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Journal of Chromatography A, 692 (1995) 121–129

JOURNAL OF CHROMATOGRAPHY A

Qualitative and quantitative determination of illicit heroin street samples by reversed-phase high-performance liquid chromatography: method development by CARTAGO-S

Kathrin Grogg-Sulser, Hans-Jörg Helmlin, Jean-Thomas Clerc*

Institute of Pharmacy, University of Berne, Baltzerstrasse 5, CH-3012 Berne, Switzerland

Abstract

The development of a method involving reversed-phase HPLC using CARTAGO-S, a spreadsheet computer program as a method development tool is described for the simultaneous qualitative and quantitative determination of heroin in illicitly manufactured street samples containing by-products originating from opium and also commonly occurring adulterants that have been found in Switzerland. The method utilizes simple sample dissolution followed by reversed-phase HPLC on a 3-μm ODS-1 column with acetonitrile–water–phosphoric acid–hexylamine as the mobile phase. UV detection and quantification were carried out at 210 nm. The optimum linear gradient elution consisted of four steps with a total duration of 36 min. Excellent agreement between predicted and measured retention times with differences ranging from 0.45% to 6.8% was found. For calibration, all compounds showed a good linear relationship between peak area and concentration. The low UV cut-off of this mobile phase allows the detection of heroin and related compounds at its major UV absorption at 210 nm. With this method, the limits of detection absolute ranged from 10 ng for noscapine, corresponding to 0.09% of the sample mass, in the best case to 100 ng for cocaine in the worst case, depending on molar absorptivities.

1. Introduction

In recent years in Switzerland, there has been a strong increase in illicit drug consumption and in the number of overdoses and deaths related to drugs, especially heroin. The subject of this study was to analyse heroin street samples by HPLC and to study both qualitative and quantitative aspects.

Heroin is produced by acetylation of morphine originating from opium. In clandestine laboratories the purification of morphine and also of heroin is seldom efficient. The heroin produced contains significant amounts of the opium alkaloids and their acetylated products. In addition, most illicit heroin street samples are adulterated and/or diluted several times before reaching the user. Adulterants such as analgesics, local anaesthetics and caffeine are pharmacologically active substances that mimic the bitter taste of heroin, whereas carbohydrates such as lactose, mannitol and sucrose are inactive and are often used for dilution purposes.

Various methods for the analysis of heroin described in the literature encompass a diversity of chromatographic techniques such as GC with flame ionization detection [1–5], GLC [6] and GC–MS [7]. HPLC overcomes the problems associated with GC such as adsorption, heat instability, transesterification and solubility [8–

* Corresponding author.

0021-9673/95/$09.50
SSDI 0021-9673(94)00755-1

10], which result in poor precision and accuracy. The successful separation of opiates in illicit heroin by HPLC has been described by several workers [1,11–17].

This paper reports a fast and accurate HPLC procedure for the simultaneous determination of heroin, cocaine, acetylcodeine, noscapine, papaverine, 6-monoacetylmorphine, morphine, paracetamol, caffeine, benzocaine, lidocaine and procaine in illicitly manufactured heroin street samples using UV detection. The qualitative determination of codeine and 3-monoacetylmorphine is also reported.

Method development for the gradient separation of thirteen components found in illicit heroin street samples was performed with CARTAGO-S [18], a spreadsheet-based computer program, which is under development in our laboratory.

Because no single chromatographic procedure can overcome the co-elution of compounds, each sample was subjected to both HPLC and GC–MS. In addition, TLC screening was performed for sugars according to a routine method of the laboratory [19].

