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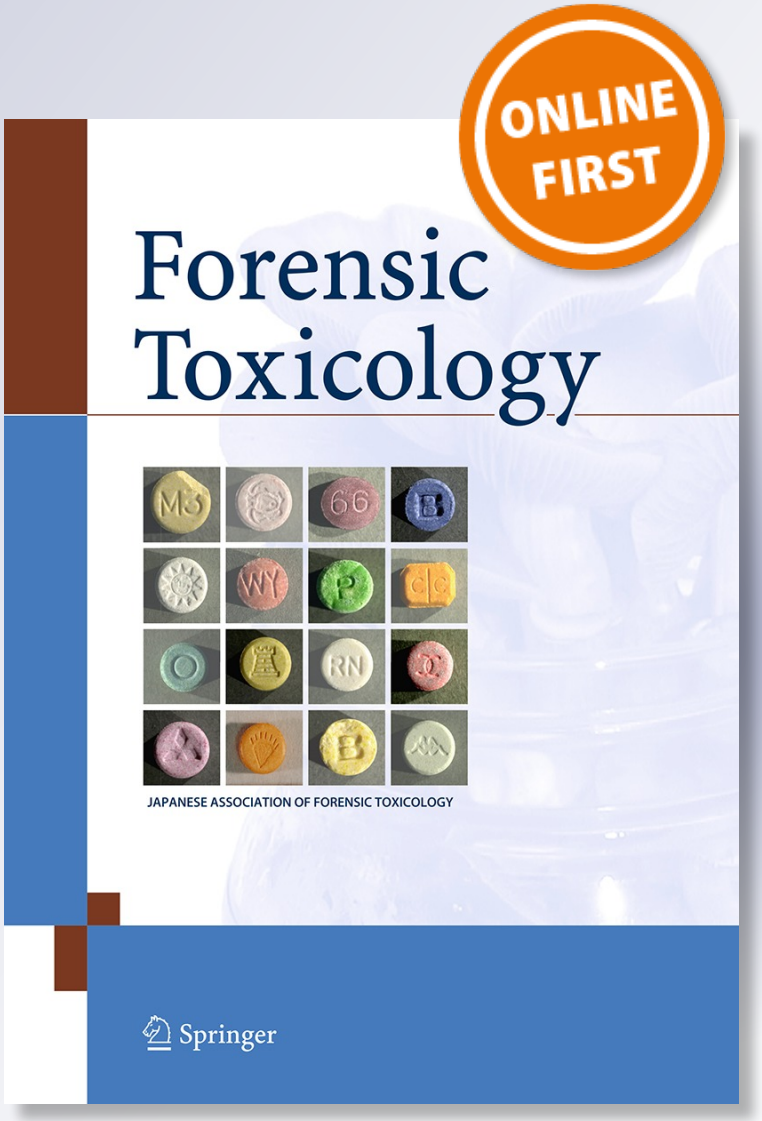

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SHORT COMMUNICATION

Single injection quantification of cocaine using multiple isotopically labeled internal standards

Lana Brockbals[1] · Morten Karlsen[2] · John Ramsey[1] · Bram Miserez[1]

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Abstract The purity of street cocaine is an important parameter in drug research and for forensic purposes, as it can be used to group samples, determining their origin, and to assess the monetary value of a drug sample. Current methods require batch processing of samples, as calibration curves need to be run. In this paper, a method is presented for the quantification of cocaine by single injection, avoiding the need for a calibration curve by using multiple, differently isotopically labeled internal standards. Using this fast method, results can be reported immediately after analysis and fully open-access mass spectrometric analysis becomes possible. The method was fully validated, with recoveries compared to certified standards of 99–101 % and to an accredited method from an independent laboratory of 86–117 %. Precision was tested both interday and intraday on three levels and all relative standard deviations were lower than 6.1 %. A linear response was found down to a purity of 1.3 %. The total analysis time for a single sample was approximately 30 min. The method was applied to 106 cocaine samples collected from a large UK music festival. Cocaine purity ranged from 1.3 to 78.8 %, with a mean of 43.1 %. This was comparable to other studies of UK cocaine samples. Our new approach has the potential to be applied to simple quantification of a variety of analytes in biological and non-biological samples.

