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# A Case Against

# Using Internal Standards

by Randall Bra

**for EPA Method TO-15 In**

**mston-Cook and Edward**

**Involving Toxic Compound**

**Bramston-Cook**

Ran  
unce



# ds in Ambient Air

dom measurement errors are compounded when  
ertainties for Internal Standard areas are added.

[ ... ]

*area i*



×  $[IS]$

# I. ABSTRACT

Internal standards are frequently based on a number of factors related to the selection of internal standards in the EPA Method TO-15 is to correct for the need for complicating system operation in addition.

# II. BACKGROUND

Purpose of Internal Sta

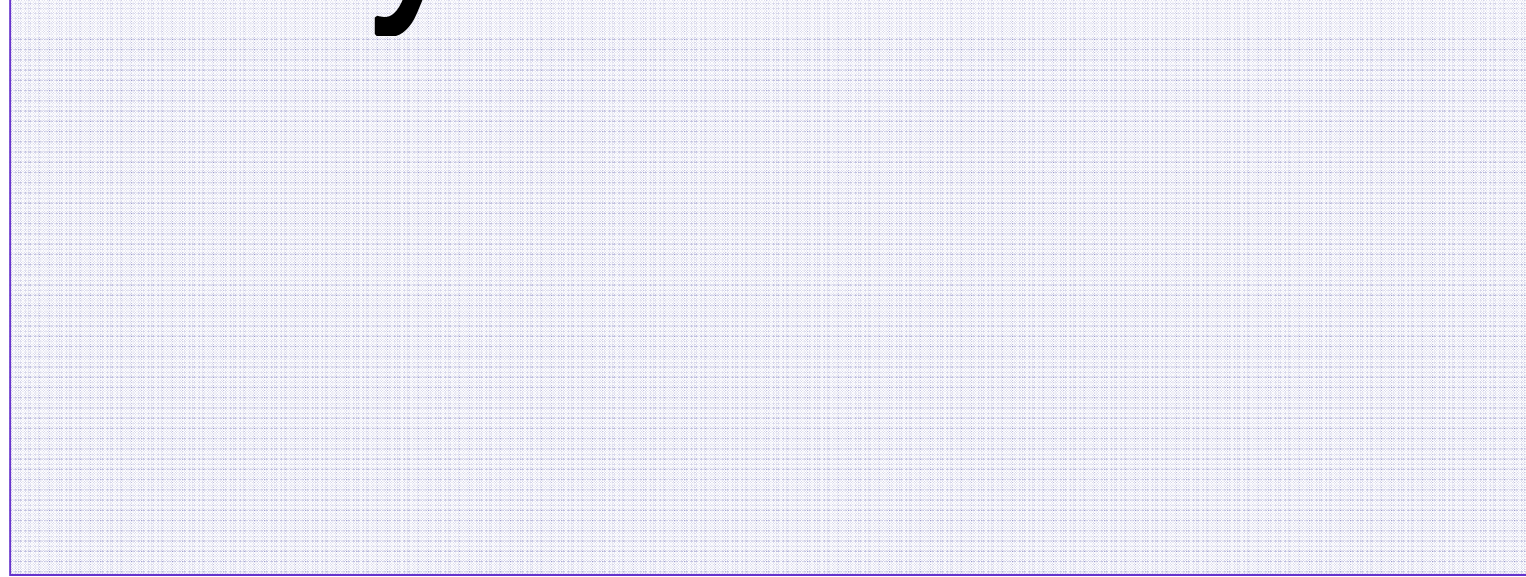
y employed in many analytical methods, especially involving gas chromatography. The criteria for choice of internal standards are related to the method and analytes of interest. Criteria for choice of internal standards and how their usefulness can be impacted are discussed. The potential for instability of the mass spectrometer. Inherent ability with a high precision and accuracy. Operations with the addition of internal standards. Data are provided that

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**ID**

standards

matography. Their selection and use are  
nal standards are reviewed. Methods of addi-  
primary application of internal standards in  
performance mass spectrometer alleviates the  
t demonstrate improved results without this



Mandate F: Not interfere in or be interfered with by  
Coelution of Bromochloromethane v

The example on the left shows no distortion when concentration a  
bromochloromethane, as shown on the right, the internal standard

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y any analyte or matrix component to enable identification with adjacent analytes creates peak distortions and invalid results.

are below 10 ppbV. However, when the major peak - Tetrahydrocannabinol is split into multiple peaks, with significantly reduced area, the results are invalid.



For c  
mea  
the f

ifying ions to be picked

furan - elutes right on top of  
ed peak retention times very

*Sta*



example, an error of 10% relative for all area measurements gives an elevated error of 20% for final result, thus impacting the detection limit.

$$\text{lev of } [analyte\ i] = \sqrt{\left(\frac{stdev(area\ i)}{Area\ i}\right)^2 + \left(\frac{stdev}{A}\right)^2}$$

$$[analyte\ i] = \frac{area\ i}{RRF\ i \times area\ std\ i}$$

$$RRF\ i \equiv \frac{area\ std\ i}{[analyte\ std\ i] \times c\ i}$$

$$\left( \frac{area\ IS\ in\ sample}{area\ IS\ in\ sample} \right)^2 + \left( \frac{stdev\ (area\ std\ i)}{area\ std\ i} \right)^2$$

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*IS in sample*

$\times [IS]$

---

*area IS in std*

---

$+ \left( \frac{\textit{stdev}(\textit{area IS in std})}{\textit{area IS in std}} \right)^2$

# Purposes of Internal Standards

- . Mathematical correction
  - Sample preparation
    1. Sample
    2. Purging
    3. Injection
- . Monitor performance
  - Chromatography
    4. Leaks
    5. Column
    6. Retention
  - Detector performance
    7. Changes

## Mandates for Choice of

- A. Not present in sample
- B. High purity, especially
- C. Chemically and physically