2. Experimental

2.1. Instrumentation

The HPLC system consisted of a chromatograph with two M 6000-A pumps and of a Model 660 solvent programmer (Waters, Milford, MA, USA), a Model 7125 injector (Rheodyne, Cotati, CA, USA) fitted with a 20-μl sample loop, an LC 55 spectrophotometer (Perking-Elmer, Küsnacht, Switzerland), and a Shimadzu Chromatopac C-R1 B data processor (Burkhard Instrumente, Zurich, Switzerland). Separations were performed at 25°C on a 125 × 4 mm I.D. column packed with 3-μm Spherisorb ODS-1 (Phase Separations, Deeside, UK), filled by Macherey–Nagel (Oensingen, Switzerland), with a 4 × 4 mm I.D. precolumn, packed with 5-μm LiChrospher 100 (Merck, Darmstadt, Germany). The mobile phases were (A) water containing 5.0 ml (8.5 g) of orthophosphoric acid (85%) and 0.56 ml (0.44 g) of hexylamine per 1000 ml and (B) acetonitrile–water (9:1; v/v) containing 5.0 ml (8.5 g) of orthophosphoric acid (85%) and 0.56 ml (0.44 g) of hexylamine per 1000 ml. The flow-rate was 0.7 ml/min. The eluents were filtered through a membrane filter (regenerated cellulose, 0.45 μm; Schleicher and Schuell, Dassel, Germany) and degassed by sonication. Methanol was used for washing the column. The detection wavelength was set at 210 nm.

2.2. Chemicals and reagents

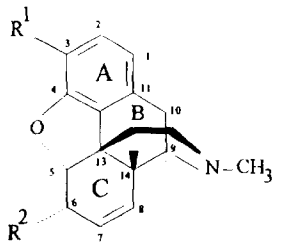
Hexylamine (99%, purum) was provided by Fluka (Buchs, Switzerland). Water used for HPLC was doubly distilled. All other chemicals and solvents were of analytical-reagent or HPLC grade, purchased from Merck. Morphine · HCl (**1**), cocaine · HCl (**11**), codeine (**4**), papaverine · HCl (**13**), procaine · HCl (**2**), lidocaine · HCl (**6**), paracetamol (**3**) and benzocaine (**8**), all of Pharmacopoeia Eur II grade, were obtained from Siegfried (Zofingen, Switzerland). Caffeine (**7**), pure, was obtained from Merck and noscapine (**12**) from Mepha Pharma (Aesch, Switzerland). Heroin (**10**), 6-monoacetylmorphine (**5**) and acetylcodeine (**9**) were prepared by the method of Wright [20] and 3-monoacetylmorphine (**14**) by the method of Welsh [21]; their purities were established by GC–MS. The structures of **1**, **4**, **5**, **9**, **10** and **14** are shown in Fig. 1.

2.3. Heroin street samples

Heroin street samples (ca. 30 mg) were collected by assistants of Contact (a governmental subsidized socio-medical institution for the support of drug addicts) in Berne directly from the users.

2.4. Gradient elution optimization

CARTAGO-S exploits the fact that the logarithm of the capacity factor varies approximately linearly with the concentration of the organic modifier. The parameters of this linear relationship are determined for each compound



	R^1	R^2
Morphine *(1)*	OH	OH
Heroin (10)	$COOCH_3$	$COOCH_3$
6-Monoacetylmorphine *(5)*	OH	$COOCH_3$
3-Monoacetylmorphine *(14)*	$COOCH_3$	OH
Codeine *(4)*	OCH_3	OH
Acetylcodeine *(9)*	OCH_3	$COOCH_3$

Fig. 1. Structures of heroin and related compounds.

using at least two isocratic runs. These are then used to predict the retention behaviour in piecewise linear gradients. The program is implemented in a spreadsheet and can be used for chromatogram simulation and for optimization.

2.5. Chromatographic conditions

The optimum elution profile consisted of four steps with a total duration of 36 min: first a gradient from 9% to 14% B in 4 min, followed by an isocratic step with 14% B for 13 min; the third step was a gradient from 14% to 45% B in 11 min and the fourth step was isocratic conditions at 45% B for 8 min (see also Fig. 2). The times given are corrected for the gradient delay time (i.e., the time lag between initiation of the gradient at the pumps and its onset at the sample inlet). The absorbance of the column effluent was monitored at 210 nm. The re-equilibration time after each run was 20 min.

