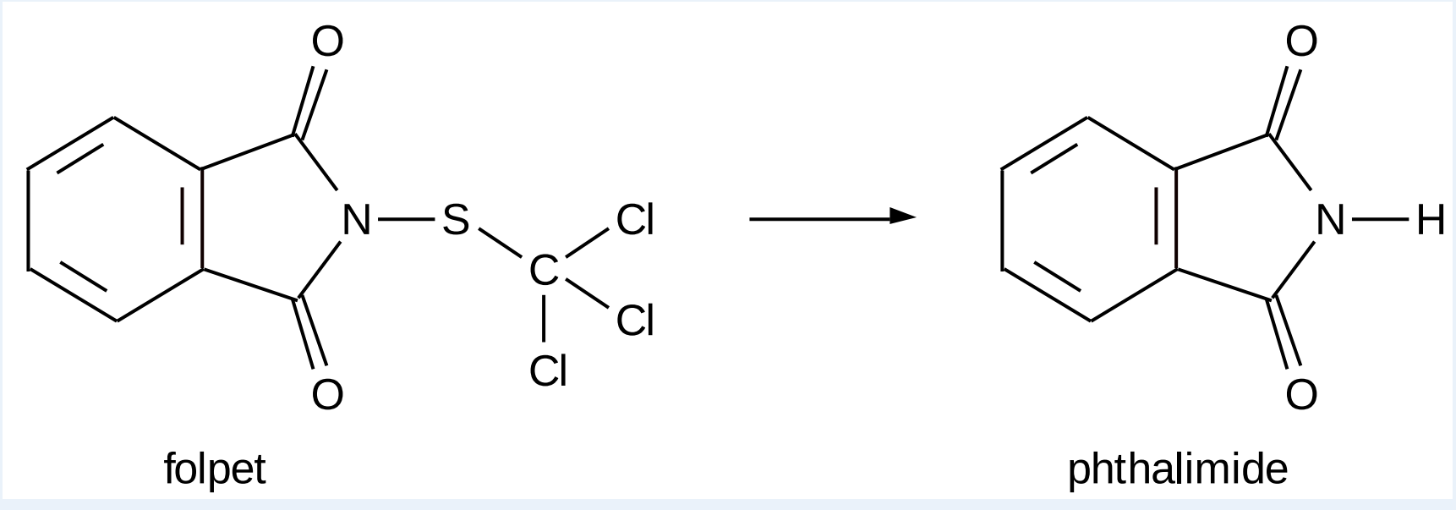
Phthalimide –
Metabolite of Folpet or unavoidable artefact

Nitsopoulos A., Wollein G., Friedle A.
Labor Friedle GmbH, Von-Heyden-Str. 11, 93105 Tegernheim (Regensburg), Germany

