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Comparison of different GC-MS configurations for the determination of prevalent drugs and related metabolites

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Abstract: Cocaine, cannabis, heroin, and other opioids are among the prevalent drugs in Europe. The use of these drugs is demonstrated by the determination of either parent drugs or related metabolites in a variety of biological samples. Various analytical methodologies can be applied for this purpose, all of which might show relevant differences in analytical performance. In this work we used different GC-MS configurations for the quantitation of cocaine, cocaethylene, benzoylecgonine, morphine, and Δ9-tetrahydrocannabinol with the aim of comparing the analytical performance of different GC-MS instruments, different injectors, ion sources, ionization modes, mass analyzers, operating modes, and acquisition modes, in order to find the optimal configuration in terms of sensitivity and precision. Other important factors, such as the derivatization process for GC analysis or the injection mode, were also investigated for the same purpose. A comparative study of different methods used for the calculation of the limits of detection was also performed, in order to compare them in terms of the obtained values and their veracity. Differences found in the results obtained with different configurations showed different limits of detection and different precision. These results allowed us to indicate advantages and limitations, which depended on the configuration of the GC-MS used. Finally, differences up to seven orders of magnitude were found in the LOD values obtained with different methods, some of them being too small to show any measurable peak.

Keywords: GC-MS; cocaine; heroin; cannabis; opioids.

Introduction

Cocaine (COC), cannabis, and opioids are among the most widely abused substances globally. In particular, COC and cannabis are the most frequently abused drugs in Europe, being regularly used in combination with alcohol. Spain has been on the top of European countries in prevalence of COC and cannabis abuse during the last 15 years [1] and, although the prevalence of opioids use is smaller, their abuse is increasing among people over the age of 34 [2]. COC is a powerfully addictive stimulant drug that increases levels of dopamine in brain. Among its negative health effects, heart attacks and strokes are the most serious [3]. On the other hand, cannabis contains Δ9-tetrahydrocannabinol (THC), which is a mind-altering chemical. THC acts on specific brain receptors altering perception, sense of time, producing mood swings, cognitive difficulties, and impaired movement and memory. Cannabis also affects brain development and, when used by teenagers, may reduce thinking, memory, and other cognitive functions [3]. Moreover, according to Lopez-Quintero et al., cannabis can also produce addiction in 8.9% of its users [4]. Opioids include the illegal substance heroin and other legal pain relievers, such as oxycodone, hydrocodone, codeine, or morphine (MOR). Although pain relievers are generally safe when recommended dose indications are followed, sometimes opioids are taken in greater quantities, since they produce euphoria. With regular use, heroin and opioid pain relievers may produce dependence and, when abused, they can lead to death from overdose [3].

COC is rapidly and almost completely metabolized to benzoylecgonine (BEN). Therefore, in biological samples, the use of COC is demonstrated by the determination of BEN. However, when COC is used in combination with alcohol, a process of transesterification occurs between COC and ethanol, forming cocaethylene (CET) [5]. Thus, CET may also be determined in biological samples as prove of COC use. Regarding the use of cannabis, THC is usually the compound to be determined when screening for cannabis users [6, 7]. Heroin is primarily metabolized to 6-monoacetylmorphine, which is quickly converted to MOR [8]. Codeine (a pain reliever) is also converted to MOR [9]. Therefore, MOR might indicate the use of heroin and other opioids.

However, according to Konstantinova et al. [8], other heroin metabolites may need to be considered in order to demonstrate heroin use.

Different analytical methodologies have been applied to the determination of drugs of abuse and their metabolites. They have mainly been based on liquid chromatography (LC) and gas chromatography (GC) for being separation techniques

with high resolving power [7]. Due to its high-specific nature and high sensitivity, mass spectrometry (MS) is usually the detection system used in combination with LC and GC for the determination of drugs of abuse. Tandem MS (MS/MS) implies more than one stage of mass analysis, resulting in higher selectivity when studying fragmentation of particular ions and is, in principle, more desirable than a single step of MS. The polar character of most of these drugs and their metabolites makes LC-MS more suitable than GC-MS for the analysis of these samples, because a derivatization step is often needed prior to GC analysis [7]. However, LC-MS shows certain drawbacks, being prone to matrix effects, especially when applying electrospray ionization (ESI). These matrix effects may cause alteration in response due to the presence of co-eluting compounds that may increase (ion enhancement) or reduce (ion suppression) ionization of the analyte [6]. This considerably affects important parameters, such as limit of detection (LOD), limit of quantification (LOQ), linearity, accuracy, and precision [7]. In order to evaluate and compensate matrix effects, expensive isotope-labeled compounds [10] or analogous compounds are required as internal standards [11]. GC-MS is also an important technique in the analysis of samples for the determination of drugs of abuse and may be less expensive than LC-MS because it consumes fewer reagents. Electron impact (EI) is the most widely used ion source in GC-MS, providing low LODs and high specificity [7]. Moreover, the fragmentation of drugs by EI is very reproducible. Wu et al. [12] developed a GC-EI/MS in a single quadrupole in selected ion monitoring (SIM) mode for the quantitation of amphetamines, ketamine, and opiates, including MOR and metabolites. A different GC-EI/MS methodology, also performed in a single quadrupole in SIM mode, was done by Cordero et al. [13] for the quantitation of amphetamines, opiates including MOR, diazepam, COC, and CET. Minoli et al. [14] developed a multiple reaction monitoring (MRM) method in a GC-EI/MS/MS with a triple quadrupole analyzer for the quantitation of THC and its main metabolite. Another way of achieving GC-EI/MS/MS is using ion traps as performed by Emidío et al. [15] for the quantitation of cannabinoids or by Gambelunghe et al. [16] for the quantitation of steroids, COC, and its metabolite, BEN. Chemical ionization (CI) has also been used, in positive (PCI) or negative (NCI) modes, in GC-MS for being more selective than EI [7]. Cognard et al. [17] developed a GC-PCI/MS/MS with an ion trap for the quantitation of COC and three metabolites including CET. The main metabolite of THC was also determined using a GC-NCI/MS/MS system with a triple quadrupole [18].

In view of the consulted literature, it seems clear that numerous different approaches are valid for the identification and quantitation of drugs of abuse and their metabolites by GC-MS, combining different ion sources and mass analyzers. Moreover,

different companies offer their own products, which might also vary in analytical performance, although their configurations are similar. Furthermore, certain mass spectrometers can be operated in different acquisition modes, which can affect the analytical performance, according to the manufacturers. The aim of this work was to develop different GC-MS methods using different instruments, with different injectors, ion sources, ionization modes, mass analyzers, operation modes, and acquisition modes, for the quantitation of COC, CET, BEN, MOR, and THC in order to address comparative studies in terms of their analytical performance.

