Application Note

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µSPEed extraction followed by HPLC-DAD analysis of opium alkaloids in poppy seed tea

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SUMMARY

The innovative µSPEed technique has been applied using digiVOL Digital Syringe (Figure 1) for accurate and precise extraction and pre-concentration of the main opium alkaloids (morphine, codeine, thebaine, papaverine, noscapine and oripavine) from poppy seed tea using a PS/DVB-RP cartridge. The infusion sample has been loaded 10 × 250 µL and the elution was with a small amount of methanol with 10% formic acid (2 × 125 µL), pre-concentrating the analytes 10-fold before analysis by HPLC-DAD, demonstrating the high preconcentration capacity of this microextraction technique.

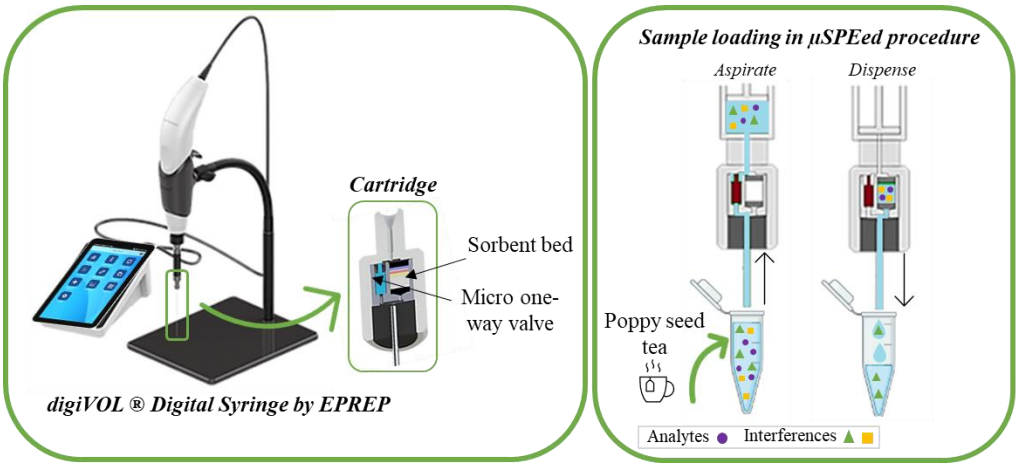

Figure 1. digiVOL Digital Syringe with cartridge structure and scheme of sample loading in µSPEed procedure.

INTRODUCTION

Opium alkaloids (OAs) are secondary metabolites that are present in milky latex sap of opium poppy plant (*Papaver somniferum L.*). The main OAs present in the latex are morphine, codeine, thebaine, papaverine, noscapine and oripavine (Figure 2). For this reason, this traditional plant is widely used for medicinal purpose due to its pharmacological properties [1,2].

OAs can be introduced into the food chain from poppy seed of this plant. These seeds are increasingly used in some food, such as bakery products, as toppings for salads or yoghurts, or to produce teas and oil [3,4].

