ENVIRONMENTAL ANALYSIS

TRACE LEVEL DETERMINATION OF 4-MBC METABOLITES IN URINE SAMPLES USING THE AGILENT 6495 LC/MS/MS

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ABSTRACT

A robust, sensitive method for the routine determination of 4-MBC metabolites in pre-treated urine samples has been developed on the Agilent 6495 LC/MS/MS. Using matrix stripping liquid chromatography, this method achieves excellent linearity and precision over the calibration range of 0.2 – 50 µg/L.

INTRODUCTION

4-methylbenzylidene camphor (4-MBC) is a UV-filter that is frequently used in cosmetics and sunscreens. Studies have raised safety concerns that 4-MBC may act as an endocrine disruptor and may also affect the thyroid. In response to these concerns, the Scientific Committee on Consumer Products (SCCP) issued an opinion in June 2008. It concluded that 4-MBC can be considered safe in finished cosmetic products (whole body application) at a concentration of up to 4% [1].

The analysis of urine samples involves the measurement of the two major metabolites of 4-MBC, namely 3-(4-carboxybenzylidene)-6-hydroxycamphor (MBC-OH) and 3-(4-carboxybenzylidene)-camphor (MBC-CX).

This solution note details an analytical method for the determination of these two metabolites in pre-treated urine samples using matrix stripping liquid chromatography on the Agilent 6495 LC/MS/MS.

ANALYTICAL TECHNIQUE

Standards

- 4-MBC Metabolite Standards: 3-(4-carboxybenzylidene)-camphor (MBC-CX) and 3-(4-carboxybenzylidene)-6-hydroxycamphor (MBC-OH)
- Internal Standards – deuterated analogues of MBC-CX and MBC-OH

Instrumentation

- Agilent 1290 Infinity LC System – analytical gradient separation
- Agilent 1260 Infinity Binary Pump – loading and matrix stripping
- Agilent 1200 Infinity Series Quick-Change Valves: 6 port, 2 position valve for column switching
- Agilent 6495 Triple Quadrupole MS with Jet Stream Ion Source

Agilent HPLC Operating Conditions	
Analytical column	Agilent Poroshell 120 Phenyl-Hexyl, 2.1 x 100 mm, 2.7 µm
Matrix stripping column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 x 30 mm, 1.8 µm
Injection volume	10 µL
Flow rate	0.4 mL/min
Run time	9.6 minutes

Column switching was carried out with a 6 port 2 position valve located in the thermostatted column compartment. The same solvent setup was used for both pumps systems.

