



ΑΑΔΕ

Ανεξάρτητη Αρχή
Δημοσίων Εσόδων



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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General Chemical State Laboratory, 2nd Chemical Service of Athens

National Reference Laboratory for Food Contact Materials

Email: fcm.gcsl@aade.gr



Capacity - Initial Decisions

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

Laboratory Name:	
Address:	
Contact person:	
Email:	
For how long have you been testing printed FCM or printing ink components?	
Do you use (a) multianalyte method(s) for the quantification of photoinitiators that have migrated into food or food simulant?	YES <input type="checkbox"/> NO <input type="checkbox"/>
What analytical instrumentation(s) do you use? (GC/MS, HPLC/DAD, LC/MS/MS, etc..)	
Is your method validated?	YES <input type="checkbox"/> NO <input type="checkbox"/>
Is your method accredited?	YES <input type="checkbox"/> NO <input type="checkbox"/>
What substrates (food or food simulant) have you analysed using this method?	

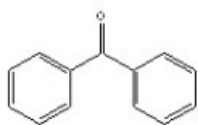
Comments:

Please complete and return this form by e-mail (consumer.health@edqm.eu) before **15 February 2018**

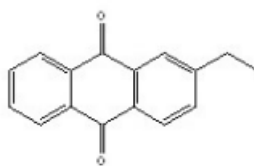
- Call for interest for the peer review of a method (via the working group): 14 laboratories responded positively.
- Study's scope: determination of interlaboratory reproducibility (precision).
- Both GC-MS/MS and LC-MS/MS based analysis.
- Matrices: liquid simulant, food.
- Examine suitable extraction/purification step for food substrate.
- Analytes: 6 photoinitiators, 3 degradation products, 1 plasticiser.

Analytes – Matrices

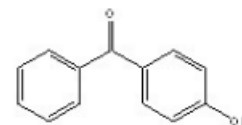
- 2 matrices: 95% EtOH and oat flakes
- 10 analytes: 6 photoinitiators, 3 decomposition products, 1 plasticiser



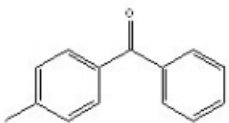
Benzophenone, BP



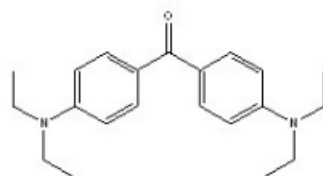
2-ethylanthraquinone, EA



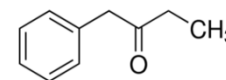
4-Hydroxybenzophenone, 4-HBP



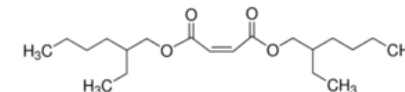
4-Methylbenzophenone, 4-MBP



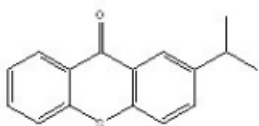
4,4'-bis(diethylamino)benzophenone, DEAB



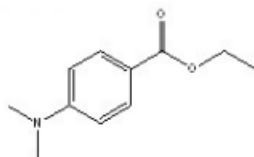
1-Phenyl-2-butanone, 1P2B



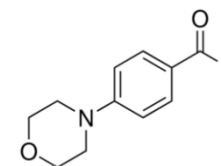
Bis(2-ethylhexyl) maleate



2-isopropyl-9H-thioxanthen-9-one, 2-ITX



Ethyl-4-dimethylaminobenzoate, EDMAB



4-(4-morpholinyl) benzaldehyde , 4,4 MB

Selection/Development of Method



TESTING RESIDUES ORIGINATING FROM PRINTING INKS

QUESTIONNAIRE ON METHOD CHARACTERISTICS

PART A: GENERAL INFORMATION

Laboratory Name	<laboratory name>
Contact person	<contact person>
Email	<email address>
Address	<address>

Method Name	<method name in english>
Method Number	<specify internal SOP number>
Publication of the method (DOI)	<not published / DOI>

Matrix	<e.g. dry food, simulans E, ...>
Number of analytes	<number of analytes (without internal standards)>

Number of internal standards	<number of internal standards>
Internal standards (list)	<list of all internal standards>

Concentration Range (mg/kg, mg/dm ²)	<for food in mg/kg and for FCM in mg/dm ² >
Calibration Matrix (solvent, matrix surrogate, matrix matched)	<e.g. matrix matched, matrix surrogate, solvent>
Calibration by standard addition (yes/no)	<yes / no>

