Food Chemistry 242 (2018) 443–450

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Straightforward analytical method to determine opium alkaloids in poppy seeds and bakery products

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ARTICLE INFO

Keywords:
Morphine
Codeine
Thebaine
Noscapine
Papaverine
Narceine
LC-MS/MS

ABSTRACT

A straightforward method to determine the content of six opium alkaloids (morphine, codeine, thebaine, noscapine, papaverine and narceine) in poppy seeds and bakery products was developed and validated down to a limit of quantification (LOQ) of 0.1 mg/kg. The method was based on extraction with acetonitrile/water/formic acid, ten-fold dilution and analysis by LC-MS/MS using a pH 10 carbonate buffer.

The method was applied for the analysis of 41 samples collected in 2015 in the Netherlands and Germany. All samples contained morphine ranging from 0.2 to 240 mg/kg. The levels of codeine and thebaine ranged from below LOQ to 348 mg/kg and from below LOQ to 106 mg/kg, respectively. Sixty percent of the samples exceeded the guidance reference value of 4 mg/kg of morphine set by BfR in Germany, whereas 25% of the samples did not comply with the limits set for morphine, codeine, thebaine and noscapine by Hungarian legislation.

1. Introduction

The seeds of the poppy plant (*Papaver somniferum* L.) are used predominantly in Central Europe in food, such as bakery products, toppings for dishes, in fillings of cakes and desserts, and to produce edible oil. The seeds themselves hardly contain any opium alkaloids, but they can be contaminated with the opium alkaloids from the latex as a result of poor harvesting practices, insect damage or use of poppy seeds arising as by-product from pharmaceutical production of opium alkaloids using *P. somniferum* L. The phenanthrene derivatives morphine, codeine and thebaine are the most well-known opium alkaloids present in latex. (Fig. 1) (EFSA, 2011).

Consumption of poppy seeds contaminated with opium alkaloids can result in detectable amounts of free morphine in blood as well as measurable concentrations in urine, sufficient to interfere with drug abuse testing (Lachenmeier, Sproll, & Musshoff, 2010). It can also lead to adverse health effects, especially in babies, infants, the elderly and people with severe health issues (Sproll, Perz, & Lachenmeier, 2006). Morphine, codeine and thebaine are the most toxic opium alkaloids, although there is very limited data for thebaine (EFSA, 2011).

Currently, there is no harmonised European legislation on opium alkaloids in poppy seeds for food purposes, although Hungary has national maximum regulatory limits for opium alkaloids in poppy seeds: morphine (30 mg/kg), codeine (20 mg/kg), thebaine (20 mg/kg) and for the sum of morphine and noscapine (40 mg/kg) (EFSA, 2011). In 2005, BfR (Bundesinstitut für Risikobewertung), the German Federal Institute for risk assessment, derived a provisional reference value of 4 mg/kg for morphine in poppy seed for use in food, which since then is used as action limit in Germany, but not for legislative purpose (BfR, 2005). In Belgium poppy seeds can only be used on bakery products, but they are forbidden in food in general (EFSA, 2011).

The European Food Safety Authority (EFSA) carried out a risk assessment in 2011 on opium alkaloids from poppy seeds intended for human consumption and established an acute reference dose (ARfD) of 10 μg morphine/ kg bw (EFSA, 2011). Occurrence data were provided by Germany, Hungary, Austria and the Netherlands and indicated that morphine was the major alkaloid in poppy seed samples, in concentrations up to 630 mg/kg. The occurrence data on bakery products and baking ingredients that were provided to EFSA confirmed previous studies stating that food processing decreases the alkaloid content down to 10% of the original concentration (Lachenmeier et al., 2010). Soaking, heat treatment and grinding of the seeds were the most effective methods to reduce the opium alkaloid concentrations (Sproll, Perz, Buschmann, & Lachenmeier, 2007). However, EFSA recommended to collect more occurrence data of other opium alkaloids, besides morphine, for a more accurate risk assessment. In line with the EFSA opinion, the European Commission published in 2014a Recommendation on good practices to prevent and to reduce the presence

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http://dx.doi.org/10.1016/j.foodchem.2017.08.045
Received 31 May 2017; Received in revised form 26 July 2017; Accepted 15 August 2017
Available online 18 August 2017
0308-8146/

