HBM4EU

science and policy
for a healthy future

HBM4EU project

Determination of Glyphosate, methods and application

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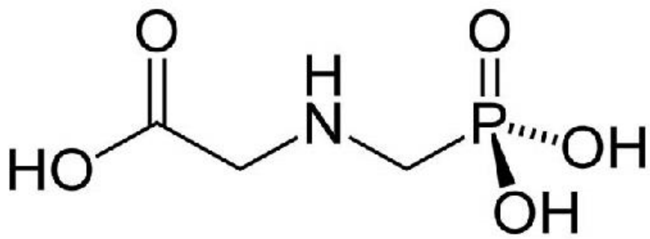
1. Background
2. Biomarkers
3. Literature
4. HSL Method
5. Issues
6. Summary/ conclusions

Glyphosate

- Widely used
- Considered harmless for years
- Complicated to analyse
  - Highly polar
  - Low MW (169 amu)



IARC classification (2015)

- 2A – Probably carcinogenic to humans
- No universal agreement

Metabolism

- *Little to no metabolism occurs*
- *Small amount converted to AMPA*

Matrices

- *Urine – Most common*
- *Blood (serum/ plasma) – Few reports (acute poison cases)*

Urine biomarkers

- *Unchanged parent compound*
- *Aminomethyl phosphonic acid (AMPA)*
  - *Similar toxicology to Glyphosate*

Available HBM studies prior to IARC classification

References	Analytical method, LOD/LOQ	Participants	Urine concentrations [µg/l]	
			Mean	Maximum
Acquavella et al. (2004)	HPLC following ion exchange LOD 1 µg/L	**48** male farmers from Minnesota and South Carolina (USA), **their spouses** and **79** children	**3.2**	**233** (farmer) **29** (child)*
Curwin et al. (2007)	Immunoassay (fluorescent microbeads) LOD 0.9 µg/L	**48** women, **47** men, **117** children from "farm" and "non-farm" households in Iowa	**1.1–2.7** (in different groups)	**18** ("farm child")*
Mesnage et al. (2012)	HPLC–MS LOD 1 µg/L LOQ 2 µg/L	**1** farmer, his wife and **3** children, presumably Europe	**n.a.** (only single values available)	**9.5** (farmer) **2** (child)*
Hoppe (2013)	GC–MS/MS following derivatisation LOQ 0.15 µg/L	**182** citizens from 18 European countries	**0.21**	**1.82**
Markard (2014)	GC–MS/MS (presumably) LOQ 0.15 µg/L	**40** male and female German students	**n.a.** (22 samples above LOQ)	**0.65**
Krüger et al. (2014)	ELISA partly validated against GC–MS LOD/ LOQ not given	**>300** (mostly from Germany)	**≤2**	**5**
Honeycutt and Rowlands (2014)	ELISA LOQ 7.5 µg/L	**35** women, men and children from USA	**n.a.** (13 samples above LOQ)	**18.8**

Neimann *et al* (2015) J. Verbr. Lebensm. 10:3–12

Not all in peer reviewed publications

Missing details about analytical methods

Missing LoQ/ LoD

GC-MS

- *Derivatisation*
    - *Trifluoroethanol, Trifluoroacetic acid, Heptafluoro butanol, Trifluoroacetic anhydride…etc*
    - *Often 2 derivitisation steps*
    - *Hazardous*
    - *Some require very cold working temperatures*

Example methods

- *Hoppe et al (2013)*
    - *2 step derivitisation*
    - *Low LoQ of 0.15 µg/L*
    - *Applied to 182 samples (18 countries)*

LC-MS/MS

- Can be analysed without derivitisation
- Direct injection possible
- Chromatography can be troublesome
  - Lack of retention
  - Poor peak shape

Example methods

- Jensen et al (2016)
- Simple extraction
  - Dilute urine with formic acid
  - Low LoQ of 0.1 µg/L

Typical results

- *Studies in some agreement*
    - *Low levels – typically < 20 µg/L*
- *LoQs*
    - *0.1 – 1 µg/L*
    - *No method / instrumentation consistently lower*

No ‘gold standard’ method

- *Wide variety of methods*
- *Most have strengths and weakness*
- *Lack of quality in some reported methods*

