Εφαρμογές Φασματοσκοπίας Πυρηνικού Μαγνητικού Συντονισμού στη Φαρμακευτική Χημεία

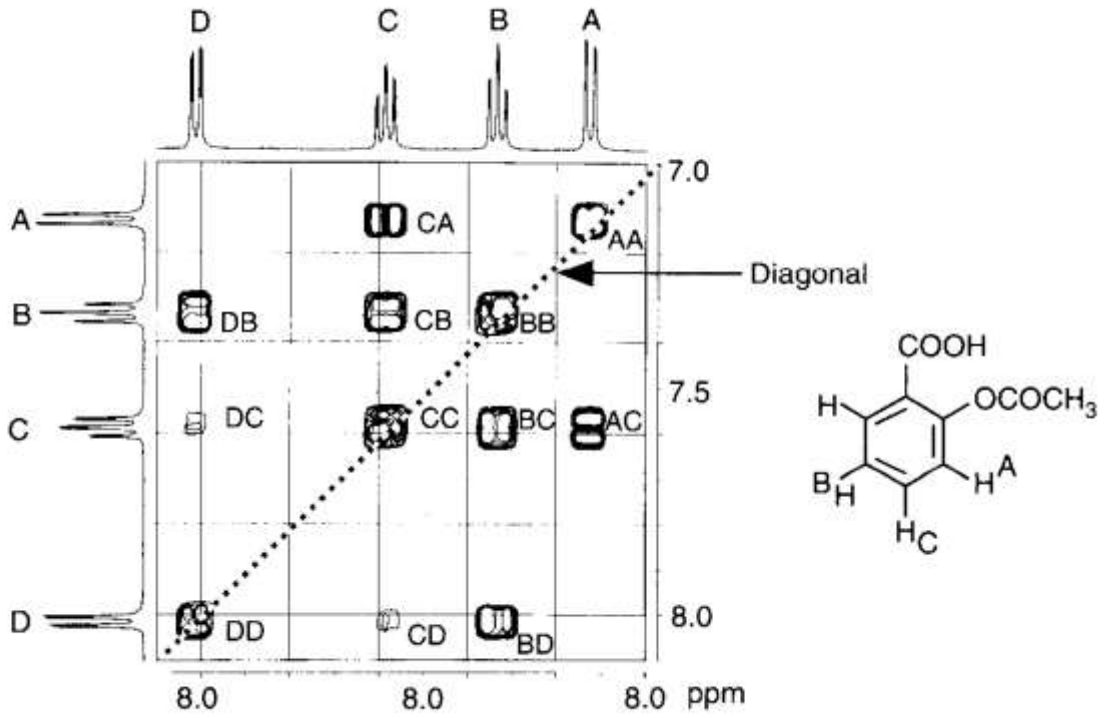
Α. Σπύρος, Εργαστήριο NMR
Ι. Λυκάκης, Εργ. Οργ. Χημείας

aspyros@chemistry.uoc.gr
lykakis@chem.auth.gr

Στα πλαίσια του προγράμματος ΠΕΓΑ

Φασματοσκοπία 2D NMR

Fig. 8.17
The proton–proton correlation spectrum of the aromatic region of aspirin.



Φάσμα ^{13}C NMR

An example of a ^{13}C spectrum

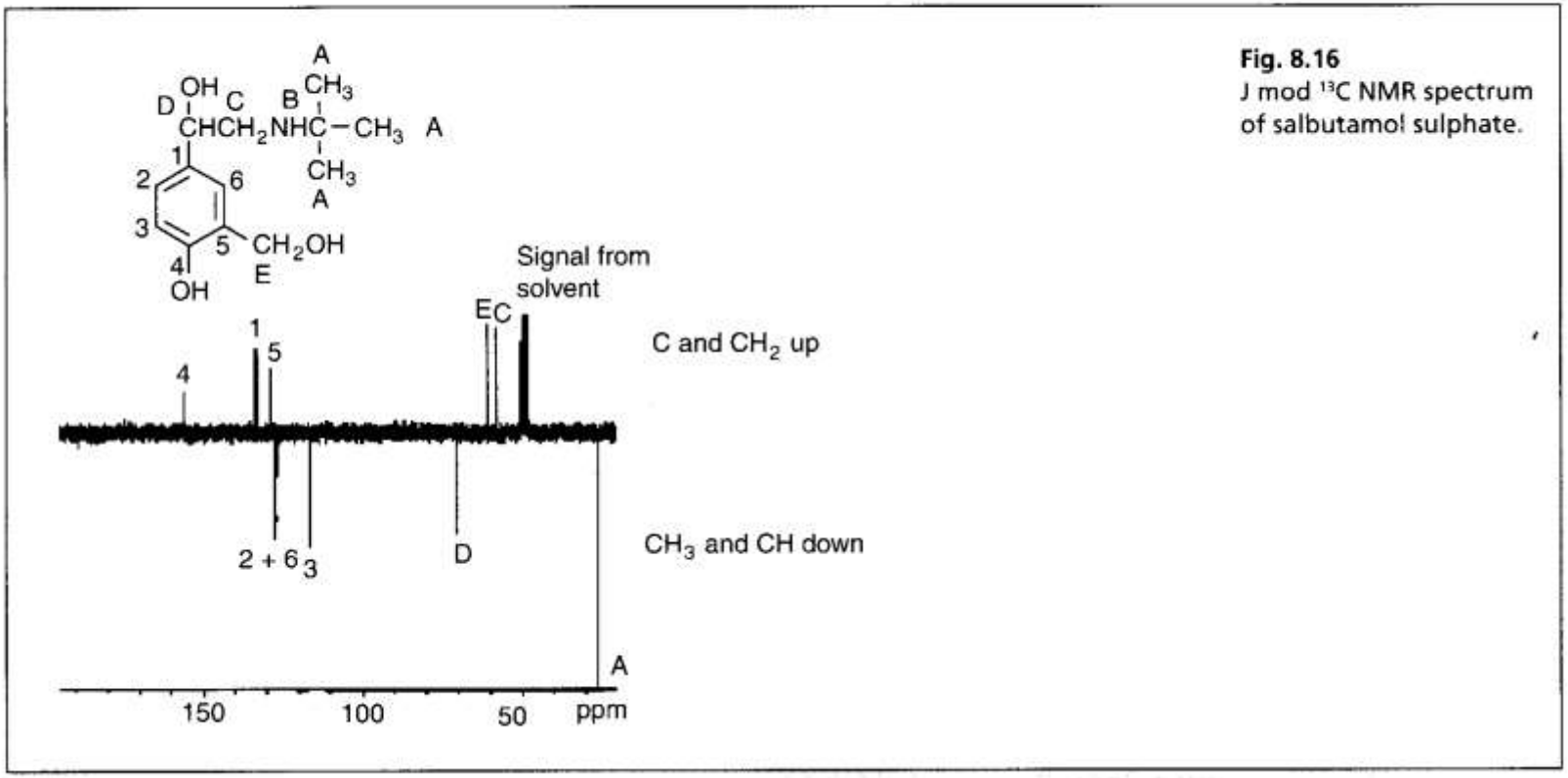


Fig. 8.16
J mod ^{13}C NMR spectrum of salbutamol sulphate.

Σύσταση αναλγητικής ταμπλέτας

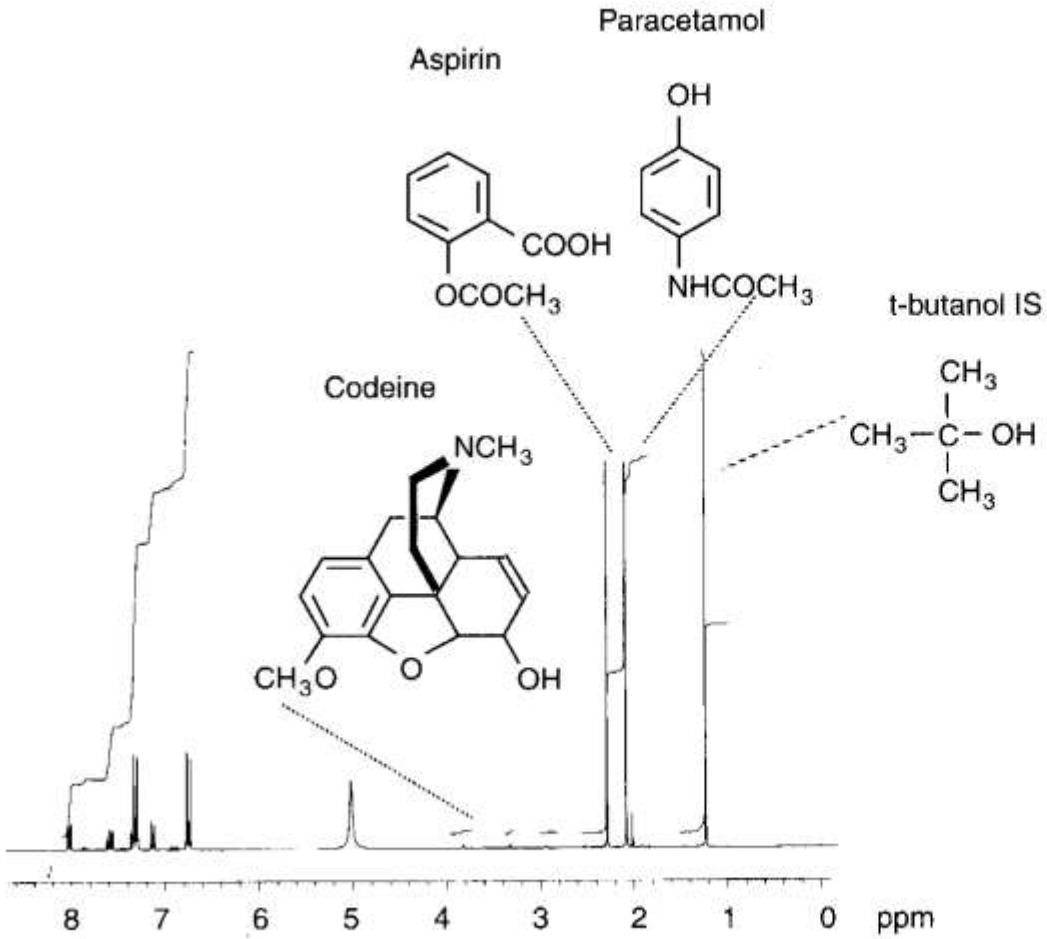


Fig. 8.19
NMR spectrum obtained from a tablet containing aspirin, paracetamol and codeine with 8 mg of t-butanol added as an internal standard.

