Supporting Information

Stereoselectivity in electrosprayed microdroplets: asymmetric synthesis of warfarin by diamine organocatalysts

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1. General Information

Reactions in tetrahydrofuran (THF) were conducted using THF with HPLC-grade purity. All the reactions performed in acetonitrile (ACN) were conducted using ACN with MS-grade purity. All the commercially available reagents and solvents were used as purchased, without further purification.

Enantiomeric ratios were determined on a WATERS or a JASCO HPLC system equipped with a UV detector. Column: Chiralpak IB (250x4.6 mm L.xI.D. 5µm); mobile phase: n-hexane/ethyl acetate 70/30 + 0.1% TFA v/v; flow rate: 1 ml/min, UV detection 280 nm. At the end of each e-HPLC analysis, a standard solution of warfarin racemic mixture was analyzed in the same conditions as control.

The device employed for the microdroplet reaction is reported in Figure S1 and described below. The electrospray ionization (ESI) probe of a quadrupole-time of flight (Q-TOF, Ultima) mass spectrometer (Micromass, Manchester, UK) was disassembled from the source block of the instrument and placed under a fume hood to prevent the release of volatile substances. The ESI probe was however kept connected to the mass spectrometer to ensure the application of the electric voltage (kV) to the capillary and the stream of the heated desolvation gas (N_2). These parameters are extremely important in the microdroplet formation process since they control the droplet sizes and the desolvation grade.
This apparatus was in turn adapted for the different experiments performed in this study.
In the system A, the reactants and catalyst were mixed and infused via a syringe pump in the ESI silica capillary of the source probe through a PEEK tubing (ID = 0.010"). In the system C and system D, a stainless-steel T junction was interposed between the syringe pump device and the ESI probe. As a result, different solutions were separately injected through two PEEK tubs directly connected to the T junction (see following figures). Lastly, in system B, the single capillary mounted inside the ESI probe (ID = 0.150 mm) was replaced by two parallel silica capillaries connected to different syringes through two PEEK tubes (ID = 0.010").
The microdroplets generated through the three above-described systems were collected on a 1 cm thick layer of silica placed inside a 1 ml capacity Eppendorf tip. According to this experimental setup, the distance between the spray needle and the silica layer is estimated to be approximately 5 cm. A tungsten wire biased with the ESI source cone voltage (60 V) was inserted in the Eppendorf tip at ca 1 cm from the ESI capillary needle to ensure the high voltage field for droplets coulombic explosion.

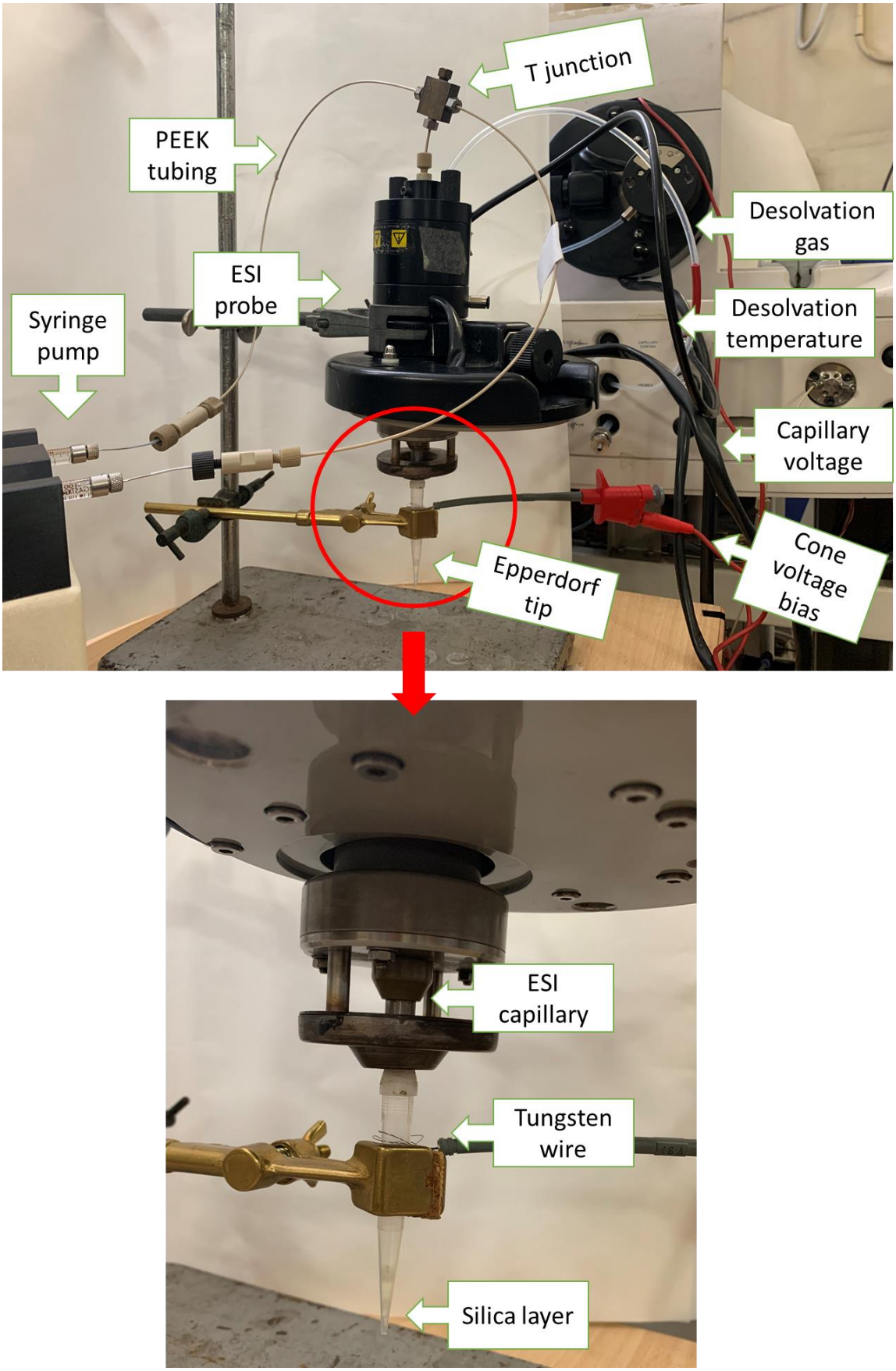
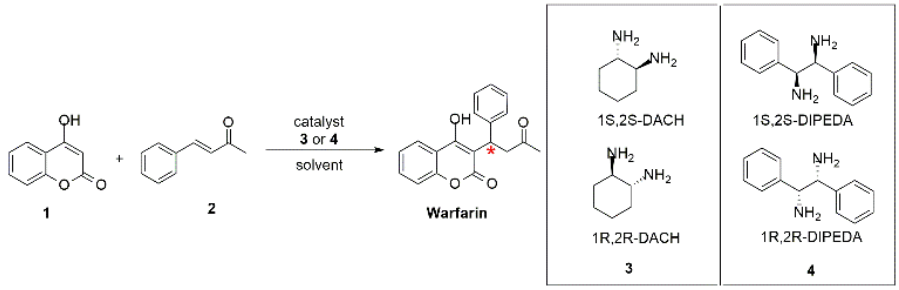


Figure S1. Photograph of the apparatus employed for the Warfarin synthesis in the microdroplet environment.

2. Experimental Procedures

Bulk-Reaction Conditions



Bulk-Reactions 10^{-2}M

0.12 mmol of 4-hydroxycoumarin **1** (19.6 mg) and 0.15 mmol of benzylideneacetone **2** (21.5 mg) are dissolved in 8 ml of solvent (ACN or THF). Then, 0.01 mmol of catalyst 1,2-diaminocyclohexane **3** (DACH, 1.3 mg) or 1,2-diphenylethylendiamine **4** (DIPEDA, 2.5 mg) dissolved in 2 mL of solvent are added and the reaction mixture (**reaction mixture A**) is left stirring (magnetic stirring) for 5 hours at the temperature indicated in Table 1 of article (room temperature, 60 °C or 82 °C). After 5 hours the solvent is evaporated. When the yield is reported in Table 1 (see article), reaction crude was purified by flash chromatography with n-hexane/ethyl acetate 70/30 mixture and the enantioenriched product was analyzed by HPLC. When the yield is not reported in Table 1, reaction crude is directly analyzed by HPLC after filtration.