✉ Bram Miserez
bmiserez@sgul.ac.uk

[1] TICTAC Communications Ltd, St George's University of London, Cranmer Terrace, London SW17 0RE, UK

[2] Chiron AS, Stiklestadveien 1, 7041 Trondheim, Norway

Keywords Cocaine analysis · Single injection GC–MS analysis · Cocaine purity · Multiple isotopically labeled internal standards · ICAL-GC–MS · Rapid analysis

Introduction

Open access (STAT, Statim; immediately) mass spectrometry (MS) analysis, with the possibility to run samples singularly in any order instead of in batches, would speed up the reporting time in most analytical laboratories, as samples no longer need to be batched before an analysis is performed. This concept is impeded by the necessity for calibration curves, as they are only economically viable when several samples need to be run. Isotopic internal calibration (ICAL) liquid chromatography–tandem mass spectrometry (LC–MS/MS) has been described as a way to circumvent the need for calibration curves, and thus, make open access mass spectrometric analysis possible [1]. A method for the quantification of clozapine and norclozapine (*N*-desmethylclozapine) in serum has been published by Couchman et al. [1], using multiple, isotopically labeled internal standards (ISs) (clozapine-D_4, clozapine-D_8 and norclozapine-D_8). A calibration curve can be run in with each sample using multiple isotopically labeled ISs. The method was fully validated and shows the potential for open access MS by using ICAL-LC–MS/MS.

In forensic toxicology, gas chromatography–mass spectrometry (GC–MS) remains the preferred method for both qualitative and quantitative analysis. ICAL-GC–MS could be used and has many advantages: no matrix effects, identification of other compounds in the sample, shorter analysis time and economic gains. As a calibration curve is no longer needed, analysis time per sample is reduced, while at the same time, the cost of making a calibration

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curve, preparation time and calibrating standards needed for calibration can all be reduced. As collecting samples to run in a batch is no longer needed, the reporting time is also reduced to a little more than the time for a single GC–MS run. In ICAL-GC–MS, the identification of other compounds in the sample, such as cutting agents or adulterants, is still possible, as quantification can be performed in full scan or by timing a selected ion monitoring method. Another advantage of ICAL, in both LC–MS and GC–MS, is the exact matrix matching of the calibrators, as they are run within the sample. Thus, ion suppression or other matrix effects are always perfectly corrected.

Cocaine is one of the most commonly abused substances and has been banned in the United Nation Convention on Psychotropic Substances of 1971 [2]. A clandestine trade has existed in cocaine for years and the United Nations estimated the value of the global cocaine to be 85 billion US dollars in 2009 [3]. When studying a cocaine seizure, the purity of cocaine is often very important, as the purity determines the value of the seizure and hence often the penalties for those convicted. What is more, samples can be grouped by purity or by cutting agents present, to trace them back to a street dealer for example.

Over recent years, several methods for the determination of the purity of street cocaine have been published and several bodies have published guidelines or standard methods. Analysis by GC–MS was proposed by Broséus et al. [4], Evrard et al. [5] and Magalhães et al. [6]. Further, published research articles by Floriani et al. [7] and Scheider and Meys [8] in addition to the United Nation Office on Drugs and Crime [9] suggested high-performance liquid chromatography coupled with a diode array detector as a reliable qualitative and quantitative separation technique for the analysis of cocaine.

In this study, ICAL-GC–MS is used for the first time and a method for the quantification for cocaine is presented. Three isotopically labeled ISs are used for calibration; cocaine-D_3, cocaine-$^{13}C_6$, and cocaine-$^{13}C_6D_3$. Validation included precision, robustness, linearity and accuracy, by comparison to reference standards and a validated method. The method was successfully applied to street samples from a major UK music festival and purity of cocaine was found to be between 1.3 and 78.8 %, with an average of 43.1 % (purity expressed as cocaine base).

Materials and methods

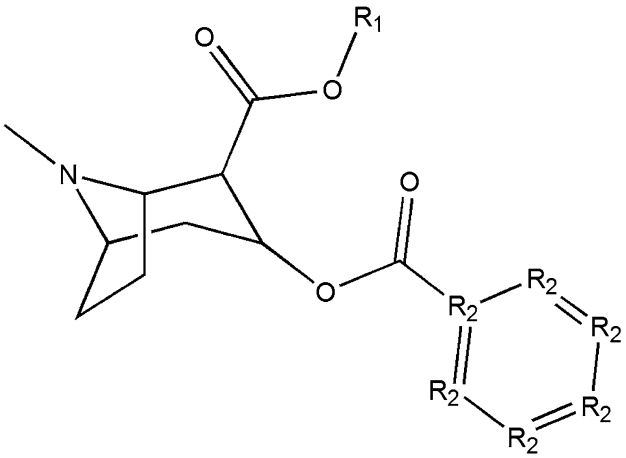
Cocaine purity units

Cocaine purity in this paper is expressed as weight percentage of cocaine base (weight of cocaine base divided by weight of the entire sample) and expressed as "%". Street cocaine powder is cocaine hydrochloride which is water soluble and usually snorted or injected whereas crack cocaine, which is usually smoked, is cocaine free base. As such, the purity of a cocaine hydrochloride sample, expressed as cocaine base, as used in this paper, is maximally 89.5 %, as the other 10.5 % weight in the sample is taken up by hydrochloride.

