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The application of portable microchip electrophoresis for the screening and comparative analysis of synthetic cathinone seizures

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ABSTRACT

Variation in the chemical composition of illicit tablets and powders is common among samples within a given drug seizure. Using microchip electrophoresis (ME), multiple tablets can be screened in a cost-effective and timely manner. This method could be used in conjunction with reporting methods that focus solely on statistical sampling to infer homogeneity or otherwise of a larger subset of tablets. Some frequently observed synthetic cathinones, often present in illicit tablets seized in New Zealand, were chosen for analysis. An ME device (Agilent Bioanalyzer 2100) was used to electrophoretically separate synthetic cathinones. The background electrolyte was composed of a 50 mM sodium tetraborate buffer with 50 mM sodium dodecyl sulphate at pH 9.66. Analytes were derivatised prior to analysis for 3 min at 90 °C, employing fluorescein isothiocyanate isomer I (FITC). A characteristic fluorescent profile was obtained for each tablet, in terms of the number of constituents, relative peak height ratios and migration times. The repeatability of the developed method was assessed for a wide range of tablets and relative standard deviations of 0.4–5.2% and 1.6–5.5% were calculated for migration times and peak height ratios, respectively. The use of microchip tablet profiles in the forensic case comparison of illicit drug seizure samples in realistic scenarios is discussed.

1. Introduction

Due to the dynamic and resilient nature of the illicit drug market, an evolving number of synthetic stimulants are pressed into tablets [1]. Visually similar tablets from a given batch or seizure do not always contain the same constituents or relative proportions. This can be attributed to the lack of quality control at manufacturing or tabletting sites. Further, tablets from a given seizure do not always originate from the same source and/or manufacturing site. For instance, the tablets/powders may have been pressed at different locations or distributed into various packets [2].

The availability of 3,4-methylenedioxymethamphetamine (MDMA) has declined in recent years, a trend which has been accompanied by the increased use of other stimulants, such as synthetic cathinones: 4-methylethcathinone (4-MEC), 4-methylmethcathinone (4-MMC), β-keto-3,4-methylbenzodioxylbutamine (bk-MBDB) and β-keto-3,4-methylenedioxymethamphetamine (bk-MDMA). These stimulants are often used as a substitute for ecstasy and typically sold via the internet [3–5]. Little is known of their detailed pharmacology; however, they have very few known medicinal uses. Synthetic cathinones are imported into New Zealand in powder form where they are often combined and distributed as ecstasy, of which 4-MEC is a predominant ingredient. Given that these tablets normally contain 4-MEC mixed with other stimulants, they are commonly referred to as MEC tablets [6].

The forensic examination of visually similar tablets is typically performed using combinations of a confirmatory analytical technique such as gas chromatography–mass spectrometry (GC–MS), colour tests and statistical methods.

Various methods are used to determine the sample size for analysis. Classical, arbitrary sampling methods have been used including, the square root rule, 10% of the seizure population, or even single samples [7]. Alternative methods based on frequentist or Bayesian statistical approaches are also employed. The frequentist approach makes use of a hypergeometric sampling population table, whilst the Bayesian model makes use of prior knowledge and applies Bayes' theorem to select a suitable sample size for analysis [8–10]. In comparison to classical methods, the frequentist and Bayesian methods are more cost-effective and

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timely and still provide adequate information for the purposes of law enforcement and judicial inquiry. Following the analysis of a representative sample of the seizure population, inferences are made regarding the rest of the population with an associated quantifiable degree of probability or likelihood. Although the presented statistical methods are based on sound mathematical principles, they are abstract and not easy to explain to a lay jury, and may be misunderstood. Accordingly, the development of simplistic approaches is needed for the presentation of statistical analysis of illicit drugs in court.

GC–MS is the industry standard for the analysis of drug exhibits. Forensic analysis requirements vary between judicial systems, depending on resources and legal framework. However, most jurisdictions cannot justify the analysis of multiple tablets, beyond the requirements of statistical methods, using confirmatory techniques such as GC–MS. In addition, some laboratories are unable to make use of GC–MS due to financial constraints. There is a need for fast and cost-effective techniques for the analysis of a larger sample size. This would improve accuracy, particularly for bulk seizures. The use of a screening tool capable of generating a profile for multiple tablets could be used to help infer the homogeneity within seizures. This would provide timely information to investigators and aid in courtroom visualisation of forensic drug analysis evidence.

Colour/spot tests are routinely employed in forensic drug laboratories during the examination of illicit drug exhibits. While they can be used to presumptively identify illicit drugs prior to GC–MS confirmation, they do not have the required specificity to distinguish between some synthetic cathinones and/or simultaneously identify those in a mixture. In addition, they can suffer from interference resulting in false positives/negatives and are dependent on the colour discrimination of the analyst [11].