Ποσοτική ανάλυση με τη Φασματοσκοπία NMR

- Stated content/tablet = aspirin 250 mg, paracetamol 250 mg, codeine phosphate 6.8 mg
- Weight of 1 tablet = 0.6425 g
- Weight of tablet powder taken for analysis = 0.1228 g
- Weight of t-butanol internal standard added = 8.0 mg
- Area of internal standard peak = 7.2
- Area of aspirin CH_3 peak = 5.65
- Area of paracetamol CH_3 peak = 6.73
- Codeine phosphate CH_3 peak = 0.115
- MW t-butanol = 74.1
- MW aspirin = 180.2
- MW paracetamol = 151.2
- MW codeine phosphate = 397.4
- Number of protons in t-butyl group = 9
- Number of protons in methyl groups of aspirin, paracetamol and codeine = 3.

$$\text{Amt. of drug} = \frac{\text{Area signal for drug protons}}{\text{Area signal for int. std. protons}} \times \text{mass of int. std. added} \times \frac{\text{MW drug}}{\text{MW int.std.}} \times \frac{\text{No. protons from int. std.}}{\text{No. protons from drug}}$$

Calculation example 8.2

Weight of aspirin and paracetamol expected in the tablet powder $= 250 \times \frac{0.1228}{0.6425} = 47.97$ mg

Weight of codeine expected in the tablet powder $= 6.8 \times \frac{0.1228}{0.6425} = 1.300$ mg

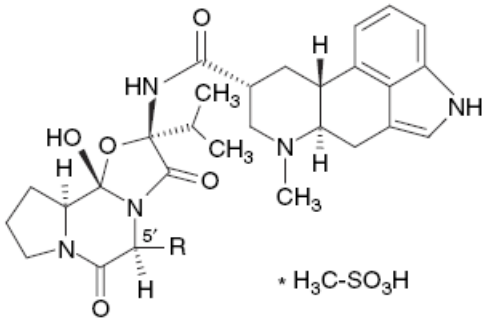
Calculation for aspirin

Substituting into the formula given above:

mg of aspirin present in extract $= \frac{5.65}{7.2} \times 8 \times \frac{180.2}{74.1} \times \frac{9}{3} = 45.80$ mg

Percentage of stated content $= \frac{45.8}{47.97} \times 100 = 95.48\%$

Hydergina, γεροντική άνοια



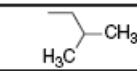
	—R
Dihydroergocornine	
Dihydroergocristine	
α-Dihydroergocryptine	
β-Dihydroergocryptine	

Figure 4 Structural formula of codergocrine mesylate.

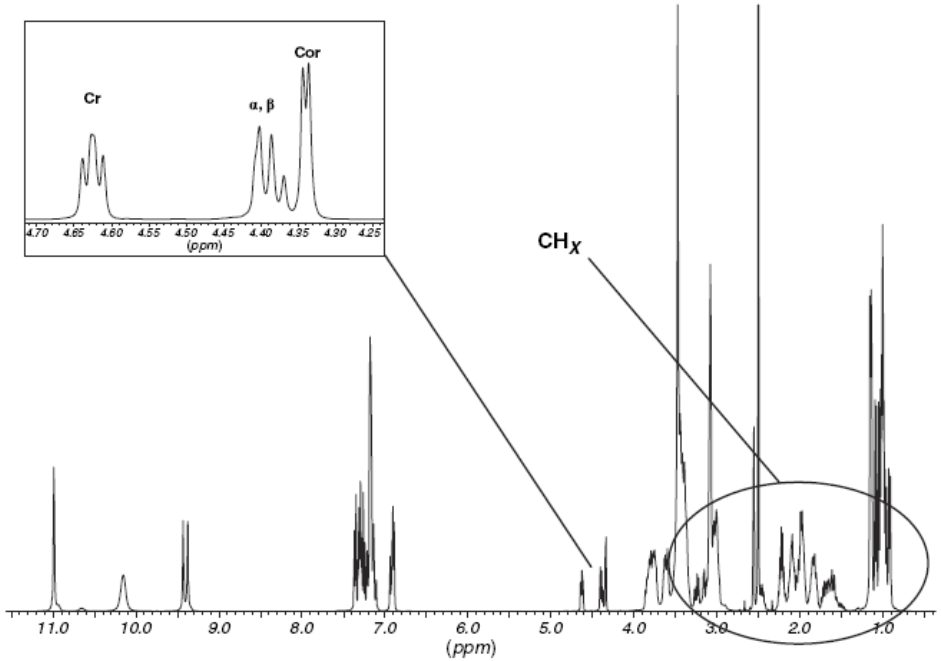
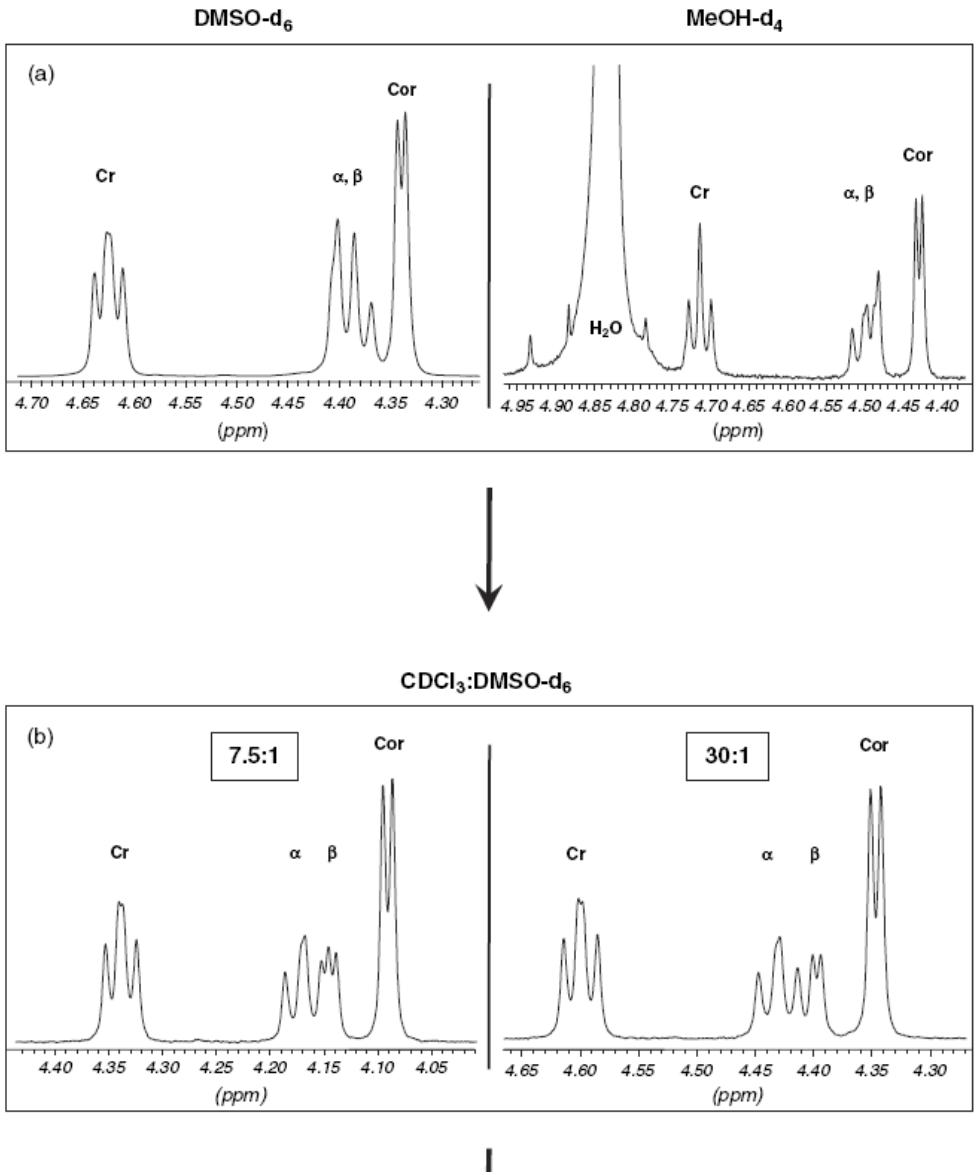
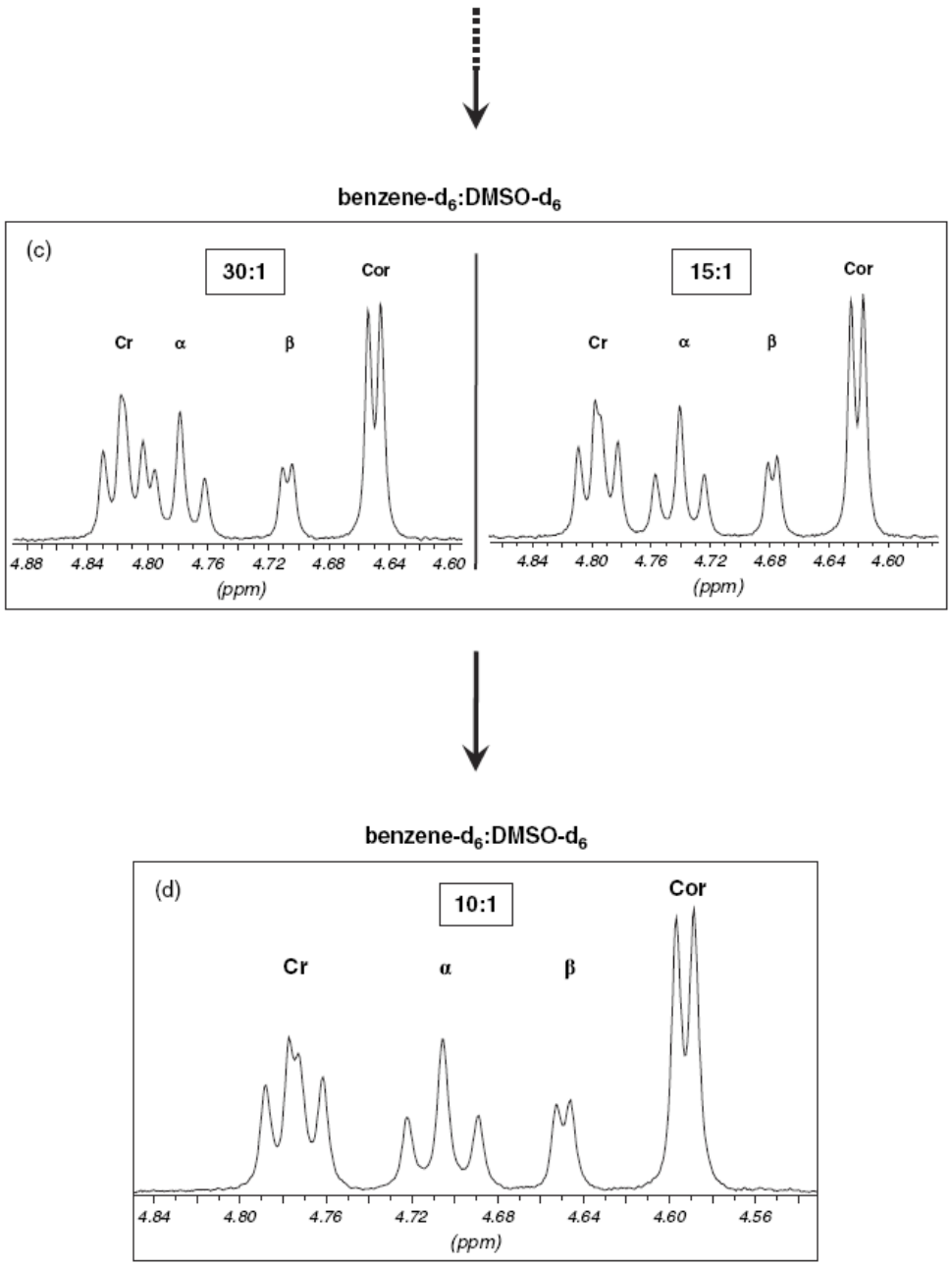
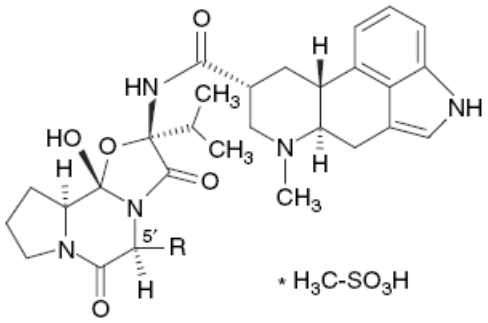