As the samples were not tested for the presence of hydrochloride, it was deemed more useful to express all purity as % cocaine, and not % cocaine hydrochloride, as theoretically, a sample containing cocaine base can have a purity over 100 %. This is the usual practice adopted by UK forensic science laboratories. However in the current literature, cocaine purity can be expressed as % cocaine base or % cocaine hydrochloride. For all cited numerical figures, where numbers indicate % cocaine hydrochloride, it has been recalculated to % cocaine base.

Chemicals

Methanol (Chromasolv® for HPLC ≥ 99.9 %) and methyl-*t*-butyl ether (MTBE, Chromasolv® for HPLC, ≥99.9 %) were bought from Sigma-Aldrich (Dorset, UK). The ISs were kindly provided by Chiron AS (Trondheim, Norway) and consisted of three differently isotopically labeled internal standards dissolved in methanol: cocaine-D_3, cocaine-$^{13}C_6$ and cocaine-$D_3^{13}C_6$ with masses 306, 309, and 312 Da, respectively. Structures are shown in Fig. 1. All



Analyte	R_1	R_2
Cocaine	CH_3	C
Cocaine-D_3	CD_3	C
Cocaine-$^{13}C_6$	CH_3	^{13}C
Cocaine-$^{13}C_6D_3$	CD_3	^{13}C

Fig. 1 Structures of the internal standards and cocaine

were present at different concentrations: cocaine-D_3 at 1 mg/mL, cocaine-$^{13}C_6$ at 0.4 mg/mL and cocaine-$D_3^{13}C_6$ at 0.2 mg/mL. A certified cocaine standard (purity 96.1 ± 2.6 %, Australian Government Measurement Institute, Lindfield, Australia) was used for accuracy testing.

Instruments

For accurate weighing of the samples, a balance with readability to 0.00001 g was used (Sartorius Genius ME235P, Göttingen, Germany). For GC–MS measurements, an Agilent Technologies 7890 GC system with 5976C GC MSD single quadrupole MS (Agilent Technologies, Santa Calara, CA, USA) was used. The GC was equipped with a split-splitless injector and an HP5-MS column (30 m length, 0.25 µm film thickness, 0.25 mm internal diameter, Agilent Technologies). Fourier transform infrared (FT-IR) analysis of street samples was carried out on a Bruker Alpha (Bruker, Billerica, MA, USA) with attenuated total reflectance (ATR) option.

Methods

GC–MS

For the final method presented here, 10 mg of a cocaine sample was weighed out and dissolved in 10 mL of methanol, the recommended solvent for cocaine analysis [6]. Twenty microliters of this solution and 20 µL of IS solution were added to 100 µL of MTBE in a GC-vial with 6 mm polyspring insert (300 µL, silanized; National Scientific, Rockwood, TN, USA). This was analysed by GC–MS using the following settings: injection 10 µL; split ratio 1:5; injector temperature 225 °C; gas helium at 1 mL min^{-1}; MS source temperature 230 °C; MS quadrupole temperature 150 °C; gradient: 0–4 min 80 °C, 4–11 min increase by 40 °C/min to 290 °C; MS settings: 3–10 min scan *m/z* 40–400, 10–10.5 min scan *m/z* 300–320, 10.5–11 min scan *m/z* 40–400.

Quantification of cocaine was performed by comparing the cocaine peak area to the calibration curve constructed using the three ISs. As the isotopic labels (see Fig. 1) were far apart, the molecular ion could be used for quantification. Practically, extracted ion chromatograms at *m/z* 303, 306, 309 and 312 were made. All were integrated. A calibration curve with the areas of the peaks at *m/z* 306, 309 and 312 was made. A linear curve, fitted through zero, was used and the concentration of the cocaine in the sample calculated. A linear curve, not fitted trough zero, was also tested, but gave slightly poorer results in accuracy.

ATR-FT-IR spectroscopy

Street samples were initially identified by single bounce ATR-FT-IR spectroscopy. An aliquot of the sample was loaded onto the ATR crystal and analysed (average of 16 spectra taken). The resulting spectrum was compared to the TICTAC FTIR Spectral Library (TICTAC Communications Ltd, London, UK).

