Chiral determination of ketamine and norketamine in hair based on CE separation

Nadia Porpiglia

Department of Diagnostics and Public Health, Unit of Forensic Medicine, University of Verona, Verona, Italy

nadia.porpiglia@univr.it

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Ketamine

General features

- Phencyclidine derivative
- Acts at the NMDA receptor Ca^{2+} channel pore
- Interaction with μ and κ opioid receptors

- Dissociative anaesthetic
- Mostly used in veterinary surgery and paediatric emergency
- Main advantage: profound analgesia vs. maintaining of cardiopulmonary function

Ketamine's misuse

Abuse overview

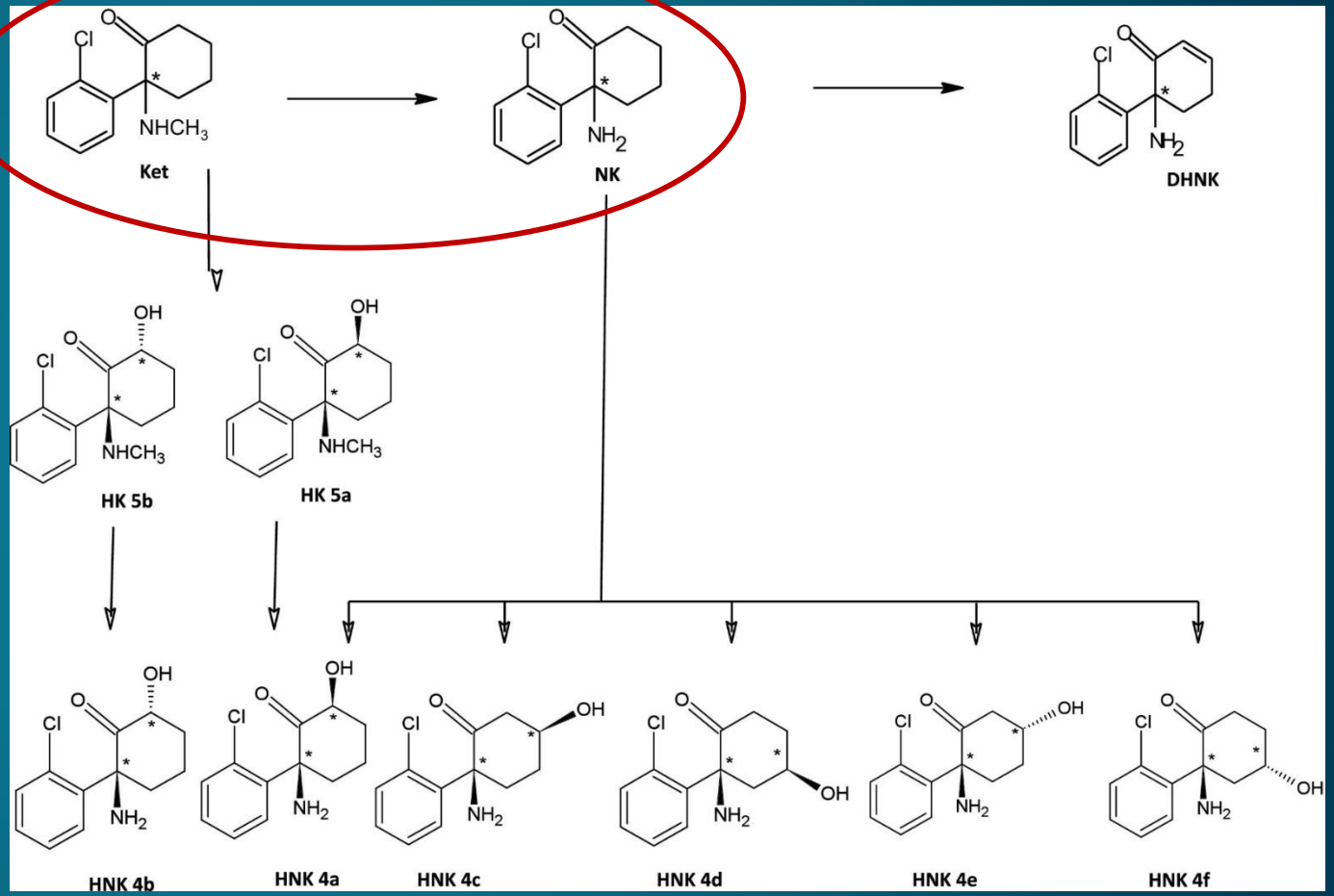
- Abused since 1980s
- "Special K", "Vitamin K", "Lady K"
- Attempt to cleanse 'doors of perception' (*K-hole*)
- Club, dance and raves parties' drug
- Drug-facilitated sexual assault