Internal Standards

Correction for systematic errors

Losses

Extractions

Efficiencies

Reaction volumes

Response of complete system

Instrument degradation

Retention time shifts

Response

Changes in sensitivities

## Use of Internal Standards

Requirements

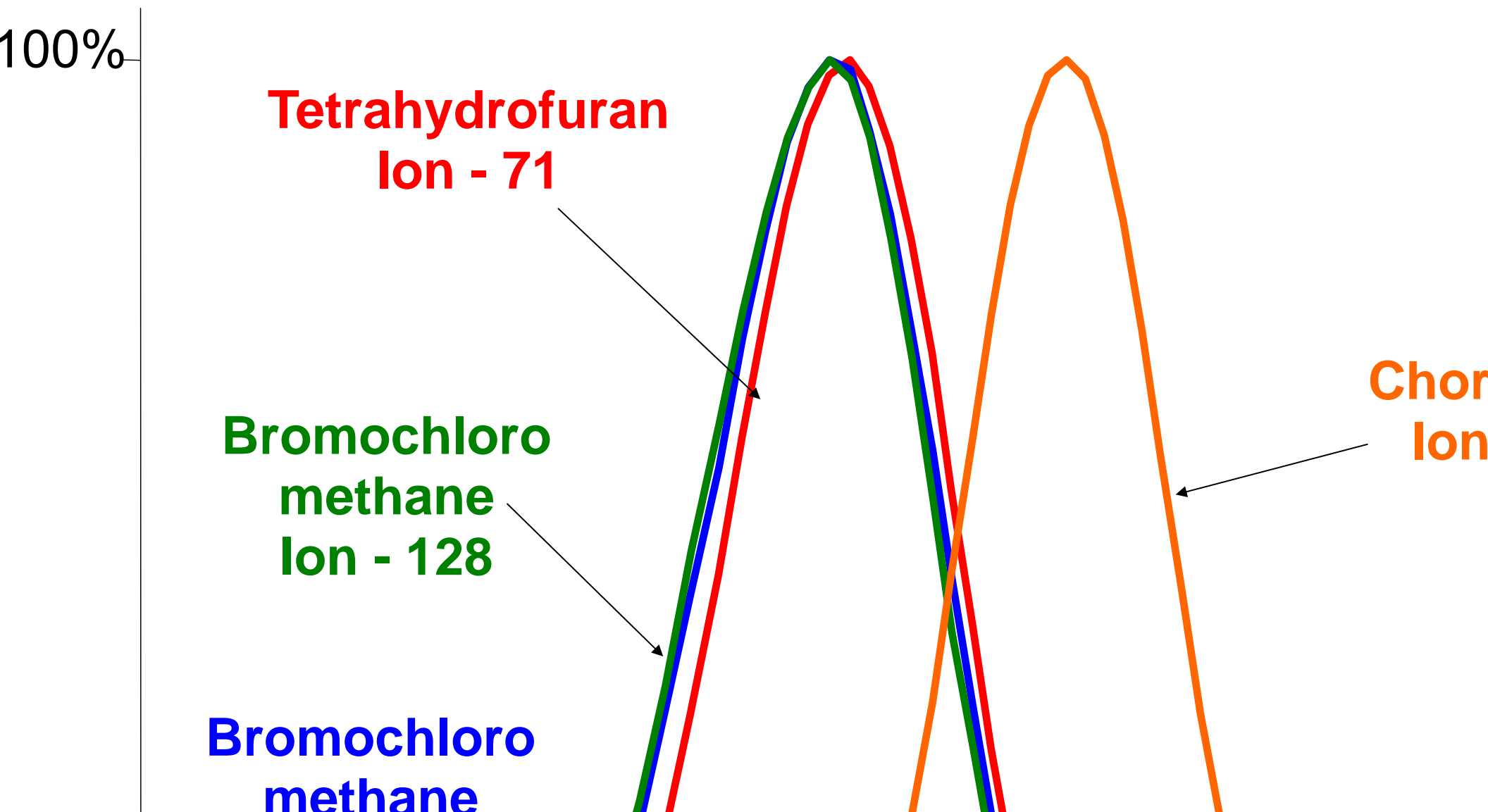
Chemically free of targets

Physically similar to related analytes



bromochloromethane, as shown on the right, the internal standard peaks are normalized for easier visual comparisons.

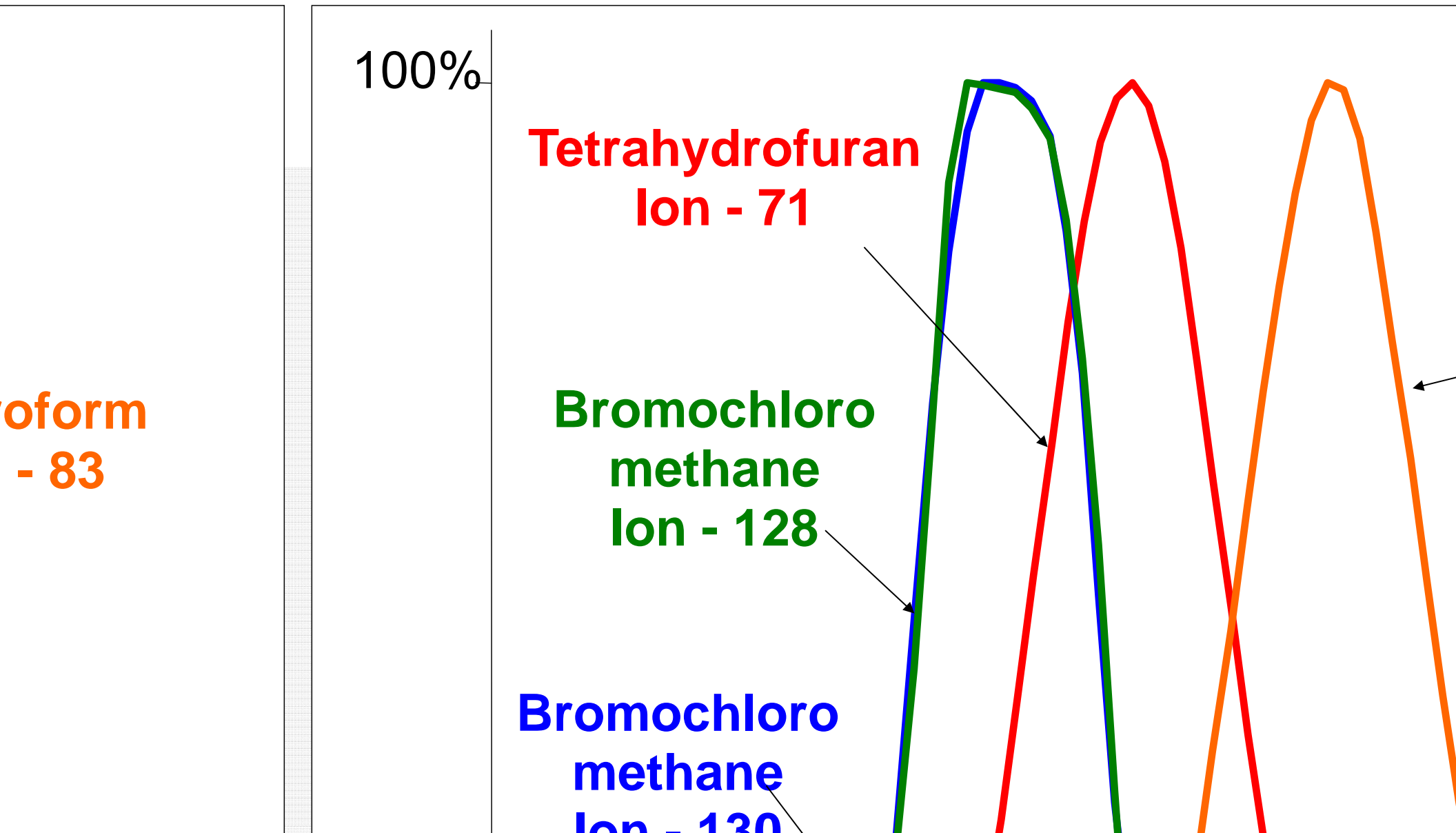
## Low Level for Tetrahydrofuran





is split into multiple peaks, with significantly reduced areas, and

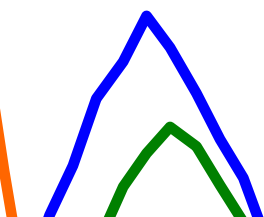
## High Level for Tetrahy



and peak retention times vary.

