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Experience from the ERNDIM Purine and Pyrimidine Scheme

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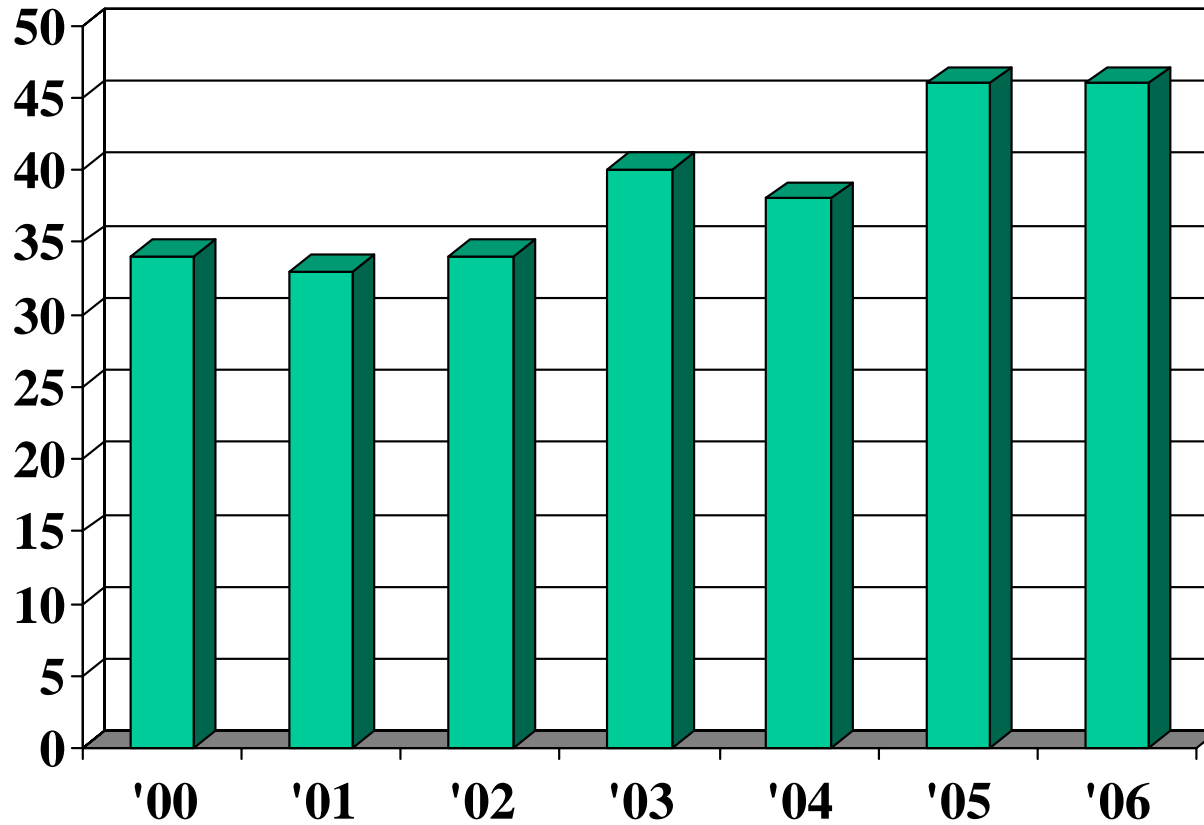
Erndim P&P Scheme

- Purpose:
- Monitoring of the analytical quality of the quantitative assay of purines and pyrimidines in urine in laboratories involved in the screening and diagnosis of patients with inherited metabolic disorders.

History

- 2000-2001: pilot schemes
- 2002 – onwards: established scheme
- Scheme originally designed, planned and co-ordinated by Albert van Gennip and Cas Weykamp

Participants



Experience from P&P Scheme

- Recovery

Year	2000	2001	2002	2003	2004
Recovery (%)					
Min	74	63	78	81	83
Max	153	124	113	134	127
Overall	107	100	97	98	92

Experience from P&P Scheme

- Precision

Year	2000	2001	2002	2003	2004
Intralab CV (%)					
Min	4.8	4.5	3.7	3.6	2.6
Max	54.8	28.6	31.7	27.8	20.5
Overall	16.0	11.9	11.9	8.9	9.0

