



# **Pesticide Testing According to the European Pharmacopoeia (Ph.Eur.) – Legal Requirements and Practical Approach**

Dr. Bernhard Klier  
PhytoLab GmbH & Co. KG  
91487 Vestenbergsgreuth  
Germany

**GA2011 Antalya,  
4-9 September, 2011**

# Content

Introduction

Pesticide residues, Ph.Eur. 7th edition

Limits (EU 396/2005, allocation of products)

Methods and scope of testing  
(matrix effects, prospects)

Summary

# Introduction

Raw products of herbal origin are naturally subjected to a considerable variation. Of the roughly **400 plants** and parts of plants on the herbal market, 30 to 40 plants are cultivated on a large scale. Only about 25 % of the total amount of herbal drugs, but nevertheless the vast majority of plant species (70 - 80%), are gathered from the wild.

It is therefore important, when addressing the problem of pesticide residue analysis in herbal drugs, to take into account the **vast diversity of different matrices** as well as **the particular circumstances in the countries of origin**, which are mostly outside the EU.

# Pesticide residues, Ph.Eur. 7<sup>th</sup> editon

# Introduction

In the European Pharmacopoeia (Ph.Eur.) pesticide residues are described under chapter 02 „Methods of analysis“. The Ph.Eur. Monograph „2.8.13. Pesticide residues“ contains „Definition“, „Limits“, „Sampling“ and „Qualitative and quantitative analysis of pesticide residues“ of herbal drugs.

The requirements for **“Herbal Drugs”** and **“Extracts”**, as well as **“Herbal Drugs for Homoeopathic Preparations”** and **“Mother Tinctures for Homoeopathic Preparations”** are referred within the scope of their monographs in chapter 05 „General texts“:

# General monographs

## Herbal Drugs:

TESTS ...

**Pesticides (2.8.13).** Herbal drugs comply with the requirements for pesticide residues. The requirements take into account the nature of the plant, where necessary the preparation in which the plant might be used, and where available the knowledge of the complete record of treatment of the batch of the plant.

## Extracts:

TESTS

Where applicable, as a result of analysis of the herbal drug or animal matter used for production and in view of the production process, tests for

microbiological quality,  
heavy metals,  
aflatoxins and  
pesticide residues

in the extracts may be necessary.

# Homoeopathic preparations

## Herbal Drugs for Homoeopathic Preparations

TESTS ...

**Pesticides (2.8.13).** Herbal drugs for homoeopathic preparations comply with the requirements for pesticide residues.

...

Where justified, the test for pesticides may be performed on the mother tincture according to the requirements of the general monograph *Mother tinctures for homoeopathic preparations (2029)*.

## Mother Tinctures for Homoeopathic Preparations

**Pesticides (2.8.13).** Where applicable, the mother tincture for homoeopathic preparations complies with the test. This requirement is met if the herbal drug has been shown to comply with the test.

Justification is provided in cases where the test for pesticides is performed on the mother tincture, instead of on the herbal drug according to the requirements of the general monograph *Herbal drugs for homoeopathic preparations (2045)*.

## Pesticide residues 2.8.13, Ph.Eur. 6.2

The monograph pesticide residues 2.8.13 had been introduced to Ph.Eur. 1997 (USP 24 <561>, 2000)

In June 2006 the Ph.Eur. Pesticide Expert group has been mandated to update the Ph.Eur monograph 2.8.13 referring to the publication „Pesticide residues in medicinal drugs and their preparations ([PHARMEUROPA Vol.17 No. 1, Jan. 2005](#)).

Maximum limits for frequently found pesticides based on positive findings, 90th percentiles and quantitation limits have been proposed.

# Pesticide residues 2.8.13, Ph.Eur. 6.2

## Definition of pesticide

(in herbal drugs and herbal drug preparations)

## Limits for herbal drugs in table 2.8.13.-1

(calculation for herbal drug preparations)

## Sampling and sample preparation of herbal drugs 2.8.20

(general chapter)

## Qualitative and quantitative analysis of pesticide residues

(validated analytical procedures)

## Pesticide residue 2.8.13 (new)

- Expanding number of substances in table 2.8.13-1 to **115 pesticides (70 MRL`s)**
- Cross reference to new European Food Law
- Formula for calculation of residues in herbal drug preparations
- Sampling according Ph.Eur. 2.8.20.
- Method for determination of pesticides has been deleted
- Method validation criteria acc. to SANCO/10232/2006 **(SANCO/10684/2009)**
- Considering of natural occurring constituents by interpretation of results (e.g. disulfide)

## Advantages in practise

No fixed method in Ph.Eur. (different methods are used in pesticide residue laboratories depending on substances and instruments).

Harmonised validation procedures for methods used:

Method validation and quality control procedures for pesticide residue analyses in food and feed: **SANCO/10684/2009** (update).

List of **frequently found pesticides** expanded (34 to 115 substances).

Simple evaluation of pesticide residues in herbal drug preparations (extracts).

Reference to harmonised maximum residue limits (MRL`s) for food and feed in Europe: **Commission Regulation (EC) No. 396/2005**.

# Limits

## Pesticide residues 2.8.13

What **Maximum Residue Limits** (MRLs) are applying to pesticides that are not listed in **Ph.Eur. Table 2.8.13.-1**?

**Commission Regulation (EC) No. 396/2005**, including annexes and successive updates

Pesticides not listed in European Union texts apply to **Default MRL of 0,01mg/kg**. Calculation with ADI-value is possible (fao/who).

**REGULATION (EC) NO 396/2005 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL**  
**of 23 February 2005**  
**on maximum residue levels of pesticides in or on food and feed of plant and animal origin and**  
**amending Council Directive 91/414/EEC**  
*(Text with EEA relevance)*

## Regulation (EC) No. 396/2005

Harmonised pesticide MRLs in Europe (number of existing MRL`s could be reduced from 500.000 **national** to 145.000 **EU-harmonised**).

old EU directives

396/2005

**EU-MRLs:**

about 250 pesticides

about 550 p.