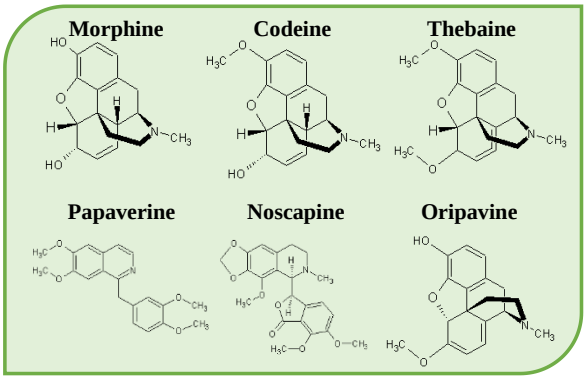

Figure 2. Structure of main opium alkaloids

Poppy seeds hardly contain any OAs, but they can be contaminated with the OAs present in the latex of this plant due to harvesting practices or insect damage. Consumption of contaminated food can lead to false positives in drug tests and may cause adverse health effects, such as nausea and vomiting, drowsiness, respiratory problems, and dependence, especially for the most vulnerable people and including more serious cases of intoxication, especially with the consumption of tea made from poppy seeds5. For this reason, the European Food Safety Authority (EFSA) has recommended the analysis of OAs in different matrices and has established an acute reference dose (ARfD) of 0.01 µg of morphine/kg body weight1. However, due to a lack of control in recent years, numerous food alerts have been reported, 34 since 2005, with high levels of morphine in poppy seeds, up to almost 400 ppm [6]. Therefore, due to the potential health risks described above, the European Commission recently published Regulation (EU) 2021/2142, which has entered vigour on July 1, 2022, that sets maximum levels for OAs, expressed in morphine equivalents (morphine + 0.2 × codeine), in bakery products (1.5 mg/kg) and in poppy seeds (20 mg/kg) [7].

For the analysis of these compounds, liquid chromatography coupled to a tandem mass detector (HPLC-MS/MS) is mainly used. However, many routine food quality control laboratories have diode array detectors (DAD). Therefore, there is a need to develop methodologies with a sample preparation step that allows not only purifying the extract to remove possible matrix interferences, but also pre-concentrating the extract to allow lower instrumental detection limits. To date, there are many works that use solid-phase extraction (SPE), but in addition to requiring more time and solvent consumption, none of them allows to achieve high preconcentration factors of the extract.

The innovative µSPEed technique makes it possible to decrease time and solvent consumption, obtaining methodologies more in line with green chemistry, as well as to obtain high preconcentration factors, contributing to low instrumental limits thanks to high preconcentration capacity. In addition, there is a large variety of commercial cartridges that allow their use with many types of analytes of different natures. The most suitable for opium alkaloids resulted to be the PS/DVB-RP polymer-based cartridge. Therefore, this application note describes a µSPEed-based analytical methodology for the extraction of opium alkaloids from poppy seed teas. Using a PS/DVB-RP cartridge to load 10 × 250 µL of sample and the target analytes were eluted with 2 × 125 µL of methanol [8].

PROCEDURE

Sample preparation

Teas were elaborated with poppy seeds (Figure 3). They were prepared according to International Standard ISO 3103 protocol [9]. For this purpose, 2 g of poppy seeds was infused with boiling water (100 ˚C) for 5 min. After, the sample was cooled to room temperature and filtered through a nylon syringe filter (0.45 µm) before µSPEed process.

Figure 3. Opium poppy seeds.

µSPEed Extraction Workflow

All extractions were performed on PS/DVB-RP (3 µm/300 Å) µSPEed cartridge by EPREP such as show Figure 4. First, cartridge was activated with an aspiration-dispense cycle of 250 µL of methanol followed by conditioning with 250 µL of water. Then, infusion sample was loaded onto µSPEed cartridge (ten aspiration-dispense cycles of 250 µL) and eluted with two aspiration-dispense cycles of 125 µL, pre-concentrating the analytes 10-fold. All steps were carried out at 15 µL/sec in extract-discard mode (each volume aspirated in all cycles was then discarded, after the dispense step, in a waste vial). Figure 4 shows the µSPEed extraction workflow followed and the aspiration-elution working mode.

