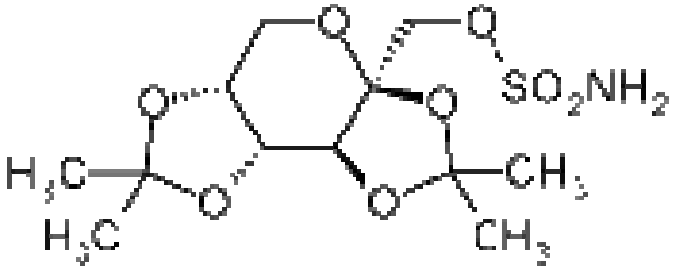
# Pharmacokinetic profiles of antiepileptic drugs by using dried blood spot and Liquid Chromatography-Tandem Mass Spectrometry

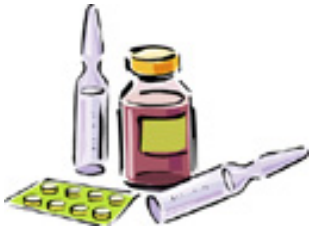
**Giancarlo la Marca**

**Mass Spectrometry Lab, Meyer Children's Hospital**

**Dept of Pharmacology, University of Florence**

# TOPIRAMATE (TPM) IS AN ANTIEPILEPTIC DRUG



**2,3:4,5-Bis-O-(1-methylethylidene)-beta-D-fructopyranose sulfamate**

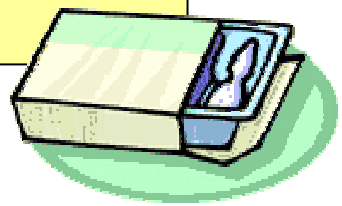
**CAS number 97240-79-4**

**ATC code N03AX11**

**Chemical data Formula $C_{12}H_{21}NO_8S$**

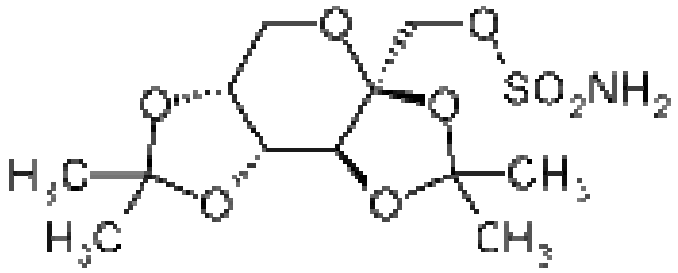
**Mol. mass 339.363 g/mol**

1) APPROVED AS MONOTHERAPHY OR ADJUNCTIVE TREATMENT OF PARTIAL AND GENERALISED SEIZURES IN ADULTS AND CHILDREN



2) TREATMENT OF LENNOX-GASTAUT SYNDROME

# TOPIRAMATE (TPM)




**Chemically, topiramate is a sulfamate-substituted monosaccharide, related to fructose.**

**Topiramate is quickly absorbed after oral use.**

**Most of the drug (70%) is excreted in the urine as unchanged drug. The remainder is extensively metabolized by hydroxylation, hydrolysis, and glucuronidation.**

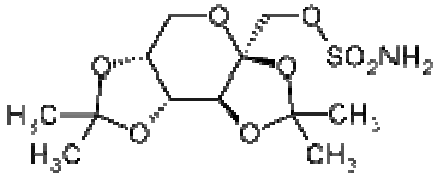
**Six metabolites have been identified in humans, none of which constitutes more than 5% of an administered dose.**

# THE ANTICONVULSANT ACTIVITY OF TPM:

**Inhibitory activity against glutamate receptors including *N*-methyl-d-aspartate (NMDA), kainate (KA) and α-amino-3-hydroxy-5-methylisoxazole propionic acid (AMPA) receptors.**

**Other studies indicate that TPM can influence the activity of voltage-activated $Na^+$ and $Ca^+$ channels, can reduce some carbonic anhydrase isozymes or the mitochondrial permeability transition pore.**

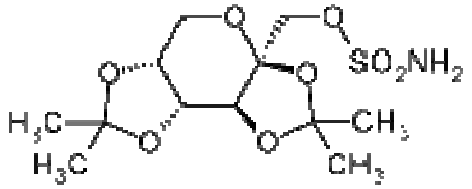
# TOPIRAMATE (TPM)




## Doses

- IN ORDER TO AVOID EARLY SIDE-EFFECTS (E.G. COGNITIVE DYSFUNCTION) THE INITIAL DOSE NORMALLY IS LOW AND INCREASED IN SLOW STEPS.
- THE USUAL INITIAL DOSE IS 25 TO 50 MG DAILY IN 2 SINGLE DOSES.
- RECOMMENDED INCREMENTS ARE 25 TO 50 MG EVERY 1 OR 2 WEEKS.
- COMMON DOSES FOR MAINTENANCE TREATMENT ARE 100 TO 200 MG DAILY.
- THE HIGHEST DOSE POSSIBLE IS 1,000 MG DAILY IN DIVIDED DOSES.