Practical aspects

- Powder, liquid or tablet
- Various administration routes

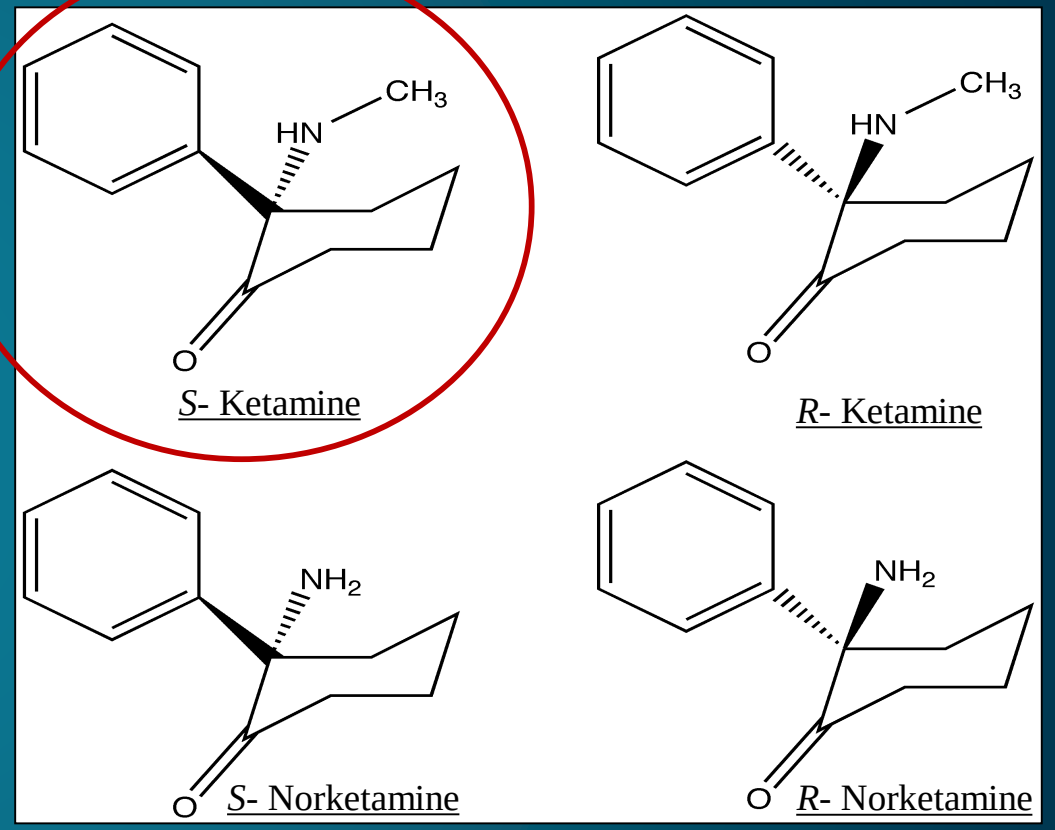
Ketamine and its metabolites

- Metabolised in liver:
  - Demethylation and hydroxylation of ciclohexanone ring
  - Major pathway N-*demethylation* to norketamine
- Norketamine's activity: 20-30% of parent drug
- Mainly excreted in urine



A chiral substance

- Chiral centre at the cyclohexanone ring
- ***S*-ketamine**
  - Greater affinity to NMDA receptor than *R*-K
  - More potent than racemic mixture and *R*-K
  - Faster recovery time than its racemate
  - Smaller dose required, i.e. fewer side effects
  - Available in Europe and USA



Aims of the study

- To establish an enantioselective CE method for both ketamine and norketamine
- To adapt the method for hair analysis to investigate chronic ketamine abuse in different administrative and forensic contexts
- To apply the method to real-case specimens