Materials and methods

- Reagents and standards

All reagents were of trace analysis grade. n-Hexane (95%), used for washing the injection syringe before and after the sample injection, was from J. T. Baker (Deventer, The Netherlands). Cocaine, cocaethylene, benzoylecgonine, morphine, and (−)-trans-Δ9-tetrahydrocannabinol were from Cerilliant (Cerilliant Corp., Round Rock, TX). N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA, ≥98.5%) for GC derivatization was from Sigma-Aldrich (Buchs, Switzerland). Figure 1 shows the structures of the analyzed compounds after derivatization with MSTFA.

Different stock solutions (100 Qg/mL, 10 Qg/mL, 1 Qg/mL, 0.1 Qg/mL and 0.01 Qg/mL) were prepared in MSTFA in vials suitable for GC and the vials were incubated at 100 ºC for 30 min for silylation of polar groups and stored at -20 ºC. Dilutions from the stock solutions were prepared in MSTFA, depending on the analysis requirements. Derivatized standards were directly injected into the GC system.

- Instruments

Two different GC-MS systems were used in this work. The first system was a Thermo TRACE GC Ultra gas chromatograph coupled to a mass spectrometer equipped with a TSQ Quantum XLS triple quadrupole analyzer (Thermo Fisher Scientific Inc., Bremen, Germany) operated in EI ionization mode at -70 eV of electron energy, in SIM and MRM acquisition modes, called EIQqQ(SIM) and EI-QqQ(MRM), respectively. The current of the filament was 150 µA. Injections were performed in a programmed temperature vaporization (PTV) injector in splitless mode. The split flow before the injection was 10 mL/min and the splitless time was 2 min. Under optimal conditions, the

initial temperature in the injector was 130 ºC and it was increased up to 320 ºC at 14.5 ºC/s. Then, this temperature was maintained for 2 min with a constant septum purge. A cleaning phase of the injector was included after the transfer phase, consisting of a temperature increase in the injector at 14.5 °C/s up to 330 ºC. Then, the temperature was held at 330 ºC for 5 min. The flow was also increased up to 50 mL/min during the cleaning phase. Temperatures of the MS transfer line and the MS source were set at 300 ºC and 240 ºC, respectively, and Ar was used as collision gas in the collision cell for MRM experiments at 1 mTorr. Data recording was started 5 min after the chromatographic run began and was stopped 2.2 min later. XcaliburTM 2.1.0.1140 (Thermo Fischer Scientific Inc., San Jose, CA, USA) was used to record and process the acquired data.

The second system was an Agilent 6890N gas chromatograph coupled to an Agilent 5975MSD (Agilent Technologies, Palo Alto, CA, USA) operated in both, EI and PCI ionization modes, in the SIM acquisition mode, called EI-Q(SIM) and EI-PCI(SIM), respectively. The electron multiplier was operated in the Gain factor mode (gain factor = 1). Injections were performed in an isothermal injector in splitless mode. The split flow was set at 10 mL/min before the injection and the splitless time was 2 min. The injector temperature was kept at 250 ºC with a constant septum purge. The current of the filament was 150 µA and the temperature of the MS transfer line was set at 300 ºC. The MS source was set at 250 ºC while the quadrupole was set at 150 ºC.

An aliquot of 1 µL was injected in both instrumental systems. A capillary column HP-5MS (30 m × 0.25 mm i.d., 0.25 µm film thickness) purchased to Agilent Technologies (Palo Alto, CA, USA) was used for the separation. The same column and temperature program were used in both systems. An initial temperature of 150 °C for 2 min was programmed in the oven and it was increased at 120 °C/min to 260 °C. Then, the temperature was increased 1 ºC/min to 264 ºC and finally 120 ºC/min up to 300 ºC. The final temperature was held for 2 min. Helium was used as carrier gas at a constant flow rate of 0.8 mL/min.

- Evaluation of the analytical performance

- Working range and r^2 value

The linear working range was calculated from eight different concentrations of the analytes in the range from 0.01 to 25 µg/mL. These concentrations were: 0.01, 0.05, 0.1,

0.25, 1, 5, 10, and 25 µg/mL and the r^2 values were obtained from the calibration curves calculated with these concentrations.

- Precision

The precision for the different methods was evaluated in terms of repeatability and intermediate precision. Repeatability was calculated at two different concentrations, 0.1 and 1 µg/mL, in five repeated analyses, while the intermediate precision was calculated in five non-consecutive days over one week, at the same two concentrations.

- Limits of detection

Seven different approaches were used for the calculation of the LOD values. Methods 1 and 2 are based on the standard deviation of the response, methods 3 and 4 are based on the noise signal, methods 5 and 6 use the signal-to-noise value provided by the software for data analysis and method 7 is based on visual evaluation of the analysis. Methods 1, 3, and 5 use a proportion to calculate the LOD, while methods 2, 4, and 6 use the calibration curve. For the LOD values calculated based on standard deviation (methods 1 and 2), five repeated analyses were performed and the standard deviation of the peak areas was used in the calculations. As for the noise-based methods (3 and 4), the noise and the peak heights were manually calculated.

Method 1. Calculation based on the standard deviation of the response, in which 3 times the standard deviation of repeated analyses of samples with a low concentration of the analyte was used to proportionally calculate the LOD. The area obtained with the sample with low concentration of the analyte was used in this proportion.

$$LOD = 3 \times SD \times C / A$$

where SD is the standard deviation of repeated analyses, C is a low concentration, and A is the average of the peak areas obtained in the analyses.

Method 2. Calculation based on the standard deviation of the response and the slope, calculated by plotting the value of three times the standard deviation in the calibration curve and obtaining the LOD value based on the slope of the curve.

$$LOD = 3 \times SD / S$$

where SD is the standard deviation of repeated analyses and S is the slope of the calibration curve.

Method 3. Calculation based on the signal-to-noise ratio measured by the analyst, in which the signal of samples at low concentration was compared to the noise around the peak in order to proportionally establish the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio of 3 was selected.

$LOD = 3 \times C / S/N$

where C is a low concentration and S/N is the signal-to-noise ratio.

Method 4. Calculation based on the noise signal measured by the analyst and the slope, in which the value obtained by multiplying three times the noise height was plotted in a calibration curve to obtain a concentration value based on the slope of the curve. First an area value was estimated for the height of three times the noise using samples of low concentration. Then, the estimated area was plotted in the curve to obtain a concentration corresponding to the LOD value.

$N_A = 3 \times N \times A / H$

$LOD = 3 \times N_A / S$

where NA is the estimated area for three times the noise height, N is the noise height, A is the area of the peak at low concentration, H is the height of the peak at low concentration, and S is the slope of the calibration curve.

Method 5. Calculation based on the signal-to-noise ratio provided by the software, in which that value obtained for a sample of low concentration was used to proportionally calculate the LOD, established as a signal-to-noise ratio of 3.