# TOPIRAMATE (TPM)

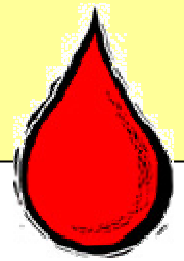



## SIDE EFFECTS

- CHANGE IN TASTE (CARBONATED BEVERAGES, ESPECIALLY DIET SODAS AND BEER, TASTE PARTICULARLY BAD)
- FEELINGS OF PINS AND NEEDLES IN THE HEAD AND EXTREMITIES
- COGNITIVE DEFICIENCY (PARTICULARLY WORD-FINDING DIFFICULTY)
- LETHARGY
- RENAL (KIDNEY) STONES
- IMPAIRMENT OF FINE MOTOR SKILLS
- VISION ABNORMALITY AND TRANSIENT OR PERMANENT VISION LOSS
- WEIGHT LOSS
- MENSTRUAL DISORDER

THE CLINICAL EFFECT OF THIS DRUG CORRELATES BETTER WITH BLOOD LEVEL THAN WITH DOSES.

THIS MAKES THERAPEUTIC MONITORING IMPORTANT.



# TOPIRAMATE (2-25 µg/mL) has been dosed mostly by

**HPLC UV detection: Sample volume required 1ml serum-2ml blood, LOQ 0.4µg/mL). liquid-liquid extraction and subjected to derivatization with 9-fluorenylmethyl chloroformate**

*Bahrami et al, 2005, J Chromatogr B*

**HPLC-MS :(0.5 ml serum; LOQ 1µgmL, LOD 0.3 µg/mL)**

*Masucci et al, 1998, J Mass Spectrom.*
*Christensen et al, 2002, Ther Drug Monit.*
*Britzi et al, 2003, Ther Drug Monit.*

**GC/NPD liquid-liquid extraction and nitrogen phosphorus detection**

*Riffitts et al, 1999, J Pharm Biomed Anal.*
*Tang et al, 2000, Ther Drug monit.*

