COUNCIL OF EUROPE MULTIANALYTE METHODS FOR THE DETERMINATION OF SUBSTANCES MIGRATING FROM PRINTED FCMs

A COLLABORATIVE STUDY

Council of Europe: *ad hoc* Working group on printing inks

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QUESTIONNAIRE ON TESTING PRINTED FCM

Would you be interested in participating in the peer review of a method for the analysis of photoinitiators in food/food simulant?

YES ☐   NO ☐

- Call for interest for the peer review of a method (via the working group): 14 laboratories responded positively.

- Study's scope: determination of interlaboratory reproducibility (precision).

- Both GC-MS/MS and LC-MS/MS based analysis.

- Matrices: liquid simulant, food.

- Examine suitable extraction/purification step for food substrate.

- Analytes: 6 photoinitiators, 3 degradation products, 1 plasticiser.

Analytes – Matrices

- 2 matrices: 95% EtOH and oat flakes

- 10 analytes: 6 photoinitiators, 3 decomposition products, 1 plasticiser
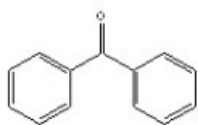
Benzophenone, BP

2-ethylanthraquinone, EA

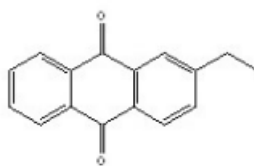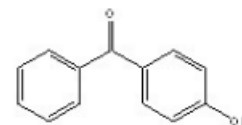4-Hydroxybenzophenone, 4-HBP
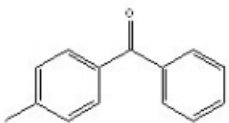
4-Methylbenzophenone, 4-MBP
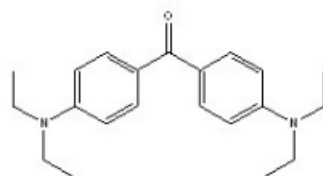
4,4'-bis(diethylamino)benzophenone, DEAB
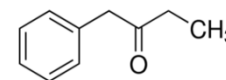
1-Phenyl-2-butanon, 1P2B
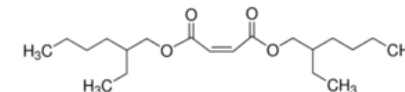
Bis(2-ethylhexyl) maleate
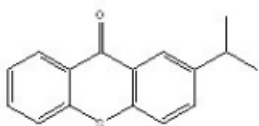
2-isopropyl-9H-thioxanthen-9-one, 2-ITX
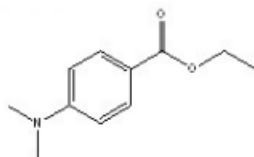
Ethyl-4-dimethylaminobenzoate, EDMAB
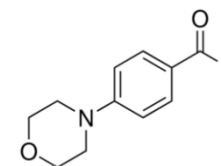
4-(4-morpholinyl) benzaldehyde , 4,4 MB

- Questionnaire for existing methods within the working group. Compilation of responses.

- Discussion and decision on details of detection methods (LC-MS/MS and GC-MS/MS). First draft of analytical protocol.

- Comments and initial evaluation by the laboratories – adjustments of the draft.

- **Feasibility study** with samples of known concentration distributed to participating laboratories.

- Presentation of results and green light for the study.

- Three different extraction procedures from dry food (Carrez, QuEChERS, SweEt). Discussion on their advantages and disadvantages.

Registration of Participants

STATE	PARTICIPATING LABORATORY	DETECTION			EXTRACTION		
		LC-MS/MS	GC-MS/MS	GC-MSD	CARREZ	QuEChERS	SweEt
			×			×	
			×			×	×
		×			×		
		×	×				×
		×			×	×	
			×			×	
			×			×	
		×		×	×	×	
			×			×	
		×			×		
		×		×	×	×	
			×			×	
		×			×		
				×		×	

Decision not to include in the study the SweEt extraction protocol since not enough laboratories were willing to report results based on it.

Experimental Design


```
0 (Laboratory)          i ---

1 (Day/Operator)    j ---              ...

2 (Repeats)    k ---        ...        ...        ...

        Y_{i11}  Y_{i12}  Y_{i1K}  Y_{i21}  Y_{i22}  Y_{i2K}  Y_{iJ1}  Y_{iJ2}  Y_{iJK}
```

Layout for a three factor fully-nested experimental design

Statistical analysis: Elena Regourd (EDQM)

Assam Pryseley, Koen Mintiens, Katia Knapen, Yves Van der Stede & Geert Molenberghs. Estimating precision, repeatability, and reproducibility from Gaussian and non- Gaussian data: a mixed models approach. *Journal of Applied Statistics.* 2010, Vol. 37, 10, pp. 1729-1747 (in accordance with ISO 5725)

2 matrices – 2 levels for each matrix → 4 samples
10 analytes in each sample → 40 results

For every sample & every analyte

- 3 different days and

- 2 repetitions each day

Every laboratory had to submit 240 results + linearity, LOD and QC

Dry Food Sample Preparation

NIPH, Czech Republic

UNIVERSITY OF CHEMISTRY AND TECHNOLOGY, PRAGUE
Faculty of Food and Biochemical Technology
Department of Food Analysis and Nutrition

Homogeneity test – for both concentration levels

10x 100 g → 2 x 5g of oats was weighted in Eppendorf tube for QuEChERS sample preparation
one way Anova F –test

2x 100 g → 6 x 5g of oats samples through the jar depth – to determine homogeneity in one glass jar

Peer-Review

- Laboratory analysis: November 2019 – February 2020
  - Matrix match calibration curve (range 10-200 µg/kg or 10-200 µg/L).
  - Each laboratory was asked to perform analyses in 3 different days, 2 analyses each day).