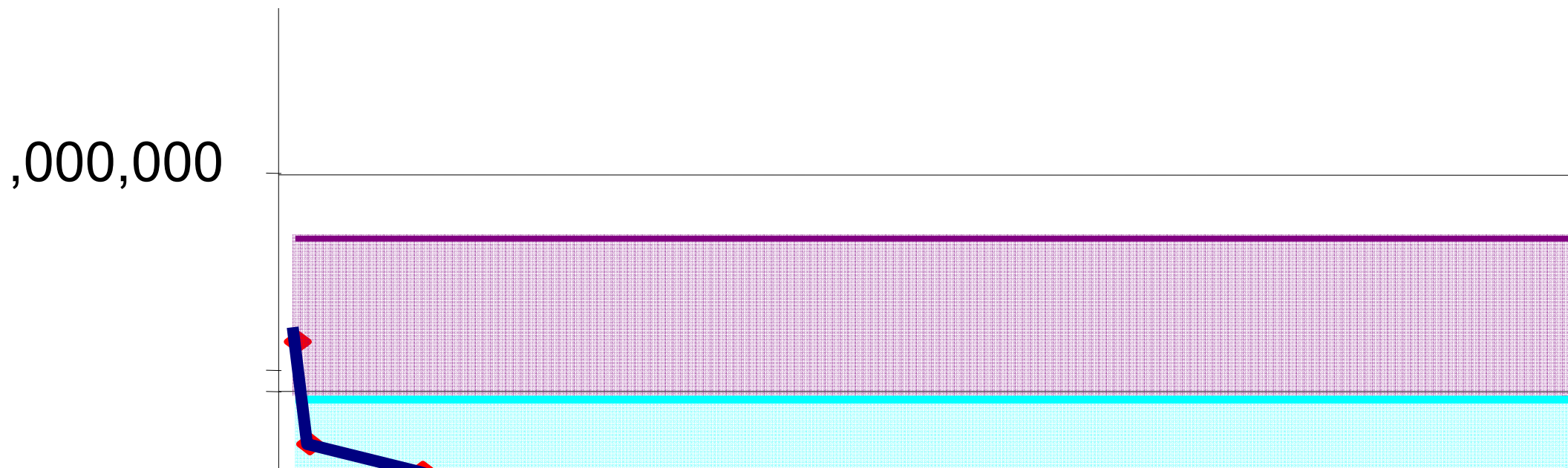
# hydrofuran

**Choroform**  
**Ion - 83**



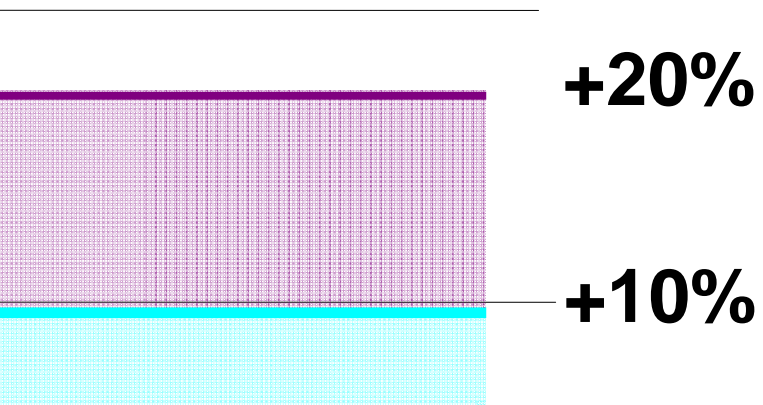
Stability achieved with high performance ma

Control Chart for difluorodichlorome  
in Daily Checks over 14 days



Mass spectrometers negates the advantages of adding  
even over extended time.

thane



**Typical Ana**

Dichlorodifluoro

Toluene



ng in internal standards,

<b>alytes</b>	Variation over 14 days
r methane	$\pm 9\%$
e	$\pm 15\%$

- D. Perform similar c
- E. Chromatographic
- F. Not interfere in o

## Listed TO-15 Internal S

- . Bromochloromethane
- . Chlorobenzene-d5
- . 1,4-Difluorobenzene

## Mandate C: Chemically an

Internal standards do not m

Internal Standards for TO-15

chromatography to assigned analytes

ally separated from targets, or possess unique MS ions

r be interfered with by any analyte or matrix component to enable ident

## Standards

e

e

d physically similar to related analytes.

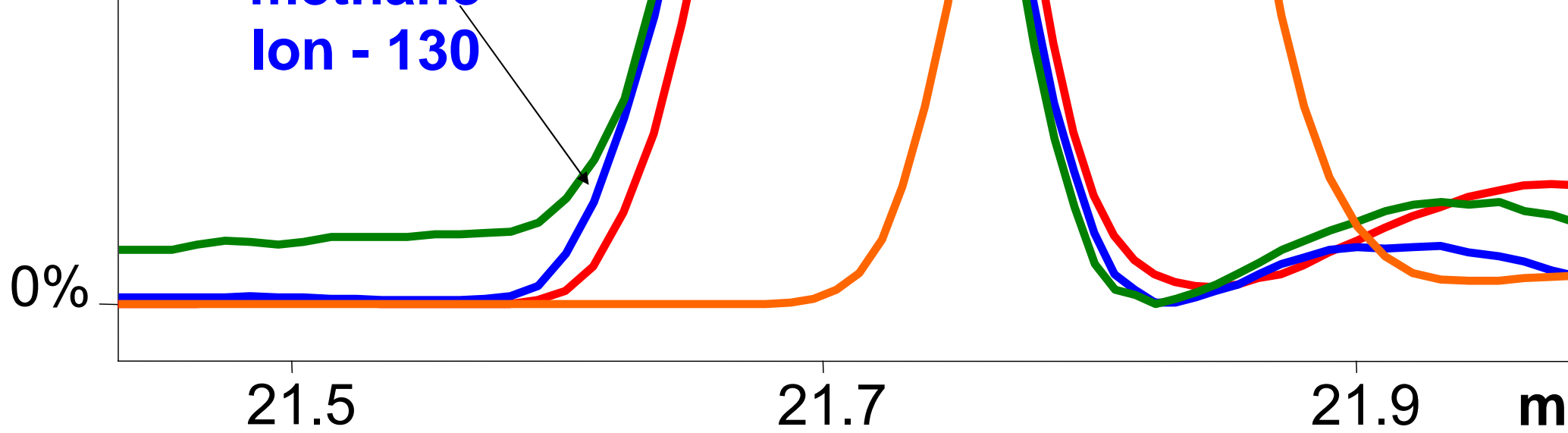
atch boiling points for early and late eluters.

do not match boiling points for many of

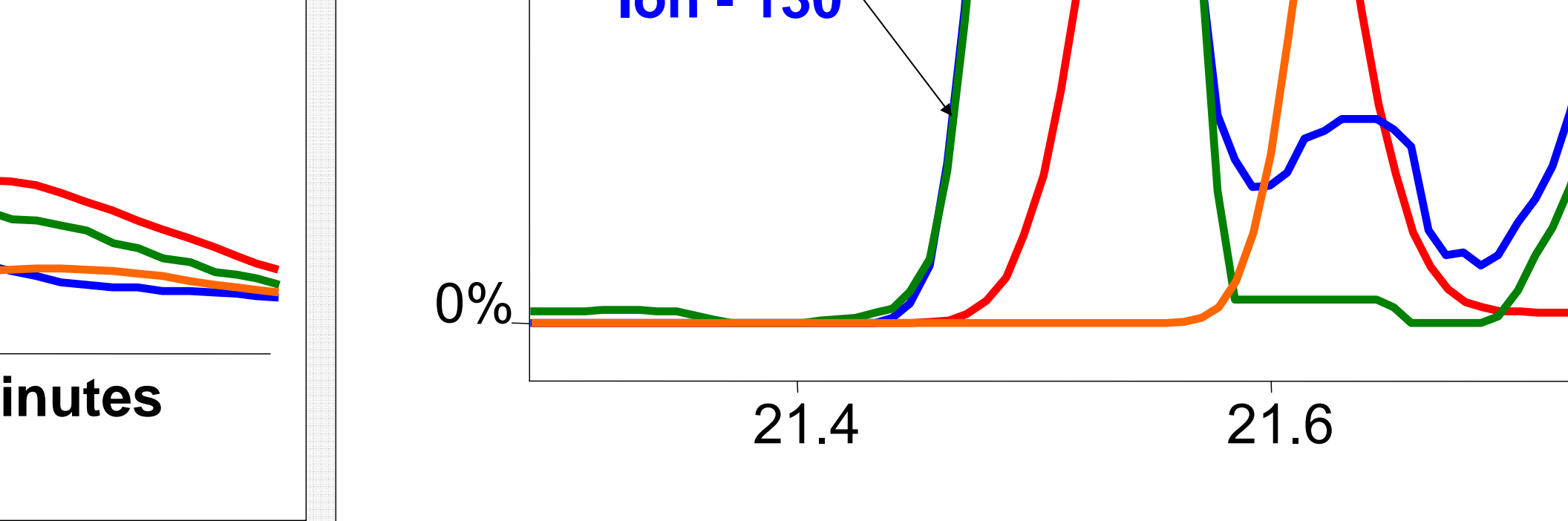


Identifying ions to be picked

**Mandate D: Perform similar chromatography**  
Internal standards do not cover retention times for



Why to assigned analytes  
for early and late eluters.



