Determination of illicit drugs and related substances by high-performance liquid chromatography with an electrochemical coulometric-array detector

G. Achilli[a], G.P. Cellerino[a,*], G.V. Melzi d'Eril[b], F. Tagliaro[c]

[a]*EuroService srl, Piazza Maggiolini 3, 20015 Parabiago, Italy*
[b]*Dipartimento di Biochimica, II Facoltà di Medicina e Chirurgia (Varese), Università di Pavia, Pavia, Italy*
[c]*Istituto di Medicina Legale, Università di Verona, Policlinico, 37134 Verona, Italy*

Abstract

A general method for the simultaneous determination of fifteen common drugs (6-acetylmorphine, 3,4-methylenedioxymetamphetamine, buprenorphin, cocaine, codeine, dihydrocodeine, ethylmorphine, heroin, hydrocodone, lidocaine, methadone, morphine, naloxone, procaine and thebaine) was developed using reversed-phase HPLC and electrochemical detection. The separation of the drugs was achieved by using as the mobile phase 20 mM monobasic sodium phosphate–acetonitrile (90:10) with a gradient to 50% of the organic modifier, on a silica based C_{18} column (150 × 4.6 mm I.D.) of 3 μm particle size and by the selectivity supplied by an array of eight coulometric electrodes at increasing potential. It was possible to identify and to determine fifteen different drugs in the same chromatographic run in 50 min. The method was tentatively applied to the determination of drugs in extracts of human hair.

Keywords: Electrochemical detector; Detectors, LC; Drugs, illicit

1. Introduction

The identification and quantitation of the drugs of abuse in biological samples requires a large commitment of manpower and time within forensic science laboratories, especially when instrumental techniques are to be used for confirmation purposes after the usual screening immunoassays. Attempts have been made to develop methods of wide application and of high specificity to confirm the presence of drugs of abuse and to determine their concentration.

Chromatographic methods provide an inexpensive yet powerful approach for the identification and quantitation of the controlled substances. Liquid chromatography is particularly well suited for the determination of a number of the commonly controlled substances which are relatively non-volatile or thermally unstable. However, to our knowledge, most of the methods published in recent years have been used to determine only one or a few drugs. The highest number of simultaneous determinations (five drugs) was found in a paper published in 1980 [1]. Moreover, most of the investigations were performed with biological fluids and only very few involved

* Corresponding author.

hair. The presence of a drug or of its metabolites in biological fluids is limited by the mechanism of its elimination from the body. Drugs of abuse and their metabolites are retained for a much longer time in hair: this allows an additional opportunity to determine the past drug history of the subject [2]. Our purpose was to develop a method capable of identifying the largest number of drugs of abuse in a single run, minimizing the analysis time and maximizing the specificity. To achieve this, we combined the specificity of electrochemical array detection [3] with the flexibility of gradient HPLC to separate and determine fifteen drugs of great forensic interest, including cocaine, codeine ethylmorphine, hydrocodone, lidocaine morphine, naloxone, procaine and thebaine. The application of the method was tested on samples of human hair subjected to standard sample preparation techniques.

2. Experimental

2.1. Chemicals

The eluent mixtures used in the gradient separations are identified as mobile phase A and B. Mobile phase A consisted of 20 mM monobasic sodium phosphate–acetonitrile (90:10) (pH 7.00) and mobile phase B consisted of 20 mM monobasic sodium phosphate–acetonitrile (50:50) (pH 7.00). The mobile phases were filtered through 0.2-μm PTFE lyophilic filters (Millipore, Bedford, MA, USA) and degassed by sonication under vacuum for 10 min prior to use. The water used for this method was purified with a Milli-Q R/O water-purification system (Millipore) and filtered through 0.2-μm PTFE lyophilic filters.

