Electronic Supplementary Material (ESI) for Lab on a Chip.

SUPPORTING INFORMATION

Enantioselective reaction monitoring utilizing two-dimensional heart-cut liquid chromatography on an integrated microfluidic chip

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1. Additions to the instrumental setup and operation principle

The instrumental setup to realize two-dimensional HPLC-MS on a single glass chip is schematically shown in Figure 1 in the main publication. Special clamps described earlier[1] were used for fluidic chip to tube connections.

At a flowrate of 1.5 $\mu L \cdot min^{-1}$ in the first dimension and 5 s transfer time the maximal transfer volume calculates to about 100 nL. Due to compression of the solvent in the closed off arms of the on-chip cross in transfer mode, the actual transfer volume will be somewhat smaller.

The transfer process can also be operated in a fully automated mode by triggering the 6-port valve when a threshold of the detected fluorescence intensity is exceeded. In injection mode, the pressure at the on-chip cross increases linearly, since both arms of the cross are closed off. This is monitored by the integrated pressure sensor and can be used to observe the successful injection and furthermore, to automatically end the injection when a certain pressure threshold is reached. The injection scheme for the second dimension is very reproducible. The exact amount of transferred analyte is only dependent on the reproducibility of the first dimensional chromatography run, if a runtime based switching mechanism is utilized.

For a successful operation the solvents in first and second dimension should, as in classical 2D-LC, match in solvent strength and miscibility. In the current study the solvents consisted of ACN/H_2O (50/50 vol%) in the first and MeOH/H_2O (70/30 vol%) in the second dimension. Both solvent compositions are fully miscible. The solvent in the second dimension has a higher elution strength than the solvent from the first dimension. This allows the formation of a defined injection plug on the column head of the second column and hence, a high quality separation.