Validation and quality control

Method validation included accuracy, precision, linearity, and sensitivity. Accuracy was tested both with a reference standard and a comparison with an ISO 17025 accredited forensic laboratory (LGC Forensics, Teddington, UK). Five milligrams of the reference standard was weighed out and dissolved in 10 mL of methanol and processed as a sample, with a theoretical concentration of 48 %. The solution was also diluted 1/2 with methanol, to achieve a theoretical concentration of 24 %. Both samples were analysed in triplicate. For the comparison with the accredited laboratory, ten street samples previously analysed by LGC Forensics, were analysed blind by the newly developed method.

Both intraday and interday precisions were tested by running three street-seized cocaine samples ten times on one day and twice on five consecutive days. Purity of the tested samples were 17, 49, and 78 %, to test the entire range.

Linearity was tested by diluting three high concentration samples (purity measured as 83, 79 and 75 %). All dilutions were performed in triplicate and concentration ranged from 83 % to below 1 %. This also allowed estimation of the lower limit of quantification.

As a quality control (QC), the same homogenized street sample of high purity (68 %) was analysed after every tenth sample, and as the first and last sample. For the QC sample, an acceptance criterion was set as z value <2 (based on a batch of ten analyses of this sample). Prior to each batch, a solvent blank (only MTBE) and a blank (20 µL IS solution in 100 µL MTBE) was run.

Street samples

Street samples were collected at a music Festival in 2014. Powders were analysed by ATR-FT-IR spectroscopy as described above. Samples with a spectrum showing features matching cocaine or a known cutting agent of cocaine (benzocaine for example) were included in this study ($n = 106$). All samples were analysed for purity by the described GC–MS method. Powders identified as a cutting agent were further analysed by subtracting the spectrum for the cutting agent and identifying the remaining spectrum.

All powder samples not identified by ATR-FT-IR spectroscopy as cocaine or a main cutting agent, were analysed by routine GC–MS for identification purposes but none were shown to contain cocaine.

Results and discussion

Validation

Accuracy

Accuracy was tested using a commercial primary standard (PS) and by analysing ten samples previously analysed by an ISO 17025 accredited forensic laboratory. Using the PS, two samples were made, with a theoretical concentration of 24–48 %. Analysis of the samples was performed in triplicate and recoveries were all within 99–101 % accurate, showing extremely good accuracy.

Ten real street samples, previously analysed by an ISO 17025 accredited laboratory (LGC Forensics, Teddington, UK), were analysed, both in triplicate (comparing the average of the three analyses) and in a single run. The purity of the samples ranged from 6 to 82 %. Recovery values for the new method using a single run for each sample were between 86 and 117 %. The comparison to the accredited method is shown in Fig. 2. Analysis in triplicate showed no improvement. The generally accepted accuracy criterion of being within 15 % of the nominal value was not met for one sample; a sample with purity of 6 % according to the accredited method was 7 % according to our method. This difference is however acceptable. Eight out of ten samples were within 10 % deviation and five within 3 %.

Comparison to a standard is incomplete (as no matrix is present), but comparison to an existing method is always flawed by inaccuracies in the existing method. Hence, it can be concluded the accuracy of the new method is sufficiently acceptable.

The agreement of the results by the present new method with those previously analyzed by an ISO 17025 accredited forensic laboratory (Fig. 2), and the excellent linearity of an IS calibration curve of different kinds of stable isotopes (Fig. 3d) show that the isotopes do not almost affect the peak areas.

Precision

Precision of the new method was tested using three street samples, with purities of 17, 49 and 78 %. Results are expressed as relative standard deviation (RSD). The low sample (purity 17 %) showed an intraday precision of 6.1 % and an interday precision of 5.4 %. For the medium purity sample (purity 49 %), precision was 3.0 % intraday and 5.0 % interday. For the high purity sample (purity 78 %), precision was 3.3 % intraday and 4.1 % interday.

The values for both the medium and high purity samples are deemed excellent (RSD $\leq$ 5 %). For the low purity sample, the interday value was lower than the intraday value, which was unexpected. The values are however still acceptable, as they remain well below 10 %.