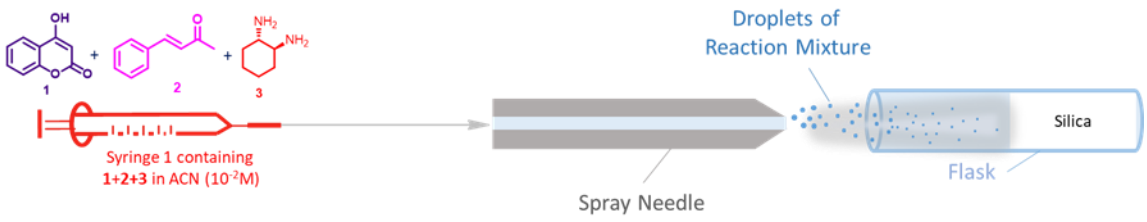
Bulk-Reactions 10^{-4}M

0.1 mL of **reaction mixture A** are diluted with 9.9 mL of solvent (ACN or THF) and left stirring for 5 hours at room temperature. After 5 hours the solvent is evaporated, and the reaction crude is directly analyzed by HPLC after filtration.

Microdroplet Conditions

The reagent solutions are charged into a 500 µL syringe and fluxed for 5 hours by a syringe infusion pump in order to collect a manageable quantitative of the crude. Once the 500 µL of the reagent solutions are finished, they are manually recharged into the syringe. In total, 4.8 mL of solutions are infused for each experiment. Reaction crude is collected on a silica layer. At the end of each experiment, reaction crude is recovered by washing the system with 3 mL of ACN, 3 mL of MeOH and 3 mL of DCM.
Syringe pump: 15 µL/min, capillary voltage: +3.5 kV, desolvation gas: 300 L/hr, desolvation gas temperature: 150 °C (for entries 1-9 + entry 11 Table 2, See article) or 50 °C (for entries 10, 12 and 13 Table 2, See article).

Figure S2. Schematic representation of System A.



As soon as DACH catalyst **3** is added, the **reaction mixture A** (10^{-2}M) is taken by syringe and promptly undergoes to microdroplet conditions. After about 5 hours, the experiment is stopped, the reaction crude is recovered and dried. The reaction crude is analyzed by HPLC without purification.

Figure S3. Schematic representation of System B.

A 10^{-2}M solution of **1** and **2** in ACN is placed in **syringe 1**; a 10^{-3}M solution of DACH catalyst **3** in ACN is placed in **syringe 2** and both the solutions are subjected to microdroplet conditions through two different capillary columns inserted into the same spray needle.
After 5 hours, the experiment is stopped, the reaction crude is recovered and dried. The reaction crude is analyzed by HPLC without purification.

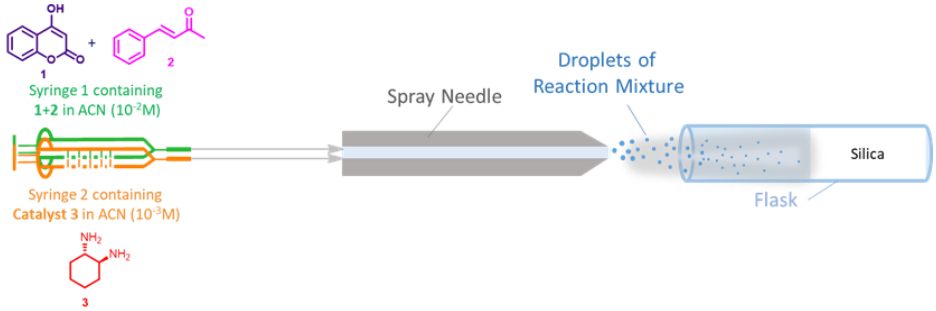
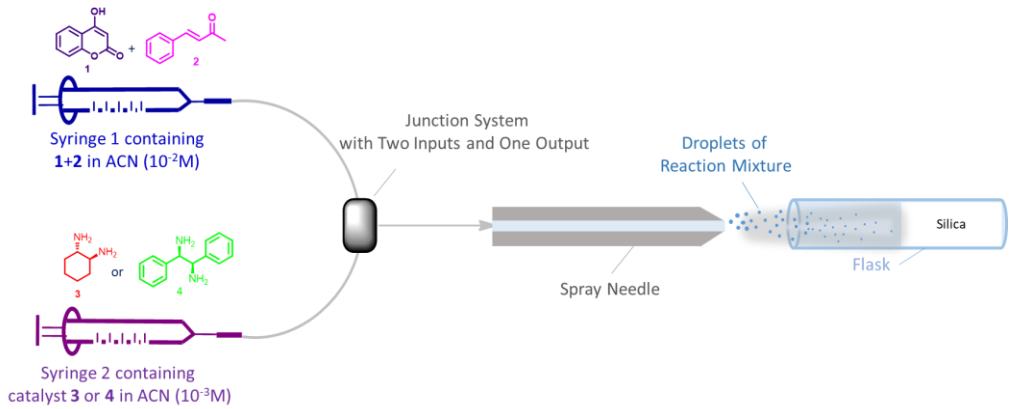
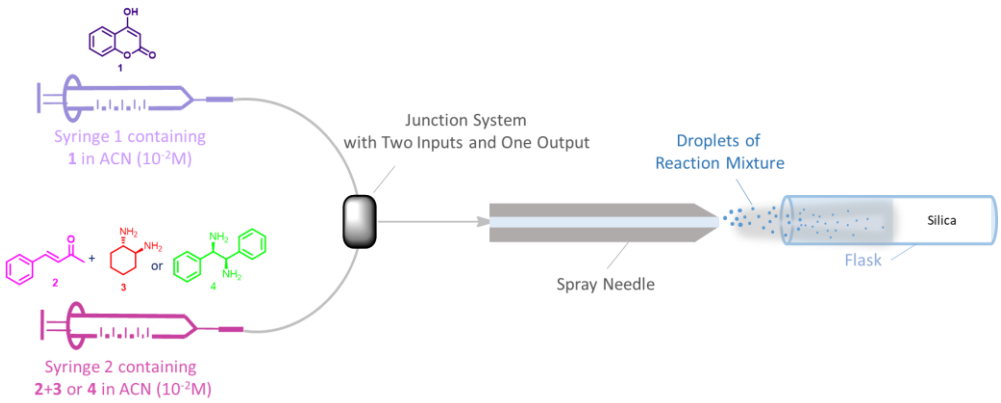


Figure S4. Schematic representation of System C.



A 10^{-2}M solution of **1** and **2** in ACN is placed in **syringe 1** and a 10^{-3}M solution of catalyst DACH **3** or DIPEDA **4** in ACN is placed in **syringe 2.** The two solutions meet in a "T junction" at zero void volume and undergo to microdroplet conditions through the spray needle.
After 5 hours, the experiment is stopped, and the reaction crude is recovered and dried. When conditions reported in the entries 3 and 7 of Table 2 (see article) are used, the reaction crude was purified by thin-layer chromatography (TLC) eluting with *n*-hexane/ethyl acetate 70/30 mixture. Methanol was used to recover the product from SiO_2 after separation, the extract in MeOH was filtered, dried and analysed by HPLC.

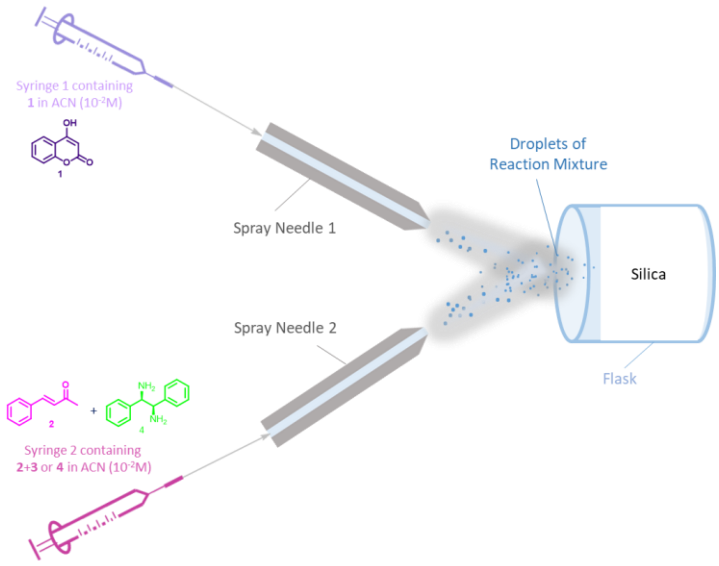
Figure S5. Schematic representation of System D.