Experience from P&P Scheme

- Linearity

Year R	2000	2001	2002	2003	2004
Min	0.8495	0.8560	0.9528	0.9793	0.9629
Max	0.9897	0.9988	0.9990	0.9991	0.9979
Mean	0.9524	0.9864	0.9874	0.9934	0.9917

Experience from P&P Scheme

- Interlab CV

Year	2000	2001	2002	2003	2004
Interlab CV (%)					
Min	13.1	8.2	7.1	5.3	5.2
Max	119.7	294.5	421.9	297.9	594.5
Mean	75.3	126.0	94.2	72.9	86.5

Experience from P&P Scheme

- Recovery: OK
- Intra lab CV: OK
- Linearity: OK
- Interlab CV: not OK
 - Enormous interlab variation

Experience from P&P Scheme

- Recovery: OK
- Intralab CV: OK
- Linearity: OK
- Interlab CV: not OK
 - Enormous inter lab variation
- Many different methods
- Standardisation useful
- Critical evaluation of methods used

Some examples

- Interlab CV (%) specific compounds

Year	2000	2001	2002	2003	2004
<u>Adenine</u>	<u>98.9</u>	<u>126.4</u>	<u>287.6</u>	<u>274.3</u>	<u>536.7</u>
<u>Thymine</u>	<u>92.9</u>	<u>104.1</u>	<u>421.9</u>	<u>297.9</u>	<u>594.5</u>
<i>Deoxy- Inosine</i>	83.0	131.5	26.2	18.5	12.1
<i>Thymidine</i>		118.6	17.3	14.1	14.3
Pseudo- uridine	17.1	15.1	13.9	13.6	10.7

Underlined: not OK; *italic*: improved; **bold**: OK

Methodology

In historical order:

- Thin layer chromatography
- High pressure liquid chromatography
- LC-tandem mass spectrometry (LC-MS/MS)

Methodology

In historical order:

- Thin layer chromatography
- *Not quantitative, informative, cumbersome*

- High pressure liquid chromatography
- *Quantitative, relatively fast*

- LC-tandem mass spectrometry (LC-MS/MS)
- *Quantitative, very fast, method of the future*

