THE GOLD STANDARD FOR FORENSIC ANALYSIS

^{13}C

^{13}C Labelled internal standards(IS) are superior to DEUTERATED standards in UPLC-MS/MS analysis of drugs

The same RETENTION TIMES and RESPONSE FACTORS as the natives lead to
NO BIAS WITH ION SUPPRESSION

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Why use an internal standard (IS):

The IS is added prior to any manipulations in the analysis procedure, to adjust for loss and fractionation, and thereby to improve qualitative and quantitative detection.

Important properties for a perfect IS:
-Similar chemical and physical properties as the analyte
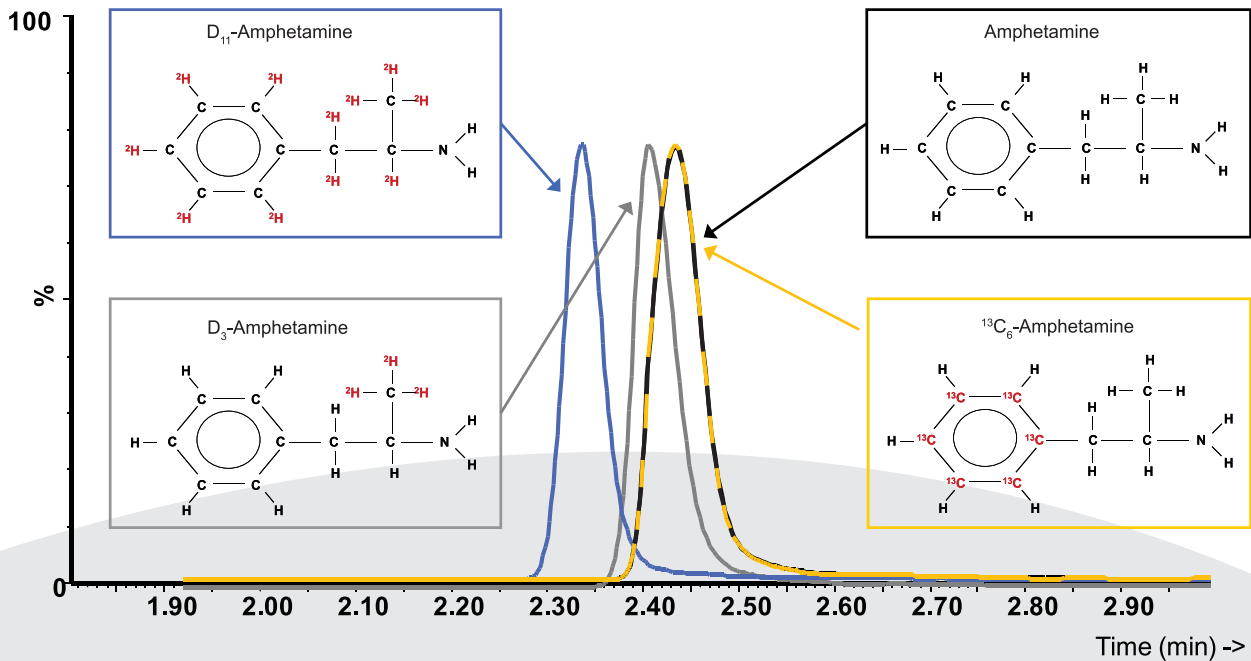-Absent in the sample
-Detected separately from the analyte

Where can loss and fractionation occur:
Loss and fractionation can occur during sample preparation and instrumental analysis. In LC-(ESI)MS/MS analysis of drugs, ion suppression in the MS-source is a major concern and may lead to loss of analyte and/or internal standard.
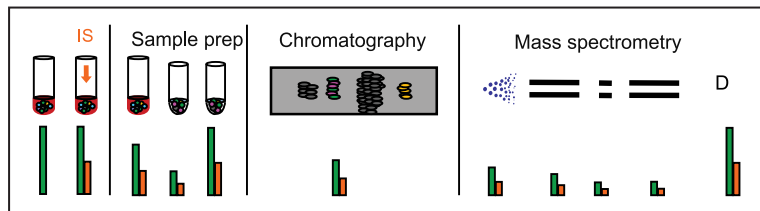
Results:

Chromatography

$^{13}C_6$-Amphetamine and native amphetamine CO-ELUTE perfectly on UPLC-(ESI+) MS/MS

D_3 -Amphetamine overlap but do NOT CO-ELUTE with the native on UPLC-(ESI+) MS/MS

D_{11}-Amphetamine and amphetamine are almost BASELINE SEPARATED on UPLC-(ESI+) MS/MS

