

How to investigate the influence of hyperlipidemic samples on bioanalytical assays

Dr. Martina Wein





EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Committee for Medicinal Products for Human Use (CHMP)

Guideline on bioanalytical method validation

excipients can be studied by the determination of the MF or by a dilution study of a study sample with a high concentration with blank matrix not containing the excipient.

In addition to the normal matrix it is recommended to investigate matrix effects on other samples e.g. haemolysed and hyperlipidaemic plasma samples. If samples from special populations (such as renally or hepatically impaired populations) are to be analysed it is also recommended to study matrix effects using matrix from such populations.

Guidances requesting investigation of hyperlipidaemic plasma samples (ANVISA)



Agência Nacional de Vigilância Sanitária

www.anvisa.gov.br

Ministério
da Saúde



Resolution - RE n. 899, of May 29, 2003

D.O.U. 02/06/2003

The Deputy of the Collegiate Board of Directors of the Brazilian Sanitary Surveillance Agency, in the use of the attribution vested in him by Administrative Order n. 238, of March 31, 2003,

3.1. Specificity

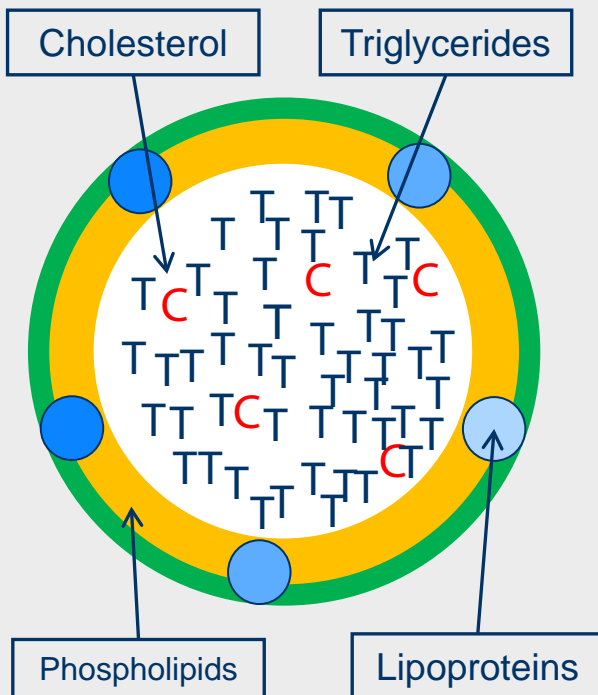
3.1.1. Samples of the biological matrix must be analyzed (blood, plasma, serum, urine, or other) obtained from six individuals, four normal samples, a lipemic sample and hemolized sample, under controlled conditions in terms of time, feeding and other important factors for the study. Each blank sample must be tested using the procedure and the chromatographic conditions proposed. The results must be compared with those obtained with aqueous solution of the analyte, in concentration close to the LLQ.

- Lipaemic samples contain >300 mg/dL triglycerides*
 - Usually only few study samples are affected
 - Lipaemic plasma is commercially available but only few sources
 - Is there a substitute to mimic lipaemic plasma?
-
- **Might intralipid be a suitable substitute?**
 - **Systematic evaluation of plasma samples spiked with various drugs (small molecules) containing intralipid**

* Renz H (Ed). Integrative Klinische Chemie und Laboratoriumsmedizin: Pathophysiologie - Pathobiochemie – Hämatologie. De Gryter, 2003.

Intralipid is a brand name for an emulsion of soy bean oil, egg phospholipids and glycerin for human use.

It is used as parenteral nutrition for patients who are unable to get nutrition via an oral diet. It is available in a 10%, 20% and 30% concentration.



Intralipid is also widely used in optical experiments to simulate the scattering properties of biological tissues.

It has similar properties and particle sizes as chylomicrons (=the particles that transfer fatty acids from the digestive tract to the liver)

Source: Wikipedia

Substance	Log P*	Extraction	Substance	LogP*	Extraction
BI 01	0.3	SPE	BI 10	5.4	SPE
BI 02	0.8	SPE	Etretinate	6.5	PP
BI 03	0.8	SPE	BI 11	6.8	SPE
BI 04	0.8	SPE	Tamoxifen	7.1	PP
BI 05	2.3	PP	Orlistat #	8.6	PP
BI 06	3.1	SPE	Fulvestrant	8.9	PP
BI 07	4.0	PP	Probucol	8.9	PP
BI 08	4.4	SPE	Anacetrapib #	9.3	PP
BI 09	5.2	PP			