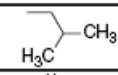
Figure 5 ^{1}H NMR spectrum of codergocrine mesylate in DMSO-d_6 (400 MHz). The inset shows an expanded view of the H5′ region which contains the signals of dihydroergocristine (Cr), α- and β-dihydroergocryptine (α, β), and dihydroergocornine (Cor).


DMSO-d6
MeOH-d4
(a)
Cr
α, β
Cor
4.70 4.65 4.60 4.55 4.50 4.45 4.40 4.35 4.30
(ppm)
H2O
Cr
α, β
Cor
4.95 4.90 4.85 4.80 4.75 4.70 4.65 4.60 4.55 4.50 4.45 4.40
(ppm)
CDCl3:DMSO-d6
(b)
7.5:1
Cr
α
β
Cor
4.40 4.35 4.30 4.25 4.20 4.15 4.10 4.05
(ppm)
30:1
Cr
α
β
Cor
4.65 4.60 4.55 4.50 4.45 4.40 4.35 4.30
(ppm)


benzene-d6:DMSO-d6
(c)
30:1
Cor
Cr
α
β
15:1
Cor
Cr
α
β
4.88 4.84 4.80 4.76 4.72 4.68 4.64 4.60
(ppm)
4.84 4.80 4.76 4.72 4.68 4.64 4.60
(ppm)
benzene-d6:DMSO-d6
(d)
10:1
Cor
Cr
α
β
4.84 4.80 4.76 4.72 4.68 4.64 4.60 4.56
(ppm)

^{13}C NMR



	—R
Dihydroergocornine	
Dihydroergocristine	
α-Dihydroergocryptine	
β-Dihydroergocryptine	

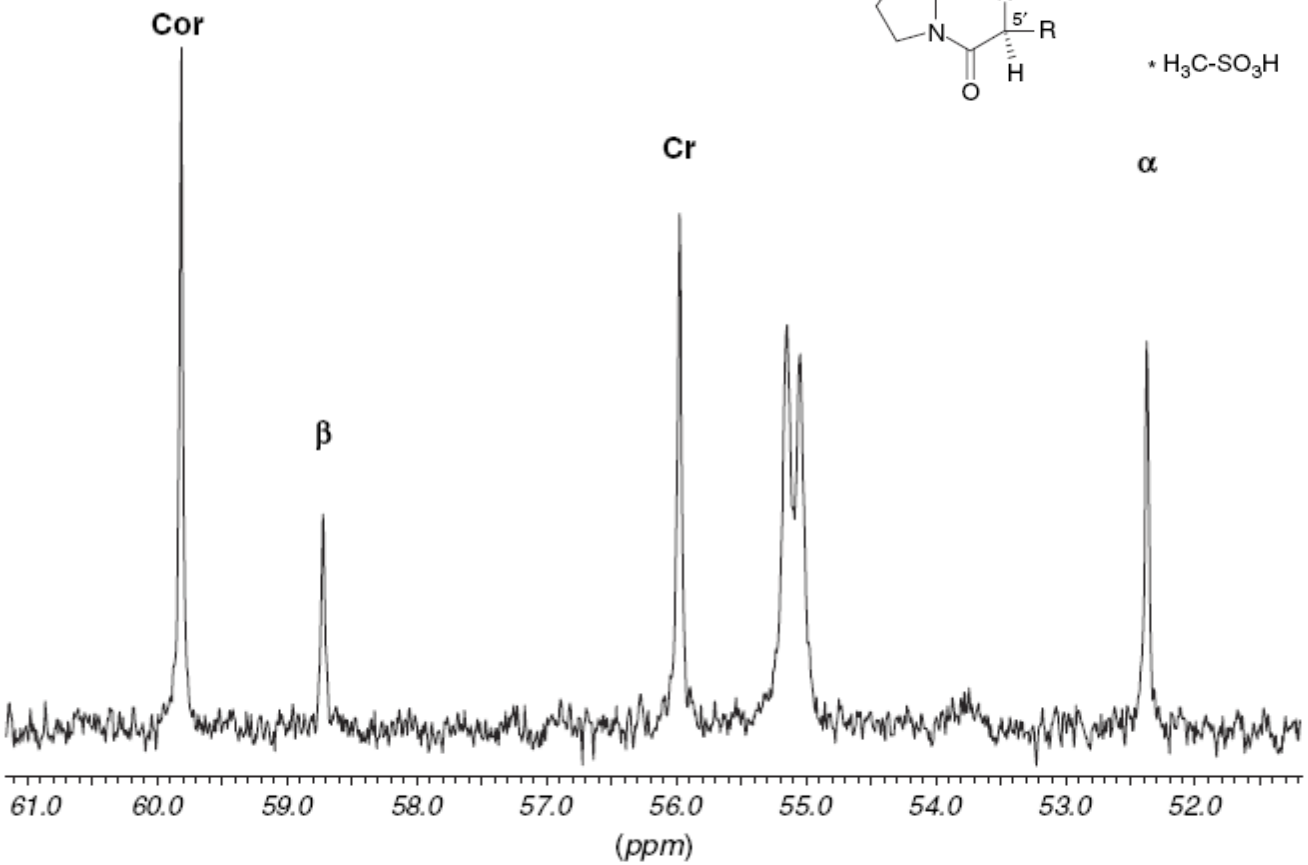


Figure 7 C5′ region of the ^{13}C NMR spectrum of codergocrine mesylate in DMSO-d_6 (400 MHz).

