Toward Newborn Screening for Cerebrotendinous Xanthomatosis (CTX); Identifying Informative Markers for CTX in Newborns

Andrea DeBarber, PhD
Research Assistant Professor
Physiology & Pharmacology Department
Associate Director, Bioanalytical Shared Resource Facility
Oregon Health & Science University

debarber@ohsu.edu

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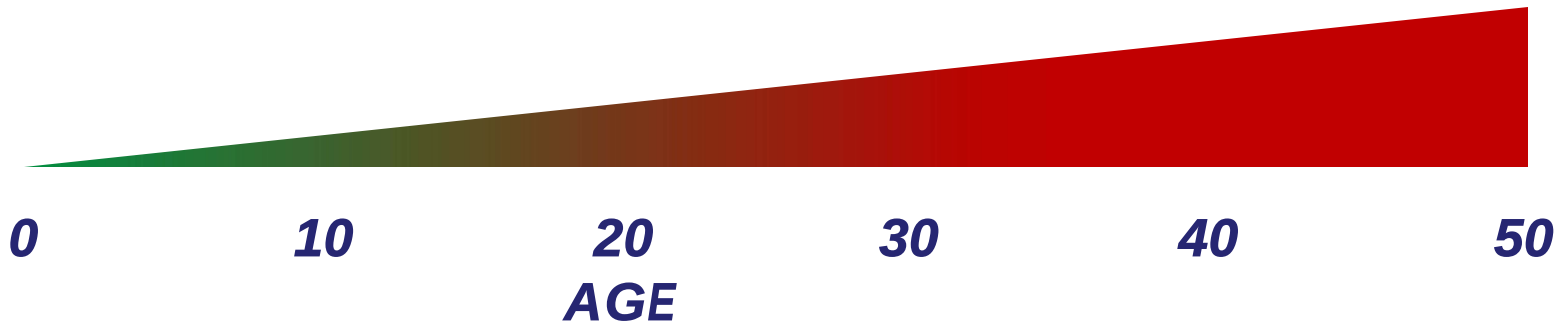
- **AB SCIEX - Michal Star-Weinstock, Babu Purkayastha**
  (patent application filed using QAO methodology to test for CTX)

Clinical Phenotype of CTX:



***J Inherit Metab Dis* 25 (2002) 501-513** PT Clayton *et al*
***Arch Neurol* 57 (2000) 520-524** A Verrips *et al*

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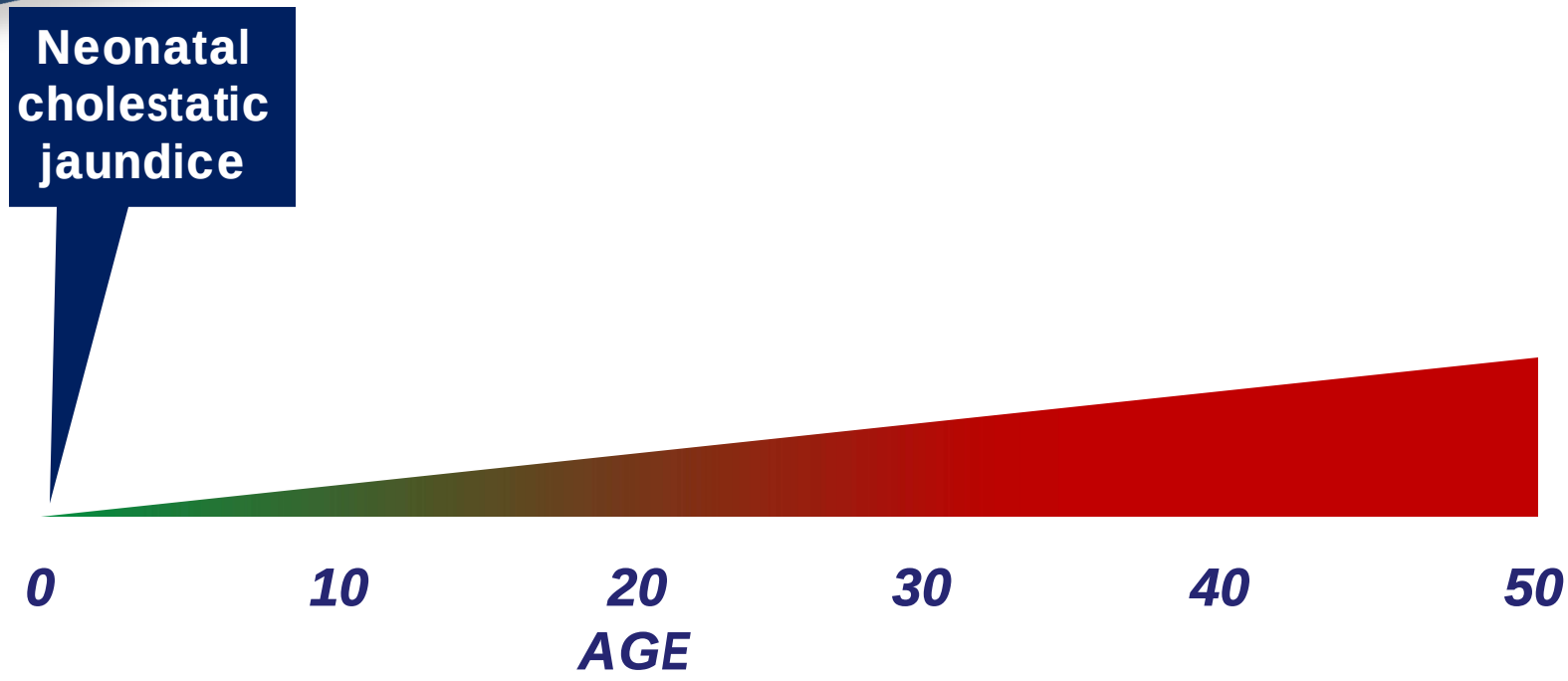
Clinical Phenotype of CTX:



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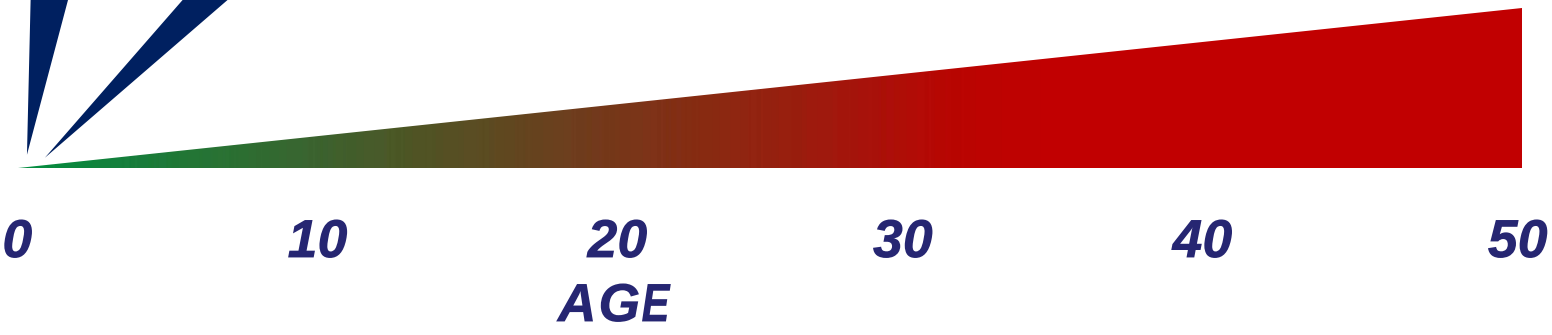
Clinical Phenotype of CTX:

Neonatal cholestatic jaundice



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Clinical Phenotype of CTX:

Neonatal cholestatic jaundice

0 10 20 30 40 50

AGE

Mean age symptom onset 14-19 years

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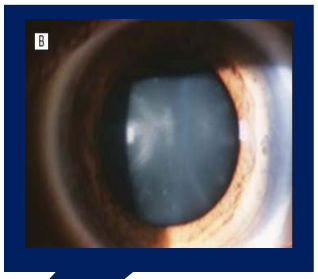

