Differentiation of Enantiomers in Complex Systems by NMR Spectroscopy and Chiral Solvating Agents (CSA) : Applications and Methodology

SeRMN

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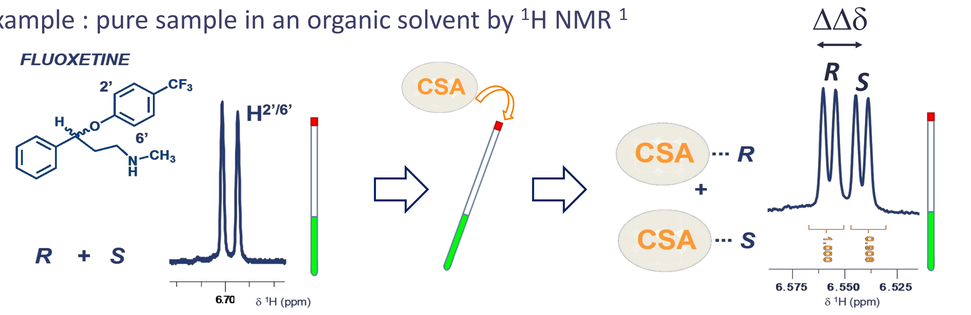
INTRODUCTION

Complex Systems

- ✓ Mixture of compounds:
  - · body fluids (urine, plasma, …)
  - · extracts of plants/tissues
  - · crude of reactions
- ✓ Pure enantiomeric mixtures with complex ^{1}H spectrum

Enantiodifferentiation by CSA & NMR Spectroscopy

Example : pure sample in an organic solvent by ^{1}H NMR [1]



Enantiodifferentiation of racemic fluoxetine in $CDCl_3$ using ABTE-18 as CSA

Advantages

- ✓ Simple
- ✓ Fast
- ✓ Robust
- ✓ No derivatization
- ✓ No purification

Fields of Application

- ✓ Pharmacology
- ✓ Chiral Metabonomics [2]
- ✓ Natural Products
- ✓ Toxicity Studies
- ✓ …

CHIRAL METABONOMICS [2] - NEW APPLICATION

Towards Enantiospecific Metabolic Profiling

Metabolic Profile: *"description of the small molecule composition of a biological sample"*

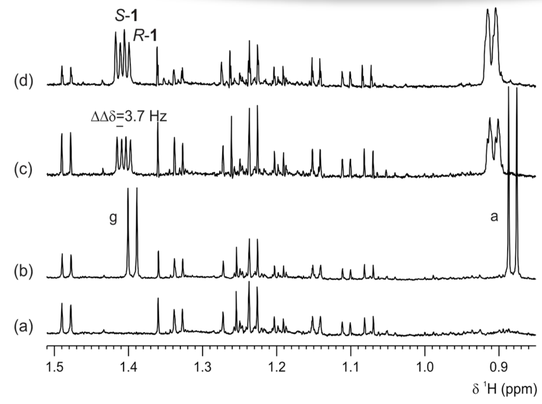
Outline of Analysis Strategy

Most of endog./exog. metabolites are chiral molecules

Does not differentiate enantiomers of chiral metabolites

BLIND TO POTENTIAL ENANTIOMERIC RATIO DIFFERENCES

Enantiodifferentiation of *R,S*-ibuprofen within human urine by ^{1}H NMR and a CSA



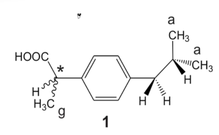
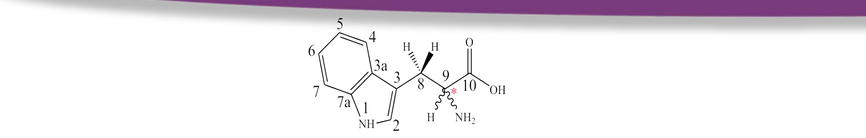


Figure 1. Expanded region of ^{1}H NMR spectra of (a) human urine of a healthy volunteer, (b) after the addition of racemic ibuprofen **1**, (c) the former sample in presence of βCD, (d) after spiking sample (c) with *S*-**1**. Spectra were acquired at 300.0 K at a magnetic field strength of 14.1 T.