* Source: Internal investigation for all BI drugs and www.drugbank.ca for all others

analysed without internal standard

SPE: Solid phase extraction

PP: Protein precipitation

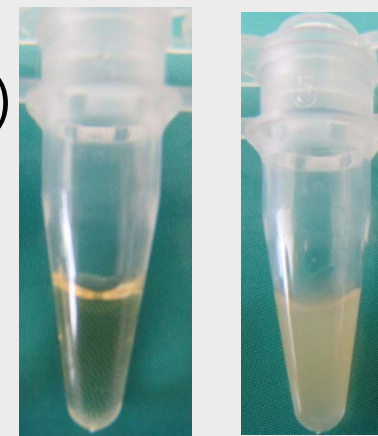
- Regular QC samples
- Lipaemic QC samples (400 mg/dL triglyceride)
- Intralipid QC samples (plasma/intralipid 20%, 98/2)
= equivalent to 400 mg/dL triglyceride



lipaemic

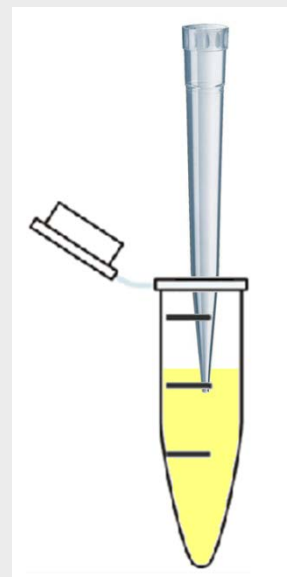
intralipid

- QC low and QC high (QC.2 and QC.4)
- 6 replicates
- Analysed with Cal + 2 QC sets (low, mid, and high level)
- Acceptance criteria: accuracy and precision

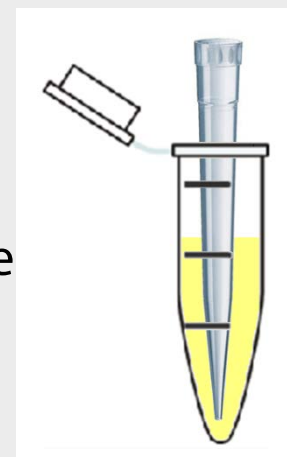


20 Nov 2013

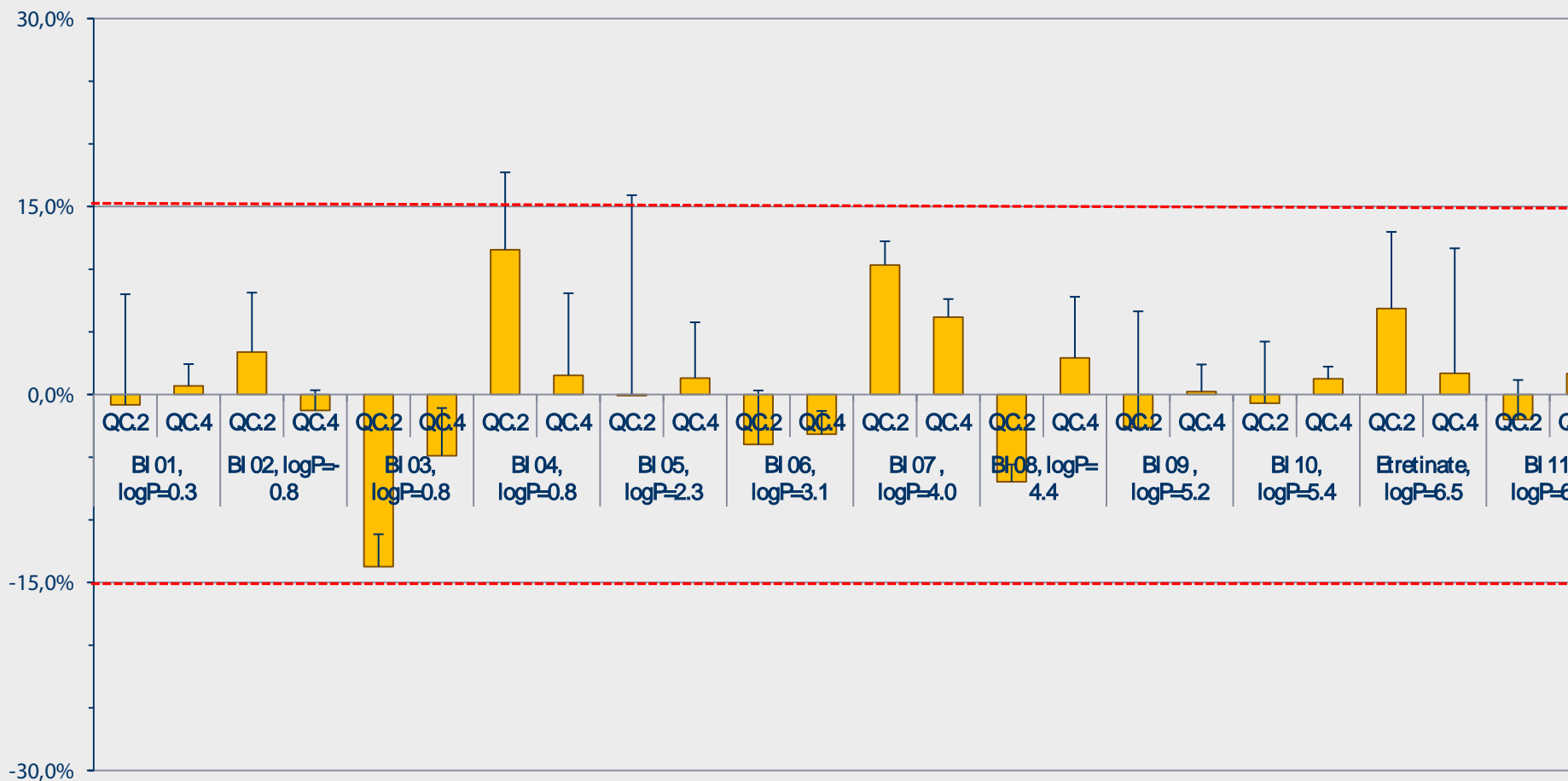
- Regular sample transfer:
 - Vortex sample
 - Centrifugation
 - Take sample just below the surface (below the fatty layer)