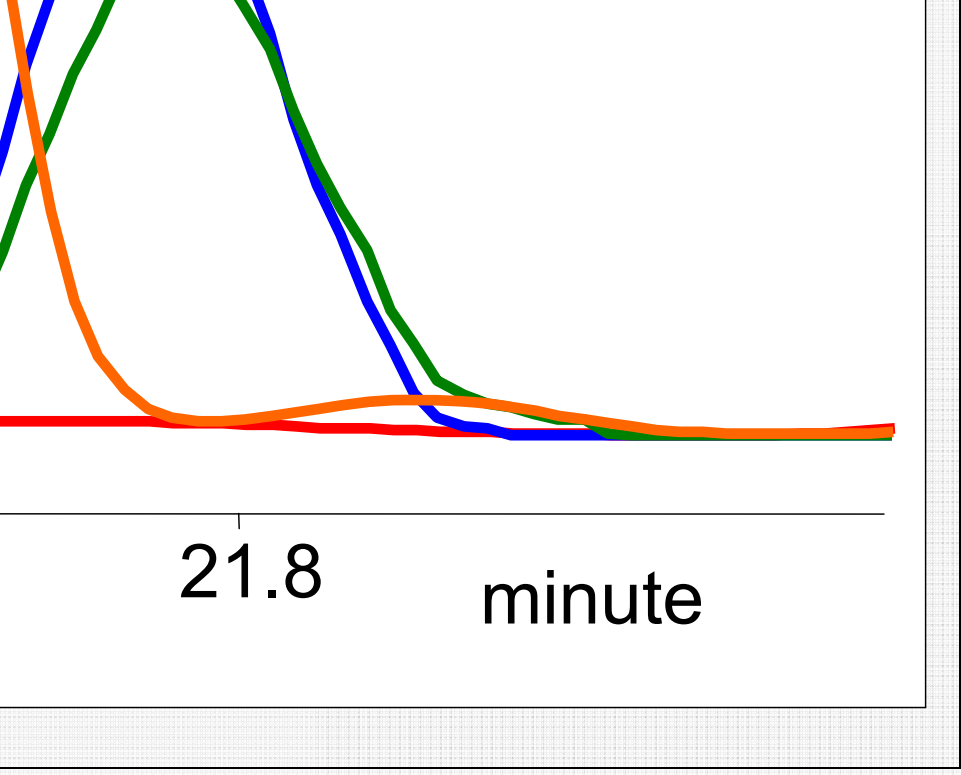
minutes

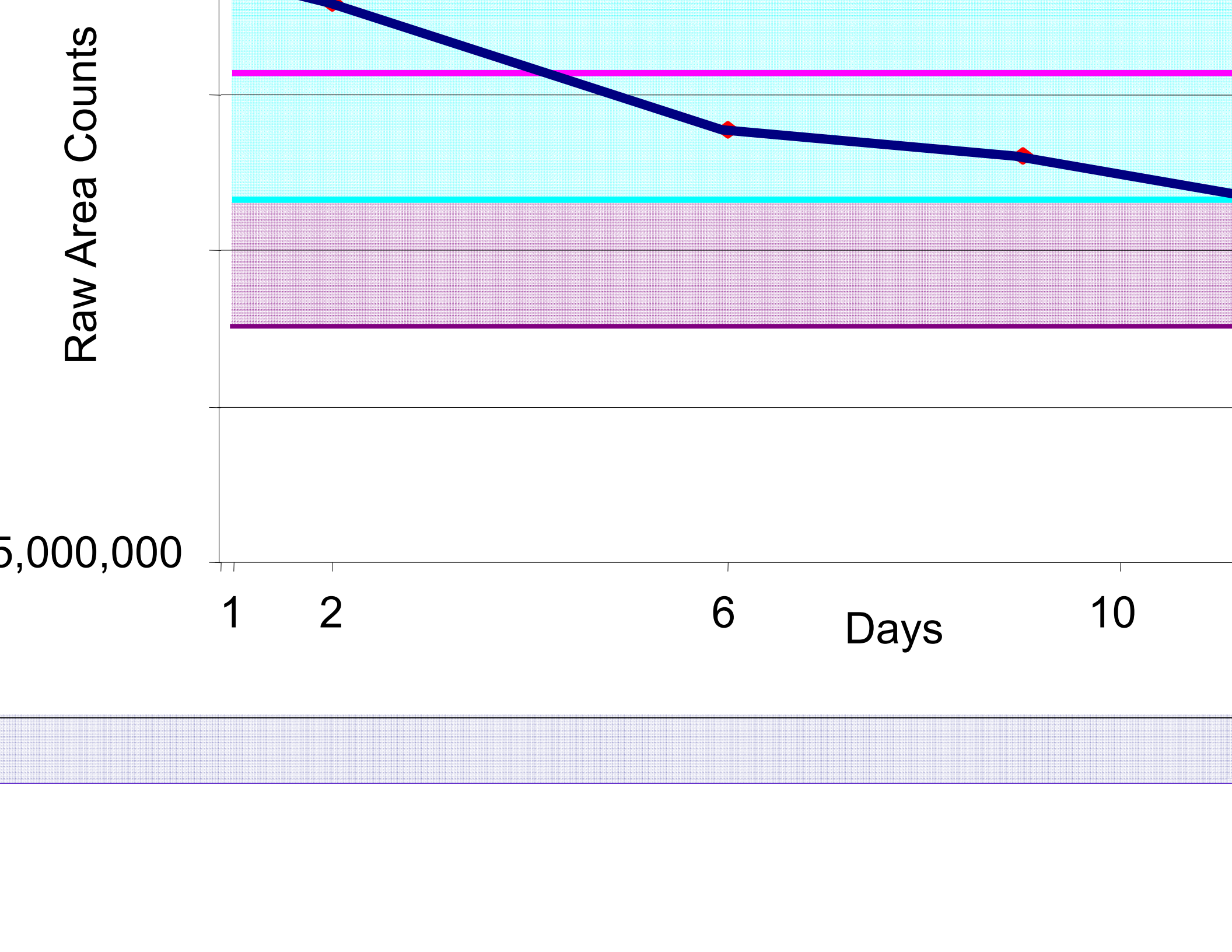
0%

21.4

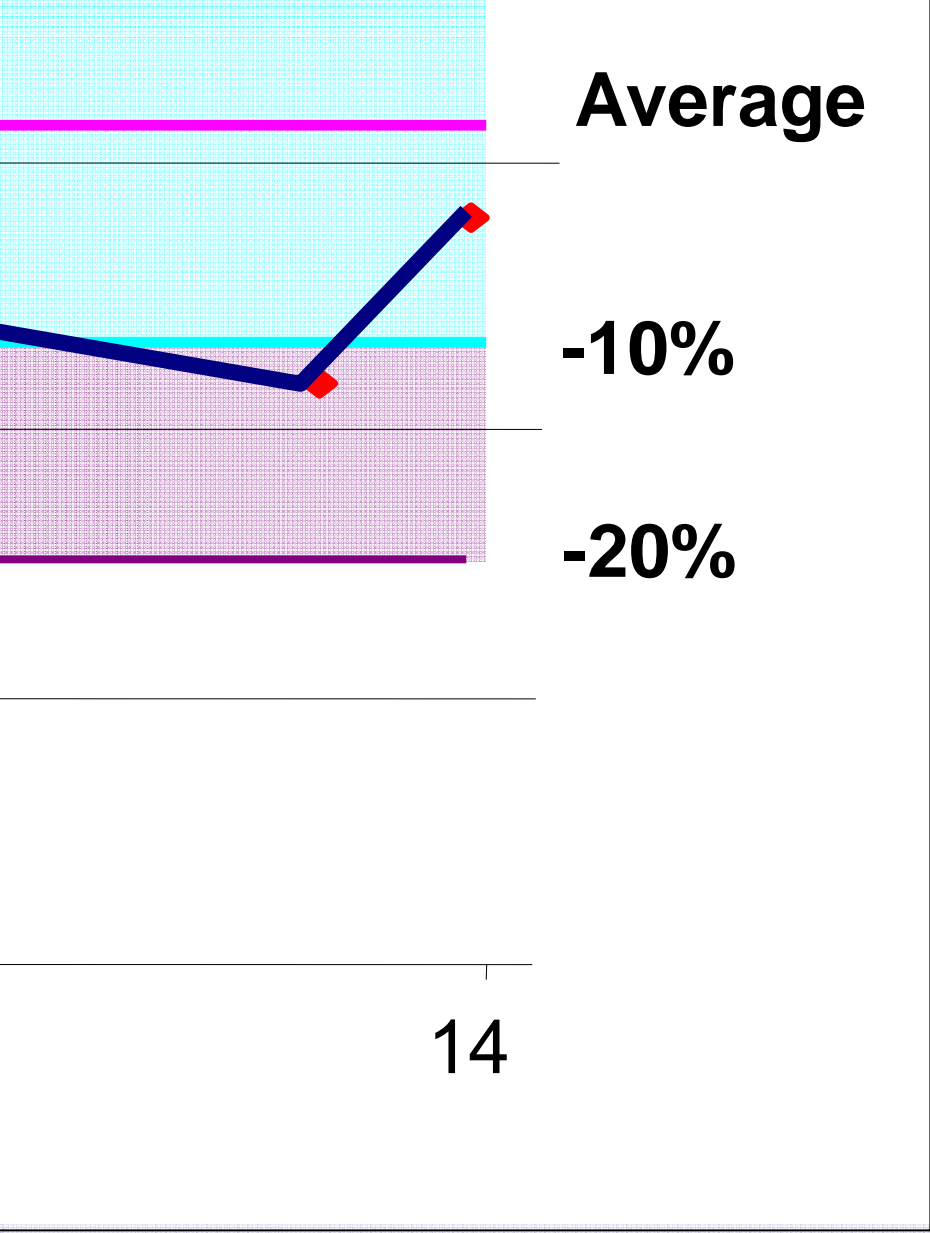
21.6

1011-150







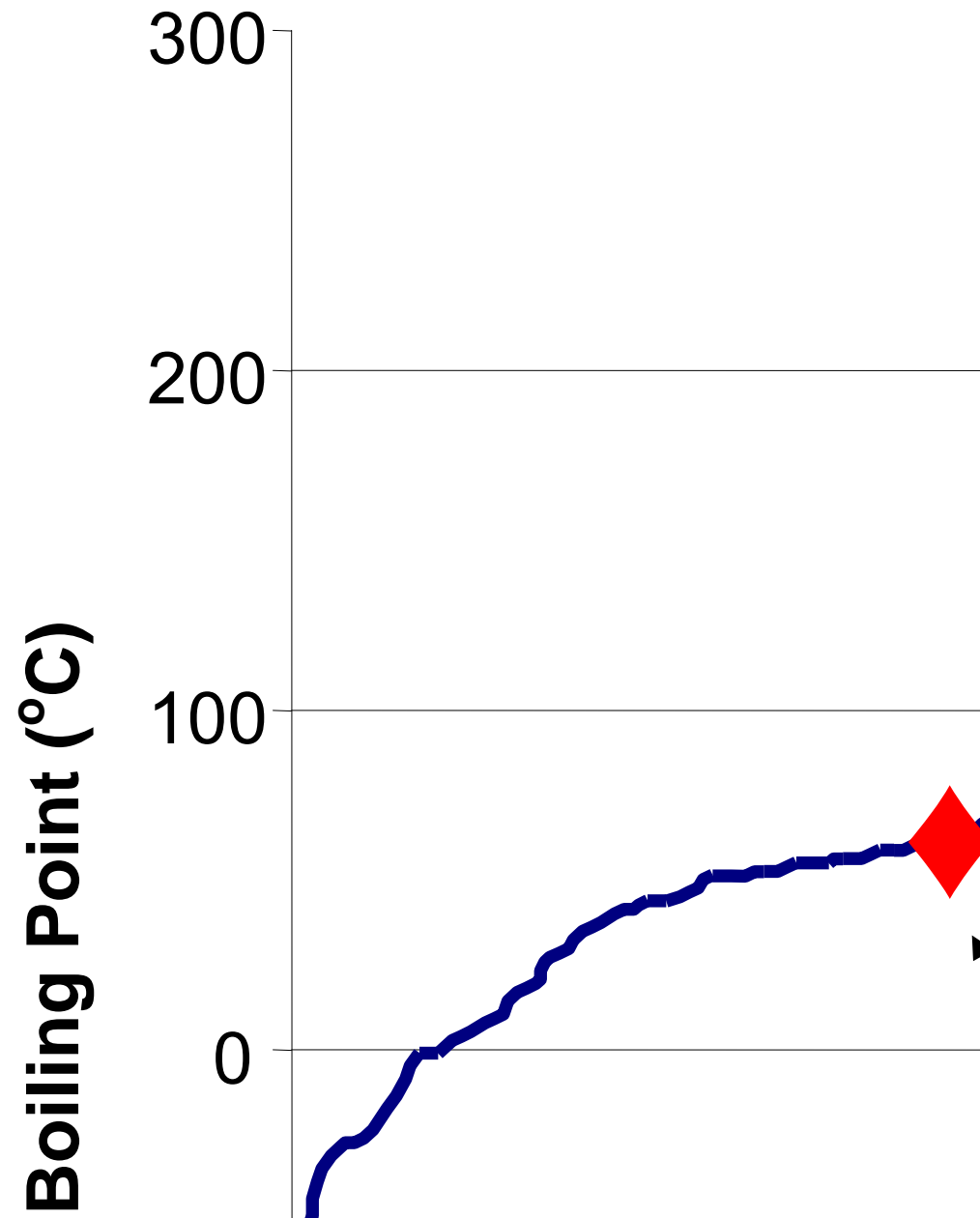


HexaChloroBu
Bromochlorome
14 Difluoroben
Chlorobenzene

butadiene	$\pm 5\%$
ethane (IS)	$\pm 10\%$
benzene (IS)	$\pm 7\%$
acetone-d5 (IS)	$\pm 8\%$



# Internal Standards for TO-15 the target ana



do not match boiling points for many of  
analytes in Method TO-15.

