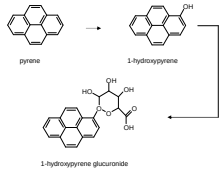
Fast and simple method for the determination of urinary 1-Hydroxypyrene

Xavier Cahours*, Maryline Blanchet, Miguel Rey
SEITA, Imperial Tobacco Group
4, rue André Dessaux, 45404 Fleury-les-Aubrais, France
Tel : +33 238723938
Fax : +33 238723825
xavier.cahours@fr.imtop.fr

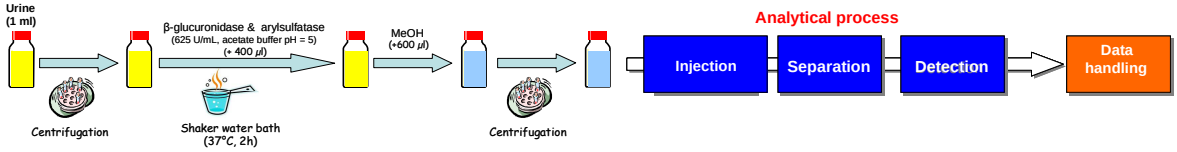
INTRODUCTION

The incomplete combustion of organic matter produces Polycyclic Aromatic Hydrocarbons (PAHs) with Pyrene as a dominant compound. These compounds are found in cigarette smoke, fossil fuels (oil, petrol,…), food that has been barbecued, roasted or smoked, or other types of environmental and occupational exposure.
Metabolism of Pyrene involves the formation of 1-Hydroxypyrene (1-OH-Py) as a phase I metabolite which undergoes phase II metabolism with conjugation to glucuronic acid (1-OH-Py-Glu). 1-OH-Py is a urinary PAH metabolite that has often been used as a biomarker for recent exposure to multiple PAHs. The glucuronide levels in urine account for more than 80% of total Pyrene metabolite in human urine.

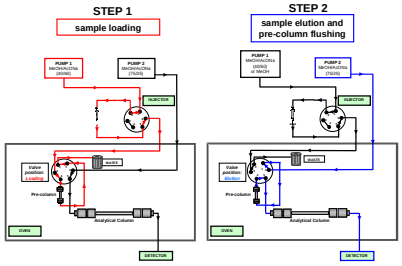


METHOD DEVELOPMENT

Several analytical methods have been described in the literature to determine 1-OH-Py metabolites in urine. The most widely used method was published by Jongeneelen et al. [9]. Their protocol includes enzymatic hydrolysis of the conjugated metabolite, solid phase extraction and analysis with high performance liquid chromatography. Over the past decade, different Research teams have been working on the development of other analytical methods in order to improve sensitivity and decrease analysis time. Nevertheless, all these methods require a time consuming sample clean up procedure.
The present paper reports a simple and fast method for the measurement of metabolites of Pyrene in urine. This method can quantify the total amount of Pyrene metabolites corresponding to glucuronic acid and sulfate conjugates as well as free 1-OH-Py. The method described in this work removes the need for sample clean up step using column switching system. It allows the direct injection of an enzymatically treated urine sample. Purification and analysis were carried out by liquid chromatography with fluorescence detection.



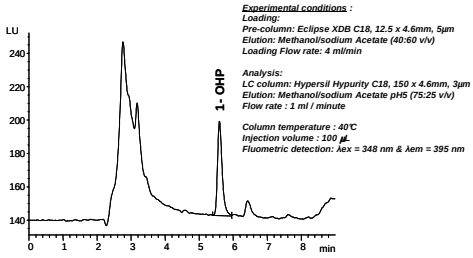
Schematic diagram of the analytical system



Switching program

Time (min)	Valve position	Loading pump		Event
		Mobile phase composition	Flow rate	
0.0	1-2	MeOH/AcONa (40/60 v/v)	4 mL/min	Sample loading
0.5	1-6	MeOH/AcONa (40/60 v/v)	4 mL/min	Analyte transfer
0.6	1-6	MeOH	1 mL/min	System flushing
2.5	1-2	MeOH	1 mL/min	Pre-column flushing
7.0	1-6	MeOH	1 mL/min	System flushing (anti-stacking effect)
7.1	1-2	MeOH	1 mL/min	
7.2	1-6	MeOH	1 mL/min	
7.3	1-2	MeOH	1 mL/min	
7.4	1-6	MeOH	1 mL/min	
7.5	1-2	MeOH	1 mL/min	
7.6	1-2	MeOH/AcONa (40/60 v/v)	1 mL/min	Pre-column reconditioning

HPLC chromatogram of hydrolyzed urinary sample



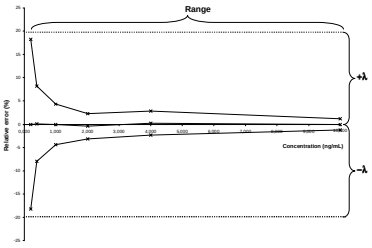
METHOD VALIDATION

The optimal conditions give an analysis of total 1-OH-Py in less than 10 minutes with a good selectivity. In order to validate the method, precision was determined measuring the matrix effect, accuracy and intermediate precision. In our conditions, the within-day precision (expressed as the relative standard deviation of replicate analysis) was less than 1% at each concentration. The inter-assays precision, or intermediate precision, was obtained by analyzing one urine (three successive enzymatic hydrolysis) on three days, using a separate calibration each day. The obtained between-day precision was less than 2% at each concentration without matrix effect.