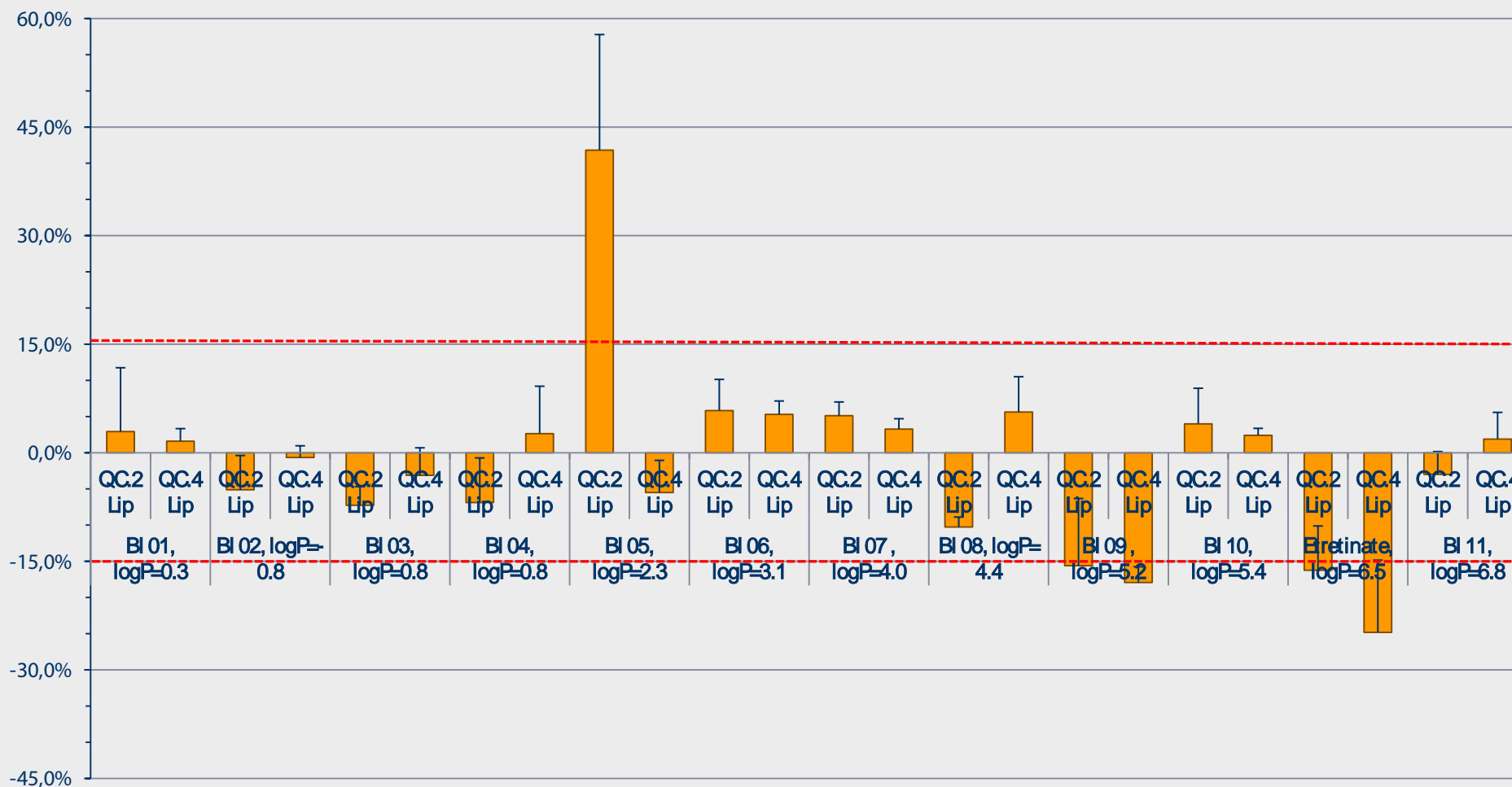
- Transfer of mixed sample:
 - Vortex sample
 - Take sample from the middle or bottom of the tube



