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Cation-exchange liquid chromatography: A convenient and "eco-friendly" alternative method for quantitative determination of herbal and fungal alkaloids

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Abstract

Aim: The customs laboratory Cologne, Germany, is acknowledged according to the voluntary European "Eco-Management and Audit Scheme" (EMAS). Thus, one of our goals is to improve environmental performance not only concerning energy and resource consumption of the facility in general, but also concerning the ecological impact of analytical methods. Therefore, a simple cation-exchange liquid chromatography method for the analysis of herbal and fungal alkaloids has been developed, which avoids laborious sample pre-treatment, derivatisation procedures and harmful organic eluents like acetonitrile.

Methods: Alkaloids from hallucinogenic fungi, khat and opium were separated by cation-exchange liquid chromatography using acidic water-based phosphate buffers with low amounts of ethanol as eluents. Samples were extracted either by 100 mM hydrochloric acid (khat) or 1 mM hydrochloric acid (opium). Acidified methanol was used for extraction of hallucinogenic mushrooms due to degradation of fungal alkaloids in acidic aqueous solution.

Results and Discussion: The method has already been validated for the analysis of khat alkaloids, psilocin and psilocybin from hallucinogenic fungi (Laussmann, T. and Meier-Giebing S., Forensic Sci. Int. 195 (2010) 160-164). In the present paper we describe an improved gradient for the separation of fungal alkaloids and the validation of the method for the analysis of morphine in opium.

Conclusion: The presented method is simple and "eco-friendly". It has been validated for the analysis of the relevant alkaloids from hallucinogenic fungi, khat and opium.

1. Introduction

The voluntary European "Eco-Management and Audit Scheme" (EMAS), based on regulation (EC) No 1221/2009, is a management tool for companies and other organisations to evaluate, report and improve their environmental performance [1]. The customs laboratory Cologne, Germany, is registered according to EMAS. Therefore, one of our goals is to improve analytical methods in order to save harmful chemicals and, subsequently, chemical waste.
The well-elaborated methods for testing morphine in opium published by the United Nations [3] are either based on gas chromatography after sample derivatisation by silylating agents or on reversed-phase liquid chromatography using significant amounts of organic eluents. Sample derivatisation is time-consuming and involves the risk of partial loss of analytes due to e. g. incomplete reaction. Additionally, only small sample amounts around 10 mg are used for derivatisation which might lead to lower reproducibility due to sample inhomogeneity. The reversed phase HPLC methods use eluents which contain either acetonitrile or methanol. Thus, for a double determination of one sample, usually about 50 ml of these harmful or even toxic organic solvents are consumed. Cation-exchange liquid chromatography in combination with acidic water-based phosphate buffers and low amounts of ethanol as eluents has been

shown to be a fast, convenient and "green" alternative to analyse alkaloids of herbal and fungal origin [2]. Additionally, no sample pre-treatment or derivatisation is required. In the present study, we describe an improved gradient for the separation of fungal alkaloids and the validation of this method for the analysis of morphine in opium samples.

2. Material and Methods

2.1. Chemicals and standards

Potassium dihydrogen phosphate, phosphoric acid (85%), sodium chloride, tryptamine and ethanol (non-denatured) were obtained from Merck (Darmstadt, Germany) and were of p.a. quality. Morphine standard was purchased from Lipomed (Arlesheim, Switzerland). Coffein was from Sigma-Aldrich (Steinheim, Germany). The chemicals and standards required for the analysis of khat and fungi have been described previously [2].

2.2. High-performance liquid chromatography

A VWR Hitachi LaChrom HPLC system comprising an organizer, an autosampler (L-2200), a high pressure pump (L-2130), a column oven (L-2350) and a diode array detector (L-2455) was used in all experiments. A Luna 5 mm SCX 100A 150 mm x 4.60 mm cation exchange column (Phenomenex, Aschaffenburg, Germany) was employed for separation. Mobile phase A consited of 50 mM KH_2PO_4, 5 % ethanol (pH 3.0). Mobile phase B consisted of 50 mM KH_2PO_4, 200 mM NaCl and 20 % ethanol (pH 3.0). The injection volume was 10 µL (fungi) or 5 µL (opium). Separation was performed at a flow rate of 1.5 mL/min (35 ºC) (fungi) or 2.0 mL/min (35 ºC) (opium) with the following gradients: fungi: 0 min: 100 % A, 4.5 min: 100 % B (linear gradient), 7 min: 100 % B; opium / morphine: 0 min: 55 % A, 45 % B, 16 min: 100 % B (linear gradient), 20 min: 100 % B. Psilocybin and psilocin were quantified at 220 nm. Morphine was quantified at 215 nm. The method was calibrated with 7 solutions of morphine standard in 1 mM HCl containing between 5.0 µg/mL and 100 µg/mL morphine and 25 µg/mL tryptamine (internal standard). Calibration for the analysis of khat and fungi has been described previously [2].