2. Optical setup

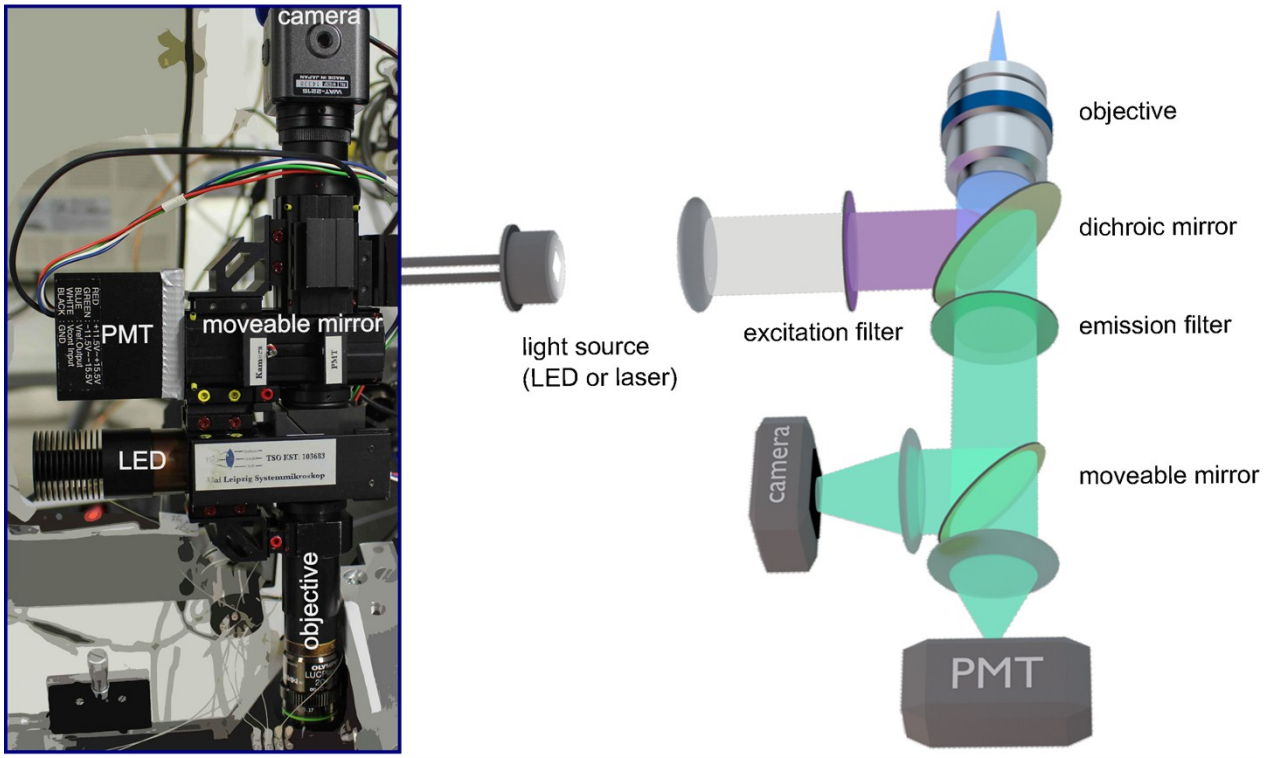

Figure S-1 Optical setup. Left: Picture of the utilized custom-built portable epi-fluorescence microscope. Right: Schematic illustration of the light path.

A portable custom-built epi-fluorescence microscope allowed simultaneous optical and mass spectrometric detection. It was utilized for the optical detection of the first dimensional separation and to monitor the fluidic situation at the on-chip cross. Two different configurations were applied. The first configuration is shown in Figure S-1. It includes a 365 nm excitation LED, a 350/50 excitation filter, a 380 nm DCLP (dichroic mirror) and a 390 nm emission filter. It was utilized to detect the model analytes 7-amino-4-methylcoumarin, pirkle's alcohol and anthracene and to observe the on-chip transfer of sample from the first to the second column.

In the second configuration a fiber guided 325 nm laser was utilized in combination with a 420-480 nm emission filter. This setup was utilized to detect the components of the warfarin synthesis. The detection point is marked in Figure 1 in the main publication. It is at the end of the first column close to the on-chip cross. The stationary phase material gives a slight background fluorescence, which allows to detect benzalacetone (**2**) via indirect fluorescence detection. At the same time 4-hydroxycoumarin (**1**) and warfarin (**3**) are detected via direct fluorescence detection (see Figure 3 in the main publication). The spectroscopic characteristics of all components are shown in Figure S-2.

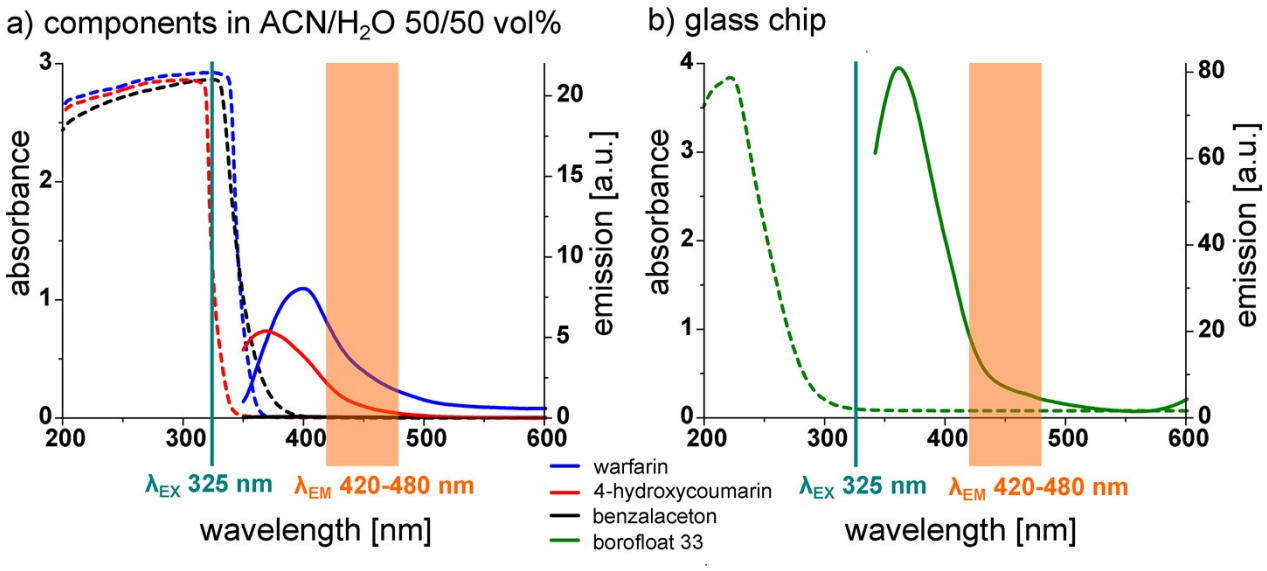

Figure S-2 Spectroscopic characteristics of the warfarin reaction components and the chip material. Dotted line: absorbance; solid line: emission at 325 nm excitation)