2.6. Calibration

Quantitative determinations of heroin and selected adulterants were performed on the basis of the peak areas using the external standard method.

Calibration graphs were constructed from aqueous solutions (10 mmol ortho-phosphoric acid; pH 2.5) of pure standard substances at known concentrations selected to correspond to those in illicit heroin street samples. The concentrations for calibration range with a linear response are shown in Table 2. Each calibration graph was constructed from triplicate determinations of at least four concentration levels. The injection volume was 15 μl. The average value of the area for each calibration point was used to study the linearity of the response.

2.7. Sample preparation

Homogenized heroin street samples were accurately weighed (ca. 15 mg) into a 20-ml volumetric flask and dissolved in aqueous acid (10 mmol orthophosphoric acid; pH 2.5) using an ultrasonic bath for 10 min. Samples containing more than 30% heroin have to be diluted before analysis. Filtration prior to injection was necessary (Spartan 13/30, 0.2 μm; Schleicher and Schuell).

2.8. Precision and reproducibility

For checking the precision and accuracy of the method, a heroin sample was prepared by grinding together known amounts of heroin, cocaine · HCl, morphine · HCl, 6-monoacetylmorphine, acetylcodeine, noscapine · HCl, papaverine · HCl, caffeine, paracetamol, lidocaine, procaine · HCl and benzocaine. Five accurately weighed amounts of this control sample were then treated as described and each sample was analysed in duplicate.

Aliquots of a calibration mixture were partitioned and frozen at −18°C. The stability of the calibration was determined by injection a control mixture at the beginning of each week or at other times as required in order to check calibration and efficiency of the chromatographic system.