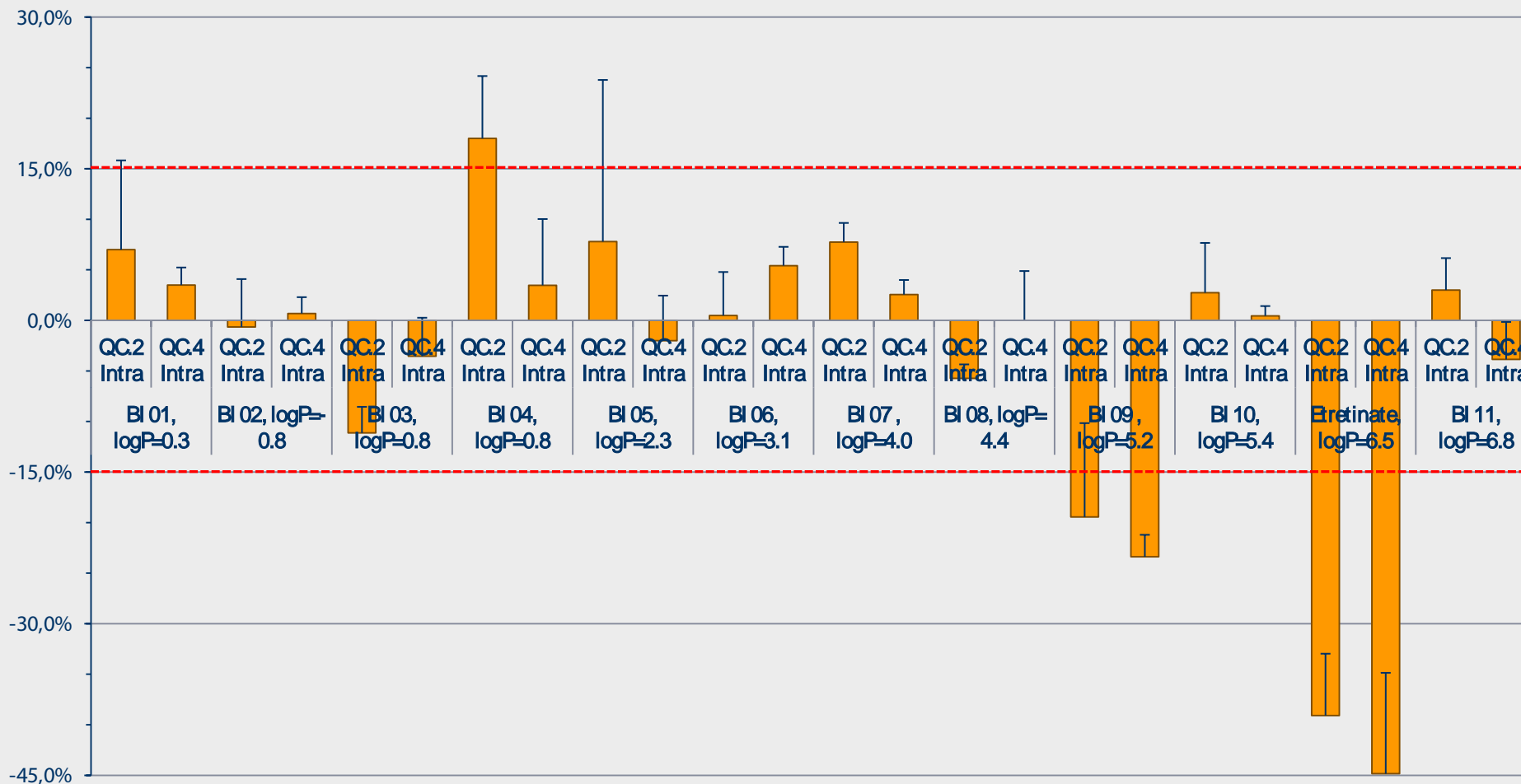
QC- Deviation to Nominal



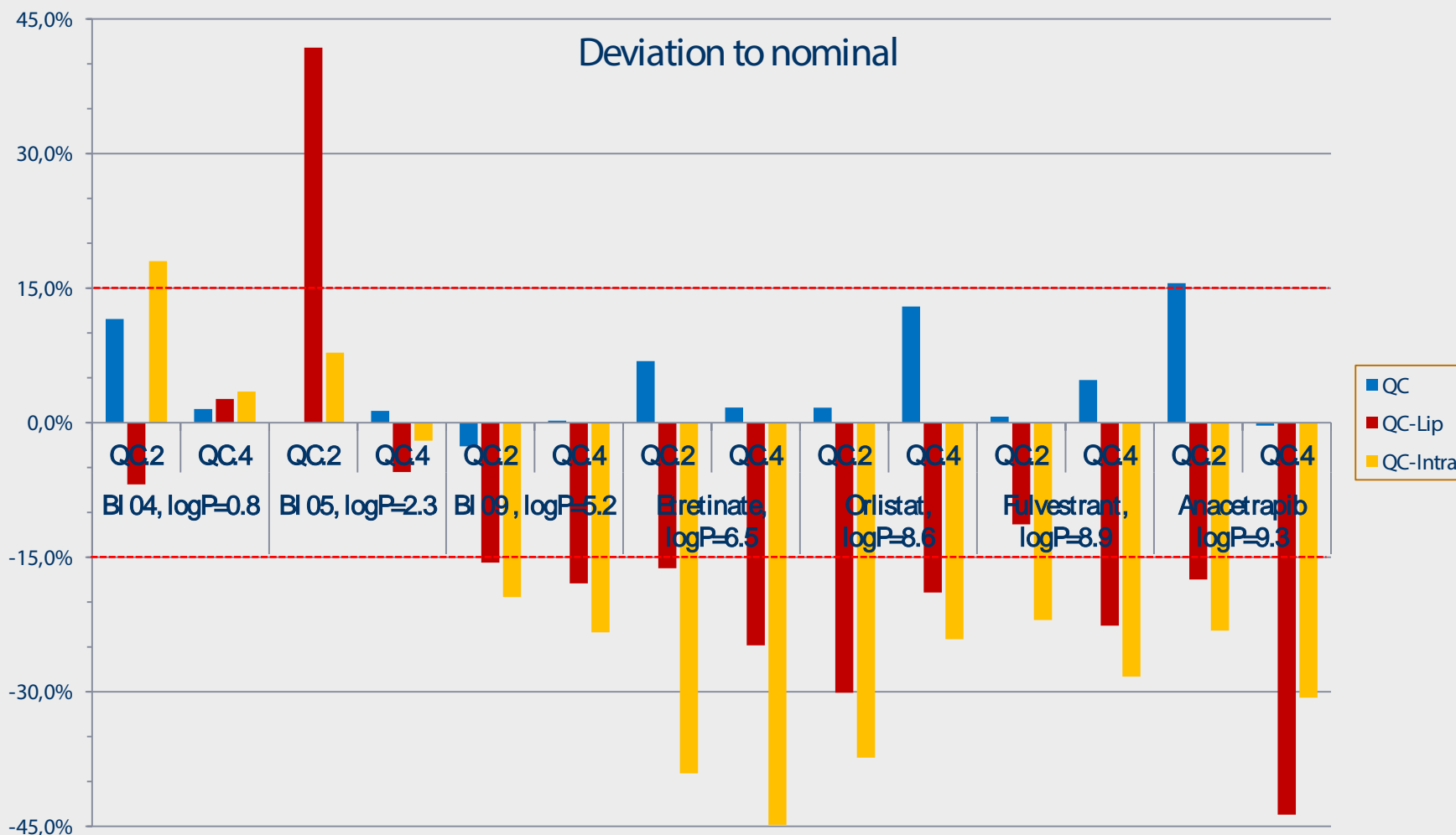