3. Reaction time dependent solvent screening

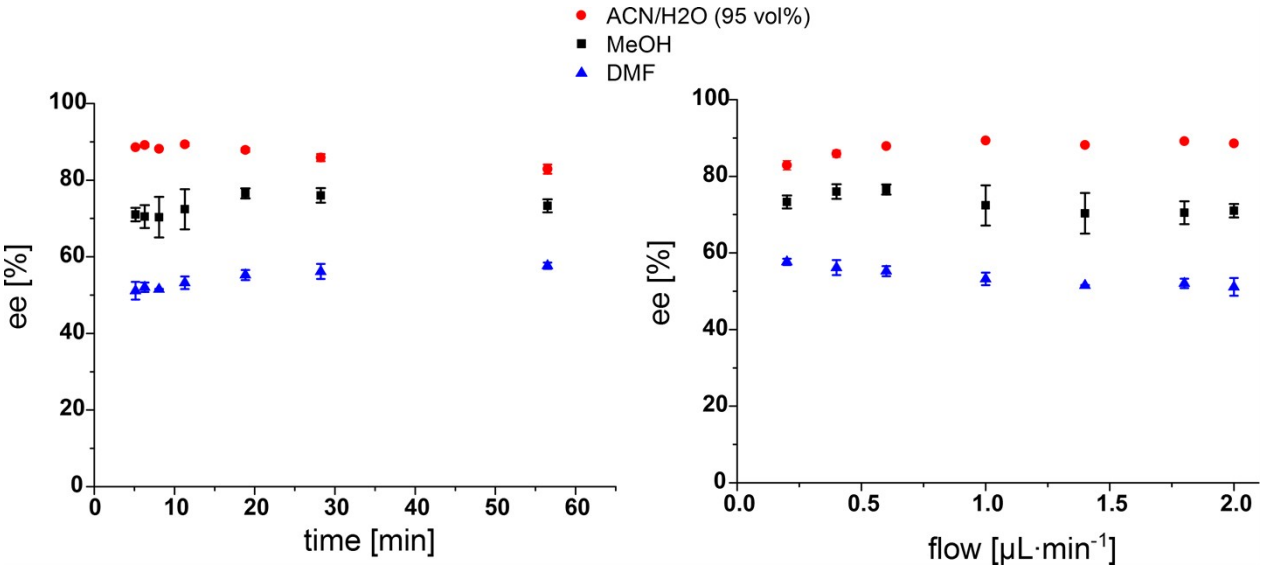

Figure S-3. Reaction time and flow rate dependent measurements of the enantiomeric excess for different solvents.

To investigate whether the enantiomeric excess (*ee*) of the warfarin synthesis reaction is dependent on the reaction time, different reaction times between 5 and 57 min were employed by varying the reactant flow rates and the enantiomeric excess was determined. The resulting plots of the reaction time and the equivalent flow of the reaction solution versus the enantiomeric excess are shown in Figure S-3.