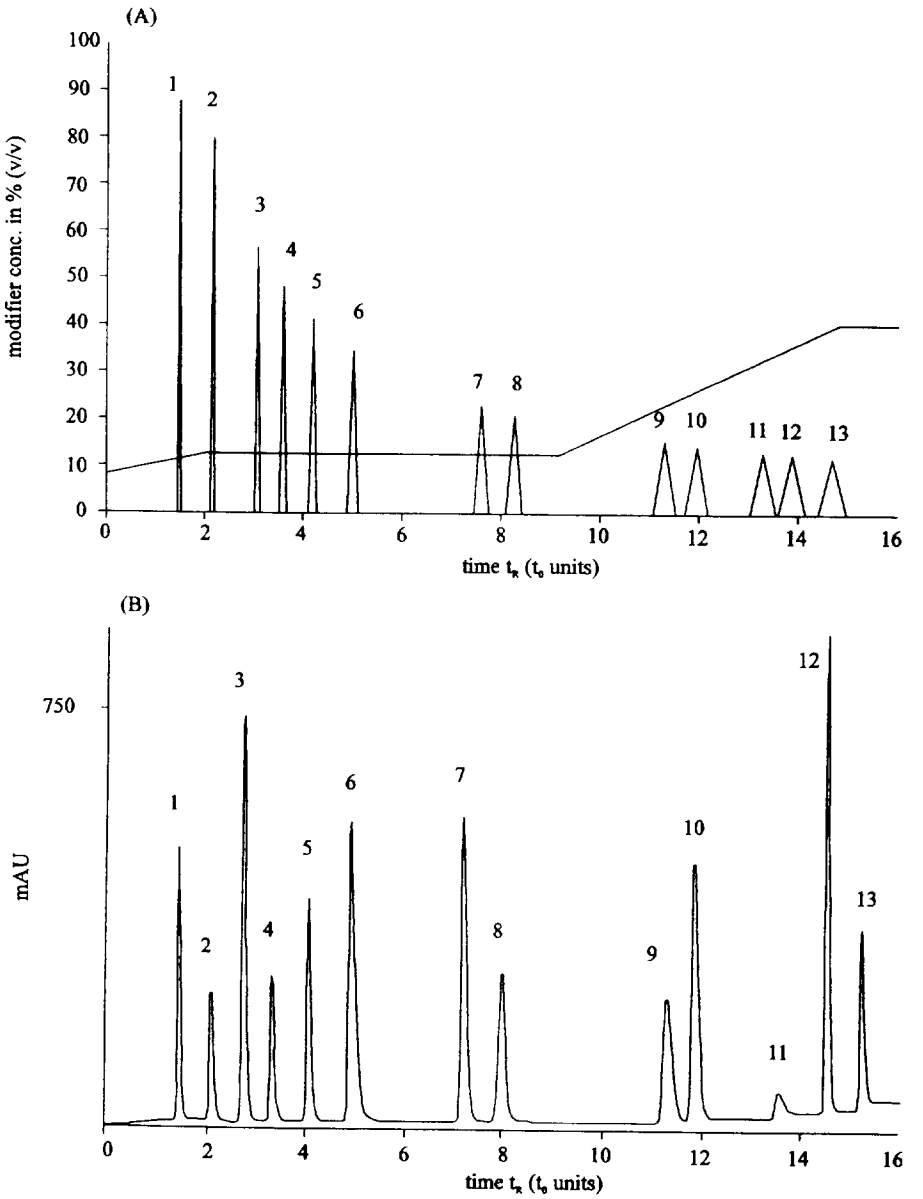


Fig. 2. (A) Chromatogram predicted by CARTAGO-S and (B) chromatogram of reference substances. Peaks: 1 = morphine; 2 = procaine; 3 = paracetamol; 4 = codeine; 5 = 6-monoacetylmorphine; 6 = lidocaine; 7 = caffeine; 8 = benzocaine; 9 = acetylcodeine; 10 = heroin; 11 = cocaine; 12 = noscapine; 13 = papaverine.

3. Results and discussion

3.1. HPLC system

By using CARTAGO-S, time-consuming experimental evaluation of optimum piecewise linear gradient elution was prevented and optimum chromatographic conditions were achieved within a reasonable time for thirteen components with a wide range of polarities. Table 1 shows the results of predicted retention times, t_R (t_0 units = t_R/t_0), by CARTAGO-S, measured t_R, elution time (minutes), precision of t_R within-day and week-to-week and resolution

Table 1
Results of t_R predicted by CARTAGO-S, measured t_R, elution time, precision of t_R within-day and week-to-week and resolution

Substance	Predicted t_R (t_0 units)	Measured t_R (t_0 units)	Elution time (min)	R.S.D. of t_R (%) ($n = 5$)		R_S
				Within-day	Week-to-week	
Morphine (**1**)	1.5	1.5	2.9	0.77	7.7	
Procaine (**2**)	2.2	2.2	4.3	0.46	2.0	4.5
Paracetamol (**3**)	3.1	2.9	5.7	0.92	3.8	4.7
Codeine (**4**)	3.6	3.4	6.7	0.45	2.5	3.1
6-Monoacetylmorphine (**5**)	4.2	4.1	8.0	0.51	2.4	4.0
Lidocaine (**6**)	5.0	4.8	9.4	0.36	2.1	3.5
Caffeine (**7**)	7.6	7.3	14.3	0.42	4.0	9.1
Benzocaine (**8**)	8.3	8.1	15.9	0.18	2.1	3.2
Acetylcodeine (**9**)	11.3	11.2	21.9	0.42	3.0	7.2
Heroin (**10**)	11.8	11.8	23.1	0.22	2.3	1.4
Cocaine (**11**)	13.0	13.4	26.3	0.15	1.3	5.0
Noscapine (**12**)	13.6	14.5	28.4	0.12	1.2	3.9
Papaverine (**13**)	14.3	15.1	29.6	0.07	1.2	2.5

Chromatographic conditions as described in Experimental.

between selected substances. Excellent agreement between predicted and measured t_R with differences ranging from 0.45% for benzocaine to 6.8% for paracetamol was found. For within-day determinations, the relative standard deviations (R.S.D.s) for t_R ranged from 0.07 to 0.92% and for week-to-week precision the R.S.D.s ranged from 1.17 to 4%. However, small errors in concentration of one or more compounds of the organic or aqueous phase are propagated to errors in t_R, so care should be taken when preparing the mobile phase. The notoriously difficult separation of 3- from 6-monoacetylmorphine was adequate for qualitative separation, the experimental retention times being 7.66 and 8.04 min, respectively.