Ion suppression

There is a competition during electron spray ionization between the compound of interest and co-eluting compounds to get ionized. A compound co-eluting with an analyte may reduce the amount of analyte molecules that are ionized (ion suppression). The degree of ion suppression depends on different factors like concentration of co-eluting compound(s), concentration of analyte, flow rate, injection volume, etc.
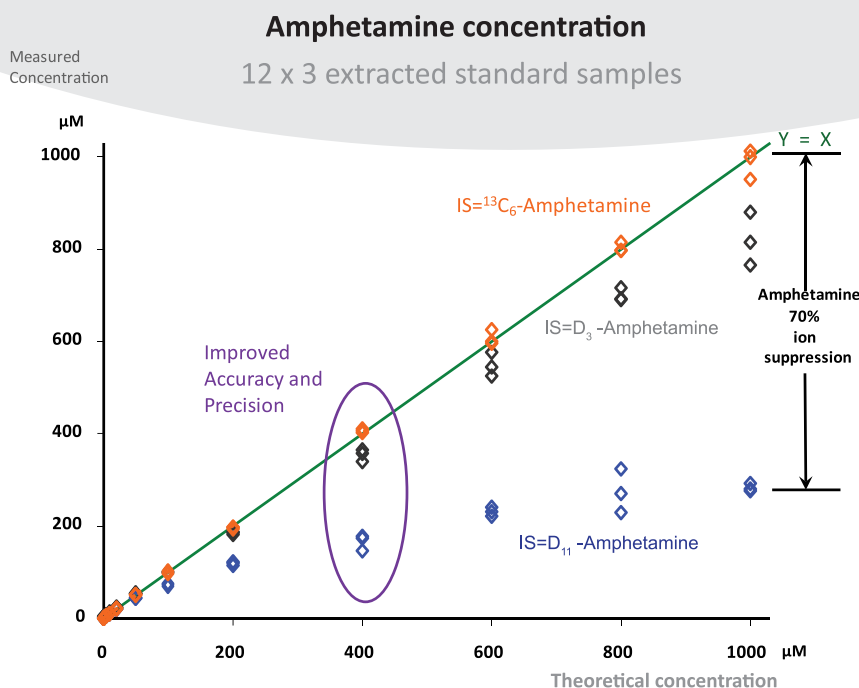
Amphetamine concentrations in 12 validation samples, each added three ISs.
Linear calibration curves were used for calculations. Three injections of each
validation sample was performed on the UPLC-MS/MS. Amphetamine concentra-
tions in the 12 samples were from 0.1μM up to 1000μM.

The figure shows:

Linear calibration curve from 0.1 μM to 1000 μM when $^{13}C_6$-amphetamine was used as IS.

Increased linear range of calibration curve was observed when D_3-amphetamine was used, compared to using D_{11}-amphetamine as IS.

Explanation/Additional information:

Amphetamine peak responses were flattening with increasing amphetamine concentrations because of ion suppression (data not shown).

Both $^{13}C_6$-amphetamine and D_3-amphetamine peak responses were reduced with increasing amphetamine concentrations because of ion suppression caused by amphetamine (data not shown). However, since $^{13}C_6$-amphetamine was the only IS perfectly co-eluting with amphetamine, it was the only IS able to correct for all the ion suppression of amphetamine and therefore the only IS that gave a linear calibration curve from 0.1 to 1000μM.