^{13}C NMR [3]: OVERCOME THE OVERLAPPING PROBLEM - METHODOLOGY

Enantiodifferentiation by ^{13}C NMR & CSAs



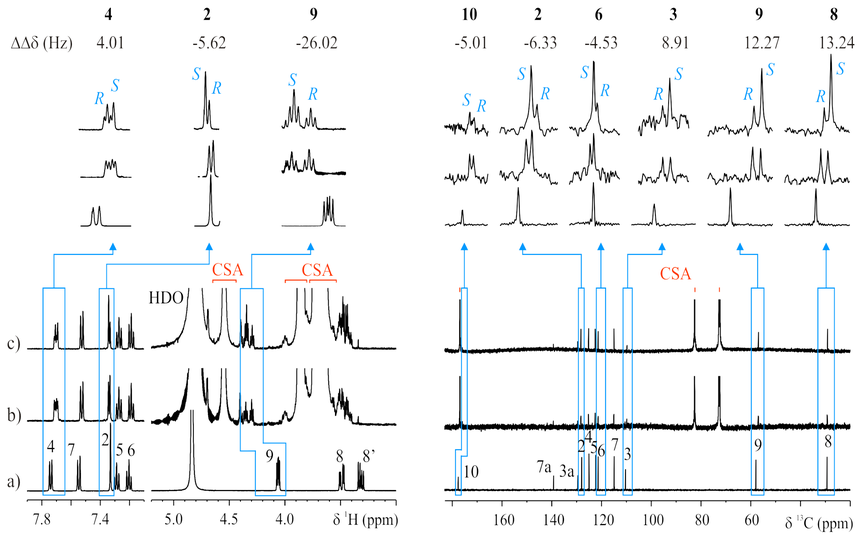


Figure 2. a) 2.3 mM Racemic TRP in D_2O; b) with 5.4 equivalents of 18C6TCA added and c) after spiking the sample with L-TRP. Experiments performed in a 500 MHz spectrometer equipped with a TCI cryoprobe.

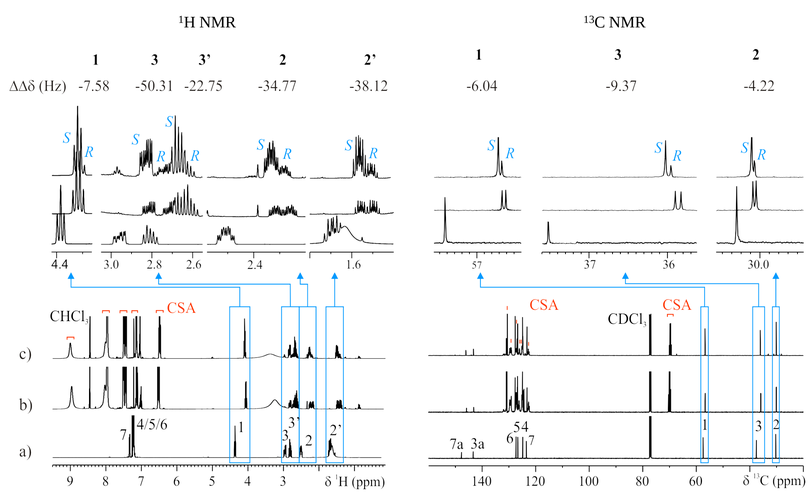


Figure 3. a) 50 mM Racemic AMI in $CDCl_3$; b) with 4.5 equivalents of *R*-PA added and c) after spiking the sample with *S*-AMI. Experiments performed in a 500 MHz spectrometer equipped with a TCI cryoprobe.