With this system, more than 300 analyses have now been performed without significant changes in t_R and resolution power, proving that no column degradation had occurred due to the organic mobile phase as reported [16].

It is well known that basic compounds may show a pronounced tailing effect on certain reversed-phase columns owing to interactions with the residual polar silanol groups of the stationary phase [22,23]. The addition of an amine modifier to the mobile phase as a masking agent for the silanol groups improves the peak shape and changes the capacity factor (k'). With the addition of orthophosphoric acid to the mobile phase, an acidic eluent with a pH of ca. 2.5 is obtained, so that the components of interest, such as heroin and other basic substances, are protonated [24].

A chromatogram predicted by CARTAGO-S and the corresponding real chromatogram are shown in Fig. 2 and a chromatogram for an illicit heroin sample is shown in Fig. 3. All compounds are sufficiently separated and have symmetrical, sharp, non-tailing peaks.

With some samples, small peaks eluting just after 6-monoacetylmorphine, heroin, papaverine and noscapine were observed. It is well known that noscapine and also thebaine, codeine and morphine give degradation and rearrangement products due to heat and acetylation [25–27]. Injection of a concentrated solution of noscapine that had been acetylated at 100°C for 2 h showed peaks at the same t_R, indicating that these substances originate from noscapine. Further investigations need to be carried out to prove this assumption.

3.2. Calibration

Working in the linear range of the detector, the calibration graph was linear from 68 to 306 μg/ml for heroin (Table 2). As the concen-

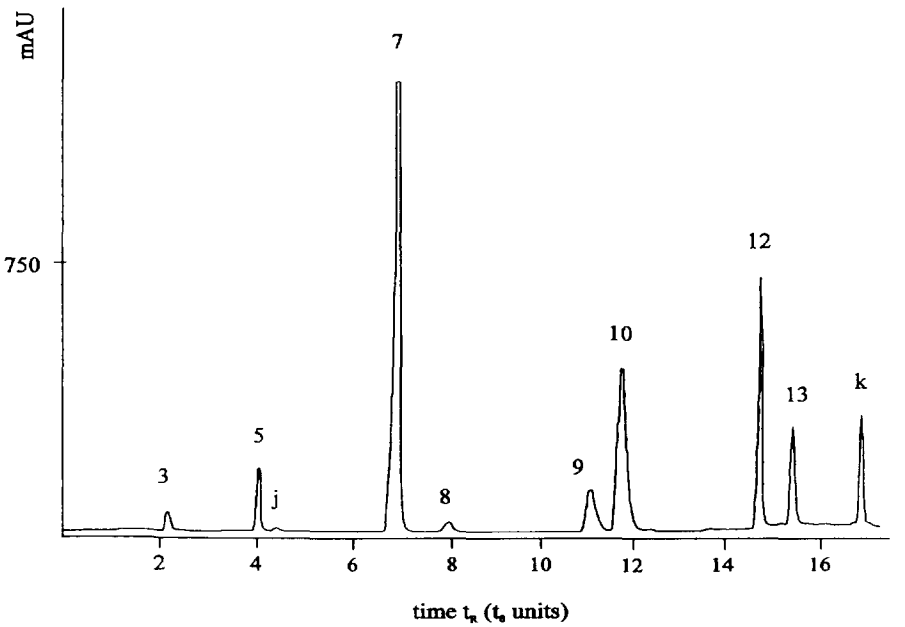


Fig. 3. Chromatogram of a heroin street sample. Peaks: 3 = procaine; 5 = 6-monoacetylmorphine; 7 = caffeine; 8 = benzocaine; 9 = acetylcodeine; 10 = heroin; 12 = noscapine; 13 = papaverine; j and k, unidentified, but probably due to noscapine degradation or rearrangement products.