$LOD = 3 \times C / (S/N)_{software}$

where C is a low concentration and $(S/N)_{software}$ is the signal-to-noise ratio provided by the software.

Method 6. Calculation based on the signal-to-noise ratio provided by the software, in which the value obtained by multiplying three times the noise height was plotted in a calibration curve to obtain a concentration value based on the slope of the curve. First an area value was estimated for the height of three times the noise using samples of low concentration. Then, the estimated area was plotted in the curve to obtain a concentration corresponding to the LOD value.

$N_{software} = H / (S/N)_{software}$

$N_A = 3 \times N_{software} \times A / H$

$LOD = 3 \times N_A / S$

where $N_{software}$ is the noise height measured by the software, H is the height of the peak at low concentration, $(S/N)_{software}$ is the signal-to-noise ratio provided by the software, NA is the estimated area for three times the noise height, A is the area of the peak at low concentration, and S is the slope of the calibration curve.

Method 7. Experimentally calculated or based on visual evaluation, in which samples of known concentrations were used to calculate the LOD of the analytes. The LOD was established at the lowest concentration at which the analyte could be reliably detected.

Results and discussion

Different experimental conditions were studied in order to obtain the best separations and the maximum responses for the analytes in all the systems used. This is important when analyzing biological samples when the amount of drug or its metabolites is low. The experimental conditions studied were: the MS ionization and fragmentation characteristics of the analyzed compounds, their chromatographic separation, the derivatization process, and the conditions in the injector (for the PTV). Then, the analytical performance of the different GC-MS configurations was also studied and compared under the selected conditions.

- Selection of the MS working parameters

In order to study the MS parameters of the selected drugs and metabolites, samples were derivatized with MSTFA, according to a method found in the literature [19]. COC and CET do not undergo derivatization because they don't have free functional groups that can react with the derivatization agent. BEN and THC accept one trimethylsilyl group each, which substitutes the hydroxy groups. MOR has two hydroxy groups and therefore has two derivatization points (Figure 1).

First, PCI and EI ionization modes were studied for the analysis of the samples. Only one or two major ions were produced in the PCI mode because of the lower energy used. As can be seen in Figure 2, the ionization of these compounds produced $[M+H]+$ and $[M+C_2H_5]+$ ions in all cases: COC (m/z 304 and 332), CET (m/z 318 and 346), BEN (m/z 362 and 390), THC (m/z 387 and 415) and MOR (m/z 430 and 458). However,

$[M+C_2H_5]+$ ions were less abundant than [M+H]+ ions and only in the case of THC the $[M+C_2H_5]+$ ion was selected for the MS. Other major fragments were also produced. Table 1 shows the selected ions for the PCI mode, as well as the quantitative ion and the ion ratio. Figure 3 shows the MS spectra of the analytes after EI, which were richer in fragments. In view of the spectra produced, different ions were selected for SIM and MRM modes. As can be seen in Table 1, the molecular ions were selected for MS in almost all the cases for being very selective. Only in the case of BEN the molecular ion was discarded as precursor ion in MRM mode, for showing very low abundance. In this case, the fragment of m/z 240 was selected as precursor ion for three transitions. Other characteristic and abundant ions were chosen as selected ions for SIM or precursor ions for MRM in order to have, at least, two of them for each compound. The quantitative and qualitative ions (SIM) and transitions (MRM), the ion and transition ratios and the collision energies are also shown in Table 1. The energy in the collision cell for those experiments in MRM mode was also studied for all the transitions, in order to obtain the maximum signal for all the compounds. First, the energies were studied in the range from 5 to 30 eV (5, 10, 15, 20, 25, and 30 eV). Then a fine study was done around the optimal energy in a range of 8 eV, one by one. The results obtained for these energies can be found in Figure 4, which depicts graphics for each transition and each compound representing the peak areas obtained at different energies. The energy providing the highest peak areas was selected as the optimal for that transition.

- Selection of the separation conditions

The conditions for the GC separation of the analytes were selected using the EI-QqQ(MRM) configuration described in the previous section. The compounds were previously derivatized following the above mentioned derivatization procedure [19]. The chromatogram obtained for the separation of the five compounds studied under the selected conditions is shown in Figure 5. We observed how a rapid increase of the temperature in the first temperature ramp allowed the quick elution of the first analytes. Then, in order to baseline-separate CET and BEN, a slow temperature increase of 1 ºC/min from 260 ºC to 264 ºC was required. After this, the temperature could be rapidly increased again in order accelerate the elution of THC and MOR. As expected, we did not observe differences in resolution between the two systems used. In the MRM mode, the noise obtained was very low compared to that obtained in the SIM mode, being almost zero most of the time. In fact, it was observed that the noise in SIM mode was six orders of magnitude more intense than in MRM mode, as Figure 6 shows. This is

characteristic of tandem MS, in which the majority of ions, including matrix ions, are eliminated before they reach the electron multiplier, producing very clean chromatograms.

- Study of the derivatization conditions

Different processes can be found in the literature for the derivatization of compounds in order to suppress active points in the molecules and increase volatility for GC analysis. Among them, silylation of polar groups with MSTFA is very practical, since it only implies the drying of the sample, followed by the addition of the reagent and application of heat. MSTFA can be applied for the derivatization of different compounds and different protocols can be found in the literature [18-22]. For this reason, we studied the derivatization of the drugs and metabolites with MSTFA combining two different times (i.e. 30 and 60 min) and two different temperatures (i.e. 80 and 100 ºC) with the EI-QqQ(MRM) configuration. As shown in Figure 7A, there are differences in the analyte signals, measured as peak areas, related to the derivatization conditions used and the studied compounds. For example, the signal of THC was decreased down to 75% when it was derivatized at 80 ºC for 30 min or 100 ºC for 60 min, compared to the derivatization at 100 ºC for 30 min, which was found the optimal for this analyte. For CET and MOR, the highest signals were obtained at 100 ºC for 30 min. COC presented more or less the same signal for all the derivatization conditions and BEN found the optimal at 100 ºC for 60 min. However, under these conditions the signals of the other four compounds were the lowest. For these reasons, we selected the derivatization at 100 ºC for 30 min for further experiments.

- Study of the injection process in the PTV system

The triple quadrupole system is equipped with a PTV injector which can be operated in different modes. In order to study the different possibilities, this system was again operated in the MRM mode, with the optimal derivatization process studied above. The evaluated injection modes were: constant temperature, in which the temperature remains constant during the transfer phase; constant temperature with a pulse of pressure that forces the analytes to transfer towards the column; PTV, in which the temperature is programmed during the transfer phase; and PTV with control of the injection pressure. The temperature in the PTV injection port in the constant temperature mode was 320 ºC, as well as in the constant temperature mode with pulse of pressure.