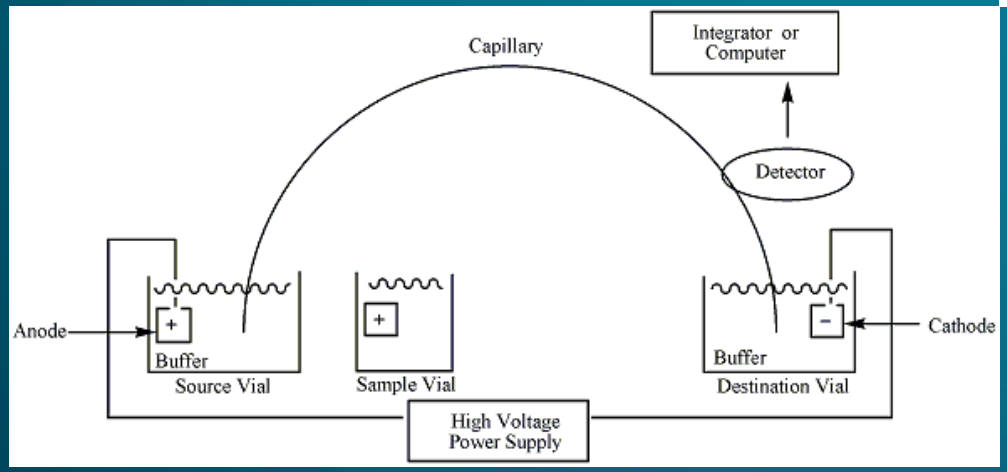
Why hair?

- Substances present in blood trapped in hair follicle
- Non- invasive and non-offensive
- Insignificant quantity of hair is cut
- Collection of second sample in disputed cases
- Contamination from perspiration and environment
- Wide detection window
- Abuse pattern can be measured
- Very low amount

CE Method Development

Optimising experimental conditions:

- Buffer: ionic strength, pH, enhancing additive
- Temperature, voltage, polarity
- Internal standard (I.S.): **lamotrigine**
- Increase in sensitivity



Running buffer and chiral selector

BUFFER COMPOSITION AND CONCENTRATION	pH	CHIRAL SELECTOR	ORGANIC MODIFIER
50 mM Tris	2.5	β-CD (5, 10, 20 mM)	ACN, n-prOH, MeOH (5, 10, 20 %)
		DM-β-CD (5, 10, 20, 30 mM)	MeOH (5, 10, 20 %)
		HP-β-CD (1, 2.5, 5, 10 mM)	MeOH (5, 10, 20 %)
		S-β-CD (5 mM) Reverse Polarity	
75 mM Tris	5	CM-β-CD (7.5 mM)	
50, 100, 150 mM KH_2PO_4	2.5, 4.5	β-CD (10 mM)	
		γ-CD (10, 20, 30 mM)	
50 mM Triethylammonium phosphate	2.5	HS-γ-CD (2.5, 5 %) Reverse Polarity	

Complete separation of K enantiomers

Complete separation of K enantiomers, partial separation of NK enantiomers

Non-baseline separation of K enantiomers

Complete separation of K **AND** NK enantiomers

Running buffer and chiral selector

Adjusting to sample stacking….

BUFFER COMPOSITION AND CONCENTRATION	pH	CHIRAL SELECTOR	POLARITY
50, 100 mM $NH_4H_2PO_4$	2.5, 3	HS-γ-CD (0.3, 2, 2.5 %)	Reverse
15 mM Tris	2.5	HS-γ-CD (2 %)	Reverse
15 mM Tris	2.5	HS-γ-CD (0.1, 0.3, 0.5 %)	Normal