**Capillary Electrophoresis with indirect UV detection**

*Klockow-Beck et al, 1998, J Chromatogr B*

**Immunometric method (0.2 ml serum, LOQ 1µg/mL, LOD 0.3 µg/mL)**

*DeGrella et al, 1988, Am Biotech Lab*

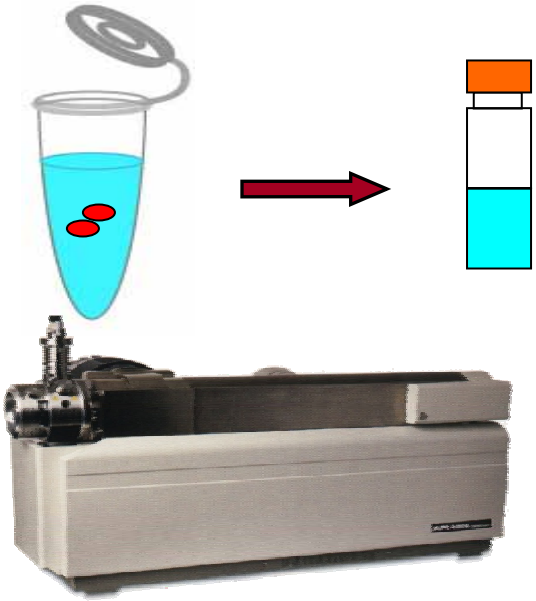
# SAMPLE PREPARATION FOR LC-ESI-MS/MS ANALYSIS

About 6 µL of entire blood,
about 3 µL of serum

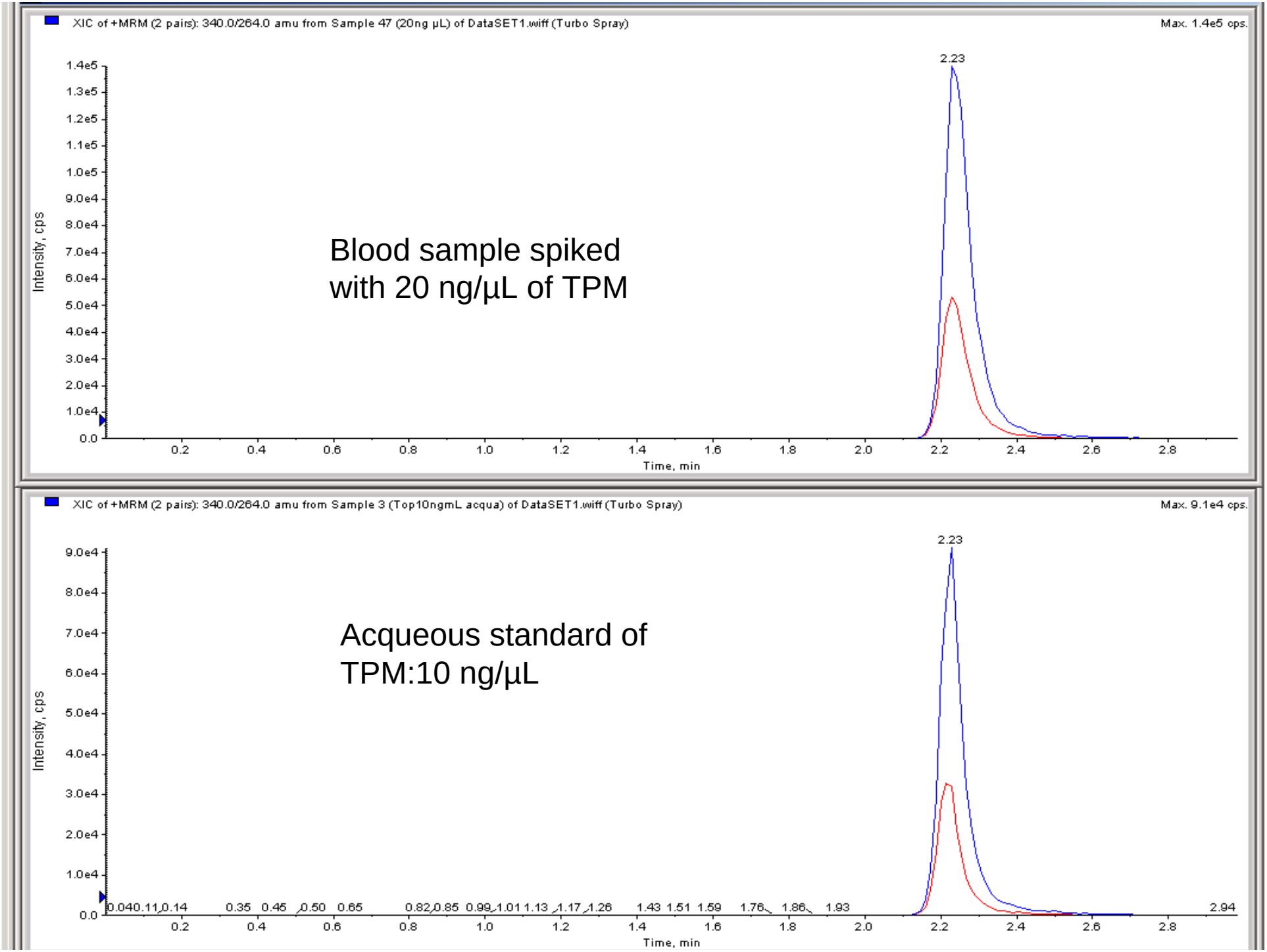
Blood Spot on paper

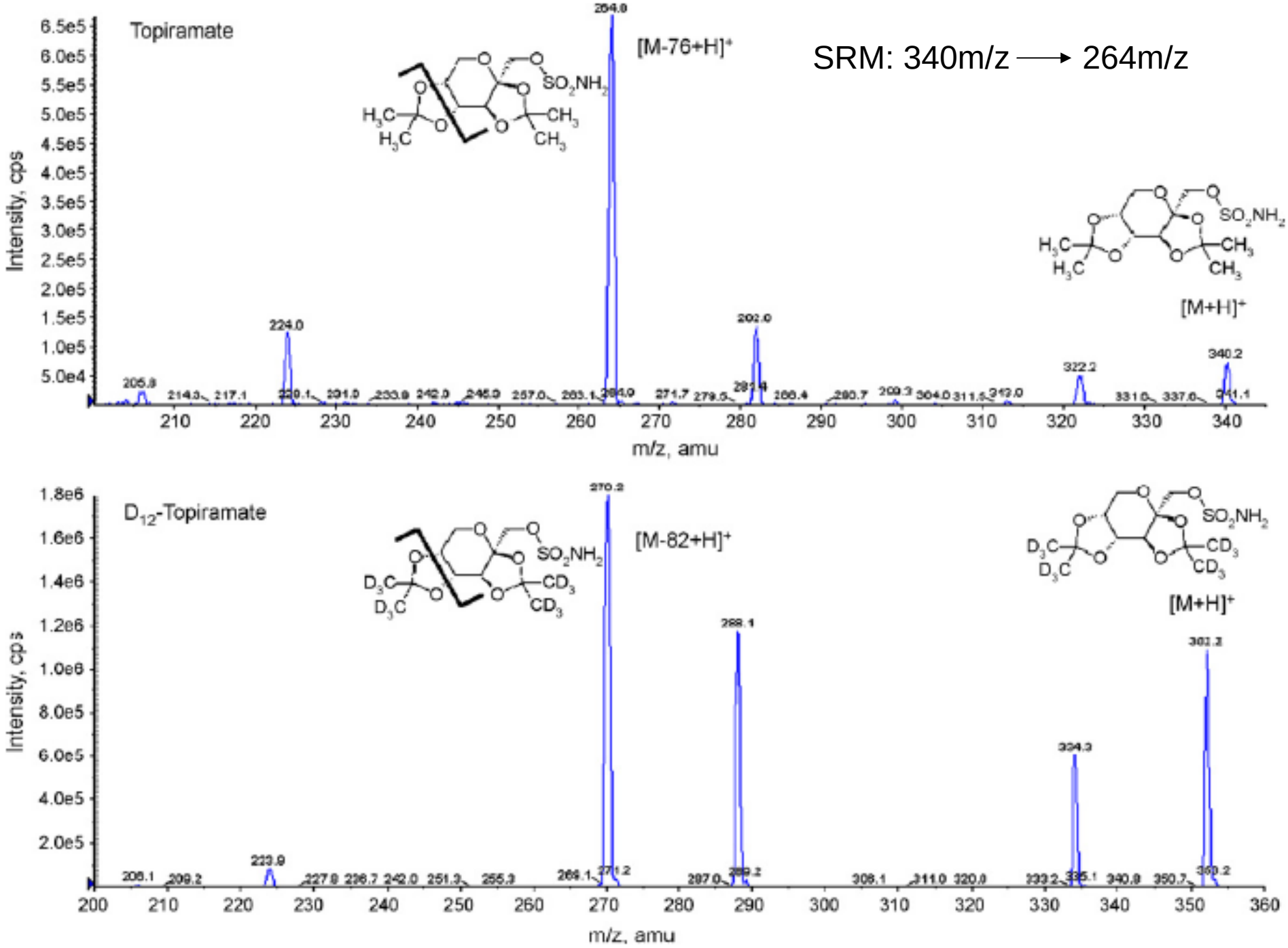