0 ***10*** ***20*** ***30*** ***40*** ***50***

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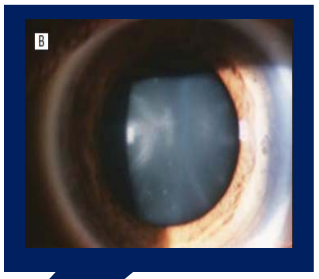
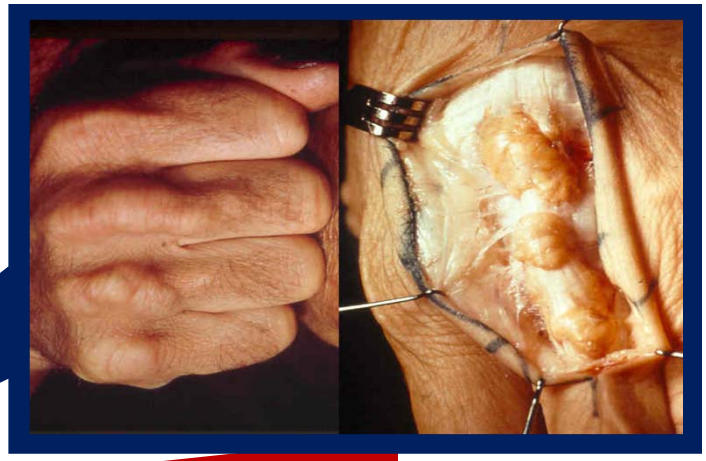
Clinical Phenotype of CTX:

Neonatal cholestatic jaundice





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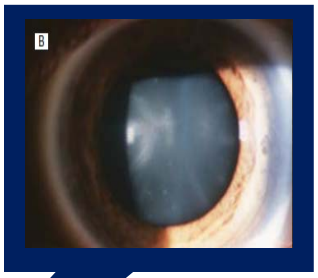

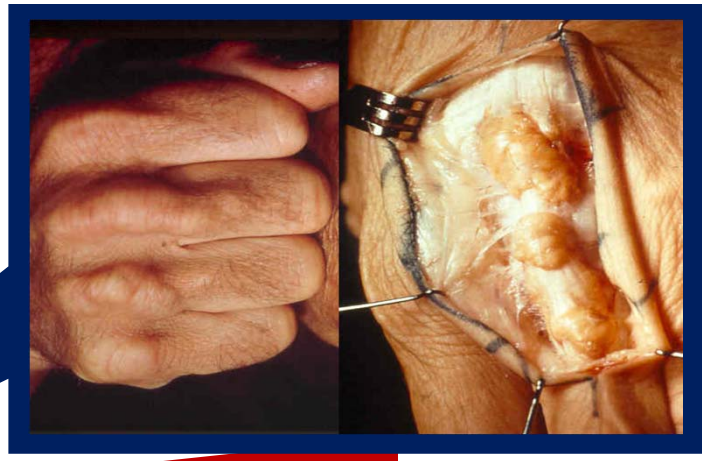

0 ***10*** ***20*** ***30*** ***40*** ***50***

AGE

Mean age symptom onset 14-19 years

Mean age diagnosis 35-37 years

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Clinical Phenotype of CTX:

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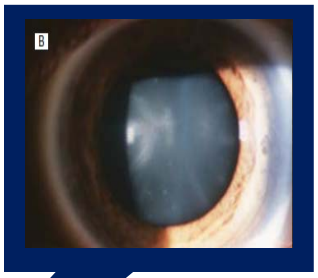

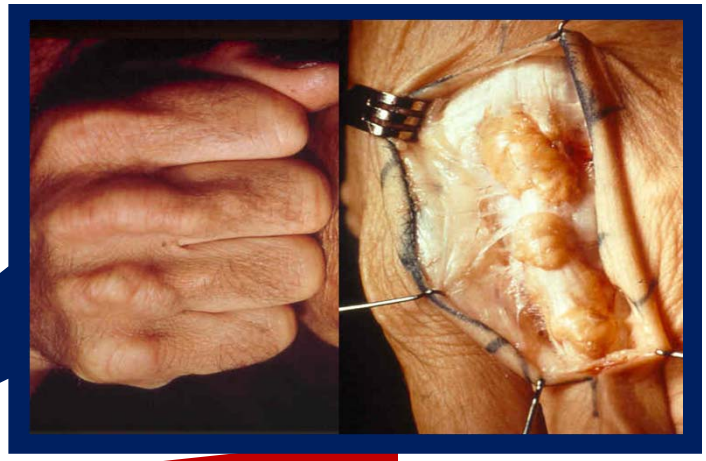

neurological symptoms (95-97%): **cognitive impairment, cerebellar signs (ataxia) & pyramidal signs (spasticity)**

0 10 20 30 40

AGE

Mean age symptom onset 14-19 years

Mean age diagnosis 35-37 years

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Effective Oral Treatment for CTX:

- **Supplementation deficient bile acids[1,2]**
- **Treatment pre-symptomatic children prevents disease complications[3,4]**
- **Unfortunately treatment after many years of disease progression does not reverse neurological deterioration[2]**

1. N Engl J Med (1984) 311: 1649-1652 VM Berginer, *et al*
2. Neurol Sci (2001) 190: 29-33 M Mondelli, *et al*
***3. J Inherit Metab Dis* (2008) Suppl:S 241-5** G Pierre, *et al*
4. Pediatrics (2009) 123: 143-147 VM Berginer *et al*

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Is CTX a Good Candidate Disorder to Screen for in Newborns?

Nomination requirements:

- ***Clinical characteristics***
- ***High-throughput lab test***

Optimally dried bloodspot (DBS)
ESI-MS/MS biochemical test

http://www.hrsa.gov/advisorycommittees/mchbadvisory/heritabledisorders/nominatecondition/index.html

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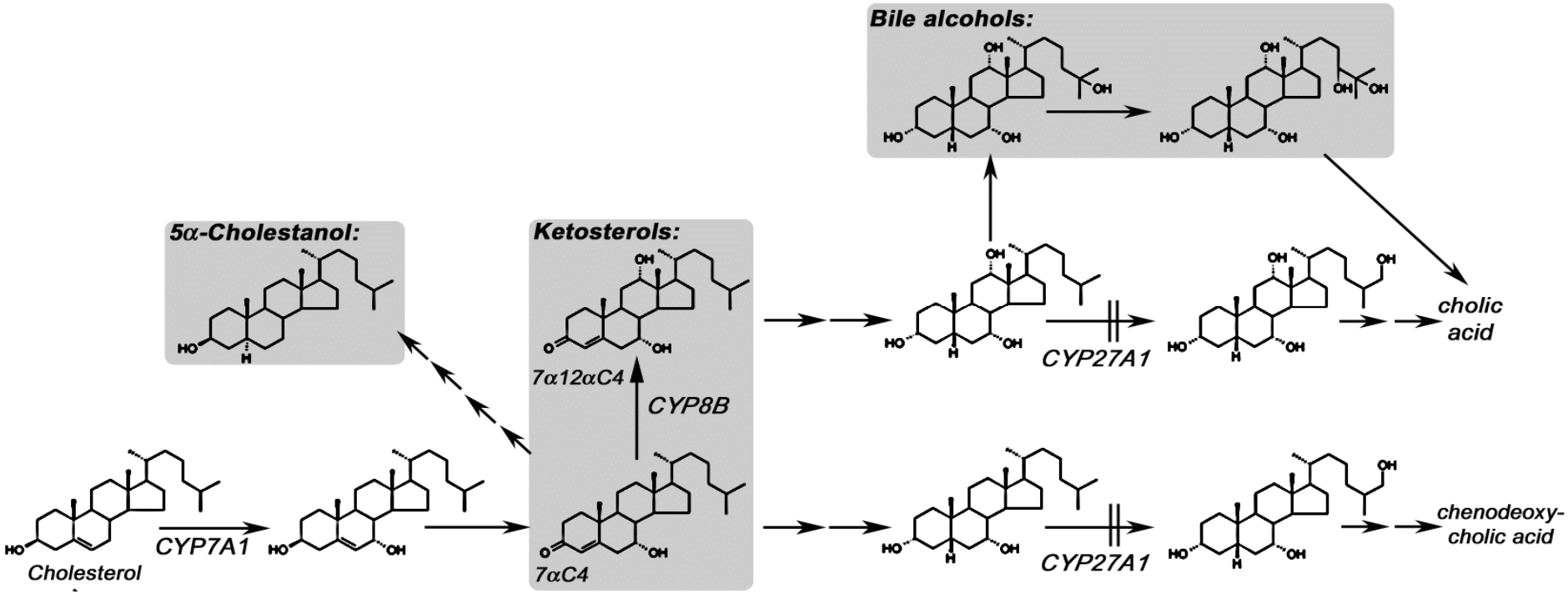
Biochemical Phenotype of CTX:



***J Lipid Res* 22 (1981) 191-200** I Bjorkhem, *et al*
***J Lipid Res* 42 (2001) 291-300** A Honda, *et al*

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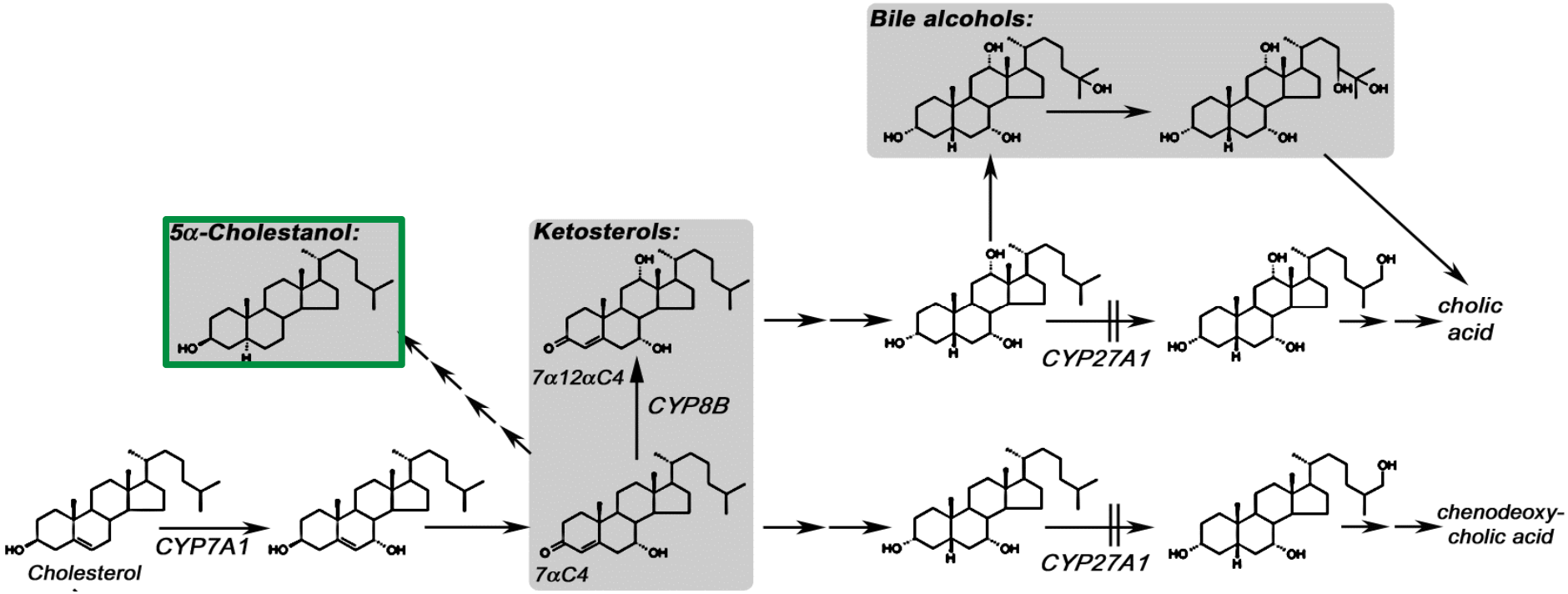
Biochemical Phenotype of CTX:



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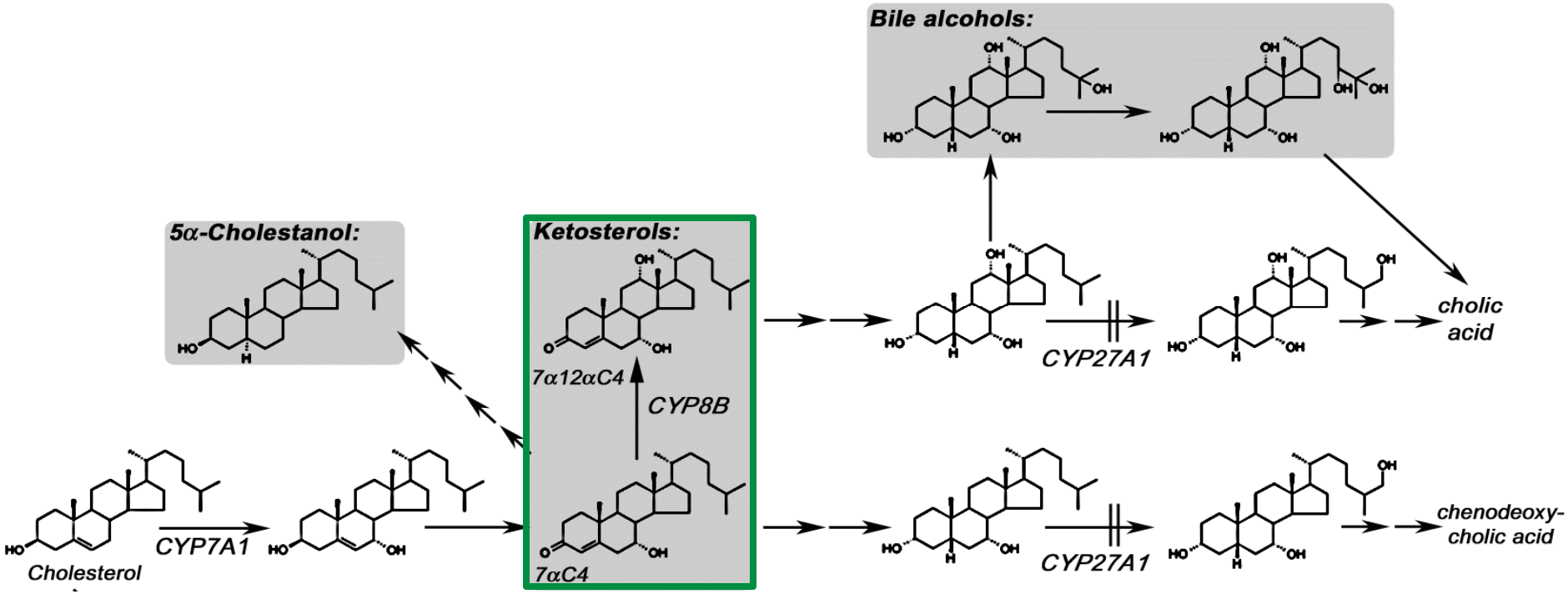
Biochemical Phenotype of CTX:



***J Lipid Res* 22 (1981) 191-200** I Bjorkhem, *et al*
***J Lipid Res* 42 (2001) 291-300** A Honda, *et al*

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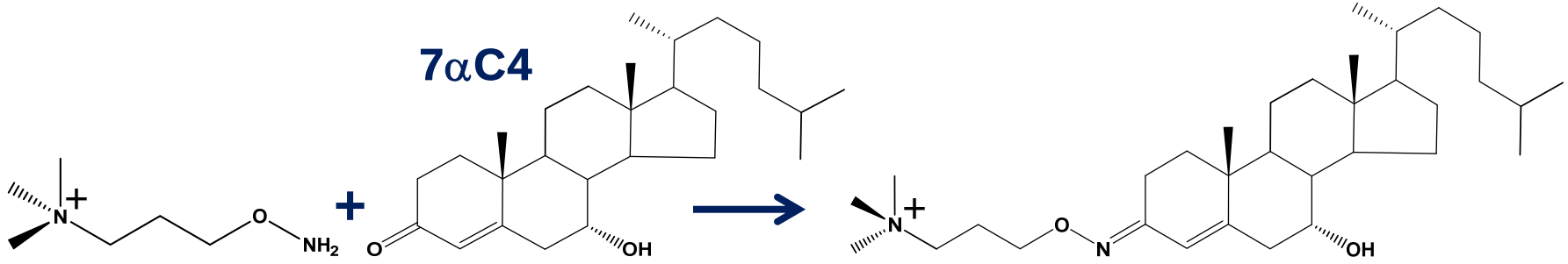
Derivatization to Characterize Diagnostic Utility of Ketosterols:

- **Derivatization with quaternary amonoxy (QAO) *O*-(3-trimethylammonium-propyl) hydroxylamine reagent**
- **Improve LLOQ & synthesize IS**