**national MRLs:**

about 850 pesticides

none

**default MRL**

none

0,01 mg/kg

# Regulation (EC) No. 396/2005

Annex I	List of commodities	Reg. (EC) No 178/2006
Annex II	Existing EU MRLs	Reg. (EC) No 149/2008 ( March 2008) + Reg. (EC) No 839/2008 (Update, 30 August 2008)
Annex III	Temporary MRLs	
Annex IV	Substances for which no MRLs are required	
Annex VII	Fumigants	Reg. (EC) No 260/2008

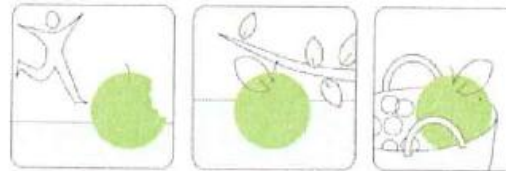
Application date of Regulation (EC) 396/2005 including annexes:  
1 September 2008.

# Allocation of herbal products

# Public database on EU-MRLs and active substances

## Active substances

Directive 91/414/EEC



## Pesticide EU-MRLs

Regulation (EC) No  
396/2005

### Active substance

Active substance updated on  
16/03/2009

Directorate General for  
Health & Consumers

Products

Pesticides

MRLs updated on 18/02/2009

### Disclaimer

This database is made available solely for the purpose of information. It has no legal value. The Commission declines all responsibility or liability whatsoever for errors or deficiencies in this database. Neither the Commission nor any person acting on behalf of the Commission is responsible with regard to the improper use of the document and its contents. The official MRLs are those published in the Official Journal of the European Union ([Plant Protection - Pesticide Residues - Community Legislation](#)).

[http://ec.europa.eu/sanco\\_pesticides/public/index.cfm](http://ec.europa.eu/sanco_pesticides/public/index.cfm)

## 396/2005 Regulation (EC) No. 396/2005

In the context of this regulation MRLs for all pesticides in each commodity listed in Annex I (315) have been fixed (145.000).

Allocation of many products to several categories seems to be possible depending on its usage:

e.g. peppermint            →            fresh herb  
   (→            herbal infusion ?)

But only one single MRL for one product is allowed.

# Allocation of products (396/2005 Annex I)

## 1. FRUIT FRESH OR FROZEN

(d) Other small fruit & berries

Blueberries (Bilberries cowberries (red bilberries))

Cranberries

Currants (red, black and white)

Gooseberries (Including hybrids with other ribes species)

Rose hips

## 2. VEGETABLES FRESH OR FROZEN

(f) Herbs

Chervil

Parsley

Sage (Winter savory, summer savory, )

Rosemary

Thyme ( marjoram, oregano)

# Allocation of products (396/2005, Annex I)

## 6. TEA, COFFEE, HERBAL INFUSIONS, COCOA

(iii) Herbal infusions (dried)
(a) Flowers
Camomille flowers
Hybiscus flowers
Others
(b) Leaves
(c) Roots
(d) Other herbal infusions

## 7. SPICES

## 8. HOPS

## 9. SUGAR PLANTS

Sugar beet (root)

Sugar cane

Chicory roots

Others

## Allocation List of EHIA

There are 315 products listed in Annex I of Regulation 396/2005 but not all herbal drugs being currently on the market are named.

In many categories the subcategory „**others**“ is added. Allocation of products which are not listed in Annex I (e.g. nettle, hypericum, ...) is becoming difficult.

The European Herbal Infusion Association (EHIA) published an allocation list for about 400 plants and parts of plants. Based on the inventory list of EHIA all 400 products are allocated to the categories of Annex I:

Updated January 2008

EHIA INVENTORY LIST OF HERBALS CONSIDERED AS FOOD					Allocation according to Reg. (EC) 178/2006				
English name	Latin	German	Allocation		Code-Number	Remarks			
name of the plant	name of the plant	name of the plant	plant part used	Category			Group	Subgroup	
* Agrimony	herb	Agrimonia eupatoria	Odemennig	Kraut	tea, coffee, herbal infusions and cocoa	herbal infusions	other herbal infusions	639000	maybe subgroup leaves 0632000
Alfalfa	herb	Medicago sativa	Alfalfa	Kraut	tea, coffee, herbal infusions and cocoa	herbal infusions	other herbal infusions	639000	maybe subgroup leaves 0632000
Allspice	fruits	Pimenta officinalis	Piment	Früchte	spices	spices	allspice	820010	
Almond	flowers	Prunus dulcis var. dulcis	Mandel	Blüten	tea, coffee, herbal infusions and cocoa	herbal infusions	flowers, others	631990	
Almond	seeds	Prunus dulcis var. dulcis	Mandel	Samen	fruit fresh and frozen; nuts	nuts	almonds	120010	
* Aloe vera	leaf sap	Aloe barbadensis / Aloe vera	Aloe vera	Blattgel	tea, coffee, herbal infusions and cocoa	herbal infusions	other herbal infusions	639000	
* Alpine ladies mantle	herb	Alchemilla alpina	Silbermantel	Kraut	tea, coffee, herbal infusions and cocoa	herbal infusions	other herbal infusions	639000	maybe subgroup leaves 0632000
* Angelica	roots	Angelica archangelica	Engelwurz	Wurzeln	tea, coffee, herbal infusions and cocoa	herbal infusions	roots, others	633990	
* Angelica	stems	Angelica archangelica	Engelwurz	Stengel	vegetables fresh or frozen	leaf, vegetables and fresh herbs	celery leaves	256030	
Anise	fruits	Pimpinella anisum	Anis	Früchte	spices	spices	anis	810010	
Annato	seeds	Bixa orellana	Annatto	Samen	spices	spices	seeds, others	810990	
Apple	fruits	Malus domestica	Apfel	Früchte	fruit fresh and frozen; nuts	pome fruit	apples	130010	
Apple mint	leaves	Mentha rotundifolia	Apfelminze	Blätter	vegetables fresh or frozen	leaf, vegetables and fresh herbs	mint	256080	regrouping herbal infusions 0632990

EHIA European Herbal Infusions Association

SonninstraÙe 28 • D-20097 Hamburg City Sued

 Tel: +49/40/23 60 16-33/-21 • Fax: +49/40/23 60 16-10/-11 • E-Mail: ehia@wga-hh.de • <http://www.ehia-online.org>

# Methods of analysis and scope of testing

## Scope of testing

According to Ph.Eur. monographs all pesticides in herbal drugs must comply, but which pesticides must be tested?