2.2. Apparatus

The HPLC system consisted of a CoulArray from ESA (Chelmsford, MA, USA). The instrument consisted of a refrigerated autosampler which is capable of variable-volume injections with a 100-μl loop. Gradient operation was provided by two Model 580 HPLC pumps (ESA)

capable of operating from 0.05 to 10 ml/min. The output of the pumps was connected to a dynamic gradient mixer. The analytical column (150 × 4.6 mm I.D.) used was a stainless-steel column packed with 3-μm particle size of silica-based C_{18} material (NBS; ESA). The detection system consisted of two coulometric array modules, each containing four electrochemical detector cells. The detectors, porous graphite working sensors with palladium reference and counter electrodes, were arranged in series after the analytical column. The column was housed in a thermal chamber maintained at 37°C. The autosampler, pumps, detectors, temperature-controlled box and all associated electronic circuitry were controlled by CoulArray software installed on a Model 486 computer equipped with a 350-Mbyte hard disk and a 1.4-Mbyte floppy disk drive. This computer system performed data acquisition, storage, analysis and report generation. An appropriate software package was used for summary reports of the final data (Lotus 1–2–3; Lotus, Cambridge, MA, USA).

2.3. Chromatographic method

A gradient chromatographic method providing complete resolution of the fifteen compounds chosen was developed. The gradient used in the separation, expressed as the percentage of mobile phase B, was 7% isocratic until 11.5 min after the injection, then an increase in B to 50% at 19.5 min, isocratic at 50% B until 24.5 min, then an increase in B to 90% at 34.5 min and finally isocratic. The mobile phase B concentration was then returned to the initial value of 7% and 7.5 min were allowed for column re-equilibration. The flow-rate was 0.8 ml/min. The cell potentials constituted an increasing array: 450 mV at electrode 1, 550 mV at electrode 2, 650 mV at electrode 3, 750 mV at electrode 4, 850 mV at electrode 5, 900 mV at electrode 6, 950 mV at electrode 7 and 1000 mV at electrode 8. The indicated potentials are referred to the solid-state palladium reference electrode built in the coulometric cells; their absolute value is about 250 mV lower than the corresponding potential measured by using an Ag/AgCl reference elec-

trode. At the end of each analysis, all cell potentials were increased to 1200 mV for 60 s to prevent long-term adsorption of material on the electrode surface. The electrodes were then allowed to stabilize for 7.5 min before the next injection. This stabilization time was simultaneous with the re-equilibration of the column.

2.4. Standard and sample preparation

Pure standards of 3,4-methylenedioxymethamphetamine (MDMA), buprenorphin, cocaine, codeine, dihydroxycodeine, ethylmorphine, heroin, hydrocodone, lidocaine, methadone, morphine, naloxone, oxycodone, procaine and thebaine, were obtained from Sigma Chimica (Milan, Italy). Stock standard solutions were prepared at 1 mg/ml in methanol and stored at −18°C. A fifteen-component working standard solution was prepared at suitable concentrations by combining and diluting with mobile phase A an aliquot of each of the stock standard solutions. This working standard solution was prepared every day and kept refrigerated at 4°C, then 10 μl were injected into the system.

Hair samples were prepared according to the method described previously [4]. In summary, hair samples (20–200 mg) were incubated overnight in 2 ml of 0.25 M HCl at 45°C and the resulting mixture was neutralized and extracted with a commercial liquid–liquid method (Toxi-Tubes A; Analytical Systems, Laguna Hills, CA, USA); the dried residue was reconstituted with mobile phase A and then 10 μl were injected into the system.

2.5. Assay performance

The assay linearity and detection limit were determined by adding known amounts of the drugs (from 1 to 10 000 μg/l) to hair extracts of subjects who had never used the investigated compounds. These extracts were also previously tested to confirm the absence of drugs of abuse and therefore their suitability for use as blanks. The within-run reproducibility was evaluated by replicate analyses ($n = 10$) of each standard solution added to the extracts already described as

blanks and treated as samples. The between-run reproducibility was tested by preparing and analysing standard extracts in duplicate on ten separate days.