2 Spots are punched to obtain 2 circles with diameter of 3.2 mm. They are put into a vial eppendorf

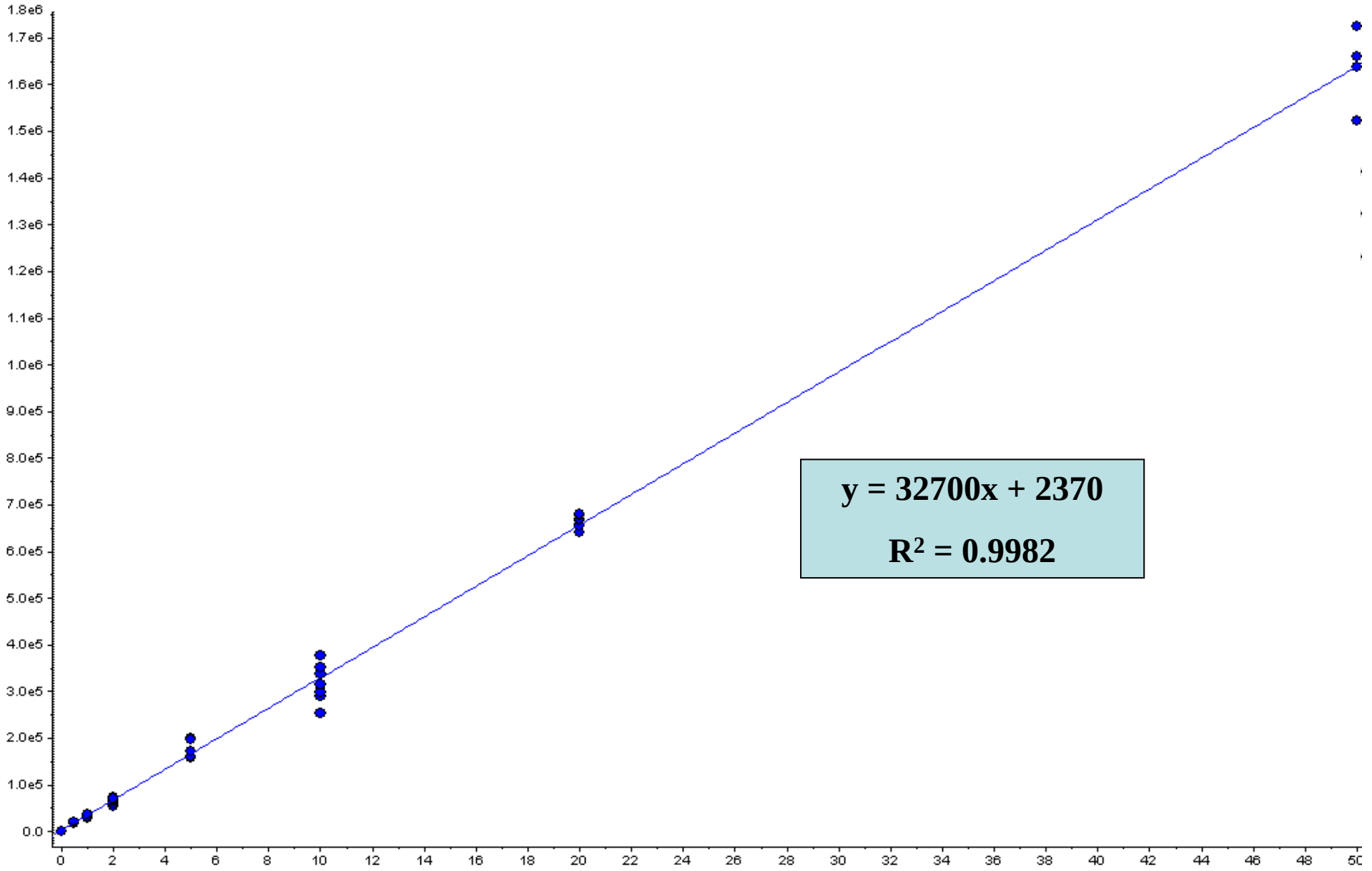
200 µL of $H_2O$-$CH_3CN$ +0.05% formic acid 30:70 20 min Orbital Shaker