There are **550 compounds and metabolites** listed in EU 396/2005, but totally existing **about 1.650 chemical pesticides** (Pesticide Manual 2003).

In analytical laboratories **400-600** analytes could be detected with:

- multi methods
- group specific methods
- single residue methods.

There is an analytical gap of more than 1000 substances.

## Scope of testing

In Ph.Eur. 2.8.13. (s.below) a reduced testing is allowed if the herbal drug has been cultivated according to good agricultural and collection practises (GACP).

Residue analysis on a regular basis of used pesticides is recommended.

The competent authority may grant total or partial exemption from the test when the complete history (nature and quantity of the pesticides used, date of each treatment during cultivation and after the harvest) of the treatment of the batch is known and can be checked precisely according to good agricultural and collection practice (GACP).

## Scope of testing

For all other herbal drug the following recommendation on pesticide analysis could be given:

1. Analysis of frequently found pesticides (multi methods)
2. Analysis of pesticides whose presence is suspected for any reason (group specific and single methods)

# Scope of testing, methods

## Multi methods:

GC-methods (e.g. DFG S19)

→ about 200-300 substances

LC-MS/MS (e.g. Quechers)

→ about 200-300 substances

## Group specific methods:

Dithiocarbamates, Phenoxyalkancarboxylic acids, Phenylureas, Carbamates, ...

→ about 60 substances

## Single methods:

Pyridat, Chlormequat, Glyphosate, Paraquat, Nicotine ...

→ about 20 substances

## Scope of testing, methods

Analysis of all pesticides with one (multi-) method (e.g. LC-MS/MS) is not possible.

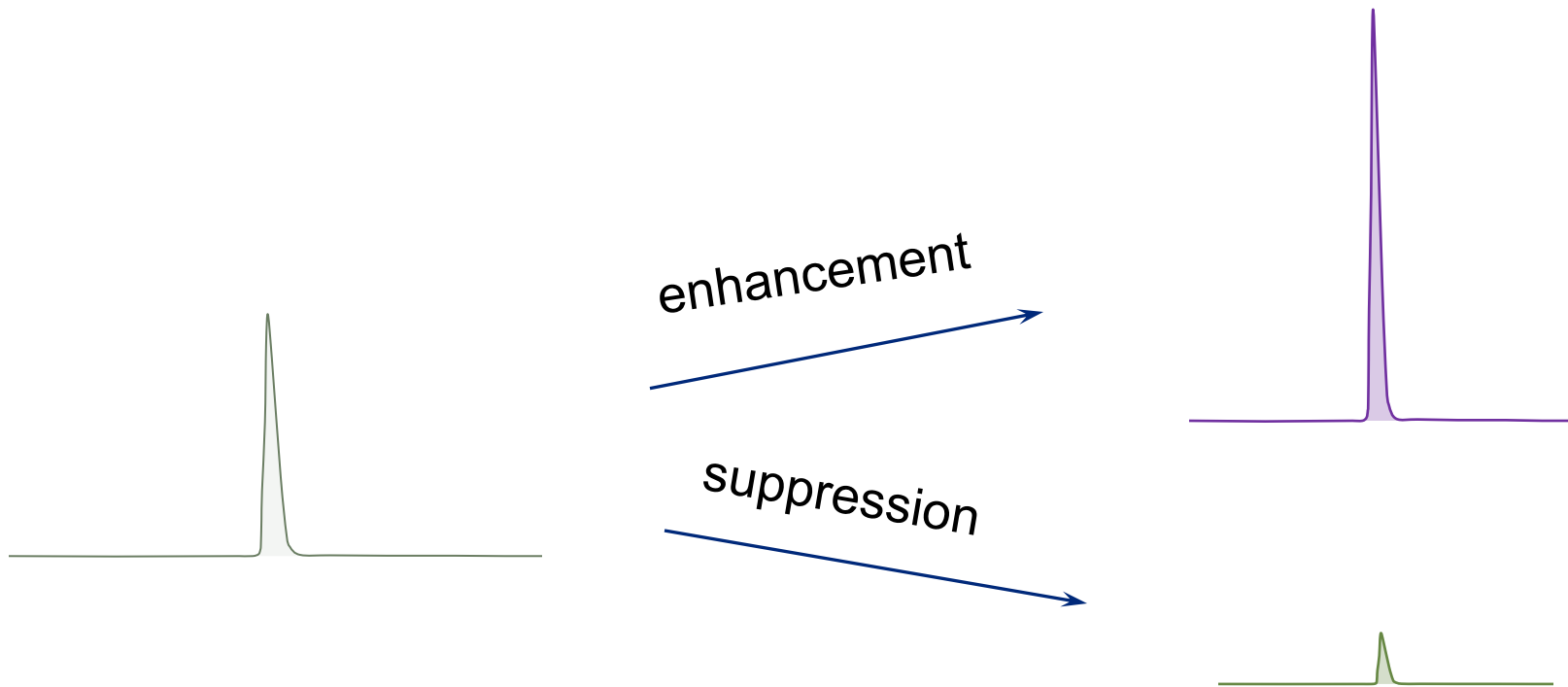
For example: 550 compounds of EU 396/2005  
88% analysed  
38% only LC-MS/MS  
16% only GC-MS/MS  
34% both possible

As herbal drugs consist of a **complex matrix** methods of analysis must be validated accurately and the suitability of multimethods should be tested (false positive / false negative).