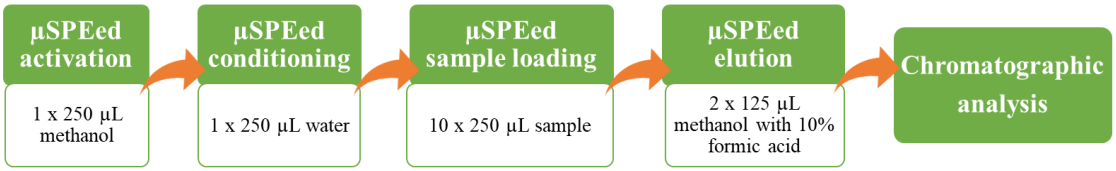

Figure 4. Diagram of µSPEed procedure

Chromatographic analysis

The separation (Figure 5) and determination of each of six opium alkaloids was carried out with an Agilent 1260 Infinity II HPLC system (Agilent Technologies, Madrid, Spain) coupled to diode array detector (G7117C 1260 DAD HS). The column used was an InfinityLab Poroshell 120 EC-C18 column (3.0 mm i.d. × 150 mm, 2.7 µm particle size) equipped with an InfinityLab Poroshell 120 EC-C18 guard column (3.0 mm i.d., 2.7 µm particle size) both from Agilent Technologies (Madrid, Spain) at 30 °C. The mobile phase used was milli-Q water containing 0.1% trifluoroacetic acid and acetonitrile in gradient elution mode with a total analysis time of 9 min. The wavelength used was 212 nm for all analytes.

RESULTS

Analytical Parameters

Good performances of the method were demonstrated in terms of precision and accuracy using the proposed methodology with the µSPEed technique. The results obtained were evaluated according to the criteria established for precision and accuracy in the SANTE/11312/2021 document [8].

Accuracy was evaluated in terms of recovery percentage (%) for standard solutions at 0.2 µg/L prepared in water and for three herbal infusions (rosemary, echinacea and valerian). The herbal infusions were spiked with an appropriate amount of atropine and scopolamine standard solution to achieve 0.2 µg/L concentration level. The 0.2 µg/L spiked level was selected according to the Commission Regulation (EU) 2021/1408 maximum level concentration established for liquid herbal infusions [4]. The standard solutions and samples were subjected to the µSPEed process before the HPLC-MS/MS analysis. An additional sample of water or infusion (simulated sample, post-extraction doping) was purified con the µSPEed procedure and

doped immediately after the µSPEed procedure to estimate the recovery percentage (%). After HPLC-MS/MS analysis, the areas obtained from the sample doped before the µSPEed process and the samples doped after the µSPEed process were compared. The results of six samples purified (n=6) with µSPEed and injected in triplicate are shown in **Table 1**. According to the validation guideline, the recovery percentages should be between 70-120%.[8] The results obtained with the proposed methodology showed good accuracy with recovery percentages between 95-102% for atropine and between 85-102% for scopolamine in the standard solutions and infusions of rosemary, echinacea and valerian.

On the other hand, the precision of the method was evaluated (expressed as relative standard deviation, RSD%) in the standard solutions and in the infusions. The precision was evaluated in terms of intra-day precision (repeatability), six replicates in one day (n = 6), and inter-day precision (reproducibility), three replicates in three different days (n = 9), all tests at 0.2 µg/L (for each analyte). According to the validation guidelines, RSD values should be ≤20%. **Table 1** shows the intra- and inter-day RSD values for the standard solutions and the three matrices studied, which are all below 20%.[8] Specifically, the intra-day values are ≤8% for both atropine and scopolamine, and the inter-day values are ≤9% for atropine and ≤8% for scopolamine in all matrices studied.