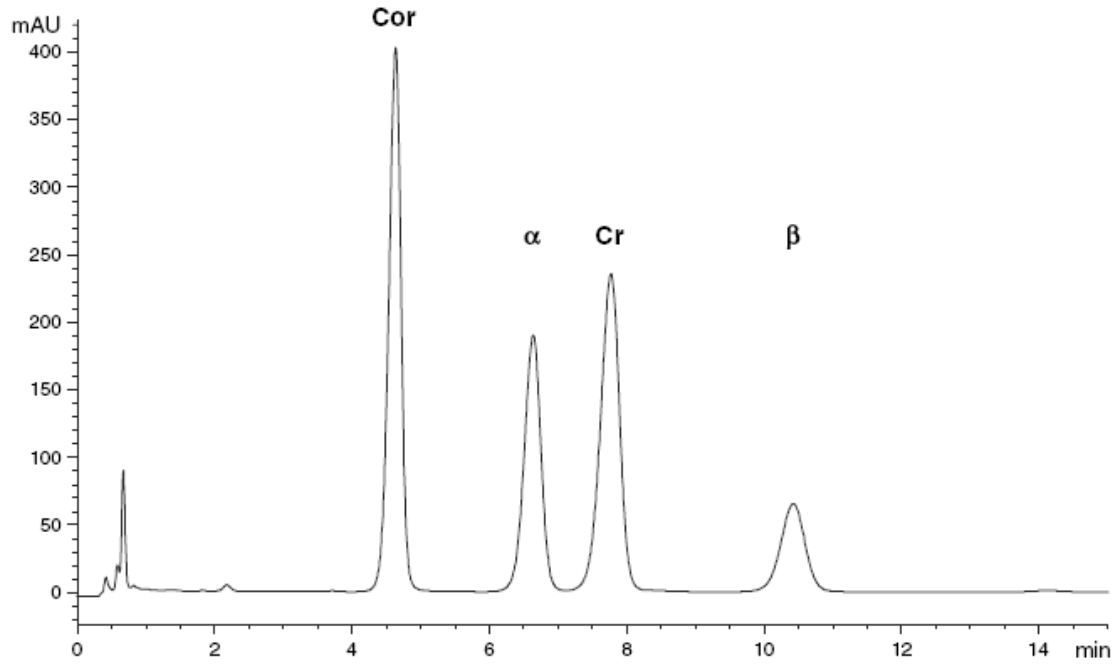


Figure 8 HPLC chromatogram of codergocrine mesylate obtained by the EP method.[18]

Table 2 Results obtained by the four applied methods – for each component of codergocrine mesylate, the relative amount is given in mass percentage: dihydroergocornine (Cor), dihydroergocristine (Cr), α-dihydroergocryptine (α), and β-dihydroergocryptine (β).

	Cor (%)	Cr (%)	α (%)	β (%)
HPLC PhEur 6.0[18]	34.11 ± 0.09	32.76 ± 0.10	22.15 ± 0.04	10.98 ± 0.04
HPLC USP 30[19]	34.00 ± 0.11	32.85 ± 0.08	22.33 ± 0.11	10.83 ± 0.13
^{1}H NMR	33.57 ± 0.08	32.67 ± 0.04	22.80 ± 0.06	10.96 ± 0.06
^{13}C NMR	33.79 ± 0.27	32.97 ± 0.49	22.20 ± 0.20	11.04 ± 0.16

Ανάλυση σκευάσματος που περιέχει PVP ως έκδοχο

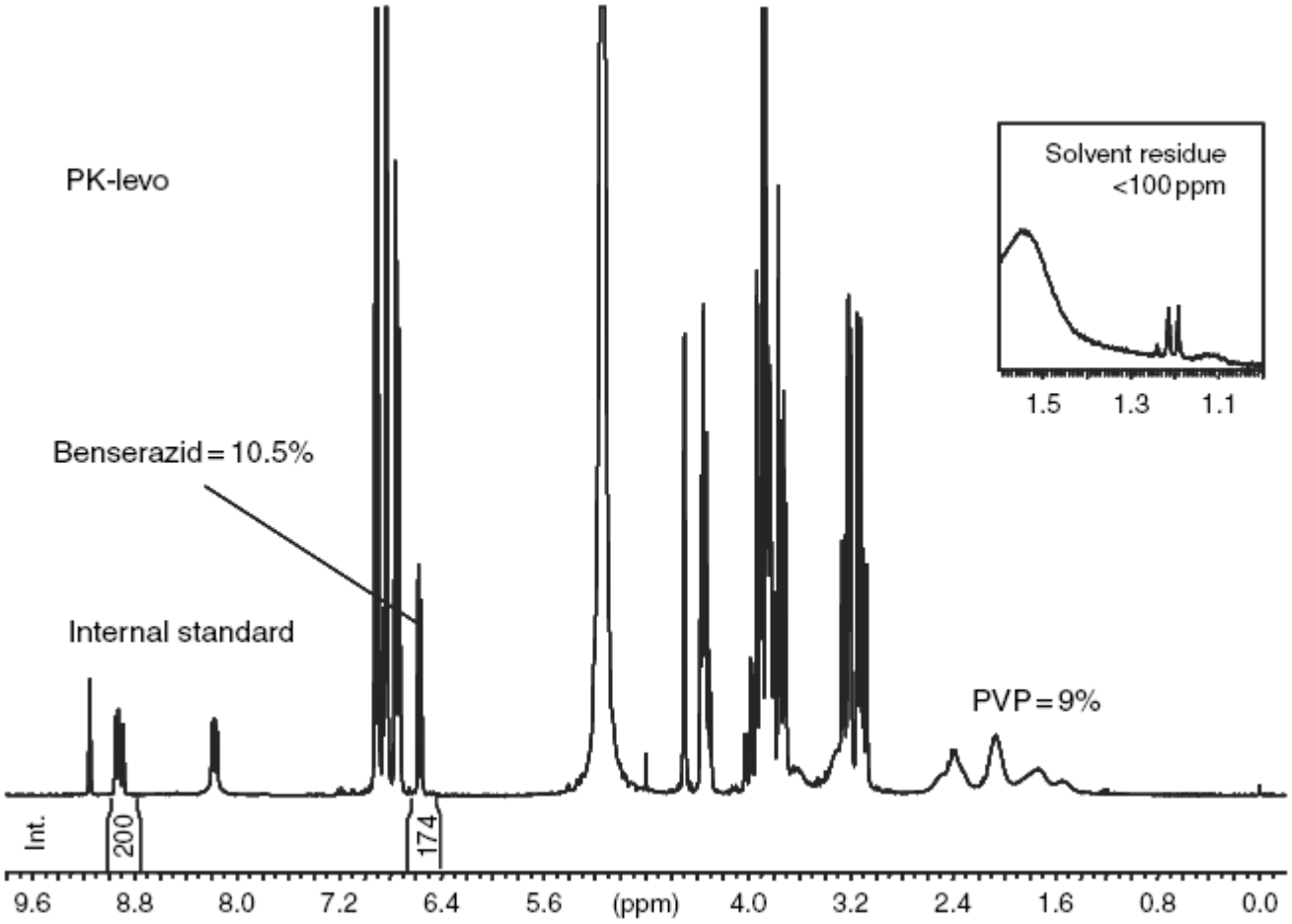


Figure 32 ^{1}H NMR of a drug formulation containing PVP.