## Matrix effects

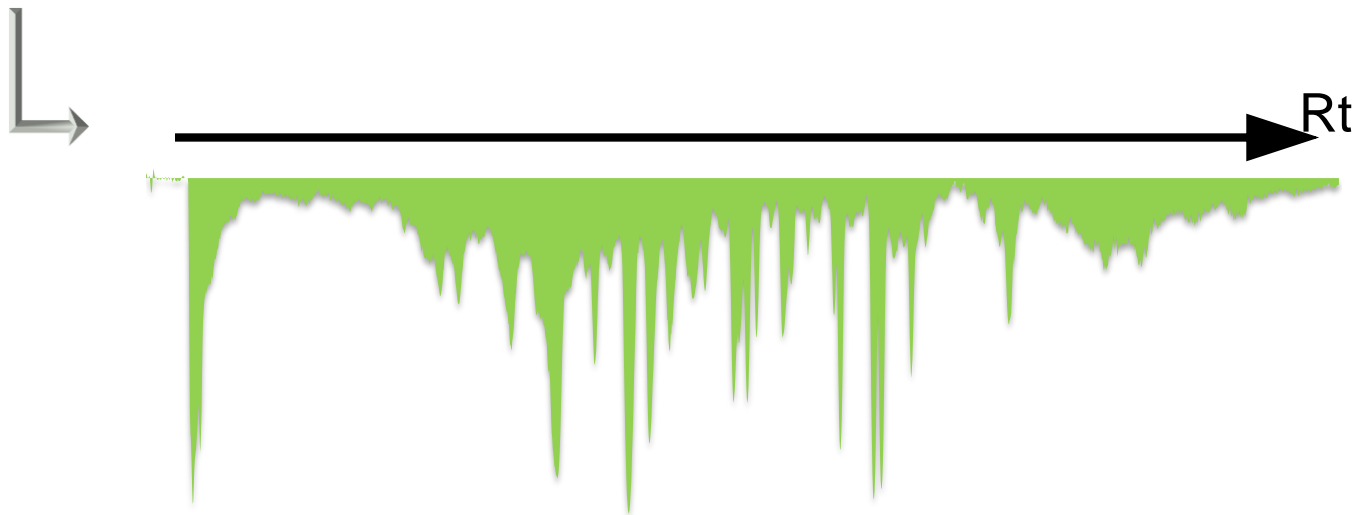
→ Matrix effects cause changes in signal intensity:

(G. Kempe, 6th International Fresenius conference, 24th May 2011)



## Measurement of matrix effects in LC-MS/MS

Determination of matrix effect profiles with **postcolumn infusion**  
(G. Kempe, 6th International Fresenius conference, 24th May 2011)



with continuous measurement the matrix load in the entire chromatogram could be made visible.

## Requirements on multimethods

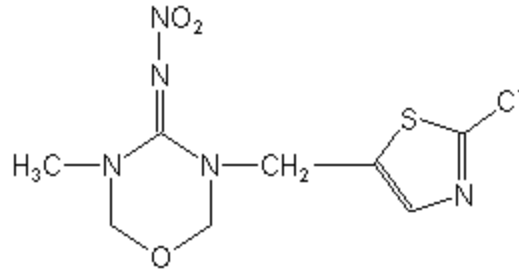
- in LC-MS/MS there are mainly peak suppressions observed:
  - in LC-MS/MS each sample has to be calibrated necessarily in the same matrix or use of internal (labelled) standard
- in the GC-MS/MS both, suppression or fortification of the signal, is possible
- unclear findings should be confirmed by standard addition
- but false positive results caused by matrix substances („same“ transitions, „same“ Rt):
  - change chromatography or **HRMS**

## Prospects - HRMS

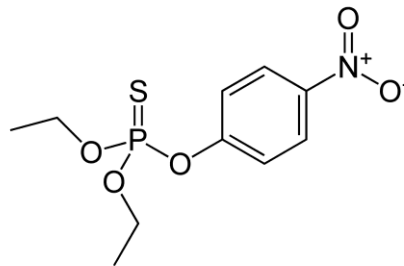
1. Confirmation of positive results
2. Analysis of „known unknowns“  
(closing the analytical gap?)

## Isobaric Pesticides

Thiamethoxam:  $[M+H]^+ = C_8H_{11}ClN_5O_3S$  (292.02656)

