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ORIGINAL ARTICLE

Tutorial on method verification: A routine method for the determination of heroin

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Abstract Method verification is crucial in ensuring that a routine quantitative method remains fit for analysis. Verification is less comprehensive than validation because fewer aspects are covered. In addition, the aspects to be verified must have a significant impact on the analytical readings. In this paper, a verification process is presented in the form of tutorial in order to aid narcotics laboratories in performing this task in a more competent manner. Although heroin is used as an example in this tutorial, the overall procedure can be extended to other drug compounds as well. The procedure presented here, however, serves as a minimum requirement. Additional aspects should be included to ensure that the overall verification process is able to meet the criteria set by the clients as well as the legal practitioners.

1. Introduction

An accurate and reliable method for determining the exact amount of illicit drug can never be compromised because the court of law relies on analytical results to make a fair judgement. In this regard, analytical methods for the quantification of narcotic drugs are important. The reported net weight will determine the fate of the accused. In any country, an analytically sound method for the determination of heroin is pivotal, seeing as how this illicit product remains the most widely abused substance throughout the world.

Illicit heroin is processed clandestinely and later diluted with a variety of diluents before it is sold on the streets. Previous studies established that at least seven major components, in addition to diacetylmorphine (or heroin), are quantifiable in the sample matrix seized in Malaysia.[1,2] In particular, opium alkaloids and caffeine constituted the major part of the sample matrix. Diluents especially caffeine added at the wholesale and retail levels have been found to have significantly diluted heroin to 1–50% in the bulk.

Many analytical methods discussed in the literature can be adopted for the quantification of heroin. Among all, gas chromatography coupled with flame ionization detector (GC–FID) remains the most ideal choice because it is rapid and versatile.[2,3] Alternatively, quantification of heroin is also possible with gas chromatography–mass spectrometry (GC–MS).[4–6] Other less routinely used techniques such as high performance liquid chromatography (HPLC),[7] Fourier transform infrared spectrophotometry (FTIR),[8] diffuse reflectance near-infrared spectroscopy (DR-NIR)[9] and micellar electrokinetic capillary chromatography (MECC) are among the options used by the researchers.[10,11]

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Certainly, each laboratory is fully responsible for establishing a method that is well suited for the local samples. Verification is performed if a laboratory prefers to directly adopt a published method. In other instances, it is carried out as a revision for a particular method that has already been in place for use. In this regard, basic aspects of method validation or verification must be fulfilled.[12,13] Although guidelines are available, some laboratories may still have lacked the relevant skills in performing method verification. Hence, the author would like to present a brief tutorial on this key matter by using heroin as a target analyte (6-monoacetylmorphine hydrochloride was included in the entire study but it is not discussed in this paper). It is hoped that with this tutorial, novices can benefit from the steps covered and be able to perform a more reliable verification task in their respective laboratories.

2. Materials and method

2.1. Standards and solvents

Heroin hydrochloride was commercially obtained from Sigma Aldrich. 2,2,2 Triphenylacetophenone was used as an internal standard (IS) and it was purchased from Aldrich Chemical Company. HPLC grade methanol and analytical reagent grade chloroform were both purchased from Fisher Scientific. Both solvents (9:1 chloroform:methanol) were employed to prepare a 0.18 mg/mL IS solution.

2.2. Gas chromatography–flame ionization detector (GC–FID)

An Agilent 6890 GC–FID system was used for analysis. Other parameters for the system are summarized in Table 1.

2.3. Statistical analysis

All GC data (peak area or concentration in mg/mL for heroin base) were statistically analyzed with Microsoft Excel and Minitab 15.

Table 1 GC–FID operating conditions for the quantification of heroin.[a]

Parameter	Condition
Column	J&W HP-5 (5% phenyl 95% methyl siloxane)
Dimensions	Length: 30 m I.D.: 250 μm Film thickness: 0.25 μm
Carrier gas	Helium
Pressure	134.7 kPa
Total flow	37.6 mL/min
Injection volume	1 μL
Split ratio	40.5:1
Flow rate	0.8 mL/min
Injector temp.	280 °C
Isothermal	260 °C for 11.30 min
Detector temp.	280 °C
H_2 flow	30 mL/min
Air flow	300 mL/min
He makeup flow	25 mL/min
Total run time	11.30 min

[a] This method has been in place for years and the author could not trace the origin of this method.

3. Selectivity & specificity

Selectivity/specificity depicts how well the target analyte such as heroin, can be separated from other commonly found components in a complex matrix. The matrix is usually country dependent. For instance, most heroin samples seized in Malaysia share a similar matrix background that is constituted mainly by caffeine, chloroquine, acetylcodeine etc. Therefore, a method must be validated/revised to ensure it functions well with the latest sample matrix. In this tutorial, a sample matrix containing nine components (including the target, diluents, alkaloid impurities etc.) in the presence of the IS (0.18 mg/mL) was cocktailed and analyzed by the GC system. Selectivity was checked by examining if all these components were well separated from one another on a chromatogram. Fig. 1 proves sufficient selectivity for the target analyte and IS, on which both also demonstrate good peak shapes. The names of these compounds are detailed in Table 2.

4. Precision studies

Area ratios (heroin relative to IS) were employed to evaluate precision. Precision expressed as the relative standard deviation (RSD); is useful to measure how reliable or consistent a method is in repeatedly analyzing a single sample without bias. Although most method verification procedures tend to include a standard solution to estimate the precision, this however does not reflect the performance of the method with real case samples. Alternatively, a standard solution as well as heroin samples containing the target analyte at routine concentration levels were analyzed to examine the intra-day precision (repeatability, $n = 10$) and inter-day precision (reproducibility, $n = 10$). The heroin standard (0.3600 mg/mL heroin) achieved RSDs = 0.40% and 0.29% for the intra-day and inter-day precision, respectively. Likewise, the heroin in the samples obtained RSDs = 0.34% and 1.02%. The performance is excellent as the results are very much lower than 5% which is the maximum RSD conventionally reconcilable by most narcotics laboratories.

Further statistical tests should be performed to ensure the reliability of the data. The intra-day and inter-day data of the heroin standard solution were tested for equal variances. Both Levene's test (p-value = 0.113) and F test (p-value = 0.377) showed no significant variance between the two data sets at a significant level p-value < 0.05 (meaning that both sets have equal variances). In other words, the system is able to give the same range of variances despite analysis being carried out on the same day or over a specified period of time.

In addition, control charts were plotted for the intra-day and inter-day data of the heroin present in the samples (whereby two independent weights were respectively used for intra-day and inter-day precision studies). Moving range was employed to detect the subsequent difference between two continuous data points. The charts (Fig. 2) display no systematic errors (e.g. four data points on one side). Random errors illustrated by the trend on each control chart are also acceptable.

5. Limit of detection (LOD) & limit of quantification (LOQ)

LOD is the lowest level of analyte that can be detected by the system. Conventional procedures tend to use 3 signal-to-noise