The initial temperature in both PTV modes was 130 ºC, which was increased at 14.5 ºC/s up to 320 ºC. The pressure pulse created in the injection port in the constant temperature and PTV modes with pulse of pressure was 150 kPa. As can be seen in Figure 7B, the PTV mode was found to be the injection mode providing the highest signals for the analytes. Compared to this mode, the constant temperature mode resulted in signals which decreased down to 42%. The PTV with control of pressure showed good signal intensities, in general, although the signal for BEN was decreased down to 73%. The constant temperature with pressure pulse mode also produced smaller signals, down to 48%, compared to the PTV mode. Therefore, the PTV mode was selected as the injection mode in the triple quadrupole system.

- Analytical performance of the different GC-MS configurations

Table 2 shows the analytical performance of the different systems operating in different modes, in terms of linear working range, repeatability, and intermediate precision, studied under the optimal conditions.

The r^2 values were good, showing that data were well fitted to their respective lines. These values were lower in the EI-QqQ(MRM) configuration. This is explained by the number of components in the mass spectrometer that are involved when MRM mode is used. In this case a first quadrupole, a collision cell, and a third quadrupole are working simultaneously, while in SIM mode only one quadrupole is involved in the analysis. The higher the number of components working in the mass spectrometer, the higher the variability expected in the results and the dispersion in the obtained data.

Two different concentrations were used to study the repeatability (0.1 and 1 Qg/mL) and it was expected to obtain better values for repeatability with the higher concentration. However, this only happened in the EI-QqQ(MRM) configuration. The rest of the values were similar showing very good repeatability values for the performed analyses. It is remarkable that the values obtained with the EI-QqQ(MRM) configuration at the low concentration were worse than the values obtained with the other configurations. Again, the high number of components involved in the mass spectrometer for performing MRM has a direct consequence in the variability of the registered data.

The results for the intermediate precision showed a good consistency in the data obtained during the days of analysis. Once again, the variability of the data obtained with the EI-QqQ(MRM) configuration was higher than the variability in the EI-QqQ(SIM) configuration, because of the reasons explained above. In this case, the variability of

data was lower at the higher concentration level, as it was expected at the beginning of the experiment. In addition, the intermediate precision was also studied in the PCI-Q(SIM) configuration with the detector operating in Absolute mode. This was done because, according to the software, the sensitivity of the detector can be adjusted to different modes. Gain factors have the advantage of not changing as the electron multiplier (EM) ages and produce much higher signal reproducibility for any instrument and better consistency between instruments. The detector can also be operated in the former Relative or Absolute modes, which do not offer any of these advantages. Results were compared to those obtained with the EM operated in the Gain factor mode and can be seen in Table 2. The numbers in brackets were obtained in Absolute mode and were, in general, much higher than those obtained in Gain factor mode (without brackets). Some of these values could even be considered unacceptable, in certain cases. Therefore, it is clear than the precision was greatly improved when the detector was operated in the Gain factor mode and its use is highly recommended. In the triple quadrupole system, the EM gain was set as default to 300,000 in SIM mode and 2,000,000 in MRM mode.

Limits of detection were calculated according to seven methods described in the experimental section. Standards at 0.1 Qg/mL were used in all the assays, for being considered a low concentration of all the analytes. Table 3 shows the obtained results. These results showed important differences in the obtained LOD values, which depended on the method used for their calculation. Differences also depended on the instrumental configuration used for the analyses. In general, LOD values obtained with methods using the standard deviation were higher than those obtained using the signal-to-noise ratio or three times the noise height. LOD can be calculated as 3sb/b, where sb is the standard deviation of the signal produced in a blank sample and b is the slope of the calibration equation. However, in chromatography, a blank sample does not produce any measurable peak, so it is impossible to calculate sb. Alternatively, samples in which the analyte is spiked at a concentration near the LOD values are injected and the value of sb is calculated from the standard deviation of the area [23, 24]. Only when the PCI-Q(SIM) configuration was used the situation was the contrary and the LOD values obtained using the standard deviation were lower than when using other methods. This was due to the low sensitivity obtained with this ionization mode, which provided much lower signal-to-noise ratios than EI ionization. The LOD values obtained with the PCI-Q(SIM) configuration using noise-based calculations methods were the highest values obtained, being much higher than those obtained experimentally. From these results, it is possible to deduce that the concentration used to calculate the LOD or the intensity of

the response of the approach used will determine first and foremost which calculation method will provide the lowest LOD values. Developing this idea, very low concentrations or methods providing poor responses will obtain better (lower) LOD values if a calculation method based on the standard deviation is used instead of a noise-based method, as in the case of the PCI-Q(SIM) configuration. For higher concentrations or configurations with higher intensities of response, the lowest LOD values will be obtained using the signal-to-noise ratio or three times the noise height instead of the standard deviation, as in the case of the EI ionization.

Software for data analysis use the signal-to-noise ratio to calculate the LOD. These softwares tend to get the noise height from the least noisy parts in the chromatograms, providing really low noise values. Therefore, the LOD values calculated by software are, by far, the lowest LODs that an analyst can obtain but those are, at the same time, too far from a real situation. For instance, because of the low noise obtained in the MRM analyses, the LODs obtained in these analyses were extremely low, down to 10 pg/mL, being six orders of magnitude lower than the values obtained experimentally. Clearly, the injection of standards at the concentrations corresponding to the LODs calculated by software did not show any visible peak. For this reason, it is extremely important to always explain how the LODs were calculated. This will give to the reader much more information about the obtained value and will allow to critically evaluate it. As these software use noise-based methods for the calculation of the LODs, the values obtained for PCI ionization were again the highest values obtained.

Finally, most of the LOD values calculated by plotting areas in calibration curves were lower than those obtained proportionally and, in general, closer to the values obtained experimentally. Regarding this, the LOD calculation based on visual evaluation is the method that provides the closest value to the real LOD. Following this method, the analyst is facing a real situation with real samples in which it is possible to evaluate the lowest concentration at which the analyte can be reliably detected. Moreover, according to the International Conference on Harmonization, if the limit of detection is calculated by other methods different than visual evaluation, a suitable number of samples known to be near or prepared at the LOD concentration should be injected in order to confirm that value [25, 26]. Therefore, any estimated LOD value (noise, standard deviation, and software-based methods) must eventually be visually verified [25]. In fact, visual evaluation has been found to be the most appropriate method for the analysis of LOD values in other works, providing more realistic values [27].

Although the reasons for the high variability in the LOD values obtained with the different methods have been explained and a comparison of different GC-MS configurations has been done, it is important to note that current mass spectrometers, mainly those working in tandem MS, provide very low noise signals, compared to other mass analyzers from 15 years ago. This often makes quite difficult to establish comparisons between different configurations since the noise height is, in most of the cases, very close to zero. This is especially clear in new high resolution MS analyzers or in tandem MS systems. In fact, in the last years, the meaning of using the signal-to-noise ratio as a measure for the evaluation of the performance in MS has been frequently discussed [28, 29].

