

Efficient application of monolithic silica column to determination of illicit heroin street sample by HPLC

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Abstract

In this paper, an HPLC method is proposed for a routine, rapid and simple analysis of heroin samples confiscated from the illicit market, based on a new type of packing for HPLC columns (monolithic silica). Acetonitrile and pH 3.5 phosphate buffer solution were used under both isocratic and gradient conditions. Under our analytical conditions, all the components of a typical mixture of an illicit heroin sample proved to be fully separated into well-resolved peaks in 7 min. Analytical linearity and accuracy of the method were also studied for all analytes using tetracaine hydrochloride as the internal standard.

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1. Introduction

Heroin is mainly found on the illicit market as a product of morphine acetylation. In clandestine laboratories, purification of morphine and heroin is seldom efficient. Therefore, other alkaloids from opium (and their acetylated derivatives) can be found in what is sold in the illicit market as clandestine heroin. Other substances such as analgesics, local anesthetics and carbohydrates are usually used as adulterants and diluents [1]; in particular, in recent years we have often found heroin samples confiscated from the illicit market in which significant quantities of caffeine and paracetamol are present.

Illegal heroin assumption still represents a major problem in our society and therefore the analysis of heroin samples confiscated from the illicit market still remains one of the main roles for forensic toxicologists; consequently, even if several valid HPLC and GC analytical procedures are nowadays available, the possibility of finding new alternative, simple, rapid, sensitive and low-cost analytical methods, which might be applied alone or in association with procedures already used, must be taken into account.

Up to now, various HPLC methods for the analysis of clandestine heroin have been reported (see for example [1–6]) and most of them use conventional HPLC columns.

Monolithic silica is a new type of packing for HPLC columns that has recently been introduced. These monolithic columns differ from conventional HPLC columns in that they are formed from a single piece of porous silica gel, possessing a higher porosity (macropores and mesopores), which creates a larger surface, and can therefore be operated at higher flow rates regardless of back-pressure. These particular features lead to excellent separations in a fraction of the time needed by a standard particulate column [7,8].

On this basis, the aim of the present work was the development and the optimization of an HPLC method using a monolithic silica column, which could be applied for routine, rapid and simple analysis of illicit heroin samples.

2. Experimental procedures

2.1. Standards and chemicals

Water used for HPLC was doubly distilled. All salts used for buffer solution were of analytical grade and organic solvents were of HPLC grade. Potassium dihydrogenphosphate, phosphoric acid solution and sodium hydroxide were

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obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile was also provided by Sigma-Aldrich (St. Louis, MO, USA). Standards of controlled drugs were purchased from Salars (Como, Italy).

Tetracaine hydrochloride, used as the internal standard and other pure standards of therapeutic drugs were also purchased from Sigma-Aldrich.

Milligram amounts of clandestine preparations of heroin were submitted for analysis to our laboratories by the Italian Police, as required by the law on narcotics (D.P.R. 309/90).

2.2. Instrumentation

Analytical HPLC analysis was performed on a Beckman System Gold apparatus under the following conditions: Merk Chromolith RP_18e column (100 × 4.6 mm I.D.); eluant A: 15 mM phosphate buffer (pH 3.5), eluant B: acetonitrile; 90% A over 3 min, flow 3.0 ml/min; gradient from 90% to 75% A over 5 min, flow 5.0 ml/min; reconditioning 90% A over 3 min, flow 3.0 ml/min; UV detection 210 nm.

2.3. Sample preparation

Stock solutions of tetracaine hydrochloride, used as the internal standard, were prepared at a concentration of 1 mg/ml in 15 mM phosphate buffer (pH 3.5) and stored at 4 °C.

Illicit heroin samples were prepared by dissolving 20 mg in 4 ml of the internal standard solution. The resulting mixture was kept at room temperature for 30 min and then filtered. Aliquots of 100 µl of the filtered solution were diluted to 1 ml with water and then injected.

3. Results and discussion

The aim of this study was to assess the advantages of a monolithic silica column for the HPLC analysis of heroin samples confiscated from the illicit market, with a view of their use in routine control.

De Leenheer and colleagues, recently reported an interesting, fast LC/SSI-ion trap MS method for the simultaneous determination of opium alkaloids present in illicit heroin samples, based on the use of a monolithic silica column [9]. The resulting TIC chromatogram of a mixture of opium alkaloids, albeit very short, efficient and suitable for MS determination, showed peaks which were not fully separated.

We decided to use a mobile phase composed of 15 mM phosphate buffer at pH 3.5 and acetonitrile; under optimized conditions, the elution program started with 90% buffer (and 10% acetonitrile), at a flow rate of 3.0 ml/min. After 3 min, the relative amount of acetonitrile changed linearly to 25% (and buffer changed to 75%) in 5 min, at a flow rate of 5 ml/min. The reconditioning time was 3 min, with 90% buffer (and 10% acetonitrile) at a flow rate of 3.0 ml/min. Therefore, the total analysis time for each sample, including re-equilibration and reporting, was 11 min.

