RSC Advances Supporting Information

Extending pharmacological dose-response curves for salsalate with natural deep eutectic solvents

E. Rozema[a,§], A.D. van Dam[b,§], H.C.M. Sips[b], R. Verpoorte[a], O.C. Meijer[b], S. Kooijman[b,#] and Y. H. Choi[a,#]

[a]*Natural Products Laboratory, Institute of Biology, Leiden University, 2333 BE Leiden, The Netherlands, E-mail: y.h.choi@biology.leidenuniv.nl*

[b]*Department of Medicine, Division of Endocrinology, Post Zone C7Q. Leiden University Medical Center, 2300 RC Leiden, The Netherlands, E-mail: s.kooijman@lumc.nl*

[§]*These authors contributed equally to this work*

[#]*These authors share last authorship*

Experimental section

Materials and methods

Solvent preparation

The following NADES were prepared: malic acid:choline chloride:water (molar ratio, 1:1:2, MCH), glucose:choline chloride:water (2:5:5, GCH), lactic acid:glucose:water (5:1:3, LGH), 1,2-propanediol:choline chloride:water (1:1:1, PCH), 1,2-propanediol:malic acid:water (1:1:3, PMH), fructose:glucose:sucrose:water (1:1:1:11, FGSH) and sucrose:choline chloride:water (1:4:4). The following NADES components were used DL-malic acid (≥ 99.9%), choline chloride (99%), sucrose (Fisher Scientific, Waltham, MA, USA), fructose, DL-lactic acid, D-glucose-monohydrate (Boom Laboratorium, Meppel, The Netherlands), DL-lactic acid and 1,2-propanediol (p.a. ≥ 99.5%) (Sigma, St. Louis, MO, USA). The components were weighed and brought in a bottle with cap with a stirring bar. The bottle was heated in a water bath at 50 °C and stirred until a clear liquid was formed.[1]

Solubility testings,

The solubility of salsalate was tested in the seven NADES mentioned, which was performed by adding 10 mg salsalate per mL NADES while stirring at room temperature and if not yet dissolved also in a water bath at 40 °C, and solubility visually inspected. The maximum solubility was determined by saturating PCH with an excess of salsalate in a bottle while stirring at 40 °C in a water bath for three hours and two hours of leaving for precipitation of undissolved compound.[1] Three replicates were diluted and the maximum solubility was determined by HPLC-DAD at 280 nm using a calibration curve.

Salsalate stability

Salsalate was dissolved to 25 mg/mL in DMSO (Biosolve, Valkenswaard, The Netherlands) and PCH and transferred to a HPLC vial and analyzed after preparation and after one and three months storage at room temperature. References of salicylate and methyl salicylate (Sigma) were dissolved to a concentration of 25 mg/mL and profiles compared with that of salsalate in PCH.

HPLC analysis

An HPLC-UV-DAD spectrometer was utilized (Agilent Technologies 1200 series, Santa Clara, California). A reversed phase Curosil PFP column (Phenomenex, Torrance, CA, USA) was used. 1 μL of sample was injected on the reversed column and an isocratic eluent, with methanol for HPLC (≥ 99.9 purity) (CHROMASOLV@, Sigma Aldrich) as solvent A (60%) and 1% aqueous acetic acid (Biosolve) as solvent B (40%), was applied for 15 minutes. The flow was set at 1.5 mL/min and signals were detected by photo diode array detection (DAD) at wavelengths of 220, 240, 254, 260, 280 and 320 nm.

Cell culture

T37i cells were cultured and differentiated as described before.[2] Differentiated cells were treated for 8 h with increasing volume percentage of dimethylsulfoxide (DMSO) or PCH, or with salsalate (SML0070, Sigma) dissolved in DMSO and PCH applied as 0.33% solution in culture medium.

Cell viability assay

Cell viability was measured using PrestoBlue® Cell Viability Reagent (A13261, Life Technologies) according to manufacturer's protocol.

Glycerol measurement

After 8 h of incubation with salsalate in DMSO or PCH supernatant was collected. Glycerol concentrations were assessed with an enzymatic assay (Instruchemie, Delfzijl, The Netherlands).

RNA purification and qRT-PCR

RNA was extracted from cells using Tripure RNA Isolation reagent (Roche) according to the manufacturer's protocol. Total RNA (1 μg) was reverse transcribed using Moloney Murine Leukemia Virus (M-MLV) Reverse Transcriptase (Sigma) for qRT-PCR according to the manufacturer's instructions to produce cDNA. Gene expression was analyzed using a Bio-Rad CFX96 thermocycler. Expression was normalized to β2-microglobulin and 36b4 mRNA content and expressed as fold change compared to control (DMSO without salsalate).

