Aloin determination by 2D-LC with MS/MS detection in foodstuffs containing *Aloe vera*

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1. INTRODUCTION

Since several years, various foodstuffs containing Aloe vera are commercialized. The structure of the Aloe vera leaf presents a central part containing the juice or jelly and an external layer containing laxative and irritating anthracene derivatives, e.g. aloin. When preparing aloe vera juice, the most important step is a cautious control of separation of the jelly from the outer part of the leaf, in order to avoid contamination of the edible part by the anthracene derivatives.

UE and Switzerland legislation give a limiting value for the maximum content of aloin in foodstuffs. This value is fixed at 0.1 mg/kg, expressed in total aloin content (i.e A+B isomers). In this context, food control authority need reliable analytical methods for the determination of aloin in various foodstuffs.

2. ANALYTICAL METHODS

Aloin analyses were carried out by two-dimensional liquid phase chromatography coupled with tandem mass spectrometry detection (HPLC-MS/MS). This approach allows to reach an excellent sensitivity and specificity. This can be carried out on all products of the market announcing aloe like an ingredient such as dairy products, aloe juices or drinks containing aloe juice, herb tea, liquid extracts or pills. Preliminar results with a single chromatographic separation showed important matrix effects. The addition of a second chromatographic separation as clean-up allowed to overcome this problem.

Sample preparation

Liquid sample: 20 mL sample + 400 µL ammonium acetate 100 mM. Adjust pH to 5. Filtration on Nylon 0.45 µm membrane. For samples containing pulp, a preliminary centrifugation step is performed.

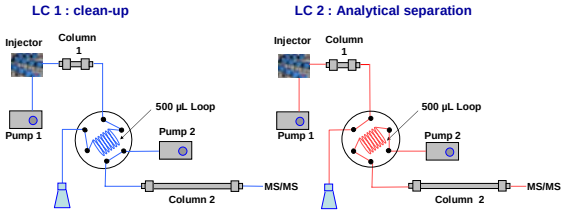
Dairy products: 15 g of yoghourt + 375 µL ammonium acetate 100 mM. Complete to 3.75 g with acetonitrile and shake vigorously. Take a 1.5 ml aliquote and centrifuge to 10'000 g during 15 min. Take the supernatant by avoiding fat and filtrate on Nylon 0.45 µm.

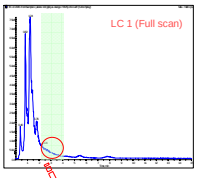
Powdered samples: Weigh between 50-300 mg of powder. Add ammonium acetate 100 mM. Put in ultrasonic bath until dissolution and filter on Nylon 0.45 µm.

2D-HPLC

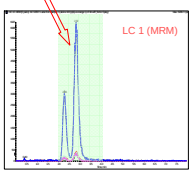
Column 1:	Macherey-Nagel Nucleodur 100-3 C18-ec 30mm x 2 mm
Flow rate:	0.25 ml/min
Injection:	5 µl extract
Mobile phase:	A : Ammonium acetate 2 mM pH 5.0 B : Acetonitrile
Gradient pump 1:	time (min) / A/B (%): 0 to 4.1 : 95 / 5 18 to 22 : 5 / 95 22.1 to 25 : 5 / 95

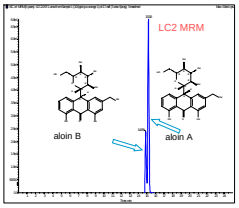
Column 2:	Phenomenex Synergi 4 µ POLAR-RP 80A 150mm x 2mm
Flow rate:	0.25 ml/min
Valve switching:	2.1 - 4.1 min
Mobile phase:	A : Ammonium acetate 2 mM pH 5.0 B : Acetonitrile
Gradient pump 2:	time (min) / A/B (%): 0 to 4.1 : 78 / 22 15 to 20 : 5 / 95 20.1 to 25 : 78 / 22





Time [min]	Valve position	Step
0 to 2.1	LC2	Sample injection
2.1 to 4.1	LC1	Loading of the 500 µL loop
4.1 to 25	LC2	Transfer from the loop on the column 2 and rinsing of column 1





Chromatograms obtained in the first and second dimension for an orange juice spiked at 100 µg/kg aloin

ESI-MS/MS conditions (ABI 3200 QTRAP)

Source: Turbolon spray operated in negative ESI mode ***MS/MS:*** Operated in MRM mode

Substances	Retention time [min]	Transitions	Dwell time [msec]	Coll. Energy [V]
Aloin A	14.9	417.2 > 297.1	150	-28
Aloin B	15.2	417.2 > 268.2	150	-44
		417.2 > 251.2	150	-56

Capillary voltage: -4500 V, Source heater: 550°C, Curtain gas: 20 psi, Nebulizer gas: 50 psi,Auxiliary gas: 40 psi, Declustering potential: -55 V, Entrance potential: -5 V

3. PERFORMANCES

Quantitation limit for S/N=10 : 1.3 µg/L total aloin content or 0.9 µg/L aloin A and 0.4 µg/L aloin B

Linearity range : 10 to 350 µg/L

The method was fully validated by spiking two different matrices at 3 concentrations, each level was analyzed in 4 replicates. These scheme was repeated over 3 days (results shown below)

JUICE

Concentration [µg/kg]	Trueness	Precision (intra-day)	Precision (inter-days)	Confidence interval (t=1.94)
25.5	90 %	4 %	16 %	29 %
102	84 %	3 %	8 %	13 %
255	81 %	3 %	11 %	17 %

DAIRY PRODUCT

Concentration [µg/kg]	Trueness	Precision (intra-day)	Precision (inter-days)	Confidence interval (t=1.94)
25.5	90 %	4 %	16 %	29 %
102	84 %	3 %	8 %	13 %
255	81 %	3 %	11 %	17 %

4. RESULTS

Aloe juices

Producer	Aloin [mg/kg]				
A	7.4	6.58	<0.01	<0.025	
	36.4	<0.01	<0.01	6.3	
B	1.7	2.0	1.6	3.1	1.5
C	5.6	8.0	0.36		
D	0.36	2.1			
Other	0.61	47.4	<0.01	4.16	
	<0.01	1.76			

Pills

Producer	Aloin [mg/kg]
A	15.7
B	7.1
C	<0.01
D	0.46
E	<0.01

Dairy products

Producer	Aloin [mg/kg]		
A	<0.01		
B	<0.01	<0.01	
C	0.012	<0.03	0.055
D	0.059	<0.01	<0.03
	0.013	0.07	<0.025
E	<0.031		
F	0.04		
G	<0.025	<0.01	

5. CONCLUSION

- The method was successfully applied in a survey of *aloe vera* products sold in Geneva
- Numerous aloe juices and pills show aloin concentrations widely above the MRL
- For a same producer, very widespread results were observed showing poor quality control of manufacturing practice
- Dairy production show better results and no concentration above MRL is observed