Conclusions

In this work, a comparison of different GC-MS configurations for the quantitation of COC, CET, BEN, MOR, and THC was carried out. As expected, different configurations showed differences in the intensity of the response and this is of importance when dealing with samples with low amounts of analytes. Important factors, such as the derivatization of the analytes for GC analyses were also studied. The derivatization with MSTFA was proven to be effective and we found the optimal conditions for this derivatization at 100 ºC for 30 min. Regarding the injection mode, a PTV injector provided higher signals than an injector operated in a constant temperature mode. The optimization of these parameters is important and results in an increase of sensitivity.

The complexity of the mass analysis process affects the linearity of the data and the analytical precision, as demonstrated the results obtained with the triple quadrupole analyzer working in MRM mode. Therefore, a system working in SIM mode (single or triple quadrupole) provided better repeatability values than a triple quadrupole operated in the MRM mode, although the intensity of the response obtained could be lowered. Since the results obtained in MRM mode provided acceptable repeatability, we recommend the use of MRM when possible, in order to gain selectivity and sensibility. In general, we did not observe important differences in performance between systems from different suppliers.

A comparative study on different ways of calculating the LOD showed extremely large differences in the values obtained for the same analytes in the same systems. These results showed that the LOD values greatly depend on the sample concentration

and the system used. If the signal-tonoise ratio is big, a calculation method using the standard deviation will provide lower LOD values than a noise-based method. When having a lower signal-to-noise ratio, better LOD values will be obtained with a noise-based calculation method. On the other hand, it is important to consider that the noise values obtained by current mass spectrometers are very low. Hence, sometimes the comparison of different configurations is not possible. In fact, using the signal-to-noise ratio in MS is frequently considered meaningless. The decision on the LOD calculation method to be used will rely on the analyst, since all the described methods are accepted. Nevertheless, we suggest to calculate LOD values based on visual evaluation, since this method provides realistic values for a given concentration with a certain system.

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Figure Captions:

Figure 1. Structures of the studied compounds, after derivatization.

Figure 2. MS spectra of the studied compounds produced in the PCI ionization mode.

Figure 3. MS spectra of the studied compounds produced in the EI ionization mode.

Figure 4. Study of the energies in the collision cell for the five studied compounds and for the different selected transitions. Dots indicate the highest response obtained. PA, peak area.

Figure 5. Chromatogram for the separation of 10 µg/mL of cocaine (COC), cocaethylene (CET), benzoylecgonine (BEN), /9-tetrahydrocannabinol (THC), and morphine (MOR) with the optimized conditions: capillary column HP-5MS (30 m × 0.25 mm i.d., 0.25 µm film thickness). An initial temperature of 150 °C for 2 min was programmed in the oven, which was increased at 120 °C/min to 260 °C, then 1 ºC/min to 264 ºC and finally 120 ºC/min to 300 ºC. The final temperature was held for 2 min. Helium was used as carrier gas at a constant flow rate of 0.8 mL/min.

Figure 6. Comparison of the noise in the chromatogram obtained in SIM mode (A) and MRM mode. Separation conditions as in Figure 5.

Figure 7. Study of the derivatization process (A) and the injection process (B) under the optimal separation and MS detection conditions. CT, constant temperature.

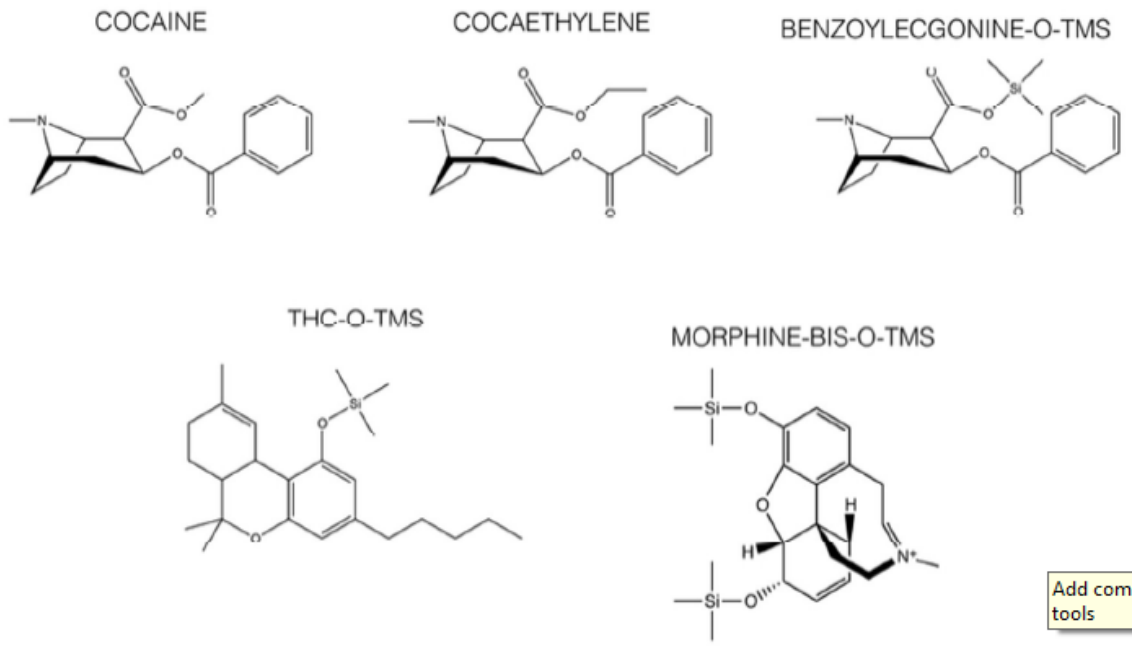


Figure 1. Structures of the studied compounds, after derivatization. 71x39mm (300 x 300 DPI).