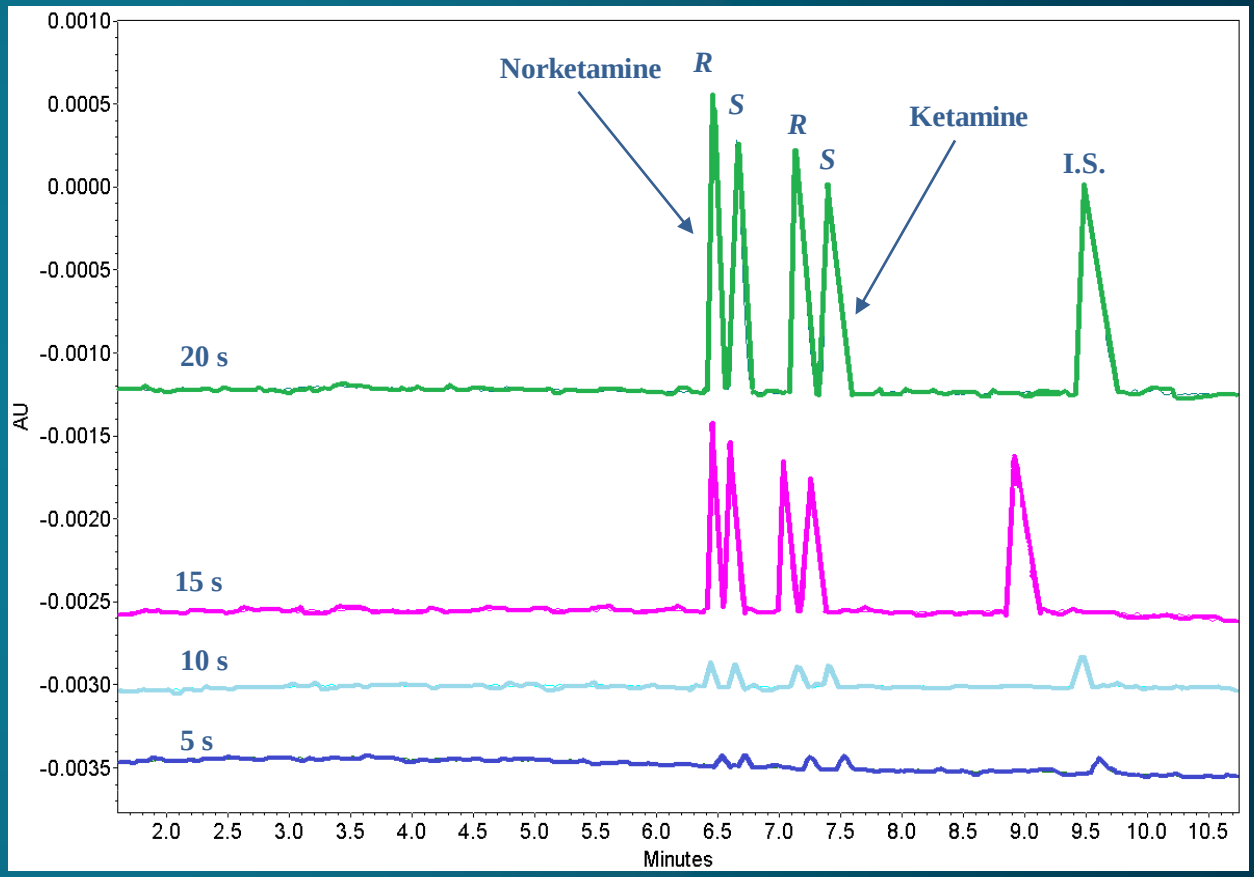
15 mM Tris pH 2.5 HS-γ-CD 0.1 % Normal Polarity

- **Resolution (R-USP): 2.0 for NK enantiomers, and 2.4 for K enantiomers**

Analytical sensitivity increase

Sample Stacking

- Field Amplified Sample Stacking (FASS)
  - plug of running buffer with NO CDs (0.5 psi, 40 s) prior to
- Large Volume Injection (LVI)
- Electrokinetic Injection 7 kV, 20 s
- LOD: 0,08 ng/mg (S/N = 3)
- LOQ: 0,25 ng/mg (S/N = 10)



Hair sample: extraction procedure

~50 mg hair + 500 µL 0.1 M HCl → 1 hr sonication and overnight incubation at 37°C → Neutralisation with 1 M NaOH

Extraction with hexane/ethyl acetate (50:50) → Dryness of organic phase → Reconstitution with 250 µL distilled water