3. Results

Under the above conditions, a mixture containing the fifteen standards was separated and quantified. A typical separation of a 10-μl sample containing the fifteen components as external standard is shown in Fig. 1. For clarity only six traces are shown. These traces correspond to electrodes 1 (450 mV), 2 (550 mV), 3 (650 mV), 4 (750 mV), 5 (850 mV) and 7 (950 mV). The total analysis time was less than 50 min. Confirmation of peak identification was carried out in two steps. First, the extracts of normal hair were spiked with the appropriate standard. Second, the ratio R between each standard was matched with the actual peaks of the spiked extracts (R is the ratio of the signal distribution between dominant and sub-dominant channels expressed as a
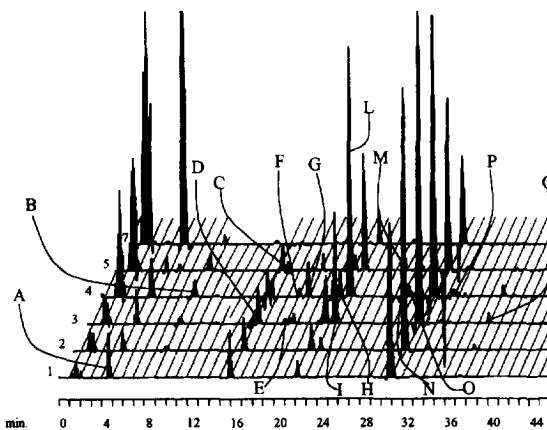

Fig. 1. Six-channel chromatogram of the fifteen external standards. The numbers represent the electrodes and the letters represent each compound. Applied potentials: electrode 1, 450 mV; electrode 2, 550 mV; electrode 3, 650 mV; electrode 4, 750 mV; electrode 5, 850 mV; and electrode 7, 950 mV. External standards: A = morphine; B = procaine; C = codeine; D = MDMA; E = dihydrocodeine; F = 6-acetylmorphine; G = ethylmorphine, H = cocaine; I = naloxone; L = methadone; M = hydrocodone; N = thebaine; O = lidocaine; P = heroin; Q = buprenorphine.

percentage, where 1 corresponds to the maximum match and 0 corresponds to no match) [5]. The detection characteristics of these fifteen external standards are presented in Table 1. The precision (with- and between-run), the detection limits and the dominant potentials are also reported (the dominant potential is that electrode potential where the maximum signal occurs for a given substance if eluted through a given coulometric array). The retention time repeatability during the precision studies (carried out over a 10-day span) was found to be excellent (R.S.D. < 2.0%) for all standards. This was due to the strict control of both the gradient profile and the column temperature. The calibration graphs for the standards for all drugs were linear throughout the range tested (better than 0.998, $p < 0.001$). The minimum detectable amount (at a signal-to-noise ratio of 3:1), as reported in the Table 1, was very low for all the analytes. In the worst case (cocaine) the sensitivity was less than 26.4 μg/l, whereas in the best case (hydrocodone) it was less than 1.5 μg/l. The within-run concentration variability (R.S.D.) ranged from 1.8% (hyrocodone) to 5.2% (MDMA); the between-run concentration variability ranged from 3.5% (codeine) to 6.1%
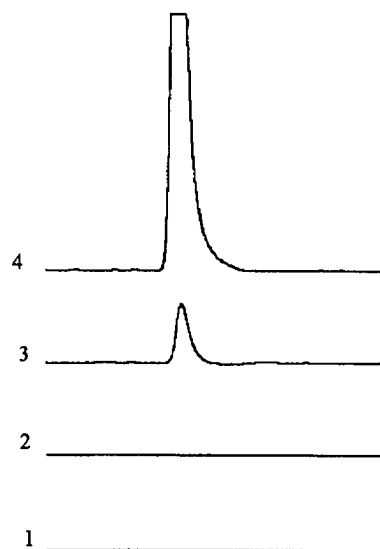

Fig. 2. Comparison of the 450-mV signals of four injected samples (full-scale deflection = 1 μA). 1 = Mobile phase blank; 2 = normal hair extract; 3 = hair extract found positive for morphine (corresponding to 133 ng); 4 = external standard of morphine standard extracted.

(MDMA). The analytical recovery for morphine from hair was found to be consistent with previously published data [6].