Data and statistical analysis

Statistical analysis was performed using the Graphpad Prism Software. EC50 95% confidence intervals were calculated and statistical significance between DMSO and PCH was determined by t-tests.

References

1 Y. Dai, J. van Spronsen, G. J. Witkamp, R. Verpoorte and Y. H. Choi, *Anal. Chim. Acta*, 2013, 766, 61-68.

2 M. C. Zennaro, D. Le Menuet, S. Viengchareun, F. Walker, D. Ricquier and M. Lombes, *J. Clin. Invest.*, 1998, 101, 1254-1260.

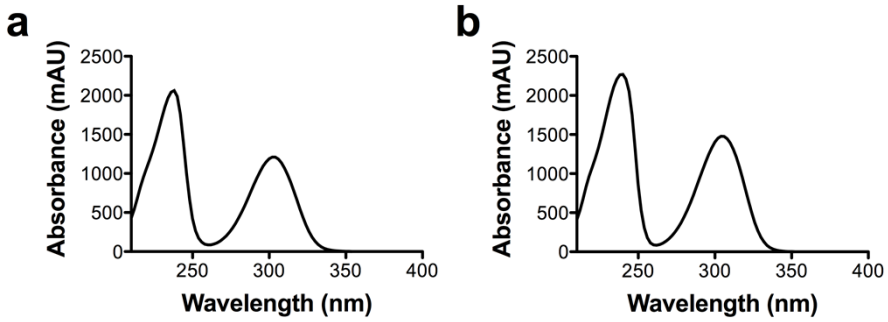

Fig S1. UV visible spectra of peaks of salicylate (5.0 min.) (a) and methylsalicylate (5.0 min) (b) dissolved in PCH, both with maximum absorbance at 238 and 302 nm.

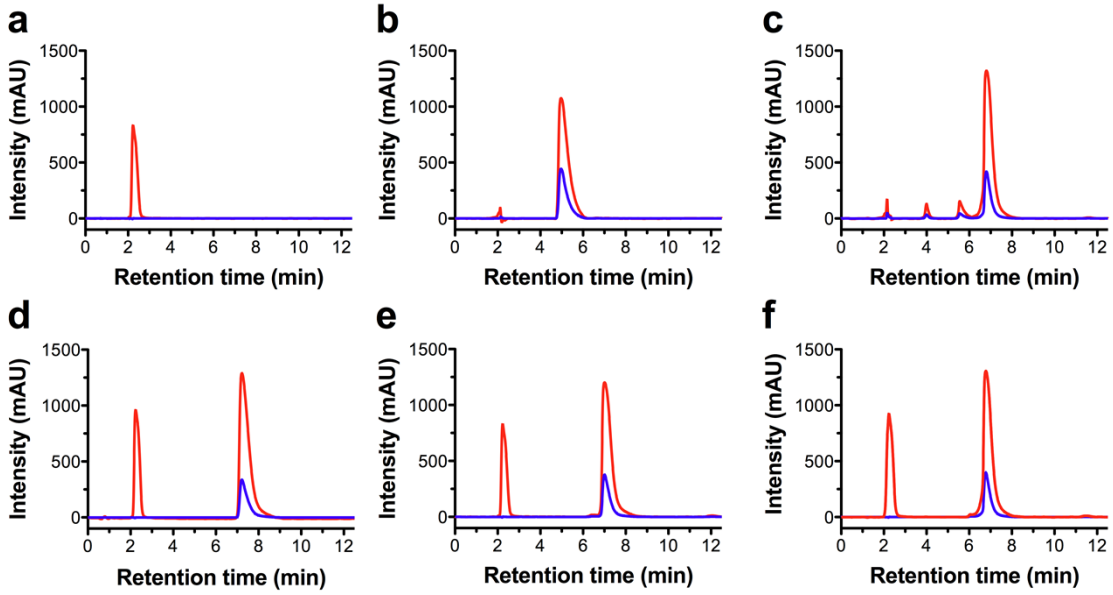

Fig. S2. HPLC spectra of DMSO (a), PCH (b), salsalate dissolved in PCH (25 mg/mL) after three months storage (c), salsalate in DMSO (25 mg/mL) after preparation (d) and after one (e) and three (f) months storage at room temperature. Detection at 220 nm (red), 280 nm (blue).