injection 5µL on Phenomenex Synergi 4u POLAR-RP 80A 4µm, 2 x 150 mm coupled to TIS-tripleQuad (PE Sciex) MS/MS system


XIC of +MRM (2 pairs): 340.0/264.0 amu from Sample 47 (20ng µL) of DataSET1.wiff (Turbo Spray)
Max. 1.4e5 cps.
Intensity, cps
Time, min
2.23
Blood sample spiked
with 20 ng/µL of TPM
XIC of +MRM (2 pairs): 340.0/264.0 amu from Sample 3 (Top10ngmL acqua) of DataSET1.wiff (Turbo Spray)
Max. 9.1e4 cps.
Intensity, cps
Time, min
2.23
Acqueous standard of
TPM:10 ng/µL


Topiramate
[M-76+H]+
SRM: 340m/z ⟶ 264m/z
[M+H]+
Intensity, cps
m/z, amu
D12-Topiramate
[M-82+H]+
[M+H]+
Intensity, cps
m/z, amu

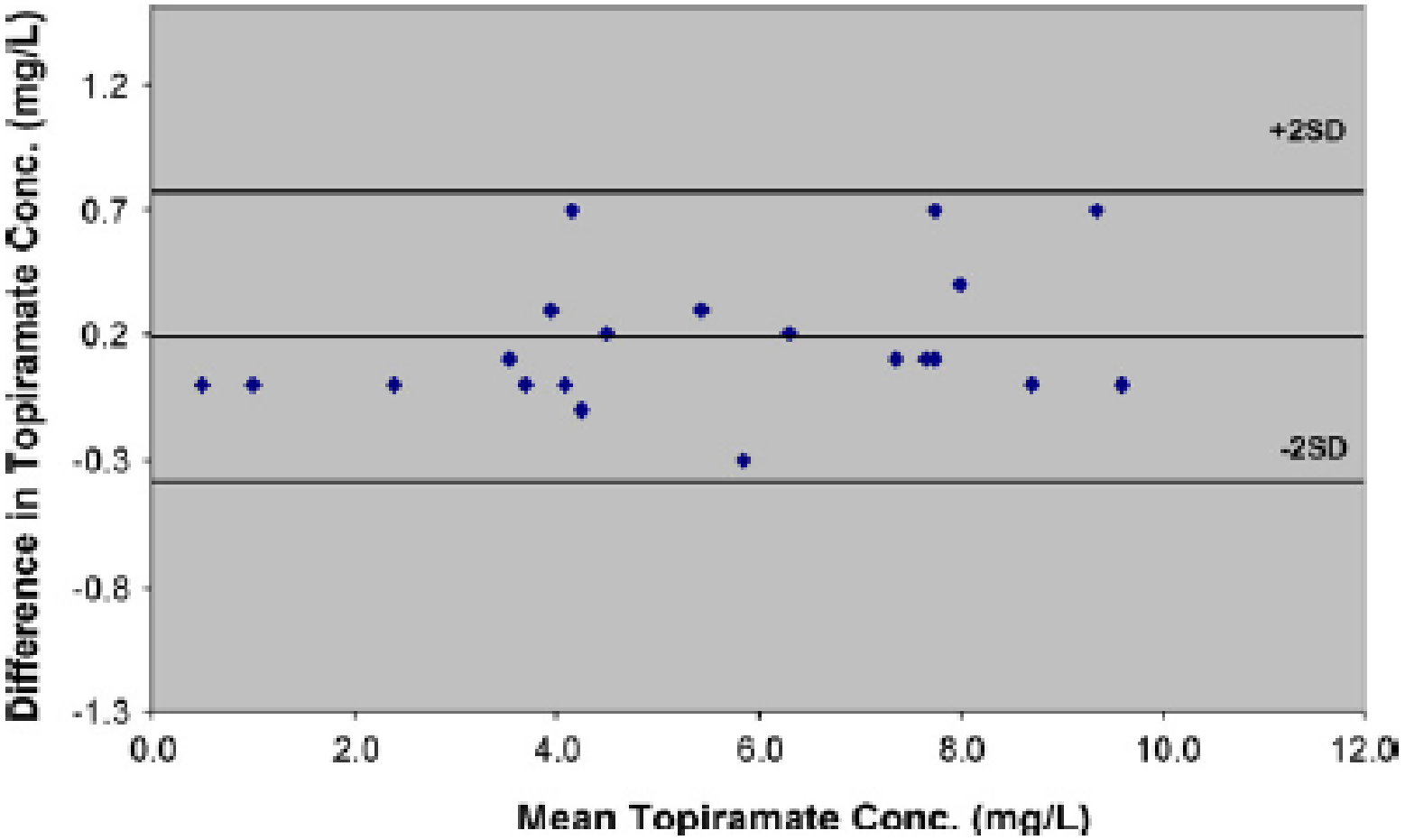

1.8e6
1.7e6
1.6e6
1.5e6
1.4e6
1.3e6
1.2e6
1.1e6
1.0e6
9.0e5
8.0e5
7.0e5
6.0e5
5.0e5
4.0e5
3.0e5
2.0e5
1.0e5
0.0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
46
48
50
y = 32700x + 2370
R2 = 0.9982

| INTRADAY | | | | |
|---|---|---|---|---|
| Expected Concentration n=7 (ng/µL) | Mean | SD | %CV | Accuracy |
| 0 | 0 | 0 | | |
| 0.5 | 0.51 | 0.05 | 10.32 | 101.24 |
| 1 | 0.91 | 0.11 | 12.37 | 91.06 |
| 2 | 1.95 | 0.22 | 11.27 | 97.30 |
| 5 | 5.42 | 0.56 | 10.36 | 108.45 |
| 10 | 9.63 | 1.25 | 12.97 | 96.33 |
| 20 | 20.20 | 0.43 | 2.12 | 100.99 |
| 50 | 49.92 | 2.58 | 5.16 | 99.84 |

| INTERDAYS (n=4) | | | | |
|---|---|---|---|---|
| Expected Concentration n=5 (ng/µL) | Mean | SD | %CV | Accuracy |
| 0 | 0 | 0 | | |
| 0.5 | 0.57 | 0.05 | 9.49 | 113.16 |
| 1 | 1.10 | 0.10 | 9.09 | 110.49 |
| 2 | 2.05 | 0.15 | 7.40 | 102.45 |
| 5 | 4.81 | 0.38 | 7.76 | 96.23 |
| 10 | 9.33 | 0.85 | 9.12 | 93.33 |
| 20 | 19.95 | 0.88 | 4.35 | 99.76 |

**LOD (S/N > 3) in DBS 3.32 ng/mL**
**LOQ (S/N > 10) in DBS 16.6 ng/mL**

## Analisi comparativa con metodo di riferimento FPIA (21 campioni)




**TPM serum=DBS/(100 - HT)**

**100**

$$\left(\mathrm{DBS}_{[\mathrm{analyte}]}/[1\text{-hematocrit}]\right)\times(1-f_{BC})=\mathrm{plasma}_{[\mathrm{analyte}]}$$

where $f_{BC}$ is the fraction of analyte bound to blood cells,
could be obtained by an *in vitro* test.