Extraction Type	<e.g. QUECHERS, LLE, ...>
Extraction Description	<short description in words>
Extraction Solvent	<extraction solvent>
Extraction ratio sample to solvent	<in g sample / ml solvent; e.g. 10 g sample / 10 ml solvent>
Extraction conditions	<extraction conditions like shaking, microwave or ASE>
Cleanup 1	< e.g. dSPE, freezing out, ...>
Cleanup 2	< e.g. dSPE, freezing out, ...>
Concentration step (x ml > y ml)	<e.g. 5 ml > 1 ml>

Instrument (Brand and Type)	<Brand and type. E.g. Thermo GC 1310, Thermo Quantum GC>
Column	<For GC including phase, length, inner diameter and film thickness. For LC including phase, length and particle size>
Mobile Phase 1	<For GC H ₂ , He. For LC solvent A.>
Mobile Phase 2	<For LC solvent B>

- 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



Please complete and return this form to Consumer Health Protection, DBO, EDQM **before 26 August 2019**

by post: EDQM, 7, Allée Kastner, CS30026 F-67081 Strasbourg
by fax: +33 (0)3 88 41 27 71
by email: consumer.health@edqm.eu

REGISTRATION DETAILS

PARTICIPANT DETAILS* (Delivery address)		INVOICING DETAILS (if different from participant/delivery details)	
First Name		First Name	
Last Name		Last Name	
Company/ Institution		Company/ Institution	
Name of Unit/ Section (to be mentioned in the attestation of the participant)		Address	
Address (No PO Boxes)			
Postcode		Postcode	
Town		Town	
Country		Country	
VAT No (EU only)		VAT No (EU only)	
Tel		Tel	
Fax		Fax	
E-mail		E-mail	
*Purchase Order Reference (to be mentioned on the invoice)			

*Please note that all related information, documentation or material (e-mails, protocols, samples, reports, attestations of participation) will be sent to the above-mentioned registered participant at the above-mentioned address.

MCA PEER REVIEW n°	MCA PEER 001
Name of Study	Printing inks
Date of sample shipment	01/10/2019
Deadline for result submission	05/12/2019
Participation	<input type="checkbox"/> YES <input type="checkbox"/> NO
Our laboratory will report results following the method(s) of extraction	<input type="checkbox"/> Carrez <input type="checkbox"/> QuEChERS <input type="checkbox"/> SweEt
Our laboratory will report results following the method(s) of detection	<input type="checkbox"/> LC/MS-MS <input type="checkbox"/> GC/MS-MS <input type="checkbox"/> GC-MSD

FEES: MCA PEER 001 samples will be provided free of charge.

Date	Name	Signature

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.

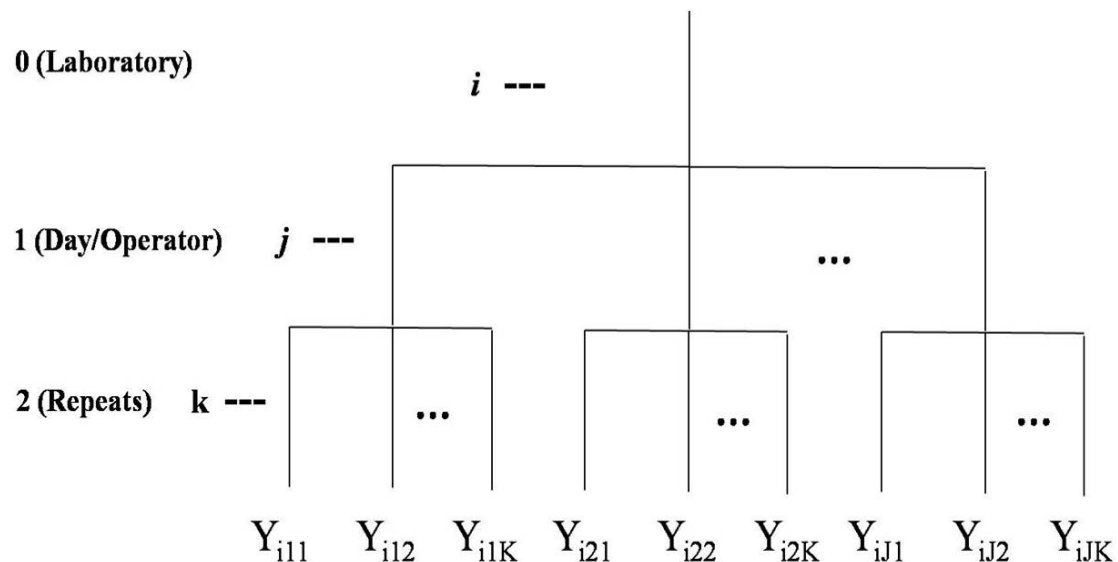
Experimental Design

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



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)