Other techniques available for screening multi-constituent tablets include high performance liquid chromatography (HPLC). In comparison to HPLC, capillary electrophoresis (CE) is fast, cost-effective and simple. Further, it is more suitable for miniaturisation and portability [12]. As a result, many CE-based microchip devices have been developed and applied for the portable analysis of DNA, proteins and small molecules [13–15]. This technique, a simple yet powerful separation tool, has also been reportedly applied for the analysis of illicit drugs in various matrices [16–19]. These devices achieve rapid results, are cost-effective and require little maintenance. Recent growth in the area of microchip technologies has improved the 'functionality' and robustness of portable systems, therefore enhancing their capability for high-throughput analyses.

This paper evaluates the use of a ME device, the Agilent Bioanalyzer 2100, for the rapid screening of MEC tablets. The analysis of amphetamine-type stimulants, derivatised by fluorescein isothiocyanate isomer I, has been previously reported by our group [20]. In this study, our previous method was adapted for the analysis of synthetic cathinones commonly encountered in illicit tablet seizures in New Zealand. Furthermore, the use of characteristic ME 'profiles' obtained for MEC seizure tablets from completed casework was used to assess the homogeneity of visually similar tablets from the same seizure. Throughout this publication, the electropherograms obtained are referred to as ME 'profiles' to demonstrate the use of ME for comparative screening rather than identification.

2. Materials and methods

2.1. Apparatus

All experiments were performed on an Agilent 2100 Bioanalyzer using the Agilent 2100 Expert software (Agilent technologies, Waldbronn, Germany). Detection was by laser emitting diode-induced fluorescence (λ_{ex} 470 nm, λ_{em} 525 nm). Separations were carried out using standard DNA 500 microchips obtained from Agilent Technologies (Forest Hill, Australia). The chips were fabricated from soda lime glass. The micro-channels which interconnect 12 sample wells have a depth of 10 μm and width of 50 μm. The effective separation length was 15 mm.

2.2. Chemicals and reagents

Sodium dodecyl sulfate (SDS; ≥98.5%), sodium tetraboratedecahydrate (≥99.5%) and fluorescein isothiocyanate isomer I (FITC) (>90%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Analytical reagent grade methanol and acetone was obtained from Merck (Darmstadt, Germany). Nile blue chloride was purchased from Sigma–Aldrich (Sydney, Australia). Individual primary drug standards of 4-methylethcathinone, 4-methylmethcathinone, *N*-ethylamphetamine and *N*-ethylcathinone were purchased from the National Measurement Institute (Sydney, Australia). Individual primary drug standards of β-keto-3,4-methylbenzodioxylbutamine, β-keto-3,4-methylenedioxymethamphetamine, 1-(3,4-methylenedioxyphenyl)-2-bromopropane and seizure tablets were supplied by the Institute of Environmental Science and Research Ltd (ESR) in Auckland, New Zealand.

2.3. Electrolyte preparation

Electrolytes and sample stock solutions were prepared daily in distilled water. The separation electrolyte consisted of 50 mM sodium tetraborate buffer and 50 mM SDS (pH 9.66). The electrolyte was mixed, sonicated for 5 min and filtered through a 0.45 μm nylon membrane syringe filter prior to loading on the chip. All electrolyte solutions and stock solutions of target analytes were kept in the refrigerator at 4 °C and fresh solutions were prepared daily. A concentration of 10 μg/mL nile blue chloride dye was diluted in the electrolyte and primed through the micro-channels prior to analysis for laser focussing.

2.4. Sample preparation

2.4.1. FITC stock solution

A 50 μg/mL stock solution of FITC was prepared in analytical reagent grade acetone and stored in a 10 mL glass sample tube wrapped in aluminium foil at −18 °C.

2.4.2. Buffer

A solution of 50 mM sodium tetraborate was prepared in distilled water and filtered through a 0.45 μm nylon membrane filter for use in the derivatisation procedure.

2.4.3. MEC tablets

Previous seizure tablets were provided by ESR. One tablet of each source and variety (i.e. visually similar) was available for analysis. Each tablet was homogenised using the method described below and confirmatory analysis was carried out using GC–MS.

2.4.4. Tablet homogenisation

Tablets were crushed into a fine powder using a mortar and pestle. Approximately 3–4 mg of powder was added to 1 mL methanol in a sample tube and thoroughly mixed by shaking. The solution was left to settle prior to derivatisation.

2.5. Fluorescent derivatisation procedure

To 100 μL of the homogenised tablet or target analyte solution, 100 μL each of FITC stock solution and buffer were added in a flat bottomed glass insert (placed inside a crimped 1.7 mL microtube).