**STUDI DI FARMACOCINETICA NEI NEONATI O NEI BAMBINI PICCOLI**

**ETICAMENTE E TECNICAMENTE IMPOSSIBILI**

*Epilepsia*, **(*):1–7, 2009
doi: 10.1111/j.1528-1167.2009.02302.x

FULL-LENGTH ORIGINAL RESEARCH

# Topiramate concentrations in neonates treated with prolonged whole body hypothermia for hypoxic ischemic encephalopathy

*Luca Filippi, †‡Giancarlo la Marca, *Patrizio Fiorini, *Chiara Poggi, §Giacomo Cavallaro, †Sabrina Malvagia, ‡Domenico E. Pellegrini-Giampietro, and ¶Renzo Guerrini

*Neonatal Intensive Care Unit, Department of Critical Care Medicine, "A. Meyer" University Children's Hospital, Florence, Italy; †Metabolic Unit, Department of Pediatric Neurosciences, "A. Meyer" University Children's Hospital, Florence, Italy; ‡Department of Pharmacology, University of Florence, Florence, Italy; §Institute of Pediatrics and Neonatology, Fondazione IRCCS Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, University of Milan, Milan, Italy; and ¶Department of Neurology and Neurosurgery, "A. Meyer" University Children's Hospital, Florence, Italy

TPM concentrations were measured at the beginning of hypothermia, before the first dose of TPM ($T_0$), and at $T_{0.5}$, $T_1$, $T_{1.5}$, $T_2$, $T_4$, $T_6$, $T_8$, $T_{12}$, $T_{16}$, $T_{20}$, and $T_{24}$ (before the second dose), at $T_{26}$, $T_{30}$, $T_{34}$, $T_{40}$, and $T_{48}$ (before the third dose), and at $T_{48.5}$, $T_{49}$, $T_{49.5}$, $T_{50}$, $T_{52}$, $T_{54}$, $T_{56}$, $T_{60}$, $T_{64}$, $T_{68}$, and $T_{72}$. All of these four newborns were also on PB.

**Table 2. Mean ± SD topiramate pharmacokinetic profiles of newborns who reached the virtual steady state. Data are plotted that distinguish between newborns in DH or MH and between newborns receiving PB or not**

| | All newborns (n = 9) | Newborns with DH (n = 3) | Newborns with MH (n = 6) | p-value | Newborns with PB (n = 4) | Newborns without PB (n = 5) | p-value |
|---|---|---|---|---|---|---|---|
| $C_{max}$, mg/L, mean ± SD | 17.96 ± 4.2 | 17.87 ± 6.4 | 18.71 ± 3.2 | 0.219 | 15.38 ± 5.3 | 19.87 ± 1.9 | 0.060 |
| $C_{min}$, mg/L, mean ± SD | 10.35 ± 2.5 | 10.54 ± 3.2 | 10.77 ± 1.9 | 0.199 | 8.70 ± 2.9 | 11.67 ± 0.9 | 0.032 |
| $T_{max}$, h, mean ± SD | 3.80 ± 2.2 | 4.00 ± 1.1 | 4.08 ± 2.7 | 0.333 | 3.13 ± 2.4 | 4.40 ± 2.2 | 0.216 |
| $AUC_{0-24}$, mg/L/h, mean ± SD | 343.2 ± 72.2 | 318.1 ± 101.6 | 366.2 ± 48.1 | 0.096 | 302.4 ± 89.7 | 375.8 ± 37.4 | 0.068 |
| $C_{avg}$, mg/L, mean ± SD | 14.29 ± 3.0 | 13.25 ± 4.2 | 15.26 ± 2.0 | 0.096 | 12.60 ± 3.7 | 15.66 ± 1.6 | 0.068 |
| $T_{1/2}$, h, mean ± SD | 35.58 ± 19.3 | 48.82 ± 4.6 | 29.03 ± 23.8 | 0.080 | 26.46 ± 17.7 | 42.88 ± 19.1 | 0.113 |
| CL/F, ml/kg/h, mean ± SD | 15.42 ± 4.6 | 15.72 ± 7.3 | 13.87 ± 1.9 | 0.084 | 17.92 ± 6.2 | 13.42 ± 1.4 | 0.078 |

DH, deep hypothermia; MH, mild hypothermia; PB, phenobarbital; $C_{max}$, maximal plasma concentration; $C_{min}$, minimal plasma concentration; $T_{max}$, time of peak concentrations; $AUC_{0-24}$, area under plasma concentration-time curve from 0 to 24 h; $C_{avg}$, average plasma concentration; $T_{1/2}$, half-life; CL/F, apparent oral clearance.

# A new rapid micromethod for the assay of phenobarbital from dried blood spots by LC-tandem mass spectrometry

*†‡Giancarlo la Marca, *‡Sabrina Malvagia, §Luca Filippi, ¶Francesca Luceri, #Gloriano Moneti, and *‡**Renzo Guerrini

*Mass Spectrometry and Pharmacology Laboratory, Pediatric Neurology Unit and Laboratories, Department of Neurosciences, A Meyer Children's Hospital, Florence, Italy; †Department of Pharmacology, University of Florence, Florence, Italy; ‡Pediatric Neurology Unit and Laboratories, A. Meyer Children's Hospital, Florence Department of Neurosciences, University of Florence, Florence, Italy; §Neonatal Intensive Care Unit, A. Meyer University Children's Hospital, Florence, Italy; ¶General Laboratory, Azienda Ospedaliera Careggi, Florence, Italy; #CISM, Mass Spectrometry Center, University of Florence, Florence, Italy; and **IRCCS Fondazione Stella Maris, Pisa, Italy