Ανάλυση εκχυλισμάτων φαρμακευτικών φυτών: *Aloe Vera*

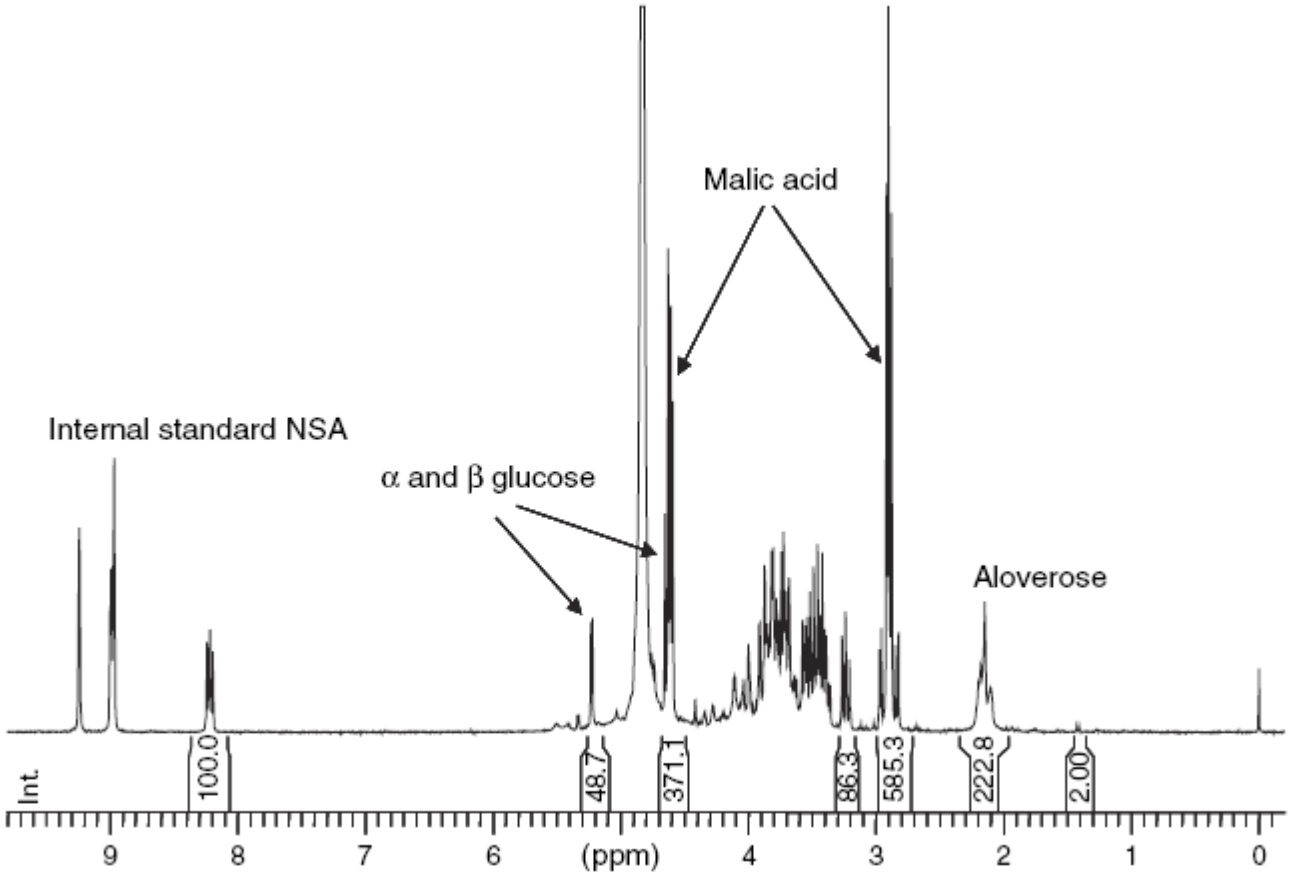


Figure 15 ^{1}H NMR spectrum of fresh *A. vera* gel + NSA as internal standard.

Solid state ^{13}C NMR: Φαρμακευτικά έκδοχα

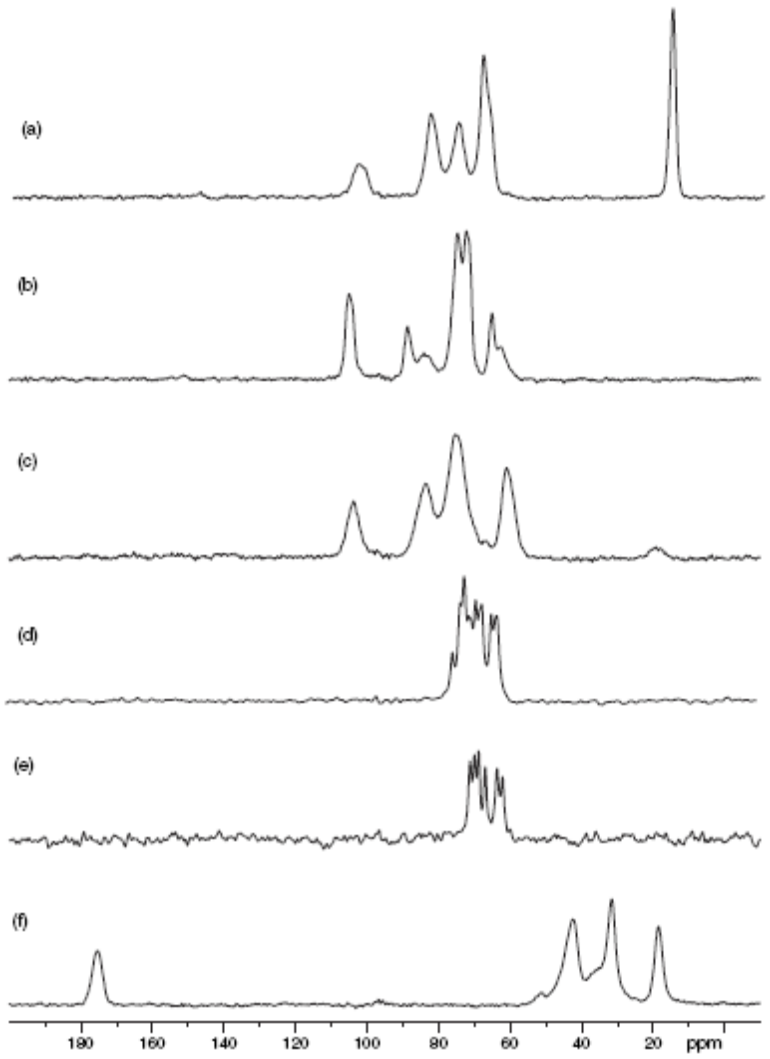


Figure 9 ^{13}C CPMAS NMR spectra of the most commonly used excipients: (a) AQUALON N-22 (ethy cellulose), ser. 41252-Hercules, (b) AVICEL (microcrystalline cellulose), PH-105, ser. 5527C, (c) BENECEL (hydroxypropylmethy cellulose HMPC), ser. VK 8494-Hercules, (d) SORBITOL (corion instant), ser. M261140-MERCK, (e) MANNITOL PARTECH 200-MERCK, and (f) COLLIDONE CL (crospovidone PVP), ser. 89-1609-BASF.

Ανάλυση συνθετικών πολυπεπτιδίων

Table 1 Composition of the peptides reported in this study

Peptide	Number of AA	Sequence
Oxytocin	9	H-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH2
Desmopressine acetate	9	Mpa-Tyr-Phe-Gln-Asn-Cys-Pro-D-Arg-Gly-NH2
Gonadorelin acetate	10	Glp-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2
Gonadorelin diacetate*	10	Glp-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2
Buserelin acetate	10	Glp-His-Trp-Ser-Tyr-D-Ser(tBu)-Leu-Arg-Pro-Gly-NHEt
Goserelin	10	Glp-His-Trp-Ser-Tyr-D-Ser(tBu)-Leu-Arg-Pro-Azagly-NH2
Protirelin	3	Glp-His-Pro-NH2
Tetracosactide	24	H-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro-OH

*Gonadorelin diacetate contains 4 mo of water (4AQ) per mole of Gonadorelin diacetate.

Ανάλυση συνθετικών πολυπεπτιδίων

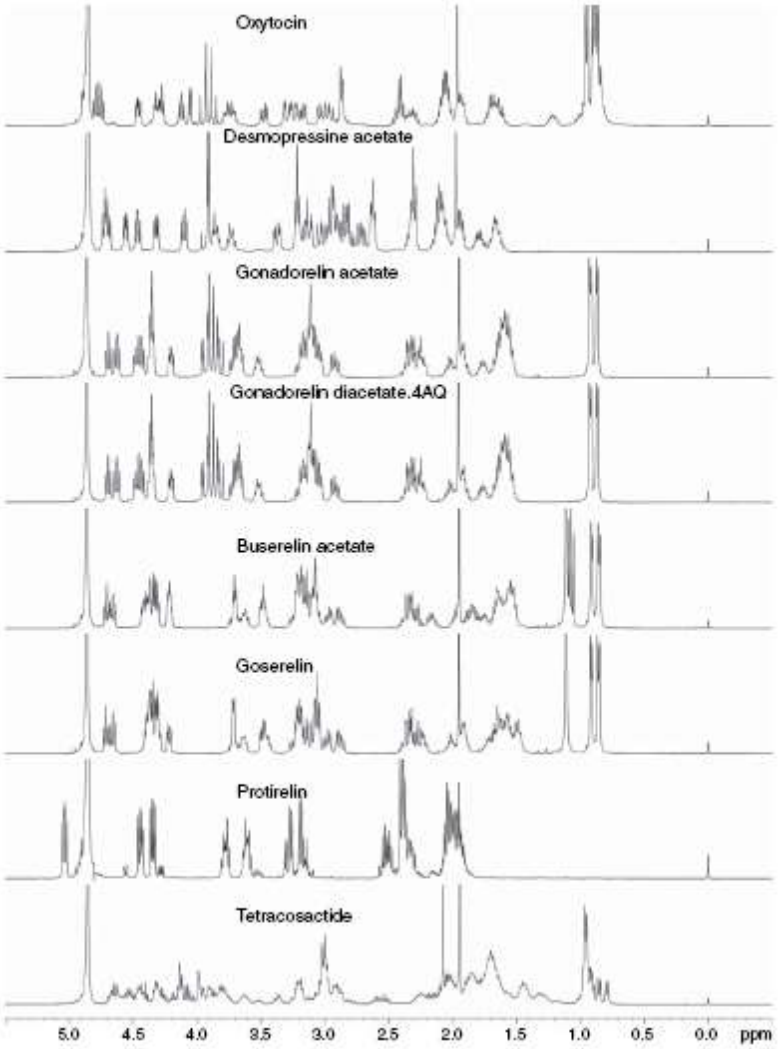


Figure 1 Aliphatic region of the 400 MHz NMR spectra of the eight different peptides.