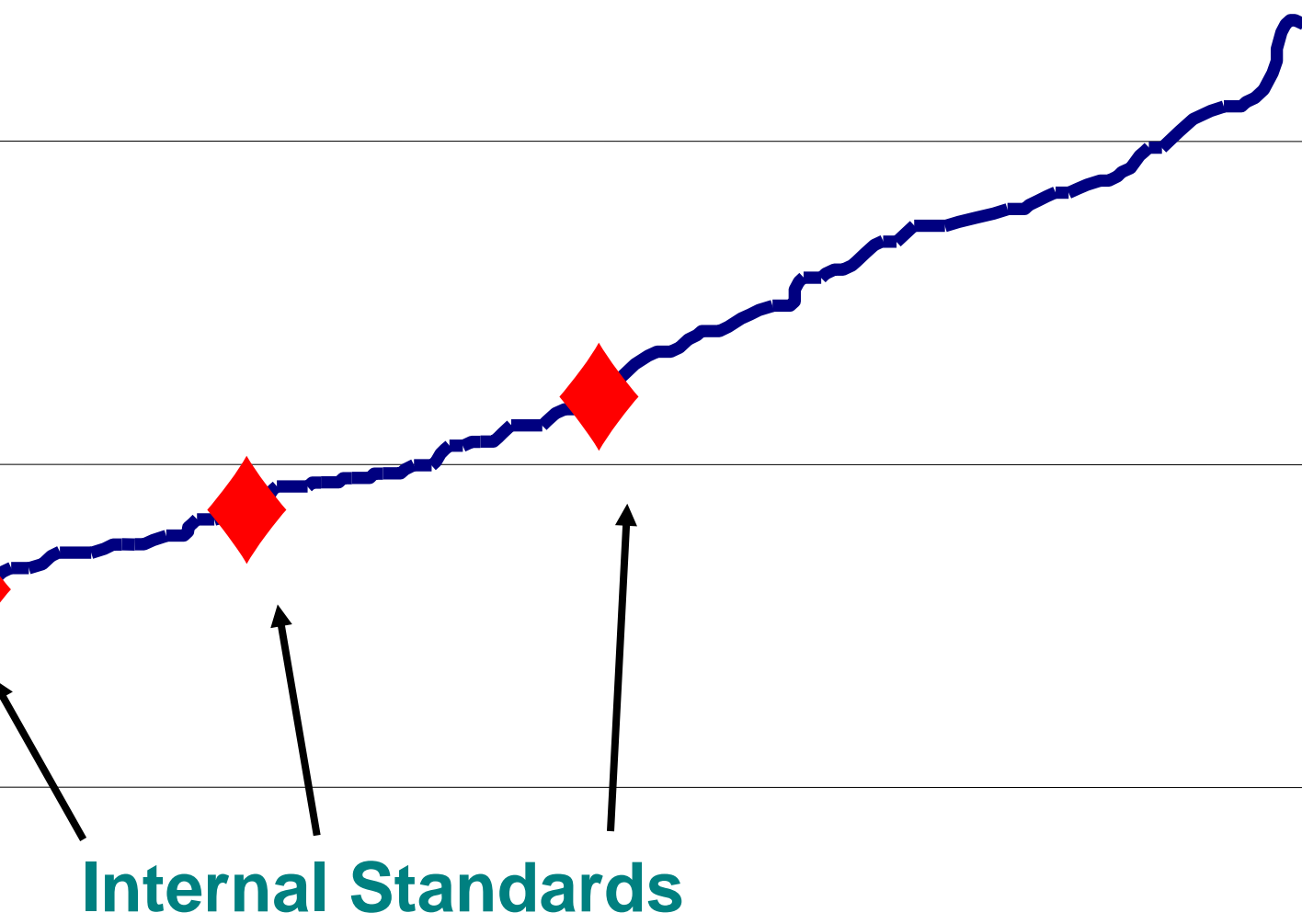
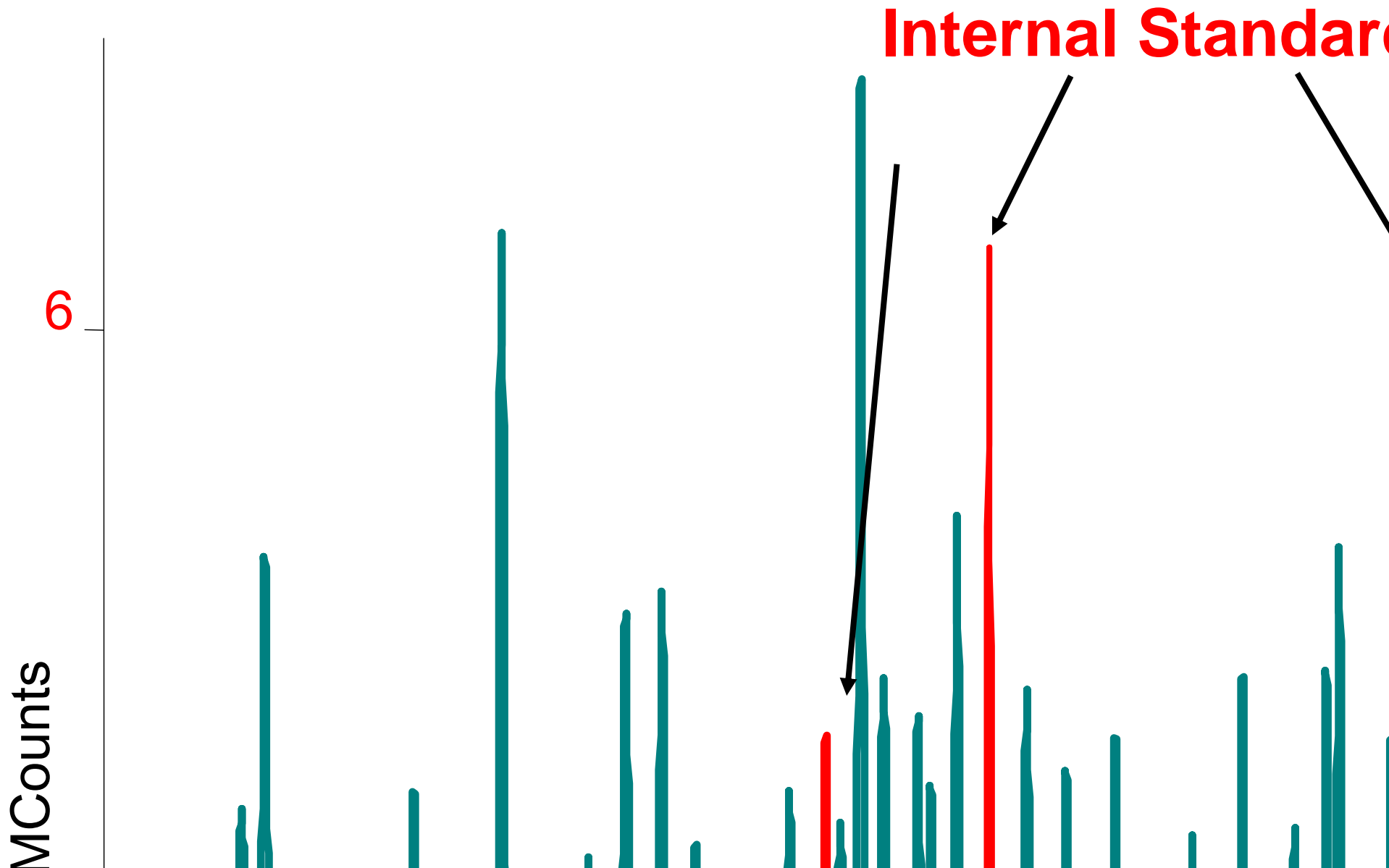
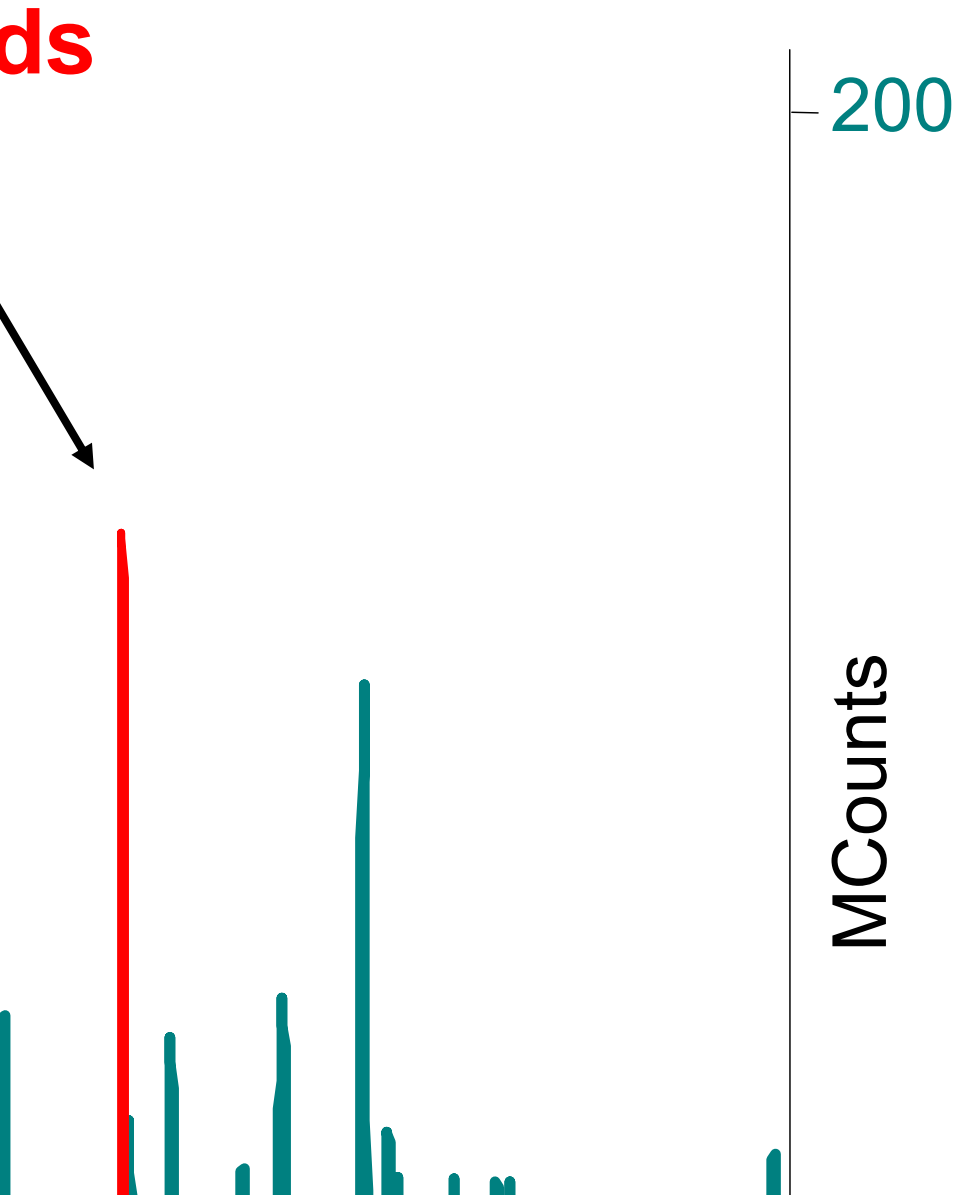