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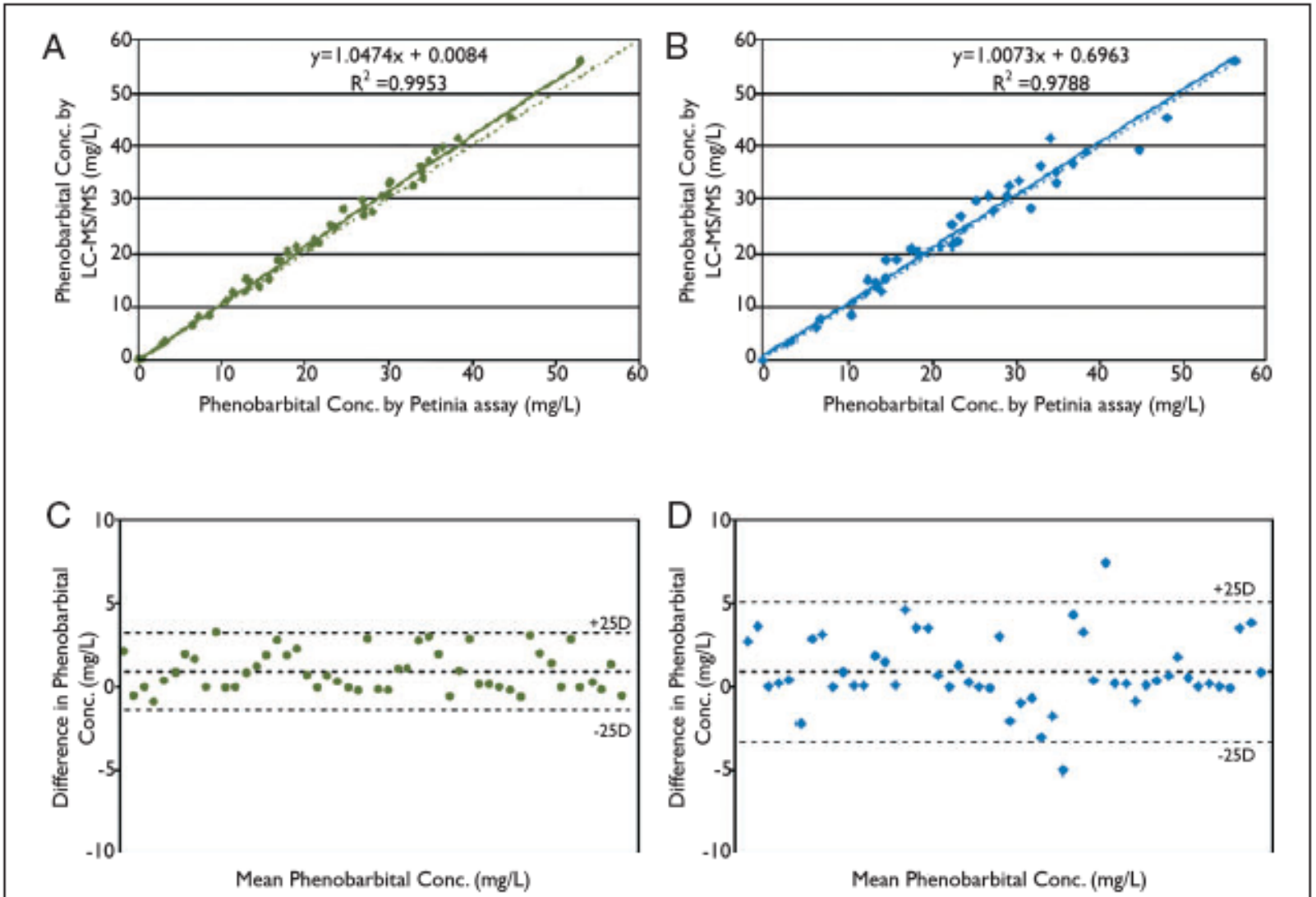


**Figure 1.**
LC-MS/MS versus Petinia: (**A**) and (**C**) adjusted for measured haematocrit. (**B**) and (**D**) adjusted for theoretical haematocrit (40% in adults; 55% in newborns). The dashed line is the line of identity, and the solid line is the regression line.

# CONCLUSION

**The analysis performed on DBS allows a great advantage in terms of costs, affordability, easiness of sampling, especially in young children.**

**Because of improved specificity, sensitivity and decreased sample volume requirements, this LC-MS/MS method should be particularly useful for monitoring TPM therapy in pediatric patients, for PK and research studies.**