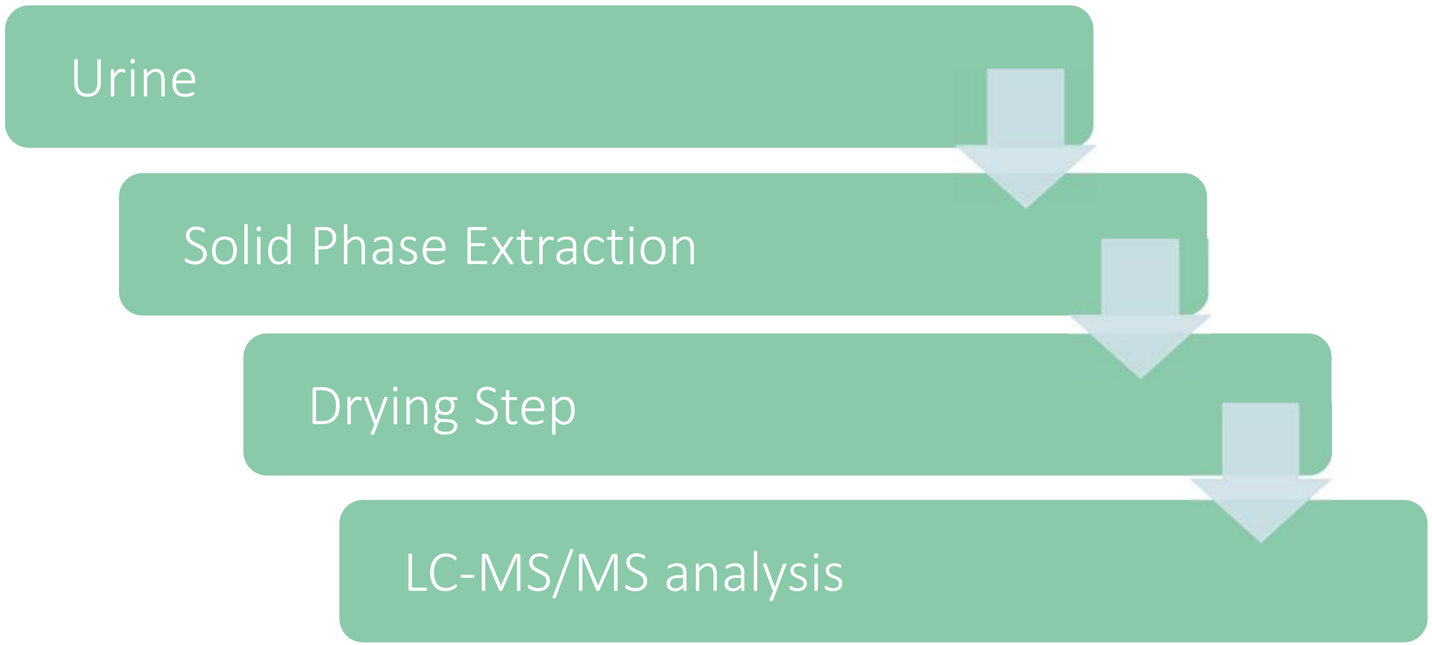
ELISA

- *Few publications using this method*
- *Lack of data on performance*
- *1 study has LoD of 7.5 µg/L*

Food Analysis

- *Nagatomi et al (2013)*
- *Beer, Barley Tea...*
- *SPE and acid extraction*

Overview



Sample Treatment

200 µl urine sample in 800 µl water

Glyphosate-2-^{13}C,^{15}N (Internal Standard)

SPE

Strata SAX, 100 mg/1 mL (Phenomenex)

Condition:	*Water*
	Sample load
Wash:	*Water*
Elution:	*10% Formic acid in methanol*

Preparing for instrument analysis

Evaporate elution solvent using nitrogen stream

Reconstitute in 100 µl of 0.1% formic acid

Liquid Chromatography

System:	*Shimadzu HPLC*
Gradient:	*0.1% Formic acid*
	Acetonitrile
Column:	*Zorbax XDB-C8, 150 x 4.6 mm, 5µ (Agilent)*
flow rate:	*0.4 mL/min*
Injection vol:	*10 µL*
Run time:	*16 minutes*