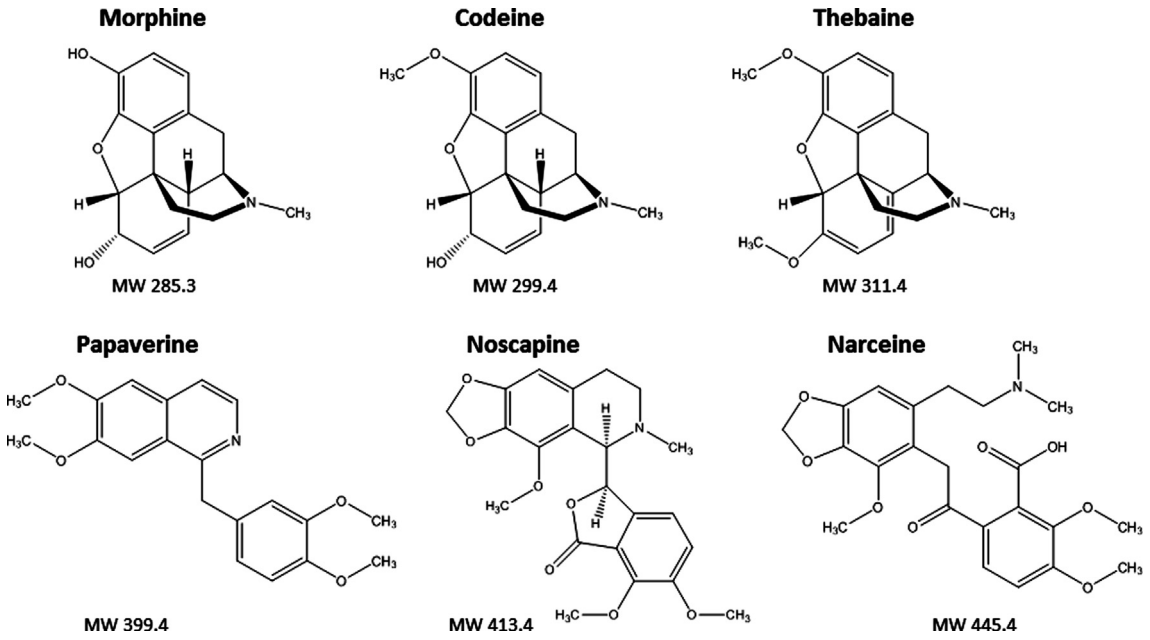

Fig. 1. Structure of the most common opium alkaloids.

of opium alkaloids in poppy seeds and poppy seed products (EU., 2014).

The determination of opium alkaloids has been traditionally carried out by gas chromatography-mass spectrometry (GC–MS) or by liquid chromatography-diode array detection (HPLC-DAD) (Acevska et al., 2012; Bosch, Sanchez, Rojas, & Ojeda, 2007). The major constraint of GC methods is the complex and costly sample preparation needed for derivatization of the analytes prior to GC analysis. The analysis of opium alkaloids by HPLC-DAD is hindered by the similar chromatographic behaviour of the minor alkaloids papaverine and noscapine and the likely presence of substantial matrix interferences, thus requiring purification steps.

Liquid chromatography in combination with tandem mass spectrometry (LC-MS/MS) and in combination with a "dilute-and-shoot" approach is an efficient method for routine analysis because it requires little or no sample clean-up, and the high specificity of mass selective detection avoids matrix interferences and compensates for separation problems. LC-MS/MS is extensively used in forensic research on opium alkaloids (Barroso, Gallardo, Vieira, Queiroz, & Lopez-Rivadulla, 2011; Eckart et al., 2015; Newmeyer et al., 2015).

The aim of this work was the development and validation of a straightforward "dilute-and-shoot" method, based on LC-MS/MS to be used for routine applications to determine opium alkaloids in poppy seeds and food commodities containing poppy seeds. The method was applied in a survey, which was carried out on samples taken at different points of the commercial food chain in the Netherlands and in retail in Germany at the end of 2015.

2. Materials and methods

2.1. Chemicals and reagents

Stock solutions of codeine, morphine, morphine-d_3 and thebaine at 1 mg/mL in methanol, noscapine hydrochloride hydrate and papaverine hydrochloride were purchased from Sigma-Aldrich (Sigma-Aldrich, Zwijndrecht, The Netherlands). Narceine trihydrate was purchased from Sequoia Research Products (Pangbourne, UK). Orivapine could not be obtained and was therefore not included in the method. Individual stock solutions of narceine, noscapine and papaverine at 2 mg/mL in methanol were prepared. All stock solutions were kept at −18 °C.

Mixed standard solutions of opium alkaloids at 100 and 10 μg/mL and of the internal standard (IS) morphine-d_3 at 1 μg/mL in water were prepared. Acetonitrile and methanol, both UPLC grade, were purchased from Actu-all Chemicals (Oss, the Netherlands), and formic acid (98–100%) and acetic acid (99%) from Merck (Amsterdam, the Netherlands).

2.2. Samples

A total of 41 samples were purchased from retail in the Netherlands (16), Germany (8) and Italy (1) at the end of 2015. In the Netherlands another 16 samples were collected from trader warehouses. The total sample set comprised of 32 blue and 3 white poppy seed samples, 3 ground blue poppy seed samples, 1 ready-to-use poppy seed filling for bakery and 2 ready-to-eat bakery products containing poppy seeds. Survey was designed to be conducted in the Netherlands, which is the affiliation country of the authors. Germany was included due to its proximity to the Netherlands and the existence of a provisional reference value for morphine in poppy seeds (BfR, 2005).

The samples were stored at the temperature recommended on the label until processing and unground to prevent degradation of morphine (Lachenmeier et al., 2010). For further details on samples see Supplementary information SS2. Poppy seeds from *Papaverum rhoeas*, *P. dubium* and *P. argenome* were purchased from De Bolderik (Werver-shoof, the Netherlands).

2.3. Optimized analytical method

Ten grams of sample were extracted with 100 mL of acetonitrile/water/formic acid (80/19/1, v/v/v) using a rotary shaker tumbling machine (Edmund Bühler GmbH, Hechingen, Germany) for 30 min at moderate speed. The sample was left to settle at room temperature for 30 min. Then, 50 μL of extract, 25 μL of IS morphine-d_3 at 1 μg/mL and 425 μL of water were pipetted in Mini-Uniprep™ PTFE filter vials (Whatman™, GE Healthcare UK limited, Buckinghamshire, UK) and filtered. Samples were re-extracted by decanting the extraction solvent, adding 100 mL fresh extraction solvent and repeating the procedure as described above. The two extracts for each sample were stored at -18 °C until LC-MS/MS analysis.

The opium alkaloids were separated and identified by LC-MS/MS