Keywords: Propofol

1. Introduction

Quantitation of drugs or metabolites in biological fluids is generally based on the responses of the pure reference compounds to a dedicated analytical method and a particular detection system. The response of a compound includes two stages of the analytical process: (i) extraction efficiency from the biological matrices, (ii) specific sensitivity of the detection mode. Whereas gas chromatography–mass spectrometry (GC–MS) with single ion monitoring (SIM) detection is based on molecular response of each compound, according to preselected ions, gas chromatography–atomic emission detection (GC–AED), is based on elemental response of a preselected element present in the molecule to be assayed. Consequently, GC–AED offers an interesting detection system giving response factors which depend only on the number of atoms of the element chosen for the detection of the molecule [1,2]. Hence, the signal for any compound of known structure may be directly related to the concentration of this molecule in the sample.

Moreover, for metabolites appearing during the metabolisation process of a drug, GC–MS is a suitable tool to detect and determine their structures, but not to quantitate them in the absence of available compound. This is due to the specific response factor

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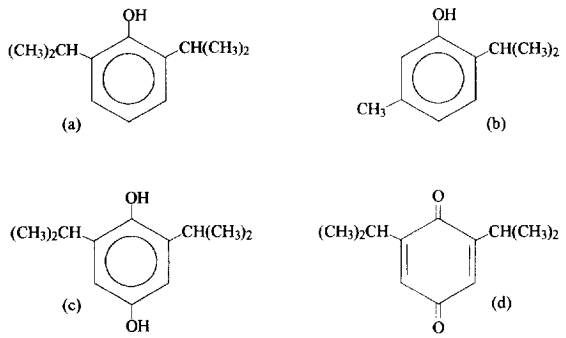

Fig. 1. Structure of propofol (a), thymol (b), 2,6-diisopropyl-1,4-quinol (c) and 2,6-diisopropyl-1,4-quinone (d).

of the compound, which depends on the preselected ion used for the detection. In such a case, GC–AED is likely to give better results for the estimation of the metabolites, since their structures do not influence the response of the detector.

The aim of this work was to assess both techniques for the assay of propofol by monitoring carbon at 193.030 nm compared with the previously obtained results using GC–MS [3]. Additionally, the tentative assay of two metabolites was evaluated (Fig. 1).

2. Experimental

2.1. Drugs and chemicals

Pure propofol was kindly supplied by Zeneca Pharma (Cergy, France). Thymol, glucose-6-phosphate (G6P), glucose-6-phosphate dehydrogenase (G6PDH) and nicotinamide adenine dinucleotide phosphate ($NADP^+$) were obtained from Sigma (St. Quentin Fallavier, France). Chloroform and ethyl acetate were chromatography grade and were purchased from Merck (Darmstadt, Germany).

2.2. Calibrated standard solution

The calibration for the analysis in the incubation medium was done by spiking 1 ml of the reconstituted medium, in the absence of NADP, so that no metabolisation occurred, with the appropriate volume of a standard solution of pure propofol. Five replicates each of six different final concentrations of 1, 5, 10, 20, 35, and 50 μM were prepared. Each sample was injected once, after extraction according to the procedure described below.

Two batches of control samples at 2.5, 15, and 40 μM were prepared in duplicate in the same way, and included in the sequence.

2.3. Metabolite formation and extraction procedure

Metabolite formation was obtained from rat liver microsomes, which had been prepared as described previously [4]. Incubations were performed at 37°C, in a final volume of 1 ml containing 0.1 M potassium phosphate buffer (pH 7.4) with NADPH-regenerating system (0.5 mM $NADP^+$, 5 mM G6P, 1 unit of G6PD), microsomal fractions. After a 3-min preincubation, the reaction was initiated by adding propofol (45 μM) and terminated after 3, 6, 9, 12, 15 and 18 min incubation periods by adding 600 μl chloroform–ethyl acetate (70:30, v/v), and 100 μl of a solution of thymol at 25 $\mu g\,ml^{-1}$. Then, propofol and metabolites were extracted by vortex-mixing for 5 min followed by centrifugation (3900 g, 10 min). The organic layer was then separated in two equal parts, and subsequently analysed by injection of 1 μl aliquot into the column of the GC–MS and of the GC–AED system.