2.3. Sample preparation

Opium: Samples were deep-frozen at -80 °C und homogenised with a laboratory mill Grindomix GM 200 (Retsch, Haan (Rheinland), Germany) for 30 s at 10,000 rpm (interval mode). 100 mL of internal standard solution (1 mM HCl and 25 µg/mL tryptamine) were added to 50 mg of homogenised sample. The suspension was incubated in an ultrasonic bath for 60 min. Subsequently, an aliquot of the suspension was filtered via a paper filter and a 0.2 µm syringe filter (Macherey–Nagel, Düren, Germany) for HPLC analysis. Fungi: Sample extraction is performed by 10 mM HCl in methanol and has been described previously [2]. The conventional and well-established extraction of fungi by methanol could not be replaced by less harmful aqueous extraction since massive degradation of alkaloids has been observed when using e.g. 10 mM HCl in water.

3. Results and Discussion

Cation-exchange liquid chromatography has already been applied for the analysis of herbal and fungal alkaloids. The method is fully validated for the analysis of hallucinogenic fungi and khat. [2].

This method was now optimized and validated for the analysis of morphine in opium. Accuracy: The results obtained by the new method were compared with results obtained by a well-established alternative method (gas chromatography after silylation of the sample [3]) in our laboratory. A seized opium sample was measured in replicate (n=6) by both, the GC method and the HPLC method presented. The measured concentration of morphine was 10.9 g/100g ± 0.9 g/100g with the GC method and 11.7 g/100g ± 0.2 g/100g with the described cation-exchange HPLC method. Selectivity and specificity: A representative chromatogram is shown in Fig. 1. Peak purity of the measured alkaloids and the internal standards was controlled by correlation of the UV spectra with library spectra. No peak impurities could be detected. Linearity and limits of detection and quantification: Linearity, limits of detection and limits of quantification were calculated from the calibration curve (calibration range: 1 g/100g to 20 g/100g morphine) by replicate (n=6) measurements of calibration standards according DIN 32645 using a statistics software (SQS 99, Dr. Kleiner Software, Moos, Germany). The calibration curve was found to be linear. The limit of detection for morphine in opium was calculated to be 0.38 g/100g. The limit of quantification for morphine in opium was calculated to be 1.39 g/100g. Precision: Repeatability data of the method were calculated from 6 determinations of control material performed by the same person on the same day. Relative standard deviation (RSDs) was 0,42 %. The intermediate precision (between days repeatability) was calculated from replicate determinations (n=28) of control material on different days. RSD was 2.1 %.

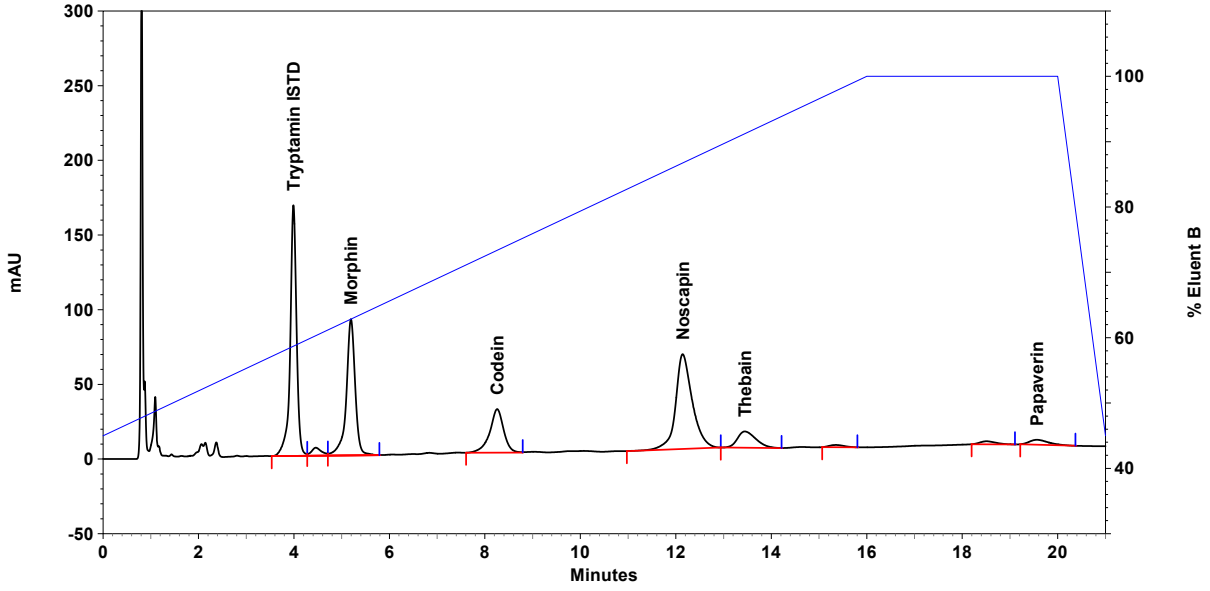

Fig. 1. Representative chromatogram (215 nm) of opium alkaloids from a seized opium sample. Alkaloids were separated on a Luna 5 mm SCX 100A 150 mm x 4.60 mm cation exchange column (Phenomenex). Mobile phase A: 50 mM KH_2PO_4, 5 % ethanol (pH 3.0). Mobile phase B: 50 mM KH_2PO_4, 200 mM NaCl and 20 % ethanol (pH 3.0).