Figure 2. Many target analytes before the first internal standard and a

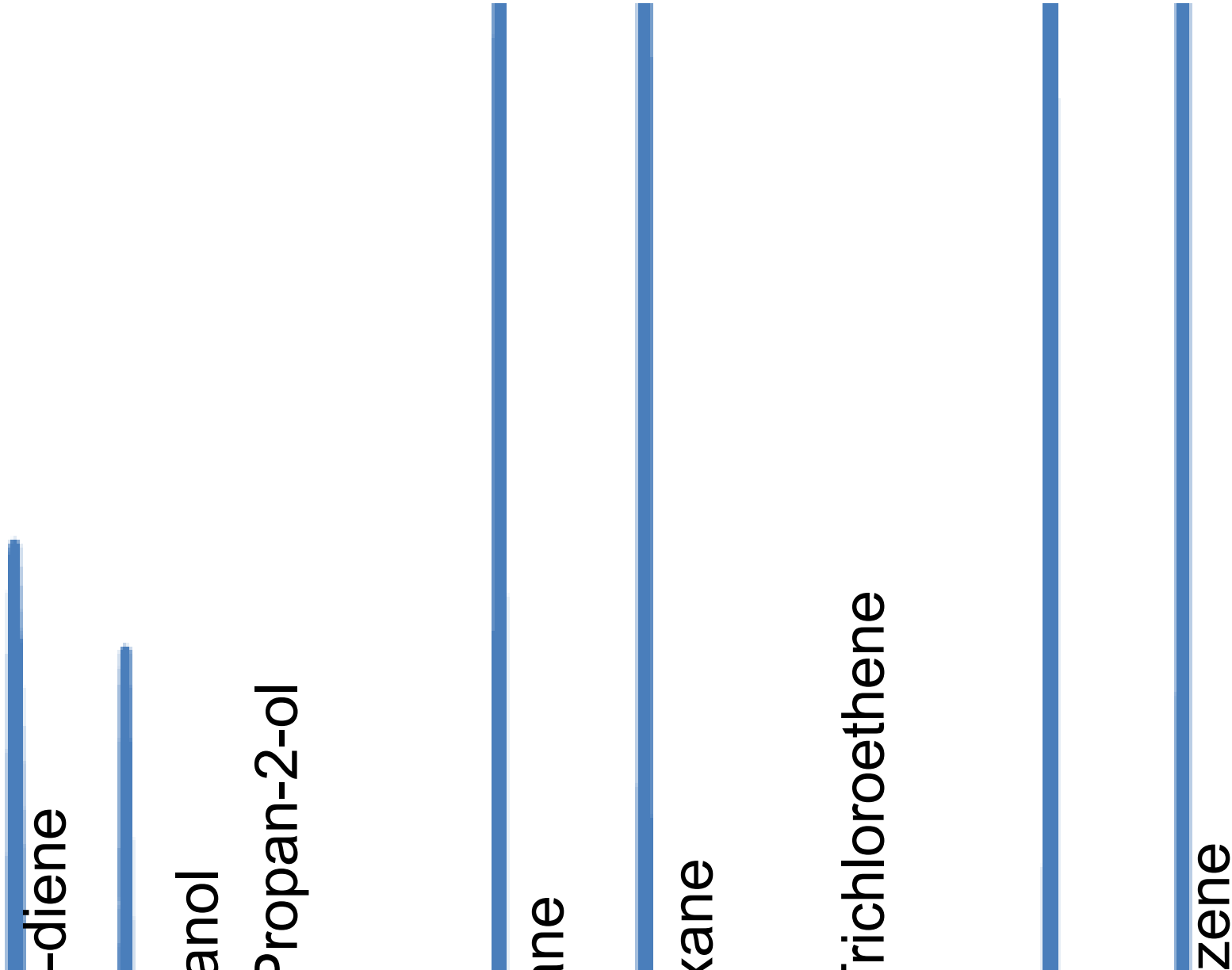


es elute  
after the last one.



# Mandate B: High pu

Internal standards can have ,many target analytes



rity, especially of targets

s detectable with high performance mass spectrometers.

<b>Analyte</b>	<b>Conc (pm</b>
Buta-1,3-diene	
Ethanol	
Propan-2-ol	
Cycohexane	



**Concentration**  
(mol/mol)

20

72

203

18

### III. SUMMARY

1. Mandated internal standards differ in chemical properties.
2. Internal standards can be used for measurements.
3. Corrections for trapping can be lost if trap is not closed. Other case is detected.
4. Specific chromatograms when internal standards are used.



standards were purposefully selected for not being pr  
properties with both low boiler and high boiler target an

n impart significant impurities into the measurement

ng performance are not possible with mandated int  
old enough and heavies may not be released when  
with available internal standards.

phic and spectrometric conditions with analytes ca  
rd coelutes with an analyte, especially at high analy

present in real samples, but they  
analytes.

nt, especially at low level

ternal standards. Early eluters can  
the trap is not hot enough. Nei-

n cause distortion of peak shapes  
yte concentrations.