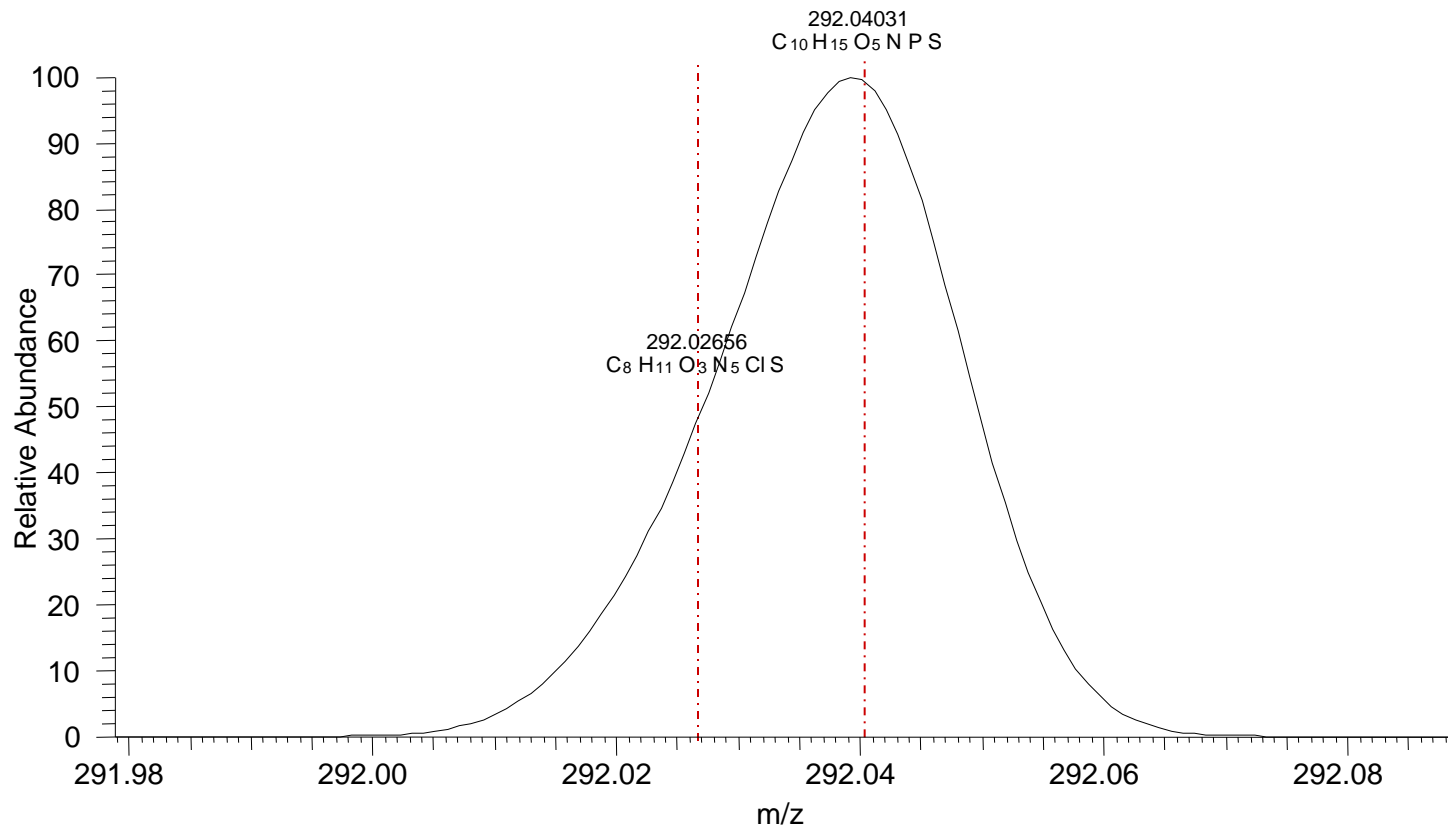


Parathion-ethyl:  $[M+H]^+ = C_{10}H_{15}NO_5PS$  (292.04031)



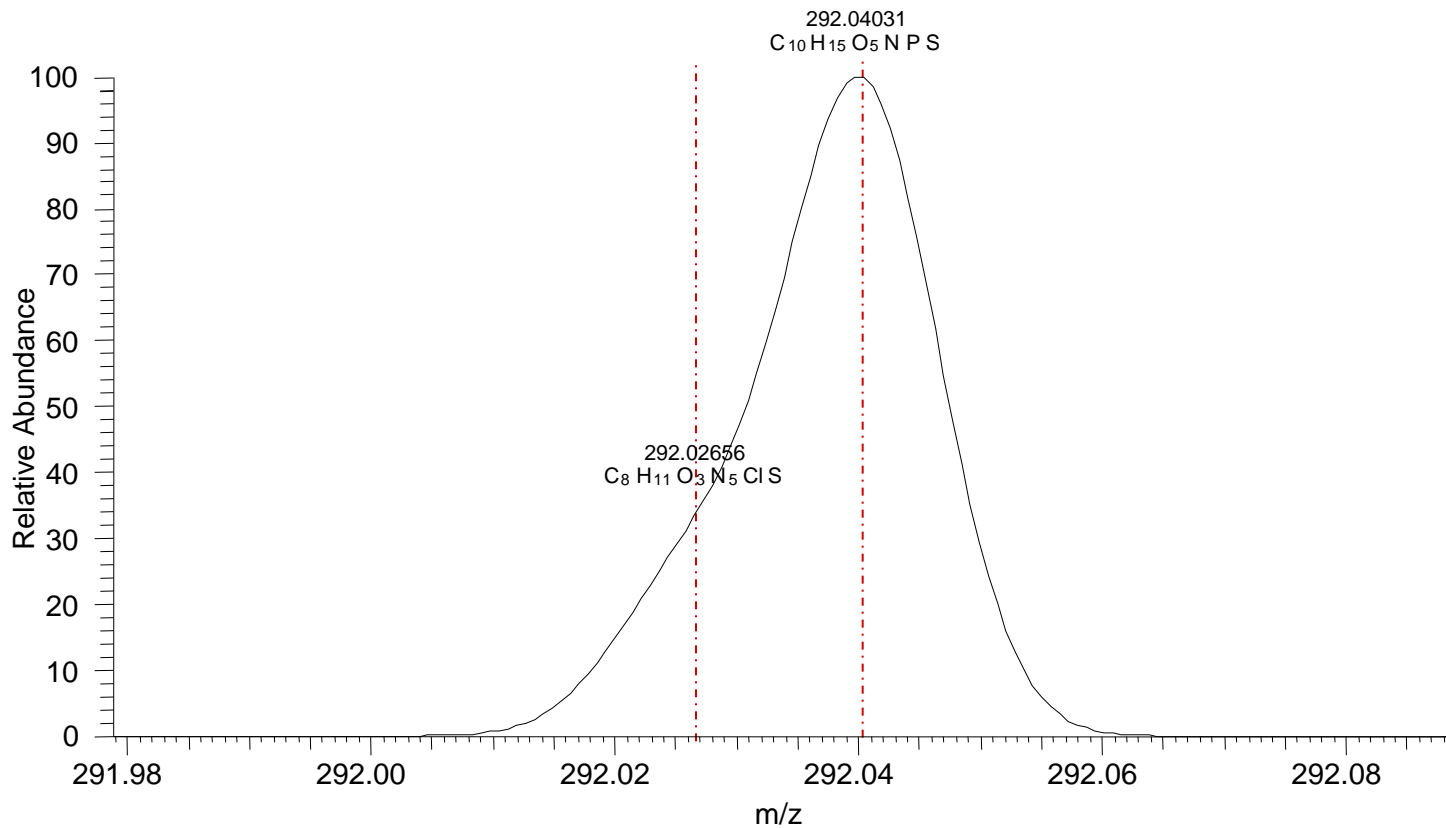
# Simulated Resolution = 15,000 (Mix 1:3)