Useful info

Method originally developed for 2 analytes (fluroxypyr)

Higher flow rate helps with peak shape

- *Low back pressure because of column dimensions*

Mass Spectrometry

Negative MRM

m/z =	*168/63*
CUR:	*50*
CAD:	*High*
Voltage:	*-4500*
Temp:	*500°C*
GS1:	*70*
GS2:	*50*

Useful info

Lower gasses resulted in no signal

Very sensitive to contamination/ charging

HBM4EU project — *Glyphosate: HSL Method*

Calibration

Linear range 0 – 20 µg/L, Matrix matched (r^2 > 0.999)

Quality Control

8 µg/L spike level

- *Mean = 8.5 µg/L, n = 40*
- *Intra assay CV = 3.5%, N = 10*
- *Inter assay CV = 10.0%, N = 40 over 4 runs*
  - *<10% long term N = 226 , >25 runs*

Stability

2 years at -20°C

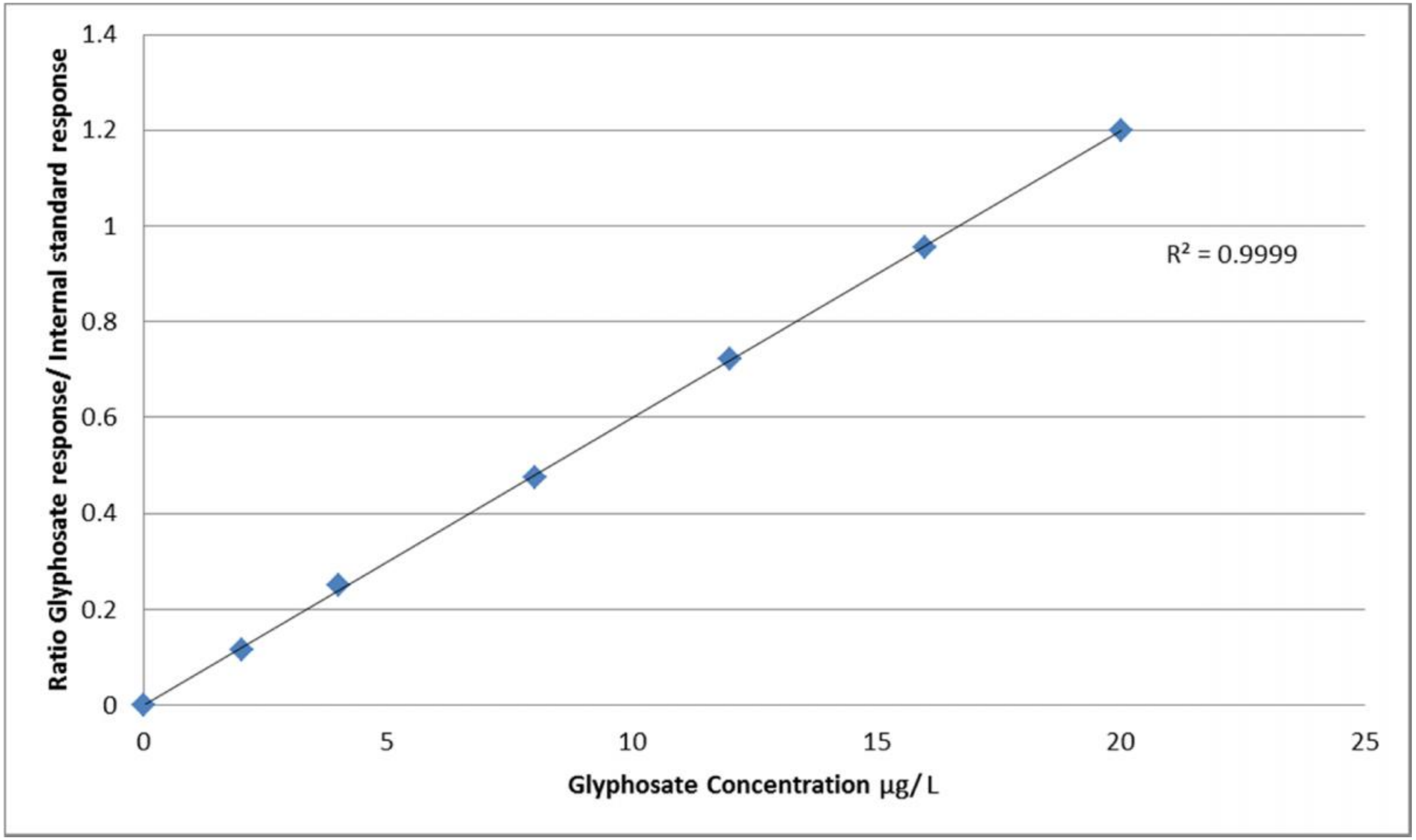
Short term storage (24 hours) tested at 37°C, RoomTemperature, 4°C