Introduction

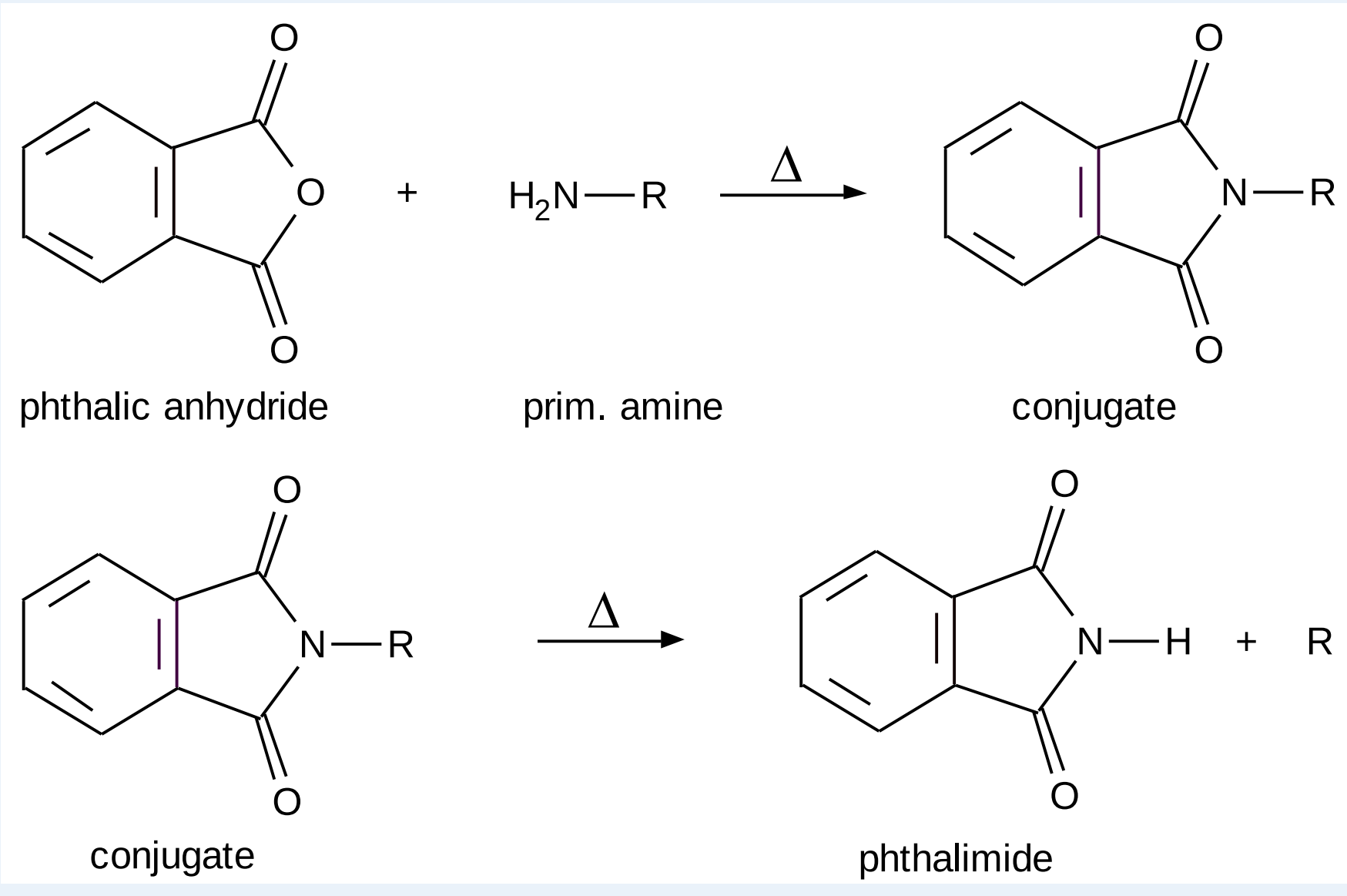
Based on Regulation (EU) 2016/156 dated 18 January 2016 a new definition of residue concerning the fungicide folpet was given, entered into force 26 August 2016: “Sum of folpet and phthalimide, expressed as folpet.”
Folpet belongs to the group of phthalimide fungicides, which quickly decay at higher temperatures, e.g. in a gaschromatographic injector. The bond breaking takes place on the nitrogen (N), so that the final product is phthalimide.



equ. 1

In plants **folpet** is also metabolized to **phthalimide** as the major metabolite (equ. 1). As part of the upcoming redefinition, we determinated phthalimide in our multi-method (QuEChERS) and often observed evidence in fresh fruit and vegetables, partially high concentrations in dried products - without the slightest indication of folpet.
We followed up this observation and found an interesting correlation between the presence of phthalimide (PI) and phthalic anhydride (PSA), an ubiquitous environmental chemical, which can easily react under specific conditions (esp. higher temperatures) with primary amino groups (like present in amino acids, peptides, etc.) to form phthalimid (equ. 2).
As a consequence, it is obvious that the common analytical technique for measuring folpet and PI - the hot injection GC-detection - is a potential source for the generation of false positive levels of PI.
An alternative analytical approach - the LC-MS/MS-technique using atmospheric pressure chemical ionisation (APCI) - is discussed, in order to avoid such false positive results.

Conclusions



equ. 2

Phthalimide can be (I) a **metabolite** of folpet or phosmet, (II) **reaction product** of folpet during GC injection, (III) an **artefact** resulting from thermic reaction of the ubiquitously occurring phthalic anhydride with primary amino compounds of the matrix either in GC-injector or (IV) of **process-induced origin** especially in dried products . We showed that it is possible to exclude formation of phthalimide during analysis, using LC-MS/MS-technique with atmospheric pressure chemical ionisation (APCI) instead of GC-MS/MS, but there is no chance to differ phthalimide as folpet metabolite from process-induced origin.