A 10^{-2}M solution of **1** in ACN is placed in **syringe 1**; a 10^{-2}M solution of benzylideneacetone with catalyst DACH **3** or DIPEDA **4** in ACN is placed in **syringe 2.** The two solutions meet in a "T junction" at zero void volume and undergo to microdroplet conditions through the spray needle.

After 5 hours, the experiment is stopped and the reaction crude is recovered and dried. The reaction crude was purified by thin-layer chromatography (TLC) eluting with *n*-hexane/ethyl acetate 70/30 mixture. Methanol was used to recover the product from SiO_2 after separation, the extract in MeOH was filtered, dried and analyzed by HPLC.

Figure S6. Schematic representation of System E.



A 10^{-2}M solution of **1** in ACN is placed in **syringe 1**; a 10^{-2}M solution of benzylideneacetone with catalyst DIPEDA **4** in ACN is placed in **syringe 2.** The two spray needles are located at an angle of 60 degrees between them, at the distance of 5 cm from the silica cap.

After 5 hours, the experiment is stopped and the reaction crude is recovered and dried. The reaction crude was purified by thin-layer chromatography (TLC) eluting with *n*-hexane/ethyl acetate 70/30 mixture. Methanol was used to recover the product from SiO_2 after separation, the extract in MeOH was filtered, dried and analyzed by HPLC.

3 Chromatograms

Enantiomeric ratios were determined on Chiralpak IB column (250x4.6 mm L.xI.D. 5µm); mobile phase: n-hexane/ethyl acetate 70/30 + 0.1% TFA v/v; flow rate: 1 ml/min, UV detection 280 nm.

Figure S7. Bulk Reaction 10^{-2}M in THF, catalyst *S,S*-DACH (Table 1, entry 1, see article); *e.r.* warfarin (+)/(-) = 20/80

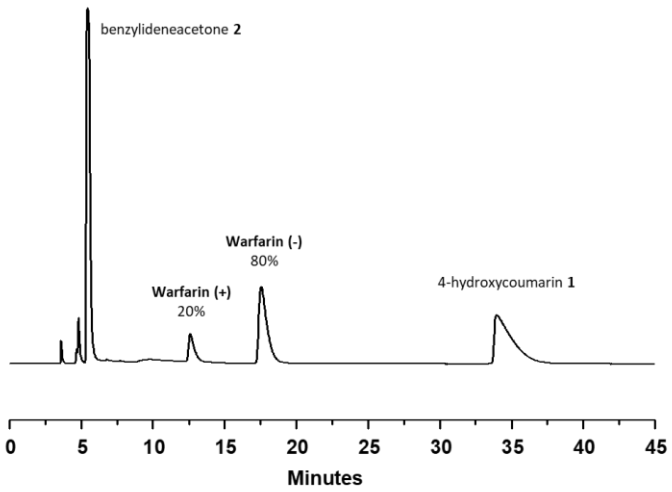


Figure S8. Bulk Reaction 10^{-4}M in THF, catalyst *S,S*-DACH (Table 1, entry 2, see article); *e*.r.. warfarin (+)/(-) = 24/76

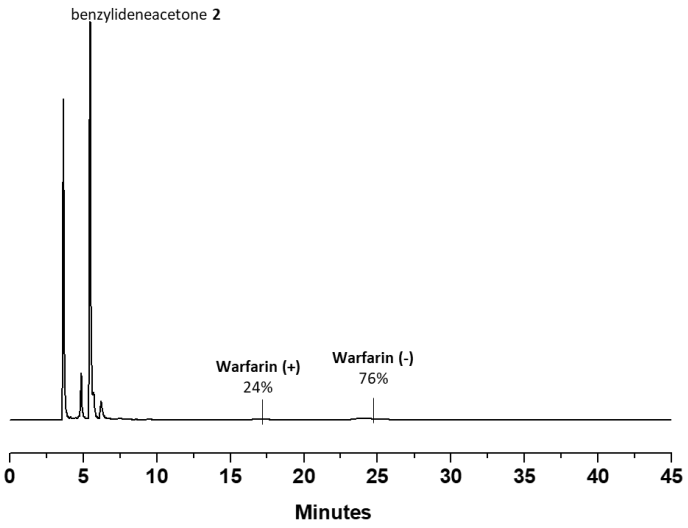


Figure S9. Bulk Reaction 10^{-2}M in ACN, catalyst *S,S*-DACH (Table 1, entry 3, see article); *e*.r. warfarin (+)/(-) = 15/85

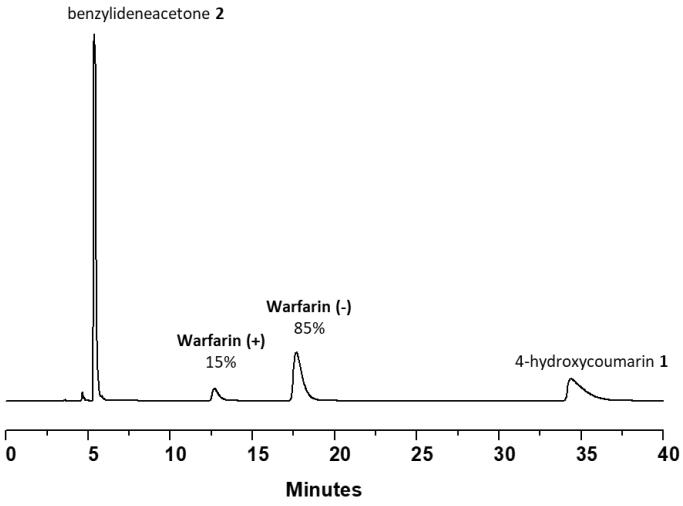


Figure S10. Bulk Reaction 10^{-4}M in ACN, catalyst *S,S*-DACH (Table 1, entry 4, see article); *e*.r. warfarin (+)/(-) = 18/82

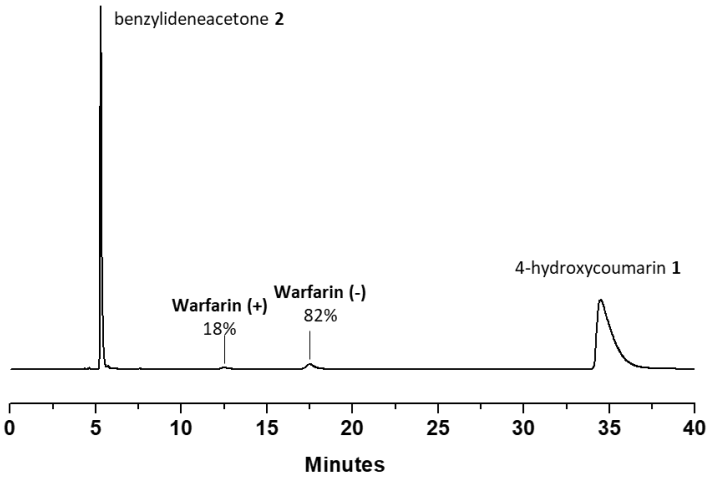


Figure S11. Bulk Reaction 10^{-2}M in ACN, 60°C, catalyst *S,S*-DACH (Table 1, entry 5, see article); *e*.r. warfarin (+)/(-) = 20/80

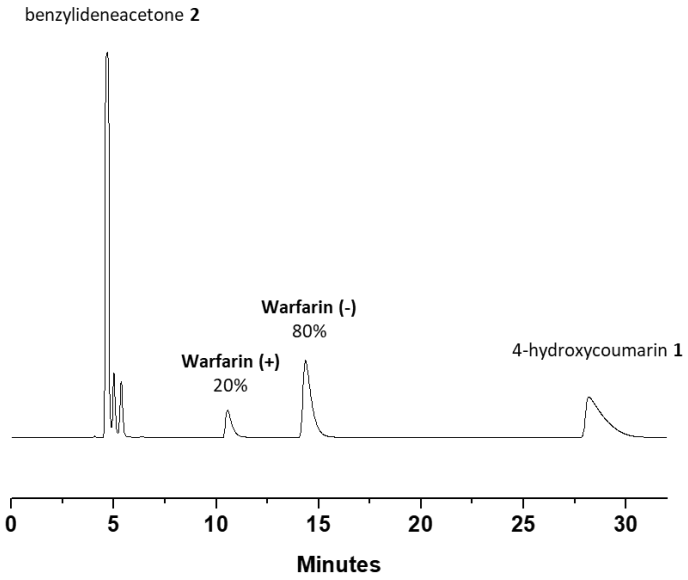


Figure S12. Bulk Reaction 10^{-2}M in ACN, 82°C, catalyst *S,S*-DACH (Table 1, entry 6, see article); *e*.r. warfarin (+)/(-) = 33/67