D_{11}-Amphetamine and amphetamine were almost baseline separated. D_{11}-Amphetamine was not suppressed by amphetamine in validation samples with high amphetamine concentrations and therefore not able to correct for the ion suppression of the ampheta- mine standard.
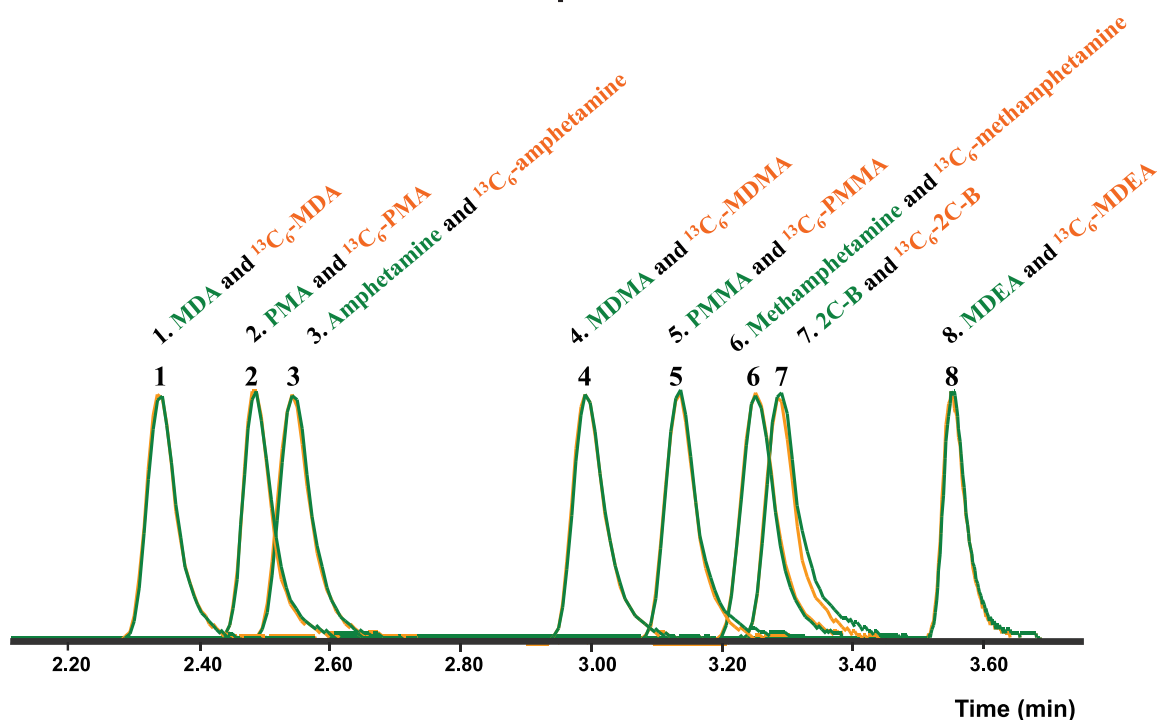
Conclusions:

$^{13}C_6$-Amphetamines are superior to deuterium labelled amphetamines as internal standards

$^{13}C_6$-Amphetamines give better:

 -Ion suppression correction

 -Accuracy

 -Precision

Available ¹³C labelled amphetamine standards:

Chromatogram peaks labelled:
1. MDA and ¹³C₆-MDA
2. PMA and ¹³C₆-PMA
3. Amphetamine and ¹³C₆-amphetamine
4. MDMA and ¹³C₆-MDMA
5. PMMA and ¹³C₆-PMMA
6. Methamphetamine and ¹³C₆-methamphetamine
7. 2C-B and ¹³C₆-2C-B
8. MDEA and ¹³C₆-MDEA

Time (min) axis: 2.20, 2.40, 2.60, 2.80, 3.00, 3.20, 3.40, 3.60

Amphetamine	
9676.9-50-ME	DL-{$^{13}C_6$}-Amphetamine sulphate
9157.9-100-ME	DL-Amphetamine sulphate
Methamphetamine	
9677.10-50-ME	DL-{$^{13}C_6$}-Methamphetamine hydrochloride
9160.10-100-ME	DL-Methamphetamine hydrochloride
MDA	
10022.10-50-ME	DL-{$^{13}C_6$}-MDA hydrochloride
9162.10-100-ME	DL-MDA hydrochloride
MDEA	
10024.12-50-ME	DL-{$^{13}C_6$}-MDEA hydrochloride
9166.12-100-ME	DL-MDEA hydrochloride
MDMA	
10023.11-50-ME	DL-{$^{13}C_6$}-MDMA hydrochloride
9164.11-100-ME	DL-MDMA hydrochloride
2C Phenetylamines	
10098.10-50-ME	{$^{13}C_6$}-4-Bromo-2,5-dimethoxyphenethylamine hydrochloride ({$^{13}C_6$}-2C-B)
10097.10-100-ME	4-Bromo-2,5-dimethoxyphenethylamine hydrochloride (2C-B)
10100.10-50-ME	{$^{13}C_6$}-2,5-Dimethoxyphenethylamine hydrochloride ({$^{13}C_6$}-2C-H)
10099.10-100-ME	2,5-Dimethoxyphenethylamine hydrochloride (2C-H)
10102.10-50-ME	{$^{13}C_6$}-2,5-Dimethoxy-4-iodophenethylamine hydrochloride ({$^{13}C_6$}-2C-I)
10101.10-100-ME	2,5-Dimethoxy-4-iodophenethylamine hydrochloride (2C-I)
PMA	
10025.10-50-ME	DL-{$^{13}C_6$}-PMA hydrochloride
9693.10-100-ME	DL-PMA hydrochloride
PMMA	
10026.11-50-ME	DL-{$^{13}C_6$}-PMMA hydrochloride
9691.11-100-ME	DL-PMMA hydrochloride

Delivered as solutions in Methanol, 50 or 100µg/mL.

The results are presented by the courtecy of T. Berg and D.H. Strand, The Norwegian Institute of Public Health. Ref: Berg & Strand, Journal of Chromatogr. A, 1218 (2011), 9366