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Monitoring compliance to therapy during addiction treatments by means of hair analysis for drugs and drug metabolites using capillary zone electrophoresis coupled to time-of-flight mass spectrometry

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ABSTRACT

Capillary electrophoresis coupled to time-of-flight mass spectrometry was used in the present work for the determination of therapeutic and abused drugs and their metabolites in the hair of subjects undergoing addiction treatments, in order to monitor their compliance to therapy.

For this purpose a rapid, qualitative drug screening method was adopted based on capillary electrophoresis hyphenated with time-of-flight mass spectrometry, which had earlier been developed and validated for the forensic-toxicological analysis of hair, limitedly to illicit/abused drugs [1]. Sampling of hair was carried out in order to refer to a time window of about two months from the date of sampling (i.e. 2 cm ca. from cortex). A single extraction procedure was applied, allowing the determination in the hair matrix of "drugs of abuse" referred to the past abuses, and therapeutic drugs prescribed in the detoxification program as well as their metabolites. Analyte identification was based on accurate mass measurements and comparison of isotope patterns, providing the most likely matching between accurate mass value and elemental formula. Small molecules (<500 Da) of forensic and toxicological interest could be identified unambiguously using mass spectrometric conditions tailored to meet a mass accuracy ≤5 ppm. In the present study, the proposed approach proved suitable for the rapid broad spectrum screening of hair samples, although needing further confirmation of results by using fragmentation mass spectrometry.

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1. Introduction

Among the analytical approaches currently used in clinical and forensic toxicology, the determination of controlled drugs in hair has recently been included, particularly in workplace drug testing, post-mortem toxicology, and certification of physical fitness to drive vehicles [2]. However, in the last decades, another important field where hair analysis has been proposed, and sometimes criticized, is the monitoring of therapeutic drugs (TDM) [3–5]. In principle, the possibility of determining prescribed drugs and/or drug metabolites in the hair during maintenance programs could provide valuable information not only on the patient's compliance to the therapy, but also on the drug absorption and metabolism. For selected drugs, literature data show a correlation between drug concentration in the hair matrix and the "area under the absorption curve" [6]. However, other reports, although based on less controlled works, show a poor quantitative correlation between drug dosage and its concentration in hair [6].

In modern forensic toxicology, it is well known that, if blood and urine are still the conventional specimens to document recent drug intake, hair testing can be used to increase greatly the time-window of detection from several hours (for blood) or few days (for urine) up to some months (for hair). This is based on the assumption that the drugs present in blood diffuse and are incorporated into the hair matrix, which grows at a fairly constant rate (about 1 cm/month), where drugs and metabolites can stay unaltered for long time protected from metabolism and degradation. Hair collection is non-invasive and can be carried under close supervision. Finally, hair samples can be stored for long time and transported at room temperature with minimal precautions.

All these advantages are well known in the forensic field where hair analysis has obtained full acceptance, but so far not fully understood in clinical pharmacology and toxicology where hair testing could provide precious information on therapy compliance and poly drug use/abuse over a period of weeks-to-months before sample collection.

In the toxicological analysis of hair, one of the most challenging problems is the need for maximizing analytical sensitivity and

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identification power, without sacrificing the analytical spectrum, in minute amounts of samples, which for aesthetical reasons cannot exceed 100–200 mg.

Although historically, several techniques were applied for this purpose, by far of gas-chromatography combined with mass spectrometry (GC–MS) has for many years played the major role, particularly for its ability to offer, through integration of retention and mass spectral data, the way to unambiguous molecule identification and accurate quantification. Nevertheless, gas-chromatographic techniques are not suitable for highly polar, non volatile and thermally unstable substances, such as many of the therapeutic drugs and their metabolites. In some cases these drawbacks can be overcome by means of derivatisation, but this may introduce delicate analytical steps, particularly for quantification, and weaken the suitability of this technique for general-purpose analysis.

The possibility of a sound coupling of mass spectrometric detectors with liquid chromatography (LC–MS) has more recently offered a more flexible analytical tool, combining the specificity and sensitivity of MS detector with the almost universal separation capability of HPLC. In this perspective, many efforts have been made recently in order to improve instrument performance and the number of substances of toxicological interest detectable in a single analytical run [7,8].

Strangely enough, notwithstanding clear evidence of the possibility of coupling capillary electrophoresis with mass spectrometry (CE–MS) and its characteristics of robustness, speed of analysis, separation efficiency, and minimal sample requirements, only few groups have proposed this technology as a valid alternative to LC–MS for the determination of illicit and/or prescribed drugs in hair [9–12]. In recent reports, the interfacing of CE with time-of-flight (TOF)–MS has proved the advantages, in terms of most accurate peak definition, of combining a fast-scanning, high resolution mass detection, such as TOF–MS, with a high efficiency separation technique, such as CE. Furthermore, the high TOF sensitivity and selectivity in full scan permits the acquisition of a broad spectrum of information in a single run, under fairly "generic" analytical conditions with the possibility of post-experiment data evaluation. Furthermore, the high resolution (10 000 as FWHM) and mass accuracy (up to 5 ppm) typical of TOF–MS permits to achieve molecule identification on the basis of accurate mass and isotopic pattern, thus in principle overcoming the need for molecule fragmentation [13].

As a result of such considerations, TOF–MS appeared eligible for broad spectrum toxicological screening of compounds for which the MS fragmentation spectra are unavailable as well as standards and chromatographic retention data, which makes their recognition by using chromatography coupled to low resolution mass spectrometry hardly possible.

In order to test in practice the peculiar advantages of high resolution mass spectrometry, in the present work, five volunteers following different drug rehabilitation programs were tested with hair analysis for abstinence from illicit drugs and compliance to therapy.