- *Room temperature also tested for 4 days*

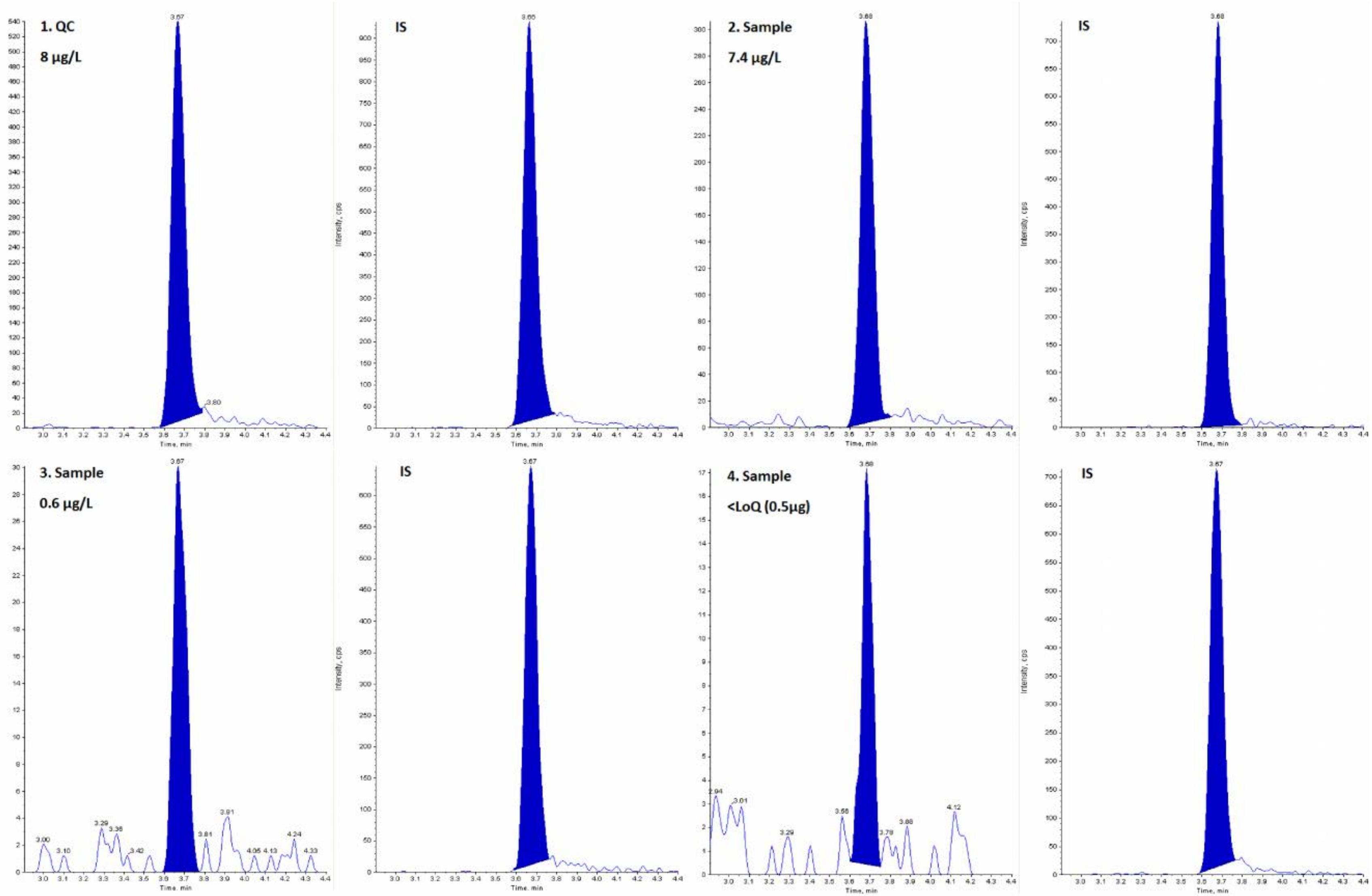
LoQ

0.5 µg/L

Standard Calibration



Glyphosate: HSL Method



Background

- Ireland
- Small - 50 participants
- 20% > LoQ
- 0.8 – 1.35 µg/L

Occupational

- Amenity horticulture workers
  - Ireland
  - Amenity horticulture workers
  - Spot urine samples (Pre and post shift)

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Glyphosate: HSL Method

References	Type of study	Country	Analytical Method	LOD/LOQ ($\mu g\ L^{-1}$)	Participant numbers	Urine conc. ($\mu g\ L^{-1}$) Statistic & Value	Max
*Current study	Occupational exposure in horticulture amenity gardening.	Ireland	LC–MS/MS[2]	LOD 0.5	19 workers	AM 1.35	10.66
Mesnage et al. (2012)	Family exposure in agricultural setting	Europe	HPLC–MS[3]	LOD 1 LOQ 2	1 farmer & spouse & 3 children	N/A	9.5
Curwin et al. (2007)	Farm families and 'non-farm' families	United States	Immunoassay (fluorescent microbeads)	LOD 0.9	47 fathers, 48 mothers and 117 children	AM 1.1-2.7	18
Acquavella et al. (2004)	Occupational and residential exposures in agricultural setting.	United States	HPLC following ion exchange	LOD 1	48 farmers, 48 spouses & 79 children.	GM 3.2	233
Jayasumana et al. (2015)	Investigate glyphosate levels in Sri Lankan Agricultural Nephropathy (SAN) patients	Sri Lanka	ELISA[4] validation done in comparison with GC–MS		30-3 groups of 10). Group 1 Group 2 Group 3	Median 56.8 73.5 3.3	≥80