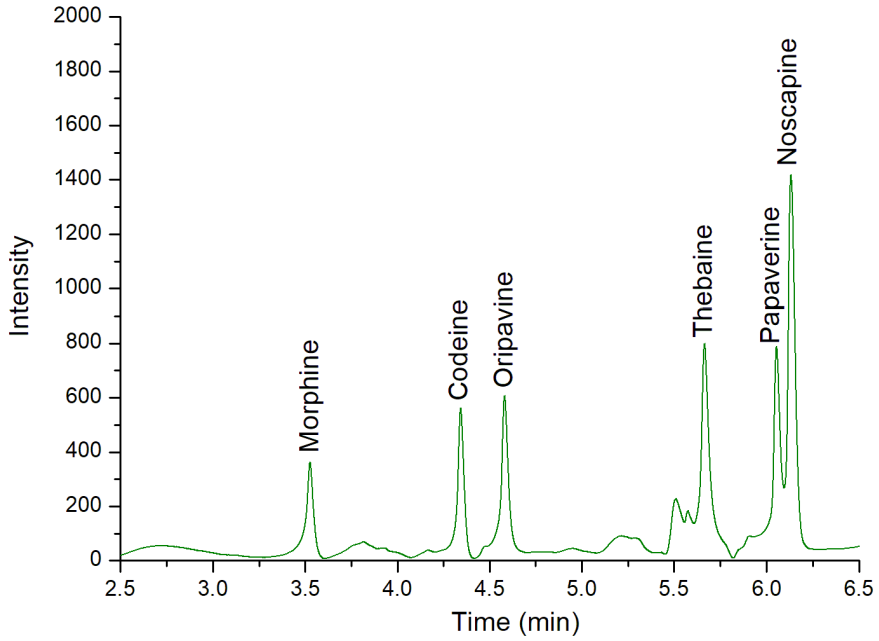

Figure 5. Chromatographic separation of six OAs at 8 µg/mL under optimised conditions with the HPLC-DAD method at 212 nm.

CONCLUSION

The µSPEed technique has proven to be a suitable technique for the extraction and purification of the main opium alkaloids, morphine, codeine, thebaine, papaverine, noscapine and oripavine, in poppy seed tea samples. The method has provided adequate recovery rates between 85-104% and good intra- and inter-day precision (≤ 11%) for all analytes. Moreover, this technique is simple, fast and environmentally friendly, as it uses significantly lower volumes of organic solvents than other conventional techniques such as solid-phase extraction. Moreover, the solvents used are not very harmful and hazardous, as the sample is loaded in water and eluted with a small aliquot of methanol with formic acid (2 x 125 µL). In this sense, the good performance of µSPEed was demonstrated with its application on poppy seed tea samples for OAs analysis, contributing to the requirements of food safety and green analytical chemistry.

ACKNOWLEDGEMENTS

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Table 1. Accuracy (recoveries ± SD %) and intra-day and inter-day precision (RSD %) obtained using the µSPEed procedure proposed following by HPLC-DAD analysis for the determination of opium alkaloids in standard solutions prepared in water at 0.4 µg/mL and poppy seed tea at 0.2, 0.4 and 0.8 µg/mL concentration.

Analyte	Standard solution			Poppy seed tea samples		
	Accuracy (recoveries ± SD %)	Intra-day precision (RSD %)	Inter-day precision (RSD %)	Accuracy (recoveries ± SD %)	Intra-day precision (RSD %)	Inter-day precision (RSD %)
Morphine	89 ± 3 ML	3 ML	7 ML	95 ± 1 LL	1 LL	2 LL
				91 ± 5 ML	6 ML	7 ML
				94 ± 2 HL	3 HL	3 HL
Codeine	92 ± 8 ML	9 ML	8 ML	100 ± 1 LL	1 LL	2 LL
				85 ± 6 ML	7 ML	8 ML
				97 ± 4 HL	4 HL	5 HL
Thebaine	88 ± 6 ML	6 ML	6 ML	96 ± 2 LL	3 LL	3 LL
				97 ± 2 ML	2 ML	5 ML
				100 ± 4 HL	4 HL	4 HL
Papaverine	91 ± 8 ML	8 ML	11 ML	103 ± 5 LL	4 LL	9 LL
				94 ± 6 ML	6 ML	11 ML
				97 ± 5 HL	5 HL	11 HL
Noscapine	89 ± 8 ML	9 ML	11 ML	101 ± 2 LL	2 LL	3 LL
				104 ± 4 ML	4 ML	8 ML
				97 ± 4 HL	4 HL	5 HL
Oripavine	100 ± 1 ML	1 ML	7 ML	92 ± 6 LL	7 LL	5 LL
				86 ± 3 ML	4 ML	5 ML
				100 ± 2 HL	2 HL	2 HL

Accuracy: n = 6; Intra-day precision: n = 6, 1 day; Inter-day precision: n = 9, 3 days. LL: low level (0.2 µg/mL); ML: medium level (0.4 µg/mL); HL: high level (0.8 µg/mL).

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