

Overview of HIP-1, HIP-2 and HIP-3 Intercomparison exercises of HPLC Phytoplankton Pigments Analysis.

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What does the JRC GEM Laboratory do?

Produces *in-situ* measurements (e.g. phytoplankton pigments) for satellite data products validation and bio-optical algorithm development

Ensures quality to the analysis and quantifies uncertainties data through Intercomparison with other laboratories

Why we do not focus only on the Chl a pigment?

Collect the maximum number of information from the samples that we could analyze today only

2009-ongoing

The JRC organized 3 HPLC Intercalibration exercises of Phytoplankton Pigments (HIP exercises)

Overall objectives

Quantify single laboratory uncertainties

Quantify differences among European laboratories applying published methods

Create a reference community for HPLC pigments analysis in Europe

Benefits derived from the 3 exercises

Identify weakness in the methods or procedures

Improve the quality of results for single laboratories

**Trace and document in time the condition of the single lab
respect the others**

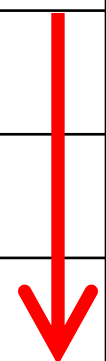
**During each exercise, focus on problems that emerged in
the previous HIPs**

The laboratories involved from 2009

Exercise	Participants	Laboratories	Country	Method compared	Distributed natural samples
hip 1	3	DHI	Denmark	1	108
		LOV	France		
		JRC	European Commission		
hip 2	6	DHI	Denmark	2	270
		LOV	France		
		JRC	European Commission		
		NIVA	Norway		
		IMAR	Portugal		
		Stockholm Un.	Sweden		
hip 3	6	DHI	Denmark	3	306
		LOV	France		
		JRC	European Commission		
		NIVA	Norway		
		IMAR	Portugal		
		GKSS	Germany		

The distributed mix standard and the analysis costs (supported by JRC)

Exercise	Year	Number of participants	Analysis, Standards & Shipments	Cost for natural samples
HIP 1	2009	3	7301	?
HIP 2	2010	6	6600	?
HIP 3	2011	6	5000	???



Performance Metrics (Method Validation)

Quality Level of the analysis:

State of Art > Quantitative > Semi-Quantitative > Routine

Analysis of Standard Mix

Evaluation of the HPLC Method using known samples

Analysis of Natural Samples

Evaluation of the HPLC Method and of the
Samples Extraction Procedure

**Basic step in lab
self evaluation**

**Focusing on
the HPLC
method**

**Evaluating the
difference introduced
by the extraction
procedure**

STEP 3: UNCERTAINTIES ON NATURAL SAMPLES

Average APD across the 12 batches of natural samples Primary Pigments

Chl. A, 6,8%

10 sample series (25 mm)	MV chlorophyll a	tot chlorophyll b	tot chlorophyll C	b-carotene	but-fucoaxanthin	19-hex-fucoaxanthin	Alloxanthin	Diadinoxanthin	Diatoxanthin	Fucoaxanthin	Peridinin	Zeaxanthin	AVERAGE
APD(JRC)	9.3	15.4	17.4	14.7	13.6	12.8	14.3	18.1	24.9	13.8	11.4	17.4	15.3
APD(DHI)	6.5	5.8	14.9	19.6	5.5	7.3	8.7	7.8	13.5	8.0	16.4	15.3	10.8
APD(LOV)	4.6	16.0	5.6	15.3	11.0	5.8	5.6	11.1	13.0	6.1	13.4	9.0	9.7
avg(apd)	6.8	12.4	12.7	16.5	10.0	8.6	9.5	12.3	17.2	9.3	13.7	13.9	11.9
MIX_106													
avg(apd)	2.0	6.7	10.3	1.9	5.3	8.8	4.1	6.1	7.1	1.5	7.8	9.8	7.2

Average APD across the 12 batches of natural samples Primary Pigments

A reference subset is defined (labs D,L and J):

the labs with an APD > 25% for more than 3 Pp are excluded from the subset

Reference
Subset: Chl.A <5 %

APD		Tot Chlorophyll A	Tot Chlorophyll B	Tot Chlorophyll C	Carotenes	but-fucoanthin	19-hex-fucoanthin	Alloxanthin	Diadinoxanthin	Diatoxanthin	Fucoanthin	Peridinin	Zeaxanthin	MV chlorophyll a
C8 (Van Heukelem)	D	3.9	10.8	5.8	14.2	2.3	5.1	9.4	4.1	15.6	3.5	22.7	12.7	5.7
	L	5.0	11.3	7.2	32.0	9.5	5.4	11.6	5.4	8.3	6.3	10.6	31.0	7.8
	J	4.6	7.1	10.9	26.3	9.7	10.1	20.0	5.2	16.0	8.9	17.0	20.2	4.6
C18 (Jeffrey)	I	23.8	>100	27.3	82.1	101.3	25.2	35.3	28.9	26.8	19.8	36.2	41.3	26.0
	N	15.1	17.3	78.0	39.6	31.8	20.4	88.1	43.6	56.7	28.4	>100	45.5	
	U	>100	>100	>100	100.0	>100	>100	>100	>100	>100	>100	>100	>100	
Samples CV% (subset)		7.7	9.2	7.3	9.6	6.2	5.7	21.0	8.2	13.7	9.4	13.4	8.4	8.3
Mix_107 CV% (subset)		1.1	0.8	1.6	1.8	0.8	0.6	0.9	0.9	1.4	0.7	2.1	1.6	1.2

Two laboratories ask to resubmit their data after the first comparison results. There are significant progress, in one case

APD	Tot Chlorophyll A	Tot Chlorophyll B	Tot Chlorophyll C	Carotenes	but-fucoxanthin	19-hex-fucoxanthin	Alloxanthin	Diadinoxanthin	Diatoxanthin	Fucoxanthin	Peridinin	Zeaxanthin
N	15.1	17.3	78.0	39.6	31.8	20.4	88.1	43.6	56.7	28.4	>100	45.5
N'	21.3	10.3	90.9	40.6	31.2	16.7	86.0	39.1	55.2	23.6	>100	39.5
U	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
U'	27.5	> 100	94.2	100.0	57.8	10.7	70.5	52.1	54.8	27.2	64.2	99.5

(in RED the resubmitted data)

The HIP exercises give a picture of the State of Art of European Accredited Labs for HPLC pigments analysis

By providing uncertainty values for the analysis performed

By leading to improvement of results for single labs over time

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