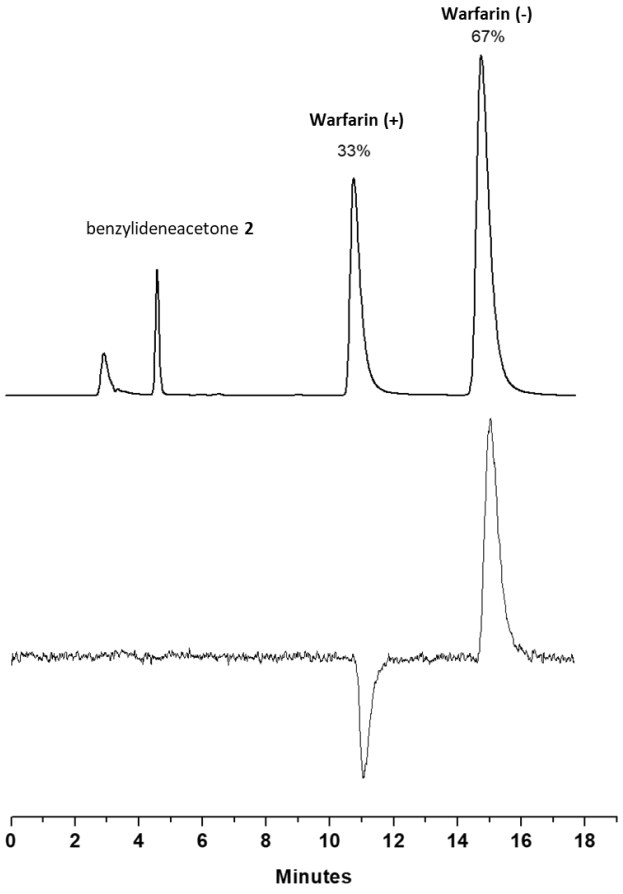


Figure S13. Bulk Reaction 10^{-2}M in ACN, Catalyst *R,R*-DIPEDA (Table 1, entry 7, see article); *e*.r. warfarin (+)/(-) = 80/20

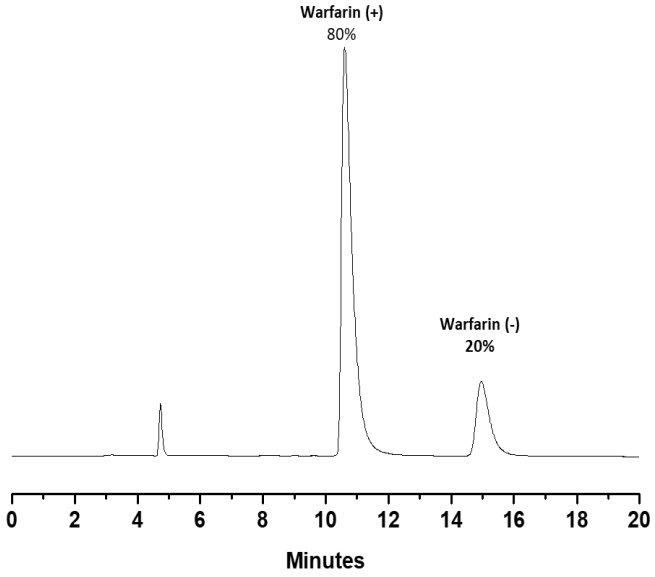


Figure S14. Bulk Reaction 10^{-2}M in ACN, 82°C, Catalyst *R,R*-DIPEDA (Table 1, entry 8, see article); *e.r.* warfarin (+)/(-) = 64/36

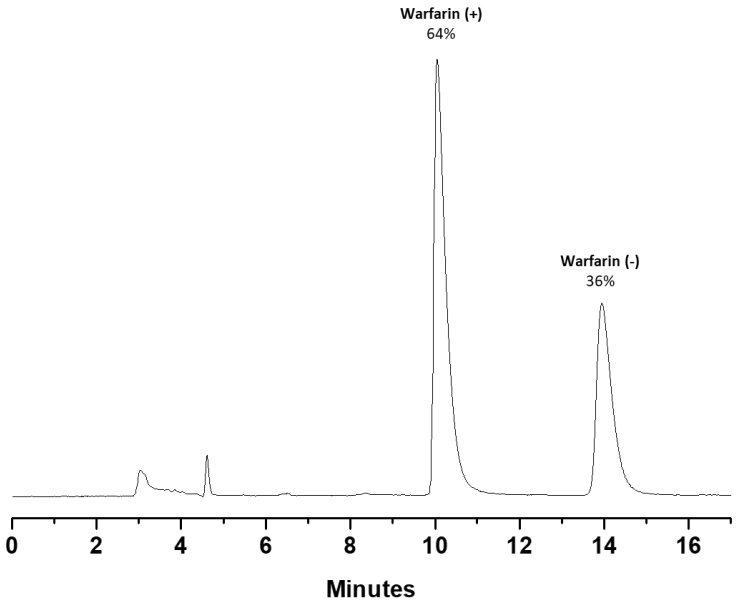


Figure S15. Microdroplet Reaction 10^{-2}M in ACN, System A, Catalyst *S,S*-DACH (Table 2, entry 1, see article); *e*.r. warfarin (+)/(-) = 23/77

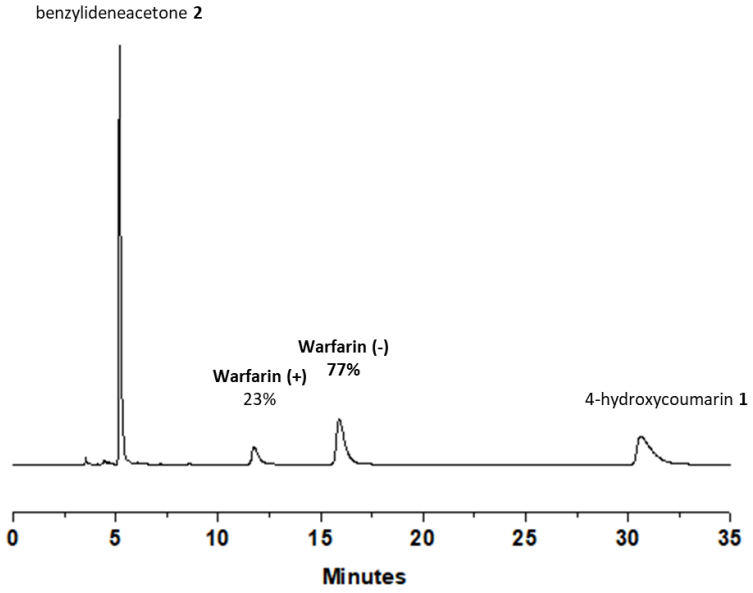


Figure S16. Microdroplet Reaction 10^{-2}M in ACN, System B, Catalyst *S,S*-DACH (Table 2, entry 2, see article); *e.r.* warfarin (+)/(-) = 38/62

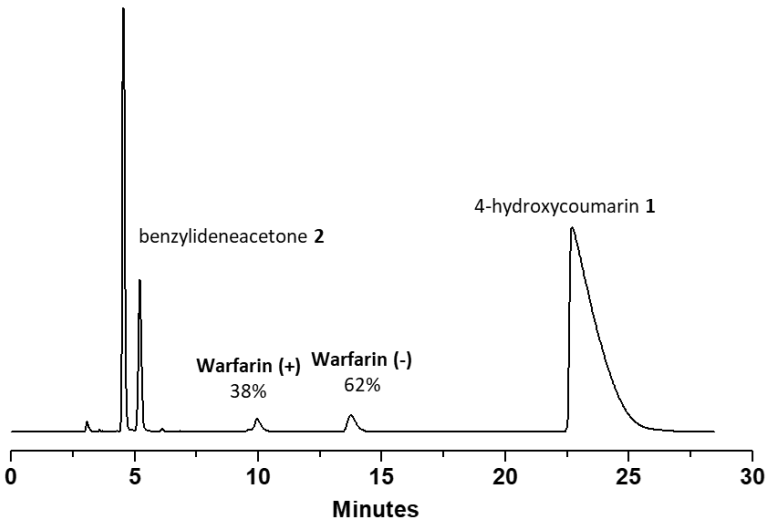


Figure S17. Microdroplet Reaction 10^{-2}M in ACN, System C, Catalyst *S,S*-DACH (Table 2, entry 3, see article); *e.r.* warfarin (+)/(-) = 25/75

