Detection of nutmeg misuse by GC-MS screening of urine

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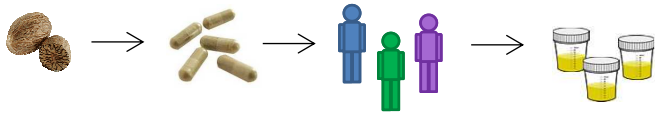
Background and Aim

High doses of **nutmeg (*myristica fragrans*) can be used as a psychoactive drug** due to the phenylpropenes contained in the seeds. During controlled abstinence, e.g. in forensic psychiatry or in prisons, **nutmeg misuse has to be distinguished from an ingestion of spices**. Typically, intake of one to four nutmegs leads to psychotropic effetcs, whereas about 0.15 g are used for seasoning of food.
The aim of this study was to develop an evaluation model for the estimation of time and amount of nutmeg intake and for the differentiation from ingestion of other spices. Therefore, urine samples were analyzed for metabolites of safrole, myristicin and elemicine by GC-MS.

Methods

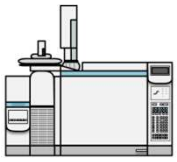
Studies and samples



Study 1: **Three** volunteers ingested **1.5 g** of freshly ground nutmeg in capsules. No symptoms were reported. Urine samples were collected for 3 days.
Study 2: Two volunteers ingested **0.15 g** of nutmeg during lunch.
Blank samples were from 18 volunteers without nutmeg intake.
Authentic samples were from two patients in forensic psychiatry.

Sample preparation

- Acidic hydrolysis (HCl)
- LLE (Dichloromethane: 2-Propanol: Ethylacetate, 1:1:3, v:v:v.)
- Acetylation (Ac_2O)
- GC-MS screening (full scan)
- Library search (Maurer, Pfleger, Weber)
- Semiquantitation of safrole-, myristicin- and elemicine-metabolites
- Creatinine (Jaffé-reaction, UV)



Agilent 7980 A GC, 5975C MSD, HP-5MS column

Results and Discussion

Metabolism

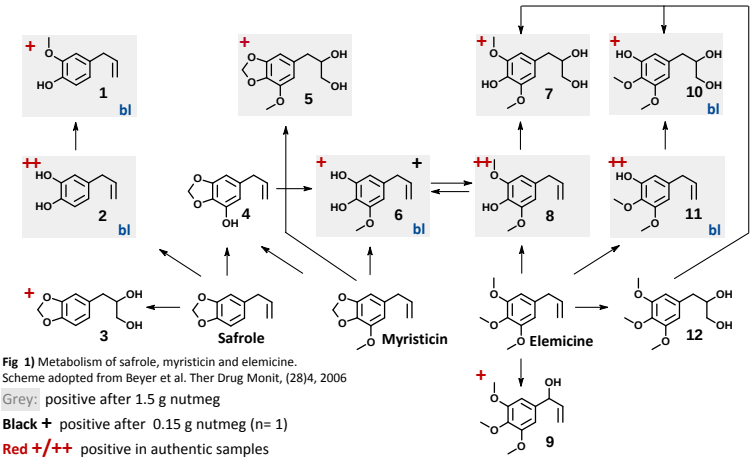


Fig 1) Metabolism of safrole, myristicin and elemicine.
Scheme adopted from Beyer et al. Ther Drug Monit, (28)4, 2006
Grey: positive after 1.5 g nutmeg
Black + positive after 0.15 g nutmeg (n= 1)
Red +/++ positive in authentic samples
bl: detectable in **blank** samples

Study 1: Up to eight different metabolites were positive* for at least 18 hours
Study 2: Only metabolite 6 was positive in one of the two participants.
Authentic samples: Normalized area ratios 0.5 to 14 x maximum of study 1. Two additional metabolites (3 and 9) were detected.
Blank samples: Probably due to ingestion of other spices (e.g. black pepper or garden lovage) five metabolites (1, 2, 6, 10 and 11) were detected (below reporting threshold).
* Positive reporting threshold: 3 x maximum of blank samples

Decision scheme

negative

positive

- No metabolite positive* –> no indication of nutmeg consumption
- Only safrole and myristicin-metabolites positive (1, 2, 5, 6, 7, 8) –> uptake of nutmeg or high amounts of other spices
- Elemicine-metabolite(s) positive (+/- safrole/myristicin-metabolites) –> nutmeg uptake confirmed, intake as spice can not be ruled out
- Metabolites of all three compounds, lower than in study 1 –> consumption of higher amounts of nutmeg probable
- Metabolites of all three compounds, higher than in study 1 –> nutmeg abuse likely

Elimination kinetics

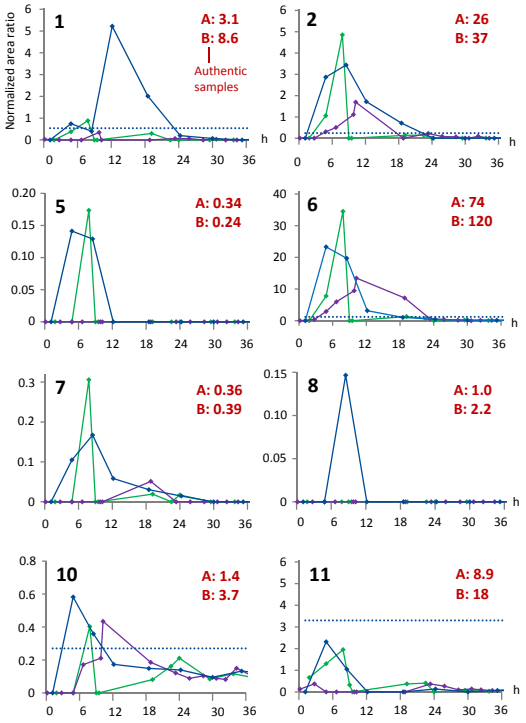


Fig. 2) Elimination of safrole, myristicin and elemicine metabolites in three volunteers (study 1) and values of two **authentic samples** (**A** and **B**).
For quantitative estimations, area ratios of the analytes to the internal standard (MDMA-d5) were normalized by dividing by the creatinine concentration.
Positive reporting threshold: 3 x maximum ratio of blank samples

Nutmeg used for the study:
myristicin 1.5 %, elemicin 0.045 %,
safrole below limit of detection (GC-MS)

Conclusion

The intake of high doses of nutmeg can be differentiated from the ingestion of spices via standard GC-MS analysis of urine and application of a chemometric evaluation model.

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