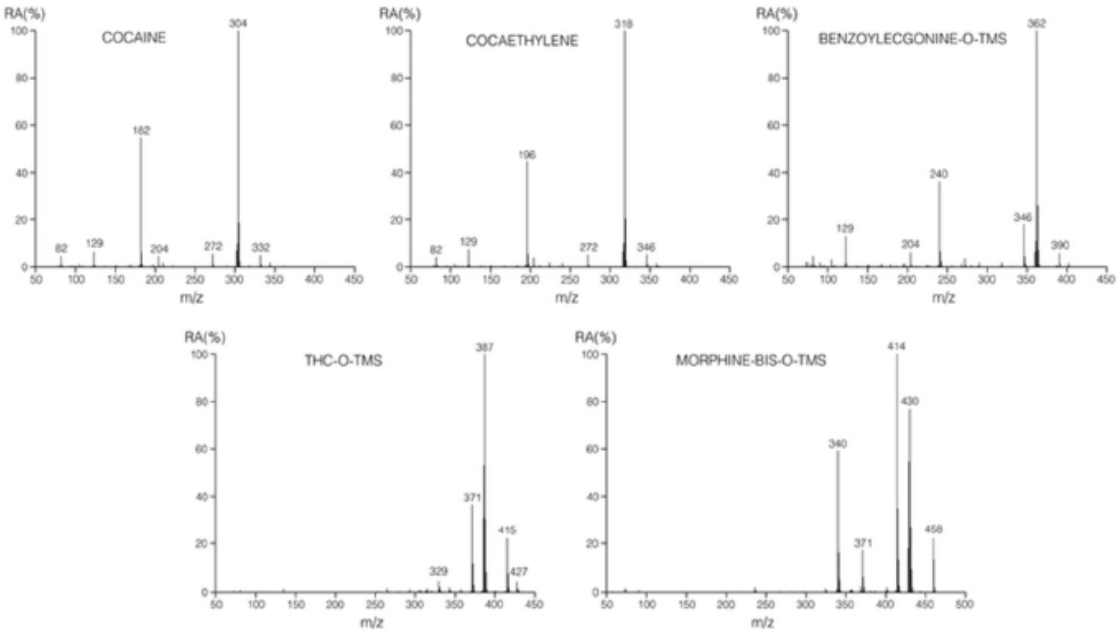


Figure 2. MS spectra of the studied compounds produced in the PCI ionization mode. 69x37mm (300 x 300 DPI).

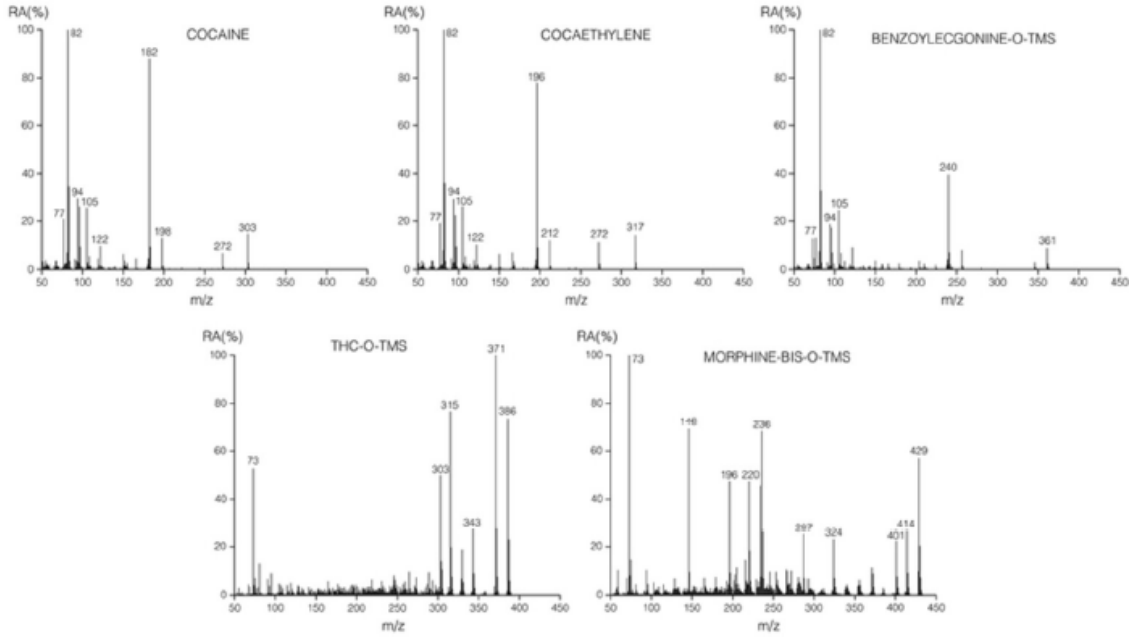


Figure 3. MS spectra of the studied compounds produced in the EI ionization mode. 70x39mm (300 x 300 DPI).

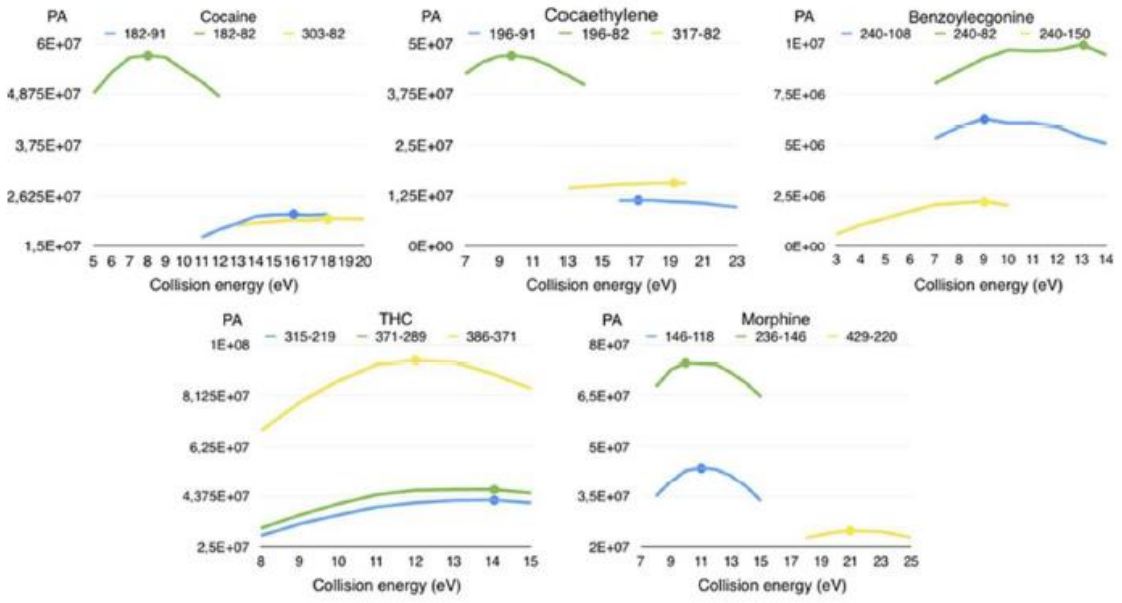
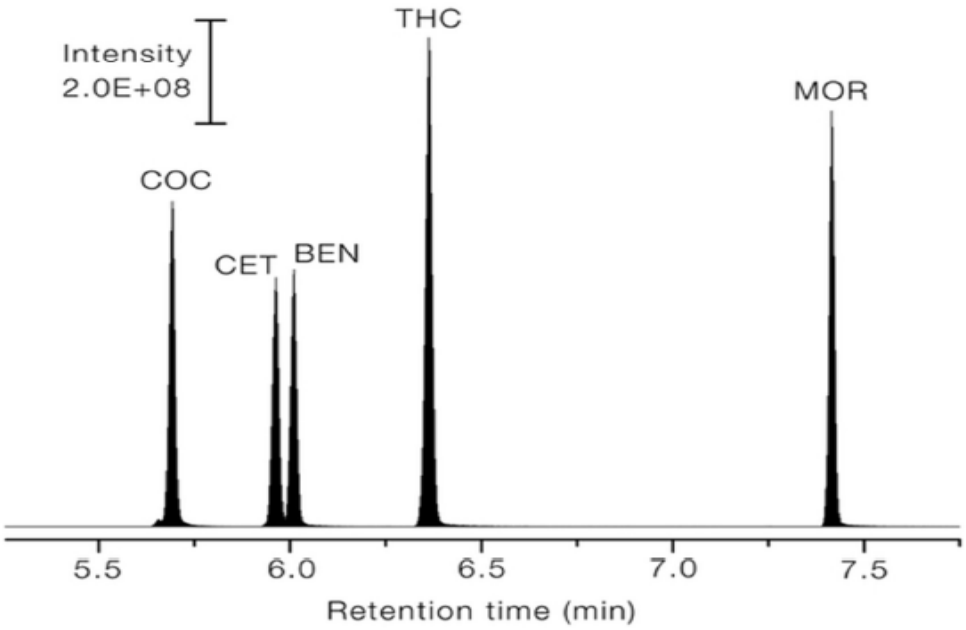