Lipaemic - deviation to nominal



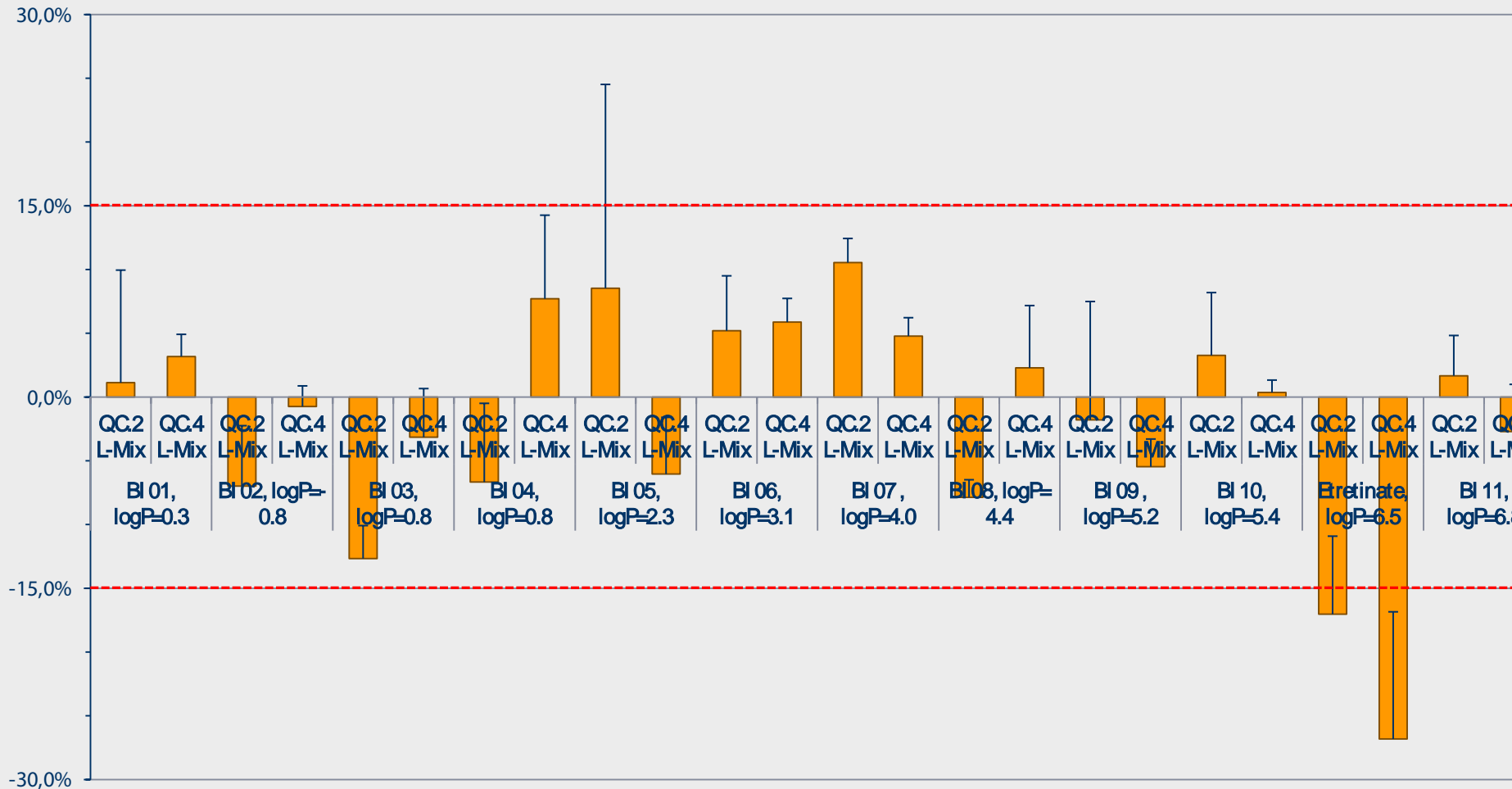
Intralipid - deviation to nominal