2.4. Apparatus and chromatographic conditions

The chromatographic separation was performed by injection in splitless mode (valve time: 40 s), of 1 μl of the extract in an HP1 capillary column (25 m× 0.32 mm I.D.; film thickness: 0.17 μm). Helium flow-rate was adjusted to 1.08 $ml\,min^{-1}$ at initial oven temperature, and was maintained constant during the run by the electronic pressure control system. The injector temperature was 260°C and the oven temperature was programmed as follows: 80°C for 0.8 min, 15°C min^{-1} up to 200°C and 20°C min^{-1} up to 280°C for 2 min. The transfer line temperature was set to 280°C. In these conditions thymol, propofol, 2,6-diisopropyl-1,4-quinone and 2,6-diisopropyl-1,4-quinol eluted at 9.6, 11, 11.6 and 15.2 min, respectively.

2.5. Detection

The AED detection system comprised an atomic emission source, a diode array spectrometer (Model 5921A, Hewlett-Packard, Palo Alto, CA, USA) and a microwave induced plasma coupled to a gas chromatograph (Model HP 5890, Series II plus). An automatic sampler (Model HP 7673A) and a workstation (Model HP 5895) completed the system. The emitting source was a 99.9999% pure helium plasma generated inside a silica discharge tube (I.D.=1 mm), placed in a Beenekker resonant cavity (microwave induced plasma: 2.75 GHz, 70W). Carbon was monitored on the AED system at the vacuum-UV wavelength of 193.030 nm using the manufacturer's recommended conditions. Helium at 60 ml min^{-1} was used as make-up gas, and the reagent gases were O2: 99.998% and H2: 99.998% at 1 and 4 kPa, respectively.

The GC–MS detection used an apparatus (Model GCD, HP) working in SIM mode under electronic ionisation at 70 eV. The propofol and thymol molecules were detected by monitoring ions at *m/z* 163 and 135, respectively, while 2,6-diisopropyl-1,4-quinone and 2,6-diisopropyl-1,4-quinol were detected by using ions at *m/z* 149 and 179. Each of these ions, corresponds to the M−15 ($M-CH_3$) ions from molecular ions, except for 2,6-diisopropyl-1,4-quinone which corresponds to M−43 ($M-C_3H_7$), and are the major ions of the spectra [3].

3. Results and discussion

3.1. Calibration curves

3.1.1. GC–AED

A linear relationship [$y=0.00752$ ($\pm 2e^{-5}$) $x-0.0158$ (± 0.0068); $r=0.9999$; $n=5$] [($F_{cal}=170076$). F_{tab} (0.05; 1;4=7.71)] was obtained by an unweighted linear regression analysis between the ratio of the signals from propofol to thymol, measured at 193.03 nm and the ratio of each compound concentration. In order to obtain a calibration line independent of the molecular weight of the compound, the ratio of the quantities of carbon arising from both molecules were substituted to the concentration ratio. Table 1 shows the results for mean individuals concentrations calculated from the corresponding calibration line.

Table 1
Mean concentration of propofol, standard deviation, precision and accuracy from five different calibration curves established by GC–AED technique

Spiked (nmol ml^{-1})	Mean (nmol ml^{-1})	S.D. (nmol ml^{-1})	Precision (%)	Accuracy (%)
1	1.16	0.096	8.3	16.1
5	5.11	0.099	1.9	2.2
10	9.92	0.194	2.0	0.8
20	19.90	0.130	0.7	0.5
35	35.03	0.304	0.9	0.1
50	49.83	0.149	0.3	0.4

S.D.: standard deviation.

3.1.2. GC–MS

A linear relationship [$y=0.007457$ (± 0.00136) $x-0.0272$ (± 0.0109); $r=0.9988$; $n=5$] [($F_{cal}=2076$). F_{tab} (0.05; 1;4=7.71)] was obtained by an unweighted linear regression analysis between the ratio of the area of ions *m/z* 163 of propofol to ion *m/z* 135 of thymol, and the concentration of propofol. Table 2 shows the results for mean individuals concentrations calculated from the corresponding calibration line.

3.1.3. Residuals

The distribution of the residuals (Fig. 2) shows random variations, the number of positive and negative values being approximately equal. GC–MS exhibits larger residuals for high concentrations, while GC–AED presents constant values throughout

Table 2
Mean concentration of propofol, standard deviation, precision and accuracy from five different calibration curves established by GC–MS technique

Spiked (nmol ml^{-1})	Mean (nmol ml^{-1})	S.D. (nmol ml^{-1})	Precision (%)	Accuracy (%)
1	1.28	0.130	10.2	27.6
5	5.14	0.180	3.5	2.8
10	9.69	0.356	3.6	3.1
20	19.03	0.566	3.0	4.8
35	36.43	0.535	1.5	4.1
50	49.43	0.371	0.8	1.1

S.D.: standard deviation.