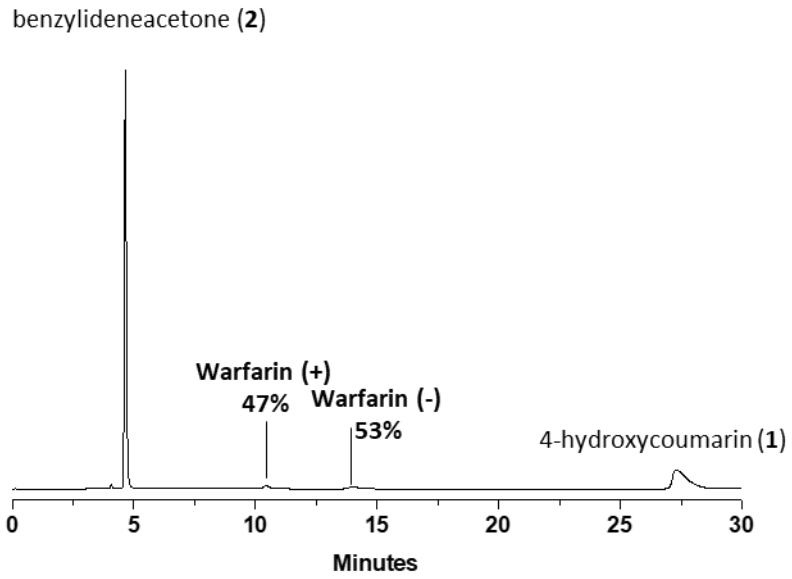


Figure S18. Microdroplet Reaction 10^{-2}M in ACN, System C – distance of the ESI source 8.5 cm from collection system. Catalyst *S,S*-DACH (Table 2, entry 4, see article); *e.r.* warfarin (+)/(-) = 47/53

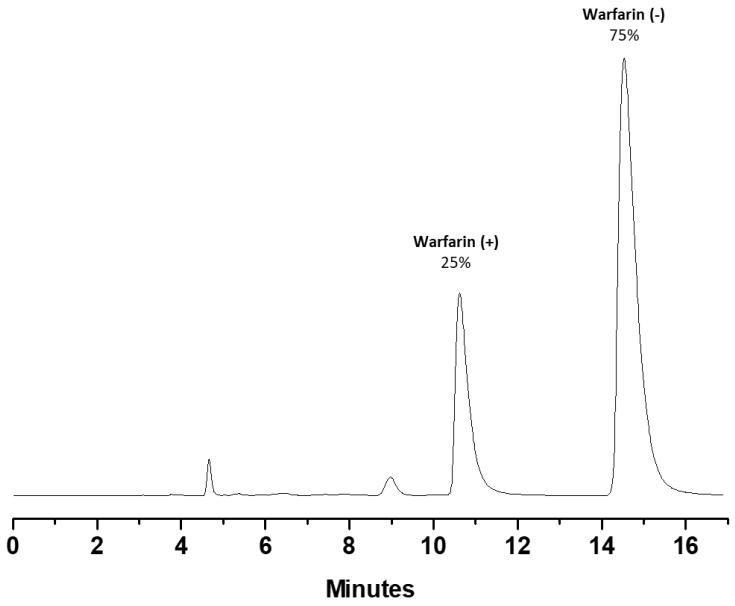


Figure S19. Microdroplet Reaction 10^{-2}M in ACN, System C – NO ESI. Catalyst *S,S*-DACH (Table 2, entry 6, see article); *e.r.* warfarin (+)/(-) = 31/69

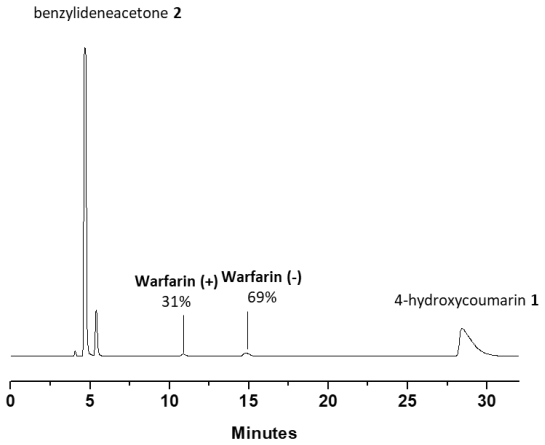


Figure S20. Microdroplet Reaction 10^{-2}M in ACN, System C, Catalyst *R,R*-DIPEDA (Table 2, entry 7, see article); *e.r.* warfarin (+)/(-) = 74/26

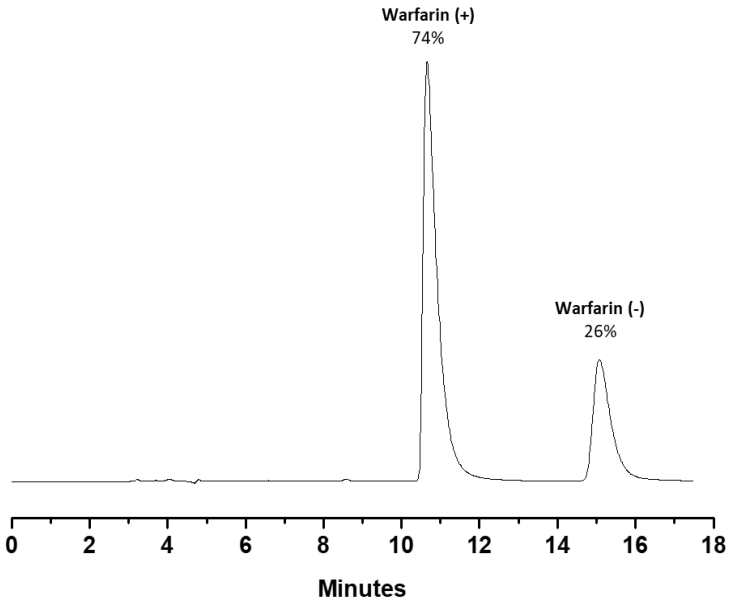


Figure S21. Microdroplet Reaction 10^{-2}M in ACN, System D, Catalyst *S,S*-DACH (Table 2, entry 8, see article); *e.r.* warfarin (+)/(-) = 25/75

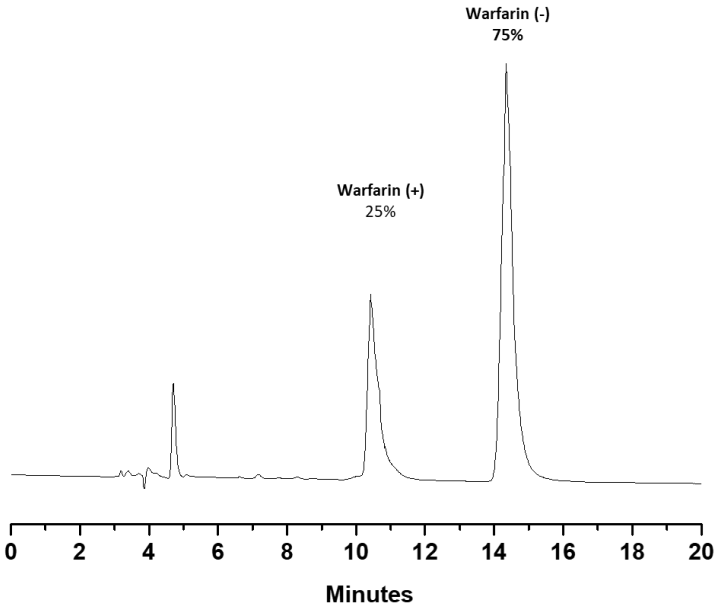


Figure S22. Microdroplet Reaction 10^{-2}M in ACN, System D, Catalyst *R,R*-DIPEDA (Table 2, entry 9, see article); *e.r.* warfarin (+)/(-) = 78/22

