A novel UHPLC-DAD/MS method for the fast high-resolution analysis of destruxins, a fungal depsipetide derivative class

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INTRODUCTION

- Destruxins (dtxs) are structurally closely related cyclic hexadepsipeptides secreted by the entomopathogenic fungus *Metarhizium anisopliae* (1).
- Besides their role in fungal pathogenicity, pharmacological activities as the prevention of osteoblasts (2), ion-channel formation (3), or effects on heart muscle contraction (4) have been reported.
- To monitor dtxs in fungal culture broth a fast and selective off-line SPE UHPLC-DAD/MS method based on previously reported assays (5) was established.
- This method shall serve as basis for further assay development in food matrices.

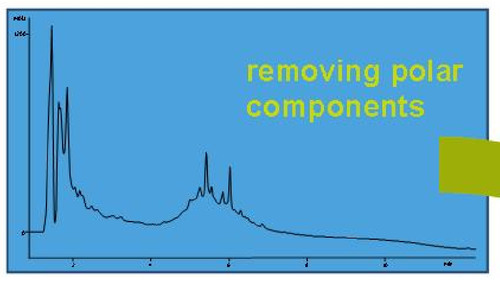
RESULTS

- For dtx isolation, concentration and purification from culture broth prior to HPLC analysis solid phase extraction was chosen.
- Optimal purification was achieved by a washing-step with 45% methanol (v/v), removing most of the polar components. The highest amount of dtxs was obtained by using 85% methanol (v/v) for elution.
- The improved UHPLC-DAD assay allowed separation of the dtx congeners within 7 min (total run time 12 min) with higher resolution compared to previous reported HPLC-DAD assays (5).
- Besides the available reference compounds dtxA, dtxB, dtxE, dtxE-diol, 18 dtx derivatives were tentatively identified by analyzing TOF-MS data.

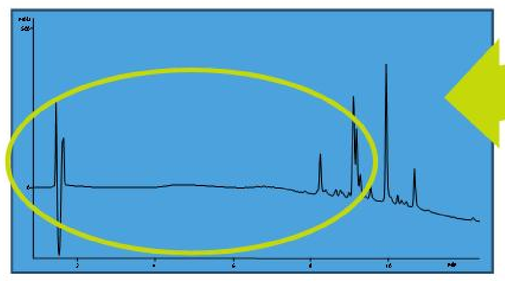
SAMPLE PREPARATION

STEP 1: 1 ml culture broth on RP-SPE

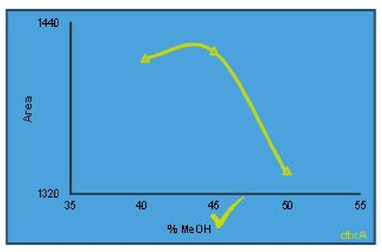
STEP 2: washing with 1 ml 45% methanol



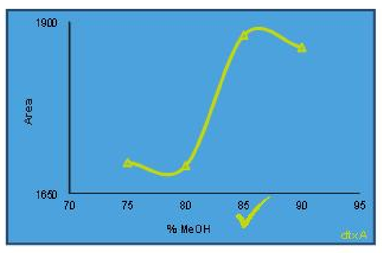
STEP 3: eluting with 1 ml 85% methanol



Wash solvent

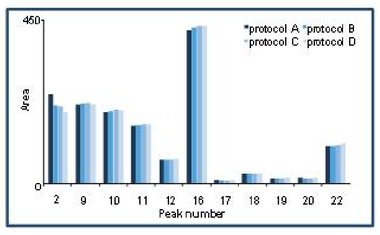


Elution solvent



Centrifugation parameters

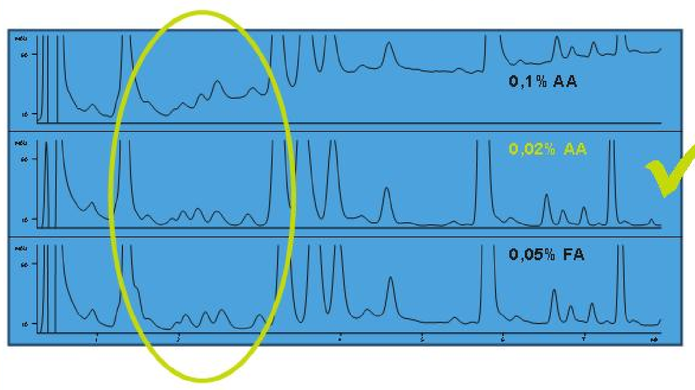
protocol	conditioning 1 ml MeOH / 1 ml H_2O	sample 1 ml culture broth	washing 1 ml 45% MeOH	eluting 1 ml 85% MeOH
A	2 min / 31 g	5 min / 11 g	5 min / 11 g	5 min /11 g
B	1 min / 80 g	2 min / 80 g	2 min / 80 g	4 min /31 g
C	1 min /126 g	2 min /126 g	2 min /126 g	3 min /61 g
D ✓	1 min /247 g	2 min /247 g	2 min /247 g	2 min /102 g



