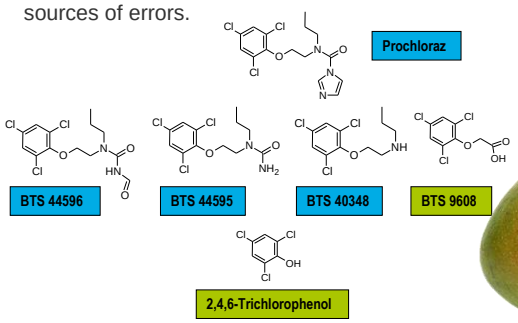
Analysis of Prochloraz (Sum) via its Metabolites

Dorothea Mack, Anja Barth, Ellen Scherbaum and Michelangelo Anastassiades

E-Mail: Michelangelo.Anastassiades@cvuas.bwl.de

Introduction

The current residue definition for prochloraz requires the use of procedures involving a hydrolysis step. However very few laboratories routinely employ such procedures. Therefore, the analysis of total prochloraz via certain characteristic metabolites containing the 2,4,6-trichlorophenyl (TCP) moiety would be a viable alternative. We have tested the analytical behavior of several TCP-containing metabolites including the two metabolites (BTS 44595 and BTS 44596) that were included in the residue definition proposed in a recent reasoned opinion by EFSA. Both GC and LC-MS/MS analysis require attention in order to avoid different sources of errors.



Analytical methods

GC-MS:

GC-MS enables the analysis of prochloraz and TCP. However analysis is tricky since prochloraz and its metabolites degrade to some extend to TCP during injection. This leads to an overestimation of TCP. Combining TCP results derived via GC with LC-MS/MS results of prochloraz and its metabolites will lead to an overestimated amount of prochloraz (sum).

LC-MS-MS:

In principle prochloraz and all tested metabolites can be analyzed by LC-MS/MS. Prochloraz and the metabolites BTS 44595, BTS 44596 and BTS 40348 are analyzed in the ESI positive mode whereas the acidic metabolites TCP and BTS 9608 require the ESI negative mode. Analysis is complicated by the fact that BTS 9608 and BTS 44596 show in-source fragmentation to 2,4,6-trichlorophenol. For BTS 44595 an in-source fragmentation to BTS 40348 has been observed.

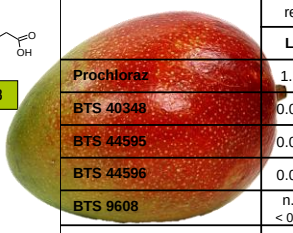
2,4,5-T and 2,4,5-TP show in-source fragmentation to the isomeric 2,4,5-trichloro-phenol and are thus potentially interfering. Interestingly, fluroxypyr and triclopyr, although not containing any TCP moiety, also experienced in-source fragmentation to products that gave signals at the MRM-traces of 2,4,6-TCP.

A good chromatographic separation of all affected components and the attention to retention times is necessary for the prevention of identification and quantification errors.

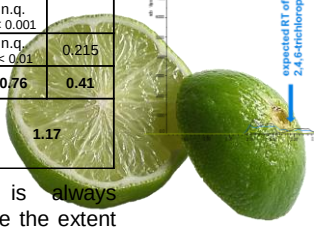
An additional source of error was the degradation of BTS 44596 to TCP in acetonitrile solutions. Acidification with acetic acid resulted in good stability.

Real samples

To get a picture of the residue situation in real samples with a history of prochloraz treatment, different types of samples (mango, lime, papaya, mushroom, pineapple, avocado, and pomelo) were analyzed using both GC-MS (CI-neg.) and LC-MS/MS. The main metabolites in the fruit and vegetable samples studied were BTS 44595, BTS 44596 and BTS 40348 components. BTS 9608 and free TCP were, if at all, only present at very low levels. To demonstrate potential sources of errors when combining various results we calculated “prochloraz (sum)” in different ways.



	Mango results mg/kg		Lime results mg/kg	
	LC	GC	LC	GC
Prochloraz	1.35	0.313	0.60	n.d.
BTS 40348	0.071		0.10	
BTS 44595	0.004		0.005	
BTS 44596	0.030		0.020	
BTS 9608	n.q. < 0.001		n.q. < 0.001	
2,4,6-TCP	n.d.	0.372	n.q. < 0.01	0.215
sum calc. as prochloraz	**1.48**	**1.02**	**0.76**	**0.41**
sum calc. as prochloraz (TCP from GC, all other metabolites and prochloraz from LC)	**2.19**		**1.17**	



GC-degradation in the injector is always influenced by matrix effects, therefore the extent of TCP occurrence may vary. BTS 40318 has been only recently available, therefore limited data is available for this metabolite at the moment.

Our conclusion:

As long as the separation of all the metabolites and other substances affected by in-source fragmentation is achieved, LC-MS/MS is the method of choice for the analysis of prochloraz (sum).

Observations at a glance:

Compound	QuEChERS amenable	Degradation rate to TCP in GC	LC-MS/MS behaviour		
			Amenable to ESI pos.	Amenable to ESI neg.	In-source fragmentation
Prochloraz	✓	medium	✓		not observed
BTS 44595	✓	very high	✓		YES to BTS 40348
BTS 44596	✓	high	✓		YES to TCP
BTS 40348	✓	high	✓		not observed
BTS 9608	✓ (skip cleanup)	minor		✓	YES to TCP
TCP	✓	-		✓	not observed

References

More Details: http://www.eurl-pesticides.eu/
List of observations

EFSA Journal 2011; 9(7):2323

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