Linearity and sensitivity

To assess linearity, dilutions of three high purity street samples were used. Ten milligrams of each sample was weighed and dissolved in 10 mL methanol. This methanol solution was diluted in steps six of 1/2. Twenty microliters

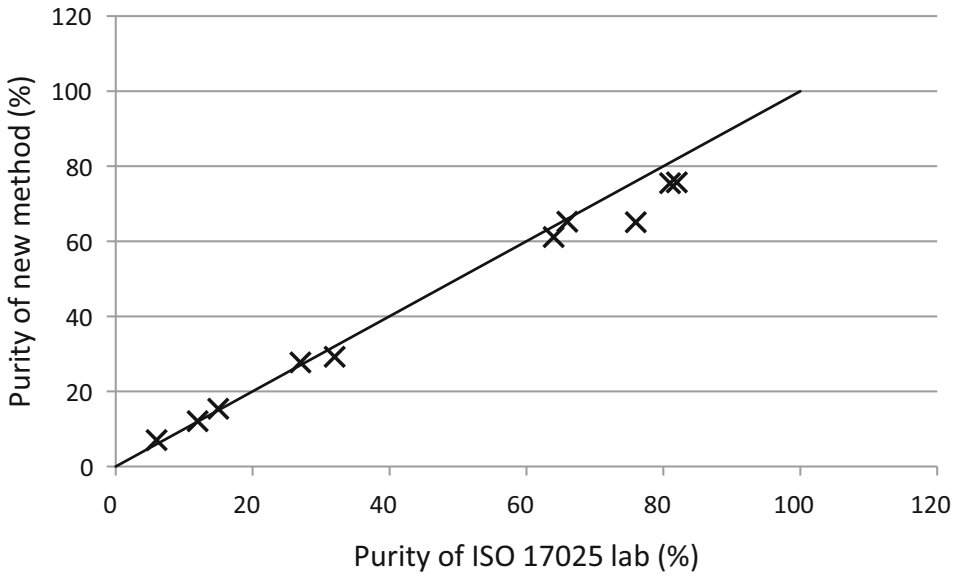


Fig. 2 Accuracy of the new method compared to an ISO17025 accredited method. Full line: ideal fit ($y = x$), cross means real sample