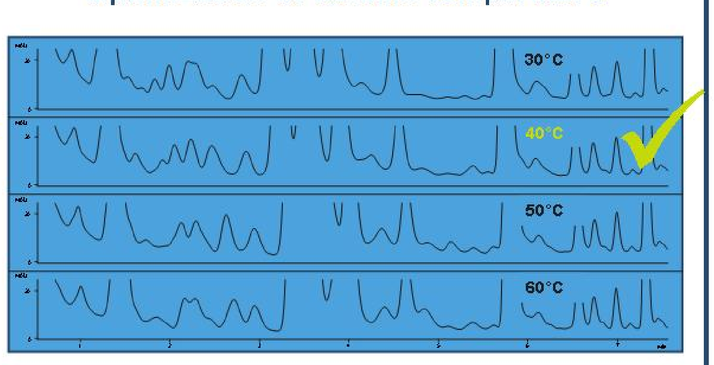
■ **Sample preparation:** Preparation was carried out by a solid phase extraction (SPE) on a Strata C18-E reversed phase material (Phenomenex, Aschaffenburg). The column was equilibrated with 1 ml MeOH and 1 ml H_2O by centrifugation (Heraeus Labofuge 400, Thermo Scientific, Osterode) for 1 min at 247 g (1400 rpm). Then a 1 ml aliquot of the culture filtrate was extracted by centrifugation for 2 min at 247 g. Optimal purification was achieved by a washing-step with 45% methanol (2 min, 247 g), removing most of the polar components. For elution a 85 % methanolic solution was used and the analytes eluted by centrifugation for 2 min at 102 g.

HPLC METHOD DEVELOPMENT

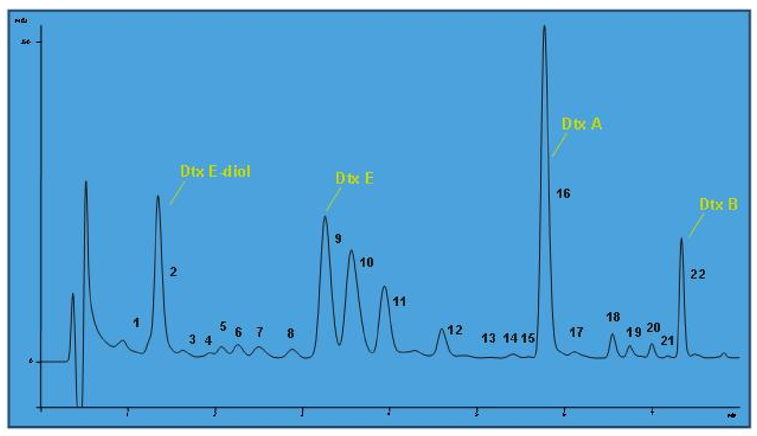
Optimization of solvents



Optimization of column temperature

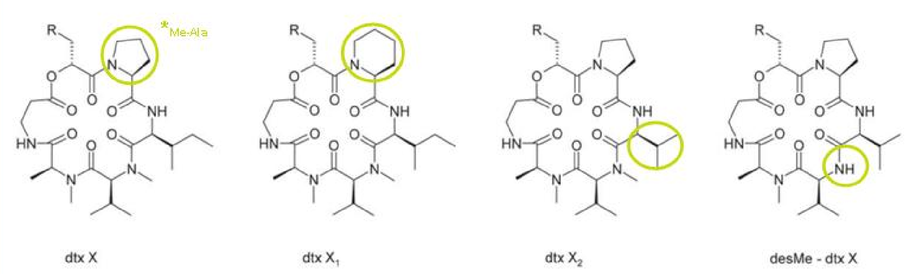


Optimized HPLC-DAD assay



peak number	[MH]+	basic structure	rest (R)	destruxin
2	612,3638	X	OH OH	E-diol
4	596,3644	desMe-X	CH_3 OH	Desmethyl C
5	580,3334	X_2	O	E_2
6	610,3446	X_2	CH_3 CO_2H	D_2
7	626,3745	X_1	OH OH	Ed_1
8	624,3625	X	CH_3 CO_2H	D*
9	594,3525	X	O	E
10	624,3653	X	CH_3 CO_2H	D*
11	630,3294	X	Cl OH	Cl
12	564,3392	X_2	CH_2	A_2*
13	638,3787	X_1	CH_3 CO_2H	D_1
14	564,3395	X_2	CH_2	A_2*
15	566,3545	X*	CH_2	A_3*
16	578,3559	X	CH_2	A
17	566,3545	X*	CH_2	A_3*
18	580,3718	desMe-X	CH_3 CH_3	Desmethyl B
19	580,3696	X	CH_2	Dihydro A
20	580,3719	X_2	CH_3 CH_3	B_2
21	592,3692	X_1	CH_2	A_1
22	594,3837	X	CH_3 CH_3	B

* suspected dtx



■ **HPLC-DAD setup:** An Agilent 1200 UHPLC-DAD system (Agilent, Waldbronn) was utilized to separate and detect the dtx congeners. A Zorbax Eclipse XDB-C18 rapid resolution column (2,1*50 mm, 1,8 µm particle size, Agilent) was used as stationary phase, with a water (A) / acetonitrile (B), each containing 0,02% acetic acid, gradient at a flow rate of 0,3 ml/min serving as mobile phase. Column oven temperature was set at 40°C. The gradient was t= 0 min 75% A, t= 3 min 60% A, t= 4 min 50% A, t= 5 min 35% A, t= 6 min 5% A, t= 6,5 min 2% A, t= 12 min 2% A. Between runs the column was equilibrated with 75% A for 12 min. The injection volume was 5 µl, chromatograms were recorded at 210 nm.

■ **TOF-MS setup:** A Bruker micrOTOF-QII mass spectrometer (Bruker Daltonics, Bremen) was used to detect and identify dtx congeners. Experiments were performed in positive ESI-mode with the following parameters: capillary energy 4500 V, nebulizer gas 23,2 psi, dry gas 6,0 l/min at a temperature of 200 °C, scan range 50-1500 m/z.
Fragmentation was performed in automatic mode with a collision energy of 10-25 V.

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