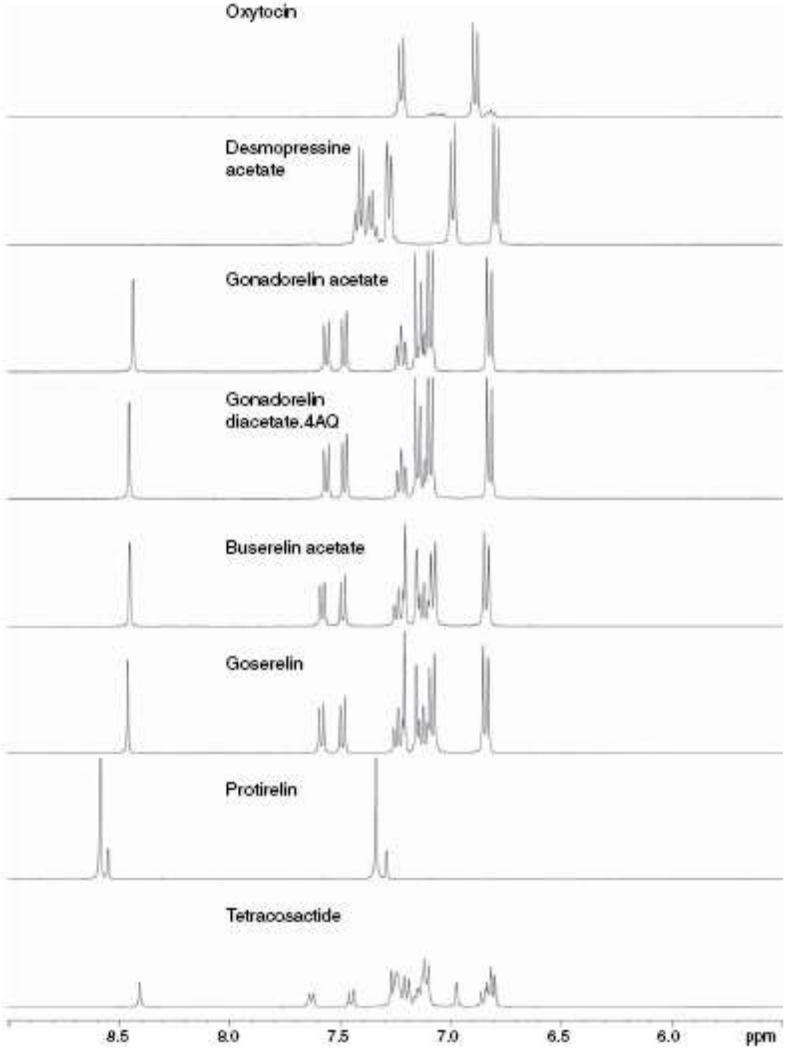


Figure 2 Aromatic region of the 400 MHz NMR spectra of the eight different peptides.

Σύζευξη αναλυτικών τεχνικών στη φαρμακευτική ανάλυση

Figure 3 The metabolic profiler LC–MS and NMR system from Bruker.

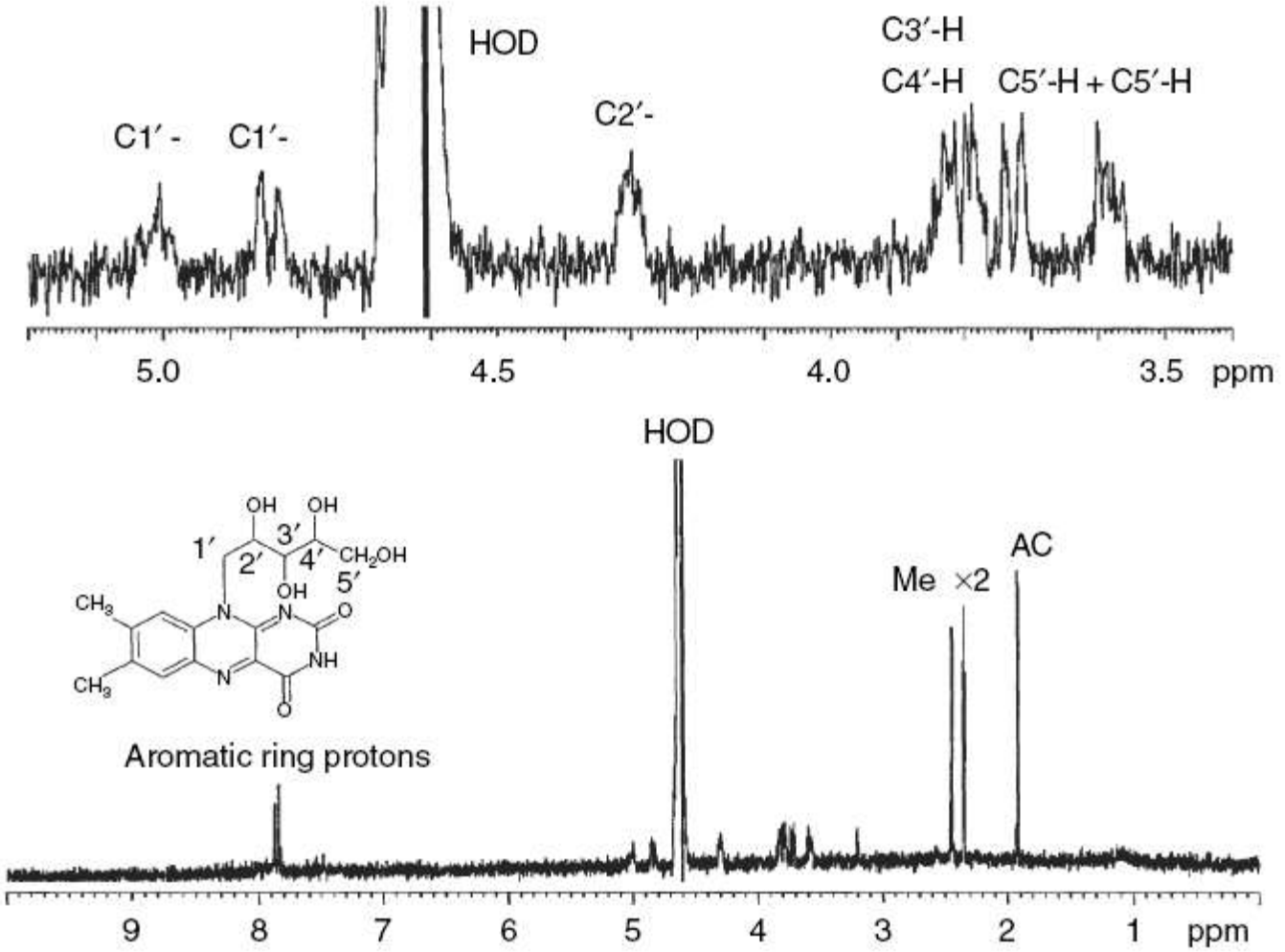


Figure 4 Stopped-flow ^{1}H NMR spectrum for riboflavin obtained following LC–NMR–MS with chromatography on a PS-DVB column at 200°C with D_2O as the mobile. The inset shows details of the small signals between 3.5 and 5.1 ppm.[44]

