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Differentiation of opium and poppy straw using capillary electrophoresis and pattern recognition techniques

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ABSTRACT

Opium samples from four different locations and poppy straw from different plant varieties have been assayed using micellar capillary electrophoresis incorporating a sweeping technique. Individual alkaloids (morphine, codeine, papaverine, noscapine, thebaine, oripavine, reticuline and narceine) were quantitatively determined in the different samples by a validated capillary electrophoresis method. Unsupervised pattern recognition of the opium samples and the poppy straw samples using hierarchical cluster analysis (HCA) and principal component analysis (PCA), showed distinct clusters. Supervised pattern recognition using soft independent modelling of class analogy (SIMCA) was performed to show individual groupings and allow unknown samples to be classified according to the models built using the CZE assay results.

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1. Introduction

Opium is extracted from *Papaver somniferum* by making incision cuts to the ripening poppy capsules [1]. The white milky latex, which exudes is allowed to dry overnight. The reddish–brown deposit is collected as crude raw opium by scraping from the capsule. The raw opium contains more than 30 different alkaloids, the most abundant being morphine (4–21%), codeine (0.8–2.5%), noscapine (4–8%), papaverine (0.5–2.5%) and thebaine (0.5–2%) [1].

Poppy straw is the dried heads and stalks of *P. somniferum*. The alkaloids are commercially extracted using a method developed by Kabay in the 1920s [2]. Poppy straw is the principal source of both morphine and thebaine [3]. *P. somniferum* has been genetically modified by gene insertion to either enhance alkaloid production or to "switch off" the genes responsible for production of non-required alkaloids [4,5]. Tasmanian Alka-

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loids have used these techniques to produce individual poppy varieties to produce significant quantities of individual alkaloids. The variety named "Norman", produces no morphine while producing thebaine and oripavine used in the production of the strong painkillers oxycodone and buprenorphine. Other gene modified poppies produce enhanced quantities of papaverine and noscapine along with morphine [4,6].

Assay methods for the determination of the various alkaloids present in opium and poppy straw include thin layer chromatography (TLC) [7–14], gas liquid chromatography (GLC) [8,15–20], supercritical fluid chromatography (SFC) [21,22], high performance liquid chromatography (HPLC) [23–32] and capillary zone electrophoresis (CZE) [33–42]. The GLC methods require derivatisation for some of the alkaloids, which significantly increase analysis time. The TLC and SFC methods lack the sensitivity required to determine the principle alkaloids of interest in the opium and poppy straw samples. The HPLC methods also lack the resolution required for the quantitative determination of the alkaloids. Poor CZE separation is shown for certain alkaloids in opium [35] and pH has to be strictly controlled [34,38]. Other methods are unable to operate at the optimum detection wavelengths [33,34,37]. Various cyclodextrin additives have been used to obtain the required separation [33,41,42].

Pattern recognition consists of two general areas; supervised or unsupervised. In unsupervised pattern recognition, information on the individual groupings is likely to be unknown, but is not a pre-requisite. In supervised pattern recognition, however, the groupings of samples must be known to allow predictions to take place [43]. Examples of unsupervised pattern recognition are hierarchical cluster analysis (HCA) and principal component analysis (PCA). Soft independent modelling of class analogy (SIMCA) is used for supervised pattern recognition. The method developed is based on the pattern recognition decision tree in Chemometrics: A Practical Guide [44].

The main objective of HCA is to display data in natural clusters showing patterns in two-dimensional space [44]. The results are presented in a dendrogram, which is qualitative in nature and permits visualization of clusters and correlations amongst samples. In HCA, the Euclidean distances among samples or variables are transformed into similarity indices ranging from 0 to 1. A small distance corresponds to a large index and means a large similarity [45].

PCA is a data compression method based on correlation amongst variables [45]. The aim of PCA is to group correlated variables, and replace them by new sets called principal components (PC). PCs are completely uncorrelated and are built as simple linear combinations of the original variables. PCs contain most of the data set variability, but in a much lower-dimensional space. The first principal component, PC1, is defined as the direction of maximum variance of the whole data set. PC2 is the direction that describes the maximum variance in the orthogonal subspace to PC1. The subsequent components are taken orthogonally and describe the maximum remaining variance. When redundancy is removed, only the first few principal components are required to describe the information contained in the original data set. The algorithms for PCA calculations can be found in standard chemometric books [46].

SIMCA uses PCA to create a model for the shape and position in space from the data [45]. A box is created for each sample class and unknown samples are assigned if possible. To construct the boxes a training set of samples is required, with known identities. Principal components for each class are calculated separately. The number of principal components for each class may vary and the SIMCA model is completed by defining a boundary region for each PCA model. The class of an unknown is determined mathematically, by projecting the measurement vector into each SIMCA model. The unknown may be a member of more than one class, and this shows a lack of discrimination power to differentiate between classes [45].

Attempts to identify opium origins involving GLC to determine concentration ratios of five alkaloids [16] used a triangular plot to graphically differentiate opium from five different locations. Pattern recognition techniques have been applied to determine the source of opium from India, using a fingerprint of the free amino acids in the sample and applying multiple discriminant analysis to the quantitative data [47]. PCA has been used in conjunction with HPLC quantitative analysis of various alkaloids, in 27 opium samples from known locations [48]. Four distinct clusters were evident, but three samples did not fit into any classification.

A requirement exists for methods to distinguish poppy cultivars in new growing areas as well as methods to determine the source of opium seized from illicit trafficking [49]. It is the purpose of this research to develop:

(a) a suitable CZE separation to quantitatively determine the concentration of specific alkaloids present in opium and poppy straw,
(b) a suitable statistically based method to differentiate the origin of samples by the discriminatory matching of the components.

2. Materials and methods

2.1. *Instrumentation*

The capillary electrophoresis was carried out using an Agilent HP^{3D}CE system (Waldbron, Germany) equipped with a diode array detector. Instrument control and data manipulation was performed using CE ChemStation software version 6.01. Capillaries (60 cm × 0.5 μm, i.d.) were supplied by Composite Metal Services Ltd. (Ilkley, England).

The multivariate statistics was performed using Pirouette Lite Classify software from Infometrix (Woodville, WA, USA).

2.2. *Chemicals*

Codeine, thebaine, papaverine and noscapine were obtained from Sigma (Poole, England). Narceine was supplied by Koch-Light Laboratories Ltd. (Colnbrook, England) and morphine and dihydrocodeine for use as an internal standard (IS) were from Macfarlan Smith Ltd. (Edinburgh, Scotland). Methanol (HPLC grade), sodium dodecyl sulphate, glacial acetic acid and di-sodium hydrogen orthophosphate were from Fisher

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