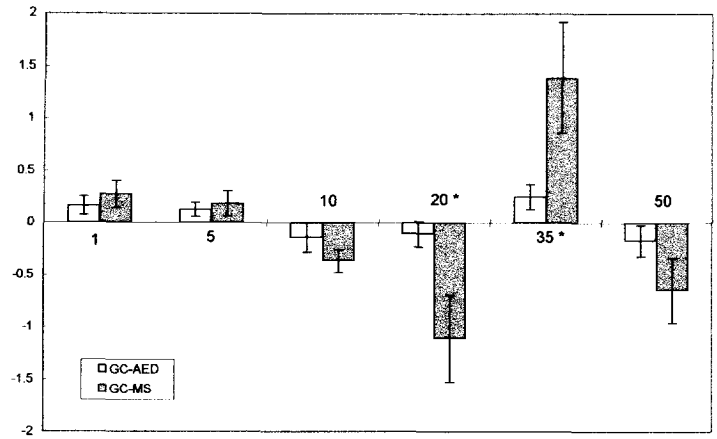

Fig. 2. Distribution of residuals (measured-target value) obtained by GC–AED and GC–MS. Standard deviation is indicated. (*) indicates significant difference between values at the $p<0.05$ confidence interval.

the concentration range. A Student's t-test applied to the five values obtained from both techniques for each concentration, showed no significant difference, except for the point at 20 and 35 μM.

3.2. Propofol assay by GC–MS and GC–AED

3.2.1. Control samples

In order to apply these techniques to the assay of propofol, the control samples spiked at 2.5, 15, and 40 μM were analyzed before and after injection of the kinetics samples in the same batch and their concentrations were calculated using the mean calibration lines previously established for each technique. The calculated concentrations (nominal values), precision, and accuracy are presented in Table 3. All the quality control samples are within ±7% of their respective nominal value. According to the recommendations of the conference for "Analytical Methods Validation" reported by Shah et al. [5], (no more than two results out of the acceptability limit: 20% deviation), the batch of analysis was accepted.

3.2.2. Propofol concentrations

The samples from two replicate incubation media, were analyzed in the same way and the concentrations of propofol were calculated by reference to the mean calibration line of each technique. Propofol concentrations followed a parallel decrease from the initial concentration (45 μM) to the last incubation time (17.5 μM at $t=18$ min), and for both kinetics.

3.2.3. Limit of quantitation

The limit of quantitation, established from three times the signal of a blank sample, was calculated as 0.1 μM for GC–MS and 0.3 μM for GC–AED.

Table 3
Concentration and accuracy for duplicate quality control samples at 2.5, 15 and 40 mmol l^{-1}, included in the analysis sequence, by GC–AED and GC–MS techniques

Spiked (nmol ml^{-1})	AED				MS			
	Ct_1	Acc. (%)	Ct_2	Acc. (%)	Ct_1	Acc. (%)	Ct_2	Acc. (%)
2.5	2.60	+3.9	2.49	−0.6	2.68	+7.1	2.67	+6.9
15	15.07	+0.4	14.99	0.0	15.40	+2.7	14.19	−5.4
40	39.12	−2.2	38.95	−2.6	39.20	−2.0	41.85	+4.6

Ct_1 and Ct_2: controls. Control concentration are expressed in nmol ml^{-1}. Acc.: accuracy.

3.2.4. Metabolite appearance

Since both metabolites were not available as pure compounds, quantitation of these molecules was not possible by GC–MS, as no response factor could be measured.

The plot of the area ratios of specific ions of each metabolite and of propofol during incubation is shown in Fig. 3. The increase of the signals of metabolites, and the decrease of the signal of propofol at increasing incubation time, are clearly visible. Nevertheless, at any time the sum of 2,6-diisopropyl-1,4-quinone and 2,6-diisopropyl-1,4-quinol production and of unchanged propofol did not reach the theoretical value (dash line) corresponding to the initial signal due to propofol. Assuming that 2,6-diisopropyl-1,4-quinone and 2,6-diisopropyl-1,4-quinol are the only metabolites formed during incubation (no other peaks detected by GC–MS), this sum should be constant throughout the incubation, if the individual signals of all compounds were directly dependant of the concentrations.

However, the plot of the signal measured at 193.030 nm by GC–AED for both metabolites and for propofol (Fig. 4), shows a better agreement with the theoretical value (dashed line) since the response depends only on the number of carbon atoms in each molecule.