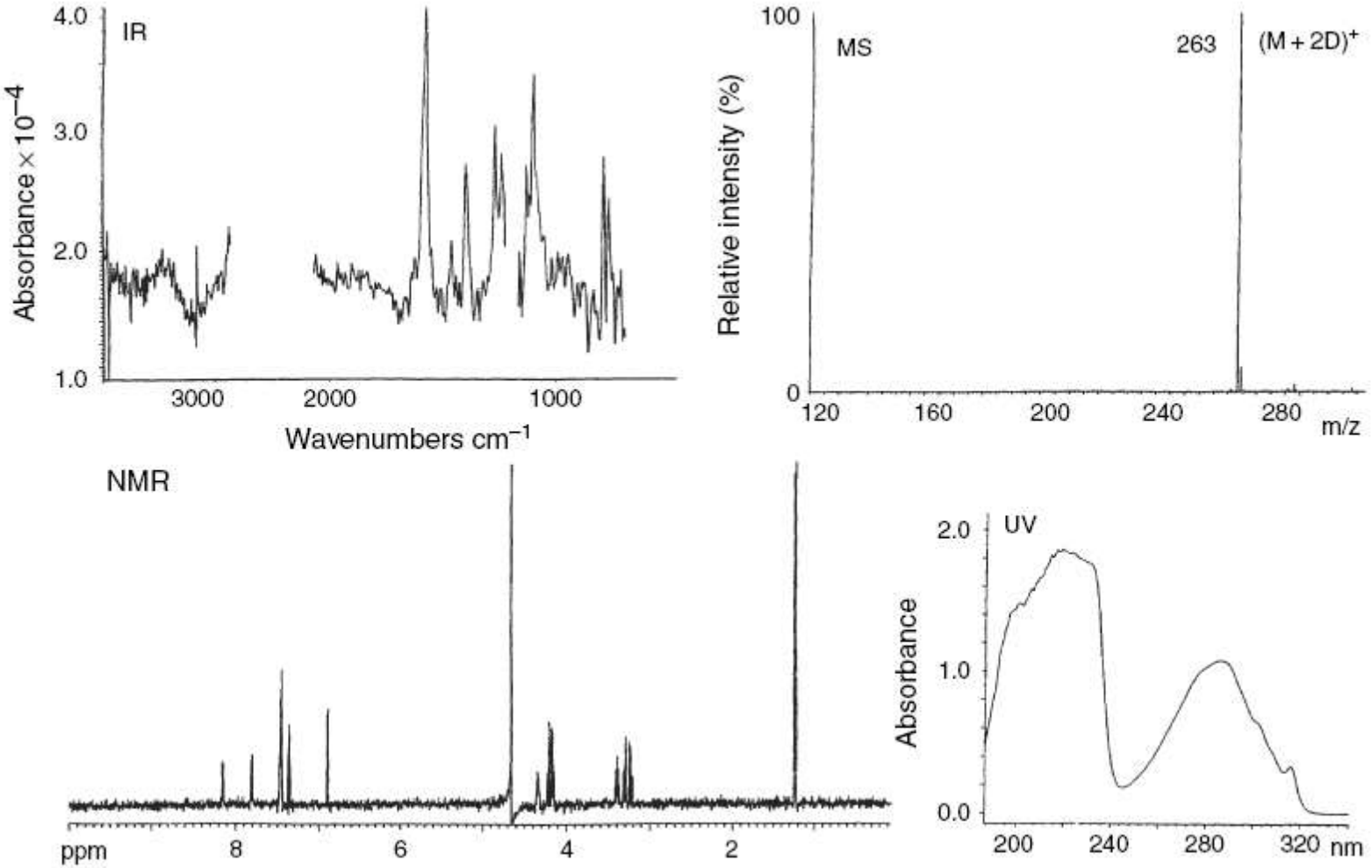


Figure 1 UV, IR, NMR and MS spectra obtained for propranolol (215 µg) using the UV–IR–NMR–MS–FIA system.[10]

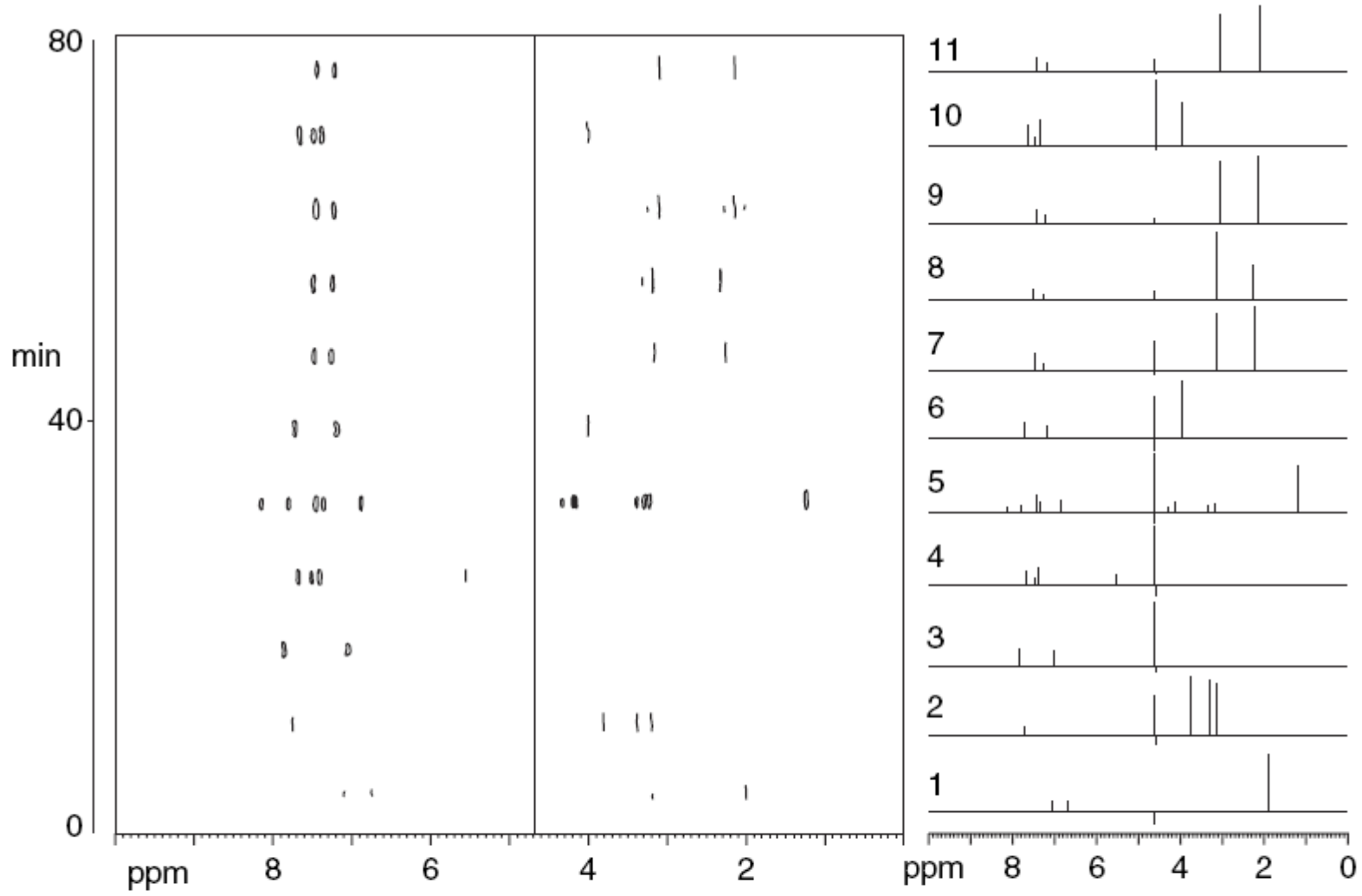


Figure 2 Pseudo 2D ^{1}H NMR spectral data for the model library of analytes obtained using the UV–IR–NMR–MS–FIA system described in ref. 10. Key: (1) acetaminophen (140 µg), (2) caffeine (165 µg), (3) 4-aminobenzoic acid (185 µg), (4) α-hydroxyhippuric acid (200 µg), (5) propranolol (215 µg), (6) 4- aminohippuric acid (270 µg), (7) 4-aminoantipyrine (295 µg), (8)4-dimethylaminoantipyrine (545 µg) (9), antipyrine (840 µg), (10) hippuric acid (365 µg) and (11) antipyrine (210 µg).[10]

On-line NMR for monitoring pharmaceutical reactions

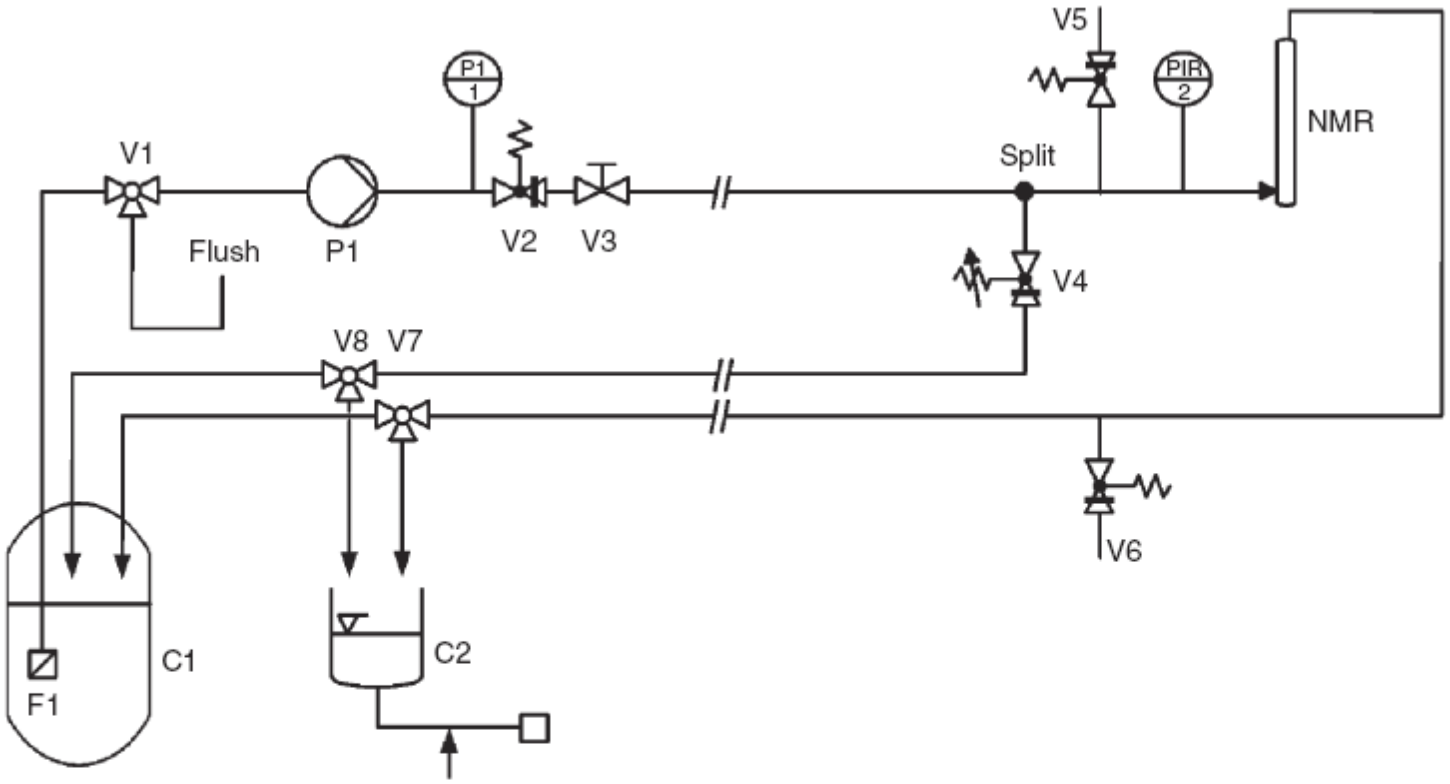


Figure 3 Typical setup for online NMR measurements. C1: laboratory reactor, F1: inlet filter, V1: (tee) purging valve, P1: thermostated dosing pump, V2: back pressure regulator, V3: shut off valve, V4: variable back pressure regulator for split adjustment, V5, V6: pressure relief valves, PI1, PIR2: pressure transducer, NMR: thermostated flow probe of NMR spectrometer, V7, V8: tee valves, C2 container on balance for mass flow control. All tubing 1/16″ OD.