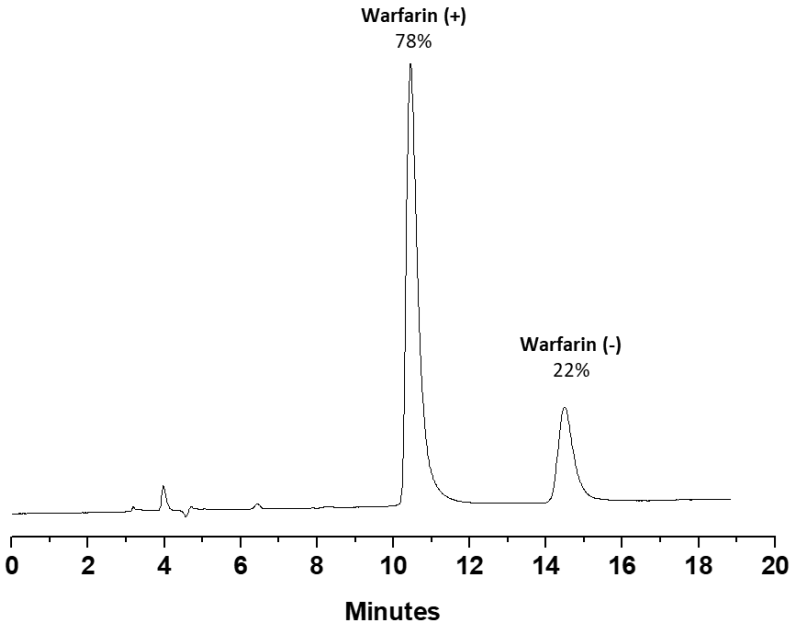


Figure S23. Microdroplet Reaction 10^{-2}M in ACN, System D, room temperature., Catalyst *R,R*-DIPEDA (Table 2, entry 10, see article); *e.r.* warfarin (+)/(-) = 76/24

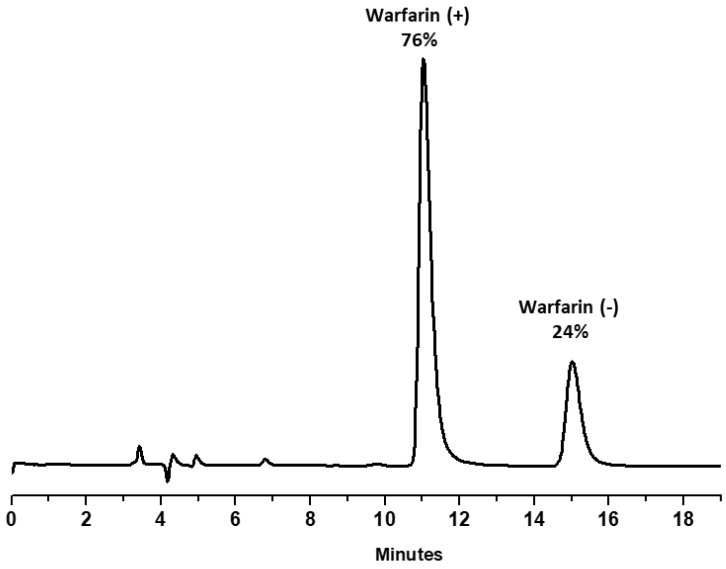


Figure S24. Microdroplet Reaction 10^{-2}M in ACN, System D – distance of the ESI source 1 cm from the collection system., Catalyst *R,R*-DIPEDA (Table 2, entry 11, see article); *e.r.* warfarin (+)/(-) = 77/23

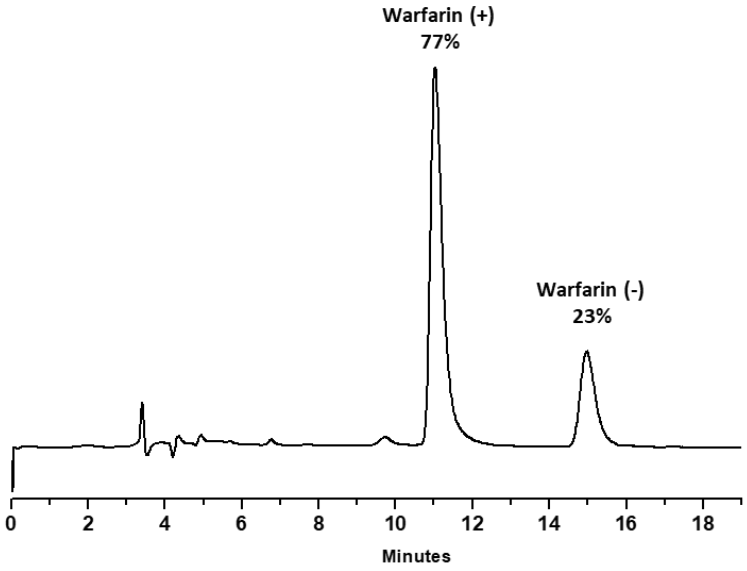


Figure S25. Microdroplet Reaction 10^{-2}M in ACN, System D – room temperature, distance of the ESI source 1 cm from the collection system., Catalyst *R,R*-DIPEDA (Table 2, entry 12, see article); *e.r.* warfarin (+)/(-) = 75/25

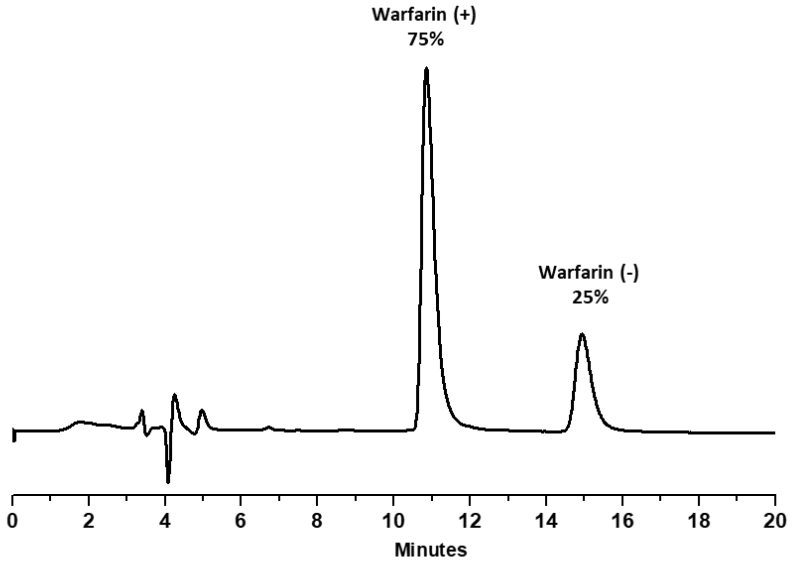
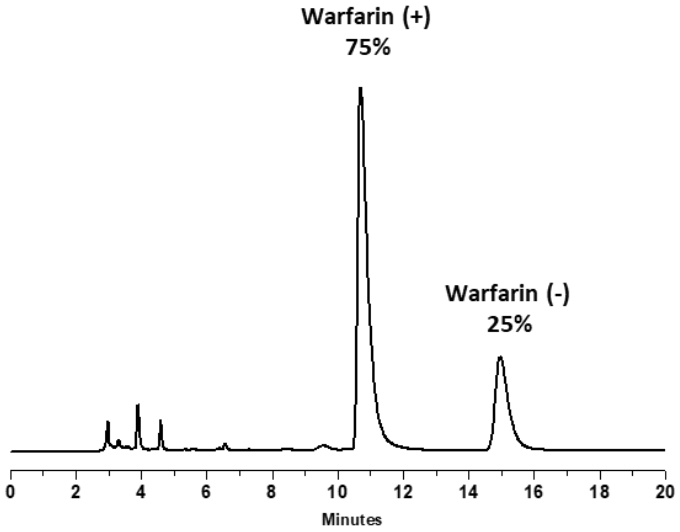