Figure 4. Study of the energies in the collision cell for the five studied compounds and for the different selected transitions. Dots indicate the highest response obtained. PA, peak area. 67x36mm (300 x 300 DPI).



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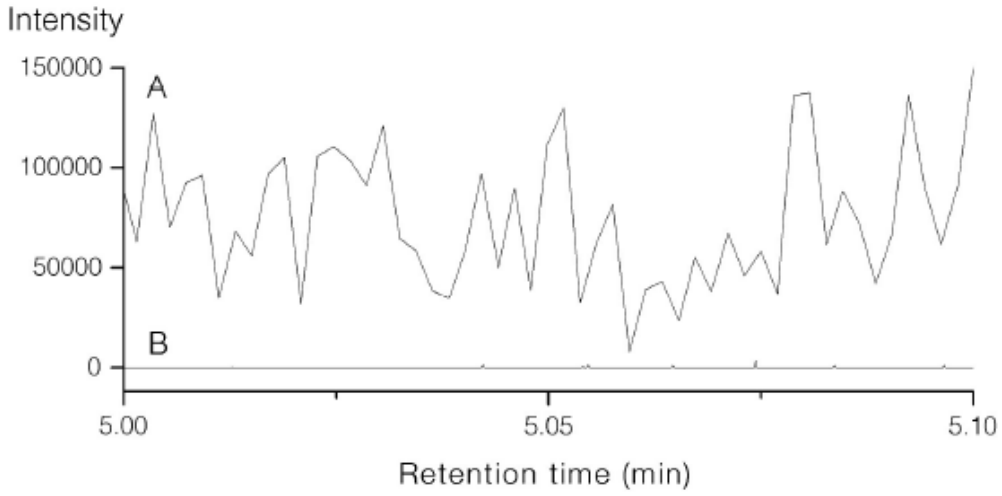

Figure 6. Comparison of the noise in the chromatogram obtained in SIM mode (A) and MRM mode. Separation conditions as in Figure 5.

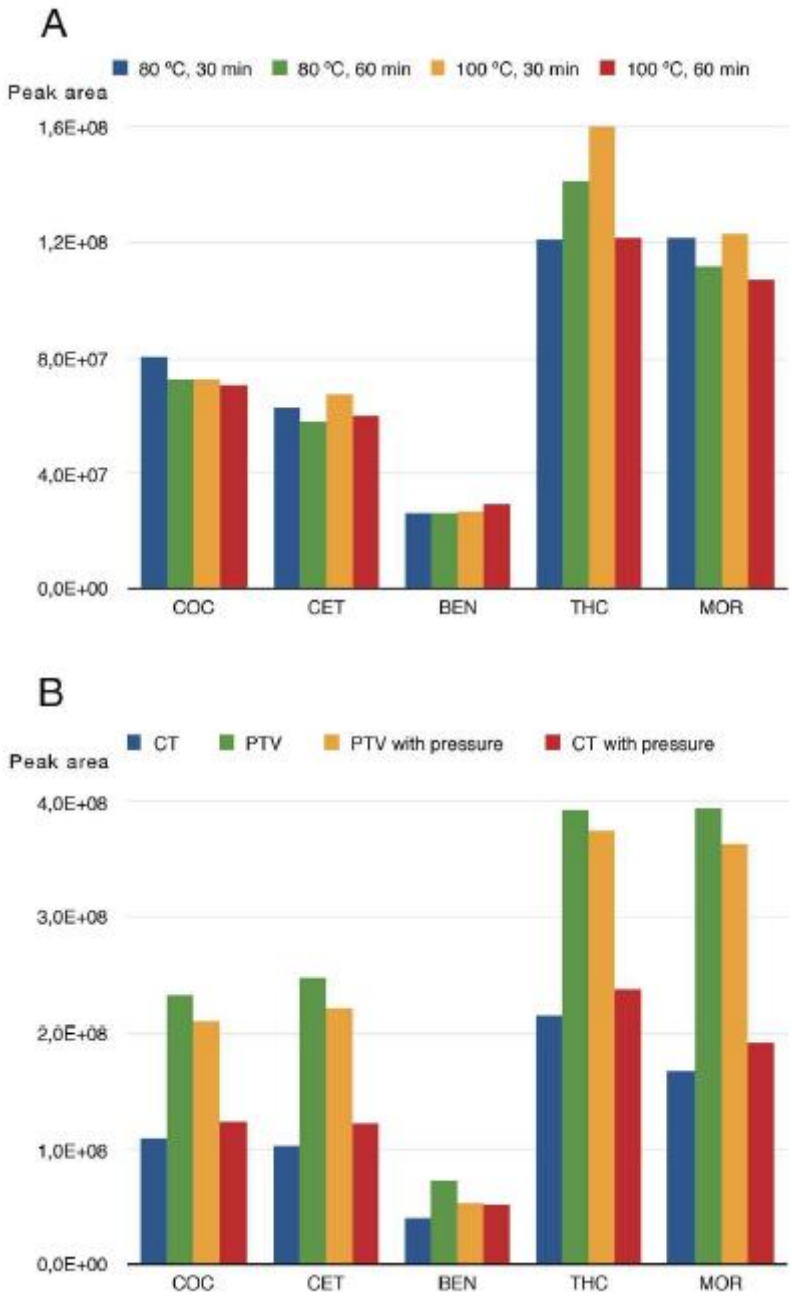

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Table 1. MS parameters of the analyses performed for the determination of the studied drugs and metabolites.