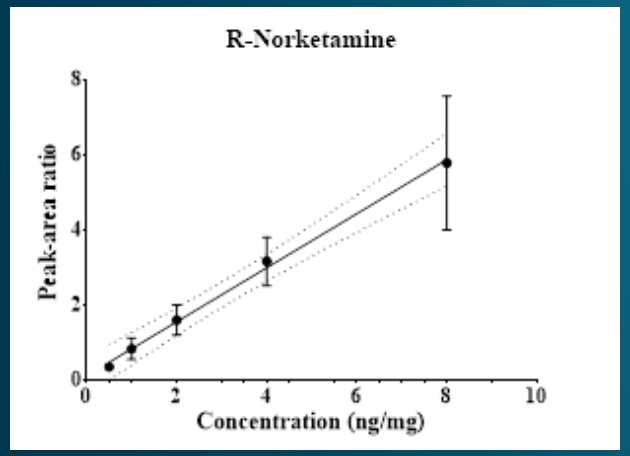
Method validation

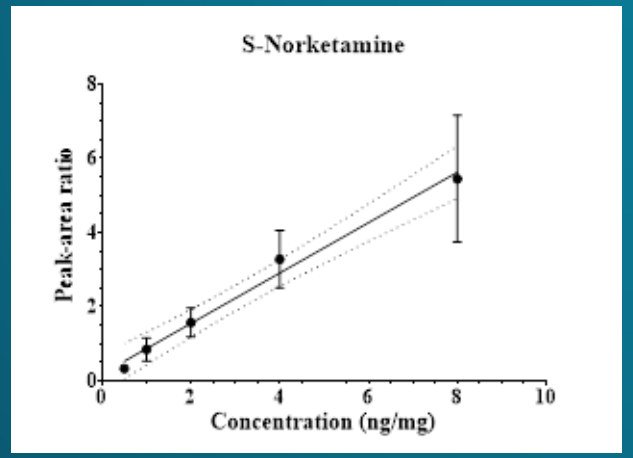
- Peak assignment: R-NK, S-NK, R-K, S-K
- Resolution (R-USP): 2.0 for NK enantiomers, and 2.4 for K enantiomers
- LOD: 0,08 ng/mg (S/N = 3)
- LOQ: 0,25 ng/mg (S/N = 10)
- Matrix effect and recoveries percentages
- Intra- and inter-day precision and accuracy, %RSD and %RE respectively
- Linearity: estimated with a five point- calibration curve

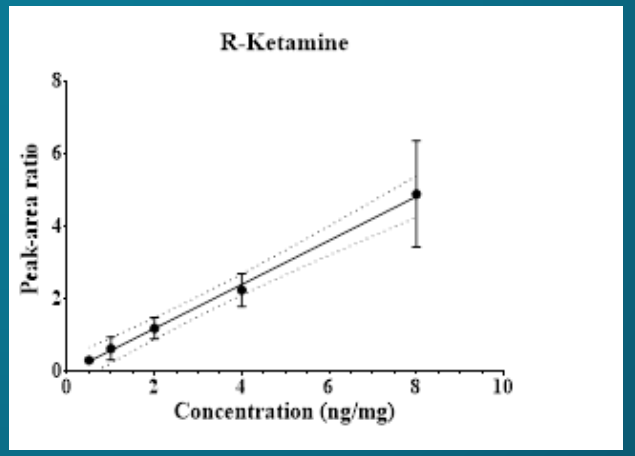
Linearity

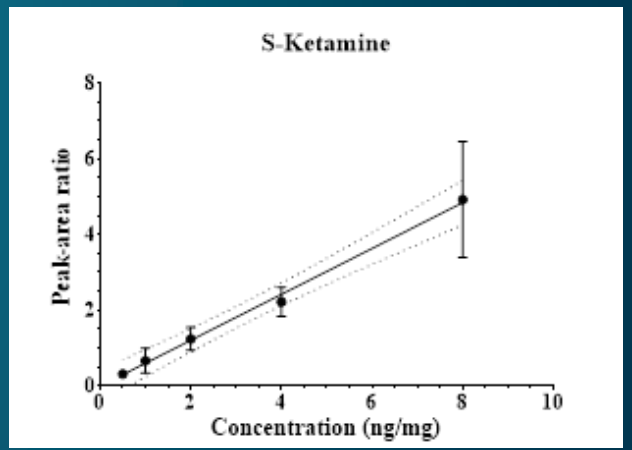
Enantiomer (n=5)	Regression equation	Correlation coefficient (R^2)
***R*-norketamine**	$y = (0.72 \pm 0.04)x + (0.12 \pm 0.05)$	0.997
***S*-norketamine**	$y = (0.68 \pm 0.02)\ x + (0.20 \pm 0.11)$	0.988
***R*-ketamine**	$y = (0.61 \pm 0.09)\ x - (0.02 \pm 0.08)$	0.998
***S*-ketamine**	$y = (0.61 \pm 0.11)\ x - (0.01 \pm 0.05)$	0.996