Resolution 15,000



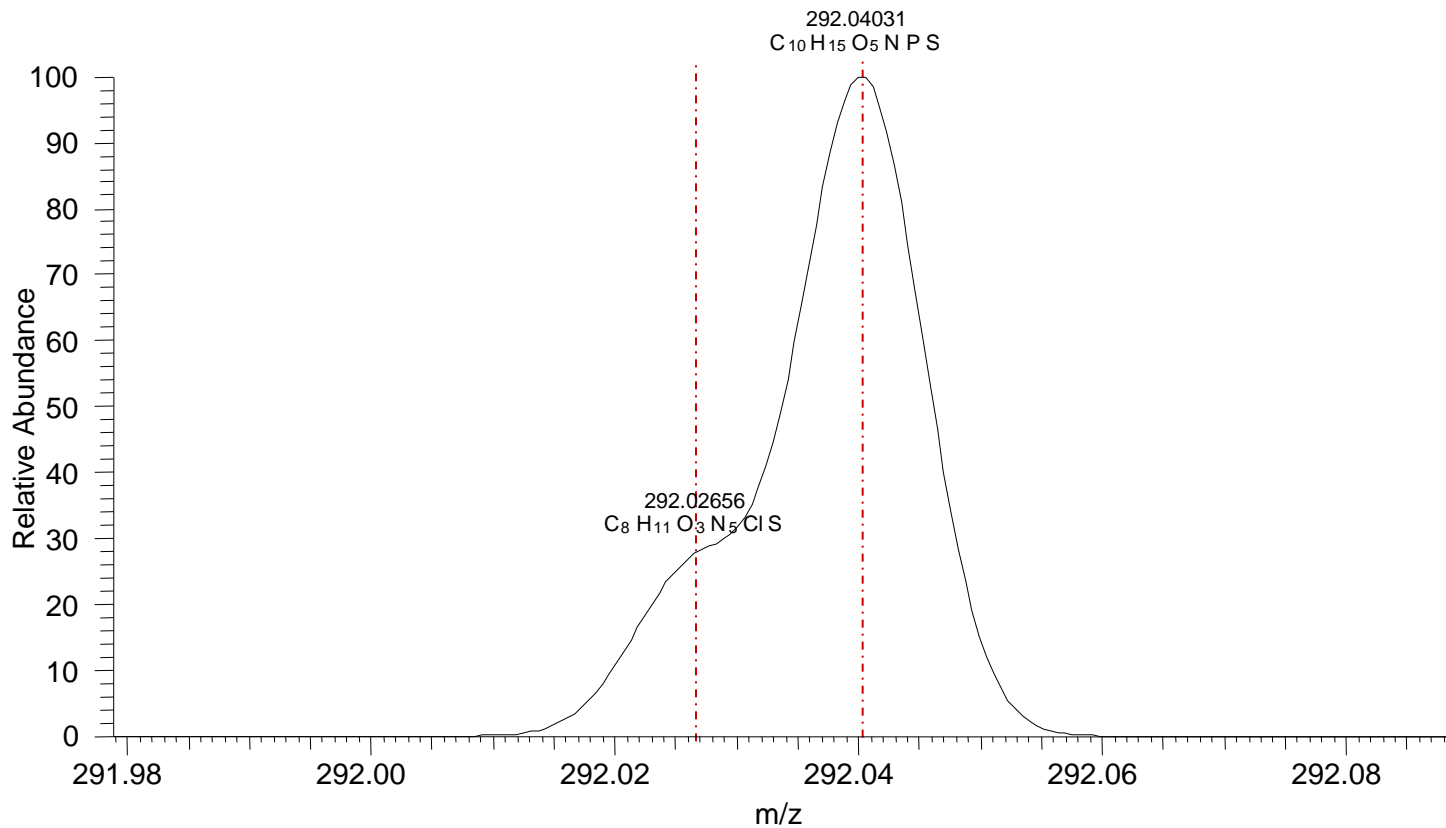
# Simulated Resolution = 20,000 (Mix 1:3)