- 11 Laboratories submitted results (7 LC-MS/MS, 3 GC-MS/MS, 1 GC-MSD).

- Linearity achieved ($R^2 > 0.99$).

- LOD varied but in general < 10 µg/kg or 10 µg/L.

- Recoveries calculated based on the target values of spiking.

- All results submitted were taken into account for the calculation of precision except for a laboratory that asked to exclude results of a particular day based on failure of the quality control of that day.

LC-MS/MS - matrix 95% EtOH

Analyte	n	Sample A1					Sample A2				
		Mean (µg/L)	Rel. Repeat.	Rel. Reprod.	HorRat	REC	Mean (µg/L)	Rel. Repeat.	Rel. Reprod.	HorRat	REC
BP	6	16.6	7%	13%	0.6	119%	108.5	5%	11%	0.5	117%
4-MBP	6	20.5	6%	14%	0.6	130%	138.2	4%	11%	0.5	128%
4-HBP	6	15.5	9%	17%	0.8	103%	101.3	4%	15%	0.7	100%
2-ITX	6	16.6	15%	17%	0.8	105%	112.1	4%	19%	0.9	105%
EDMAB	6	15.3	7%	17%	0.8	109%	97.8	3%	20%	0.9	102%
DEAB	6	15.8	20%	25%	1.1	102%	100.2	4%	22%	1.0	92%
1P2B	3	14.9	5%	12%	0.5	96%	100.1	5%	10%	0.5	98%
44MB	5	13.7	5%	17%	0.8	91%	93.0	7%	21%	1.0	90%
EA	5	17.1	14%	26%	1.2	113%	118.9	6%	21%	1.0	117%
DEHM	5	15.3	7%	27%	1.2	101%	110.9	8%	12%	0.5	105%

GC-MS/MS - matrix 95% EtOH

Analyte	n	Sample A1					Sample A2				
		Mean (μg/L)	Rel. Repeat.	Rel. Reprod.	HorRat	REC	Mean (μg/L)	Rel. Repeat.	Rel. Reprod.	HorRat	REC
BP	3	16.6	3%	11%	0.5	105%	108.5	3%	7%	0.3	102%
4-MBP	3	20.5	7%	11%	0.5	118%	138.2	3%	11%	0.5	125%
4-HBP	3	15.5	7%	11%	0.5	94%	101.3	6%	13%	0.6	95%
2-ITX	3	16.6	5%	14%	0.6	97%	112.1	5%	8%	0.4	103%
EDMAB	3	15.3	5%	10%	0.5	95%	97.8	2%	6%	0.3	97%
DEAB	3	15.8	7%	11%	0.5	93%	100.2	6%	9%	0.4	99%
1P2B	3	14.9	6%	7%	0.3	99%	100.1	4%	7%	0.3	98%
44MB	3	13.7	9%	11%	0.5	88%	93.0	6%	8%	0.4	93%
EA	3	17.1	6%	6%	0.3	95%	118.9	3%	11%	0.5	102%
DEHM	3	15.3	8%	9%	0.4	93%	110.9	6%	13%	0.6	110%

Combined LC and GC - Matrix 95% EtOH

Analyte	n	Sample A1					Sample A2				
		Mean (μg/kg)	Rel. Repeat.	Rel. Reprod.	HorRat	REC	Mean (μg/kg)	Rel. Repeat.	Rel. Reprod.	HorRat	REC
BP	9	16.6	6%	14%	0.6	114%	108.5	4%	13%	0.6	112%
4-MBP	9	20.5	6%	14%	0.6	126%	138.2	4%	11%	0.5	127%
4-HBP	9	15.5	9%	16%	0.7	100%	101.3	5%	14%	0.6	98%
2-ITX	9	16.6	14%	17%	0.8	103%	112.1	4%	17%	0.8	104%
EDMAB	9	15.3	7%	17%	0.8	104%	97.8	3%	17%	0.8	100%
DEAB	9	15.8	17%	23%	1.0	99%	100.2	5%	19%	0.9	94%
1P2B	6	14.9	5%	10%	0.5	98%	100.1	4%	9%	0.4	98%
44MB	8	13.7	6%	15%	0.7	90%	93.0	6%	17%	0.8	91%
EA	8	17.1	12%	24%	1.1	107%	118.9	6%	20%	0.9	111%
DEHM	8	15.3	7%	24%	1.1	98%	110.9	7%	12%	0.5	107%