*Rudzok et al. (2016)	Environmental exposures	Germany	GC–MS/MS[5]	LOQ 0.15	250 samples 2–6 year old children.	Median 0.14	95th%ile 0.97
Conrad et al. (2017)	Environmental exposures	Germany	GC–MS/MS	LOQ 0.1	399 samples adults	Median 0.18	2.80
*Hoppe (2013)	European study – back-ground/dietary exposures	Europe	GC–MS/MS	LOQ 0.15	182 urine samples, 18 European countries	AM 0.21	1.82
*Markard (2014)	Environmental exposures	Germany	GC–MS/MS (presumed)	LOQ 0.15	10 male 10 female	N/A	0.65
Krüger M et al. (2014)	Human & animal to investigate environmental exposures.	Europe	ELISA partly validated against GC–MS	LOD/LOQ unknown	14 human samples	≤2	5
*Honeycutt and Rowlands (2014)	Environmental exposures	United States	ELISA	LOQ 7.5	35 (male, female and children).	NA	18.8

N/A: Information not available due to single measurements or not given in literature.
*Literature that has not gone through peer review or is unpublished reports.
Partial details from this table was obtained in (Niemann et al., 2015).

Connolly et al (2017) International Journal of Hygiene and Environmental Health, 220(6): 1064-1073

Occupational

Amenity horticulture workers

- Ireland
- Amenity horticulture workers
- Pre, post and following morning void samples
- All voids for 24 hours collected from some participants