Resolution 20,000



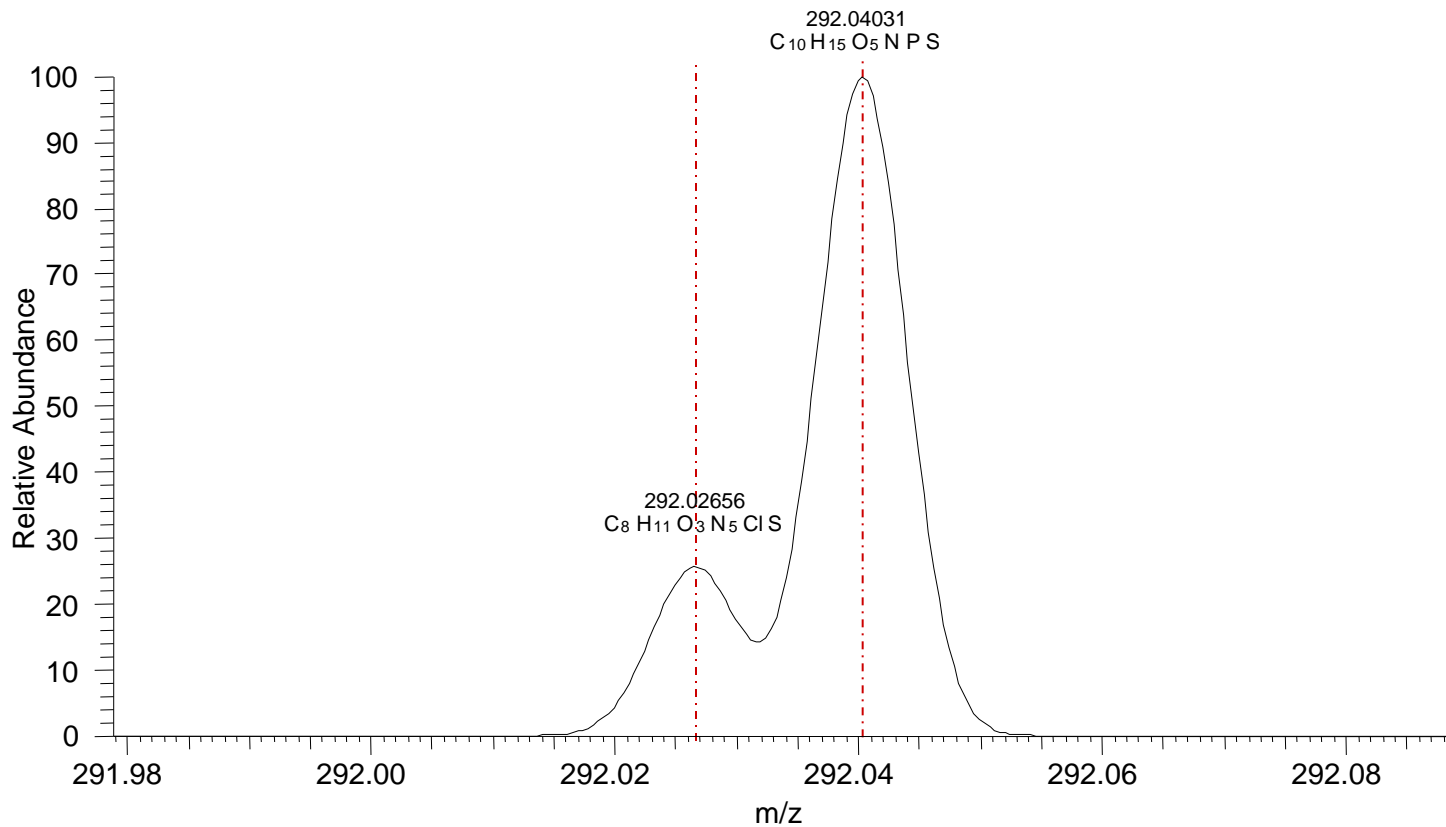
# Simulated Resolution = 25,000 (Mix 1:3)

Resolution 25,000



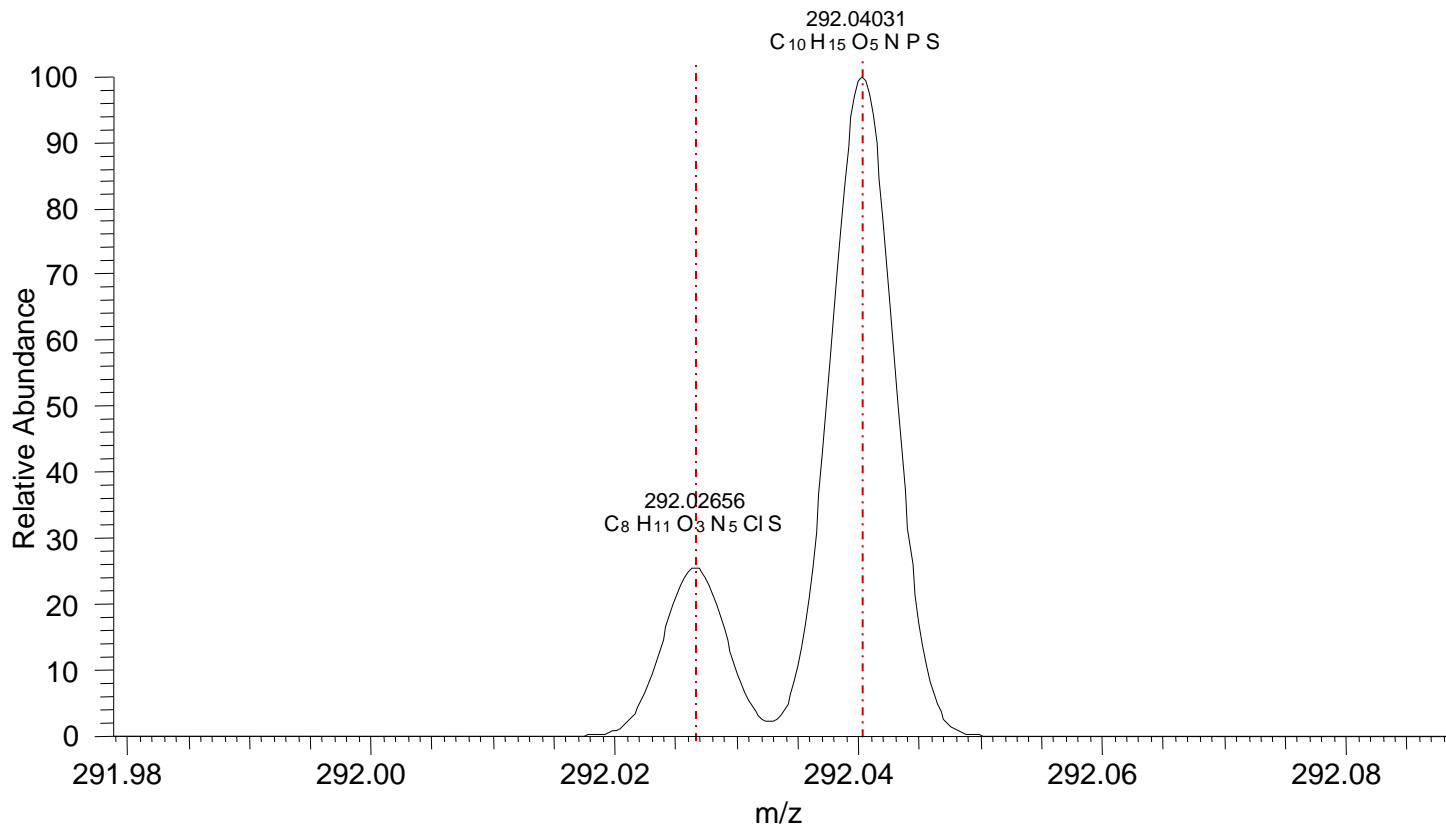
# Simulated Resolution = 35,000 (Mix 1:3)