In our view, therefore, folpet's new residue definition - without concurrent proof of the parent substance - is not suitable for limit value monitoring and carries the risk of false positive findings - a huge problem especially for organic products which go through a heating and drying process like tea or herbs.

Results and Discussion

Analytical methods

For sample extraction and clean up we used the QuEChERS standard method. The final extract (acetonitrile) was re-dissolved in methanol before injection into the GC- resp. LC-systems.

GC-MS/MS	LC-MS/MS
· GC-MS/MS 7000B (Agilent Technologies) · GC column: HP-5MS UI (30m x 0,25mm x 0,25µm) · Inj. volume: 1µL · Oven: 50°C (1min) ⇨35°C/min to 100°C (0min)⇨8°C/min to 320°C (3min)⇨Backflush · PTV: 60°C (0,14min) ⇨700°C/min to 280°C (15min) · Ionisation: EI+ · MRM-transitions: PI (Quantifier) 147 ⇨ 103 PI (Qualifier) 147 ⇨ 76 PI-D4 (ILIS) 151 ⇨ 107	· LC-MS/MS 6495 (Agilent Technologies) · LC column: Poroshell 120 SB-C18 (2,1 x 100mm ; 2,7 Micron) · Eluent A: water ; Eluent B: methanol · LC gradient: 0min (0%B) ⇨0,3min (0%B)⇨ 18min (100%B) ⇨20min (100%B) ; post time 5min · Flow: 0,3mL/min · Ionisation: APCI neg. · MRM-transitions: PI (Quantifier) 146 ⇨ 42 PI (Qualifier) 146 ⇨ 146 PI-D4 (ILIS) 150 ⇨ 42

Phthalic anhydride and its corresponding phthalic acid are both industrial chemicals, which are used broadly, e.g. as raw materials in the production of alkyde resins and softeners. Residues of these chemicals can be detected in varnishes, paper coatings, synthetic materials (PVC) and newspaper ink. Based on countless analyses of house dust we can observe that phthalic anhydride occurs virtually ubiquitous.
The correlation between this widely used industrial chemical and the presence of PI in food products can be explained by reaction equ. 2 (see above) of phthalic anhydride with compounds of the food matrix containing primary amino-groups (amino acids, peptides etc.) under heating conditions as prevailing in the GC-injector (280 °C) during the analysis.

Correlation PSA levels in the sample and formation of PI in the hot GC-injector

Following up on these observations we analyzed about 60 samples, both organic and non-organic origin, primarily herbs or spices but also other products resulting in an interesting correlation between PI and the approximately 10 times concentrated phthalic anhydride (Fig. 1).
After that a test with different levels of PSA spiking in dried parsley was done for verification of our observation.
A direct proportional correlation between the formation of PI and previous added PSA could be shown (Fig. 2).