Additionally, the analysis of the two fungal alkaloids psilocybin and psilocin was improved by application of a linear gradient instead of the isocratic elution protocol described previously [2]. The linear gradient leads to stronger retention of psilocybin and thus a better separation from the injection peak (Fig. 2).

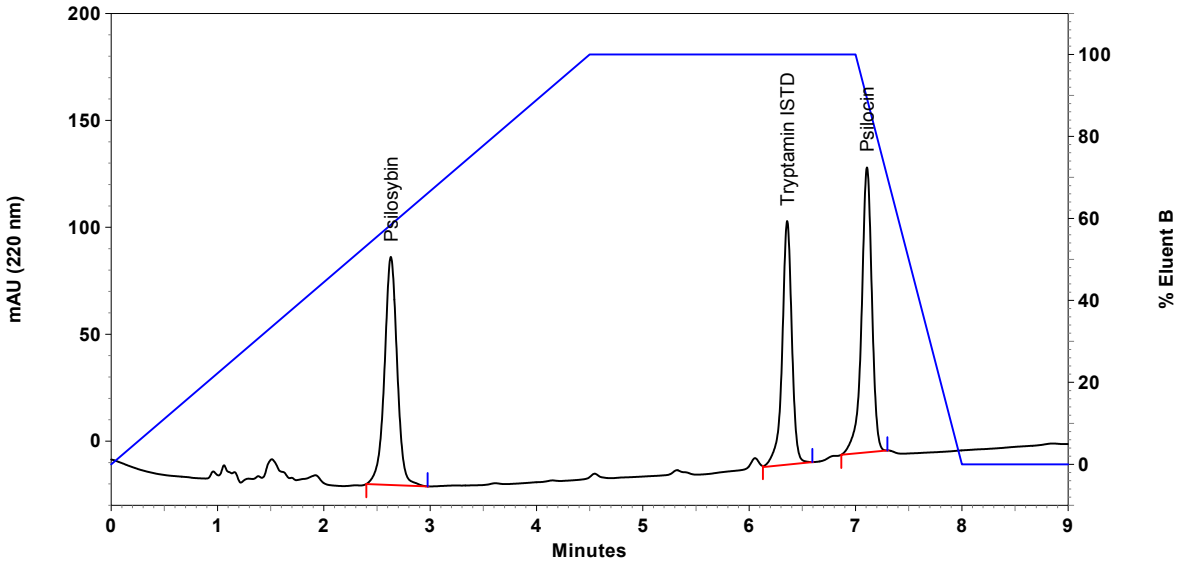

Fig. 2. Representative chromatogram (220 nm) of fungal alkaloids from a seized sample of *Panaeolus cyanescens*. Alkaloids were separated on a Luna 5 mm SCX 100A 150 mm x 4.60 mm cation exchange column (Phenomenex). Mobile phase A: 50 mM KH_2PO_4, 5 % ethanol (pH 3.0). Mobile phase B: 50 mM KH_2PO_4, 200 mM NaCl and 20 % ethanol (pH 3.0).

The presented cation-exchange liquid chromatography method is routinely used by German customs laboratories and a number of interesting findings concerning hallucinogenic mushrooms and khat could be achieved [2]. For example, it has been demonstrated that freeze-drying of khat and mushrooms is the method of choice to preserve psychotropic alkaloids. Additionally, several different seized mushroom species have been analysed with *Panaeolus cyanescens* having about three times higher total alkaloid content compared to the well-known hallucinogenic mushroom *Psilocybe cubensis*. Seized "grow-boxes" for hallucinogenic mushrooms were found to produce enough material for up to 17 hallucinogenic doses. In the future it is planned to use this convenient, eco-friendly method not only for the analysis of herbal and fungal alkaloids but also for the quantification of synthetic compounds like amphetamine and its derivatives.

4. Conclusion

Due to the different separation principle, application of cation-exchange chromatography should generally be taken into account as an alternative to established RP methods when analysing alkaloids. It might be a special advantage that the samples can be extracted, for example, by acidic aqueous solutions avoiding possible co-extraction of interfering compounds that might occur when organic extraction solvents are employed. Additionally, cation-exchange HPLC with aqueous solvents avoids ecologically harmful organic eluents that are usually necessary in RP HPLC which is a step forward to achieve a "green lab" with respect to health, safety and environmental regulations.

5. References

[1] http://ec.europa.eu/environment/emas/index_en.htm

[2] Laussmann T, Meier-Giebing S. Forensic Sci. Int. 2010;195:160-164

[3] United Nations International Drug Control Programme, Recommended methods for testing opium, morphine and heroin, United Nations, New York, 1998