Fig. 2, shows the trace of the dominant channel

Table 1
Chromatographic and electrochemical behaviour of the tested drugs

Identification	Compound	Retention time (min)	Dominant potential (mV)	Detection limit (pg)	Within-run R.S.D. (%)	Between-run R.S.D. (%)	Concentration (ng per 10 μl)
A	Morphine	4.66	350	17	3.2	3.8	17.00
B	Procaine	8.75	750	31	4.3	4.9	10.00
C	Codeine	15.53	750	18	2.8	3.5	25.00
D	MDMA	15.95	450	107	5.2	6.1	10.00
E	Dihydrocodeine	18.57	750	48	3.5	4.8	17.00
F	6-Acetylmorphine	18.98	650	261	4.8	5.8	10.00
G	Ethylmorphine	19.28	750	201	3.7	4.5	13.00
H	Cocaine	21.58	750	264	3.2	4.8	29.00
I	Naloxone	22.28	650	61	2.7	4.0	12.50
L	Methadone	23.12	750	25	3.0	3.8	50.00
M	Hydrocodone	28.08	750	15	1.8	3.8	20.00
N	Thebaine	28.73	750	70	2.8	3.6	32.50
O	Lidocaine	29.66	550	91	2.4	4.3	20.00
P	Heroin	33.00	750	130	3.6	5.5	5.00
Q	Buprenorphine	37.75	650	120	2.9	4.8	5.00

(450 mV) for morphine in a sample of hair from a morphine user. Fig. 2 also shows the traces of the blank, of the hair blank and of the external standard of morphine. As a result of the combined effect of the extraction and the selectivity of the array detection, the peak of the drug is well resolved without co-eluted peaks of interfering substances. The quantitation of morphine was also possible as recovery data were available from a previous study [4] and was performed by using the external standard method. The morphine concentration determined with this method was of 133 ng in the extracted sample, and is consistent with the result obtained for the same sample using radioimmunoassay, 120 ng.

4. Discussion and conclusion

The use of coulometric array detection for the determination of neurochemicals in tissues and biological fluids has already been reported [7–10]. More recently, it has been used for the determination of 36 phenolic constituents in natural beverages and plant extracts [11] and for the measurements of phenols and herbicides in water [12]. In this work, we demonstrated that by combining reversed-phase chromatography with highly selective array coulometric detection it is possible to separate and to determine a large number of drugs in a single run in 50 min. The method yields a highly precise determination of drugs of different families in a single run. The coulometric efficiency of each element of the array allows a complete voltammetric resolution of analytes as a function of their reaction po-

tential. Some peaks can be resolved by the detector even if they are unresolved when they leave the chromatographic column. For the samples analysed here, we were able easily to differentiate morphine-positive samples from controls. In conclusion, the proposed method for the simultaneous determination of several drugs in hair provides a fast and reproducible sampling procedure with good selectivity and sensitivity over a wide concentration range.

References

[1] R.G. Peterson, B.H. Rumack, J.B. Sullivan and A. Makowski, J. Chromatogr., 188 (1980) 420.
[2] F. Tagliaro, M. Chiarotti and Z. Deyl (Editors), Hair Analysis as a Diagnostic Tool for Drugs of Abuse Investigation, Forensic Sci. Int., 63 (1993) 1–309.
[3] W.R. Matson, P. Langlais, L. Volicer, P.H. Gamache, E. Bird and K.A. Mark, Clin. Chem., 30 (1984) 1477.
[4] F. Tagliaro, C. Antonioli, Z. De Battisti, S. Ghielmi and M. Marigo, J. Chromatogr., 674 (1994) 207.
[5] V. Rizzo, G. Melzi d'Eril, G. Achilli and G.P. Cellerino, J. Chromatogr., 536 (1991) 229.
[6] M. Marigo, F. Tagliaro, C. Poiesi, S. Lafisca and C. Neri, J. Anal. Toxicol., 10 (1986) 158.
[7] K.J. Swartz and W.R. Matson, Anal. Biochem., 185 (1990) 363.
[8] C.N. Svendsen and E.D. Bird, Neurosci. Lett., Suppl., 35 (1989) 49.
[9] W.R. Matson, P.G. Gamache, M.F. Beal and E.D. Bird, Life Sci., 41 (1987) 905.
[10] P.H. Gamache, M.L. Kingery and I.N. Acworth, Clin. Chem., 39 (1993) 1825.
[11] G. Achilli, G.P. Cellerino, P. Gamache and G. Melzi d'Eril, J. Chromatogr., 632 (1993) 111.
[12] G. Achilli, G.P. Cellerino, G. Melzi d'Eril and S. Bird, J. Chromatogr. A, 697 (1995) 357.