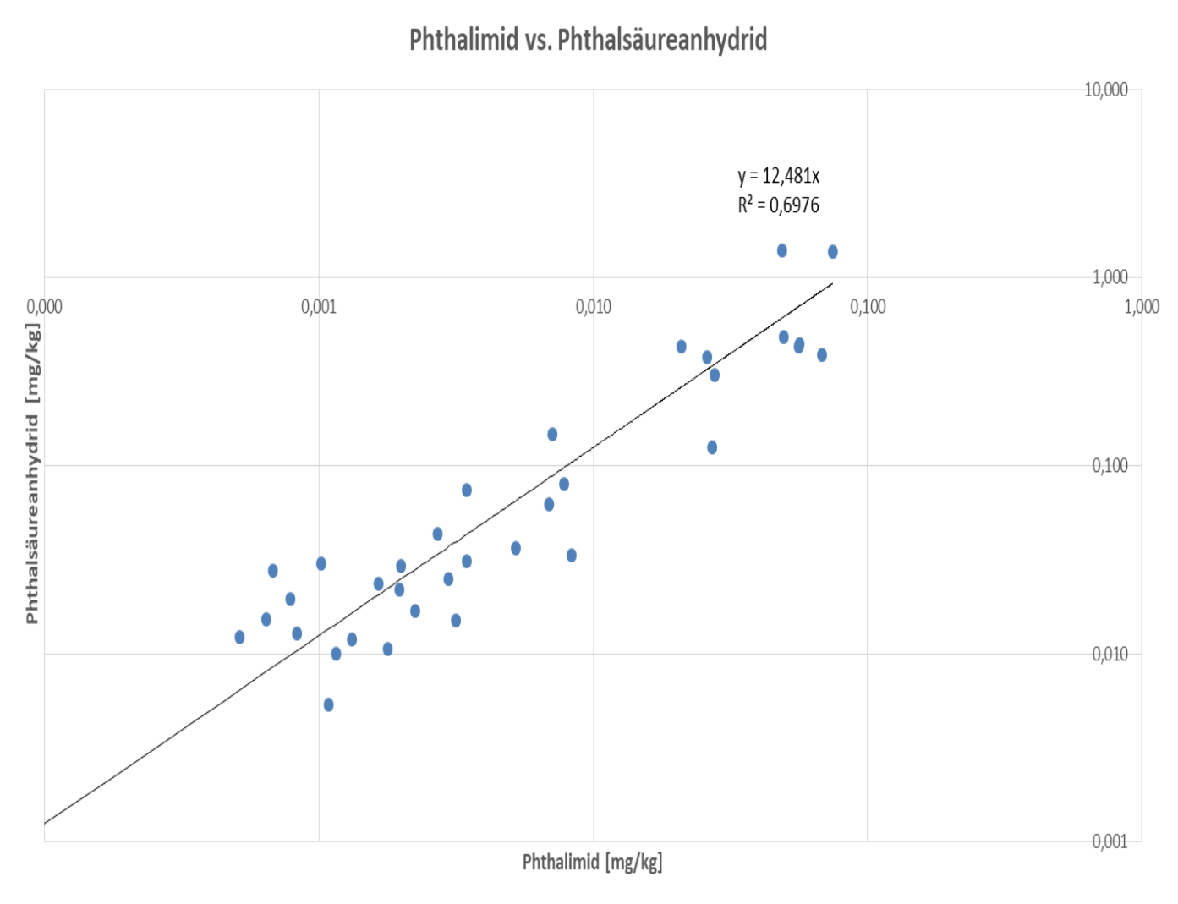


Fig. 1

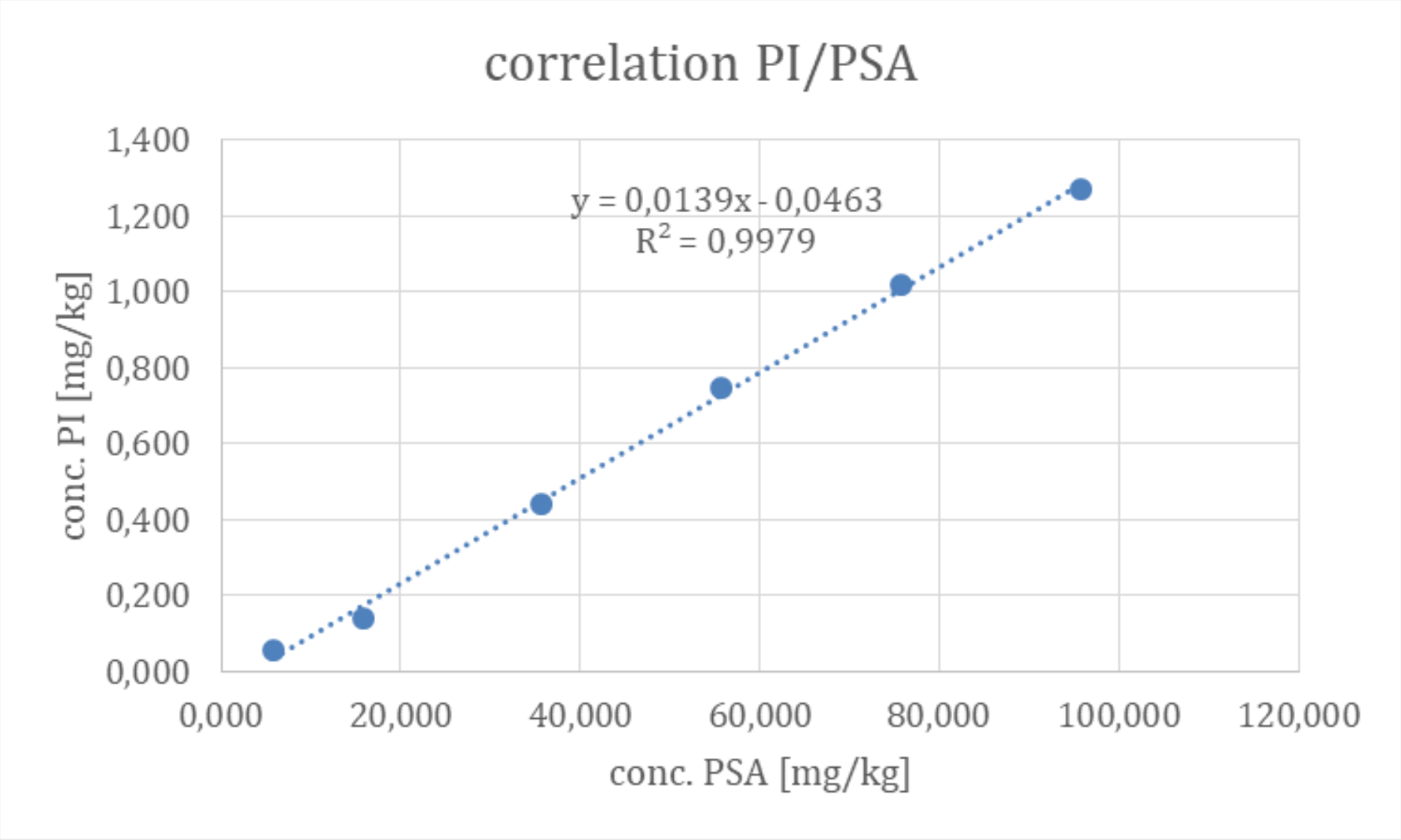


Fig. 2

Influence of different analysis techniques on formation of phthalimide

The intention of the next step was to find an alternative analysis technique avoiding the artefact formation of PI resulting from thermic reaction of the ubiquitously occurring phthalic anhydride with primary amino compounds of the matrix in the hot GC-injector.
We decided to use LC-MS/MS-technique with atmospheric pressure chemical ionisation (APCI, Fig. 3) instead of GC-MS/MS to prevent the formation of PI during analysis.