R/S Molar Ratio Measurement

Table 1. Theoretical and measured (by the ratio of the signal integrals) *R/S* molar ratio values of mixtures of *R,S*-AMI. Measured values correspond to three different experiments: 1D ^{1}H (zg, r.d. 2s), 1D ^{13}C with NOE contribution (zgpg, r.d. 2 s) and 1D ^{13}C without NOE contribution (zgig, r.d. 8 s). The observational error for each measurement in percentage is indicated. The three mixtures were prepared from a 50 mM racemic AMI solution and the CSA used was 4,5 equivalents of *R*-PA.

Theoretical *S/R* ratio[a] (*R:S*)	Measured *R/S* ratio and Error[b]							
	^{1}H			^{13}C				
				pg			*ig*	
	H atom	meas.	error (%)	C atom	meas.	error (%)	meas.	error (%)
1 (50:50)	H-1	0,98	1,62 *	C-1	1,01	1,25	1,00	0,11
	H-2	1,01	1,28 *	C-2	1,00	0,35	1,01	0,99
	H-2'	0,97	2,92 *	C-3	1,00	0,31	0,99	0,71
	H-3	0,77	22,90 **p	C-3a	0,98	2,40	1,03	2,66
	H-3'	-	- */**					
3 (25:75)	H-1	3,08	2,50 *	C-1	3,00	0,14	3,00	0,03
	H-2	4,34	44,57 *	C-2	3,00	0,05	3,03	1,00
	H-2'	3,38	12,73 *	C-3	3,06	1,98	3,02	0,62
	H-3	-	- **	C-3a	2,91	2,87	3,10	3,20
	H-3'	-	- */**					
9 (10:90)	H-1	6,98	22,44 *	C-1	9,05	0,57	8,81	2,10
	H-2	11,11	23,41 *	C-2	8,92	0,90	8,95	0,58
	H-2'	10,11	12,30 *	C-3	8,97	0,29	9,01	0,11
	H-3	-	- **	C-3a	- *		- *	
	H-3'	12,58	39,80 *					

[a] From weighted values

[b] Observational error (fm-fr)*100/fr

*Signal partially split

**(p) Signal (partially) overlapped with another signal of the spectrum

CONCLUSIONS

There is an interest in the differentiation (and identification) of enantiomers in complex systems, such as complex mixtures. This is the case of enantiodifferentiation studies of biological samples. Recently, a new area in metabonomics was described named under "Chiral Metabonomics", in which enantiomeric molecules are differentiated in a metabolic profile; the experiment was conducted using a CSA and ^{1}H NMR spectroscopy.[2]

The differentiation of enantiomers in complex samples by ^{1}H NMR is often impeded by overlapping, due to the small δ range of proton and to the multiplicity of the signals. Though experimental times are longer than for ^{1}H, observing ^{13}C nuclei is a convenient information-rich alternative in many situations and particularly when studying complex systems, since 1D {^{1}H} ^{13}C NMR experiment:

- overcomes the main overlapping drawback of the ^{1}H NMR experiment due to the intrinsic high dispersion of the ^{13}C and to the easiness of obtaining a proton decoupled spectrum.
- provides valuable/complementary information to the ^{1}H NMR experiment and extends the possibilities of enantiodifferentiation to fully deuterated and nonproton containing compounds.
- has a huge potential for the enantiomeric study of complex mixtures (Chiral Metabonomics).

Download the poster here

[1] Pérez-Trujillo, M.; Virgili, A., Tetrahedron-Asymmetr. **2006**, *17*, 2842-2846. [2] Pérez-Trujillo, M. ; Lindon, J.C.; Parella, T., Keun, H.; Nicholson, J.K.; Athersuch, T.J. Anal. Chem. **2012**, *84*, 2868-2874. [3] Pérez-Trujillo, M. ; Monteagudo, E.; Parella, T. **2013** *Submitted.*

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