trations of papaverine and acetylcodeine in actual samples vary from 0 to 6.5% from 0 to 14% [28], respectively, in relation to heroin, calibration for these substances was set in the concentration range expected in illicit heroin. In contrast, the concentration of noscapine can vary from 0 to 200% and that of 6-monoacetylmorphine from 0 to 223% [19] in relation to heroin,

Table 2
Regression data for selected compounds and linear range

Substance	Calibration range (μg/ml)	Slope	Intercept	S_y	S_x	n	r^2
Morphine (**1**)	3.7–36.7	4382	5538	2130	59	11	0.9984
6-Monoacetylmorphine (**5**)	4.7–46.8	3197	3735	1668	36	11	0.9988
Heroin (**10**)	68–306	4021	−84893	9335	4021	6	0.9995
Acetylcodeine (**9**)	4.1–41	4139	−6959	767	21	9	0.9998
Noscapine (**12**)	2.9–43.2	7329	358	2608	56	12	0.9994
Papaverine (**13**)	4.9–49	2992	2677	1463	30	11	0.9990
Cocaine (**11**)	21.7–260	482	−2163	839	3.6	7	0.9996
Benzocaine (**8**)	50–150	1554	6728	1121	15	5	0.9997
Procaine (**2**)	43–134	982	399	1521	21	5	0.9986
Lidocaine (**6**)	43–134	3433	4870	2231	31	5	0.9975
Caffeine (**7**)	12.6–101	8326	−45993	21287	277	6	0.9956
Paracetamol (**3**)	34.3–147	4126	−51395	9303	111	4	0.9985

Experimental conditions as described in Experimental.
S = Standard deviation; n = number of measurements; r = correlation coefficient.

which often makes further dilution of the sample solution necessary in order to determine noscapine and 6-monoacetylmorphine. For all compounds an excellent linear relationship between peak area and concentration was observed (Table 2).

Another advantage of this separation system is the low UV cut-off range of the mobile phase, which allows the detection of heroin and related compounds at its major UV absorption maximum of 210 nm. At this wavelength heroin and related compounds show up to a ten-fold increased sensitivity in comparison with detection at 280, 284, 279 and 228 nm as applied by other workers [1,11,12,15,17]. The limits of detection absolute on-column ranged from 10 ng for noscapine, corresponding to 0.09% of the sample mass, to 100 ng for cocaine, which shows poor absorption at 210 nm. Papaverine also shows less absorption at 210 nm. Using diode-array or multiple-wavelength detection would overcome this problem by quantifying each compound at its optimum wavelength. In addition, using a diode-array detector for checking purity or carrying out detection at different wavelengths would increase the separation power significantly [15]. Fig. 4 shows the overlaid UV spectra of heroin, noscapine and cocaine, measured in the mobile phase.

3.3. *Precision, reproducibility and recovery*

A control solution was injected at the beginning of each week or at other times to determine the reproducibility of calibration. To investigate the precision and recovery of sampling, five aliquots of a heroin sample that was made up in this laboratory were analysed. The results are given in Table 3. Calibration proved to be stable over a long period, making this method extremely valuable for long-term routine analyses of illicit heroin samples. Good results for precision and recovery were also obtained with this method. Analysing a pure standard solution containing heroin after 2 and 4 h, no 6-monoacetylmorphine could be detected, proving that heroin has a sufficient short-term stability in the chosen solvent [29].