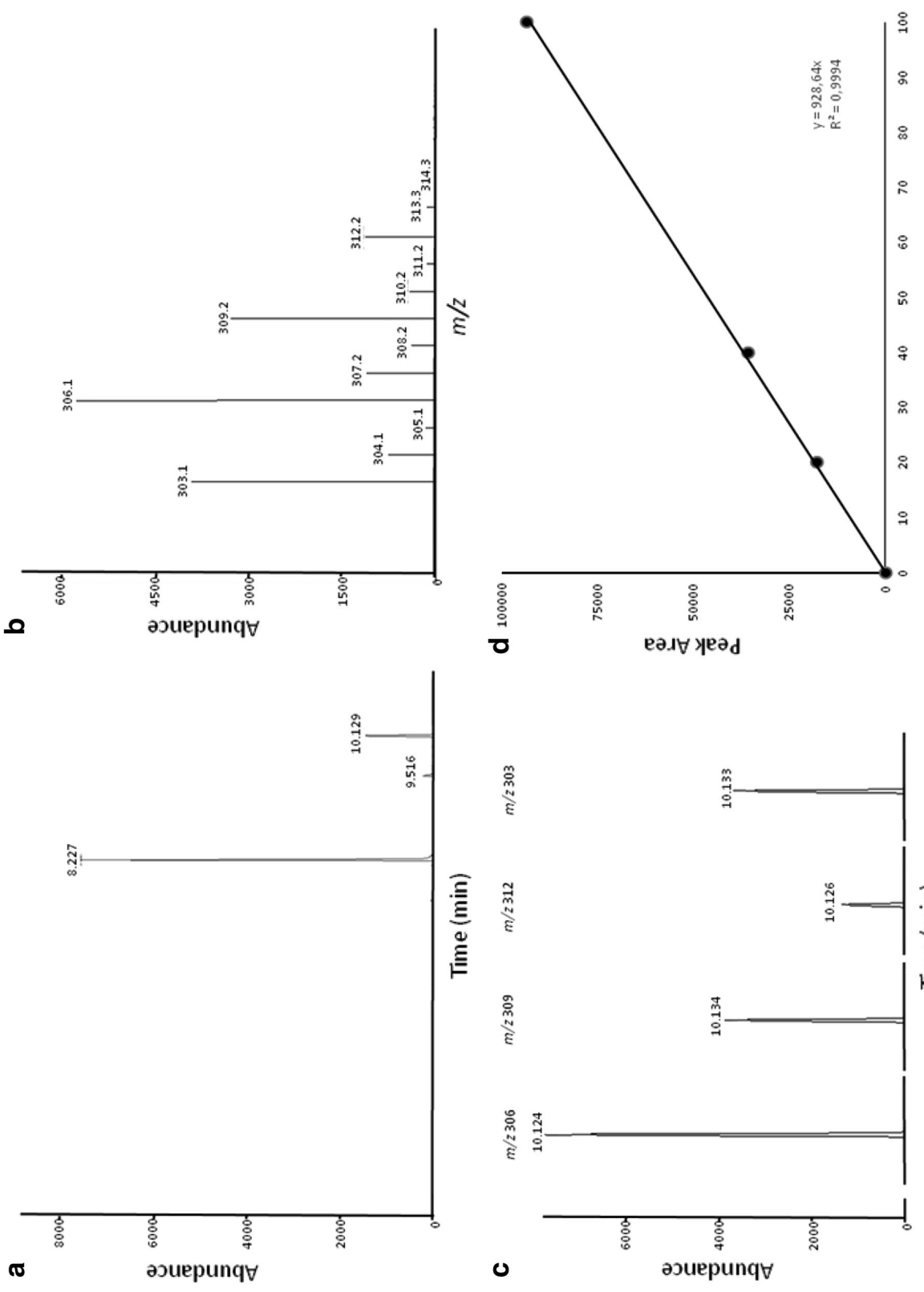


Fig. 3 Gas chromatography–mass spectrometry analysis of a cocaine sample. **a** Total ion chromatogram, peak identification: 8.227 min benzocaine; 9.516 min tetramisole; 10.129 min cocaine. **b** Mass spectrum at 10.129 min. **c** Extracted ion chromatograms at *m/z* 306 (cocaine-D_3), 309 (cocaine-$^{13}C_6$), 312 (cocaine-$^{13}C_6D_3$) and 303 (cocaine). **d** Calibration curve based on peaks shown in **c**

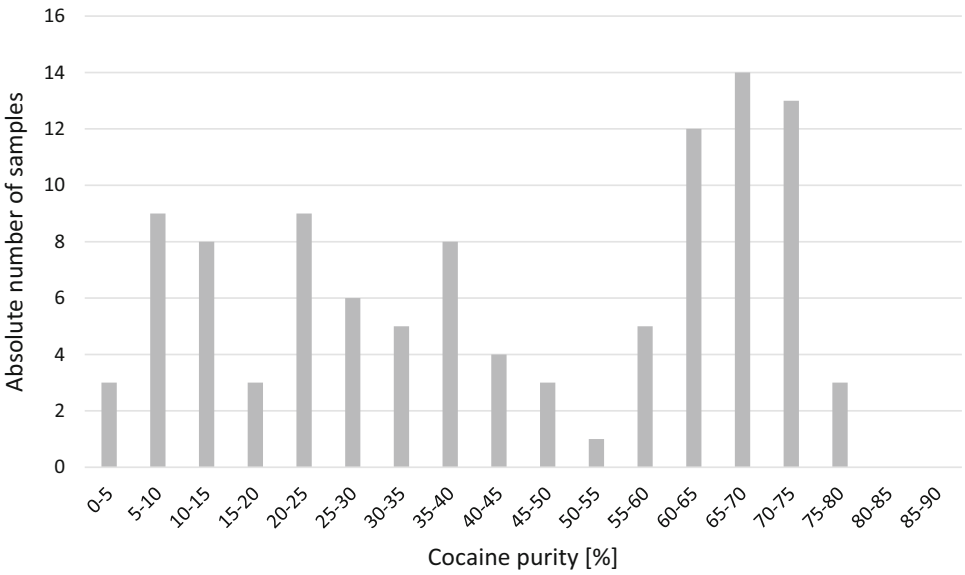


Fig. 4 Purity distribution of cocaine samples from a major UK music festival, expressed as cocaine free base ($n = 106$)

of the resulting solutions was added to 100 µL of MTBE and 20 µL of IS solution to determine linearity and sensitivity.

For all three samples, linearity was shown from the highest dilution (83, 79 and 75 % purity) down to 1/64 dilution (1.3, 1.2, and 1.2 %, respectively), with R^2 values all above 0.99 and in two cases above 0.999 when forced through zero. Signal-to-noise ratios at these concentrations were 10; the lower limit of quantification for this method was determined as 1.5 % purity.

Robustness

Typical problems with quantification of cocaine in street samples are their inhomogeneity and the picking up of water from the atmosphere [4, 10]. Sample inhomogeneity is especially problematic for large samples, as small samples can be homogenized in a pestle and mortar. For an accurate result, samples can be dried overnight at 80 °C, resulting in a dry cocaine sample. This might of course result in a higher purity, as samples handled on the streets are not completely dry either.