The analytical strategy was chosen taking advantage of the versatility and simplicity of capillary zone electrophoresis (CZE) as a separation tool and of the identification capability of high resolution TOF–MS. On these grounds, an original CZE–TOF method, previously developed for the major drugs of abuse [1], was used in the present study, with minor changes including the broadening of analytical spectrum to include many therapeutic drugs and drug metabolites in addition to the usual panel of abused substances.

2. Materials and methods

2.1. Chemicals and reagents

Methanol, isopropanol, hydrochloric acid, sodium hydroxide, ammonium formate and ammonia used for the preparation of CZE buffers, for the sheath liquid and for the hair extraction were HPLC or "analytical" grade and were purchased from Carlo Erba (Milan, Italy). Folcodine were provided by Salars (Como, Italy). Folcodine, used as internal standard, was provided by Salars (Como, Italy). Tween 20 was obtained by Sigma (St. Louis, MO, USA). Commercially available ready-to-use solutions for liquid–liquid extraction (Toxi-Tubes A) of basic compounds from biological samples were supplied by Varian (Lake Forest, CA, USA).

The ultra pure water used in the present study was obtained from an aqua MAX-Ultra 370 Series water purification system (Young Lin Instrument, Anyang, Korea).

2.2. Instrumentation

A P/ACE 5500 capillary electropherograph (Beckman Coulter, Fullerton, CA, USA) fitted with naked fused-silica capillaries (75 μm internal diameter, 100 cm total length, Composite Metal Services, The Chase, Hallow, Worcestershire, UK) was used throughout the present study. The analytical method used is fully described elsewhere [1]. However, in short, it can be summarized as follows: separation voltage: 15 kV (forward polarity), temperature: 20 °C. Field-amplified sample stacking (FASS) injections were carried as follows: the injection end of the capillary was dipped into water for 1 s (external rinse step), then a plug of water was hydrodynamically injected for 1 s at 3.45 kPa psi and then the sample was injected electrokinetically for 30 s at 7 kV.

The cathodic end of the capillary was connected to an orthogonal electrospray (ESI) source of a MicrOTOF mass spectrometer (Bruker Daltonics, Bremen, Germany). The on-line coupling was obtained with a commercial coaxial sheath liquid interface (Agilent Technologies, Palo Alto, CA, USA) orthogonally positioned to the MS ion source. Capillary voltage was 4 kV, source temperature 200 °C. Nitrogen was used as both drying and nebulizing gas (drying gas flow rate: 5 L/min, nebulizer pressure: 60 Pa). The mass spectrometer was operated in the positive ion scan mode from 50 to 800 *m/z*, with an acquisition scan rate of 20 spectra/s. The typical resolution of the instrument was 10 000 (FWHM). A sheath liquid consisting of a mixture of isopropanol/water (50/50 (v/v) added with 0.5% (v/v) formic acid) was delivered at a flow rate of 4 μL/min by a syringe pump (Cole-Parmer, Vernon Hill, IL, USA). External calibration was obtained by infusing for 1 min before each electrophoretic run a solution composed of 10 mM sodium hydroxide in isopropanol (1:1, v/v), and 0.2% of formic acid using seven calibration points. Data elaboration was carried out with the help of the microTOF Data Analysis software (Bruker Daltonics, Version 3.2).

2.3. Compound identification

The criteria for unknown compound identification were as follows.

The candidate peak selected in the plot of total ion electropherogram undergoes "ion extraction" (with a mass width ± 0.02 Da) at the apex of the selected peak. The Data Analysis software provides a list of candidate of molecular formula (±10 ppm tolerance). The candidate formulas are compared to the experimental masses not only on the basis of accurate mass determination but also by comparing theoretical with the measured isotopic pattern. This latter parameter, described by the so-called *sigma value* is calculated automatically by a patented algorithm (SigmaFit™, Bruker Daltonics), accounts for the difference between theoretical and measured isotopic pattern; the lower the value, the better the matching. In this work, compounds with *sigma* >0.04 are excluded from the candidates list. For improving compound recognition, a database of chemical formulas developed by our group [13] was used.

In the present work, migration time is also not applied for the identification, due to the lack of reference standards for many compounds (mainly metabolites), but in future migration parameters (e.g. electrophoretic mobilities) could in principle be usefully used.

2.4. Sample collection and preparation

The hair samples for the present study were obtained from a group of subjects under pharmacological treatment of drug addiction who preliminarily gave informed consent to the enrolment in the study. Subjects were on purpose chosen with different addiction histories, different pharmacological treatments and different duration of the treatment, in order to build up a study group as heterogeneous as possible. Available clinical and therapeutic information for each subject are summarized in Table 1, where the type of addiction, the drugs assumed, with both scientific and trade names, and the duration of treatment at the date of sampling are reported.

Hair samples were cut with an average length of 2.0–2.5 cm from the scalp, in order to evaluate (on the basis of an average growth rate of 1.0–1.2 cm/month) a period of about two months before collection for each subject. Each sample was collected from the scalp of the vertex posterior of the head. Hair samples (100 mg ca) were then washed with an aqueous solution of 0.3% Tween-20 (20 mL, twice), in order to remove any potential contaminants present on the surface. Then hair were cut into small fragments and incubated overnight in 1 mL 0.1 M HCl at 45 °C. Finally, the incubation mixtures were neutralized with equimolar NaOH and extracted into organic phase with Toxi-Tubes A (Varian). The organic layers were evaporated under a stream of air and then the dried residues were reconstituted in 250 μL of ultrapure water.

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