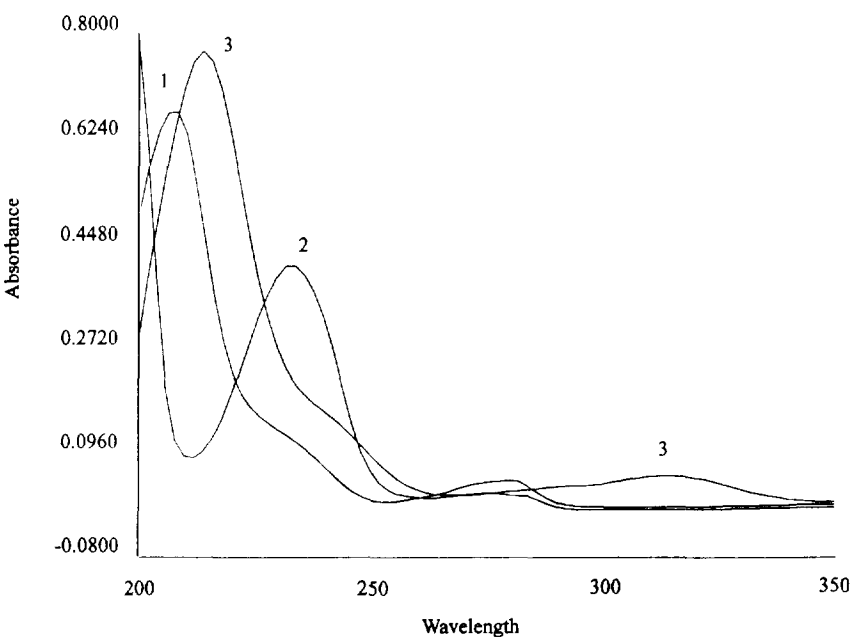


Fig. 4. UV absorption of (1) heroin, (2) cocaine and (3) noscapine, measured in the mobile phase. Wavelength in nm; absorbance in mAU.

Table 3
Results of precision and recovery of sampling

Substance	Reproducibility of calibration ($n = 8$) (8 weeks)			Replicate sampling ($n = 5$)			Recovery (%) ($n = 5$)
	Mean (μg/ml)	S.D. (μg/ml)	R.S.D. (%)	Mean (μg/ml)	S.D. (μg/ml)	R.S.D. (%)	
Cocaine (**11**)	63.15	1.2	1.9	68.15	1.1	1.6	97.9
Heroin (**10**)	83.27	1.6	1.9	245.00	4.6	1.9	101.6
Caffeine (**7**)	37.84	1.1	2.9	31.91	0.7	2.2	100.2
Paracetamol (**3**)	69.81	1.9	2.7	85.36	1.5	1.7	96.3
Procaine (**2**)	86.16	2.4	2.8	78.53	1.6	2.1	102.3
Benzocaine (**8**)	100.72	2.2	2.2	88.52	2.6	3.0	97.6
Lidocaine (**6**)	87.53	2.3	2.7	81.29	1.6	1.8	97.5
Morphine (**1**)	28.94	0.9	3.1	7.78	0.25	3.2	103.7
Noscapine (**12**)	35.32	0.9	2.5	26.89	0.98	3.6	99.4
Papaverine (**13**)	39.65	1.3	3.3	8.12	0.33	4.0	96.1
Acetylcodeine (**9**)	40.19	0.8	2.0	19.02	0.38	2.0	103.5
6-Monoacetylmorphine (**5**)	35.71	0.8	2.2	7.57	0.37	4.9	102.9

Experimental conditions as described in Experimental.

4. Conclusions

An excellent separation of heroin and selected basic impurities and adulterants was obtained via reversed-phase liquid chromatography. Inexpensive methodology suitable for small laboratories was developed which allowed the simultaneous determination of heroin, morphine, 6-monoacetylmorphine, papaverine, acetylcodeine, noscapine and cocaine and also various adulterants such as paracetamol, caffeine, procaine, benzocaine and lidocaine over a wide concentration range. Simple sample pretreatment minimizes the time required and variations due to the extraction technique.

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