4. Temperature screening

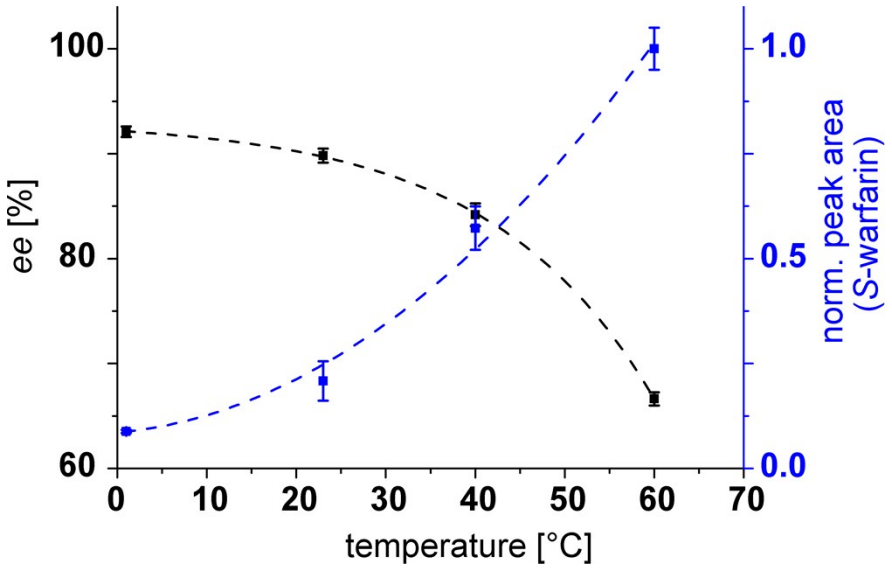

Figure S-4. On-chip temperature screening for the asymmetric warfarin synthesis. Solvent: 95% ACN/H_2O. Reaction time: 11 min. Separation parameters as described in the caption of Figure 4 in the main publication.

In order to investigate the effect of the reaction temperature on the *ee*-value, the micro-flow reactor chip was placed in a thermostat. The effluent was analyzed with the developed 2D-HPLC-MS-chip system. The results are shown in Figure S-4, displaying the determined *ee*-values and the normalized peak area of (S)-wafarin as the main product. An increase in reaction temperature reduces the *ee*-values drastically, while the overall turnover increases.

5. Experimental section

5.1 Microchip design and fabrication

The utilized microfluidic glass chips were produced by iX-factory (Dortmund, Germany) to our specifications by common photolithography, wet-etching and bonding methods. The material is BOROFLOAT® 33, with outer dimensions of 10x45x2.2 mm. The channels are 155 µm wide and 45 µm deep.

The layout is shown within Figure 1 and 2 in the main publication. The chip was designed for multiple purposes and as not all fluidic channels are used for the current 2D-HPLC setup two channels were connected via a T-piece, as depicted in Figure 1 in the main publication.

The particulate columns were integrated via a slurry packing approach[2,3] with porous polymer monoliths as particle retaining elements, as described previously.[4] The first dimensional column is packed versus one on-chip retaining monolith and extends partly into the connection tubing. The second dimensional tubing is immobilized within the chip between two porous frits.

An electrospray emitter in the shape of a sharp cut corner and with hydrophobic coating was manufactured at the chip end as described previously [2] .

The utilized microchip reactor was purchased from Micronit (Enschede, Netherlands). It contains two inlets, a meandered reactor channel and one outlet. The powder blasted channels are 150 µm deep and wide. The total reaction volume calculated to 11.3 µL.

52 Chemicals

The utilized chemicals are listed below.

Chemical	Purity	Source
Methanol	gradient grade	VWR International LLC (Radnor, USA).
Acetonitrile	gradient grade	VWR International LLC (Radnor, USA).
Ultrapure water	18.2 MΩcm	Smart2Pure purifying system (TKA Wasseraufarbeitungssysteme GmbH, Niederelbert, Germany)
Chloroform	p.A.	Carl Roth GmbH + Co. KG (Karlsruhe, Germany)
n-Heptane	p.A.	Carl Roth GmbH + Co. KG (Karlsruhe, Germany)
Tetrahydrofuran	≥ 99.9%	Carl Roth GmbH + Co. KG (Karlsruhe, Germany)
N,N-Dimethylformamid	≥ 99.9%	Carl Roth GmbH + Co. KG (Karlsruhe, Germany)
4-Hydroxycoumarin	98%	Sigma-Aldrich GmbH (Taufkirchen, Germany)
Benzalacetone	≥ 99%	Sigma-Aldrich GmbH (Taufkirchen, Germany)
Warfarin (racemic)	analytical standard	Sigma-Aldrich GmbH (Taufkirchen, Germany)