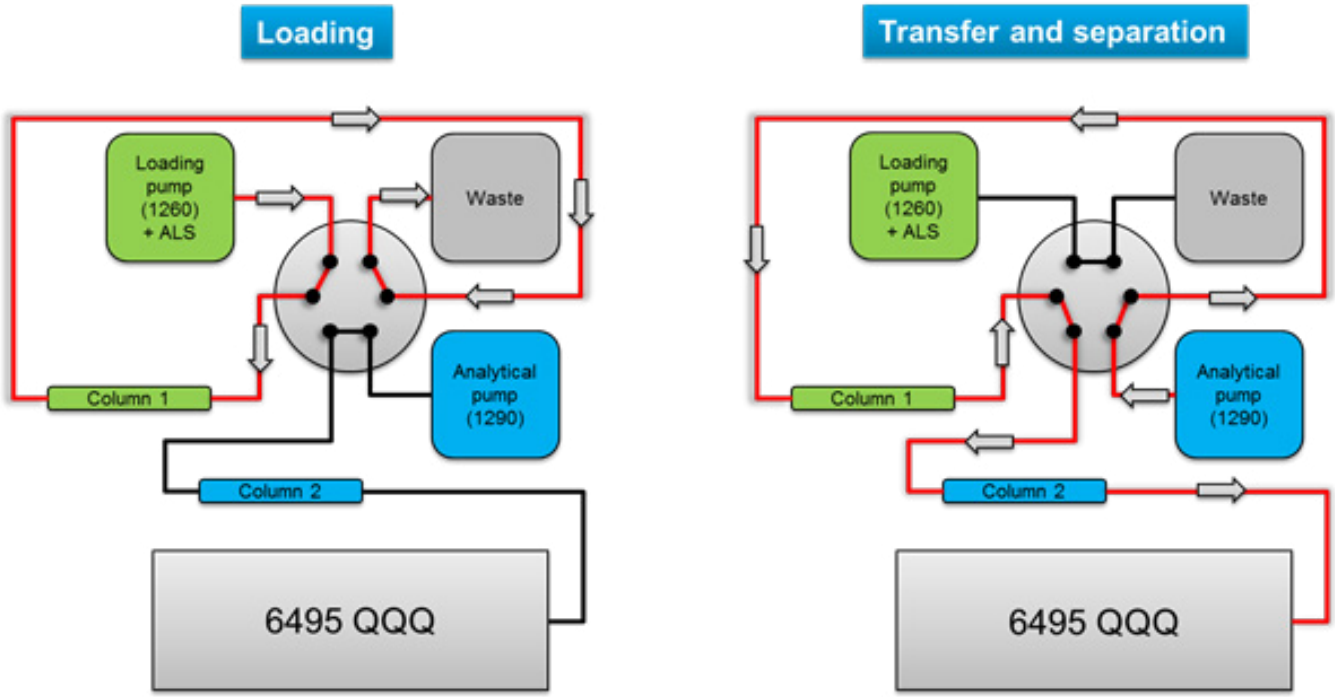

Figure 1. Schematic diagram for the two-column HPLC system with back-flush arrangement.

RESULTS AND DISCUSSION

Benefits of Matrix Stripping

The matrix stripping approach allows the first section of the chromatographic run to be 'cut out' and channelled to waste. This provides a cleaner spray chamber for a longer period of time, since the polar matrix components, which tend to elute early, do not enter the ion source. This is especially useful when analysing complex and/or dirty samples.

Linearity

The linearity of the instrument was evaluated for both metabolites (MBC-CX and MBC-OH) using the internal standard calibration approach, over the calibration range of 0.2 – 50 µg/L. The calibration curves are shown in Figure 2.