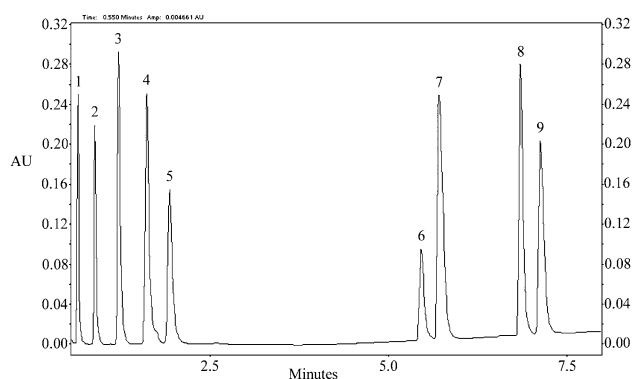


Fig. 1. Typical chromatogram of the separation of a mixture of (1) morphine, (2) paracetamol, (3) codeine, (4) caffeine, (5) monoacetylmorphine, (6) acetylcodeine, (7) heroin, (8) papaverine and (9) noscapine.

Fig. 1 shows a typical chromatogram obtained using a standard mixture of the main components usually found in clandestine heroin: under our analytical conditions, all the components of the mixture in 7 min proved to be fully separated into well-resolved peaks and migrated in the following order: morphine, paracetamol, codeine, caffeine, monoacetylmorphine, acetylcodeine, heroin, papaverine and noscapine.

Fig. 2 shows the chromatogram obtained from the injection of a sample of a clandestine preparation of heroin under the same analytical conditions, containing tetracaine hydrochloride as the internal standard: the chromatogram confirms the good separation of heroin from the other components.

The analytical linearity of the method was also studied for all analytes in the range 0.8–4.0 mg/ml. Linear regression equations were calculated by using the least-square method between analyte-to-IS peak area ratio and analyte concentration, with excellent correlation coefficients ($R^2 = 0.9997–0.9999$). The resulting equations are shown in Table 1.

The analytical accuracy was calculated by comparing the HPLC results of a real sample with those from a gas chromatographic method currently used in our laboratory [10]. The results of both methods are reported in Table 2.

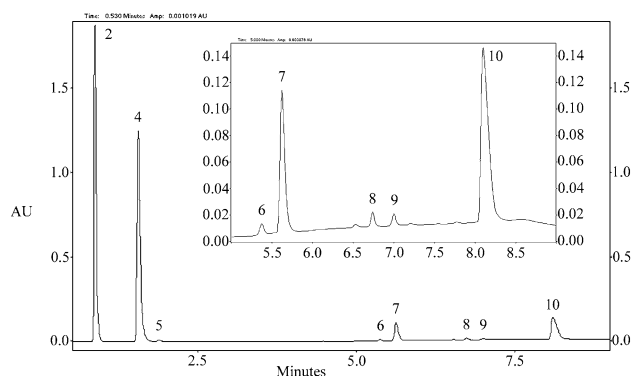


Fig. 2. Typical chromatogram obtained from real illicit heroin preparation containing (2) paracetamol, (4) caffeine, (5) monoacetylmorphine, (6) acetylcodeine, (7) heroin, (8) papaverine, (9) noscapine and (10) tetracaine (internal standard).

Table 1
Linearity equations and correlation coefficients

	Linearity equations	R^2
Morphine	$y = 0.2271x + 0.0164$	0.9998
Paracetamol	$y = 0.3714x + 0.0473$	0.9999
Codeine	$y = 0.6173x - 0.0119$	0.9999
Caffeine	$y = 0.4824x + 0.0388$	0.9999
Monoacetylmorphine	$y = 0.2272x + 0.006$	0.9999
Acetylcodeine	$y = 0.2512x + 0.0287$	0.9999
Heroin	$y = 0.2648x - 0.00165$	0.9997
Papaverine	$y = 0.5211x - 0.0265$	0.9998
Noscapine	$y = 0.3294x - 0.0193$	0.9999

Table 2
Comparison between HPLC and GC analytical accuracy for real sample

	HPLC (%)	GC (%)
Monoacetylmorphine	0.58	0.59
Heroin	8.01	8.16
Acetylcodeine	0.59	0.60
Papaverine	0.40	0.39
Noscapine	0.55	0.57

4. Conclusions

In the present paper, we report a new HPLC method, which make use of monolithic silica column for the analysis of heroin samples confiscated from the illicit market; the resulting chromatograms showed a good separation of heroin from the typical adulterants and original impurities. The monolithic silica column proved to be a useful tool for providing a routine, rapid and simple method, which can be used as a powerful complementary technique, particularly in

the case of global characterization of various illicit heroin samples for comparative purposes.

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