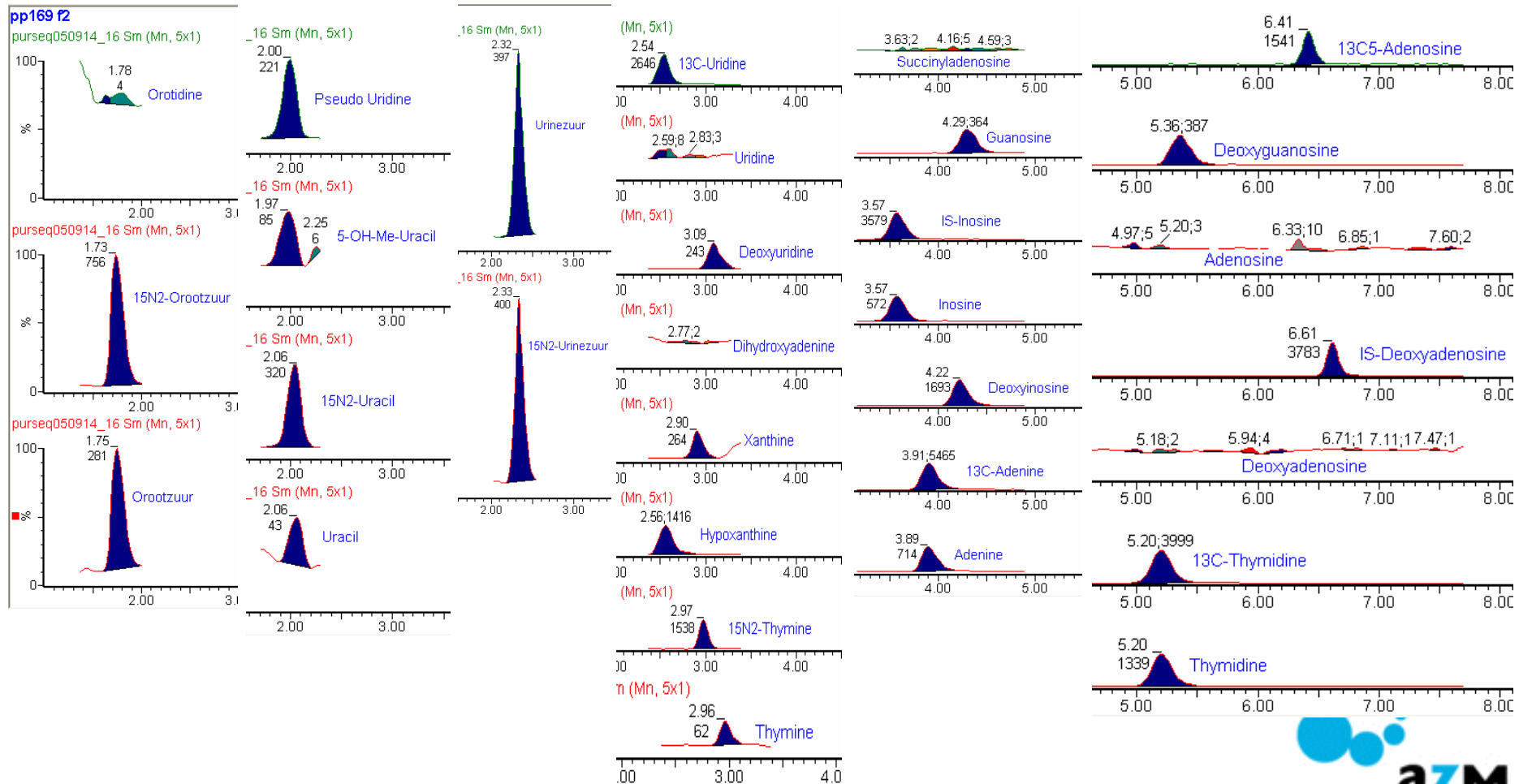
Practical experience

- Dept. of Biochemical Genetics,
Maastricht university hospital
- HPLC and LC-MS/MS

Comparison HPLC vs LC-MS/MS

	HPLC	LC-MS/MS
Sample preparation	Dilute and filter	Dilute and filter
Run time (inj – inj)	90	22
Detection	UV Lambda = 254 + 280 nm	ESI MRM
Specificity	Moderate	High
Quantification	External standard	Stable isotope IS
Detection limit	$\pm 10 \mu\text{M}$	Low μM
Beware	UV absorption not very specific	Ion suppression. Isobars

P&P screening using LC-MS/MS



Comparison HPLC vs LC-MS/MS

std laag	Component	n	Added	Ref	All	UV	Tandem MS EMZ				
							GEM	STDEV	VC%	n	Rec %
	5-OH-Me-Uracil	pp102	17	21	19	0	14	11	78	9	84
	Adenine	pp101	16	13	15	0	16	3	19	8	89
	Adenosine	pp103	20	22	20	21	18	2	11	9	92
	Deoxyadenosine	pp103	30	45	29	26	41	9	22	9	136
	Deoxyguanosine	pp104	20	21	18	18	19	2	13	10	94
	Deoxyinosine	pp104	30	29	30	0	30	7	24	10	95
	Guanosine	pp104	20	16	17	15	14	3	19	10	71
	Hypoxanthine	pp101	48	59	58	59	66	7	11	8	99
	Inosine	pp104	20	24	21	20	25	5	22	10	126
	Orootzuur	pp104	30	28	30	37	27	3	11	10	89
	Orotidine	pp103	11	12	12	24	15	6	39	9	133
	Pseudo Uridine	pp103	26	80	77	77	82	28	34	9	91
	Thymidine	pp101	50	46	48	61	41	5	13	8	89
	Thymine	pp102	33	31	37	37	34	7	19	9	102
	Uracil	pp102	78	82	87	109	86	14	16	9	100
	Uridine	pp103	17	20	17	16	14	2	13	9	83
	urinezuur	pp103	180	nb	246	197	257	61	24	5	100
	Xanthine	pp101	58	56	58	62	55	9	16	8	95

Comparison HPLC vs LC-MS/MS

- Results in ERNDIM P&P Scheme
 - LC-MS/MS and HPLC results comparable
- Advantage of LC-MS/MS
 - Analysis time
 - High accuracy
- When in doubt:
 - Use other technique to confirm
 - Analyse fragmentation spectrum

Conclusions

- Intra lab variations seems OK
 - Inter lab variation very large
 - Standardisation
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- Results LC-MS/MS and HPLC comparable
 - LC/MS/MS method of the future.