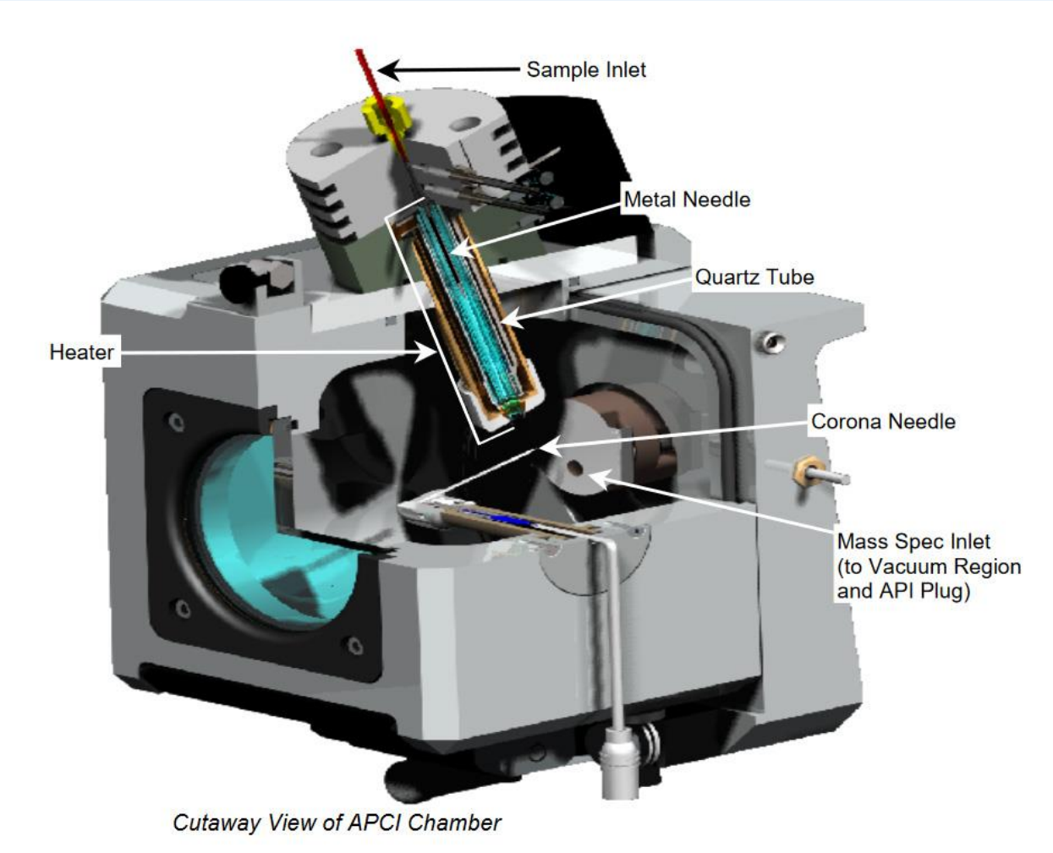


Fig. 3

Samples of dried parsley were spiked with 1ppm PSA and measured both with the GC-MS/MS and the (APCI)LC-MS/MS system after QuEChERS extraction and clean-up.
The following chromatograms show that there is a strong and precise signal of PI in case of GC-MS/MS arising from previous artefact formation. In case of the (APCI)LC-MS/MS there is no detection of PI (Fig. 4).

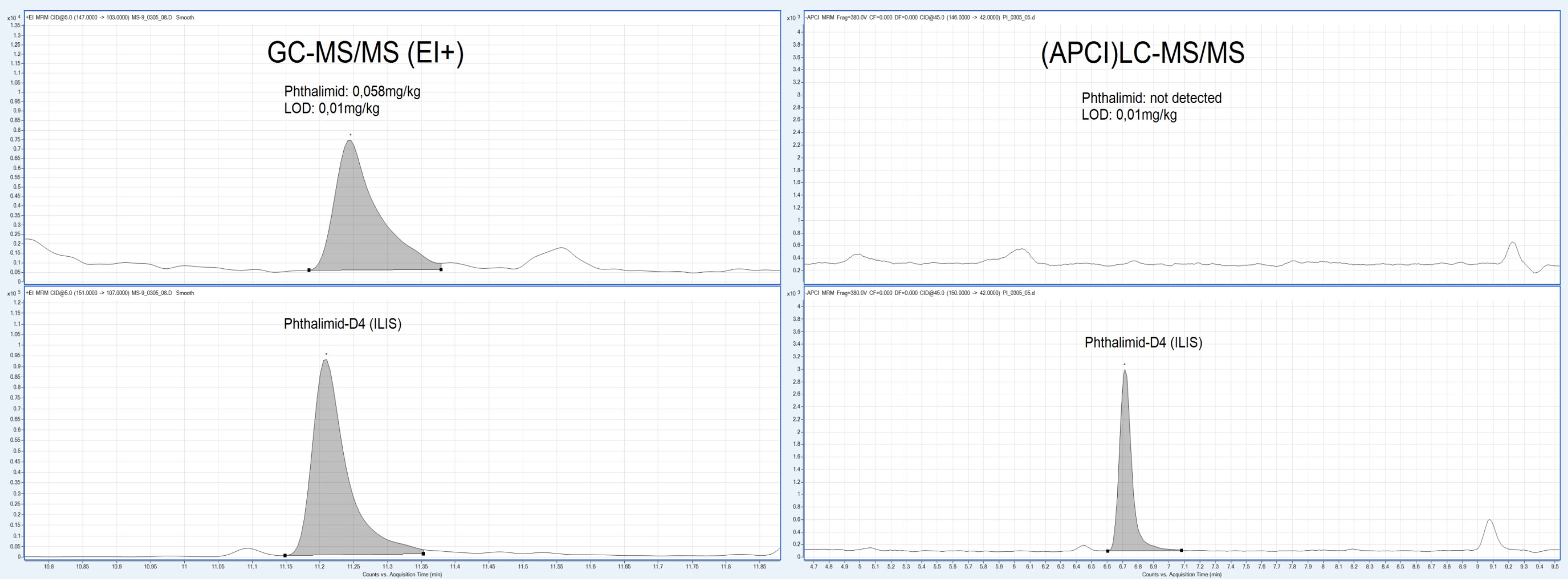


Fig. 4

Influence of drying temperature on formation of phthalimide

To find out, if there is an independent way on formation of PI as an artefact of PSA, except during hot GC injection, products like dried parsley were affected additionally by high processing temperatures. Increasing temperatures facilitate the formation of PI in such products. It is likely, that the pre-cursor molecule is the PSA (as it is present in the used parsley sample at a level of approx. 6mg/kg). The formation of PI is increasing strongly at temperatures above 200°C (Fig. 5).
In black tea, which was investigated during the same test design, a similar increase of the level of PI at a temperature of 250°C was evident.