There was no evidence of the formation of cocaine methyl ester from our use of methanol to dissolve the samples. Pyrolysis of cocaine free base to anhydroecgonine methyl ester (AEME) has been reported in the GC–MS analysis [11]. In our work, AEME was detected in trace amounts in almost all samples, but its presence did not significantly influence the accuracy of the results.

Specificity

In GC–MS, correct identification of the compound of interest is normally achieved using GC retention time and the mass spectrum. However, as this method uses a very small scan range at the retention time of cocaine (scanning *m/z* 300–320 only), comparison of the mass spectrum is impossible. However, the GC retention time stays intact and the isotopically labeled ISs elute almost at the same retention time as cocaine. Another point of identification is the *m/z* value. Despite the fact that the entire mass spectrum is not recorded, the molecular ion at *m/z* 303 is still a point of identification. A GC–MS analysis of a cocaine street sample can be seen in Fig. 3.

Two other points to make for the specificity of the method are that the samples have already been identified by an orthogonal technique, ATR-FT-IR spectroscopy, as cocaine or benzocaine and that the GC gradient used is in use by our laboratory (a private forensic laboratory specialized in identification of drugs of abuse and new psychoactive substances) and no compound eluting at the same retention time as cocaine has ever been observed in drug samples.

Comparison to other methods

When comparing the newly developed method with existing, published methods to determine cocaine purity, sample preparation and analysis modes were found to be similar in terms of chemicals used, number of steps and run time on the instrument [6, 7]. However, great differences in the speed of preparation, due to a 1 h long derivatisation step, could also be observed upon comparison with Broséus et al. [4]. Additionally, they describe the use of a 7-point external calibration curve, which results in the need to prepare extra samples in combination with a total run time of 28 min per sample, leading to a further reduction of the speed of analysis in comparison to the developed single-