Resolution 35,000



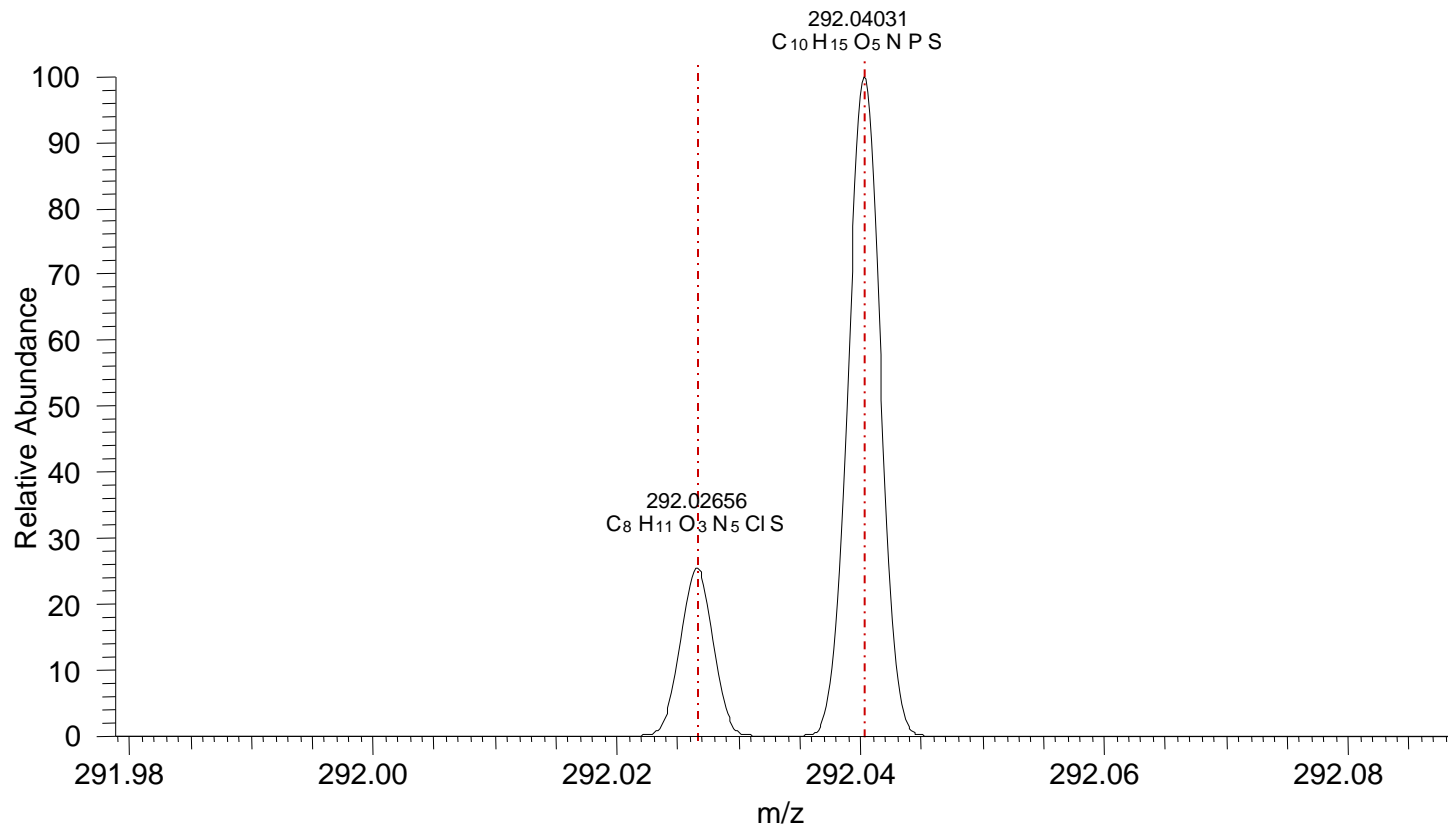
# Simulated Resolution = 50,000 (Mix 1:3)

Resolution 50,000



# Simulated Resolution = 100,000 (Mix 1:3)

Resolution 100,000



# Summary

## Summary

There is no fixed pesticide method prescribed in Ph.Eur. but method validation requirements .

MRLs of 115 pesticides are listed in Table 2.8.13.-1. With cross reference to Regulation (EC) 396/2005 there are harmonised limits given for estimation of pesticide residues in medicinal herbs.

Unambiguous allocation is possible with EHIA Allocation List.

Scope of testing depends on origin of material and method of analysis.

Strong matrixeffects in herbal drugs multimethod analysis lead to false negative and/or false positive results.



**Thank you for your interest!**