Combined LC and GC - Matrix: oat flakes

Analyte	n	Sample B1 (QuEChERS)					Sample B2 (QuEChERS)				
		Mean (μg/kg)	Rel. Repeat.	Rel. Reprod.	HorRat	REC (%)	Mean (μg/kg)	Rel. Repeat.	Rel. Reprod.	HorRat	REC (%)
BP	5	14.3	17%	43%	2.0	71%	107.8	5%	17%	0.8	108%
4-MBP	6	18.4	5%	22%	1.0	92%	90.0	11%	13%	0.6	90%
4-HBP	6	19.1	7%	23%	1.0	95%	91.6	7%	12%	0.5	92%
2-ITX	6	19.1	19%	35%	1.6	95%	86.9	13%	30%	1.4	87%
EDMAB	6	10.8	3%	27%	1.2	54%	57.7	7%	19%	0.9	58%
DEAB	6	17.2	10%	32%	1.5	86%	82.1	18%	38%	1.7	82%
1P2B	4	16.6	7%	31%	1.4	83%	76.0	6%	16%	0.7	76%
44MB	5	17.4	7%	27%	1.2	87%	87.1	4%	11%	0.5	87%
EA	5	15.4	29%	35%	1.6	78%	85.7	8%	15%	0.7	87%
DEHM	4	13.1	8%	27%	1.2	73%	62.5	6%	19%	0.9	69%

TIMELINE OF THE PROCESS

Meeting 11 May 2017 → Decision to peer-review a multianalyte method 2-3 March 2018 → Meeting 23-24 May 2018 → Meeting 19 Feb 2019

• Teleconference 13 September 2018

Feasibility phase 2 April-31 May 2019 → Peer review phase 19 Nov – 2 Feb 2020 → Final report 23 Sep 2020

• Teleconference 17 June 2019

• Teleconference 15 June 2020
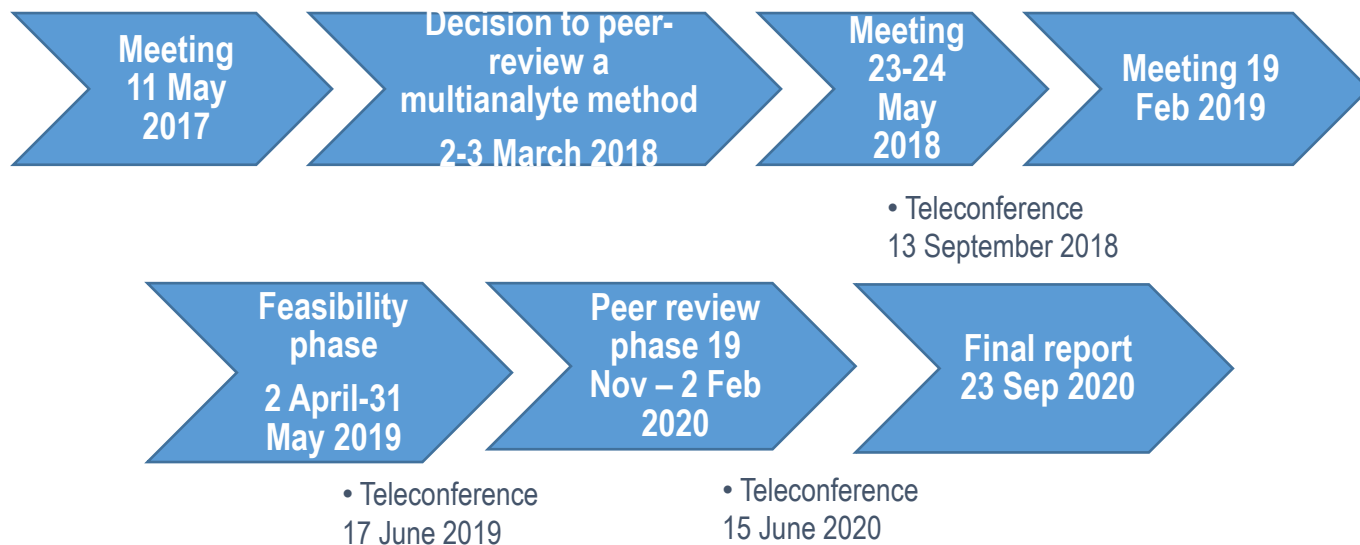
The method is available on-line at
https://freepub.edqm.eu/publications/

Number of downloads by 19/10/2022: 1471

Use of the protocol requires verification instead of full validation data

FOOD CONTACT MATERIALS AND ARTICLES
Substances migrating from printing inks to food or food simulants

GC-MS/MS and LC-MS/MS multi-analyte methods for the determination of photoinitiators and a plasticiser

EDQM
European Committee for Food Contact Materials and Articles (CD-P-MCA) 2021

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THANK YOU!!

https://www.edqm.eu/en/food-contact-materials-and-articles

Support to the working group – communication between partners
Financed sample preparation/distribution – statistical analysis
Publication of the method

General Chemical State Laboratory

https://www.aade.gr/gcsl