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



- Laboratory analysis: November 2019 – February 2020
 - Matrix match calibration curve (range 10-200 $\mu\text{g}/\text{kg}$ or 10-200 $\mu\text{g}/\text{L}$).
 - 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 $< 10 \mu\text{g}/\text{kg}$ or $10 \mu\text{g}/\text{L}$.
- 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

		Sample A1					Sample A2				
Analyte	n	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

		Sample A1					Sample A2				
Analyte	n	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

		Sample A1					Sample A2				
Analyte	n	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%

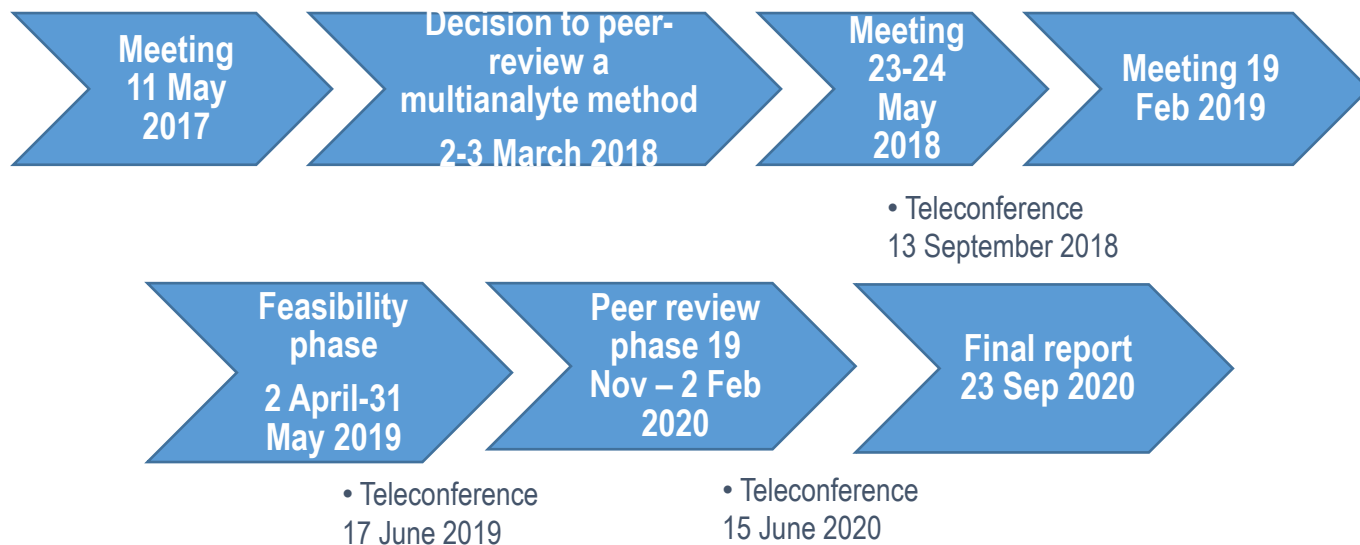


Combined LC and GC - Matrix: oat flakes

		Sample B1 (QuEChERS)					Sample B2 (QuEChERS)				
Analyte	n	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



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

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COLLABORATORS



STATE	PARTICIPATING LABORATORY	Contact Person
BELGIUM	NRL – SCIENSANO	Kathy van den Houwe
CZECH REPUBLIC	NRL - National Institute of Public Health (NIPH)	Jitka Sosnoscova
EU	EURL - European Commission - DG Joint Research Centre	Stefanka Bratinova
FRANCE	NRL - Laboratoire de Bordeaux (SCL)	Philippe Paulin
GERMANY	OCL - Chemical and Veterinary Analytical Institute – Münsterland-Emscher-Lippe CVUA	Fabrian Brenz
GERMANY	OCL - Lower Saxony State Office for Consumer Protection and Food Safety (LAVES)	Oliver Schmidt
GREECE	NRL - General Chemical State Laboratory (GCSL)	Eugenia Lampi
GREECE	Hellenic Research & Innovation Centre (HRIC)	Stratos Komaitis
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SPAIN	NRL - Spanish Agency for Food Safety and Nutrition AESAN (AECOSAN)	Juana Bustos
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SPAIN	University of Zaragoza (ZARAGOZA)	Cristina Nerin
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SWITZERLAND	OCL - Laboratoire Cantonal de St Gall	Juerg Daniel
UNITED KINGDOM	NRL - Fera Science Ltd (FERA)	Claire McKillen

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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>