Spiked hair samples - range: 0.5 – 8.0 ng/mg









95% confidence intervals

Recovery and matrix effect

Enantiomer	Expected amounts in ng/mg (set A)	Amount measured in set B (ng/mg)	Matrix effect (%) n=5	Amount measured in set C (ng/mg)	Extraction recovery (%) n=5
***R*-norketamine**	**0.13**	0.10 (±0.03)	77	0.10 (±0.02)	79
	0.50	0.34 (±0.04)	68	0.32 (±0.03)	64
	2.00	1.27 (±0.15)	64	1.47 (±0.09)	74
***S*-norketamine**	**0.13**	0.11 (±0.04)	89	0.11 (±0.03)	91
	0.50	0.33 (±0.03)	66	0.32 (±0.03)	64
	2.00	1.27 (±0.15)	64	1.50 (±0.08)	75
***R*-ketamine**	**0.13**	0.14 (±0.08)	112	0.09 (±0.03)	73
	0.50	0.38 (±0.06)	76	0.25 (±0.04)	49
	2.00	1.26 (±0.17)	63	1.09 (±0.14)	54
***S*-ketamine**	**0.13**	0.15 (±0.09)	119	0.11 (±0.04)	89
	0.50	0.39 (±0.06)	79	0.25 (±0.04)	51
	2.00	1.31 (±0.18)	66	1.05 (±0.15)	53

- Set A: standard solutions
- Set B: hair samples spiked AFTER extraction
- Set C: hair samples spiked BEFORE extraction

$$\textbf{Recovery (\%)} = \frac{C}{A} \cdot 100$$

$$\textbf{Matrix effect (\%)} = \frac{B}{A} \cdot 100$$

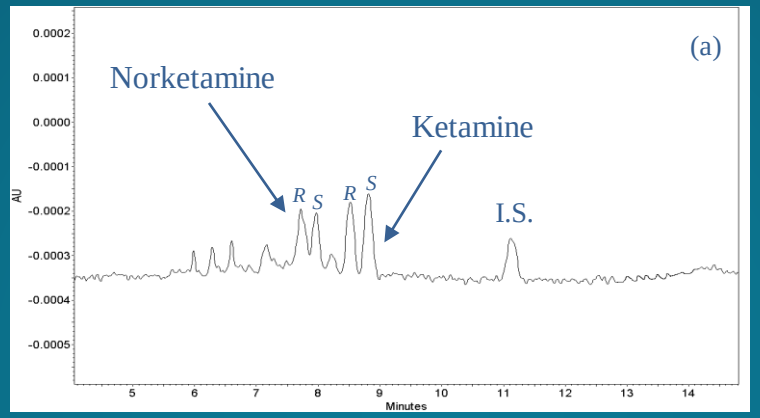
Precision & accuracy

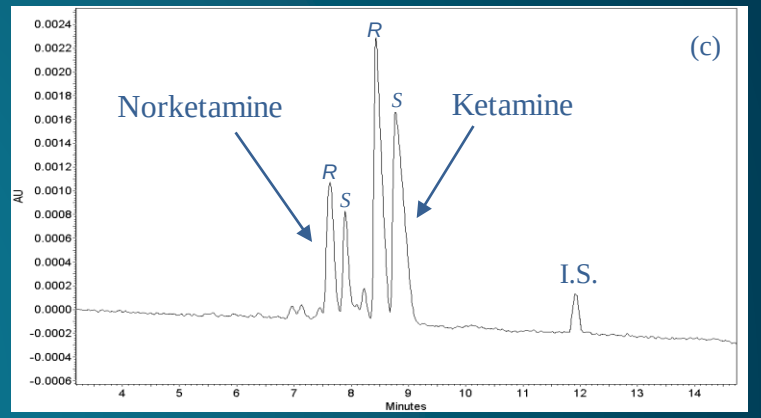
Enantiomer	Amount added (ng/mg)	Amount measured (ng/mg)	Area Precision (%RSD)		Area Accuracy (%RE)		Migration Time Precision (%RSD)	
			Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
***R*-norketamine**	0.25	0.24 (± 0.03)	11.6	14.0	-16.4	-16.6	0.99	0.24
	1.00	1.00 (± 0.20)	14.5	14.7	-14.6	-17.4	0.75	0.21
***S*-norketamine**	0.25	0.25 (± 0.03)	11.4	14.6	-17.0	-17.2	1.02	0.25
	1.00	1.00 (± 0.20)	14.3	14.8	-13.8	-15.9	0.78	0.22
***R*-ketamine**	0.25	0.23 (± 0.06)	12.9	14.4	-22.6	-22.8	1.14	0.31
	1.00	1.07 (± 0.20)	13.4	12.7	-17.7	-21.3	0.85	0.28
***S*-ketamine**	0.25	0.25 (± 0.06)	14.4	17.9	-22.3	-22.9	1.17	0.34
	1.00	1.10 (± 0.21)	15.4	15.7	-22.7	-29.2	0.88	0.30