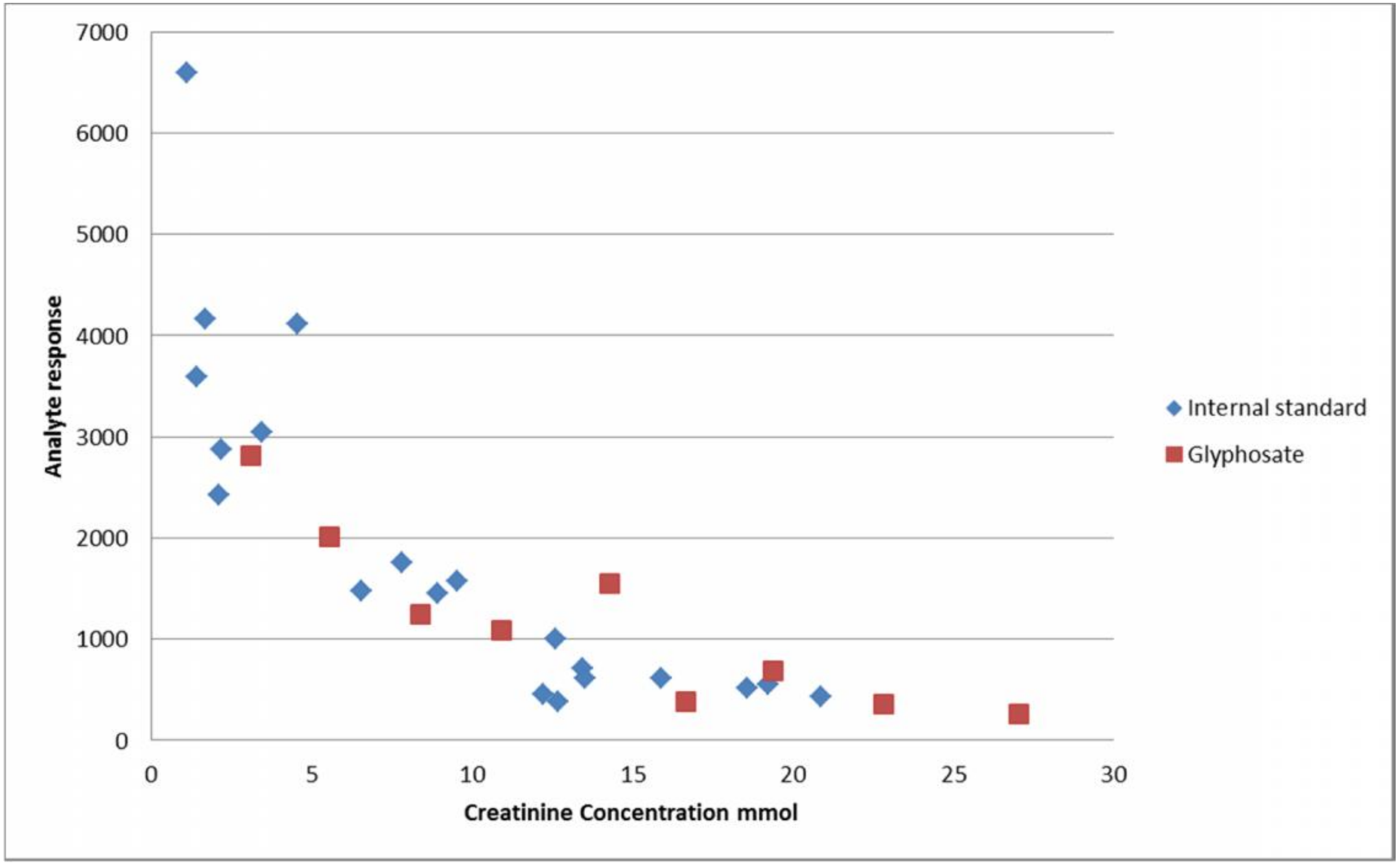
Findings

- Results were similar to previous study
- Best sampling time is up to 3 hours post exposure
- Half-life information gathered

Issues

- *Loss of peak shape over time*
- *Loss of sensitivity over time*
  - *Both often fixed with a new column or source clean*
- *Charging*
  - *Glyphosate particularly sensitive*
  - *Cleaning of Q1 needed*
- *Ion suppression*
  - *Better clean-up procedure needed*

Ion suppression



Summary

Glyphosate interest high

Quality data is needed

Decent methods exist but not without issues

Agreement on method approach

Sharing methods/ experiences can help improve processes

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Thank you for your attention.

Any questions?