***Analytical Chemistry* 84 (2012) 9310-9317** M Star-Weinstock, *et al*

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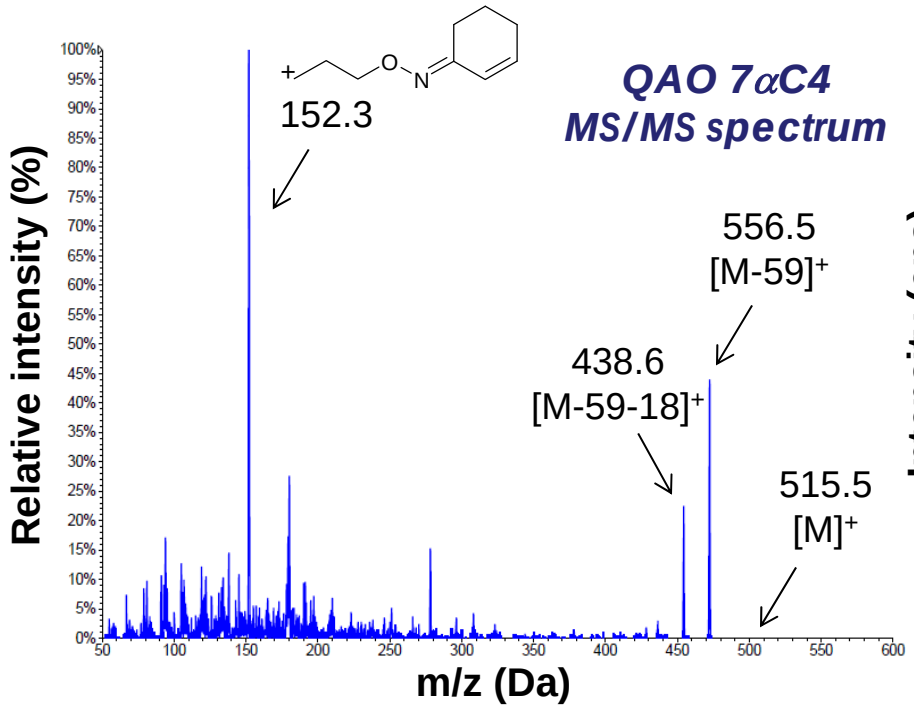
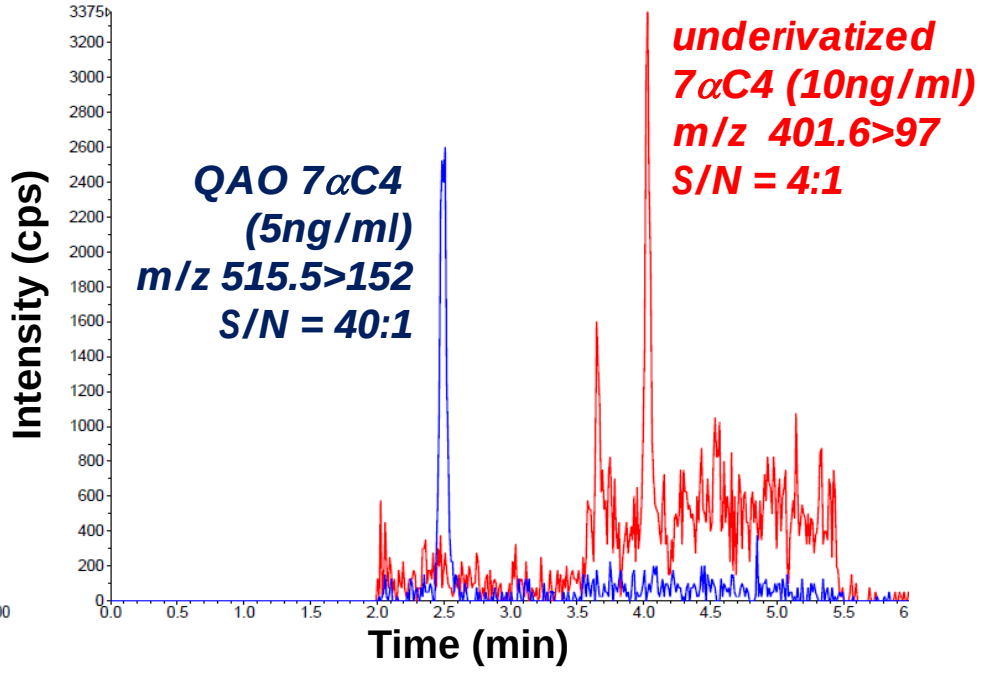
QAO Derivatization Signal Enhancement:





***Analytical Chemistry* 84 (2012) 9310-9317** M Star-Weinstock, *et al*

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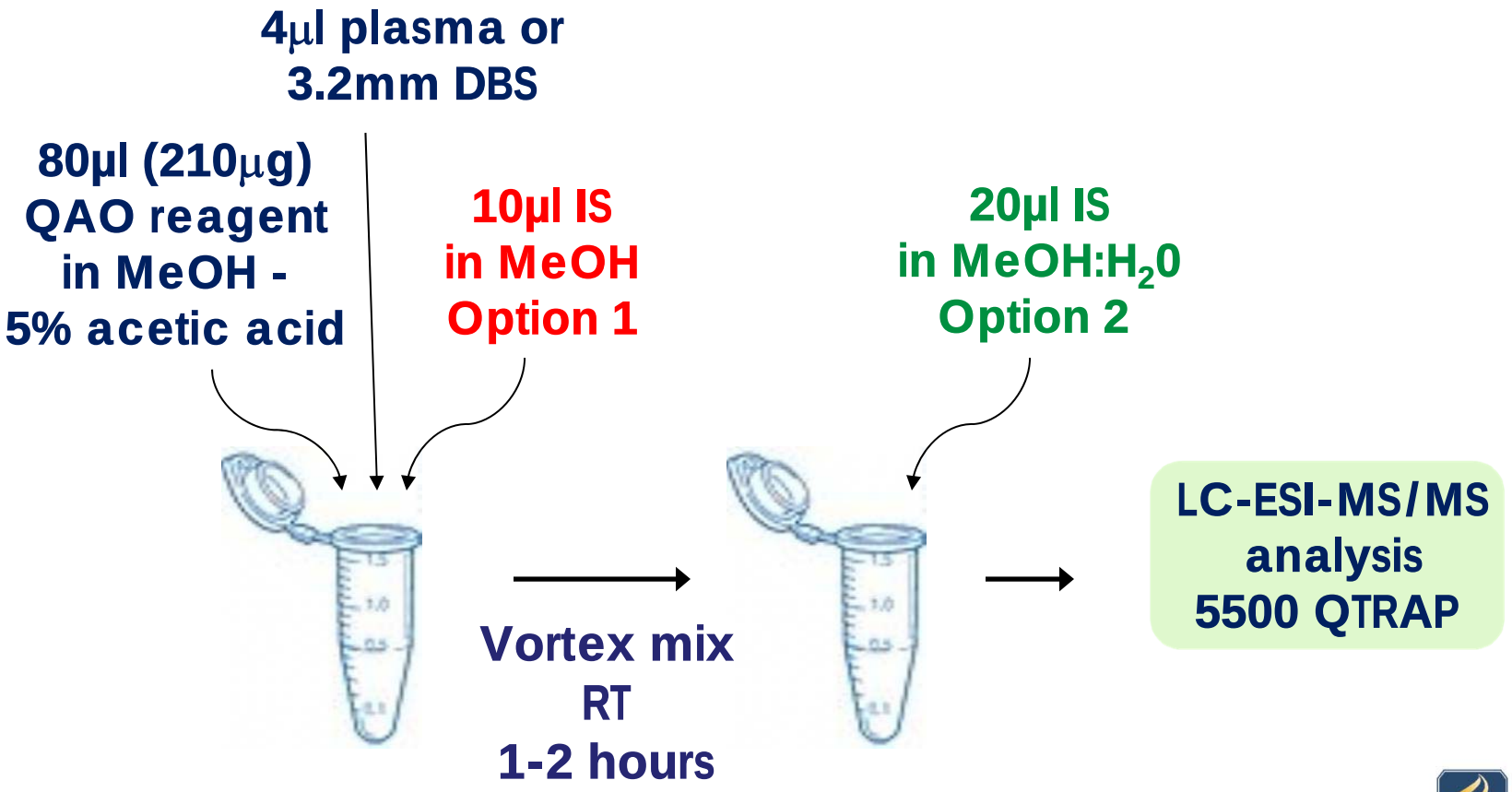
QAO Derivatization Workflow:



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Quantification 7α C4 in Plasma:

- **QAO Derivatization 7αC4 in 4μl plasma**
- **LC-ESI-MS/MS analysis 4-6 min**
- **LOD 7αC4 spiked DCS plasma 5ng/ml**
- **Satisfactory linearity, precision & accuracy across range 20-250ng/ml**
- **QAO 7αC4-d_7 IS (Option 1)**

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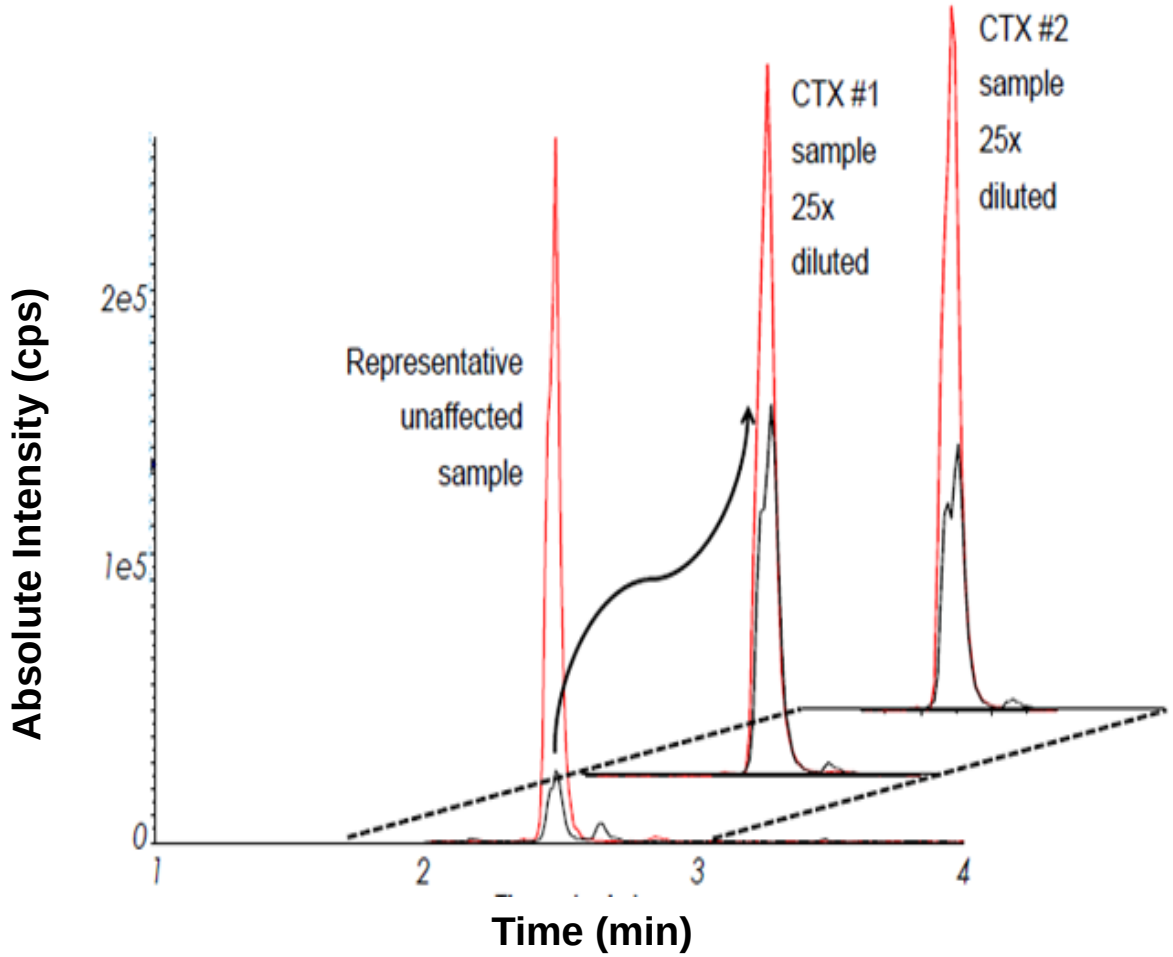
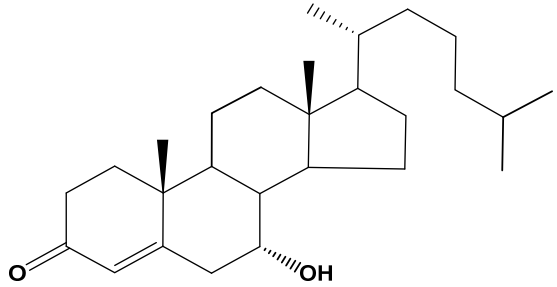
QAO Derivatized 7α C4 in Plasma:





QAO 7α C4
m/z 515.5 > 152.3
QAO 7α C4-d_7
m/z 522.5 > 152.3

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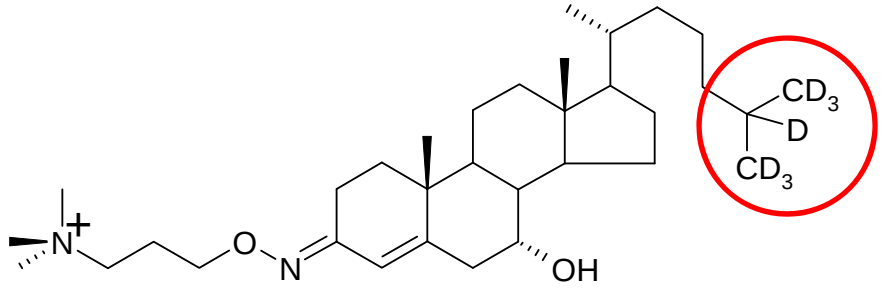
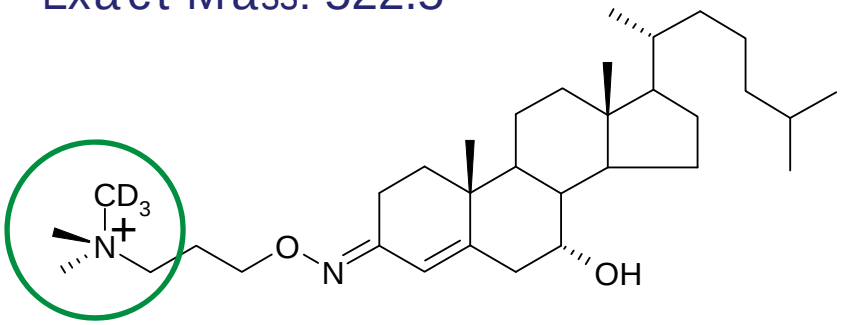
Synthesis IS using stable-isotope enriched QAO reagent:

Internal Standard (IS) options:



Exact Mass: 522.5

IS *Option 1*:
Analyte-d_7 +
QAO-d_0 reagent



Exact Mass: 518.5

IS *Option 2*:
Analyte-d_0 +
QAO-d_3 reagent

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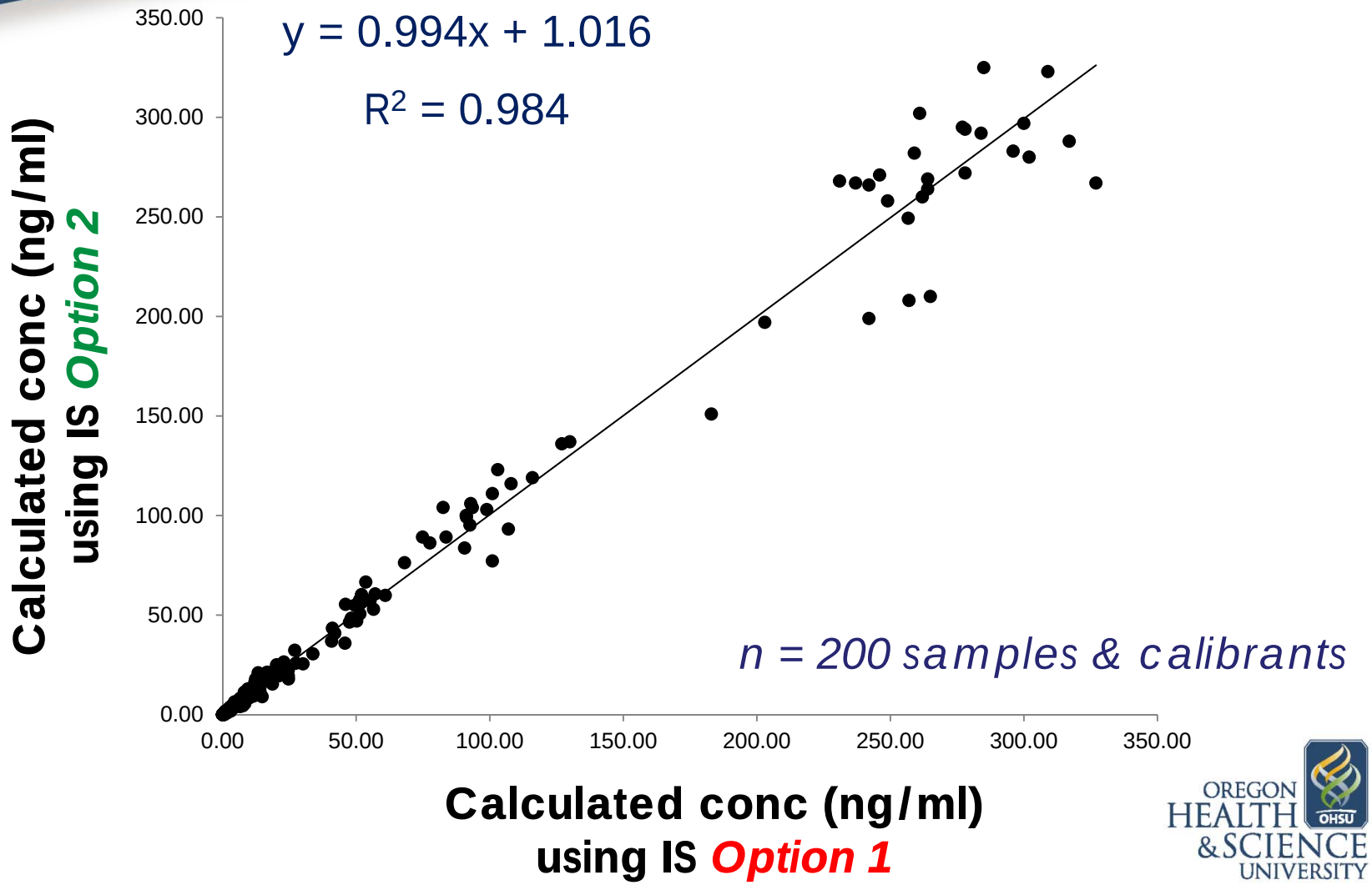
Synthesis IS using stable-isotope enriched QAO reagent:



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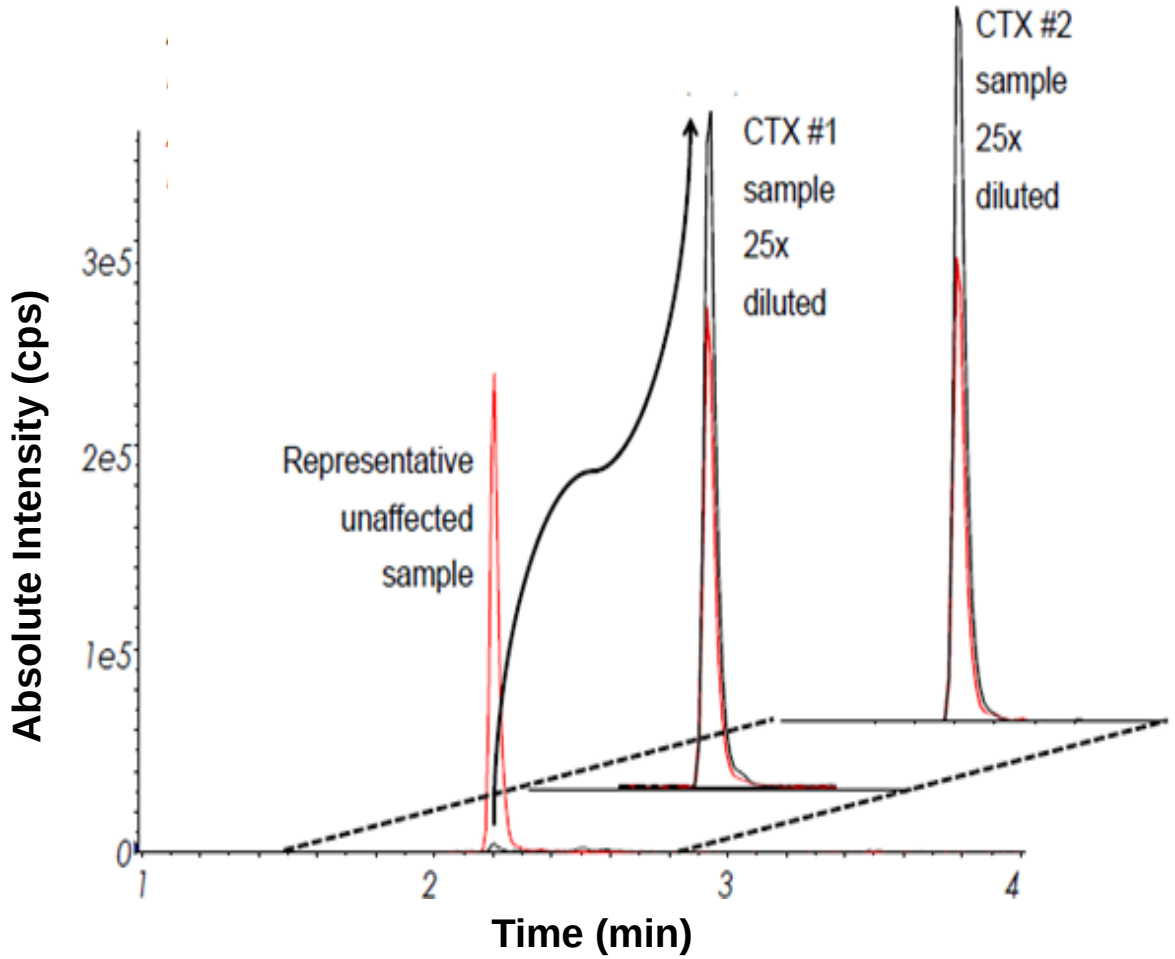
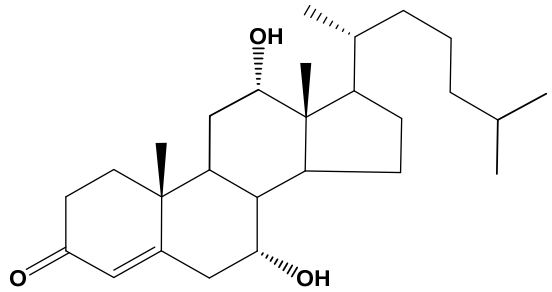
QAO Derivatized 7α 12α C4 in Plasma:





QAO 7α12αC4
m/z 531.5 > 152.3
QAO-d_3 7α12αC4
m/z 534.5 > 152.3

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7α C4 & 7α 12α C4 as Improved Markers for CTX:

Plasma concentrations:

	7α C4 ng/ml	7α12α C4 ng/ml	5α-cholestanol µg/ml
CTX patients (n=10)	1174 ± 711 [204-1828]	1545 ± 1141 [57-2420]	31 ± 19 [8.4-66]
Unaffected individuals (n=20)	6.4 ± 5.3 [1.6-22]	0.4 ± 0.27 [0.1-0.8]	1.3 [0.8-1.8]

Mean ± SD and [range of results] given

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7α C4 & 7α 12α C4 as Improved Markers for CTX:

>183-fold

Plasma concentrations:

	7α C4 ng/ml	7α12α C4 ng/ml	5α-cholestanol µg/ml
CTX patients (n=10)	1174 ± 711 [204-1828]	1545 ± 1141 [57-2420]	31 ± 19 [8.4-66]
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Mean ± SD and [range of results] given

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7α C4 & 7α 12α C4 as Improved Markers for CTX:

>3862-fold

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Mean ± SD and [range of results] given

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7α C4 & 7α 12α C4 as Improved Markers for CTX:

>24-fold

Plasma concentrations:

	7α C4 ng/ml	7α12α C4 ng/ml	5α-cholestanol µg/ml
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Mean ± SD and [range of results] given

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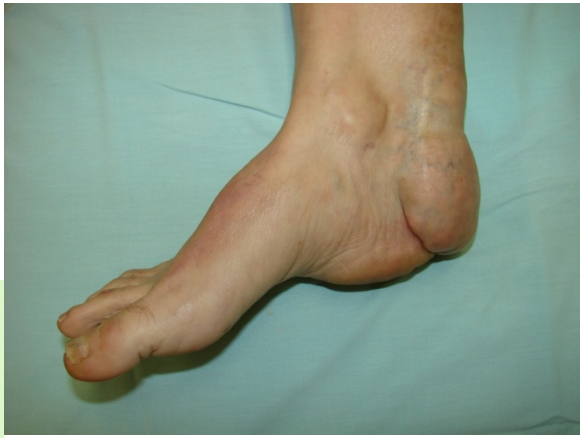
Case Study; A Better Blood Test for CTX?