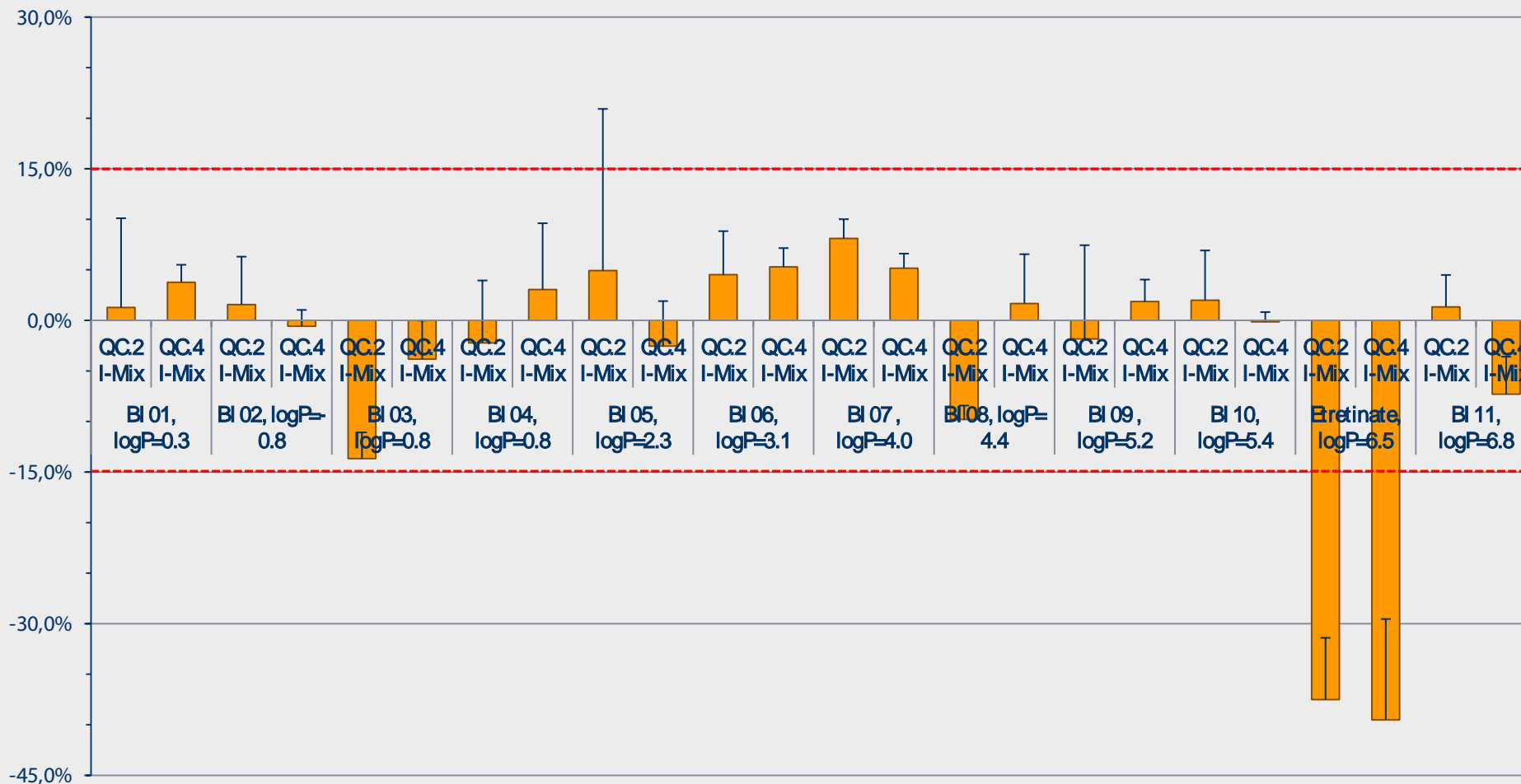
Lipaemic vs intralipid samples – Regularly analysed