(*S*)-Warfarin	> 98%	Biomol GmbH (Hamburg Germany)
(1S,2S)-1,2-Diphenylethylenediamine	97%	Sigma-Aldrich GmbH (Taufkirchen, Germany)
Ethylenediamine diacetate	98%	Sigma-Aldrich GmbH (Taufkirchen, Germany)
Anthracene	97%	Sigma-Aldrich GmbH (Taufkirchen, Germany)
7-Amino-4-methylcoumarin	99%	Sigma-Aldrich GmbH (Taufkirchen, Germany)
(S)-(+)-1-(9-Anthryl)-2,2,2-trifluoroethanol	>98%	Sigma-Aldrich GmbH (Taufkirchen, Germany)
(R)-(-)-1-(9-Anthryl)-2,2,2-trifluoroethanol	>98%	Sigma-Aldrich GmbH (Taufkirchen, Germany)
Formic acid	MS grade (98%)	Sigma-Aldrich GmbH (Taufkirchen, Germany)
Acetic acid	100%	Sigma-Aldrich GmbH (Taufkirchen, Germany)
1,3-butanediol diacrylate (98%)	98%	Sigma-Aldrich GmbH (Taufkirchen, Germany)
3-(trimethoxysilyl)propyl methacrylate	98%	Sigma-Aldrich GmbH (Taufkirchen, Germany)
2,2-dimethoxy-2-phenylacetophenone	99%	Sigma-Aldrich GmbH (Taufkirchen, Germany)
Trichloro(1H,1H,2H,2H-perfluorooctyl)silane	97%	Sigma-Aldrich GmbH (Taufkirchen, Germany)

5.3 Materials

The utilized materials and instruments are listed below.

Material	**Source**
ProntoSIL C18 SH, 5 µm	BISCHOFF Analysentechnik u. -geraete GmbH, Leonberg, Germany
CHIRALPAK IB, 5 µm	Chiral technologies europe S.A.S., Illkirch, France
6-port valve C72MPKH-4676D	VICI AG, Schenkon, Switzerland
Nano injection valve C74MPKH-4674-.005D	VICI AG, Schenkon, Switzerland
Ultimate 3000 HPLC pump NCS-3500RS (micro-flow configuration with loading pump)	Thermo Scientific, Dionex Softron GmbH, Germering, Germany
PEEK capillaries 360 µm OD; 50, 75 and 100 µm ID	VICI AG, Schenkon, Switzerland
XYZ linear translation stage	OWIS GmbH, Staufen i.Br., Germany
6520 Q-TOF	Agilent Technologies Inc., Santa Clara, USA
pressure sensor	DURATEC Analysentechnik GmbH, Hockenheim, Germany
neMESYS low pressure syringe modules NEM-B101-02	cetoni GmbH, Korbußen, Germany
Microchip reactor R150.332.2	Micronit Microfluidics BV, Enschede,

	Netherlands
350/50 bandpass excitation filter	AHF analysentechnik AG, Tübingen, Germany
390 longpass emission filter	AHF analysentechnik AG, Tübingen, Germany
380 DCLP (dichroic mirror)	AHF analysentechnik AG, Tübingen, Germany
420-480 bandpass filter	AHF analysentechnik AG, Tübingen, Germany
365 nm LED, M365L2	Thorlabs Inc. Newton, New Jersey, USA
Color camera WAT-221S	Watec Co., Ltd. Japan
photosensor module H9306-04	Hamamatsu Photonics Deutschland GmbH, Herrsching am Ammersee, Germany
Omnichrome Series 74 325nm HeCd laser 3074R-M-A01	Melles Griot, part of IDEX Health & Science, LLC., Lake Forest, USA

6. References

(1) Lotter, C.; Heiland, J. J.; Stein, V.; Klimkait, M.; Queisser, M.; Belder, D. *Anal. Chem.* **2016**, *88*, 7481–7486.
(2) Lotter, C.; Heiland, J. J.; Thurmann, S.; Mauritz, L.; Belder, D. *Anal. Chem.* **2016**, *88*, 2856–2863.
(3) Thurmann, S.; Dittmar, A.; Belder, D. *J. Chromatogr. A* **2014**, *1340*, 59–67.
(4) Thurmann, S.; Mauritz, L.; Heck, C.; Belder, D. *J. Chromatogr. A* **2014**, *1370*, 33–39.