- ***Clinical phenotype:***

  Older female
  Cataracts/xanthoma

- ***Biochemical phenotype:***

  Cholestanol 8.4 mg/ml [unaffected <6-8 mg/ml][1,2]
  7αC4 1548 ng/ml [unaffected <22 ng/ml]
  7α12αC4 795 ng/ml [unaffected <0.8 ng/ml]

- ***Genotype:***

  Homozygous c.1421G>A mutation *CYP27A1*

***1. Ann Intern Med* 75 (1971) 843-851** G Salen, *et al*
***2. J Biochem* 80 (1976) 223-228** Y Seyama, *et al*

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Quantification 7α 12α C4 in DBS:

- **QAO Derivatization 7α12αC4 in 3.2 mm DBS punches**
- **LC-ESI-MS/MS analysis 4-6 min**
- **LOD 7α12αC4 spiked blood 10ng/ml**
- **Satisfactory linearity, precision & accuracy across range 50-250ng/ml**
- **QAO-d_3 7α12αC4 IS**

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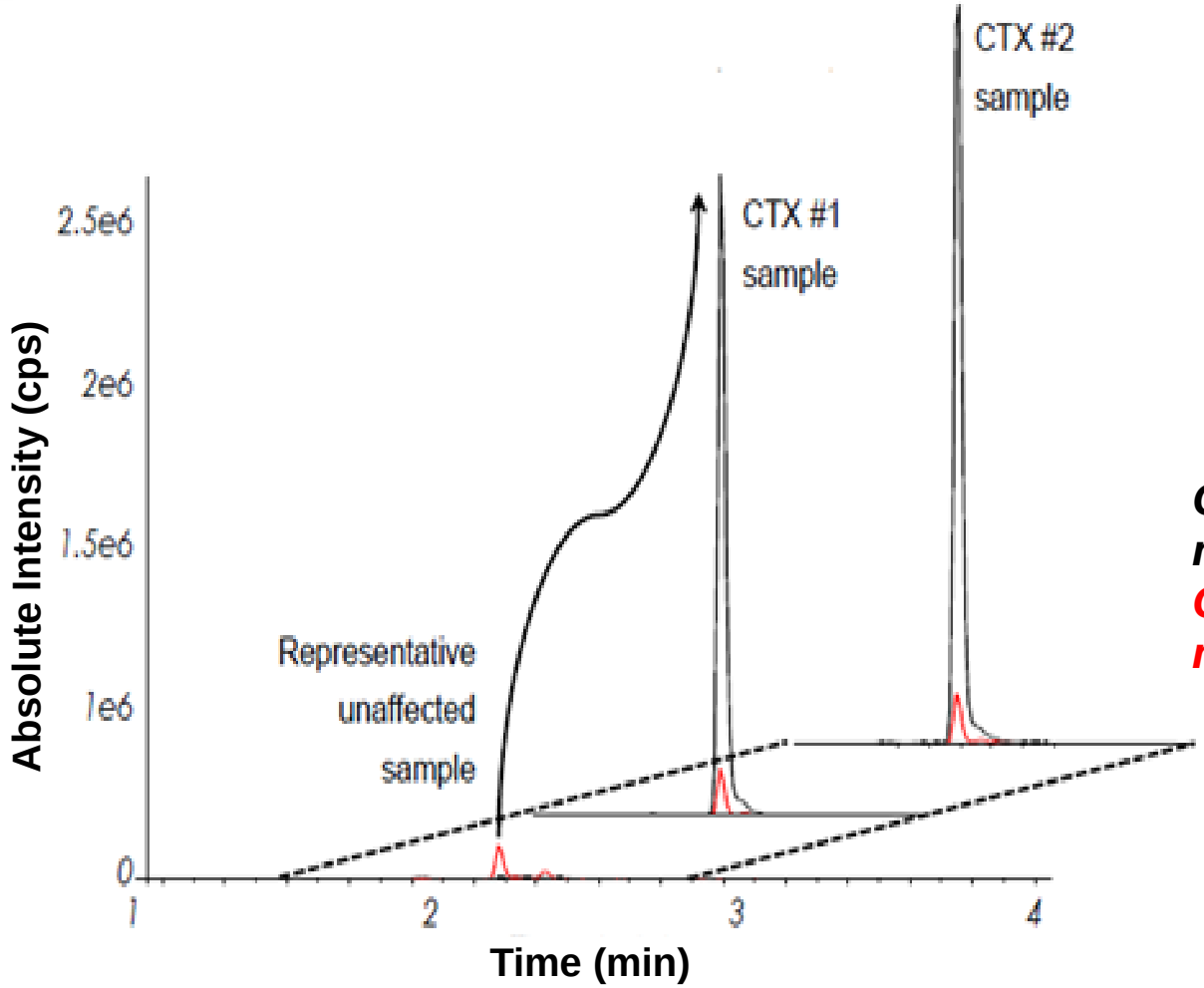
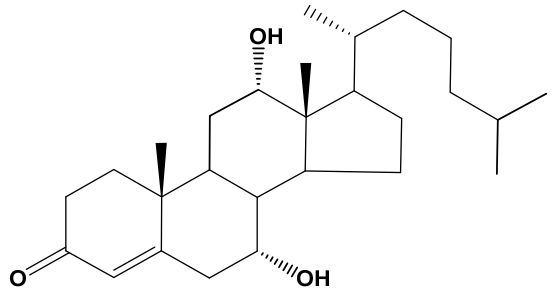
QAO Derivatized 7α12α C4 in Adult DBS:





QAO 7α12αC4
m/z 531.7 > 152.3
QAO-d_3 7α12αC4
m/z 534.7 > 152.3

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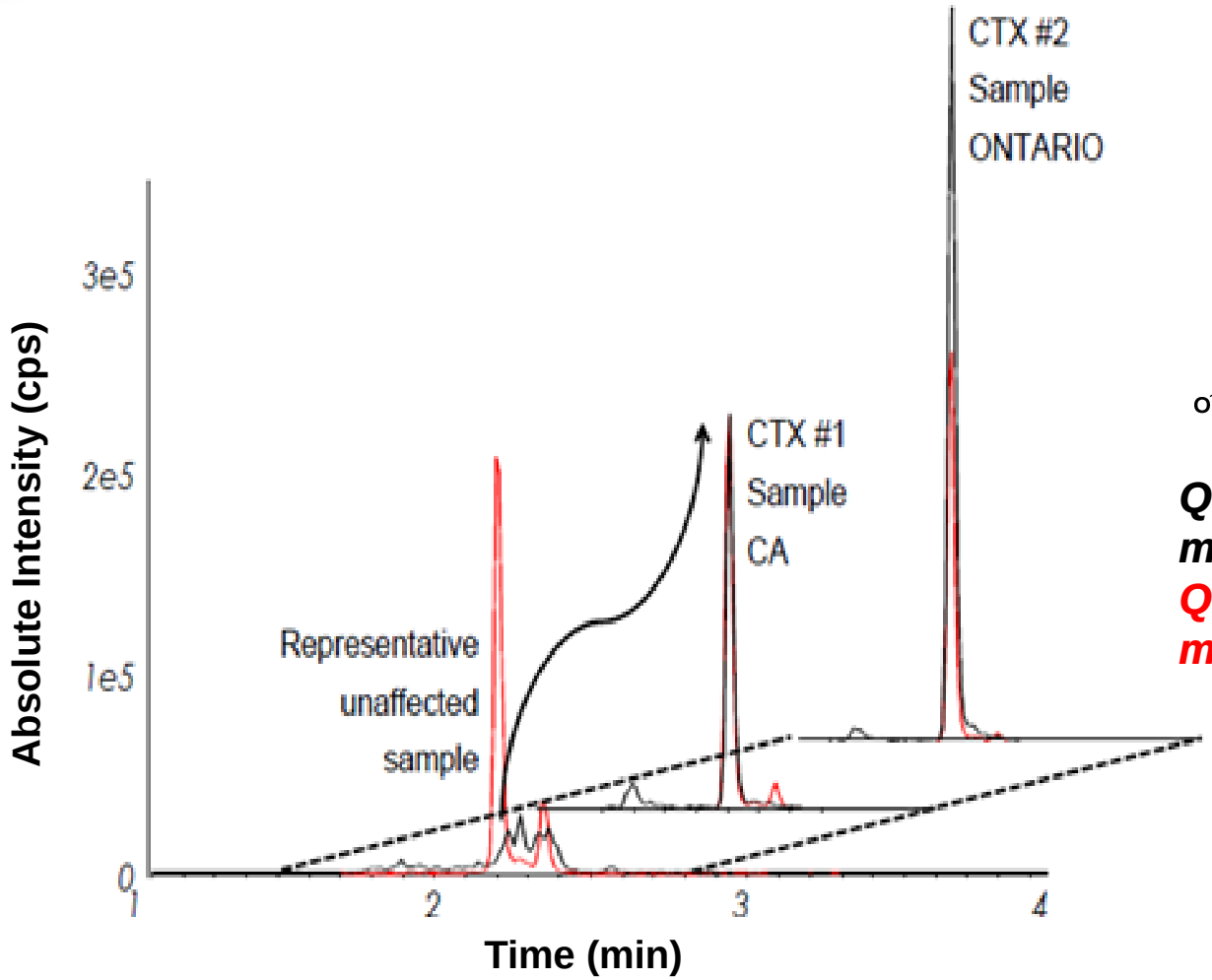
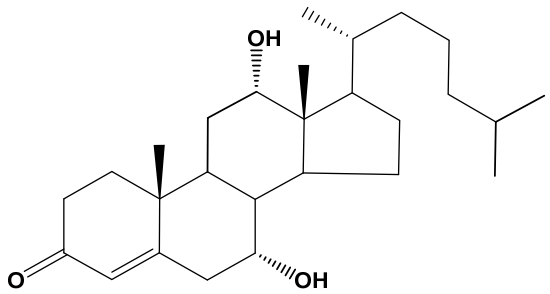
QAO Derivatized 7α12α C4 in Newborn DBS:





QAO 7α12αC4
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m/z 534.7 > 152.3

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7α 12α C4 as a DBS Marker for CTX:

DBS concentrations:

		7α12α C4 ng/ml		cholestanol µg/ml
CTX adults	***n=4***	1385 ± 467 [852-1990]	***n=3***	17.4 ± 6.5 [6.8-24.4]
Unaffected adults	***n=3***	1.0 ±1.3 [0-2.5]	***n=6***	2.3 ± 0.9 [1.4-3.8]
CTX newborns	***n=2***	120-214	***n=2***	8.4-8.7
Unaffected newborns	***n=6***	16.4 ± 6.0 [12.3-26.3]	***n=4***	4.7 ± 1.8 [2.5-7.0]

Mean concentration ±SD and [range of results] given

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7α 12α C4 as a DBS Marker for CTX:

10-fold

DBS concentrations:

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Mean concentration ±SD and [range of results] given

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7α 12α C4 as a DBS Marker for CTX:

2-fold

DBS concentrations:

		7α12α C4 ng/ml		cholestanol µg/ml
CTX adults	***n=4***	1385 ± 467 [852-1990]	***n=3***	17.4 ± 6.5 [6.8-24.4]
Unaffected adults	***n=3***	1.0 ±1.3 [0-2.5]	***n=6***	2.3 ± 0.9 [1.4-3.8]
CTX newborns	***n=2***	120-214	***n=2***	8.4-8.7
Unaffected newborns	***n=6***	16.4 ± 6.0 [12.3-26.3]	***n=4***	4.7 ± 1.8 [2.5-7.0]

Mean concentration ±SD and [range of results] given

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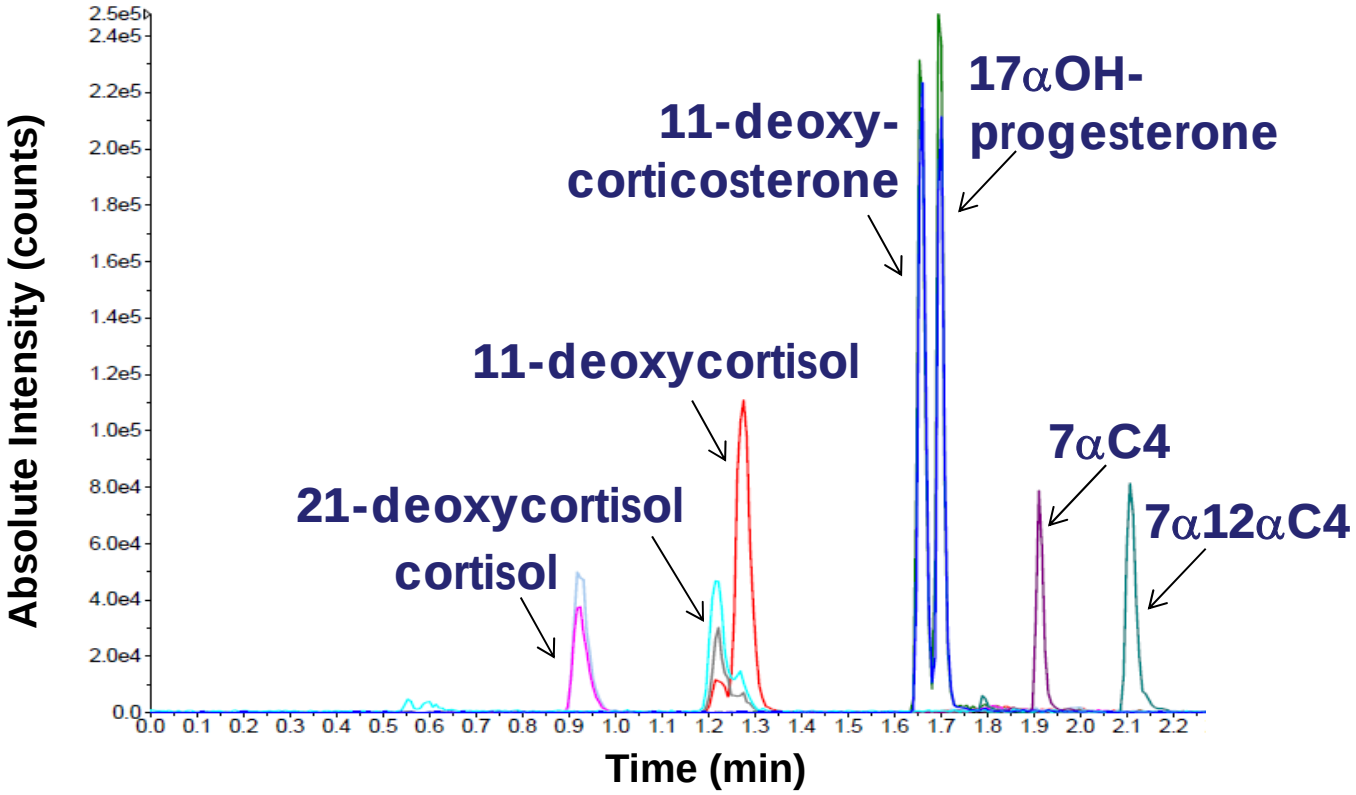
1st Step Toward ESI-MS/MS Based Newborn Screening for CTX:



cortisol
m/z 363>121

21-deoxycortisol
m/z 347>311

11-deoxycortisol
m/z 347>97

11-deoxycortico-sterone
m/z 331>97

17α OH-progesterone
m/z 331>97

7α C4
m/z 401.6>97

7α 12α C4
m/z 417.6>97

***Clin Chem* 50 (2004) 621-625** JM Lacey, *et al*
***Steroids* 76 (2011) 1437-1442** N Janzen, *et al*

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