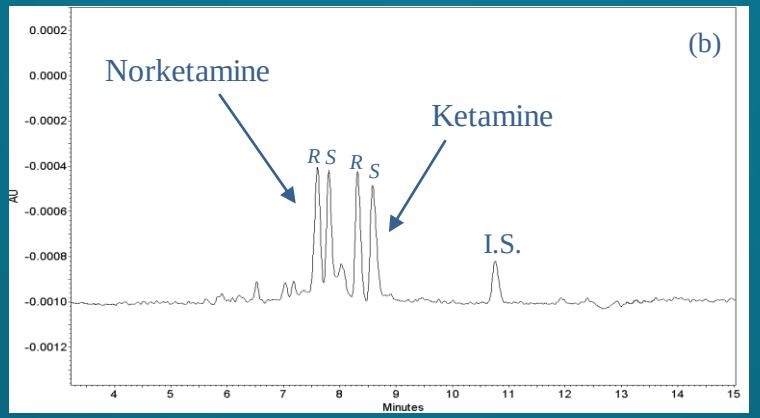
n = 6 (repeated for 6 days)

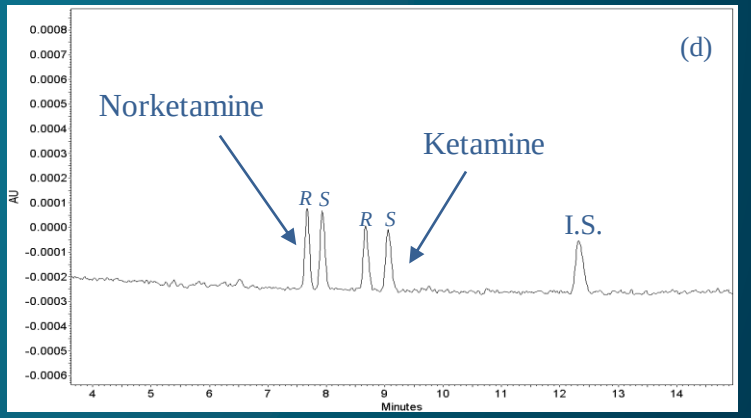
Real-world samples

- n=12 samples
- 0.33 -107 ng/mg
- samples from young individuals applying for re-granting of the driving license for DUI of drugs (a-c)
- standard solution of ketamine, norketamine and I.S. (d)









Porpiglia, N. et al. (2016), Forensic Sci Int; 266:304–310

Conclusions

- An enantioselective CE method for the separation of ketamine and norketamine was developed and validated
- The method is suitable for forensic analysis of hair samples
- It can be applied for the analysis of real samples collected from ketamine abusers
- Because of the availability in the market of both racemic ketamine and its *S*-enantiomer, chiral analysis may reveal the type of drug taken by the individual
- On the other hand, chiral analysis of ketamine could also reveal an enantioselective metabolism of the drug
- Based on our recent experience:
  - racemic ketamine is currently abused
  - no evidence of enantioselective metabolism has been found

Thanks for listening!

Grazie!

Formulas

$R(USP) = 2(t_2 - t_1)/(w_1 + w_2)$

Precision: %RSD = (SD/average)·100

Accuracy: %RE = (nominal value – expected value/expected value) · 100