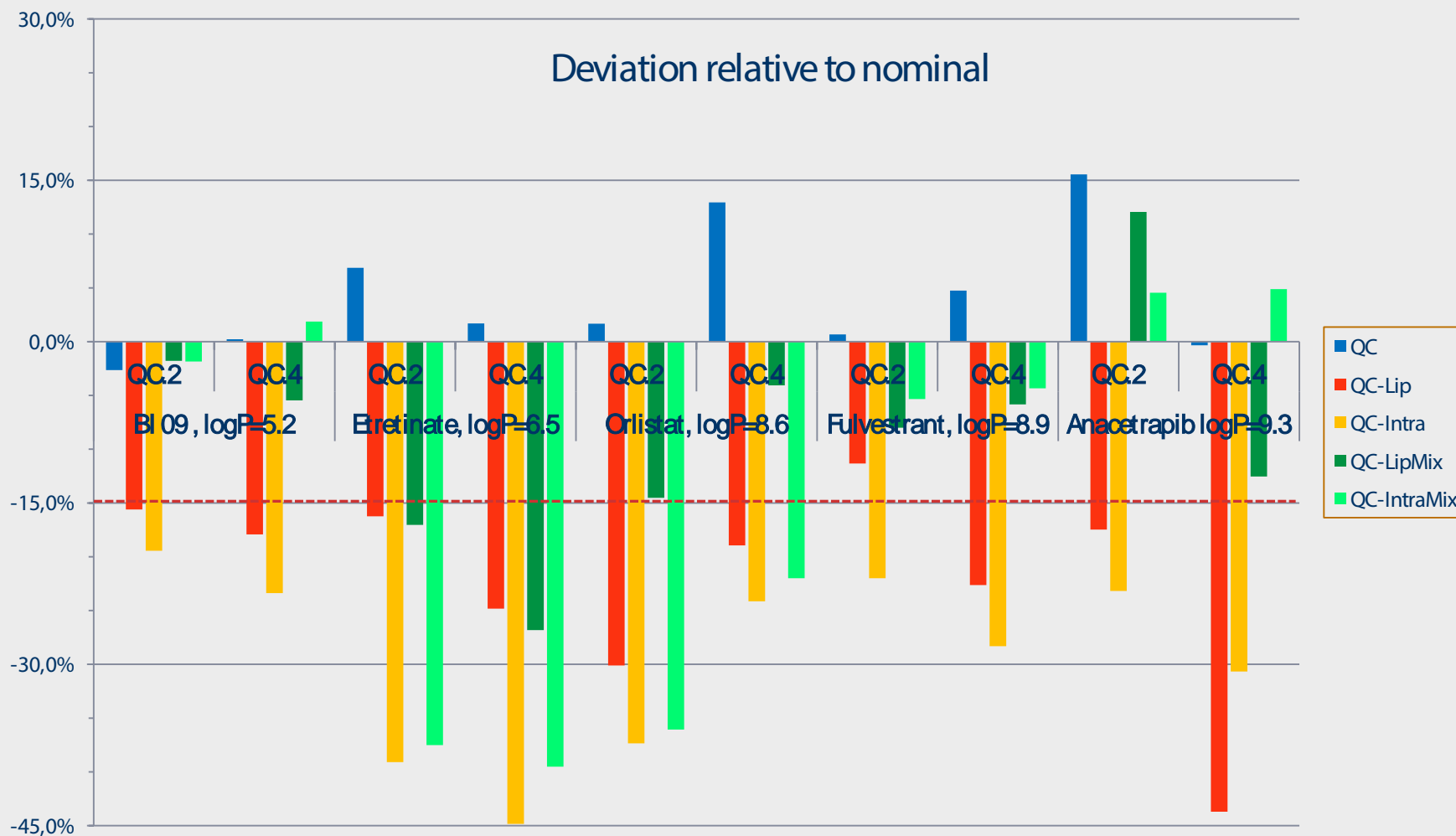
Lipaemic mix - deviation to nominal



Intralipid Mix - deviation to nominal



Relevant substances with deviation <-15%



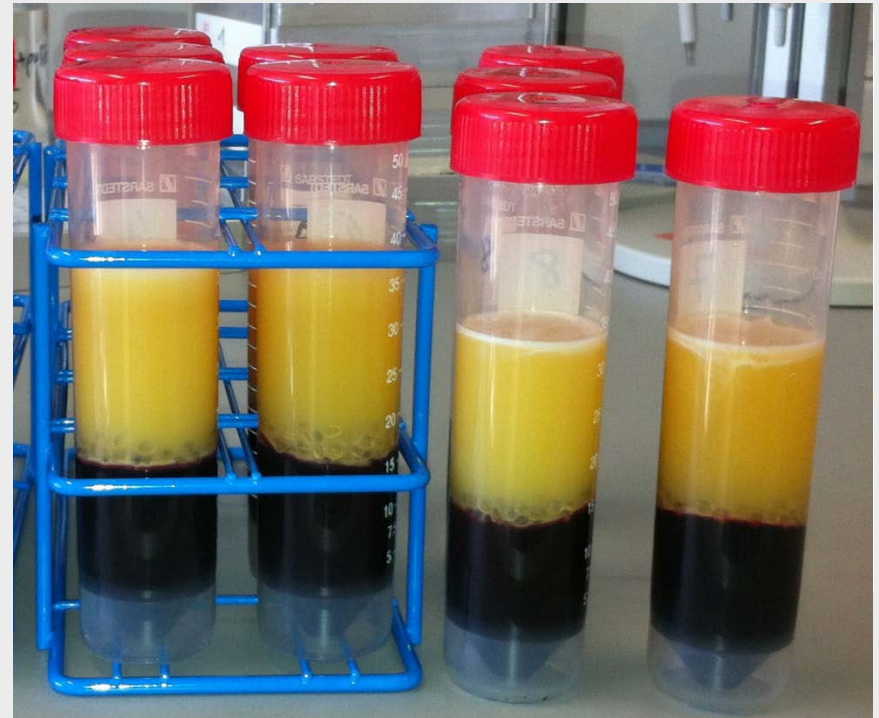
- We investigated 15 drugs with a logP between 0.3 and 9.3
 - **If deviation was <-15% in lipaemic samples, deviation was also <-15% in samples containing intralipid**
 - Mixing of lipaemic samples prior to sample transfer might already be sufficient to compensate the matrix effect of lipaemic samples
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- **It is possible to mimic lipaemic samples with blank plasma samples containing 2% intralipid (20%)**
 - Due to some false positive results with intralipid samples, investigation should be repeated with lipaemic samples if intralipid indicates a matrix effect