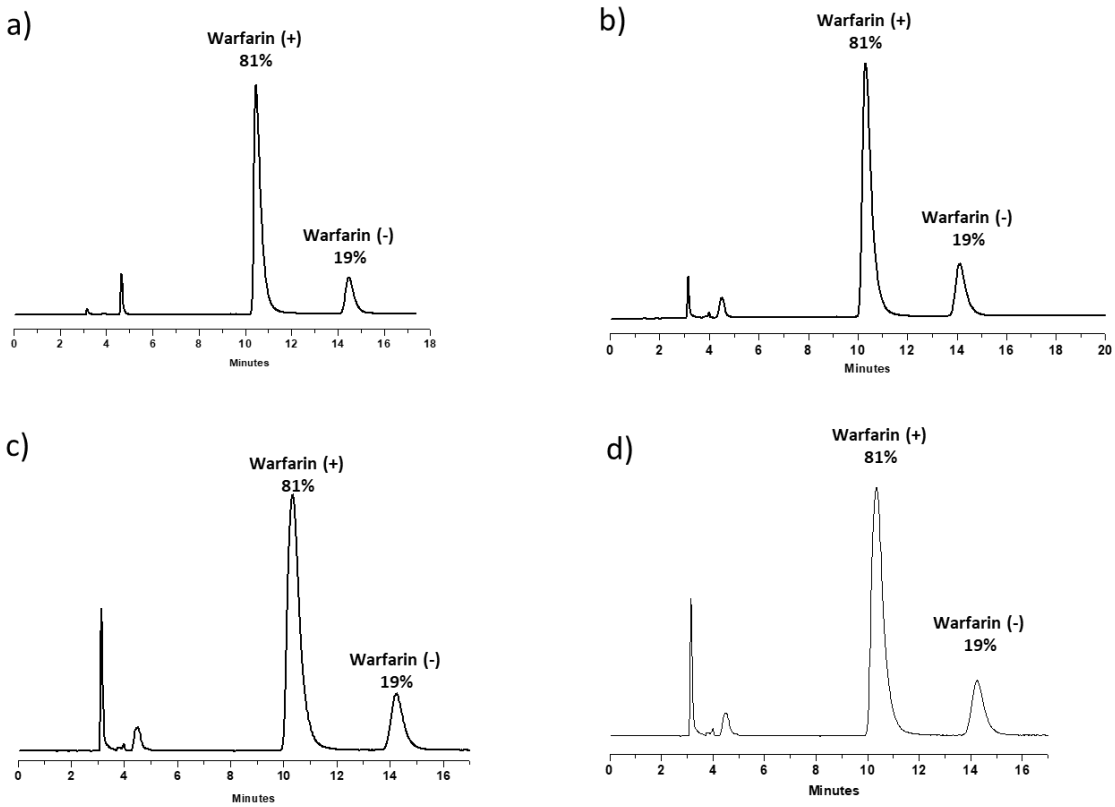
Figure S26. Microdroplet Reaction 10^{-2}M in ACN, System E, Catalyst *R,R*-DIPEDA (Table 2, entry 13, see article); *e.r.* warfarin (+)/(-) = 75/25



4 Racemization Experiments

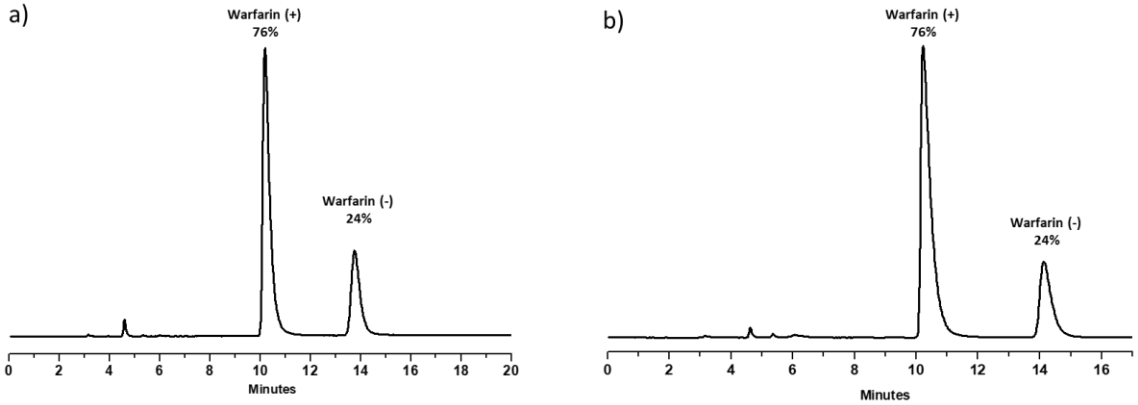
Racemization experiments in bulk conditions were performed on a scalemic mixture of pure warfarin (*r.e.* 81/19). The scalemic mixture of warfarin in CAN is left at reflux temperature for 5 hours. The chromatograms are registered on the product at room temperature, after 1 hour at 82 °C, after 3 hours at 82 °C and after 5 hours at 82 °C.

Figure S27. Chromatograms of a scalemic mixture of warfarin at r.t. (a), after 1 h at 82 °C (b), after 3h at 82 °C (c), after 5 h at 82 °C (d).



Racemization experiments in microdroplet conditions were performed on a scalemic mixture of pure warfarin (*r.e.* 76/24). The scalemic mixture of warfarin is dissolved in CAN and subjected to microdroplet conditions at 85 °C (desolvation gas temperature (DSG) setting at 150 °C). The collected product is analyzed by HPLC.

Figure S28. Chromatograms of a scalemic mixture of warfarin at r.t. (a) and after being subjected to microdroplet conditions at 85 °C (DSG = 150 °C) (b).



5 Mass Spectrometric experiments

Figure S29. ESI-(+) mass spectrum of the (**1**+**2**+**3**) solution submitted to reaction through the **System A**.

