PRODAROM
Syndicat National des Fabricants de Produits Aromatiques

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The International Fragrance Association

COMMISSION SCIENTIFIQUE ET TECHNIQUE OF PRODAROM (CST)
IFRA ANALYTICAL WORKING GROUP (AWG)

LC-MS QUANTIFICATION METHOD FOR QUANTIFICATION OF ATRANOL AND CHLOROATRANOL IN MOSS EXTRACTS

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Contents

1 - Object and scope of application

This procedure describes a method for the determination of atranol and chloroatranol content in processed lichen extracts.

This method allows measuring low levels of aldehydes. The range of investigation starts from a few ppm to 100 ppm for each aldehyde (maximum level recommended by IFRA). This additional method reinforces the UV-HPLC method in terms of specificity.

2 - Principle

The concentrations of atranol and chloroatranol in moss extracts are determined by LCMS with electrospray ionization in negative mode. The dosage is performed by external standardization.

3 - Material and Equipment

3-1 - Common laboratory material

- Pasteur pipettes
- Graduated flasks of: 10mL, 20mL, 25mL, 50mL, 100mL
- Filters PTFE or RC 0.45µm and 0.2µm
- Syringes for single use of 2mL
- Vial 2 mL
- Measured pipettes (gauged with 2 features): 1mL, 2mL, 5mL

3-2 – Analytical balances

3-3 - Ultrasound bath

3-4 - LC instrumentation

- Pumping system allowing programmed solvent gradients
- Solvent degassing system
- Injection system with programmable volume
- Oven with column

Examples of instruments: - Agilent HPLC 1260
- Waters Aquity UPLC H Class

3-5 - MS Detection System (SQD or TOF)

Examples of instruments: - Agilent LCMS SQ 6120
- Waters LCMS Xevo G2 TOF
- Thermo LC ultimate 300 Q exactive benchtop
- Agilent LC-TQD 6420

3-6- Ionization System

- Electrospray ionization (ESI) in negative mode

3-7 - Columns

Recommended columns: column C18 type

Examples of recommended commercial columns:

- Zorbax SB-C18 Agilent, 1.8 μm, 2.1 mm x 50 mm
- Aquity UPLC HSS T3 Waters, 1.8 μm, 2.1 mm x 100 mm
- Eclipse plus C18 RRHD 1.8μm, 2.1mm X 50mm
- Kinetec C18 2.6μm, 2.1mm X 100mm

4 - Reagents

4-1 - Reference standard substances for the calibration:

Atranol - 2,6-dihydroxy-4-methyl-benzaldehyde
CAS RN 526-37-4
$C_8H_8O_3$
MW = 152.16 g/mol

Chloroatranol - 3-Chloro-2,6-dihydroxy-4-methyl-benzaldehyde
CAS RN 57074-21-2
$C_8H_7ClO_3$
MW = 186.59 g/mol

4-2 - Solvents for elution on HPLC column:

- Water, LCMS grade (H_2O)
- Acetic Acid LCMS grade
- Formic acid LCMS grade
- Ammonium formate LCMS grade
- Methanol LCMS Grade (MeOH)
- Acetonitrile LCMS grade (ACN)

4-3 - Experimental procedure

4-3-1 - LC conditions

Mobile phase:
According to the systems available in each company, differents mobile phase are proposed, in binary mixture with linear gradient mode of elution.

- Solvent A: aqueous phase with adducts
- Solvent B: organic phase with adducts

Organic phases could be

- MeOH,
- ACN, or
- Mixture 50/50 of MeOH/ACN

Adducts could be formic or acetic acid in a proportion range between 0.1 to 0.5 %

As examples

Mixture 1

- Solvant A: H_2O +0.1% formic acid + 5mM ammonium formate (pH = 3.8)
- Solvant B: 50/50 MeOH/ACN + 0.1% formic acid +5mM ammonium formate

Mixture 2

- Solvant A: H_2O + 0.5 % acetic acid
- Solvant B: MeOH + 0.5 % acetic acid

Mixture 3

- Solvant A: H_2O + 0.2 % acetic acid
- Solvant B: ACN + 0.2% acetic acid

A single gradient of elution is proposed for these 3 possible binary mixtures

Time (min)	Solvent A (%)	Solvent B (%)
0	85	15
10	15	85
12	0	100
15	0	100

Re-equilibrate the column to the initial conditions

Injection volume: 0.4 to 1 µl (adapted to the column diameter)

Oven temperature: 40-55°C

Flow: 0.4-0.5 mL/min

4-3-2 - MS conditions

Mass spectrometry detection is performed in SIM mode (Single Ion Monitoring), with a negative electrospray ionization. Examples of parameters:

- Ionization Mode -ESI- Negative mode
- Fragmentor 130
- Temperature drying gas 350 - 400°C
- Drying gas flow (N_2) L/mn 12
- Nebulize pressure (psig) 30- 35
- Sample cone 15 V
- Extraction cone 4
- Capillary voltage -0.5 to -1.1 kV

Compounds	**Rt (min)**	**Quantifiers $[M-H]^-$**	**Qualifiers**
Atranol	2.5-5.5	151	152 123 79
ClAtranol	5-7	185	186 157 93

Retention time is a column length dependent parameter.

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