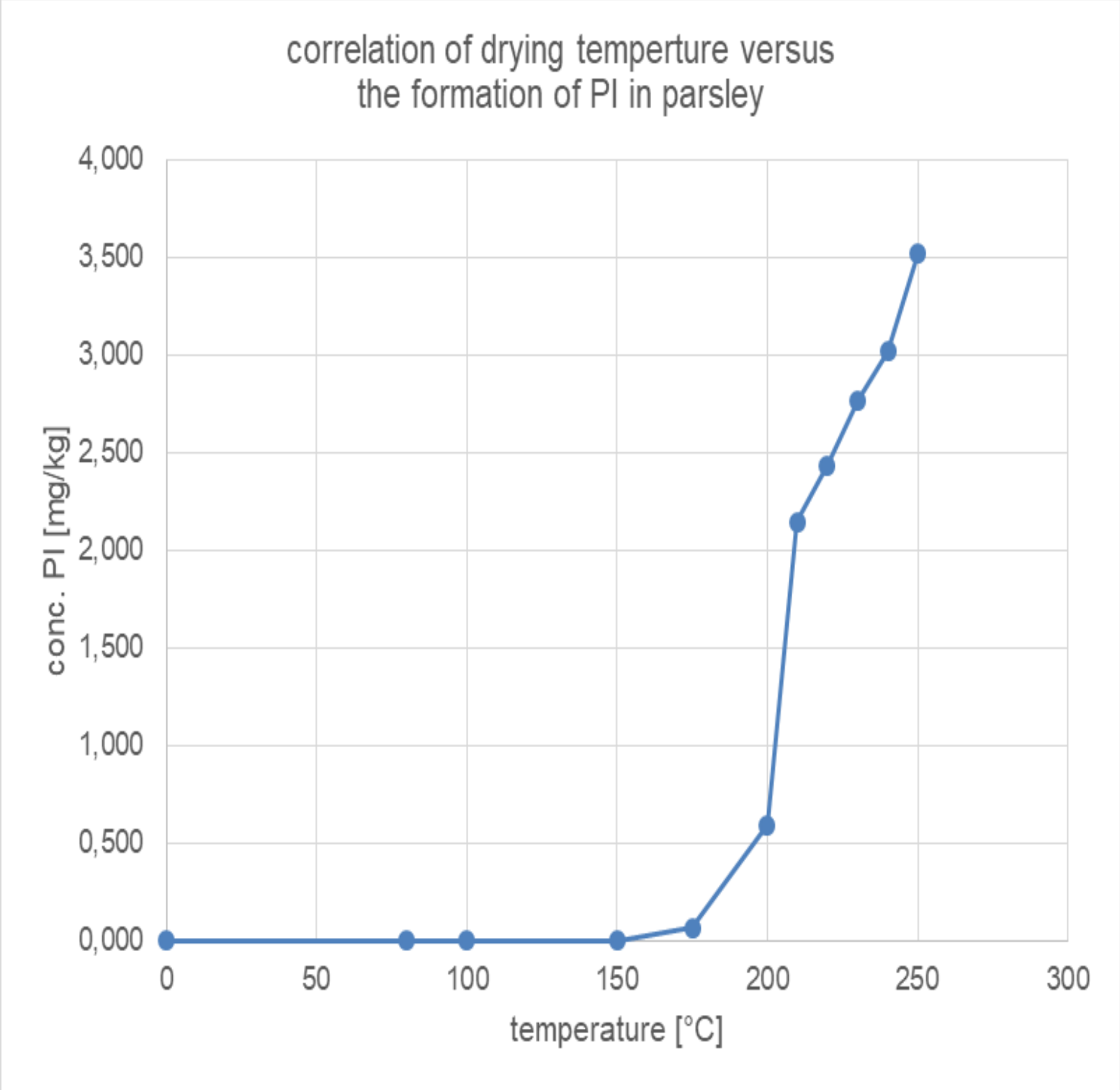


Fig. 5

It is not possible to differ phthalimide as folpet metabolite from process-induced origin - folpet's new residue definition is a problem especially for organic products