Accuracy index & Limits of quantitation

Model	Accuracy Index	Range Index	Precision Index	Trueness Index	Lower and upper limits of quantitation (ng/mL)
Linear regression	0,94	0,98	0,86	0,99	<0.2 ; 10
Weighted linear regression	0,87	0,98	0,77	0,89	<0.2 ; 10
Linear regression after logarithm transformation	0,95	0,92	1,00	0,94	0.8 ; 10
Weighted linear regression after logarithm transformation	0,79	0,98	0,68	0,73	<0.2 ; 10
Linear regression after square root transformation	0,95	0,98	0,92	0,96	<0.2 ; 10
Weighted linear regression after square root transformation	0,76	0,74	0,96	0,62	1,0 ; 4
Quadratic regression	0,94	0,98	0,87	0,97	<0.2 ; 10
Weighted Quadratic regression	0,72	0,98	0,56	0,69	<0.2 ; 10

Linear accuracy profile
(±20% acceptability limits)



Matrix effect on the 1-OH-Py

Concentration in [ng/mL]	Matrix	Signals mean	Bias	Bias %
0.4	MeOH / Water	504	-	-
0.4	Urine 1	507	+ 3	**+ 0.60**
0.4	Urine 2	501	- 3	**- 0.60**
0.4	Urine 3	509	+ 5	**+ 0.99**
0.4	Urine 4	503	- 1	**- 0.20**
4.0	MeOH / Water	5057		
4.0	Urine 1	5126	+ 69	**- 1.37**
4.0	Urine 2	5086	+29	**- 0.56**
4.0	Urine 3	5157	+ 100	**- 1.970**
4.0	Urine 4	5110	+ 53	**- 1.04**
10.0	MeOH / Water	12770		
10.0	Urine 1	12871	+ 101	**- 0.79**
10.0	Urine 2	12734	- 36	**+ 0.28**
10.0	Urine 3	12784	+ 14	**- 0.11**
10.0	Urine 4	12882	+ 112	**- 0.88**

Within- and between-dy precision

Day	Analysis	Conc.1 (0.40 ng/mL)	Conc.2 (2.00 ng/mL)	Conc.3 (8.0 ng/mL)
1	1	0.453	2.08	9.77
	2	0.454	2.08	9.77
	3	0.447	2.04	9.72
	4	0.456	2.11	9.85
	5	0.455	2.11	9.87
	6	0.452	2.09	9.36
	7	0.455	2.10	9.89
	8	0.455	2.10	9.36
	9	0.446	2.08	9.70
2	1	0.457	2.09	9.84
	2	0.455	2.09	9.90
	3	0.444	2.07	9.82
	4	0.457	2.09	9.88
	5	0.458	2.13	9.85
	6	0.453	2.12	9.86
	7	0.454	2.13	9.84
	8	0.459	2.13	9.90
	9	0.450	2.13	9.90
3	1	0.446	2.06	9.54
	2	0.439	2.05	9.56
	3	0.444	2.04	9.59
	4	0.444	2.04	9.56
	5	0.441	2.04	9.57
	6	0.446	2.04	9.61
	7	0.442	2.04	9.57
	8	0.446	2.07	9.82
	9	0.450	2.08	9.85
Between-dy	Mean	0.450	2.085	9.738
	RSD (%)	1.4	1.5	1.3

Day 1	Conc.1 (0.40 ng/mL)	Conc.2 (2.00 ng/mL)	Conc.3 (9.5 ng/mL)
Mean	0.452	2.096	9.802
RSD (%)	0.9	0.9	0.7

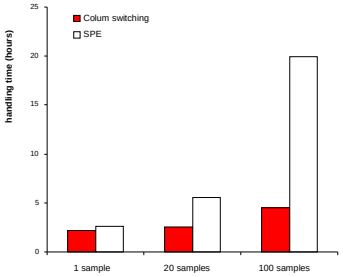
Day 2	Conc.1 (0.40 ng/mL)	Conc.2 (2.00 ng/mL)	Conc.3 (9.5 ng/mL)
Mean	0.454	2.106	9.873
RSD (%)	1.0	1.2	0.3

Day 3	Conc.1 (0.40 ng/mL)	Conc.2 (2.00 ng/mL)	Conc.3 (9.5 ng/mL)
Mean	0.443	2.052	9.638
RSD (%)	1.0	0.8	1.0

CONCLUSION

In conclusion, this study presents an improved HPLC method for a rapid and sensitive analysis of urinary metabolites of Pyrene using a column-switching method. Its main advantage is the simplicity of the procedure which requires only a few simple operations with common and relatively low-cost laboratory instruments. Furthermore, no gradient elution mode is used, 1-OH-Py-Glu hydrolysis is obtained in only 2 hours and sample handling is reduced drastically by excluding time consuming off-line extraction.
For one hundred samples, the handling time of on-line method is four times less than off-line method. Of course, these times are directly linked with the cost of the analysis. The switching method offers also the advantage that the reduction of handling operations decreases the associated risk of error. Other advantage of the method, the urine sample is low, less than 1 mL, which facilitates biological sampling and transport.

Handling time comparison between the proposed column-switching method and a conventional off-line method using Support Phase Extraction



Blind test on different concentrations of reference materials

Samples	Calculated concentration	Theorical concentration (reference materials)*	% Accuracy
A	3.07	3.40	90.1
B	1.63	1.80	90.6
C	16.1	18.0	89.4
D	Blank	Blank	NA
E	3.20	3.40	94.1
F	0.36	0,340	105.9
G	3.02	3.40	88.7
H	5.54	6.00	92.3

*ClinCal®-Urine calibrator, RECIPE, Germany