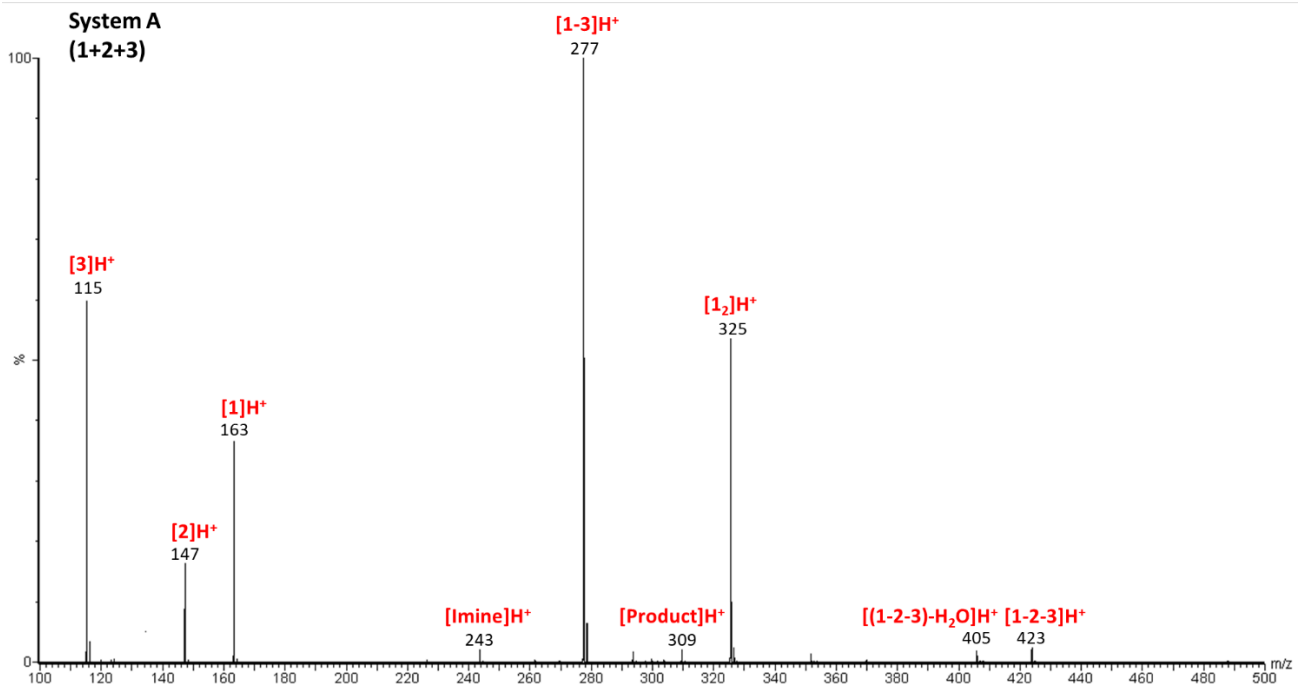


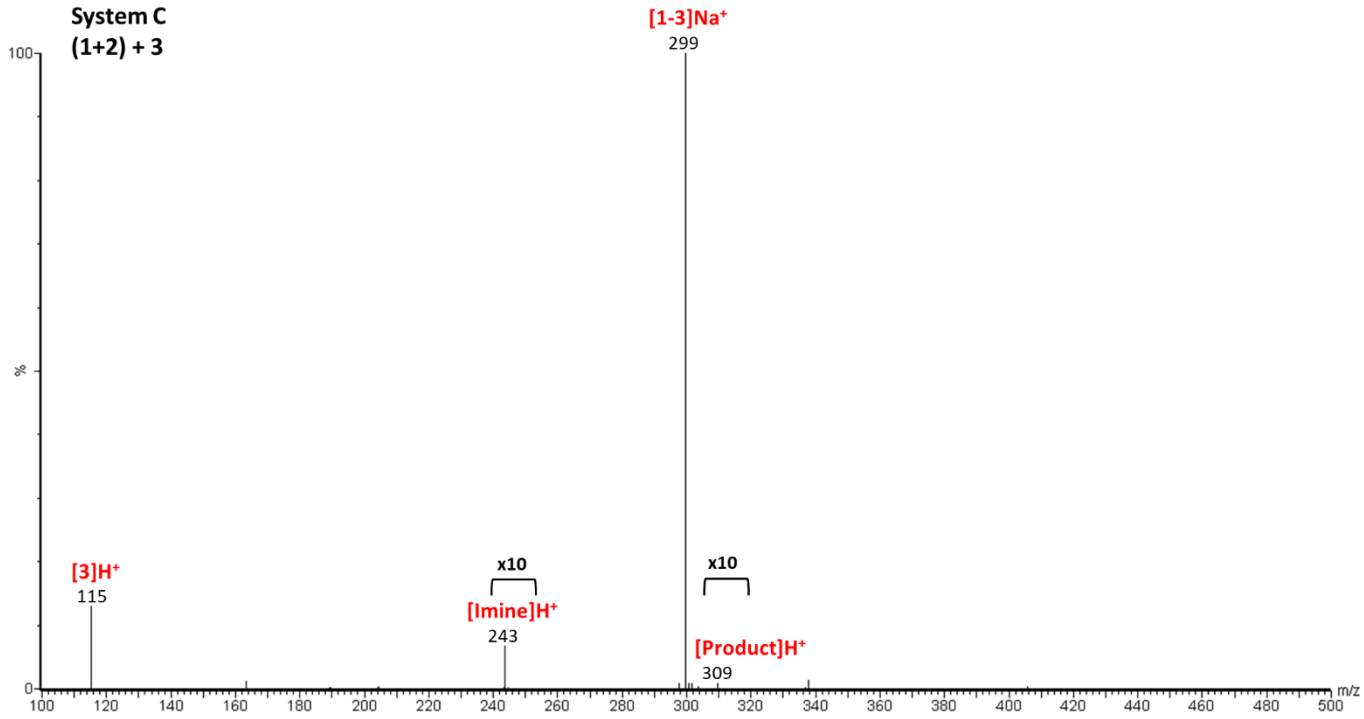
Figure S29 displays the positive electrospray mass spectrum (ESI-MS) recorded before the infusion of the (**1**+**2**+**3**) solution through the **System A**.

Major ionic peaks are assigned to the protonated catalyst **3** (*m/z* = 115), the protonated reactant **1** (*m/z* = 163) together with the corresponding dimer (*m/z* 325), the protonated adduct between **1** and **3** compounds (*m/z* = 277), and the protonated reactant **2** (m/z = 147).

Minor ion at *m/z* 243 is attributable to the imine species formed by the reaction of the reactant **2** with the catalyst **3**. This intermediate is notoriously involved in the reaction mechanism leading to the formation of the final product that is detected at a low intensity as the protonated species at *m/z* 309. Also in this case, it is not possible to exclude the contribution of the solution reaction in the formation of the final product.

To evaluate the effective contribution of the microdroplet environment in generating the reaction intermediate and then the final product, a mixture containing both **1** and **2** (**1**+**2**) was infused separately from a solution of the catalyst **3** through the **System C** (Figure S30).

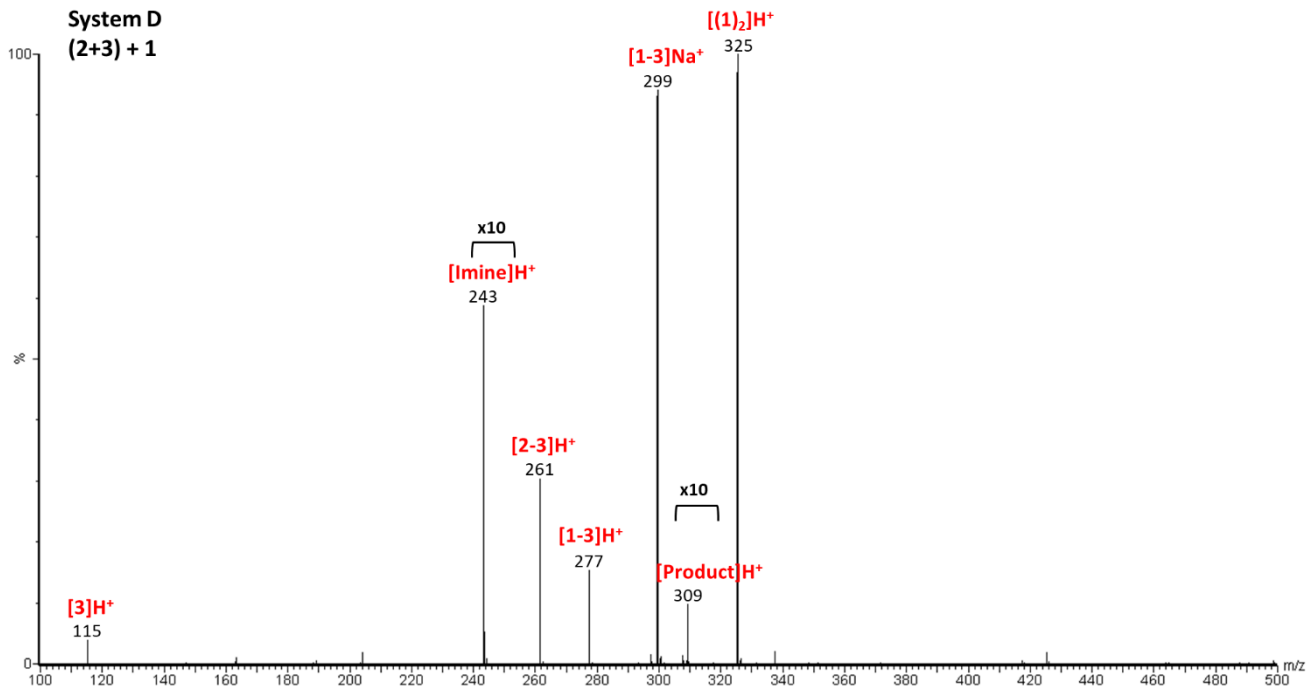
Figure S30. ESI-(+) mass spectrum of the (**1**+**2**) and **3** solutions submitted to reaction through the **System C**.



In this case, the MS can be considered representative exclusively of the reaction occurring in the microdroplet environment since the two reactants (**1** and **2**) encounter the catalyst only reaching the T junction. Therefore, the imine intermediate at *m/z* 243 is necessarily formed in the confined volume of the microdroplets generated during the electrospray process. Little amount of the protonated product at *m/z* 309 was also detected.

To improve the efficiency of the reaction, a mixture containing both **2** and **3** (**2**+**3**) was infused separately from a solution of the reactant **1** through the **System D** (Figure S31).

Figure S31. ESI-(+) mass spectrum of the (**2**+**3**) and **1** solutions submitted to reaction through the **System D**.



In this case, the imine intermediate at *m/z* 243 can be obtained not only from an accelerated reaction in the microdroplet environment, but also in solution during the residence time of the (**2**+**3**) mixture in the syringe used for the injection. The increase of the intermediate concentration because of the two parallel processes determines a predictable increase of the product detected at *m/z* 309. If the ionic intermediate can be formed from different pathways (solution + microdroplets), the final product only arises from a microdroplet reaction since the 4-hydroxycoumarine **1** encounters the imine intermediate only at the level of the T junction.