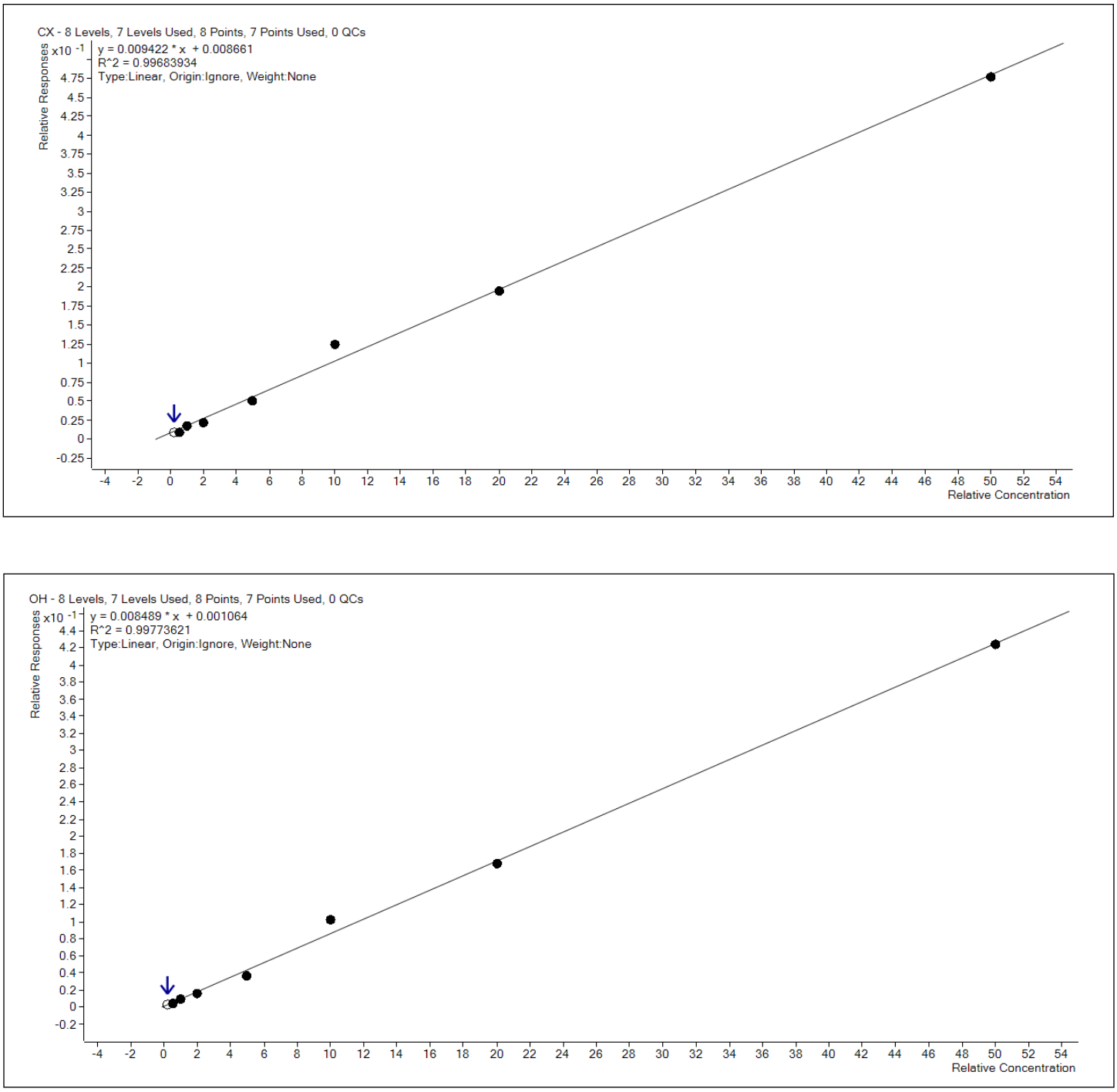

Figure 2. Calibration curves for CX and OH metabolites, standards at 0.2, 0.5, 1, 2, 5, 10, 20 and 50 µg/L.

PRECISION

The method precision was evaluated by analysing six replicate injections at low concentrations. The precision data at three different concentration levels is shown in Table 1.

4-MBC Metabolite	Recovery @ 0.5 µg/L		Recovery @ 2 µg/L		1 µg/L Standard	
	Average Area	% RSD	Average Area	% RSD	Average Area	% RSD
CX	3575.2	11.7	11289.5	6.6	6955.3	2.3
OH	3378.0	6.4	10599.9	2.9	6051.7	3.5

Table 1. CX and OH metabolite precision results.

SAMPLE RESULTS

Six pre-treated urine samples were analysed using this method and the results are shown in Figure 3. Sample 2 tested positive for both metabolites, with a concentration of 0.9 µg/L for the CX metabolite and 1.0 µg/L for the OH metabolite.

Sample			CX Results			CX-d4...	OH Results			OH-d4...
Name	Data File	Acq. Date-Time	RT	Area	Calc. Conc.	Area	RT	Area	Calc. Conc.	Area
Sample_1	Sample_1.d	1/22/2015 12:04 AM	4.194	0	0.0	466200	4.924	0	0.0	605311
Sample_2	Sample_2.d	1/22/2015 12:15 AM	4.194	7847	0.9	460278	4.851	5388	1.0	587868
Sample_3	Sample_3.d	1/22/2015 12:25 AM	4.261	0	0.0	144037	4.913	0	0.0	326207
Sample_4	Sample_4.d	1/22/2015 12:36 AM	4.251	0	0.0	138710	4.908	0	0.0	324130
Sample_5	Sample_5.d	1/22/2015 12:46 AM	4.225	0	0.0	234229	4.939	0	0.0	429566
Sample_6	Sample_6.d	1/22/2015 12:57 AM	4.225	0	0.0	238575	4.944	0	0.0	420047

Figure 3. Sample results for 6 pre-treated urine samples.

CONCLUSIONS

A robust, sensitive method has been developed for the determination of 4-MBC metabolites in pre-treated urine samples using the Agilent 6495 LC/MS/MS. This method achieves excellent linearity and precision over the calibration range of 0.2 – 50 µg/L.

REFERENCES

1. SCCP/1184/08 - Scientific Committee on Consumer Products, Opinion on 4-Methylbenzylidene camphor (4-MBC), COLIPA no S60, adopted by the SCCP at its 16th plenary of 24 June 2008.

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