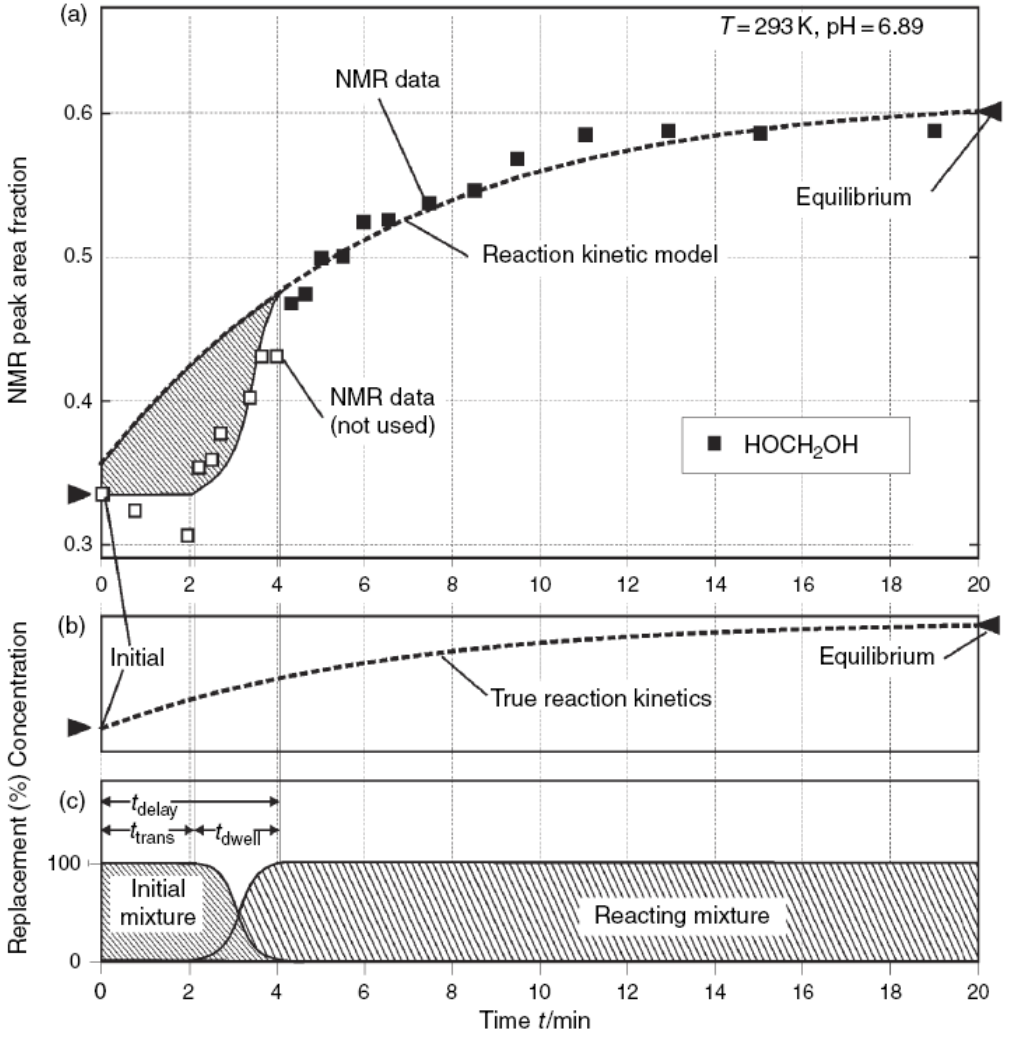


Figure 4 NMR data from a typical reaction kinetic experiment and evaluation. (a) Changes of NMR peak area fractions vs time (dilution at $t = 0$) and fit by a reaction kinetic model. Open symbols are not used for the fit. (b) Concentration changes during reaction in the reactor (qualitative). (c) Replacement of solution according to the RTD function measured independently. The delay time t_{del} is the sum of the transfer time t_{trans} and the residence time t_{dwell} in the probe.

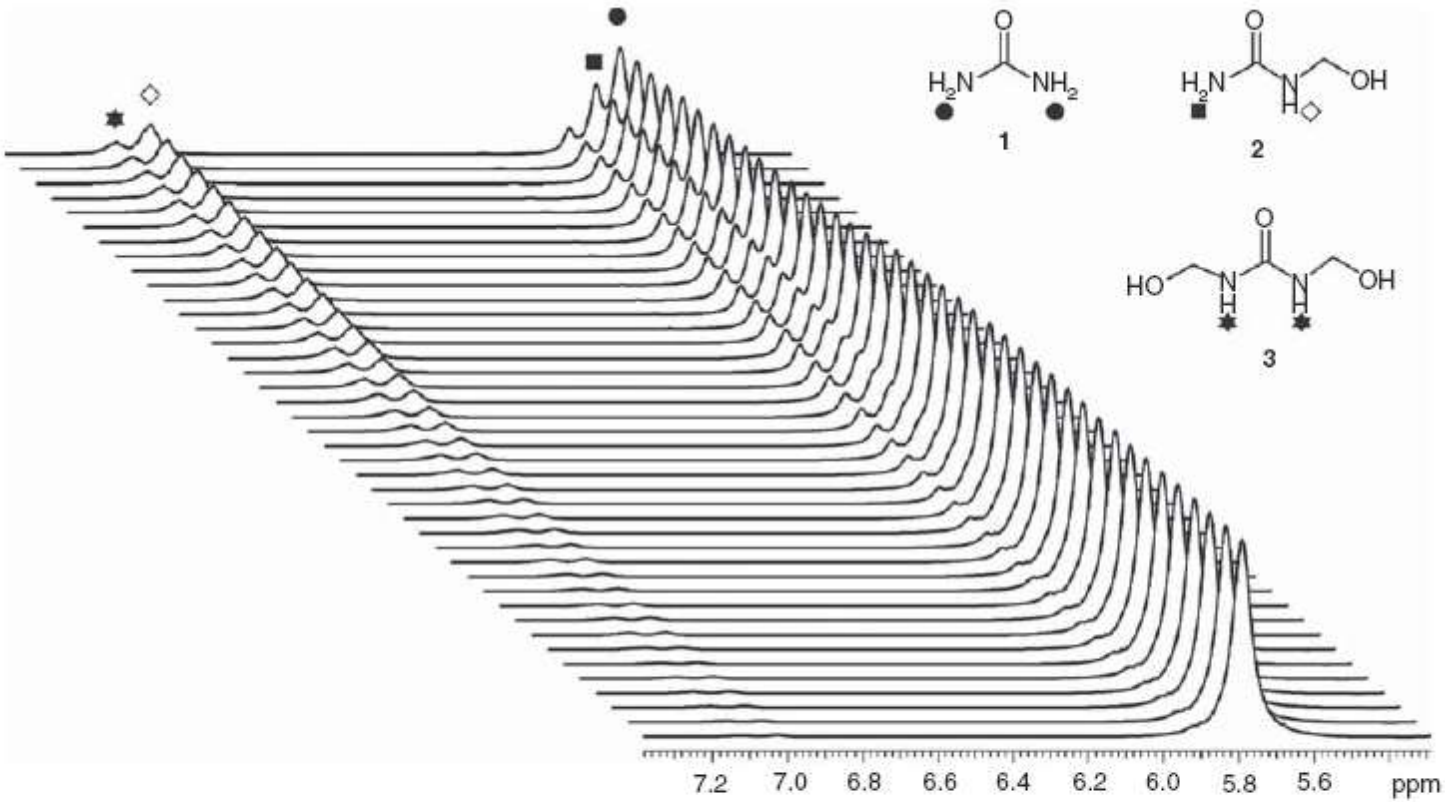


Figure 8 Observation of the reaction of urea with formaldehyde over a course of 2 h at a temperature of 343 K and pH 8.5. The molar ratio of formaldehyde to urea was 2:1. Consumption of urea (●) and formation of two different hydroxymethyl ureas (✱,◇) can be distinguished clearly making use of the amide protons. Protons of methylene groups cannot be used for quantification because of overlap with formaldehyde signals.