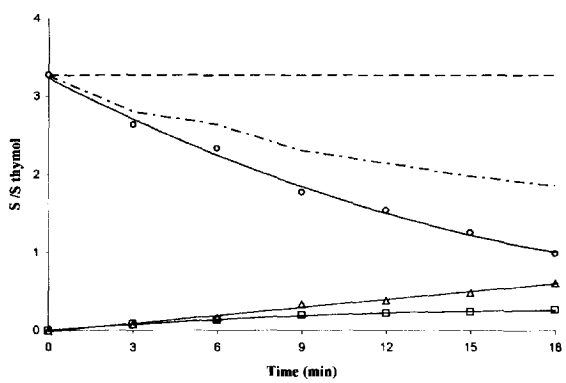

Fig. 3. Microsomal kinetics of propofol and its metabolites 2,6-diisopropyl-1,4-quinol and 2,6-diisopropyl-1,4-quinone obtained by GC–MS. (○) Ratio of m/z 163 (propofol) to m/z 135 (thymol). (△) Ratio of m/z 179 (2,6-diisopropyl-1,4-quinol) to m/z 135 (thymol). (□) Ratio of m/z 149 (2,6-diisopropyl-1,4-quinone) to m/z 135 (thymol). (– · – · –) Sum of signals of propofol (○), 2,6-diisopropyl-1,4-quinol (△) and 2,6-diisopropyl-1,4-quinone (□). Dashed line represents the initial value (- - -).

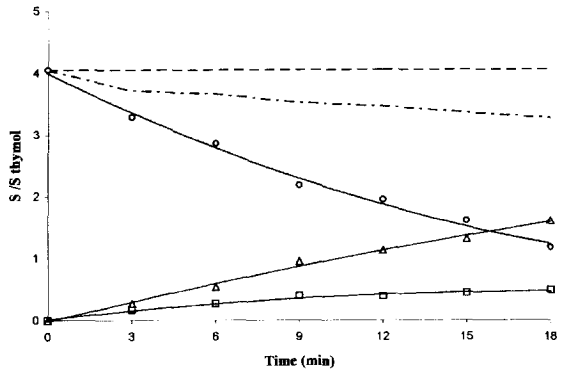

Fig. 4. Microsomal kinetics of propofol and its metabolites 2,6-diisopropyl-1,4-quinol and 2,6-diisopropyl-1,4-quinone obtained by GC–AED. (○) Ratio of C193 nm (propofol) to C193 nm (thymol). (△) Ratio of C193 nm (2,6-diisopropyl-1,4-quinol) to C193 nm (thymol). (□) Ratio of C193 nm (2,6-diisopropyl-1,4-quinone) to C193 nm (thymol). (– · – · –) Sum of signals of propofol (○), 2,6-diisopropyl-1,4-quinol (△) and 2,6-diisopropyl-1,4-quinone (□). Dashed line represents the initial value (- - -).

In order to verify this hypothesis, eight samples of increasing concentrations of propofol ($C_{12}H_{18}O$) and thymol ($C_{10}H_{14}O$) ranging from 2.8 to 168.3 nmol ml^{-1} and 3.3 to 199.7 nmol ml^{-1} respectively, were analyzed by the GC–AED method. These samples were prepared by dilution of stock solution of pure products in chloroform, and were each injected four times.

A linear relationship was obtained between the intensity of the signals measured at 193.030 nm and the amount of each compound expressed as the quantity of carbon entering the plasma. The slopes (a) and the intercepts (b) of the calibration lines have been compared by using a Student's t-test. The results showed that both calibration lines were not significantly different for the slopes [(t_{cal}=2.05). t_{tab} (0.05; 14=2.15)] as well as for the intercept [(t_{cal}= 0.08). t_{tab} (0.05; 14=2.15)].

4. Conclusion

Propofol was better quantified by GC–AED as residuals between measured concentrations and target values were always less than 0.2 nmol ml^{-1} for the six different concentrations, while GC–MS

exhibited larger residual values at high concentrations. The mode of detection by the specific wavelength of carbon atom radiation may be responsible for the more accurate results.

The identification and detection of the metabolites yielded excellent results for GC–MS, through the study of the mass spectra and the use of SIM detection. However, any attempt at quantification yielded errors, since the response of each compound was largely dependent on the ion chosen for its detection.

On the contrary, the monitoring of the signal of carbon atoms from the AED plasma for the detection of compounds of similar structures have proved to be dependent only on the number of carbon atoms in the molecule. Taking these results into account, for new metabolites of known formulae, it is possible to derive the quantity of material reaching the plasma from their AED signals. Assuming that the solvent system has the same extraction efficiency for the metabolites as for the parent molecule, one would be able to transform the signals from these compounds to concentrations in the biological matrices. Nevertheless, even in the absence of this parameter, the estimation of proportion of the metabolites in the medium is better with GC–AED than with GC–MS.

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