	RT (min)	PCI-Q(SIM)			EI-Q(SIM)			EI-QqQ(SIM)			EI-QqQ(MRM)				
		Selected ions	Quantitative ion?	Ion ratio[a]	Selected ions	Quantitative ion?	Ion ratio[a]	Selected ions	Quantitative ion?	Ion ratio[a]	Precursor ions (m/z)	Product ions (m/z)	Collision energy (eV)	Quantitative transition?	Transition ratio[b]
COC	5.71	304	Yes		182	Yes		182	Yes		182	82	8	Yes	-
		182		1.13 ± 0.10	303		5.24 ± 0.18	303		4.51 ± 0.55	182	91	16		2.77 ± 0.27
											303	82	18		3.47 ± 0.28
CET	5.93	318	Yes		196	Yes		196	Yes		196	82	10	Yes	-
		196		1.29 ± 0.10	317		4.80 ± 0.22	317		5.90 ± 0.35	196	91	17		3.31 ± 0.16
											317	82	19		3.00 ± 0.12
BEN	6.01	362	Yes		240	Yes		240	Yes		240	82	13	Yes	-
		240		1.62 ± 0.25	361		3.58 ± 0.22	361		5.02 ± 0.41	240	108	9		3.22 ± 0.15
											240	150	9		4.69 ± 0.18
THC	6.34	387	Yes		371	Yes		371	Yes		386	371	12	Yes	-
		371		2.54 ± 0.30	315		1.48 ± 0.13	315		1.27 ± 0.02	315	219	14		2.43 ± 0.26
		415		3.80 ± 0.86	386		1.15 ± 0.07	386		1.53 ± 0.02	371	289	14		2.40 ± 0.26
MOR	7.42	414	Yes		429	Yes		236	Yes		236	146	10	Yes	-
		340		1.82 ± 0.24	146		1.49 ± 0.33	146		1.26 ± 0.04	146	118	11		1.58 ± 0.08
		430		1.51 ± 0.21	236		1.10 ± 0.08	429		1.02 ± 0.07	429	220	21		3.92 ± 0.09

[a] Quantitative ion peak area divided by the qualitative ion peak areas. n=5. Calculated for concentrations of 1 g/mL for each analyte.
[b] Quantitative transition peak area divided by the qualitative transition peak areas. n=5. Calculated for concentrations of 1 g/mL for each analyte.

Table 2. Linear working range, correlation coefficients (r2) and precision (measured as repeatability and intermediate precision) for the studied drugs and metabolites with the different GC-MS working modes used.

	Concentration range (µg/mL)	r^2	Repeatability at 0.1 µg/mL (%RSD)[a]	Repeatability at 1 µg/mL (%RSD)[a]	Intermediate precision at 0.1 µg/mL (%RSD)[b]	Intermediate precision at 1 µg/mL (%RSD)[b]
			EI-QqQ(MRM)			
COC	0.01 - 25	0.9930	8.1	5.5	11.2	8.5
CET	0.01 - 25	0.9906	10.1	2.3	14.0	9.7
BEN	0.01 - 25	0.9881	8.9	3.2	12.4	10.1
THC	0.01 - 25	0.9987	4.5	4.7	9.9	7.8
MOR	0.01 - 25	0.9985	3.8	5.0	10.2	8.4
			EI-QqQ(SIM)			
COC	0.01 - 25	0.9994	4.6	4.9	6.1	7.9
CET	0.01 - 25	0.9992	7.4	7.6	8.8	6.9
BEN	0.01 - 25	0.9966	4.6	5.6	7.4	6.7
THC	0.01 - 25	0.9918	1.0	1.8	6.1	6.7
MOR	0.01 - 25	0.9983	1.8	2.4	5.5	6.6
			EI-Q(SIM)			
COC	0.01 - 25	0.9955	4.5	4.5	6.1	2.4
CET	0.01 - 25	0.9948	4.6	6.4	9.0	2.1
BEN	0.01 - 25	0.9964	4.2	4.6	7.5	6.5
THC	0.01 - 25	0.9987	3.9	4.5	5.2	7.6
MOR	0.01 - 25	0.9969	2.6	2.1	8.9	6.6
			PCI-Q(SIM)			
COC	0.01 - 25	0.9976	1.8	4.5	4.8 (12.4)[c]	5.6 (12.8)[c]
CET	0.01 - 25	0.9969	3.2	3.1	3.1 (9.5)[c]	2.6 (14.5)[c]
BEN	0.01 - 25	0.9969	3.4	6.1	6.2 (4.9)[c]	4.4 (15.9)[c]
THC	0.01 - 25	0.9950	5.0	4.1	5.5 (6.4)[c]	2.4 (13.8)[c]
MOR	0.01 - 25	0.9949	2.7	3.8	5.5 (7.3)[c]	5.2 (8.7)[c]

COC, cocaine; CET, cocaethylene; BEN, benzoylecgonine; MOR, morfine.
[a]n=5
[b]n=5 non-consecutive days over one week.
[c] Numbers in parentheses were obtained from analyses in which the detector was operated in the old Absolute mode.

Table 3. LOD values (ng/mL) for the studied drugs and metabolites, calculated according to seven different methods for the different systems and working modes used.

	SD-based		Noise-based		Software based		Experimental
	Method 1 3 x SD (proportional)[a]	Method 2 3 x SD (calibration curve)[a]	Method 3 S/N (3:1) proportional	Method 4 3 x noise (calibration curve)	Method 5 S/N (3:1) (proportional)	Method 6 3 x noise (calibration curve)	Method 7
	EI-QqQ(MRM)						
COC	24.3	13.2	2.7	1.9	0.0019	0.00011	11.0
CET	30.2	18.6	10.0	6.8	0.0065	0.00034	8.0
BEN	26.8	15.2	4.4	3.0	0.0011	0.000075	12.0
THC	13.5	11.5	4.7	3.4	0.0003	0.000013	5.0
MOR	11.4	11.0	1.2	1.6	0.0004	0.0000434	4.0
	EIQqQ(SIM)						
COC	13.9	11.6	5.4	5.8	0.48	0.14	6.0
CET	22.2	17.1	10.6	9.2	0.72	0.083	10.0
BEN	13.8	8.4	6.9	4.4	0.65	0.078	8.0
THC	2.9	4.6	6.1	8.0	0.20	0.068	2.0
MOR	5.5	6.8	7.3	10.7	0.52	0.31	4.0
	EI-(SIM)						
COC	13.4	6.0	11.7	4.8	1.4	0.55	7.0
CET	13.8	5.6	17.3	6.1	1.2	1.06	5.0
BEN	12.5	4.9	13.1	4.1	1.8	0.61	4.0
THC	11.8	4.1	9.4	3.6	1.2	0.73	2.0
MOR	7.9	1.8	18.6	5.5	3.4	1.44	5.0
	PCI-Q(SIM)						
COC	5.5	4.6	88.0	81.8	45.4	24.6	20.0

[a]Calculated as 3 times the SD at 0.1 4g/mL; n=5.