- Susanna Kluge, Karen Köhler, and Jan Kasper
- Aline Wenzel, Alexander Held, and Dr. Sebastian Otto
- Dorothea Haidlauf, Leonie Thalheim, and Patrick Faber

- Special thanks to 12 volunteers who donated lipaemic blood after a really high fat breakfast

- 4 kg „Weißwurst“ (Bavarian sausages)
- 1 kg „Leberkäse“ (a type of meat loaf popular in Germany and Austria)
- 800 g Cheese
- 500 g Salad with strips of sausages with mayonnaise dressing
- 100 g Butter
- ½ Glass Nutella (hazelnut cream)
- 500 g Peanuts
- 29 Croissants

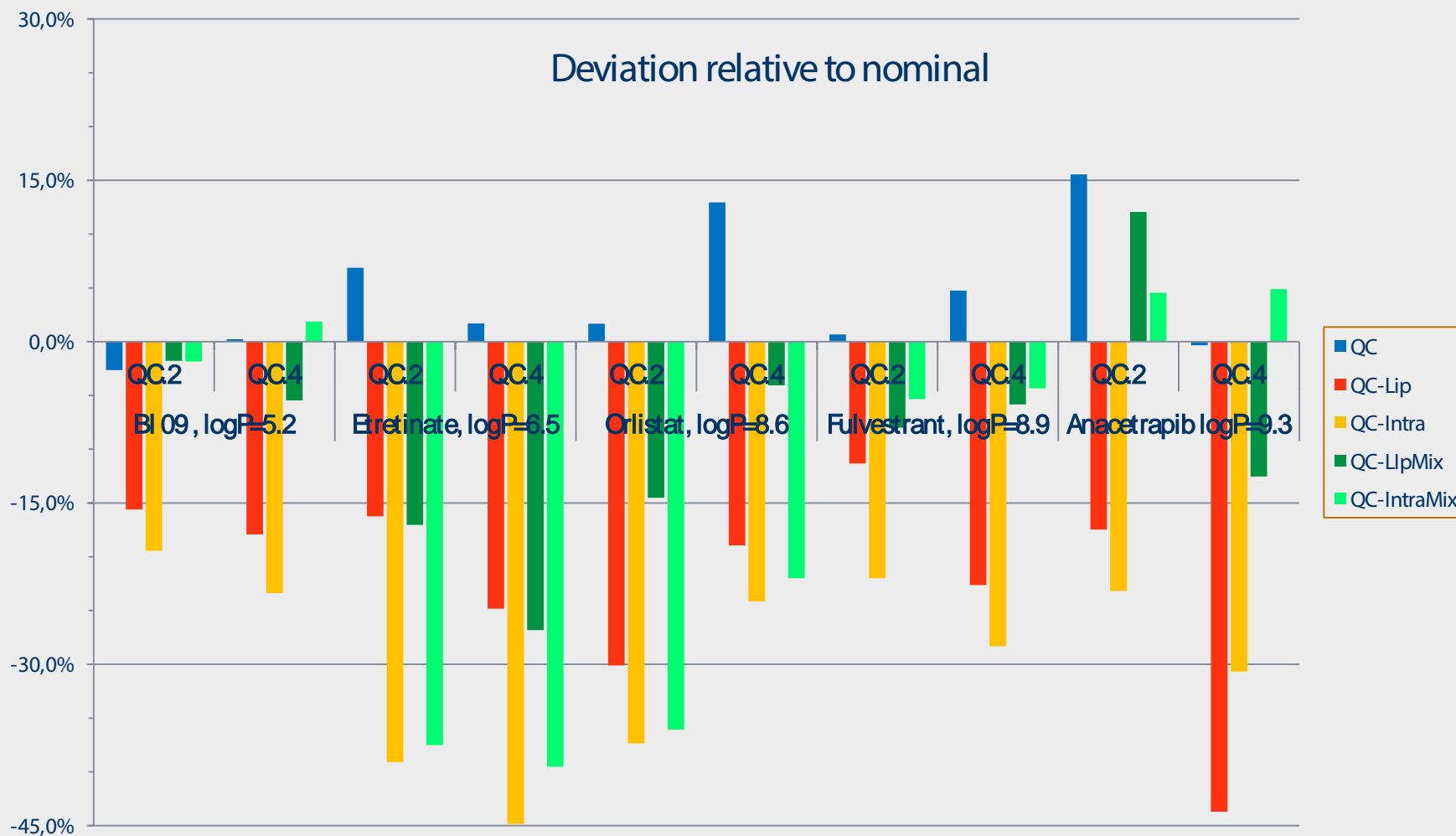
➤ About 9.2 kg of fat food



Thank you for
your attention

BACK UP

Relevant substances with deviation >15%



Relevant substances with deviation >15%