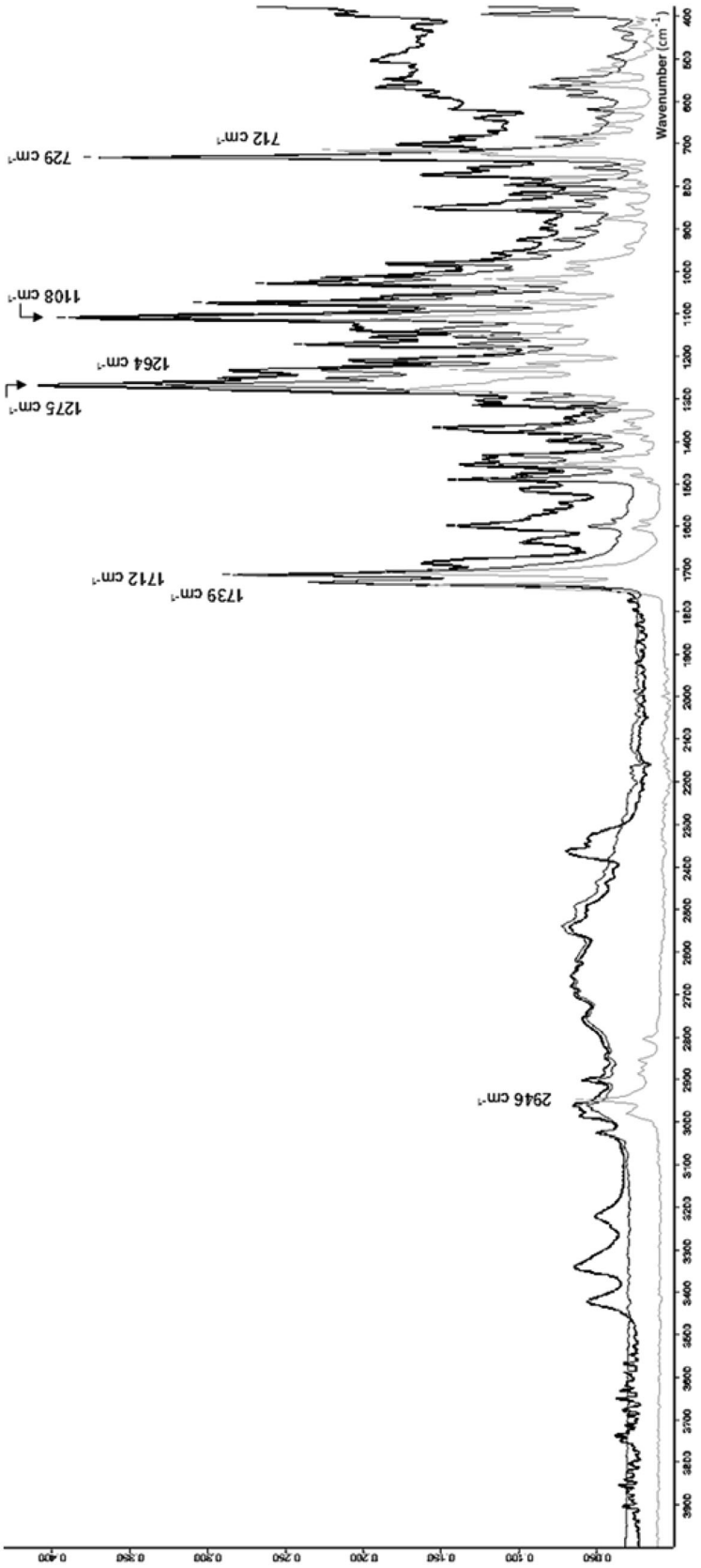


Fig. 5 Attenuated total reflectrance-Fourier transform-infrared spectroscopy analysis of a street sample (*black*) and comparison to cocaine-HCl (*dark grey*) and cocaine base standard (*light grey*)

injection quantification. Analysis time for our new method, from weighing the sample to GC–MS result can be less than 20 min.

In terms of precision and accuracy most studies used reference standards for their method validation [4, 6, 7], which makes it difficult to compare, as for the current method, street-seized cocaine samples were used to be able to assess the matrix effect and to develop a mode of analysis directly fit for routine work. Generally, accuracy values between 95 and 108 % were determined by Brosèus et al. [4]. and between 96 and 101 % by Floriani et al. [7]. In comparison to these, the confirmatory accuracy values of the newly developed method (99–101 %), are showing much more precise values, thus supporting the excellent suitability for routine use.

Street samples

Cocaine samples (106) from a UK music festival in 2014 were analysed using the newly developed method and purity ranged between 1.9 and 78.8 %, expressed as cocaine base. Mean purity was 43.1 % although it is notable that there are very few samples with this purity. As seen in Fig. 4, there were samples in the entire range of purity, with a peak around 70 % purity.

Compared to a recent study on cocaine in Finland, these findings show different results [4]. The analysed Finish samples showed an absence of samples with medium (45–55 %) purity. High purity samples were deemed to be sold as bought from producing countries, and low purity samples to be diluted greatly. In our UK samples, this was not the case: samples with all purities, from very low to quite pure were found.

As the samples were all festival ones, all samples where ATR-FT-IR spectroscopy could identify cocaine showed the presence of cocaine-HCl rather than cocaine base. An example is shown in Fig. 5. As such, a purity of 78.8 % cocaine base was very high compared to the maximal 89.5 % cocaine base content of a cocaine hydrochloride sample.

The high purity of this sample, but also the fact that 40 % of the samples have a purity higher than 60 %, seems a contradiction to the expectation to find low purity drug samples at festivals [12]. The wide spread of cocaine purity found in our sample set, 1.9 to 78.8 %, resembles the UK cocaine purity of 0.9–89.5 %, as reported by the European drug report [13]. This large variation in cocaine purity is a serious risk to consumers, as it is impossible for them to test the purity before use and as such, accurate dosing becomes impossible [14]. Mean purity for UK cocaine powder was 34 % in 2013, the year the report of EMCDDA in 2015 is based on. This is considerably lower than the 43.1 % that our samples showed. However, cocaine purity is rising in the EU since 2009, and it is possible the analysed festival samples just show this trend.

Conclusions

A fast, single injection quantification method for cocaine was successfully developed. The method used ICAL-GC–MS in the positive electron ionization mode for the first time and provided excellent validation results: accuracy between 99 and 101 %, precision between 3.0 and 6.1 % (%CV, interday and intraday on 10 repeated analyses). The method was shown to be linear down to a cocaine concentration of 1.3 % (% cocaine base). When applied to 106 samples from a UK festival, the concentration of the samples was shown to be between 1.2 and 78.8 %. This large variation is expected from previous studies on cocaine purity, but the large amount of high purity samples is surprising for a festival. Our new method has the potential to be applied to simple quantification of a variety of compounds in biological and non-biological matrices.

Acknowledgments Paul Bunt of Avon & Somerset Police for providing the cocaine samples from a music festival. Peter Caine of LGC Forensics for provision of the blind pre analysed cocaine samples for comparison. Bram Miserez thanks the EU-International Training Network SEWPROF (Marie Curie-FP7-People grant #317205). The financial support of the European Union is gratefully acknowledged.

Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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