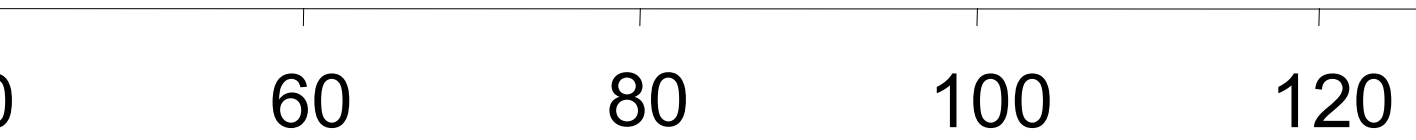
-100

0

20

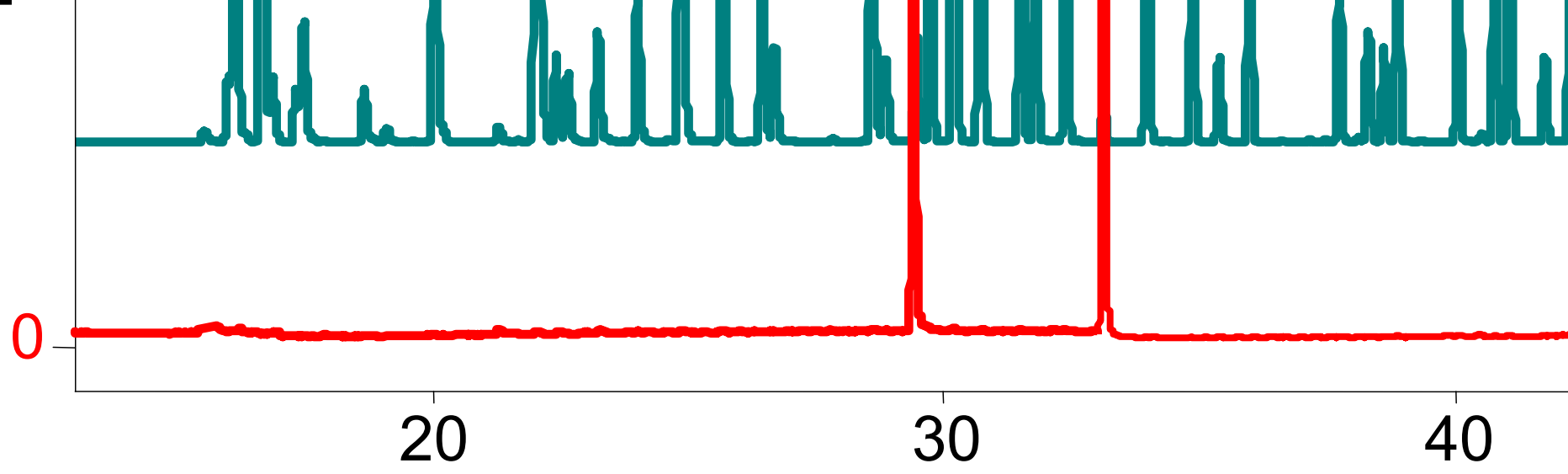
40

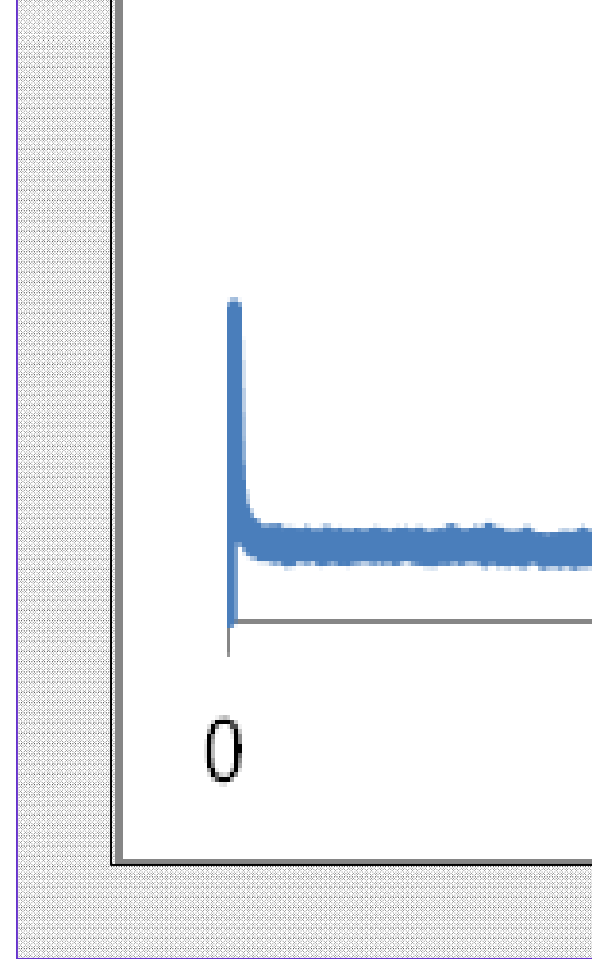
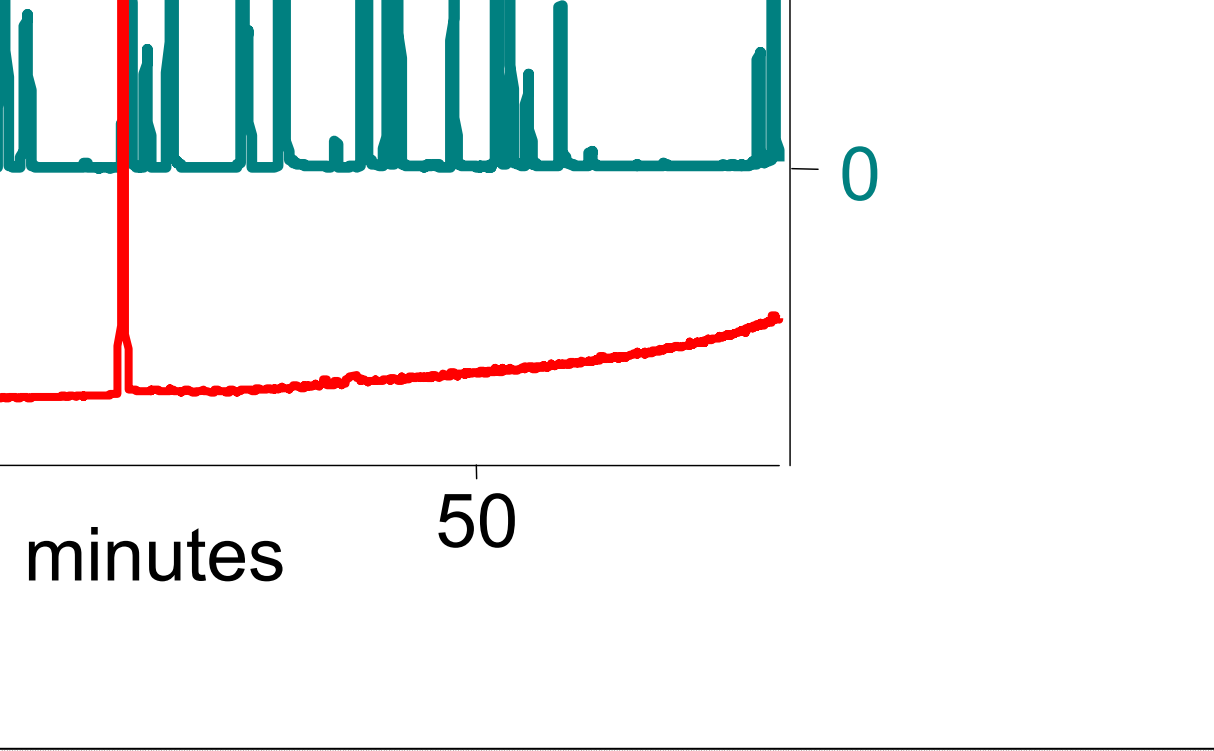
**TO-15 Comp**

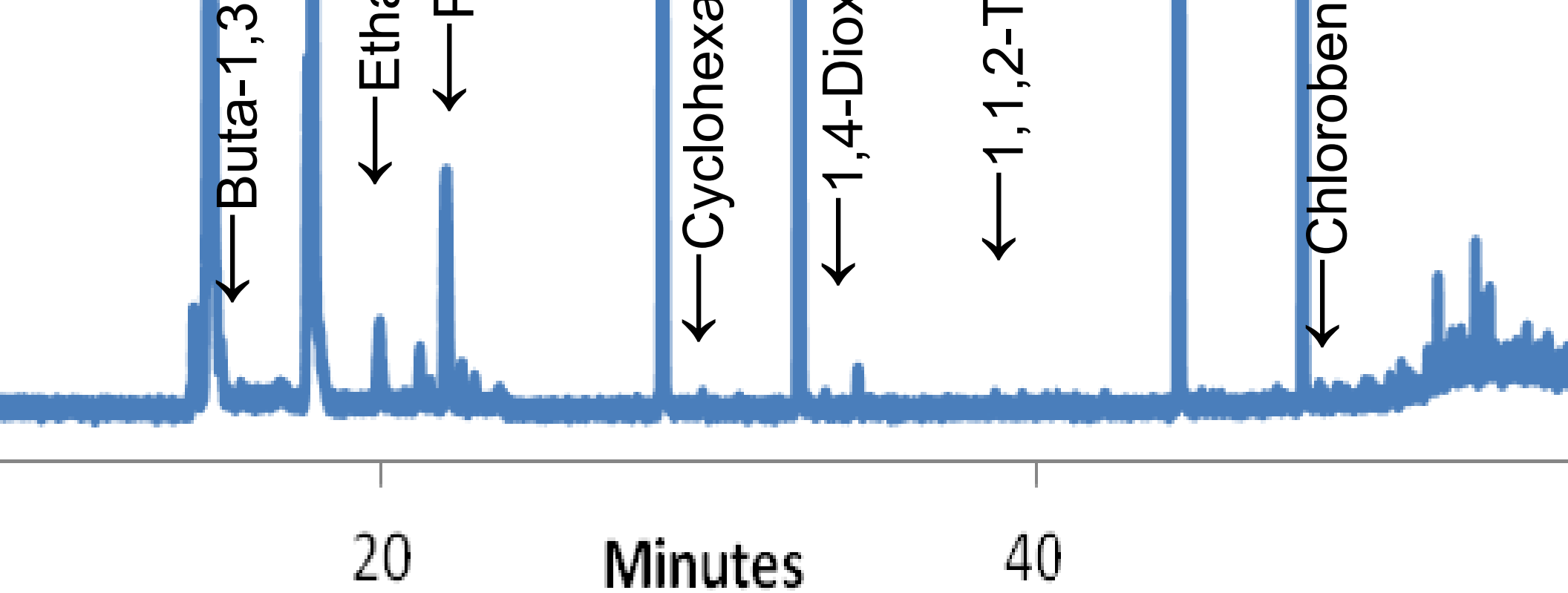


# Compound Number by Boiling Point











1,4-Dioxane

1,1,2-Trichloroethane

Chlorobenzene

60

66
17
18

5. Added random errors and impacts the measurement.
6. High performance manufacturing internal standards.

of internal standard peak areas compounds the un-  
measured detection limits.

mass spectrometers are stable for extends periods an

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uncertainty of the measurement,

and do not need corrections from